FORTITUDE OF NUTRITIONAL CAPACITY, TOTAL POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF DIVERSE VARIETIES OF ALMOND (*PRUNUS DULCIS* MILL. D.) FROM SELECTED AREAS

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Abstract. Natural antioxidant use has increased as a consequence of consumer desire for natural and organic foods. Almonds (*Prunus dulcis* Mill. D.) are an important food item both nutritionally and medicinally. The health advantages attributed to these, and the value-added products developed from them have been linked to the antioxidant activities of phenolic compounds in nuts. The nutritional characteristics, total phenolic contents, and antioxidant activity of almond with brown skin and without skin were examined in the current study. The findings of the nutritional facts support the possibility of using almonds with brown skin in the food sector and they propose that almonds are a significant source of energy, protein, fiber, and lipid. The results of phenolic content of almond that of with skin ranged from 240.6±3.2–295.5±4.2 and without skin were ranged from 232.3±2.8–278.4±3.6 mg GAE/100 g. The inhibition of DPPH radical scavenging capacity varied over 37.60–65.09% of almond with skin and 28.83–45.51%, without skin at concentration of 5 mg/ml. The reducing power (absorbance 700 nm) increased as the concentration increase in all varieties however these activities were higher in skin almond of all varieties. This study showed that almonds are excellent sources of nutrients and antioxidants that have a potential for value addition and nutraceutical development.
Keywords: *almond, ecology, nutrients, polyphenols, DPPH, RPA*

Introduction

The almond nuts *Prunus dulcis* (Mill.) D. A. Webb. cv. Pakistani, *Prunus dulcis* (Mill.) D. A. Webb. cv. American, *Prunus dulcis*. cv. Irani, and *Prunus dulcis* cv. Kaghzi are abundantly available in Pakistan and these are species of *Purnus* belonging to the family *Rosaceae* (*Fig. 1*). In the globe, 26 species of almonds collectively make up a separate and recognizable taxonomic category. Due to its rusticity and its adaptation to drought and arid climate almond tree becomes the main nut tree in the Mediterranean zones. Natural and artificial selection have been combined to create widely used variants (Perez de Los et al., 2021). Around 1.7 million metric tons of almonds are produced worldwide

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each year and 80% of the almonds grown worldwide are in California (FAO-Stat, 2020). Over 50% of the weight of almond seeds is made up of lipid, which is found in intracellular oil bodies. 22–25% of the seed is made up of proteins, while 11–12% is made up of dietary fiber (House et al., 2019).

Figure 1. *Almond tree, fruit, and Seeds*

Due to its numerous health advantages, such as anti-inflammatory, antihepatotoxic, immunity-boosting and antioxidant properties, almond oil is used in a variety of industries, including food, cosmetics, and alternative medicine (Lin et al., 2018; Moore et al., 2020). Additionally, the lipid composition of almond oil is rich, with monounsaturated (63.42–78.03%) and polyunsaturated (14.41-27.01%) fatty acids (Summo et al., 2018; Oliveira et al., 2019). Moreover, this priceless nut provides a good dietary source of antioxidants like tocopherols, polyphenols, hydrolysable tannins, proanthocyanin, stilbene, phenolic acids, prunasin and flavonoids (catechin, protocatechuic acid, prenylated benzoic acid, 2-prenylated benzoic acid, 2-prenyl-4-O-dglucopyranosyl-oxy-4-hydroxybenzoic acid) (*Fig. 2*). These are exclusively present in the vegetative sections of the almond plant (Xiang et al., 2023; Zhang et al., 2023; Aires et al., 2018; Kahlaoui et al., 2019).

Antioxidants are substances that, when present in small quantities compared to an oxidizable substrate, can greatly slow down or prevent the substrate's oxidative destruction. Lipids, DNA, and proteins are the substances in human systems that are most vulnerable to oxidative harm. According to Ghanaian et al. (2019) damage to these oxidizable substrates has been linked to the possible onset and progression of a variety of chronic disorders, including rheumatoid arthritis, inflammatory bowel disease and Parkinson's disease. Dietary antioxidants play a significant role in the sophisticated antioxidant defense that the human physiology has developed in reaction to these potentially harmful impacts (Aziz et al., 2023b; Aziz et al., 2024). A growing variety of chronic illnesses, including cardiovascular disorders are being linked to oxidative damage as a common pathogen (Uddin et al., 2020; Riaz et al., 2021).

Consumers are known to believe that medications made from plant sources are safer and healthier than those made from synthetic materials, hence efforts to replace synthetic antioxidants with natural additions have increased (Raymond, 2019). Because of this, investigations on the screening of medicinal plants having functional chemicals that give antioxidant capabilities have recently been crucial in order to uncover novel and effective sources (Al-Dabbas, 2017; Saeed et al., 2023). Diverse breed of almonds have more disparity in genes opposing to aridity, saltiness, pests, diseases and other factors such as quality, yield, taste & flavor. In order to compare the nutritional facts and the antioxidant

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activity of these different varieties of almond with skin and without skin were selected for this study. The goals of this study were (1) to establish the nutritional content of various almond varieties with skin and without skin (2) to measure its total phenols (3) to ascertain its free radical scavenging capacity (4) to estimate its reducing power capacity and (5) to compare the nutritional and antioxidant activity of various varieties of almonds.

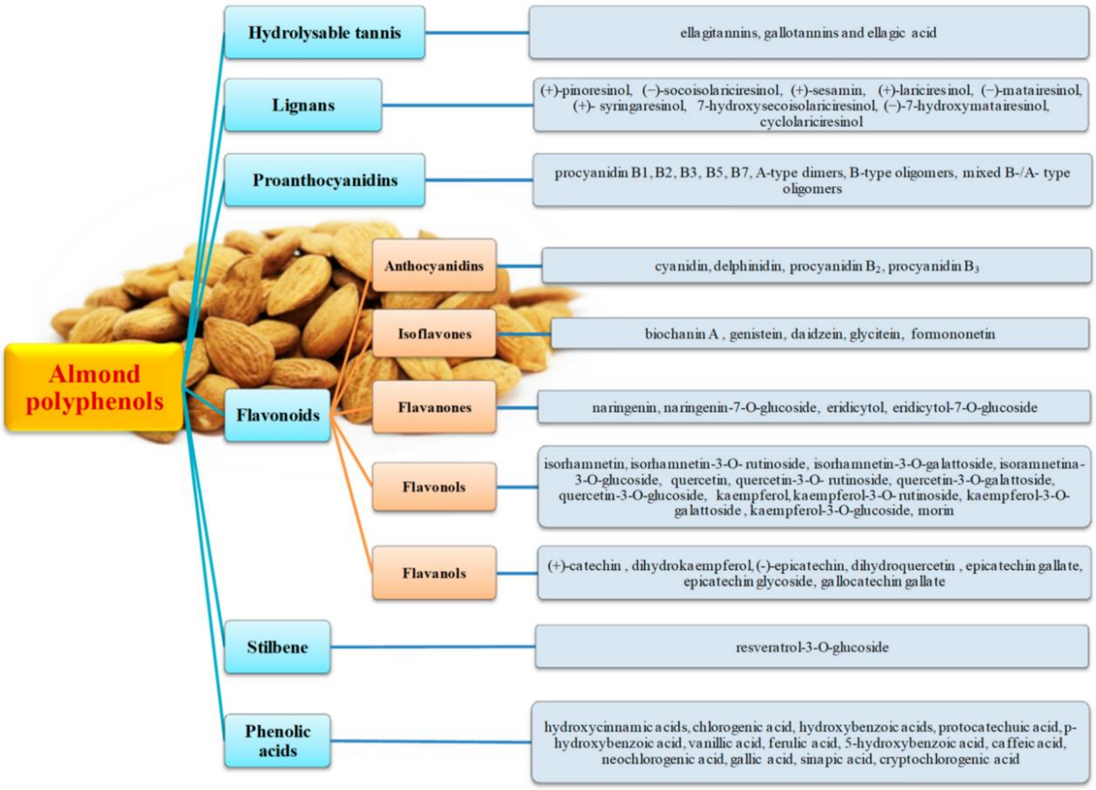

Figure 2. *Main polyphenols present in whole almonds (Source: Barreca et al., 2020).*

Materials and Methods

Collection of samples

Almond samples (Pakistani, American, Irani and Kaghazi) were purchased from local dry fruit market of Lahore, Pakistan in January 2023 which was harvest in October-November 2022. Almonds that had been manually shelled were dried in ambient temperatures and they were kept in bottles with tight closures which were used throughout the experiment.

Chemicals and reagents

All the reagents used in the current research were analytical grade from Merck or Sigma. 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH), Folin-Ciocalteu reagent were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals (analytical grade) that are anhydrous sodium carbonate, sodium hydroxide, sodium nitrite, ferrous chloride, ammonium thiocyanate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate used in this study were purchased from Merck (Darmstadt, Germany).

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Fortitude of nutritional capacity

According to the AOAC (2016) methods, the moisture, ash, fat, fiber, protein and carbohydrate were determined.

Extracting antioxidants from almonds

Using 200 mL of methanol and water the grinded almonds (50 g) were extracted in an orbital shaker at room temperature for 6 hours. By filtering through filter paper (Whatman No. 1), the residues were removed from the extracts; the residues were then extracted once again using new both solvents. The extracted materials from both extractions were combined and a rotary evaporator was used to remove the solvent under vacuum and at 45°C. The obtained semi-solid crude concentrated extracts were saved and kept at -4°C until employed in further studies i.e. polyphelic content and antioxidant study. (Sultana et al., 2009).

Determination of total phenolics content

The quantity of TP was determined with Folin-Ciocalteu reagent following the method as described by Singlaton and Rossi (1965) with slight modification (Saeed et al., 2021). In this experiment, 0.1 mL of almond extract was completely mixed with 0.5 mL Folin-Ciocalteu reagent after 5 minutes. Then 1.4 mL of 7.5% sodium carbonate (Na_2CO_3) was added and the mixture was left to react for 90 min at room temperature. The absorbance was measured at 760 nm using spectrophotometer (UV-1700, Shimadzu Japan). The results were expressed as gallic acid equivalents (GAE) mg/100 g of dry matter.

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the extracts was evaluated using a method similar to that previously utilized (Brand-Williams, 1995) with a few minor modifications made by Saeed et al. (2018). Briefly, 100 µl almond extracts containing 5 mg/mL of dry mass, then added 2.9 ml of freshly prepared DPPH free radical solution (0.004% in methanol). After 30 minutes of incubation at 50 °C, the reaction mixture's absorbance was measured at 517 nm with a spectrophotometer (1700, Shimazdu, Japan). The following equation was used to compute the percent DPPH radical scavenging activity:

$$I\% = 100 - (A_{blank} - A_{sample})/A_{blank} \quad \text{(Eq.1)}$$

In this equation A_{blank} denotes the absorbance of control while A_{sample} is the absorbance of the test reaction mixture.

Reducing power

The reducing power activity of almond with brown skin and without skin were measured using the Oyaizu 1986 technique. Briefly phosphate buffer (1 mL, 0.2 M, pH 6.6), potassium ferricyanide (1.0 mL, 10 mg/mL), and almond extracts (1 mg/mL) were combined and incubated at 50 °C for 20 minutes. Then trichloroacetic acid (1.0 mL, 100 mg/mL) was added to the mixture and centrifuged for 5 minutes at 10,000 ×g. The supernatant (1.0 mL) was mixed with distilled water (1.0 mL) and ferric chloride (0.1 mL, 1mg/mL) and then the absorbance was measured at 700 nm.

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Statistical analysis

Every experiment had at least three replicates. The significance of findings between treatments was determined using one-way ANOVA. SPSS v.11.5 was used for all statistical analysis (Steel et al., 1997).

Results and Discussion

Almonds are tree nuts that are recognized as a healthy snack. They are rich in protein, monounsaturated fatty acids, dietary fiber (insoluble/soluble fiber at a 4:1 ratio), vitamin E, riboflavin, essential minerals (manganese, magnesium, copper, phosphorus) and a daily intake of 30-50 g is recommended as part of a healthy diet (Gama et al., 2018). The nutritional components (moisture, ash, fat, fiber, protein, and carbohydrates) of various varieties of almond with skin and without skin available in Pakistan were determined and the findings are reported in *Tables 1-4.*

Table 1. *Nutritional Facts of American Almond*

Sr. No.	Parameters	% Value (with skin)	% Value (without skin)
1	Moisture	4.01 ± 0.90	5.39 ± 1.20
2	Ash	2.00 ± 0.14	2.07 ± 0.15
3	Fat	51.00± 3.15	47.80± 2.9
4	Fiber	10.82 ± 1.35	1.98± 0.12
5	Protein	19.10 ± 2.18	21.76±2.28
6	Carbohydrates	13.17± 1.49	21.00±2.26

Data are represented ± SD

Table 2. *Nutritional Facts of Pakistani Almond*

Sr. No.	Parameters	% Value (with skin)	% Value (without skin)
1	Moisture	4.65 ± 0.95	4.63 ± 0.95
2	Ash	2.68 ± 0.17	2.63 ± 0.17
3	Fat	55.00 ± 3.20	49.79 ± 3.12
4	Fiber	12.25 ± 1.40	1.88 ± 0.11
5	Protein	18.06 ± 2.14	21.28 ± 2.26
6	Carbohydrates	7.35 ± 1.28	20.24 ± 2.21

Data are represented ± SD

Table 3. *Nutritional Facts of Irani Almond*

Sr. No.	Parameters	% Value (with skin)	% Value (without skin)
1	Moisture	5.28 ±1.08	5.23±1.16
2	Ash	2.40 ± 0.15	2.12 ± 0.14
3	Fat	54.90 ± 3.20	49.00± 3.12
4	Fiber	12.21 ± 1.40	2.01 ± 0.10
5	Protein	18.36 ± 2.15	20.11 ± 2.22
6	Carbohydrates	6.84 ± 1.24	21.54 ± 2.25

Data are represented ± SD

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Table 4. *Nutritional Facts of Kaghzi Almond*

Sr. No.	Parameters	% Value (with skin)	% Value (without skin)
1	Moisture	5.02 ±1.05	4.79 ± 0.97
2	Ash	2.77 ± 0.18	2.28 ± 0.13
3	Fat	54.60 ± 3.16	48.90 ± 3.12
4	Fiber	12.59 ± 1.45	2.05 ± 0.12
5	Protein	18.63 ± 2.17	20.36 ± 2.24
6	Carbohydrates	6.38 ± 1.20	21.69 ± 2.25

Data are represented ± SD

Moisture content of the investigated samples were ranging from 4.01± 0.90–5.28±1.20 in almond with brown skin and 4.63±1.16%–5.39±1.23% in almond without skin. The ash content data indicated a non-significant amongst the studied samples, with the lowest value of 2.07±0.15% without skin and 2.00±0.15% with skin found in American almond. The ash concentration was lower than that reported by Simsek and Kizmaz (2017) in Turkish almond (2.45–4.42%). In terms of crude fat, almond kernels are known to be unusually high in oil, ranging from 48 to 67 g/100 g (Sakar et al., 2017; Simsek et al., 2018).

The protein levels obtained in this study varied from 18.06±2.14–19.10±2.18% in almonds with skin and 20.11±2.22–21.76±2.28% in almonds without skin and non-significant difference were found. According to these findings, almonds' high protein content might be utilized as a dietary supplement to combat protein deficit as well as for patients suffering from hypertension. According to Socias Company and Gradziel (2017) protein content of almond ranged from 10 to 35% on average.

The crude fiber content in almond with skin ranges from 10.82±1.35–12.59±1.45%, whereas it was in almond without skin, ranging from 11.88±0.12%–2.05 ± 0.15%. In our study the value of fiber content of in almond without skin was lower than observed by Falaye and Elezuo (2018). There was a significant difference between of fiber between skin and without skin almonds. This variation could be explained by environmental effect such as climatic conditions including weather and temperature variation, soil characteristics, growing conditions and also agronomic practices (Ibourki et al., 2022). The carbohydrates content of the almond was found to be in a range of 7.35±1.2–21.69±2.50 and total carbohydrates content in almond with skin usually ranged from 14 to 28% (Roncero et al., 2020). Almond carbohydrates are mostly composed of soluble sugars (primarily sucrose), starch, and other polysaccharides such as cellulose and non-digestible hemicelluloses.

During this investigation, the energy value was also evaluated. Among the investigated samples, the energies values were greater in almonds without skin than in almonds with skin (*Fig. 3*). The lowest value (586.65±8.04 kcal/100 g (with skin); 614.20±9.35 kcal/100 g (without skin) was found in American almonds, while the highest (596.03 ± 8.66 kcal/100 g) was found in Pakistani almonds with skin and in without skin 601.60±9.02 kcal/100 g, followed by Irani almonds (594.9± 8.62 with skin; 608.33±9.22 without skin) and Kaghzi Almonds (591.44±8.53 with skin; 607.76±9.20 without skin). Our findings were remarkably similar to those published by the US Department of Agriculture, Agricultural Research Service (USDA, 2010) and Summo et al. (2018). The almond samples have a high protein and lipid content, which offers energy and power to the human body (Siddiqua et al., 2021).

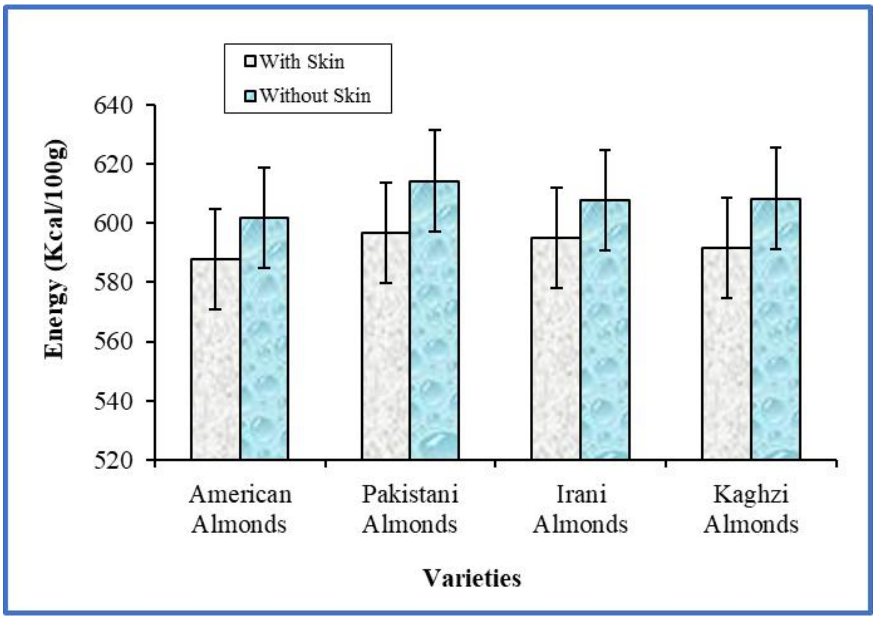

Figure 3. *Energy values of various varieties of almonds with skin and without skin. *Energy values was higher in almond without skin in all varieties*

Total phenol content

Using gallic acid as a reference, the total phenol content of almonds with skin and without skin was determined. The phenolic content of almond with skin ranged from 240.35±4.34–283.50±5.80 and without skin from 232.30±4.50–262.30±5.38 mg GAE/100 g (*Table 5*). Our results are accordance to Valdes et al. (2022) who depicted that almond are rich in total phenolic content (5.64 mg GAE g^{-1}), moreover these results are accordance to given literature (Gracia et al., 2021; Timon et al., 2022), but lower than Salem et al. (2022) who reported total phenolic content of almond with shells (21.46 mg GAE/g). Our results were similarly lower than those of Yildiz et al. (2014) who revealed that the TPC values in various almonds ranged from 48.36 to 66.97 mg GAE/g. The most active natural antioxidants in plants are phenolic compounds which act as antioxidants due to their propensity to donate hydrogen or electrons and are stable radical intermediates. In general, polyphenolic compounds are abundant in plant exterior layers such as the peel (skin), shell and hull which protect inner materials (Farhat et al., 2020). Our data clearly demonstrated that almond nuts are rich in total phenolic content.

Table 5. *Total polyphenolic content (mg GAE/100 g) of various varieties of Almonds*

Extracts	**Water Extract**		**Methanol Extract**	
	With skin	**Without skin**	**With skin**	**Without skin**
American Almond	264.2±3.6	252.2±0.2	268.3±1.5	262.2±0.3
Pakistani Almond	282.5±3.7	260.6±0.5	295.5±4.2	278.4±3.8
Irani Almond	246.7±3.4	242.6±0.5	256.2±1.8	249.8±3.4
Kaghzi Almond	240.6±3.2	232.3±2.8	248.3±1.5	242.2±3.2

Data are represented as mean ± SD

Assay for radical scavenging using DPPH

Plants are often studied for their antioxidant capacity (Rasheed et al., 2021), and their antioxidant capacity relates to its level of total polyphenolic and total flavonoids contents (Masood et al., 2023). The presence of antioxidants in methanolic and water extract extracts of almonds with brown skin and without skin was studied in this experiment and the findings are shown in *Figures 4 and 5*. Efficacy of the employed almond varieties of potent antioxidant components followed the order: methanolic extract with skin Pakistani variety (65.09% I) > American variety (56.62% I) > Irani variety (50.72% I) > Kaghzi variety (42.25% I). While methanolic extract without skin Pakistani variety (61.50% I) > American variety (52.60% I) > Irani variety (47.46% I) > Kaghzi variety (37.62% I). Water extract with skin Pakistani variety (55.61% I) > Amerikan variety (48.6% I) > Irani variety (42.71% I) > Kaghzi variety (37.6% I). While water extract without skin Pakistani variety (45.14% I) > Amerikan variety (40.14% I) > Irani variety (36.67% I) > Kaghzi variety (28.83% I). These results are same as described by Ozer (2017) who estimated the percentages of DPPH scavenging activity of almond 45.5 ± 0.6 in water extract. Our findings are also consistent with the findings in the literature on the DPPH radical scavenging activity of almond nuts (Nasreen et al., 2012; Win et al., 2019).

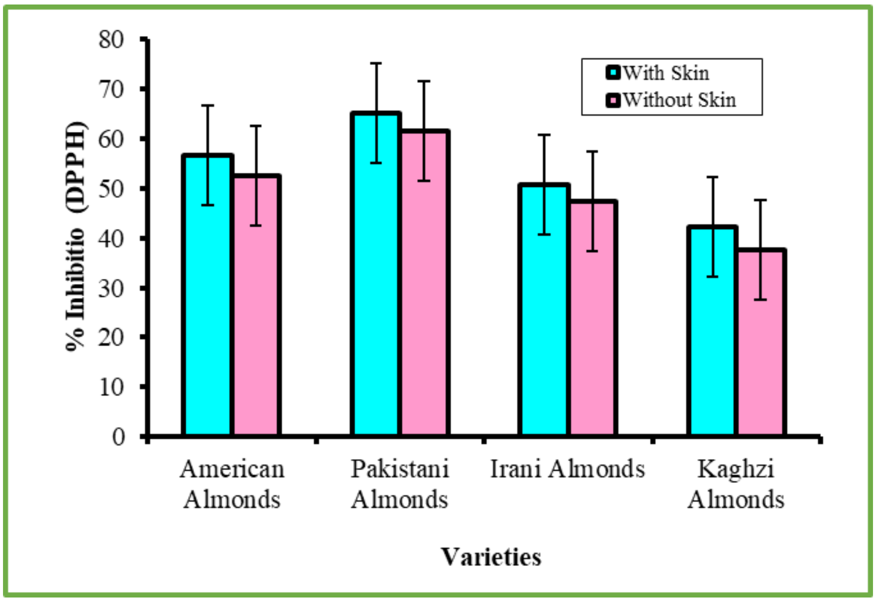

Figure 4. *% Inhibition of methanol extract of almond with skin and without skin*

This activity was determined using the DPPH, which is a violet-colored stable organic free radical with an absorption maximum between 515 and 528 nm. When proton is received from hydrogen donor chemicals (phenolics), it loses its chromophore and turns yellow (Benkhira et al., 2022). DPPH free radical scavenging capability increase when phenolic compound content or degree of hydroxylation rises (Saeed et al., 2021; Javed et al., 2022). The current study's findings show that almond methanol and water extract have considerable antioxidant capacity. These actions are caused by the existence of various active molecules in almond nuts, which have antioxidant properties and may have many therapeutic uses.

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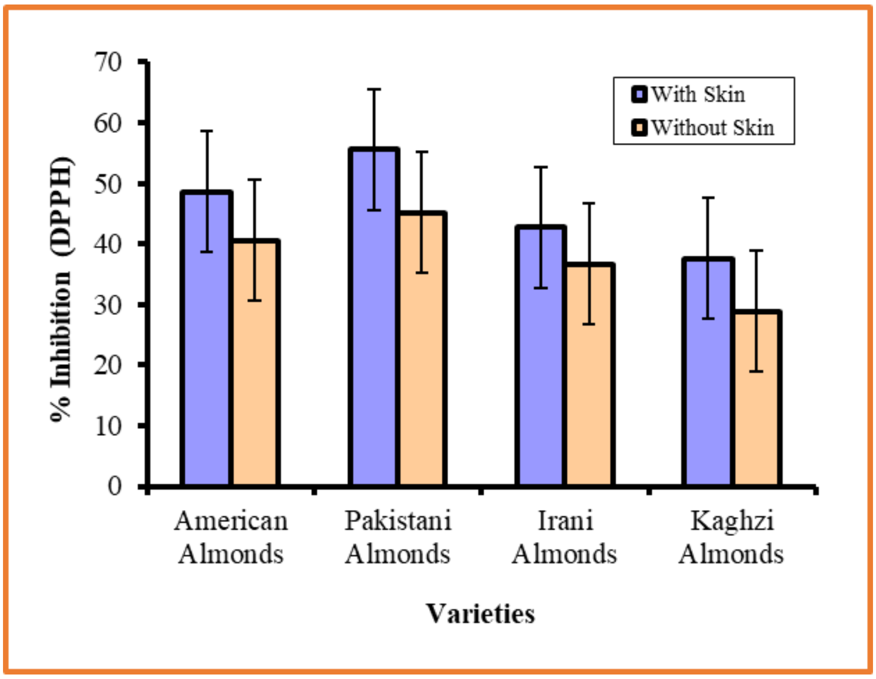

Figure 5. *% Inhibition of water extract of almond with skin and without skin*

Reducing power assay

The reducing antioxidant power assay is a popular method for evaluating the presence of antioxidant molecules in a sample, in which antioxidants function as reductants in a redox-linked colorimetric reaction (Jomi et al., 2022). Mechanistically, the reductive capacity of Fe^{+3}/Fe^{+2} conversions in the presence of almond extracts was examined in this approach, with the resulting colour shift from yellow to blue green (Hejna et al., 2021). The reducing potential of the compounds present in the reaction media directly influences the intensity of colour. Increased colour intensity correlates with stronger absorption, which correlates with higher antioxidant activity (Thasneem et al., 2022). The extracts' reducing potential at concentrations ranging from (1 to 5 mg/mL) increased in a concentration-dependent way. At a concentration 5 mg/mL the reducing potential in methanol extract varied from 0.101 to 1.751 (absorbance values) in almond with skin (*Fig. 6a*), whereas it ranged from 0.092 to 1.489 in almonds without skin (*Fig. 6b*). Similarly, the reducing potential in aqueous extract ranged from 0.073 to 1.351 in almond with skin (*Fig. 6c*), whereas it ranged from 0.061 to 1.238 in almond without skin (*Fig. 6d*). Methanolic extracts had a stronger reduction capability than water extracts in both almonds skin or without skin. The statistical analysis revealed that the reduction potential varied significantly depending on the extraction solvent and types studied. Esfahlan and Jamei (2012) reported the reducing power absorbance of various almond species ranging from 0.389 to 0.883, while Isfahlan et al. (2010) reported the reducing power of almond shell extracts extracted with methanol to be 0.151 to 0.228, both of which were lower than the values found in the current study. The high polyphenols content of almond's extracts appears to be responsible for electron donating to end the radical chain reaction (Meshkini, 2016; Naveed at al., 2022; Aziz et al., 2023; Atta et al., 2023; Saleem et al., 2023).

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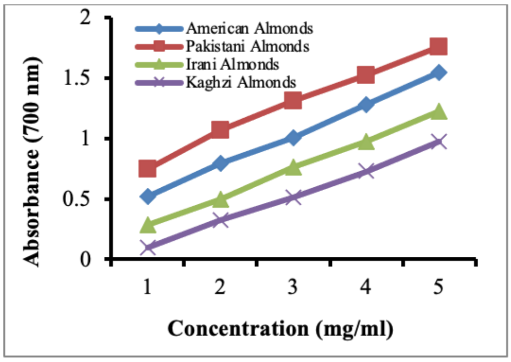

Fig. 6a. RPA of methanol extract of almond with skin

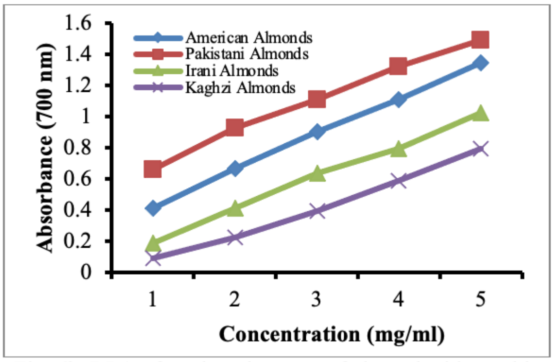

Fig. 6b. RPA of methanol extract of almond without skin

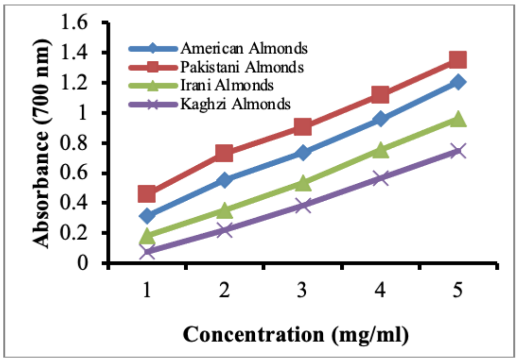

Fig. 6c. RPA of water extract of almond with skin

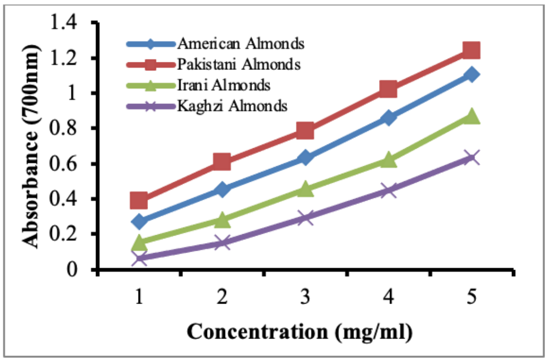

Fig. 6d. RPA of water extract of almond without skin

***Figure 6.** RPA of methanol and water extracts of almond with and without skin*

Conclusion

The potential of skinned almonds in their native state as a natural antioxidant in meals was investigated in this study. The data obtained in this study demonstrated that almonds with skin contain the highest nutrients and antioxidant activity due to the presence of phenolic content in good quantity. We conclude that the use of almonds with skin has more health benefits and further research on the isolation of bioactive components can reveal the exact potency of almonds recommended.

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