

COMPARATIVE ANALYSIS OF NUTRITIONAL AND ANTIOXIDANT CHARACTERISTICS OF DIFFERENT PAKISTANI AND EXOTIC ALMONDS

ABSTRACT

Health boosting potential of almonds is attributed to its antioxidant potential and rich nutritional profile containing a blend of multifarious nutrients and phytochemicals. These traits considerably vary amongst different genotypes and varieties. Hence, current research was planned to explore the basic nutritional composition and antioxidant potential of four different almond genotypes *i.e.* Patasa & Talwar (Pakistani) and Abdul Wahidi & Katha (Afghani). Amongst, Talwar and Abdul Wahidi showed better nutritional profile followed by Patasa and Katha. Proximate analysis revealed higher quantities of oil in Talwar and Abdul Wahidi *i.e.* 51.12 ± 0.97 and $50.64 \pm 0.86\%$, respectively. Katha contained higher ash contents. Likewise, Abdul Wahidi and Talwar were also high in fiber content as 13.11 ± 0.39 and 12.45 ± 0.37 , correspondingly. Substantial amount of protein was quantified in Abdul Wahidi. Talwar almonds were proved as a good source of potassium and magnesium, 14.02 ± 1.12 mg/g and 3.10 ± 0.24 mg/g, respectively. Likewise, Abdul Wahidi contained higher amount of phosphorous, 9.17 ± 1.10 mg/g. However, Patasa were high in calcium. HPLC based quantification showed that vitamin-E (α -tocopherol) was highest in Patasa trailed by Abdul Wahidi and Talwar. However, Katha almonds were low in vitamin-E. *In vitro* analysis exhibited Talwar and Abdul Wahidi with highest flavonoids contents. Whereas, Patasa and Abdul Wahidi almonds had promising amounts of total phenolics. Antioxidant assays (DPPH and ABTS) explicated better radical scavenging activity by Talwar and Abdul Wahidi almonds whilst Katha almonds showed better performance for FRAP assay.

Keywords: Almonds, nutritional composition, antioxidant potential

1. INTRODUCTION

Scientific studies support the positive role of plant-based foods in reducing oxidative stress-mediated metabolic disorders arising due to imbalance between free radicals and cellular antioxidants' defense mechanism. In this context, numerous fruits, especially dry fruits and nuts have been explored possessing health-boosting potential. The dry fruits have been consumed since the primordial times and are known for their rich nutritional profile and unique bioactive diversity. Versatility in genotypes, varietal differences, variable climatic conditions and geographical locations are the major determinants of difference in nutritional composition and physical traits of the same fruit. Almond fruit is valued globally due to its delicious taste, fascinating nutritional profile and health boosting perspectives [1].

Almond (*Prunus dulcis*) belongs to the family Rosaceae, grown in warm dry regions of the world. Due to subgenus *Amygdalus*, almond is also known as *Prunus amygdalus*. Though almonds are not considered as a major crop in Pakistan, still it is being grown at an adequate scale [2]. Several almond genotypes are being grown in Pakistan, nonetheless, Patasa and Talwar are the promising genotypes based on their market. Amongst other almond genotypes, Abdul Wahidi and Katha Badam (Afghani genotype) also have high market value and popularity. Variable physical and sensorial properties make the almonds quite suitable ingredient to be used in an array of designer food products with health boosting potential. Nutritional mapping is also vital to categorize almonds for their health promoting potential [3].

As far as the nutritional profile is concerned, almonds contain about 4.4% readily available sugars particularly glucose, fructose and sucrose which are an instant energy source for the body. Besides, almonds also contain dietary fiber and significant amount of vitamins & minerals that enhance its nutritional worth [4]. It contains a range of minerals such as sodium, iron and zinc *etc.* but the major minerals abundantly found in almond kernel include potassium, magnesium, calcium and phosphorous. Almond kernel is high in oil which constitute around 49-50% of the total kernel mass. Almond oil is known to be high in omega-6 monounsaturated fatty acids (MUFA) along with sufficient amount of polyunsaturated fatty acids (PUFA) which are recommended by the dietitians and nutritionists for the prevention of atherosclerosis and other related diseases [5, 6]. Moreover, kernel and its peel possess a variety of nutraceutical moieties that impart antioxidant characteristics and disease ameliorating potential. The fruit kernel

contains flavonoids, polyphenols, phenolic amides, dietary fiber and different vitamins especially vitamin-E (tocopherol) *etc.* [7, 8]. A wide range of polyphenolics and flavonoids are present in almonds in significant quantities. All of these biological active constituents make almond a strong antioxidant ingredient of diet [9, 10]. The quantity of nutrients and bioactive constituents in almonds depends upon the variety and environmental conditions. Nutritional composition is one of the most important factors for selection of almonds to be used for a particular purpose. These compositional traits vary amongst varieties and even variations are found within the same variety [11].

Keeping in view the above-mentioned facts, the instant research was designed for comparative assessment of promising almond genotypes *i.e.* Patasa (Pakistani), Talwar (Pakistani), Abdul Wahidi (Afghani) and Katha (Afghani) with special reference to their basic nutritional composition and antioxidant potential.

2. MATERIALS AND METHODS

2.1. Procurement of raw material

All types of almonds were purchased from local market of Faisalabad, Punjab, Pakistan. Almonds of selected types were graded and cleaned. The samples were kept in polyethylene bags at room temperature. The nut samples were shelled to obtain the kernels for extraction to perform different analyses. Standards, analytical and HPLC grade reagents were procured from Sigma Aldrich, Japan and Merck, Germany.

Proximate analyses

The proximate analyses (basic nutritional composition) of almond samples were carried out for moisture content, crude protein, crude fat, total ash contents and nitrogen-free extract following the standard protocols devised by [12].

Moisture content: 10 grams of crushed sample were kept in Hot-Air Forced Oven, (Model: DO1/30-02, PCSIR, Pakistan) at $105 \pm 05^{\circ}\text{C}$ temperature until the weight of samples was found constant. Following expression was used to calculate moisture percentage in the samples;

$$\text{Moisture (\%)} = \frac{\text{Fresh sample weight in grams} - \text{Dried sample weight in grams}}{\text{Fresh sample weight in grams}} \times 100$$

Crude protein: Kjeltex apparatus, (Model: D-40599, Behr Labor Technik, Germany) was used to estimate the crude protein contents in almond samples. Acid digestion of the samples were done in the presence of digestion mixture followed by distillation with base and boric acid. Subsequently, titration against 0.1N acid was done. Nitrogen was calculated as per mathematical expression given below;

$$\text{Nitrogen (\%)} = \frac{\text{Vol. of 0.1N Sulphuric acid used} \times 0.0014 \times 250}{\text{Weight of sample} \times \text{Volume of aliquot}} \times 100$$

$$\text{Crude protein content (\%)} = \text{Nitrogen (\%)} \times 5.18$$

(Conversion factor 5.18 is specified for almonds)

Crude fat: Crude fat content in almond samples was assessed through Soxtec Apparatus (Model: H/2/1045-FOSS Tecator-Denmark). The crushed dried almond sample was put into the thimble. The sample was siphoned for six times with n-hexane solvent. On completion of six cycles, the sample was dried at 105⁰C temperature until constant weight. Crude fat contents were quantified using the following formula;

$$\text{Crude fat contents (\%)} = \frac{\text{Initial weight of almond sample (g)} - \text{Final weight of almond sample (g)}}{\text{Initial weight of almond sample (g)}} \times 100$$

Crude fiber: Crude fiber was analyzed through Fiber-tech apparatus (FT-122/Fibertec-FOSS-Denmark). The defatted and dried sample put to acid digestion, washing with distilled water, alkali treatment followed by a second washing. The treated sample was dried until a constant weight with subsequent incineration until grayish-white color.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of dried sample after digestion (g)} - \text{Weight of ash (g)}}{\text{Initial sample weight (g)}} \times 100$$

Total ash: Charring of dried sample was done in crucible followed by complete incineration in a muffle furnace until grayish-white ash obtained.

$$\text{Ash content (\%)} = \frac{\text{Residues weight after incineration (g)}}{\text{Weight of sample taken initially (g)}} \times 100$$

Nitrogen free extract: Nitrogen free extract in almond samples was determined by using the equation as given below;

$$\text{NFE (\%)} = 100 - (\text{moisture\%} + \text{crude protein\%} + \text{crude fat\%} + \text{crude fiber\%} + \text{ash\%})$$

2.2. Mineral profile

Calcium and potassium were quantified through flame photometer while magnesium and phosphorous were determined through Atomic Absorption Spectrophotometer as per procedures described by AOAC [12]. Standard solutions for each mineral element were prepared by adding 0.5N HCL to 250 μ L of standard stock solution to a volume 25 mL. Afterward, a diacid mixture was prepared by mixing Nitric Acid (HNO₃) and Perchloric Acid (HClO₄) in a 7:3 ratio, respectively. 0.5 g almond sample was added to the mixture and digested with continuous heating until turned colorless, followed by addition of double distilled water to make the volume 100 mL. Finally obtained solution was used to quantify the minerals by using Flame Photometer (Model: BK-FP64-Biobase) and Atomic Absorption Spectrophotometer (Model: AA-6880, Shimadzu-Japan). A 1000 ppm aqueous solution prepared from commercially available stock solution (Merk) was used to prepare calibrated standards for comparison. Highly purified deionized water was used to prepare working standards.

2.3. Preparation of almond extracts

Solvent extraction: For the extraction of bioactives (flavonoids & phenolics), the guidelines of Sireesha, Reddy [13] were followed with slight modification. Purposely, the crushed almond samples were mixed with ethanol-water (50:50) solvent and kept on orbital shaker at 420 rpm for two hours followed by overnight stay time. Afterward, the mixture was filtered and the filtrate was centrifuged at 5000 rpm for ten minutes. The supernatant was taken and solvent was evaporated through a rotary evaporator. The finally obtained extracts were stored in a -40°C freezer.

Almond oil extraction (supercritical fluid extractor): SFE-150 system (SF Tech. Incorporation Delaware, USA) was used to obtain almond kernel oil as per method of Santos, Lorenzo [14] with some modifications. 100 g almond powder was filled in the 150 mL extraction vessel tube of the system. The supercritical CO₂ was directed to enter the tubular extraction vessel set at 45°C. Hexane was used as a co-solvent. The pressure was varied to obtain maximum amount of oil, but average pressure was set at 3000 psi. After giving a rest time of 3 hours, extracts were gathered in a glass tube.

2.4. HPLC quantification of Vitamin-E (tocopherol)

Vitamin-E in the almond oil samples was quantified through HPLC following the method described by Alves, Casal [15] with slight modifications. 2 mL of almond oil was sonicated at 70°C for ten minutes followed by addition of 10 mL of ethanolic KOH (50%) for saponification. After a stay time of 30 minutes, 10 mL of glacial acetic acid was added to neutralize the effect of KOH. The sample was added with 0.5 g of MgSO₄ and mixed well, followed by Millipore filtration (0.45 µm) and dilution with hexane. 0.5 mL heptane was added to the residue and injected in the system. A 50 ppm (50 µg/mL) vitamin-E standard solution was prepared in ethanol. A small amount of acetone was added to aid the dissolution. Methanol-water was used as a mobile phase in a ratio of 95:5 (v/v) with a set flow rate at 1 mL/min. The quantification was done through Zorbax Eclipse-XDB, C₁₈ column (150 × 4.6 mm, 5 µm). Diode Array Detector (DAD) was used and wavelength was set at 293 nm.

***In vitro* studies**

2.5. Phytochemical screening analysis

Flavonoids: The flavonoid contents were spectrophotometrically measured by following the method of Ismail, Chan [16]. Purposely, 1 mL almond extract was poured into a flask and volume was made 5 mL by the addition of distilled water followed by the addition of 5% (w/v) sodium nitrite (0.3 mL). After five minutes, 0.6 mL of 10% (w/v) AlCl₃ was added trailed by 6 minutes stay time. Then, 2 mL of NaOH (1 M) was mixed and 2.1 mL of distilled water was added. Quercetin was used as standard. Absorbance was taken through UV/Visible spectrophotometer at 510 nm wavelength. The results were mentioned in quercetin equivalent in milligrams per gram (QE/g) of extract.

Total phenolic contents: The total phenolics were determined using the Folin-Ciocalteu method as mentioned by [17]. The test was based on a phosphotungstic acid reduction to phosphotungstic blue. Aromatic phenolic groups rise in the number and so the absorbance. Purposely, 250 µL Folin Ciocalteu's reagent was taken in the test tube, 50 µL of almond extract and 750 µL sodium carbonate solution (20%) were added followed by the addition of distilled water to make the volume up to 5 mL. After a stay time of two hours, absorbance was taken through UV/Visible spectrophotometer at 765 nm. The absorbance of control having all reagent except sample extract was also measured. The total polyphenols were calculated using following expression as gallic acid equivalent in mg gallic acid equivalent per gram of extract.

$$C = c \times V/m$$

C = Total phenolic contents (mg per g of extract, in gallic acid equivalent)

c = Gallic acid concentration (mg/mL)

V = Extract volume (mL)

m = Weight of almond extract (g)

2.6. Antioxidant assays

DPPH assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of almond extracts was assessed as per the procedure of Chan and Ismail [18]. 4 mL of almond extract was taken and 1 mL of DPPH was added and incubated for 30 minutes at room temperature. The absorbance was taken through a spectrophotometer at 520 nm wavelength. Following expression was employed to calculate percent inhibition;

$$\text{Absorbance reduction (\%)} = [(AB-AA)/AB] \times 100$$

AB: absorption of blank sample (time = 0 minutes)

AA: Extract solution's absorbance (time = 30 minutes)

FRAP (Ferric reducing antioxidant power): FRAP assay was carried out by adopting the method of [19]. Almond extract's ability to form blue colored ferrous by reducing ferric tripyridyltriazine was measured to assess the reducing power of samples. For preparing FRAP reagent, 2.5 mL of TPTZ (10 mM), 25 mL of 0.1 M acetate buffer and 2.5 mL of ferric chloride (20 mM) were mixed followed by incubation at 30°C for ten minutes. 100 µL of almond extract or standard was immediately mixed with 1.5 µL of FRAP reagent and distilled water (100 µL). Absorbance was taken through UV/Visible spectrophotometer at 593 nm. Using FeSO₄ (0-500 µmol per mL), a calibration curve was obtained. The results were mentioned as µmol Ferrous sulphate per gram of sample (µmol FeSO₄/g).

ABTS Assay: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of almond extracts was assessed following the procedure prescribed by Kim, Moon [20]. For preparing ABTS radical, 5 mL ABTS solution (14 mM) was mixed with the same volume of potassium persulfate solution (4.9 mM) and kept for 16 hours in dark. Further dilution of the mixture was done with the respective solvent. To prepare 1 mL of the final reaction solution, 50 µL of almond extract and 950 µL of ABTS solution was mixed for thirty seconds and given a stay time for 5 minutes. Through a UV/Visible spectrophotometer, absorbance was recorded at 734 nm. Variable Trolox concentrations (780-1000 µL/mL) was used to make calibration curve.

The expression used to show the ABTS radical scavenging activity was $\mu\text{mol Trolox equivalent antioxidant capacity (TEAC)}$ per gram of sample extract.

3. RESULTS AND DISCUSSION

3.1. Proximate analysis:

Momentous variations were recorded in moisture, crude fiber, crude protein contents and nitrogen free extract (NFE) of the kernels of selected almond genotypes. Crude fat and ash contents did not show considerable variations. It is indicated that Patasa showed the highest moisture percentage *i.e.* 5.10 ± 0.15 followed by Katha and Talwar as 4.98 ± 0.15 and $4.74 \pm 0.14\%$, respectively (Table 1). Abdul Wahidi has the lowest moisture contents ($4.68 \pm 0.13\%$). Patasa, Talwar, Katha and Abdul Wahidi contain 49.57 ± 0.84 , 51.12 ± 0.97 , 49.42 ± 0.89 and $50.64 \pm 0.86\%$ fat contents, respectively. Similarly, 2.99 ± 0.09 , 2.98 ± 0.10 , 3.00 ± 0.11 and $2.97 \pm 0.08\%$ ash contents were found in Patasa, Talwar, Katha and Abdul Wahidi, respectively. Highest fiber contents were found in Abdul Wahidi ($13.11 \pm 0.39\%$) trailed by Talwar ($12.45 \pm 0.37\%$). Patasa contains $11.98 \pm 0.38\%$ fiber. Lowest value of fiber was found in Katha as $11.04 \pm 0.35\%$. Highest amount of protein was found in Abdul Wahidi *i.e.* $22.95 \pm 0.64\%$ followed by Patasa and Talwar as 21.45 ± 0.62 and $21.33 \pm 0.64\%$, respectively. Lowest quantity of protein was presented by Katha *i.e.* $20.32 \pm 0.63\%$. Katha showed highest NFE value as $11.24 \pm 2.13\%$ followed by Patasa and Talwar *i.e.* 8.91 ± 2.09 and $7.38 \pm 2.22\%$, respectively. Lowest value of NFE was found in Abdul Wahidi kernels as $5.65 \pm 2.10\%$.

The results found in current study are generally in agreement with previously reported findings of Barreca, Nabavi [4] who analyzed the proximate composition of nineteen different almonds varieties. The moisture content of investigated varieties ranged between 3.1 to 6.5%. Similarly, the values found for fat, ash, fiber and protein contents were 46.3 to 47.7%, 2.5 to 4.6%, 11.00 to 12.9% and 14.1 to 23.3%, respectively. The nitrogen free extract was ranged 5.98 to 13.5%. Significant variations were reported for moisture, protein and NFE contents. The presently recorded values are well in line with previously reported outcomes of House, Hill [21] who elucidated the biochemical profile of five commercial American almond varieties. Similarly, the results of currently investigated chemical properties also relate to the work of Summo, Palasciano [22].

Table 1. Means for proximate composition of selected almond genotypes

Genotype	Moisture (%)	Crude fat (%)	Ash (%)	Crude fiber (%)	Crude protein (%)	NFE (%)
Patasa	5.10±0.15 ^a	49.57±0.8 ^a	2.99±0.09 ^a	11.98±0.38 ^{bc}	21.45±0.62 ^{ab}	8.91±2.09 ^{ab}
Talwar	4.74±0.14 ^{ab}	51.12±0.9 ^a	2.98±0.10 ^a	12.45±0.37 ^{ab}	21.33±0.64 ^{ab}	7.38±2.22 ^{ab}
Katha	4.98±0.15 ^{ab}	49.42±0.8 ^a	3.00±0.11 ^a	11.04±0.35 ^c	20.32±0.63 ^b	11.24±2.13 ^a
Abdul Wahidi	4.68±0.13 ^b	50.64±0.8 ^a	2.97±0.08 ^a	13.11±0.39 ^a	22.95±0.64 ^a	5.65±2.10 ^b

Column values sharing similar alphabetical letters are statistically alike

Health promoting potential of almonds can be attributed to presence of healthy constituents in the kernel matrix, like high amount of good quality oil, protein, fiber and presence of significant amount of minerals. The differences amongst different genotypes, varieties and cultivars are mainly attributed to genetic variations along with other factors such as season difference, weather conditions, agronomic practices and different geographical locations Rabadan [23].

3.2. Mineral profile:

No substantial variations were observed in the mineral contents of almond genotypes under study except calcium. Mineral determination (Table 2) showed that potassium is the most abundant mineral found in selected almond genotypes. Highest quantity of potassium was found in Talwar almonds (14.02±1.12 mg/g) followed by Katha and Patasa *i.e.* 13.89±1.11 and 13.81±1.17 mg/g, respectively, whilst Abdul Wahidi showed 13.76±1.05 mg/g potassium contents. Phosphorous was also dominant in all genotypes where Abdul Wahidi acquired highest amount of phosphorous *i.e.* 9.17±1.10 mg/g followed by Patasa, Talwar and Katha almonds as 9.12±1.09, 8.91±1.07 and 8.83±1.02 mg/g, respectively. Highest magnesium contents were observed in Talwar (3.10±0.24 mg/g) trailed by Katha (2.97±0.22 mg/g), Patasa (2.96±0.22 mg/g) and Abdul Wahidi (2.89±0.20 mg/g). The highest amount of calcium was recorded for Patasa almonds as 3.01±0.22 mg/g followed by Abdul Wahidi and Katha almonds as 2.83±0.23 and 2.59±0.21 mg/g, respectively. Lowest calcium contents were found in Talwar *i.e.* 2.31±0.18 mg/g.

Barreca, Nabavi [4] reported quite similar results of mineral contents for 23 different almond genotypes being grown in America. The broad range of calcium in American grown almonds was found as 0.9 to 3.3 mg/g. Similarly, wide ranges for phosphorous and potassium were also recorded as 1.91 to 3.0 and 5.25 to 15.5 mg/g, respectively. Magnesium contents in twenty three almond types were reported as 1.21 to 3.0 mg/g. Although the values for mineral contents found in current study fall in the similar ranges as those reported previously, but slightly higher

amounts of calcium, phosphorous and potassium were found in Pakistani and Afghani almond genotypes. The findings of present study are also at par with another research previously conducted by Amorello, Orecchio [24] to explore the mineral profiling of almonds of different geographical origins. Moreover, current findings are well supported by the results of Drogoudi, Pantelidis [25] who explored the mineral profile of Italic, French and Greece almond cultivars. The explicated values are in line with currently found ones. However, the presently found potassium contents are a bit higher than found in Spanish cultivars, studied by [11]. Amongst analyzed almond genotypes, Talwar was found to contain highest amounts of potassium and magnesium with better quantity of phosphorous. Abdul Wahidi had highest quantity of phosphorous. Inclusion of these genotypes in daily diet may help in preventing physiological malfunctions. The outcomes clearly depict that almond nut is a potential source of micronutrients (minerals) which may vary due to varietal variations in combination with some other factors.

Table 2. Means for mineral contents in almond genotypes (mg/g)

Genotypes	Potassium	Phosphorus	Magnesium	Calcium
Patasa	13.81±1.17 ^a	9.12±1.09 ^a	2.96±0.22 ^a	3.01±0.22 ^a
Talwar	14.02±1.12 ^a	8.91±1.07 ^a	3.10±0.24 ^a	2.31±0.18 ^b
Katha	13.89±1.11 ^a	8.83±1.02 ^a	2.97±0.22 ^a	2.59±0.21 ^{ab}
Abdul Wahidi	13.76±1.05 ^a	9.17±1.10 ^a	2.89±0.20 ^a	2.83±0.23 ^{ab}

Column values sharing similar alphabetical letters are statistically alike

3.3. Vitamin-E (α -tocopherol) quantification:

HPLC quantification of vitamin-E (α -tocopherol) showed considerable variations among the studied almond genotypes. The mean values of vitamin-E for different almond oils are enclosed in Table 3, showing highest quantity of vitamin-E in Patasa *i.e.* 0.51±0.02 mg/g trailed by Talwar and Abdul Wahidi as 0.43±0.01 and 0.38±0.02 mg/g, respectively. Katha oil contained lower tocopherol contents (0.33±0.01 mg/g) as compared to other studied genotypes.

The obtained values for vitamin-E in current study are at par with the previously reported outcomes of Bernoussi, Boujemaa [26] who found average quantity of vitamin-E as 0.37 mg/g which is close to currently recorded values. Ojeda-Amador, Fregapane [27] explored the vitamin-E contents of four different almond cultivars. Their reported values ranged from 0.42 to 0.54 mg/g. The findings of present study are in line with these reported quantities. Similarly, the current outcomes are supported by the results reported by Wang, Wang [28] for vitamin-E concentration in five different wild almond species being grown in China. They reported the

values ranged from 0.43 mg/g to 0.68 mg/g. Overall, the values for vitamin-E (α -tocopherol) for almond genotypes under current study fall in the previously reported range but wild almond species contained higher amounts of analyzed vitamin-E. On the other hand, average quantity of vitamin-E (0.20 mg/g) in almonds reported by [29] falls close to lowest quantities found in present study. The currently recorded values of vitamin-E fall close to the results expounded by Kodad and Alonso [30] who analyzed the vitamin-E contents in the oil of different almond cultivars being grown in Spain, Morocco and Argentina. Vitamin-E is a fat-soluble vitamin well known for its strong antioxidant potential. The presently studied all almond genotypes were found to contain promising quantities of vitamin-E (α -tocopherol) especially Patasa and Talwar. Abdul Wahidi also contained enough vitamin-E contents. Hence, these almonds are suitable for the development of functional products with health boosting potential.

Table 3. Means for vitamin-E in almond genotypes (mg/g)

	Patasa	Talwar	Katha	Abdul Wahidi
Vitamin-E	0.51±0.02 ^a	0.43±0.01 ^b	0.33±0.01 ^d	0.38±0.02 ^c

In vitro studies

3.4. Phytochemical screening analysis

Total flavonoids: In the spectrophotometric determination of total flavonoid contents, significant variations were observed. The mean values illustrated in Figure 1 explicate that Talwar contained highest quantity of total flavonoids *i.e.* 26.34±1.90 mg QE/g trailed by Abdul Wahidi (23.54±1.31 mg QE/g) and Katha (21.20±1.34 mg QE/g). Patasa had lowest amount (19.23±1.02 mg QE/g) of total flavonoids amongst analyzed genotypes. Current findings regarding total flavonoids in Pakistani and Afghani almond genotypes are comparable with those reported by [31]. They recorded the total flavonoids in twenty-three different almond cultivars being grown in Turkey. A broad range was found as 11.71 to 51.15 mg QE/g. The values found in current study fit well in the reported range. Similarly, Hanine, Zinelabidine [32] explicated the total flavonoids in seven almond cultivars being cultivated in Morocco. They described the range of analyzed phytochemicals as 14.1 to 25.7 mg QE/g. Present findings are at par with this earlier reported range as current values fall sufficiently close to the previous ones. Current findings are also comparable with the outcomes of Keser, Demir [33] who analyzed the flavonoids contents in Turkish almonds and reported the average value as 19.54 mg QE/g. Flavonoids are diverse

phytonutrients which are responsible for antioxidant power of plant food matrix. All almond genotypes under present study were found to have good quantities of flavonoids specially Talwar (Pakistani) and Abdul Wahidi (Afghani) which contained higher quantities as compared to other two.

Total phenolic contents: The studied almond genotypes had momentous variations in total phenolic contents. A broader range was recorded for different almonds under study (Figure 1). Abdul Wahidi contained highest quantities of total phenolics *i.e.* 21.45 ± 1.14 mg GAE/g followed by Patasa (18.10 ± 1.01 mg GAE/g) and Talwar (15.87 ± 1.00 mg GAE/g). Katha was observed to have lowest amount (12.67 ± 0.91 mg GAE/g) of total phenolic contents amongst analyzed genotypes. Current findings regarding total phenolic contents of selected among genotypes are in corroboration with the results presented by Bottone, Montoro [34]. They explored the metabolites in three Italian almonds varieties and recorded the total phenolics in Toritto, Fascionello and Pizzuta as 14.38, 17.72 and 19.38 mg GAE/g. These values closely match with those calculated for Pakistani and Afghani almonds under study. Similarly, [35] explicated the quantities of total phenolic contents in different almond varieties being grown in Serbia. Alike current outcomes, they also reported that almonds with different genetic makeup contained significantly different concentration of phytochemicals. Current results are supported by the work of Oliveira, Meyer [36] who found considerably different values of total phenolics for different almond cultivars. The results are not comparable with current values due to difference in units. Results recorded in present study are also in accordance with those earlier explained by Hanine, Zinelabidine [32]. Phenolic contents are strong antioxidants and known to play a crucial role in body's physiological functioning. Amongst currently investigated almond genotypes, all were found good in total phenolic contents especially Abdul Wahidi and Patasa contained higher amounts so can be promoted for the development of almond based functional and designer products.

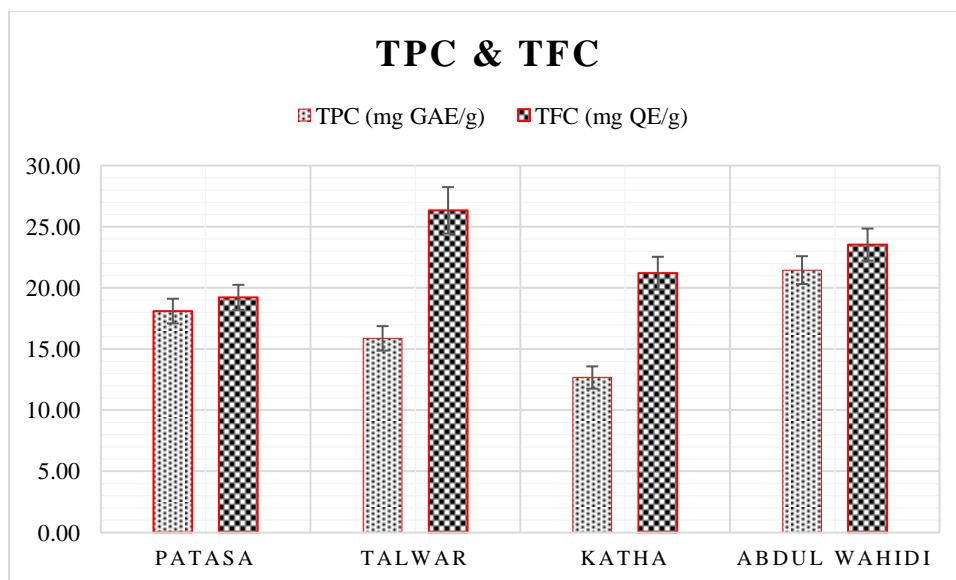


Figure 1. Phytochemical screening analysis of selected almond genotypes

3.5. Antioxidant assays

DPPH assay: Momentous variations in DPPH activity existed between selected almond genotypes. Figure 2 depicts that Abdul Wahidi almonds showed highest DPPH inhibition ($85.34 \pm 4.52\%$) trailed by Talwar ($73.54 \pm 4.63\%$). Lower DPPH inhibition potential was found in Patasa and Katha *i.e.* 67.78 ± 3.77 and $62.21 \pm 4.48\%$, respectively.

The outcomes of current study are in close agreement with findings of Bottone, Montoro [34] who tested the DPPH inhibition of almond extracts obtained from three Italian cultivars. The explicated values were 77.67, 83.09 and 78.47%. The calculated values of free radical inhibition for almond genotypes under current study are close to these earlier described results. Similarly, present outcomes are also comparable with radical scavenging activity of fourteen Moroccan almond cultivars investigated by [32]. The cultivars were found to possess variable radical scavenging potential with a minimum value 46.3 to a maximum 84.3%. In another research, free radical inhibition of different almond extracts was explicated by Aires, Morais [37]. They reported that the range was lower as compared to currently calculated values *i.e.* 25 to 45%. Presently recorded results are in corroboration with another study in which Turkish almonds were assessed for antioxidant potential through DPPH assay [33]. Present outcomes are supported by [36] who reported almond extracts from eight Portuguese cultivars to have variable DPPH radical scavenging potential.

FRAP (Ferric reducing antioxidant power): Chain reactions mediated by free radicals are terminated due to electron donating ability of extracts containing antioxidants. This ability of extracts is determined by FRAP. The tested ferric reducing antioxidant power of almond extracts were significantly different amongst investigated almond genotypes. Means for FRAP assay illustrated in Figure 2 depict that Talwar showed maximum reducing power ($55.34 \pm 3.49 \mu\text{mol FeSO}_4/\text{g}$) trailed by Katha *i.e.* $44.21 \pm 3.18 \mu\text{mol FeSO}_4/\text{g}$). Abdul Wahidi and Patasa showed bit lower values *i.e.* 36.31 ± 1.92 and $32.10 \pm 1.78 \mu\text{mol FeSO}_4/\text{g}$, correspondingly.

The current results regarding ferric reducing power of almond extracts are in corroboration with the outcomes explained by [31]. They analyzed the ferric reducing power of twenty-three Turkish almond varieties. A broad range between maximum and minimum values were reported as 20.88 and 76.32 $\mu\text{mol FeSO}_4/\text{g}$, respectively. The results recorded for different almonds in present study fall well between those reported for Turkish almonds. Present study is also supported by Oliveira *et al.* (2018) who explored the reducing power of eight Portuguese almond varieties and explained significant variations in the values among different varieties. The results explicated by Moosavi Dolatabadi, Dehghan [38] also back the current values. They investigated the ferric reducing power of eighteen almond genotypes and observed considerable variations in results similar to present study.

ABTS Assay: The free radical ABTS is scavenged due to electron/hydrogen donating ability of the antioxidant moieties present in the sample extract. Momentous variations were observed in this regard in the tested almond extract's samples. Mean values shown in Figure 2 reveal that Talwar extract had maximum electron donating ability *i.e.* $83.56 \pm 5.26 \mu\text{mol TE/g}$ trailed by Abdul Wahid and Patasa as 78.65 ± 4.17 and $73.01 \pm 4.06 \mu\text{mol TE/g}$. Katha had lower value ($68.45 \pm 4.93 \mu\text{mol TE/g}$).

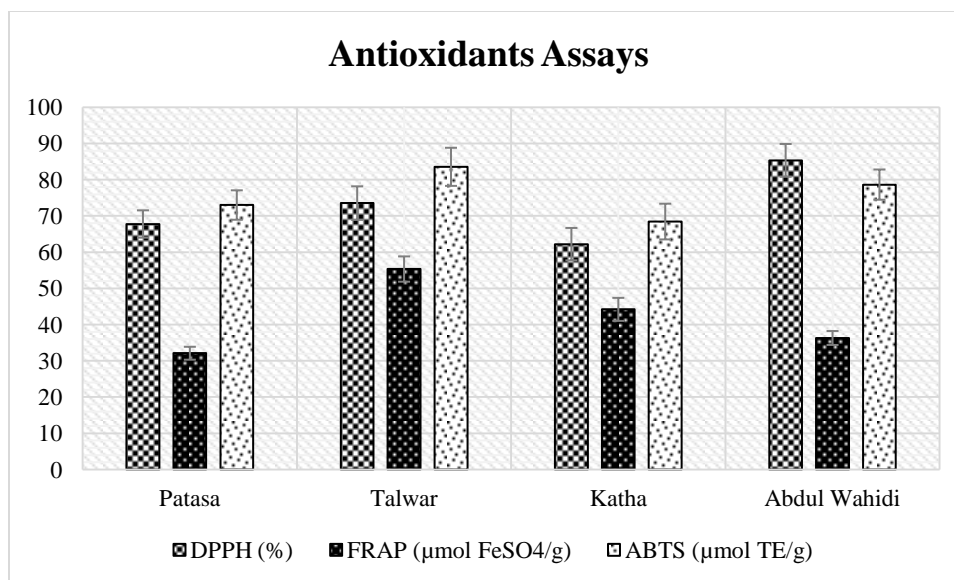


Figure 2. Antioxidant assays for selected almond genotypes

Earlier, eighteen Iranian almond genotypes were found to have a wide range of ABTS free radical scavenging ability *i.e.* 10 to 90 µmol TE/g [38]. The values recorded for genotypes under current study fits well in the reported range. Similarly, the current findings regarding ABTS free radical scavenging activity of almond genotypes are well supported by previously recorded results of Miraliakbari and Shahidi [39]. Quite higher values of ABTS scavenging ability as compared to current results were reported for three Moroccan almond genotypes *i.e.* 216.94, 233.10 and 238.07 µmol TE/g [40]. Alike presently recorded results, Bottone, Montoro [34] also reported variations in electron donating capability of extracts obtained from Italian almond cultivars. Likewise, Oliveira, Meyer [36] also explicated significant variations in the values calculated for ABTS free radical scavenging through electron donation of extracts obtained from eight Portuguese almond cultivars.

4. CONCLUSION & OUTLOOK

It is evident from the conducted study that genetic variations significantly affect nutritional and bioactive's composition of almond kernels. All of the currently investigated almond genotypes are found to contain a good blend of an array of nutrients along with health promoting phytochemicals with considerable antioxidant potential. Nonetheless, Talwar (Pakistani) and Abdul Wahidi (Afghani) almonds possess better nutritional profile as compared to rest of two genotypes. Hence, the later could be promoted to be used in health boosting designer food products and medical purposes. Hence, these can be further explored for a deep insight of nutrients and bioactive components with prophylactic potential.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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