Original article

Comparative analysis of antioxidant activity, toxicity, and mineral composition of kernel and pomace of apricot (Prunus armeniaca L.) grown in Balochistan, Pakistan

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A R T I C L E   I N F O

Article history:
Received 12 December 2020
Revised 22 January 2021
Accepted 1 February 2021
Available online 16 February 2021

Keywords:
Prunus armeniaca L
Phenolic content
Antioxidant activity
Minerals
Toxicity

A B S T R A C T

The present study aims to investigate some physical attributes, total phenolics content, total flavonoids content, mineral composition, bioluminescence toxicity assay and antioxidant activity in terms of DPPH, HPS, TAC and FRAP assays in the kernel and pomace samples of six apricot cultivars grown in Balochistan, Pakistan. TFC and TPC determined by the AlCl3 and Folin-Ciocalteu assays in apricot kernel extracts of six cultivars varied from 1797.5 (Chagali) to 4778.9 (Badoghur) mg QUE/100 g DW and from 1750.0 (Chagali) to 5005.8 (Badoghur) mg GAE/100 g DW. Apricot kernels exhibited higher antioxidant activity than pomace; antioxidant activity in terms of IC50 in kernels ranged from 24.88 to 98.61 μg/ml for DPPH, 334.84 to 516.63 μg/ml for HPS, from 22.02 to 110.80 μg/ml for TAC and from 96.27 to 163.35 μg/ml for FRAP. The apricot kernels showed higher TPC, TFC, bioluminescence toxicity to V. logei and antioxidant activity than the pomace. The correlation analysis demonstrated substantial contributions of polyphenols and flavonoids to antioxidant assays. The sample type was the leading factor affecting the amounts of K, Na, Ca, Fe, and Mn in the tested samples; mineral contents were higher in pomace than kernels. The highest inhibition to V. logei was found in the kernels of Badoghur (IC50 = 1.61 mg/ml). The PCA analysis showed significant contributions of phenolic and flavonoid contents towards antioxidant bioluminescence toxicity assays. Our results suggest Badoghur, Shakarpara and Sardai kernels are rich sources of secondary metabolites and possess remarkable antioxidant and antiluminescence activity and can make a significant contribution to the treatment and prevention of chronic health problems.

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1. Introduction

Recently research trends are focused on studying healthy foods and natural products due to their potential use as biologically active compounds and their consecutive connection in treating and mitigating diseases (Mata et al., 2016). The polyphenolic compounds are the significant constituents of natural sources, having substantial antioxidant prospects (Carocho and Ferreira, 2013). Fresh and dried fruits are valuable components of our day to day diet and are rich sources of antioxidants. The use of antioxidants-rich fruits has been described to control the myriad of degenerative illnesses that influence humans health (Hussain et al., 2013). These antioxidants are incredibly efficacious against oxidative damage associated with health disorders, including the aging process, neurological syndromes, cancer, cardiovascular diseases, cataract formation, inflammation, and immune system (Biglari et al., 2008).

One good source of natural phenolics for humans is apricot (Prunus armeniaca L.), fruit of the Rosaceae family of Rosales order. It is mainly grown in the Mediterranean regions (Hacseferogullari et al., 2007). Apricot fruit has become a product of considerable interest due to its richness in health beneficial nutrients (vitamins, fibers, carbohydrates, minerals, etc.) and phytochemicals (carotenoids, polyphenolics, and glycosides) (Wani et al., 2017). Fresh apricot fruits are described to contain an elevated amount of phenolic compounds, for instance, chlorogenic acids, neochlorogenic acids, dimers and trimers of proanthocyanidins, various glycosides...
of kaempferol and quercetin, and cyaniding-3-glycoside as primary pigments (Sőjka et al., 2015). These secondary metabolites are essential contributors in antioxidants, anti-inflammatory, antimicrobial, anti-mutagenic, cardio-protective, and hepatoprotective (Erdogan-Orhan and Kartal, 2011). Besides, the apricot fruit, its kernel either bitter or sweet, constitutes about 34% of the seed and represents around 15% of the fruit (Mandal et al., 2007), is a useful by-product. Sweet kernels are consumed directly as a vital source of oil, protein, and fibers and a substitute for almond in their dried state for flavor and taste in bakery products. Additionally, apricot kernels are used to produce cosmetics, benzaldehyde, oils, active carbon, perfume, and aroma (Guner et al., 1999).

The largest producers of apricot fruit are Asia and Europe (Bhat et al., 2013). The multiple ecological zones for apricot production in Pakistan are the Malakand division of Khyber Pakhtunkhwa province, Northern areas (Gilgit-Baltistan), and upper parts of Balochistan province (Jasra and Rafi, 2002). Balochistan province is the largest grower of fruits in Pakistan, hence famous as the “fruit basket of Pakistan.” Due to favorable climatic conditions, deciduous fruits in Balochistan province have particular lead above other fruit growing areas. The climate’s diverse nature is not less than a blessing for producing various fruits varying from temperate to tropical and sub-tropical (FAO, 1993).

However, the phytochemical composition and the important activities of apricot fruit have been widely researched from other world regions. Although there are no data on the antioxidant capacity and phytochemical quantification in indigenous apricot varieties grown in the province is available in the literature. Consequently, the current study aimed to evaluate and compare antioxidant activity, mineral composition, phytochemical quantification, and toxicity towards V. logei bacteria of apricot pomaces and corresponding kernels using similar cultural practices in the northern part of Balochistan.

2. Materials and methods

2.1. Chemicals and materials

Commercial standards for Catechin hydrate, DPPH (1,1-diphenyl-2-picrylhydrazlyl), quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) were from Sigma-Aldrich (St. Louis, MO, USA). Gallic Acid and Trolox (6-hydroxy-2,5,7,8-tetramethoxy-2-carboxylic acid) were from Acros (New Jersey, USA). Follin–Ciocalteu reagent was purchased from the Central Drug House (New Delhi, India). Alpha-Tocopherol (2R)-2,5,7,8-Tetramethyl-2-[4R,8R]-4,8,12-trimethyltridecyl)chroman-6-ol was from Sigma-Aldrich (St. Louis, MO, USA). L-Ascorbic acid, soluble starch, lactose and glycerol were from Merck (Darmstadt, Germany). L-Ascorbic acid, soluble starch, lactose and glycerol were from Merck (Darmstadt, Germany). Yeast extract, peptone and bacteriological agar were from Biokar Diagnostics. All other reagents and chemicals used were of analytical quality.

2.2. Apricot samples

The fresh, firm, and healthy apricot fruits of six different varieties, namely Badoghr, Charmaghz, Chagali (wild), Nari, Sardai and Shakarpara, were directly harvested from Pishin, Fort of Saffulah and Sinjawi areas of Balochistan province in June and July. The studied varieties were grafted on bitter almond rootstock without proper phytosanitary treatment except well rotten farmyard green manure once a year. Horticultural experts authentically identified fruits at Agriculture Research Institute (ARI), Quetta, Balochistan, Pakistan. After washing with tap water and distilled water, fruits were dried with a muslin cloth and were refrigerated (~25 °C) unless used.

2.3. Determination of some physical properties of apricots

Thirty apricot/variety were randomly taken to measure physical traits (pulp weight, fruit weight, pit weight, and pulp/pit ratio (PPR)) by a digital electronic balance (Kern 440, Kern and Shon, Germany) with 0.01 g sensitivity.

2.3.1. Sample preparation

The pits from the pomaces (skin + pulp) of fresh apricot fruits were manually removed while intact kernels (brown skin + white matter) were obtained physically hammering the pits coat.

2.3.2. Preparation of crude phenolic extraction

Phenolic compound extraction was conducted as explained previously (Soong and Barlow, 2004) with minor modification. Samples of kernels and pomace were freeze-dried (~50 °C, 24 h) and grounded using Anex grinder (Germany).Powdered kernels were defatted by well mixing with n-hexane (1:5 w/v, 5 min × 3) in ultra-turrax T-25 basic homogenizer (IKA, Staufen, Germany). To remove all traces of solvent, the defatted kernel samples were air-dried for 12 h and stored at ~20 °C in polyethylene bags until extraction. Powdered fruit sample (each 20 g) was extracted with 200 ml of aqueous methanol (80% v/v) using an orbital shaker (IKA KS 2608-Staufen Germany) at an agitation speed of 150 rpm for 12 h under dim light, then filtered through Whatman No. 1 filter paper and the residues were re-extracted twice with a fresh portion of solvent under the same conditions. The filtrate was pooled and desoventized in a rotary evaporator (Stuart, Stone Staffordshire, UK) at 35 °C. The resulting dried extracts were dissolved in 80% aqueous methanol and stored (4 °C) until analysis.

2.3.3. Determination of moisture and dry matter content

Dry matter content (DMC) and moisture content (MC) were assessed by the oven-drying technique as explained earlier (Chemists, 1990).

2.4. Determination of extraction yield

The yield of the pomace and the kernel extracts was measured on a dry weight basis (DWB) by Eq. (1):

\[
\text{Yield}(\%) = \left( \frac{\text{Weight of extract}}{\text{Weight of kernel or pomace taken}} \right) \times 100 \]

2.4.1. Determination of total phenolics content

Total phenolics content (TPC) was measured by the Folin–Ciocalteu method according to (Singleton and Rossi (1965)). 0.5 ml (containing 0.05 g/ml) of diluted fruit extract was mixed with 0.5 ml Folin–Ciocalteu’s reagent and deionized water (8.0 ml), incubated for ten minutes after that added that added 7.5% saturated sodium carbonate (w/v, 1.5 ml) and mixed well. After two hours, absorbance was read at 750 nm by Shimadzu-1700 UV–VIS spectrophotometer (Japan) and compared to a calibration curve of prepared gallic acid solutions in the range of 10–250 mg/L. The results were expressed as mg gallic acid equivalent (GAE) mg/100 g dry weight (DW).

2.4.2. Quantification of total flavonoids content

The aluminum chloride colorimetric method was used to assess total flavonoids content (TFC) following the procedure as described earlier (Zhiheng et al., 1999). 0.5 ml (containing 0.02 g/ml) of diluted fruit extract was mixed with 4 ml of distilled water in 10 ml volumetric flasks followed by the addition of 3 ml of 5% Sodium nitrite. After six min, 0.3 ml of 10% saturated AlCl₃ was added and left to stand for another 5 min before 2 ml of 1.0 M NaOH was mixed. The volume of the reaction mixture was brought

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up to 10 ml by distilled water and mixed well. The absorbance of the reaction mixture was read at 510 nm and compared to a calibration curve of prepared quercetin (QU) solutions in the limit of 10–250 mg/L. Final values of TFC were presented as mg QUE/100 g DW.

2.4.3. Antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted as described before (Chen et al., 2016) with minor modifications. Fresh DPPH solution (0.1 mM) in 100 ml of methanol was prepared from the stock DPPH solution (10 mM in methanol) in amber bottles for 2 h before stabilizing the absorbance. An aliquot of 2 ml extract of each concentration (20–120 µg/ml) was mixed with 2 ml of DPPH solution and vortexed for a min. The reaction mixture was incubated for 40 min under dim light at room temperature. The decrease in absorbance (Abs.) was measured at 517 nm against the blank comprised of extract and methanol only. Ascorbic acid and trolox were used as standards. The radical scavenging activity was determined by Eq. (2) given below and linear regression of concentration against absorbance was used to calculate IC50 (50% inhibitory concentration) for DPPH and expressed as µg/ml.

\[
\text{DPPH scavenging effect} (%) = \frac{(\text{Control Abs.} - \text{Test Abs.})}{\text{Control Abs.}} \times 100
\]

Hydrogen peroxide scavenging (HPS) activity was conducted according to the method of Ruch et al. (1989). A hydrogen peroxide solution of 43 mM is briefly prepared in phosphate buffer (0.1 M, pH 7.4) from a 0.5 M intermediate stock solution. The latter was prepared from 35% H2O2. Sample of various concentrations (50–500 µg/ml) was dissolved in 3.4 ml of 0.1 M sodium phosphate buffer (pH 7.4) and then thoroughly mixed with 0.6 ml of 43 Mm hydrogen peroxide solution (made in the same buffer). The reaction mixture was left for 15 min at room temperature under dark. The amount of unreacted hydrogen peroxide was assessed spectrophotometrically at 230 nm. An additional blank sample without hydrogen peroxide solution (made in the same buffer). The reaction mixture was incubated for 40 min and then thoroughly mixed with 0.6 ml of 43 Mm hydrogen peroxide solution (made in the same buffer). The reaction mixture was incubated for 90 min at 95°C. Precisely mixed 1.0 ml extract of various concentrations (50 to 300 µg/ml) to 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min in a water bath and sharply cooled. After that, 2.5 ml of 10% (w/v) of trichloroacetic acid were mixed, centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with equal volumes of distilled water and 0.5 ml of ferric chloride (0.1%, w/v). Allowed the reaction mixture to stand for 10 min at room temperature to develop Perls’ Prussian blue color. The absorbance was noted at 700 nm against the blank (80% methanol). The sample concentration providing 0.5 of absorbance (IC50) was measured from the graph of extract concentration against absorbance values. Ascorbic acid and catechin were used as standards.

2.5. Bio luminescence (BL) assays

Acute and chronic toxicity was evaluated by measuring the bioluminescence inhibition of the marine bacterium V. logei (wild strain) after different times of exposure to the different concentrations of the kernel and pomace extracts. The freeze-dried bacterium was prepared and reconstituted as previously described (Girotti et al., 2002). Wallac Victor 1420 Multilabel Counter luminometer (Wallac, Sweden) and 96 well microplates were used to measure luminescence in relative light units (RLU). For acute toxicity test, the assay was performed by adding to each well 20 µl BL bacteria in culture broth and 180 µl of the sample in 3% NaCl solution. In comparison, 150 µl BL bacteria and 150 µl of the sample in 3% NaCl was used for the chronic toxicity test. Similarly, a blank was prepared by adding 3% NaCl and BL bacteria. The emitted light was recorded at intervals of 5 min for 1 h in acute and for 20 h at intervals of 17 min in chronic tests. Before starting the assays, the intensity of the light emission was optimized. Results were expressed as a percentage of inhibition with respect to the blank emission using Eq. (5) mentioned as under, while IC50 (mg/ml) (representing 50% light inhibition) was calculated from the linear regression analysis.

\[
\text{Luciferinence} (%) = \frac{L_{c,t} - L_{s,t}}{L_{c,t}} \times 100
\]

where \( t \) represents the time of measurements (1 h, in the acute assay) and (20 h, in the chronic assay), \( L_{c,t} \) is the luminescence of blank and sample emission at \( t \) hour respectively.

2.6. Mineral analysis

The mineral composition was estimated following the method, as reported before (Sahito et al., 2002). One gram of oven-dried sample of (Pomace and kernel) was taken in digestion flask (Pyrex) having 5 ml of absolute H2SO4. The flasks were heated at hotplate (Jenway, 1000, Barloworld Scientific Ltd, UK) at 80–90 °C for about 60 min, further increasing the temperature to 160 °C. Meanwhile, 5 ml of absolute HNO3 and 2 ml of 30% (v/v) H2O2 were mixed to the flasks and left the mixture for boiling/heating until a clear solution was obtained. After effervescence had faded away, the flasks were cooled, mixed well with a small volume of deionized water and filtered through Whatman No. 42 (<0.45 µm Millipore) filter paper. Finally, diluted to 50 ml with deionized water. In the same way, a blank was also prepared. All analyses were carried out in triplicate on atomic absorption spectrophotometer (Thermo electronic corporation, Cambridge, UK). Standard calibration curves were constructed for individual elements, and results expressed in mg/100 g DW.
2.7. Statistical analysis

Research data results were presented as means ± SD having triplicate analysis (n = 3). One-way analysis of variance (ANOVA) with tukey’s honestly significant difference (HSD) test was carried out to evaluate the significance of differences between means using the R-Studio software v.3.6.3 at a significance level of P < 0.05. Pearson correlation coefficient (r) was used to investigate the relationship between total phenolics and antioxidant assays at a significant level of P < 0.01 and 0.05. Multivariate analysis in terms of principal component analysis (PCA) was carried out on the combined TFC, TPC, antioxidant activities, mineral composition and BL assays using the R-Studio software v.3.6.3.

3. Results

3.1. Physical properties of apricot

Results for the physical properties of studied apricot varieties have been shown in Table 1. The recorded values for fruit weight, pomace weight, pit weight, and pomace/pit ratio (PPR) ranged from 12.39 to 40.28 g, 10.30 to 36.02 g, 2.10 to 4.27 g, and 3.95 to 8.44, respectively. All the physical attributes investigated were statistically significant (p < 0.05) among the studied varieties.

3.2. Moisture content and dry matter

The MC and DMC results of the kernel and pomace of the varieties examined are provided in Table 2. The MC in pomace and kernels varied from 78.40 to 84.27% and 3.87 to 4.93%, respectively. The highest MC in the pomaces was noted in Sardai (84.27%), followed by Chagali (82.93%), Shakarpara (82.67%), Badoghur (82.27%), Charmaghz (79.27%), and lowest in Nari (78.40%). Whereas in kernels, higher MC was observed for Sardai (4.93%), which was followed by Chagali (4.67%), Nari (4.60%), Charmaghz (4.53%), Badoghur (4.27%), lowest in Shakarpara (3.87%). The values did not differ significantly from each other except Nari and Sardai. The DMC in the pomace samples was significant (p < 0.05) among tested varieties and varied between 15.73% and 21.60% noted respectively in Sardai and Nari varieties, however, the DMC in kernel ranged between 95.07% and 96.13% observed respectively in Sardai and Shakarpara varieties, did not differ significantly except Sardai and ShakarPara.

3.3. Extraction yield

The data observed for extraction yield was partially significant (P < 0.05) for pomace extracts (PE) and kernel extracts (KE) among varieties shown in Table 2. The almost higher extraction yield was noted in the pomace of Charmaghz (14.18%), while the lowest values were observed in Chagali (9.92%) kernel.

3.4. Total phenolics content

As evident from Fig. 1A, the TPCs (mg GAE/100 g) of the apricot PE and KE differed significantly (P < 0.05) among the six varieties. In PE, higher TPC values were recorded for Chagali (4369) and Badoghur (4078) that were followed (with decreasing order) by Shakarpara (3719), Sardai (3307), Nari (2922), and lowest in Charmaghz (2192). Whereas of the KE, the Badoghur variety possessed the highest values of (5005), followed (with decreasing order) by Shakarpara (4478), Sardai (3843), Nari (3079), Charmaghz (2556) and lowest in Chagali (1750). The TPCs tended to be highest in the kernels, followed by the pomace for all varieties except the Chagali variety.

3.5. Total flavonoids content

Results shown in Fig. 1B portrayed the measured concentrations of TFC based on mg QE/100 g DW. The results revealed no significant differences (p < 0.05) among the examined varieties irrespective of the pomace and kernel samples. Of the KE, the highest amount was observed for Badoghur (4778), followed (in decreasing order) by Shakarpara (4553), Sardai (3723), Nari (3020), Charmaghz (2840). Nonetheless, the lowest content was noted in the Chagali (1797). Whereas in the PE, the Chagali variety contained the highest values of (4376), which was followed (in decreasing order) by Badoghur (3812), Shakarpara (3565), Sardai (3295), Nari (2881) and Charmaghz (2284).

3.6. Assessment of antioxidant activity (AoA) in apricot PE and KE

Four in vitro antioxidant chemical assays were performed to evaluate AoA in apricot KE and PE of six varieties (shown in Table 3). The results were expressed as a minimum inhibitory concentration (IC50). Generally, the lower the IC50 higher is the antioxidant activity. Significant differences were noted among varieties for DPPH, HPS and FRAP but not for TAC (P < 0.05). DPPH IC50 values in the investigated KE and PE ranged from 24.88 to 98.61 µg/ml and from 23.13 to 96.97 µg/ml, respectively. Badoghur kernel possessed the most significant activity with IC50 of (24.88 µg/ml). The kernels of Shakarpara, Sardai, Nari, Charmaghz and Chagali apricots had DPPH activities of 49.97, 52.61, 79.96, 91.78, 98.61 µg/ml, respectively. The PE of Chagali apricots had the highest DPPH activity at (23.13 µg/ml) compared to that component of other apricots (33.46, 60.92, 78.05, 93.25 and 96.97 µg/ml) for Badoghur, Shakarpara, Sardai, Nari and Charmaghz, respectively. The PE of Chagali apricots had the lowest IC50 (23.13 µg/ml) compared to that component of other apricots (33.46, 60.92, 78.05, 93.25 and 96.97 µg/ml) for Badoghur, Shakarpara, Sardai, Nari and Charmaghz, respectively. HPS values varied from 334.84 to 516.63 µg/ml in KE and from 312.93 to 489.43 µg/ml in PE. The KE from Badoghur (334.84 µg/ml) showed the lowest IC50. However, the highest value at (516.63 µg/ml) for Chagali. In contrast, in PE, the highest (312.93 µg/ml) and lowest

| Varieties     | Parameters       | Fruit weight (g) | Pulp weight (g) | Pit weight (g) | Pulp/Pit Ratio |
|---------------|------------------|------------------|-----------------|----------------|----------------|
| Badoghur      |                  | 40.28 ± 3.8a     | 36.02 ± 3.79a   | 4.27 ± 0.04a   | 8.44 ± 0.89a   |
| Chagali       |                  | 12.39 ± 1.3a     | 10.30 ± 1.34a   | 2.10 ± 0.04a   | 4.92 ± 0.65c   |
| Charmaghz     |                  | 26.71 ± 3.0b     | 22.94 ± 3.05b   | 3.77 ± 0.04b   | 6.09 ± 0.62b   |
| Nari          |                  | 18.41 ± 2.9c     | 15.50 ± 2.86c   | 2.92 ± 0.05c   | 5.32 ± 1.01c   |
| Shakarpara    |                  | 14.20 ± 1.1d     | 11.34 ± 1.16d   | 2.87 ± 0.04d   | 3.95 ± 0.37d   |
| Sardai        |                  | 24.66 ± 1.7e     | 21.93 ± 1.74e   | 2.72 ± 0.05e   | 8.06 ± 0.66a   |

Data are means ± SD (n = 30); on fresh weight basis, different lowercase letters in superscript within similar column mark significant differences at (P < 0.05), as determined by Tukey’s HSD test among apricot varieties g: Gram.
Comparison of moisture content, dry matter and extraction yield between the kernel and pomace parts in apricots grown in Balochistan.

Table 2
Comparison of moisture content, dry matter and extraction yield between the kernel and pomace parts in apricots grown in Balochistan.

| Fruit tissue | Variety | Moisture content (%) | Dry matter (%) | Extraction yield (%) |
|--------------|---------|----------------------|----------------|----------------------|
| Kernel       | Badoghur| 4.27 ± 0.12<sup>a</sup> | 95.73 ± 0.1<sup>ab</sup> | 9.92 ± 0.78<sup>bc</sup> |
|              | Chagali | 4.53 ± 0.31<sup>ab</sup> | 95.47 ± 0.3<sup>bc</sup> | 6.85 ± 0.71<sup>b</sup> |
|              | Charmaghz| 4.67 ± 0.12<sup>bc</sup> | 95.53 ± 0.1<sup>bc</sup> | 8.02 ± 0.83<sup>bc</sup> |
|              | Nari    | 4.60 ± 0.20<sup>c</sup>   | 95.40 ± 0.2<sup>c</sup>   | 8.33 ± 0.79<sup>c</sup>   |
|              | Shakarpara| 3.87 ± 0.12<sup>d</sup>   | 96.13 ± 0.1<sup>d</sup>   | 10.47 ± 0.63<sup>d</sup>   |
|              | Sardai  | 4.93 ± 0.12<sup>d</sup>   | 95.07 ± 0.1<sup>d</sup>   | 11.53 ± 0.63<sup>d</sup>   |
| Pomace       | Badoghur| 82.27 ± 0.1<sup>b</sup>   | 17.73 ± 0.1<sup>b</sup>   | 12.43 ± 0.89<sup>b</sup>   |
|              | Chagali | 82.93 ± 0.1<sup>b</sup>   | 17.07 ± 0.1<sup>b</sup>   | 10.70 ± 0.48<sup>b</sup>   |
|              | Charmaghz| 79.27 ± 0.1<sup>c</sup>   | 20.73 ± 0.1<sup>c</sup>   | 14.18 ± 0.69<sup>c</sup>   |
|              | Nari    | 78.40 ± 0.2<sup>c</sup>   | 21.60 ± 0.2<sup>c</sup>   | 12.47 ± 0.38<sup>c</sup>   |
|              | Shakarpara| 82.67 ± 0.1<sup>c</sup>   | 17.33 ± 0.1<sup>c</sup>   | 13.53 ± 0.53<sup>c</sup>   |
|              | Sardai  | 84.27 ± 0.3<sup>c</sup>   | 15.73 ± 0.3<sup>c</sup>   | 11.37 ± 0.53<sup>c</sup>   |

Data are mean ± SD (n = 3). Different lowercase letters in superscript within similar column mark significant differences (P < 0.05), as determined by Tukey's HSD test among apricot varieties.

Fig. 1A. TPCs of apricot kernel and pomace extracts of six selected apricot varieties. Error bars show mean ± standard deviation (n = 3). Different lowercase letters represent significant difference at p < 0.05, as determined by Tukey’s HSD test among apricot varieties. V1-Badoghur; V2-Chagali; V3-Charmaghz; V4-Nari; V5-Shakarpara; V6-Sardai.

Fig. 1B. TFCs of apricot kernel and pomace extracts of six selected apricot varieties. Error bars show mean ± standard deviation (n = 3). Different lowercase letters represent significant difference at p < 0.05, as determined by Tukey’s HSD test among apricot varieties. V1, V2, V3 … represents the varieties as shown in Fig. 1A.

Besides, TAC values in the PE and KE range from 26.55 to 91.59 µg/ml and from 22.02 to 110.80 µg/ml, respectively. The Badoghur PE depicted the highest antioxidant activity with the lowest IC<sub>50</sub> at 26.55 µg/ml, followed by Chagali, Shakarpara and Sardai at IC<sub>50</sub> 28.66, 46.13 and 51.48 µg/ml, respectively. Though of the KE, best TAC activity was shown again by Badoghur (22.02 µg/ml) variety, followed by Shakarpara (26.13 µg/ml), Sardai (36.42 µg/ml), Charmaghz (63.36 µg/ml), Nari (72.61 µg/ml) and Chagali (110.80 µg/ml). However, percentage inhibition of Shakarpara PE and Charmaghz KE at the lowest concentration (60 µg/ml) was negative (data not shown), which might be the insufficient formation of phosphomolybdenum complex. Descending order of FRAP activity in apricot KE was: Shakarpara > Badoghur > Nari > Sardai > Charmaghz > Chagali. Whereas the observed order of IC<sub>50</sub> in the PE was Chagali > Badoghur > Shakarpara > Nari > Charmaghz > Sardai. The lowest IC<sub>50</sub> in the KE was recorded in Shakarpara at 96.27 µg/ml, which indicates the highest FRAP activity, followed by Badoghur and Nari with IC<sub>50</sub> of 102.67 and 106.77 µg/ml, respectively. Although in the PE, maximum and minimum activity was exhibited by Chagali and Sardai with IC<sub>50</sub> values of 112.32 and 244.10 µg/ml, respectively. Furthermore, the ratio of AoA in the KE to that in the PE varied from 0.67 to 4.26 (DPPH assay), 0.77 to 1.65 (HPS assay), 0.58 to 3.86 (TAC assay) and 0.47 to 1.46 (FRAP assay) (Table 3). Our results are comparable with Xu et al. (2016), who also found that the antioxidant potential of apple seeds was higher than peels. Therefore, these results showed that apricot kernels exhibited stronger antioxidant potential than the pomace.

### 3.7. Bioluminescence assay

In acute toxicity assay, neither PE nor the KE did show any bacterial luminescence inhibition; increased the bacterial luminescence than the blank at room temperature at the studied concentrations (data not shown). Whereas in chronic toxicity assay, only KE of the studied apricot varieties was able to inhibit the bacterial emission with IC<sub>50</sub> values of 1.61, 1.63, 2.03, 1.92, 1.93 and 1.90 mg/ml, respectively calculated for Badoghur, Chagali, Charmaghz, Nari, Shakarpara and Sardai varieties (Fig. 2). All these values are insignificant (P < 0.05).

### 3.8. Mineral composition of apricot pomace and kernel

The results (mg/100 g) as mentioned in Table 5 of the main mineral and trace elements of the investigated apricot varieties, revealed that all analyzed samples contained a higher amount of K followed by Ca, Fe and Na. The estimated contents of K, Na, Ca, Fe, Mn, Co and Cd range from 1740.11 to 1539.20, 27.55–13.04, 217.46–77.87, 60.49–14.53, 2.80–1.11, 0.48–0.10, 3.57–0.27 and 0.04–0.01 mg/100 g DW for pomace while 2082.17–418.68, 22.38–9.67, 167.51–16.68, 26.46–7.97, 2.78–1.58, 0.75–
Comparison of antioxidant activity of kernel and pomace extracts and different standard compounds assessed by DPPH, HPS, TAC and FRAP assays (IC50 difference. V1, V2, V3
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Data are mean ± SD (n = 3). Different lowercase letters in superscript within each line mark significant differences (P < 0.05), as determined by Tukey’s HSD test among apricot varieties. ‘Kernel/pomace’ represents the ratio of antioxidant activity in the kernel to that in the pomace.

Table 3
Comparison of antioxidant activity of kernel and pomace extracts and different standard compounds assessed by DPPH, HPS, TAC and FRAP assays (IC50 [µg/ml]) in apricots grown in Balochistan.

| Antioxidant index | Varieties         | Badoghur | Chagali | Charmaghz | Nari | Shakarpara | Sardai |
|-------------------|------------------|----------|---------|-----------|------|-------------|--------|
|                   | IC50 (µg/ml)     |          |         |           |      |             |        |
| DPPH              | Kernel           | 24.88 ± 1.5| 98.61 ± 0.6| 91.78 ± 0.2| 79.96 ± 1.0| 49.97 ± 0.9| 52.61 ± 0.8|
|                   | Pomace           | 33.46 ± 1.8| 23.13 ± 0.4| 96.97 ± 1.4| 93.25 ± 2.6| 60.92 ± 2.2| 78.05 ± 0.5|
|                   | Kernel/Pomace    | 0.74 ± 0.04| 4.26 ± 0.10| 0.95 ± 0.04| 0.86 ± 0.01| 0.82 ± 0.04| 0.67 ± 0.01|
|                   | Ascorbic acid    | 10.26 ± 1.5|          |           |      |             |        |
|                   | Trolox           | 15.77 ± 1.1|          |           |      |             |        |
| HPS               | Kernel           | 334.84 ± 0.3| 516.63 ± 1.0| 400.47 ± 0.9| 385.00 ± 1.0| 350.50 ± 0.2| 374.79 ± 5.5|
|                   | Pomace           | 435.16 ± 1.8| 312.93 ± 0.3| 489.43 ± 0.9| 472.44 ± 0.7| 414.06 ± 1.3| 432.45 ± 0.9|
|                   | Kernel/Pomace    | 0.77 ± 0.06| 1.65 ± 0.00| 0.82 ± 0.00| 0.81 ± 0.00| 0.85 ± 0.00| 0.87 ± 0.01|
|                   | Ascorbic acid    | 282.06 ± 1.3|          |           |      |             |        |
|                   | Alpha tocopherol | 198.62 ± 0.9|          |           |      |             |        |
| TAC               | Kernel           | 22.02 ± 2.6| 110.80 ± 7.8| 63.36 ± 8.5| 72.61 ± 4.0| 26.13 ± 4.3| 36.42 ± 2.0|
|                   | Pomace           | 26.55 ± 3.3| 28.66 ± 1.0| 90.67 ± 3.5| 91.59 ± 3.5| 46.13 ± 6.9| 51.46 ± 1.2|
|                   | Kernel/Pomace    | 0.84 ± 0.2| 3.86 ± 0.2| 0.70 ± 0.1| 0.79 ± 0.1| 0.58 ± 0.1| 0.71 ± 0.1|
|                   | Ascorbic acid    | 19.75 ± 7.5|          |           |      |             |        |
|                   | Quercetin        | 9.49 ± 2.6|          |           |      |             |        |
| FRAP              | Kernel           | 102.67 ± 1.0| 163.35 ± 3.6| 119.25 ± 0.7| 106.77 ± 1.4| 96.27 ± 0.4| 112.76 ± 1.3|
|                   | Pomace           | 135.06 ± 2.2| 112.32 ± 3.6| 185.76 ± 4.7| 167.46 ± 3.3| 151.92 ± 2.5| 244.10 ± 29.8|
|                   | Kernel/Pomace    | 0.76 ± 0.02| 1.46 ± 0.05| 0.63 ± 0.02| 0.64 ± 0.01| 0.63 ± 0.01| 0.47 ± 0.06|
|                   | Ascorbic acid    | 68.42 ± 1.0|          |           |      |             |        |
|                   | Catechin         | 63.34 ± 0.7|          |           |      |             |        |
extracted lower amounts of phenolics compared with ethanol or acetone solvents.

Dissimilarity in the TPC of maximum and minimum ranked varieties i.e., Badoghur and Chagali was 2.9-folds in the KE and 2.0-folds in PE. In comparison, 1.9-folds and 2.7-folds variation in the KE and PE was observed in TFC between tested varieties containing uppermost and the lowest amounts of TFC, respectively. The observed variations in TPC and TFC among the tested varieties portray the genotype and fruit part’s effect. The higher levels of phenolics and flavonoids in the kernels than the pomace are because the number of phenolic increases as the fruits reach the maturity state since phenolics take part in seed germination (Dueñas et al., 2009). This means that TPC and TFC in the kernels are correlated to the cultivar, but not to the difference in the maturation period because both Badoghur and Chagali are early-ripened varieties. The recorded contents of TPC and TFC in the kernels were higher than those reported by (Chen et al., 2020; Gomaa, 2013; Kalia et al., 2017; Korekar et al., 2011) in the kernels of apricot cultivars from Pakistan, Indian and Egyptian domestic varieties, which could be due to the defatted composition of kernels used in the present study.

Nonetheless, the observed TPC and TFC values in the PE were lower than previously reported by Ali et al. (2011) and higher than recorded by Wani et al. (2017) in the apricot cultivars belonging from Pakistan and India, respectively. As reported by Korekar et al. (2011), different factors like genetic diversity, geographical location, growing conditions, harvesting time and soil composition could be the reasons for variations in the phenolics among the kernels and pomaces of apricot varieties from Balochistan province. Moreover, the choice of extraction solvent, the chemical composition of the substance and analytical methods under consideration also lead to mixed results among different authors.

The antioxidant potential of apricot KE and PE was measured spectroscopically using DPPH, HPS, TAC and FRAP assays. Due to diverse modes of action of antioxidants and complex sample behavior like different polarities, functional groups, and chemical etiquettes, more than one type of antioxidant activity quantification assay is recommended in the in-vitro antioxidant analysis. All these assays are simple, rapid and need a UV–vis spectrophotometer along with few reagents to perform. Pearson’s correlation analysis (Table 4) disclosed negative correlations between TPC and TFC assays with IC50 values of HPS, DPPH, TAC and FRAP assays, it demonstrates that the samples with substantial polyphenol content manifest lower IC50 values, agreeing that phenolics are seemed to contribute to the antioxidant activity of the extracts.

Both flavonoids and phenolic acids have been proved for their reductive capacities and potent antioxidants. Additionally, the DPPH, HPS, TAC and FRAP assays displayed comparable results, higher significant correlations were observed between FRAP and HPS assays \( r = 0.977 \) followed by FRAP and DPPH assays \( r = 0.933 \) of KE, which presumably be due to their common reaction mechanism based on their electron transfer capabilities. A similar approach was also shown in apricot fruit (Ali et al., 2011; Wani et al., 2017) and in apricot kernels (Gomaa, 2013; Zhang et al., 2018).

Assays based on light-emitting bacteria were used to study antioxidant perspectives of bioactive compounds of artificial and natural origination. Bioluminescent bacteria are sensitive to the action of different substances like hydrocarbons, heavy metals, and toxic compounds, which influences bacterial growth. These bacteria naturally have the capacity to emit light and therefore the presence of poisonous substances brings to inhibition of bacteria luminescence. All the same, the present study findings revealed that only the KE were effective to inhibit the bacterial BL only in chronic toxicity assay, which suggests the presence of toxic substances with a slow penetration mechanism in bacteria cells that might cause the inhibition of quorum sensing or cell growth regulation. Phenolic compounds can show their microbiode effect via different mechanisms of action. Such compounds can suppress various microbial virulence factors (e.g., neutralization of bacterial toxins, inhibition of host ligand interaction and suppression of biofilm formation), lower the membrane fluidity, suppress the synthesis of nucleic acids and energy metabolism or the cell wall (Görniak et al., 2019; Quideau et al., 2011). However, this could not be explained only based on flavonoids and phenolic acids.

As kernels of almonds, apricot, plums, peaches, apples and other members of the Rosaceae family are known for containing a toxic cyanogenic glycoside called amygdalin (Bolarinwa et al., 2014; Yildirim et al., 2014), which is hydrolyzed by β-glucosidase into hydrogen cyanide (HCN), benzaldehyde and d-glucose (Walker and Krieble, 1909). The toxicity of HCN is due to cyanide ion, which stops bacterial cellular respiration by inhibiting cytochrome C oxidase enzyme located in the bacterial membranes (Organization for the Prohibition of Chemical Weapons, 2017). At the same time, benzaldehyde, upon reaction with phenolic compounds forms phenolic benzaldehyde, a toxic compound (Friedman et al., 2003; Ghazavi et al., 2008). In this context, the quantification and identification of secondary metabolites having the anti-luminescence potential to the V. logei of apricot kernels could significantly benefit, particularly in discovering new antivirulence compounds.

| Table 4 | Pearson’s Correlation Coefficient (r) between assays. |
|---|---|
| **Antioxidant index** | **Coefficient correlation Pearson (r)** | **TPC** | **TFC** | **IC50 DPPH** | **IC50 HPS** | **IC50 TAC** |
| Kernel | TFC | 0.992** |
| | IC50 DPPH | \(-0.975^m\) | \(-0.946^m\) | \-0.899** | \-0.918ns | 0.807** |
| | IC50 HPS | \-0.951** | \-0.971** | 0.900** | \-0.701** | 0.939** |
| | IC50 TAC | \-0.830** | \-0.858** | 0.701** | 0.977** | 0.873** |
| | TFC | 0.990** |
| Pomace | IC50 DPPH | \-0.945** | \-0.945** |
| | IC50 HPS | \-0.834** | \-0.901** | 0.716** |
| | IC50 TAC | \-0.933** | \-0.917** | 0.829** | 0.752** |
| | IC50 FRAP | \-0.618** | \-0.608** | 0.933** | 0.560** | 0.454** |

**Correlation is significant at the level 0.01, *Correlation is significant at the level 0.05 ns: Nonsignificant, TPC: Total phenolics content.**

IC50 DPPH: Inhibitory concentration, the amount of sample needed to decrease by 50% initial DPPH Concentration.

IC50 HPS: Inhibitory concentration, the amount of sample required to decrease by 50% initial HPS concentration.

IC50 TAC: Inhibitory concentration, the amount of sample required to decrease by 50% initial TAC concentration.

IC50 FRAP: Inhibitory concentration, the amount of sample required to provide 0.5 of absorbance of Ferric reducing antioxidant power.
The concentration of different element content in the kernel and pomace tissues of apricot grown in Balochistan.

Apart from phytochemicals, the information about the concentration of essential minerals is of great significance since the activity of more than one-third of all human proteins relies on them (Konczak and Rouille, 2011). Our findings revealed that among others, K, Na, Fe and Ca are significant apricot minerals, which

| Varieties   | Na     | K      | Fe      | Mn      | Co     | Cd     | Cu     | Pb     |
|-------------|--------|--------|---------|---------|--------|--------|--------|--------|
| Kernel      |        |        |         |         |        |        |        |        |
| Badoghur    | 18.01 ± 0.05 | 830.18 ± 7.47 | 55.07 ± 2.37 | 26.46 ± 0.69 | 2.78 ± 0.10 | 0.75 ± 0.10 | 0.55 ± 0.03 | 0.60 ± 0.20 |
| Chagali     | 22.38 ± 0.38 | 642.14 ± 7.06 | 73.13 ± 2.19 | 22.52 ± 0.55 | 2.10 ± 0.33 | 0.63 ± 0.23 | 0.35 ± 0.02 | 0.03 ± 0.01 |
| Charmaghz   | 12.98 ± 8.14 | 788.10 ± 29.16 | 167.51 ± 0.84 | 16.68 ± 0.35 | 1.58 ± 1.57 | 0.30 ± 0.21 | 0.60 ± 0.02 | 0.09 ± 0.02 |
| Nari        | 11.04 ± 0.07 | 2082.17 ± 8.33 | 49.98 ± 3.19 | 7.97 ± 1.48 | 1.64 ± 1.63 | 0.39 ± 0.19 | 0.49 ± 0.04 | 0.06 ± 0.03 |
| Shakarparsa | 9.67 ± 0.65 | 793.25 ± 11.90 | 166.68 ± 6.28 | 5.67 ± 0.46 | 1.58 ± 1.57 | 0.30 ± 0.21 | 0.43 ± 0.04 | 0.09 ± 0.02 |
| Sardai      | 16.20 ± 0.28 | 418.68 ± 5.02 | 153.66 ± 1.54 | 15.95 ± 1.35 | 2.08 ± 0.39 | 0.70 ± 0.13 | 0.53 ± 0.01 | 0.05 ± 0.03 |
| Pomace      |        |        |         |         |        |        |        |        |
| Badoghur    | 27.55 ± 0.33 | 1539.83 ± 61.59 | 131.28 ± 10.37 | 60.49 ± 0.60 | 3.80 ± 0.14 | 0.42 ± 0.14 | 3.57 ± 0.12 | 0.03 ± 0.01 |
| Chagali     | 13.04 ± 0.10 | 1698.47 ± 22.08 | 215.99 ± 5.62 | 22.05 ± 0.44 | 1.36 ± 1.34 | 0.26 ± 0.10 | 0.27 ± 0.02 | 0.04 ± 0.02 |
| Charmaghz   | 18.57 ± 0.74 | 1650.08 ± 47.85 | 217.46 ± 2.49 | 33.03 ± 1.29 | 2.55 ± 0.28 | 0.31 ± 0.13 | 0.55 ± 0.03 | 0.01 ± 0.01 |
| Nari        | 18.04 ± 0.13 | 1692.80 ± 30.47 | 215.30 ± 15.50 | 27.37 ± 0.66 | 1.46 ± 0.48 | 0.33 ± 0.09 | 0.61 ± 0.05 | 0.02 ± 0.01 |
| Shakarparsa | 19.56 ± 0.35 | 1746.11 ± 12.18 | 136.34 ± 9.82 | 25.85 ± 0.98 | 1.11 ± 1.06 | 0.48 ± 0.20 | 0.79 ± 0.01 | 0.04 ± 0.02 |
| Sardai      | 20.60 ± 0.20 | 1539.20 ± 61.59 | 77.87 ± 3.66 | 14.53 ± 0.64 | 1.45 ± 1.00 | 0.10 ± 0.01 | 0.63 ± 0.04 | 0.03 ± 0.01 |

Data are mean of three replicates ± SD (n = 3). Different lowercase letters in superscript mark significant differences (P < 0.05), as determined by Tukey’s HSD test among apricot varieties. ND: Not Detected.

| Component   | PC 1     | PC 2     | PC 3     |
|-------------|----------|----------|----------|
| Eigen value | 5.900    | 3.549    | 2.328    |
| Total variance (%) | 39.33 | 23.66 | 15.52 |
| Cumulative Eigen value | 5.900 | 9.449 | 11.777 |
| Cumulative variance (%) | 39.33 | 62.99 | 78.51 |
| TPC         | 0.377    | -0.126   | -0.171   |
| TFC         | 0.381    | -0.079   | -0.193   |
| Na          | -0.145   | -0.433   | 0.186    |
| K           | -0.125   | -0.139   | -0.429   |
| Ca          | -0.136   | -0.117   | -0.262   |
| Fe          | -0.005   | -0.594   | 0.069    |
| Mn          | 0.057    | -0.289   | 0.464    |
| Cu          | 0.257    | -0.085   | 0.307    |
| Co          | 0.019    | -0.456   | 0.040    |
| Cd          | 0.273    | -0.021   | 0.275    |
| DPPH        | -0.357   | 0.205    | 0.135    |
| TAC         | -0.360   | 0.119    | 0.176    |
| HPS         | -0.371   | -0.111   | 0.213    |
| FRAF        | -0.304   | -0.117   | -0.098   |
| BL toxicity assay | 0.153 | 0.342 | 0.388 |

The principal component analysis (PCA) is an efficient statistical method to minimize data dimensionality. The entire data on the TFC, TPC, mineral contents, antioxidant and bioluminescence toxicity assays were subjected to PCA (15 total variables). The results of the PCA are summarized in (Table 6). Three significant principal components (PCs), were obtained explaining more than 78% variance in data. PC 1 with maximum data variation (39.33%) displayed the highest data loadings for flavonoids followed (in decreasing order) by phenolics, Cd and BL toxicity assay. PC 2 showed high loadings all for BL assay, DPPH and TAC assays whereas, PC 3 revealed higher loadings in favour of Mn, BL assay, Cu, Cd, and HPS assay together with significant contributions from DPPH and TAC assays. The PCA results indicated that flavonoids and phenolics contribute significantly towards BL assay and DPPH, TAC, and HPS activities. Overall, flavonoids and phenolic contents exhibited strong associations with the AoA and BL toxicity assays.

5. Conclusion

It was inferred from the present work that apricot grown in the Balochistan province is a rich source of phytochemicals, antioxidants and BL toxicity assays of apricot samples.
V. logei. The cultivar and sample type were the predominant factors that influenced the contents of phenolic, flavonoid and antioxidant activity. Comparatively, apricot kernels were found superior to corresponding pomaces in the investigated parameters. Hence, kernels of these varieties could be essential ingredients as functional foods. Correlation analysis confirms the role of phenolics to contribute to antioxidant activity. Bioluminescence study showed an anti-virulence perspective of the kernel. However, a detailed investigation of particular bioactive compound(s) could be crucial, particularly in discovering novel antibacterial compounds in apricot kernels.

6. Limitations

The present work has limitations. It mostly reports the activity of crude kernel and pomace extracts, which acts synergistically with phenolic compounds, anthocyanins, carotenoids, carbohydrates, betalains, vitamins and enzymes. Therefore, identifying active secondary metabolites of the kernel and pomace parts of the fruit would be requisite for better interpretation of the reported results.

Funding

This study was financially supported by Higher Education Pakistan (HEC) under the IRSIP (International research support initiative program) scholarship (No. 1-8/HEC/HRD/2019/8769, PIN: IRSIP 43BMS G3) for six months at the University of Bologna, Italy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All authors express their appreciation to Higher Education Pakistan (HEC) for funding the research work under the IRSIP (International research support initiative program) scholarship (No. 1-8/HEC/HRD/2019/8769, PIN: IRSIP 43BMS G3) for six months at the University of Bologna, Italy.

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