Phytochemical profiling, antioxidant and HepG2 cancer cells’ antiproliferation potential in the kernels of apricot cultivars

Yongsheng Chen a, Abdullah Ahmed Al-Ghamdi b,⇑, Mohamed S. Elshikh b, Munir H. Shah c, Monerah A. Al-Dosary b, Arshad Mehmood Abbasi d,⇑

a Department of Food Science and Engineering, Jinan University, Guangzhou 510632, China
b Department of Botany and Microbiology, Faculty of Sciences, King Saud University, Riyadh, Saudi Arabia
c Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
d Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus 22060, Pakistan

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ABSTRACT

Phytochemical composition, in vitro antioxidant and antiproliferative activity against HepG2 cells were studied in the kernels of apricot cultivars grown in the northern areas of Pakistan. Relatively, the kernel of Habbi cultivar/AP-12 depicted significant potential to scavenge DPPH and ABTS + free radicals as well as oxygen radical absorbance capacity along with highest contents of total flavonoids, phenolics, chlorogenic and syringic acids on dry weight basis. The average concentration of quercetin ranged 0.072–1.343 mg/100 g, and of EGCG from 0.713 to 6.521 mg/100 g with maximum concentration in Hulappa/AP-3 and Kho Chali-Khatta 3/AP-17, respectively. Amygdalin content was highest (1145 mg/100 g) in the kernel of Balaani/AP-14. Highest inhibition of HepG2 cells was found in the kernel of Waflu Chuli/AP-9 (EC50 = 15.70 ± 3.77 mg/mL). The PCA showed significant contributions of polyphenols and flavonoids towards biochemical assays, while CA revealed similarities and associations among various cultivars. Our study revealed that Habbi, Waflu Chuli, Thukdeena and Balaani kernels are rich sources of bioactive compounds and possess significant antioxidant and anticancer activity and can contribute considerably in the prevention and treatment of chronic health disorders.

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1. Introduction

In the present era, natural products are gaining increasing attention and several workers are focused on the beneficial effects of plant based antioxidants (Korekar et al., 2011). Natural antioxidants such as phenolic, vitamins, minerals, and carotenoids are contributing substantially in the prevention of oxidation and quenching of free radicals species (Hussain et al., 2013). These antioxidants are highly effective against oxidative damage related health disorders including cardiovascular diseases, neurological syndromes, aging process, cancer, cataract development, weakness in immune system and inflammation (Biglari et al., 2008, Eunok and Min, 2009). Different parts of fruits either in fresh and dry form are rich source of natural antioxidant including polyphenols (flavonoids, phenolic acids, lignins, etc.), carotenoids and vitamins A, C and E (Hussain et al., 2013), which have been reported to control degenerative diseases in human (Abbasi et al., 2015).

Prunus armeniaca L. (apricot) belongs to family Rosaceae of Rosales order and is mainly cultivated in the Mediterranean regions (Haciseferoğulları et al., 2007). It has been estimated that domestication of apricot in Iran, Turkey, Afghanistan, Middle Asia and Western China is approximately 5000 years old (Sochor et al., 2010). According to (Faostat, 2013) in 2016 apricots production were more than 3.8 million tons and Turkey was the world’s top apricot producing state, followed by Iran and Uzbekistan. Pakistan is the sixth largest producer of apricot with annual production over 177,630 tons (Faostat, 2013). Gilgit-Biltistan and Khyber Pakhtunkhwa with a contribution of over 60% in annual production are the main apricot growing regions in Pakistan (Ali et al., 2014).

Currently, being a rich source of health beneficial nutrients (carbohydrates, vitamins, minerals, fibres, etc.) and phytochemicals (polyphenolics, carotenoids and glycosides) apricot has become a...
product of great interest (Wani et al., 2017), particularly for the researchers focused on its functional properties. Apricot fruit is rich in polyphenolics which include epicatechin, catechin, epigallocatechin, chlorogenic, ferulic, and p-coumaric acids, neochlorogenic acid, cyanidin-3-glucosides, quercetin-3-glucosides, kaempferol-3-rutinoside, rutin (Campbell et al., 2013), minerals (Hagedues et al., 2011), carotenoids (Zaghoudi et al., 2015), and fatty acids and sterols (Erdogan-Orhan and Kartal, 2011, Dragovicuzelac et al., 2005). These phytochemicals are the key contributors in antioxidant, anti-mutagenic, anti-microbial, hepatoprotective, cardio-protective and anti-inflammatory properties of apricot beside their role in taste, colour and nutritive value (Erdogan-Orhan and Kartal, 2011). In addition, use of apricot in the treatment of infertility, eye inflammation, hemorrhages, spasm (Wani et al., 2017), vaginal and skin infections (YiGIT et al., 2009) is also mainly attributed to phytochemicals present in its fruit, kernels, leaves and bark.

Apricot kernels either sweet or bitter, comprises around 34% of the seed (Mandal et al., 2007) and rich source of dietary proteins (Nout et al., 1995), oil, fiber (Hacseferogullari et al., 2007) and deadly cyanogenic glycoside amygdalin (Gomez et al., 1998). It was investigated that apricot kernels contain more amount of natural antioxidants compared to flesh (Soong and Barlow, 2004) and their oil content is used in snacks, pharmaceutical and cosmetic industries (Güler et al., 1999, Rati et al., 1997). More than 60 apricot cultivars are grown in the Pakistan northern areas. The fruits and leaves of these cultivars contain high concentration of natural antioxidant and anticancer compounds (Ali et al., 2011). Nevertheless, phytochemical composition and effective properties of apricot fruit have extensively been studied. However, present study was based on the anticipation that apricot kernels are good source of secondary metabolites beneficial for health along with strong antioxidant and anti-proliferative potential. Consequently, main objectives of the current investigation were to scrutinize (1) phytochemical profile, and (2) antioxidant and antiproliferative potential in the kernels of apricot cultivars. Moreover, this is the first study focusing on phytochemical profiling and bioactivities in the kernels of different cultivars of apricot grown using same cultural practices in the northern parts ‘Gilgit-Baltistan’ of Pakistan.

2. Materials and methods

2.1. Sampling

Kernels of 19 apricot (AP) cultivars were collected from the northern Pakistan (Gilgit-Baltistan) and stored at −25 °C until extraction. Moisture content was determined by oven-drying technique as described earlier (Wolfe and Liu, 2003).

2.2. Extraction

By using method of Chen et al phenolic compounds was extracted from apricot kernels (Chen et al., 2014). Shortly, kernel’s powder (2 g) in triplicate was extracted thrice with 100 mL chilled acetone (80%) for 3 min, then filtered under vacuum. The filtrate was evaporated at 45 °C under pressure and re-formed with distilled H2O to 10 mL and kept at −25 °C until further investigation.

2.3. Assessment of phenolic content

Folin-Ciocalteu colorimetric method was employed to assess total phenolics content (TPC) following the method as reported earlier (Chen et al., 2015) using Gallic acid as standard. In brief, extract or standard solutions were blended with distilled water and Folin-Ciocalteu reagent, respectively for 6 min. Afterwards, mixtures were neutralized using sodium carbonate solution (7%) for 90 min before taking absorbance at 760 nm using Infinite M200 PRO microplate reader (Tecan, Switzerland). Final values of TPC were displayed as mg GAE/100 g DW.

2.4. Quantification of total flavonoids

Total flavonoids content (TFC) was assessed by NaNO2-AlCl3 colorimetric scheme (Chen et al., 2017a). Sample or standard solutions were mixed with water in volumetric flask, and NaNO2 working solution was added. After 5 min, working solution of AlCl3 was added and mixture was kept for 6 min. Afterwards, NaOH solution and distilled water were supplemented to each volumetric flask, respectively. Reaction mixture absorbance was read at 510 nm by Infinite M200 PRO microplate reader. Catechin was used as standard, and the final concentrations of TFC were presented as mg CE/100 g DW.

2.5. Estimation of phenolic acids and flavonoids by HPLC

Phenolics and flavonoids composition was measured using high performance liquid chromatographic method (Pang et al., 2016). Briefly, the phenolic compounds in the AP kernel’s extract were alienated in a Waters Carbon18 column on a Thermo Scientific Ultimate 3000 HPLC system at 1.0 mL/min flow rate; 30 °C temperature; 20 μL injected volume at finding wavelength of 280 nm. The binary mobile phase comprised 996:4 V/V of water/acetic acid (solvent A) and acetonitrile (solvent B). The mobile phase gradient for solvent A was: 0–40 min (95–75%); 40–45 min (75–65%); 45–50 min (65–50%); 50–55 min (50–95%) and 55–60 (95%). Chromatographic peak identity was distinguished according to the retention time for each compound. Measured levels of phenolic acids and quercetin were presented as mg/100 g DW.

2.6. Quantification of amygdalin (Vitamin B-17) content

Vitamin B17 (amygdalin) content in apricot kernels was identified using the method of García-A et al. (2016). Briefly, 2 g sample powder was extracted with methanol (100 mL) for 6 h in a water bath (60 °C) and filtered under vacuum. Filtrate was evaporated at 40 °C under vacuum, and reconstituted with chromatographic methanol to 10 mL for HPLC analysis. The chromatographic conditions were set as: solvent A (water) and B (methanol) at ratio of 65:35; 1.00 mL/min flow rate; 30 °C temperature; 20 μL injected volume at a detection wavelength of 218 nm.

2.7. Antioxidant assays

2.2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted as explained before (Chen et al., 2016). Concisely, 2 mL solution of DPPH (0.1 mM) and 2 mL extract of each concentration were mixed and incubated in the dark for 30 min at room temperature. Absorbance of samples, blank (ethanol instead of DPPH) and control (ethanol instead of the extract) was measured at 517 nm. The equation given below was used to calculate DPPH radical scavenging activity and linear regression analysis of absorbance against concentration was done to compute EC50 (representing 50% reduction) for DPPH value.

\[
\text{DPPH radical scavenging activity} = \left( \frac{\text{Abs. of Control} - \left( \text{Abs. of Sample} - \text{Abs. of Blank} \right)}{\text{Abs. of Control}} \right) \times 100\%
\]

The ABTS radical scavenging activity in apricot kernels was performed according to the method already reported. (Chen et al., 2016). In this assay green ABTS+ is generated under appropriate
oxidant, and is blocked in the presence of antioxidants (Lee and Yoon, 2008). 1.75 mL of potassium per-sulphate (2.45 mM) with 100 mL of ABTS (7 mM) in the ratio of 625:11 (V/V) were used to prepare ABTS+ stock solution. Working solutions were prepared by diluting the stock solution with 0.05 M phosphate buffer (pH 7.4) till the absorbance was read 0.70 ± 0.02 at 734 nm. Different concentrations of extracts and working solutions of 1:19 (V/V), were blended at room temperature and stored for 10 min in dark. Absorbance at taken 734 nm for 30 min against ethanol using Infinite M200 PRO microplate reader. The percentage ABTS radical scavenging activity (%) was calculated using equation mentioned as under. Linear regression analysis was done to compute EC50 expressed as mg/mL.

**ABTS radical scavenging activity**

\[
\text{ABTS radical scavenging activity} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100
\]

Total antioxidant capacity of apricot kernels was quantified using ORAC assay following the procedure reported previously (Chen et al., 2017b). Initially, a mixture of 200 µL fluorescein (0.96 µM) and 20 µL Trolox or sample extract in 96 well plate was incubated at 37 °C for 20 min. Subsequently, 20 µL of freshly prepared AAPH (119.4 mM) was added to each well. Fluorescence was measured immediately at 485 and 535 nm (excitation and emissions, respectively) at 37 °C for 35 cycles (after every 4.5 min) through Fluoroskan Ascent fluorescent spectrophotometer Infinite M200 PRO. The ORAC value was expressed as µM Trolox equ./g DW.

2.8. Antiproliferation assay

The antiproliferation ability of apricot kernels was estimated following the method as reported previously (Chen et al., 2019). HepG2 liver cancer cells were first grown in DMEM medium containing fetal bovine serum (10%), hydrocortisone (0.05 µg/mL), N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (10 mM), penicillin (50 units/mL), 5 streptomycin (0 µg/mL) and gentamycin (100 µg/mL) and kept at 37 °C and 5% CO2. HepG2 cells were seeded in a 96-well microplate (2.5 x 104 cells/well) and cells were nourished with fresh medium containing different concentrations of the kernels’ extract and control containing growth medium only. Cell viability was determined after incubation of 72 h at 37 °C using the methylene blue method. Final values were expressed as EC50 (mg/mL).

2.9. Statistical analysis

Data were presented as mean ± SD for triplicates analysis. ANOVA and Duncan’s test (p < 0.05) were executed also via SPSS, Inc., Chicago, IL, USA. For detail information on data set and to gain insight into the distribution of phytochemicals and their bioactivities multivariate analysis in terms of principal component analysis (PCA) and cluster analysis (CA) was done using varimax normalized rotation on the data set and Ward’s method respectively as explained previously (Patras et al., 2011).

3. Results and discussion

Total phenolics, flavonoids, phenolic compounds, quercetin, amygdalin (vitamin B17) contents, antioxidiant capacity and antiproliferative effect on HepG2 liver cancer cells were examined in the kernels of nineteen apricot cultivars. These cultivars are commonly grown in different areas of Gilgit-Baltistan region. Fruits of these cultivars varied in size, taste, texture, flavour and aroma. Likewise, their kernels ranged from extremely bitter, slightly bitter and sweet in taste. Moisture content affects significantly on the composition and concentration of phytochemicals in the fruits, vegetables, grains and seeds (Ali et al., 2011). The highest percentage of moisture content was found in the kernels of Borow Chuli (AP-10), followed by Yaqar Chuli (AP-4), Kho Chali-Khatta-1 (AP-15) and Brio Chuli (AP-6) at 5.73, 5.37, 5.29 and 5.19%, respectively (Table 1). On the whole, average percentage moisture content in the kernels of studied samples was 4.44 ± 1.21% (Table 3), with the highest concentration in the kernels of Borow Chuli (AP-10) and lowest in Kho Chali-Khatta-2 (AP-16).

### 3.1. Total phenolics and flavonoids

Measured levels of total phenolics content (TPC) and total flavonoids content (TFC) in the kernels of apricot are presented in Fig. 1(A and B), respectively. TPC ranged 209.4 ± 0.91 to 1060 ± 0.20 mg GAE/100 g DW (Fig 1A) with an average value of 59.41 ± 0.54 mg GAE/100 g on dry weight basis (Table 3). Habbi (AP-12) kernel had maximum TPC at 209.4 ± 0.91 mg GAE/100 g DW, followed by Thukdeena (AP-11) and Wafuu Chuli (AP-9) at 134.3 ± 1.20 and 131.5 ± 1.86 mg GAE/100 g DW, respectively. However, Sukior Chuli (AP19) exhibited the lowest levels of TPC at 10.60 ± 0.20 mg GAE/100 g DW. It was observed that TPC varied significantly (p < 0.05) in all cultivars excluding Borow Chuli (AP-10) and Sukior Chuli (AP-19). Results illustrated in Fig. 1B depicted that the kernels of Habbi (AP-12) had highest TFC (182.6 ± 1.04 mg CE/100 g DW) among all the studied samples, which was significantly different at p < 0.05. Likewise, TFC detected in the kernels of Wafuu Chuli, Thukdeena and Staa Chuli (126.7 ± 0.57, 94.24 ± 0.85 and 57.31 ± 0.55 mg CE/100 g DW, respectively) were higher than other cultivars (p < 0.05), whereas Sukior Chuli (AP-19) kernel had the lowest level of total flavonoids on dry weight basis (1.266 ± 0.35 mg CE/100 g DW). On the whole, average concentration of TFC was 32.20 ± 0.39 mg CE/100 g DW (Table 3).

Difference in the phenolics content of uppermost and lowest ranked cultivars i.e. Habbi and Sukior Chuli was 19-folds, whereas 182-folds variation was noted in the case of total flavonoids between cultivars containing maximum and minimum levels of TFC. This portrays substantial disparity in the phenolics and flavonoids contents in the kernels of apricot cultivars. Such variations have also been reported among the kernels of almond cultivars, (Yildirim et al., 2010), black and hybrid berries varieties and apricot genotypes (Connor et al., 2005). Measured levels of TPC and TFC in the kernels of apricot from the northern areas of Pakistan were analogous to reported previously in apricot kernels (Korekar et al., 2011, Kalia et al., 2017, Gomaa, 2013) from Indian and Egyptian native varieties and to apricot fruits investigated earlier in China, Pakistan, India, Czech Republic, Turkey and Italy (Fan et al., 2018, Wani et al., 2017, Ali et al., 2011, Sochor et al., 2010, Kalyoncu et al., 2009, Leccese et al., 2008). We suggest that genetic diversity, growing conditions, geographical position, soil composition and harvesting time as reported earlier (Korekar et al., 2011) might be accountable for variations in the phenolics and flavonoids contents among the kernels of apricot cultivars from the northern areas of Pakistan as well.

Flavonoids to phenolics contribution intended on the basis of milliM ranged from 7.01 to 56.47% (Fig 1C). The highest contribution was calculated for the kernels of Wafuu Chuli (56.47%), followed by Habbi (51.12%), Thukdeena (41.10%), Staa Chuli (34.36%) and Jangli (40.27%), while Sukior Chuli had lowest contribution at 7.01%. These values were different considerably (p < 0.05) and representing flavonoids as major phenolics in the kernels of the most of the cultivars.
3.2. Polyphenolics composition of apricot kernels

Health beneficial properties of food and medicinal plants are mainly attributed to polyphenolics among others secondary metabolites. The apricot kernels have rarely been analysed systematically so far, particularly with reference to polyphenolics profiling. Measured levels of phenolic compounds viz. caffeic acid, epigallocatechin Gallate (EGCG), chlorogenic, p-Coumaric, syringic, sinapic, and ferulic acids and quercetin were identified and quantified in the kernels of 19 apricot cultivars by HPLC method are given in Table 1 along with chromatographs (Fig. S1).

Chlorogenic acid is one of the key phenolic acids that have been quantified in the fruits and leaves of apricot and other Prunus species (Dulf et al., 2017, Wani et al., 2017, Bors and Michel, 2002). In the present study, chlorogenic acid was recognised and calculated in the kernels of maximum cultivars except Kho Chuli, Karfo Chuli, Pharingh Chuli and Kho Chali-Khatta-1. In these cultivars, chlorogenic acid was below the detection limit. Average concentration of the chlorogenic acid in the studied samples varied from 1.60 ± 0.33 to 34.98 ± 1.86 mg/100 g on dry weight basis in Hulappa (AP-3) and Habbi (AP-12), respectively. Comparatively, these values were lower than reported in apricot fruit (Wani et al., 2017, Sochor et al., 2010). Caffeic acid is a powerful antioxidant and prevent early aging (Magnani et al., 2014). This acid was quantified in the kernels of three cultivars viz. Hulman, Wafiu Chuli and Thukdeena only ranging 0.30 ± 0.02 to 0.80 ± 0.09 mg/100 g DW. However, these values were comparatively lower than quantified levels in the fruits of apricot from India (Wani et al., 2017). Syringic acid was quantified in the kernels of five cultivars only, with maximum concentration in Habbi (AP-12) at 0.76 ± 0.07 mg/100 g on dry weight basis. p-Coumaric acid was identified only in the Murgulam kernel (AP-2), whereas ferulic and sinapic acids were detected only in 5 and 4 cultivars, respectively. Nevertheless, measured levels of phenolic acids in the kernels of apricot kernels were relatively lower than reported in its leaves and fruits (Fan et al., 2018, Wani et al., 2017, Hussain et al., 2013, Sochor et al., 2010). This might be due to the more accumulation of phenolic acids in the outer layers of fruits and leaves compared to endosperm (Idehen et al., 2017).

Quercetin and catechin have been reported as the major flavonoid antioxidants in fruits, predominantly in apricot (Fan et al., 2018, Hussain et al., 2013, Sochor et al., 2010). Quercetin inhibits prostate and lung cancer cell growth in human (Xing et al., 2001) and reduces cardiac complaints (Knekt et al., 2000). In the present study, quercetin was quantified in the kernels of nine cultivars (Table 1) with descending order: AP-3 > AP-4 > AP-16 > AP-2 > AP-1 > AP-8 > AP-19 > AP-17 > AP-18. Average concentration of quercetin content varied from 1.34 ± 0.31 to 0.06 ± 0.00 mg/100 g DW, which was moderately lower than enumerated in the fruits of apricot (Sochor et al., 2010).

The Epigallocatechin Gallate (EGCG) or epigallocatechin-3-gallate content was determined in the kernels of all cultivars except Kho Chuli (AP-5) and Brio Chuli (AP-6). The measured levels of EGCG varied from 0.71 ± 0.05 to 6.52 ± 0.11 mg/100 g DW in the kernels of Hulappa (AP-3) and Kho Chali-Khatta 3 (AP-17), respectively. Though, green tea has been reported as a rich source of EGCG (Du et al., 2012, Fujiki and Suganuma, 2012), however a study conducted in different types of fruits including apricot, vegetables, legumes, staple and processed foods commonly consumed in the Netherlands has reported no EGCG content (Arts et al., 2000). In the present study, detection of EGCG content in apricot kernels is of significant worth, because this compound has been reported as one of the most powerful antioxidant and anticancer agent (Du et al., 2013, Lambert et al., 2010). Therefore, apricot kernels with EGCG content could be a valuable addition in functional foods with noteworthy anticancer potential.
3.3. Amygdalin (Vitamin B-17) content in apricot kernels

Amygdalin (D-mandelonitrile-β-D-gentiobioside) is a commonly known bioactive phytochemical in the kernels of apricot, almonds, apples, plums, peaches and other members of Rosaceae family (Bolarinwa et al., 2014, Yıldırım et al., 2014). Maximum tolerance dose limit of amygdalin in human intravenous injection is approximately 0.07 g/kg (Song and Xu, 2014). In the traditional system, amygdalin has been reported in the treatment of diabetes, asthma, leprosy and bronchitis (Bolarinwa et al., 2014, Halenár et al., 2013). Though, amygdalin is toxic to human if consumed in excessive amount but is also used as anticancer agent in the form of vitamin B-17 and laetrile (Yıldırım and Askin, 2010). It stimulates the apoptosis process in cancerous cells and improves deceleration of cell cycle by delaying cell proliferation and growth (Saleem et al., 2018). Recent investigations have proven that on hydrolysis amygdaline release toxic hydrogen cyanide (HCN) both in cancer and normal cells, hence may not be safe for human. However, studies on the anticancer potential of amygdalin reported that it decrease the expression of integrin’s, catenin levels and prevents Akt-mTOR pathway and subsequently inhibits the adhesion of lung cancer cells, bladder cancer cells and breast cancer cells and constrain metastases of cancer cells. In renal cancer cells, amygdaline augments expression of p19 protein expression, which prevents cell transfer from G1-phase to S-phase, and thus inhibit cell proliferation (Liczbiński and Bukowska, 2018). As reported in Table 3, average amygdalin content in the kernels of apricot cultivars was 164.7 ± 11.1 mg/100 g DW. Highest concentration of amygdalin that is 1145 ± 58.8 mg/100 g DW was estimated in the kernel of Balaani (AP-14), whereas measured levels of amygdalin content ranged 32.14 ± 3.61 to 1145 ± 58.8 mg/100 g DW in the kernels of Staa Chuli (AP-18) had the lowest concentration viz. 32.14 ± 3.61 mg/100 g DW (Table 1). The difference of amygdalin content in t highest and lowest ranked cultivars was 35 folds. The amygdalin content detected in the kernels of Kho Chali-Khatta-3 (AP-17) and Waflu Chuli (AP-9) was also above 200 mg/100 g on dry weight basis. Considerably higher amount of amygdalin was also determined in the kernels of Habbi (AP-12), Murgulam (AP-2), Kho Chali-Khatta-1 (AP-15) and Brio Chuli (AP-6) at 172.2 ± 6.13, 161.2 ± 20.9, 157.6 ± 30.3 and 155.1 ± 8.78 mg/100 g DW, respectively. On the whole, kernels of bitter cultivars depicted high amygdalin content compared to sweet or less sweet cultivars and these findings were comparable with previous reports (Yıldırım and Askin, 2010, Yıldırım et al., 2014). However, measured levels of amygdalin in the apricot kernels of Turkish cultivars (Yıldırım et al., 2014), in bitter almond genotypes (Ferrara et al., 2010, Yıldırım and Askin, 2010) were higher than detected in the present study.

3.4. Antioxidant potential in the kernels of apricot

The antioxidant capacity of apricot kernels were determined using DPPH, ABTS and ORAC assays, because a single assay is not sufficient to evaluate the free radicals scavenging capability of all
antioxidants. DPPH and ABTS scavenging activities were expressed as median effective dose (EC50). A sample extract with lowest EC50 value had strong antioxidant activity.

DPPH assay is one of the most simple and commonly used antioxidant technique (Cai et al., 2003) that estimates the capacity of antioxidant compounds to react and scavenge the DPPH free radical (Sochor et al., 2010). In the studied sample average EC50 value for DPPH activity was 20.38 ± 1.08 mg/mL (Table 3). This indicates that apricot kernels have significant potential to scavenge the free radicals. In all samples of apricot kernels, EC50 ranged 1.52 ± 0.02 to 90.70 ± 2.70 mg/mL (Fig. 2A). Highly significant DPPH activity was estimated in the kernels of Habbi (AP-12), followed by Waflu Chuli (AP-9), Thukdeena (AP-11), Staa Chuli (AP-18) and Murgulam (AP-2) at EC50 1.52 ± 0.02, 2.08 ± 0.04, 2.48 ± 0.09, 5.26 ± 0.21 and 5.32 ± 0.10 mg/mL, respectively. However, there was no significant difference in these values at p < 0.05. These findings revealed that DPPH scavenging potential in the apricot kernels fluctuate significantly across the cultivars. In addition, the EC50 value calculated for DPPH scavenging ability in the apricot kernels was significantly higher than pitaya flesh and peel (Wu et al., 2006), raspberry (Liu et al., 2002) and bacaba fruit, whereas comparable to apricot kernels (Korekar et al., 2011, Zhang et al., 2018) and fruit (Fan et al., 2018, Wani et al., 2017, Hussain et al., 2013).

According to Wu et al. (2006), ABTS assay is useful in both organic and inorganic solvent systems and to evaluate the antioxidant capacity and is considered as a better method compared to DPPH assay (Thaipong et al., 2006). The average effective concentration (EC50) in the kernels of apricot cultivars determined by ABTS assay was 52.31 ± 0.80 mg/mL (Table 3). Descending order of ABTS activity in studied sample was: AP-12 > AP-11 > AP-9 > AP-8 > AP-2 > AP-18 > AP-14 > AP-15 > AP-7 > AP-13 > AP-5 > AP-14 > AP-1 > AP-6 > AP-17 > AP-10 > AP-3 > AP-19 (Fig. 2B). The lowest EC50 was detected in Habbi (AP-12) at 8.91 ± 0.21 mg/mL, which indicating highest ABTS activity in the cultivar, followed by Thukdeena (AP-11) and Waflu Chuli (AP-9) with EC50 = 11.74 ± 0.16 and 14.18 ± 0.23 mg/mL, respectively (p < 0.05). Sukior Chuli (AP-19) exhibited maximum EC50 = 161.1 ± 4.90 mg/mL, which indicates the lowest ABTS activity. Comparatively, apricot kernels showed significant potential to scavenge the ABTS radical than its peel and pulp (Fan et al., 2018, Wani et al., 2017, Leccese et al., 2008). However, variation in the DPPH and ABTS activity in the kernels across the apricot cultivars may be due to genetic variations and environmental factors. Moreover, Lahouar et al. (Lahouar et al., 2014) reported that in vitro DPPH and ABTS assays confirm an impression of the antioxidant capacity in the plant samples only, instead of the contribution of specific antioxidants. Therefore, the antioxidant capacity in the kernels of apricot was further confirmed using ORAC assay.

The ORAC assay is one of the most extensively utilized method to determine the antioxidant potential in fruits, vegetables, medicinal plants and nutraceuticals (Madhujith and Shahidi, 2007). This assay offers ability of sample extract to scavenge peroxy radicals...
by transferring a hydrogen atom (Slavin et al., 2009). The oxygen radical absorbance capacity of apricot kernels was studied for the first time using Trolox control by ORAC assay. Measured values of ORAC in the kernels of nineteen cultivars of apricot varied from 518.0 ± 61.6 to 4892 ± 82.25 µM TE/100 g DW (Fig. 2C) and about 9-folds difference was observed between minimum and maximum values. As shown in Table 3, mean value of ORAC in all samples was 1991 ± 144.3 (µmol Trolox equiv./100 g DW). Habbi kernel (AP-12) depicted the highest capacity to inhibit oxygen radical at 4892 ± 82.25 µM TE/100 g DW, followed by Balaani (AP-14), Kho Chuli (AP-5), Kho Chali-Khatta-1 (AP15), Thukdeena (AP-11) and Wafulu Chuli (AP-9) at 4199 ± 558.3, 3144 ± 85.05, 2826 ± 111.3, 2728 ± 125.6, 2683 ± 453.6 µM TE/100 g DW, respectively. ORAC values in AP-12, AP-14 and AP-5 varied significantly (p < 0.05). Whereas, Pharingh Chuli (AP-8), Sukior Chuli (AP-19) and Borow Chuli (AP-10) were among the cultivars having the lowest ORAC values (776.2 ± 84.0, 658.0 ± 86.4 and 518.0 ± 61.6 µM TE/100 g DW, respectively).

3.5. HepG2 cancer cells' antiproliferation potential of apricot kernels

Antiproliferative activity of apricot kernels was studied against HepG2 human liver cancer cells for the first time and results are summarized in Table 2. The HepG2 cells' inhibition was calculated as the median effective dose (EC50) and the lowest EC50 value indicates higher antiproliferative activity. Wafulu Chuli (AP-9), Thukdeena (AP-11), Habbi (AP-12), Balaani (AP-14), Kho Chali-Khatta-1 (AP-15), Kho Chali-Khatta-2 (AP-16), Kho Chali-Khatta-3 (AP-17) and Staa Chuli (AP-18) depicted relatively more inhibition of HepG2 cell growth. Wafulu Chuli (AP-9) exhibited highest HepG2 cells inhibition with EC50 = 14.71 ± 0.82 mg/mL followed by Habbi and Staa Chuli (EC50 = 15.70 ± 3.71 and 28.05 ± 0.39 mg/mL, respectively). However, these values didn't show any significant difference (p < 0.05). Relatively low antiproliferative activity was noted in Thukdeena, Balaani, Kho Chali-Khatta-1, Kho Chali-Khatta-2 and Kho Chali-Khatta-3. However, in the kernels of other cultivars EC50 could not be intended precisely and measured values were over 90 mg/mL, which might be due to the deviation from the extrapolation assessment.

Our findings revealed that the kernels of Wafulu Chuli and Habbi cultivars possess significant potential to inhibit the HepG2 cancer cells. Furthermore, antiproliferative activity in these cultivars was analogous to the reported levels in bitter almond cells. Furthermore, antiproliferative activity in these cultivars possess significant potential to inhibit the HepG2 cancer cells. Although, antiproliferative activity of apricot kernels was belong to the region having phytochemical content, antioxidant and anti-proliferative activity are independent of their geographical origin (Ben Mohamed et al., 2018) and was not significant. Consequently, HepG2 cells’ inhibition by the kernels of apricot couldn’t be elucidated only on the basis of phenolics and flavonoids. This exposed that some unique phytochemicals in apricot kernels could be responsible for the inhibition of HepG2 tumor cells. In this context, the identification and quantification of secondary metabolites having anticancer potential in the kernels of apricot could be of significant value, particularly to discover the novel anticancer drugs.

On the whole, significant variations were observed in phenolics, flavonoids and amygdalin contents along with antioxidant and anti-proliferative activity of apricot kernels. These variations mainly attributed to growing environment and genotype relations, geo-climatic factors, harvesting time, and plant physiology along with extraction and analytical techniques used during the study (Chappell et al., 2017, Bolarinwa et al., 2014). Though, phytochemical content, antioxidant and anti-proliferative activity are independent of their geographical origin (Ben Mohamed et al., 2018) but in our study apricot cultivars were belong to the region having a significant diversity. Hence, genetic diversity may contribute significantly in the composition and properties of apricot kernel.

3.6. Principle component and cluster analysis

The principal component analysis (PCA) and cluster analysis (CA) are effective statistical tools to reduce the dimensionality in data. The entire data related to the phytochemicals and bioactivity assays were employed for PCA and CA except phenolic acids and quercetin, as their values were below the detection limit in sizeable number of samples. Results of PCA, extracted by varimax normalized rotation on the dataset and are shown in Table 4. Three significant principal components (PCs), were extracted elucidating significantly higher than raspberries (Liu et al., 2002) and strawberries (Meyers et al., 2003). Speciously, the association between phytochemical content and EC50 of HepG2 cells’ proliferation was not significant. Consequently, HepG2 cells’ inhibition by the kernels of apricot couldn’t be elucidated only on the basis of phenolics and flavonoids. This exposed that some unique phytochemicals in apricot kernels could be responsible for the inhibition of HepG2 tumor cells. In this context, the identification and quantification of secondary metabolites having anticancer potential in the kernels of apricot could be of significant value, particularly to discover the novel anticancer drugs.
more than 87% variance in data with Eigen values greater than unity and cumulatively. PC 1 with maximum variance of data (58.30%) showed highest loadings for phenolics, flavonoids, chlorogenic acid and anti-proliferation activity together with significant contributions from ORAC, ABTS and DPPH activities. PC 2 revealed elevated loading in favour of amygdalin only while PC3 exhibited higher loading for EGCG and some significant contributions from ABTS and DPPH activities (Table 4). The PCA results therefore indicated that phenolics, flavonoids and chlorogenic acid are contributing significantly towards anti-proliferation activity as well as ORAC, ABTS and DPPH activities whereas amygdalin showed insignificant contributions towards these bioactivities. However, EGCG also contributes towards ABTS and DPPH activities as it exhibited shared contributions towards PC1 and PC3. This analysis indicated that some other metabolites may also contribute towards free radical scavenging activities. Overall, the phenolic and flavonoid contents showed significant associations with the bioactivity assays.

Cluster analysis (CA) was carried out to check multiple relationships, similarities or grouping among various cultivars of apricot on the basis of phytochemical contents and bioactivity data. The results of CA based on Ward’s method are presented in Fig. 3. In general, intimacy in the clusters reflects resemblances among the varieties based on their phytochemical and bioactivity data. First cluster comprises AP-1, AP-7, AP-13, AP-2, AP-4 and AP-16, which has close association with second cluster of AP-5, AP-15 and AP-8 cultivars. In third cluster, AP-1, AP-7 and AP-13 were grouped together and were closely associated with another mutual cluster fourth comprising of AP-2, AP-4, AP-16, AP-5 and AP-15. However, AP-8 showed weak association in these clusters. AP-6, AP-17 and AP-3 were grouped in fifth cluster. Similarly, a strong cluster was found for AP-11, AP-12, AP-18 and AP-9 were found together in sixth cluster, while AP-14 showed more or less independent behaviour and was not strongly associated with any other cultivar. AP-10 and AP-19 showed a mutual cluster showing maximum heterogeneity with rest of the varieties thus indicating their unique and characteristic association among apricot cultivars. On the whole, CA revealed similarities and associations among various cultivars of apricot; however AP-14 and AP-8 showed more or less independent behaviour as they were not strongly associated with any other variety.

4. Conclusions

Phenolic acids, quercetin, epigallocatechin gallate (EGCG), amygdalin contents, in vitro antioxidiant and antiproliferative activity against HepG2 cells were studied in the kernels of apricot cultivars grown in northern areas of Pakistan. Habbi, Waflu Chulia, Balaani and Thukdeena were among the top ranked cultivars with elevated levels of phytochemical contents, and significant antioxidiant and antiproliferation potential against HepG2 cells. Therefore, kernels of these cultivars could be valuable ingredients as functional foods to promote consumer’s health. PCA confirm the role of polyphenolics towards bioactivity assays, while CA revealed significant associations in apricot cultivars based on their composition and bioactivities. However, due to less association between phytochemicals content and EC50 of HepG2 cells’ proliferation detailed profiling of health beneficial secondary metabolites along with in vitro/in vivo studies could be of significant value, particularly to discover the novel anticancer drugs base on apricot kernels.

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Declaration of Competing Interest

All authors declare that there is no competing and financial interest.
Appendix A. Supplementary material

Supplementary data to this article can be found at https://doi.org/10.1016/j.sjbs.2019.06.013.

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