INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 6, p. 346-354, 2018

RESEARCH PAPER OPEN ACCESS

Assessment of phytochemical constituents, antioxidant activity, cytotoxicity and antiproliferative activity of apricot

M. Asghar*[1], M. Gulfraz[1], M. J. Asad[1], T. Masud[2]

[1]*University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan*

[2]*Department of Food Technology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan*

Key words: Apricot, MTT, HPLC, FTIR, Flavonoids, Phenolic acids, Antioxidants

http://dx.doi.org/10.12692/ijb/12.6.346-354 Article published on June 29, 2018

Abstract

Medicinal herbs are having decisive role to improve the human health standard because of predominant bioactive constituents. Prunus *armeniaca* (Apricot) is a highly nutritious fruit with a rich composition of health promoting components with a unique taste. Apricot samples grown in Gilgit Baltistan were assessed for their biochemical attributes, FTIR interferogram profiling, Phytochemicals screening, antioxidant activity, Cytotoxicity, and MTT based antiproliferative activity. HPLC data of fruit extract showed the presence of phenolic acids and flavonoid contents, appreciable quantities of vanillic acid, gallic acid and catechin. These secondary metabolites exhibited rich biochemical and functional activity, significant antioxidant potential and free radicals scavenging activity. FTIR spectra's were used to obtain information on functional groups and chemical structure of apricot samples showed significant antioxidant activity as well antiproliferative activity against cervical cancer cells (HeLa-S3). Anti-cancer results are also supported by antitumor potato disc assay by significant inhibition of tumor formation.

* **Corresponding Author:** M. Asghar ✉ qamasghar@gmail.com

Introduction

Apricot (*Prunus armeniaca*) is classified under the genus *Prunus* belongs to the family of *Rosacea*, cultivated in different parts of the world including northern areas of Pakistan. It is amongst the most popular of fresh fruits worldwide due to its combination of color, flavor, and nutritional value. Apricot fruit is well known for its high content bioactive compounds and strong antioxidant capacity. Apricot fruit regarded as a source of potent phytonutrients rich in polysaccharides, fatty acids, phenolic compounds, flavonoids, polyphenols, carotenoids, vitamins and minerals (Ali *et al.,* 2011).

Diseases that are commonly observed in the humans are due to the oxidative stress in the process of aerobic respiration may be due to ROS. Both metabolic products and antioxidant enzymes of apricot have a variety of preventative potential, helps to reduce the cellular oxidative damage. Apricot contains a large number of secondary metabolites that have several functional groups with different polarization. The metabolites are chemically diverse compounds and are often specific to different types.

Important phytochemical constituents have been studded using powerful analytical techniques including Fourier Transform Infrared spectroscopy (FTIR), High performance liquid chromatography (HPLC), *in-vitro* and *in-vivo* methods, different immunological and biological assays.

Pharmacological studies of apricot fruit express antioxidant and radical scavenging properties have antimicrobial activity and antitussive effects (Yigit *et al.*, 2001). These activities provide the basis for these compounds of greater importance to be used in pharmaceuticals (Muthocomaran *et al.*, 2011).

Many authors reported prophylactic properties of apricot, but very less activity has been reported to be comprehensive and effective for bioactivity and antioxidant capacity of apricots from Pakistan that may be used in the development of modern natural products. The objective of the present study was framed to characterize the effectiveness of apricot phytochemicals collected from Gilgit Baltistan region as a potential source of antioxidants and antitumor compounds.

Material and methods

Chemical composition

Apricot (P*runus armeniaca* L.) fully ripened fruits were collected from Gilgit Baltistan region in Northern Areas of Pakistan. The percent composition of moisture, crude fat, crude protein, ash content, crude fiber, and nitrogen free extract were performed in triplicate as described by (AOAC, 2005).

Total phenolic content expressed as gallic acid equivalent (GAE) was determined by using Folin-Ciocalteu's reagent method. Flavonoid contents were determined using aluminum chloride colorimetric method as described by (Kaneria *et al.,* 2012). Tannins in fruit extract was measured using tannic acid standard curve results by addition of Folin Phenol reagent and Na_2CO_3 in samples. Absorbance of samples was measured at 725nm.

To determine saponin, filtered sample was re-extracted, and combined extract was concentrated on water bath at 90°C. The concentrated extract was than extracted with diethyl ether. Purification process was carried out twice with n-butanol containing aqueous sodium chloride (05%). Layer of sodium chloride was discarded, and the extract was oven dried, weighed and content of saponin was determined as percentage (Akindahunsi *et al.*, 2006).

Flavanols and Phenolic Acids Determination (HPLC)

Flavanols and Phenolic acids were identified, and their quantities were evaluated using HPLC method on a Waters breeze HPLC system consist of Waters 1525 High pressure binary pump, Waters 2996 PDA detector, Waters 2489 dual Lambda UV-Vis detector, RP-C18 Nova Pack Waters USA column (250mm x 4.6mm, 5u particle size) and thermostatically controlled Waters column heater. Analysis was done for quercetin, myricetin, kaempferol and phenolic acids like vanillic acid, *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, chlorogenic acid, and gallic acid from the samples.

Flavanols and phenolics extraction and hydrolysis was carried out following the method as reported by (Tokusoglu *et al.* 2005). Fruits sample 5g, Acidified methanol 25ml (01 percent (v/v) HCl) and 0.5mg per ml BHT was added in a refluxing flask then 5ml HCl 1.2M was added to the mixture followed by stirring for 2 hours at 90°C under reflux. The extract was cooled to room temperature. After cooling the mixture was centrifuged for 10 min at 5000 rpm. Upper layer was recovered and sonicated 5 minutes long to remove the air. The resulting mixture was filtered through 0.45µm filter paper and injected for HPLC analysis. The mobile phase consists of trifluoracetic acid 50 per cent (0.3 percent), acetonitrile 30 percent and methanol 20 percent was mixed and homogenized for 30 minutes. Mobile phase was mixed to homogenize and was filtered through 0.45-micron nylon filters, sonicated and delivered at the flow rate of 1.0ml per min. Isocratic elution was used and detected at 360nm

Fourier Transform Infrared Spectroscopy

Samples were also evaluated and identified using chemical and physical methods along with FT-IR spectroscopy, Powdered samples were used for FT-IR spectroscopy. The interferogram of the samples were obtained by analyzing the sample at Thermo nicolet IS5 FTIR containing Dimond ATR. Fourier transform infrared spectrum with wavelengths ranging from 4,000-400 cm-1 at resolution of 4 cm-1 was used to obtain information on chemical structure. Dry nitrogen was used to purge the interferometer and the detector chamber to prevent spectral interference and the removal of carbon dioxide and water vapors. The background spectrum was taken before the experiment.

Antioxidant activity

Antioxidants present in extract reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ), forming an intense blue color Fe^{2+}-TPTZ complex with an absorption maximum at 593nm. The color developed is directly proportional to Ferric reducing antioxidant power of sample. DPPH radical scavenging activity was determined by the method of Antioxidant assay described by (McCune and Johns, 2002). Various concentrations of extract diluted in methanol and DPPH (0.2mM in methanol) were mixed well and incubated at room temperature for 30min in dark. Reduction of absorbance was recorded at 517nm. Total antioxidant activity of sample was determined by the scavenging of blue-green ABTS radicals, and was reported as ascorbic acid equivalent. BHT solution was used as the standard mixture (Arnao *et al.*, 2001).

The hydroxyl radical scavenging activity of extract was calculated as fenton reaction. Phosphate buffer 0.2M (pH 7.2), ferrous sulphate (0.02 M), fruit extract and 1, 10-phenanthroline (0.04M) were delivered in a test tube. Hydrogen peroxide (7mM) was used to initiate the fenton reaction. After 5 minutes of incubation at room temperature absorbance was taken at 560nm (Vinson *et al.*, 2000).

Antitumor Potato Disc Assay

Antitumor activity of fruit extracts was checked by the potato disc method as reported by (Ferrigini *et al.*, 1982). Potato disc were soaked in 2 percent $MgCl_2$ solution for sterilization and rinsed in distilled water. Anti-cancer potential of apricot extract was estimated by mixing agro-bacterium culture mixed with pant extract and poured on potato discs. After the incubation period of twenty-one days dies were stained with lugol solutions and number of tumors were counted. The Percent inhibition by using various concentration of fruit extract was calculated

Cytotoxic Brine Shrimp Assay

Cytotoxicity against brine shrimp for the extract of samples was evaluated by the method reported by (Meyer *et al.,* 1992). Sterile vials were used and in each vial 0.8ml of each extract solution i.e. 2000ug/ml, 1000ug/ml, 100ug/ml poured in to Vials then solvent was evaporated from vials and sediment of vials was resuspended by using 4 ml of normal saline. The assay was performed by adding twenty (20) shrimps in vial having total volume 15ml, these vials were incubated at temperature of 25-28°C. After the completion 24 hours of incubation, number of survived shrimps was counted with help of magnifying hand glass lens 10X. Result which is corresponding with deaths rate was calculated.

MTT Based Antiproliferative Assay

Cellular toxicity was determined using an assay (3,4,5-dimethyl-thiazol-2-yl) -5-5-diethyltetraziolium bromide. Reduction of MTT to the purple formazine due to mitochondrial dissociation of active metabolic cells provide the base for this test analyses (Zhao and Zhao, 2004). Culture media RPMI 1640 with 10 percent heat inactivated fetal bovine serum was used to seed HeLa-S3 cells at a concentration of 10 x 10^4 in a 24 wells plate. Methanol extracts prepared from apricot was introduced into cell culture system using different concentrations as 0-300µg per ml. Assay plates were incubated for 24 hours and the amount of reduced MTT was measured by measuring the intensity of color developed. The absorption was then measured at 570 nm and experiment was repeated at least three times.

Statistical analysis

All analyses were performed in three replications, and the data was expressed as mean ± standard deviation (SD). Dose-response relation between the different concentrations of extracts was calculated by regression analysis.

Analysis of variance (ANOVA) was used to evaluate the statistical significance ($p < 0.05$) confidence level were considered statistically significant.

Results and discussions

Proximate analysis and biochemical evaluation

In this study, we evaluated the nutritional significance and biological activity of apricot based on their chemical composition, phenolic contents, flavonoids, tannins and saponins. Proximate analysis has vital role in chemical screening of apricot. The chemical composition including moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract of dry fruit and kernel is given in (Table 1). The present results of fruit proximate composition are in harmony with the investigations of Vinson and co-workers (Vinson *et al.*, 2000). Higher energy density is represented by the higher protein and lipid contents. The crude Protein content of kernel 22.34 (%) is in accordance with previous findings of (Akindahunsi *et al.*, 2006) and their concentrated forms can be a good source of protein, essential for life as component of human body.

Table 1. Chemical composition of apricot fruit (%).

	Moisture	Crude Protein	Crude fat	Crude Fiber	Ash	NFE
Fruit	8.16±0.41	3.12±0.16	2.18±0.19	3.16±0.12	3.94±0.14	3.67±0.66
Kernel	4.78±0.32	22.34±1.36	52.62±2.41	2.97 ± 0.51	2.27±0.6	16.7±1.63

Each value is expressed as mean ± standard deviation (n = 8).

Mostly phenolic compounds in fruits exhibit antioxidant activity there of inhibit oxidative degradation of different substances by scavenging of reactive species, including free radicals. In apricot total phenolic contents, flavonoids and antioxidant is the crucial factor reported by several cultivars. Total Phenolic content of sample (72.26 ± 2.26 mgGAE $100g^{-1}$) is lower than (263.40 ± 9.82 mgGAE $100g^{-1}$) reported by (Dag *et al.*, 2011). Fruit sample (85.18 ± 1.2 mgQE $100g^{-1}$) was particularly rich in flavonoid contents (Table 2). Flavonoids fight continuously against polluted atmosphere and are remarkable reactive species (ROS). Quantity of saponins was found higher (1.41 ± 0.23 mg $100g^{-1}$) in apricot, but relatively lower in *D. lotus* (0.98 ± 0.08 µg/100g), A. hypogaea (0.88 ± 0.36µg/ 100g) and *J. regia* (0.54 ± 0.02µg $100gm^{-1}$). Saponins also reduces heart diseases and higher amount of tannins was also found (as tannic acid equivalent 4.49 ± 0.64mg $100g^{-1}$) in apricot. Tannins performs antimicrobial activity as they do not allow the microbes to connect with cell wall. Tannins help in protein transportation and attach to polysaccharides in cell membrane (Chen and Blumberg, 2008).

Table 2. Total phenolic content and radical scavenging activity of Apricot.

Phenolic mgGAE/100g	Flavonoid mgQE/100g	Tannins (%)	Saponin (%)	FRAP µmol Fe^{+2}/g	DPPH (%)	ABTS (%)
72.6±2.26	85.18±1.2	0.038±1.6	0.002±1.11	38.6±2.8	79.3±1.9	72.33±2.4

Each value is expressed as mean ± standard deviation (n = 6).

Fourier Transform Infra-Red Spectroscopy (FTIR)

Properties of the samples were identified using chemical and physical methods in parallel with FT-IR. Interferograms were taken in frequency region 4000-400 per cm for samples using Thermo-nicolet IS5 Fourier Transform Spectrometer. In qualitative analysis it is observed that aromatic domain bands presenting phenolic compounds at frequency range of 1654–1651cm (Fig. 1). Weak carboxylic C-O band of polyphenols at frequency range of 1465–1456cm was also found in interferometer. The absorption peak at frequency range of 1747–1746cm was due to –C=O stretching vibration in carbonyl compounds, may be due to the presence of high content of polyphenols in the samples. The hydroxyl (-OH) absorption band at frequency range of 3700–3200cm also showed in spectral analysis (Fig. 2). All the spectral values were expressed in percent transmittance. FT-IR spectroscopy is an excellent identification and analytical tool for analysis of raw foods and samples. One of the major advantage of FTIR is also repetitive analysis without any specific reagents for analysis required. The FT-IR generated time domain Interferogram for sample was then Fourier Transformed to obtain a frequency domain Infrared Spectrum showed similar spectral characteristic.

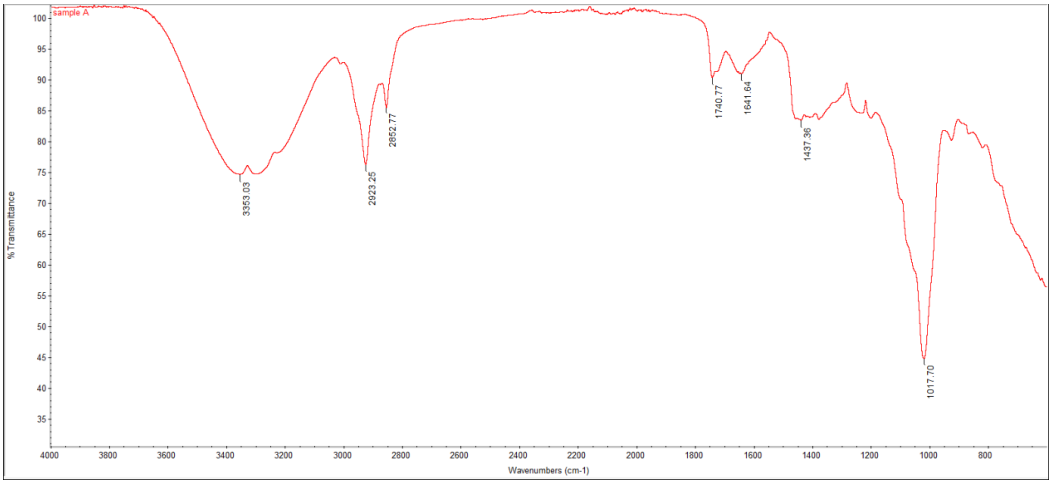

Fig. 1. FTIR spectra of apricot extract.

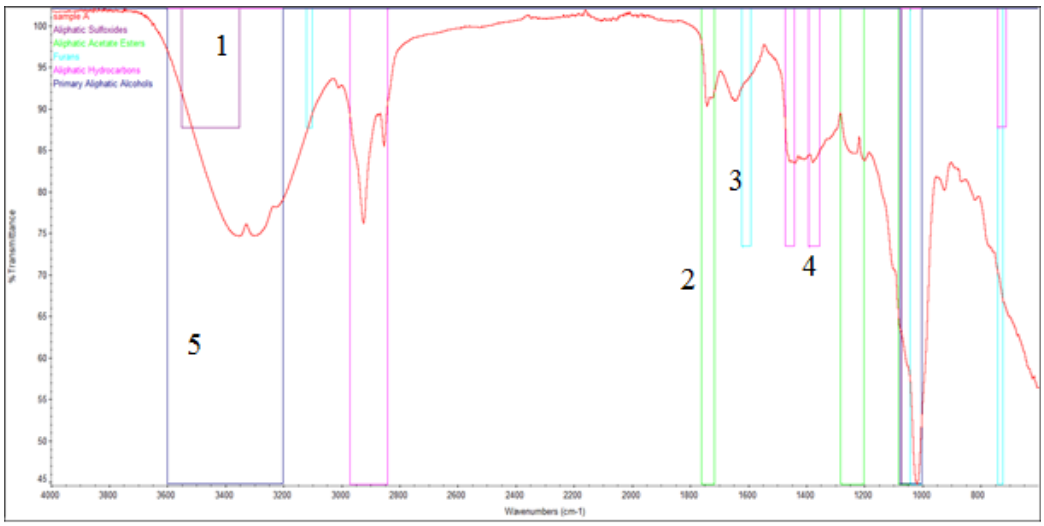

Fig. 2. Functional groups of apricot extract FTIR spectra.

1. Aliphatic Sulfosides
2. Acetate esters
3. Furans
4. Aliphatic Hydrocarbons
5. Primary aliphatic alcohols

HPLC phenolic profile

The phenolic acids such as caffeic acid, chlorogenic, neochlorogenic, p-coumaric and ferulic acids derivatives are the most commonly found in apricot (Sass-Kiss *et al.,* 2005). Chlorogenic acid and rutin were found to be the predominant phenolics in this study (Table 3). The highest catechin content per 100 gram of fruit sample was found (4.89 ± 1.43mg). Contents of *p*-coumaric acid (0.033 ± 0.02) and Quercetin (0.188 ± 0.08) were generally found to be higher. In the study of (Dragovic-Uzelac *et al.,* 2010) were reported gallic acid contents of apricot grown in Croatia as (3.47, 2.35 and 2.43mg per kg).. (Sultana *et al.,* 2007) in their study reported p-coumaric acid content in apricot fruit as (23.6mg per kg), ferulic acid as (13.9mg per kg), gallic acid content as (4.54mg per kg) and caffeic acid as (6.70mg per kg). These differences in phytochemical constituents may be due to the differences in climatic conditions and geographical factors.

Table 3. HPLC determination of polyphenols of apricot (100gm).

Gallic acid	Vanillic acid	Catechin	Cumeric acid	Rutin	Quercetin
0.512±0.22	2.359±0.46	0.571±0.38	0.033±0.02	4.89±1.43	0.188±0.08

Values are expressed in terms of Mean ± SD after three replications.

Antioxidant Activity

DPPH is a stable free radical in a methanolic solution used to determine the free radical scavenging capacity because of its simplicity and fast assay. Apricot fruit sample extract DPPH radical scavenging activity (40.71 ± 0.19) percent with the IC_{50} value of 97.41µg giving a significantly higher potential to protect against cellular damage caused by high levels of free radicals (Table 4). Similar antioxidant activity was also determined in dried apricots by (Molyneux, 2004). Higher DPPH radical scavenging activity value indicates greater antioxidant activity can be utilized by the food industry interested in fruits and vegetables with high content of bioactive compounds with the aim of producing supplements having better therapeutic effects.

Table 4. DPPH radical scavenging activity of apricot (% inhibition).

Fruit extracts conc. (µg/ml)	Gallic acid	Apricot extract
20	11.13±0.19	14.81±1.14
40	16.42±0.23	24.82±0.16
60	30.38±0.53	35.69±0.32
80	39.06±0.73	40.71±0.19
IC_{50} µg	102.42	97.41

Values are expressed in terms of Mean ± SD after three replications.

The total antioxidant potential of apricot extract (72.33 ± 2.4µmol ascorbic acid g^{-1}) performed by using ABTS radical cation showed significant antioxidant activity, displayed higher scavenging effect against BHT (Table 5).

The antioxidant activity is also based on the ability to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent, reduces yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue-colored ferrous complex (Fe(II)-TPTZ). FRAP Reducing power of apricot (38.6 ± 2.8µmol Fe^{+2} g^{-1}) indicates that the antioxidant compounds of apricot are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process.

Cytotoxicity Brine Shrimp Assay

Since antitumor activity of the plant extract can be tested by cytotoxic method, therefore cytotoxic potential of apricot samples was estimated by using brine shrimp's death rate.

Results obtained is presented in (table 6). Various fractions of samples added to test tubes containing brine shrimp in normal saline. Brine shrimp lethality rate as (93±2.83) at concentration of (1000µg**)** and LD_{50} value with reference to (r^2=0.9674) as (407.95µg) indicated that the brine shrimp survival rate depended on amount of plant extract, similar results were also reported by (Kanegusuku *et al.,* 2001).

Table 5. ABTS Antioxidant activity of apricot (% inhibition).

Extract (µg/ml)	BHT	GAFS
100	28±1.23	22±0.87
200	37.5±1.82	36.2±1.84
300	56±1.33	48.5±1.44
400	64±1.66	61±0.88
500	73.4±2.4	72.3±2.4
IC_{50}	285.4	318.23

Values are expressed in terms of Mean ± SD after three replications.

Antitumor Potato Disc analysis

Antitumor potato disc test is used as a tool to check the antitumor activity of herbs against crown galls caused by *A. tumefaciens* (Galsky *et al.,* 1980). Basic mechanism of uncontrolled cell division in plant tissue and animal tissue is similar (McLaughlin, l99l). Results for Antitumor potato disc test (78.4 ± 1.82) percent inhibition using 1000µg/ml and IC_{50} value 198.52µg showed highest potential to control tumor formation (Table 6). Observations of our current work showed that extract of Prunus *armeneaca* in different concentrations carry antitumor potential and are supported by finding of (Hussain *et al.* 2009). They reported that potato disc assay is reliable method for the screening of antitumor potential of plant extracts.

These sorts of research are important for selection and isolation of chemicals processing antitumor activity.

Table 6. Cytotoxicity and Antitumor analysis of apricot.

Apricot extract µg/ml	Cytotoxicity % death rate	Antitumor activity % inhibition
10	13 ± 1.16	14.5 ± 0.06
100	22 ± 1.74	46.7 ± 2.1
300	38 ± 2.37	63 ± 2.37
600	64 ± 1.63	67.7 ± 2.63
1000	88 ± 2.41	56.2 ± 1.14
LD_{50}/IC_{50}	468.19	320.16

Values are expressed in terms of Mean ± SD after three replications.

Antiproliferative Activity Using MTT Method

Cytotoxic agents can be of immune cells, chemicals, natural phytochemicals or proteins etc. Cells may lose their membrane integrity (necrosis) on exposure to a cytotoxic agent which may result stop dividing of the cell and undergo apoptosis. In this study MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylt etrazolium bromide) method used to measure the antiproliferative activity of apricot samples in terms of necrotic death and metabolic inactivity. Anticancer activity of apricot samples against cervical cancer cells (HeLa-S3) was studied (Table 7).

Apricot extract exhibited significant antiproliferative activity against the HeLa-S3 cells in a dose dependent manner as it inhibited up to (86 percent) cell growth at concentration of (300 µg per ml) in 24 hours. Dose dependent cytotoxicity of standard drug methotrexate on HeLa-S3 cells showed (80 percent inhibition) at concentration of (0.025µg per ml) and more than 90 percent inhibition at concentration of (1.25µg per ml).

The highest cytotoxic activity was found in apricot could be due to presence of higher anticancer phytochemicals.

Table 7. Antiproliferative activity of apricot.

Hours	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	300 µg/ml
03	96+1.14	90±1.39	83±2.11	74±1.47	66±1.98
06	94±0.66	43±1.22	38±1.42	26±0.65	17±0.33
24	55±2.32	31±1.86	26±2.18	22±1.54	17±1.63

Values are expressed in terms of Mean ± SD after three replications.

Conclusions

Significant contribution of apricot phytochemicals to biological activity including antioxidant potential, antitumor and antiproliferative activity measured in apricot, we conclude that these phytochemicals can be important source of dietary antioxidants and its co-administration may be responsible for the health benefits.

References

Akindahunsi AA, Oyetayo FL. 2006. Nutrient and antinutrient distribution of edible mushroom *Pleurotus tuberregium* (Fries) Singer. Elsevier **39,** 548-553.

Ali S, Masud T, Abbasi KS. 2011. Physico-chemical characteristics of apricot (*Prunus armeniaca* L.) grown in Northern Areas of Pakistan. Scientia Horticulture **130,** 386-392.

Arnao MB, Cano A, Acosta M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry **7,** 239-244.

Association of Official Analytical Chemists – AOAC. 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. (Ed.,18th)., Gaithersburg AOAC.

Chen CY, Blumberg JB. 2008. Phytochemical composition of nuts. Asia Pacific Journal of Clinical Nutrition **17(1),** 329-332.

Dag A, Kerem Z, Yogev N, Zipori, Lavee S, and Ben-David E. 2011. Influence of time of harvest and maturity index on olive oil yield and quality. Scientia Horticulturae **127(3),** 358-366.

Dragović-Uzelac V, Savić Z, Brala A, Levaj B, Kovačević and Biško A. 2010. Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the Northwest Croatia. Food Biotechnology **48(2),** 214-221.

Ferrigini NR, Putna JE, Anderson B, Jacobsen LB, Nichols DE, Moore DS, McLaughlin JL. 1982. Modification and evaluation of the potato disc assay and antitumor screening of Euphorbia seeds. Journal of Natural Products **45,** 679-686.

Galsky AG, Wilsey JP, Powell RG. 1980**.** Crown gall tumor disc bioassay. Plant Physiology **65,** 184-185.

Hussain J, Khan AL, Rehman NU, Hamayun M, Shah T, Nisar M, Bano T, Shinwari ZK, Lee IJ. 2009. Proximate and nutrient analysis of selected vegetable species: A case study of Karak region, Pakistan. African Journal of Biotechnology **8(12),** 2725-2729.

Kanegusuku M, Benassi JC, Pedrosa RC, Yunes RA, Filho VC, Maia AA, Marcia M, Monache FD, Niero R. 2001. Cytotoxic, hypoglycemic activity and phytochemical analysis of *Rubus imperialis* (Rosaceae). Naturforsch **57,** 272-276.

Kaneria M, Kanari B, Chanda S. 2012. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. *Asian Pacific* Journal of *Tropical* Biomedicine **2(3),** 195-202.

McCune LM, Johns T. 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. Ethnopharmacology **82,** 197-205.

McLaughlin JL. 1991**.** Crown-gall Tumors in Potato Discs and Brine Shrimp Lethality: Two Simple Bioassays for Higher Plant Screening and Fractionation. Methods in Plant Biochemistry **39,** 387-396.

Meyer-Alber A, Hartmann H, Sumpel F, Creutzfeldt W. 1992. Mechanism of insulin resistance in CCl4-induced cirrhosis of rats. Gastroenterology **102,** 223-229.

Molyneux P. 2004**.** The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Journal of Science and Technology **26(2),** 211-219.

Muthukumaran P, Padmapriya P, Salomi S, Umamaheshwari R, Kalaiarasan P, Malarvizhi C. 2011. In Vitro Anti-Microbial Activity of Leaf Powder, Asian Journal of Pharmacology **1(4),** 108-110.

Sass-Kiss A, Kiss J, Milotay P, Kerek MM, Toth-Markus M. 2005. Differences in anthocyanin and carotenoid content of fruits And vegetables. Food Research International **38,** 1023-1029.

Sultana B, Anwar F, Przybylski. 2007**.** Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica* and *Eugenia jambolana*. Food Chemistry **104,** 1106-1114.

Tokusoglu O, Unal MK, Yemis F. 2005. Determination of phytoalexin in peanut and pistachios by HPLC-DAD and GC-MS. Journal of Agriculture Food Chemistry **53,** 5003-5009.

Vinson JA, Zubik L, Bose P, Samman N, Proch J. 2000**.** Dried fruits. Excellent *in vitro* and *in vivo* antioxidants. Journal of the American College of Nutrition **24,** 44-50.

Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD Tenover FC. 2001. Novel carbapenem-hydrolyzing b-lactamase, KPC-1, from a Carbapenem resistant strain of *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy **45,** 1151-1161.

Zhao J, Zhao Y. 2004. Effects of curcumin on proliferation and apoptosis of human cervical carcinoma HeLa cells in vitro. Chinese Journal of Cancer Research **16,** 225-8.