Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E: ISSN 2278-4136
P: ISSN 2349-8234
JPP 2014; 3 (3): 23-28
Received: 13-07-2014
Accepted: 16-08-2014

Sawan Sharma
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Gouri Satpathy
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Rajinder K. Gupta
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.

Correspondence:
Rajinder K. Gupta
Food Processing Laboratory, University School of Biotechnology, GGS Indraprastha University, Dwarka-110078, India.
Email: rkg67ap@yahoo.com;
Tel: +91-11-25302321,
Fax: +91-11-25302305

Nutritional, phytochemical, antioxidant and antimicrobial activity of *Prunus armenicus*

Sawan Sharma, Gouri Satpathy, Rajinder K. Gupta*

Abstract
The present study has been undertaken to understand the various constituents of the dry apricot (*Prunus armeniac* L.) fruit pulp which provide health benefits. Antioxidant, antimicrobial, nutritional activities, phytochemicals; total phenolics, flavanoids, saponins, alkaloids, vitamin E, sorbitol, phytosterol, fatty acids and eugenol which have different therapeutic uses were studied. The nutritional results reveals that the fruit pulp is a good source of energy, dietary fiber, protein and minerals such as potassium, magnesium, calcium, zinc, iron and copper. The phytochemical results revealed that the prepared extract is a moderate source of antioxidants. The extract shows good antimicrobial activity against gram positive bacteria which shows that dry apricot is a good source of health promoting constituents which can be used for therapeutic purpose.

Keywords: Apricot, Nutritional, Phytochemical, Antibacterial, Antioxidant, GC MS.

1. Introduction
Apricot (*Prunus armeniaca* L.) belongs to Rosaceae family. Its property of being rich in vitamins and minerals makes it acceptable worldwide among consumers. In local language it is also called khubani and is one of the most important fruits grown in the world. Consumers cherish the aroma and a flavour of good quality apricots, with the sugar content being one of the most appreciable quality characteristics [1]. Apricot is a small tree growing at 8-12 m, with a trunk up to 40 cm in diameter and a dense, spreading canopy. The leaves are 5 cm long, 4-8 cm wide and ovulate in shape, with a rounded base, a pointed tip and a serrated margin. The flowers are 2-4.5 cm in diameter, with five white to pinkish petals, produced singly or in pairs. The fruit is similar to a small peach, 1.5-2.5 cm in diameter, from yellow to orange in colour, often tinged red on the side which is exposed to the sun; its surface can be smooth glabrous or velvety with very short hairs i.e.; pubescent. The flesh is not very juicy but it is firm. Being rich in vitamins and minerals, apricot is one of the most familiar crops grown worldwide. Apricot trees grow in certain regions where the environmental conditions are favorable [2]. Carbohydrates, vitamins C and K, β-carotene, niacin and thiamine are found in fresh apricot fruit, and the fruit is also found to be a good source of organic acids, phenols, volatile compounds, esters and terpenoids [3, 4]. Being phytochemically rich it also an efficient source of antioxidants which restrict the destructive effects of oxidant reactions produced by free radicals. Many synthetic antioxidants such as butylated hydroxyl toluene (BHT) have been used for industrial processing but their carcinogenic effect and involvement in liver damage is a big health concern [5]. For this reason, the need for antioxidants from natural source has received much attention and efforts have been made to identify compounds that can act as antioxidants to replace synthetic ones. Fruits exhibit a wide range of antimicrobial, anti-proliferation and anti-carcinogenic activities. These biological activities can be attributed to their antioxidant properties [6]. The presence of vitamin C, vitamin E, carotenes, polyphenols and flavonoids also contribute to its antioxidant activity [7]. Apricot is considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease [8]. The fruit also contains volatile compounds, esters, dietary protein along with good amounts of oil and fibers [9]. In general, *Prunus armeniaca* (L.) is used as medicine in the treatment of skin diseases [10, 11] and parasitic diseases [12, 13]. As a part of search for new biological activity of a plant extracts, preliminary bio-screening [14] was performed to evaluate the antibacterial and antifungal activity of the extracts.

2. Material and method

2.1 Material

Dry apricot fruit was used as a sample for various analysis. The sample of dry apricot was collected from local market of Jammu in the month of November. The dry pulp was separated from the seed using knife and dried in oven at 40 ºC. The dried pulp was grinded and stored in air tight container to avoid any further gain in moisture as the fruits are highly hygroscopic in nature.

2.2 Reagents

All analytical grade chemicals, acids and solvents, media and other chemicals used in the present study were purchased from different sources. Aluminium Chloride (Fisher), Ascorbic Acid (SRL), Acetone, Ethanol, Ethyl acetate, ICP Multielement Standard (Qualigens), Folin-Ciocalteu's Phenol reagent (SRL), Gallic acid (HiMedia), Dimethyl Sulfoxide (SRL), Sodium Hydroxide(SRL), Ferrous chloride (Thomas Baker), Petroleum Ether (Loba Chemie). (SRL), BHT, PBS, potassium persulphate (Fisher), Aluminium chloride (Fisher), Distilled water, Dichloromethane (Fisher), Methanol (Thomas Baker), 2,2'-Azino-bis (3- ethylbenzthiazoline-6-sulphonic acid) (Sigma), Trolox (Aldrich), TPTZ (Fluka), Ammonia Solution (SRL), Ferrozine (SRL).

2.3 Extract preparation

The fruit extracts were prepared using method described previously by Haq et al. [15], with minor modifications. The sample was collected and dried in an oven at 40 °C, and powdered using an electric blender. The powder (10 g) was soaked in 100 ml of methanol and kept them in shaker at room temperature in dark for three days. Each sample was filtered through Whatman no. 2 filter paper and the filtrate was then evaporated over water bath at 40 °C overnight and plant extract was further stored at 4 °C.

2.4 Nutritional analysis

Total ash content and moisture content was determined by gravimetrical method at 105 ºC [23] and at ≤525 ºC by AOAC method Ref. 942.05 respectively. The total nitrogen content was determined using Kjeldahl method Ref. 976.05 [17]. A gravimetric method was used for determination of total dietary fiber after the enzymatic digestion of starch and protein in fat and moisture free sample. Crude fat content was determined by extracting the sample in petroleum ether and total carbohydrate content was also measured [24]. Total calorific value (Kcal/100g) was calculated using formula [(Protein +carbohydrate)*4 + (fat)*9] and carbohydrate (%) was calculated using formula 100- (moisture% +ash% +fat% +protein%).

Minerals, trace elements and heavy metals in the examined material were determined by using Optima 2100 DV ICP-OES (Perkin-Elmer, USA), after prior mineralization in an Anton Paar Multiwave microwave digester (Anton Paar Ltd., Hertford, UK) as per Ref 956.52 [17]. As a standard, the certified multi-element standard solution ''ICP Multi-element Standard IV" (Merck, Darmstadt, Germany) was used for the instrument's response. The correlation coefficients for the calibration curves obtained were more than 0.99.

2.5 Secondary metabolites determination

Identification of the secondary metabolites in the sample was done by using GC-MS. Carrier gas in GC-MS was helium An Agilent 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for the sample. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library. The detected compounds have been identified by NIST'05 mass spectrum library and more than 70% matching value were reported.

2.6 Antioxidant activity determinations

2.6.1 ABTS radical scavenging assay

Scavenging of blue-green ABTS radicals by the sample extract determined the antioxidant activity of the extract and was shown as ascorbic acid equivalent [24]. It is produced by reacting of ABTS solution (7mM in water) with 2.5mM potassium per sulfate (final concentration) for 16 h at ambient temperature in the dark (stock solution). Then the ABTS stock solution was diluted with methanol to bring down the absorbance of stock solution to 0.7 at 734 nm as the solution was very dark blue in colour initially. 1 mg of the extract was dissolved in 5 ml of 70% aqueous methanol. 50 µl of extract solution was added to 2.0 ml of diluted ABTS solution with Absorbance 0.7. Decreasing absorbance of the solution was measured after 5 min of incubation at room temperature in the dark. All determinations were carried out in triplicate. Standard solution was made using ascorbic acid. All stock were prepared fresh daily.

$$\%\text{scavenging activity} = \{A_{control} - A_{sample})/A_{control}] * 100$$

2.6.2 FRAP antioxidant Assay

FRAP Antioxidant Assay is mainly based upon the ability of the extract to reduce the ferric ions and the assay was based upon the methodology of Benzie and Strain 1996 [23]. 10mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 250 mM sodium acetate buffer (pH 3.6) were the main components of FRAP reagent. FRAP reagent was freshly prepared by mixing FeCl3 solution TPTZ solution and acetate buffer in a ratio 1:1:10.A 100 µl of the methanol extract of the fruit was mixed with 900 µl of FRAP reagent and kept for 4 min at 37 ºC and later absorbance was taken at 593 nm. BHT dissolved in DMSO was used as a standard in FRAP assay.

2.7 Phytochemical analysis

2.7.1 Total phenolic content analysis

Total phenolic content was determined as per the method described by Singleton and Rossi (1965) [19]. The sample extracts were oxidized with Folin–Ciocalteu reagent and neutralization was done using sodium carbonate. The results were expressed as gallic acid equivalents (GAE, mg 100 mg^{-1}extract). Reaction absorbance was taken at 765nm against DMSO blank.

For total phenol estimation standard curve was made using different concentration of Gallic acid.

2.7.2 Flavonoid content determination

Flavonoid contents in the extracts were determined by a colorimetric method described by Jia, Tang, and Wu (1999) [20]. The extract (250 µl) was mixed with 1.25 ml of

distilled water and 75 µl of 5% $NaNO_2$ solution. After 5 min, 150 µl of 10% $AlCl_3.H_2O$ solution was added. After 6 min, 500 µl of 1 M NaOH and 275 ml of distilled water were added to prepare the mixture. Absorbance of the well mixed solution was taken at 510 nm. For flavonoid estimation standard curve is made by using different concentration of catechin. And result was determined in catechin equivalent (CE).

2.7.3 Total alkaloid content determination
2.5 g crude sample was mixed with 100 ml 10% acetic acid in ethanol and incubated for 4 h at room temperature. Further sample was filtered and concentrated to ¼ of original volume using water bath. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation occurred. Precipitates were filtered on a pre-weighted whatmann filter paper. Crude alkaloid was weighed after the filter paper dried (Herborne, 1973) [21].

2.7.4 Total saponin content determination
The saponin content was calculated as per method described by Obadoni & Ochuko [22]. To determine saponins, 5 g of sample powder was mixed with 50 ml of 20% aqueous ethanol. The sample was continuously stirred over a hot water bath for 4 h at about 55 °C for heating. The mixture was filtered and the residue was extracted again with another 50 ml of 20% ethanol. The combined extracts were reduced to 10 ml over water bath at 90 °C. The concentrate was transferred into a separating funnel and 20 ml of diethyl ether was added and shaken vigorously. Ether layer was discarded and the aqueous layer was recovered. The purification process was repeated. 15 ml of n-butanol was added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride, remaining solution was heated over water bath. The samples were dried in the oven to a constant weight and the saponin content was calculated in percentage.

2.8 Antimicrobial activity determination
Agar well diffusion method: Microwells were made on culture media in 6 mm in diameter with the help of gel puncture machine. The microwells were filled with 100 µl of methanolic extracts The Petri dishes used for antibacterial screening were incubated at 37 ºC for 24-48 hours. The activity was measured in terms of zone of inhibition (mm) appearing around the microwells [25, 26].

3. Results and discussion
3.1 Nutritional analysis
Nutritional analysis of the dried apricot shows a good potential health benefits (Table 1). Moisture and ash content of fruits play a very important role in affecting the nutritional composition of the fruit. Moisture content of the food directly affects the appearance, texture, its storage ability in case of dry fruits. As the low fat content observed in the sample i.e., 0.006% which signifies it as a good and even found to be healthy. Low in fat content means low calories which will avoid obesity. The presence of protein will serves as building block of cells, muscles, cartilage, skin, hormones, enzymes, vitamins.

Table 1: Nutritional profiling of dried apricot fruit.

Parameter	Dried Apricot Fruit
Moisture	5.71%
Ash	11.01%
Fat	0.006%
Protein	3.31%
Dietary fiber	2.81%
Carbohydrate	79.964%
Energy	333.144 k cal

ICP-OES analysis reveals the presence of different essential minerals; magnesium, calcium, iron, zinc, copper etc. in dried apricot fruit (Table 2). Calcium is crucial for teeth's and bones maintenance and for overall health. Many studies show that calcium plays a key role in the prevention of bones related problems like osteoporosis, etc. [28]. Magnesium is needed for enzyme action, balanced hormones, a healthy nervous system and cardiovascular system. Recent studies confirm that magnesium plays a key role in the preventing cardiovascular diseases [29]. Iron is needed for the production of red blood cell and enzymes help in fighting against anemia. Zinc supplementation in diabetes mellitus proved to have antioxidant effect.

Table 2: Mineral analysis of dry apricot fruit

S. No.	Mineral	Concentration (mg/kg)
1	Ca	447.2
2	Cu	80.19
3	Fe	37.25
4	Mg	355.3
5	P	365.75
6	Cr	1.682
7	Mn	1.919
8	n	13.94
9	Ni	1.126
10	Pb	0.268
11	Se	0.770

3.2 Determination of Antioxidant activity

3.2.1 ABTS radical scavenging assay

The antioxidant potential of dry apricot methanolic extract was determined against ascorbic acid as percent inhibition of ABTS free radicals. The antioxidant activity (IC_{50} value) as determined by ABTS assay was found to be moderate in the extract i.e.; 15 mg/ml.

3.2.2 FRAP assay

In FRAP assay, ferrous complex with an intense blue colour was measured at a wavelength of 593 nm. Color intensity is related to the amount of antioxidant reductants present in the sample. FRAP activity was found to be moderate in dry apricot fruit extract i.e., 0.68 mg/100 mg.

3.3 Phytochemical analysis of dry pulp

Phytochemicals are known to be the major bioactive compounds which provide health benefits. Today food industries are very interested in using the plant extracts having good total phenolic content. Phenolic compounds like flavonoids, phenolic acids, and tannins are the major contributors of the antioxidant capacity of plants. Diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities are possessed by these antioxidant compounds. These activities contribute to their antioxidant activity (Chung, Wong, Huang, & Lin, 1998) [30]. Thus, the total phenolic content of the fruit extract was also evaluated, using the Folin–Ciocalteu's method. Flavonoid content of the fruit was evaluated by aluminum chloride method and result was expressed in catechin equivalent.

The fruits showed a reasonable amount of crude alkaloids and negligible amount of saponins (Table 3). Alkaloids are majorly helpful for healing of wounds, ulcers and have some other medicinal properties. Saponins, on the other hand, are anti-nutritional factors and can reduce the uptake of certain nutrients. So their presence is least amount in the fruit proved its important properties.

Table 3: Phytochemical content of dry apricot fruit and its extract

Analyte	Content
Total Phenolic	14 µg GAE/mg extract
Total Flavonoid	7 µg CE/mg extract
Alkaloids	2.7% in dry fruit
Saponins	0.002% in dry fruit

3.4 GC-MS profiling

GC-MS analysis of the dry apricot fruit showed the presence of 2-Furanmethanol, Hydroquinone, Isosorbide, L-glutamic acid, n-acetyl mannoseamine, Sorbitol, n-hexadecanoic acid majorly. 2-furanmethanol has its application in production of resins for bonding foundry sands for production of cores and molds. Hydroquinone has its effects in skin whitening so can be used in cosmetic products. Sorbitol and Isosorbide has pharmaceutical properties their presence in any food refer them to be a good source of medicine. Isosorbide can act as compound which can act as relaxing compound for heart vessels so it can be used in drugs for coronary diseases. L-glutamic acid a non-essential amino acid works as neurotransmitter so it has it application in forming drugs for nervous system. n-Hexadecanoic acid had properties which are essential in forming cosmetic products and even act as soap releasing agents. Gamma sitosterol is found to lower the serum cholesterol. It has lots of medical, functional food, and cosmetic applications and can also contribute towards the antimicrobial and antioxidant properties. Since it has saturated fat reducing and cholesterol lowering activity, and thus help in reducing the risk of coronary diseases**.** Oleic acid was also detected in the dried fig extract that is used as an emollient. Isoamyl Laurate detected is also used as conditioner for hair.

Table 4: GC-MS profiling of the dry apricot fruit.

RT	% area	Compound name	Cas#
4.848	1.25	2-Furanemethanol	000098-00-0
10.041	7.21	4H-pyran-4-one,2,3-dihydroxy-3,5-dihydroxy-6-methyl	028564-83-2
11.487	5.85	2-furancarboxaldehyde, 5-(hydroxymethyl)	000067-47-0
11.891	0.29	1,2-benzenediol,3-methyl	0000488-17-5
12.171	0.77	Hydroquinene	000123-31-9
12.385	1.19	Isosorbide	000652-67-5
12.676	0.63	2-methoxy-4-vinylphenol	007786-61-0
13.697	0.39	L-glutamic acid	000056-86-0
17.140	1.84	Propylamine, N-[9-borabicyclo [3.3.1]non-9-yl]	1000160-82-3
19.944	1.21	Hexadecanoic acid, methyl ester	000112-39-0
20.112	0.79	Sorbitol	000050-70-4
20.325	4.44	n-hexadecanoic acid	000057-10-3
20.605	0.18	Hexadecanoic acid , ethyl ester	000628-97-7
21.592	1.05	10,13octadecadienoicacid,methyl ester	056554-62-2
21.648	1.86	9-octadecanoic acid (Z)-, methyl ester	000112-62-9
21.861	0.35	Octadecanoic acid, methyl ester	000112-61-8
22.063	9.88	Oleic acid	000112-80-1

22.209	2.05	Octadecanoic acid	000057-11-4
22.389	0.86	9,17-octadecadienal,(z)	056554-35-9
22.725	0.34	9,12-octadecadienoic acid(z,z)	000060-33-3
23.891	0.16	Octadec-9-enoicacid	10000190-13-7
24.845	0.52	Cyclopropaneoctonal,2-octyle	056196-06-6
24.934	0.09	1-eicosene	003452-07-1
25.103	0.91	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylediester	023470-00-0
25.181	0.21	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylediester	023470-00-0
26.538	3.37	9-Octadecenal,(Z)	023470-00-0
26.606	1.56	Nonanoic acid,9-(3-hexenylidenecyclopropylidene)-,2-hydroxy-1-(hydroxymethyl)ethylester,(Z,Z,Z)	055268-58-1
34.322	0.25	Stigmasterol	000083-48-7
35.634	0.43	gamma-Sitosterol	000083-47-6

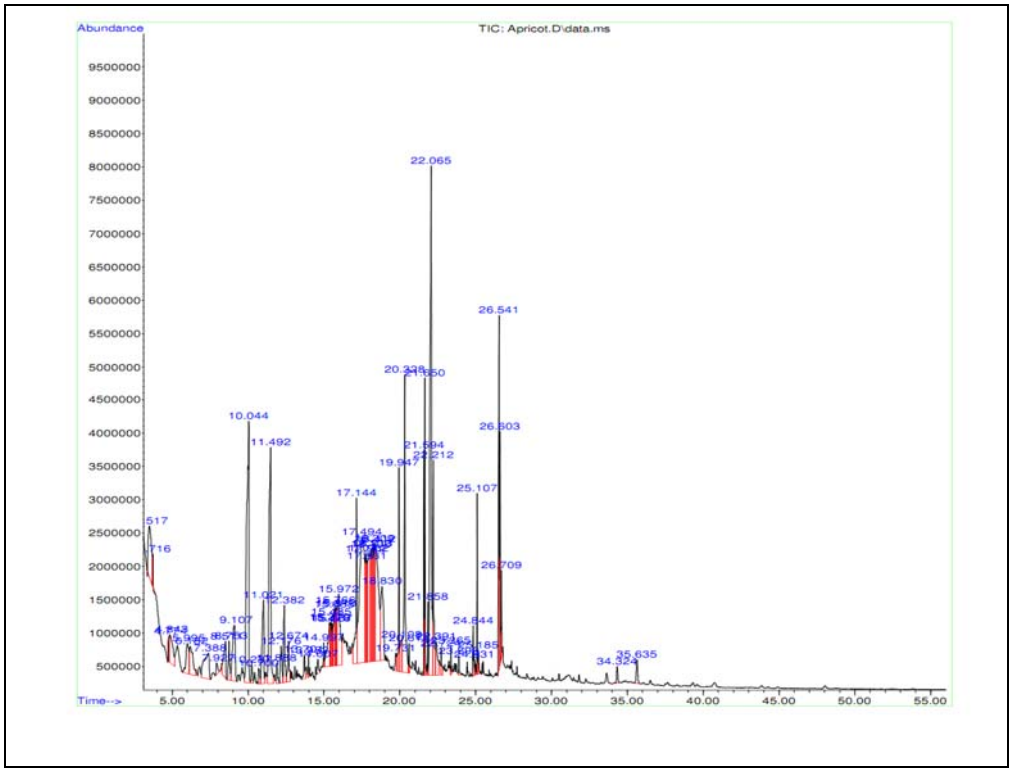

Fig 1: Chromatogram of methanolic extract of dry apricot fruit.

3.5 Antimicrobial activity determination

Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis and Proteus mirabilis were used to screen the antibacterial activity of the dry apricot sample. Agar well diffusion method was used to assess the activity against the bacteria by measuring the zone of inhibition. Inhibiting concentration used for the sample here is 200 mg/ml of DMSO.

Methanol extract of dry *Prunus armenicus* showed good inhibiting activity against two bacterial species i.e. *Bacillus subtilis* and *Staphylococcus aureus (Table 5).* The inhibitory action was shown because of the phytochemical compound present in the apricot fruit extract.

Table 5: Antibacterial activity of dry apricot extract (200mg/ml)

Bacterial Strain	Zone of inhibition
Gram positive	
Staphylococcus aureus	22mm
Staphylococcus epidermidis	_
Bacillus subtilis	19mm
Gram negative	
Proteus mirabilis	_

4. Conclusion

The various experiments conducted in the fruit revealed it to be a very good source of phytochemicals: total phenoloics, flavonoids and bioactive compounds which have health benefits and are widely sought after. The bioactive compounds identified in GCMS analysis are reported for their wide

application in different food and pharmaceutical industries. The extract also indicated its potential as an antioxidant, antimicrobial and therapeutic agent which could play a very important role in drug development and as a health supplement.

5. Acknowledgement
We are very grateful to University Grants commission for the financial support under the Special Assistance Programme (SAP) from 2011-2016.

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