Nutritional composition, phytochemical screening, antioxidant, GC-MS, and FTIR analysis of methanolic extract of *Prunus armeniaca*

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Abstract:

With the aim of looking for alternatives to chemicals being used in the aquaculture, food industry, nutraceutical and medicinal industries which are responsible for environmental degradation in one way or another, the present study has been done to investigate the phytochemical composition of apricot, *Prunus armeniaca* kernel extracted with methanol collected from Kargil, Ladakh (India). Apricot kernels were analysed for their proximate nutritional properties, qualitative and quantitative phytochemistry and antioxidant properties. GC-MS and FT-IR analysis were employed to identify the various secondary compounds and their functional groups present in the extract. The results revealed apricot kernel possesses 20.61% crude protein and 56.08% crude fat composed mainly of unsaturated fatty acids which is indicative of its rich nutritional, nutraceutical and medicinal properties. The qualitative phytochemical screening showed the presence of alkaloids, flavonoids, phytoestrogens, glycosides and phenolic compounds, while the quantitative phytochemistry results showed total phenolics (63.56931 ± 1.686 mg/100 g GAE) and total flavonoids (176.51 ± 3.396 mg/100 g QE) present in the extract. Free radical scavenging activity by the DPPH scavenging method also showed a good antioxidant property of the extract. A total of 7 compounds were identified by GC-MS analysis with Pentadecanoic acid, 14-methyl-, methyl ester (5.07%), trans-13-Octadecenoic acid, methyl ester (78.44%) and 10-Methyleicosane (10.93%) being the major compounds. The FT-IR spectrum revealed the presence of amines, alkanes, alkenes, alcohols, phenol, esters and carboxylic acids.

Keywords:

Antioxidant activity, FT-IR, Medicinal properties, *Prunus armeniaca*, Phytochemistry, Secondary metabolites.

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Introduction:

The excessive use of chemicals in the food, aquaculture, pharmaceutical, and cosmetics industries has a negative impact on the consumers and degrades the environment by producing free radicals. One of the most prevalent physiological stressors in an organism is oxidative stress, which is caused by free radical damage over time and results in a number of degenerative illnesses (Nair et al. 2012). This has prompted efforts to look for alternatives in the form of natural products from plants with the least side effects. Interest in aromatic and medicinal plants has grown among personnel in many industries and organizations lately (Dalia et al. 2016). For their nutritional, commercial, and pharmacological uses, plant seeds and kernels continue to be significant source of proteins and vital fatty acids. Thus, we may exploit the health benefits of specific plants and parts, such as the apricot kernel (*P. armeniaca*), in various formulations and lower the risk of various common ailments.

Apricot is a strong tree that grows in the arid, temperate regions of the North-western Himalayas, notably in the valleys of Himachal Pradesh, Jammu & Kashmir, Uttarakhand and Ladakh regions in India (Rai et al. 2016). *Prunus armeniaca* is the most commonly cultivated apricot species, also called ansu apricot or Siberian apricot or Tibetan apricot, a species of *Prunus*, in the subgenus *Prunus*. Apricot possesses an excellent profile of macro & micro molecules (sugar, organic acids, minerals, vitamins) and secondary metabolites (phenolic compounds, flavonoids and carotenoids) which play an important role in maintaining the nutritive value (Rai et al. 2016). Different parts of the apricot contain a variety of phytocompounds that are used in traditional medicine for ages to cure common illnesses such as cough, asthma, bronchitis, anaemia and fever (Erdogan and Kartal 2011). These were utilized as feed supplements (Asma et al. 2007) and have antioxidant, anti-asthmatic, and anti-spasmodic activity (Yigit et al. 2009; Erdogan and Kartal 2011). Phenols are the most abundant phytocompound in apricots, with gallic, ferulic, caffeic, 4-aminobenzoic, pro catechin, salicylic, and p-coumaric acid being the most important ones, and quercetin, glycoside, rutin, resveratrol, and vanillin are the important flavonoids (Sochor et al. 2010).

Apricot kernel, due to the presence of some important bioactive molecules like carotenoids, catechins and caffeic acid, stimulates increased digestion and metabolism of nutrients causing higher efficiency in the utilization of feed and in turn results in higher growth in chickens (Dragovic. et al. 2007). The kernels are also used to produce apricot oil, which is a principal component in the generation of cosmetic products, medications, and aromas, and devoured as

starters (Guner et al. 1999; Alpaslan and Hayta, 2006; Durmaz and Alpaslan, 2007). It also has a very high content of mono - and polyunsaturated fatty acids, which have an important role in human nutrition and health as these compounds help decrease blood cholesterol levels and maintain blood pressure levels (Turan et al. 2007). The apricot kernels can be used as a potential anticancer agent due to their toxicity effect on various cell lines (Akcicek et al. 2005; Chang et al. 2006). The apricot kernel has been reported to have more antioxidant properties and phenolic compounds than the flesh (Soong and Barlow, 2004) and maximum amount of phenolics and flavonoid contents were present within the kernel skin (Monagas et al. 2007; Mandalari et al. 2010). Moreover, it can also be used as an effective antimicrobial agent seeing its antibiotic activity against a variety of microbes and an effective remedy for skin diseases (Abtani et al. 2008; Yigit et al. 2009; Geng et al. 2016).

The reported protein content of apricot kernel ranged from 14.1 to 45.3% with essential amino acids constituting 32–34% of the total amino acids. The major essential amino acids are arginine and leucine, and the predominant nonessential amino acid is glutamic (Kamel, 1992). The Carbohydrate content of apricot kernel varies from 17.3% to 27.9% (Beyer, 1990; Tunçel, 1990; Kamel, 1992). The oil content of the kernels varies from 27.7 to 66.7% (Kamel, 1992). The major fatty acids are oleic and linoleic (Mandal et al. 2007; Ul'chenko et al. 2009).

However, there is still a lack of information on the secondary metabolite contents of the methanolic fraction of apricot kernel extract. Thus, the present study was designed to investigate the preliminary phytochemical constituents and antioxidant properties by gold standard methods complemented with advanced analytical techniques like GC-MS & FT-IR to highlight the importance of this extract in the nutraceutical, pharmaceutical and aquaculture industry as a nutritive supplement.

Materials and Methods:

Plant collection

Apricot (*Prunus armeniaca*) kernels have been collected from the main market of Kargil, Ladakh. The kernels were washed with distilled water and shade dried before being ground to powder. Samples of the apricot kernel have been submitted to the Herbarium in the Department of Botany,

Panjab University, Chandigarh for authentication and a voucher number has been acquired (21691).

Proximate composition

Standard proximate analytical techniques in accordance with AOAC (2012) have been used to examine the dry grounded kernel's proximate composition. Crude protein was determined by the Kjeldahl method, moisture percentage by oven drying for 24 hours at 104°C, crude oil was determined by Soxhlet extraction method, crude fibre by acid/alkali digestion, ash content by incineration at 650°C for 4 hours in a muffle furnace and Nitrogen-free extract (NFE) was calculated by subtracting the total protein, lipid, fibre, moisture and ash values from 100.

Plant extraction

The grounded apricot kernels were extracted with methanol using a continuous hot Soxhlet extractor for 48 hours. The extracts were concentrated using a rotary evaporator under a vacuum. The sticky semi-solid leftover extract was then stored at 4°C till further use.

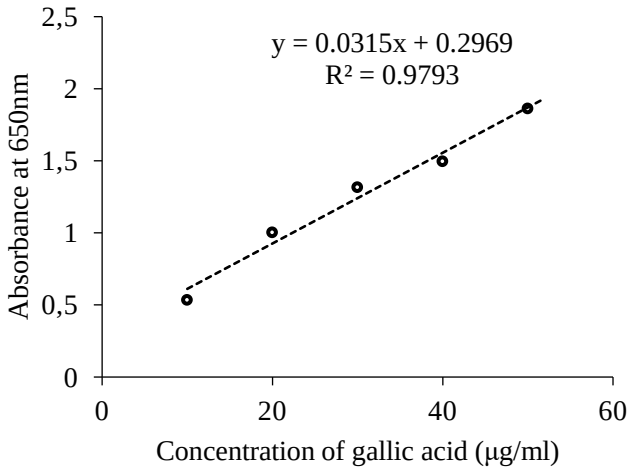
Preliminary Phytochemical analysis

The qualitative phytochemistry of methanolic extract of apricot kernel has been evaluated following Fahal et al. (2018) using different methods for each test.

Quantitative phytochemical analysis

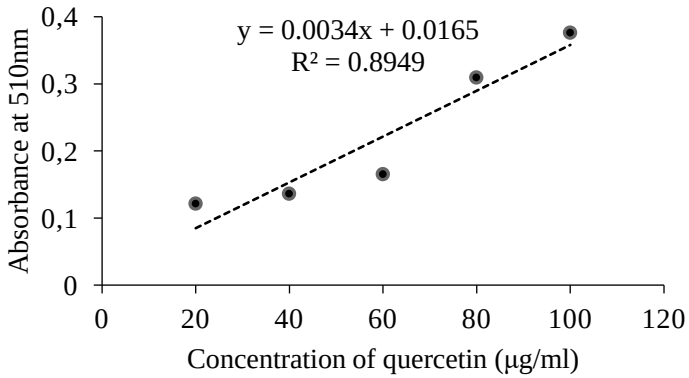
Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Baba and Malik, 2015) with slight modification. Briefly, 200 µL of crude extract (1 mg/mL) was made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve given below and the results were expressed as mg GAE/g dry weight.



Total flavonoid content

The total flavonoid content of crude extract was determined by the aluminum chloride colorimetric method (Dulf et al. 2016). In brief, 50 µL of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve given below, and the result was expressed as mg QE/ g dry weight.



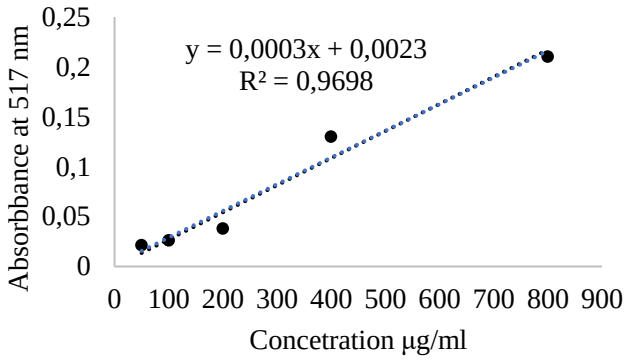
Antioxidant activity by DPPH assay

Antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, with some modifications (Krakowska et al. 2017). In brief, 200 µL of each extract (50–800

µg/mL) was mixed with 3.8 mL DPPH solution and incubated at room temperature for 1 hour in the dark. The absorbance of the mixture was then measured at 517 nm. Ascorbic acid was used as a positive control. The quantity of sample extracted into 1 ml of solution required to reduce the initial DPPH concentration by 50% is known as the IC_{50}, which is used to express antioxidant value. The percent inhibition versus concentration plot was used to calculate IC_{50}. The ability of the sample to scavenge DPPH radical was determined from the equation:

$$\text{DPPH inhibition } \% = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

A standard calibration curve was prepared with ascorbic acid as shown below.



GC-MS analysis

GC-MS analysis of the extract was carried out by following the method of Hema et al. (2011) using a Thermo Trace 1300GC coupled with a Thermo TSQ 800 Triple Quadrupole MS equipped with a TG 5MS (30m X 0.25mm, 0.25µm) composed of 5% diphenyl; 95% dimethyl polysiloxane. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.5 ml/min and an injection volume of 1.0 µl was employed at injector temperature 250°C; ion-source temperature 230°C. The oven temperature was programmed as an initial temperature of 60°C for 2 minutes with an increase of 15°C/min up to 200°C with a holding time of 6 minutes, ending with 6 minutes isothermal at 220°C. The total GC run time was 21.78 minutes. Detection was performed in full scan mode from m/z 50 to 700. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The software adopted to handle mass spectra and chromatograms was an XCalibur 2.2SP1 with Foundation 2.0SP1. The interpretation of the mass spectrum of GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component

was compared with the spectrum of the known components stored in the NIST library. The names, molecular weights, and structures of the components of the test materials were ascertained.

FT-IR analysis

The characteristic functional group of compounds present in the extract was determined by using Fourier transform infrared (FTIR). The extract was dissolved in dichloromethane (DCM) solvent before being used for FTIR analysis. The IR spectrum was obtained using a Perkin-Elmer FTIR spectrometer and was scanned from 4000 to 500 cm-1.

Results and Discussion:

Preliminary phytochemical analysis has been widely employed by researchers to evaluate the therapeutic potential of plants. Phytochemical screening tests are crucial for finding new sources of chemicals with medicinal and industrial value in order to make the best and most responsible use of the available natural resources. Apricot kernel and its oil have long been used in folk medicines for various illnesses believed to be due to the presence of various phytocompounds in apricot fruit and kernel such as vitamin C, vitamin K, β-carotene, niacin, thiamine, organic acids, phenols, volatile compounds, esters, and terpenoids (Michalcová et al. 2016).

Proximate composition

The proximate composition analysis of apricot kernel revealed that it contains crude protein (20.61%), moisture content (5.81%), crude oil (56.08%), crude fibre (3.2%), ash content (2.75) and NFE (Nitrogen Free Extract) 11.55%. A similar study (Dwivedi and Ram, 2006) reported the fat content in the bitter kernels to be as high as 54.24%. Protein content was found to vary from 17.75 to 22.56%, carbohydrate from 21.16 to 35.26%, crude fibre from 0.84 to 4.71%, and dietary fibre from 6.03 to 22.24% which is almost in line with the results of the present study. Sharif et al. (2015) also reported similar results for the proximate composition of apricot kernel as moisture (4.87±.23), crude protein (17.31±2.51), crude fat (55.50±4.66), crude fibre (3.19±.10), ash (2.23±.05) and NFE 16.90±1.55%, respectively. Similarly in another study Gupta et al. (2012) revealed the crude oil content (45.6 – 46.3%), moisture content (4.0 – 4.1%), ash content (2.2 – 2.4%) in the apricot kernel from different locations of Himachal Pradesh, India.

Qualitative phytochemical analysis

The phytochemical analysis is the primary step to look for the chemical compounds which can be isolated (Vijayalakshmi and Ravindran, 2012). The kind of solvent employed in the extraction

technique has a big impact on the success of isolating biologically active chemicals. Therefore, while screening plant parts for their phytochemistry, a variety of solvents can be used (Tiwari et al. 2016). The qualitative phytochemical investigation of apricot kernel extracted with methanol in this study showed the presence of various phytocompounds including flavonoids, phenolic compounds, alkaloids, glycosides, protein and oil as shown in Table 1. Similar results have been reported from time to time (Femenia et al. 1995; Mandal et al. 2007; Turan et al. 2007; Dwivedi and Ram, 2008; Ul'chenko et al. 2009). Qin et al. (2019) also reported that apricot kernel extract contains a broad spectrum of biologically active substances such as flavonoids, polyphenols, glycosides, vitamins, and metal ions.

Table 1. Qualitative phytochemical analysis of apricot kernel methanolic extract.

Phytocompounds	**Methods**	**Results**
Flavonoids	Shinoda test	+
Phenolic compounds	Lead acetate test	+
Protein	Biuret test	-
	Millon's test	-
Fixed oils	Spot test	+
	Saponification test	+
Alkaloids	Wagner's test	+
	Mayer's test	+
Terpenoids	Salkowski test	-
	Libermann burchard Test	-
Saponins	Foam test	-
Tannins	Ferric chloride test	-
Reducing sugars	Fehling's test Molish's test	- -
Glycosides	Borntrager test Legal test	+ +

The (+) and (-) signs in the table mean the presence and absence of the compounds.

Quantitative phytochemical analysis

Furthermore, the quantitative phytochemical analysis showed that the apricot kernel extract contains a high content of flavonoids and phenolic compounds. Gallic acid and quercetin were used as standards for flavonoids and phenolic compounds. Total phenolic compounds were 63.569 ± 1.686 mg gallic acid equivalent/100 g dry weight of the sample, and the total flavonoid content was 176.519 ± 3.39 mg quercetin equivalent/100 g dry weight of the sample. Chen et al. (2020) reported similar results for a wide variety of apricot kernels with an average value of 59.41 ± 0.54 mg GAE/100 g on a dry weight basis. However, in other studies (Korekar et al. 2011 and Kamel et al. 2018), the total phenolic content and total flavonoid content were reportedly high, ranging from 92.2 to 162.1, with a mean value of 128.5 mg of GAE/100 g DW kernel for total phenolic and 226.18 total flavonoids (µg rutin equivalent/g dry extract) respectively. The difference in content of total phenolic and total flavonoid content can be attributed to the fact that genetic variation in species, geographical conditions, seasonal changes, harvesting time, extraction techniques, and extraction solvent can result in the detection of different levels of phytocompounds' as reported by Korekar et al. (2011). Phenolic compounds, along with flavonoids and tannins, are the major compounds contributing to the antioxidant property of a plant, which is attributed to its anti-inflammatory, antibacterial, and anti-cancer activities (Sharma et al. 2014). The nutraceutical and pharmaceutical potential of medicinal plants is perhaps due to the presence of secondary metabolites like alkaloids, flavonoids, phenolics, saponins, tannins and phytosterols (Britto and Sebastian, 2012).

Antioxidant activity by DPPH assay

The DPPH assay is a simple and gold standard 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). The IC_{50} value for aprciot methanolic extract in the present study was 2.76 mg/ml. In a similar study Han et al. 2013 reported the IC_{50} value for apricot sweet kernel to be 4.3 mg/L extract. The difference in antioxidants IC_{50} in present study and other studies can be attributed to several factors including temperature, duration, solvent composition, and solvent to solid ratio influences the antioxidant capacity of extracts (Karacabey et al. 2008; Khan et al. 2010). The DPPH % inhibition activity of apricot kernel extract along with ascorbic acids as a standard was evaluated and found an exponential incline in inhibition activity with an increase in the

concentration of extract, with a concentration of 800 µg/ml being the highest concentration, showing the highest percentage of inhibition (33.76%) and the lowest at the concentration level of 50 µg/ml (7.39%) as shown in Figure 1. The free radical scavenging ability of the extract can be attributed to the presence of a high quantity of flavonoids because flavonoids are the main source of antioxidants in plants (Singh Gill et al. 2010; Egbung et al. 2013). The kernel extract of bitter apricot had a modest radical scavenging activity against DPPH and in the ferric-reducing antioxidant power (FRAP) (Liu et al. 2009).

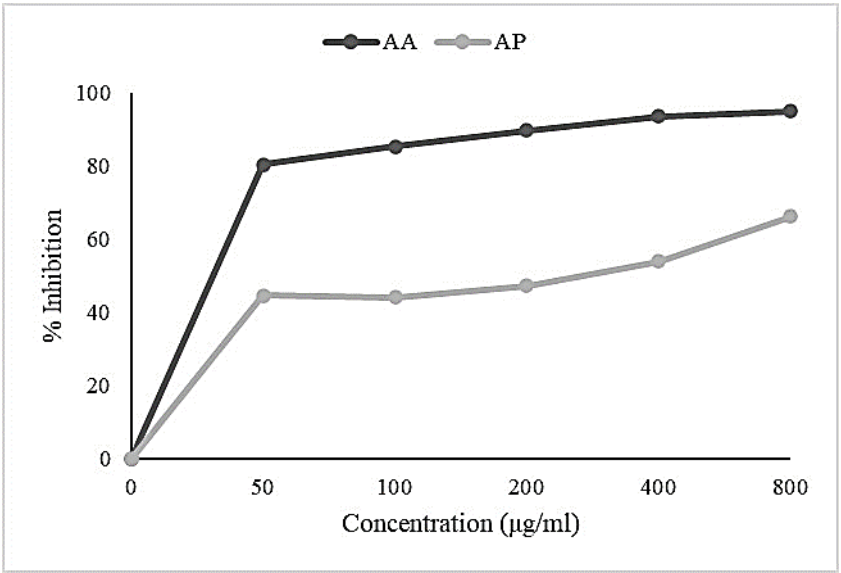


Figure 1. DPPH free radical scavenging activity inhibition percentage is shown by apricot kernel (AP) methanolic extract along with ascorbic acid standard (AA) at different concentration levels (50, 100, 200, 400 & 800 ug/ml).

GC-MS analysis

A total of 7 compounds were identified in the methanolic extract of apricot kernel by GC-MS as shown in the chromatogram (Figure 2). The identified compounds along with their molecular weight, chemical formula and nature as reported in the literature are presented in Table 2. The major compounds identified along with their peak percentage areas were Pentadecanoic acid, 14-methyl-, methyl ester (5.07%), trans-13-Octadecenoic acid, methyl ester (78.44%) and 10-Methyleicosane (10.93%). The corresponding mass spectra and structures of each compound found were elucidated from the NIST webbook (supplemetry file 1). In other similar studies GC-MS analysis showed the presence of six phytosterol (β-sitosterol as predominant) and four

tocopherols (ϒ-tocopherol as predominant) isomers, as well as oleic acid, linoleic acid and palmitic acid in apricot kernel extracted with petroleum ether (Turan et al. 2007; Ul'Chenko et al. 2009). However, none of these compounds were detected in our study which could be due to the solvent's polarity used in the extraction process as we used a more polar solvent methanol rather than petroleum ether. This suggests that the type of solvent in extraction can affect the finding of different compounds in an extract. Furthermore various other factors such as different species, geographical conditions, seasons and extraction techniques can result in the detection of different compounds by GC-MS. Krishnamoorthy and Subramaniam (2014) reported the compound trans-13-Octadecenoic acid, methyl ester, which is the prominent compound found in the present study, possesses anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, hypocholesterolemic, 5-alpha reductase inhibitor and insectifuge activities. As revealed by Syeda et al. (2011), the compound pentadecanoic acid, 14-methyl-, methyl ester possesses antibacterial and antifungal activities as well. 10-Methyleicosane, an alkane, has been reported to possess a high antioxidant capacity (Vinjamuri, 2017).

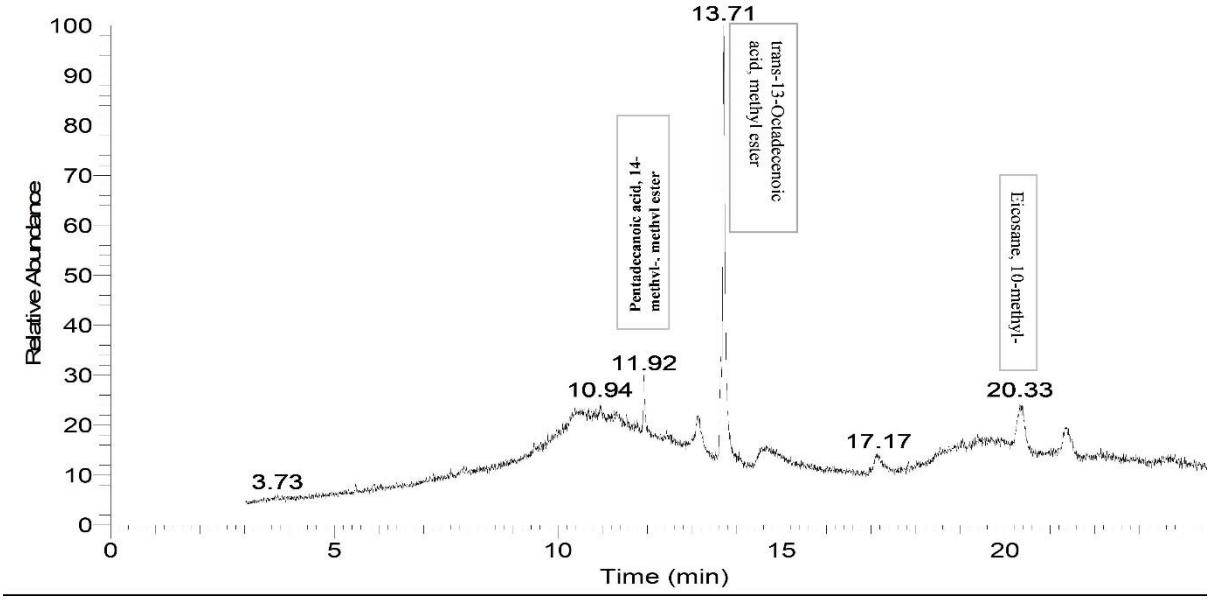
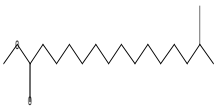
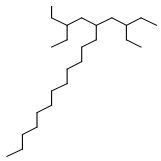
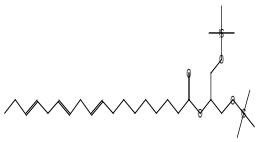
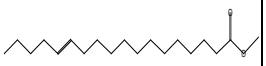
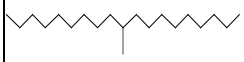
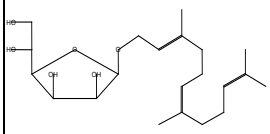
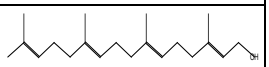


Figure 2. GC-MS chromatogram of apricot kernel methanolic extract.

Table 2. Phytocompounds revealed by GC-MS analysis of apricot kernel methanolic extract.

Name of compound	**% Area**	**RT**	**MW**	**Molecular Formula**	**Nature**	**Structure**
Pentadecanoic acid, 14-methyl-, methyl ester	5.07	11.92	270	$C_{17}H_{34}O_2$	Palmitic acid methyl ester	
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	2.95	13.14	366	$C_{26}H_{54}$	Higher alkanes	
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	0.08	13.20	496	$C_{27}H52O4Si2$	Alkaloid derivative	
trans-13-Octadecenoic acid, methyl ester	78.44	13.71	296	$C_{19}H_{36}O_2$	Fatty acid ester	
10-Methyleicosane	10.93	20.33	296	$C_{21}H_{44}$		
á-D-Mannofuranoside, farnesyl-	2.12	21.38	384	$C_{21}H_{36}O_6$	Sugar moiety	
trans-Geranylgeraniol	0.39	21.42	290	$C_{20}H_{34}O$		

RT = retention time, MW = molecular weight, MF = molecular formula.

FT-IR analysis

The FT-IR technique is a powerful tool in elucidating the functional groups of phytocompounds, helping in their identification (Smith 1979). The FT-IR analysis of apricot kernel methanolic

extract revealed 11 bond stretches (Figure. 3) corresponding to various functional groups. Results were analysed by comparing the peak values with the standard IR spectrum chart by Sigma-Aldrich and identified amines, alkanes, alkenes, primary alcohols and phenols as major functional groups (Table 3), which probably represents different classes of phyto-compounds including alkaloids, flavonoids and phenolic compounds. The peaks were recorded at 3388.06 cm^{-1}, 3013.4 cm^{-1}, 2924.16 cm^{-1}, 2853.33 cm^{-1}, 1744.87 cm^{-1}, 1630.1 cm^{-1}, 1458.7 cm^{-1}, 1357 cm^{-1}, 1139.8 cm^{-1}, 1053.1 cm^{-1} and 996.3 cm^{-1}. C-H stretch corresponds to alkane groups, as shown by the peaks at 2924.16 cm^{-1}, 2853.33 cm^{-1}, and 1458.7 cm-1 (Maitera et al. 2016). While the peaks at 3388.06 cm^{-1} and 3013.4 cm^{-1} are attributed to N-H and C-H/O-H, which are assigned to the stretching vibration of amine moieties and primary alcohols (Poojary et al. 2015). Similar results have been reported by Fadhil (2017) as the apricot kernels are mainly composed of alkanes, alkenes, ketones and amines. Thakur et al. (2019) in line with the current study's findings also reported the presence of N-H (amines), C=O (ketones), O-H (carboxylic acid) and C-H (alkanes) in the apricot kernel press cake.

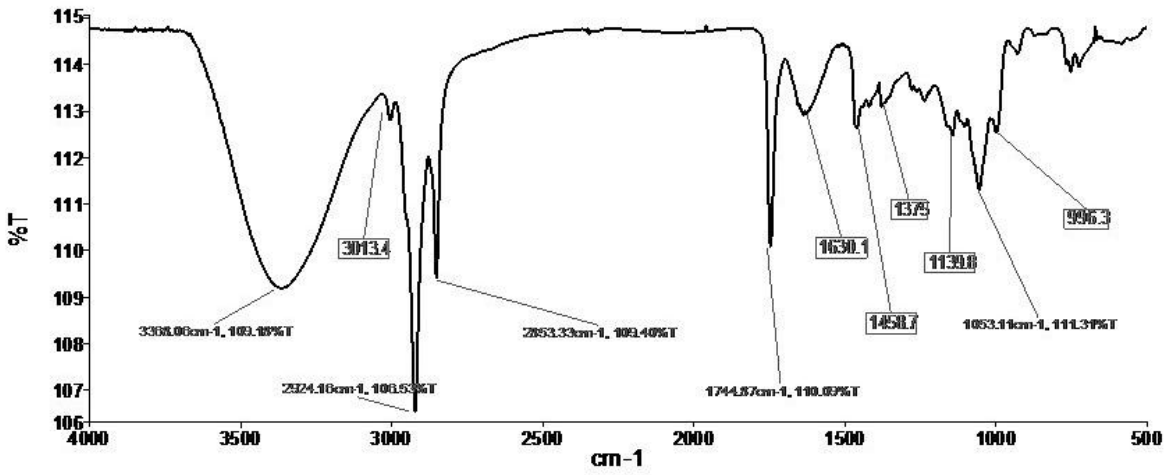


Figure 3. FT-IR chromatogram of apricot kernel methanolic extract.

Table 3. FT-IR analysis of apricot kernel methanolic extract revealing their functional groups.

Sr. No.	Peak Value	Bond	Functional group
1	3388.06	N-H	Amine
2	3013.4	O-H	carboxylic acid
3	2924.16	C-H	Alkane
4	2853.33	C-H	Alkane
5	1744.87	C=O	esters/δ-lactone
6	1630.1	C=C	Alkene
7	1458.7	C-H	Alkane
8	1357	O-H	Phenol
9	1139.8	C-O	aliphatic ether
10	1053.11	C-O	primary alcohol
11	996.3	C=C	Alkene

Conclusions:

From the results of the present study, it can be concluded that the plant part comprises a high amount of protein, oil and many pharmacologically important phytocompounds known as secondary metabolites. The findings of the current study indicate that the plant part contains significant amounts of protein, oil, and several secondary metabolites of pharmacological importance. The presence of these secondary metabolites justifies the use of apricot kernel for various traditional medicines. Moreover, the GC-MS analysis revealed the presence of various volatile compounds which might be contributing to the pharmacological activities. The main shortcoming of the present study is that we used only a single solvent extract (methanolic) for all the phytochemical analysis and performed a single antioxidant assay which might have limited the identification of variety of secondary metabolites. However, with the revelation of preliminary phytochemistry and antioxidant properties of the methanolic kernel extract further investigations

are needed to elute and purify biologically important bioactive compounds from this plant part. Furthermore, different solvents and techniques can be incorporated to elute a maximum number of phytocompounds available in the plant part. The industrial application of the apricot kernel extract concerning medicinal properties, nutraceutical, pharmaceutical and food industries can be evaluated in future experiments.

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