

Research Article

Assessment of phytochemical screening by Fourier Transform Infrared spectroscopic analysis of peach (*Prunus persica*) seed biomass from Uttarakhand region of India

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Abstract

Prunus persica (Peach) has several medicinal and nutritive properties such as antioxidant, antimalarial, anticoagulant, antifungal, ant-allergic, etc. The present study focuses on the exploratory for phytochemicals constituents screening of seed extracts of *Prunus persica* from Uttarakhand region of India by Fourier Transform Infrared spectroscopy (FTIR) analysis. The extraction of seed was carried out using Soxhlet apparatus in different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, and water. The characteristics of physical parameters of dried seed of *P. persica* were reported as total ash (14.250±0.126 %), acid insoluble ash (6.800±0.036 %), water-soluble ash (11.714±0.123 %), and sulphated ash value (2.274±0.025 %); whereas, the extractive values were also recorded as alcohol soluble extractive (1.917±0.011 %), and water-soluble extractive (10.580±0.048 %). The highest values of nutritive components (366.778±0.257 %) were followed by, carbohydrate (45.500±0.336 %), crude protein (29.360±0.551 %), available carbohydrate content (28.002±0.439 %), moisture content (12.547±0.022 %), crude fibre (11.602±0.123 %), crude fat (7.482±0.068 %), and total nitrogen (4.695±0.032 %). The maximum extraction yield was recorded in the aqueous extract solution (11.15 %), followed by petroleum ether (2.8 %), ethyl acetate (2.1 %), ethanol (1.6 %), and chloroform (0.4 %). Besides, this the most effective chemical bonding groups of FTIR spectra analyzed in a sample of *P. persica* seed were N-H, O-H (3600-3400 1/cm), C-H (3000-2800 1/cm), N-H, C-C, C=O (1680-1550 1/cm), C-H (1470-1350 1/cm), CO-OR (1400-1000 1/cm), C-H (850-550 1/cm), and C-I (500-400 1/cm), respectively. Therefore, this study provides useful insights into the beneficial properties of *P. persica* seed biomass from the Uttarakhand region of India, which may be further used for the production of several pharmaceuticals and nutraceutical products.

Keywords: Extraction process, FTIR analysis, *P. persica* seed, Phytochemical screening

INTRODUCTION

The *Prunus persica* is commonly known as Peach, which is cultivated since the 19th century in India. Its seed has been reported for its several medicinal properties such as antioxidant, antimalarial, anticoagulant, antifungal, ant-allergic, etc. (Cao *et al.*, 2014; Kant *et al.*, 2018). Traditionally, its leaves were used for healing sores and wounds, for the treatment of coughs, bronchitis, and abdominal disorders. *P. persica* leaves have a broad range of vital nutrients and antioxidants for the healthy functioning of the human body (Kim *et al.*, 2014; Maatallah *et al.*, 2020). Peach seed extract is found to be useful against different disorders like chronic diarrhoea, dysentery, and chronic hepatitis. Due to the relatively lower incidence of adverse reac-

tions of leave extract in comparison with synthetic agents, it is widely exploited as an attractive eco-friendly alternative for antimicrobial drugs (Rani *et al.*, 2016).

P. persica is deciduous tree up to 10m in height naturally distributed throughout temperate regions originally from Asia and Southern Europe (Gaur, 1999). It belongs to the family Rosaceae and commonly known as "Aaru" in Hindi and "Peach" English. Peach has an important place in human nutrition and can be used as fresh, dried, or processed fruit. Peaches are nutritionally and economically essential, and they are one of the most popular fruits consumed worldwide (Zhao *et al.*, 2015).

The bark of peach is greyish or ashy acuminate glabrous usually utilized in cough, whooping cough, chronic bronchitis, sedative, stomachic, demulcent,

anti-scorbutic, diuretic (Raturi *et al.*, 2011). Its flowers are pinkish-white sessile, short, and pedicelled. Green color leaves are very useful as astringent, demulcent, diuretic, expectorant, febrifuge, laxative, and parasiticide and are seductive (Raturi *et al.*, 2011; Zhao *et al.*, 2015). The fruits have usually a clear ventral suture, do not retain floral residues next to the pedicel, and are characterized by a membranous exocarp with an outer fleshy mesocarp consisting mainly of parenchyma cells (Kim *et al.*, 2014). The mesocarp surrounds a shell (the pit or stone) of hardened endocarp with a seed inside and due to this genus *Prunus* is also referred to as "stone fruit". In almonds, the consumed portion is the seed within the pit, while the edible part in most stone fruits includes the mesocarp, and eventually, the exocarp (Shukla *et al.*, 2012). Peaches, along with cherries plums and apricots are stone fruits (drupes). The single, broad seed is red-brown, around 1.3–2.0 cm long, oval and is enclosed by a wood-like husk. Varieties of heirlooms are available, such as the Indian Peach or Indian Blood Peach, and these arrive in the late summer ranging from red to white to purple. The purpose of this study was aimed at the phytochemical estimation of *P. persica*. In addition, the nutrient values and proximate compositions of *P. persica* were also assessed.

MATERIALS AND METHODS

Collection of *P. persica* seed: For the present study, the *P. persica* seed was collected from the Rani Pokhri Rishikesh (30°10'52.4"N 78°12'38.2"E), Uttarakhand. The collected sample of *P. persica* (seed) was verified by the Botanical Survey of India, Dehradun with accession No.116191. A verified voucher specimen has also been submitted in medicinal plants herbarium of the Department of Chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar. The collected seeds were washed and shade dried followed by grinding for the preparation of fine mash. The fine mesh was used in further analysis of physical evaluation (ash, extractive value) and proximate analysis viz., moisture content or loss of drying, total nitrogen, crude protein, crude fat, crude fibre, total carbohydrate content, available carbohydrate content, and nutritive values.

Preparation of seed extracts: The extraction of seed extracts was carried out using Soxhlet extraction method by different solvents viz., petroleum ether (C₆₋₁₆H₁₂₋₃₄), chloroform (CH: CHCl₃), ethyl acetate (EA: C₄H₈O₂), ethanol (PE: C₂H₅OH) and aqueous (AQ: H₂O) in the way of increasing polarity. Extraction of the seed done through at least 60 cycles of siphoning was completed with each solvent and extraction was continued until siphon tube became colourless. Seed extracts were concentrated using a rotary vacuum evaporator and refrigerated for further use (Nakagawa *et al.*, 2018).

Physical parameters estimation of *P. persica* seed: The estimation of physical characteristics of *P. persica* seed viz., ash, total ash, acid insoluble, water

-soluble ash, and sulphated ash was done, and on the other hand, the extractive values were determined in the form of water-soluble and alcohol soluble also examined. The values of physical characteristics of *P. persica* seed are reported in ash value and extractive value and their sub-parameters. These parameters were subjected to quantitative test analysis using the standard method (Kumar and Chaudhary, 2017).

Proximate analysis and nutritive values of *P. persica* seed: The proximate analysis of the above mashed *P. persica* seed was performed by the percentage (%) estimation of moisture content, crude protein, crude fibre, total nitrogen content, and crude fat, following standard method AOAC (2005). Further, the rest parameters were calculated using the equation:

$$\text{Total Carbohydrate} = 100 - (\% \text{ Ash} + \% \text{ Moisture} + \% \text{ Crude fibre} + \% \text{ Crude protein}) \quad \dots\dots\dots(\text{Eq. 1})$$

The nutritive value was expressed in kilocalories/100 g of the dry weight of leaves, which was calculated as nutritive value (NV).

$$\text{NV(Kcal/100 gm)} = (4 \times \% \text{ protein}) + (9 \times \% \text{ Crude fat}) + (4 \times \% \text{ total Carbohydrate}) \quad \dots\dots\dots(\text{Eq. 2})$$

Total nitrogen (%) was determined by the well-known Kjeldahl method, and their relative crude protein (RCP) calculated by following formula as:

$$\text{RCP} = (\% \text{ N} \times 6.25) \quad \dots\dots\dots(\text{Eq. 3})$$

Phytochemical analysis of *P. persica* seed extract: Phytochemical analysis for various phytoconstituents (alkaloids, carbohydrate, glycosides, inulin, protein, steroids/triterpenoids, fixed oils and fats, flavonoids, phenolic, compound and tannins, gums and mucilage) of the seed extracts was determined using standard qualitative methods. The obtained extracts were screened for the presence of different metabolites viz., tannins, saponins, phlobatanins, flavonoids, terpenoids, cardiac glycosides, and alkaloids (Kumar and Chaudhary, 2017).

Analysis of FTIR peaks of *P. persica* seed sample

The Fourier Transform Infrared Spectrophotometer (FTIR) is feasibly the utmost influential instrument for recognizing the forms of element bonds (functional groups) existing in the samples. The wavelength of light immersed is distinctive of the chemical bond as can be seen in the illustration bands. By understanding the infrared immersion spectrum, the chemical bonds in a molecule can be analyzed. Dried powder of *P. persica* seed was used for the FTIR examination. 10 mg of the dried extract powder was summarized in 100 mg of KBr pellet, to make clean sample discs. The powdered sample of translucent was loaded in FTIR spectroscope, with an analyzed range from 400 to 4000 cm⁻¹ (Ashokkumar and Ramaswamy, 2014).

RESULTS AND DISCUSSION

Estimation of physical parameters (%) of *P. persica* seed: The results of the physical characteristics of *P. persica* seed are presented in Table 1.. In physical parameters the selected parameters are described as

total ash value (14.250±0.126 %), acid insoluble ash (6.800±0.036 %), water-soluble ash (11.714±0.123 %), and sulphated ash value (2.274±0.025 %); whereas, the extractive values are recorded as alcohol soluble extractive (1.917±0.011 %), and water-soluble extractive (10.580±0.048 %), respectively. The findings of the present study are in line with Ashraf *et al.*, (2011) who reported similar results of nutritional and physicochemical studies on fruit pulp, seed, and shell of indigenous *Prunus persica*. Zhang *et al.* (2020) also reported the results of the physical characteristics of *P. persica* by UPLC-Q-TOF/MS-based metabolomics methods.

Assessment of proximate composition of *P. persica* seed: The proximate examination encompasses the mass percentages of moisture, ash, volatile matter, and fixed carbon, etc. Table 2 shows the proximate composition of *P. persica*. It is characterized by nutritive values (366.778±0.257 %), followed by carbohydrate (45.500±0.336 %), crude protein (29.360±0.551 %), available carbohydrate content (28.002±0.439 %), moisture content (12.547±0.022 %), crude fibre (11.602±0.123 %), crude fat (7.482±0.068 %), and total nitrogen (4.695±0.032 %). Similarly, Kumar (2016) also investigated that the different proximate values of *P. persica* during the study of nutritional constituents and sensory evaluation of bakery products prepared from seed and bark of *Prunus persica* (peach). The results by Kumari *et al.* (2017) also showed that the seed of *P. persica* is a good source of protein, fat and crude fiber, high carbohydrate and other constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also

Table 1. Characterization of physical parameters of the dried seed of *P. persica*.

Parameter	Sub-parameter	% [†]
Ash value	Total ash value	14.250±0.126
	Acid insoluble ash	6.800±0.036
	Water soluble ash	11.714±0.123
	Sulphated ash value	2.274±0.025
Extractive value	Alcohol soluble extractive value	1.917±0.011
	Water soluble extractive value	10.580±0.048

[†]Values presented in the table are the mean ± SD of three replicates.

Table 2. Proximate composition of dried seed of *P. persica*.

Parameter	% [*]
Moisture content or loss of drying	12.547±0.022
Total nitrogen	4.695±0.032
Crude protein	29.360±0.551
Crude fat	7.482±0.068
Crude fibre	11.602±0.123
Total carbohydrate content	45.500±0.336
Available carbohydrate content	28.002±0.439
Nutritive value [*]	366.778±0.257

^{*}Values are expressed as mean±SD of the three replicates.

^{*}Nutritive value is calculated in kcal/100 g dry weight of leaves. SD: Standard deviation.

provide raw materials for many industries.

Evaluation of extractive yield of *P. persica* seed:

The percentage extractive yield of *P. persica* seed was presented in Table 3.. The maximum extraction yield was recorded in the aqueous extract solution (11.15 %), followed by petroleum ether (2.8%), ethyl acetate (2.1 %), ethanol (1.6 %), and chloroform (0.4 %). The findings of the present study are according to Altemimi *et al.* (2017) who reported extractive yield from selected plant biomass with reference to phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. During the present experimental study, the different yield of extracts from the selected plant material in various solvents confirms the solubility of plant chemical ingredients in different solvents thus signifying the best solvent for extraction of phytochemicals. Altemimi *et al.* (2015) has reported the maximum extractive value from *P. persica* seed in solvents of high polarity which is indicative of the occurrence of polar compounds in greater quantity.

Phytochemical analysis of *P. persica* seed: The assessment of qualitative phytochemical examination of *Prunus persica* seed showed the occurrence of different compositions viz., alkaloids, steroids, terpenoids, flavonoids, phenolic, tannins, saponins, glycosides, carbohydrates, and proteins in different extracts as presented in Table 4. These tests were performed by the different solutions of petroleum ether (PE), chloroform (CH), ethyl acetate (EA), ethanol (ET), and aqueous (AQ). Similarly, Kumar and Chaudhary (2017) also investigated the different phytochemical compositions of *P. persica* using various tests. From their findings, it was confirmed from the phytochemical analysis of *P. persica* that several carbohydrates, inulin, and flavonoids were present its extract. The other study also confirmed that plant extract compounds are responsible for several biological functions in the human body, therefore have a great pharmaceutical application (Tungmunnithum *et al.*, 2018).

Phytochemical screening by FTIR spectroscopic analysis of *P. persica* seed:

The determination of phytochemical constituents of *P. persica* using FTIR spectroscopic analysis indicated the presence of highly chemical bonding compounds viz., N-H, O-H, C-H, N-H, C-C, C=O, C-H, CO-OR, C-H, C-I (overlapping and stretching of amides, crude protein, carbohydrates; alkyl chain; unconjugated alkenes, carbohydrates, crude protein; bending cyclic alkenes; alicyclic compounds, aromatic hydrocarbons and saccharides of seed cuticle organo-iodides. The wavenumber group (1/cm) of the spectrum of *P. persica* seed biomass are presented in Table 5. The selected peaks were ranged from 400 to 4000 cm⁻¹ in respect of overlapping and stretching of amides, crude protein, carbohydrates, alkyl chain, unconjugated alkenes, carbohydrates, bending cyclic alkenes, alicyclic compounds, aromatic hydrocarbons, saccharides of seed cuticle, and organoiodides. The interpretation of

Table 3. Percentage yield and colour of concentrated different seed extracts of *P. persica*.

Extract	Weight of sample	Weight of extract (gm)	%Yield (w/w)	Colour
Petroleum ether (PE)	200gm	5.6	2.8	Light yellow
Chloroform (CH)	200gm	0.8	0.4	Brownish
Ethyl acetate (EA)	200gm	4.2	2.1	Brownish
Ethanol (ET)	200gm	3.2	1.6	Dark Brown
Aqueous (AQ)	200gm	22.3	11.15	Dark Black

Table 4. Phytochemical constituents of *P. persica* seed extract.

Phytoconstituents		Test performed					
		PE	CH	EA	ET	AQ	
Alkaloids	Mayer's Test	-	+	+	-	-	
	Wagner's Test	-	-	-	-	-	
	Hager's Test	-	-	-	-	-	
	Tannic acid Test	-	-	-	-	-	
Carbohydrate	Molisch's Test	-	+	+	+	+	
	Benedict's Test	-	-	+	+	+	
	Selivanoff's Test	-	-	-	-	-	
Glycosides	Anthraquinone glycosides	Borntrager's Test	-	-	-	-	
		Hydroxy-nthraquinones	-	-	-	-	
		Keller-Killiani Test	+	+	+	+	++
Inulin	Cardiac glycosides	Legal's Test	-	-	+	+	+
		Baljet's Test	-	-	-	+	+
Protein	Heat Test	+	+	++	++	++	
	Biuret Test	-	-	-	-	-	
	Xanthoproteic Test	-	+	+	++	++	
Steroids/ Triterpenoids	Salkowski Test	-	+(T)	+(T)	+(S)	-	
Fixed oils and Fats	Spot Test	-	-	-	-	-	
	Shinoda Test	+	-	+	-	-	
Flavonoids	Alkaline reagent Test	+	+	++	++	++	
	Zinc hydrochloride Test	-	-	-	+	++	
Phenolic	Ferric chloride Test	-	-	++	++	-	
Compound and Tannins	Test for Chlorogenic acid	-	-	-	-	-	
Gums and Mucilage		-	-	-	-	-	

+: Present; - : Absent; PE: Petroleum ether; CH: Chloroform; EA: Ethyl acetate; ET: Ethanol; AQ: Aqueous; and (T): Triterpenoids; (S): Steroids.

marked FTIR peaks of the spectrum of *P. persica* seed biomass (quantity = 1/transmittance) are shown in Fig. 1. The present study observed the most effective chemical bonding groups in the analyzed sample of *P. persica* seed viz., N-H, O-H (3600-3400 1/cm), C-H (3000-2800 1/cm), N-H, C-C, C=O (1680-1550 1/cm), C-H (1470-1350 1/cm), CO-OR (1400-1000 1/cm), C-H (850-550 1/cm), and C-I (500-400 1/cm). Ashok kumar and Ramaswamy (2014) did the phytochemical screening using FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants, whereas Jiao *et al.* (2019) also studied that the preparation of a chitosan-chlorogenic acid conjugate and its application as edible coating in postharvest preservation of peach

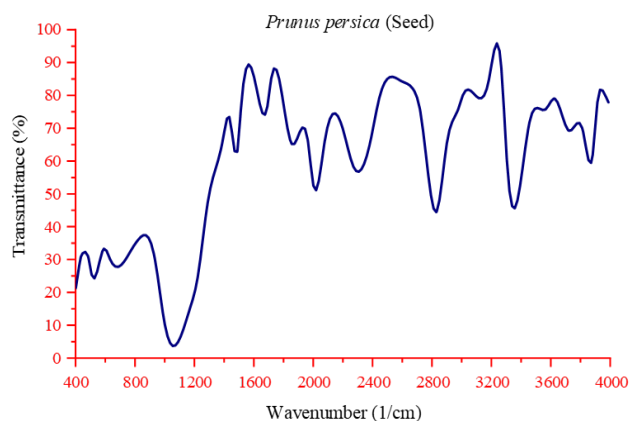
**Fig. 1.** FTIR peaks of *Prunus persica* seed biomass.

Table 5. Interpretation of marked FTIR peaks of the spectrum of *P. persica* seed biomass (Quantity = 1/Transmittance).

Wavenumber group (1/cm)	Analyzed peak (1/cm)	Transmittance (%)	Bonding groups	Compounds	Reference
3600-3400	3566.21	75.82	N-H, O-H	Overlapping and stretching of amides, Crude protein, Carbohydrates	Jiao et al. (2019)
3000-2800	2899.76	75.41	C-H	Alkyl chain	Sivapriya and John (2019)
1680-1550	1621.92	81.27	N-H, C-C, C=O	Unconjugated alkenes, Carbohydrates, Crude protein	Ashokkumar and Ramaswamy (2014)
1470-1350	1435.34	73.44	C-H	Bending cyclic alkenes	Sivapriya and John (2019)
1400-1000	1001.08	10.22	CO-OR	Alicyclic compounds, Aromatic hydrocarbons	Victoria Fernandez et al. (2019)
850-550	590.23	27.3	C-H	Saccharides of seed cuticle	Ashokkumar and Ramaswamy (2014)
500-400	401.20	25.33	C-I	Organoiodides	Jamila et al. (2019)

fruit. Kumar *et al.* (2020) discussed different marked peaks associated with the presence of phytochemically active compounds in sesame plant biomass. Acquah *et al.* (2016) studied the FTIR spectra of *P. persica* seed and found that it had numerous bonding groups which were associated with the presence of different phytochemical compounds. Out of them, the majority of identified peaks were 600, 900, 1190, 1200, 1440, 1500, 1620, and 1800 1/cm, respectively. The most occurring bonding groups were C-O and C=O associated with compounds containing conjugated aromatic ketones and stretching vibration in flavone, respectively.

Conclusion

This study assessed the phytochemistry and nutritional values of *P. persica* seed biomass in Uttarakhand. Since different climatic regions have a significant effect on the phytochemistry of the same plant, therefore, our work presents new findings for this region. It was found that *P. persica* seed biomass was characterized by reasonable quantities of alkaloids, steroids, terpenoids, flavonoids, phenolic, tannins, saponins, glycosides, carbohydrates, and proteins. Also, the nutritive values (nutritive components of 366.778±0.257, carbohydrate of 45.500±0.336 %, crude protein of 29.360±0.551 %, the available carbohydrate content of 28.002±0.439 %, moisture content of 12.547±0.022 %, the crude fibre of 11.602±0.123 %, crude fat of 7.482±0.068 %, and total nitrogen of 4.695±0.032 %) of *P. persica* were also higher in this region than other previously carried studies out in various researches. These results might be due to climatic variations of Uttarakhand region, which may actively affect the plant response and its phytochemical compositions. FTIR analysis results suggested that the phytochemical extracts of *P. persica* seed had several bonding groups responsible for the presence of various phytoactive compounds which further may be used for the production of pharmaceutical and nutraceutical products.

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Conflict of interest

The authors declare that they have no conflict of interest.

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