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AN EXPERIMRNTAL ASSESMENT OF ANTIMICROBIAL PROPERTY OF PHYTOCHEMICAL DERIVATIVES EXTRACTED FROM THE PEACH (*Prunus persica* L. Batsch) LEAVES

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ABSTRACT

This study focuses on the preliminary phytochemical screening, nutritive value, and antimicrobial activity of *Prunus persica* leaves extracts. Proximate analysis of the *P. persica* leaves shows the moisture content (10.615%), crude fiber.534%), crude fat (4.443%), total protein (27.468%), total carbohydrate (41.566%), and nutritive value (316.123kcal/100g). The qualitative phytochemical studies show the presence of tannins, saponins, tannins, and flavonoids. The antimicrobial activity of *P. persica* leaves extracts was performed against three bacteria (*Escherichia coli* MTCC-40, *Pseudomonas aeruginosa* MTCC-2474, *Staphylococcus aureus*- MTCC-1144) and two fungal strains (*Penicillium* sp. and *Aspergillus* sp.) The ethyl acetate leaves extract of *P. persica* showed the highest antibacterial activity and the highest antifungal activity compared to other extracts (ethyl acetate > ethanol> chloroform). Overall, this study suggests that *P. persica* has good nutritive value, is an excellent source of phytochemicals, and shows good antimicrobial activity.

Keywords: Antimicrobial activity, Nutritive Values, Phytochemical Screening, Proximate composition, *Prunus persica.*

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INTRODUCTION

Over the last few decades, plant-derived substances are the first choice of researchers to develop high-potential medicines. As per reported data, different medicinal plants provide more than 25 percent of medicines, either directly or indirectly.[1] Various epidemiological studies have revealed that the consumption of natural products is beneficial for health against a range of chronic diseases like cancer, diabetes, hepatitis, and cardiovascular disease.[2,3] Fruits and vegetables show good health-promoting properties due to the presence of a variety of antioxidants.[4,5] *Prunus persica* L. is generally called Aaru in Hindi, and Peach in English belongs to the Rosaceae family. The peach crop is essential regarding nutrition and economy, and we use peach as fresh, dry, and frozen fruits[6]. P. *persica* is a widespread source of bioactive compounds such as proteins, vitamins, sugar, and minerals, and it has an excellent nutritive value and antioxidant properties[7]. Dietary intake of peach as fruit can reduce the generation of reactive oxygen species (ROS) responsible for alteration in organs. Peach leaves are a vital source of freshly prepared glucans, which have effective antimicrobial, antifungal, and anti-inflammatory properties.[8,9] In contrast with a synthetic drug, its extract has a minimum adverse effect on human health and may be used as an alternate source for the antimicrobial drug.[10] Currently, the researchers are showing interest in developing eco-friendly natural product-based antimicrobial agents to overcome the problems related to chemical treatment.[2,8,10,11] In this study, we extracted phytochemical derivatives from peach and found their antimicrobial activity against three bacteria viz., *Escherichia coli* MTCC-40, *Pseudomonas aeruginosa* MTCC-2474 *Staphylococcus aureus*- MTCC-1144 and two fungal strains viz., *Penicillium* sp.

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and *Aspergillus* sp. This study provides valuable insights into the benefits of antimicrobial properties of phytochemical derivatives obtained from the peach leaves from the Uttarakhand region (India) which we may further use to produce several pharmaceuticals and nutraceutical products.

EXPERIMENTAL

Material and Methods

Sampling Details

We collected the leaves of *P. persica* from the Rishikesh, Uttrakhand in May 2016. BSI, Dehradun verified the morphology of the same with accession number 116191. The specimens collected were first washed, dried, and then grind into the fine mass used for the proximate and physicochemical analysis.

Preparation of *P. persica* Leaves Extracts

We extracted leaves in petroleum ether, chloroform, ethyl acetate, ethanol, and water using a Soxhlet apparatus. For extraction, siphoning up to 60 cycles with each solvent was carried out until the siphon tube became colorless.[12] Obtained extracts were concentrated using a rotary vacuum evaporator and stored in a refrigerator till further use.

Physical Parameters of *P. persica* Leaves

Physical parameters involved the analysis of ash content i.e., total ash, acid-insoluble ash, water-soluble ash, and ash in the form of sulphates. Further, we determined the extraction values in the form of water and alcohol soluble ash. We used quantitative analysis according to the standard protocol mentioned elsewhere for this analysis[12].

Nutritive and Proximate Analysis of *P. persica* Leaves

We estimated the moisture, crude protein, total nitrogen, and crude fat content of the peach leaves using the standard method.[12] In addition, the rest of the parameters were calculated using eqn.-1:

$$\text{Total carbohydrates} = 100 - (\%\ \text{ash} + \%\ \text{moisture} + \%\ \text{crude fibre} + \%\ \text{crude protein}) \quad (1)$$

The nutritional value was expressed in kilocalories/100g of the dry weight of the leaves, and calculated with the following eqn.-2:

$$\text{Nutritive value (Kcal/100 gm)} = (4\times\%\ \text{protein}) + (9\times\%\ \text{crude fat}) + (4\times\%\ \text{total carbohydrate}) \quad (2)$$

Total nitrogen (%) was determined by the well-known Kjeldahl method and their relative crude protein was calculated according to the formula (%N×6.25).

Phytochemical Analysis of *P. persica* Leaves Extract

The phytochemical analysis of leaf extract using the standard methods mentioned was carried out in the presence of different metabolites such as tannin, saponin, alkaloids, phlobatanins, flavonoids, etc.[13]

Antimicrobial Screening of *P. persica* Leaves

Three ubiquitous bacterial species, i.e., *Escherichia coli* (MTCC-40), *Pseudomonas aeruginosa* (MTCC-2474), *Staphylococcus aureus* (MTCC-1144), and two fungal species, i.e. *Penicillium* sp. and *Aspergillus* sp. used for the evaluation of the antimicrobial activity of *P. persica* leaves extract. We used Muller Hilton Agar using the agar well diffusion method to evaluate the antibacterial activity. Each bacterial sample was spread on Muller Hilton agar plates, followed by cutting five wells using a cork borer. Leaves extracts were dissolved in DMSO at the concentration of 50 mg/ml, and 45 µl of each extract was poured into each well. The plates were incubated at 37° C for 24 h. Similarly, Sabouraud dextrose agar (SDA) was inoculated with fungal cultures by seed culture technique. Each solidified plate of SDA medium was punctured with five well, filled with 45 µl of each extract, and incubated at 27° C for seven days. The antifungal and antibacterial potential was measured as a zone of inhibition, excluding the 6mm diameter of the well.

RESULTS AND DISCUSSION

Estimation of Physical Parameters of *P. persica*

Physical analysis of *P. persica* leaves revealed that it contains 15.908% total ash, i.e., physiological, and non-physiological ash[14]. Table-1 shows the results. Acid insoluble ash is generally present in the form of

silica, especially siliceous earth.[15] While the sulphated ash contains a variable amount of VOCs, igniting can lose it[16]. The results obtained were concurrent with the findings of Zhang *et al.* who elucidated the physical properties of *P. persica* using UPLC-Q-TOF/MS-based metabolomics techniques.[17]

Table-1: Characterization of Physical Parameters of Dried Leaves of *P. persica*

Parameter	Sub-parameter	Results (%) *
Ash value	Total ash value	15.908±0.131
	Acid insoluble ash	7.729±0.049
	Water-soluble ash	10.817±0.108
	Sulphated ash value	1.684±0.017
Extractive value	Alcohol-soluble extractive value	1.880±0.005
	Water-soluble extractive value	11.575±0.056

*Values presented in the table are the mean ± SD of three replicates.

Proximate Analysis of *P. persica*

Table-2 shows the proximate analysis results. The analysis revealed moisture within the acceptable limit.[18] Results conclude that the leaves of *P. persica* are an excellent source of protein, fats, carbohydrates, and fibers. The high fiber and carbohydrate content are beneficial. Furthermore, the *P. persica* leaves have a high nutritive value, i.e., ~316.123 kcal/kg.

Table-2: Proximate Composition of Dried Leaves of P. *persica*

Parameter	Results (%)*
Moisture content or loss of drying	10.615±0.022
Total nitrogen	4.395±0.032
Crude protein	27.468±0.551
Crude fat	4.443±0.068
Crude fiber	11.534±0.123
Total carbohydrate content	41.566±0.336
Available carbohydrate content	30.032±0.439
Nutritive value*	316.123±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.

Extractive Yield of *P. persica* Leaves

Extraction results revealed that the aqueous extract provides a maximum yield (9.75%) than other extracts (Table-3). Thus, we preferred an aqueous solvent for the extraction of phytochemicals from the *P. persica* leaves. The findings were concurrent with (Altemimi *et al*.) concluded that high polarity of solvent enhances the extraction process.[20]

Table-3: The Percentage Yield and Colour of Concentrated Different Leave Extracts of *P. persica*

S. No.	Weight of sample	Weight of extract (gm)	%Yield (w/w)	Color	Extract
1	200gm	1.2	0.60	Light Brown	Petroleum ether (PE)
2	200gm	0.9	0.45	Brownish	Chloroform (CH)
3	200gm	5.1	2.55	Brownish	Ethyl acetate (EA)
4	200gm	5.6	2.8	Dark Brown	Ethanol (ET)
5	200gm	19.2	9.75	Dark Black	Water (AQ)

Phytochemical Analysis of *P. persica* Leaves

The qualitative phytochemical analysis of the different extracts of peach leaves shows different compounds are present in them (Table-4). The phytochemical analysis shows that carbohydrates, inulin, and flavonoids are present in all five extracts, while tannins and phenolic compounds are present only in ethyl acetate and ethanol extracts. The presence of flavonoid and tannins are responsible for free radical scavenging activity because flavonoids, phenolics, and tannins are the major group of compounds that acts as primary antioxidants or free radical scavengers.[19,21] Chloroform and ethyl acetate extracts are rich in alkaloids,

triterpenoids, and petroleum ether while ethanol extract contains steroids. The petroleum ether extract is also rich in fixed oils and fats.[22] Moreover, the phytochemical screening of the *P. persica* was carried out using the FTIR spectroscopic method revealing the peaks for the stretching of amides, crude protein, carbohydrates, alkyl chain, unconjugated alkenes, carbohydrates, crude protein, bending cyclic alkenes, alicyclic compounds, aromatic hydrocarbons, saccharides of seed cuticle and organo-iodides.[23] (Acquah *et al.*)[24] estimated the FTIR spectra of *P. persica* kernels and established closeness assemblies related to the occurrence of various phytochemical complexes.

Table-4: Phytochemical Constituents of *P. persica* Leave Extract

Phytoconstituents and tests performed			Phytoconstituents and Tests 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-anthraquinones	-	-	-	-	-
	Cardiac glycosides	Keller-Killiani Test	++	+	++	+	++
		Legal's Test	-	+	+	++	++
		Baljit's Test	-	-	-	+	+
Inulin			++	++	++	+++	+++
Protein	Heat Test		-	-	-	-	-
	Biuret Test		-	-	-	-	-
	Xanthoproteic Test		-	+	+	+	++
Steroids/ Triterpenoids	Salkowski Test		+(S)	+(T)	+(T)	+(S)	-
Fixed oils and Fats	Spot Test		+	-	-	-	-
Flavonoids	Shinoda Test		-	-	+	-	-
	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.

Antimicrobial Activity of *P. persica* Leaves

We tabulated antibacterial activity results (Table-5) in terms of zone of inhibition. The results reveal that ethyl acetate extract has more prominent activity against gram-positive bacteria. The extract showed a 15.07±0.07mm zone of inhibition for *E. coli,* 25.10±0.06mm for *P. aeruginosa,* and 13.17±0.12mm for *S. aureus*. Results throw light on triterpenoids present in ethyl acetate extract being responsible for their antimicrobial activity. The petroleum ether, ethanol, and aqueous extracts remained inactive during antibacterial activity, while chloroform extracts showed good activity (12.17±0.12 mm) against *E. coli*. We used the commonly used antibiotic ofloxacin as a positive control. The antifungal screening results showed (Table 6) that the ethyl acetate extract gave a maximum zone of inhibition against *Penicillium* spp. (16.07±0.03 mm) followed by ethanol extract (10.10±0.10 mm) and chloroform extract (09.07±0.07 mm). Whereas *Aspergillus* sp. were only susceptible to ethyl acetate extract having a zone of inhibition (10.03±0.03 mm) and ethanol extract (7.03±0.03 mm). Here, we used ketoconazole (antifungal) as a positive control gave zone of inhibition (42.030±0.23mm) against *Penicillium* sp. and (42.553±0.26mm)

against *Aspergillus* sp. (Gacem *et al.)*[25] performed similar work. The antimicrobial activities of leave extracts are due to the presence of secondary metabolites such as saponins, flavonoids, tannins, carbohydrates, etc.[26]

Table-5: Antibacterial Activity of the Leaves Extracts of *Prunus persica*

Plant extracts	*E. coli*	*P. aeruginosa*	*S. aureus*
Ofloxacin	26.173±0.54mm	24.214±0.05mm	39.172±0.09mm
Petroleum ether	---	---	---
Chloroform	12.17±.012mm	---	---
Ethyl acetate	15.07±0.07mm	25.10±0.06mm	13.17±0.12mm
Ethanol	---	---	---
Water	---	---	---

Values are expressed as mean±SD of the three replicates

Table 6: Antifungal Activity of the Leaves Extracts of *Prunus persica*

Plant extracts	*Penicillium* sp.	*Aspegillus* sp.
Fluconazole	42.030±0.23mm	42.553±0.26mm
Petroleum ether	---	---
Chloroform	9.07±0.07mm	---
Ethyl acetate	16.07±0.03mm	10.03±0.03mm
Ethanol	10.10±0.10mm	7.03±0.03mm
Water	---	---

Values are expressed as mean±SD of the three replicates

CONCLUSION

Phytochemical analysis indicates that the leaves extract has phytoconstituents like terpenoids, flavonoids, glycosides, carbohydrates, etc. Thus, it can be used as a natural antimicrobial drug. In addition to this, proximate analysis reveals that it can be used as a nutritive supplement. Leaves extract showed exceptionally good antimicrobial activity. The presence of these phytochemicals in leaves might contribute to the therapeutic potential of *P. persica*. The current study will be useful for the integrated management of harvested biomass for energy recovery.

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