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RESEARCH ARTICLE

Evaluation of nutritive value, phytochemical screening, total phenolic content and *in-vitro* antioxidant activity of the seed of *Prunus domestica* L.

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ABSTRACT

Prunus domestica L. is a member of the *Rosaceae* family that shows many biological activities including antioxidant, antimicrobial, antihaemolytic, anti-inflammatory, hepatoprotective activity and many other activities. In the current study, we evaluated nutritive value, phytochemical screening, total phenolic content and antioxidant activity by DPPH and FRAP method for the different extracts obtained by successive soxhlet extraction using the different solvents based on their polarity. Results show that it is a good source of energy. Phytochemical screening revealed the presence of many secondary metabolites which include alkaloids, carbohydrates, glycosides, protein, steroids and terpenoids, fixed oils and fat as well as phenolic compounds. The highest total phenolic content was found in the ethyl acetate fraction. Highest antioxidant activity by DPPH method is reported in ethyl acetate fraction (IC50 =1837.399±0.377µg/ml) while the ferric reducing antioxidant power was maximum for diethyl ether (56.032±0.985µM/ml FRAP value = 0.325±0.002).

Introduction

Various fruits constitute an important part of the human diet. It is accepted worldwide that a diet enriched with fruits and vegetables lowers the hazard of persistent sicknesses like cardiovascular diseases, cancer and leading a long and healthy life (1). These food products have been regarded as nutraceuticals or functional foods. The foods which are a good source of antioxidants (like phenolic acids, flavonoids etc.) have high demand in today's market as a nutraceutical. Fruits are the source of low fat and low calories while dried fruits have high protein contents, high mineral contents (2).

Prunus domestica L. of the family *Rosaceae* is generally regarded as plum (2, 3). The family *Rosaceae* is the 19th largest family of plants (4) and includes about 3000 species whereas the genus *Prunus* includes about 400-430 species; but only 89 are enlisted in the Genetic Resources Information System. In India, about 36 species of *Prunus* have been reported of which 18 species can be used for the cultivation of different purposes (5-9).

It is used in combination with other drugs to treat leucorrhoea, irregular menstrual and miscarriage followed by debility (10). Plums and prunes show laxative stomachic effect. The bark of *P. domestica* is used as febrifuge while roots are utilized as astringent (11). *P. domestica* is the source of calcium, magnesium, vitamin A, potassium and fibre (12). This species possesses a discrete place in the Indian medicinal system due to its numerous health benefits (2). It is abundant in different bioactive phytochemicals such as anthocyanins, pectins and carotenoids, lignans, abscisic acid, glucoside, flavonoids, flavonoids glycosides, bipyrrole, dihydroflavonols and carbohydrates (12-19). These bioactive compounds are found to be varied in concentration depending upon various pre-harvesting factors (20). Plums are an excellent source of nutrients with a significant contribution to human nutrition. Due to many important phytochemicals, this fruit has an effective antioxidant activity (21). In addition to this, different extracts of *P. domestica* show many important activities namely antibacterial, anticancer, antihyperlipidemic, blood pressure-lowering activity, anxiolytic activity and antidiabetic activity (2, 22, 23).

The fruit of the *P. domestica* is juicy and fleshy which is eaten while the seed is usually discarded. Therefore, the current study aims to evaluate the

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nutritive value, qualitative phytochemical screening and antioxidant activity of the seed (*i.e.*, kernel) of *P. domestica*.

Materials and Methods

Reagents and Chemicals

Petroleum ether (SD fine), methanol (Merck), diethyl ether (SD fine), ethyl acetate (Merck), double distilled water, folin's reagent (Merck), sodium hydroxide (Fisher), sodium acetate trihydrate (Merck), nitric acid (Fisher), gallic acid (Merck), sodium carbonate monohydrate (Merck), sulphuric acid (Fisher), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Alpha acer), ascorbic acid (Merck) and 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ) (SRL chem). All other reagents which were used in this study were of analytical grade.

Sample collection

For the collection of the seeds, plums were purchased from the local garden situated in Haridwar, Uttarakhand. Seeds were dried under the shade. The plant was recognized and confirmed by the Botanical Survey of India (BSI), Dehradun under accession number 374. For the authentication of the plant material two herbarium specimens were prepared, one of which is deposited in the Botanical Survey of India.

Physical Parameters and Proximate Analysis

Evaluation of the physical parameters and proximate analysis for the calculation of the nutritive value was done according to the Indian pharmacopeia and earlier study on *Bombax ceiba* (24, 25).

Preparation of Extracts

Soxhlet extractor was employed for the extraction. Briefly, 200 gms of the powdered plant material was put in the thimble of the soxhlet extractor. Extraction was done using the solvents petroleum ether, diethyl ether, ethyl acetate, methanol and double-distilled water in the increasing order of the polarity. Approximately 72 cycles of siphoning were conducted for each solvent or the extraction was continued till the siphoning tube appears colourless. After the extraction, the extracts of different solvents were concentrated under reduced pressure using the rotary evaporator and stored in a refrigerator for further examination.

Phytochemical Screening

Phytochemical screening of the different extracts for the presence of the various phytoconstituents was carried out by standard qualitative methods (26, 27). Each concentrate was screened for the nearness of natural dynamic mixes like alkaloids, sugars, glycosides, amino acids, proteins, triterpenoids and steroids, flavonoids, phenolics, gums. Furthermore mucilages, naphthoquinones and so forth.

Total Phenolic Content

The folin-ciocalteau method was adopted for the determination of the total phenolic content of each extract with some modifications. Briefly, 100 µl extract dilution at a concentration (1000 µg/ml) or gallic acid (10-400 µg/ml) put in a test tube, containing 7.9 ml of the distilled water. Distilled water also serves as blank. Now, added 500 µl of the folin's reagent to the volumetric flask and shaken properly and incubated for 8 min at room temperature. Now, 1.5 ml of 20% Na_2CO_3 was added to the above mixture to make the final volume 10 ml and incubated for 2 hrs at room temperature. Absorbance was measure at 765 nm with UV-Vis double beam spectrophotometer (Systronics 2205). Calculations were carried out using the calibration curve of gallic acid. The total phenolic content of each extract was expressed as mg of gallic acid equivalents (GAE) per gms dried weight (mg GAE/g dw) (28).

Antioxidant Activity

DPPH Free Radical Scavenging Assay

All the obtained extracts of the seed of the *P. domestica* were evaluated for their antioxidant power by the DPPH method according to standard methodology with some modification (29). Briefly, 1 ml of the extract (100 – 5000 µg/ml) was mixed with 0.004% DPPH solution and leave at room temperature for 1 hr. After 1 hr absorbance was measured at 517 nm using the Systronics 2205 double beam UV-Visible spectrophotometer. The change in the color from pink to yellow is directly proportional to the scavenging of the DPPH radical. Ascorbic acid was used as standard. 1 ml of the solvent and 3 ml of the DPPH solution serve as blank.

$$\% \text{ Inhibition} = \frac{\text{Absorbace of Blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times \mathbf{100}$$

Ferric Reducing Antioxidant Power (FRAP Assay)

FRAP assay was carried out according to standard methodology with some modification (30). Stock solution include 300 mM acetate buffer (pH 3.6), 40 mM HCl solution, 20 mM $FeCl_3.6H_2O$ (Ferric chloride hexahydrate) solution, 10 mM TPTZ (2,4,6-tri-(2-pyridyl)-1,3,5-triazine) solution. Working FRAP reagent was prepared by mixing Acetate buffer, ferric chloride hexahydrate solution and TPTZ solution in the 10:1:1 ratio. Antioxidant potential was evaluated by reacting 1 ml of extract (500 µg/ml) and 10 ml of working FRAP reagent. The absorbance of the coloured product was taken at 593 nm after incubating at 37 °C after 30 min. 1 ml methanol and 10 ml working FRAP reagent act as the control. Ascorbic acid was used as standard.

Results and Discussion

Physical Parameters and Proximate Analysis

Table 1 and 2, show the results of the physical parameters and proximate analysis respectively. The study revealed total ash 4.115±0.118%, acid insoluble ash 1.032±0.072%, water-soluble ash 1.296±0.049% and sulphated ash 4.020±0.075%. While water-soluble and alcohol soluble extractive values are found to be 26.746±1.265% and 26.759±0.983% which show that extraction with water and alcohol is approximately equally exhaustive. Proximate analysis, revealing the

moisture content 10.553±0.132%, total nitrogen content 3.133±0.036%, protein 19.580±0.227%, crude fat 22.441±0.087%, crude fibre 27.870±0.440%, total carbohydrates 43.292±0.035%, available carbohydrates 15.422±0.419%. Different ash values measure the presence of substances like silica, silicates, oxalates, carbonates, phosphates, oxides and volatile inorganic substances indicative of the richness of the mineral contents in the particular plant part. Plum seeds are a good source of protein, carbohydrates, fat as well as fibre, which constitutes the major essential for the living being. On the basis of the above values, the nutritive value of the seed was calculated and its nutritive value is found to be 341.991±2.106 Kcal/100 gm. High nutritive value is suggestive that plum seed can be the source of the feed and fodder.

Table 1. Physical evaluation of the seed of *P. domestica*

Parameter	Value (%)
Total ash	4.115±0.118
Acid insoluble ash	1.032±0.072
Water-soluble ash	1.296±0.049
Sulphated ash	4.020±0.075
Water-soluble extractive value	26.746±1.265
Alcohol soluble extractive value	26.759±0.983

Each experiment was performed in triplicate. All the values are represented as the mean ± SD.

Table 2. Proximate analysis of the seed of *P. domestica*

Parameter	Value (%)
Moisture Contents or Loss on Drying	10.553±0.132
Total Nitrogen Contents	3.133±0.036
Protein	19.580±0.227
Crude Fat	22.441±0.087
Crude Fibre	27.870±0.440
Total Carbohydrate Contents	43.292±0.035
Available Carbohydrate Contents	15.422±0.419
Nutritive Value (Energy value)	341.991±2.106 Kcal/100gm

Each experiment was performed in triplicate. All the values are represented as the mean ± SD.

Extractive Yield

Table 3, displays the yield of the extract of different solvents based on the total material. Petroleum ether, diethyl ether, ethyl acetate, methanol and water yield 13.752%, 10.045%, 7.251%, 11.934% and 14.625% respectively concerning the total material. Fig. 1, represents the yield of the different obtained extracts based on total extract.

Table 3. Extractive yield of the extract obtained by successive sohxlet extraction using the different solvents

Solvent	Yield (%)
Petroleum ether	13.752
Diethyl ether	10.045
Ethyl acetate	7.251
Methanol	11.934
Aqueous	14.625

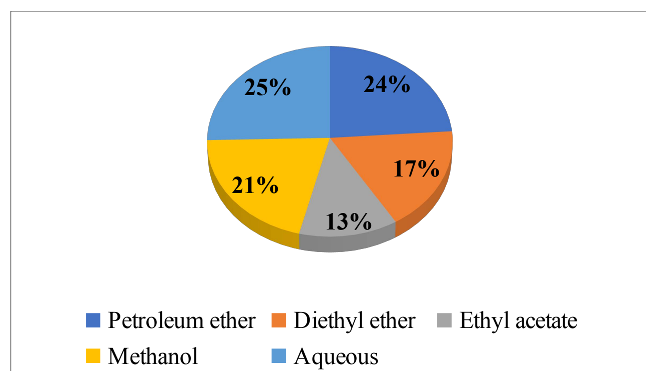


Fig. 1. % yield of the different extracts concerning the total extract.

Phytochemical Screening

Table 4, representing the results of the qualitative phytochemical screening. Phytochemical screening has shown the presence of carbohydrates, glycosides, protein, steroids and terpenoids, fixed oil and fats, flavonoids and phenolic compounds. Bioactivity and the therapeutic value of the plant extract are attributed to the phytoconstituents of the plant extract.

Total Phenolic Content

Table 5, emphasizes the total phenolic content of the different fractions and found to be in the order ethyl acetate > methanol > water> diethyl ether > petroleum ether in an amount of 28.366±0.577 > 27.700±1.000 > 23.366±1.154 > 19.366±0.577 > 10.033±0.577. All the total phenolic contents are expressed in terms of mgGAE.gm-1dw. For the calculation of the total phenolic content calibration curve of gallic acid was also prepared with equation $y=0.001x+0.0013, R^2=0.9992$.

Antioxidant Activity

DPPH Free Radical Scavenging Assay

Table 6, depicts the results of the DPPH free radical scavenging assay. The IC50 (concentration of the plant extract required to scavenge the 50% of DPPH concentration) values of diethyl ether, ethyl acetate, methanol and aqueous fractions are 3730.567±0.914 µg/ml, 1837.399±0.377 µg/ml, 2520.596±0.398 µg/ml, 3490.380±0.777 µg/ml respectively whereas the IC50 value of the petroleum ether fraction was beyond our dilutions prepared and IC50 value of ascorbic acid was determined in the same way as that of the sample and it is found to be 13.296±0.075 µg/ml. Fig. 2, shows the variation of the percentage inhibition against the concentration of the plant extract and standard.

Ferric Reducing Antioxidant Power (FRAP Assay)

Table 7, representing the results of the FRAP method. Fig. 3, shows the comparison among the FRAP values of the different fractions. Fig. 4, shows the standard calibration curve of the ascorbic acid giving the equation $y = 0.0041x + 0.0996, R^2 = 0.9915$ and the results are expressed in µM/ml. Diethyl ether has the highest ferric reducing antioxidant power 56.032±0.985 µM/ml, followed by petroleum ether 20.341±0.645 µM/ml, ethyl acetate 18.634±0.243 µM/ml, methanol 15.788±0.745 µM/ml and aqueous 14.731±0.879 µM/ml. The FRAP value of the standard ascorbic acid is found to be 472.601±0.140 µM/ml.

Conclusion

Various experiments conducted on the seed of this plant revealed it to be an excellent source of energy because of its excellent nutritional value. Phytochemical screening shows the presence of many

Table 4. Qualitative phytochemical screening of the *P. domestica* seed extracts

Phytoconstituents and Test Performed			Extract				
			PE	DEE	EA	MeOH	H_2O
Alkaloids	Mayer's Test		-	-	-	-	+
	Wagner's Test		-	-	-	-	+
	Hager's Test		-	-	-	-	-
Carbohydrates	Molisch's Test		-	+	+	+	+
	Fehling's Test		-	+	+	+	+
	Benedict's Test		-	-	+	+	-
	Seliwanoff's Test		-	-	+	+	-
Glycosides	Anthraquinone Glycosides	Bontrager's Test	-	-	-	-	-
		Test for Hydroxy-anthraquinones	-	-	-	-	-
	Cardiac Glycosides	Keller-Killiani Test	+	+	+	+	+
		Legal's Test	-	-	-	-	-
		Baljet's Test	-	-	-	-	-
	Saponin Glycosides	Froth Formation Test	-	-	-	+	+
	Flavanol Glycosides	Mg and HCl Reduction	-	-	-	-	-
Protein	Heat Test		-	-	-	-	-
	Biuret Test		-	-	-	-	+
	Xanthoproteic Test		+	+	+	+	+
Amino Acid	Ninhydrin Test		-	-	-	-	-
Steroids and Terpenoids	Salkowski Test		+	+	-	+	-
Fixed Oils and Fats	Spot Test		+	+	+	+	-
Flavonoids	Shinoda Test		-	-	-	-	-
	Zn-HCl Test		-	-	-	-	-
Phenolic Compounds and Tannins	Lead Acetate Test		-	-	-	+	+
	Ferric Chloride Test		-	-	-	-	-
	Test for Catechin		-	-	-	-	-
	Test for Chlorogenic acid		-	-	-	-	-
Gums and Mucilage			-	-	-	-	-
Naphthoquinone	Juglone Test		-	-	-	-	-
	Dam-Karrer Test		-	-	-	-	-

Where, PE = Petroleum ether, DEE = Diethyl ether, EA = Ethyl acetate, MeOH = Methanol and H_2O= Water. Where: (+ve) = present and (-ve) = absent.

secondary metabolites. The total phenolic content and the antioxidant study revealed that the seed of the *P. domestica* has antioxidant potential. Total phenolic content in plant extract is responsible for its various biological activities including antioxidant activity. We have found a strong correlation between the total phenolic content and the antioxidant activity by the DPPH method. The antioxidant potential is attributed to phenolic content, plays an important role in drug development and supplements, which could be very important in preventing or decreasing the rate of oxidative stress.

Table 5. Total phenolic content of the different seed extracts of *P. domestica*

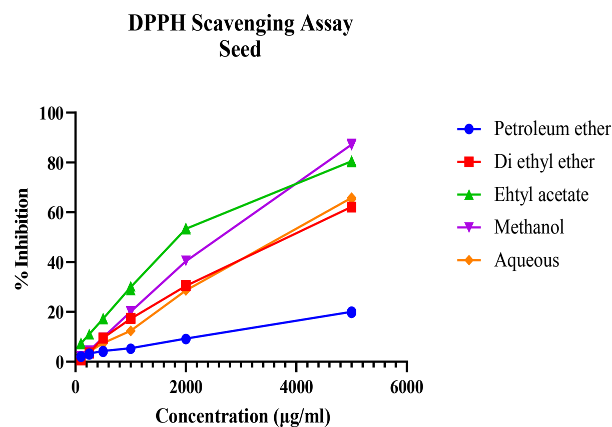
Solvent /extract	Total phenolic content (mgGAE.gm-1dw)
Petroleum ether	10.033±0.577
Diethyl ether	19.366±0.577
Ethyl acetate	28.366±0.577
Methanol	27.700±1.000
Aqueous	23.366±1.154

Each experiment was performed in triplicate. All the values are represented as the mean ± SD.

Table 6. DPPH free radical scavenging assay of the different seed extracts of *P. domestica* and ascorbic acid (standard)

Solvent/extract	IC50 (µg/ml)
Ascorbic acid (standard)	13.296±0.075
Petroleum ether	Beyond the Dilution
Diethyl Ether	3730.567±0.914
Ethyl acetate	1837.399±0.377
Methanol	2520.596±0.398
Aqueous	3490.380±0.777

Each experiment was performed in triplicate. All the values are represented as the mean ± SD.



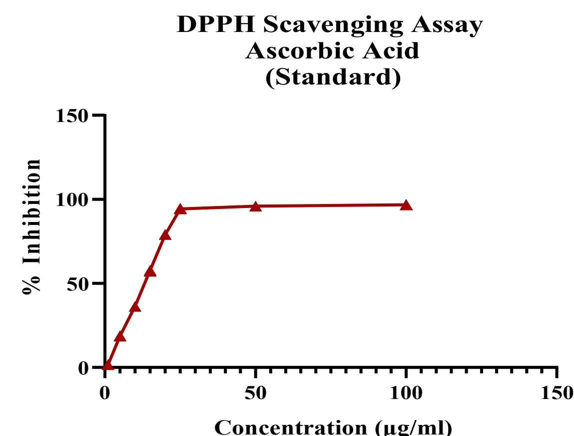


Fig. 2. Variation of %inhibition against concentration of different extract and standard by DPPH assay.

Table 7. Ferric reducing antioxidant power (FRAP) of the different seed extracts *P. domestica* and ascorbic acid (standard)

Solvent/Extract	Ferric reducing antioxidant power (µM/ml)	FRAP value
Ascorbic acid (standard)	472.601±0.140	2.000±0.000
Petroleum ether	20.341±0.645	0.180±0.001
Diethyl ether	56.032±0.985	0.325±0.002
Ethyl acetate	18.634±0.243	0.173±0.002
Methanol	15.788±0.745	0.162±0.001
Aqueous	14.731±0.879	0.155±0.002

Each experiment was performed in triplicate. All the values are represented as the mean ± SD.

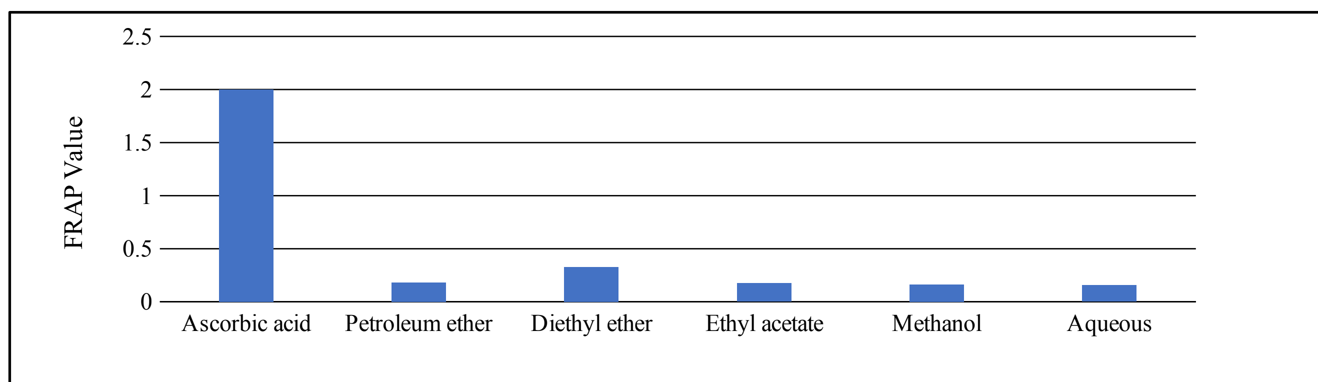


Fig. 3. Comparison of FRAP values of different seed extracts and ascorbic acid (standard).

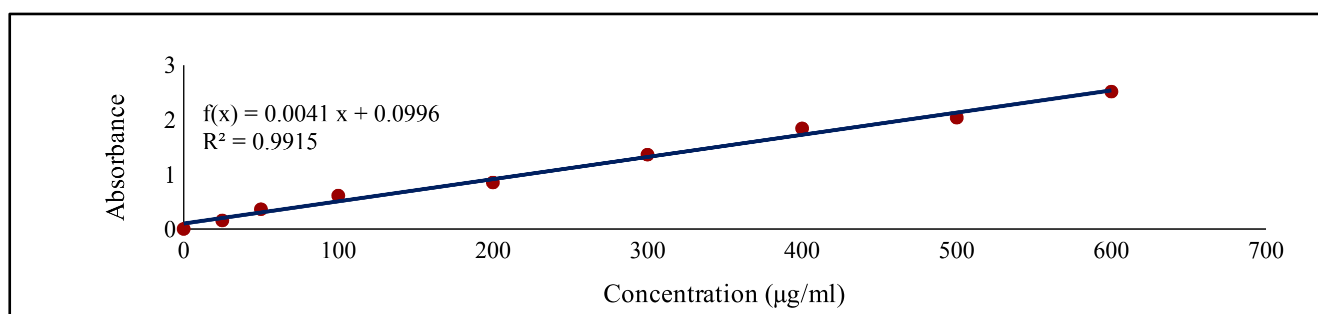


Fig. 4. Calibration curve of ascorbic acid.

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Authors' contributions

Concept and planning of the work was supervised by RKS and AS. Experimental work related to the physical parameters, proximate analysis, nutritive value, preliminary phytochemical screening, total phenolic content and antioxidant activity was done by K. RS with K has participated in the design and study of the antioxidant activity. Manuscript writing and statistical calculations were done the K. Corrections in the final manuscript were also done by K. All the authors have read the manuscript and given their approval for the final manuscript.

Conflict of interests

Authors do not have any conflict of interests to declare.

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