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NUTRITIVE VALUE, PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC, *IN-VITRO* BIOLOGICAL ACTIVITIES, AND ICP-MS ANALYSIS OF *PRUNUS DOMESTICA* L. (LEAVES)

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ABSTRACT

Several studies on *Prunus domestica* L. are reported on the fruit of this plant species. Therefore, the current study was carried out on the leaves of *P. domestica* to evaluate the nutritive value, qualitative phytochemical screening, total phenolic content, antioxidant activity, antibacterial activity, protein denaturation inhibitory activity, and elemental analysis using ICP-MS technique. The study revealed that it has a significant nutritive value and the presence of many secondary metabolites. TPC was highest in diethyl ether fraction. Antioxidant activity was also highest in diethyl ether. Four extracts have shown antibacterial activity except for petroleum ether extract. Petroleum ether, methanolic, and aqueous extracts are found to be active in the selected concentration range for protein denaturation inhibition activity.

Keywords: Phytochemical, antioxidant, antibacterial activity, PDIA, ICP-MS

INTRODUCTION

The burgeoning uses of chemicals and artificial things deteriorating the global health which adversely affecting the health of human beings, the herbs-based product

such as herbal medicines do not have any adverse effect on the planet as well as the living organisms **[1]**. It is accepted worldwide that a diet enriched with fruits and vegetables lowers the hazard of persistent sicknesses **[2]**. Vegetables, fruits, and beverages constitute an important part of the human diet and contain a significant number of secondary metabolites such as phenolics that are widely distributed in the plant kingdom. Phenolic and flavonoids act as antioxidants (cell reinforcement) are being preferred since they have a therapeutic and preventive nature **[3]**. Increased consumption of fruits has been associated with the diminished pervasiveness of degenerative sicknesses, like cardiovascular illnesses or disease, because of their cell reinforcement movement (*i.e.,* antioxidant potential) **[4]**.

P. domestica is an important medicinal plant that is broadly distributed around the world **[5]**. It is one of the members of the family *Rosaceae* and genus *Prunus* and it is commonly known as plum **[6,7]**. In India, *P. domestica* is found in Kashmir, Uttarakhand, Meghalaya, and Manipur, further extends to China, Russia, Africa, Europe, and North America **[8]**. The fruits of the *P. domestica* show a wide variation in texture, color, size and in flavor **[9]**. People like plum fruits due to their flavors, color and aromatic features **[10]**.

P. domestica is also enriched with many naturally occurring bioactive phytochemicals such as anthocyanins, pectin, carotenoids, lignans, abscisic acid, glucoside, flavonoids, flavonoid glycosides, bipyrrole, dihydroflavonols and carbohydrates. In addition to this, *P. domestica* is also the source of vitamin A, calcium, magnesium potassium and fiber **[11]**.

Due to the presence of the above natural secondary metabolite's different parts of this plant shows many biological activities like antimicrobial activity, antioxidant, anticancer, antihyperlipidemic activity, anxiolytic activity, laxative, hepatoprotective activity, larvicidal activity, repellent activity, and many other activities **[5,12]**.

Thus, considering the above medicinal and nutritional facts of *P. domestica,* the current study was planned to evaluate the nutritive value, secondary metabolites by phytochemical screening, total phenolic content, antioxidant potential, antibacterial activity, and PDIA of *P. domestica* leaves and to give the collective scientific data.

EXPERIMENTAL

Sample Collection and Authentication

The plant was identified and authenticated under accession number 374 by Botanical Survey of India (BSI), Dehradun, India.

Reagents and Chemicals

All the reagents which were used in the analysis were of analytical grade.

Physical Evaluation

The dried leaves of *P. domestica* examined to evaluate the different ash values and extractive values. The ash value included the estimation of the total ash, acid insoluble ash, water-soluble ash, and sulphated ash. In addition to this, extractive value includes the determination of the alcohol-soluble extractive value and water-soluble extractive value. All these parameters were evaluated according to the standard pharmacopeial methods **[13]**.

Proximate Analysis and Nutritive Value

The proximate analysis includes the determination of moisture content, total nitrogen content, crude protein content, crude fat, crude fiber, total carbohydrates, and available carbohydrates **[14-20]**. The total carbohydrate content and available carbohydrates and nutritive value were determined using the formulae below:

$$\textbf{Total carbohydrtes} = 100 - (\%\ of\ ash + \%\ of\ moisture + \%\ of\ crude\ fat + \%\ of\ crude\ protein)$$

$$\textbf{Available carbohydrates} = (\%\ of\ total\ carbohydrate\ content - \%\ of\ crude\ fiber)$$

$$\textbf{Nutritive value}\ (kcal\ per\ 100gms) = (4 * \%\ of\ protein) + (9 * \%\ of\ crude\ fat) + (4 * \%\ of\ carbohydrate)$$

Extraction of Plant Material

The successive Soxhlet extraction method was adopted for the extraction. Briefly, 200gms of the powdered leaves were put into the thimble of the Soxhlet extractor and extraction was carried out using 5 different solvents (PE, DEE, EA, MeOH and DDW). The extractive yield of each concentrated extract was calculated according to the following formula **[21]**:

$$\%\ yield = \frac{weight\ of\ extract}{weight\ of\ plant\ sample} * 100$$

Phytochemical Screening

Phytochemical screening of the various extracts for the presence of the different phytoconstituents was done by standard qualitative methods **[22, 23]**.

Total Phenolic Content

The folin-ciocalteau method with some alterations was used to evaluate the total phenolic content of each extract **[24]**. Gallic acid was used for the preparation of the calibration curve.

Antioxidant Activity

a) DPPH Scavenging Assay: All the extracts from the leaves were screened for antioxidant activity according to the method described by (Brand-William et *al.* 1995) with some alteration **[25]**. The percentage (%) inhibition of DPPH by extract or standard (ascorbic acid) was calculated by the following formula:

$$\%\ Inhibition = \frac{Abs\ of\ blank - Abs\ of\ sample}{Abs\ of\ blank} * 100$$

Antioxidant activity index was calculated using the following formula **[26]**:

$$\textit{Antioxidant activity index (AAI)} = \frac{\textit{Final DPPH concentration } (\mu g \textit{ per ml})}{\textit{IC50 value } (\mu g \textit{ per ml})}$$

b) FRAP Assay: Benzie and Strain method was employed for the estimation of the ferric reducing antioxidant power of the different extracts of the leaves with some modification **[27]**.

Antibacterial Activity

Agar well diffusion method was employed for antibacterial activity **[28]**. Two Gram-positive (*B. cereus* and *S. aureus*) and two Gram-negative **(***E. coli* and *S. typhi)* pathogens. The sterilized Muller Hinton Agar (MHA) was poured into Petri plates and allowed to solidify. The fresh overnight cultures having turbidity equivalent to Mac Farland standard (0.5) was swabbed uniformly on the MHA plates. After that, five wells were punctured using a cork borer. Then 200µl of each extract (100mg/ml) in DMSO was put in each well and incubated at 37℃ for 18 to 24 hours. Standard discs of commercially available antibiotics were used as standard.

Protein Denaturation Inhibition Activity (PDIA)

PDIA was performed according to Chandra et *al.* with a little modification **[29]**. Briefly, 2ml of the plant extract (in DMSO) at varying concentrations was mixed with the 2.8 ml of the phosphate buffer of pH 6.4 and left for 5min to attain the equilibrium. No significant inhibition was observed at lower concentrations. After that 0.2ml of the egg albumin fresh hen's egg was mixed and shaken vigorously. After 15min the reaction mixture was heated at 70±2°C for 5min. After cooling the turbidity of the resultant solution was measured at 660nm using Systronics (2205) UV-Vis double beam spectrophotometer. Sodium diclofenac (*Vavoran* SR 100mg tablet) was used as the standard reference drug.

$$\%\ \textit{protein denaturation inhibition} = \frac{\textit{Abs of blank} - \textit{Abs of sample}}{\textit{Abs of blank}} * 100$$

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

0.2gms of the sample was mixed with 3ml of the Conc. HNO_3 of the EMSURE grade. After that sample was digested in microwave digester for 1 hour. After cooling at room temperature each sample was diluted with distilled water upto 25 ml. After the dilution, each sample was analysed by iCAP-MS for various elements.

RESULTS AND DISCUSSION

Physical Evaluation

Table 1, shows the results of the physical evaluation of the leaves of *P. domestica*. The total ash value is found to be 12.076±0.048%. Total ash is the measure of the diagnostic purity index. Physiological and non-physiological ash,

both the ashes can be represented by total ash. Acid insoluble ash is found to be 1.453±0.025%, which is indicative of non-physiological components such as silica and silicates especially siliceous earth in drugs. Water-soluble ash and sulphated ash are found to be 4.111±0.328% and 17.195±0.743% respectively. The lignin content which is not lost by the treatment with sulphuric acid can be predicted by sulphated ash **[30]**. Water-soluble and alcohol soluble extractive values are 20.016±0.201 and 12.786±0.406 respectively.

Proximate Analysis

Results of the proximate analysis are shown in **Table 2**. Moisture content is measured to be 7.195±0.487% (acceptable limit 6% - 15%)[50]. Total Kjeldahl nitrogen is found 2.251±0.019%. The crude protein is found to be 15.756±0.117%. Crude fat and crude fiber are 5.195±0.293% and 14.537±0.532% respectively. Total carbohydrate content and available carbohydrate content are 59.778±0.161% and 45.211±0.357% respectively. Because carbohydrates are one of the major classes of naturally occurring organic compounds, they are essential to plant and animal life, as well as providing essential raw materials for a variety of industries **[31]**. The nutritive value is found to be 348.889±3.205 Kcal/100gms.

Extractive Yield

Extractive yield of the different extracts obtained shown in **Table 3**. Highest yield is obtained when water is used as solvent followed by methanol and petroleum ether respectively. Least amount of yield is found when diethyl ether was used as solvent.

Phytochemical Screening

Results of the phytochemical screening are shown in **Table 4**. It is clearly seen that there are present of many secondary occurring metabolites in the leaves of the *P. domestica.*

Total Phenolic Content

Phenolic compounds have a great contribution in health beneficial effects. Additionally, they serve in plant defence mechanisms to counteract reactive oxygen species (ROS) to save the molecular damage and harm from microorganisms, insects, and herbivores **[32]**. **Table 5** emphasizes the results for the total phenolic content of various extracts. The highest total phenolic content is found in diethyl ether extract followed by methanol, ethyl acetate, petroleum ether. While the least amount of the total phenolic content is found in aqueous extract.

Antioxidant Activity

Antioxidants have a vital role in food preservation by repressing the oxidation process and contributing to wellbeing advancement rendered by many dietary supplements, functional foods, or

nutraceuticals **[33]**. Antioxidants for the treatment of cellular rupture are getting famous since they inhibit or delay the oxidation process by stopping both the initiation and propagation of the chain oxidation reaction **[34]**.

a) DPPH Free Radical Scavenging Assay: DPPH method was first introduced in 1958 and is one of the most common methods used for the assessment of antioxidant activity **[35]**. **Table 6** shows the results of the DPPH method. The highest potent extract is diethyl ether with IC50 102.760±0.396µg/ml and antioxidant activity index 0.389±0.002. Higher the AAI value, higher will be antioxidant potential.

b) FRAP Assay: FRAP method is another important method for the assessment of antioxidant activity. In this method $[Fe^{3+}(TPTZ)_2]^{III}$ is reduced into $[Fe^{2+}(TPTZ)_2]^{II}$. The reduced form of the iron is blue. The results of the FRAP are reproducible over a wide range of concentrations **[27]**. **Table 7** shows the results of the FRAP method. Ferric reducing antioxidant power is found highest in diethyl ether 287.971±1.282 µM/ml, followed by methanol 141.990±0.612µM/ml, ethyl acetate 48.559±0.588µM/ml, petroleum ether 24.147±1.282µM/ml and aqueous 20.324±0.882µM/ml extracts respectively. Ascorbic acid was used as standard and its ferric reducing antioxidant power is measured to be 482.382±0.000µM/ml.

Antibacterial Activity

Table 8 shows the results of the antibacterial activity of different fractions of *P. domestica* leaves. The maximum activity was seen in the ethyl acetate fraction followed by aqueous extract. The ethyl acetate fraction showed the antibacterial activity against all the tested bacterial strains, the maximum activity was seen against *S. typhi* followed by *E. coli, B. cereus* and *S. aureus* respectively. The methanolic extract has activity against *S. typhi* only. The diethyl ether fraction was active against *B. cereus* and *S. aureus*.

Protein Denaturation Inhibition Activity (PDIA)

One of the well-documented causes of arthritic and inflammatory diseases is the denaturation of the tissue protein. Denaturation of the protein *in-vivo* may cause the formation of the autoantigen in many arthritic diseases **[29]**. The results of the PDIA are summarized in Table 9. Three out of five extracts have given dose-dependent responses. Highest PDIA is observed in aqueous extract (IC50 = 2175.959±1.880µg/ml) followed by petroleum ether (IC50 = 3705.693±1.430µg/ml) and methanol (IC50 = 3759.744±2.291µg/ml) respectively. While the standard diclofenac sodium has an IC50 value 93of

9.351±0.745µg/ml. Diethyl ether and ethyl acetate did not give any significant inhibition in the tested concentration range.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Results of the ICP-MS analysis are shown in the **Table 10**. A total of 12 metals were analysed for their presence. Potassium was the dominant element in all the tested metals followed by the magnesium, calcium, iron. Which shows that leaves of plum are good source of micronutrients.

Table 1: Physical evaluation of the leaves of the *P. domestica*

Parameter	Value (%)
Total ash	12.076±0.048
Acid insoluble ash	1.453±0.025
Water-soluble ash	4.111±0.328
Sulphated ash	17.195±0.743
Water-soluble extractive value	20.016±0.201
Alcohol soluble extractive value	12.786±0.406

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

Table 2: Proximate analysis of the leaves of the *P. domestica*

Parameter	Value (%)
Moisture contents or loss on drying	7.195±0.487
Total nitrogen content	2.521±0.019
Protein	15.756±0.117
Crude fat	5.195±0.293
Crude fiber	14.537±0.532
Total carbohydrate content	59.778±0.161
Available carbohydrate content	45.211±0.357
Nutritive value (Energy value)	348.889±3.205(Kcal/100gm)

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

Table 3: Extractive yield of the different extracts obtained by different solvents

Solvent/extract	Yield (%)
Petroleum ether	10.820
Diethyl ether	5.332
Ethyl acetate	7.135
Methanol	13.412
Aqueous	15.479

Table 4: Qualitative phytochemical screening of the *P. domestica* leaves 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		-	-	-	-	-
	Alkaline reagent Test		-	+	+	+	-
	Zn-HCl Test		-	-	-	-	-
Phenolic Compounds and Tannins	Lead Acetate Test		-	+	+	+	+
	Ferric Chloride Test		-	+	+	+	+
	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.

Table 5: Total phenolic content of the leaves extracts of *P. domestica*

Solvent /extract	Total phenolic content (mgGAE.gm-1dw)
Petroleum ether	32.367±1.528
Diethyl ether	170.033±0.577
Ethyl acetate	62.367±0.577
Methanol	87.700±1.732
Aqueous	42.033±1.555

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

Table 6: DPPH free radical scavenging assay of the leaves extracts of *P. domestica*

Solvent/extract	IC50 (µg/ml)	Antioxidant Activity Index (AAI)
Petroleum ether	912.851±0.789	0.044±0.000
Diethyl ether	102.760±0.396	0.389±0.002
Ethyl acetate	772.526±0.610	0.052±0.000
Methanol	126.537±0.971	0.316±0.002
Aqueous	970.835±0.705	0.041±0.000

Each experiment was performed in triplicate and results are expressed as mean ± SD of three values. All the calculations were done using the GraphPad Prism 9 software to calculate the IC50 values.

Table 7: Ferric reducing antioxidant power (FRAP) of the leaves extracts *P. domestica*

Solvent/Extract	Ferric reducing antioxidant power (µM/ml)	FRAP value (µM)
Ascorbic acid	482.382±0.000	2.000±0.000
Petroleum ether	24.147±1.282	0.244±0.001
Diethyl ether	287.971±1.282	1.254±0.005
Ethyl acetate	48.559±0.588	0.336±0.002
Methanol	141.990±0.612	0.694±0.002
Aqueous	20.324±0.882	0.231±0.006

Each experiment was performed in triplicate and results are expressed as mean ± SD of three values.

Table 8: Antibacterial activity of the leaves extracts of *P. domestica*

Extract/Standard	Zone of inhibition (in mm)			
	B. cereus	*S. aureus*	*E. coli*	*S. typhi*
Petroleum ether	---	---	---	---
Diethyl ether	7.133±0.321	9.000±0.200	---	---
Ethyl acetate	12.967±0.058	6.833±0.208	18.067±0.115	19.000±0.100
Methanol	---	---	---	11.967±0.152
Aqueous	9.033±0.451	10.067±0.208	9.833±0.289	6.900±0.100
Chloramphenicol	22.833±0.289	23.000±0.600	16.067±0.666	30.533±0.751
Gentamicin	20.500±0.500	24.667±0.577	17.833±0.289	23.733±0.461

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

Table 9: Protein denaturation inhibition activity of leaves extracts of *P. domestica*	
Extract	**IC50 (µg/ml)**
Diclofenac sodium (Standard)	**939.351±0.745**
Petroleum ether	**3705.693±1.430**
Diethyl ether	**-----**
Ethyl acetate	**-----**
Methanol	**3759.744±2.291**
Aqueous	**2175.959±1.880**

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

Table 10: ICP-MS analysis of the leaves of *P. domestica*		
S. No.	**Metal**	**Concentration(mg/kg)**
1.	**Potassium (as K)**	**21909.26**
2.	**Magnesium (as Mg)**	**5945.22**
3.	**Calcium (as Ca)**	**2496.66**
4.	**Iron (as Fe)**	**414.66**
5.	**Manganese (as Mn)**	**110.67**
6.	**Chromium (Cr)**	**3.30**
7.	**Zinc (as Zn)**	**14.30**
8.	**Nickel (as Ni)**	**1.00**
9.	**Copper (as Cu)**	**6.68**
10.	**Arsenic (as As)**	**0.55**
11.	**Lead (as Pb)**	**1.79**
12.	**Selenium (as Se)**	**0.86**

CONCLUSION

The fruit of the *P. domestica* is juicy and fleshy and is the only edible part of this plant, being the reason for most of the studies to be focused on this part. The study has revealed that the leaves of *P. domestica* have a significant nutritive value, many naturally occurring phytoconstituents and a significant amount of total phenolic content. The leaves also showed different pharmacological activities namely, antioxidant, antibacterial, and PDIA. Therefore, from the current study leaves of *P. domestica* can be suggested as the potential source of energy (nutritive value), secondary metabolites and bioactivity.

CONFLICT OF INTEREST

Authors do no have any conflict of interest.

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