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Phytochemical Screening, Antioxidant Evaluation and Estimation of Antidepressant Potential of *Prunus domestica* Leaves Extracts

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Abstract: Depression affects 20% of the population worldwide, and today's stressful life is responsible for depression. Synthetic drugs are the major mode of treatment for depression; however, these drugs have various disadvantages. To overcome these disadvantages, researchers are working on alternative or traditional herbal medicines. In the quest for a solution, the present study was conducted to evaluate the antidepressant potential of *P. domestica* leaves extract. Various plant extracts (methanol, petroleum ether, and chloroform) were prepared using the Soxhlet extraction method. Based on the phytochemical screening, the chloroform and methanol extracts were subjected to tests for antioxidant and antidepressant activity. The DPPH and H_2O_2 methods were used to evaluate the antioxidant potential, in which the methanol extract showed better antioxidant potential than the chloroform extract; the IC_{50} values for the methanol and chloroform extracts were found to be 12.94 and 73.03, respectively. The antidepressant activity was assessed in Wistar rats using the tail suspension and forced swim tests, with two doses (200 mg/kg and 400 mg/kg) of each extract. The methanol extract exhibited maximum antidepressant activity compared to the control. The study revealed that both doses show almost equal response compared to the control.

Keywords: Antidepressant, Forced swim test, Tail suspension test, *Prunus domestica*

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Introduction

Depression may be defined as a condition of a patient having a feeling of mood disturbance, despondency, disinterest, and may or may not be associated with insomnia, headache, lethargy, laziness, low libido, and loss of appetite (Mann, 2005; Tierney *et al*., 2006). Depression affects the social, economic, and personal life of the patients and their close relatives (Hansson, 2002). Worldwide, depression is the most prevalent disease (Kruijshaar *et al*., 2005). As per

estimation, it is presumed that depression will be ranked as the second leading disease by the year 2030 (Smith *et al*., 2008). At the Global level, approximately 20% people are affected by depression, and females are more prone to this disease compared to males (Kendler *et al*., 2001; Singh *et al*., 2025). Synthetic drugs are the major mode of treatment for depression; however, synthetic drugs have various disadvantages. To overcome these disadvantages, researchers are working on alternative or traditional herbal medicines. *Prunus domestica* leaves possess great therapeutic potential. The plant is cultivated in northern India, including Uttarakhand, Himachal Pradesh, Punjab, and Jammu and Kashmir (Chopra, 2009). The ripe fruit exhibits various medicinal properties, enhancing immunity and improving eyesight. The various natural compounds reported in *P. domestica* include flavonoids, proanthocyanidins, steroids, terpenes, phenylpropanoid esters, phenolic acids, coumarins, carotenoids, and carbohydrates (Poonam *et al*., 2011). The plant exhibits good antioxidant, anti-inflammatory, anti-cholinesterase, anti-tumor, antidiabetic, and anti-hyperlipidemic properties (Rho *et al*., 2007; Shin *et al*., 2010; El-Beltagi *et al*., 2019). Traditionally, local people used it for treating various diseases. *P. domestica* leaves have the potential for further research, especially their effect on the CNS. Therefore, we want to investigate the anti-depressant potential of *P. domestica* leaves using well-established animal models. As we know, solvent selection influences the extraction of chemical constituents (Thouri *et al*., 2017), therefore, in our present study, we aim to determine the optimal solvent system for extracting the chemical constituents responsible for antidepressant activity.

Materials and Methods

Plant Material:

The leaves of *Prunus domestica* were collected from Ramgarh, Nainital, India. The plant sample was identified and authenticated by NISCAIR, Delhi (voucher no. NISCAIR/RHMD/Consult/ 2021-3802-03-2).

Materials:

Imipramine was procured from Triko Pharmaceuticals, Rohtak, Haryana. DPPH, H_2O_2, Chloroform, methanol, and ethyl acetate were procured from Sigma Aldrich India.

Extracts Preparation:

The collected leaves were washed with water and dried in the shade for a few days, then a coarse powder was prepared using a grinder. The prepared powder was packed in a thimble. The plant sample was extracted using different solvents (petroleum ether; chloroform and methanol).

Antioxidant activity:

The antioxidant potential was assessed using the DPPH assay and the H_2O_2 method.

DPPH assay: The antioxidant potential is evaluated based on the free radical scavenging capacity of the extract. A 100 µg/ml methanolic DPPH solution was prepared. Rutin was used as a standard, with a 1 mg/ml methanolic rutin solution, from which various concentrations (2-12 µg/ml) were prepared. These were mixed with an equal volume of DPPH, kept in the dark for 30 min, and then the absorbance was measured at 517 nm. The same procedure was applied to the plant extract. The antioxidant potential was calculated as described by Guglani *et al*. (2022);

$$\% \text{ FRS capacity} = [A_{Co}-(A_{Test}-A_B)]/A_{Co} \times 100$$

Where A_{Co} = Absorbance of DPPH, A_{Test} = absorbance of Extract/Rutin + DPPH, A_B = absorbance of Extract/Rutin without DPPH. All the sampling was done in triplicate.

H_2O_2 method: H_2O_2 assay is based on Dehpour's method. A 40 mM H_2O_2 solution was added to pH 7.4 phosphate buffer, and aliquots (0.0625 to 2 mg/ml) of extract and ascorbic acid were prepared. H_2O_2 was added to these samples, and absorbance at 560 nm was measured

spectrophotometrically. Scavenging activity was calculated as per Bhatti *et al*. (2015):

$$\% \text{ FRS}[H_2O_2] = 1 - \frac{\text{Absorbance Control}}{\text{Absorbance Extract}} \times 100$$

Antidepressant activity:

Forty-eight Wistar rats were randomly selected and placed in a cage for acclimatization. They were given standard feed and water, which was withdrawn a day before the experiment. The study was conducted at the Department of Pharmaceutical Sciences, SJCBT Bhimtal, following the protocol (IAEC protocol no Kudops/13).

Vehicle and standard drug:

A 5% Tween 80 aqueous solution served as the vehicle for delivering imipramine, extract fraction, and isolate orally. It was prepared by dissolving these in the Tween 80 solution.

Experimental design:

Wistar rats were randomly divided into eight groups of six animals. Group 1 (Control) received vehicle (2 ml, p.o.), Group 2 (Standard) received imipramine (15 mg/kg, p.o.), Groups 3 and 4 received 200 and 400 mg/kg of chloroform extract, respectively. Groups 5 and 6 received 200 and 400 mg/kg of methanol extract of *P. domestica* leaves, respectively (Singh *et al*., 2025).

Evaluation of antidepressant activity:

Forced swim test:

When the rats were subjected to excessive tension, it goes into depression. This test involves placing the rat in a plexiglass chamber filled with water and forcing them to swim in the water. The rat is placed in water for 6 min after giving the dose, and the total swimming duration is recorded. Total swimming time refers to the period during which the rats attempt to maintain their nose above the water surface, characterized as immobility time (Richa *et al.*, 2017).

Tail suspension test:

In this test, the rats were suspended in the air with the help of a string attached to a metal pillar. The rat's tail was fixed 1 cm above the tail tip using sticky tape. The rat is maintained at a distance of 54 cm above the ground. The rat is dosed 45 min before the test starts, and then it is suspended in air for 5 min. After hanging the rat, a change in the rat's behaviour is noticed. When the rats do not struggle or hold a position, they are assumed to be immobile. Both the total immobility time and the immobility occurrences are noted (Shashikumara *et al.*, 2017).

Statistics:

A multiple comparison analysis of Variance (ANOVA) was carried out to compare the control, standard, and test samples, followed by Sidak's test. The data (n=6) were presented as mean ± standard deviation (S.D.) (McHugh, 2011).

Results and Discussion

Various extracts were prepared separately in different solvents (Petroleum ether, chloroform, and methanol) successively using the Soxhlet method. The yields were found to be 1.63, 2.21, and 5.50% for petroleum ether, chloroform, and methanol, respectively. The phytochemical screening of all extracts was performed to determine the presence of bioactive compounds (Guglani *et al.*, 2020). The study revealed that the chloroform and methanol extracts are rich in phytoconstituents such as flavonoids (Table 1), which are responsible for antidepressant activity (Pannu *et al.*, 2021). Several previously published studies have reported that a substantial number of flavonoid and phenolic compounds exhibit antidepressant properties (Butterweck *et al.*, 2000). Therefore, only the phytoconstituent-rich crude extracts (chloroform and methanol extracts) were subjected to antioxidant and antidepressant activity.

DPPH method:

In the DPPH study, the methanol extract demonstrated better antioxidant potential than the chloroform extract, with IC_{50} values of 12.94 and 73.03 for the methanol and chloroform extracts, respectively. The IC_{50} value for the

Table 1: Phytochemical Analysis of various extracts

Phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract
Saponins	-	-	+
Proteins	-	-	+
Carbohydrates	-	-	+
Anthraquinone glycosides	-	-	-
Steroids	-	+	-
Triterpenoids	-	+	-
Coumarins	-	-	-
Flavonoids	-	+	+
Tannins	-	-	+
Cardiac glycosides	-	-	-
Cyanogenetic glycosides	-	-	+
Fixed oils	+	-	-
Alkaloids	-	-	-

+ present, - absent

Table 2: Antioxidant activity of both extracts in the DPPH method

Treatment	Concentration (µg/ml)	Mean percentage inhibition ± S.D.	IC_{50} Values (µg/ml)
Rutin	2	25.45 ± 0.221	5.61
	4	39.50 ± 0.410	
	6	53.14 ± 0.365	
	8	68.29 ± 0.125	
	10	77.64 ± 0.116	
	12	89.32 ± 0.548	
Chloroform extract	5	18.50 ± 0.125	73.03
	10	23.14 ± 0.254	
	20	28.79 ± 0.412	
	40	36.87 ± 0.260	
	80	54.68 ± 0.459	
	160	85.74 ± 0.874	
Methanol extract	5	43.90 ± 0.122	12.94
	10	46.88 ± 0.325	
	20	53.90 ± 0.145	
	40	62.17 ± 0.854	
	80	75.97 ± 0.756	
	160	97.15 ± 0.874	

standard (rutin) was found to be 5.61. The results are given in Table 2.

H_2O_2 method:

In the H_2O_2 assay, the methanol extract exhibits slightly better antioxidant potential than the chloroform extract, with IC_{50} values of 1.05 and 1.32 for the methanol and chloroform extracts, respectively. The IC_{50} for standard (ascorbic) was found to be 0.86 (Table 3).

The antidepressant potential of *P. domestica* leaves was determined using the forced swim test

Table 3: Antioxidant activity of both extracts in H_2O_2 Method

Treatment	Concentration (mg/ml)	Mean percentage inhibition of H_2O_2 radical ± S.D.	IC_{50} Values (mg/ml)
Ascorbic acid	0.0625	19.17 ± 0.110	0.86
	0.125	22.25 ± 0.125	
	0.25	28.60 ± 0.214	
	0.50	38.65 ± 0.325	
	1	56.80 ± 0.148	
	2	90.14 ± 0.325	
Chloroform extract	0.0625	7.90 ± 0.456	1.32
	0.125	10.01 ± 0.528	
	0.25	16.08 ± 0.285	
	0.50	21.98 ± 0.452	
	1	40.35 ± 0.365	
	2	72.14 ± 0.459	
Methanol extract	0.0625	11.50 ± 0.521	1.05
	0.125	14.12 ± 0.125	
	0.25	20.14 ± 0.328	
	0.50	31.78 ± 0.654	
	1	47.80 ± 0.458	
	2	85.47 ± 0.663	

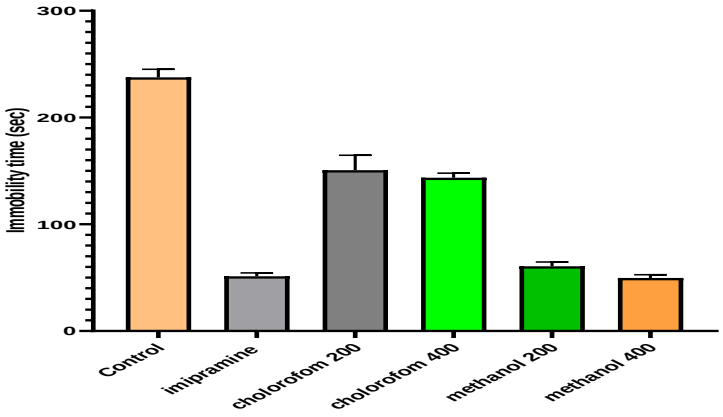

Fig. 1: Various treatments on immobility time using the tail suspension test (One-way ANOVA followed by Sidak's multiple comparisons tests, n=6, ****p<0.0001, ***p<0.001, *p<0.05.

and the tail suspension test. In forced swim test, the methanol extract exhibited maximum antidepressant activity compared to control group, a significant difference (<0.0001) was observed between control and methanol extract, the methanol extract showed a dose dependent antidepressant activity, at a dose of 400 mg/kg the methanol extract minimizes the immobility time slightly better than 200 mg/kg, the extract showed an almost equal response to the standard drug. However, chloroform extract has shown a mild antidepressant effect. The chloroform extract significantly (<0.0001) reduces the immobility time compared to the control. However, when the antidepressant activity of the chloroform extract and methanol extract was compared, the methanol extract showed better antidepressant activity, reducing the immobility time by almost three times (Fig. 1).

In the tail suspension test, the chloroform extract of both doses exhibited low antidepressant activity compared to the standard drug; however,

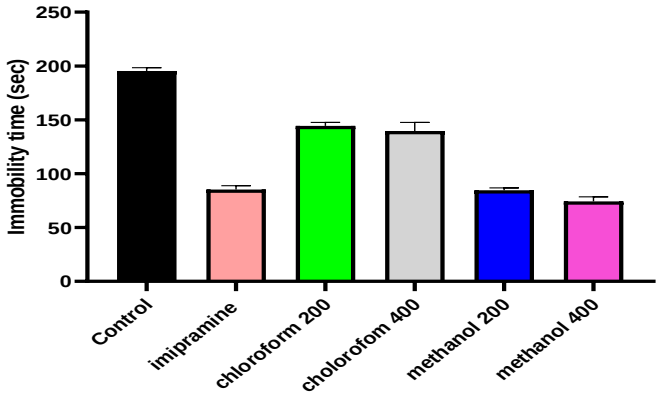

Fig. 2: Various treatments on immobility time using the tail suspension test (One-way ANOVA followed by Sidak's multiple comparisons tests, n=6, ****p<0.0001, ***p<0.001, *p<0.05.

both doses significantly ($p < 0.0001$) reduced immobility time compared to the control group. When the antidepressant effect of chloroform extract was compared to that of the methanol extract, both dosages (200 and 400 mg/kg) significantly reduced the immobility time, at 84.70±2.08 and 74.33±4.16, respectively. The methanol extract exhibited a dose-dependent reduction in immobility time, with higher doses yielding slightly better results than lower doses. The methanol extract at both doses showed a nonsignificant difference ($p>0.05$) compared to the standard drug. The high dose showed even better results than the standard (Fig. 2).

Conclusion

Earlier reports suggest that flavonoids and phenolic compounds exhibit antidepressant action. In light of our findings, it is proposed that the antidepressant action of the plant *Prunus domestica* is attributed to these bioactive groups of phytoconstituents. Ultimately, it can be proposed that it is essential to separate the primary bioactive constituents responsible for antidepressant action in future studies utilizing advanced chromatography methods. The study revealed that both doses show almost equal response compared to the control.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Pharmaceutical Sciences, SJCBT Campus Bhimtal, Kumaun University, Nainital, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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