Research Paper

Investigating Antioxidant and Antidepressant Potential of Methanolic Extract and its Ethyl Acetate Fraction of *Prunus Persica* Leaves on Rat Model

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ABSTRACT

Traditionally, *Prunus persica* has been utilized for treating mental disorders for a long time, but to date, it has not been approved as a drug due to a lack of pharmacological investigations on this plant. The current examinations have been planned to assess the phytochemical, *in-vitro* antioxidant activity, and *in-vivo* antidepressant activity of plants using well-established animal models. *P. persica* leaves were collected from Ramgarh, Uttarakhand, and the plant extract (ME) was prepared in methanol) using soxhlate apparatus. First, phytochemical screening was done to determine the main chemical constituents, which show that the extract contains carbohydrates, alkaloids, and flavonoids. Previous reports suggest that plants' flavonoid and phenolic compounds are responsible for antidepressant activity. The antidepressant activity of ME was estimated using a tail suspension and forced swim test at doses 200 and 400 mg/kg. At a dose of 400 mg/kg, ME has shown significant antidepressant activity compared to (standard) Imipramine. The plant extract shows a dose-dependent antidepressant activity. The ME was further processed to make an ethyl acetate fraction, and two doses of ethyl extract (Et 25 and Et 50) were further evaluated for their phytochemical, antioxidant, and antidepressant activity. The ethyl acetate fraction has shown dose-dependent antidepressant activity. The fraction has shown better antioxidant potential than the standard.

Keywords: *Prunus persica*, Antidepressant, Forced swim test, flavonoids, Tail suspension.

INTRODUCTION

The world is changing, and we live a fast-paced life, which is affecting our lifestyle. As a result of this, people have daily encounters with oxidants. The reactive oxidant species are the biggest cause of illness, and the ROS causes various types of disease, which include depression, hypertension, diabetes, and cancer (1, 2). As per the World Health Organization, depression is the most predominant mental issue, and it is a clinical and social issue that influences approximately 340 million individuals globally (3). The symptoms include mood change, lack of interest in work, low confidence, sleep disturbance, loss of appetite, tiredness, and lack of concentration (4). The global depression prevalence is about 20% (5), and females are approximately two times more prone to depression than males (6). Usually, the course of the illness is intermittent, and most patients who recuperate from significant depression scenes become discouraged thereafter (7). Generally, for treating depression, synthetically

manufactured tricyclic antidepressants are recommended. Synthetic antidepressants have high efficacy, but these medications also produce various undesirable effects, e.g., dry mouth, headache, mydriasis, constipation, drowsiness, restlessness, and temporary fatigue (8). Frequently, the withdrawal of antidepressant drugs results in sexual dysfunction (9, 10). Subsequently, scientists are investigating natural medicines for novel antidepressant drugs with more safety and high efficacy. Plants have been explored for developing medicaments for a long time because of the easy and suitable drug development techniques (11). As ROS is the major cause of depression, antioxidant compounds play a pivotal role in treating depression. Plants are major sources of antioxidant compounds and have now been practiced for treating depression. The flavonoids are mainly responsible for their antioxidant properties.

The plant *Prunus persica* (*P. persica*) is an individual from the Rosaceae family and a quickly developing tree found Spain in China, and India. Various natural compounds belonging to different classes, such as flavonoids, proanthocyanidins, steroids, terpenes, phenolic acids, carotenoids, gibberellins, and carbohydrates, were earlier reported by researchers in *P. persica*. Various pharmacological functions e.g. cholinesterase inhibitory, anti-inflammatory, analgesic, anti-photoaging, antioxidant, anticancer, security against skin carcinogenesis, and spasmogenic actions of *P. persica* are already known (12-18). In the traditional drug delivery system, for a long time, *P. persica* is a well-known plant for its utilization in mental disorders (19). However, to date, the antidepressant potential of *P. persica* is still awaited. Therefore, in present study we are striving to estimate the antioxidant and antidepressant potential of the methanolic extract (ME), and its ethyl acetate fraction using tail suspension (TST), and forced swim test (FTS).

MATERIALS AND METHODS

Chemicals and Reagents

Imipramine was purchased from Triko Pharmaceuticals, Rohtak, Haryana. Methanol, DPPH, $H_2O_2$was purchased from CDH, and ethyl acetate were procured from Merck, Mumbai, India. All analytical grade reagents were used in the present study.

Collection and identification and Extraction of leaves

The *P. persica* leaves were collected from Ramgarh, Uttarakhand. The Plant identification and authentication was done at NICAIR, Delhi, by Dr Sunita Garg, former Chief Scientist, the herbarium was accessioned as NISCAIR/RHMD/Consult /2021-3802-03-2, New Delhi. The collected *P. persica* leaves were dried and coarsely powdered before extraction. The extraction was done successfully using the Soxhlet apparatus, and methanol was used as the extraction solvents. The extract was dried using a rotary evaporator and stowed in a desiccator (20, 21).

Phytochemical screening

In the phytochemical screening the fundamental qualitative chemical test were performed as per the stanadrsd method (20). The term fundamental qualitative chemical test screening identifies different classes of essential and optional metabolites in crude medications. The greater part of these chemical tests is related to colored reactions that are specific to specific substances. These tests incorporate general tests for alkaloids, glycosides, proteins, triterpenoids, steroids, sugars, coumarins, tannins, and flavonoids (22).

Antioxidant activity

Following method were used for the determination of antioxidant potential *P. persica* leaves extract.

a. DPPH assay

The antioxidant potential of *P. persica* leaves extract was determined using a DPPH assay, in which the extract's Free Radical Scavenger capacity (FRS) was determined against rutin (standard). First, a DPPH stock solution (100 µg/mL) in methanol was prepared, after preparing the DPPH solution, a 100 µg/mL methanolic rutin (stock solution) was prepared, and various aliquots (2-12 µg/mL) of rutin was prepared in methanol. Then, an equal volume of aliquots was mixed with DPPH solution and placed in a dark condition for half an hr. After half an hr, the absorbance of the sample is determined at 517 nm in UV/Visible spectrophotometer using methanol as blank. The method was repeated using the ME. The FRS capacity of samples was calculated using the following equation (23, 24).

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

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

Hydrogen peroxide method

The extract's FRS capacity was determined using the Dehpour-modified method. First, A phosphate buffer of pH 7.4 was prepared, and 40 Mm H_2O_2 solution was prepared from the prepared buffer. Then different aliquots (0.0625, 0.125, 0.25, 0.50, 1 and 2 mg/ml) of the extract were mixed to the H_2O_2 solution. Similarly, ascorbic acid (standard) was processed. Then the absorbance of all solutions was estimated using UV spectrophotometer at 560 nm, and the % FRS was calculated using the given formula, the experiment was repeated in triplicate (25).

$$\% \text{ FRS } [H_2O_2] = 1 - \frac{\text{Absorbance}^{\text{Control}}}{\text{Absorbance}^{\text{Extract}}} X100$$

Antidepressant activity studies

Animals

The *in-vivo* antidepressant activity was performed at Department of Pharmaceutical Sciences, SJCBT campus Bhimtal, Kumaun University (IAEC protocol no. Kudops/137). Thirty-six Wistar rats (200-250 gm body weight) were randomly selected for the experiment. A standard diet and water were provided to the animals, which was withdrawn 18 hrs before starting the experiment.

Experimental design

The experimental design of the present investigations comprised 36 animals of either sex which were randomly grouped into six group (each containing six animal). Group I (Control) given vehicle 2 mL, p.o.), Group II (standard) given Imipramine (15 mg/kg, p.o.), group III and IV was given 200 and 400 mg/kg methanol extract respectively, whereas group V and VI was given 25 and 50 mg/kg doses of ethyl acetate fraction of methanol extract, respectively.

Vehicle Preparation

A 5% aqueous solution of tween-80 was used to administer Imipramine (standard), various test doses of methanolic extract, and its ethyl acetate fraction. All the doses standard, Control, and test samples were prepared by separately dissolving in a prepared vehicle (1.5-2.0 mL) per oral route.

Antidepressant activity Test

The antidepressant potential is determined with the help of a Tail Suspension method, and forced swim test (FST).

Tail suspension test

In this test, the rat is suspended above the surface with the help of sticky tape by sticking the tail on a string positioned in the metal pillar. The height of the string is maintained at 58 cm from the surface. The apparatus is composed of a three-walled rectangular compartment. The test animals are hung on the string for six minutes, and the behavioral changes are observed. The rats are considered immobile when they do not struggle or remain in a position. The immobility events and total immobility time are recorded (26).

Forced swim test

The forced swimming causes depression in animals, for testing the antidepressant activity rats are forcibly allowed to swim in a cylindrical vessel (150 cm X 50 cm) made up of plexiglass.

The vessel is filled with water at a height of 110 cm, and the water temperature is maintained at 25^0 C (27). The test is conducted for 6 minutes; the rat's immobility time is determined. The total swimming time, in which the rat swims in water, putting his nose above the water surface is known as immobility time, is recorded.

Statistics

In this study, ANOVA was performed, followed by Sidak's multiple comparison test. where n=6. All the data are presented as mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION

P. persica ME was successfully prepared using the Soxhlet apparatus with the help of methanol as solvent. Soxhlet extraction methodology was picked for the partition of various phytoconstituents from plant material. The percentage yield of the plant sample was found to be 6.47 % w/w.

Phytochemical Analysis

The plant's methanol extracts were analyzed for bioactive phytoconstituents utilizing specific standard reagents. The results of phytochemical analysis of *P. persica* are given in table 1.

Table 1: Phytochemical analysis of *P. persica* leaves ME

Class of phytoconstituents	Methanol extract
Carbohydrates	+
Proteins	+
Fixed oils	-
Alkaloids	-
Steroids	-
Saponins	+
Tannins	+
Triterpenoids	-
Flavonoids	+
Cardiac glycosides	-
Anthraquinone glycosides	-

Cyanogenetic glycosides	+

+: present, -: absent

The DPPH and H_2O_2 assay were performed for the estimation of antioxidant potential of *P. persica* ME. In the DPPH assay, the study shows rutin has an IC_{50} value of 5.61. However, the IC_{50} was found 17.76 for ME, the results are shown in the table 2).

Table. 2 Percentage inhibition and IC_{50} Values of extract in DPPH assay

	Concentration (µg/ml)	**% inhibition of DPPH radical ± 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	
Methanol extract	5	43.28 ± 0.159	17.76
	10	45.21 ± 0.228	
	20	52.17 ± 0.330	
	40	60.46 ± 0.458	
	80	72.88 ± 0.980	
	160	96.45 ± 0.756	
Ethyl acetate fraction	5	44.21 ± 0.228	10.48
	10	47.87 ± 0.329	
	20	54.25 ± 0.456	
	40	63.45 ± 0.256	
	80	75.87 ± 0.650	
	160	95.48 ± 0.412	

In the H_2O_2 assay, the study shows that rutin has an IC_{50} value of 0.86. However, it was found to be 1.13 and 0.99 for methanolic and its Ethyl acetate fraction, respectively. the results are given in the table 3).

Table 3. H_2O_2 Method *P. persica*

Treatment	**Concentration (mg/ml)**	**% Inhibition of H_2O_2 ± 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	
	0.0625	11.01 ± 0.204	1.13
	0.125	13.98 ± 0.321	

Methanol extract	0.25	19.87 ± 0.458	
	0.50	30.14 ± 0.650	
	1	45.97 ± 0.985	
	2	80.14 ± 0.148	
Ethyl Acetate Fraction	0.0625	14.80 ± 0.854	0.99
	0.125	18.78 ± 0.149	
	0.25	23.47 ± 0.478	
	0.50	35.87 ± 0.542	
	1	50.14 ± 0.459	
	2	85.64 ± 0.880	

Antidepressant activity studies

The antidepressant activity of methanolic extract was evaluated using tail suspension and forced swim method, and. The method named tail suspension test is a well-known animal protocol employed to investigate depression or stress in experimental animals. This test is based upon the principle that if the experimental animal is exposed to a minimum period of inescapable stress or depression then the rat will become immobile.

In FTS, the duration of immobility is determined to assess the antidepressant potential of the methanolic leaves extract [28]. The fundamental phytochemical screening show methanolic extract of *P. persica* confirmed methanol extracts contained bioactive classes of phytoconstituents. Therefore, the methanol extract was subjected to biological evaluation at two dose levels i.e. 200 or 400 mg/kg. the results of both studies are given in Tables 4 and 5 respectively.

In the tail suspension study, compared to Control, the standard drug significantly (<0.0001) reduces the immobility time. The study shows that the extract significantly reduces the immobility time compared to the Control. However, samples at different doses show similar results as a standard drug; no significant difference was found among standard, extract 200 mg, and 400 mg, the results are shown in the table 4 and figure 1).

Table 4: Effect ME, standard and control on immobility time using tail suspension test

Treatment	Dose (mg/kg)	Immobility time (sec) Mean^n ± S.D.
Control	Vehicle	201.45 ± 8.25
Standard	15	81.24 ± 6.33
Methanol extract	200	93.24 ± 9.14
	400	83.14 ± 7.39

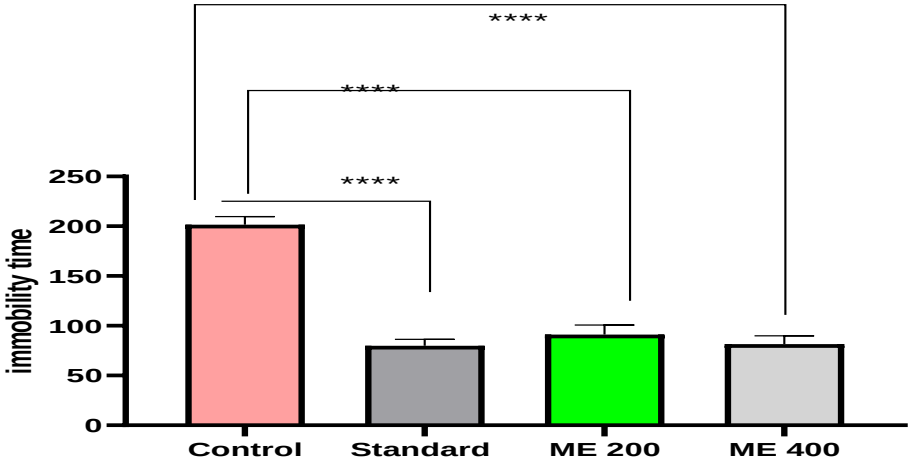

Figure (1) Effect of different treatment on immobility time using tail suspension test

(One way ANOVA followed by Sidak's multiple comparisons tests, n=6, ****p<0.0001, ***p<0.001, *p<0.05)

The study shows that the standard drug significantly (<0.0001) reduces the immobility time from 249 to 50 sec, similarly both dosage of the extract significantly (<0.0001) reduces the immobility time to 77.00 ± 5.06 and 55.16 ± 2.14 respectively. However, both dosages show similar results compared to standard drug (Imipramine). The study shows that both extracts show similar antidepressant activity, meaning the dose does not affect the antidepressant activity, the results are shown in the table 5 and figure 2).

Table 5: Effect ME, control and standard on immobility time using forced swim test

Treatment	Dose (mg/kg)	Immobility time (sec) Mean^n ± S.D.
Control	Vehicle	249.00 ± 5.55
Standard	15	50.50 ± 3.15
	200	77.00 ± 5.06
Methanol extract	400	55.16 ± 2.14

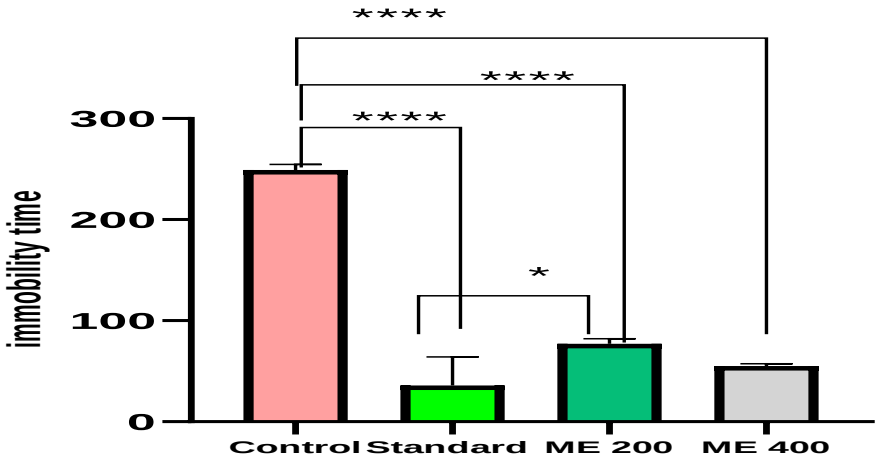

Figure (2) Effect of different treatment on immobility time using forced swim test

(One-way ANOVA followed by Sidak's multiple comparisons test, n=6, ****p<0.0001, ***p<0.001, *p<0.05)

The bioactive methanol extract of the plant was then mixed with water and stirred for 1 hr, after 1 hr. the sample was further treated with ethyl acetate and heated for 30 minutes at 50^0 C with continuous stirring. This procedure continuously repeated till the methanolic extract completely entered the ethyl acetate solvent. Then the ethyl acetate fraction was concentrated using rota evaporator, and the percentage yield of fraction was recorded. After this, we found the ethyl acetate and the remaining methanol fractions. The percentage yield of ethyl acetate fraction was found to be 27.45%. The fundamental phytochemical screening of both fractions obtained from bioactive methanol extract indicating the presence of following bioactive classes of phytoconstituents (Table 6).

Table 6: Preliminary phytochemical screening of ethyl acetate fraction and remaining methanol extract.

Class of phytoconstituents	Ethyl acetate fraction	Remaining methanol extract
Carbohydrates	-	+
Cyanogenetic glycosides	+	-
Saponins	+	-
Flavonoids	+	-
Tannins	+	-
Proteins	-	+

+: present, -: absent

The phytochemical analysis shows ethyl acetate fraction contains glycoside and flavonoids; therefore, the ethyl acetate fraction of the plant was further subjected to biological evaluation in rats at various dose levels i.e., 25 or 50 mg/kg (Figure 8-10). The ethyl acetate fraction has shown better antioxidant potential with the IC_{50} values of 10.48, and 0.99 in DPPH and H_2O_2 assay, respectively (Table 2 and 3). The ethyl acetate fraction of *P. persica* leaves exhibited maximum antidepressant activity using both models at both doses. The multiple comparison analysis shows that both extracts show similar antidepressant activity as the standard drug in a dose-dependent manner.

In tail suspension method we found that standard drug significantly reduces the immobility time (<0.0001) compared to the Control, and both concentration of ethyl acetate significantly reduces the immobility time compared to the standard. The study shows ethyl acetate fraction at both dosage (25 and 50 mg/kg) have similar results compared to the standard. Both the dosage is not significantly different to each other, the results are shown in the table 7 and figure 3).

Table 9: Effect of Ethyl acetate fraction, Control and Standard on Immobility time using Tail suspension test.

Treatment	Dose (mg/kg)	Immobility time (sec) Mean^n ± S.D.
Control	Vehicle	201.45 ± 8.25
Standard	15	81.24 ± 6.33
Ethyl acetate fraction	25	95.24 ± 4.58
	50	84.46 ± 3.64

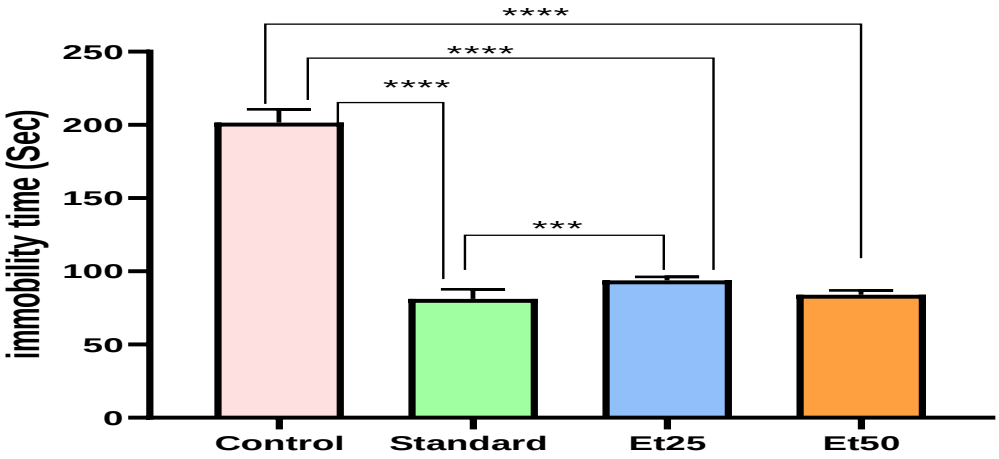

Figure (3) Effect of different treatment on Immobility time using Tail suspension test

(One-way ANOVA followed by Sidak's multiple comparisons test, n=6, ****p<0.0001, ***p<0.001, *p<0.05)

In the FTS study, we found very impressive results, and the study shows that compared to Control, the standard drug shows a significant (<0.0001) reduction in immobility time. Standard drug reduces immobility time five-fold than Control. The ethyl acetate fractions (Et 25 and Et 50) show similar results to the standard, and the multiple comparison studies show that both dosages significantly (<0.0001) reduce the immobility time in a dose-dependent manner compared to the Control. The higher dose of ethyl acetate (Et 50) shows similar results to the standard drug, the results are shown in the table 8 and figure 4).

Table 8: Effect Ethyl acetate fraction, Control, Standard on immobility time using forced swim test.

Treatment	Dose (mg/kg)	Immobility time (sec) Mean^n ± S.D.
Control	Vehicle	249.00 ± 5.55
Standard	15	50.50 ± 3.15
Ethyl acetate fraction	25	65.50 ± 3.39
	50	49.17 ± 2.31

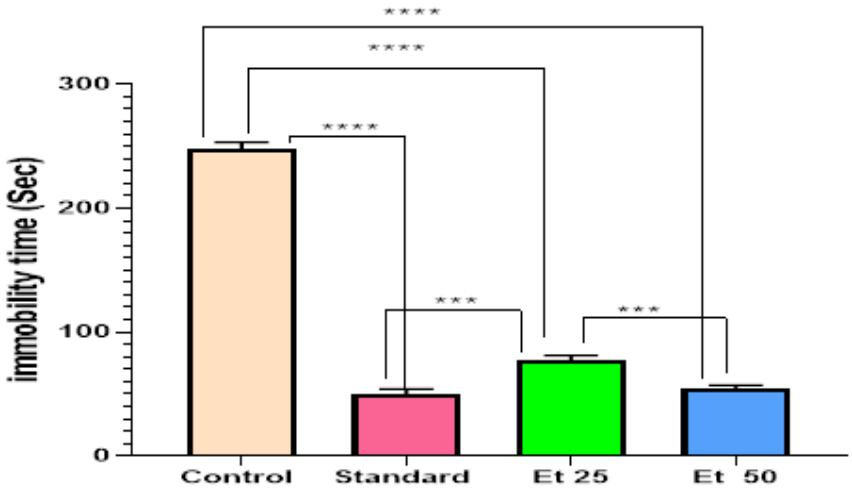

Figure (4) Effect of different treatment on Immobility time using forced swim test

(One-way ANOVA followed by Sidak's multiple comparisons test, n=6, ****p<0.0001, ***p<0.001, *p<0.05)

CONCLUSION

Various previous studies shows that flavonoids and phenolic compounds are responsble for antidepressant activity (27, 29). The phytochemical investigation shows presence of flavonoids and phenolic compounds in the *P. presica* leaves extracts. The study shows the leaves extract shows a significant antidepressant actvity on rats. The leaves extract show similar antidepressant potential to the imipramine drug. Similarly the ethy acetate fraction show better antidepressant activity. Finally, from our study we can conclude that the *P. presica* methanolic extract show good antidepressant effect and its ethyl acetate fraction show even better results compared to the methanolic extract. From our study we can conclude that both methanolic etract and its ethyl acetate fraction show good antidepressant activitity, and further study can be performed to find out which chemical constituents are mainly responsible for its anitdepressant activity.

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