RESEARCH PAPER

In vivo investigations of antidepressant profile of *Moringa oleifera* seeds using well established experimental models

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Abstract

The petroleum ether (60–80°C) extract, chloroform, methanol, and water extracts of *Moringa oliefra* seeds were prepared using the repetitive and sequential Soxhlet extraction procedure. Only the chloroform and methanol extracts included more bioactive classes of phytoconstituents, according to the results obtained by preliminary phytochemical study on many crude extracts. Thus, only extracts of chloroform and methanol were tested using well-established models for the antidepressant profile of plant seeds. The only test ingredient among the many extracts of *Moringa oliefra* seeds that exhibits the strongest significant antidepressant action at a higher dose level of 400 mg/kg is the methanol extract, which is equivalent to the commercially available medication imipramine. For additional purification, the most active biologically methanol extract under current investigations was successively fractionated utilizing solvents in ascending order of increasing polarity, namely n-hexnae, ethyl acetate, and 1-butanol. Only the ethyl acetate fraction, which is the test component, has the strongest significant antidepressant impact among the other fractions of Moringa oliefra seeds at a higher dose level of 50 mg/kg, which is comparable to the commercially available medication imipramine. According to scientific reports, the naturally occurring dietary phenolic compound can be used to treat a number of illnesses, including oxidative stress, microbial infections, liver disorders, heart disorders, inflammatory problems, fever, mental health issues, obesity, viral infections, and hypertension. According to the aforementioned findings, flavonoids and phenols are thought to be the main bioactive components of plant matter. Flavonoids and phenolic chemicals are responsible for the CNS actions of plant material.

Keywords: *Moringa oleifera* seeds, antidepressant, imipramine, FST, TST, OFT

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INTRODUCTION

A review of relevant literature indicates insufficient knowledge on the central nervous system effects of Moringa oleifera Lam. Moringa has significant neuroprotectant effects. Cerebral ischemia results from a disruption of cerebral blood flow, resulting in lipid peroxidation and reperfusion. During reperfusion, reactive oxygen species are produced. Because of its antioxidant effects, Moringa is able to provide protection to the neural tissue through decreasing reactive oxygen species (Baker et al., 1998; Kirisattayakul et al., 2013). Research on the neuroprotective effects of M. oleifera is at its beginning. The evidence from experimental model of dementia-induced rats (intracerebroventricular bilateral infusion of a cholinotoxin), (Sutalangka et al. 2013) found that an aqueous M. oleifera leaf extract is a effective cognitive enhancer and neuroprotector. An extract dose between 100 and 400 mg/kg were administered for seven days. It was found that the extract increased antioxidant enzymes catalase and superoxide dismutase as well as brain levels of lipid peroxidation. Active ingredients of the extract were not identified. Multiple phytochemicals, such as polyphenols and flavonoids, are abundant in the M. oleifera leaves, seeds, flowers, pods, and stems (Mbikay, 2012). According to Behl et al. (2022), polyphenolic components are responsible for the control of mental health like behavior, brain plasticity, cognition, depression, and mood.

There is however, no pharmacological research in the existing literature evaluating the antidepressant properties of *Moringa oleifera* Lam. seeds, so it is worth to investigate the antidepressant activity of *Moringa oleifera* Lam. seeds.

MATERIALS AND METHODS

Plant collection and authentication

In December 2024, *Moringa oleifera* seeds were gathered at the Nawanshahr local market in Punjab, India. With reference number 0275, dated 08/01/2025, Dr. K. Madhava Chetty, Assistant Professor in the Department of Botany at Sri Venkateswara University in Tirupati, Andhra Pradesh, India, recognized the plant material.

Preparation of various extracts

The different un-characterized extracts and fractions of plant materials were prepared as per standard pattern of scientific literature using the Soxhlet and reflux devices (Kumar and Kumar, 2015). To identify the various groups of phytoconstituents present, phytochemical screening was performed on all plant seed extracts, including petroleum ether extract (PEE), chloroform extract

(CE), methanol extract (ME), and water extract (WE) (Farnsworth, 1966).

Antidepressant activity profile of plant seeds

Animals

For the current studies, laca mice (either sex) weighing 20–25 g were acquired from the Bilwal Medchem and Research Laboratory Pvt. Ltd., Jaipur, Rajasthan, India. All the animals were given a standard laboratory diet pellets and had access to unlimited drinking water. All the experiments were performed in accordance with the approval from the Institutional Animal Ethics Committee (IAEC) of Bilwal Medchem and Research Laboratory Pvt. Ltd., Jaipur, Rajasthan. It is to be noted that IAEC has properly sanctioned all the experiments under Reg. No. 2304/PO/Rc/S/2024/CPCSEA (Protocol no. BMRL/IAEC/2025-18; dated 1/02/2025). The animals were gradually exposed to laboratory condition for an hour daily seven days prior to the start of the experiment. All the experiments were carried out between 9 AM to 12 PM according to the protocols laid down by the Committee for the Purpose of Control & Supervision on tests on Animals. Six animals were used in each group of experiment. The animals were starved throughout the night before being employed.

Vehicle and standard drug

The use of distilled water containing 2% Tween 80 was used in order to make various test dosages of the plant seed extract, fractions and isolated components. Imipramine was utilized as a standard antidepressant drug.

Experimental design for the assessment of antidepressant activity

There were two experimental protocols created, with six animals in each group. Antidepressant activity was examined using three animal models: the open field test, the tail suspension test, and the forced swimming test. Following an appropriate washing interval, each experimental procedure is repeated on different extracts, fractions, or isolates of different plant seeds. In a similar manner, following an appropriate washing interval, each experimental process is repeated for a separate experimental model.

The objective of experimental procedure I, which consist six Groups, was to define the antidepressant properties of various plant seed crude extracts.

Group 1 The control group was received with the vehicle (0.25 ml, p.o.).

Group 2: Imipramine (15 mg/kg, i.p.) was treated to the standard group.

Group 3 and 4: The test groups were dosed with CE of 200 and 400 mg/kg, respectively.

Group 5 and 6: The test groups were dosed with ME of 200 and 400 mg/kg, respectively.

The objective of Experimental Protocol II, which consist six groups, was to define the antidepressant effect of various fractions derived from plant seed methanol extract.

Group 1: The control group was received with the vehicle (0.25 ml, p.o.).

Group 2: Imipramine (15 mg/kg, i.p.) was treated to the standard group.

Groups 3 and 4: The test groups were dosed with EAF of 25 and 50 mg/kg, respectively.

Groups 5 and 6: The test groups were dosed with BF of 25 and 50 mg/kg, respectively.

Antidepressant animal models

Well-known animal models for antidepressants, including the forced swim test (FST), open field test (OFT) (Randhawa et al., 2014), and tail suspension test (TST) (Shashikumara et al., 2017), were used to compile the previous studies. The process for each model is detailed in Figure 1.

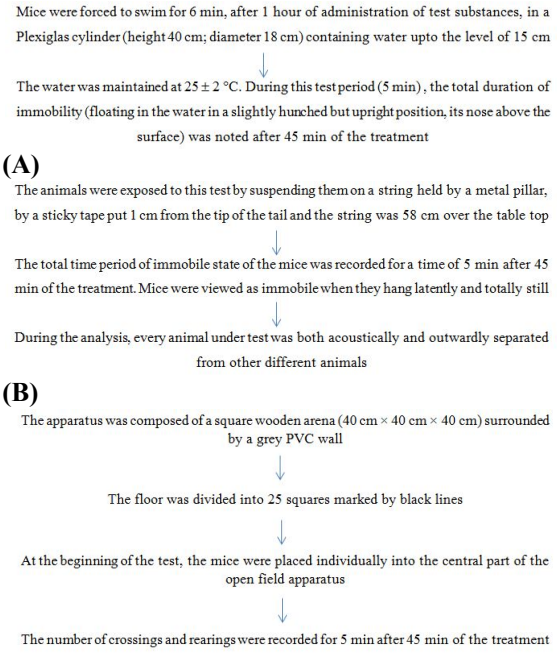

Figure 1: Animal model procedure (A) Forced swim test; (B) Tail suspension test; (C) Open field test.

Statistics

Results are expressed as mean ± standard deviation (SD). The test medications were compared with the standard drug and control using Student-Newman-Keul's test and one-way analysis of variance (ANOVA) (Scheffer, 1980).

RESULTS AND DISCUSSION

Percentage yield of various extracts of *Moringa oliefra* seeds

To create petroleum ether (60–80°C) extract, chloroform, methanol, and water extracts, different extracts of Moringa oliefra seeds were extracted using the repetitive and sequential Soxhlet extraction procedure. The Soxhlet extraction technique was chosen for this study because it has several significant advantages, including: the sample to be extracted comes into contact with the fresh solvent after each siphoning, which helps to

consistently extract phytoconstituents from the sample; the system temperature stays relatively high because some of the heat from the distillation flask reaches the extraction cavity; The entire extraction process doesn't require filtration; multiple Soxhlet assemblies are connected in series to boost sample throughput; the assembly setup is low-cost; the straightforward methodology, which requires minimal specialized training, has the potential to extract more sample mass than the majority of the most recent techniques (microwave extraction, supercritical fluids, etc.) (Luque de Castrohe and Garcia-Ayuso, 1998). The percentage (w/w) yields of many extracts, including petroleum ether, chloroform, methanol and water were found to be 2.40, 5.46, 15.30 and 8.70 w/w respectively.

Preliminary phytochemical screening of various extracts of *Moringa oliefra* seeds

The measurement of several kinds of primary and secondary metabolites found in crude medicines is associated with the phrase preliminary phytochemical screening. The majority of these chemical tests are substance-specific color responses. Alkaloids, glycosides, proteins, triterpenoids, steroids, carbohydrates, coumarins, tannins, flavonoids, and other substances are all tested for in these tests (Kumar and Kumar, 2015).

Lipids were found in petroleum ether extract, alkaloids and terpenoids in chloroform extract, anthraquinone glycoside, cardiac glycosides, flavonoids, saponins, tannins, amino acids in methanol extract, and carbohydrates and proteins in water extract, according to the preliminary phytochemical screening. Only the chloroform and methanol extracts included more bioactive classes of phytoconstituents, according to the findings of an initial phytochemical screening of several crude extracts. Thus, only extracts of chloroform and methanol were tested using well-established models for the antidepressant profile of plant seeds.

Antidepressant Activity of *Moringa oliefra* seeds

The seeds of Moringa oliefra, a plant with a long history of use and potential for medical use, are found all over India. In the early days of Ayurveda, these plants were used as herbal remedies to treat a variety of illnesses linked to mental sadness. These plants have long been used in traditional medicine, although their antidepressant properties have not yet been proven. Therefore, it was thought to be worthwhile to look into the antidepressant properties of Moringa oliefra seeds.

The forced swim test, tail suspension test, and open field test are three well-established experimental models that were used to evaluate the antidepressant efficacy of Moringa oliefra seeds. Mice are subjected to vigorous swimming in a confined space from which they are unable to flee during these examinations. Mice initially try to get out of the small area and maintain their adaptable state. Mice adapt to the characteristic behavior of immobility and stability after a few moments. As a measure of the test drugs' antidepressant activity, the duration of immobility is noted (Weiss et al., 1981). The open field test was employed in the current investigations because to its ease of use, affordability, lack of animal training, and environmental friendliness. This test involves exposing animals to their external surroundings, evaluating behavioral changes, and primarily examining locomotor deficits (Haramoto et al., 2008).

The forced swim test, tail suspension test, and open field test were used to assess the antidepressant effects of Moringa oliefra seed extracts in methanol and chloroform on mice. The mean immobility time of the experimental animals following oral dosages of test substances 200 or 400 mg/kg, the commercially available medication imipramine (15 mg/kg, p.o.), and vehicle, p.o., is displayed in Tables 1-3.

The only test ingredient among the many extracts of Moringa oliefra seeds that exhibits the strongest significant antidepressant action at a higher dose level of 400 mg/kg is the methanol extract, which is equivalent to the commercially available medication imipramine. It was discovered that chloroform extract, a medium polar solvent, had a modest antidepressant effect. Although it was not comparable to a normal medication, chloroform extract, a medium polar test material, considerably decreased the mean amount of time mice spent immobile at doses of 200 and 400 mg/kg when compared to the control. The most bioactive plant seed extract, according to these findings and discussion, is a polar extract known as methanol extract. Additionally, there was no indication of the locomotor profile of mice in OFT in the polar extract; this suggests that the psychostimulant effect was not linked to the decrease in immobility induced by the polar test substance, methanol extract.

For additional purification, the most bioactive methanol extract was successively fractionated utilizing solvents in ascending order of increasing polarity, namely n-hexnae, ethyl acetate, and 1-butanol. Using recognized animal models, the in vivo antidepressant efficacy of all fractions and the residual bioactive extract was screened.

Table 1: Antidepressant profile of crude extracts of *Moringa oliefra* seeds in mice using Forced swim test.

Sample	Dose (mg/kg)	Mean value of immobility time (sec)^{n} ± S.D.
Control	Vehicle	215.40 ± 14.10^{a}
Imipramine	15	40.74 ± 4.87*
Chloroform	200	170.56 ± 15.48^{*a}

extract	400	150.48 ± 10.45*a
Methanol extract	200	65.26 ± 6.70*a
	400	43.87 ± 4.10*

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6.

Table 2: Antidepressant profile of crude extracts of *Moringa oliefra* seeds in mice using Tail Suspension test.

Sample	Dose (mg/kg)	Mean value of immobility time (sec)n ± S.D.
Control	Vehicle	195.12 ± 9.63a
Imipramine	15	45.10 ± 3.45*
Chloroform extract	200	160.45 ± 10.56*a
	400	143.14 ± 7.39*a
Methanol extract	200	70.36 ± 5.46*a
	400	51.23 ± 4.80*

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6.

Table 3: Spontaneous locomotor activity of crude extracts of *Moringa oliefra* seeds in mice using Open field test.

Sample	Dose (mg/kg)	Mean number of crossings ± S.D.
Control	Vehicle	55.12 ± 3.50
Imipramine	15	52.50 ± 2.75
Chloroform extract	200	54.23 ± 3.15
	400	51.48 ± 4.10
Methanol extract	200	49.70 ± 3.48
	400	49.90 ± 3.78

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6.

Percentage yields of various fractions of bioactive methanol extract of *Moringa oliefra* seeds

The percentage yields of various fractions including ethyl acetate, 1-butanol, n-hexane and the remaining bioactive extract were determined to be 25.19, 16.35, 1.20, and 53.20% w/w, respectively with the respect to methanol extracts.

Phytochemical screening of various fractions of bioactive methanol extract of *Moringa oliefra* seeds

The percentage yields of various fractions including ethyl acetate, 1-butanol, n-hexane and the remaining bioactive extract were determined to be 25.19, 16.35, 1.20, and 53.20% w/w, respectively with the respect to methanol extracts.

In vivo antidepressant activity of various fractions of methanol extract of *Moringa oliefra* seeds

Using the forced swim test, tail suspension test, and open field test, the ethyl acetate fraction and 1-butanol fraction derived from the bioactive methanol extract of Moringa oliefra seeds were tested for antidepressant efficacy in mice. The mean immobility time of the experimental animals following oral doses of test medications of 25 or 50 mg/kg, marketed drug imipramine (15 mg/kg, p.o.), and vehicle, p.o., is displayed in Table 4-6.

Only the ethyl acetate fraction, which is the test component, has the strongest significant antidepressant impact among the other fractions of Moringa oliefra seeds at a higher dose level of 50 mg/kg, which is comparable to the commercially available medication imipramine. 1. It was discovered that the butanol fraction had a slight antidepressant effect. 1. At doses of 25 and 50 mg/kg, the butanol fraction test material considerably decreased the mean amount of time mice spent immobile in comparison to the control, but it was not comparable to a normal medication. The ethyl acetate fraction of plant seeds is the most bioactive fraction, according to these findings and discussions. Additionally, the ethyl acetate fraction did not exhibit any indication of the locomotor profile of mice in OFT, indicating that there was no correlation between the psychostimulant effect and the decrease in immobility caused by the ethyl acetate fraction (Table 4-6).

Table 4: Antidepressant profile of various fractions obtained from bioactive methanol extract of *Moringa oliefra* seeds in mice using Forced swim test.

Sample	Dose (mg/kg)	Mean value of immobility time (sec)n ± S.D.
Control	Vehicle	215.40 ± 14.10a
Imipramine	15	40.74 ± 4.87*
Ethyl acetate fraction	25	55.12 ± 5.40*a
	50	42.40 ± 4.23*
1-Butanol fraction	25	130.25 ± 10.14*a
	50	115.70 ± 9.70*a

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6

Table 5: Antidepressant profile of various fractions obtained from bioactive methanol extract of *Moringa oliefra* seeds in mice using Tail Suspension test.

Sample	Dose (mg/kg)	Mean value of immobility time (sec)[n] ± S.D.
Control	Vehicle	195.12 ± 9.63[a]
Imipramine	15	45.10 ± 3.45[*]
Ethyl acetate fraction	25 50	60.12 ± 4.50[*a] 48.79 ± 4.23[*]
1-Butanol fraction	25 50	125.10 ± 7.87[*a] 108.78 ± 8.14[*a]

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6.

Table 6: Spontaneous locomotor activity of various fractions obtained from bioactive methanol extract of *Moringa oliefra* seeds in mice using Open field test.

Sample	Dose (mg/kg)	Mean number of crossings ± S.D.
Control	Vehicle	55.12 ± 3.50
Imipramine	15	52.50 ± 2.75
Ethyl acetate fraction	25 50	49.70 ± 4.25 50.23 ± 3.56
1-Butanol fraction	25 50	53.14 ± 4.32 54.40 ± 3.47

*P<0.05 vs control; and P<0.05 vs standard substance by the Student-Newman-Keul's test and one-way ANOVA test; n=6.

CONCLUSION

According to scientific reports, the naturally occurring dietary phenolic compound can be used to treat a number of illnesses, including oxidative stress, microbial infections, liver disorders, heart disorders, inflammatory problems, fever, mental health issues, obesity, viral infections, and hypertension. According to the aforementioned findings, flavonoids and phenols are thought to be the main bioactive components of plant matter. Flavonoids and phenolic chemicals are responsible for the CNS actions of plant material.

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