RESEARCH PAPER

Antidepressant-Like, Antioxidant, and Phytochemical Evaluation of Ethanolic Leaf Extract of *Mirabilis jalapa* in Wistar Albino Rats

Vinay Kumar[1]*, Pankaj Kumar Shankhdhar[1], Pushpendra Kannojia[1]

1. BIU College of Pharmacy, Bareilly International University, Rohilkhand Medical College and Hospital Campus, Pilibhit Bypass road, Bareilly- 243006, Uttar Pradesh, India

Corresponding Author:
Name: Vinay Kumar
Email: vinayrajpoot5533@gmail.com
Phone No.: 6397781202

Abstract

Background: Depression is a major neuropsychiatric disorder, and medicinal plants may provide useful sources of antidepressant lead compounds. This study evaluated the phytochemical composition, antioxidant potential, and antidepressant-like activity of the ethanolic leaf extract of Mirabilis jalapa L. in Wistar albino rats.

Methods: Leaves were successively extracted with petroleum ether and ethanol by Soxhlet extraction. Preliminary phytochemical screening, thin-layer chromatography, total flavonoid content, and total phenolic content were determined. Antidepressant activity was assessed using the Open Field Test, Tail Suspension Test, and Spontaneous Locomotor Activity test. Rats received normal saline, imipramine 15 mg/kg, or ethanolic extract at 200 or 400 mg/kg orally. Data were analysed using one-way ANOVA followed by Dunnett's post-hoc test; $p < 0.05$ was considered significant.

Results: The ethanolic extract yielded 6.11% w/w, higher than the petroleum ether extract (3.76% w/w). Phytochemical screening detected alkaloids, flavonoids, phenols, tannins, terpenoids, carbohydrates, and saponins. Total flavonoid and phenolic contents were 51.1 µg/mL and 31.3 µg/mL, respectively. In the Open Field Test, 400 mg/kg extract significantly increased rearing, rearing duration, distance travelled, and center entries. In the Tail Suspension Test, both doses reduced immobility time in acute and chronic models, with greater activity at 400 mg/kg. Spontaneous locomotor activity was reduced dose-dependently, indicating CNS depressant/sedative activity.

Conclusion: The ethanolic leaf extract of M. jalapa showed notable antioxidant phytochemical content and dose-dependent antidepressant-like activity in rats. Its effects may be associated with flavonoids, phenolics, alkaloids, and terpenoids, possibly through monoaminergic and antioxidant mechanisms. Further isolation, neurochemical, toxicity, and clinical studies are warranted.

Keywords: Mirabilis Jalapa; antidepressant activity; tail suspension test; open field test; flavonoids; phenolic compounds.

How to cite this article: Vinay Kumar1* Pankaj Kumar Shankhdhar1 Pushpendra Kannojia1. Antidepressant-Like, Antioxidant, and Phytochemical Evaluation of Ethanolic Leaf Extract of Mirabilis jalapa in Wistar Albino Rats. Int J Drug Deliv Technol. 2026;16(89s):968-973. DOI: 10.25258/ijddt.16.89s.101.

Introduction

Depression is one of the most common and disabling neuropsychiatric disorders worldwide. It is characterized by persistent sadness, loss of interest or pleasure in daily activities, fatigue, sleep disturbance, poor concentration, feelings of worthlessness, and, in severe cases, recurrent thoughts of death or suicide. The disorder affects emotional, cognitive, behavioral, and physical functioning, thereby reducing quality of life and increasing the risk of disability and suicide. Depression may occur as a single depressive episode or as recurrent depressive disorder and may coexist with anxiety disorders, chronic medical illnesses, and substance-use disorders [1].

According to the World Health Organization, depression is a leading cause of disability worldwide [1]. Although several pharmacological treatments are available, including selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors, a substantial proportion of patients do not achieve adequate response. Common limitations of conventional antidepressants include delayed onset of therapeutic action, sexual dysfunction, weight gain, gastrointestinal disturbances, sedation, and discontinuation symptoms. Treatment-resistant depression also remains an important clinical challenge [3]. These limitations have encouraged continued research into safer, more effective, and more affordable therapeutic alternatives.

The pathophysiology of depression is complex and involves genetic, neurobiological, psychological, and environmental factors. The classical monoamine hypothesis proposes that

depression is associated with reduced serotonergic, noradrenergic, and dopaminergic neurotransmission [2]. However, contemporary research also emphasizes hypothalamic–pituitary–adrenal axis dysregulation, neuroinflammation, altered neuroplasticity, impaired hippocampal neurogenesis, mitochondrial dysfunction, and oxidative stress. Increased reactive oxygen species production and reduced antioxidant defence may contribute to neuronal damage and disturbed neurotransmitter signalling [2,4]. Therefore, natural products with antioxidant and neuroprotective properties may have potential value in the management of depressive disorders.

Herbal medicine, also known as botanical medicine or phytomedicine, refers to the therapeutic use of plant parts such as leaves, roots, seeds, bark, flowers, and fruits. Herbal medicines have been used for more than 2,000 years and remain an important component of primary healthcare for a large proportion of the world's population [5]. In India, traditional systems such as Ayurveda, Siddha, and Unani rely extensively on plant-based remedies. Medicinal plants contain diverse secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides. Many of these phytoconstituents possess antioxidant, anti-inflammatory, neuroprotective, anxiolytic, and antidepressant-like properties [6,7].

Several medicinal plants have been investigated for potential antidepressant effects. Hypericum perforatum (St. John's wort), Withania somnifera (Ashwagandha), Bacopa monnieri (Brahmi), Crocus sativus (saffron), Rhodiola rosea, and Valeriana officinalis are commonly used in traditional practice for mood support, stress reduction, and relaxation [7–9]. Experimental and clinical studies suggest that plant-derived compounds may modulate monoaminergic neurotransmission, influence GABAergic activity, reduce inflammation, and protect neurons from oxidative injury. These findings support the scientific evaluation of medicinal plants as potential sources of new antidepressant lead molecules [7–9].

Mirabilis jalapa Linn., commonly known as the four o'clock plant or Marvel of Peru, is a medicinal plant belonging to the family Nyctaginaceae. It is widely distributed in tropical and subtropical regions and is known by vernacular names including Gulabbas in Hindi, Krishnakeli in Sanskrit, and Sandhyamalati in Bengali. Different parts of the plant, particularly leaves, roots, seeds, and flowers, have been used in folk medicine for wounds, boils, skin infections, abdominal disorders, diarrhoea, fever, inflammation, and pain. Traditional use also suggests calming and sedative effects, indicating possible activity on the central nervous system [10–12].

Phytochemical investigations have shown that M. jalapa contains alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, saponins, proteins, carbohydrates, and steroids. The plant also contains bioactive proteins, including ribosome-inactivating proteins, associated with antiviral and cytotoxic activities [10,13]. Previous pharmacological studies have reported antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antinociceptive, antidiabetic, wound-healing, and hepatoprotective effects of different extracts of M. jalapa [10,13–15]. The presence of flavonoids and phenolic compounds is particularly relevant because these constituents may reduce oxidative stress and modulate neurotransmitter systems involved in depression.

Although several pharmacological activities of M. jalapa have been reported, its antidepressant potential has not been extensively studied. In particular, there is limited scientific evidence regarding the effect of its leaf extract on behavioural despair, exploratory activity, anxiety-like behaviour, and spontaneous locomotion in experimental animals. Therefore, the present study was designed to evaluate the phytochemical composition, antioxidant potential, and antidepressant-like activity of the ethanolic leaf extract of M. jalapa in Wistar albino rats. The Open Field Test was used to assess exploratory and anxiety-related behaviour, the Tail Suspension Test was used to evaluate behavioural despair, and the Spontaneous Locomotor Activity test was used to determine whether the observed behavioural effects were associated with general CNS stimulation or sedation.

2. Materials and Methods

2.1 Collection and authentication of plant material

Fresh leaves of Mirabilis jalapa L. were collected from Bareilly, Uttar Pradesh, India. The plant was identified and authenticated by comparison with standard herbarium specimens and botanical references at the Department of Botanical Science, M.J.P. Rohilkhand University, Bareilly [16]. The authenticated specimen was preserved for future reference.

2.2 Preparation of plant extract

Shade-dried leaves were powdered mechanically. Approximately 100 g of air-dried, finely powdered plant material was placed in a 500 mL Soxhlet apparatus for successive solvent extraction. The material was first extracted with petroleum ether for about 18

h to remove non-polar constituents such as fats, waxes, and lipophilic compounds. After complete drying, the remaining marc was extracted with ethanol for approximately 72 h to obtain polar and semi-polar phytoconstituents [16].

The petroleum ether and ethanolic extracts were concentrated under reduced pressure using a rotary evaporator. The concentrated extracts were dried, weighed, and stored in airtight containers at room temperature until further analysis. The percentage yield was calculated using the following formula:

Percentage yield = (Weight of dried extract / Weight of plant powder) × 100.

2.3 Preliminary phytochemical screening

Preliminary qualitative phytochemical screening of the petroleum ether and ethanolic extracts was performed using standard chemical tests. Carbohydrates were detected by Molisch, Fehling's, Benedict's, and Barfoed's tests. Alkaloids were detected using Dragendorff's, Wagner's, Mayer's, and Hager's reagents. Flavonoids were identified by the Shinoda, lead acetate, and alkaline reagent tests. Saponins were detected by the foam test, while phenols and tannins were detected using standard precipitation and colour reactions [17–19].

2.4 Thin-layer chromatography

Thin-layer chromatography (TLC) was performed on silica gel G plates. TLC plates were prepared using silica gel G suspended in distilled water, applied to a uniform thickness of 0.25 mm, dried, and activated at 110°C for 30 min. Diluted extracts were spotted 2 cm above the lower edge of the plate using a capillary tube. The plates were developed in suitable mobile phases, and Rf values were calculated as the ratio of the distance travelled by the solute to the distance travelled by the solvent front [20,21].

Rf = Distance travelled by solute / Distance travelled by solvent.

2.5 Estimation of total flavonoid content

Total flavonoid content was determined by the aluminium chloride colorimetric method with minor modification [22]. Briefly, 1 mL of extract solution (1 mg/mL) was mixed with distilled water to make 5 mL. Sodium nitrite solution (5% w/v, 0.3 mL) was added, followed after 5 min by 0.6 mL of 10% aluminium chloride solution. After 6 min, 2 mL of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm. Quercetin was used as the reference standard, and results were expressed as quercetin equivalents.

2.6 Estimation of total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu method [23]. Briefly, 1 mL of extract solution (1 mg/mL) was mixed with 0.5 mL of Folin–Ciocalteu reagent and 1.5 mL of sodium carbonate solution. The mixture was incubated for 30 min, and absorbance was measured at 750 nm. Gallic acid was used to prepare the calibration curve, and total phenolic content was expressed as gallic acid equivalents (GAE).

2.7 Experimental animals

Adult Wistar albino rats of either sex, weighing 175–200 g and aged 9–12 weeks, were used for the pharmacological evaluation. Animals were housed under standard laboratory conditions at 22–30°C with a 12 h light/dark cycle. They were provided with standard pellet diet and distilled water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [24,25].

2.8 Experimental design

Animals were randomly divided into four groups (n = 5 per group). The normal-control group received normal saline (10 mL/kg, p.o.). The positive-control group received imipramine (15 mg/kg). Treatment Group I received ethanolic M. jalapa extract at 200 mg/kg, p.o., and Treatment Group II received ethanolic M. jalapa extract at 400 mg/kg, p.o. The extract was administered once daily for seven consecutive days [16].

2.9 Open Field Test

The Open Field Test was used to evaluate locomotor activity, exploratory behaviour, emotionality, and anxiety-related responses. The apparatus consisted of a white polywood arena measuring 72 × 72 cm with 36 cm high walls. The floor was divided into equal squares using visible red lines. Thirty minutes after the final treatment, each rat was placed individually at the center of the apparatus and allowed to explore freely for 6 min [26,27].

Behavioural parameters recorded included the number of rearings, rearing duration, distance travelled, and number of center entries. Increased rearing and distance travelled were considered indicators of enhanced exploratory and locomotor activity, whereas increased center entries were interpreted as reduced anxiety-like behaviour [26,27].

2.10 Tail Suspension Test

Antidepressant-like activity was evaluated using the Tail Suspension Test, based on the method described by Steru et al. [28]. Each rat was suspended by the tail using a clamp placed approximately 1 cm from the tail tip. Animals were suspended in a rectangular box measuring

35 × 23 × 53 cm, with the head positioned approximately 5 cm above the floor to prevent support. The test was conducted in a darkened and sound-controlled room to minimize external disturbances.

Each session lasted 6 min, and immobility duration was recorded during the final 4 min. An animal was considered immobile when it remained passively motionless, except for movements required for respiration. Reduced immobility time was interpreted as an antidepressant-like effect. Each animal was used only once to avoid stress adaptation and behavioural variability [28,29].

2.11 Spontaneous Locomotor Activity

Spontaneous locomotor activity was measured using a digital photoactometer to differentiate between CNS stimulant and sedative effects. After administration of the test extracts, animals were allowed 1 h for absorption and onset of pharmacological action. Each rat was then placed individually in the photoactometer chamber, and photobeam interruptions were recorded for 5 min [30,31].

Locomotor activity was expressed as the total number of photobeam interruptions at 0, 30, 60, 90, and 120 min after treatment. A decrease in photobeam counts was interpreted as possible sedative or CNS depressant activity, whereas an increase indicated CNS stimulant activity [30,31].

2.12 Statistical analysis

All results were expressed as mean ± standard deviation (SD). Statistical differences among groups were analysed using one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc multiple-comparison test. A probability value of $p < 0.05$ was considered statistically significant [16].

3. Result and Discussion

3.1 Extraction yield

The ethanolic extract produced a higher yield (6.11% w/w) than the petroleum ether extract (3.76% w/w), indicating that ethanol extracted more polar and semi-polar constituents from M. jalapa leaves.

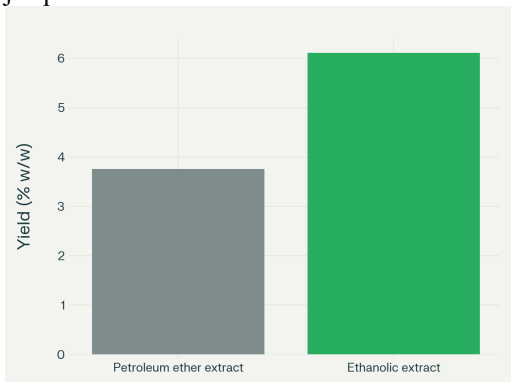


Figure 1. Percentage yield of petroleum ether and ethanolic extracts of M. jalapa leaves.

3.2 Phytochemical screening and antioxidant content

The ethanolic extract contained alkaloids, flavonoids, phenols, tannins, terpenoids, carbohydrates, and saponins. TLC showed Rf values of 0.79 for the petroleum ether extract and 0.85 for the ethanolic extract. The ethanolic extract showed total flavonoid content of 51.1 µg/mL and total phenolic content of 31.3 µg/mL, indicating substantial antioxidant phytochemical content.

3.3 Open Field Test

Compared with the normal control, imipramine significantly increased all Open Field Test parameters. The 200 mg/kg extract produced moderate increases in rearing and rearing duration, whereas 400 mg/kg significantly increased rearing, rearing duration, distance travelled, and center entries. The high-dose response was comparable with imipramine.

Group	Rearing count	Rearing duration (s)	Distance travelled (cm)	Center entries
Normal control	12.6 ± 0.83	19.3 ± 0.65	2167.3 ± 66.44	8.2 ± 0.29
Imipramine 15 mg/kg	23.6 ± 2.11	34.6 ± 1.72	2573.1 ± 91.43	15.6 ± 0.79
Extract 200 mg/kg	19.77 ± 0.58	29.3 ± 1.18	2254.3 ± 96.54	10.3 ± 0.73
Extract 400 mg/kg	23.2 ± 0.62	36.7 ± 0.66	2487.3 ± 59.44	12.9 ± 0.47

Table 1. Effect of ethanolic M. jalapa extract on rat behaviour in the Open Field Test. Values are mean ± SD; n = 5.

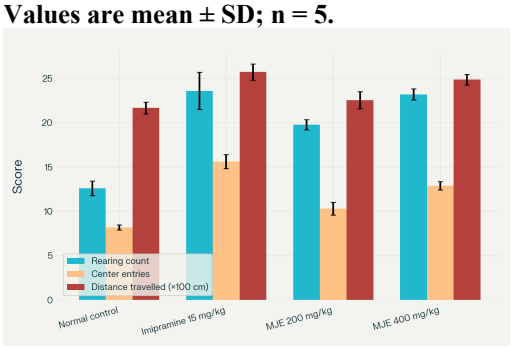


Figure 2. Effect of ethanolic M. jalapa extract on exploratory and locomotor parameters in the Open Field Test.

3.4 Tail Suspension Test

In the acute Tail Suspension Test, normal-control rats showed high immobility (275.50 ± 24.48 s), reflecting behavioural despair. Both extract doses reduced immobility time in acute and chronic tests, with 400 mg/kg producing the

greatest reduction and an effect comparable with imipramine.

3.5 Spontaneous Locomotor Activity

The ethanolic extract reduced spontaneous locomotor activity in a dose-dependent manner. At 90 min, the 400 mg/kg group showed the lowest photobeam count (51.76 ± 7.85), while imipramine also markedly reduced activity. This indicates CNS depressant or sedative activity at the tested doses.

Group	0 min	30 min	60 min	90 min	120 min
Normal control	311.65 ± 7.11	307.52 ± 6.52	301.29 ± 5.98	307.65 ± 7.54	301.11 ± 12.35
Imipramine 15 mg/kg	308.53 ± 8.27	114.94 ± 9.76	55.76 ± 4.76	36.11 ± 4.87	60.11 ± 3.87
Extract 200 mg/kg	318.65 ± 9.11	179.58 ± 7.28	134.39 ± 10.21	119.60 ± 9.41	141.93 ± 11.65
Extract 400 mg/kg	311.65 ± 8.54	144.62 ± 9.64	107.69 ± 5.28	51.76 ± 7.85	104.33 ± 7.35

Table 2. Effect of ethanolic M. jalapa extract on spontaneous locomotor activity in rats. Values are mean ± SD photobeam counts.

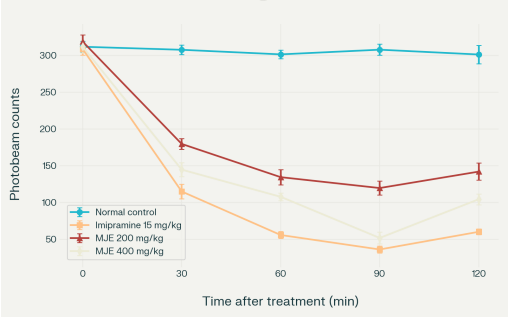


Figure 3. Time-course effect of ethanolic M. jalapa extract on spontaneous locomotor activity.

4. Discussion

The higher yield of the ethanolic extract suggests that polar and semi-polar constituents, including flavonoids and phenolics, are abundant in M. jalapa leaves. The presence of alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins provides a plausible phytochemical basis for the observed central nervous system activity.

In the Open Field Test, increased rearing, distance travelled, and center entries indicate enhanced exploration and reduced anxiety-like behaviour. The 400 mg/kg dose approached the effect of imipramine, supporting a dose-dependent antidepressant-like response.

The reduction in immobility time in the Tail Suspension Test is a classical indicator of antidepressant-like activity. Because this effect occurred alongside reduced spontaneous locomotion, the Tail Suspension Test result is unlikely to be explained simply by generalized motor stimulation; rather, it may reflect a specific effect on behavioural despair.

The reduction in spontaneous locomotor activity suggests sedative or CNS depressant properties. Flavonoids may contribute through GABAergic modulation, while phenolics and other antioxidants may reduce oxidative stress associated with depressive-like states. The extract's high flavonoid and phenolic content therefore supports a possible antioxidant-neuroprotective contribution to its behavioural effects.

Overall, the findings support the traditional use of M. jalapa in neurological and mood-related disorders. However, the exact active constituents and molecular mechanisms remain unknown. Future work should include bioassay-guided fractionation, LC-MS/GC-MS identification, neurotransmitter estimation, receptor-binding studies, chronic toxicity evaluation, and clinical validation.

5. Conclusion

The ethanolic leaf extract of *Mirabilis jalapa* showed significant phytochemical richness, antioxidant potential, and dose-dependent antidepressant-like activity in Wistar rats. The 400 mg/kg dose effectively increased exploratory behaviour and reduced immobility time, with effects comparable to imipramine. Reduced locomotor activity also suggested mild CNS depressant activity. These findings support the traditional use of *M. jalapa* in mood-related disorders.

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