

UPI Journal of Pharmaceutical Medical, and Health Sciences

Content Available at www.uniquepubinternational.com ISSN: 2581-4532



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Research Article

EVALUATION OF INVIVO ANTIDEPRESSANT AND INVITRO ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF CALENDULA OFFICINALIS

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DOI: <https://doi.org/10.37022/jpmhs.v8i2.136>

Article History	Abstract
Received: 21-05-2025 Revised: 11-06-2025 Accepted: 26-07-2025	<p><i>Calendula officinalis</i>, commonly known as marigold, is a well-known medicinal herb used traditionally for its anti-inflammatory, wound healing, and antimicrobial activities. The present study aimed to evaluate the in vivo antidepressant and in vitro antioxidant activities of the ethanolic extract of <i>Calendula officinalis</i>. The antidepressant activity was assessed using standard animal models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) in Wistar albino rats. The extract showed a significant reduction in immobility time, indicating potential antidepressant effects comparable to standard drugs. In vitro antioxidant activity was evaluated through DPPH free radical scavenging and reducing power assays. The extract demonstrated strong antioxidant potential, suggesting the presence of bioactive compounds with free radical neutralizing ability. The findings of this study highlight the dual therapeutic potential of <i>Calendula officinalis</i> in combating oxidative stress and depressive disorders. The presence of flavonoids, phenolics, and other phytoconstituents may contribute to its pharmacological effects. These results support the traditional use of <i>Calendula officinalis</i> in herbal medicine and indicate its promise as a natural source for developing antidepressant and antioxidant therapies. Further studies including isolation of active compounds and clinical trials are warranted. Overall, the study establishes a scientific basis for the medicinal value of this plant extract.</p>
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<p>Keywords: <i>Calendula officinalis</i>, antidepressant, antioxidant, DPPH free radical scavenging, flavonoids.</p>	

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Introduction

Calendula officinalis, commonly known as marigold, is a well-known medicinal herb used traditionally for its anti-inflammatory, wound healing, and antimicrobial activities. The plant is rich in flavonoids, carotenoids, and phenolic compounds, which are known for their antioxidant and neuroprotective effects [1]. Despite its extensive traditional use, limited scientific research is available on its antidepressant activity. Exploring its pharmacological profile could provide insights into its broader therapeutic applications.

Given the increasing global burden of depression and the limitations of current pharmacotherapy, natural plant-based alternatives are gaining attention [2]. This study focuses on investigating the antidepressant-like effects and antioxidant capacity of the ethanolic extract of *Calendula officinalis*. The outcomes may provide evidence supporting the plant's use as a complementary or

alternative therapy. Moreover, this research could pave the way for the development of safer and more effective herbal-based antidepressant formulations.

The present study aimed to evaluate the in vivo antidepressant and in vitro antioxidant activities of the ethanolic extract of *Calendula officinalis*. The antidepressant activity was assessed using standard animal models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) in Wistar albino rats.

Materials and Methods

Calendula officinalis plant was retrieved in May 2023, from the nearby market in Machilipatnam. All chemicals, reagents, and solvents used were of analytical grade and sourced from reliable suppliers. Proper documentation and quality control of supplies were maintained throughout the study.

Extraction

The fresh *Calendula officinalis* plant was gathered, its leaves and roots removed, washed, let to air dry at room temperature, and then roughly pulverised in a combination. Following a 72-hour maceration with 95% ethanol, the powdered material was extracted using hot percolation. The concentrated substance was employed for additional research after being dried using a desiccator and anhydrous calcium chloride.

Antidepressant Activity

For in vivo antidepressant evaluation, animal models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) were employed to measure behavioral changes. In vitro antioxidant activity was assessed using DPPH radical scavenging and reducing power assays. The extract was tested at various concentrations to determine dose-dependent effects. Instruments were calibrated before each experiment, and all procedures were carried out under standardized laboratory conditions. These techniques ensured reliability and reproducibility of the results [3].

Study Design

20 male Wistar albino rats are employed for the depressant activity (bodyweight 180-200g), which were acquired from the National Institute of Nutrition in Hyderabad. Twenty Wistar albino rats were randomly divided into four groups (n=5). The animals were acclimatized for 7 days before the experiment under controlled environment (12-hour light/dark cycle, 25 ± 2 °C) with free access to standard pellet diet and water.

Rats were modeled using a chronic unpredictable mild stress (CUMS) procedure. The administration method was traditional and behavioral indicators were used to evaluate the anti-depressant effect of *Calendula officinalis* extract.

Rats were randomly selected as:

Group 1	5 subjects	Control-placebo	Saline water
Group 2	5 subjects	Candida extract	150mg/gm
Group 3	5 subjects	Candida extract	300mg/gm
Group 4	5 subjects	Imipramine	15mg/gm

A stimulus factor was randomly selected every day, and the same stimulus factor could not appear continuously to ensure the randomness and unpredictability of the stimulus factor.

Stressors used in the Chronic Unpredictable Stress paradigm are:

1. Thermal stimulus: 10 min at 45°C
2. Ice-water bath 4°C: ice water bath for 5 min
3. Tail pinch: Clamp the tail root of the rat about 1 cm for 2 min

4. Water deprivation: Water was deprived for 24 h
5. Fasting: Feed deprivation for 24 h

Behavior Test

Body weight: During the CUMS induction period, the weights of the rats were recorded at 0, 3, and 7 weeks. This was used to evaluate CUMS modeling and the effect on rat body weight after administration.

1. Forced swimming test (FST) [4]
2. Tail Suspension test (TST) [5]
3. Locomotor activity [6]

Evaluation of In-Vitro Antioxidant Activity

Hydrogen Peroxide Scavenging Process

The recently released article tested the extracts' capacity to scavenge hydrogen peroxide. A 40 mM hydrogen peroxide solution was made in phosphate buffer (pH 7.4) using a spectrophotometer, the quantity of hydrogen peroxide was ascertained by absorption at 230 nm. In distilled water, extracts (0.05, 0.1, 0.3, and 0.5 mg/ml) were added to a solution of hydrogen peroxide (0.6 ml, 40 mM). After ten minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution that contained phosphate buffer but no hydrogen peroxide. The extracts' and standard compounds' percentage of hydrogen peroxide scavenging was measured [8].

DPPH Free Radical Scavenging Activity

A 0.1 mM DPPH solution in methanol was made, add 3.0 ml of the extract solution in methanol at various concentrations (0.05, 0.1, 0.3, and 0.5 mg/ml) was mixed with 1.0 ml of this solution. The absorbance was measured at 517 nm thirty minutes later. Extract was not added to a blank that was prepared. As a standard, ascorbic acid was utilized at different concentrations (0.05, 0.1, 0.3, and 0.5 mg/ml). Higher free radical scavenging activity is shown by a lower absorbance of the reaction mixture [9].

Hydroxyl Radical Scavenging Assay

A single sample (1 ml) was added to the reagents, which contained 0.3 ml of sodium salicylate (20 mM), 0.7 ml of H₂O₂, 0.05, 0.1, 0.3, and 0.5 mg/ml of FeSO₄. The reaction mixture's absorbance was measured at 562 nm following a 1-hour incubation period at 37°C. The capacity to scavenge Hydroxyl radicals was computed [8].

Results and Discussion

Phytochemical Analysis

The present phytochemical analysis of Ethanolic extract of *Calendula officinalis* showed positive results for Flavonoids, Saponins, Tannins, Carbohydrates and Resins.

Antidepressant activity of *Calendula officinalis*

In present study *Calendula officinalis* (150 and 300 mg/kg) produced significant dose dependant antidepressant effect in behavior despair tests. Animals subjected to the antidepressant drug treatment struggle more even in desperate situations and they spend less time with immobility [10]. Behavioral tests such as the Forced Swim Test and Tail Suspension Test showed significant

reductions in immobility time, indicating potential antidepressant-like effects. In vitro assays like DPPH radical scavenging confirmed the extract's strong antioxidant capacity. These results suggest the extract may act through both neurochemical and oxidative stress-related pathways. The findings align with the traditional use of *Calendula officinalis* in promoting mental well-being. The dual action of *Calendula officinalis* in enhancing mood and combating oxidative stress highlights its therapeutic potential in depressive disorders. The extract showed dose-dependent efficacy and a promising safety profile in animal models.

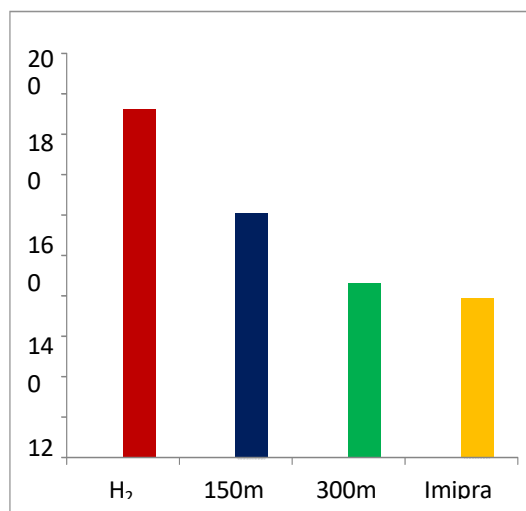


Figure 1. Effect of Ethanolic extract of *Calendula officinalis* on duration of immobility in the Tail Suspension Test

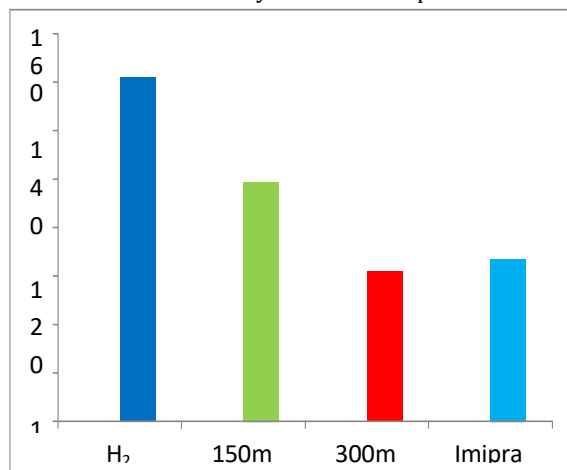


Figure 2. Effect of Ethanolic extract of *Calendula officinalis* on immobility in the Forced Swim Test

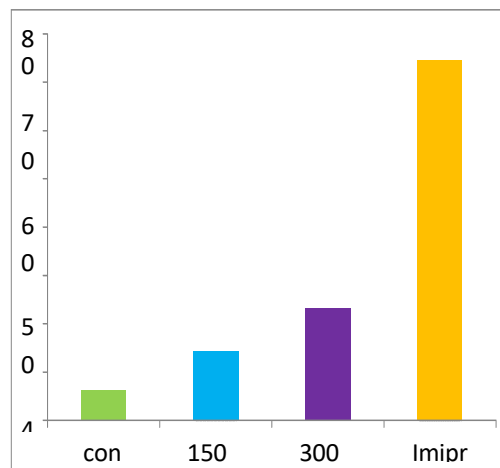


Figure 3. Effect of Ethanolic extract of *Calendula officinalis* on locomotor activity by photoactometer

In vitro* Antioxidant activity of Alcoholic extract of *Calendula officinalis

The Antioxidant activity was performed by Hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, DPPH method. The results of antioxidant values are expressed as IC₅₀ against various free radicals. The calculated IC₅₀ values for Alcoholic extract of *Calendula officinalis* and reference standard Ascorbic acid. Free radical scavenging activity of the compound is concentration dependent, as the concentration of the test compound increases, the radical scavenging activity increases and lower IC₅₀ value reflects better protective action. The antioxidant activity of the Alcoholic extract of *Calendula officinalis* could be attributed to presence of Alkaloids, tannins, triterpene, resins.

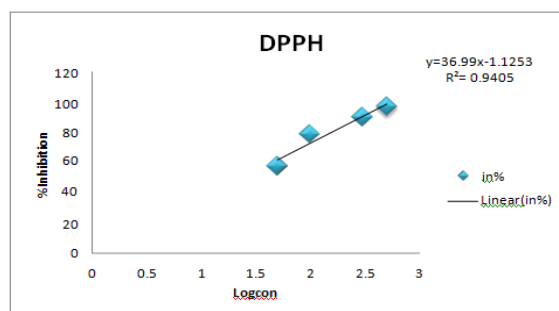


Figure 4. DPPH method of Ethanolic extract of *Calendula officinalis*

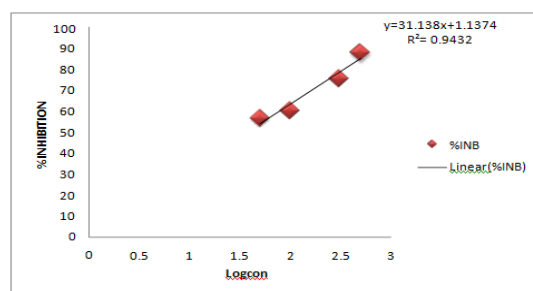


Figure 5. Hydrogen peroxide method of Ethanolic extract of *calendula officinalis*

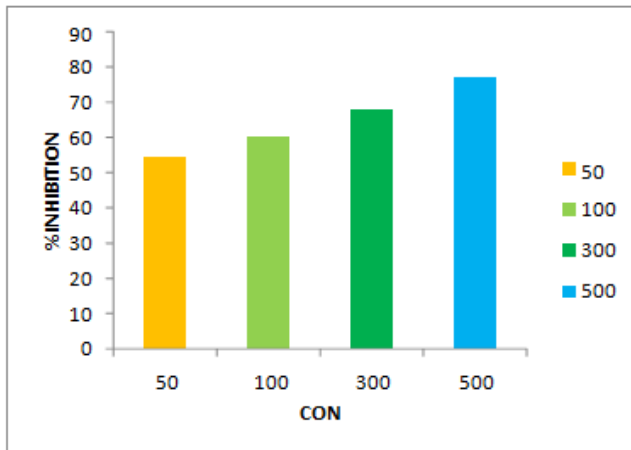


Figure 6. Hydrogen radical scavenging method of Ethanolic extract of *C.officinalis*

Conclusion

The present study evaluated the ethanolic extract of *Calendula officinalis* for its in vivo antidepressant and in vitro antioxidant activities. Behavioral tests such as the Forced Swim Test and Tail Suspension Test showed significant reductions in immobility time, indicating potential antidepressant-like effects. In vitro assays like DPPH radical scavenging confirmed the extract's strong antioxidant capacity. These results suggest the extract may act through both neurochemical and oxidative stress-related pathways. The findings align with the traditional use of *Calendula officinalis* in promoting mental well-being.

The dual action of *Calendula officinalis* in enhancing mood and combating oxidative stress highlights its therapeutic potential in depressive disorders. The extract showed dose-dependent efficacy and a promising safety profile in animal models. Its rich content of flavonoids and phenolic compounds likely contributes to both observed effects. This study supports the continued exploration of herbal medicines as complementary or alternative treatments for depression. Further studies, including clinical trials, are needed to confirm its efficacy and mechanism of action in humans.

Acknowledgement

The authors are thankful to the management of Avanthi Institute of Pharmaceutical Sciences for their support in carrying out this work.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both are contributed equally

Financial Support

None

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