



## Research Article

## EVALUATION OF IN VIVO ANTIDEPRESSANT ACTIVITY AND IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *MOMORDICA CHARANTIA*

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### ABSTRACT

Depression is a prevalent mental disorder often associated with oxidative stress. Traditional medicinal plants, such as *Momordica charantia*, have been explored for their neuropharmacological effects due to the presence of bioactive phytochemicals. While the fruit of *Momordica charantia* is well-studied, its leaves remain underexplored for central nervous system effects. This study investigates the antidepressant and antioxidant potential of its leaf extract to validate its ethnomedicinal use. To evaluate the in vivo antidepressant activity and in vitro antioxidant potential of the ethanolic leaf extract of *Momordica charantia* (ELMC), a plant traditionally used in herbal medicine. Fresh leaves of *Momordica charantia* were collected, shade-dried, and extracted using ethanol by Soxhlet extraction. The extract was subjected to In vitro antioxidant assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (Ferric Reducing Antioxidant Power) assays. In vivo antidepressant activity evaluation using animal models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) in Swiss albino mice. The extract was administered at doses of 100 mg/kg and 200 mg/kg orally for 14 days. Imipramine (15 mg/kg) was used as the standard antidepressant. The ethanolic leaf extract of *Momordica charantia* showed significant antioxidant activity with IC<sub>50</sub> values of 59.5 µg/mL in the DPPH assay and 73.06 µg/mL in the hydrogen peroxide scavenging assay, along with a dose-dependent increase in FRAP values. In vivo, the extract produced a significant reduction in immobility time in both the Forced Swim Test and Tail Suspension Test ( $p < 0.05$ ), demonstrating antidepressant-like activity comparable to imipramine. No signs of toxicity or mortality were observed during the acute oral toxicity study up to 2000 mg/kg. The ethanolic leaf extract of *Momordica charantia* demonstrated potent antioxidant and antidepressant activity, supporting its traditional use in mental health disorders. These effects may be attributed to the presence of phenolic compounds and flavonoids. Further studies are warranted to isolate and characterize the active constituents and elucidate their mechanisms of action.

**Keywords:** *Momordica charantia*, Antidepressant, Antioxidants, Tail suspension test, Forced swim test, DPPH assay.

### INTRODUCTION

Depression is a multifactorial neuropsychiatric disorder that profoundly affects emotional, cognitive, and physical functioning and represents a major contributor to global disability (Politi et al., 2008; Kulkarni and Dhir, 2007). Clinically, it manifests as persistent low mood, anhedonia, fatigue, sleep and appetite disturbances, impaired concentration, psychomotor changes, and recurrent suicidal ideation (Duman and Aghajanian, 2012). Despite the availability of several classes of antidepressant drugs, including monoamine oxidase inhibitors and selective serotonin reuptake inhibitors, therapeutic outcomes are

often limited by delayed onset, partial remission, and adverse effects, highlighting the need for safer and more effective alternatives (Kulkarni and Dhir, 2007; Politi et al., 2008). Growing evidence indicates that oxidative stress plays a significant role in the pathophysiology of depression. Excessive production of reactive oxygen species and compromised antioxidant defenses contribute to neuronal damage and synaptic dysfunction (Behl and Moosmann, 2002; Diniz et al., 2014). Consequently, agents possessing both antioxidant and neuroprotective properties may offer enhanced therapeutic benefits (Misra et al., 2011; Maurya and Devasagayam, 2010). *Momordica charantia* L.

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(Cucurbitaceae), commonly known as bitter melon, is widely used in traditional medicine for its antidiabetic, anti-inflammatory, and antioxidant properties (Grover and Yadav, 2004; Walters and Becher-Walters, 1988). The plant is rich in flavonoids and phenolic compounds with proven antioxidant activity (Bohm *et al.*, 1998; Harborne, 1998). However, the antidepressant potential of its leaves remains poorly explored. Therefore, the present study aimed to evaluate the antidepressant activity of the ethanolic leaf extract of *Momordica charantia* L using validated behavioural models, along with its *in vitro* antioxidant potential, to substantiate its therapeutic relevance in depressive disorders. *Momordica charantia* (family: Cucurbitaceae), commonly known as bitter melon or karela, is a perennial climbing plant extensively cultivated in tropical and subtropical regions. It has a long history of use in traditional medicinal systems for the management of metabolic, inflammatory, and infectious disorders (Walters and Becher-Walters, 1988; Grover and Yadav, 2004). Various parts of the plant, including fruits and leaves, are traditionally employed for their therapeutic benefits, particularly in diabetes and oxidative stress-related conditions (Ahmed *et al.*, 1998; Oyedemi *et al.*, 2010).

Phytochemical studies have demonstrated that *Momordica charantia* contains a diverse range of bioactive constituents such as flavonoids, phenolic compounds, alkaloids, glycosides, saponins, and triterpenoids (Harborne, 1998; Bohm *et al.*, 1998). These compounds are known to possess significant antioxidant activity, contributing to free radical scavenging and protection against oxidative damage (Misra *et al.*, 2011; Maurya and Devasagayam, 2010). Extracts of *Momordica charantia* have shown strong antioxidant potential in standard *in vitro* assays, including DPPH and ABTS methods (Prieto *et al.*, 1999; Re *et al.*, 1999; Shan *et al.*, 2012). Oxidative stress is closely associated with neuropsychiatric disorders such as depression. The antioxidant and neuroprotective properties of flavonoids present in *Momordica charantia* may help mitigate neuronal damage and synaptic dysfunction (Behl and Moosmann, 2002; Diniz *et al.*, 2014). Although the fruit has been extensively studied, the leaves remain comparatively underexplored, supporting their investigation as a potential source of antidepressant and antioxidant agents.

## MATERIAL AND METHODS

### Selection and Authentication of Plant material

Fresh leaves of *Momordica charantia* were collected from a home-cultivated source and authenticated by Dr. P. Satyanarayana Raju, Department of Botany, Acharya Nagarjuna University, Guntur.

### Preparation of Ethanolic Extract

The leaves were shade dried, powdered, and subjected to Soxhlet extraction using 95% ethanol as solvent. The

extract was concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use. (Li *et al.*, 2017).

## Experiment in animals

### In Vivo Antidepressant Activity

Healthy Swiss albino mice (20–25 g) were procured and maintained under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C, and ad libitum access to food and water). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CCSEA guidelines.

### Drugs & Chemicals used

The drug used within this research is amitriptyline. Plant extract was suspended alcohol administered in orally. Amitriptyline was dissolved in normal saline and administered orally. All chemicals and reagents used in the study were of analytical grade. Standard drugs used included fluoxetine (for antidepressant activity) and ascorbic acid (for antioxidant assays)

### Drug treatment

Animals were divided into four groups (n=6 per group):

- Group I: Control (vehicle, 0.5% CMC)
- Group II: Standard (Fluoxetine, 10 mg/kg, p.o.)
- Group III: Ethanolic leaf extract of *Momordica charantia* (100 mg/kg, p.o.)
- Group IV: Ethanolic leaf extract of *Momordica charantia* (200 mg/kg, p.o.)

## Experiment methodology

### Forced swim test (FST)

The Forced Swim Test was performed to evaluate antidepressant-like activity, following established protocols for behavioural screening of antidepressants (Kulkarni and Reddy, 1996; Dhingra and Sharma, 2006). Male mice were individually placed in a transparent cylindrical glass container (20 cm diameter × 40 cm height) containing fresh water to a depth of 19 cm, maintained at 25 ± 3 °C. The test consisted of two sessions. During the pre-test session, untreated mice were forced to swim for 15 min without behavioural scoring to allow habituation and to establish a stable immobility profile. Twenty-four hours later, the test session was conducted. Animals received their respective treatments 60 min, 4 h, or 24 h prior to testing and were allowed to swim for 5 min. The total duration of immobility was recorded using a stopwatch, and sessions were video-recorded for further analysis. Immobility was defined as the absence of active movements, with mice making only minimal motions to keep their heads above water. Active behaviours such as vigorous swimming, head dipping, circling, wall climbing, and four-limbed paddling were

considered escape behaviours (Kulkarni and Reddy, 1996). After each trial, animals were towel-dried, placed briefly under a rodent heater, and returned to their home cages.

### Tail suspension Test

The Tail Suspension Test was conducted as another validated behavioral model for assessing antidepressant activity (Dhingra and Sharma, 2006; Kulkarni and Dhir, 2007). Sixty minutes after treatment administration, mice were suspended individually by the tail using adhesive tape, approximately 1 cm from the tail tip, from a horizontal bar positioned 50 cm above the surface. Each session lasted for 6 min, during which immobility time was recorded using a stopwatch. Mice were considered immobile when they remained motionless, exhibiting only movements necessary for respiration. The entire session was video-recorded and analyzed in a blinded manner to minimize observer bias. The TST is conceptually related to the FST and is widely used for screening antidepressant agents (Kulkarni and Reddy, 1996; Politi *et al.*, 2008).

### Open Field Test

The Open Field Test was performed to assess locomotor activity and to exclude any nonspecific motor effects of the test extract (Kulkarni and Reddy, 1996). One hour after drug administration, mice were placed individually at the centre of an open field apparatus consisting of a dark wooden box (68 × 68 × 45 cm) with the floor divided into 16 equal squares. The apparatus was illuminated by a 60 W bulb placed above the arena. The number of peripheral crossings (ambulation), central crossings, and total locomotor activity were recorded over a 5-min period. After each trial, the apparatus was cleaned with alcohol to eliminate olfactory cues. Behavioural sessions were video-recorded for accurate assessment.

### In vitro assessment of antioxidant activity

#### Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Scavenging Assay

This assay evaluates the ability of the plant extract to neutralize hydrogen peroxide, which is a reactive oxygen species capable of causing cellular damage. A 20 mM hydrogen peroxide solution was prepared using phosphate buffer (pH 7.4). Different concentrations of the plant extract (25, 50, 100, 200, and 400 µg/mL) were prepared in distilled water. To each test tube, 0.6 mL of hydrogen peroxide solution was added and incubated for 10 minutes at room temperature. After incubation, the absorbance was measured at 230 nm using a UV-Visible spectrophotometer.

A blank containing only phosphate buffer was used for baseline correction. Ascorbic acid was used as the standard antioxidant reference.

Formula used for Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Scavenging Assay:

$$\%H_2O_2 \text{ Scavenging Activity} = (A_c - A_s) / A_c \times 100$$

Where:

- **A<sub>c</sub>** = Absorbance of control (hydrogen peroxide without extract)
- **A<sub>s</sub>** = Absorbance of sample (hydrogen peroxide + extract)

All experiments were carried out in triplicate, and results were expressed as Mean ± SEM.

#### DPPH Free Radical Scavenging Assay

The DPPH assay measures the ability of the extract to donate hydrogen atoms or electrons to neutralize free radicals. Different concentrations of the plant extract (200–1000 µg/mL) were prepared. 1 mL of extract solution was mixed with 1 mL of DPPH solution (0.3 mM in ethanol). The mixtures were shaken well and incubated in the dark at room temperature for 30 minutes. After incubation, absorbance was measured at 516 nm. The negative control consisted of 1 mL ethanol + 1 mL DPPH solution without extract. Each concentration was tested in triplicate.

Formula used for DPPH Free Radical Scavenging Assay:

$$\%DPPH \text{ Radical Scavenging Activity} = (A_c - A_s) / A_c \times 100$$

Where:

- **A<sub>c</sub>** = Absorbance of DPPH control (negative control)
- **A<sub>s</sub>** = Absorbance of DPPH with plant extract

Results were expressed as Mean ± SEM.

#### Statistical analysis

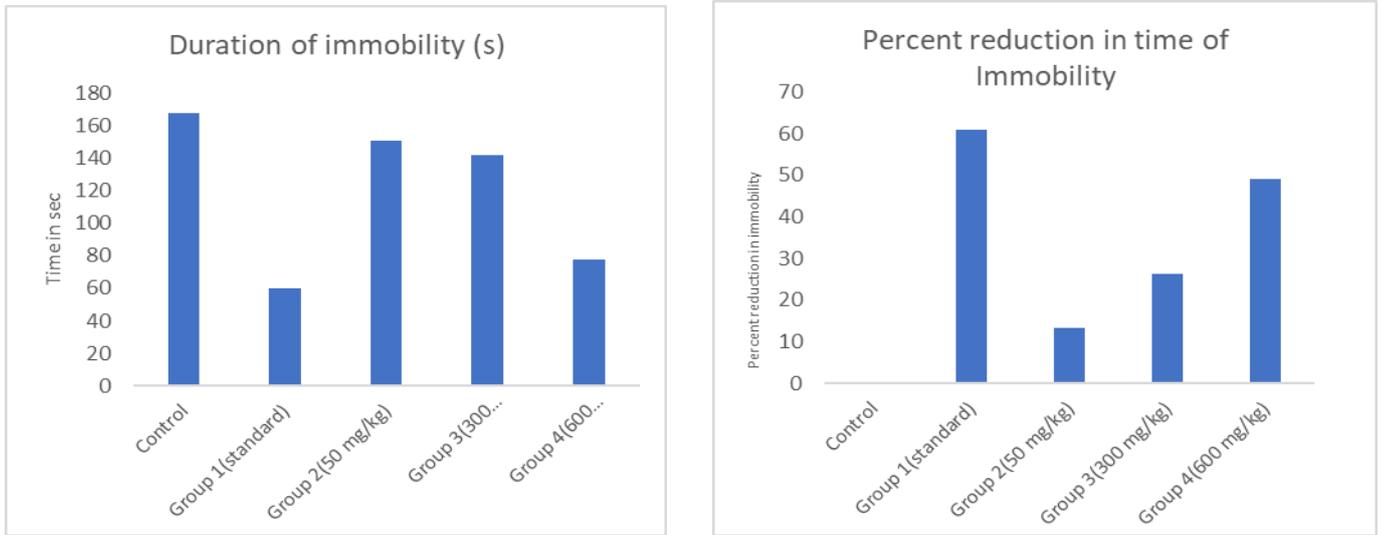
All results were expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

**Table No 1.** Effect of *Momordica charantia* ethanolic leaf extract on mice by TST.

Treatment group	Duration of immobility (s)	Percent reduction in time of Immobility
Control	167.8 ± 0.98	-
Group 1(standard)	60.1 ± 1.1	60.89%
Group 2(50 mg/kg)	151 ± 2.30	13.19%

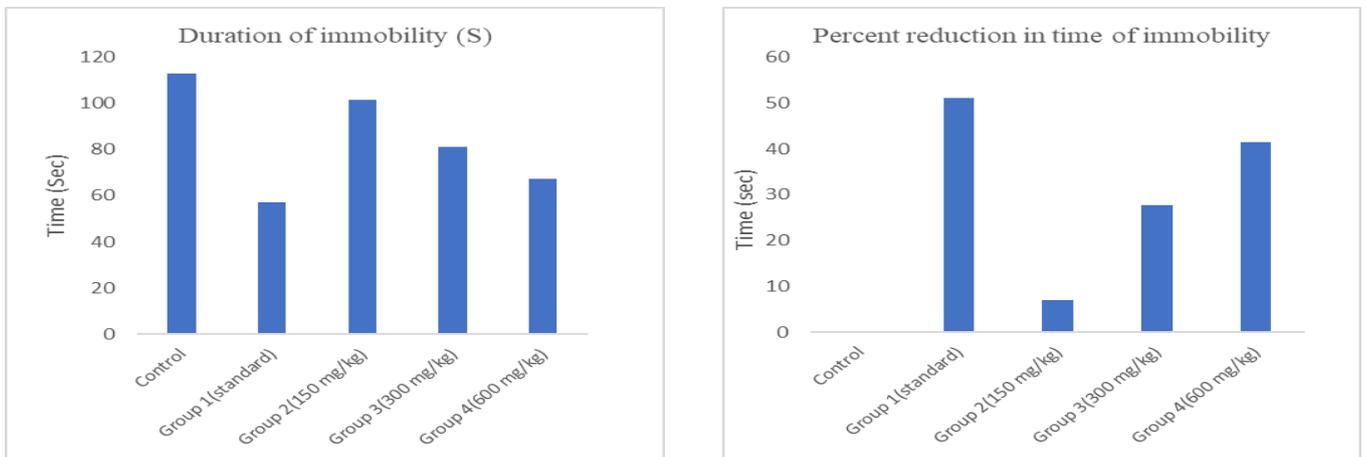
Group 3(300 mg/kg)	142±1.0	26.29%
Group 4(600 mg/kg)	77.5±1.64	49.14%



**Figure 4.** Duration Of Immobility In TST.

**Table 2.** Effect of *Momordica charantia* ethanolic leaf extract in mice forced swim test.

Treatment groups	Duration of immobility(s)	Percent reduction in time of immobility
Control	112.67± 0.44	-
Group 1(standard)	57.25± 0.38	51.02%
Group 2(150 mg/kg)	101.25 ± 0.32	6.95%
Group 3(300 mg/kg)	81.09 ± 0.14	27.68%
Group 4(600 mg/kg)	67.08± 0.22	41.41%

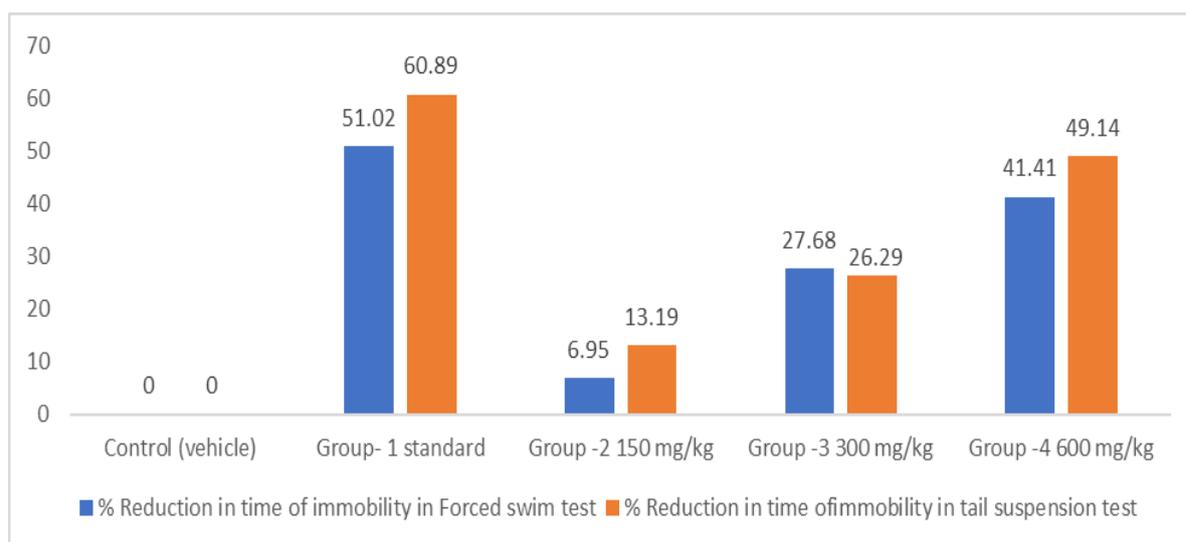


**Figure 5.** Duration of Immobility in Forced swim test.

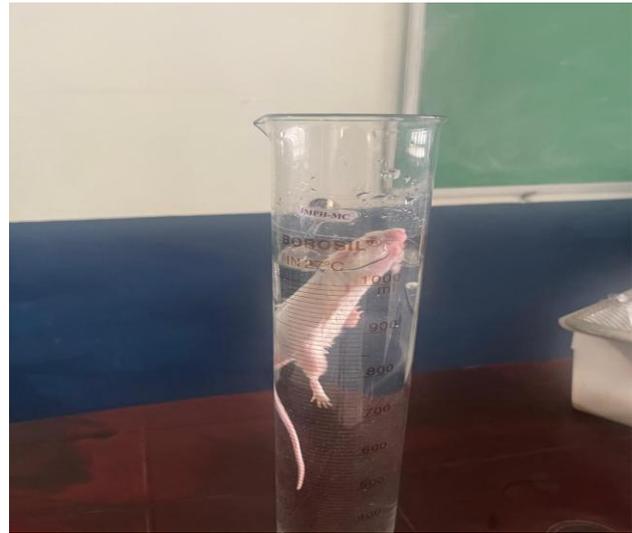
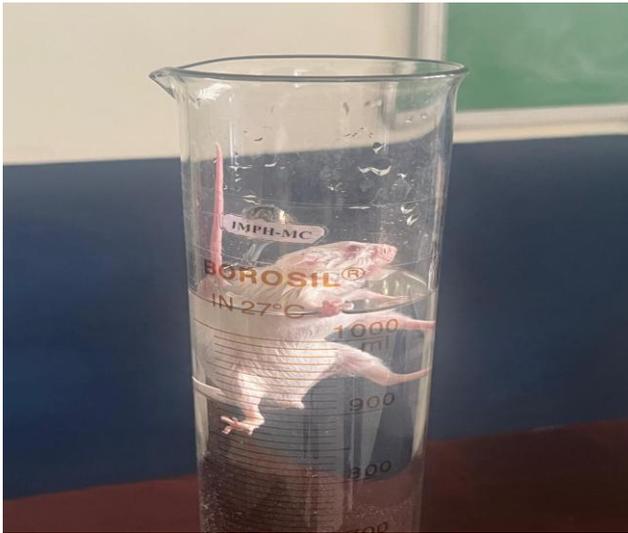
Comparative evaluation of percentage decrease in immobility time between the Forced Swim Test and Tail Suspension Test:

**Table 3.** Comparative evaluation of percentage decrease in immobility time between the Forced Swim Test and Tail Suspension Test.

Treatment group	% Reduction in time of immobility in Forced swim test	% Reduction in time of immobility in tail suspension test
Control (vehicle)	-	-
Group- 1 standard	51.02%	60.89%
Group -2 150 mg/kg	6.95%	13.19%
Group -3 300 mg/kg	27.68%	26.29%
Group -4 600 mg/kg	41.41%	49.14%

**Figure 6.** Comparative evaluation of percentage decrease in immobility time between the Forced Swim Test and Tail Suspension Test.**Table 4.** Effect of *Momordica charantia* ethanolic leaf extract on locomotion and exploratory behaviours in mice open field test.

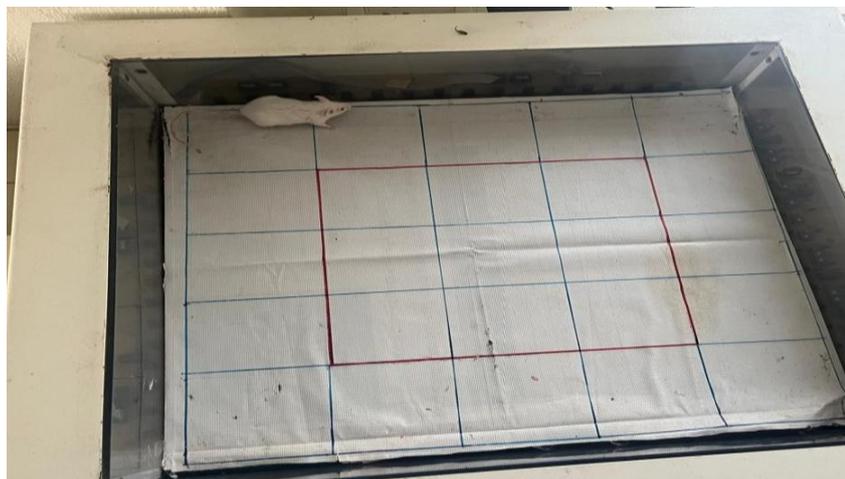
Treatment group	Number of crossings Peripheral squares	Number of crossings Central squares	Number of crossings Total squares	Percent change in total locomotion
Control	11.47± 0.32	7.2 ± 2.3	83.26 ± 0.47	0
Group 1 standard	33.15± 0.60	5.5 ± 1.3	110.32±0.87	-2.5
Group 2 (125 mg/kg)	16.49± 0.36	6.2± 2.9	92.67± 1.20	-9.16
Group 3 (250 mg/kg)	22.54 ± 1.17	3.0 ± 0.7	98.9 ± 0.45	5.8
Group 4 (500 mg/kg)	23.10± 0.17	3.8 ± 0.8	100.27 ±0.37	18.6



**Figure 1.** Forced Swimming Test.



**Figure 2.** Tail Suspension Test.



**Figure 3.** Open Field Test

**Table 5.** Effect of ethanolic leaf extract of *Momordica charantia* on DPPH radical scavenging.

Tested material	Concentration ( $\mu\text{g/ml}$ )	% Inhibition	IC 50 ( $\mu\text{g/ml}$ )
Ethanolic extract of <i>Momordica charantia</i>	25	30.81 $\pm$ 0.001	59.5 ( $\mu\text{g/ml}$ )
	50	48.36 $\pm$ 0.002	
	100	59.21 $\pm$ 0.005	
	200	66.23 $\pm$ 0.005	
Ascorbic acid	25	52.83 $\pm$ 0.001	22.5 ( $\mu\text{g/ml}$ )
	50	60.75 $\pm$ 0.002	
	100	69.12 $\pm$ 0.007	
	200	77.71 $\pm$ 0.005	

ELMC IC<sub>50</sub> = 59.5  $\mu\text{g/ml}$  Ascorbic acid IC<sub>50</sub> = 22.5  $\mu\text{g/ml}$ **Table 6.** Effect of ethanolic leaf extract of *Momordica charantia* on DPPH radical scavenging.

Tested material	Concentration	%inhibition	IC 50 ( $\mu\text{g/ml}$ )
Ethanolic extract of <i>Momordica charantia</i>	20	26.38 $\pm$ 0.21	73.06 $\mu\text{g/ml}$
	40	37.71 $\pm$ 0.19	
	60	46.23 $\pm$ 0.01	
	80	57.76 $\pm$ 0.03	
	100	63.09 $\pm$ 0.05	
Ascorbic acid	20	55.47 $\pm$ 0.11	19.85 $\mu\text{g/ml}$
	40	65.33 $\pm$ 0.15	
	60	70.97 $\pm$ 0.07	
	80	81.02 $\pm$ 0.22	
	100	93.07 0.02	

ELMC IC<sub>50</sub> = 73.06  $\mu\text{g/ml}$ . Ascorbic acid IC<sub>50</sub> = 19.85  $\mu\text{g/ml}$ 

The findings of the present study provide evidence that ethanolic leaf extract of *Momordica charantia* possesses significant antioxidant and antidepressant potential. The dual activity can be correlated with the phytochemical profile of the plant, particularly phenolic compounds and flavonoids, which exert both neuroprotective and free radical scavenging effects. The antidepressant-like activity observed may be partly mediated through antioxidant mechanisms, reducing oxidative damage in neural tissues and maintaining neurotransmitter balance. These results are consistent with earlier reports on the neuroprotective role of plant-derived antioxidants. However, further studies are necessary to isolate the active compounds, elucidate the precise mechanisms of action, and evaluate the extract in chronic models of depression. Toxicological studies are also required to ensure the safety of long-term use.

## CONCLUSION

The ethanolic leaf extract of *Momordica charantia* exhibits significant antioxidant and antidepressant activities in validated animal models. These properties suggest

therapeutic potential in depressive disorders. Further studies are needed to isolate active constituents and elucidate underlying mechanisms.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

After receiving approval from the Institutional Animal Ethics Committee (IAEC) of the Chalapathi Institute of Pharmaceutical Sciences, Guntur, all studies were carried out. The protocol of the experiment was approved by the

Institutional animal ethics committee (03/IAEC/CLPT/2024-25). All animal experimental procedures were in compliance with the rules of the committee for the supervision and regulation of animal experiments (CCSEA), ministry of social justice and empowerment, Government of India.

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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