

Evaluation of Antidepressant Activity of *Emblica officinalis* in Albino Wistar Rats

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ABSTRACT

Background: Major depressive disorder is a multifactorial neuropsychiatric condition characterized by incomplete therapeutic response and tolerability issues associated with currently available antidepressant drugs. This has stimulated interest in plant-derived agents with multi-targeted biological activity. *Emblica officinalis* (Amla), a traditional *Rasayana* drug, is rich in polyphenols and related phytoconstituents with reported antioxidant and neuromodulatory properties, suggesting potential relevance in depressive disorders. **Aim:** To evaluate the antidepressant-like activity of the ethanolic extract and successive polarity-based solvent fractions of *Emblica officinalis* using validated rodent behavioural models. **Materials and Methods:** Fresh fruits of *Emblica officinalis* were subjected to Soxhlet extraction with ethanol, followed by successive fractionation into petroleum ether, benzene, ethyl acetate, acetone, and methanol fractions. Adult albino Wistar rats were treated orally for 10 consecutive days. Antidepressant-like activity was assessed using the Forced Swim Test, Tail Suspension Test, and Cook's Pole Climbing Apparatus. Behavioural parameters included immobility time, latency to climb, and conditioned avoidance responses. Data were analysed using one-way ANOVA followed by Tukey's *post hoc* test. **Results:** Imipramine produced a marked reduction in immobility in both despair-based models, validating the experimental design. Among the test samples, the ethyl acetate fraction demonstrated the most pronounced antidepressant-like activity in the Forced Swim and Tail Suspension tests, followed by the methanol fraction and the crude ethanolic extract. In the Cook's Pole Climbing Apparatus, the crude ethanolic extract produced the greatest modulation of conditioned avoidance and escape behaviour, with comparatively smaller but significant effects observed for the methanol and ethyl acetate fractions. **Conclusion:** Medium-polarity fractions of *Emblica officinalis*, particularly ethyl acetate and methanol fractions, exhibited consistent antidepressant-like effects in despair-based behavioural models, while the crude ethanolic extract showed broader behavioural modulation. These findings support further phytochemical standardisation and mechanistic evaluation of *Emblica officinalis* fractions as potential leads for plant-based antidepressant research.

Keywords: Behavioural despair, Depression, *Emblica officinalis*, Forced swim test, Tail suspension test, Wistar rats.

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INTRODUCTION

Depression is a prevalent and debilitating mental health disorder that contributes substantially to global disability and socioeconomic burden worldwide (Ferrari *et al.*, 2013; Whiteford *et al.*, 2013; World Health Organization, 2017). Its pathophysiology is complex and multifactorial, involving disturbances in monoaminergic neurotransmission (Nestler *et al.*, 2002), dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis

(Pariante and Lightman, 2008), and neuroinflammatory processes (Maes *et al.*, 2011; Miller and Raison, 2016). Additional factors include oxidative stress (Lopresti *et al.*, 2014), mitochondrial dysfunction, and impaired neuroplasticity (Duman and Aghajanian, 2012; Duman and Monteggia, 2006).

Despite the availability of multiple pharmacological classes of antidepressants, such as tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, and atypical agents, significant therapeutic limitations persist (Malhi and Mann, 2018). A considerable proportion of patients experience delayed onset of action, incomplete response or remission, relapse, and adverse effects that compromise long-term adherence (Malhi and Mann, 2018). Collectively, these challenges underscore the need for safer, more effective, and multi-targeted therapeutic strategies for the management



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of depressive disorders (Malhi and Mann, 2018; World Health Organization, 2017).

Medicinal plants have historically played an important role in the discovery and development of psychotropic agents and continue to attract interest as potential sources of novel antidepressant compounds (Russo and Borrelli, 2005; Sarris *et al.*, 2015). *Emblica officinalis* (Amla), belonging to the family *Phyllanthaceae*, is widely used in the Ayurvedic, Siddha, and Unani systems of medicine and is classified as a *Rasayana* drug. Traditionally, Amla is valued for its rejuvenative, adaptogenic, and antioxidant properties (Baliga and Dsouza, 2011; Sundaram *et al.*, 2014).

Phytochemical investigations have demonstrated that the fruit of *Emblica officinalis* is rich in bioactive constituents, including gallic acid, ellagic acid, emblicanin A and B, flavonoids, and hydrolysable tannins (Bajpai *et al.*, 2005; Jain *et al.*, 2016). These constituents exhibit potent antioxidant and anti-inflammatory activities and have been reported to influence monoaminergic neurotransmission and cellular stress pathways, which are mechanisms highly relevant to the neurobiology of depression (Gupta *et al.*, 2012; Khan *et al.*, 2023).

Several preclinical studies have reported antidepressant-like effects of crude extracts of *Emblica officinalis* in experimental animal models, including reductions in behavioural despair and modulation of neurotransmitter systems (Choudhary *et al.*, 2014; Dhingra *et al.*, 2013; Rao *et al.*, 2021). However, the majority of these studies have primarily focused on whole extracts, with limited emphasis on delineating the contribution of individual phytochemical fractions. Given that polarity-based solvent fractionation allows differential enrichment of phytoconstituents, this approach represents a rational strategy for identifying pharmacologically active components and facilitating future standardisation (Kabra *et al.*, 2022; Uttu *et al.*, 2021). Nevertheless, systematic comparison of polarity-based solvent fractions of *Emblica officinalis* across multiple validated behavioural paradigms of depression remains limited (Khan *et al.*, 2023).

The present study was therefore designed to evaluate the antidepressant-like activity of the ethanolic extract and successive polarity-based solvent fractions, including petroleum ether, benzene, ethyl acetate, acetone, and methanol, using three validated behavioural models: the Forced Swim Test (FST), the Tail Suspension Test (TST), and the Cook's Pole Climbing Apparatus (CPCA). By comparing the behavioural effects of the crude extract with those of individual solvent fractions, this study aimed to identify fractions exhibiting optimal antidepressant-like activity and to infer the phytochemical nature of the active constituents.

MATERIALS AND METHODS

Plant Material

Fresh, ripe fruits of *Emblica officinalis* (Amla), belonging to the family *Phyllanthaceae*, were procured from a local market in Aligarh, Uttar Pradesh, India. The plant material was identified based on macroscopic and organoleptic characteristics described in standard pharmacognostic and Ayurvedic texts. The fruits were thoroughly washed with distilled water, manually deseeded, and shade-dried at room temperature, between 25 and 30°C, for 7 days. The dried fruit pulp was pulverised using a mechanical grinder to obtain a coarse powder, which was stored in airtight containers protected from light and moisture until further use.

Preparation of Ethanolic Extract and Solvent Fractions

300 g of the powdered plant material were subjected to continuous hot extraction in a Soxhlet apparatus using 99% absolute ethanol for approximately 18 hr, until the siphon solvent became colourless. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40 to 45°C to obtain a semi-solid crude ethanolic extract, which was weighed and stored at 4°C.

For successive solvent fractionation, 25 g of the dried ethanolic extract was suspended in 250 mL of distilled water and partitioned sequentially using solvents of increasing polarity in the following order: petroleum ether, benzene, ethyl acetate, acetone, and methanol. Each solvent was used in three successive extraction cycles (3×200 mL). The pooled organic layers were separated using a separating funnel, concentrated under reduced pressure, and dried to yield their respective fractions. All fractions were stored in amber-coloured airtight vials at 4°C until further use.

Drugs, Chemicals, and Vehicle

Imipramine hydrochloride was used as the reference antidepressant drug and was obtained from a commercially available tablet formulation. All solvents used for extraction and fractionation, including ethanol, petroleum ether, benzene, ethyl acetate, acetone, and methanol, were of Analytical Reagent (AR) grade and procured from Merck, India. A 0.5% w/v Carboxymethylcellulose (CMC) suspension prepared in normal saline was used as the vehicle for oral administration of all test substances. The dosing volume was standardised at 1 mL/100 g body weight.

Preparation of Dosing Solutions

Imipramine was administered orally at a dose of 10 mg/kg/day for 10 consecutive days. A fresh suspension (1 mg/mL) was prepared daily by crushing the tablet, dispersing it in warm distilled water, and suspending it in 0.5% CMC. The ethanolic extract and each solvent fraction of *Emblica officinalis* were administered orally at a dose of 500 mg/kg/day for 10 consecutive days. Fresh

suspensions (100 mg/mL) were prepared daily in 0.5% CMC, vortexed thoroughly, and administered by oral gavage using a blunt-ended metallic cannula.

Experimental Animals

Adult albino Wistar rats of either sex, weighing 200 to 300 g, were procured from the Central Animal House, Jawaharlal Nehru Medical College, Aligarh Muslim University. Animals were housed in polypropylene cages under standard laboratory conditions, including a temperature of $22\pm 2^\circ\text{C}$, relative humidity of 50 to 60%, and a 12 hr light/dark cycle, with free access to standard pellet diet and drinking water ad libitum. All animals were acclimatised for at least 7 days prior to experimentation.

Ethical Approval

All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2018). Ethical clearance was obtained from the Institutional Animal Ethics Committee, Central Animal House, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh (IAEC approval dated 09/12/2023).

Dose Selection and Experimental Design

The dose of *Emblica officinalis* extract and its solvent fractions (500 mg/kg, p.o.) was selected based on published literature reporting the use of comparable doses in rodent neuropharmacological screening studies (Dhingra *et al.*, 2013; Gupta *et al.*, 2012; Rao *et al.*, 2021). Considerations were also applied regarding dose translation from animal to human studies (Table 1) (Reagan-Shaw *et al.*, 2008).

Animals were randomly allocated into eight groups, with 5 rats per group. All treatments were administered once daily for 10 consecutive days. Behavioural testing was performed on Day 10, one hour after the final dose, between 09:00 and 13:00 hr. The order of group testing was randomised, and all behavioural assessments were conducted by an observer blinded to treatment allocation.

Behavioural Tests

Forced Swim Test (FST)

The Forced Swim Test was performed according to the method originally described by (Porsolt *et al.*, 1977) with minor modifications. Each rat was placed individually in a transparent cylindrical tank, measuring 40 cm in height and 18 cm in diameter, containing water maintained at $25\pm 1^\circ\text{C}$ to a depth of 15 cm. Each session lasted 6 min, where the initial 2 min were considered an acclimatisation period and immobility time was recorded during the final 4 min. Immobility was defined as the absence of active escape-directed movements, with only minimal movements necessary to keep the head above the water surface.

A reduction in immobility time compared with the control group was interpreted as an antidepressant-like effect (Porsolt *et al.*, 1977).

Tail Suspension Test (TST)

The Tail Suspension Test was conducted following the method described by (Steru *et al.*, 1985), with minor adaptations for rats. Animals were suspended individually by the tail using adhesive tape fixed approximately 1 cm from the tail tip to a horizontal bar positioned 58 cm above the surface. The total duration of the test was 6 min, during which immobility time was recorded. Immobility was defined as the absence of limb and body movements. A decrease in immobility time relative to the vehicle-treated control group was considered indicative of antidepressant-like activity (Steru *et al.*, 1985).

Cook's Pole Climbing Apparatus (CPCA)

Conditioned avoidance behaviour was assessed using the Cook's Pole Climbing Apparatus as described previously (Cook and Weidley, 1958; Cook, 1966). The apparatus consisted of a sound-attenuated chamber equipped with a vertical pole (30 cm height \times 2 cm diameter), a buzzer as the conditioned stimulus, and a grid floor capable of delivering a mild electric foot shock as the unconditioned stimulus.

Animals were trained for 10 trials per day for 10 consecutive days. Each trial consisted of a 5 s buzzer signal, and climbing the pole during this period was recorded as a conditioned avoidance response. Failure to climb the pole during the buzzer period resulted in the delivery of a mild foot shock. The inter-trial interval was maintained at 1 min. On Day 10, behavioural assessment was performed by recording the latency to climb the pole in seconds and the number of conditioned avoidance responses during 10 trials. An increase in latency to climb and a reduction in conditioned avoidance responses compared with the control group were interpreted as modulation of conditioned avoidance and escape behaviour (Cook and Weidley, 1958; Slikker and Griffith, 1976).

Statistical Analysis

Statistical analysis was primarily performed using GraphPad Prism, Version 10.1.2. IBM SPSS Statistics for Windows, Version 27, was used for confirmatory analysis. Data were expressed as mean \pm SEM. Group differences were analysed using one-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons. A p -value < 0.05 was considered statistically significant.

RESULTS

Overview

A total of 40 albino Wistar rats completed the study ($n=5$ per group). All animals tolerated the 10-day oral treatment schedule

without mortality or observable signs of toxicity. Behavioural data are expressed as mean±SEM. Group-wise comparisons for each behavioural endpoint were analysed using one-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test, with $p<0.05$ considered statistically significant.

Forced Swim Test (FST)

Rats in the vehicle control group exhibited prolonged immobility (132.0±5.9 s), indicating a depressive-like behavioural phenotype. Imipramine (10 mg/kg, p.o.) produced a robust antidepressant-like effect, significantly reducing immobility time by approximately 52% compared with control (62.6±6.6 s; $p<0.001$), as shown in Figure 1. Among the *Emblca officinalis* preparations, the ethyl acetate fraction demonstrated the greatest reduction in immobility (83.0±4.2 s; $p<0.001$ vs control), followed by the methanol fraction (87.4±4.6 s; $p<0.001$) and the crude ethanolic extract (93.6±4.4 s; $p<0.05$). In contrast, the petroleum ether (108.0±5.9 s), benzene (104.0±6.1 s), and acetone fractions (113.0±7.7 s) produced comparatively weaker reductions in immobility (Figure 1). Overall group differences were significant ($F(7, 32)=65.97, p<0.0001$).

Tail Suspension Test (TST)

In the Tail Suspension Test, vehicle-treated rats exhibited prolonged immobility, reflecting a depressive-like behavioural state. One-way ANOVA revealed a significant difference in immobility time among experimental groups ($F(7, 32)=175.7, p<0.0001$; Figure 2). Imipramine (10 mg/kg, p.o.) produced a marked and statistically significant reduction in immobility time (51.0±4.4 s), confirming the validity and sensitivity of the model. Among the test samples, the ethyl acetate fraction produced the greatest reduction in immobility (60.4±2.1 s), followed by the methanol fraction (65.0±1.6 s) and the crude ethanolic extract (70.0±1.6 s), all of which were significantly different from the vehicle control (Figure 2). The petroleum ether, benzene, and acetone fractions showed relatively smaller reductions and exhibited limited antidepressant-like activity. Overall, the rank order of antidepressant-like activity in the TST was:

Imipramine>Ethyl acetate fraction>Methanol fraction>Ethanolic extract.

Cook's Pole Climbing Apparatus (CPCA)

In the Cook's Pole Climbing Apparatus test, vehicle-treated rats exhibited short latency to climb the pole and a high number of Conditioned Avoidance Responses (CARs), indicating intact conditioned avoidance behaviour. One-way ANOVA revealed highly significant differences among treatment groups for both latency to climb ($F(7, 32)=106.1, p<0.0001$; Figure 3) and number of conditioned avoidance responses ($F(7, 32)=110.7, p<0.0001$; Figure 4). Imipramine (10 mg/kg, p.o.) produced the maximum increase in latency to climb (9.40±0.55 s) along with the lowest number of CARs (2.40±0.55), confirming the sensitivity and validity of the model. Among the *Emblca officinalis* preparations, the crude ethanolic extract produced a marked modulation of conditioned avoidance and escape behaviour, evidenced by a significant increase in latency to climb (8.60±0.55 s; Figure 3) and a significant reduction in CARs (3.20±0.45; Figure 4) compared with the control group. The methanol fraction (latency: 7.40±0.55 s; CARs: 4.60±0.55) and ethyl acetate fraction (latency: 6.40±0.55 s; CARs: 5.60±0.55) also produced significant but comparatively smaller effects. In contrast, the petroleum ether, benzene, and acetone fractions produced relatively modest changes and remained closer to control values. Overall, the efficacy pattern in the CPCA followed the order: Imipramine ≈ Ethanolic extract>Methanol fraction>Ethyl acetate fraction.

Overall Efficacy Pattern

Across all three behavioural paradigms, imipramine produced the most pronounced antidepressant-like effects. Among the test samples, mid-polarity fractions, particularly ethyl acetate and methanol, demonstrated the most consistent and robust activity in the Forced Swim Test and Tail Suspension Test, whereas the crude ethanolic extract showed the strongest modulation of conditioned avoidance behaviour in the CPCA. Less polar fractions exhibited comparatively weak or inconsistent effects (Table 2).

Table 1: Study design and treatment groups.

Group	Treatment	Dose	Route
I	Vehicle control (0.5% CMC)	1 mL/100 g	Oral
II	Imipramine (standard drug)	10 mg/kg	Oral
III	Ethanolic fruit extract of <i>Emblca officinalis</i> (Amla)	500 mg/kg	Oral
IV	Petroleum ether fraction of <i>Emblca officinalis</i>	500 mg/kg	Oral
V	Benzene fraction of <i>Emblca officinalis</i>	500 mg/kg	Oral
VI	Ethyl acetate fraction of <i>Emblca officinalis</i>	500 mg/kg	Oral
VII	Acetone fraction of <i>Emblca officinalis</i>	500 mg/kg	Oral
VIII	Methanol fraction of <i>Emblca officinalis</i>	500 mg/kg	Oral

DISCUSSION

The present study systematically evaluated the antidepressant-like activity of the ethanolic extract of *Emblica officinalis* (Amla) and its polarity-based solvent fractions using three validated behavioural paradigms in rodents. The findings demonstrate that *Emblica officinalis* exhibits significant antidepressant-like effects, with marked variation across fractions depending on solvent polarity. Across all behavioural tests, imipramine produced the most robust effects, thereby confirming the sensitivity and validity of the experimental models employed. Among the test samples, mid-polarity fractions, particularly the ethyl acetate and methanol fractions, displayed the most consistent and pronounced antidepressant-like activity. These were followed by the crude ethanolic extract, whereas non-polar fractions showed comparatively weaker effects (Gupta *et al.*, 2012; Kabra *et al.*, 2022; Rao *et al.*, 2021).

Interpretation of Forced Swim and Tail Suspension Test Findings

The Forced Swim Test (FST) and Tail Suspension Test (TST) are well-established behavioural despair models widely used for screening antidepressant activity and are particularly sensitive to agents that enhance monoaminergic neurotransmission (Porsolt *et al.*, 1977; Steru *et al.*, 1985). In the present study, imipramine significantly reduced immobility time in both tests, which is shown in Figures 1 and 2. This result is consistent with its established mechanism of inhibiting serotonin and noradrenaline reuptake (Ali and Engidawork, 2022; Porsolt *et al.*, 1977; Steru *et al.*, 1985).

Among the *Emblica officinalis* preparations, the ethyl acetate fraction produced the greatest reduction in immobility time in both the FST and TST, as shown in Figures 1 and 2, followed by the methanol fraction and the crude ethanolic extract. The consistency

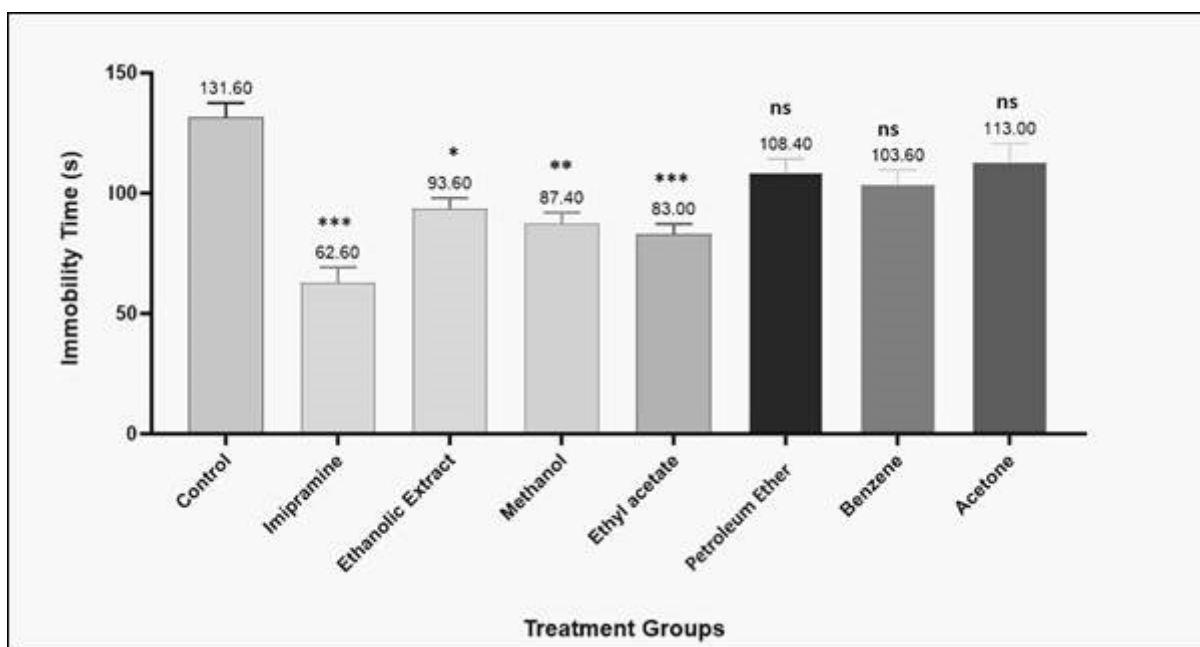


Figure 1: Effect of *Emblica officinalis* extract and solvent fractions on immobility time in the Forced Swim Test (FST) in rats. Values are expressed as Mean±SEM (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle control; ns=not significant. FST: Forced Swim Test; SEM: Standard Error of Mean.

Table 2: Effect of *Emblica officinalis* extract and solvent fractions on behavioural parameters in rats.

Group	Treatment	FST	TST	CPCA	CPCA
	(Dose, p.o.)	Immobility (s)	Immobility (s)	Latency (s)	CARs (n)
I	Vehicle control (0.5% CMC)	132.0±5.9	100.0±3.8	2.4±0.5	9.6±0.5
II	Imipramine (10 mg/kg)	62.6±6.6***	51.0±4.4***	9.4±0.5***	2.4±0.5***
III	Ethanolic extract	93.6±4.4*	70.0±1.6*	8.6±0.5**	3.2±0.4**
IV	Petroleum ether fraction	108.0±5.9 ns	80.0±2.0 ns	5.2±0.4 ns	6.6±0.5 ns
V	Benzene fraction	104.0±6.1 ns	74.8±1.5 ns	4.6±0.5 ns	7.6±0.5 ns
VI	Ethyl acetate fraction	83.0±4.2***	60.4±2.1***	6.4±0.5*	5.6±0.5*
VII	Acetone fraction	113.0±7.7 ns	84.8±1.9 ns	3.4±0.5 ns	8.4±0.5 ns
VIII	Methanol fraction	87.4±4.6***	65.0±1.6**	7.4±0.5**	4.6±0.5**

Values are expressed as Mean±SEM (n=5). One-way ANOVA followed by Tukey's *post hoc* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs vehicle control; ns = not significant.

of these effects across two independent despair-based paradigms strengthens the interpretation of a genuine antidepressant-like effect rather than nonspecific behavioural stimulation. In contrast, the petroleum ether, benzene, and acetone fractions produced relatively modest effects, suggesting that non-polar constituents contribute minimally to the antidepressant profile of *Emblca officinalis*.

The superior performance of the mid-polarity fractions is pharmacologically plausible, as polyphenols, flavonoids, tannins, and phenolic acids, which are well-documented constituents of *Emblca officinalis*, are preferentially enriched in ethyl acetate and methanol fractions. These phytoconstituents have been reported to modulate monoaminergic neurotransmission, attenuate oxidative stress, and suppress neuroinflammatory pathways, all of which are centrally implicated in the pathophysiology of depression (Baliga and Dsouza, 2011; Lopresti *et al.*, 2014; Maes *et al.*, 2011; Miller and Raison, 2016; Sundaram *et al.*, 2014).

Cook's Pole Climbing Apparatus: Complementary Behavioural Insights

The Cook's Pole Climbing Apparatus (CPCA) provides complementary behavioural information by assessing conditioned avoidance responses and latency to escape, thereby reflecting motivational and cognitive components of behaviour. In the present study, imipramine significantly increased latency to climb and markedly reduced Conditioned Avoidance Responses (CARs), as shown in Figures 3 and 4. These findings are consistent with its known suppressive effects on avoidance behaviour (Ali and Engidawork, 2022).

The crude ethanolic extract produced a latency to climb comparable to imipramine, indicating a substantial modulatory influence on conditioned escape behaviour. The methanol and ethyl acetate fractions also significantly increased latency and reduced avoidance responses, although to a lesser extent. These observations suggest that different behavioural endpoints assessed in the CPCA may be differentially influenced by the phytochemical composition of the extract and its fractions.

Although suppression of conditioned avoidance responses is classically associated with antipsychotic activity, several antidepressants, particularly tricyclic antidepressants such as imipramine, are also known to inhibit avoidance behaviour owing to their complex central pharmacodynamic actions (Ali and Engidawork, 2022; Slikker and Griffith, 1976). Accordingly, CPCA outcomes in the present study were interpreted as supportive rather than standalone evidence of antidepressant-like activity. Importantly, these CPCA effects occurred alongside consistent reductions in immobility in both the FST and TST, arguing against nonspecific motor impairment or sedation as the primary explanation for the observed behavioural changes (Cook, 1966; Slikker and Griffith, 1976).

Polarity-Based Fractionation and Pharmacological Implications

A notable strength of the present investigation is the use of polarity-based fractionation, which permits inference regarding the chemical nature of the bioactive constituents. The consistent superiority of ethyl acetate and methanol fractions across behavioural paradigms strongly suggests that

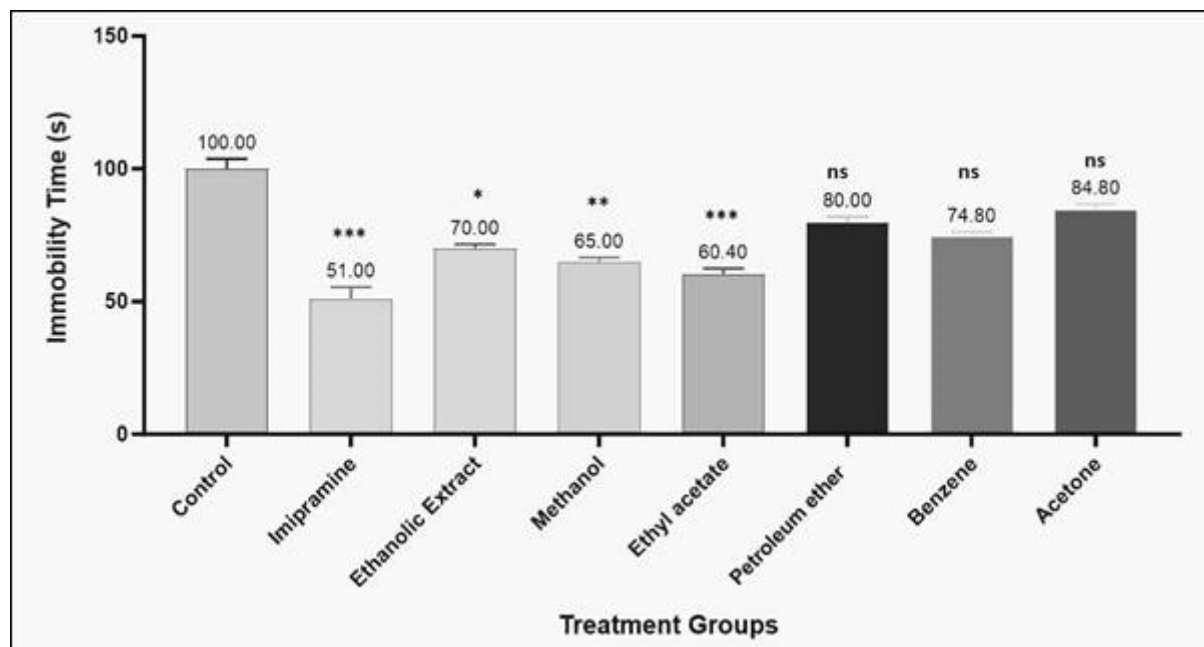


Figure 2: Effect of *Emblca officinalis* extract and solvent fractions on immobility time in the Tail Suspension Test (TST) in rats. Values are expressed as Mean±SEM ($n=5$). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. vehicle control; ns=not significant. TST: Tail Suspension Test; SEM: Standard Error of Mean. Imipramine ≈ Ethanolic extract>Methanol fraction>Ethyl acetate fraction.

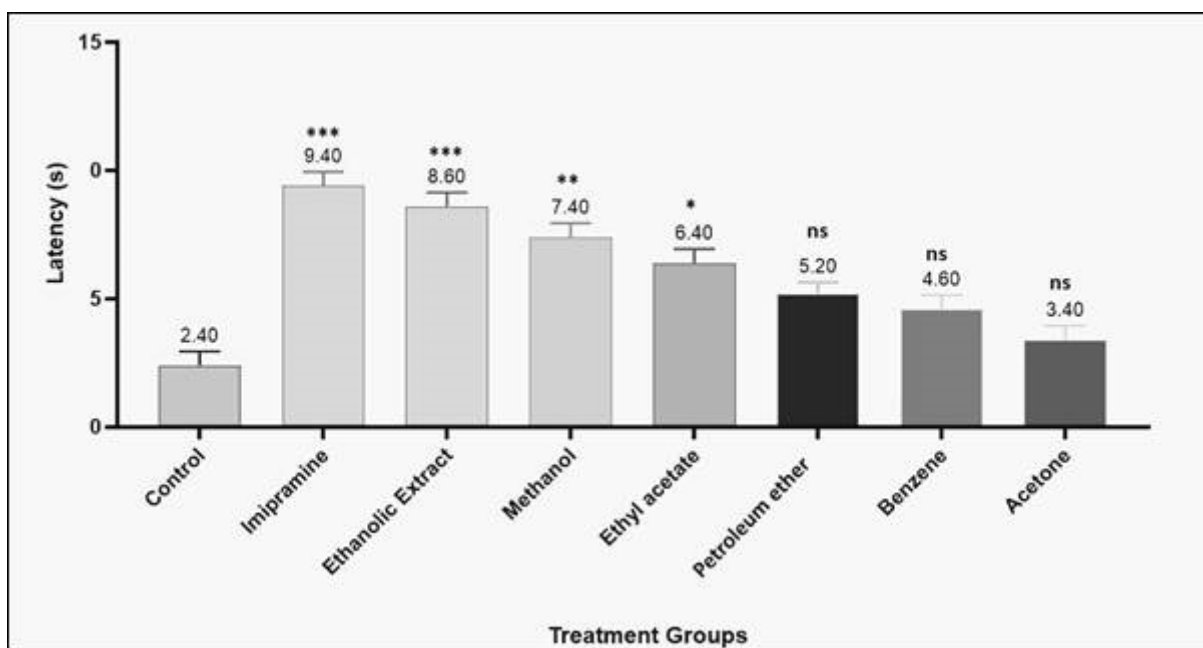


Figure 3: Effect of *Emblica officinalis* extract and solvent fractions on latency to climb in the Cook's Pole Climbing Apparatus (CPCA) test in rats. Values are expressed as Mean±SEM (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle control; ns=not significant. CPCA: Cook's Pole Climbing Apparatus; SEM: Standard Error of Mean.

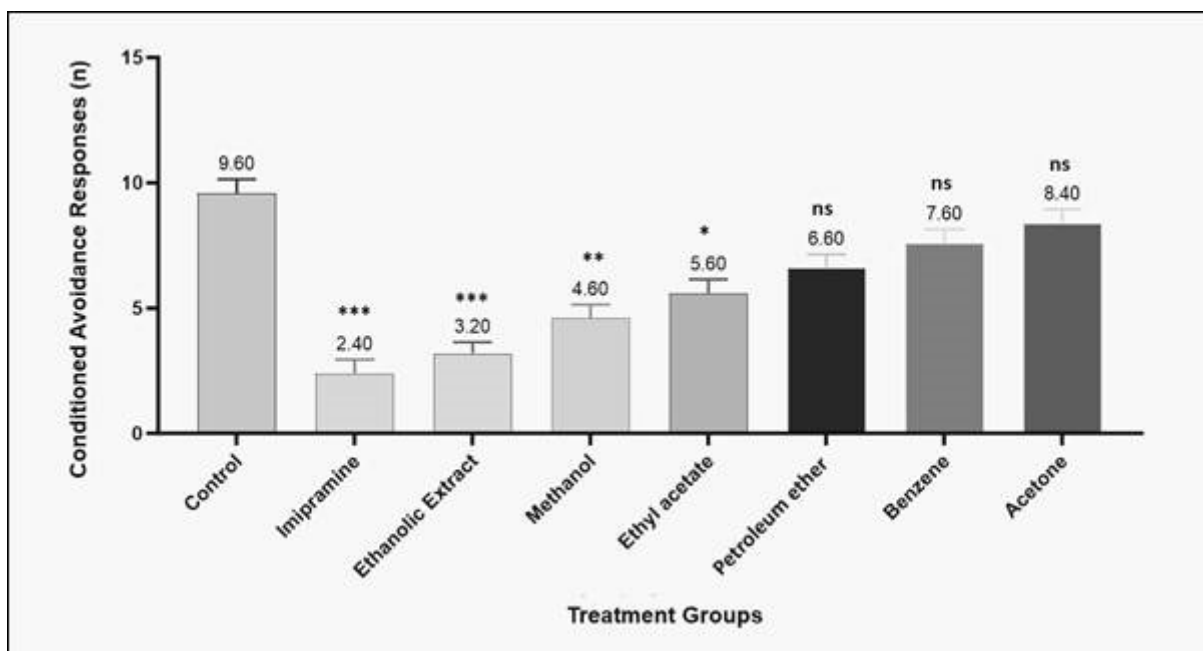


Figure 4: Effect of *Emblica officinalis* extract and solvent fractions on conditioned avoidance responses in the Cook's Pole Climbing Apparatus (CPCA) test in rats. Values are expressed as Mean±SEM (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle control; ns=not significant. CPCA: Cook's Pole Climbing Apparatus; CARs: Conditioned Avoidance Responses; SEM: Standard Error of Mean.

medium-polarity phytoconstituents are primarily responsible for the antidepressant-like effects of *Emblica officinalis*. In contrast, non-polar fractions exhibited minimal or inconsistent activity, indicating limited involvement of lipophilic constituents.

The crude ethanolic extract demonstrated robust and reproducible effects across all tests, supporting the traditional use of whole-plant preparations. However, the enhanced activity observed with specific fractions highlights the potential value

of fraction-guided isolation and standardisation for future phytopharmaceutical development.

Limitations and Future Directions

Despite the encouraging findings, certain limitations warrant consideration. The study relied exclusively on behavioural models without accompanying biochemical or neurochemical analyses to elucidate underlying mechanisms. Only a single dose

of each extract and fraction was evaluated, precluding assessment of dose-response relationships, and detailed phytochemical characterisation of individual fractions was not performed. Additionally, the relatively small sample size per group, although consistent with standard pharmacological screening studies, may limit broader generalisability.

Future investigations should focus on comprehensive phytochemical profiling of the active fractions, identification of bioactive constituents, and evaluation of dose-response relationships. Incorporation of biochemical markers related to monoaminergic transmission, oxidative stress, and neuroinflammation, along with testing in chronic stress-based models of depression, would further strengthen mechanistic understanding and translational relevance.

CONCLUSION

Embolica officinalis exhibited significant antidepressant-like activity in validated rodent behavioural paradigms. Ethyl acetate and methanol fractions demonstrated the most consistent effects in despair-based models, while the crude ethanolic extract showed broader behavioural modulation. These findings support further phytochemical standardisation and mechanistic evaluation of *Embolica officinalis* as a potential source of antidepressant agents.

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ABBREVIATIONS

ANOVA: Analysis of Variance; **CARs:** Conditioned Avoidance Responses; **CMC:** Carboxymethylcellulose; **CPCA:** Cook's Pole Climbing Apparatus; **FST:** Forced Swim Test; **HPA:** Hypothalamic-Pituitary-Adrenal Axis; **p.o.:** Per Oral (Oral Administration); **SEM:** Standard Error of the Mean; **TST:** Tail Suspension Test; **CNS:** Central Nervous System; **MDD:** Major Depressive Disorder.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

- Dr. Shariq Ahmad Azmi: Conceptualization, Investigation, Formal Analysis, Writing - Original Draft.
- Prof. Syed Ziaur Rahman: Methodology, Supervision, Resources.
- Prof. Mehtab Parveen: Validation, Writing - Review & Editing.

SUMMARY

This study systematically evaluated the antidepressant-like activity of *Embolica officinalis* fruit extract and its polarity-based fractions in Wistar rats. Medium-polarity fractions, specifically ethyl acetate and methanol, demonstrated the most significant reduction in immobility in despair-based models, whereas the crude extract showed broader modulation in conditioned avoidance behaviour. These findings suggest that medium-polarity phytoconstituents are primary drivers of the plant's antidepressant-like effects and warrant further phytochemical characterization.

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