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Behavioural Evaluation of *Pancratium maritimum L.* (Amaryllidaceae) Ethanol bulb Extract Reveals Dose-Dependent Antidepressant-Like Effects in Murine Models.

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Abstract

Depression remains a major global health challenge., Limitations associated with current pharmacotherapies drive the search for novel, safer antidepressant agents from natural sources. *Pancratium maritimum* is a medicinal plant rich in bioactive secondary metabolites. This study evaluated the antidepressant-like effects of the ethanol extract of *Pancratium maritimum* bulbs in murine models and characterized its phytochemical constituents. Swiss albino mice were treated intraperitoneally with the extract at doses of 150, 300, and 600 mg/kg, while imipramine (15 mg/kg) served as the positive control. Antidepressant activity was assessed using the forced swim test (FST) and tail suspension test (TST). Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids/steroids, carbohydrates, and cardiac glycosides. In the TST, the extract significantly reduced immobility time from 134.06 ± 0.03 s (control) to 127.43 ± 0.03 s, 114.27 ± 0.06 s, and 86.04 ± 0.04 s at 150, 300, and 600 mg/kg, respectively. Similarly, in the FST, immobility time decreased from 148.76 ± 5.6 s (control) to 129.74 ± 4.59 s, 118.56 ± 4.56 s, and 69.25 ± 3.2 s across the same dose range. Imipramine produced marked reductions in immobility (69.22 ± 0.02 s in TST and 38.31 ± 3.5 s in FST). These findings demonstrate a clear dose-dependent antidepressant-like effect of *Pancratium maritimum* bulb ethanol extract, likely mediated by its rich phytochemical profile. The study provides novel evidence supporting *Pancratium maritimum* as a promising source of natural antidepressant agents.

Keywords: *Pancratium maritimum*; antidepressant-like activity; forced swim test; tail suspension test; phytochemical screening

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Introduction

Depression is a major neuropsychiatric disorder characterized by persistent low mood, anhedonia, cognitive impairment, and altered psychomotor activity, and it represents a leading cause of global disability (WHO 2017; Malhi and Mann, 2018; Fan *et al* 2025). Despite the availability of several pharmacological treatments, including tricyclic antidepressants and selective monoamine reuptake inhibitors, current therapies are often associated with delayed onset of action, incomplete remission, and undesirable adverse effects (Rush *et al*., 2006; Andrade *et al*., 2015; Uyar and Gonul 2025). Medicinal plants remain a valuable reservoir of bioactive compounds, many of which exert central nervous system effects through antioxidant, neuroprotective, and neuromodulatory mechanisms (Scapagnini *et al.,* 2012; Yin *et al*., 2024). In this perspective, members of the Amaryllidaceae family have gained significant interest due to their rich alkaloid content and various biological functions (Meerow and Snijman, 1998; Cedrón *et al*., 2010). The genus Pancratium is one of the most widely distributed genera within the Amaryllidaceae family, occurring across Africa, Southern Europe, the Middle East, and parts of Asia (Meerow *et al*., 2006). Among its species, *Pancratium maritimum* commonly known as sea daffodil or marine narcissus, is a bulbous perennial plant adapted to coastal sand dune ecosystems of the Mediterranean and Black Sea regions (Davis, 1984; Perrone *et al*., 2015). Beyond its ecological importance, *Pancratium maritimum* has an extensive track record in phytochemical investigation due to the unique diversity of secondary metabolites revealed in its bulbs. The first phytochemical investigations on the species were carried out in the mid-twentieth century by Du Merac (1954), who reported the presence of characteristic Amaryllidaceae alkaloids. Subsequent studies have consistently demonstrated that *Pancratium maritimum* produces a wide spectrum of alkaloids belonging to the lycorine, lycorenine, galanthamine, crinine, haemanthidine, tazettine, montanine, pancratistatin, narciclasine, and phenanthridone structural groups, all biosynthetically derived from norbelladine precursors (Cedrón *et al*., 2010).

In addition to alkaloids, *Pancratium maritimum* bulbs and aerial parts contain several non-alkaloidal phytochemicals, including flavonoids, chromones, phenolic acids, phenylpropanoids, and alginates (Ali *et al*., 1990; Youssef *et al*., 1998; Sanaa *et al*., 2010; Rokbeni *et al*., 2016; Youssef *et al*., 2022). These constituents contribute to the wide range of biological activities reported for the extracts and isolated compounds from the plant. Prior investigations have shown the cytotoxic, antibacterial, antiviral, antimalarial, antioxidant, antinociceptive, amoebic, and acetylcholinesterase inhibitory activities of *Pancratium maritimum* extracts and purified alkaloids (Çakici *et al*., 1997; Sener *et al*., 2003; Hetta and Shafei, 2013; Rokbeni *et al*., 2016; Leporini *et al*., 2018; Masi *et al*., 2022). Notably, antioxidant and anti-inflammatory activities have been emphasized, highlighting the ability of *P. maritimum* ethanol bulb extracts to reduce reactive oxygen species and exert protective effects in cellular models without inducing cytotoxicity (Cicio *et al*., 2023).

Despite the extensive phytochemical and pharmacological profiling of *Pancratium maritimum*, its potential effects on depression-related behaviours remain poorly explored. Several Amaryllidaceae alkaloids, including galanthamine-type and lycorine-type compounds, are known to interact with central neurotransmitter systems and to exhibit neuroactive properties, as evidenced by acetylcholinesterase inhibition and other neuromodulatory actions reported for *Pancratium maritimum* constituents (Orhan and Sener, 2003; Bozkurt *et al*., 2019). Therefore, the present study aimed to evaluate the antidepressant effects of the ethanol extract of *Pancratium maritimum* bulbs using established murine behavioural paradigms, namely the forced swim test and tail suspension test.

Materials and Methods

Drugs, Chemicals, and Reagents

The following substances were used in the study; imipramine hydrochloride, normal saline (0.9%), distilled water, 70% ethanol, Molisch's reagent, concentrated sulphuric acid, ferric chloride solution, lead sub-acetate solution, chloroform, glacial acetic acid, ammonium solution, and other analytical-grade chemicals.

Equipment and Apparatus

Laboratory equipment used, include weighing balance, mortar and pestle, evaporating dish, water bath, stopwatch, glassware (beakers, test tubes, pipettes), syringes and needles, filter paper, and personal protective equipment.

Plant Collection and Authentication

Fresh bulbs of *Pancratium maritimum* were collected from the Galma River edge along the shores of Oxbow Lake, Zaria, Nigeria. Plant identification and authentication were carried out at the Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria. A voucher specimen (KASU/BSH/675) was deposited at the Department.

Preparation and Extraction of Plant Material

The collected bulbs of *Pancratium maritimum* were thoroughly cleaned, air-dried, and pulverized into a fine powder using a mortar and pestle. Two hundred grams (200 g) of the powdered bulb material were subjected to cold maceration using 1 L of 70% ethanol for 48 hours with intermittent shaking. The extract was filtered, and the filtrate was concentrated on a water bath at 50 °C until a

brownish, semi-solid residue was obtained. The dried ethanol bulb extract was stored at 4°C for subsequent chemical analysis. Fresh solutions of the extract were prepared with distilled water prior to each experimental procedure.

Experimental Animals

Swiss albino mice (18–39 g) of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Toxicology, Kaduna State University. The animals were housed in polypropylene cages under standard laboratory conditions with a natural light–dark cycle and allowed free access to standard pellet feed and water ad *libitum*. All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals, as outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH, 1996).

Phytochemical Screening

Preliminary phytochemical screening of the extract was carried out in the Department of Pharmacognosy and Drug Development using standard qualitative methods as described by Trease and Evans (1996) and Edeoga *et al*. (2005).

Antidepressant Activity Studies

Twenty-five (25) mice were randomly assigned into five groups (n = 5 per group). Group I received normal saline (0.9%) and served as the negative control. Groups II, III, and IV received ethanol bulb extract of *Pancratium maritimum* at doses of 150, 300, and 600 mg/kg body weight, respectively. Group V received imipramine hydrochloride (15 mg/kg) as the positive control. All treatments were administered intraperitoneally, and a 30-minute interval was allowed before behavioural testing.

Tail Suspension Test

The tail suspension test was performed according to Can et al., (2012) with slight modification. Mice were suspended individually by the tail using adhesive tape fixed approximately 1 cm from the tail tip and hung 16 cm above a flat surface. Each test session lasted 5 minutes, during which the duration of immobility was recorded using a stopwatch. Immobility was defined as the absence of initiated movements, with the animal hanging passively and completely motionless.

Forced Swim Test

The forced swim test was conducted using cylindrical containers (30 cm height × 20 cm diameter) filled with water at room temperature (23–25 °C) to a predetermined level. Mice were gently placed into the water and observed for 6 minutes. The total duration of immobility was recorded, defined as the time during which the animal remained floating without struggling and made only minimal movements necessary to keep the head above water (Yankelevitch-Yahav *et al*., 2015).

Statistical Analysis

The experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test for multiple comparisons. Differences were considered statistically significant at $P < 0.05$.

Results

Phytochemical Composition of Pancratium maritimum Ethanol Bulb Extract

Qualitative phytochemical screening of the ethanol bulb extract of *Pancratium maritimum* revealed the presence of multiple bioactive secondary metabolites (Table 1). The extract tested positive for carbohydrates, saponins, flavonoids, tannins, terpenoids/steroids, alkaloids, cardiac glycosides, and phenolic compounds. Anthraquinones were not detected in the extract.

Effect of the Extract on Immobility Time in the Forced Swim Test

In the forced swim test, treatment with the extract resulted in a significant and dose-dependent decrease in immobility time compared with the control group (Table 2, Figure 1). At doses of 150 mg/kg and 300 mg/kg, the extract significantly reduced immobility duration ($P < 0.05$), while the 600 mg/kg dose elicited a more substantial reduction ($P < 0.01$). Imipramine (15 mg/kg) produced a marked and statistically significant decrease in immobility time relative to the control group.

Effect of the Extract on Immobility Time in the Tail Suspension Test

Administration of the extract caused significant reduction in immobility time in the tail suspension test when compared with the normal saline–treated control group (Table 2, Figure 2). Mice treated with 150 mg/kg and 300 mg/kg of the extract showed a statistically significant decrease in immobility duration ($P < 0.05$). The highest dose (600 mg/kg) produced a more pronounced reduction in immobility time ($P < 0.01$), demonstrating a clear dose-dependent effect. The standard antidepressant drug imipramine (15 mg/kg) produced the greatest reduction in immobility time.

Table 1: Phytochemical constituents of the extracts

Phytoconstituents	Inferences
Carbohydrates	+
Saponins	+
Flavonoids	+
Tannis	+
Terpenoids/steroids	+
Alkaloids	+
Anthraquinones	–
Cardiac glycosides	+
Phenolic compounds	+

Presence (+), absence (–).

Table 2: Effect of the Extract on immobility time in Forced swim test and Tail Suspension test

Treatment	Dose (mg/Kg)	Tail Suspension test Duration of Immobility (Sec)	Forced Swim test Duration of Immobility (Sec)
Normal saline	Control	134.06±0.03	148.76±5.6
Extract	150 mg/kg	127.43±0.03*	129.74±4.59*
Extract	300 mg/kg	114.27±0.06*	118.56±4.56*
Extract	600 mg/kg	86.04±0.04**	69.25±3.2**
Imipramine	15 mg/kg	69.22±0.02**	38.31±3.5**

Value is expressed as mean ± SEM (n=5). *P < 0.05, **P < 0.01 as compared with the control group.

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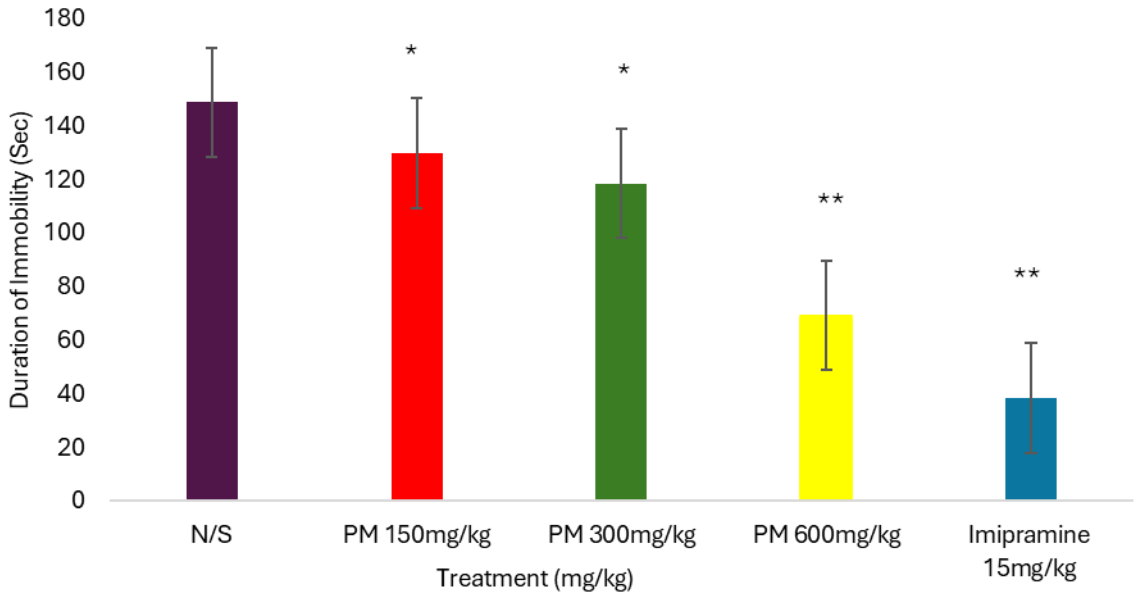

Figure 1: Antidepressant effect of *Pancratium maritimum* extract on force swim test. Data expressed as mean ± SEM; significance indicated as *P < 0.05, **P < 0.01 as compared with the control group. N/S= Normal Saline, PM= *Pancratium maritimum* Extract

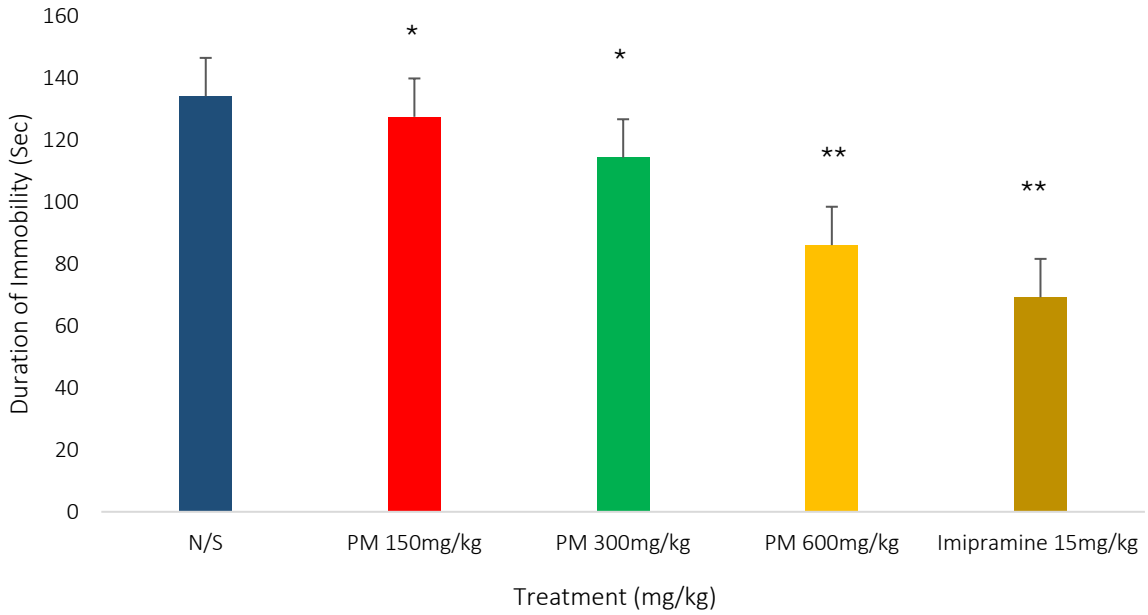

Figure 2: Antidepressant effect of *Pancratium maritimum* extract on Tail suspension test. Data expressed as mean ± SEM; significance indicated as *P < 0.05, **P < 0.01 as compared with the control group. N/S= Normal Saline, PM= *Pancratium maritimum* Extract

Discussion

The present study provides the behavioural evidence that the ethanol extract of *Pancratium maritimum* bulbs elicits significant antidepressant-like effects in murine models, as demonstrated by reductions in immobility time in both the forced swim test (FST) and tail suspension test (TST). These paradigms are widely accepted for evaluating antidepressant efficacy, as decreased immobility reflects enhanced active coping strategies and motivational drive rather than nonspecific motor stimulation, a conclusion supported by studies demonstrating consistent pharmacological responsiveness and construct relevance of active versus passive behaviours in these tests (Berrocoso, *et al*., 2013; Della Valle *et al*., 2025).

Comparison of the behavioural outcomes in both the tail suspension and forced swim tests demonstrated a consistent pattern of antidepressant-like activity for the ethanol bulb extract of *Pancratium maritimum*. In both models, the reduction in immobility time increased progressively with dose. Although the extract did not achieve the same magnitude of effect as imipramine, the highest dose exhibited a substantial behavioural response, suggesting significant central nervous system activity. The dose-dependent pattern observed across both models strengthens the pharmacological relevance of the extract, particularly at 600 mg/kg, where behavioural outcomes approached those produced by the reference antidepressant imipramine. Importantly, consistency between the FST and TST outcomes suggests that the extract may exert robust central nervous system effects. The behavioural effects can be interpreted in light of the phytochemical profile identified in the extract. Qualitative screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids/steroids, and cardiac glycosides., Amaryllidaceae alkaloids represent a particularly relevant class, as *P. maritimum* is well known for producing structurally diverse alkaloids derived from norbelladine precursors (Cedrón *et al*., 2010). Previous phytochemical investigations of *P. maritimum* bulbs have consistently identified lycorine-type, crinine-type, galanthamine-type, and phenanthridone alkaloids (Berkov *et al*., 2004; Bozkurt *et al*., 2019; Cicio *et al*., 2023). Several of these alkaloids exhibit acetylcholinesterase inhibitory activity and neuromodulatory properties, suggesting plausible interactions with monoaminergic and cholinergic neurotransmission, systems that are centrally implicated in the pathophysiology of depression (Orhan and Şener 2003; Soltan *et al.,* 2015).

Beyond the alkaloids, the presence of flavonoids and phenolic compounds may further explain the behavioural outcomes. Depression is increasingly recognized to be associated with increased oxidative stress and neuroinflammation, as well as impaired neuronal plasticity, and recent preclinical studies showed that plant-derived antioxidants, such as date fruit extracts and flavonoids, may ameliorate depressive-like behaviours by reducing oxidative damage, suppressing inflammatory responses, and protecting neuronal integrity (Asdaq *et al*., 2025). Phoenix dactylifera (Ajwa date) extract, for example, exhibited antioxidant and neuroprotective effects that improved behavioural outcomes and attenuated oxidative stress and neuroinflammation in a chronic mild stress model of depression (Asdaq *et al*., 2025), whereas the flavonoid luteolin reduced neuroinflammation and depressive-like behaviours by modulating microglial polarization and inflammatory signalling (Yuan *et al*., 2025). Ethanol extracts of *Pancratium maritimum* bulbs have previously been shown to significantly reduce reactive oxygen species production without inducing cytotoxicity in cellular models, highlighting their antioxidant capacity (Rokbeni *et al.,* 2016; Cicio *et al*., 2023). Such antioxidant effects may indirectly enhance monoaminergic signalling and stress resilience, thereby reducing behavioural despair in animal models. Flavonoids have been shown to modulate key intracellular signalling pathways linked to neurotrophic factors and synaptic plasticity, including Brain-Derived Neurotrophic Factor (BDNF)/TrkB-mediated cascades, which are increasingly recognized as critical mechanisms underlying antidepressant responses (Wang *et al*., 2025). For example, dietary flavonoid-rich anthocyanins enhanced BDNF expression and associated TrkB signalling to improve depressive-like behaviours in a chronic stress model, implicating synaptic function and neuroplasticity in their antidepressant-like effects (Wang *et al*., 2025). Thus, the presence of both alkaloids and polyphenolic compound in the ethanol bulb extract may provide a biologically acceptable rationale for the dose-dependent behavioural effects observed in this study.

The progressive reduction in immobility time with increasing extract doses further suggests that the antidepressant-like activity of *P. maritimum* is dose dependent, consistent with a pharmacodynamic relationship rather than an incidental behavioural change. While the extract did not surpass the efficacy of imipramine, its substantial effect at higher doses is notable given the complexity of crude plant extracts and the likelihood of multiple active constituents acting concurrently. This observation aligns with previous pharmacological studies reporting significant bioactivity of *P. maritimum* extracts across diverse biological systems, including antinociceptive, antioxidant, antimicrobial, and acetylcholinesterase inhibitory effects (Çakici *et al*., 1997; Leporini *et al*., 2018; Bozkurt *et al*., 2019). Importantly, no behavioural signs suggestive of sedation or motor impairment, indicating that the reductions in immobility may unlikely be as a result of nonspecific alterations in locomotor activity.

Conclusion

The present study demonstrates that the ethanol extract of *Pancratium maritimum* bulbs produces significant, dose-

dependent antidepressant-like effects in murine models, as evidenced by reduced immobility time in the forced swim and tail suspension tests. These behavioural outcomes validate the central nervous system activity of the extract and support its psychopharmacological relevance. The effects are plausibly linked to the rich phytochemical composition of the extract, particularly alkaloids, flavonoids, and phenolic compounds, which are known to exert neuromodulatory and antioxidant actions. Collectively, these findings provide novel experimental evidence supporting the antidepressant potential of *Pancratium maritimum* and justify further investigations aimed at isolating active constituents, elucidating mechanisms of action, and assessing long-term safety and therapeutic applicability.

Conflicts of Interest

The authors declare no conflicts of interest

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