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Research Article

Antimicrobial and CNS depressant activities of *Premna obtusifolia* R. Br. (Lamiaceae)

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Abstract
People in Bangladesh's rural areas have a long tradition of treating various diseases with medicinal plants. To investigate antimicrobial and central nervous system (CNS) depressant activity of *Premna obtusifolia* R. Br., the crude ethanolic extract of the whole plant with its various fractions (*n*-hexane, chloroform, and methanol soluble fractions) was used. The disc diffusion method was used to conduct an antimicrobial test at a dose of 400 µg/disc. Swiss albino mice's central nervous system activity was examined using the Open Field Test and Hole Cross Test at doses of 150 and 300 mg/kg BW. In the disc diffusion method, the various fractions didn't show prominent antimicrobial activity. In the hole-cross test, a significant reduction ($p < 0.05$) in activity was observed at 30 min only for the higher dose of the crude extract and the methanol-soluble fraction. In contrast, both the *n*-hexane and chloroform-soluble fractions produced significant reductions at both dose levels tested. By 60 and 90 min, all treated groups exhibited statistically significant activity reduction compared to the control. Similarly, in the open-field test, all fractions significantly decreased the number of square visits at the 30-min mark. While the crude extract also reduced visits at 60 and 90 min, this effect was not statistically significant. The other fractions, however, maintained a significant reduction at these later time points. It is evident from the results above that different *P. obtusifolia* fractions have shown strong neuropharmacological potential in various *in vivo* experimental models. Therefore, it will be a possible source for a lead molecule that may be isolated to treat various diseases.

Keywords: antimicrobial activity; bangladesh; CNS depressant; *Premna obtusifolia* characterization, encapsulation

1. INTRODUCTION

Since humanity's beginnings in the prehistoric era of civilization, plants have always been an essential source of medicine. Most developing nations have been noted to use medicinal plants, and around 80% of the world's population uses herbal remedies as their primary form of healthcare [1]. Despite the slow decline in research in this area due to the development of combinatorial chemistry, recent studies suggest that roughly 25–30% of all medications still come from nature [2]. However, because natural products include structures that have been chosen by evolutionary forces over millions of years, they are still regarded as a priceless source of new chemical entities for the creation of effective treatments for some difficult diseases [3][4].

Premna obtusifolia is a member of the Lamiaceae family (Formerly: Verbenaceae). There are 200 species in the *Premna* genus, most of which are found in tropical and subtropical Asia, Africa, Australia, and the Pacific Islands [5]. The plant has long traditional uses in the treatment of different ailments, including rheumatism, eruptive fevers, gripes and flatulence, gonorrhea, convulsion, colic, and neuralgia [6][7]. Significant anti-inflammatory effect was shown by several plant alcohol extracts of this genus [8][9]. Phytochemical investigations of plants within the *Premna* genus indicate a diverse profile of bioactivity. Both crude extracts and purified compounds have been shown to exhibit a range of therapeutic properties, including but not limited to antioxidant, antimicrobial, anti-inflammatory, cytotoxic, immunomodulatory, antidiabetic, antihyperlipidemic, hepatoprotective, and cardioprotective actions [10]. Empirical evidence further substantiates species-specific efficacy; for instance, the root of *Premna integrifolia* demonstrates pronounced antimicrobial, analgesic, and antioxidant activity [11], the leaves of *Premna flavescens* display anti-inflammatory and analgesic potential, and *Premna barbata* exhibits a confirmed antibacterial effect [12]. However, there is no established data for the antimicrobial potential of *P. obtusifolia*.

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Table 1. Evaluation of Antimicrobial activity by measuring the zone of inhibition.

Test bacteria and fungi	Diameter of zone of inhibition (mm)			
	Standard Drug*	*n*-hexane**	Methanol**	Chloroform**
Bacillus cereus	50	6	7	5
Bacillus megaterium	48	6	6	4
Bacillus subtilis	48	7	7	6
Salmonella paratyphi	47	5	8	0
Salmonella typhi	47	0	6	0
Vibrio parahemolyticus	48	0	5	0
Vibrio mimicus	49	0	8	0
Staphylococcus aureus	50	8	7	0
Escherichia coli	48	6	8	0
Shigella dysenteriae	50	0	6	0
Pseudomonas aeruginosa	48	5	7	0
Sarcina lutea	49	0	7	0
Shigella boydii	47	0	6	0
Saccharomyces cerevisiae	46	0	0	0
Candida albicans	46	0	0	0
Aspergillus niger	46	0	0	0

* 5 µg/disc; ** 400 µg/disc

Studying natural products has an advantage over developing synthetic drugs in that it produces materials with novel structural features and biological activities [13]. Lead compounds identified from the screening of natural products can be improved using conventional medicinal chemistry or by using combinatorial methods. Antibiotic resistance has made it necessary to look for potent and novel antibiotics [14]. Natural products may be an excellent option for discovering new antimicrobials. Conventional pharmacotherapy for many neurological disorders is often limited by a lack of efficacy and the induction of adverse side effects. Consequently, natural products are increasingly regarded as safer alternatives, offering a more favorable safety profile with reduced risk of adverse reactions and dependence [15][16]. Their therapeutic potential is largely attributed to neuroprotective mechanisms mediated by anti-inflammatory and antioxidant pathways [17]. The traditional use of *P. obtusifolia* includes both infectious and neurological ailments. Considering all these factors, we studied *P. obtusifolia* to find out the antimicrobial and central nervous system (CNS) depressant activities to justify its use as a traditional medicine.

2. MATERIALS AND METHODS

2.1. Materials

All the solvents utilized during the experiment were analytical or laboratory grade, which were acquired from Merck (Germany). Before use, all of the solvents—including *n*-hexane, ethyl acetate, chloroform, ethanol, and methanol—were distilled. Diazepam was purchased from Bangladesh's Square Pharmaceuticals Ltd. Whole plant of *Premna obtusifolia* was collected and identified by an expert from Bangladesh National Herbarium (DACB accession number 47665). After being thoroughly freed from the mud, the plant was allowed to dry in the shade for a few days before being carefully crushed into coarse powder by a high-capacity grinding machine. Each plant's powdered components weighed about 1 kg, and they were placed in a clean flask with a circular bottom and 2.5 L of distilled ethanol to soak. The container and its contents were stored for 30 days with sporadic

shaking and stirring. The combined mixes were then filtered through a new cotton plug and finally using a filter paper. The filtrates' volumes were then decreased using a rotary evaporator under low pressure and temperature. The weight of *P. obtusifolia* extract was 20.08 g.

2.2. Methods

2.2.1. Preparation of Aqueous Extract

The crude extract was diluted in 50 mL of water and methanol (90% + 10%) mixture. Using the modified Kupchan method, the solution was extracted using *n*-hexane and chloroform [18]. A 50 mL of *n*-hexane was added to 50 mL crude extract prepared in a separating funnel and was shaken properly. After 5 min of shaking, the organic layer was separated. This process was repeated twice. After that, the aqueous portion was taken, and 50 mL of chloroform was added to it in a separating funnel. The separating funnel was shaken properly. After 5 min of shaking, the organic layer was separated. This process was repeated twice. Finally, aqueous and organic fractions of the extract were evaporated to dryness by using Buchi Rotavapor at 50 °C and low pressure, and then kept for further analysis.

2.2.2. Experimental Animal

For the experiments, either sex of young Swiss-Albino mice weighing between 18 and 20 g was employed. They were employed to assess the neuropharmacological effects. The animals were kept in an animal facility under standard laboratory settings. Water and a regular meal were given to the animals. The animal study was conducted with prior approval of the ethical committee of Northern University Bangladesh (Reference number: NUB/DoP/RC/EC/2019/03/02).

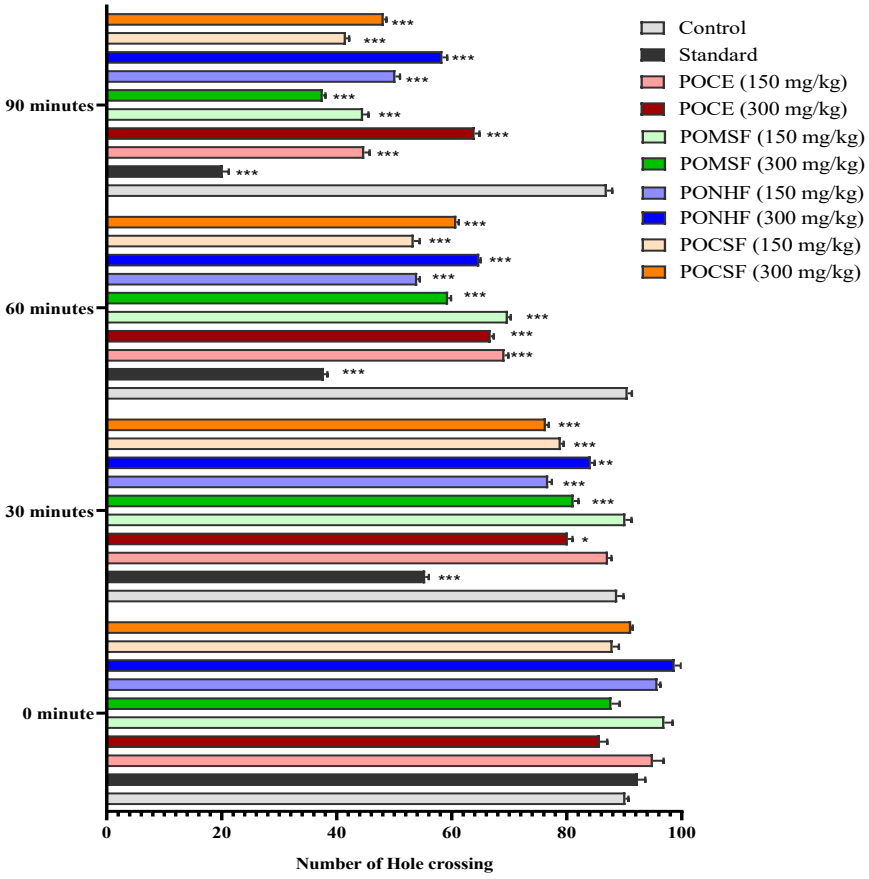

Figure 1. Effect of ethanolic extract and different fractions of *P. obtusifolia* on the hole cross test. Each value represents the mean ± SEM (n = 5); Student's test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p< 0.001$ compared with control. POCE = crude ethanolic extract of *P. obtusifolia*, POMSF= methanol soluble fraction, PONHF= *n*-hexane soluble fraction, POCSF= chloroform soluble fraction.

2.2.3. Antimicrobial Activity Screening

In this study, test substances were screened for antimicrobial activity using the disc diffusion method [19]. The nutrient agar medium was used to carry out the disc diffusion procedure. In this test, both gram-positive and gram-negative bacteria as well as fungi, were employed. The test organisms were moved from the pure cultures to the agar plates with the aid of a loop in an aseptic setting underneath the laminar air cabinet. The inoculated strains were then incubated for 24 h at 37 ºC for their maximum growth. The suspensions of the microorganisms were added to the appropriate petri plates. The petri plates were rotated multiple times to ensure a homogenous distribution of the test organisms in the fluid. After that, the petri plates were left at room temperature to allow the agar to solidify. Three types of discs were prepared for the antimicrobial screening: Ciprofloxacin standard disc, blank disc (solvent), and sample disc. To ensure that the contents from the discs had enough time to diffuse into the surrounding agar medium, the plates were then maintained in a refrigerator at 2 –8 °C for 24 h. The plates were then turned over and left in the incubator for 24 h at 37 °C. Following incubation, the antimicrobial efficacy of the test materials was assessed by measuring the millimeter-scale diameter of the zones of inhibition. All the measurements were performed for thrice.

2.2.4. CNS Activity

CNS depressant activity of different extracts of *Premna obtusifolia* was evaluated using hole cross and open field test in mice at doses of 150 and 300 mg/kg BW orally. Each of the four groups in use here contains five mice. Diazepam was used as standard medication. A special cage with a hole of 3 cm diameter, a steel partition fixed in the middle position [16]. The control group received vehicle (1% Tween 80 in water), and the test group received the crude extract at two different doses. Here, the standard group received Diazepam at a dose of 1 mg/kg BW. Each mouse was then placed on one side of the chamber, and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min at 0, 30, 60, and 90 min during the study period. After administration of respective treatments, the animals were placed on the floor of an open field divided into a series of squares. Following treatment, each animal's three-minute visits to each square were recorded at 0, 30, 60, and 90 min [20].

2.2.5. Statistical Analysis

The data were presented as mean ± SEM. The observed data were compared with the control group. Student's t-test was employed for determining the p-value. $P < 0.05$ was considered significant. GraphPad Prism 8.0 was used for statistical analysis and graph preparation.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activity

Nowadays, antimicrobial resistance is a serious threat and traditional antibiotics are not working against the pathogens [21][22]. The antimicrobial activity of different fractions of the *P. obstusifolia* was evaluated by measuring the zone of inhibition. The zones of inhibition of the test substances and the standard drug were compared in Table 1. From this investigation it was observed that *P. obtusifolia* had weak antibacterial activity but no antifungal activity. Compared to the standard drug, the zone of inhibition of the extract was very small. However, other studies revealed the potent antimicrobial activity of *Premna resinosa* [23], *Premna serratifolia* Lin. [24], and *Premna integrifolia* [25]. Species-specific compound variation may be the cause of this dissimilarity. The extraction procedure and the strain of the microorganism may also affect the results. Further investigations are warranted with different doses and strains.

3.2. Hole Cross Test

In the hole cross test, the mice gradually reduced the number of times they needed to cross between the chambers over the course of 90 min (Figure 1). The number of crossings between the chambers was higher for all groups during the pre-treatment phase. In comparison to other test substances, the ethanolic extract of *P. obtusifolia* and all other fractions demonstrated a sedative effect.

3.3. Open Field Test

During the open field test, the mice in the various test groups visited fewer squares over the course of 90 min as time went on (Figure 2). The

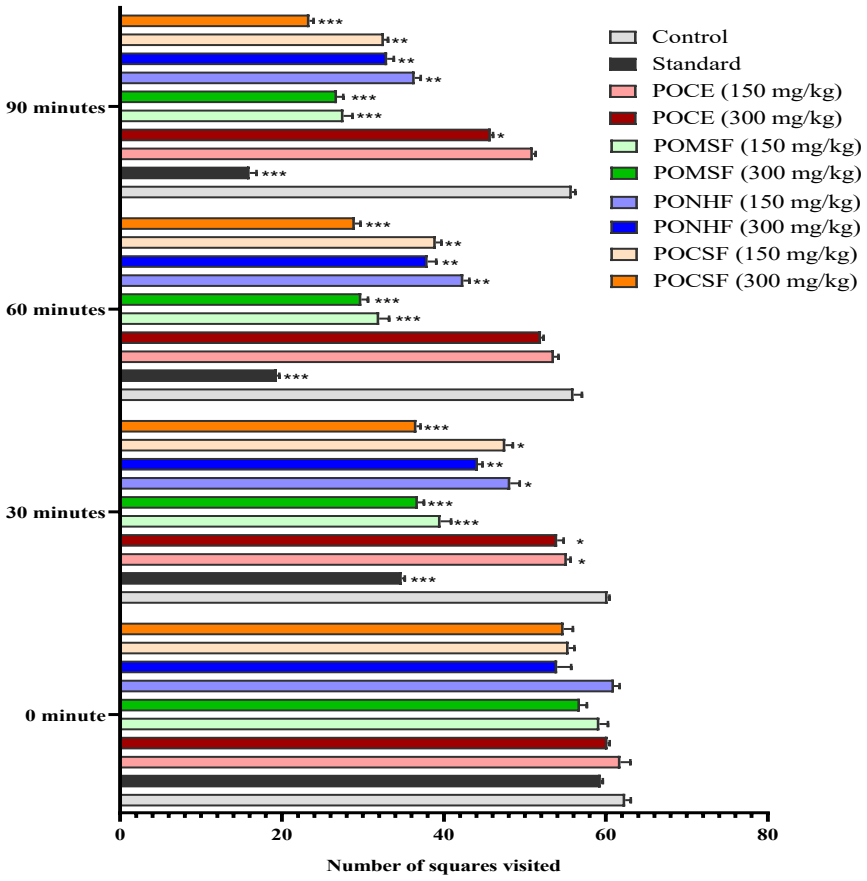

Figure 2. Effect of ethanolic extract and different fractions of *P. obtusifolia* on the open-field test. Each value represents the mean ± SEM (n = 5); Student's t-test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p< 0.001$ compared with control. POCE = crude ethanolic extract of *P. obtusifolia*, POMSF= methanol soluble fraction, PONHF= *n*-hexane soluble fraction, POCSF= chloroform soluble fraction.

ethanolic extract of *P. obtusifolia* and all its fractions were shown to reduce the number of squares that the mice visited over time when compared to the control, suggesting that the plant extract may have sedative effects. The effect on the locomotor activity of the test animals was observed after 30 min of dose administration. This time was for absorption and distribution of the active compounds to the site of action. The hole cross and open field tests were used to observe the locomotor activity of mice. The locomotor activity is a motive to act on the CNS by uplifting alertness and decreasing the locomotor activity could induce a sedative action. Locomotor activity can be used to gauge the level of CNS excitation, and a decrease in this activity is strongly related to the sedation that results from CNS depression [26]. From the initial (30 min) observation to the final (90 min) observation, practically all the *P. obtusifolia* fractions reduced the number of square visits and holes crossed, and they also had an impact on the mice's locomotor activity, which showed signs of sedative activity.

The CNS depressant activity of *P. obtusifolia* is primarily due to its ability to decrease CNS excitability, leading to reduced locomotor and exploratory activities. Extracts from *Premna* species, such as *Premna integrifolia* and *Premna orientale*, have been shown to decrease the frequency and amplitude of movements in animal studies. This reduction in spontaneous motor activity is a key indicator of CNS depressant effects [27]. The mechanism likely involves the modulation of neurotransmitters or receptors, similar to the action of barbiturates and diazepam [28]. *Premna* species contain various compounds like diterpenoids, iridoid glycosides, and flavonoids, which are known to have a range of biological activities, including CNS effects [5][29].

The study has some limitations. Firstly, the study lacked phytochemical characterization, leaving the active constituents responsible for the observed effects unidentified. Secondly, the small sample size (n=5) in the *in vivo* CNS depressant assays limits the statistical power and generalizability of the findings. Furthermore, the absence of an established dose-response relationship for either activity hinders the assessment of the extract's potency and therapeutic window. Finally, the antimicrobial evaluation was restricted to the disc diffusion method (measuring the zone of inhibition), which is a preliminary qualitative assay that does not provide quantitative data on minimum inhibitory concentration or bactericidal effects.

4. CONCLUSIONS

The present study aimed to investigate the antimicrobial activity and CNS activity of the plant with its different fractions of *P. obtusifolia*. This investigation conclusively establishes the significant sedative activity of *P. obtusifolia*, a finding consistently supported by the open field and hole cross tests. The limited antimicrobial results, however, highlight the specificity of its bioactivity. Given the increasing relevance of natural products in drug discovery, the pronounced sedative properties warrant deeper investigation. The critical next steps include a comprehensive chemical analysis to identify the active ingredients and molecular studies to unravel the specific mechanisms of action involved.

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Conflicts of Interest

The authors declare no conflict of interest.

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