Article

Bioactive Potential of *Premna esculanta*: A Study on Antioxidant, Antimicrobial and Antidiarrheal Efficacy

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Abstract: Objective: The discovery of the genus *Premna* and its traditional uses are the result of extensive information gained by living in forest or semi-forest areas and closely observing indigenous populations regarding the therapeutic qualities of plants. This investigation centered on examining the DPPH scavenging, antimicrobial and anti-diarrheal properties of ethanolic leaf extracts. This study aimed to investigate the bioactive components and antibacterial properties of ethanolic leaf extracts and their fractions. Methods: In vitro antioxidant was evaluated by DPPH scavenging assay and the disc diffusion method evaluated antimicrobial efficacy. In vivo screening for antidiarrheal was conducted, the latent period of defecation in castor oil-induced diarrhea in mice assessed antidiarrheal effects. Results: The extract showed an ability to scavenge DPPH with IC_{50} values of 0.931 µg/mL for *Premna esculanta* extract and IC_{50} 0.902 µg/mL for ascorbic acid standard. The extract demonstrated significant antimicrobial activity, with inhibition zones ranging from 12–19 mm against various microbial strains, notably *Shigella boydii*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Sarcina lutea*, *Bacillus subtilis*, and *Candida albicans*, at concentrations of 250 and 500 µg/disc. In anti-diarrheal test, Loperamide (3 mg/kg) reduced total feces and defecation by 56.62% with concentrations of 500 mg/kg. Significant changes were observed in anti-diarrheal studies. Conclusion: *Premna esculanta* leaf extract demonstrated significant antimicrobial, and antidiarrheal activities.

Keywords: DPPH; antimicrobial; anti-diarrheal

1. Introduction

The use of plants for medicinal purposes in both humans and animals dates back centuries. Many synthetic treatments, particularly those that are tropical, tend to have a range of side effects and are often too expensive for most people to afford to address this issue, people have turned to the plants available in their surroundings, despite the lack of scientific proof regarding their efficacy [1]. The health benefits of these plants come from phytochemicals, which are non-nutritive compounds that protect humans from various illnesses. The primary components include alkaloids, flavonoids, saponins, phenolic compounds, phytosterols, proteins, amino acids, gums, mucilage, and lignin. These phytochemical elements are fundamental to the development of various pharmaceutical industries and are crucial for identifying crude drugs [2]. Both epidemiological and in vitro research on medicinal plants and vegetables have strongly indicated that plant compounds with antioxidant properties may offer protective benefits against oxidative stress in biological systems [3]. One of the significant challenges in global healthcare is the urgent demand for new, effective, and affordable treatments for microbial infections, particularly in developing nations where infectious diseases account for nearly half of all deaths [4].

The World Health Organization (WHO) has initiated a control program focused on diarrheal diseases, incorporating traditional medicine alongside health education and preventive measures (Syder medicine). This program primarily relies on herbal remedies [5]. Antipyretic medications typically work by preventing or reducing the expression of COX-2, which helps lower elevated body temperatures by inhibiting the production of prostaglandin E2 (PGE2) [6]. Today, people around the globe increasingly prefer medicines derived from plants due to the undesirable side effects associated with synthetic drugs, which are often seen as more suitable for long-term treatment. Traditional plants may offer new compounds that can help mitigate the high costs and toxic effects of current medications for many rural communities in developing countries [7].

Premna esculanta (Roxb), a small, branching shrub from the Lamiaceae family, thrives in the moist, shaded areas of Bangladesh and India's primary rainforests. Tribal communities in the Chittagong Hill Tracts of Bangladesh have long used this plant to treat conditions such as gout, jaundice, lipomas (tumors), and edema. The leaves of *Premna esculanta* are applied to address arthritis and infections caused by bacteria and fungi [8].

Furthermore, this plant has been noted for its analgesic and thrombolytic properties [9], as well as its anti-inflammatory and anti-nociceptive effects [10], and its antioxidant and hepatoprotective activities [11].

This study aims to evaluate the therapeutic potential of *Premna esculanta* leaf extract by assessing its antibacterial, anti-diarrheal, antioxidant, neuropharmacological, and anti-inflammatory properties, with the goal of exploring its potential contributions to healthcare.

2. Materials and Methods

2.1. Chemical

Analytical grade chemicals, including 2,2-diphenyl-1-picryl hydrazyl (DPPH) from Sigma, Burlington, MA, USA, $AlCl_3$ (aluminum chloride), NaOH (sodium hydroxide), $Na_2HPO_4 \cdot 2H_2O$ (disodium hydrogen phosphate dihydrate) from Loba, Mumbai, India, as well as H_2O_2 (hydrogen peroxide) from Merck, Darmstadt, Germany, and $C_{12}H_{12}N_2O_2S$ (phenazine methosulfate PMS) from Sigma, USA, were utilized. All standard medications employed for in vivo pharmacological evaluations were acquired from Square Pharmaceuticals Ltd. (Dhaka, Bangladesh) and Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh.

2.2. Plant Collection and Extraction

Plant leaves from *Premna esculanta* were gathered in Bandarban, Chittagong, Bangladesh, in July 2023. The identification of the plant specimen was conducted by Dr. Mohammad Sayedur Rahman, a senior scientific officer at the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh, and the authentication number assigned was DACB-94772. The collected leaves were dried in the shade, crushed into a coarse powder, and then extracted using maceration, involving 250 g of the powdered leaves mixed with 1000 mL of 96% ethanol for 14 days. The extraction process yielded 6.3%.

2.3. Animals

Young Swiss albino mice, aged between four to six weeks and weighing approximately twenty to twenty-five grams, were obtained from Jahangirnagar University in Bangladesh. After their acquisition, they were placed in the animal facility of the pharmacology lab within the pharmacy department for a period of two to three weeks to help them adjust to their new environment. This accommodation took place at Dhaka International University in Bangladesh. All experiments were performed in a calm, secluded, and controlled setting. The animal studies for this research adhered to the ethical standards set by the Committee of Clinical Pharmacy & Pharmacology at the Department of Pharmacy, Dhaka International University, located in Satarkul, Badda, Dhaka-1212. [Ref: CPP/DIU/EC/006].

2.4. DPPH Scavenging Activity

DPPH scavenging assay, as described by Khan 2013 [12]. The experiment was carried out with ten test tubes, each containing specific amounts of plant extract and ascorbic acid at concentrations of: 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953, and 0.977 μg/mL. To prepare these concentrations, the plant extract and ascorbic acid were measured three times and then dissolved in ethanol. Ascorbic acid acted as positive control. A precise amount of DPPH was weighed and dissolved in ethanol to prepare a 0.004% (*w*/*v*) solution, which was then blended using a sonicator. Each test tube received 1 mL of the various concentrations of ascorbic acid and plant extract. Subsequently, 3 mL of the 0.004% DPPH solution was pipetted into each test tube. The test tubes were then stored in a dark environment at room temperature for 30 min to allow the reaction to occur fully. In addition, blank test tubes containing only ethanol with DPPH were prepared. After this incubation period, the absorbance of each test tube was recorded at 517 nm using a UV spectrophotometer. The percentage of inhibition was determined with the following formula: % inhibition = [(Blank absorbance − Sample absorbance)/Blank absorbance] × 100.

2.5. Antimicrobial Activity

To evaluate the extract's antimicrobial activity disc diffusion method was used which was outlined by Fakruddin [13]. Tests were performed on the extract against a range of eight gram-negative microorganisms, which

included Shigella boydii, Shigella dysenteriae, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Vibrio parahaemolyticus, Vibrio mimicus, and Escherichia coli. Additionally, there were eight gram-positive bacteria tested as well, comprising Sarcina lutea, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Saccharomyces cerevisiae, Candida albicans, Aspergillus niger, and Staphylococcus aureus. Mueller-Hinton agar medium was used to prepare test plates. 25 and 50 micrograms per of crude leaf extract per microliter were placed onto 5-mm filter paper discs (Whatman No. 1). After that the discs were totally dry. Following the imbuing process, the discs were covered with 100 µL of the culture and cultured for 24 h at 37 °C on sterilized agar plates. Discs containing 5 µg of ciprofloxacin were used as a positive control. The inhibitory zone's diameter was measured in millimeters following the incubation period. The control reference was a sterile blank disc.

2.6. Evaluation of Antidiarrheal Activity

The antidiarrheal activity was assessed using a model where mice induced with diarrhea by administration of castor oil were tested, as described by Golder [14]. Castor oil decreases the absorption of fluid content and promotes intestinal motility. While the positive control group was given the standard drug loperamide at a dose of 3 mg/kg 0.5 mL of castor oil orally to induce diarrhea 30 min later, the test groups were given the sample extract at 250 and 500 mg/kg body weight doses. After four hours, the latent period's duration and amount of feces were recorded for each mouse in its cage on blotting paper.

2.7. Statistical Analysis

The means ± standard errors of means were used to express all experimental results. One-way analysis of variance was utilized to evaluate statistical significance using Dunnett's test.

With Prism 6.0 (Graph Pad Software Inc., San Diego, CA, USA), and Excel statistical analysis was carried out. When $p < 0.05$, the study's results were deemed statistically significant.

The means ± standard errors of the means were utilized to present all experimental findings. To assess statistical significance, a one-way analysis of variance was performed, employing Dunnett's test. Statistical analyses were conducted using Prism 6.0 (Graph Pad Software Inc., San Diego, CA, USA) and Excel. Results were considered statistically significant when the *p* value was less than 0.05.

3. Results and Discussion

3.1. DPPH Scavenging Activity

The DPPH free radical scavenging assay was conducted to assess the quantitative antioxidant activity, revealing that the IC_{50} values for the ethanol crude extract of *Premna esculanta* was measured at 0.931 µg/mL, while ascorbic acid, the standard reference, had a value of 0.902 µg/mL (Figure 1). *Premna esculanta*, a plant belonging to the Lamiaceae family found in Bangladesh, was evaluated for its capacity to neutralize free radicals by reacting with DPPH free radicals. The extract's effectiveness in scavenging DPPH was compared to that of ascorbic acid, which is recognized as a potent antioxidant. The ability to act as an antioxidant is a crucial pharmacological characteristic of plants.

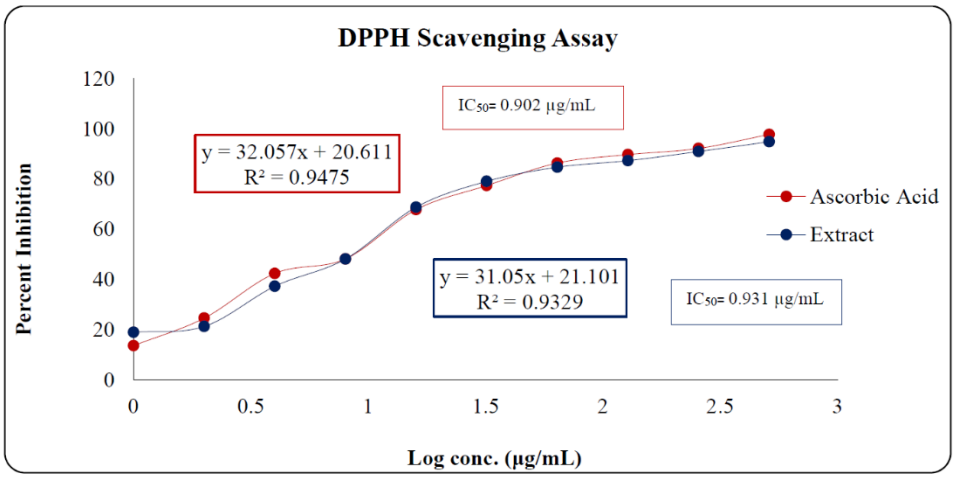

Figure 1. Comparison of absorbance vs. log concentration graph for ascorbic acid vs. *Premna esculanta.*

DPPH is commonly used to evaluate the free radical scavenging or antioxidant potential of plant extracts since it is easily neutralized by antioxidants [15]. The ability of the extract to scavenge was found to be dependent on its concentration, represented by IC_{50} (the concentration of the sample needed to reduce the initial concentration of DPPH by 50%). A lower IC_{50} value signifies greater antioxidant activity. In this study, the plant extract exhibited an IC_{50} of 0.931 µg/mL, while standard ascorbic acid had a value of 0.902 µg/mL (Figure 1).

The ethanolic extract of *Premna esculanta* demonstrates significant antioxidant activity, indicated by its DPPH scavenging ability with an IC_{50} close to that of ascorbic acid. This activity suggests the presence of bioactive phytochemicals, primarily phenolics, and flavonoids, which are known to neutralize free radicals by donating hydrogen atoms or electrons. Flavonoids and phenolics have been widely studied for their antioxidant capacities, correlating with the extract's effectiveness in reducing oxidative stress [12]. The high antioxidant potency of these compounds implies a protective role against cellular damage and potential therapeutic application in managing oxidative stress-related disorders.

3.2. Antimicrobial Activity

The antimicrobial and antifungal properties of the extract were assessed at concentrations of 250 µg and 500 µg against various bacterial and fungal strains via the zone of inhibition technique. Ciprofloxacin at 5 µg served as the positive control, while a blank sample acted as the negative control. The extract demonstrated different levels of inhibition against both Gram-positive and Gram-negative bacteria. At the 250-µg concentration, notable activity was observed against *Shigella boydii* (17 mm), *Shigella dysenteriae* (12 mm), and *Pseudomonas aeruginosa* (14 mm).

At 500 µg, the inhibition zones significantly increased for *Shigella dysenteriae* (16 mm), *Pseudomonas aeruginosa* (18 mm), and *Escherichia coli* (19 mm). For the Gram-positive strains, the extract at 500 µg showed relevant activity against *Sarcina lutea* (17 mm), *Bacillus subtilis* (17 mm), and *Candida albicans* (17 mm) (Figure 2).

The findings suggest that the extract possesses broad-spectrum antimicrobial activity, with higher concentrations yielding larger zones of inhibition. Generally, the Gram-negative bacteria displayed greater inhibition zones than the Gram-positive strains, indicating a possible difference in susceptibility [16]. Gram-negative bacteria have an outer membrane that may be more vulnerable to the extract's ingredients, which could be explained by the differences in their cell wall architectures [17].

The antifungal activity, particularly against *Candida albicans*, highlights the extract's potential as an antifungal agent [18]. The extract's efficacy at higher concentrations suggests that its active compounds could be isolated and potentially used in higher doses to combat microbial infections effectively.

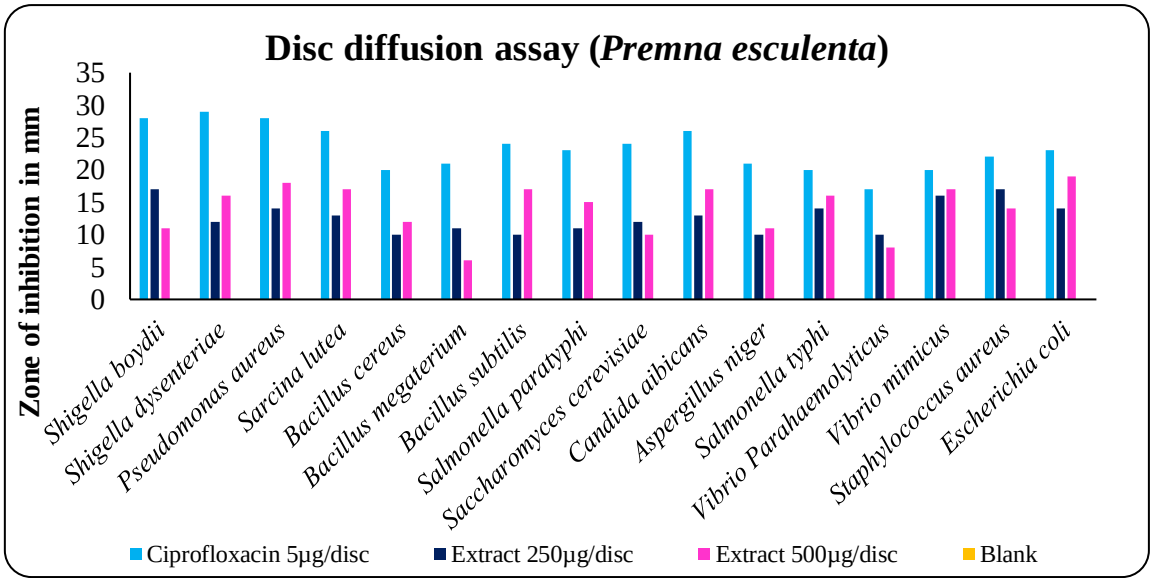

Figure 2. In vitro antimicrobial activity of ethanolic extract of *Premna esculanta.*

The antimicrobial effects of *Premna esculanta* are notable, particularly against a range of Gram-positive (+) and Gram-negative (−) bacteria, with inhibition zones of up to 19 mm for certain strains. Alkaloids, terpenoids, and saponins in the extract may be responsible for its antibacterial efficacy all of these compounds we got after the phytochemical screening. These compounds disrupt bacterial cell membranes and interface with intracellular functions, particularly in Gram-negative (−) bacteria, where their outer membrane may increase susceptibility [17].

The broad-spectrum antibacterial activity observed aligns with findings from other plant-based studies, which similarly attribute antimicrobial properties to alkaloids and terpenoids.

3.3. Antidiarrheal Activity

Premna esculanta extract at doses of 250 and 500 mg/kg body weight significantly reduced the overall number of feces and delayed the beginning of diarrhea in the castor oil-induced diarrheal mice in a dose-dependent manner when the statistical significance level was established at 0.05%. At dosages of 250 and 500 mg/kg body weight, *Premna esculanta* inhibited defecation by 32.35% and 56.62%, respectively.

In the antidiarrheal test, the predominant symptoms of diarrhea caused by oral castor oil administration were altered intestinal motility and increased bowel movements. *Premna esculanta* extract increased the latent period of defecation in mice with castor oil-induced diarrhea by 127.6 and 158.4 min at doses of 250 mg/kg and 500 mg/kg body weight, respectively. In contrast, the latent period of defecation in the standard (loperamide at 3 mg/kg dose) and control groups was 30.8 and 184 min, respectively. At doses of 250 mg/kg and 500 mg/kg, the extract significantly reduced defecation by 32.35% and 56.62%, respectively (Figure 3). A prior small-scale study on the plant's antidiarrheal properties likewise verified our findings [3].

The antidiarrheal effect of *Premna esculanta* extract, demonstrated through reduced fecal output in castor oil-induced diarrhea models, suggests bioactivity potentially linked to flavonoids, tannins, and saponins we got all of these compounds in our phytochemical screening study. Flavonoids and tannins stabilize the intestinal membrane by inhibiting fluid secretion, while saponins reduce gut motility. This plant's efficacy in increasing the latent period of defecation aligns with studies highlighting the roles of these metabolites in antidiarrheal activities [14]. The presence of such compounds supports traditional uses of *Premna esculanta* for gastrointestinal alignments.

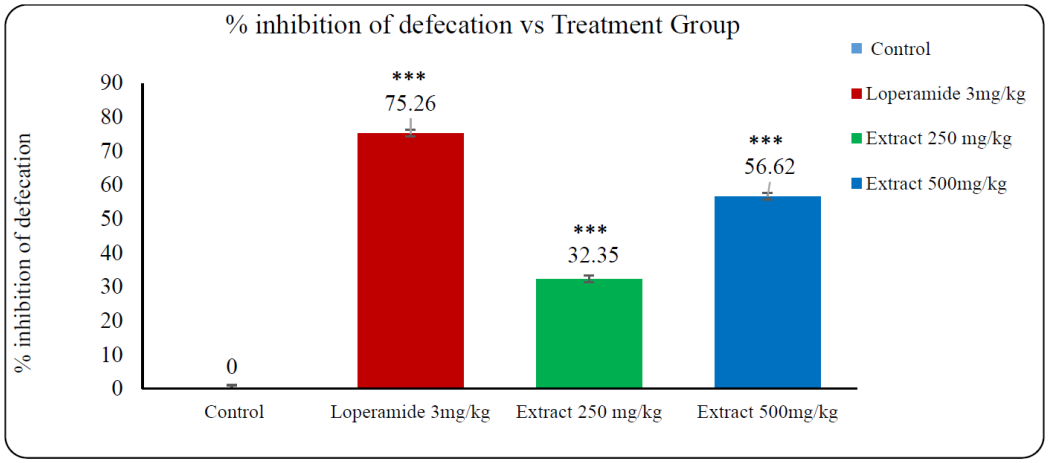

Figure 3. Percentage of inhibition defecation in case of castor-oil-induced diarrhea. *** $p < 0.001$

4. Conclusion

In this study, we evaluated the medicinal value of the *Premna esculanta* plant frequently used in traditional medicine, using thorough pharmacological studies conducted both in vitro and in vivo. The plant demonstrates significant pharmacological potential based on its evaluated properties. It exhibits robust antimicrobial, and antidiarrheal activities, indicating its efficacy in treating infections and diarrhea. Additionally, its strong antioxidant capacity suggests a protective role against oxidative stress. These findings collectively highlight the plant's promising application in medicinal treatments, supporting its potential development as a natural remedy for various health conditions. More research is necessary to clarify the underlying mechanisms and maximize their application in therapeutic settings.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of [Dhaka International University] (Ref: CPP/DIU/EC/006).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviation

DPPH	2,2-diphenyl-1-picrylhydrazyl
PGE2	prostaglandin E2
COX-2	Cyclooxygenase-2
$AlCl_3$	aluminum chloride
NaOH	sodium hydroxide
$Na_2HPO_4{\cdot}2H_2O$	disodium hydrogen phosphate dihydrate
H_2O_2	hydrogen peroxide
$C_{12}H_{12}N_2O_2S$	phenazine methosulfate PMS
UV spectrophotometer	Ultraviolet spectrophotometer
IC_{50}	Inhibition concentration of DPPH by 50%
G-positive	Gram-positive
G-negative	Gram-negative

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