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Pharmacological Potential of *Bouea oppositifolia* (Roxb.) Adelb. Leaves: Antioxidant, Antimicrobial and Membrane-Stabilizing Properties

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Abstract

The present study investigates the antioxidant, antimicrobial, and membrane-stabilizing activities of methanolic extracts of leaves of *Bouea oppositifolia* (Plum Mango), an underexplored medicinal plant from the Anacardiaceae family. Various solvent fractions, including methanol (MESF), petroleum ether (PESF), dichloromethane (DCMSF), ethyl acetate (EASF), and aqueous (AQSF), were evaluated using *in vitro* assays. Antioxidant potential was assessed via total phenolic content (TPC) and DPPH free radical scavenging assays, revealing that DCMSF had the greatest TPC (221.69 mg GAE/g extract), while AQSF exhibited the strongest radical scavenging activity (IC50 = 0.03 µg/ml). Antimicrobial activity, determined through the disc diffusion method, demonstrated moderate inhibition against bacterial and fungal strains, with AQSF showing the most pronounced activity (12 mm inhibition zone). The membrane-stabilizing efficacy of the extracts was evaluated using hypotonic and heat-induced hemolysis assays, where MESF (38.52% inhibition) and EASF (60.35% inhibition) exhibited notable protective effects against erythrocyte lysis. These findings suggest that *B. oppositifolia* leaves possess potent antioxidant, antimicrobial, and membrane-protective properties, likely attributed to their rich phenolic and flavonoid content. This study highlights the plant's pharmacological potential, requiring further phytochemical characterization and *in vivo* validation for drug development.

Key words: *Bouea oppositifolia*, antioxidant, antimicrobial, hypnotic, membrane-stabilizing.

Introduction

Plant kingdom, owing to its genetic heritage and remarkable resilience in diverse ecological systems, exhibits an enormous chemical diversity. This rich diversity has positioned them as an essential source of chemically complex bioactive compounds (Bennett, 1998). Moreover, a thoroughly recorded history of human use of any plant improves the safety profile of its bioactive phytoconstituents. The discovery and characterization of these phytochemical substances typically commence with blind biological screens of plant materials utilizing *in vitro* models or *in-vivo* investigations. These findings clarify the capacity of plant components to facilitate substantial biochemical interactions within biological systems (Fabricant and Farnsworth, 2001). Following bioactivity-guided phytochemical analysis, extracts exhibiting favorable pharmacological characteristics are examined to identify new drug candidates or lead compounds for pharmaceutical development (Sasidharan *et al.*, 2011). *Bouea*, a relatively small and recently defined genus of angiosperms, is part of the Anacardiaceae family. Among the three acknowledged species within this genus, *B.*

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oppositifolia (Roxb.) Adelb., referred to as Plum mango or Gandaria, is indigenous to Southeast Asia, encompassing Myanmar, China, Thailand, Malaysia, Laos, Indonesia, Cambodia, the Andaman Islands, and Vietnam (Lim, 2012). In Bangladesh, the plant is predominantly found in the Khagrachari and Chittagong districts. Here it is referred to as Uri Aam and it is one of the severely endangered species (Rahman, 2018). Fruits are the main reason of cultivating this plant, but it is also grown ornamentally in homes. Both unripe and ripe fruits are documented to possess substantial quantities of lipids, carbohydrates, amino acids. It also contains minerals like salt, calcium, iron, phosphorus, and potassium. in addition to vitamins like thiamin, retinol, riboflavin, carotenoids, and niacin. (Rajan and Bhat, 2020). The fruits phytochemical study result has revealed a minimum of 82 volatile chemicals in unripe fruits and 121 in fruits which are ripen. Mono- and sesquiterpene hydrocarbons are the predominant segments of the volatile profile, accounting for 32.89% and 29.28%, respectively, alongside diverse concentrations of acids, esters, alcohols, aldehydes, and ketones (Rajan and Bhat, 2017).

Despite the occasional association of various medical characteristics with the plant, none of these activities have been thoroughly explored. Consequently, a systematic *in vitro* and biological approach of the plant leaves was undertaken to assess its possible pharmacological characteristics.

Materials and Methods

Preparations of the plant crude extracts: Fresh *B. oppositifolia* (Family- Anacardiaceae) leaves were harvested from the Botanical Garden in Dhaka, Bangladesh, and a voucher specimen was submitted to the Bangladesh National Herbarium (BNH) for future reference (Accession No. DACB 56286). The leaves were meticulously washed, shade-dried, then pulverized into a coarse powder. 650g of powdered leaves were immersed in 3000 ml of methanol and allowed to macerate for 15 days, with intermittent shaking and stirring. The extract was filtered initially through a cotton plug and then through Whatman No. 1 filter paper. The filtrate was concentrated with a rotary evaporator under decreased pressure at 40°C, producing 73.5 g of crude methanolic extract. Methanol, Tween-80, premium castor oil, and acetic acid were obtained from local vendors. Standard pharmaceutical agents, such as loperamide (Square Pharmaceuticals Ltd.), morphine (Gonoshasthaya Pharmaceuticals Ltd.), acetylsalicylic acid (Essential Drugs Company Ltd.), and normal saline (Opsonin Pharmaceuticals Ltd.), were acquired. All other chemicals and solvents utilized in the study were of analytical grade.

Solvent–solvent partition of crude extract: Methanolic extracts (MEs) of leaves of *B. oppositifolia* Roxb. were subjected to solvent-solvent partitioning using the protocol designed by Beckett **(**1986). The crude extract (5 gm) was dissolved in 10% aqueous methanol. It was extracted with n-Petroleum ether, then with carbon tetrachloride and finally with chloroform followed by ethyl acetate. The test materials have been listed in Table 1.

Table 1. List of different solvents fraction.

Plant part	Sample code	Test sample
Leaves of *B. oppositifolia* Roxb.	MESF	Methanol Soluble Fraction
	PESF	Pet-Ether Soluble Fraction
	DCMSF	Dichloromethane Soluble Fraction
	EASF	Ethyl Acetate Soluble Fraction
	AQSF	Aqueous Soluble Fraction

We did not employ the carbon tetrachloride soluble fractions in the experiment's subsequent steps because we discovered negligible amounts of them.

Antioxidant activity:

Total Phenolic Content (TPC): Folin Ciocalteu strategy was implemented to figure out the leaves' methanolic extractives' TPC (Lin *et al.,* 2016). 2.5 ml of the Folin-Ciocalteu reagent, which was tenfold diluted through distilled water, and 2.5 ml of the 7.5% w/v Na_2CO_3 were mixed to each extractive (1 mg/ml). Then, the combination was allowed to sit at ambient temperature for half an hour in the dark. 760 nm absorbance was considered with a UV-Vis spectrophotometer. A calibration graph was created by setting absorbance against different gallic acid solution concentrations (from 0.3906 to 100 µg/ml) in order to measure the TPC. The TPC was reported in milligrams of GAE (gallic acid equivalent) per gram of dried leaf methanolic extractives.

DPPH free radical scavenging assay: The extractives' antioxidant capabilities have been investigated by the DPPH free radical scavenging technique (Brand-Williams *et al.,* 1995). While tertbutyl-1-hydroxytoluene (BHT) used as a positive control, and methanol has been utilized as a control group. Serial dilution technique was applied to generate various concentration of extractives and standard (500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953, and 0.977 µg/ml) from a stock solution of 1000 µg/ml. In this procedure, 2 ml of the control and methanolic solution of the extractives was combined with 3.0 ml of a methanolic DPPH solution (20 µg/ml). After amalgamation, the solutions were left in a dark place for roughly thirty minutes, and the absorbance at 517 nm was determined using a UV-visible spectrophotometer at 25°C with methanol as the blank. The inhibition of DPPH free radicals was calculated as a percentage (I%) using the following formula.

$$Inhibition\ percentage\ (I\%) = \left(1 - \frac{Asample}{Ablank}\right) \times 100$$

A_{blank} denotes the control's absorbance, which had every ingredient but no experimental sample. A_{sample} indicates the sample's absorbance. The IC_{50} value was calculated by graphing the inhibition percentage versus extractives concentration.

Determination of antimicrobial activity: The efficacy of the plant extracts in combating microorganisms was determined by employing the disc diffusion method. This process involves immersing little filter paper discs, each measuring 6 mm in diameter, in exact amounts of test substances mixed with a nutrient-rich agar medium. The discs were thereafter evenly distributed on the agar surface next to the bacteria being studied. A disc impregnated with the antibiotic ciprofloxacin acted as a reference, whereas a blank disc saturated with solvent served as a control. The plates were incubated at 37°C for 24 hours to promote optimal diffusion of the test substances inside the agar. The antimicrobial characteristics of the test materials inhibited microbial proliferation surrounding the discs, resulting in clear zones free of bacteria. The effectiveness of the test chemicals in eradicating microorganisms was evaluated by measuring the diameter of the inhibition zones, reported in millimeters (Bauer *et al.,* 1966).

Membrane stabilizing activity:

Red blood cells (RBC) collection: Human red blood cells were collected for this investigation. RBCs obtained from a male subject, weighing 70 kg, with fair skin and barren of diseases. The collected RBC were stored in a test tube containing the anticoagulant EDTA at standard settings of 23±2°C and 55±10% relative humidity.

Hemolysis induced by a hypotonic solution: ASA (0.10 mg/ml) was served as the reference drug. Every tube was loaded with 5 ml of a 10 mM sodium phosphate buffer mixture (pH 7.4) and 50 mM NaCl (a hypotonic solution) before adding 0.50 ml of an erythrocyte (RBC) suspension. The combinations underwent incubation at the ambient temperature over 10 minutes before centrifugation at 3000 rpm for about 10 minutes. After separating and cleaning

the soluble the residue, absorbance was determined for each tube. The formula below was applied to evaluate membrane stabilization:

$$Inhibition\ percentage\ of\ hemolysis = \frac{(OD1 - OD2)}{OD1} \times 100$$

OD1 represents the optical density of the control (hypotonic-buffered saline solution), while OD2 denotes the optical density of the experimental sample and standard in the hypotonic solution.

Hemolysis induced by heat: A pair of centrifuge tubes were prepared using a 5 ml of isotonic buffer, 30 µL of erythrocyte suspension, and 1 mg/ml extractives. Another two centrifuge tubes have been filled using the identical mixture, excluding the test materials. Following gently turning each tube, a set underwent incubation into a tub of water at 54°C, while the second set was held in a freezer at 0-5°C. After incubation, the blends had been subsequently centrifuged for 3 minutes at 1300 rpm to measure the absorbance of the residues. The percentage of inhibition (%) was computed using the next formula.

$$Inhibition\ percentage\ of\ hemolysis = \frac{1 - (OD2 - OD1)}{OD3 - OD1} \times 100$$

OD_1 represents the optical density of the unheated test sample, OD_2 indicates the optical density of the heated test sample, and OD_3 denotes the optical density of the heated control sample.

Results

Antioxidant activity: MESF of *B. oppositifolia* leaves, along with its distinct fractions PESF, DCMSF, EASF, and AQSF were evaluated for their TPC. The results, denoted in mg GAE/g of extract, are displayed in Figure 2 which emphasizes the phenolic concentration of each fraction. The phenolic concentration differed among the various extract fractions, spanning from 2.125 mg GAE/g to 221.6875 mg GAE/g of extract. The fraction with the highest phenolic content was DCMSF (221.69 mg GAE/g of extract), followed by EASF (207.75 mg GAE/g of extract), MESF (76.38 mg GAE/g of extract), AQSF (12.13 mg GAE/g of extract), and PESF (2.13 mg GAE/g of extract), as indicated in Figure 2.

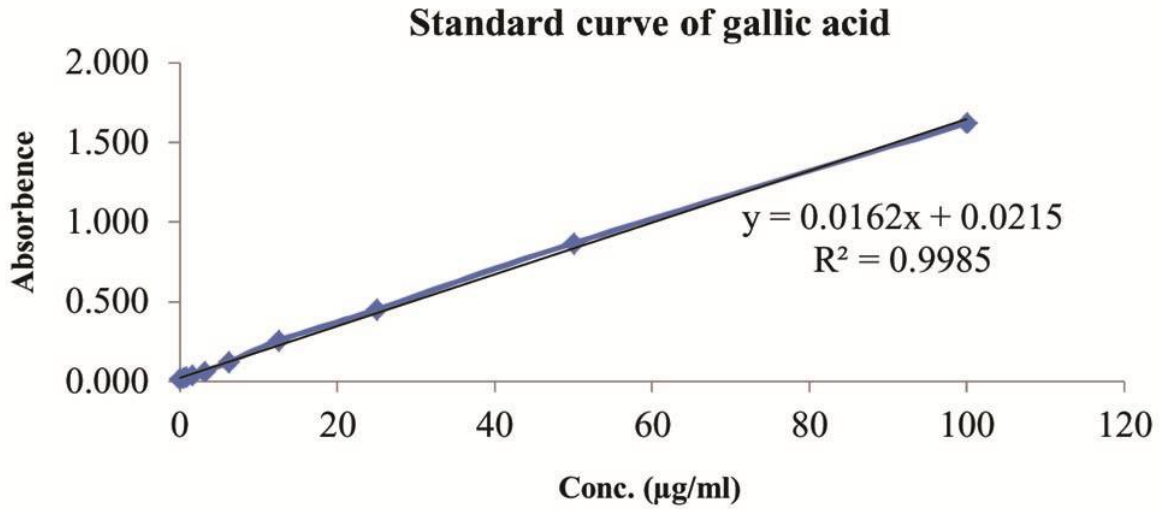

Figure 1. Standard curve of gallic acid.

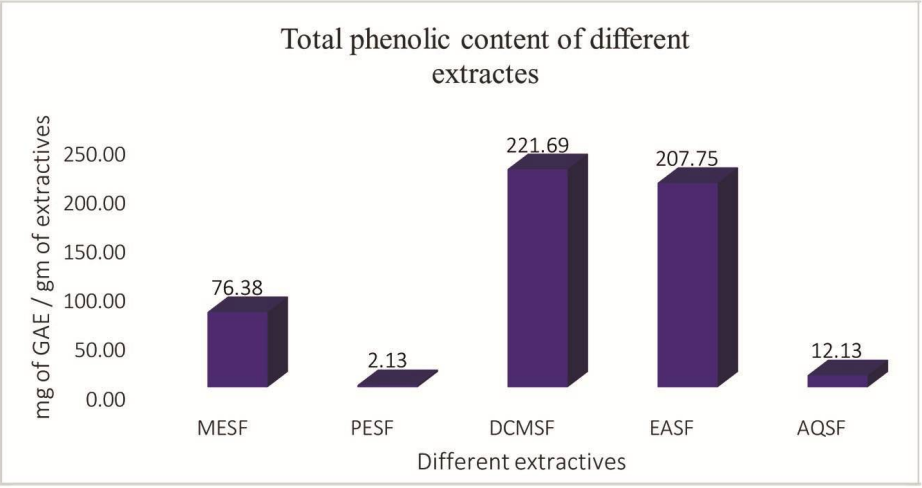

Figure 2. Total phenolic content (mg of GAE / gm of extractives) of different fractions.

Table 2. IC_{50} values of the standard and fraction of leaves of *B. oppositifolia.*

Plant part	Sample code	(IC_{50} µg/ml)	Test sample
Leaves of *B. oppositifolia*	BHT	6.86	Tert-butyl-1-hydroxytoluene (STD.)
	AA	3.05	Ascorbic Acid (STD.)
	MESF	20.71	Methanol Soluble Fraction
	PESF	557.87	Pet-Ether Soluble Fraction
	DCMSF	7.59	Dichloromethane Soluble Fraction
	EASF	0.84	Ethyl Acetate Soluble Fraction
	AQSF	0.03	Aqueous Soluble Fraction

DPPH free radical scavenging activity: MESF of *B. oppositifolia* leaves and its various extractives, namely PESF, DCMSF, EASF, and AQSF, were evaluated for free radical scavenging activity using the method established by Brand-Williams *et al.* (1995). BHT and ASA were utilized as reference standards. The antioxidant activity, as indicated by IC50 values in the DPPH technique, varied among various extracts, ranging from 0.03 µg/ml to 557.87 µg/ml. The extract of *B. oppositifolia* exhibiting the highest free radical scavenging activity was AQSF (0.03), followed by EASF (0.84 µg/ml), DCMSF (7.59 µg/ml), MESF (20.71 µg/ml), and PESF (557.87 µg/ml), in comparison to Ascorbic Acid and BHT, which were 3.05 µg/ml and 6.86 µg/ml, respectively (Table 2).

Antimicrobial activity: ME of *B. oppositifolia* leaves and its various fractions, namely MESF, PESF, DCMSF, EASF, and AQSF, were evaluated for antimicrobial activity at a concentration of 400 µg/disc for each fraction. MESF demonstrates moderate inhibition of microbial growth, with a zone of inhibition ranging from 8 mm to 10 mm. The greatest zone of inhibition seen for MESF was 10mm against *Aspergillus niger* and 11mm against *Staphylococcus aureus*, respectively. The PESF demonstrates moderate inhibition of microbial growth, with a zone of inhibition ranging from 7 mm to 8 mm. The maximum zone of inhibition induced by PESF was determined to be 12mm against *Vibrio parahemolyticus*, followed by 9mm against *Salmonella typhi*. The DCMSF demonstrates

moderate suppression of microbial growth, with a zone of inhibition ranging from 7 mm to 8 mm. The maximal zone of inhibition generated by DCMSF was determined to be 8 mm against *Bacillus subtilis* and *Bacillus cereus*, respectively. The aqueous soluble fraction demonstrates moderate suppression of microbial growth, with a zone of inhibition ranging from 9 mm to 10 mm. The maximal zone of inhibition generated by AQSF was determined to be 12mm against *Saccharomyces cerevisiae*. The study of antibacterial activity demonstrated that all fractions displayed antimicrobial effects against the pathogens indicated in Table 3.

Table 3. Antimicrobial activity of test samples of leaves of *B. oppositifolia.*

Diameter of Zone of Inhibition (mm)						
Test Microorganisms	ME	PESF	DCMSF	EASF	AQSF	Ciprofloxacin
Gram positive bacteria						
Bacillus sereus	8	0	8	8	10	35
Bacillus megaterium	10	8	0	8	9	45
Bacillus subtilis	0	7	8	8	10	42
Staphylococcus aureus	11	7	7	0	8	40
Sarcinalutea	10	0	0	8	10	40
Gram negative bacteria						
Escherichia coli	10	0	0	0	0	42
Pseudomonas aureus	11	9	0	8	0	39
Salmonella paratyphi	0	12	0	8	9	41
Salmonella typhi	0	7	7	7	0	30
Shigella boydii	10	0	7	8	10	35
Shigella dysenteriae	8	0	0	7	10	48
Vibrio mimicus	8	0	0	7	10	30
V. parahemolyticus	10	0	0	0	0	40
Fungi						
Aspergillus niger	10	0	8	0	10	37
Candida albicans	0	0	0	8	10	37
Sacharomyces cerevisiae	12	7	0	7	12	40

Hypnotic solution induced hemolysis: ME of *B. oppositifolia* leaves at a concentration of 2.0 mg/ml considerably inhibited the lysis of human erythrocyte membranes induced by a hypotonic solution, in comparison to the standard ASA (0.10 mg/ml) (Table 4). The ME and its various fractions from the leaves of *B. oppositifolia* shown effectiveness in membrane stabilization by inhibiting erythrocyte lysis caused by hypotonic solutions. MESF inhibited 38.52%, EASF inhibited 10.50%, AQSF inhibited 6.30%, PESF inhibited 4.06%, and DCMSF inhibited 2.82% of red blood cell hemolysis. ASA served as the benchmark drugs for membrane stabilizing action, demonstrating a 61.90% suppression of hemolysis under standard conditions.

Table 4. Effect of different extractives of leaves of *B. oppositifolia* on hypotonic solution-induced hemolysis of erythrocyte membrane.

Sample code Hypotonic medium	Absorbance	Concentration 50 mM	% Inhibition of haemolysis
MESF	0.849	2 mg/ml	38.52
PESF	1.325	2 mg/ml	4.06
DCMSF	1.342	2 mg/ml	2.82
EASF	1.236	2 mg/ml	10.50
AQSF	1.294	2 mg/ml	6.30
ASA	0.526	0.10 mg/ml	61.90

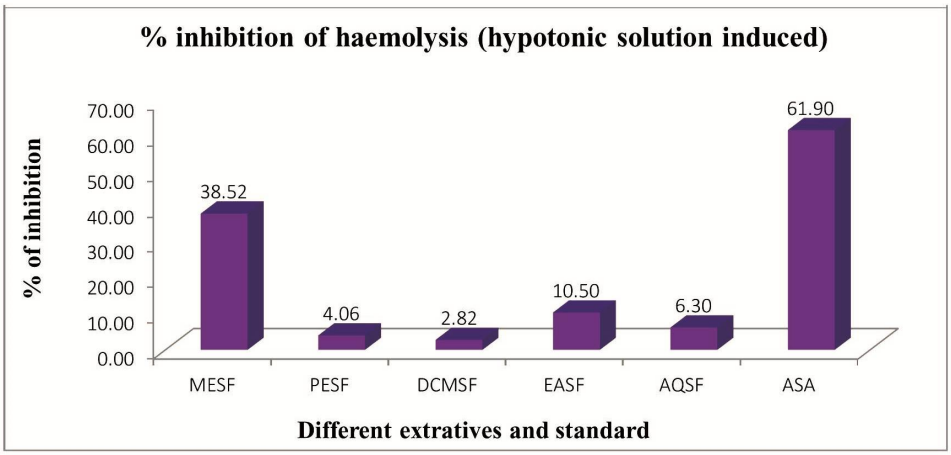

Figure 3. % inhibition of hemolysis of different extractives (hypotonic solution induced).

Heat induced hemolysis: EASF exhibited a 60.35% inhibition of hemolysis in RBCs, DCMSF demonstrated a 37.53% inhibition, MESF showed a 33.72% inhibition, AQSF resulted in a 24.48% inhibition, and PESF displayed a 21.08% inhibition. ASA served as the standard medication for membrane stabilizing action, demonstrating a 42.00% suppression of hemolysis under standard circumstances (Table 5).

Table 5. Effect of different extractives of leaves of *B. oppositifolia* on heat induced hemolysis of erythrocyte membrane.

Sample code	Absorbance		Concentration	% inhibition of hemolysis
	Heat	Cold		
MESF	1.345	0.946	2 mg/ml	33.72
PESF	1.255	0.158	2 mg/ml	21.08
DCMSF	1.047	0.213	2 mg/ml	37.53
EASF	0.685	0.118	2 mg/ml	60.35
AQSF	1.196	0.110	2 mg/ml	24.48
ASA	1.053	0.369	0.10 mg/ml	42.00
CONTROL	1.548			

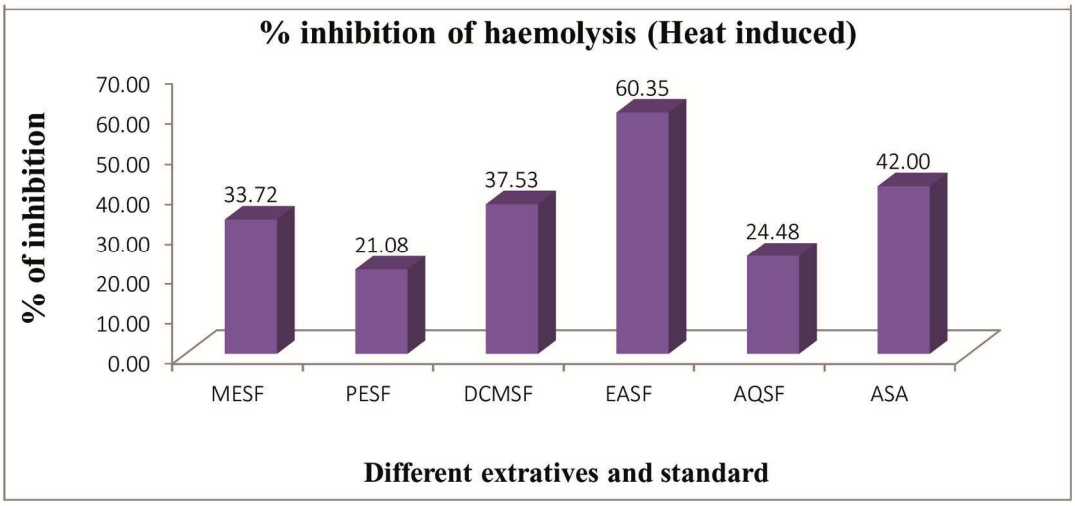

Figure 4. % inhibition of hemolysis of different extractives (heat induced).

Discussion

The present study examined *B. oppositifolia*, commonly known as plum mango, which has been the subject of various biological studies exploring its pharmacological properties. These studies have examined different parts of the plant, including seeds, leaves, and fruits, utilizing various extraction methods and assays to evaluate different *in vitro* studies.

Antioxidants are crucial for neutralizing free radicals, mitigating oxidative stress, and averting chronic and degenerative diseases, including rheumatoid arthritis, neurological disorders, aging, and cancer. Phenolic chemicals are well-known for their antioxidant processes, including free radical scavenging, metal ion chelation, and enzyme inhibition (Lin *et al.*, 2016; Samadd *et al.*, 2024). There is little has studied regarding the antioxidant capabilities of the extracts of *B. oppositifolia* leaves. Of the evaluated fractions, the DCMSF demonstrated the highest TPC at 221.69 mg GAE/g extract, followed by the EASF at 207.75 mg GAE/g extract, whereas MESF exhibited a moderate phenolic content of 76.38 mg GAE/g extract. The aqueous (12.13 mg GAE/g) and Pet-Ether (2.13 mg GAE/g) fractions exhibited markedly reduced phenolic content. These findings highlight the phenolic abundance of DCMSF and EASF, which presumably enhances their antioxidant capacity. The DPPH assay demonstrated significant differences in free radical scavenging activity among the fractions. AQSF exhibited antioxidant activity (IC50 = 0.03 µg/ml), exceeding that of typical antioxidants like Ascorbic Acid (3.05 µg/ml) and BHT (6.86 µg/ml). EASF shown significant activity (IC50 = 0.84 µg/ml), whilst DCMSF (7.59 µg/ml) and MESF (20.71 µg/ml) displayed moderate scavenging capacity. Conversely, PESF exhibited limited antioxidant activity (IC50 = 557.87 µg/ml). Prior research on *Bouea macrophylla* Griffith seeds, a different species of the same genus indicated a IC50 value of 4 µg/ml, akin to ascorbic acid, and a significant TPC (686.05 mg GAE/g), suggesting that phenolic chemicals are the principal contributors to its antioxidant activity (Fu'adah et al., 2022). Our findings indicate that the DCMSF (221.69 mg GAE/g) and EASF (207.75 mg GAE/g) of *B. oppositifolia* are abundant in phenolic compounds, which are recognized for their significant involvement in antioxidant activity. The exceptional free radical scavenging capacity of AQSF and EASF, along with their phenolic composition, highlights their medicinal potential as natural antioxidants.

Pathogenic microbes are responsible for numerous infectious conditions, particularly in distinct anatomical systems or regions (Górniak *et*

al., 2019). The primary challenge in its treatment is the resistance exhibited by pathogenic bacteria to medications. Resulting in suboptimal treatment outcomes and could culminate in therapeutic failure (Aslam and Afridi, 2018). This work examined the antibacterial efficacy of the ME and its assorted solvent fractions (MESF, PESF, DCMSF, EASF, and AQSF) obtained from the leaves of *B. oppositifolia*. The results indicated that the ME demonstrated moderate antibacterial activity, with inhibition zones between 8mm and 12mm, with the maximum inhibition of 11mm against *S. aureus*. Among the fractions, MESF demonstrated significant inhibition (10–11mm) against *S. aureus* and *A. niger*, but AQSF showed the highest inhibition (12mm) against *A. niger*. PESF exhibited moderate inhibition (12mm) against *V. parahemolyticus*, while DCMSF and EASF shown lesser inhibition, each having maximal inhibition zones of 8mm against experimental bacterial strains. In contrast, earlier research on *B. oppositifolia* ME indicated a wider antibacterial efficacy, exhibiting inhibition zones of 12.0–12.5 mm against fish pathogens including *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *Streptococcus agalactiae*. The experiments indicated minimum inhibitory concentration (MIC) values of 25 mg/ml for *A. hydrophila* and *E. ictaluri*, and 50 mg/ml for *S. agalactiae*, implying considerable bacteriostatic or bactericidal efficacy. Another study revealed the AQSF and DCMSF fractions were noted for their efficacy against gram-negative bacteria, including *E. coli* and *Pseudomonas aeruginosa* (Islam *et al.,* 2020). The current investigation revealed superior inhibition of gram-positive bacteria, including *S. aureus* and *B. subtilis*, whereas prior research highlighted greater performance against gram-negative bacteria. This discrepancy may be ascribed to differences in extraction techniques, bacterial strains evaluated, or the doses employed in antimicrobial testing. The present investigation elucidated antifungal efficacy, demonstrating substantial inhibition against *A. niger* (10–12mm), *Candida albicans*, and *S. cerevisiae*, which were overlooked in previous research.

Regarding the hemolytic activity of extracts from *B. oppositifolia*, especially in relation to membrane stabilizing effects generated by hypotonic solution, little has been studied yet. A similar study on *B. macrophylla* which is the different species of same genus integrated its ethanolic extract with yoghurt and evaluated the toxicological effects in animals. The findings demonstrated no notable toxicity at doses up to 2000 mg/kg, indicating a positive safety profile for *B. macrophylla* extracts (Rusli *et al.,* 2023). This work examined the membrane stabilizing properties of *B. oppositifolia* leaf extracts against hypotonic solution-induced hemolysis, demonstrating notable protective effects on erythrocyte membranes. Hemolysis caused by hypotonic stress transpires when RBCs encounter diminished osmotic pressure, resulting in cellular swelling and lysis. In this study, the MESF demonstrated the most significant inhibition of hemolysis (38.52%), succeeded by the EASF (10.50%), AQSF (6.30%), PESF (4.06%), and DCMSF (2.82%). In contrast, the conventional medication ASA exhibited 61.90% suppression of hemolysis at a concentration of 0.10 mg/ml.

The membrane-stabilizing efficacy of *B. oppositifolia* leaf extracts has been assessed also against heat-induced hemolysis, revealing significant protective effects on erythrocyte membranes. A study evaluating this activity revealed that the ME of *B. oppositifolia* leaves demonstrated considerable prevention of hemolysis, suggesting its capacity to stabilize cell membranes under temperature stress (Islam *et al.,* 2022). The heat-induced hemolysis assay is a prevalent technique for evaluating the anti-inflammatory properties of plant extracts, as it indicates the capacity to inhibit the lysis of RBCs under thermal stress. The efficacy of *B. oppositifolia* in this assay indicates the existence of bioactive chemicals that can improve membrane stability (Anosike *et al.,* 2012). This study assessed the membrane-stabilizing efficacy of various methanolic fractions of *B. oppositifolia* leaves in relation to heat-induced hemolysis in human erythrocytes. The results indicated that these extracts significantly inhibited RBC lysis, with the EASF exhibiting the greatest inhibition at 60.35%, followed by DCMSF at

37.53%, MESF at 33.72%, AQSF at 24.48%, and PESF at 21.08%. The results were compared to ASA, a common anti-inflammatory drug, which demonstrated 42.00% inhibition. The notable membrane-stabilizing properties of *B. oppositifolia* extracts indicate the existence of bioactive substances that can enhance erythrocyte integrity during thermal stress. The heat-induced hemolysis experiment is a recognized technique for evaluating anti-inflammatory potential, as membrane stabilization is essential for mitigating inflammation by inhibiting cell lysis (Anosike *et al.*, 2012). The documented protective effect of *B. oppositifolia* corresponds with previous research emphasizing the capacity of plant extracts to stabilize biological membranes, attributable to their abundant phytochemical content (Gunathilake *et al.*, 2018). The superior efficacy of the EASF fraction indicates that it possesses a greater concentration of active phytochemicals that contribute to improved erythrocyte membrane integrity. These findings underscore the medicinal significance of *B. oppositifolia* leaf extracts as possible natural anti-inflammatory drugs, capable of inhibiting hemolysis under thermal stress conditions. Future study should concentrate on finding specific bioactive chemicals responsible for this action and assessing their potential applications in inflammatory conditions.

Conclusion

Leaf extracts of *B. oppositifolia* show significant antioxidant, antimicrobial, and membrane-stabilizing properties, especially in the AQSF and EASF fractions. These activities are associated with the plant's rich phenolic and flavonoid content. The findings can be a potential natural source for pharmacological applications. Further research is needed to isolate, characterize and see their activity in animal models.

Author contribution

Md. Ashraful Islam, Abdul Kuddus: Analyzed and interpreted the data; wrote the paper.

Md. Khalid Hossain, Mohammad A. Rashid: Conceived and designed the experiments; Reviewed and edited the paper.

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No funds have been received to conduct this study.

Conflicts of Interest

There are no conflicts of interest that the authors have disclosed concerning the publication of this paper.

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References

Anosike, C. A., Obidoa, O. and Ezeanyika, L. U. 2012. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). *DARU J. Pharm. Sci*.. **20**, 1-7

Aslam, I. and Afridi, M. S. K. 2018. Pharmacognostic characterization of Beaumontia grandiflora (Roxb.) Wall. Leaf for taxonomic identification for quality control of a drug. *J. Appl. Res. Med. Aromat. Plants*. **8**, 53-59.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. **45**, 493-496.

Beckett, M. and Stenlake, O.I. 1986. Modified Kupchan partition. *J. Med. Chem.* **52**, 1525-1529.

Bennett, B. C. 1998. Pharmaceuticals and Forests Medicinal Resources of the Tropical Forest: Biodiversity and Its Importance to Human Health Michael J. Balick Elaine Elisabetsky Sarah A. Laird. *Bio. Sci*. **48**, 213-214.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*. **28**, 25-30.

Duyen, T.T.M., Tuan, N.T. and Hoa, T.T.T. 2022. In Vitro Antibacterial Activity of Tropical Plant Extracts Against Fish Pathogens in Vietnam. *Asian Fish. Sci*. 35.

Fabricant, D. S. and Farnsworth, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect.* **109**, 69-75.

Fu'adah, I. T., Sumiwi, S. A. and Wilar, G. 2022. The Evolution of Pharmacological Activities Bouea macrophylla Griffith In Vivo and In Vitro Study: A Review. *Pharmaceuticals*. **15**, 238.

Górniak, I., Bartoszewski, R. and Króliczewski, J. 2019. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*. **18**, 241-272.

Gunathilake, K. D. P. P., Ranaweera, K. K. D. S. and Rupasinghe, H. P. V. 2018. In Vitro Anti-Inflammatory Properties of Selected Green Leafy Vegetables. *Biomedicines*. **6**, 107.

Islam, A., Taher, M. A., Soma, M. A., Bari, M. S., Khandokar, L., Mansur, M. A. A., Hossain, M. K. and Rashid, M. A. 2022. Lupane triterpenoids, phytosterol, phenolic acid and fatty acid from leaves of bouea oppositifolia (roxb.) Meisn. *Bangladesh J. Bot*. **51**, 185-189.

Islam, M. A., Bari, M. S., Taher, M. A., Chowdhury, A., Hossain, M. K. and Rashid, M. A. 2020. Antidiarrheal and analgesic activities of bouea oppositifolia (roxb.) adelb. in experimental animal model. *Bangladesh pharm. J.* **23**, 167-171.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. and Chen, S. 2016. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules*. 21, 1374.

Rahman, M. 2018. Plant diversity in Hazarikhil Wildlife Sanctuary of Chittagong and its conservation management. *JBCBM*. 3, 43-56.

Rajan, N. S. and Bhat, R. 2017. Volatile constituents of unripe and ripe kundang fruits (*Bouea macrophylla* Griffith). *Int. J. Food Prop.* **20**, 1751-1760.

Rajan, N. S. and Bhat, R. 2020. Bioactive Compounds of Plum Mango (Bouea microphylla Griffith). In H. N. Murthy & V. A. Bapat (Eds.), *Bioactive Compounds in Underutilized Fruits and Nuts*. Springer International Publishing. pp. 529-541

Rusli, R. N. M., Naomi, R., Yazid, M. D., Embong, H., Perumal, K., Othman, F., Jasni, A. S., Jumidil, S. H., Balan, S. S., Abidin, A. Z., Kadir, K. K. A., Bahari, H. and Zakaria, Z. A. 2023. Phytochemical Analysis and Toxicity Assessment of Bouea Macrophylla Yoghurt. *Toxins*. 15, 125.

Samadd, Md. A., Hossain, Md. R., Taher, M. A., Hasan, M. M., Haque, S., Rafid, M. I., Hossain, Md. S., Islam, Md. A. and Khan, M. 2024. Multifaceted Chemico-Pharmacological Insights into *Cynometra ramiflora* L.: Unveiling its GC-MS, Cytotoxic, Thrombolytic, Anti-Inflammatory, Antioxidant, Anti-Diarrheal, Hypoglycemic, and Analgesic Potentials. *Nat. Prod. Commun*. 19, 1934578X241257377.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. and Yoga Latha, L. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *AJTCAM*. **8**, 1-10.