

First Report on the Antihemolytic and Membrane Stabilizing Potential of *Sonneratia alba* Fruit Extract

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Abstract

This investigation aimed to determine the membrane protective effect of *Sonneratia alba* fruit extract against hemolysis by employing the human red blood cell (HRBC) membrane stabilization method. Erythrocyte lysis was triggered under hypotonic stress, and the extent of protection offered by the extract was quantified by spectrophotometric estimation of released hemoglobine, the tested extract showed a concentration-dependent inhibitory effect on hemolysis, showing an IC₅₀ of 66.08µg/mL, indicating notable stability of the erythrocyte membrane. The observed activity suggests that the fruit extract possesses considerable cytoprotective property. To our knowledge, this work provide the first experimental evidence supporting the antihemolytic potential of *S.alba* fruit extract as a natural membrane stabilizing agent.

Keywords: *Sonneratia alba* fruit; Antihemolytic activity; HRBC membrane stabilization; Erythrocytes protection.

Introduction

Sonneratia alba is a mangrove species whose fruit has been utilized in traditional healthcare practices for treating various disorders, suggesting its potential therapeutic significance¹³. Mangrove plants are recognized as valuable sources of diverse bioactive secondary metabolites¹². Although the fruit of *S. alba* is traditionally regarded as medicinally important, systematic pharmacological evaluation of its biological properties remains limited⁵. Antihemolytic activity refers to the ability of a substance to protect red blood cells from membrane damage and prevent hemoglobin leakage under stress conditions. The HRBC membrane stabilization method is widely used as an in vitro model to evaluate membrane-protective and anti-inflammatory properties due to similarities between erythrocyte and lysosomal membranes. Substance that inhibit hemolysis

by stabilizing erythrocyte membranes are therefore considered to have cytoprotective potential⁷.

Study Area

The fruits were collected from the mangrove forests of Dabhol, located in Ratnagiri district, Maharashtra, India (17°35'14.15"N, 73°10'29.23"E), where *Sonneratia alba* is a dominant mangrove species. Fig1



Fig 1: Study area map

Fruit About

Figure 2. Morphology of *Sonneratia alba* fruit showing its disc-shaped structure and smooth pericarp. *Sonneratia alba* (family Sonneratiaceae), commonly known as mangrove apple, produces a globose to slightly flattened berry measuring approximately 3–6 cm in diameter. The fruit possesses a smooth outer surface that transitions from green at the immature stage to yellowish-brown upon ripening, often with a distinct central depression. It develops directly from the trunk or mature branches and encloses numerous small seeds within a differentiated pericarp comprising exocarp and mesocarp layers. Adapted to saline intertidal mangrove environments, the fruit is locally consumed in certain coastal regions. Studies have indicated the presence of several bioactive compound such as phenolics, flavonoide, tannins, saponin, sterols, and triterpenoids, along with essential minerals, including calcium, magnesium, and potassium¹³. These constituents are known to contribute to various biological properties like antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activities ^{10,14,15}.



Fig 2: *Sonneratia alba* fruit

Mechanistic Link Between Antioxidant and Antihemolytic Activity

Several studies have documented significant antioxidant activity in different *Sonneratia* species. Extracts of *S. alba* leaves, bark, stem, root, and fruit have demonstrated moderate to strong DPPH radical scavenging activity depending on solvent type and phytochemical composition (Gawali & Jadhav, 2011; Milon et al., 2012; Asad et al., 2013; Haq et al., 2014; Wijaya et al., 2023; Wonggo et al., 2024). Similarly, *S. caseolaris*, *S. apetala*, and *S. ovata* have exhibited potent antioxidant activity in DPPH, ABTS, and related assays, largely attributed to the presence of phenolics, flavonoids, tannins, and triterpenoids (Hossain et al., 2012; Laili et al., 2018; Munira et al., 2019; Audah et al., 2022; Astuti et al., 2023; Cerri et al., 2025). Overall, antioxidant efficacy within the genus is strongly correlated with its rich polyphenolic composition.

The antioxidant activity of *Sonneratia* extracts may protect erythrocyte membranes by scavenging free radicals and inhibiting lipid peroxidation, explaining their antihemolytic effects.

Plant Extraction

Fresh fruits of *Sonneratia alba* were collected from the mangrove area of Dabhol and washed thoroughly. About 10 g of fruit pulp was crushed and mixed with 50 mL DW to prepare extract, which was then stirred, filtered using Whatman NO.1 filter paper, and store at 4°C for further antihemolytic analysis.

Methodology

Membrane Stabilization (Antihemolytic) Method

Fresh human blood was obtained, and erythrocytes were separated by centrifugation at 3000 rpm for a duration of 10 minutes, followed by repeated washing with isotonic saline. A 10% suspension of erythrocytes was then prepared using in phosphate buffer (pH 7.4). The reaction mixture contained of extract at different concentrations, phosphate buffer, and erythrocyte suspension.

The mixtures were incubated at 37°C for 30 minutes and subsequently centrifuged at 3000 rpm for a duration of 10 minutes. Hemolysis was caused by adding a hypotonic solution (0.36% NaCl). The absorbance of the resulting supernatant was recorded at 540 nm to assess the extent of hemolysis. The percentage of membrane stabilization was calculated in comparison with the control sample 1,9. Figure 3

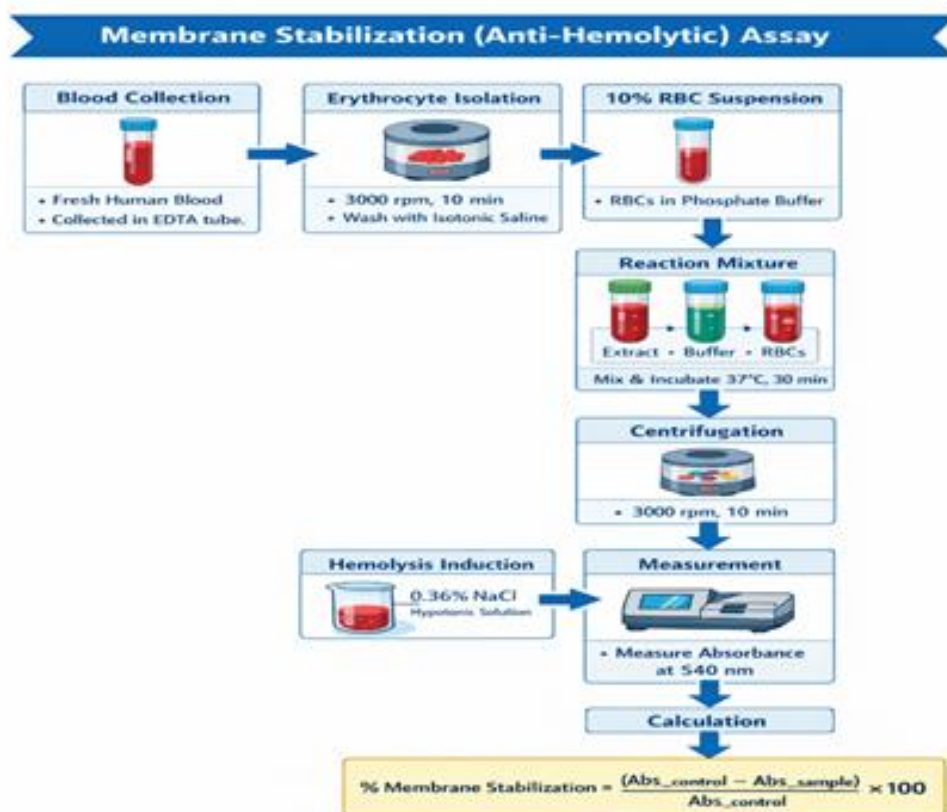


Figure3. HRBC membrane stabilization assay.

Observation

Table 1. Antihemolytic of fruit extract at different concentrations (mean $\pm$ SD, n = 3).

Concentration (μ g/ml)	Fruit sample % inhibition of hemolysis	Positive control inhibition hemolysis	% of
10	16.39%	45.12%	
50	29.36%	68.45%	
100	82.71%	94.20%	

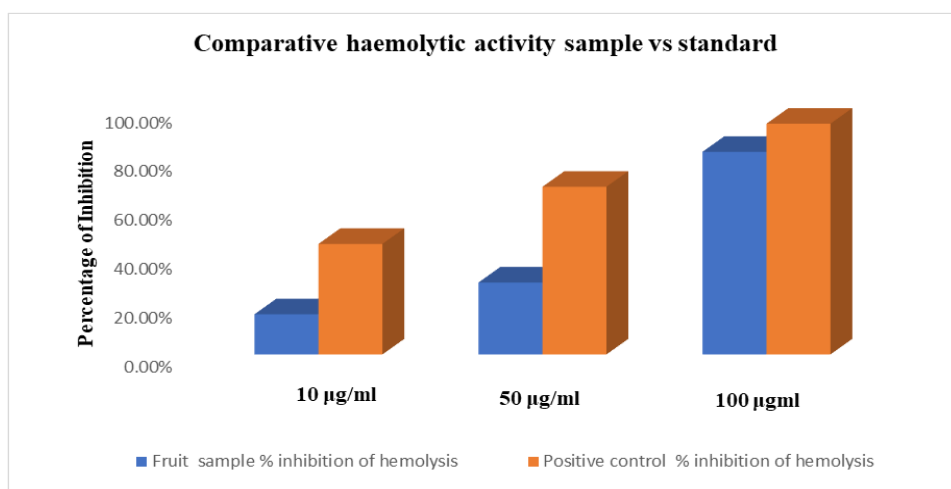


Figure 4. Concentration-dependent antihemolytic activity of fruit extract compared with standard

The antihemolytic activity of the fruit extract was assessed at different concentrations using the HRBC membrane stabilization method. As shown in Table 1 and Figure 4, the extract exhibited a concentration-dependent increase in membrane stabilization. The percentage inhibition of hemolysis increased from $16.39 \pm 1.12\%$ at 10 µg/mL to $82.71 \pm 3.10\%$ at 100 µg/mL. The positive control showed higher inhibition at all concentrations, ranging from $45.12 \pm 2.05\%$ to $94.20 \pm 1.20\%$. The IC_{50} value of the extract was calculated as 66.08 µg/mL, indicating significant erythrocyte membrane stabilizing potential.

Statistical Analysis

Experiments were performed in triplicate ($n=3$), and results are expressed as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA ($P<0.05$), and IC_{50} was calculated by linear regression.

Result

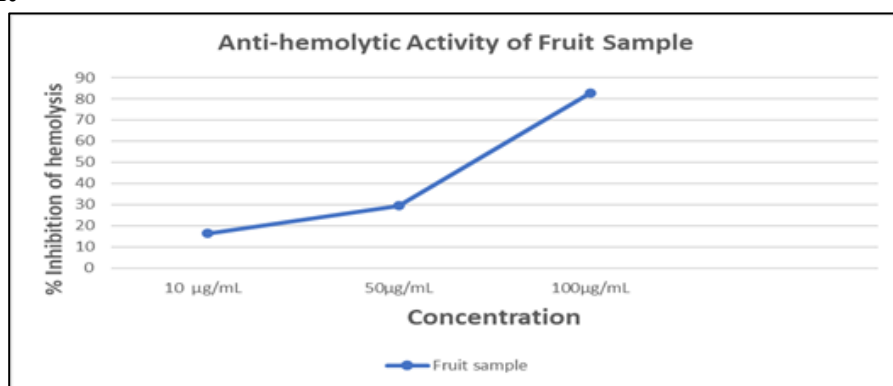


Figure 5. Dose-dependent antihemolytic activity of fruit extract showing percentage inhibition of hemolysis at different concentrations.

A concentration-dependent increase in antihemolytic effect was observed for the fruit extract. As shown in Figure 5, percentage inhibition of hemolysis increased progressively with increasing concentration, reaching maximum membrane stabilization at 100 µg/mL. The calculated IC₅₀ value was 66.08 µg/mL, indicating substantial erythrocyte membrane protective potential.

Discussion

The present study revealed significant concentration-dependent antihemolytic activity of the fruit extract in the HRBC membrane stabilization assay, with maximum protection (82.71%) observed at a concentration of 100 µg/mL, with an IC₅₀ of 66.08 µg/mL. Membrane stabilization under hypotonic stress suggests effective protection of erythrocytes against hemoglobin leakage. Although no previous reports are available on the antihemolytic activity of this fruit, earlier studies have documented strong antioxidant potential within the genus *10*. Oxidative stress is a key factor responsible for erythrocyte membrane damage, which may be reduced by the availability of bioactivity compounds *7,8,9*. These observations provide early scientific evidence supporting its cytoprotective potential. However, further in vitro and in vivo studies are necessary to better understand its therapeutic applications.

Conclusion

The present study demonstrates that the fruit extract possesses significant, concentration-dependent antihemolytic activity by effectively stabilizing erythrocyte membranes under hypotonic stress conditions. The IC₅₀ value of 66.08 µg/mL indicates substantial membrane protective potential. These findings provide preliminary scientific evidence supporting its cytoprotective relevance and justify further phytochemical characterization and in vivo investigations.

Ethical Considerations

Human blood samples were obtained from a certified diagnostic laboratory (Shri Ram Laboratory, Dapoli) after confirmation of donor health status. The samples were collected with informed consent from the donor and used strictly for in vitro research purposes. No invasive procedure was performed by the authors.

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