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Phytochemical Screening and Evaluation of Antioxidants and Membrane Stabilizing Activities of *Boehmeria glomerulifera* Miq. Leaf

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Abstract

The plant *Boehmeria glomerulifera* is native to Bangladesh which is used by traditional healers to treat a variety of illnesses. Therefore, we aimed to meticulously investigate the therapeutic potential such as antioxidant and membrane stabilizing activities of the leaf extract of this medicinal plant. Ethanol was used to extract the coarse leaf powder at room temperature. Chloroform-soluble and n-hexane-soluble fractions were obtained by solvent-solvent partitioning. Antioxidant potential was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, and the extractives' membrane stabilizing activity was assessed by their ability to prevent hypotonic solution-induced hemolysis of human erythrocytes. The membrane stabilizing values were presented as mean ± SE (n = 3). P values were determined using a one-way analysis of variance (ANOVA) test. Our study found the presence of organic compounds like alkaloids, flavonoids, glycosides, saponins, phenols and tannins in *B. glomerulifera* Miq. leaves. The free radical scavenging and membrane-stabilizing properties of the extract increased in a dose-dependent manner. In the DPPH study, the chloroform fraction displayed the highest DPPH radical scavenging activity (IC_{50} = 20.13 µg/ml) compared to crude ethanol extract and its n-hexane fraction. The crude ethanol and fractionated n-hexane extracts revealed considerable scavenging activity (IC_{50} value: 55.80 µg/mL, 72.41 µg/ml, respectively). The chloroform fraction displayed 75.9 ± 0.252% inhibition of hemolysis of RBC at high concentrations of the extract. The crude ethanol extract and its fraction n-hexane showed inhibition of hemolysis by 29.85±0.546% and 31.67 ± 0.319%, respectively, as compared to 85.99 ± 0.176% demonstrated by the standard, acetyl salicylic acid. The study found that the ethanol of *B. glomerulifera* and its fractions exhibit moderate to strong membrane stabilizing and notable antioxidant properties. However, further investigation is still required to isolate, characterize the phytoconstituent and determine their underlying mechanism of action of the pharmacological activities.

Key words: Phytochemicals, *Boehmeria glomerulifera*, antioxidants; membrane stabilizing, leaves extract.

Introduction

Traditionally, medicinal plants are utilized extensively to treat a variety of illnesses because they contain chemical compounds that serve as precursors for the isolation and development of effective medications (Schultes, 1982). The availability, affordability, low side effects, simplicity of extraction, efficacious analytical testing and

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successful drug screening of herbal treatments make them superior to chemically manufactured ones.

The flowering plant *B. glomerulifera* often called false nettle, is a member of the family Urticaceae. It is a tiny tree or deciduous shrub that has spreading branches (Jiarui *et al.*, 2003). In Bangladesh, India, Laos, Indonesia, Sikkim, Bhutan, Myanmar, Thailand, Sri Lanka, and Vietnam, the plant is abundantly found. When combined with *Amomum aromaticum*, the fresh leaves of this plant are used by traditional healers to cure anemia (Rahman *et al*., 2007). Babies with fever are advised to take a bath in boiling leaf water. It may be utilized as a decorative plant as well (Papia *et al*., 2016). However, *B. glomerulifera*'s biological activities have not been thoroughly investigated. Therefore, the present study aimed to identify the phytochemicals and explore the antioxidants and membrane-stabilizing activities of *B. glomerulifera* leaves extract. The results will facilitate the investigation of bioassay-guided fractionation, enabling the separation of the pure biological compound from the *B. glomerulifera* Miq. crude extract. It will also support preclinical testing on in vivo models to prevent inflammatory disease, including hepatitis, Alzheimer's, arthritis, cancer and Parkinson's.

Materials and Methods

Reagents and chemicals: Ethanol, chloroform, methanol, n-hexane and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used. Chemicals and reagents used in this study were of analytical grade and purchased from Merck, Germany. Reference drugs, Ascorbic acid and Acetylsalicylic acid, were obtained from Popular Pharmaceuticals Ltd. in Bangladesh.

Plant material collection and authentication: Fresh leaves of *B. glomerulifera* Miq. were collected from the hill tracts of Mirsorai, Chattogram, Bangladesh, when the plant grows at its fastest rate. The plant was taxonomically identified by experts from the Bangladesh Forest Research Institute (BFRI) Herbarium in Chittagong.

Plant extraction and fractioning: The plant material was processed by removing rotten leaves and stems after collection. Before drying, the plant material was sieved, removed the soil, and cleaned properly. After shade drying, leaves were exposed to grinding to produce a coarse powder. The powdered material (350g) was extracted with 1250 ml of ethanol in a Soxhlet apparatus. Then, using cotton and subsequently Whatman filter paper, the extracted sample was filtered. Using a rotary evaporator, at 35 °C, the ethanol solvent was evaporated and concentrated the filtrate. By using the modified Kupchan partitioning approach, the concentrated ethanol extract was subsequently fractionated (VanWagenen *et al.*, 1993). The ultimate fractionates, i.e., chloroform soluble and n-hexane soluble were used for the pharmacological screenings.

Preliminary qualitative phytochemical screening: The crude and fractionated extracts of *B. glomerulifera* were subjected to various tests to determine their chemical nature.

Test for alkaloids: The plant extract was dissolved in 100 mL of water and filtered. 2 ml of filtrate was then heated in steam with 1% HCl. Next, 1 ml of the heated liquid was combined with 6 ml of the Mayer-Wagner reagent. A precipitate of brown-red color was observed, which indicates the presence of alkaloids (Dubale *et al*., 2023).

Test for flavonoids: Concentrated H2SO4 and a few drops of a 10% (w/v) NaOH solution were incorporated to 0.05 g of extract. The flavonoids existence was specified by the formation of a yellow color (Anusmitha *et al*., 2022).

Test for saponins: Distilled water (20 ml) was taken in a graduated measuring cylinder. Then, the extract was added and mixed properly for 15 minutes. The foam layer formation suggests the existence of saponins in the plant extracts (Chen *et al*., 2022).

Test for glycosides: A drop of a ferric chloride solution and 2 mL of glacial acetic acid were combined with the test sample, which was prepared by diluting 0.05 g of extract with deionized water (5 mL). Sulfuric acid (1 ml) was gradually added to the

mixture. Glycosides' presence was confirmed after a brown ring formed at the interface (Xu *et al*., 2020).

Test for tannins: 10 mL of deionized water was combined with 5 g of extract. For five minutes, the mixture was boiled. The presence of tannins is demonstrated by the greenish precipitate that forms when two drops of 5% FeCl3 are added (Chiangnoon *et al*., 2022).

Test for steroids: 1 ml of chloroform was used to dissolve 50 mg of the extract. Sulfuric acid was carefully introduced to create a lower layer. When a steroidal ring is present, the interface appears reddish-brown (Agidew, 2022).

Test for sugars: 500 mg of the material was first combined with water. Then a few drops of fehling's reagent were added. After that, a water bath was used to heat the mixture. There are reducing sugars present when brick-red precipitate forms (Nakaziba *et al*., 2022).

Test for phenols: 0.5 ml of extract was mixed with 5 mL of the reagent Folin-Ciocalteu and 4 mL of aqueous sodium carbonate. The existence of phenols is revealed by the emergence of a blue tint (Sarika Saxena, 2015).

Test for gums: A 5 ml sample solution was combined with sulfuric acid and Molisch's reagent. The existence of gums is confirmed by the production of a reddish violet ring, which is observed at the intersection of two layers of liquid (Islam *et al*., 2023). However, the plant extracts didn't show the presence of gums.

Test for amides: A very small quantity of the test extract was mixed with NaOH (20%w/v), boiled for 15 minutes. The emission of ammonia gas transforms red litmus blue, demonstrating the presence of amides (Islam *et al*., 2023). However, the plant extracts didn't show the presence of amides.

In vitro antioxidant study of extracts by DPPH assay: Using the methodology suggested by Katalinic *et al*., we assessed the plant extracts' antioxidant activity against DPPH (Katalinic *et al*., 2006). Briefly, a solution of 0.1 mM DPPH in methanol was prepared and kept at 4 °C. Stock solution of each extract and ascorbic acid were prepared at 200 µg/ml concentration which was used for serial dilution to obtain the concentrations of 20, 40, 60, 80, and 100 µg/mL. Methanol and water were used as solvent for the serial dilution of extract and ascorbic acid, respectively. 3 ml of DPPH solution was introduced to each test tube containing 1 mL of standard ascorbic acid solution and 1 ml of each extract solution from each concentration. After 30 minute incubation at dark, the UV absorbance at 517 nm of each solution, blank (Methanol and water) and control (mixture of 3 ml DPPH and 1 ml water for standard, and mixture of 3 mL DPPH and 1 ml methanol for samples) was measured. IC_{50} values were computed by plotting the percentage of inhibition against concentration.

DPPH% Inhibition = Abs. of control solution- Abs. of sample solution/ Abs. of control solution ×100

In vitro membrane stabilizing assay: We performed the technique suggested by Shinde *et al*. to study the membrane-stabilizing properties of plant extracts (Shinde *et al*., 1999). The stock solutions of each extracts were prepared at the concentration of 1000 µg/mL that were further diluted to get the concentration of 500 µg/mL and 250 µg/ml. 0.1 mg/mL of acetyl salicylic acid was prepared in water. For the preparation of erythrocyte suspension, approximately 5 ml of blood was placed in EDTA-Na2 tube. Then an equivalent quantity of sterile Alsever's solution was added in the tube. Isotonic solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) was used to rinse the blood and isosaline was used to provide a 10% (v/v) suspension. Next, erythrocyte suspension (0.5 mL), phosphate buffer (1 ml) and hypotonic saline (2 mL) were added to each treatment tube. Later on, 1 mL of acetylsalicylic acid (0.1mg/ml) was mixed for the standard groups, 1 ml of water, ethanol, n-hexane, and chloroform was added to the control tubes respectively, and for the test group, 1 ml of each solvent fractions was mixed as marked. All the reaction mixtures were incubated at 37^0C °C for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. After cooling and filtering, supernatant was subjected to a UV-visible spectrophotometer to

measure the absorbance at 560 nm. The percentage inhibition of hypotonic solution-induced hemolysis was calculated as follows:

% Inhibition of hemolysis = (1 – Abs sample/Abs control) × 100

Statistical analysis: The membrane stabilizing data were presented in mean ± SE (n = 3), and one-way analysis of variance ANOVA (Tukey test) was performed to determine the significance of differences between the standard and test groups. P value < 0.05 was considered a significant difference and marked as*.

Results and Discussion

Preliminary qualitative phytochemical screening: The presence or absence of secondary metabolites in each solvent leaf extract was determined by a preliminary qualitative phytochemical analysis. The plant's phytochemical analysis showed that the three solvent extracts included a variety of bioactive substances, including alkaloids, flavonoids, saponins, glycosides, sugars, steroids, tannins and phenols (Table 1). None of the extracts showed the existence of gum and amides as phytoconstituents. Furthermore, saponins and gums were absent in the n-hexane extract.

In vitro antioxidant study of extracts by DPPH assay: Changing the purple solution to light yellow results from the decrease of free radicals, indicating the scavenging action (Akar *et al.*, 2017). In comparison to the standard ascorbic acid (53.86% to 85.04%), the ethanol extract and its chloroform, and n-hexane soluble fractions demonstrated a free radical scavenging activity in a concentration-dependent manner, ranging from 35.13% to 72.14%, 48.49% to 74.04% and 17.16% to 68.24%, respectively, as indicated in table 2 and figure 1.

Table 1. Phytoconstituents found in the *B. glomerulifera* leaf extracts.

Chemical groups	Ethanol extract	Chloroform extract	n-Hexane extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	-
Glycosides	+	+	+
Tannins	+	+	+
Steroids	+	+	+
Sugars	+	+	+
Phenol	+	+	+
Gums	-	-	-
Amides	-	-	-

Table 2. *In vitro* DPPH scavenging activities of *B. glomerulifera* leaf extracts.

Concentration (µg/ml)	DPPH scavenging activity (% inhibition)			
	Ascorbic acid	Ethanol extract	Chloroform	n-Hexane extract
20	53.86	35.13	48.49	17.16
40	62.56	42.73	57.13	27.67
60	69.06	5.44	64.10	37.78
80	75.48	59.04	71.18	58.04
100	85.04	72.14	74.04	68.24

According to the result, the chloroform extract exhibited the strongest inhibitory effects for free radicals (74.04%), followed by the crude ethanol extract (72.14%) and n-hexane extract (68.24%). The concentration needed for 50% inhibition, or IC_{50} values, was calculated for each extract and was 55.80 µg/ml, 20.13 µg/ml and 72.41 µg/ml for ethanol, chloroform and n-hexane, respectively. Nevertheless, the IC_{50} for the standard drug was 8.98 µg/ml (Table 3 and Figure 2). Low IC_{50} values correspond to high antioxidant activity (Tariq *et al.*, 2022). Whereas three of the plant extracts have moderate antioxidant activity, the chloroform extract exhibits the maximum antioxidant activity with the smallest IC_{50} value.

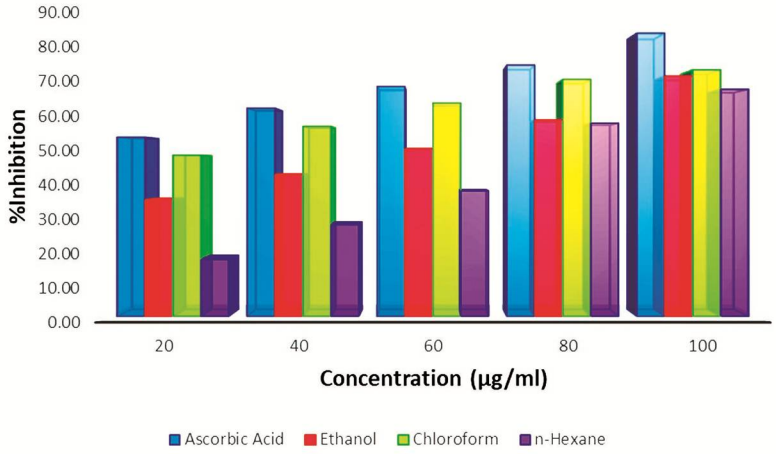

Figure 1. DPPH scavenging activity (% inhibition) of *B. glomerulifera* leaf extracts.

Table 3. IC_{50} values of different fractions and the standard drug.

Treatment groups	IC_{50} (µg/ml)
Ascorbic Acid	8.98
Crude ethanol extract	55.80
Chloroform soluble fraction	20.13
n-Hexane soluble fraction	72.41

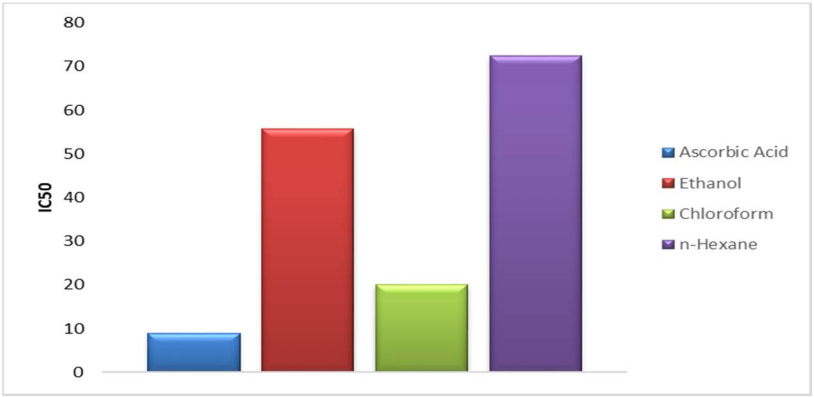

Figure 2. IC_{50} values (µg/ml) for antioxidant activity.

In vitro membrane stabilizing assay: The study investigated the impact of three solvent extracts on the total hemolysis of erythrocytes in a hypotonic solution, as shown in table 4 and figure 3. The extracts of *B. glomerulifera* showed concentration-dependent membrane stabilizing activity when compared to the standard drug, acetylsalicylic acid. The maximum concentration analyzed was 1000 µg/ml. The % inhibition of hemolysis of erythrocytes by the chloroform extract at various concentrations of 250, 500, and 1000 µg/ml were 68.97±0.505%, 70.85 ± 0.483% and 75.9 ± 0.252%, respectively. The ethanol extract suppressed hemolysis by 29.85 ± 0.546% and the n-hexane extract by 31.67 ± 0.319% at a higher dose of 1000 µg/ml. Acetyl salicylic acid, a standard drug at 0.1 mg/ml, with a % inhibition of 85.99 ± 0.176%, demonstrated greater effectiveness in preventing hypotonic-induced hemolysis of RBCs in comparison with the extract.

Table 4. Membrane stabilization activities of *B. glomerulifera* leaf extracts.

Test group	Concentration	Inhibition of hemolysis (%) ± SEM
Control	-	-
Acetylsalicylic acid	0.1 mg/ml	85.99 ± 0.176
Ethanol extract	250 µg/ml	21.31 ± 0.443*
	500 µg/ml	26.13 ± 0.404*
	1000 µg/ml	29.85 ± 0.546*
Chloroform extract	250 µg/ml	68.97 ± 0.505*
	500 µg/ml	70.85 ± 0.483*
	1000 µg/ml	75.9 ± 0.252*
n-hexane extract	250 µg/ml	17.94 ± 0.370*
	500 µg/ml	22.20 ± 0.506*
	1000 µg/ml	31.67 ± 0.319*

P value < 0.05 was considered a significant difference from the standard group and marked as *

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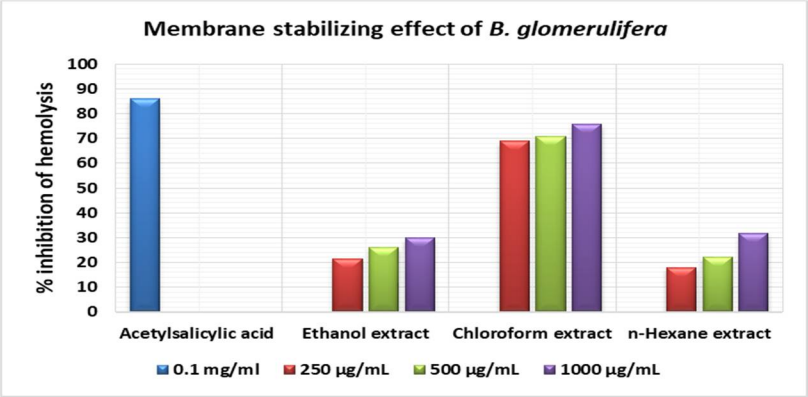

Figure 3. % inhibition of hemolysis of the *B. glomerulifera* leaf extracts.

Excessive production of free radicals in vivo leads to oxidative stress, immune system destruction, and various diseases like Alzheimer's, Parkinson's, diabetes, neurodegenerative disorders, cancer, AIDS, carcinogenesis, cardiovascular complications, aging, DNA damage, and metabolic disorders (Seyoum *et al*., 2006). Antioxidants, found in plant-derived compounds, may help combat free radicals and

reduce chronic disease risk, with their role in plant medicine's protective effect being increasingly explored (Jin *et al*., 2006). The study utilized the DPPH method to examine the antioxidant activity of *B. glomerulifera* Miq. highlighting its benefits such as sensitivity, feasibility, simplicity, and stability (Aparadh *et al*., 2012; Ozcelik *et al*., 2003). The DPPH radical scavenging property of the test samples can be monitored by observing the decrease in absorbance of DPPH solution when a hydrogen-donating antioxidant reacts with it, resulting in a lower absorbance and decolorization (Blois *et al*., 1958).

In this investigation, we found the DPPH radical scavenging action of *B. glomerulifera* leaves. The IC_{50} values were 20.13 µg/mL, 55.8 µg/ml and 72.41µg/ml for chloroform, ethanol, and n-hexane extract, respectively, demonstrating that the plant extract has a substantial free radical scavenging effect and might function well as an antioxidant. Numerous flavonoids and phenols have been shown to have a substantial role in the antioxidant activity of medicinal plants. Many phenolic compounds can act as antioxidants by hydrogenating or complexing with oxidizing species to scavenge or stabilize free radicals engaged in oxidative processes. This is made possible by the conjugated ring structures and hydroxyl groups they contain (Khan *et al*., 2012) .

Our study also examined the membrane stabilizing effect of various extracts of *B. glomerulifera* Miq. leaves. The investigations revealed that the chloroform extract has notable stabilizing properties (Figure 3). The crude ethanol extract and n-hexane extract of plant leaves demonstrated mild to moderate membrane-protecting activity. Another early investigation also confirmed the in vitro membrane stabilizing action using chloroform, carbon tetrachloride, petroleum ether, and methanol soluble extract of *B. glomerulifera* Miq., which revealed a mild to moderate range of membrane stabilization effect (Papia *et al*., 2016). The existence of flavonoids, phenols, and saponins in *B. glomerulifera* might be the cause of its membrane stabilizing properties.

Conclusion

The present study revealed that extracts from *B. glomerulifera* leaves had antioxidant properties and stabilized human red blood cell membranes in a dose-dependent way. Flavonoids, alkaloids, tannins, phenols, and saponins were found in various extracts according to a preliminary chemical analysis, and these compounds may be the drivers of the antioxidant and RBC membrane stabilizing effects. The chloroform extract in the DPPH study unveiled powerful antioxidant properties, whereas the ethanol and n-hexane extracts presented moderate efficacy. The ethanol and n-hexane extracts reasonably stabilized the erythrocyte membrane, while the chloroform extract exhibited a significant stabilization effect. These findings demonstrate the plant species' capacity to yield novel bioactive chemicals. To separate and characterized individual compounds that are responsible for these bioactivities, along with potential mechanisms of action, more research is essential.

Conflict of interest

The authors declare no conflict of interest.

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