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Phytochemical screening and evaluation of antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activities of different fractional extracts of *Grewia nervosa* (Lour.) Panigrahi

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A R T I C L E I N F O

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A B S T R A C T

Background: Grewia nervosa (Lour.) Panigrahi, a medicinal herb native to the steep forested region of Bangladesh, is utilized as a treatment for a variety of diseases or conditions, including dysmenorrhea, paratyphoid, bone fractures, jaundice, kidney stones, dyspepsia, heat stroke, cold, fever, diarrhea, hepatitis B, ingestion, and anthelmintic medication. This research aims to investigate the phyto-pharmacological qualities of this herb.
Methods: The herb was extracted using absolute ethanol followed by fractionation with n-hexane and chloroform. Following an initial investigation of phytochemicals, antioxidant, anti-inflammatory, antimicrobial, and membrane stabilization activities were assessed using the DPPH, egg albumin model, disc diffusion method, and erythrocyte hemolysis model, respectively.
Results: A preliminary phytochemical report suggests that *G. nervosa* contains reducing sugars, gums, amides, alkaloids, glycosides, flavonoids, steroids, tannins, flavonoids, and saponins. In a concentration-dependent approach, its fractional extracts displayed promising antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activity. It was seen that all fractions had a statistically significant capacity to prevent RBCs from hemolyzing, which suggests a potent membrane stabilizing function *in-vitro*.
Conclusion: Taken together, *G. nervosa* possesses important secondary metabolites and exhibits *in-vitro* antioxidant, anti-inflammatory, antimicrobial, and membrane stabilizing capacities. This endorses the conventional usage of *G. nervosa*, although additional study is needed on this potentially beneficial plant.

1. Introduction

The evolution of human civilization has been greatly influenced by medicinal plants (Ağagündüz, Şahin, et al., 2022; Fahad et al., 2021; Van Wyk & Wink, 2018). Many contemporary medications are made directly from plants, which are also the basis of many ancient remedies (Fernández et al., 2021; Oluyemisi et al., 2012). The WHO has verified that over 80% of the worldwide population uses herbal treatments to meet their medical requirements, particularly the millions who live in the vast remote regions of developing nations (Hosseinzadeh et al., 2015; Khan et al., 2020; Mitra et al., 2022). Due to their few complications and a significant safety margin in both experimental animals and humans, medicinal plants continue to hold promise as a resource for modern curative medicines for a variety of ailments (Di Lorenzo et al.,

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2015; Küpeli Akkol et al., 2020, 2021). A correlation exists between the therapeutic benefits of secondary metabolites found in medicinal plants (Iqbal et al., 2020; Vieira et al., 2020). However, the medicinal value of some plants is primarily influenced by the nature of their metabolites, both aesthetic and nutritional (Goni et al., 2021; Hassan, 2012) (see Fig. 1).

Research on the identification and development of drugs from medicinal plants now uses a multifaceted strategy integrating botanical, phytochemical, pharmacological, and biochemical approaches (Anand et al., 2019; Freitas et al., 2021). The discovery of plant-based medicines tends to result in the discovery of significant and unique lead compounds and has sparked the development of potential pharmaceuticals (Hong et al., 2015; Hossain et al., 2021; Sinan et al., 2021). Typically, medicinal herbs or their compounds are potential sources of complementary medicine and can be utilized to treat a variety of critical conditions, including pain, diabetes, Alzheimer's disease, AIDS, malaria, and several carcinomas (Ferrarini et al., 2022; Mechchate et al., 2021; Pan et al., 2013; Uddin Chy et al., 2021).

Grewia nervosa (Lour.) Panigrahi (Tiliaceae) is known as Asor-gota in Bangladesh. It is a small to medium-sized tree that occasionally resembles a shrub, with many branches. In Bangladesh, it can be discovered in the wild, especially in the rocky forested regions of Chattogram, Cox's Bazar, Sylhet, and Tangail. The leaves are alternating, short-petioled, oblong-lanceolate, somewhat serrate, and dark green (young leaves are shiny bronze), with noticeable veins and midribs, and a sharp apex. Small, long-peduncled, yellowish-white flowers with five petals and five sepals are gathered on the terminal panicle. From summer to autumn, flowers blossom. Fruit is a berry, which is tiny, smooth, blackish, and 1 cm in diameter. On the tree, flowers and fruits are occasionally seen. Seeds are responsible for the plant's reproduction. Children in the village have fun using its fruits as the bullets in a pretend pistol made of bamboo (www.floraofbangladesh.com; www.natureinfo.bd).

Traditionally, rural residents have used *G. nervosa* as an herbal remedy to treat a variety of diseases. Dysmenorrhea and paratyphoid are treated with fresh juice made from the plant's leaves until they are healed. The broken bones are externally treated using a paste made from the plant's leaves (Debnath et al., 2014). The leaves are infused and used as a cooling beverage and digestive aid (Rahman et al., 2012). An anthelmintic beverage produced from the plant's roasted and boiled leaves is given to kids. Water extract derived from boiled leaves is used to treat kidney stones and jaundice (Kalita & Deb, 2004, pp. 240–246). A study claims that the leaves of this plant can treat conditions like jaundice, dyspepsia, heat stroke, and the common cold when they are boiled with turmeric and a snail shell. Furthermore, *G. nervosa* bark's aqueous extract is used to treat Hepatitis B infection (Animesh et al., 2010). The fruit, bark, root, and leaves of *G. nervosa* have also been used as an insecticide and to cure fever and diarrhea. Chinese herbal tea has included *G. nervosa* leaves ever since antiquity (Bandara et al., 2000; Feng et al., 2008; Luo et al., 2010). The majority of studies investigating *Grewia nervosa*'s therapeutic effects have mostly concentrated on its fruits (Neha & Abdussalam, 2021). The medicinal potential of roots has not been investigated, despite the fact that leaves and stem bark have previously been employed.

Additionally, most of the research found that *G. nervosa* had antioxidant properties. Three separate *in-vitro* test methods have been used to assess the antioxidant activities of *G. nervosa*'s ethyl acetate, butanol, aqueous, and petroleum ether extracts (EAE, BE, AE, and PEE, respectively). At 500 g/mL, only EAE demonstrated a stronger antioxidant activity compared to the other extracts (Fan et al., 2010). In a different study, the anti-inflammatory efficacy shown by the methanolic bark extract (MBE) of *G. nervosa* in contrast to the positive control, ascorbic acid, seems to be promising (Meena et al., 2013). When pairwise mean comparison using the Tukey HSD test ($n = 3$) was conducted, it was discovered that the Fruit Aqueous Extract (FAE) of *G. nervosa* had substantial ($P < 0.05$, vs. aspirin group) anti-proteinase action. Most significantly, the FAE impact was at its maximum at 250 μg/ml with an IC_{50} of 285.47 μg/ml (Aziz et al., 2015). In another study, the antimicrobial property of *G. nervosa* leaf extracts in methanol, chloroform, and aqueous form against certain gram-positive and gram-negative bacteria was examined. It was discovered that the plant extracts had the greatest antimicrobial effects against gram-negative microbes. Methanolic extract (6 mg/disc) from plants demonstrated the maximum zone of inhibition (27 mm) against *Salmonella* sp. (Aziz et al., 2018). Another investigation on the membrane-stabilizing effects of *G. nervosa* found that 10 mg/ml doses of the plant's methanolic extract had the greatest effects, with values of 54.03% inhibition of hemolysis (Sarker et al., 2016).

Plants contain bioactive chemicals with a distinctive variety of chemical structures that may be developed into medicines through a low-cost, scalable procedure (Chandran & Abrahamse, 2020). Additionally, they provide less of a chance for product contamination with foreign substances, contagious viruses, or pathogens, making them typically safer (Reski et al., 2015). Meanwhile, plants are able to carry out intricate post-translational alterations that may then be further manipulated to produce humanized biomolecules (Wilson & Roberts, 2012). Our present research suggests that the plant *G. nervosa* may serve as a promising alternative phytotherapeutic tool to treat a variety of human ailments. Moreover, this project's outcome was reached based on a small number of non-clinical and preclinical studies on its crude

Fig. 1. Morphological structure of *Grewia nervosa* (Lour.) Panigrahi.

extract. It is still unknown what the active principles are and how they could interact with molecules.

It is undeniable that advanced pharmaceutical companies now have access to information about conventional and ancient medical systems. As a result, the goal of any phyto-pharmacological research is frequently to identify novel therapeutic medicines or promising plant chemicals. The right choice of particular plant species is therefore crucial in this situation. Researchers usually depend on the premise of ethno-pharmacological information and substantial literature efforts on medicinal plants because the probabilities of success are significantly lower than with any specific strategy. The difficulties in this situation also lie in coming up with acceptable ways in addition to choosing the correct plants that might meet these requirements. Considering the general information, we had G. nervosa recognized by a professional and chose certain in-vitro studies for this investigation. Previously, we acquired information regarding its historical and scientific support from dependable and legitimate resources.

We conducted this research to evaluate the ethanolic, n-hexane, and chloroform extracts of *G. nervosa*'s pharmacological and phytochemical properties. According to the findings, *G. nervosa*'s crude extracts likely include a variety of significant secondary metabolites, such as alkaloids, glycosides, steroids, tannins, flavonoids, saponins, reducing sugars, gums, and amides. In contrast to earlier studies, the extracts showed antioxidant, anti-inflammatory, anti-microbial, and membrane-stabilizing effects in a variety of test systems at various dosages.

2. Materials and methods

2.1. Collection, identification, extraction, and fractionation of plant materials

The experts at the Chattogram Herbarium of the Bangladesh Forest Research Institute (BFRI) performed the taxonomic identification of the chosen plant. The herbarium sheet was created using the established protocol and guidelines recommended by the institute's expert. In the month of April, fresh leaves were harvested from Hathazari, Chattogram. At two phases of processing, the plant material's unnecessary, undesirable components were taken out. After collecting the leaves, the decaying leaves, stalks, and other items were initially removed by hand. Prior to drying the plant components, the soil was once again removed by sieving through a net with the help of an electric fan's airflow for 5 days. The leaves were then dried in the shade at a temperature of no higher than 45 °C. They were then put through a grinder to create a coarse powder. The powder was stored in airtight containers and kept in a cool, dark, and dry place until extraction commenced. For hot extraction, about 350 g of the plant's powder was subjected to 1100 ml of absolute ethanol in a Soxhlet apparatus (Quickfit and Quarz Ltd., London, England, U.K.). The extracted solution was collected, filtered, and allowed to evaporate at 60 °C. After evaporation of the solvent, a gummy concentrate was obtained, which was designated as "hot ethanolic crude extracts (EGN)". The amount of crude extract from hot extraction was 25 g. After solvent evaporation, the yield values (7.1%) of the extract were calculated with respect to the initial amount of the powder subjected to extraction. Solvent-solvent partitioning was done using the protocol designed by Kupcha and modified by Wagenen (Ahsan et al., 2021). The EGN (25 g) was dissolved in double-distilled water (DDW) and then fractionated using a fractionating column with n-hexane (HGN) and subsequently with chloroform (CGN). Fractionation was done using 50 ml of solvent each time until 150 ml of n-hexane and chloroform were used, and each time, after vigorous shaking, the mixture was allowed to stand. Solvent layers were separated and decanted. The remaining extract was used as a fraction of hydroalcoholic (EGN). The obtained extracts were collected, filtered, and made to evaporate the solvent below 45 °C. The solvent evaporation resulted in a gummy concentrate. The gummy concentrates were weighed and taken in properly labeled and clean air-tight closure containers and stored at 4 °C.

2.2. Reagents and chemicals

Tween-80 was acquired from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra 400005, India, whereas ascorbic acid was bought from Merck KGaA, Darmstadt, Germany. The azithromycin was purchased from Aristopharma Ltd. in Bangladesh, while Beximco Infusions Ltd. supplied the sterile normal saline (0.9% NaCl). All of the solvents, reagents, and other chemicals utilized were of analytical grade. All of the solutions were prepared on the experimentation day.

2.3. Phytochemical investigation

A plant's biologically active components and their concentrations are taken into account when determining how valuable it is as a medicine. To investigate plant secondary metabolites, preliminary phytochemical group tests were conducted by the methods of Harborne (Harborne, 1984) and Trease and Evans (Trease & Evans, 2002). A brief description of the color reactions has been given below:

Test for alkaloids: A small quantity of extract was placed in a watch glass, followed by a few drops of diluted hydrochloric acid (HCl) and 1 ml of Meyer's reagent. Alkaloids can be detected by looking for white-colored precipitation.

Test for glycosides: One or two drops of concentrated sulfuric acid were added after a small amount of the extract was placed in a test tube with chloroform. The presence of glycosides is confirmed by an orange-reddish tint at the junction of the two layers.

Test for flavonoids: The alcoholic sample solution was diluted with two or three drops of concentrated HCl. Flavonoids are present when a red color develops instantly.

Test for tannins: 1 ml of a 5% ferric chloride solution was added to 5 ml of the sample solution in a test tube. The presence of tannins is indicated by orange precipitation.

Test for steroids: One or two drops of concentrated sulfuric acid were added after a small amount of the extract was placed in a test tube with chloroform. The presence of glycosides is confirmed by a red color at the junction of the two layers.

Test for saponins: A graduated cylinder was shaken for 15 min with 1 ml of the aqueous sample solution that had been diluted with distilled water to 19 ml. Saponin's presence is confirmed by 1 cm of foam on the liquid layer.

Test for reducing sugars: 5 ml of Benedict's reagent and 0.5 ml of water were combined in a beaker and heated for 5 min in a water bath. The presence of reducing sugars is verified by the red color of the precipitate.

Test for gums: In a test tube, 5 ml of the sample solution was diluted with Molisch's reagent before a few drops of sulfuric acid were added. Gums can be seen as a red-violet ring at the junction of two liquid layers.

Test for amides: A small amount of the extract, followed by a small amount of 20% NaOH, was added. It took 15 min to boil the mixture. The presence of amides is indicated by the release of NH_3 gas, which turns red litmus to blue.

2.4. Antioxidant activity

2.4.1. Qualitative assay

Thin-layer chromatography was used to investigate the preliminary phytochemical screening of the EGN. To distinguish between the polar and non-polar components of the extract, appropriately diluted stock solutions were spotted on pre-coated silica gel TLC plates. The plates were then developed in solvent systems of various polarities (polar, medium-polar, and non-polar). After drying at room temperature (59 °F to 77 °F or, 15 °C to 25 °C), the plates were sprayed with 0.004% 1, 1-diphenyl-2-picryl hydrazyl (DPPH) in methanol. After 10 min, the resolved bands were inspected for DPPH bleaching, and the color

changes (yellow on purple background) were noted. When DPPH is dissolved in methanol, a rich pink color results. Spraying it on the extract's chromatogram results in a pale yellow or yellow hue, which denotes the presence of antioxidants (Biswas et al., 2014).

2.4.2. Quantitative assay

Up to 3 ml of a 0.004% methanol solution of DPPH was added, along with 0.1 ml of EGN, CGN, and HGN at varied concentrations (20, 40, 60, 80, and 100 g/ml). Except for the control tubes, all of the reaction tubes were kept in the dark for 30 min. The solution's absorbance was measured at 517 nm after 30 min against a blank. The percentage of DPPH radical scavenging activities (%SCV) was calculated by comparing the results of the test with the control (not treated with extract) using the following formula:

$$\%SCV = \frac{A_0 - A_1}{A_0} \times 100$$

Where, SCV = Radical scavenging activity, A_0 = Absorbance of the control, and A_1 = Absorbance of the test (extracts/reference standard).

Extract concentrations providing 50% inhibition (IC_{50}) were calculated from the graph and plotted as a percentage of the SCV versus concentration curve. The test was carried out in duplicate, and ascorbic acid was used as a standard (Chakraborthy, 2009).

2.5. Anti-inflammatory activity test (egg albumin model)

As a standard, 10 mg of acetylsalicylic acid was dissolved in 10 ml of double-distilled water (DDW). To make iso-saline, 100 ml of DDW was mixed with 900 mg of NaCl. For a 5% albumin solution, 5 ml of egg albumin were added to the 95 ml of iso-saline. For the test group, 10 mg of EGN, HGN, and CGN were dissolved in a measured volume of solvent, respectively. The egg was used as the source of albumin and was reconstituted as a 5% v/v aqueous solution with iso-saline.

The egg albumin model was slightly modified to assess the crude extract's anti-inflammatory properties (Ullah et al., 2014). In essence, inhibiting protein denaturation signifies that a chemical has anti-inflammatory characteristics (e.g., an extract or a drug). For the present experiment, 15 clean centrifuge tubes were taken. Three for standard, three for control, and nine for each extract. The tubes were marked accordingly. The control tubes were added to the 2 mL of DDW, and 2 ml of acetylsalicylic acid (at the required concentrations) was mixed for the positive control group. On the other hand, for the test groups, 2 ml of test extracts were mixed as indicated. A buffer solution of 2.8 ml and 0.2 ml of albumin are fixed for all concentrations and added to the centrifuge tubes to prepare the concentration of 500 μg/ml. The remaining volume for 125 and 250 μg/ml was adjusted with distilled water up to 5 ml. The pH (5.6 ± 0.2) of all the reaction mixtures was adjusted by 1 N HCl. All the reaction mixtures were heated at 60 °C for 5 min. After cooling and filtering (with Whatmann filter paper), the colorimeter (AE11M, Absmax logT; 0-2 India) was used to measure the absorbance at 660 nm spectrophotometrically. The test was repeated three times for each extract. The following formula was used to determine the percentage of inhibition of protein denaturation:

$$\%Inhibition = [(Absorption_{Control} - Absorption_{Test}) / Absorption_{Control}] \times 100$$

2.6. Anti-microbial activity

2.6.1. Microorganisms

The following chosen microorganisms underwent independent tests to determine the antibacterial activity of the plant extracts. All the microbial species were collected from the microbiology lab of the department of pharmacy at Southern University Bangladesh, Chattogram, Bangladesh. The strains were maintained on nutrient agar slopes and subcultured. These bacteria and fungi served as test pathogens for antibacterial activity assays.

List of test bacteria

Gram-positive bacteria	Gram-negative bacteria
Lactobacillus coryniformis	*Escherichia coli*
Bacillus cereus	*Vibrio cholerae*
Lactobacillus casei	*Klebsiella pneumoniae*

2.7. Stokes disc diffusion method

For the anti-bacterial test, the required volume of nutrient medium was prepared. The medium was heated to dissolve the materials and make a clear solution. Then the media was poured into screw-cap test tubes (5 ml for slants and 15-20 ml for media plates). These test tubes, together with media, were sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 Ib/sq. inch for 30 min. After that, with the help of an inoculating loop, the test organisms from the pure cultures were transferred to the sterilized agar slants in optimum aseptic conditions. The inoculated plants were then incubated at 37 °C for 24 h. The test organisms were transferred aseptically to the labeled test tubes containing 15 ml of autoclaved media with the help of an inoculating loop. The media were poured into Petri dishes in such a way as to give a uniform depth (approximately 4 mm). Sterilized filter paper discs (5 mm in diameter) were prepared with the help of a punch machine and were placed in a blank petri dish. A sample solution of the desired concentration was applied aseptically to the discs with the help of a micropipette. The discs were left for a few minutes to complete solvent removal. For the extractions, 500 μg/10 μl/disc crude samples from different fractions of *Grewia nervosa* were used. For comparison, the standard discs were prepared with azithromycin (30 μg/10 μl/disc). Ethanol, n-hexane, and chloroform (solvents used for extraction) were subjected to test on the sterilized discs in order to determine the anti-bacterial effects of the solvents. It should be noted that before extraction, the bacterial status of the used solvents was verified. The discs (i.e., the samples and standard) were subsequently impregnated centrally into labeled Petri dishes containing bacterial media inoculated with specific organisms, respectively, with the help of sterile forceps to assure complete contact with the previously cultured medium surface. The plates were then inverted and kept in a refrigerator for about 24 h at 4 °C. This is sufficient time for the material to diffuse over a considerable area of the medium. Finally, the plates used for the antibacterial test were incubated at room temperature (59 °F to 77 °F or, 15 °C to 25 °C) for 72 h (Leelaprakash et al., 2011).

2.8. Membrane stabilization activity

As a standard, 5 mg of acetylsalicylic acid was dissolved in 10 ml of distilled water (DW). To prepare iso-saline and hypo-saline, 900 mg and 500 mg of NaCl were added to the 100 ml of DDW, respectively. Fresh whole human 1 ml blood was collected and diluted with RBC diluting fluid up to 20 ml to make a 10% RBC suspension. Then 2 ml of this solution was diluted with 18 ml of normal saline solution to get the 10% RBC suspension. For the test group, 5 mg of EGN, HGN, and CGN were mixed with a measured volume of EGN, HGN, and CGN as required according to the fractions. In this experiment, 27 pristine centrifuge tubes were used. Included are three control tubes, three standard tubes, six standard tubes, and additional centrifuge tubes for each extract. All treatment tubes contained 1 ml of a 10% RBC suspension, and the negative control tubes received 1 ml each of ethanol, n-hexane, and chloroform, while the positive control tubes received 1 ml each of acetylsalicylic acid. However, for the test group, the required concentration of the extract was combined with 1 ml of the test group as indicated. After that, 1 ml of a hypotonic solution was applied to each tube. A phosphate buffer was used to change the pH of the reaction solutions to

7.4 ± 0.2. For 30 min, a water bath heated to 56 °C was used to incubate all of the centrifuge tubes containing the reaction mixture. The tubes were cooled under running water after the incubation period. Then, the reaction mixture was centrifuged at 2500×*g* for 5 min at room temperature. The absorbance of the supernatants was measured at 660 nm after chilling and filtering with Whatmann filter paper. The test was repeated three times (Manukumar & Umesha, 2015). Membrane stabilization activity was measured by measuring the absorbance of the treatment groups and converting it into total inhibition of hemolysis.

$\%$ Inhibition of haemolysis = $[(\text{Absorption}_{\text{Control}} - \text{Absorption}_{\text{Test}}) / \text{Absorption}_{\text{Control}}] \times 100$

3. Results

3.1. Phytochemical studies

According to Table 1, the EGN contains alkaloids, glycosides, steroids, tannins, flavonoids, saponins, reducing sugars, gums, and amides. In the EGN, alkaloids were more potent, followed by tannins and flavonoids. The tests for glycosides, steroids, saponins, reducing sugars, and amides all showed low intensity, while the test for gums showed no intensity at all. Alkaloids testing showed the greatest intensity for the HGN, followed by tannins testing. The tests for glycosides, flavonoids, reducing sugars, and gums all showed low intensity, but the tests for steroids, saponins, and amides showed no intensity at all. Tests for alkaloids for the CGN showed the greatest intensity, followed by tests for tannins and flavonoids. Glycosides, steroids, reducing sugars, gums, and amides tests all showed low intensity, while saponins tests showed no intensity at all.

3.2. Antioxidant activity

In the quantitative antioxidant test, at a dose of 100 μg/ml, EGN, HGN, and CGN extracts produced significant inhibition of DPPH free radicals in comparison to the reference standard, ascorbic acid (AA). Among the crude extracts, CGN produced a better IC_{50} (Table 2). However, the inhibitory effects of all extracts were significant ($P < 0.05$) in comparison to the vehicle group.

3.3. Anti-inflammatory activity

Table 3 suggests that all extracts of *G. nervosa* exerted a concentration-dependent inhibition of protein denaturation. However, EGN exerted a better effect than the other two extracts. However, the inhibitory effects of all extracts were significant ($P < 0.05$) in comparison to the vehicle group.

Table 1
Phytochemical groups found in *Grewia nervosa* (Lour.) Panigrahi.

EGN	HGN	CGN	Chemical group
++++	++++	++++	Alkaloids
+	+	+	Glycosides
+	-	+	Steroids
++	++	++	Tannins
++	+	++	Flavonoids
+	-	-	Saponins
+	+	+	Reducing sugars
-	+	+	Gums
+	-	+	Amides

'+' means positive and '-' means negative response; multiple signs indicate greater intensity; EGN: Ethanolic extract of *Grewia nervosa*; HGN: n-Hexane extract of *Grewia nervosa*; CGN: Chloroform extract of *Grewia nervosa*.

3.4. Antimicrobial activity

The disc diffusion method was used to assess the antibacterial effects of the various extracts on six bacteria, with azithromycin serving as the reference drug. The HGN was found to have no antibacterial activity when tested for antibacterial sensitivity. Both the EGN and CGN showed activity against four different strains of bacteria, demonstrating the effectiveness of the other two fractions against bacteria (Table 4).

3.5. Membrane-stabilizing activity

According to Table 5, EGN exerted a better inhibitory effect on the hypotonic solution-induced total hemolysis of RBCs than the other two extracts. However, the inhibitory effects of all extracts were significant ($P < 0.05$) in comparison to the vehicle group.

4. Discussion

Stress leads to the production of reactive oxygen species (ROS) in living organisms, including hydroxyl (OH-), superoxide (O^{2-}), and peroxyl (ROO, OOH). Reactive oxygen species primarily target proteins, RNA, DNA, lipids, and enzymes, where they can cause malfunctions or mutations that result in serious degenerative illnesses (Ames et al., 1993). Several human illnesses, including cancer, heart disease, Alzheimer's disease, aging, inflammation, neuron disorders, atherosclerosis, and diabetic problems, have been linked to the pathophysiology of free radicals (ROS) (Burns et al., 2001; Chen et al., 2006; Diaz et al., 1997). Although humans have a natural defense mechanism to battle excessive ROS generation, excessive air and water pollution, as well as unhealthful eating habits, have reduced our ability to counteract the impacts of free radicals (Jan et al., 2015). Therefore, it is crucial to maintain the body's redox equilibrium so that certain illnesses can be prevented or delayed by neutralizing free radicals (Lobo et al., 2010). Researchers have been investigating the antioxidant potential of thousands of medicinal plants across the world for a number of years (Sharma et al., 2013). Plant secondary metabolites, including flavonoids, phenolic acids, and diterpenes, are responsible for medicinal plants' ability to scavenge free radicals or act as antioxidants (Costa et al., 2016; Soni & Sosa, 2013). Some secondary metabolites from medicinal plants, such as polyphenols and flavonoids, have been studied for their antioxidant properties (Stanković et al., 2015). Antioxidants are chemicals that primarily slow down or stop the oxidation reaction *in-vitro* and *in-vivo* by stopping the oxidation chain reaction (Yanishlieva et al., 2006). Antioxidant use in pharmacology is beneficial for enhancing existing illness therapies. Finding natural antioxidants derived from plant materials to replace synthetic antioxidants, which are prohibited owing to their carcinogenicity, has attracted a lot of attention in recent years (Sasaki et al., 2002). There are several plant chemicals that have been shown to have antioxidant and radical-scavenging properties (Khalaf et al., 2008). Flavonoids and phenols, which are widely distributed in nature and are among the antioxidant chemicals, have been extensively investigated (Amico et al., 2008; Tung et al., 2007). *G. nervosa,* which may have a role in antioxidant biological activity. In these investigations, the quantitative antioxidant test is used to determine the plant extract's antioxidant activity. Comparing the EGN, HGN, and CGN to the reference drug AA at a concentration of 100 μg/ml revealed a significant decrease in inhibition for each substance. Among all plant extracts, EGN had a high inhibition percentage at 100 μg/ml (Table 2) and a strong IC_{50} value (58.78 ± 0.07 μg/ml). Nevertheless, the CGN showed a very strong IC_{50} value (36.98 ± 0.07 μg/ml), which is relatively comparable to the IC_{50} value of ascorbic acid as a positive control (15.61 ± 0.11 μg/ml). The plant extract with the lowest IC_{50} value in the evaluation against free radicals has the strongest antioxidant potential. According to research, antioxidant activity is strongly indicated by IC_{50} values between 0.05 and 0.1 μg/ml, and it is extremely strongly indicated by IC_{50} values under 0.05

Table 2
DPPH scavenging capacity of *Grewia nervosa* (Lour.) Panigrahi.

Parameters		Percentage scavenges of DPPH			
		AA	EGN	HGN	CGN
Concentration (μg/ml)	20	54.93 ± 1.08*bcd	33.02 ± 0.95*c	15.79 ± 0.94*	30.66 ± 0.58*c
	40	65.28 ± 1.11*bcd	43.77 ± 0.98*c	26.29 ± 0.78*	42.25 ± 0.78*c
	60	73.20 ± 1.19*bcd	56.32 ± 1.23*cd	39.21 ± 1.31*	51.06 ± 0.91*c
	80	80.01 ± 0.97*bcd	68.49 ± 1.21*cd	53.58 ± 1.00*	60.33 ± 1.10*c
	100	91.16 ± 0.91*bcd	79.50 ± 1.12*cd	66.53 ± 2.01*	71.31 ± 1.08*c
IC_{50} (μg/ml)		15.61 ± 0.11	58.78 ± 0.07	74.30 ± 0.05	36.98 ± 0.07
CI (μg/ml)		6.96–34.97	46.83–69.23	69.82–85.82	26.04–45.40
R^2		0.86	0.87	0.90	0.89
NC (Vehicle)		1.11 ± 0.11			

Values are mean ± SD (n = 5); One-way ANOVA followed by Tukey post-test with multiple comparisons; Values are considered significant at P < 0.05 when compared to [a]AA, [b]EGN, [c]HGN, [d]CGN, and *NC (values are compared to the same concentration between the test sample/standard); AA: Ascorbic acid (reference standard); EGN: Ethanolic extract of *Grewia nervosa*; HGN: n-Hexane extract of *Grewia nervosa*; CGN: Chloroform extract of *Grewia nervosa*; IC_{50}: Half-minimal inhibitory concentration; CI: Confidence of interval; R^2: Co-efficient of determination; NC: Negative control (vehicle).

Table 3
Protein protection capacity of *Grewia nervosa* (Lour.) Panigrahi.

Parameters		Percentage protein protection			
		ASA	EGN	HGN	CGN
Concentration (μg/ml)	125	28.96 ± 1.19*bcd	24.09 ± 0.99*	22.56 ± 0.78*	21.30 ± 0.54*
	250	50.2 ± 1.03*bcd	42.48 ± 1.00*d	39.55 ± 1.00*	37.88 ± 0.87*
	500	87.74 ± 0.96*bcd	78.60 ± 0.97*cd	72.84 ± 0.58*	69.22 ± 0.18*
IC_{50} (μg/ml)		248.50 ± 0.08	396.60 ± 0.09	398.40 ± 0.09	468.10 ± 0.09
CI (μg/ml)		188.50–301.19	299.60–400.38	302.93–409.37	362.92–496.64
R^2		0.92	0.91	0.92	0.92
NC (Vehicle)		1.93 ± 0.54			

Values are mean ± SD (n = 5); One-way ANOVA followed by Tukey post-test with multiple comparisons; Values are considered significant at P < 0.05 when compared to [a]ASA, [b]EGN, [c]HGN, [d]CGN, and *NC (values are compared to the same concentration between the test sample/standard); ASA: Acetylsalicylic acid (reference standard); EGN: Ethanolic extract of *Grewia nervosa*; HGN: n-Hexane extract of *Grewia nervosa*; CGN: Chloroform extract of *Grewia nervosa*; IC_{50}: Half-minimal inhibitory concentration; CI: Confidence of interval; R^2: Co-efficient of determination; NC: Negative control (vehicle).

Table 4
Zone of inhibition of *Grewia nervosa* (Lour.) Panigrahi extracts against pathogenic bacteria.

Bacteria	Zone inhibition diameter in mm			
	EGN (500 μg/disc)	HGN (500 μg/disc)	CGN (500 μg/disc)	AZN (30 μg/disc)
Bacillus cereus	–	–	7	15
Lactobacillus coryniformis	10	–	7	17
Escherichia coli	8	–	9	16
Lactobacillus casei	–	–	–	16
Vibrio cholerae	6	–	–	15
Klebsiella pneumoniae	10	–	11	17

EGN: Ethanolic extract of *Grewia nervosa*; HGN: n-Hexane extract of *Grewia nervosa*; CGN: Chloroform extract of *Grewia nervosa*; AZN: Azithromycin (reference antibiotic).

mg/ml (Ramadhan et al., 2022). The experiment indicates the high antioxidant activity of the plant extracts.

Inflammation is a key biological phenomenon that regulates interactions between organisms and their surroundings (Rankin, 2004). Both acute and chronic inflammation may contribute to a variety of diseases, including arthritis, allergies, atherosclerosis, and cancer (Laveti et al., 2013). Tissue protein denaturation in some types of arthritis can lead to the generation of autoantigens. This is a symptom of an inflammatory response in people with arthritis (Mishra et al., 2011). As a result of temperature, a strong acid or base, and the presence of inorganic salt or organic solvents, proteins lose their secondary and tertiary structures, which causes inflammation (Shah et al., 2017). Drugs that reduce inflammation can prevent protein denaturation (Chandra et al., 2012). The in-vitro anti-inflammatory action of Grewia nervosa was assessed in this study using a denaturation of egg albumin as a model. The findings demonstrated that, at various doses, several plant extracts, including ethanolic, n-hexane, and chloroform extracts, prevented protein denaturation. When compared to the same standard (ASA) concentration, the extracts at 500 μg/ml showed significant anti-inflammatory activity. Among other extracts, EGN had a greater protein protection, which was comparable to the standard, along with an IC_{50} value of 396.60 ± 0.09 μg/ml.

The anti-inflammatory actions of the plant are caused by phytochemicals such as alkaloids and flavonoids (Banerjee et al., 2014). Terpenoids (González et al., 2015), glycosides, and other secondary metabolites generated from plants may also have anti-inflammatory properties (Nunes et al., 2020). In treating inflammatory diseases, polyphenols have been implicated by scientists (Sreejayan & Rao, 1996). From medicinal herbs, polyphenols, including curcumins, tannins, and flavonoids, are said to have the ability to scavenge free radicals or to suppress the enzymes lipoxygenases (LOX) and cyclooxygenases (COX), which are known to cause inflammatory responses (Lee et al., 2003; Sadik et al., 2003). In the current investigation, we also discovered a variety of secondary metabolites in *G. nervosa* extract, providing a solid basis for future research aimed at isolating lead anti-inflammatory chemicals from this therapeutic plant. The WHO estimates that traditional medicine is used for medical treatment by around 65% of the worldwide population (Nunes et al., 2020). Herbs are a possible source of secondary metabolites, which are the main ingredient in many medications used to treat various pathological conditions in people (Shah et al., 2011). A number of anti-inflammatory medicinal plant species are thought to have this effect, and the researchers claimed that this may be because their extracts include phenols, triterpenes, flavonoids, saponins, and tannins (Barbosa et al., 2016; Cabral et al., 2016; Mostofa et al., 2017). The current investigation also reveals that the EGN

Table 5
Membrane protection capacity of *Grewia nervosa* (Lour.) Panigrahi.

Parameters		Percentage membrane protection			
		ASA	EGN	HGN	CGN
Concentration (μg/ml)	125	27.42 ± 1.21*[cd]	24.32 ± 0.98*[c]	20.41 ± 0.95*	22.68 ± 0.95*
	250	46.80 ± 1.00*[bcd]	40.42 ± 1.54*[c]	35.25 ± 0.97*	38.96 ± 0.58*
	500	85.77 ± 1.16*[bcd]	72.78 ± 0.54* [c]	64.53 ± 1.08*	69.89 ± 1.11*[c]
IC_{50} (μg/ml)		295.70 ± 0.09	390.50 ± 0.09	398.30 ± 0.09	393.40 ± 0.09
CI (μg/ml)		269.69–300.96	370.82–433.31	376.32–456.91	372.63–440.30
R^2		0.91	0.90	0.91	0.91
NC (Vehicle)		2.49 ± 0.54			

Values are mean ± SD (n = 5); One-way ANOVA followed by Tukey post-test with multiple comparisons; Values are considered significant at $P < 0.05$ when compared to [a]ASA, [b]EGN, [c]HGN, [d]CGN, and *NC (values are compared to the same concentration between the test sample/standard); ASA: Acetylsalicylic acid (reference standard); EGN: Ethanolic extract of *Grewia nervosa*; HGN: n-Hexane extract of *Grewia nervosa*; CGN: Chloroform extract of *Grewia nervosa*; IC_{50}: Half-minimal inhibitory concentration; CI: Confidence of interval; R^2: Co-efficient of determination; NC: Negative control (vehicle).

contains terpenoids, saponins, and tannins. Therefore, the previously mentioned secondary metabolites of *G. nervosa* may be the reason for the herb's anti-inflammatory effects.

Widespread, incorrect, irregular, and indiscriminate use of antibiotics has led to the development of antimicrobial resistance, leaving many currently available medications worthless (Andrade et al., 2015; Baym et al., 2016; Davies & Davies, 2010). The antimicrobial compounds present in medicinal plants may inhibit the growth of bacteria, fungi, viruses, and protozoa through mechanisms distinct from those of currently used antimicrobials (Yeşilyurt et al., 2021, 2022). They may also be helpful in treating microbial strains that are resistant to current antimicrobials as well as certain conditions such as gut-brain axis-related neurological conditions (Ağagündüz, Şahin, et al., 2022; Shankar et al., 2010). Chemically complicated compounds have excellent therapeutic potential since they are less likely to develop resistance than synthetic drugs and have fewer side effects (Lewis & Ausubel, 2006; Ody, 2017). Additionally, the synergistic relationship between the therapeutic chemicals in the extracts and the ability of the medicinal plant extracts to prevent bacteria from growing is a factor (Wagner & Ulrich-Merzenich, 2009). Thousands of plant species have been studied *in-vitro* against several bacterial strains, per the literature, and a significant number of medicinal plant extracts and pure chemicals have now been shown to be effective against gram-positive and gram-negative bacteria (Mahady et al., 2008). The emergence of multi-target mechanisms, the presence of substances that can inhibit bacterial resistance mechanisms, and pharmacokinetic or physicochemical effects resulting in increased pharmacokinetic effects and decreased toxicity are just a few of the effects that contribute to the synergism action (Wagner & Ulrich-Merzenich, 2009). Due to the serious threat that antimicrobial resistance (AMR) poses to both human health and the expansion of the global economy, new antimicrobial medications are urgently needed (Laxminarayan et al., 2016). Many plant-based substances have inferior antibacterial properties, but some of them have the ability to break down antimicrobial agent resistance. Additionally, because they are present in so many foods and beverages made from plants, the majority of them are thought to be harmless to humans. Flavonoids, one of the major families of secondary metabolites in plants, are abundant in a variety of plant components, including fruit, vegetables, nuts, and tea. The most bioactive plant chemicals with notable antimicrobial action were phenolics, alkaloids, flavonoids, triterpenes, and steroids (Dzotam & Kuete, 2017; Kuete, 2010). In this study, the antimicrobial activities of EGN, HGN, and CGN were evaluated by the disc diffusion method against *Lactobacillus coryniformis*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Escherichia coli,* and *Lactobacillus casei* bacteria, using Azithromycin as standards. The HGN did not exhibit any antibacterial activity in the antibacterial sensitivity test. In the other two fractions, the EGN showed effective activity at 500 μg/disc against *Lactobacillus coryniformis*, *Escherichia coli*, *Vibrio cholerae,* and *Klebsiella pneumoniae bacteria,* while the CGN showed activity against *Bacillus cereus*, *Lactobacillus coryniformis*, *Escherichia coli*, and *Klebsiella pneumoniae* bacteria.

As a result of their adverse effects and potency, existing medications like opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are thought to not be effective in treating inflammatory illnesses in all circumstances (Bindu et al., 2020). Therefore, it would seem beneficial and important to look into other options. An effective and reasonable research technique for finding novel anti-inflammatory medications is to explore botanicals that have historically been used to treat inflammation (Aggarwal et al., 2011). So, it is very important to look for new anti-inflammatory compounds in plants with high antioxidant capacity. The instability of cell membranes is thought to be caused by the formation of free radicals such as lipid peroxides and superoxides (Kwiecien et al., 2014). Free radical scavengers such as flavonoids and other phenolic substances are said to work well (Miliauskas et al., 2004). Due to the exceptional anti-inflammatory properties of triterpenoids and flavonoids, the current study evaluates the in-vitro anti-inflammatory efficacy of G. nervosa using human red blood cell membrane (HRBC) stabilization. As the lysosomal membrane is similar to the HRBC or erythrocyte membrane, its stabilization suggests that the extract may also stabilize lysosomal membranes (Chippada et al., 2011). An in-vitro biomarker of a substance's or plant extract's anti-inflammatory action might be the stabilization of the HRBC by membrane lysis brought on by hypotonicity (Paul et al., 2021). The membrane stabilization activity of G. nervosa extracts was measured by measuring the absorbance of the treatment groups and converting it into total inhibition of hemolysis. In the current study, EGN, HGN, and CGN were examined at dosages of 500 μg/ml to assess their effectiveness in preventing the lysis of the human erythrocyte membrane brought on by hypotonic solutions when compared to conventional ASA. EGN had the greatest impact of all of them at 500 μg/ml concentrations, with values of (72.78% ± 0.54%) inhibition of hemolysis, while ASA exhibited (85.77% ± 1.16%) inhibition of hemolysis (Table 5). The ability of each fractional extract of G. nervosa to suppress RBC hemolysis was found to be statistically significant.

5. Conclusion

According to our research, *G. nervosa* has a wide range of significant secondary metabolites, including alkaloids, glycosides, steroids, tannins, flavonoids, saponins, reducing sugars, gums, and amides. In the experiments used in this investigation, *G. nervosa* significantly reduced inflammation, eliminated microbes, increased antioxidant capacity, and also had membrane-stabilizing activity. For demonstrating such activities, *G. nervosa* may be a good alternative medicinal herb to treat a variety of ailments. Furthermore, it is yet unknown how the phytochemicals in this herb interact with the observed biological processes at the molecular level. As a result, additional study is needed to isolate the lead compounds from the prospective medicinal herb and evaluate the promising mechanism(s) for each pharmacological activity utilizing analytical paradigms.

Authors contributions statement

Mehanaz Islam - CONCEPTUALIZATION, Abdullah Al Shamsh Prottay - METHODOLOGY, Irin Sultana - METHODOLOGY, Abdullah Al Faruq - METHODOLOGY, Mehedi Hasan Bappi - METHODOLOGY, Md Showkoth Akbor - SOFTWARE, Afia Ibnath Asha – FIRST DRAFT OF THE MANUSCRIPT, Md. Munnaf Hossen – FIRST DRAFT OF THE MANUSCRIPT, Henrique Douglas Melo Coutinho – PROJECT ADMINISTRATION, Muhammad Torequl Islam - SUPERVISION

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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