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ISOLATION AND CHARACTERIZATION OF BIOACTIVE POLYSACCHARIDES FROM OKRA MUCILAGE: ANTIOXIDANT, ANTIBACTERIAL, AND ANTI-INFLAMMATORY PROPERTIES

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Abstract

Plant-derived mucilage has received considerable attention due to its extensive applications in the formulation and modification of pharmaceuticals, functional foods, and nutraceuticals. The current study aimed to isolate water-soluble polysaccharides from okra pods' mucilage and investigate their biological prospects. Mucilage was extracted by immersing okra pods in distilled water for 24 hours and precipitated in 75% ethanol. The total carbohydrate content of crude mucilage, water-soluble, and ethanol-soluble polysaccharide fractions was determined using the phenol-sulphuric acid method. These fractions were then subjected to different biological assays, including antioxidant, antibacterial, *In vitro* alpha amylase inhibition, and *In vitro* and *In vivo* anti-inflammatory assays. The water-soluble fraction demonstrated notable antibacterial activity, with an inhibition zone of 3.89 ± 0.34 mm against *K. pneumonie*, while the crude fraction showed minimal activity against *Klebsiella* and *Salmonella* spp. The water-soluble polysaccharide fraction exhibited the highest percent radical scavenging activity, with the lowest EC_{50} value of 785.5 µg/mL, followed by the ethanol-soluble fraction with an EC_{50} of 987.21 µg/mL. Furthermore, the water-soluble polysaccharides demonstrated significant *In vitro* α-amylase inhibitory activity and membrane-stabilizing potential in hypotonicity-induced hemolysis of human erythrocytes. Notably, it also markedly reduced carrageenan-induced paw edema, indicating a strong anti-inflammatory effect *In vivo*. At higher concentrations, this fraction effectively reduced the inflammation, highlighting its potential as a natural anti-inflammatory agent. Our results conclude that the water-soluble polysaccharides present in the mucilage of okra pods and seeds possess promising antioxidant and anti-inflammatory properties and could be recommended for managing various inflammatory disorders. Further biochemical and structural characterization of these polysaccharides will broaden our knowledge of the potential role of these polysaccharides in combating inflammatory diseases and improving human health.

Key words: Okra, *Abelmoschus esculentus*; Mucilage; Polysaccharides; Nutraceuticals; Antimicrobial; Antioxidant; Anti-inflammatory

Introduction

The quest for novel and sustainable therapeutic agents has gained significant momentum in recent years, driven by the limitations of synthetic drugs, such as resistance, high costs, and associated side effects (Muteeb *et al.*, 2023; Weth *et al.*, 2024). Medicinal plants, with their vast repository of bioactive compounds, have been widely known as a cornerstone of traditional medicine (Buragohain *et al.*, 2024). These natural resources not only offer chemical diversity but also exhibit structural complexity and multifaceted biological activities, making them invaluable in modern drug discovery and development (Chaachouay & Zidane, 2024). Some key statistical analyses showed that about 80% in Africa, 70% in Canada, 75% in France, 71 % in Chile, and 60-70% population in China are dependent on traditional medicines (Parvin *et al.*, 2023).

Polysaccharide-rich mucilage and gums have been known for a long time (Yang *et al.*, 2023). Polysaccharides are carbohydrate polymers that are widely distributed in plants, animals, and microorganisms (Uyor *et al.*, 2024). Complex polysaccharides are created after ten or more monosaccharides unite through glycosidic linkages (Ji *et al.*, 2024). A lot of attention is given to the use of polysaccharides in food as well as medicine, as researchers have devoted a lot of time studying the biological characteristics of polysaccharides and their effectiveness in functional food products (Cakmak *et al.*, 2023). Chemical studies on mucilage have established that it is a hydrocolloid and a complex polymer of monosaccharides. A gel-like coating, also known as mucilage, is released by many plants as well as seed tissues after being submerged in water (Lira *et al.*, 2023). Many plants, such as *Abelmoschus esculentus* (okra), *psyllium* (*Plantago* species), *Lepidium sativum* (cress), *Salvia hispanica* (chia seed), *Sinapis alba* (yellow mustard), and *Linum usitatissimum* have a common constituent also known as mucilage (flax seed) (Yang *et al.*, 2023).

The anti-aging, immunoregulatory, and anti-cancer activities of plant-derived polysaccharides have been

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investigated (Xu *et al.*, 2025). Polysaccharides have been demonstrated to reduce blood cholesterol levels, which makes them potentially useful in cardiovascular disorders (Wang *et al.*, 2023). Additionally, these chemicals are said to have been effective in lowering the blood glucose levels of diabetic patients (Iqbal *et al.*, 2024; Wang *et al.*, 2024a). Antioxidants are an important class of chemicals because they rid the body of free radicals and prevent oxidation. This property helps to lower oxidative stress in the body after use (Rahaman *et al.*, 2023; Sadiq, 2023). Some investigations have shown that prolonged use of synthetic antioxidants boosts the risk of cancer and causes liver damage (Xu *et al.*, 2021). Polysaccharides derived from distinct plants have been reported as strong antioxidative agents (Akbari *et al.*, 2022). All these natural substances play a key role in the reduction of oxidative stress and free radicals, ultimately promoting good health (Xiao *et al.*, 2025; Zheng *et al.*, 2025). Additionally, anti-inflammatory properties are possessed by polysaccharides, contributing to altering immune response, stabilizing cell membranes, and inhibiting pro-inflammatory mediators (Cheng & Han, 2020). Based on these reasons, the role of polysaccharides is vital in the treatment of inflammatory diseases and also various other medical disorders (Wang *et al.*, 2022; Wang *et al.*, 2024b).

Okra (*Abelmoschus esculentus* (L) Moench) is a species of flowering plant belonging to the family *Malvaceae*. Originally, it came from Africa, but because of its edible pods, it is grown all over the world nowadays (Elkhalifa *et al.*, 2021). A slimy, white gelatinous layer is present in the seeds and pods of the fresh okra plant. Upon soaking in water, it becomes thick and is composed of polysaccharides, and has been utilized to enhance the consistency of soup and stews (Kesharwani *et al.*, 2023). The natural polysaccharides found in okra seed mucilage include galacturonic acid, L-rhamnose, monosaccharides, and D-galactose, as well as proteins and minerals (Shi *et al.*, 2022; Kesharwani *et al.*, 2023). The biological potential of okra mucilage has been extensively studied, and antitumor, antioxidant, antimicrobial, antiulcerogenic, and hypoglycemic effects have been determined (Basnet *et al.*, 2023; Fatima *et al.*, 2024; Zhang *et al.*, 2024; Ali *et al.*, 2025). Though several studies have been published on phytochemical composition and therapeutic potential of okra and its different parts, particularly mucilage, the water-soluble polysaccharides found in okra pods and seeds mucilage have not been studied well. This study aims to extract, perform biochemical analysis, and investigate the biological potential of water-soluble polysaccharides from okra pods and seeds mucilage, in terms of their antioxidant, antimicrobial, antidiabetic, and anti-inflammatory potentials. Additionally, it compares the biological activities of crude, water-soluble, and ethanol-soluble polysaccharide fractions. By investigating their antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities, this research seeks to establish okra mucilage polysaccharides as a cost-effective and sustainable resource for combating oxidative stress, microbial infections, and inflammation. Such findings will pave the way for novel therapeutic applications and contribute to the growing field of plant-based bioactives.

Materials and Experimental Approach

Plant sample acquisition and treatment: Fresh okra pods were sourced locally from an agricultural market in District Mardan during the harvesting season. The pods were cleaned by removing any damaged or unwanted parts. Afterward, the pods were rinsed repeatedly with deionized DH_2O and disinfected by soaking within a 5% hypochlorite solution (comprised of 4-6% active chlorine) for 20 minutes.

Extraction of mucilage: The mucilage extraction was executed following the method as mentioned by (Bukhari *et al.*, 2022) with some modifications. For the extraction of mucilage, 100 g of sterile okra pods were cut into small fragments and soaked in deionized distilled water overnight at ambient temperature. The mixture was strained by means of a muslin cloth, and the filtrates, containing pods and seeds, were cast off.

Ethanol fractionation: To disinfect the water-soluble polysaccharides, the attained mucilage was precipitated with 75% ethanol. To the mucilage, the ethanol was added in a ratio of 3:1 and left for 24 hours, yielding an ethanol-precipitated fraction and a non-precipitated fraction. Each fraction containing ethanol-soluble and water-soluble components, besides the crude mucilage, was collected distinctly, freeze-dried, and kept for further biological and biochemical analysis.

Total carbohydrates assay: To determine the carbohydrate content, the standard phenol-sulphuric acid method was used in okra pods and seeds mucilage, crude, and its ethanol and water-soluble polysaccharide fractions, following the demonstrated protocol of (Niemi, 2024). This assay established the presence of polysaccharides in each fraction.

Hemolytic activity: Accomplish the hemolytic potential, human red blood cells (HRBCs) were used for the seeds' mucilage crude and okra pods, along with their ethanol and water-soluble polysaccharides fractions, by following the proposed protocol of (Tehrani *et al.*, 2024). The 5 mL of blood sample was obtained from a willing volunteer and centrifuged at 1500 rpm for 15 minutes to separate the plasma. The RBCs pallet was washed several times with the autoclaved normal saline for purity. Then the obtained plasma was diluted with the autoclaved phosphate-buffered saline (PBS) at a proportion of 1:3. The three different concentrations of fractions were added individually to the RBCs suspension and incubated at 37ºC for an hour. After the mixture was incubated, it was again spun for 10 minutes at 3000 rpm. The spectrophotometric analysis was carried out at 570 nm after pouring 100 µL of the liquid phase into a micro-well plate. Triton X-100 (1%) was represented as a positive control, and PBS as a negative control. The following formula was used to compute the percentage of hemolysis:

$$\text{Hemolysis (\%)} = \frac{(\text{Mean OD of fraction} - \text{Mean OD of PBS})}{(\text{Mean OD of positive control} - \text{Mean OD of PBS})} * 100$$

Measurement of antioxidant potential: The antioxidant potential of okra pods and seeds mucilage crude, along with its water and ethanol- soluble polysaccharide fractions, was estimated through the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay following the method of (Baliyan *et al.*, 2022). A DPPH solution (0.004% in methanol) was prepared. Each fraction was tested at (250 µg/mL, 500 µg/mL, and 1000 µg/mL). For each experiment, 1 mL of the fraction was mixed with 4 mL of DPPH solution. The mixture was maintained in the dark for 30 minutes. The spectrophotometric measurement of the free radical scavenging potential was quantified at 517 nm. The L-ascorbic acid was utilized as a reference, while the negative control consisted of 0.1% DMSO. The extent of DPPH radical quenching was estimated by applying the following equation:

$$\text{DPPH scavenging (\%)} = \frac{(\text{Mean OD of blank} - \text{Mean OD of fraction})}{\text{Mean OD blank}} * 100$$

Alpha amylase inhibition assay: The suppression of pancreatic α-amylase was assessed to determine the antidiabetic potential of okra pods and seeds mucilage crude, along with its water and ethanol-soluble polysaccharide fractions, in a dose-dependent manner, following the protocol presented by (Jaber, 2023), following some modifications. Briefly, 100 µL of the extract was placed in separate vials at (1000 µg/mL, 500 µg/mL, and 250 µg/mL). Subsequently, 250 µL of porcine pancreatic α-amylase (3.2.1.1) was prepared at 0.5 mg/mL in 0.02 M sodium phosphate buffer. The reaction was commenced upon adding 250 µL starch (1%) prepared in 0.02 M N-sodium phosphate solution. The reaction mix was maintained for 10 minutes at ambient temperature and was ended by adding 500 µL of DNS (dinitrosalicylic acid). After adding DNS, the reaction solution was placed in a water bath at a high temperature for 5 minutes and then left to reach normal temperature. The absorbance was monitored at 540 nm using an ELISA plate reader (BioTek Inc. Model: ELx-800). Standard acarbose drug was represented as a reference, whereas distilled water served as a negative control. The α-amylase inhibition percentage was estimated using the following equation:

$$\alpha - \text{amylase inhibition (\%)} = \frac{(\text{Mean OD of blank} - \text{Mean OD of fraction})}{\text{Mean OD blank}} * 100$$

Antibacterial activity: The antimicrobial potential of okra pods and seeds mucilage crude, along with its water and ethanol-soluble polysaccharide fractions, was determined against the clinical isolate of five different pathogenic bacterial species (*Escherichia coli, Klebsiella pneumonia, Shigella, Staphylococcus aureus,* and *Salmonella typhi)* using the agar well diffusion technique (El Rabey *et al.*, 2024). Each bacterial species was cultured in a separate autoclaved nutrient broth for 24 hours and was inoculated in LB agar plates in a controlled environment. 5 wells of 5 mm were made in each agar plate using a sterilized tip. Among the five wells, three wells were seeded with different concentrations of each extract, while one well was seeded with 0.1 % DMSO, and the antibiotic disc was seeded in the last well as a reference drug. The test plates were then maintained at 37°C overnight, and the clear zone of inhibition (mm) around each well was analysed for each treatment. The experiment was repeated thrice.

***In vitro* erythrocyte membrane stabilization assay:** In order to identify the *In vitro* anti-inflammatory potency of okra pods and seeds mucilage crude, water-soluble, and ethanol-soluble polysaccharide fractions were subjected to the erythrocyte membrane stabilization assay according to the previously published method (Kpemissi *et al.*, 2023) with minor alterations. Each fraction was screened in a dose-proportional method at screening amounts of (250 µg/mL, 500 µg/mL, and 1000µg/mL). Briefly, fresh blood was taken from a human volunteer and centrifuged at 2000 rpm for 10 minutes. The aqueous phase was separated, and the pellet was dissolved in normal saline. The volume of erythrocyte suspension was measured, and a 40% suspension was made in isotonic solution (with pH 7.4 and 10 mM sodium phosphate buffer). Each fraction was dissolved in a hypotonic solution at different concentrations. Indomethacin was used as a reference drug. Human RBC suspension (0.1 mL) was added to 5 mL of hypotonic solution containing extract at distinct concentrations. The solution reaction was kept up at 25°C for an hour, then spun at 1300 rpm for 10 minutes. The hemoglobin content was calculated by shifting 200 µL of the aqueous phase to a microplate, and the optical density (OD) was measured at 540nm by means of an ELISA plate reader. The percent anti-inflammatory effect of the extract was estimated through the following equation:

$$\text{Percent inhibition (\%)} = \frac{(\text{Mean OD of A1} - \text{Mean OD of A2})}{(\text{Mean OD of A3} - \text{Mean OD of A2})} * 100$$

Here, *A1* represents the sample absorption in a hypotonic solution**,** *A2* represents the sample absorption in an isotonic solution, and *A3* denotes the sample absorption of the control in a hypotonic solution.

***In vivo* anti-inflammatory assay**

Experimental design: The *In vivo* anti-inflammatory activity of okra pods and seeds mucilage crude, water-soluble, and ethanol-soluble polysaccharide fractions was carried out on Balb/C mice using a previously reported method (Navarro-Leyva *et al.*, 2023). The Balb/C mice were purchased from the Veterinary Research Institute (VRI), Peshawar, and acclimated for one week. All the mice were kept in an ideal environment at 25°C with a 12-hour alternating shady and light period. Water and a standard diet were freely available to each model animal. To evaluate the anti-inflammatory effect of each fraction, mice were divided into six different groups for each

extract, with 5 animals in each group. One group was left normal, while inflammation was induced in the rest of the groups by injection of 1% carrageenan in the right paw of each mouse. After 1% carrageenan injection, the mice were left for 1 hour so that paw edema could develop. Among these, one group was left untreated, which serves as a negative control, while one was exposed to 100 mg/kg of diclofenac sodium as a positive control. The remaining three groups were dosed with the (100 mg/kg, 200 mg/kg, and 400 mg/kg) of each fraction. The volume of paw edema was recorded before and after treatment (1, 2, 3, 4, and 5 hours) of each experimental group using a screw gauge. The alleviation of paw edema volume was calculated, and the percent inhibition was determined.

Statistical analysis: All experimental procedures were repeated thrice for the sake of statistical analysis. Initial data were processed in Microsoft Excel, and One-Way ANOVA was performed by means of SPSS version 22, while graphs and IC_{50} were calculated using GraphPad Prism Version 5.0.

Results and Discussion

This study utilized water extraction and ethanol precipitation to isolate and purify polysaccharides from okra pods and seeds. The pod seeds were disinfected with 5% sodium hypochlorite (NaOCl), which contains 4-6% active chlorine content, to remove mucilage from okra pods. Then, they were immersed in autoclaved DH_2O. After immersing 100 g of okra pods and seeds in 1000 mL of DH_2O, the results showed that 19.27 g of mucilage was produced. The obtained mucilage was dried in a freeze-dryer, and the dried mucilage was fractionated with 75% ethanol. Multiple approaches were employed to characterize these polysaccharides, and their biological activities were also determined. One of the cost-effective ways that involves less sophistication in terms of equipment and operation for extracting polysaccharides from dried plant parts and other sources is water extraction and ethanol precipitation, as described in earlier (Basnet *et al.*, 2023).

Total carbohydrate content: The phenol-sulphuric acid (PSA) has long been utilized as a calorimetric assay for determining the concentrations of reducing pentose and hexose in a solution. The PSA assay works by first producing uronic acid and hydroxyurea formaldehyde by dehydrating carbohydrates with sulphuric acid (H_2SO_4). The subsequent reaction between the latter and phenol yields an orange-red hue that may be measured by taking an absorbance reading at 490 nm. As the amount of carbs in the sample increases, so does the saturation of the color.

Haemolysis assay: Rationalized by the fact that most of the therapeutic applicants are administered intravenously, where blood is the first line of interaction, we performed a haemolysis assay to investigate the potential toxicity associated with okra mucilage extracts (Greco *et al.*, 2020). The haemolytic effect of the extract obtained from okra pods and seeds' mucilage was determined against normal human erythrocytes. Haemolytic activity for each extract was expressed as percent haemolysis and recorded as ± standard deviation of the three replicates (Fig. 1). Each extract was screened in three different concentrations, and the experiment was conducted thrice. The haemolytic effect is an important aspect, as it takes place when erythrocytes come in contact with water and implant material before use. It is worth mentioning that the haemolysis limit for potential biomedical applications is set to less than 5% (Goud, 2024). Any increase above the limit results in toxicity. Table 1 shows the percent haemolysis and 50% cytotoxic concentration (CC_{50}). No significant percent haemolysis was observed for any fraction at all three screening concentrations; nevertheless, the water-soluble polysaccharide fraction was the least toxic one, which induced 4.24±0.43% haemolysis at 1000 µg/mL in human erythrocytes compared to the rest of the fractions.

Radical scavenging activity: Oxygen is an important element that plays a potential role in metabolic activities (Bungau *et al.*, 2023), along with imbalance, contributes to the development of oxidative stress. The body's natural antioxidant defence mechanism, which includes both enzymes and non-enzymatic antioxidants, often stabilizes the imbalance between prooxidants and antioxidants, a condition known as oxidative stress (Jomova *et al.*, 2024). Imbalance in reactive oxygen species (ROS) production not only compromise the nutritional value of foods through oxidation but also interfere cell's normal biomolecules including lipids, protein and DNA (Afzal *et al.*, 2023) which induces several clinical diseases such as cancer, diabetes, inflammatory diseases, aging, neurodegenerative disease and causing damage to immune system (Zhang *et al.*, 2025). When testing the ability of various substances to scavenge free radicals, the DPPH free radical inhibition assay is by far the most popular and extensively used method (Rahaman *et al.*, 2023; Zhao *et al.*, 2025). The free radical scavenging activity of okra pods and seeds mucilage crude extract, water-soluble and ethanol-soluble polysaccharide fractions was ascertained through DPPH assay, and the acquired results were compared with Ascorbic acid, which served as a positive control. Ascorbic acid had the greatest free radical inhibition potency, with a DPPH free radical inhibition efficiency of 93.4±1.23% at 1000 µg/mL. Table 2 and Figure 2 demonstrate that at 1000 µg/mL, the water-soluble polysaccharide fraction of the mucilage extract scavenges the free radicals by 70.75 ± 1.84%, followed by the ethanol-soluble polysaccharide fraction at 61.34±2.31%, and finally the crude mucilage fraction at 46.77±1.64%. The EC_{50} (half-maximal effective concentration) values for the water and ethanol-soluble polysaccharides were 530.38 and 721.68 µg/mL, respectively. The crude mucilage fraction was found to be the least effective in scavenging the DPPH free radical, as its EC_{50} was 1143.20 µg/mL. Previous literature reported that polysaccharides extracted from yam showed significant *In vitro* antioxidant potential in a dose-dependent manner by scavenging the DPPH and OH radicals by 92.73% and 84.72%, respectively, which was higher but comparable to our results. The water-soluble polysaccharides showed 70.75% antioxidant potential at 1 mg/mL, which is similar to a previous report, as their polysaccharide extract scavenges DPPH free radical by 69.57%, but their concentration was 2 mg/mL. It means that our results are potent and significantly stabilize the DPPH free radical at lower concentrations compared to previous studies (Haran *et al.*, 2024).

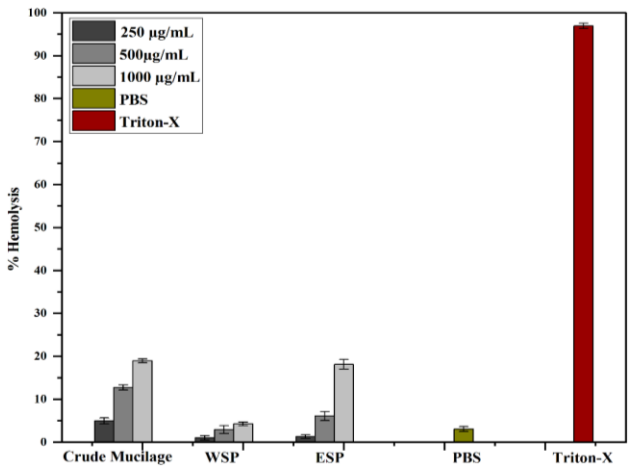

Fig. 1. Percent Hemolysis induced by okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharide (ESP) fractions at different concentrations (250 , 500, and 1000 µg/mL) with Phosphate-Buffered Saline (PBS) and Triton-X served as positive and negative controls, respectively.

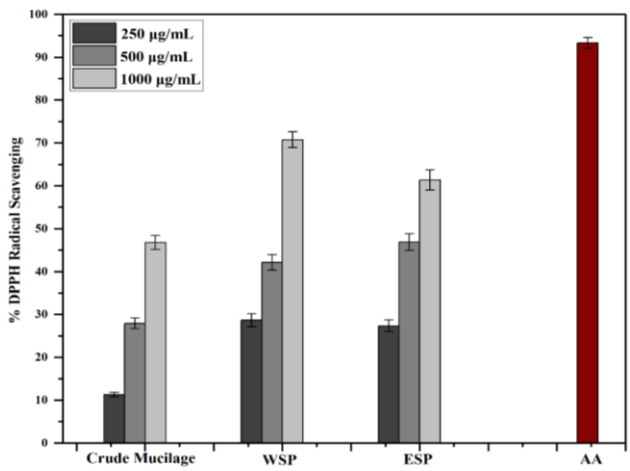

Fig. 2. Percent DPPH radical scavenging activity of okra pod crude mucilage, its water-soluble polysaccharides (WSP), and ethanol-soluble polysaccharides (ESP) at different concentrations (250, 500, and 1000 µg/mL) with Ascorbic acid (AA) served as a standard antioxidant.

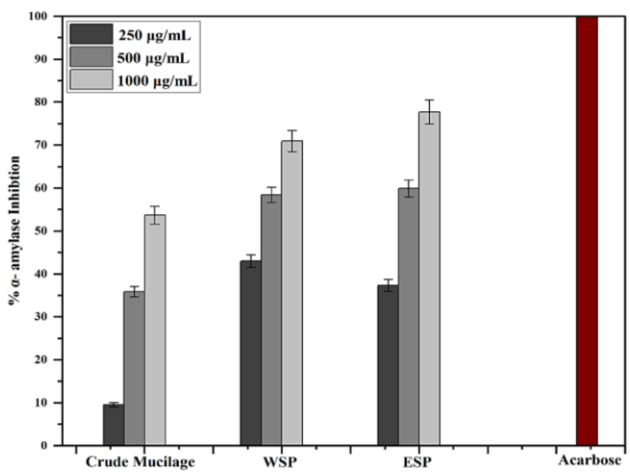

Fig. 3. Percent α-amylase inhibition by okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions at different concentrations (250, 500, and 1000 µg/mL) with Acarbose served as a standard inhibitor.

Alpha amylase inhibition activity: Development of novel antidiabetic agents from natural sources, particularly plants, is gaining the attention and interest of researchers. Alpha amylase is of prime importance, having a key role in diabetes as it helps in the digestion of polysaccharides through hydrolysis of 1-4-glycosidic linkages present in starch and glycogen to disaccharides, which leads to postprandial hyperglycemia. Hence, inhibitors of alpha amylase play a promising role in the control of diabetes (Khan *et al.*, 2024). Crude extract, water, and ethanol-soluble polysaccharide purified from okra pods and seeds mucilage were subjected to alpha amylase inhibition assay to ascertain and compare the antidiabetic potency of each fraction. The result obtained reveal that at 1000 µg/mL, water-soluble polysaccharide fraction was able to restrict the activity of alpha amylase by 77.66% followed by 70.93% by ethanol-soluble fraction, and 53.65% by crude mucilage fraction while the highest enzyme activity was inhibited by acarbose which was used as positive reference and inhibit 90% of the enzyme activity at 1000 µg/mL. The alpha amylase inhibition activity decreased when we decreased the screening concentrations to 500 µg/mL and 250 µg/mL. However, at 250 µg/mL, the water-soluble polysaccharide fraction showed the highest α-amylase inhibition, 42.95±1.5%, followed by ethanol-soluble and crude fractions, which exhibit 37.31±1.4% and 9.47±0.5% α-amylase inhibition, respectively, as shown in Table 3. Our results are in accordance with those found that the antidiabetic potential activity of okra increases with an increase in concentration (Uddin Zim *et al.*, 2021). The IC_{50} concentration calculated for each fraction showed that the water-soluble polysaccharide fraction was the most potent one, having an IC_{50} value of 346.37 µg/mL. Similarly, IC_{50} values for ethanol-soluble polysaccharide fraction and crude fraction calculated were 376.20µg/mL and 852.57 µg/mL, respectively. Suppressing the activity of alpha-amylase results in slackening the hydrolysis of carbohydrates/starch, thereby minimizing the blood glucose level (Siddique *et al.*, 2022). Literature showed that this inhibitory potential is because polysaccharides offer a competitive inhibitor to starch and thus polysaccharides bind to the catalytic region of α-amylase, thereby reducing and controlling enzymatic function (Sun *et al.*, 2020). Previous studies also confirmed that a high percentage of glucose and galactose in polysaccharides is correlated with anti-hyperglycemic effect (Teng *et al.*, 2023). In our study, it was found that acarbose, being the reference drug, significantly reduces the activity of pancreatic α-amylase; however, our extracted polysaccharides showed comparatively mild inhibition. Mild α-amylase inhibition is preferred since severe inhibition leads to aberrant microbial digestion of non-digested carbohydrates in the human colon, which could produce acarbose side effects (Ahmad *et al.*, 2022).

Antibacterial assay: We further processed the okra pods and seeds' crude extract, along with its water-soluble and ethanol-soluble polysaccharide fractions, to evaluate their antimicrobial activity against five different human pathogenic bacterial strains, including *S. typhi, K. pneumoniae, S. aureus, Shigella,* and *E. coli,* using the agar-well diffusion method. Antidiabetic discs were tested in triplicate with each portion at three different concentrations. The results revealed that at 100 µg/mL

water-soluble polysaccharide fraction showed 3.12±0.11 mm zone of inhibition against *Salmonella*, 3.89±0.34 mm against *Klebsiella*, 3.84±0.29 mm against *S. aureus*, 3.75±0.2 mm against *Shigella,* and 3.25±0.33 mm against *E. coli*. Likewise, at 100µg/mL, the ethanol-soluble fraction exhibited notable activity against *E. coli* with an inhibitory zone of 4.68±0.35 mm and 3.52±0.37 mm against *Shigella*. As shown in Table 4, the antibiotic disc was found to significantly inhibit the growth of pathogenic bacterial strains, and a prominent zone of inhibitions were observed for *Salmonella* (12±0.51 mm), *Klebsiella* (11±0.63 mm), *S. aureus* (9±0.43 mm), *Shigella* (12±0.55 mm), and *E. coli* (10±0.37 mm). The crude fraction did not show any considerable zone of inhibition at lower concentrations (25 µg/mL); however, at 100 µg/mL, it impeded the proliferation of *S. aureus* by inducing a 2.75 mm zone of inhibition. The difference in the zone of inhibition is due to different cellular and morphological structures of microorganisms, as literature showed that gram-negative bacteria (*S. typhihad* and *E. coli*) were more sensitive compared to gram-positive bacteria (*B. cereus* and *S. aureus*) to the studied concentrations of polysaccharides extracted from *Hypericum perforatum*. This cellular structure might be the possible reason that influences the sensitivity of bacterial strains to plant polysaccharides. Besides this, the source of polysaccharides might be the second reason, as different plants have different polysaccharides or might contain a high quantity of antibacterial polysaccharides (Yarley *et al.*, 2021). Plant parts play an important role because of their huge production of bioactive compounds for medicinal applications. A study conducted by (Shaeroun *et al.*, 2021) showed that Okra pods' ethanolic extract exerted inhibitory properties against test bacterial isolates (*E. coli* and *Klebsiella*), as a 3 mm zone of inhibition was recorded for each bacterial isolate (Shaeroun *et al.*, 2021).

Erythrocyte membrane stabilization assay: The process of membrane stabilization involves preventing osmotic and heat-induced lysis of biological membranes, including erythrocytes and lysosomal membranes (Ani *et al.*, 2023). The membrane integrity was assessed by treating hypotonically induced hemolysed RBC with three concentrations of okra crude mucilage, including water-soluble and ethanol-soluble fractions. Results in Table 5 show the *In vitro* membrane stabilization of each fraction, where indomethacin served as the standard drug. The percent inhibition of hemolysis in erythrocytes was found to be highest in the ethanol-soluble polysaccharide fraction, 86.15 ± 0.35%, followed by the water-soluble polysaccharide fraction, 74.52 ± 0.59%, and crude fraction, 64.80 ± 0.42%. In terms of IC_{50}, the lowest IC_{50} value was shown by the ethanol-soluble fraction, having an IC_{50} value of 380.08 µg/mL, observed by fractions of water-soluble polysaccharide (603.17 µg/mL) and crude (644.76 µg/mL). The rapid and quantitative increase in erythrocyte water content occurs when these cells are immersed in a hypotonic solution, which has a reduced osmolality. When human red blood cells (RBCs) are exposed to a hypotonic solution, it induces lysis, and this method is employed to measure the membrane stabilization because of its simplicity and reproducibility (Hess & D'alessandro, 2022). The literature also suggests that the main reason erythrocytes can't resist mechanical and osmotic stress is that their membranes have been damaged by oxidative stress (Orrico *et al.*, 2023).

Table 1. Percent hemolysis at three different concentrations and CC50 value (µg/ml) of okra pod crude mucilage, its water-soluble polysaccharides (WSP), and Ethanol-soluble polysaccharides (ESP) fractions.

	250 µg/mL	500 µg/mL	1000 µg/mL	CC_{50} µg/mL
Crude Mucilage	4.95 ± 0.71	12.72 ± 0.59	18.94 ± 0.44	2705.62
Wsp	1.01 ± 52	2.95 ± 91	4.24 ± 0.43	1754.89
Esp	1.30 ± 0.39	6.08 ± 1.04	18.11 ± 1.12	2735.85

Table 2. Percent DPPH free radical inhibition and ec50 value (µg/ml) of okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions.

	250 µg/mL	500 µg/mL	1000 µg/mL	EC_{50} µg/mL
Crude Mucilage	11.3 ± 0.5	27.89 ± 1.2	46.77 ± 1.64	1143.20
Wsp	28.65 ± 1.5	42.12 ± 1.8	70.75 ± 1.84	721.68
Esp	27.32 ± 1.4	46.89 ± 1.92	61.34 ± 2.31	530.38

Table 3. Percent α-amylase inhibition and IC_{50} value (µg/mL) of okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions.

	250 µg/mL	500 µg/mL	1000 µg/mL	IC_{50} µg/mL
Crude Mucilage	9.47 ± 0.5	35.86 ± 1.2	53.65 ± 2.1	852.57
Wsp	42.95 ± 1.5	58.35 ± 1.8	70.93 ± 2.5	346.37
Esp	37.31 ± 1.4	59.87 ± 1.92	77.66 ± 2.8	376.20

Table 5. Percent Anti-inflammatory potential and IC_{50} value (µg/mL) of okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions.

	250 µg/mL	500 µg/mL	1000 µg/mL	IC_{50} µg/mL
Crude Mucilage	15.22 ± 0.41	42.71 ± 0.29	64.80 ± 0.42	644.76
Wsp	30.02 ± 0.37	41.86 ± 0.33	74.52 ± 0.59	603.17
Esp	32.66 ± 0.52	59.87 ± 0.38	86.15 ± 0.35	380.08

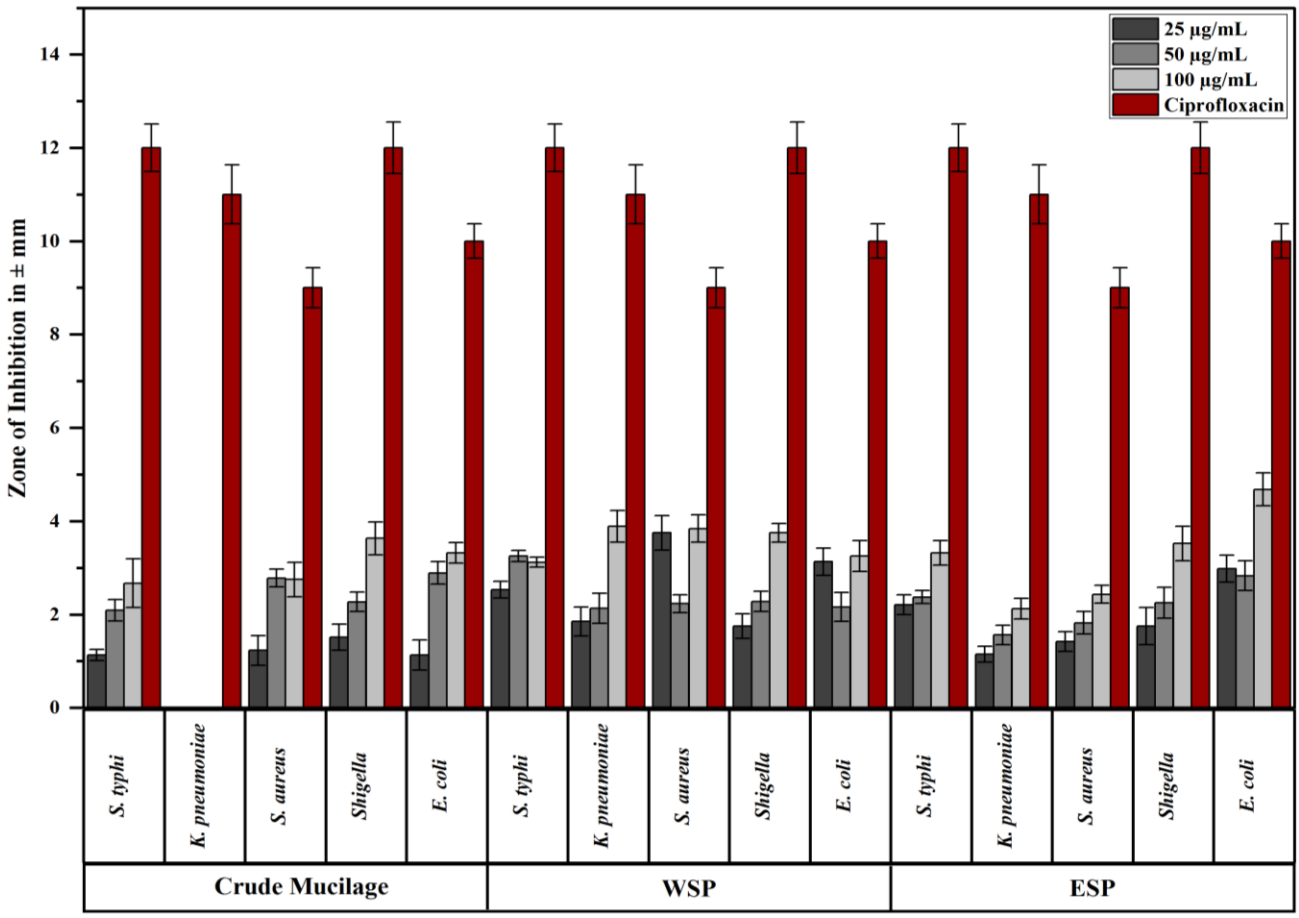

Fig. 4. Antibacterial activity of okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions against various bacterial strains at different concentrations (25, 50, and 100 µg/mL), with Ciprofloxacin served as standard antibiotic.

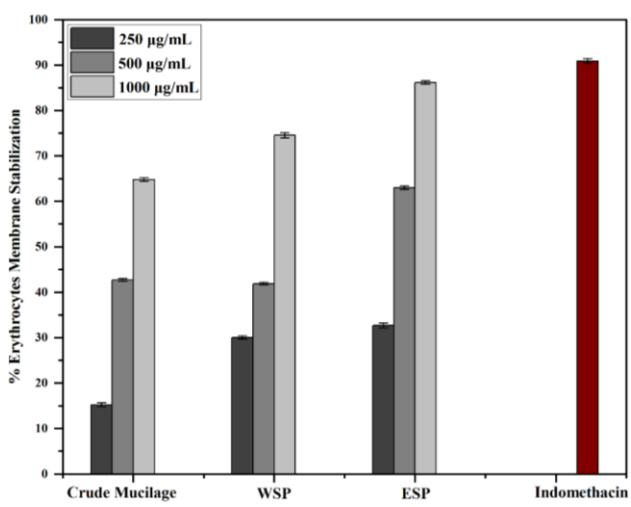

Fig. 5. Percent erythrocyte membrane stabilization by okra pod crude mucilage, its water-soluble polysaccharides (WSP) and ethanol-soluble polysaccharides (ESP) fractions at different concentrations (250, 500, and 1000 µg/mL), with Indomethacin served as a standard drug.

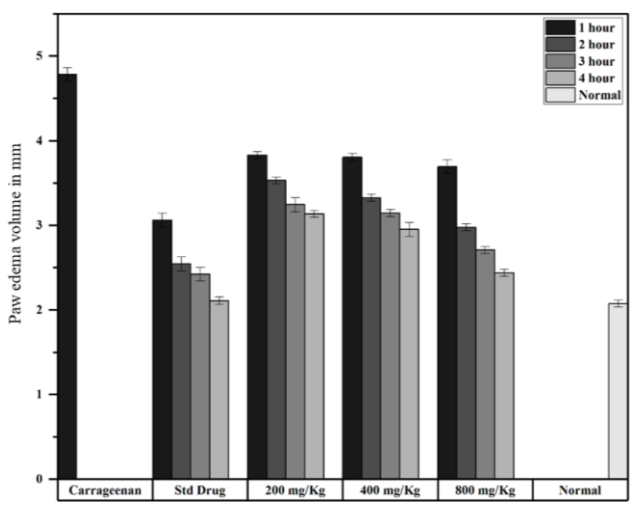

Fig. 6. Paw edema volume of Carrageenan-induced mice following the treatment with various concentrations (200, 400, and 800 mg/kg) of okra crude mucilage after 1, 2, 3, and 4 hours intervals, with Diclofenac sodium served as a standard drug.

***In vivo* anti-inflammatory activity:** Inflammation is a natural immune response that involves a series of events that enable the tissue to respond to any injury or infection. Precisely, the process of inflammation is collectively carried out by the expression of different cell types and reacting to diverse mediators through properly programmed events (Megha *et al.*, 2021). Generally, inflammation is mediated by the enhanced production of cytokines and chemokines, coupled with the recruitment of leukocytes to the site of infection. However, sustained and excessive inflammation can often lead to various clinical disorders, including psoriasis, rheumatoid arthritis, and other inflammatory bowel diseases (Ramos & Papadakis,

2019). We further processed the okra pods and seeds mucilage, crude, water-soluble, and ethanol-soluble fractions to evaluate their *In vivo* anti-inflammatory potential using the mouse paw edema model. Inflammation was induced by injecting a 1% carrageenan solution into the right paw, and an increase in edema was measured after one hour of carrageenan injection. The mechanism of carrageenan-induced inflammation is well understood, and it has been established that carrageenan works in three phases. In first phase that is after one hour of carrageenan injection, liberation of serotonin and histamine take place which is followed by the production of kinins in the second phase that is after 2 hour of carrageenan injection and induction of cyclooxygenase (COX) and production of prostaglandins in the third and final phase after 3 hours which results in causing increase in paw edema volume and hence inflammation is caused (Myers *et al.*, 2019; Ganesan *et al.*, 2024). Furthermore, the process of inflammation is also associated with enhanced production of highly reactive free radicals, which also contribute to edema (Xu *et al.*, 2024). The edema of the normal group, where no inflammation was induced, was 2.01 mm when measured with a screw gauge. The elevation in paw edema volume was recorded after one of the carrageenan injections, which shows that paw volume was increased from 2.01 mm to 4.67 mm after 4 hours of carrageenan injection; however, no significant increase was observed at the fifth hour. Diclofenac sodium was used standard anti-inflammatory drug, which effectively diminished the mice's paw edema volume to normal after 5 hours of treatment. The results showed each extract exhibited anti-inflammatory potential in a dose-dependent manner. At 800 mg/kg, the ethanol-soluble polysaccharide fraction effectively diminished the paw edema from 4.67 mm to 2.17 mm. However, the crude mucilage fraction was also able to induce a notable reduction in paw edema volume from 4.67 mm to 2.5 mm, as shown in Fig. 6. At 800 mg/kg, the water-soluble fraction reduces the paw edema volume to 2.25 mm as shown in Fig. 7. Previous literature showed that polysaccharides extracted from *Apium graveolens* have a strong anti-inflammatory role, thereby inhibiting the production of pro-inflammatory cytokines (Kumar *et al.*, 2023). Similarly, another study suggested that polysaccharides extracted from *Caesalpinia ferrea* and *Azadirachta indica* exerted the anti-inflammatory effect by inhibiting the edematogenic effect of serotonins, prostaglandins, and nitric oxide (Niu *et al.*, 2023; Tan *et al.*, 2025). Literature also showed that polysaccharides extracted from *Sedum dendroideum* induce an anti-inflammatory effect by reducing the level of TNF-α and IL1-β (De Oliveira *et al.*, 2017).

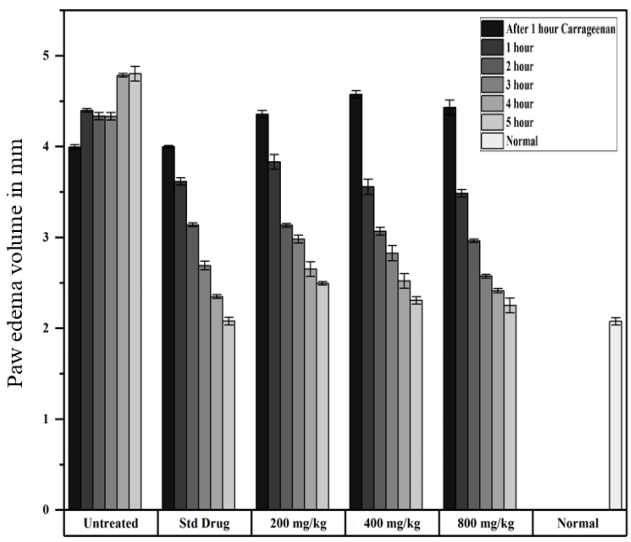

Fig. 7. Paw edema volume of carrageenan-induced mice following the treatment with various concentrations (200, 400, and 800 mg/kg) of water-soluble polysaccharide fraction after 1, 2-, 3-, 4-, and 5-hour intervals, with Diclofenac sodium served as a standard drug.

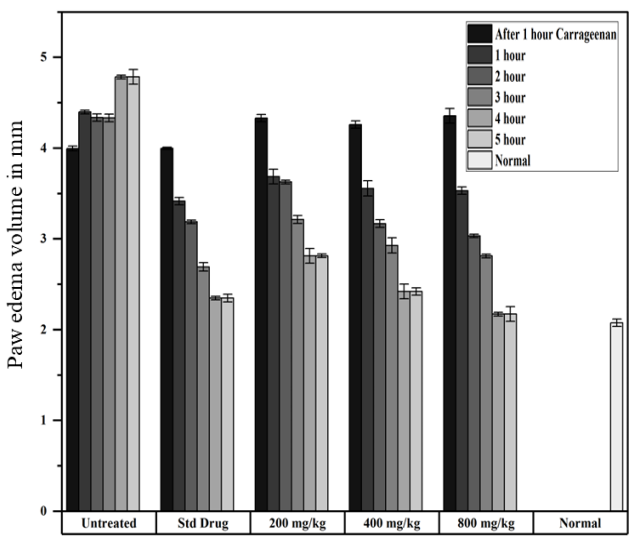

Fig. 8. Paw edema volume of carrageenan-induced mice following the treatment with various concentrations (200, 400, and 800 mg/kg) of ethanol-soluble polysaccharide fraction after 1, 2-, 3-, 4-, and 5-hour intervals, with Diclofenac sodium served as a standard drug.

Conclusion

Okra mucilage, extracted from pods and seeds, demonstrates significant potential as a cost-effective and biocompatible source of functional food ingredients due to its nutrient-rich composition. This study evaluated the crude mucilage and its water- and ethanol-soluble polysaccharide fractions, revealing notable biological activities. The mucilage exhibited strong α-amylase inhibitory activity, indicating its potential for managing hyperglycemia and supporting antidiabetic applications. Additionally, remarkable antioxidant properties were shown by both crude and fractionized extracts, highlighting their role in the neutralization of harmful free radicals. Significant antibacterial activity was also observed, specifically against pathogenic bacterial strains that are known for causing human diseases, highlighting its usefulness as a natural antimicrobial agent. Furthermore, anti-inflammatory potential was shown by the mucilage, highlighting its effectiveness in managing and treating inflammation-related disorders. Cytotoxicity assays were confirmed to be safe and non-toxic in nature for medicinal use, as they showed minimal hemolysis with aqueous okra mucilage. Through these findings, collectively, okra mucilage is showcased as a multifunctional bioactive material with antidiabetic, antioxidant, antimicrobial, and anti-inflammatory properties.

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