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Research article

Unlocking the in vitro and in vivo antioxidant and anti-inflammatory activities of polysaccharide fractions from *Lepidium sativum* seed-coat mucilage

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ABSTRACT

Inflammation coupled with oxidative stress contribute to the pathogenicity of various clinical disorders. Oxidative stress arises from an imbalance between production of reactive oxygen species (ROS) and antioxidant defense system, leading to cellular damages. The study investigated the antioxidant and anti-inflammatory effects of polysaccharides isolated from *Lepidium sativum* seed-coat mucilage. The water-soluble polysaccharides were extracted from mucilage and fractionated using gel permeation chromatography. The radical scavenging potential of various fractions was determined using DPPH, H_2O_2, and lipid peroxidation assays. The most effective EC_{50} was recorded for F53 (57.41 ± 1.34 μg/mL), followed by F20 (69.19 ± 0.61 μg/mL) and F52 (75.06 ± 0.45 μg/mL). In vitro anti-inflammatory effect was determined through human membrane stabilization assay while the in vivo effect was evaluated using a carrageenan-induced paw edema in mouse model where F53 demonstrated significant ($P = 0.05$) anti-inflammatory potential (92.60 % compared to diclofenac sodium 91.46 %). GC-MS analysis revealed the presence of galacturonic acid and glucuronic acid as main acidic monosaccharides along with varying quantities of rhamnose, arabinose, and maltose as prominent neutral monosaccharides. The study concludes that cress seed mucilage contains potent antioxidant and anti-inflammatory polysaccharides. Further studies on the mode of action of these polysaccharides could provide deeper insights into their potential use as antioxidant and anti-inflammatory agents.

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1. Introduction

Inflammation is a natural response of the host characterized by the accumulation of fluid and the recruitment of inflammatory mediators, such as white blood cells, to the site of inflammation [1,2]. However, persistent inflammation can lead to excessive production of free radicals and hyperactive molecules, resulting in oxidative stress and undesired effects on tissues and cells [3,4]. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the availability of antioxidants to scavenge them. ROS can oxidize biological molecules, modify genes and proteins, and trigger signaling cascades that contribute to the development of inflammatory diseases [5]. Antioxidants play a crucial role in stabilizing the oxidation process and delaying chain reactions initiated by high-energy molecules. While synthetic antioxidants have shown potential in combating oxidative stress, their pro-oxidant and cytotoxic nature at high concentrations makes them unsuitable for long-term use [6]. This has led researchers to shift their focus from synthetic to natural sources, such as plants, in the search for alternative antioxidant and anti-inflammatory compounds [7]. Plants are a rich source of bioactive compounds that can be used for therapeutic purposes in treating various clinical complications [8]. Plants have long been utilized in traditional medicines to treat inflammation and oxidative stress-related diseases [9]. Plant secondary metabolites contain bioactive compounds that play important roles in combating both infectious and non-infectious human diseases [10]. High molecular weight polysaccharides and low molecular weight oligosaccharides are among most frequently used compounds for this purpose [11]. The antioxidant mechanism of various polysaccharides isolated from plants and microorganisms has been determined to regulate the expression of downstream antioxidant enzymes. These antioxidant enzymes reduce the production of free radicals by inhibiting the free radical chain reactions. Also, polysaccharides significantly increase the antioxidant capacity and reduce the oxidative stress injury by suppressing iNOS mRNA expression and NO production [12].

Lepidium sativum, commonly known as garden cress and belonging to the Brassicaceae family, is a medicinally important plant originating from Egypt and distributed globally [13]. Extracts from L. *sativum* have demonstrated beneficial effects against various inflammatory disorders, including diabetes [14,15]. Moreover, *L. sativum* has shown hepatoprotective, antioxidative, antimicrobial, antidiarrheal, antispasmodic, and anti-inflammatory properties, protecting against oxidative damage [15]. Different parts of the cress plant, including the root, seeds, and leaves, have been used in traditional medicine due to their potent biological activities. Although the plant is primarily cultivated for its seeds, cress is famous for its consumption as salad, sprouts, and essential oils from its seed [16]. When cress seeds are soaked in water, they release a white gelatinous layer called mucilage, mainly composed of carbohydrates/-polysaccharides [17]. Mucilage can be used as an additive for forming suspensions or emulsions and as a thickening agent in the food industry [18]. Lepidine and mucilage are the two prominent classes of metabolites found in cress plant exudates, exhibiting nutraceutical effects [19]. Recently, seed coat mucilage of garden cress has been found to contain potent bioactive compounds with allelopathic effects [20–22]. The increasing prevalence of chronic diseases is strongly associated with oxidative stress and chronic inflammation. These pathological states arise due to imbalance between ROS production and body's antioxidant defense system which leads to cellular damage and persistent inflammatory responses. Traditional treatment often involves synthetic antioxidants and anti-inflammatory drugs, which can have significant side effects and limited long term efficacy [82]. Consequently, there is a growing interest in screening and development of natural compounds with therapeutic potential due to their safety and effectiveness. The hydroxyl group present in polysaccharides plays a crucial role in radical scavenging and anti-oxidative functions. Furthermore, the antioxidant and anti-inflammatory activities of polysaccharides are associated with several physiochemical properties such as water solubility, molecular size, monosaccharides composition and structural configuration. Polysaccharides extracted from *Polygonatum cyrtonema*, exhibited strong radical scavenging potential by stabilizing DPPH and hydrogen peroxide free radicals [81]. Based on these observations, the primary objective of the present research was to isolate and separate polysaccharides precipitated through ethanol (EPF) from the mucilage of cress seed coat. The study sought to assess the antioxidant and anti-inflammatory capabilities of these fractions through both in vitro and in vivo experiments. Subsequently, the chemical composition of the identified potent fractions was characterized using GC-MS.

2. Materials & methods

2.1. Chemicals & reagents

All the chemicals used in this study were of HPLC grade. These included 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetic acid, borax, citric acid, chlorobutanol, carrageenan, dextrose, dextran, diclofenac sodium, ethanol, glucose, hydrochloric acid, iron chloride, methanol, m-hydroxy biphenyl, orcinol, sulfuric acid, thiobarbituric acid, sodium hydroxide, sodium chloride, sodium citrate from Sigma Aldrich, Acetone, anthrone from Merck Millipore, ascorbic acid from Omicron sciences limited, Bio-Gel P-10 from Bio-Rad Laboratories, hydrogen peroxide from BDH laboratory supplies, phenol from Ambion, phosphate buffer saline from Oxoid and sodium hypochlorite from DAEJUNG.

2.2. Preparation of polysaccharides extract

To disinfect the cress seeds, they were treated with a 0.5 % sodium hypochlorite solution containing 4–6% active chlorine. Mucilage extraction was performed by soaking 100 g of disinfected cress seeds in 900 mL of autoclaved distilled water overnight on a magnetic stirrer. The seeds were separated from the solution by passing them through a muslin cloth. The resulting filtrate was then precipitated by adding 75 % ethanol and left overnight. The precipitated fraction was collected through centrifugation at 4000 rpm for

30 min. The obtained crude polysaccharides were freeze-dried using a lyophilizer and stored at −20 °C for further processing.

2.3. Gel permeation chromatography

Gel permeation chromatography (GPC) was conducted to fractionate the crude water-soluble polysaccharides from cress seed mucilage based on their size. The protocol used for GPC was followed, as described by Gómez-Ordóñez et al. [23], with slight modifications. The freeze-dried crude polysaccharides were dissolved in sterilized distilled water and fractionated using a Bio-Gel P-10 column (2.8 cm × 40 cm length) with a gel pore size of 80–180 μm. Prior to sample loading, the column was pre-calibrated using dextran. Autoclaved distilled water was used for elution, and 2 mL fractions were collected at a flow rate of 0.3 mL per minute. In total, 60 fractions were obtained from the chromatography.

2.4. Biochemical analysis

The ethanol-precipitated polysaccharides and their fractions were analyzed for total carbohydrate content using the phenol-sulfuric acid method [24]. For this, 200 μL of the sample was mixed with 500 μL of 98 % sulfuric acid and 10 μL of 80 % water-saturated phenol. The reaction mixture was gently mixed and incubated for 20 min at 37 °C. The absorbance was measured at 490 nm using a UV–visible spectrophotometer. Distilled water served as a blank, while glucose was used as a positive reference. A standard glucose calibration curve was generated using different concentrations of glucose through serial dilution, and the regression equation was determined as $y = 0.8184x + 0.0238$, with an R^2 value of 99.62. Here, “y" represents the rate of absorbance and “x" represents the concentration of carbohydrates present in the sample.

Samples with higher total carbohydrate content were further analyzed for the determination of saturated and unsaturated uronic acid, hexoses, and pentoses. Free and polymer-bound uronic acid was assessed by adding 1 mL of borax reagent prepared in 98 % H_2SO_4 to 200 μL of the sample. The reaction mixture was heated in a hot water bath for 5 min, cooled down, and the absorbance was recorded at 520 nm. Then, 20 μL of 0.15 % (w/v) m-Hydroxybiphenyl prepared in 1 M NaOH was added to the reaction mix, followed by incubation for 5 min at room temperature. The absorbance was measured at 520 nm. The difference between the two readings indicated the uronic acid content [25].

Unsaturated uronic acid content in each fraction obtained from ethanol precipitated polysaccharides was determined using protocol as described [25]. Briefly, 300 μL of 0.04 M of thiobarbituric acid (TBA) was mixed with 500 μL of the sample and then 200 μL of 0.07 M hydrochloric acid (HCl) and 50 μL of distilled water was added to the tube. The reaction tubes were mixed gently and were incubated for 30 min in hot water bath. After incubation, the tubes were allowed to cool on ice for 10–15 min. The absorbance of sample was recorded at 550 nm.

Free and polymer bound hexoses present in samples were determined by Anthrone method [26]. Anthrone solution (0.2 %) was prepared in 98 % H_2SO_4 and 0.5 mL of this solution was mixed with 0.25 mL of polysaccharides fraction. The reaction tube was mixed and kept for 5 min in hot water bath. The reaction mix was allowed to cool down and the absorbance for each fraction was recorded at 620 nm. Known amount of glucose was used as standard.

Free and polymer bound pentoses were quantified using the protocol as reported [26]. Briefly, 67 μL of orcinol solution (6 %, w/v) prepared in ethanol was poured in to 1 mL of 0.1 % iron chloride ($FeCl_3.6H_2O$) solution. The reaction mix was kept in a hot water bath for about 20 min and after incubation it was allowed to cool. The absorbance for each sample was measured at 665 nm. Xylose with a known concentration was used as standard pentose.

2.5. Determination of monosaccharide composition by GC-MS

2.5.1. Aldonitrile acetate derivatization for neutral sugars

Extraction of free monosaccharaides from the selected polysaccharide fractions was carried out by mixing 50 mg of fraction with 1 mL of methanol and 0.5 mg of sorbitol (internal standard). The samples were incubated at 80 °C for 4 h. Further the extract was evaporated to dryness and 0.3 mL of hydroxylamine hydrochloride in pyridine/methanol was added as derivatization agent prepared at 32 mg/mL concentration. The reaction mix was incubated at 75 °C for 25 min. The aldonitrile derivatives produced were acetylated by addition of 1 mL of acetic anhydride and further incubated at 75 °C for 15 min. Finally, 2 mL of dichloroethane was added and the excess of hydroxylamine hydrochloride in pyridine/methanol was removed with 1 M HCl and distilled water. The obtained dichloroethane layer was dried and dissolved in 300 μL mixture of heptane and ethyl acetate [27].

2.5.2. Uronic acids derivatization

The uronic acids present in selected polysaccharide fractions including F18, F22, F52 and F53 was derivatized according to the protocol mentioned [28]. Briefly, 200 μL of N-Methyl-2-pyrrolidone (NMP) served as solvent while 200 μL methoxyamine hydrochloride solution (20 mg/mL pyridine) was applied for the formation of methyloxime. Further, 400 μL of N, O-bis(trimethylsilyl) trifluoroacetamide was added for silylation of hydroxyl group present in acidic sugar and convert them into trimethylsilyl groups. The reaction tubes were incubated at 75 °C for 5 min. Once the derivatization completed, 50 μg of hexadecane was added as internal standard. Known amount of galacturonic acid and glucuronic acid were used as external standard.

2.5.3. GC-MS analysis

The GC-MS was used for confirmation of monosaccharaides present in the selected polysaccharide fractions obtained from cress

seed mucilage was identified through GCMS-5977B agilent technologies, USA, using the following protocol. The instrument was loaded with a DB-1 capillary column having 25 m × 0.25 mm × 0.25 μm volume. Helium (He) served as a carrier gas at a constant flow rate of 1 mL/min. The initial temperature of the oven was kept at 70 °C with a 10 °C increase after every minute. The temperature was increased to 270 °C with maximum temperature value of 300 °C while the holding for 13 min. The total analysis time was 12 min and the equilibration time was 2 min. The temperature of the injection port was 270 °C and a 1 μL volume was injected in split mode where the temperature of the heater was 250 °C, pressure was 8.808 psi and the split ratio was 20:1 [29]. The mass spectrometer recorded the entire spectrum in SCAN mode having electron ionization energy of 70 eV, ion source temperature 230 °C, MS Quad temperature 200 °C, electron multiplier voltage (EMVolts) 1200.696 V when performing selected ion monitoring, scanning from *m/z* 38 to 500 [30].

2.5.4. Method validation

A total of 10 monosaccharide standards were used for validation of analytical method. The standard monosaccharides included D-glucose, D-mannose, D-ribose, D-fucose, D-fructose, D-arabinose, D-galactose, L-rhamnose, galacturonic acid and glucuronic acid. The calibration curve for each standard monosaccharide was established by plotting the chromatographic peak area versus the concentration of the respective monosaccharide as shown in Table-1. The correlation coefficients ($R_2 > 0.998$) indicated that all the standard curves had outstanding linearity within the screen range. The limit of quantification (LOQ) and limit of detection (LOD) for each GC-MS screened sample was determined compared to the concentration of standard solution. The sensitivity of the method was confirmed as the LOD value for each monosaccharide standard was in range from 0.21 to 0.91 μmol/L.

2.6. Biological assays

2.6.1. Antioxidant assay using DPPH

The antioxidant potential of the EPF and polysaccharide fractions was estimated by utilizing 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radical [31]. The reaction was started by the addition of 500 μL of sample in three distinct concentrations and mixed with equal volume of 4 mM DPPH solution prepared in 80 % methanol. Ascorbic acid was employed as standard reference, while autoclaved deionized water was employed as blank. The reaction mixture was mixed properly and incubated in dark at 37 °C for 30 min. The absorbance for each reaction mixture was recorded at 517 nm. The percent radical scavenging potential was determined using the equation given below:

$$Percent\ \text{DPPH}\ scavenging = \frac{Ab - As}{Ab} * 100$$

Where "Ab" refers to absorbance of blank and "As" means absorbance of sample.

2.6.2. Antioxidant via hydrogen peroxide

The hydrogen peroxide (H_2O_2) free radical scavenging ability of the ethanol precipitated polysaccharide fractions from cress seed mucilage was evaluated using a spectrophotometric method developed by Ruch et al. [32]. Briefly, 100 μL of each polysaccharide fraction at three different concentrations was mixed with 300 μL of 50 mM phosphate-buffered saline (PBS), followed by the addition of 600 μL of 50 mM H_2O_2. The reaction mixture was vortexed for 10 min, and the absorbance was measured at 230 nm using a UV spectrophotometer. The percent radical scavenging potential was calculated using the standard equation:

$$Percent\ hydrogen\ peroxide\ radical\ scavenging = \frac{As - Ae}{As} * 100$$

Where "As" refers to absorbance of standard and "Ae" refers to absorbance of the extract.

2.6.3. Lipid peroxidation assay

The lipid peroxidation assay was conducted using ten albino mice weighing between 20 and 25g. Sodium pentobarbitone (35 mg)

Table 1
Linear regression equations, R^2 Value, limit of detection (LOD) and limit of quantification (LOQ) for individual neutral and acidic monosaccharide after gas chromatography-mass spectrometric (GC-MS) analysis.

Monosaccharide	Linear Regression Equations	R^2 Value	LOD in μmol/L	LOQ in μmol/L
Galactose	y = 0.0287x + 0.6912	0.998	0.81	2.13
Rhamnose	y = 0.0634x + 0.7561	0.999	0.79	3.01
Ribose	y = 0.0308x + 0.6322	0.997	0.21	0.92
Fucose	y = 0.0177x + 0.5006	0.996	0.87	1.88
Mannose	y = 0.0318x + 0.1231	0.998	0.51	1.51
Glucose	y = 0.0235x + 0.1588	0.997	0.36	0.76
Arabinose	y = 0.0211x + 0.1918	0.998	0.29	0.78
Saccharose	y = 0.0163x + 0.5701	0.991	0.85	2.21
Glucuronic acid	y = 0.0254x + 0.4005	0.995	0.91	2.93
Galacturonic acid	y = 0.0535x + 0.1376	0.999	0.43	2.14

was administered as an anesthetic to induce sedation in the mice. One liver segment was collected from each mouse during the sedation period and treated with a 0.9 % NaCl solution. The liver segments were individually homogenized using a vortex mixer with 1.15 % (w/v) ice-cold trichloroacetic acid (TCA) to obtain a 10 % tissue homogenate. Thiobarbituric acid reactive substances (TBARs) were measured using the tissue homogenate obtained. In the assay, 100 μL of the previously prepared liver homogenate was mixed with an equal amount of the sample at three different concentrations (50, 100, and 200 μg/mL). To the mixture, 30 μL of 0.1 M Tris-HCl buffer (pH 7.4) and 30 μL of freshly prepared 250 μM $FeSO_4$ as a pro-oxidant were added. The reaction mixture was incubated at 37 °C for 2 h. After incubation, 300 μL of 8.1 % sodium dodecyl sulfate (SDS) was added, followed by 600 μL of acetic acid and 300 μL of 0.8 % TBA. The mixture was further incubated at 100 °C for 1 h. The generation of the TBA-MDA (thiobarbituric acid-malondialdehyde) adduct was measured at 532 nm using a spectrophotometer, and the values were expressed as percentage of the control [33].

2.7. Anti-inflammatory assay

2.7.1. In vitro anti-inflammatory assay

The EPF and fractionated polysaccharide fractions were evaluated for their in vitro anti-inflammatory activity using the human red blood cell (HRBC) membrane stabilization assay, following a previously established protocol with slight modifications [34]. To perform the assay, HRBCs were collected from fresh human blood, and a 10 % (v/v) suspension was prepared using PBS. The suspension was then treated with three different concentrations (50 μg/mL, 100 μg/mL, and 200 μg/mL) of the polysaccharide fractions, while indomethacin was used as the reference drug. The reaction mixture consisted of 1 mL of hyposaline, 0.5 mL of the sample, and 0.75 mL of isosaline. The mixture was incubated at room temperature for 30 min. After the incubation period, the reaction mixture was centrifuged at a speed of 3000 rpm for 20 min. Approximately, 200 μL of the supernatant was transferred to an ELISA plate reader, and the absorbance was measured spectrophotometrically at 570 nm. The percent anti-inflammatory potential was calculated using the following standard equation:

$$\%MS = \frac{mean\ Abs\ of\ hyposaline - mean\ Abs\ of\ test\ sample}{mean\ ABs\ of\ control} * 100$$

Where MS = membrane stabilization and Abs = absorbance.

2.7.2. In vivo anti-inflammatory assay

For the in vivo experiment, experimental mice (Balb/C) weighing between 25 and 30 g were obtained from the Veterinary Research Institute (VRI) Peshawar. The mice were fed regular diet and mildly fasted before the start of the experiment to assess the treatment's effects. They were then divided into five groups, with each group consisting of five mice. To induce paw edema, a subplantar injection of a freshly prepared 1 % carrageenan solution in distilled water (100 μL) was administered to each of the six groups of mice (named A, B, C, D, E, and F), with each group containing five mice. After 1 h of carrageenan-induced inflammation, the mice in the groups were orally administered with selected EPF from cress at 3 distinct concentrations of 200 mg/kg, 400 mg/kg and 800 mg/kg, whereas one group of mice was given normal diclofenac sodium at a dose of 150 mg/kg, the other group was kept untreated and acted as the negative control. The volume of paw edema was measured using a micrometer screw gauge at "0 h" (before carrageenan injection) and at 1, 2, 3, 4, and 5 h after treating the edema with the different concentrations of the selected fractions [35].

2.7.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 6.0). The mean value and standard deviation were calculated from three replicates (n = 3) per treatment. The significance among the treated and control groups was determined using One-way Analysis of Variance with a p-value <0.05. Tukey's test was performed to group the homogenous means.

3. Results & discussions

3.1. Mucilage extraction and fractionation

Mucilage is a white gelatinous layer secreted by cress seeds when soaked in water and is about 6.5 %–15.0 % of the total seed weight [2]. Functional and structural properties of mucilage make it suitable choice for various applications. The dry yield of mucilage extracted from cress seed was found to be 12.45 %. We found that yield of mucilage is not affected by temperature and seed to water ratio. However, it has been found that mucilage yield has been affected by various factors, including extraction time and process [36]. The maximum quantity of mucilage is directly related to the extraction technique where solvent-based, microwave assisted, enzyme assisted and ultra-sonication extraction techniques have been followed [37]. Several studies have reported that mucilage can be extracted through agitation under aqueous condition. Beside agitation, maximum yield of mucilage can also be obtained through centrifugation [38]. We further precipitated crude mucilage in 95 % ethanol which yielded two fractions, ethanol precipitated fraction (EPF) and ethanol not precipitated fraction (ENPF). Between the two fractions, EPF showed significant antioxidant and anti-inflammatory activities in vitro, implying that it has potent free radical stabilizing activities. The biochemical analysis showed that the EPF contains monosaccharaides including hexoses, pentoses, uronic acid and unsaturated uronic acid. The EPF was then fractionated by Bio-Gel −10 column which yielded 60 fractions. Each fraction was screened for the total carbohydrates content.

3.2. Total carbohydrates content

The total carbohydrates content of each fraction is shown in Fig. 1. Fractions F1 to F16 did not contain detectable levels of carbohydrates, while a low amount of carbohydrates was detected in fraction F17. Among the 60 fractions, F18 exhibited the highest carbohydrates content (1.669 mg/mL), followed by F21 (1.51 mg/mL), F30 (1.466 mg/mL), F22 (1.036 mg/mL), F20 (0.968 mg/mL), F32 (0.804 mg/mL), F19 (0.786 mg/mL), F28 (0.765 mg/mL), and F29 (0.733 mg/mL). Comparing these fractions obtained through gel permeation chromatography, the carbohydrates content in the ethanol precipitated fraction (EPF) was found to be 12.01 ± 0.82 mg/mL. A previous study reported that the total carbohydrates content in garden cress seeds was 28.45 % [39]. The phenol-sulfuric acid method, which is a reliable and straightforward colorimetric method, was used to evaluate the sugar and polysaccharide content in the plant-derived mucilage. The carbohydrates present in each polysaccharide fraction were quantified using glucose as standard sugar. Different carbohydrates exhibit different colors upon absorption, depending on their molecular weight and sugar composition [40].

3.3. Biochemical composition

The monosaccharide units identified in the fractions included pentoses, hexoses, uronic acids, and unsaturated uronic acids (Fig. 2). Among these sugars, uronic acids were the most dominant in the ethanol precipitated fraction (EPF), while unsaturated uronic acids were present in the lowest quantity. Fraction F18 had the highest content of unsaturated uronic acids (8.25 μg/g), followed by F21 (6.81 μg/g) and F21 (6.35 μg/g). However, no unsaturated uronic acids were detected in fractions F22 and F24. In terms of uronic acids, the highest levels were found in F52 (20.66 μg/g), followed by F25 (20.09 μg/g) and F18 (19.19 μg/g). The ratio of hexoses to pentoses varied among different fractions. Fraction F24 had the highest hexoses content (13.24 μg/g), followed by F53 (12.99 μg/g) and F25 (12.59 μg/g). On the other hand, F20 exhibited a high pentoses content (11.88 μg/g), while F53 had the lowest pentoses content (4.74 μg/g). Among all the screened fractions, F52 had high levels of uronic acids (20.66 μg/g), and low levels of hexoses (2.63 μg/g) and pentoses (5.70 μg/g). Acid hydrolysis of cress seed mucilage revealed the presence of uronic acid, glucose, galacturonic acid, arabinose, rhamnose, and galactose as monosaccharide constituents [41]. Another study reported that mucilage extract from different maize varieties contained galactose (39–42 %), fructose (22–30 %), mannose (11–14 %), and glucose (1–4%) as the major monosaccharides [42]. Similarly, the monosaccharide units in *Asplenium australasicum* mucilage were found to be uronic acids, glucose, galactose, xylose, and fructose [43]. Polysaccharides from L. *sativum* seed mucilage, fractionated through size exclusion chromatography, exhibited a similar monosaccharide composition to *Lepidium perfoliatum* seed mucilage, consisting of rhamnose, galactose, arabinose, glucose, and xylose [44].

3.4. Monosaccharides composition

The results of the GC-MS analysis of selected polysaccharide fractions revealed the presence of neutral and acidic sugars, including rhamnose, fucose, arabinose, galactose, glucose, galacturonic acid and glucuronic acid as shown in supplementary data (S1, S2, S3 and S4). Among the tested fractions, F53 contained the highest percentage of galacturonic acid which accounted for 73.21 % of the total sugar followed by glucose (8.61 %), rhamnose (5.61 %) and glucuronic acid (5.41 %) as shown in Table-2. Similarly, fraction F52 composed of 63.21 % galacturonic acid, 11.67 % glucose, 7.13 % glucuronic acid, 5.21 % galactose, 4.53 % arabinose, 2.13 % xylose and 2.09 % fucose. In polysaccharide fractions F22 and F18, galacturonic acids was the main monosaccharide constituent, comprising of 36.32 % and 48.21 % of it, respectively. Notably, galacturonic acid was found to be the prominent acidic monosaccharide in almost all fractions along with glucuronic acid which indicated the presence of pectin-like and other acidic polysaccharides in each fraction. Uronic acid was the most abundant monosaccharide unit in *Corchorus olitorius* L. mucilage followed by rhamnose, galactose and

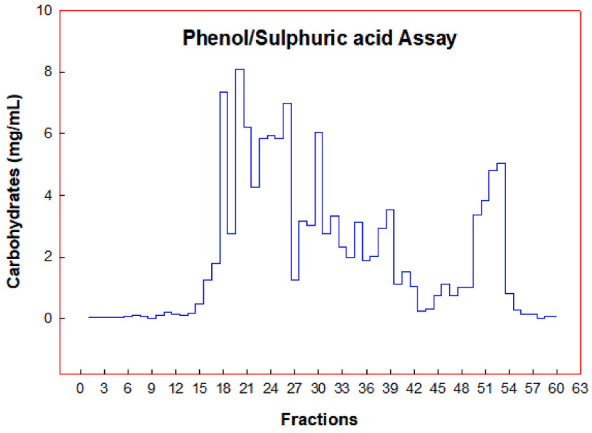

Fig. 1. carbohydrate in Bio-Gel-P = 10 fractions obtained from cress seed mucilage.

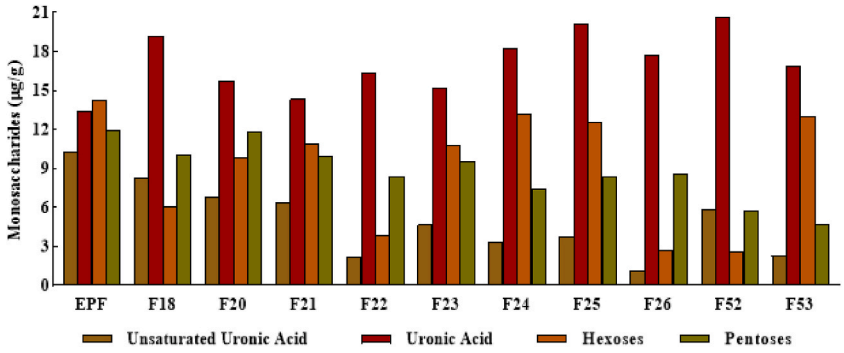

Fig. 2. Quantification of monosaccharides components present in polysaccharides fractions obtained from cress seed mucilage. EPF stands for ethanol precipitated fraction; F18-F53 are collected fractions through Bio-Gel-P-10.

Table 2
Validation of monosaccharides in cress seed mucilage polysaccharide fractions using GC-MS.

	TYPES OF MONOSACCHARAIDES	F18	F22	F52	F53
Neutral Monosaccharaides	Arabinose	4.96	11.67	4.53	3.12
	Fucose	0.54	4.56	2.09	1.96
	Xylose	traces	8.73	2.13	traces
	Galactose	5.12	13.27	5.21	2.06
	Glucose	14.23	21.21	11.67	8.61
	Rhamnose	11.71		4.03	5.61
	Sucrose	4.27	9.28	7.32	3.16
	Maltose	4.29	0.7	1.31	2.67
Acidic Monosaccharaides	Galacturonic Acid	42.21	26.34	54.58	67.38
	Glucuronic Acid	12.67	4.24	7.13	5.43

arabinose [45]. The monosaccharides composition analysis showed that *Opuntia ficus-indica* mucilage was primarily composed of fucose, arabinose, xylose, mannose, glucose, galactose and galacturonic acid [46]. Also, polysaccharides extracted from *Lepidium perfoliatum* seed gum were mainly made of rhamnose (3.40 ± 0.21), followed by galactose (12.77 ± 0.01), arabinose (31.99 ± 0.48), glucose (7.15 ± 0.33), and xylose (44.66 ± 0.37) [44].

3.5. DPPH radical scavenging potential

DPPH free radical scavenging analysis is extensively used for estimating the free radical stabilizing potential of natural extracts [47]. The antioxidant potential of EPF polysaccharides from cress seed mucilage obtained through GPC were determined using DPPH

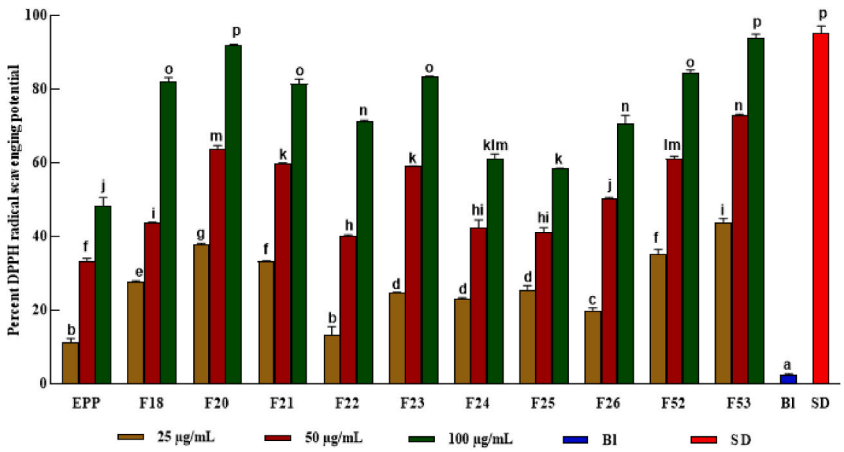

Fig. 3. Percent scavenging of the DPPH free radical activities of polysaccharides fractions extracted from cress seed mucilage. EPF stands for ethanol precipitated fraction; Bl stands for blank; SD stands for standard drug (ascorbic acid); F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

free radical assay. The antioxidant effect of each polysaccharide fraction was expressed in percent radical scavenging activities. Fig. 3 shows that different polysaccharides fractions exhibited significant antioxidant potential and an increase was observed while increasing their concentration when compared to ascorbic acid, which served as reference. At 200 μg/mL, the highest radical scavenging potential was observed in fraction F53, which scavenged the DPPH free radical by 93.83 ± 1.06 %, followed by F20 (91.95 ± 0.12 %) and F52 (84.27 ± 0.88 %). These values were comparable to ascorbic acid, which scavenged the DPPH radical by 95.01 ± 2.08 % at 100 μg/mL. The EPF inhibited the DPPH free radical by 11.17 ± 1.12 % at 50 μg/mL, 33.12 ± 0.89 % at 100 μg/mL, and 48.31 ± 2.23 % at 200 μg/mL. The 50 % DPPH radical inhibitory concentration (EC_{50}), often used to compare the DPPH stabilization potential of antioxidant compounds indicated that lower EC_{50} values represent stronger antioxidant and free radical stabilizing potential. Table-3 shows the EC_{50} values of the polysaccharide fractions. Among all the fractions, the most effective EC_{50} value was recorded for fraction F53 (57.41 ± 1.34 μg/mL), followed by F20 (69.19 ± 0.61 μg/mL) and F52 (75.06 ± 0.45 μg/mL). The antioxidant mechanism of polysaccharides can be determined through various assays, including free radical scavenging, reducing power, chain initiation blocking, peroxide degradation, continuous hydrogen abstraction and transition metal catalysis binding [48–50]. DPPH is a relatively stable free radical commonly used to evaluate the antioxidant capability of polysaccharides. Fractionated polysaccharides from cress seed mucilage effectively reduced DPPH free radicals by converting highly reactive unpaired electrons to stable electron pairs. One possible mechanism for antioxidants is the conversion of free unpaired electrons to paired ones [51]. Polysaccharide fractions obtained from cress seed mucilage demonstrated their potential to probably scavenge hydrogen or donate an electron, thereby stabilizing free radicals. The ethanol precipitated extract of *L. sativum* was previously reported to inhibit DPPH free radicals by 2.69 % in stems, 10.21 % in leaves, 11.63 % in seeds, and 12.19 % in the whole plant [52]. Although these previous findings are not consistent with our results, it is worth noting that the extract in our study mainly consists of carbohydrates in the form of mucilage. DPPH, a compound possessing a proton free radical, can be readily stabilized when exposed to proton donors [53]. Polysaccharides composed of monosaccharide units have a strong proton-donating ability, which is why the findings of our study are more significant. In a separate study, the methanolic and ethanolic extracts of *L. sativum* seeds exhibited strong antioxidant potential, with calculated EC_{50} values of 119.4 μg/mL and 134.7 μg/mL, respectively. Among the two extracts, the methanolic extract of *L. sativum* showed more promising reducing activity and radical stabilizing properties compared to the ethanol extract [54]. Our study found that the antioxidative effect of cress seed-coat mucilage polysaccharide fractions was dose-dependent and correlated with the monosaccharide composition. Fractions having highest uronic acid content showed notable free radical stabilization potential compared to those having less uronic acid content. Previous literature suggests that the DPPH free radical scavenging potential of polysaccharides is mainly dependent on its uronic acid contents [55]. Another study reported that polysaccharides extracted from *Amana edulis* exhibited strong antioxidant potential [56]. The DPPH free radical scavenging activity of the hot buffer soluble solids (HBSS) was 98 %, chelating agent soluble solids (CASS) was 97 %, dilute alkaline soluble solids (DASS) was 56.1 %, and concentrated alkaline soluble solids (CHSS) was 50.9 % [56]. In the present study, the polysaccharide fractions that were obtained through gel permeation chromatography was having higher antioxidant potential compared to ethanol precipitated fraction which suggests that low molecular weight polysaccharides may exhibit stronger proton or electron donating ability and stabilize the free radicals.

3.6. H_2O_2 radical scavenging activity

The polysaccharide fractions of cress seed mucilage, including EPF and fractions obtained through GPC, exhibited significant potential in stabilizing the H_2O_2 free radical, as shown in Fig. 4. Among all the fractions screened, F53 was found to be the most effective, stabilizing the H_2O_2 free radical by 86.54 % at 200 μg/mL. This was followed by F23 (86.36 %), F20 (81.43 %), and F52 (75.58 %). The half maximal effective concentration (EC_{50}) values also reflected the effectiveness of the fractions, with F53 having the most prominent EC_{50} value of 45.19 ± 0.18 μg/mL, followed by F23 (54.69 ± 0.77 μg/mL) and F52 (74.25 ± 1.23 μg/mL). On the other hand, F25 exhibited the least effectiveness with an EC_{50} value of 209.01 ± 3.41 μg/mL, as shown in Table-3. Hydroxyl radicals, such as H_2O_2, are highly toxic to living organisms and have the potential to cause severe damage to various biological molecules, including DNA, lipids, and proteins. Therefore, scavenging these radicals is essential for the continuation of vital life processes [57].

Table 3
Half-maximal effective concentration in μg/mL required to stabilize the free radical production by 50 %.

Fractions	DPPH FR	H_2O_2 FR	LPO FR
EPP	204.3 ± 1.48k	168.5 ± 0.93i	165.0 ± 2.21k
F18	113.7 ± 1.12g	107.4 ± 0.64e	125.5 ± 1.98i
F20	69.2 ± 0.61b	89.6 ± 0.88d	57.7 ± 1.60c
F21	79.3 ± 2.66d	121.5 ± 0.32g	81.7 ± 0.97d
F22	122.4 ± 1.32h	111.7 ± 1.98f	97.0 ± 0.33f
F23	87.6 ± 1.87e	54.7 ± 0.77b	112.1 ± 0.59g
F24	132.6 ± 0.42i	160.1 ± 0.23h	93.9 ± 1.38e
F25	142.4 ± 0.67j	209.0 ± 3.41k	155.3 ± 2.13j
F26	109.0 ± 0.93f	192.9 ± 1.09j	117.6 ± 0.17h
F52	75.1 ± 0.45c	74.3 ± 1.23c	52.6 ± 0.89b
F53	57.4 ± 1.34a	45.2 ± 0.18a	46.6 ± 0.91a

EPF stands for ethanol precipitated fraction; F18-F53 are collected fractions through Bio-Gel-P-10; FR = free radicals; LPO = lipid peroxides. The data are presented as mean along with standard error values. Means followed by different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

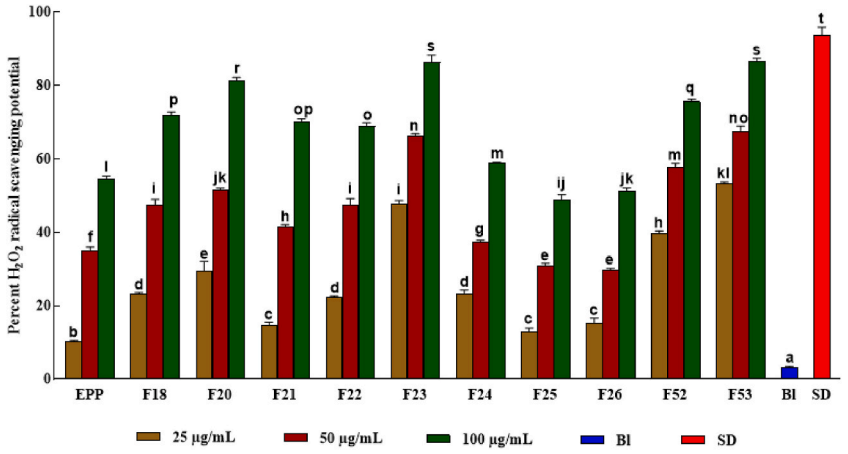

Fig. 4. H_2O_2 free radical activities of fractionated polysaccharides from cress seed mucilage. EPF stands for ethanol precipitated fraction; Bl stands for blank; SD stands for standard drug (ascorbic acid); F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

Polysaccharides have been found to possess electron and hydrogen donating abilities, allowing them to readily scavenge free radicals [58]. In our study, almost all the polysaccharide fractions from cress seed mucilage effectively stabilized the H_2O_2 free radical in a dose-dependent manner. This finding is in accordance with the previous study [59], which showed that polysaccharides extracted from *Ginkgo biloba* can scavenge hydroxyl radicals in a dose-dependent manner.

3.7. Inhibition of lipid peroxidation

The polysaccharide fractions extracted from cress seed mucilage were further evaluated for their potential to decrease lipid peroxidation using the thiobarbituric acid reactive substance (TBARS) assay [33]. The results showed that these polysaccharide fractions significantly decreased the levels of lipid peroxides in the liver of mice, as shown in Fig. 5. Among all the screened fractions, F53 exhibited the highest percent inhibition, inhibiting the TBARS free radicals by 94.54 ± 2.33 % at 200 µg/mL. The IC_{50} values, representing the minimum concentration required to inhibit 50 % of lipid peroxide radical production, were found to be the smallest for F53 with an IC_{50} of 46.64 ± 0.91 µg/mL. This was followed by F52 (52.61 ± 0.89 µg/mL), F20 (57.72 ± 1.60 µg/mL), F21 (81.70 ± 0.97 µg/mL), F24 (93.88 ± 1.38 µg/mL), and F22 (96.99 ± 0.33 µg/mL), as shown in Table-3. The TBARS assay is known to be a highly accurate and effective method for quantifying lipid peroxides [60]. In TBARS method, malonaldehyde (MDA), which is produced as byproduct, reacts with thiobarbituric acid (TBA) to produce MDA-TBA2 dimer. This dimer has pink color which can be observed at 532 nm [60]. One possible mechanism through which polysaccharides can reduce lipid peroxidation is by increasing the efficiency of antioxidative enzymes. For example, polysaccharides extracted from *Lycium barbarum* were found to have prominent antioxidant

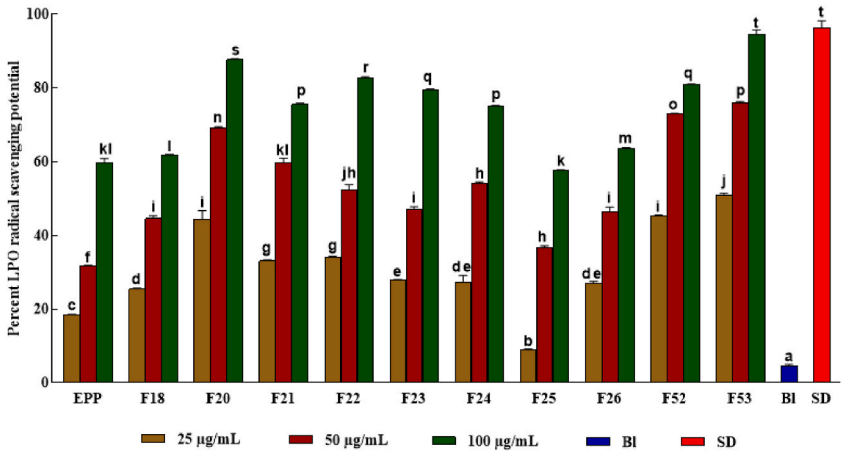

Fig. 5. Percent inhibitory potential of lipid peroxidation in liver homogenate of Balb/C mice of polysaccharides extracted from cress seed mucilage. EPF stands for ethanol precipitated fraction; Bl stands for blank; SD stands for standard drug (ascorbic acid); F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

activity in aged mice, where they decreased levels of MDA and inhibition of lipid peroxide production [61]. Similarly, polysaccharides extracted from mushrooms exhibited significant antioxidant effects [62]. In comparison to these findings, the polysaccharide fractions from cress seed mucilage, that contain uronic acid, pentoses, and hexoses as their monomeric units, also exhibited a dose-dependent inhibition of lipid peroxide production.

3.8. HRBCs membrane stabilization activity

Inflammation is a natural immune response to harmful stimuli, but dysregulation of this response can lead to acute or chronic inflammation, causing various diseases [1]. During inflammation, the activation of COX-1 and COX-2 enzymes results in the production of prostaglandins and lysosomal enzymes, which contributes to tissue damage and the progression of chronic and acute inflammation [63]. Therefore, inhibiting their activities can alleviate symptoms of inflammation including fever and pain. Furthermore, stabilization of lysosomal membrane restricts the release of lysosomal enzymes that induces inflammation. The anti-inflammatory mechanism involves activation of various enzymatic and signaling transcription factors that stabilizes lysosomal membrane and control the progression of inflammation [64]. Erythrocytes membrane stabilization assay is well-established method to screen the membrane stabilization potential of synthetic and natural products [65]. The anti-inflammatory mechanism of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with either stabilization of lysosomal membrane or restricting the release of lysosomal enzymes [66]. In vitro anti-inflammatory effect of selected cress seed mucilage EPFs was determined at three different concentrations (200 μg/mL, 100 μg/mL, and 50 μg/mL) and the EC_{50} value was calculated for each fraction as shown in Table-4. The results demonstrated that at 200 μg/mL, fraction F53 significantly stabilized human red blood cell (HRBC) membranes by 90.59 %, followed by fraction F23 (86.43 %), fraction F52 (82.26 %), and fraction F18 (78.60 %), whereas the fraction F26 showed the least significant activity (60.37 %) in stabilization of HRBC membranes. Similarly, at 100 μg/mL, fraction F53 exhibited excellent activity, stabilizing HRBC membranes by 78.76 %, followed by fraction F52 (68.28 %), fraction F23 (67.94 %), and fraction F24 (54.37 %), while fraction F26 showed the lowest anti-inflammatory activity of 34.31 %. Furthermore, at the lowest concentration of 50 μg/mL, fraction F53 again displayed the highest anti-inflammatory activity of 56.20 %, followed by fraction F52 (48.13 %), and fraction F23 (44.80 %). Regarding the EC_{50} value, which represents the half maximal effective concentration, fraction F53 showed the most effective value of 36.23 μg/mL, followed by fraction F52 (51.74 μg/mL), and fraction F23 (57.95 μg/mL). These findings revealed that fraction F53 was the most effective as it showed maximum anti-inflammatory activity in a dose-dependent manner along with an effective EC_{50} value which was followed by fraction F52 and F23. Previous literature reported that potent anti-inflammatory effect of mushroom was due to the presence of glucan polysaccharides [67]. In our study, the polysaccharide fractions extracted from cress seed mucilage were found to prevent hypotonic hemolysis in HRBCs. The possible mechanisms of membrane stabilization by the plant extracts include increasing the surface area-to-volume ratio and stabilizing tropomyosin [68].

3.9. Carrageenan induced paw edema

The induction of 50 μL of 1 % carrageenan in the right hind paw of mice results in a severe inflammatory reaction characterized by redness and swelling. The paw edema was treated with different concentrations of ethanolic polysaccharide fractions extracted from cress seed mucilage. The results demonstrated that the polysaccharide fractions effectively reduced the paw edema volume in the experimental mice at a dosage of 50 mg/kg, showing a significant difference compared to the untreated group ($p < 0.001$), as shown in Fig. 6. Fraction F52 at a dosage of 100 mg/kg demonstrated the highest reduction in paw edema volume, significantly decreasing it by 90.28 % ($p < 0.0001$) after 5 h of treatment. Following this, fraction F53 reduced the volume by 84.15 % ($p < 0.05$) (Fig. 7). On the contrary, the highest percentage reduction in paw edema volume was observed for fraction F53 at 200 mg/kg, which significantly reduced the volume by 98.10 % ($p < 0.0001$) after 5 h of treatment. This was followed by fraction F52 with a reduction of 92.60 % ($p < 0.05$), fraction F20 with a reduction of 89.60 % ($p < 0.001$), and fraction F23 with a reduction of 86.68 % ($p < 0.05$) (Fig. 8). On the

Table 4
Human RBCs membrane stabilization potential of polysaccharides fraction of cress seed mucilage.

	Percent membrane stabilization along with their EC_{50} value in μg/mL			
	50 μg/mL	100 μg/mL	200 μg/mL	EC_{50} μg/mL
EPF	12.5 ± 0.78a	34.8 ± 0.34a	67.5 ± 1.23b	159.1 ± 2.13i
F18	29.2 ± 0.12d	51.5 ± 0.27cd	78.6 ± 0.91f	91.6 ± 1.61e
F20	23.1 ± 0.23c	49.6 ± 0.61c	75.6 ± 0.53d	101.2 ± 3.54f
F21	22.7 ± 0.56c	39.1 ± 0.83b	74.9 ± 0.87d	129.3 ± 1.62h
F22	18.1 ± 0.34b	39.4 ± 1.54b	70.5 ± 0.34c	121.4 ± 2.23g
F23	44.8 ± 0.12e	68.0 ± 1.48f	86.4 ± 0.32h	58.0 ± 1.67c
F24	31.5 ± 0.23d	54.4 ± 0.91e	78.4 ± 0.51f	86.9 ± 4.12d
F25	23.9 ± 0.18c	54.1 ± 2.76de	75.5 ± 0.83e	96.9 ± 2.89f
F26	17.7 ± 1.09b	34.3 ± 0.87a	60.4 ± 0.23a	161.3 ± 3.91i
F52	48.1 ± 0.89f	68.3 ± 1.82f	82.3 ± 0.56g	51.7 ± 1.98b
F53	56.2 ± 1.82g	78.8 ± 0.73g	90.6 ± 0.52i	36.2 ± 1.21a

EPF stands for ethanol precipitated fraction; F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means followed by different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

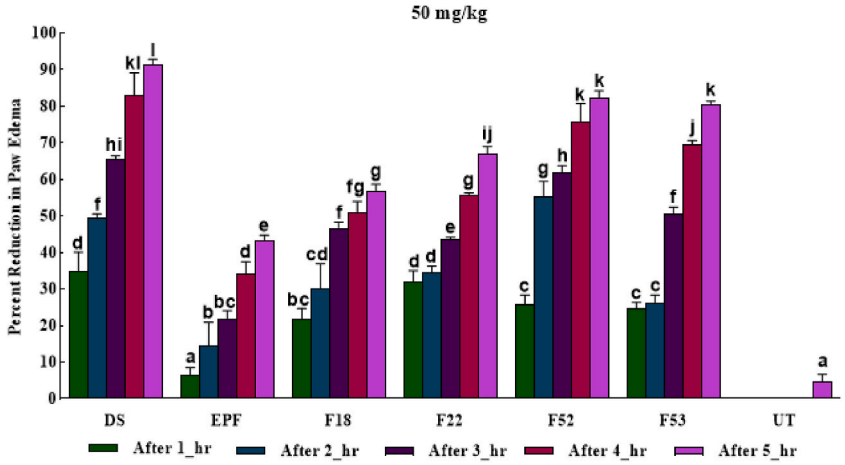

Fig. 6. Reduction in paw edema volume treated with 50 mg/kg of polysaccharides fractions of cress seed coat mucilage. EPF stands for ethanol precipitated fraction; DS stands for diclofenac sodium; UT stands for untreated rat; F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

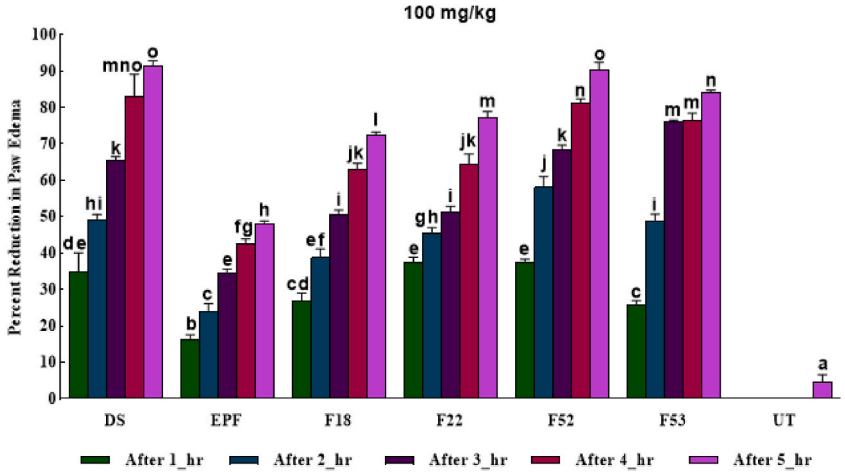

Fig. 7. Reduction in paw edema volume treated with 100 mg/kg of polysaccharides fractions of cress seed coat mucilage. EPF stands for ethanol precipitated fraction; DS stands for diclofenac sodium; UT stands for untreated rat; F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

other hand, the lowest percentage reduction in edema volume was observed in the group treated with ethanol-precipitated polysaccharides, which reduced the volume by 60.09 % ($p < 0.0001$). After 5 h of treatment, the group treated with diclofenac sodium (150 mg/kg) reduced the paw edema volume by 91.46 % ($p < 0.001$). These results indicate that polysaccharides derived from cress seed mucilage, fractionated through size exclusion chromatography, significantly reduced the edematogenic responses in mice induced by carrageenan administration. These findings are consistent with the study by Silva et al. [69], where they reported that a polysaccharide fraction from *Thuja occidentalis* significantly reduced paw edema volume in carrageenan-induced mice by 76.5 % after 3 h of treatment. Carrageenan-induced paw edema is considered as a biphasic process. Initially, fluid accumulation and vasodilation can be arbitrated by the release of inflammatory mediators (histamine, serotonin, and prostaglandins), reaching to maximum in the 6-h of carrageenan induction [70,71]. The possible mechanisms involved in stabilizing the paw edema volume include inhibiting the generation of inflammatory mediators, blocking receptor actions, and modulating interactions between inflammatory mediators and their respective receptors [70,72]. Inappropriate or excessive production of pro-inflammatory cytokines can lead to tissue damage and increased inflammation. Therefore, suppressing these pro-inflammatory cytokines is considered an active approach in managing various pathological disorders [72]. Currently available anti-inflammatory drugs often carry severe side effects that limit their long-term use [73]. Medicinal plants offer alternative therapies for inflammatory diseases due to their abundant bioactive molecules with promising therapeutic potential [74–76]. The present study concludes that polysaccharide fractions derived from cress seed

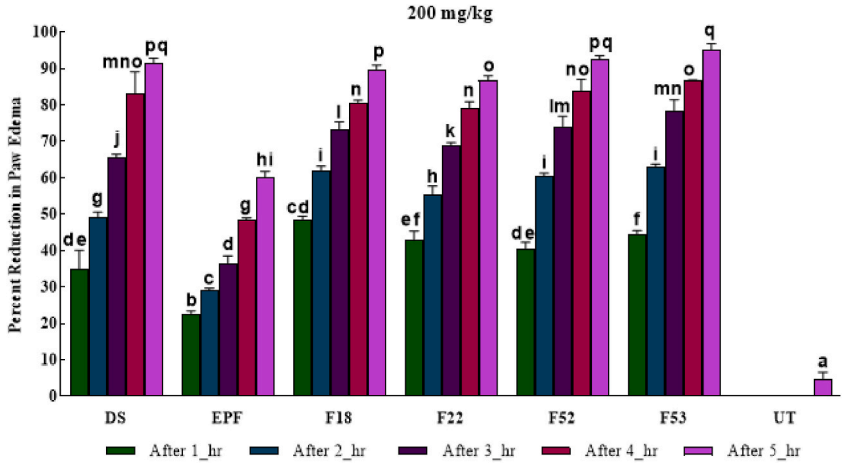

Fig. 8. Reduction in paw edema volume treated with 100 mg/kg of polysaccharides fractions of cress seed coat mucilage. EPF stands for ethanol precipitated fraction; DS stands for diclofenac sodium; UT stands for untreated rat; F18-F53 are collected fractions through Bio-Gel-P-10. The data are presented as mean along with standard error values. Means represented with different letters (i.e., a, b, c ….) are significant from each other at P = 0.05.

mucilage significantly reduced the inflammatory effects by inhibiting edematogenic effects in classic experimental models of inflammation. A previous study demonstrated that the leaf extract of *L. sativum* significantly influenced the expression of several genes associated with inflammation, including endothelial growth factor, tumor necrosis factor-alpha, and α-reductase type II in cultured human keratinocyte cells [77]. The L. *sativum* seed coat mucilage has been best known for its high anti-inflammatory effect as it significantly reduced edema in mice model. Maximum inhibitory effect was measured after 5 h of treatment of each extract which may be related to the production of prostaglandin. Though, it may not be the only reason of percent inhibition in edema, despite the fact that mucilage is capable of inhibiting the inducers of inflammation. Inflammation is a natural phenomenon that occurs in response to damage caused by pathogens or chemicals. Inflammation is useful as it responds to harmful stimuli and protects the body against their hazardous effects [78]. Plants have been utilized as a medicine for the management of many diseases as a whole in the form of crude or extracts. An extensive research has been carried out to determine the therapeutic potential of plants in terms of identification and characterization of potent constituents accountable for the therapeutic effects. The anti-inflammatory effect of medicinal plants are mainly due to the presence of secondary metabolites [79]. The phytoconsituent secreted by plant as secondary metabolites include flavonoid, polyphenolic, proanthocyanidin, alkaloid, terpenoid that accounts for anti-inflammatory effects [80].

4. Conclusion

Cress seed mucilage is a natural hydrocolloid that releases mucilage containing bioactive compounds when soaked in water. The present study concludes that ethanol precipitated fractions (EPF) of cress mucilage consisted of potent bioactive saccharides. The EPF inhibited the DPPH free radical even at 50 μg/mL, however at 200 μg/mL the inhibition was about 48.3 %. Further fractionation of EPF on Bio-Gel P-10 column separated antioxidant compounds with anti-inflammatory activity. Among the fractions from Bio-Gel P-10, fractions 20, 23 and 53 were found to be the most potent. Fraction 53 scavenged the DPPH free radical by 93.8 %, whereas F20 scavenged 92 %. Similarly, at 200 μg/mL fraction 53 stabilized H_2O_2 free radical by 87 % and fraction 20 and 23 by 81 and 86 %, respectively. Moreover, fraction 53 inhibited TBARS free radicals by 95 % and stabilized 91 % of human red blood cells (HRBC) membranes. Fraction 53 and 20 also reduced 98 % and 90 %, respectively, of paw edema volume in rats within 5 h of treatment. These results suggest that EPF from cress seed mucilage consisted of potent bioactive saccharides with no side effects. The purification and characterization of these antioxidant and anti-inflammatory compounds can play vital role in developing effective drugs.

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Ethical approval

The current study was priory approved from the Departmental Ethical Committee of the Department of Biotechnology, Abdul Wali Khan University, Mardan with Approval number No: AWKUM/Biotech/2021/2615.

Consent for publication

All authors have provided their consent to publish this paper.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

CRediT authorship contribution statement

Imdad Ullah Khan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yusra Jamil:** Investigation, Formal analysis. **Fareeha Shams:** Investigation, Formal analysis. **Salman Farsi:** Investigation, Formal analysis. **Muhammad Humayun:** Writing – original draft, Data curation. **Anwar Hussain:** Writing – original draft, Data curation. **Ayaz Ahmad:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Amjad Iqbal:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Abdulwahed Fahad Alrefaei:** Writing – review & editing, Resources, Funding acquisition. **Sajid Ali:** Writing – review & editing, Writing – original draft, Resources, Data curation.

Declaration of competing interest

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

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Supplementary Files

Supplementary file S1: Summary of GCMS data of sample F18.
Supplementary file S2: Summary of GCMS data of sample F21.
Supplementary file S3: Summary of GCMS data of sample F52.
Supplementary file S4: Summary of GCMS data of sample F53.
Supplementary file S5: GCMS spectrum of selected fractions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36797.

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