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ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF *DAUCUS CAROTA* L. SEED EXTRACTS

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ABSTRACT

Daucus carota L. is locally known as "Kali Gajar" (black carrot) and is found abundantly in Pakistan. The aim of the current study is to investigate *in vitro* antioxidant and anti-inflammatory and *in vivo* anti-inflammatory effects of *Daucus carota* L. seed extracts. The hot extraction method was used to obtain different extracts. The physiochemical testing, FTIR and mineral content estimation of the seed powder were conducted. UV Visible profiling of the extracts was also done. The *in vitro* antioxidant activity was performed by using the variety of assays included DPPH, ferric reducing power, hydrogen peroxide scavenging capacity, and phosphomolybdenum test and ascorbic acid was used as a reference. The *in vitro* anti-inflammatory activity was assessed by inhibition of protein denaturation method and the diclofenac sodium was taken as a standard. The *in vivo* anti-inflammatory activity was examined by using carrageenan-induced rat paw edema model by taking aspirin as a standard. The results of the UV Visible spectroscopy and FTIR scans showed the presence of various functional groups. The existence of many important primary and secondary metabolites was also confirmed by phytochemical analysis. The physicochemical parameters of seed powder were found to be within the recommended range as stated in USP, 2015. The present study has verified the antioxidant effect of the plant seeds and methanolic extract was found to be more active as compared to the other extracts. The study outcomes have confirmed a significant *in vitro* inhibition of the protein denaturation and *in vivo* reduction of carrageenan-induced inflammation in the rat paw. It is concluded that the plant seeds have the potential to reduce inflammation, however toxicological and clinical studies are desired in the future.

Keywords: *Daucus carota L.*, Black carrot, Medicinal plants, anti-inflammatory, anti-oxidant
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INTRODUCTION

The traditional use of natural products has set the basis for the development of modern dugs. The World Health Organization (WHO) asserted that 80% of the world's residents utilize natural products to treat health-related issues (Newman and Cragg, 2016). According to WHO, in a recent study, around 60,000 plant species have the potential for being used as medicinal plants (Dushenkov, 2016). Thus, WHO commends and supports the utilization of traditional cures because of their ability to treat the disorders with minimum side effects (Latif *et al.,* 2019). In the past few decades, massive research has been conducted on medicinal plants to find their phytochemical constituents and pharmacological activities that could be helpful in averting diseases in the future (Mulat *et al.,* 2020; El-Dahiyat *at al.,* 2020).

Daucus carota L. is an important edible vegetable and has numerous health benefits. The plant is also known as the black carrot in Pakistan and belongs to the family Apiaceae. The carrot is a herbaceous and biennial plant. It is about 30-120 cm tall and blossoming from June-August. Flowers are androgynous and are cross-fertilized by insects and flies. Fruits are 2-4 mm long and elliptical. Leaves are superbly parted, and sections are rectilinear to lanceolate and are 0.5-3.0 cm elongated. The stalk is striated or ribbed. The seeds grow from August-September. *Daucus carota* L. seeds are small and protected with a fleshy, spiny, curved mericarp. Mericarp should be detached before seeding because it contains characteristic oil which prevents seed germination. Carrot seeds are dry fruits called schizocarps, so these are not true seeds botanically (Que *et al.,* 2019; Hao *et al.,* 2020).

Flowers and roots of *Daucus Carota* L. are chief eatable components. Carrots grow within an abundant range of temperatures and hence can be found all over the world with the exemption of the very warmest region (Akhtar *et al.,* 2017). The root development is fastest at 15°C-18°C. Seeds may be cultivated at low temperatures. Low temperatures induce flowering in carrots. Carrot normally does not occur in recently abandoned fields because seeds do not stay alive for more than two years. In traditional therapeutics, various parts of the *Daucus Carota* L. are used to treat different diseases such as wound healer, antiseptic, uterine stimulant, anti-

microbial, anti-inflammatory and diuretic (Smeriglio *et al.,* 2018; Ayeni *et al.,* 2018; Ahmad *et al.,* 2019).

Carrots have many health benefits due to the presence of various phytochemicals such as polyphenols, tannins, alkaloids, and glycosaponins. B-carotene is a major phytochemical reported in carrots. β-carotene exhibits excellent anti-oxidant and anti-inflammatory properties. Around the globe, the consumption of carrots tremendously increased due to their beneficial effects on human wellbeing (Bystrická *et al.,* 2015).

The objective of this research work was to assess the *in vitro* antioxidant activities and *in vitro* and *in vivo* anti-inflammatory activities of *Daucus carota* L. seeds. Previously, limited data is available and this research would be helpful for upcoming studies (Mehnaz *et al.,* 2021).

MATERIALS AND METHODS

Chemicals: Chemicals and solvents of analytical grade were used, included n-hexane (BDH, England), Triton X (Unichem chemicals, Ireland), Quercitin (Sigma Life Science, Germany), Folin and Ciocalteu's phenol reagent (Unichem chemicals, Ireland), Anthrone reagent (Sigma Life sciences, Germany) and Gallic acid (Sinochem, China). Methanol, Chloroform, Ethanol, Potassium bromide (KBr), Hydrochloric acid (HCL), Sulphuric acid, Nitric acid ($HN0_3$), Aluminium Nitrate Crystals, Bovine serum albumin, Copper sulfate, Potassium tartrate and Glucose were procured from Merck, Germany.

Instruments: Electric balance BL 2005, Setra, USA, Oven, TYPHZ, Suszukra Pronzniowa, Poland, Rotary vacuum evaporator, 1--EI-VAP series, Heidolph, Germany, Electric carbolite furnace, Sheffield, Germany, Distillation apparatus, WSB/4 Hamilton laboratories Glass Ltd. China, Fourier transform infrared spectroscopy, ThermoNicolet, USA, Ultrasonic mixer, DSA50-CKI-1.8L, Germany, Ultraviolet spectrophotometer, Model-2550.Shimadzu Scientific Instruments, USA, Perkin Elmer Analyst 800 atomic absorption spectrophotometer,8312008, Perkin Elemer, USA, Flame photometer, Coming FP 410, Sherwood, USA.

Collection of plant material and authentication: *Daucus carota* L. seeds were collected from a local market located in Lahore, Pakistan. The part used (seeds) was authenticated by Prof Dr. Zaheer-ud-Din Khan, a taxonomist at the Department of Botany, Government College University (GCU), Lahore, Pakistan. He issued a voucher number: GC/Herb/Bot/3365. A specimen of the voucher was deposited at the Herbarium Department of GCU, Lahore.

Extraction of the plant material and storage: Seeds were sieved twice to eliminate dust and other foreign substances before being sun-dried in the open air. The dried seeds were milled into a granular powder and in a hermetic container. The continuous hot extraction was done by using the Soxhlet apparatus. The filter paper was loaded with 60g of powdered seed material, which was then positioned in the Soxhlet apparatus in the shape of a thimble. The sequential extraction was performed by using the solvents, including n-hexane, chloroform and methanol. A rotary evaporator was used to dry all of the extracts. The extracts were stored in amber glass bottles and properly labeled (López-Bascón and De Castro, 2020).

Proximate analysis: The proximate analysis was carried out using the methodology outlined in USP (2015). In proximate analysis following parameters were measured; water-soluble ash, acid insoluble ash, sulfated ash, total ash, moisture contents, water-soluble extractives and alcohol soluble extractive values (USP, 2015).

Qualitative analysis by FTIR spectroscopy: The powdered material of seeds of black carrot was evaluated for functional groups by using FTIR spectrophotometer (Thermo Nicolet iS10, USA). For this purpose, 100 mg of KBr and 1 mg of sample powder were crushed together to make pellets. Then, this blend shifted to a mold and flattened to make discs. The IR range of 4000-400 cm^{-1} used to get FTIR spectra of these discs (Pavia *et al.,* 2008).

UV/Vis Spectroscopy: UV/Vis spectroscopy was also performed for all the extracts. For the UV/Vis spectroscopy stock solution (1 mg/mL) of the respective extract was made in methanol and scans were taken in UV/Vis region (200-800 nm) scans. The maximum wavelength (λ max) was recorded for each of the extracts (Latif *et al.,* 2020).

Assessment of the mineral composition of the plant: The mineral composition of *Daucus carota* L. was estimated using an atomic absorption spectrophotometer. Approximately 1 g of ground seeds was placed in a beaker subsequently, 15 mL of a mixture containing concentrated nitric acid and hydrochloric acid in 1:3 (v/v) was added and then heated in the fuming hood. The contents were filtered after cooling at room temperature. Deionized water (100 mL) was used to make the final volume. Atomic absorption spectrophotometer was used to examine the assimilated sample. Standard stock solution (1000 ppm) was used to make working standard solutions (concentrations 0, 1, 5, 10, 15, 20, 25 and 100 ppm). The investigation was repeated three times. The number of minerals in the sample was attained from the regression equation calculated from the standard plot (Ahmad *et al.,* 2014).

Estimation of primary metabolites in seed powder: Total protein (Lowry *et al.,* 1951), total carbohydrate (Al-

Hooti *et al.,* 1998) and total lipid (Besbes *et al.,* 2004) contents of the sample material were determined to assess the presence of primary metabolites.

Estimation of the secondary metabolites in the extract: Secondary metabolites included total polysaccharides, glycosaponins (Hussain *et al.,* 2008), polyphenols (Slinkard and Singleton, 1977) and flavonoids (Chang *et al.,* 2002) were also determined.

Evaluation of in vitro antioxidant activity

DPPH assay: The antioxidant activity of seed extracts was determined using the DPPH (1, 1 biphenyl 2, pipryl hydrazyl) method which was slightly modified. 2 mL of methanol and 1 mL of DPPH solution (0.1mM) was poured in the sample solution (1 mL). After half an hour, the decline in the concentration of free radicals was measured by UV/Vis spectrophotometer at 517 nm in comparison to a blank. Control and standard (vitamin C) absorbance were also calculated. All measurements were taken three times and their mean value was used to compute the results by using following formula (Sánchez Moreno *et al.,* 1998).

$$\% \text{ Inhibition} = Ac - As/Ac \times 100$$

Where Ac = Absorbance of control
As = Absorbance of sample

Assay for ferric reducing power: To calculate ferric reducing power activity of seed extracts, the methods of Oyaizu (1986) and Ferreira *et al*. (2007) were used with a slight modification. 2.5 mL of 1% potassium ferricyanide and 2.5 mL of phosphate buffer were mixed with 1 mL of each of the sample solutions (PH 6.6, 0.2 M). After that, the solution was incubated at 50°C for 20 minutes before being cooled and added 2.5 mL of trichloroacetic acid (10%). The mixture was centrifuged at 3000 rpm for 10 minutes to get the supernatant, and then 2.5 mL of distilled water and 0.5 ml of ferric chloride (0.1%) were added in 2.5 mL of supernatant and permitted to stand for ten minutes. The absorbance at 700 nm was measured using a UV/Vis spectrophotometer and compared to a blank. As a control, ascorbic acid was employed (Oyaizu 1986; Ferreira *et al.,* 2007). Readings were taken in triplicate and average values were used for calculations of the results.

% inhibition = absorbance of sample/absorbance of control × 100.

Phosphomolybdenum antioxidant assay: This test was performed as documented by Prieto *et al.* (1999) with minor modifications. The reagent solution was consisted of sulfuric acid (0.6 M, 1.5 mL), sodium phosphate (28 mM, 1.5 mL) and ammonium molybdate (4 mM, 1.5 mL). 0.5 mL of extract solution was added to 4.5 mL of reagent solution then incubated at 95°C for 90 minutes. After cooling this mixture at room temperature, the absorbance was measured by UV/Vis spectrophotometer at 695 nm against a blank. The readings were obtained three times and the average was calculated (Prieto *et al.,* 1999).

Hydrogen peroxide radical scavenging capacity: *Daucus carota* L. seed extracts were assessed for hydrogen peroxide scavenging assay. 0.6 mL of H_2O_2 solution [40mM in PB (pH 7.4)], 2.4 mL of phosphate buffer were mixed with 1ml of sample solution (50 µg/mL) and after ten minutes, absorbance at 230 nm was taken compared to a blank(only PB). The proportion of H_2O_2 scavenging of standard and extracts was assessed and calculated by using the following equation (Ruch *et al.,* 1989).

% scavenging capacity (H_2O_2) = Absorbance (control) - Absorbance (sample) / Absorbance (control) x 100

Evaluation of the *in vitro* anti-inflammatory activity by protein denaturation inhibition method: Protein denaturation inhibition was calculated by the procedure of Mizushima and Kobayashi (1968) and Sakat *et al.,* (2010). The standard (diclofenac sodium) and solutions of the different seed extracts were made in the concentration of 0.5 mg/mL. 2 mL of phosphate buffer saline (PBS) having pH of 6.4, 2 mL of egg albumin (4% in PBS) were combined with 2 mL of the test solutions and incubated at 37°C ± 2°C for 15 minutes. Afterwards, solution was heated at 70°C in a water bath for 5 minutes. After cooling, the turbidity was checked against blank at 660 nm by UV/Vis spectrophotometer. Concentrations of 50, 100, 250, 500, 1000, 2000 (µg/mL) of diclofenac sodium were used as standard (Mizushima and Kobayashi, 1968; Sakat *et al.,* 2010).

% Activity=Absorbance of control-Absorbance of sample or standard/ Absorbance of control ×100

Evaluation of *in vivo* anti-inflammatory activity by carrageenan-induced rat paw edema model: The carrageenan-induced rat paw edema model was utilized to assess the probable impact of the methanolic extract of black carrot seeds on inflammation. Four groups of rats (n = 4) were made for this purpose. The acute inflammation was induced in the right posterior foot of rats after a sub-plantar injection of 0.1 ml of carrageenan solution (1% in normal saline). The thickness of the right appendage (mm) was measured by using the Vernier caliper instantaneously after carrageenan injection and then every 1 hour for the next 6 hours. A standard (aspirin) and methanolic seed extract in a concentration of 200 mg/kg and 400 mg/kg were administered orally one hour before sub-plantar injection of carrageenan (Winter *et al.,* 1962).

Statistical analysis: Linear regression analysis was used to estimate mineral content and metabolites (primary and secondary) of *Daucus carota* L. seeds powder and extracts. One-way analysis of variance (ANOVA) was performed for data analysis using SPSS (version. 23.00

SPSS Inc. Chicago, IL, USA) and the results are expressed as a mean ± standard deviation (SD). P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Physicochemical analysis: The physicochemical analysis of the seed powder was performed to assess the quality of the sample. The results of the physicochemical evaluation are displayed Table 1.

Table 1. Physicochemical analysis of *Daucus carota* L. seeds powder

Physicochemical property	% age content ± SD
Moisture content	8.5 ± 0.08
Total ash	10.35±0.14
Acid insoluble ash	9.2 ± 0.20
Water soluble ash	7.2 ± 0.26
Sulphated ash	11.1 ± 0.15
Alcohol soluble extractive	1.26 ± 0.20
Water soluble extractive	1.4 ± 0.30

The moisture content of a material plays a significant part in the efficacy and stability of drugs. By heating the sample to a constant weight or calculating the loss in weight on drying, the moisture content is assessed. The moisture content should be controlled or decreased to avoid bacterial attack and chemical degradation. The moisture content of the plant was found to be in the suggested range. Thus, it can be stored and used for a longer period effectively (Suthar and Solanki, 2021).

Ash values are useful in defining the purity and quality of crude drugs. The conversion of crude material to ash eliminates biological material and avoids its intrusion in analysis. Crude drug material usually results in a residue after full ignition that is called ash. Ash comprises silicates, phosphates, and carbonates of calcium, sodium, magnesium, and potassium. The mineral contents of the soil, water, and fertilizer used for growing plants greatly affect the percentage contents of ash (Munir *et al.,* 2014). Good agricultural and collection practices are required to keep these factors within the specified limits. The obtained total ash reveals the care and parameters taken into consideration during the accumulation, storage and drying of the crude plant material. Acid insoluble ash shows calcium oxalate or silica which becomes inactive during garnering the crop. Sulphuric acid is used to determine sulfated ash by some analysts to get less fusible ash than the usual one. Ash value tests are important to determine and identify the presence of any foreign matter like carbonates, silica and oxalate. Different ash values and extractive values of seed powder are determined to evaluate the purity and quality of the powdered drug. According to British Pharmacopoeia, the accepted range of ash value is 20 %. The study outcomes have shown that ash value is present in the recommended range (Kadam *et al.,* 2012). The extractive values are the indication of presence of polar compounds like phenols, tannins and glycosides (Baravalia *et al.,* 2011).

Qualitative analysis of seed powder by FTIR Spectroscopy: The grounded seed material was examined qualitatively by FTIR spectrophotometer. KBr disc method was used to obtain spectra. The resulting spectrum of the seed powder is given in figure 1. The spectrum obtained by FTIR spectroscopy of powdered seeds of *Daucus carota L.* showed a sharp band of OH stretching at 3400-3300 cm^{-1}, CO stretching, C-H stretching and C=C stretching at 1637 cm-1 and C-C stretching at 1401 cm-1 have confirmed the presence of phenolic compounds.

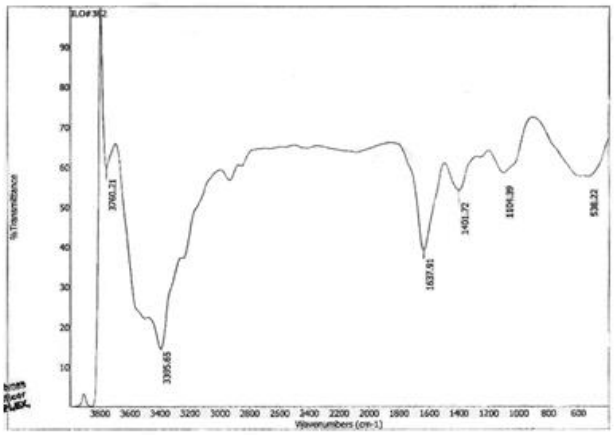

Figure 1. FTIR spectrum of *Daucus carota* L. seeds powder

UV/Vis spectroscopy: The UV/Vis spectrum of all the extracts of seeds of *Daucus carota* L. was obtained and shown in figure 2. The methanolic extract of *Daucus carota* L. showed the maximum peaks at the wavelength of about 275 nm and 320 nm. While the chloroform and n-hexane extracts have manifested the maximum peak at the wavelength of 270 nm and 290 nm respectively. The study results demonstrated that maximum peaks of all the extracts were present in the ultraviolet region. UV/Vis spectroscopy is usually performed to ascertain the presence of certain polar compounds in the extracts, i.e. polyphenols and flavonoids (Gierschner *et al.,* 2012).

Analysis of the mineral content: The mineral content analysis of seed powder of *Daucus carota* L. is given in Table 2. This analysis verified that the extract contains the maximum amount of iron followed by manganese, zinc, copper, nickel, lead, and chromium. Plants are the rich source of various minerals like magnesium, iron, phosphorous, lead, nickel, etc. The mineral content of the

plant is associated with its medicinal and therapeutic properties like anti-inflammatory, anti-cancer, anti-diabetic, or anti-oxidant activities (Kathpalia and Bhatla, 2018). Iron is an important component that is required for the formation of hemoglobin and also needed for the better functioning of central nervous system and immune system (Shubham *et al.,* 2020). Zinc is also a very important mineral that helps in the regulation of the immune system and protein metabolism. Moreover, zinc is helpful in the treatment of inflammatory diseases like arthritis (Kumar *et al.,* 2019).

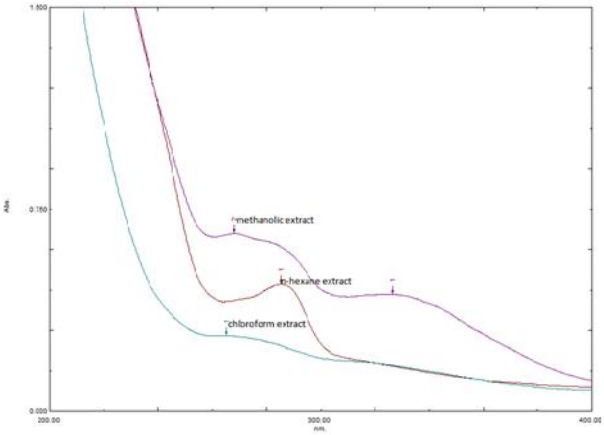

Figure 2. UV Visible spectrum of the extracts of *Daucus carota* L. seeds

Table 2. Mineral content of *Daucus carota* L. seeds powder.

Elements	Quantity(mg/g) ± SD
Iron	293.5± 0.09
Manganese	80.0± 0.13
Zinc	59.5± 0.29
Copper	23.5± 0.27
Nickel	20.0± 0.43
Lead	6.0± 0.35
Chromium	5.0± 0.20

Estimation of primary metabolites in seed powder: Plants contain natural bioactive compounds called phytochemicals. The phytochemical analysis is used to assess and classify the different constituents of plants. Hence, this analysis is used to evaluate the therapeutic and biological potential of different plant materials. Many chemical compounds are derived from medicinal plants and these chemicals are classified into primary and secondary metabolites (Velu *et al.,* 2018; Anwar *et al.,* 2022). The determination of percentage contents of three primary metabolites including total proteins, total carbohydrates and total lipids in seed powder of the plant was performed and given in Table 3. The study outcomes have demonstrated that the seeds are enriched with lipid contents followed by carbohydrates and protein. The present study confirmed the nutritional importance of the plant seeds (Krivokapić *et al.,* 2020).

Table 3. Estimation of primary metabolites in *Daucus carota* L. seeds powder.

Primary Metabolites	% age contents ± SD
Total Proteins	13.3 ± 0.30
Total Carbohydrates	31.917 ± 0.50
Total fats	49.1 ± 0.23

Estimation of the secondary metabolites in black carrot seed extracts: The outcomes for the approximation of secondary metabolites of seed powder extracts are demonstrated in Table 4. For the determination of various groups of metabolites, the linear regression equation was used. The secondary metabolite constituents include flavonoids, alkaloids, polysaccharides, essential oils, phenolic compounds, tannins, glycosaponins and terpenoids. The total polyphenols content is found to be higher as reported previously (Mehnaz *et al.,* 2021). Some of these metabolites are biologically active. For example, compounds like polyphenols and flavonoids showed anticancer, anti-inflammatory and antioxidant activities as reported in previous studies (Kumari *et al.,* 2017; Kanwal *et al.,* 2021).

***In vitro* anti-oxidant activity:** The results of antioxidant activity by DPPH model, ferric reducing power assay, hydrogen peroxide scavenging capacity, and phosphomolybdenum antioxidant assay are given in the Table 5. The present investigation has proved the antioxidant effect of the black carrot seeds. The methanolic extract has greater antioxidant activity as compared to the other extracts. The antioxidant activity of the seeds is due to the presence of polyphenols and flavonoids. The medicinal plants are a great source of natural antioxidants that are not only beneficial for human wellbeing but are also quite essential for the survival of the plants. During the metabolism, reactive oxygen species (ROS) are formed that are injurious to health. The human body tends to eliminate these free radicals in the presence of antioxidants. However, if the body cannot eliminate these radicals then these could result in chronic degenerative disorders like cancer, arthritis, and cardiac disorders (Ji *et al.,* 2020). In many recent studies, the significance of antioxidants for the prevention of different disorders has been confirmed (Ahmed *et al.,* 2019). The antioxidant activity of *Daucus carota* L.is mainly due to the redox characteristics of polyphenolic compounds. Antioxidants in plants mostly represent an extensive diversity of phenolic compounds that range from simple phenolic acids to extremely polymerized composites for example tannins. Phenolic

compounds are further characterized into fifteen classes with more than 8,000 known compounds. So, plants could be an attractive substitute to presently accessible commercial antioxidants since they are decomposable to non-noxious products (Qayyum *et al.,* 2020; Leri *et al.,* 2020).

Table 4. Estimation of secondary metabolites in different extracts of *Daucus carota* L. seeds.

Extracts mg/g (regression equation)	Total polyphenols mg/g (y = 2.8514x + 0.1308, R2 = 0.962)	Total Flavonoids mg/g (y = 2.4738x + 0.2978, R2 = 0.9974)	Total polysaccharides mg/g (y = 4.725x - 0.037, R2=0.9976)	Glycosaponins mg/g (y=7.86x+0.1106, R2=0.9917)	Proteins mg/g (y = 1.222x - 0.0035, R2 =0.9986)
Hexane extract	42 ± 0.20	178 ± 0.10	9.63 ± 0.40	37.8 ± 0.35	0.2299±0.005
Chloroform Extract	112 ± 0.15	199 ± 0.25	10.44 ± 0.45	5.6 ± 0.56	5.699±0.008
Methanol Extract	252 ± 0.10	189 ± 0.35	9.9 ± 0.55	33 ± 0.47	7.959±0.012

Table 5. Effect of *Daucus carota* L. seeds extracts on the different *in vitro* activities.

	Absorbance of extracts/standard (As)	Activity (%)
DPPH		
Control (Ac)	---	
Hexane	0.127	54.15
Chloroform	0.034	87.72
Methanol	0.009	96.75
Vitamin C	0.005	98
Ferric acid reducing power		
Control (Ac)	---	
Hexane extract	0.363	56.54
Chloroform extract	0.326	50.77
Methanol extract	0.432	67.28
Vitamin C	0.521	81.15
H_2O_2 scavenging capacity		
Control (Ac)	---	
Hexane extract	0.542	42.34
Chloroform extract	0.101	89.25
Methanol extract	0.041	95.64
Vitamin C	0.037	96.07
Phosphomolybdenum antioxidant activity		
Control (Ac)	---	
Hexane Extract	0.264	19.58
Chloroform Extract	0.291	22.91
Methanol Extract	0.168	89.28
Vitamin C	0.180	90
Protein denaturation inhibition		
Control (Ac)	---	
Hexane extract	0.363	53.46
Chloroform Extract	0.410	50.25
Methanol Extract	0.332	55.57
Diclofenac sodium	0.248	61.31

Evaluation of *in vitro* anti-inflammatory activity: Protein denaturation percentage inhibition is a measure of protein stabilization with respect to the control. The results of this test are shown in Table 5. The results showed that methanol extract had more anti-inflammatory activity than n-hexane and chloroform extracts. It is a well-known fact that denaturation of tissue proteins leads to inflammatory conditions. The production of auto-antigens in certain arthritic conditions may lead to the denaturation of proteins. Thermal treatment is used to induce denaturation of proteins which is undesired physicochemical change. Herbal products can help in reducing the inflammation by stabilizing the protein against denaturation and can be worthwhile for developing potential anti-inflammatory drug therapy (Hmidani *et al.,* 2020; Farooq *et al.,* 2022).

Evaluation of *in vivo* anti-inflammatory activity: Inflammation is a protective response against many pathological conditions. The proper management and maintenance of this crucial response is desired to improve the patients' quality of life particularly suffering from chronic inflammation. The outcomes have revealed that after carrageenan administration the right paw thickness has increased. When methanol extract of black carrot seeds was given orally at doses of 200 mg/kg and 400mg/kg, it significantly reduced the inflammation of rat paw caused by carrageenan administration at two hours, three hours, and four hours' intervals of the experiment (Figure 3). It showed that methanolic extract had dose-dependent anti-inflammatory response (Shebaby *et al.,* 2021).

Inflammation is detected in most diseases and is expected to be a defense reaction intended to remove harmful agents. The pathogenesis of numerous age-linked diseases for example heart diseases, rheumatoid arthritis, acute and chronic neurodegenerative ailments, diabetes and inflammatory bowel disease may involve chronic inflammatory progressions (Wang *et al.,* 2021). The release of chemicals from migrating cells and tissues initiate inflammation. The chemical substances that are

frequently involved in provoking inflammatory response are including histamine, platelet-activating factor, prostaglandins, bradykinin, leukotrienes and interleukin-1. The involvement of inflammatory mediators is confirmed from studies involving inhibitors of synthesis and competitive antagonists for receptors of these mediators. For example, H1 histamine antagonists are effective for some skin allergies like urticaria and hay fever indicating the significance of histamine in these circumstances. The synthetic drugs avoid the development of leukotrienes and prostaglandins by triggering the lipocortin release, which diminishes arachidonic acid release by inhibition of phospholipase A2. As a result, they lessen the inflammation of different diseases like asthma and rheumatoid arthritis. However, synthetic drugs may be associated with unwanted side effects and medicine obtained from natural sources may prove beneficial by avoiding the harmful effects (Furman *et al.,* 2019; Ashiq *et al.,* 2020; Ashiq and Ashiq, 2021) The current investigation has confirmed the anti-inflammatory activity of the plant and can therefore be helpful in the treatment of inflammatory conditions. The anti-inflammatory activity of the plant may be related to the presence of high phenolic contents (Serino and Salazar, 2019).

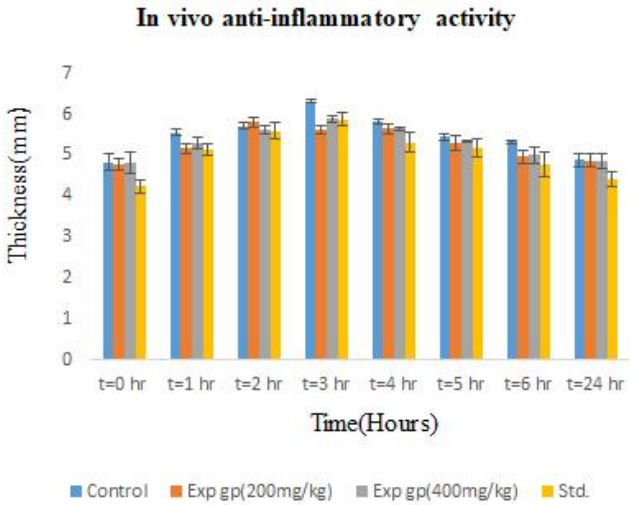

Figure 3. Comparison of *in vivo* anti-inflammatory activities of extracts, standard and control group

Conclusion: The current study confirmed that the plant seed has a potent anti-oxidant and anti-inflammatory effect. In the future, more studies are desired to identify active phytochemicals and possible pathways that involve in the reduction of oxidative stress and inflammation.

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Conflict of interest: There is no conflict of interest among the authors.

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