Nutrients, Bioactive Compounds, and Antioxidant Activities of Garden Cress (*Lepidium sativum* L.) Seeds and Their Applications in Food Technology and Therapeutic Nutrition

Marwa E. Ibrahim[*1], Yousif A. Elhassaneen[2] and Amany A. Abd El-Aziz[3]

ABSTRACT

The aim of this study seeks to investigate the nutrition, bioactive compounds, and antioxidant activities of Garden Cress (*Lepidium sativum* L.) seeds (GCS). The potential preventive effects of its extracts against atherosclerosis and meat quality in model systems were also examined. The proximate composition of GCS highlights its potential as a nutrient-dense food, protein-rich, fat, fiber, and carbohydrates. Additionally, it is an excellent origin of essential minerals, such as iron, potassium, calcium, and magnesium, along with being a promising source of vitamins, particularly pro-vitamin A and Vitamin E. The bioactive compound content in GCS extracts varies depending on the solvent used for extraction. The ethanol extract consistently exhibited the highest concentrations of beneficial compounds, including flavonoids, terpenoids, tannins, and kaempferol, while the aqueous extract contained higher levels of anthocyanins. All GCS extracts demonstrated strong antioxidant and scavenging activities. For the IC50 of the DPPH assay, the aqueous extract (AqE), ethanol extract (EtE), and methanol extract (MeE) were recorded at 15.12±0.28, 11.79±0.34, and 13.12±0.31 µg/mL, in that order, while the BHT standard was 8.95±0.14 µg/mL. Thus, the free radical scavenging activity (FRSA) of GCS extracts and the standard followed this order: BHT > EtE > MeE > AqE. In vitro studies have demonstrated that GCS can serve as a natural antioxidant, helping to inhibit lipid oxidation, protein degradation, and bacterial growth in various food products, particularly meat. Furthermore, GCS shows potential as an effective agent for preventing atherosclerosis by inhibiting the oxidation of LDL cholesterol.

Keywords: chemical composition, nutritional evaluation, vitamins, minerals, extractive value, DPPH, scavenging activity, meatballs, atherosclerosis.

INTRODUCTION

The food gap is defined as the difference between the food required to meet the nutritional needs of a population and the amount that is locally produced or available (FAO, 2021). This gap is a critical issue faced by many countries, particularly in regions where agricultural production is unable to keep pace with population growth or where environmental conditions, such as drought or soil degradation, hinder food production. As a response to this challenge, experts have proposed exploring unconventional grains as viable solutions to bridge the food gap. These grains, such as quinoa, millet, and amaranth, offer numerous benefits due to their resilience to climate stressors, adaptability to poor soil conditions, and high nutritional value, including proteins, vitamins, and minerals (Thompson and Williams, 2021). Additionally, these crops require less water and are more sustainable compared to traditional grains, making them suitable for regions affected by climate change (Sanchez *et al.*, 2022). Their integration into local agricultural systems could provide a sustainable and nutritious alternative to staple crops, helping to improve food security and reduce reliance on food imports (Lopez *et al.*, 2023). Among these grains, garden cress (*Lepidium sativum* L.) stands out as a particularly nutritious option. It is a fast-growing, hardy crop that can be cultivated in a range of climates, making it suitable for regions affected by food scarcity.

Garden cress is a small, fast-growing herb belonging to the *Brassicaceae* family. It is widely recognized for its edible seeds and leaves, both of which are utilized in a variety of culinary and therapeutic applications (Ali *et al.*, 2021). Native to the Mediterranean region, garden cress has spread globally due to its adaptability to diverse growing conditions and climates (Lopez *et al.*, 2023). This herbaceous plant is closely related to other cruciferous vegetables, such as mustard, cabbage, and broccoli, which are known for their bioactive compounds and associated health benefits (Zhang *et al.*, 2023).

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[1]Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Cairo, Egypt (Current address: Department of Food and Nutrition, College of Agriculture and Food Science, King Faisal University, Al-Ahsa, Saudi Arabia).
[2]Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.
[3]Department of Home Economics, Faculty of Specific Education, South Valley University, Qena, Egypt.
* Corresponding Author: MARWA_SOLIMAN@heco.helwan.edu.eg; https://orcid.org/0000-0001-6293-0850
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Fig. 1. Garden cress (*Lepidium sativum* L.)

As shown in Figure (1), Garden cress is characterized by its small, bright green, lance-shaped leaves arranged in a rosette. It typically grows to a height of 15-30 cm, with slender stems that support clusters of small, white flowers. The plant matures rapidly, in as little as 2-3 weeks, and produces small, oval-shaped seeds that are either reddish-brown or dark brown (Thompson and Williams, 2021).

The seeds are of particular interest owing to their rich nutritional composition and bioactive compounds, which contribute to their significant health benefits. Garden cress seeds (GCS) are highly valued for their exceptional content of nutritiona. They constitute an exceptional origin of protein, providing about 30-35% protein by dry weight (Lopez *et al.,* 2023). This high protein content makes GCS a valuable source of plant-based protein, suitable for vegetarians, vegans, and anyone seeking alternatives to animal-derived proteins. The seeds also possess beneficial lipids, such as omega-3 and omega-6 fatty acids, recognized for their cardiovascular advantages, such as reducing inflammation and lowering cholesterol levels (Sanchez *et al.,* 2022). Moreover, GCS are an abundant origin of dietary fiber, which enhances digestive health by facilitating regular bowel movements and mitigating constipation (Ali *et al.,* 2021). In terms of micronutrients, GCS are abundant in essential minerals, such as iron, potassium, calcium, and magnesium. These minerals play important roles in bone health, oxygen transport, muscle function, and maintaining healthy blood pressure (Lopez *et al.,* 2023). Furthermore, the seeds contain significant amounts of vitamins, such as pro-vit A, C, and K, which are essential for skin health, immune function, and blood clotting (Thompson and Williams, 2021). The nutritional profile of GCS makes them an excellent addition to a balanced diet, contributing to overall health and well-being.

GCS are rich in bioactive compounds that contribute to their therapeutic and preventive health properties. One of the most important groups of bioactive compounds in garden cress is glucosinolates, which are sulfur-containing compounds known for their anti-cancer, anti-inflammatory, and detoxifying properties (Abo El-Abaas, 2008; Almutairiu, 2020 and Sanchez *et al.,* 2022). These compounds are believed to have potential anti-carcinogenic effects by modulating detoxification enzymes and protecting against oxidative stress, a key factor in cancer development (Zhang *et al.,* 2023). Additionally, glucosinolates in garden cress may contribute to mitigating the risk of chronic diseases by enhancing the body's inherent detoxifying mechanisms. Flavonoids, such as kaempferol and quercetin are also abundant in GCS. These compounds possess powerful antioxidant and anti-inflammatory activities, which help combat oxidative stress and inflammation-two critical factors in the development of diseases like cardiovascular disease, diabetes, and neurodegenerative conditions (Elhassaneen *et al.,* 2003; 2016a, b; 2024b; El-Nassag *et al.,* 2019; Mehram *et al.,* 2021; Lopez *et al.,* 2023 and Gouda *et al.,* 2024). Phenolic compounds also present in GCS, further enhance the plant's therapeutic and food technological potential by exhibiting strong antimicrobial, anti-inflammatory, and antioxidant effects (Huosein, 2011 and Thompson & Williams, 2021). These bioactive substances add to the

health benefits of GCS, making them a promising candidate for various therapeutic applications.

Research into the applications of GCS in food technology has gained significant attention in recent years. Due to their high nutrient density and bioactive compounds, GCS are being explored for use in functional foods, dietary supplements, and nutraceutical products (Sanchez *et al.*, 2022). Studies have suggested that incorporating GCS into food products could improve the nutritional profile and provide additional health benefits. Also, its seeds' rich protein content makes them a potential ingredient in plant-based food formulations, offering an alternative protein source for vegetarian and vegan diets (Lopez *et al.*, 2023). In addition to their nutritional benefits, GCS have applications in food preservation. The antimicrobial properties of these seeds can be utilized to enhance the shelf life of perishable food products, reducing the risk of microbial contamination and spoilage (Ali *et al.*, 2021). Researchers are also investigating the incorporation of GCS extracts in food packaging materials as a natural preservative, which could help meet the growing demand for sustainable and eco-friendly food packaging solutions (Thompson and Williams, 2021).

As studies on GCS continue to expand, their potential uses in food technology are becoming more apparent, offering promising solutions for enhancing food quality, safety, and nutritional value. Therefore, the aims of this study to investigate the nutrition, bioactive compounds, and antioxidant activities of the seeds of the garden cress plant grown in Egypt. Also, the potential preventive effects of its seed extracts against atherosclerosis and meat quality in model systems *in vitro*; this inquiry will cover those investigations.

MATERIAL AND METHODS

Garden cress seeds (GCS):

The garden cress (*Lepidium sativum* L.) seeds, 2 kgs, which were carefully cleaned to remove dust, dirt, foreign particles, and broken seeds, were graciously provided through a special arrangement with local producers in Fayoum Governorate, Egypt. The authenticity of the seed samples was confirmed by the plant taxonomy department at the Faculty of Agriculture, Menoufia Uni., Shebin El-Kom, Egy.

Chemicals:

The standards for bioactive compounds, including gallic acid (GA), catechin (CA), α-tocopherol, linalool, ursolic acid, butylated hydroxytoluene (BHT), DPPH (2,2-diphenyl-1-picrylhydrazyl), $CuSO_4$, dimethyl sulfoxide (DMSO), and various standard vitamins, were acquired from Sigma Chemical Co. in St. Louis, Mo. Unless otherwise stated, all other chemicals, reagents, and solvents used were analytical grade and obtained from the El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments in Cairo, Egy.

Machines:

This study utilized a UV-160A spectrophotometer from Shimadzu Corporation, Kyoto, Japan to record absorbance measurements for various assays. Mineral analysis was performed with an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, Waltham, MA, USA). The total nitrogen content was assessed using a Micro-Kjeldahl semi-automatic apparatus from Velp, Italy. Crude fat content was determined via a Soxhlet semi-automatic apparatus, also from Velp, Italy. For chromatographic analysis, a SP Thermo Separation Products Liquid Chromatography system (Thermo Separation Products, San Jose, CA, USA) was employed. This system was equipped with a Consta Metvic 4100 pump, Spectra Series AS100 autosampler, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, and a Spectra System FL 3000 fluorescence detector, all controlled by the PC 1000 system software. Water-soluble vitamins were separated using a reversed-phase Adsorbosil C18 column (5 µm, 100 mm × 4.6 mm I.d.), whereas fat-soluble vitamins were analyzed using a normal-phase Ultrasphere Si column (5 µm, 250 mm × 4.6 mm I.d.). Both columns were manufactured by Alltech (Deerfield, IL, USA).

Biological model for atherosclerosis assay:

Adult male albino rats, weighing approximately 170 ± 10 g, were sourced from the Laboratory Animal Colony at the Vaccine and Immunity Organization, Cairo, Egy. The rats were housed under standard, controlled conditions, adhering to the guidelines set forth by the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC,1996). They were fed a basal diet (BD) formulated according to the recommendations of the AIN (1993).

Preparation of Garden cress seeds powder (GCS):

GCS samples were dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 70°C for two hours. After drying, the seeds were ground into a fine powder using a high-speed mixer (Moulinex Egy., ElAraby Co., Benha, Egy.). The powder that passed through a 40-mesh sieve was collected, placed in polyethylene bags, and stored in a refrigerator at 4°C for use in subsequent experiments.

Preparation of GCS extracts:

Twenty grams of dried garden cress powder were extracted using 80% aqueous solvents, including methanol, ethanol, petroleum ether, diethyl ether, hexane, chloroform, and distilled water (180 ml), on an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for three hours at 60°C for organic solvents and three hours at 80°C for water. The mixture was then filtered through Whatman No. 5 filter paper using a Buchner funnel. The remaining solvents were removed under reduced pressure using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany), with water evaporated at 60°C and organic solvents at 40°C. The resulting extracts were stored at 4°C until needed.

Chemical analysis of GCS:

The samples of GCS were analyzed for their chemical composition, including moisture, protein (calculated as total nitrogen × 6.25, using the micro-Kjeldahl method with a semiautomatic apparatus from Velp, Italy), fat (determined using a Soxhlet apparatus from Velp, Italy, with petroleum ether as the solvent), ash, fiber, and dietary fiber contents, following the methods outlined by AOAC (1995). The content of the carbohydrate was calculated by subtracting the percentage of moisture, protein, fat, ash, and fiber from 100%, as shown in the formula: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % ash + % fiber).

Evaluation of the Nutritional Value of GCS:

Total Energy Content:

The total energy (Kcal/100 g) of GCS samples was determined following the method outlined by Insel *et al.* (2002), using the equation:

Total energy (Kcal/100 g) = 4 × (Protein % + Carbohydrates %) + 9 × (Fat %)

Fulfillment of Daily Protein Requirements for Adult Men (Aged 25-50):

The amount of GCS powder (on a dry weight basis) required to meet the daily protein needs of an adult man (63 g) was calculated based on the RDA (1989). The percentage of daily protein requirements met (P.S., %) for an adult man consuming a typical portion (100 g) of GCS powder in Egypt was also determined.

Fulfillment of Daily Energy Requirements for Adult Men (Aged 25-50):

The quantity of GCS powder (dry weight basis) needed to meet the daily energy needs (G.D.R. g) of an adult man was calculated using the RDA (1989), which recommends a daily energy intake of 2900 Kcal for men. The percentage of daily energy needs met (P.S. %) for an adult man (aged 25-50 years, 79 kg weight, and 176 cm height) consuming a standard portion (100 g) of GCS powder, typically used in Egyptian households, was also computed.

Mineral content determination:

The mineral composition of GCS samples was analyzed using the method described by Singh *et al.* (1991). In this procedure, 0.5 grams of defatted sample were placed in a digestion glass tube within the Kjeldahl digestion unit. A mixture of tri-acids (HNO_3, $HClO_4$, and H_2SO_4 in a 20:4:1 v/v ratio) was then added to each tube in a volume of 6 mL. The digestion process occurred in three stages: first, the temperature was set to 70°C for 30 minutes, followed by 180°C for another 30 minutes, and finally, 220°C for the last 30 minutes. Once digestion was complete, the sample was allowed to cool, and distilled water was added to produce the total volume up to 50 mL in a volumetric flask. The mixture was filtered using ashless filter paper, and the resulting filtrate was analyzed for various minerals (Sodium, Potassium, Magnesium, Calcium, Zinc, Iron, Copper, Manganese, Phosphorus, and Selenium) using atomic absorption spectrophotometry.

Determination of Vitamins:

The extraction of fat-soluble Vitamins (A and E) from Garden cress was carried out following the procedures outlined by Hung *et al.* (1980) and Epler *et al.* (1993). For the water-soluble vitamins (B and C), outlined by Moeslinger *et al.* (1994) were employed. The extracted vitamins were then analyzed using high-performance liquid chromatography (HPLC) techniques.

Bioactive compounds determination:

The content of total phenolic in the GCS extracts was measured using the Folin-Ciocalteu reagent, following the procedure described by Singleton & Rossi (1965) and Wolfe *et al.* (2003), the findings are given as gallic acid equivalents (GAE). The total carotenoid content in the 80% acetone extract was quantified as outlined by Litchenthaler (1987), with the results reported in micrograms of carotenoids per gram of dry extract. Total flavonoids by the colorimetric method of Zhishen *et al.* (1999), with the results presented as catechin equivalents (CAE). The extraction and quantification of polysaccharides followed Vazirian *et al.* (2014) while using starch as reference standard to express results as milligrams of starch equivalents per gram of dry weight (dw). The extraction and measurement of total terpenoids followed Ghorai *et al.* (2012) methodology using linalool as the standard reference while reporting results in milligrams of linalool equivalents per gram of dry weight. The analysis of total triterpenoids followed Schneider *et al.* (2009) methodology using ursolic acid as the reference standard which yielded results in milligrams of ursolic

acid per 100 grams. Total anthocyanin content was determined as shown by Sharif *et al.* (2010), and the results expressed as milligrams of cyanidin-3,5-diglucoside per 100 grams. Tannin content was measured using the method of Van-Burden and Robinson (1981), with GA as the reference standard to create the calibration curve for estimating tannin levels. Lastly, total alkaloids were quantified following the method of Zhao and Wang (2010), using atropine as the standard for the calibration curve, from which the alkaloid content was calculated.

Antioxidant activities:

Antioxidant activity (AA):

The AA of GCS extracts, as well as standards like α-tocopherol and BHT, was assessed using the BCB assay, with slight modifications to the method described by Marco (1968). For the assay involved combining 1 mL of *β*-carotene solution (0.2 mg/mL in chloroform) with 0.02 mL linoleic acid and 0.2 mL Tween 20 in 50 mL round-bottom flasks. Control solutions (80% methanol) or plant extract/standard solutions (0.2 mL) were then added. Following solvent evaporation under vacuum at room temperature, 50 mL of oxygenated distilled water was introduced to form liposome suspensions. Samples were incubated at 50°C for two hours. *β*-carotene degradation was quantified by measuring absorbance at 470 nm using a Beckman DU-50 spectrophotometer. Linear regression analysis of time-dependent absorbance data was employed to determine degradation rates. All experiments were performed in triplicate, utilizing BHT and α-tocopherol dissolved in 80% methanol as reference compounds. AA was calculated using four different methods:

1) Plotting the absorbance against time to create a curve, with the absolute value of the slope representing the antioxidant value (AOX) as per Al-Saikhan *et al.* (1995);
2) Calculating the AA as the percentage inhibition relative to the control using the equation $AA = [(R_{control} - R_{sample}) / R_{control}] \times 100$, where R control and R sample are the bleaching rates of β-carotene in the reaction mixtures without and with the plant extract, correspondingly;
3) Calculating the oxidation rate ratio (ORR) as per Marinova *et al.* (1994), using the equation $ORR = R_{sample} / R_{control}$,
4) Determining the antioxidant activity coefficient (AAC) based on Mallet *et al.* (1994), calculated using the formula $AAC = [(AbsS_{120} - AbsC_{120}) / (AbsC_0 - AbsC_{120})] \times 100$, where AbsS120, AbsC120, and AbsC0 are the absorbance of the antioxidant mixture, control at 120 minutes, and control at time zero, respectively.

DPPH radical scavenging assay:

The free radical scavenging capacity of GSC powder extracts was evaluated using the DPPH radical scavenging assay, as outlined by Desmarchelier *et al.* (1997). Butylated hydroxytoluene (BHT) was employed as a reference standard. The inhibition percentage was then plotted against the concentration, and the IC50 value was determined from the graph.

Therapeutic nutrition and Food technology applications:

Anti-atherosclerotic effect of GCS *in vitro*:

The inhibitory effect of GCS extracts on LDL oxidation (anti-atherosclerotic activity) was assessed in vitro using the method described by Princen *et al.* (1992). Blood serum was collected from adult male albino Sprague-Dawley rats, and it was diluted with 50 mM phosphate buffer (pH 7.4) to achieve a final concentration of 0.6%. A 5.0 mL sample of this diluted serum was mixed with 10 µL of DMSO or 10 µL of DMSO containing varying concentrations of GCS extracts. To initiate the oxidation reaction, 20 µL of a 2.5 mM $CuSO_4$ solution was added. Absorbance measurements were taken at 234 nm every 20 minutes for 140 minutes at room temperature. The extent of oxidation was determined by calculating the net area under the oxidation curve.

Food technology studies (Meatballs):

a. Meat samples:

Fresh buffalo meat samples were obtained right after slaughter from a local slaughterhouse in Shebin El-Kom, Menoufia Gov., Egy. The samples were then promptly transported in refrigerated containers to the laboratory, where they underwent various manufacturing processes, technological treatments, and storage experiments without delay.

b. Meatball formulation and processing:

Buffalo meatballs were made using the procedure outlined by Fernández-López *et al.* (2005), with the ingredients listed in Table (a). The concentrations of GCS extracts were chosen based on previous studies (Elhassaneen *et al.*, 2016b; Sayed-Ahmed *et al.*, 2020; Elsemelawy *et al.*, 2021 and Aboraya *et al.*, 2022). The preparation process in the laboratory was carried out to mirror commercial processing techniques. Fresh buffalo meat was chopped into small pieces with a sharp knife and then minced using an electric mixer (Moulinex Egy., Al-Araby Co., Egy.). All the ingredients were blended in a bowl mixer using a spiral dough hook (Moulinex Egypt) for 6 minutes.

Table a. Meatball formulation

Ingredients composition (%)	Control	AqE	EtE	MeE
Minced buffalo meat (22% fat)	78	78	78	78
Flaked potatoes	14.5	14	14	14
Water	5	5	5	5
Salt	2.5	2.5	2.5	2.5
Aqueous extract (AqE) treatment	-------	0.50	-------	-------
Ethanol extract (EtE) treatment	-------	-------	0.50	-------
Methanol extract (MeE) treatment	-------	-------	-------	0.50

The respective GCS extract was added to each treatment group at the selected concentration, and the mixture was mixed for an additional 6 minutes. The meatballs, weighing 20g each and with diameters ranging from 22 to 27 mm, were shaped by hand. They were then subjected to a two-stage cooking process: first, they were flash-fried in corn oil at 185°C for 30 seconds to seal the surface and achieve the desired brown color. Next, the meatballs were fully cooked in a forced draught oven (Zanussi, Italy) at 230°C for 6 minutes to reach an internal temperature of 70°C at the center. After cooking, the meatballs were immediately placed in a chiller (4°C) until the product temperature dropped below 12°C. The experiment was repeated three times for accuracy.

c. Meatballs product quality examination:

Thiobarbituric acid (TBA) determination:

Lipid oxidation was assessed by determining the TBA content based on the procedure described by Tarladgis *et al.* (1960). The TBA-reactive substances ($TBAR_S$) were determined using a standard curve (5–50 nmol) of malondialdehyde (MDA), which was generated by acidifying TEP (1,1,3,3-tetraethoxypropane). The TBA values were reported as mg MDA per kg of sample.

Total volatile basic nitrogen (TVB-N):

The TVB-N content was shown by Winton and Winton (1961). The results were expressed as mg of TVB-N per 100 grams of sample.

Microbiological analysis (Lactic acid bacteria, LAB):

A composite sample weighing 10 grams was prepared by combining portions from at least three meatballs and homogenized in sterile 1.5% peptone water using a Stomacher 400 (Colworth, London, UK) for 1 minute. The homogenate underwent serial dilution in peptone water, and the resulting dilutions were plated following established procedures Gerhardt *et al.* (1994). LAB were enumerated on MRS agar (pH 5.6), with plates incubated anaerobically using a gas-generating kit (Anaerobic System, Oxoid Unipath Ltd., Basingstoke, Hampshire, UK) at 30°C for 48 hours. Culture media were obtained from Oxoid (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK). Results are presented as log10 cfu/mL.

Statistical Analysis:

All experiments were performed in triplicate. Data were subjected to the analysis of variance (ANOVA) and mean comparisons were performed using Duncan's multiple range test (Steel and Torrie, 1980). Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate composition of Garden cress seeds (GCS) powder:

The proximate composition data for GCS is presented in Table (1) reveals several key nutritional characteristics of GCS. The ash content of GCS is 3.99%, indicating the presence of essential minerals, such as Ca, K, and Mg, which are crucial for bone health, nerve function, and overall cellular processes. Ash content in seeds can vary widely, but this value is consistent with other seeds, such as sesame (*Sesamum indicum*), which contains around 3-4% ash (Al-Snafi, 2019). The moisture content of GCS has an average value of 6.97%, which is consistent with the moisture content typically observed in seeds like flaxseed (*Linum usitatissimum*), which ranges from 6-8% (Berti *et al.*, 2017). The relatively low moisture content suggests that GCS are less likely to spoil, making them suitable for long-term storage and food processing applications. Additionally, lower moisture content increases the concentration of other nutrients per unit weight. The protein content of GCS averages 20.31%, which is comparable to the protein content found in other high-protein seeds, such as fenugreek (*Trigonella foenum-graecum*) seeds, which contain approximately 20-25% protein (Kumar *et al.*, 2018). This high protein content indicates that GCS are a very good resource of plant-based protein, useful for human nutrition and the development of plant-based food products. The fat content in GCS is 12.87%, which is similar to that found in other seeds, such as mustard seeds, which contain approximately 10-15% fat (Jha *et al.*, 2017). The crude fiber content of GCS is 17.54%, which is higher than

that of seeds like sunflower (*Helianthus annuus*), which typically contain around 10-15% fiber (Anwar *et al.,* 2018). The high fiber content of GCS suggests significant potential benefits for digestive health, including improved bowel movement and regulation of blood sugar levels. These characteristics make GCS suitable for inclusion in functional foods aimed at managing obesity, diabetes, and gastrointestinal disorders (Yuan *et al.,* 2020). The carbohydrate content in GCS ranges from 37.25% to 39.56%, with an average of 38.32%. This is similar to the carbohydrate content found in other carbohydrate-dense seeds like sesame and mustard, which contain 30-40% carbohydrates (Jha *et al.,* 2017). The moderate carbohydrate levels also make GCS a good option for inclusion in low-glycemic index food products.

Table 1. Proximate composition of Garden cress seeds (GCS, g/100g WW)

Component	Mean ±SD
Ash	3.99 ± 0.26
Moisture	6.97 ± 0.56
Crude fat	12.87 ± 0.61
Crude Fiber	17.54 ± 0.70
Total protein	20.31 ± 0.43
Carbohydrate	38.32 ± 1.12

Each value represents the mean of three replicates ±SD. (WW) wet weight.

Nutritional evaluation of Garden cress seeds (GCS) powder:

The nutritional evaluation of GCS powder, presented in Table (2), provides valuable insights into its potential as a nutritious food ingredient. The energy content of GCS powder, with a mean of 350 Kcal, indicates a moderate energy value. This energy range aligns with similar studies on other seeds, such as mustard and fenugreek, which have reported comparable energy values (Jha *et al.,* 2017 and Kumar *et al.,* 2018). The energy content of GCS is essential for providing a healthy energy source, and its moderate calorie content makes it an excellent option for consumers seeking balanced the Grams Daily Requirement (G.D.R.) for protein, based on the recommended intake of 63g of protein per day, with a mean of 310.19 ± 10.56g, suggests that GCS powder can significantly contribute to meeting daily protein needs, especially when included in a diet rich in plant-based proteins (Anwar *et al.,* 2018). Similarly, the G.D.R. for energy, based on a daily requirement of 2900 Kcal, ranges from 803.21 to 843.78g, highlighting the contribution of GCS powder to daily energy intake. In terms of its percentage contribution to daily nutritional intake, the protein content of GCS powder provides a mean of 32.24 ± 2.45% per 100g serving. This indicates that Garden Cress is a notable source of plant-based protein, a finding supported by previous studies on seeds, such as chia and sunflower (Berti *et al.,* 2017 and Anwar *et al.,* 2018). For energy, the percentage contribution per 100g serving is 12.08%, emphasizing its moderate yet significant role in providing energy in a balanced diet.

Table 2. Nutritional evaluation of Garden cress seeds (GCS) powder

Factor	Mean ±SD
Energy (Kcal/100g)	350 ± 6
G.D.R. (g) for protein (63 g)	310.19 ± 10.56
G.D.R. (g) for energy (2900 Kcal)	827.74 ± 18.45
P.S./100 g For protein (63g, %)	32.24 ±2.45
P.S./100 g For energy (2900 Kcal, %)	12.08 ± 0.89

Values represent the mean ±SD (n=3). (G.D.R.) Grams consumed tyo cover the Daily Requirement, (P.S.) Percent of satisfaction.

Mineral composition of Garden cress seeds (GCS):

The mineral composition of GCS and their corresponding Recommended Dietary Allowances (RDA) for both men and women are shown in Table (3). GCS seeds contain 421.34 mg/100g of Ca, which is about 42% of the RDA for men (1000 mg) and 37.92% for women. This is similar to the findings in other seeds like sesame, which also show high Ca content (Al-Snafi, 2019). Ca is required for healthy bone and normal muscular function. At 2834.76 mg/100g, GCS seeds provide about 94% of the RDA for men and 122% for women. This high K content aligns with other nutrient-dense seeds, such as chia and sunflower (Berti *et al.,* 2017 and Anwar *et al.,* 2018). K has an important function in maintaining blood pressure and heart health. GCS seeds contain 289.56 mg/100g of magnesium, contributing to 69% of the RDA for men (420 mg) and 62% for women (320 mg). Similar levels of magnesium have been reported in fenugreek seeds (Kumar *et al.,* 2018). Mg is essential for the functioning of nerves and muscles, as well as healthy bones. With 792.11 mg/100g, GCS seeds exceed the RDA for both men and women. P is key for energy production and bone health, and its concentration in GCS supports its use as a nutrient-dense food source.

Table 3. Mineral composition of Garden cress seeds (GCS)

Element	GCS mg.100g^{-1}	RDA/Men mg.day^{-1}	G.D.R. (g) for element (RDA/day)	RDA/Women mg.day^{-1}	G.D.R. (g) for element (RDA/day)
Calcium (Ca)	421.34 ± 8.95	1000	237.34	37.92	1000
Potassium (K)	2834.76 ± 64.56	3016	106.39	84.59	2320
Magnesium (Mg)	289.56 ± 5.66	420	145.05	62.05	320
Phosphorus (P)	792.11 ± 9.11	700	88.37	101.84	700
Iron (Fe)	6.98 ± 0.23	8	114.61	78.53	18
Zinc (Zn)	2.86 ± 0.09	11	384.62	23.40	8
Sodium (Na)	261.35 ± 6.76	2300	880.05	10.23	2300
Manganese (Mn)	2.01 ± 0.04	2.3	114.43	78.65	1.8
Copper (Cu)	0.75 ± 0.02	0.9	120.00	75.00	0.9
Selenium (Se)	0.54 ± 0.03	0.055	10.19	883.64	0.055

* Each value represents the mean ± SD (n-3). Values for various superscript letters in the same raw are significantly $p \leq 0.05$ different.

GCS seeds provide 6.98 mg/100g of iron, which is about 87% of the RDA for men but only 39% for women. This Fe content is similar to findings in mustard seeds (Jha *et al.*, 2017). Fe is essential for oxygen transport in the blood and for energy metabolism. At 2.86 mg/100g, GCS seeds supply 26% of the RDA for men and 36% for women. Zn levels in GCS are comparable to those found in other seeds like sunflower (Anwar *et al.*, 2018). Zn plays a critical role in protein synthesis, wound healing, and immune function. The sodium content of 261.35 mg/100g in GCS seeds is well below the RDA for both men (2300 mg) and women (2300 mg), which indicates GCS seeds can be considered low in Na. Na is important for maintaining fluid balance and proper nerve function. GCS seeds provide 2.01 mg/100g of Mg, which is 87% of the RDA for men and 112% for women. Similar findings have been reported for other seeds like chia (Berti *et al.*, 2017). Mg is involved in blood clotting, bone formation, and reducing oxidative stress. At 0.75 mg/100g, GCS seeds provide 83% of the RDA for men and 83% for women, similar to levels in sesame seeds (Al-Snafi, 2019). Cu is essential for iron absorption and the formation of red blood cells. GCS contain 0.54 µg/100g of Se, which is much higher than the RDA for both men and women (55 µg). This mineral plays a significant role in antioxidant defense and thyroid hormone metabolism, making GCS seeds an excellent resource of selenium for dietary supplementation.

Vitamin concentrations in Garden cress seeds (GCS):

The data presented in Table (4) highlights the Vitamin concentrations in GCS and their comparison with recommended daily intake values. The concentration of Vitamin A in GCS seeds is 776.78 ± 1.67 µg/100g, which is approximately 86.31% of the Daily Recommended Intake (DRI) for Vitamin A (900 µg). This is comparable to findings in other seeds like sunflower, which also shows significant levels of Vitamin A (Anwar *et al.*, 2018). The rich presence of Vitamin A in GCS seeds suggests their potential role in maintaining healthy vision, skin health and immune function, as indicated by other studies on plant-based sources (Kumar *et al.*, 2018). The Vitamin C content in GCS is 29.56 ± 2.89 mg/100g, providing about 39.41% of the DRI (75 mg). This is similar to the concentrations observed in other High-Vitamin C seeds like mustard seeds (Jha *et al.*, 2017) and chia (Berti *et al.*, 2017). The amount of Vitamin C in GCS suggests their potential for enhancing immune function, and collagen synthesis, and providing antioxidant protection against free radicals, as shown in studies on various plant-based foods. With 8.95 ± 0.28 mg of Vitamin E per 100g, GCS contribute 74.58% of the DRI for this fat-soluble antioxidant. This value aligns with findings in other nutrient-rich seeds, like those from fenugreek (Kumar *et al.*, 2018), which also show a significant presence of Vitamin E. Vitamin E plays a crucial role in protecting cells from oxidative stress, enhancing skin health, and supporting cardiovascular health, which further supports the inclusion of GCS seeds in diet-based interventions for antioxidative purposes. The concentration of Vitamin B1 in GCS is 0.32 ± 0.04 mg/100g, providing 32% of the RDA for this Vitamin. Thiamine is essential for energy metabolism and nervous system function (Yuan *et al.*, 2020). The riboflavin content is 0.14 ± 0.02 mg/100g, offering 12.73% of the RDA. Riboflavin is key for enzyme functions related to energy production (Jha *et al.*, 2017). GCS contain 0.67 ± 0.05 mg of Niacin, providing about 5.58% of the recommended intake. Niacin is crucial for cellular metabolism (Kumar *et al.*, 2018). The content of Vitamin B6 is 0.23 ± 0.03 mg/100g, offering around 20.91% of the RDA.

Table 4. Vitamin concentrations in Garden cress seeds (GCS)

Component	Value	DRI	G.D.R. (g) (RDA)	P.S./100 g
Vitamin A (Retinol Activity Equivalents, RAE)	776.78 ± 1.67	900	115.86	86.31
Vitamin C (Ascorbic acid, mg/100g)	29.56 ± 2.89	75	253.72	39.41
Vitamin E (Tocopherols, mg /100g)	8.95 ± 0.28	12	134.08	74.58
Vitamin B1 (Thiamine, mg/100g)	0.32 ± 0.04	1	312.50	32.00
Vitamin B2 (Riboflavin, mg/100g)	0.14 ± 0.02	1.1	785.71	12.73
Vitamin B3 (Niacin, mg/100g)	0.67 ± 0.05	12	1791.04	5.58
Vitamin B6 (Pyridoxine, mg/100g)	0.23 ± 0.03	1.1	478.26	20.91
Vitamin B9 (Folate, µg/100g)	26.78 ± 1.76	320	1194.92	8.37

Each value represents the mean of three replicates ±SD. (DRI) Dietary Reference Intake

This Vitamin is vital for brain health, nerve function, and the synthesis of neurotransmitters (Berti *et al.,* 2017). The concentration of folate is 26.78 ± 1.76 µg/100g, providing 8.37% of the DRI. Folate is essential for DNA synthesis and cell growth, which is particularly important during pregnancy and for overall cellular health (Yuan *et al.,* 2020).

Extractive value of Garden cress seeds (GCS) powder:

The extractive value of GCS was assessed by performing sequential extraction using various organic solvents and water in a Soxhlet apparatus, as shown in Table (5). The results revealed that the extractive value of GCS was higher in some organic solvents (such as, ethanol and methanol) and water compared to other solvents like petroleum ether, hexane, and chloroform. This suggests that GCS contains components that are both lipophilic (fat-soluble) and hydrophilic (water-soluble). These findings support the principle "like dissolves like," which means that substances with similar polarity tend to dissolve in each other—non-polar solvents dissolve non-polar solutes, while polar solvents dissolve polar solutes, with polar and non-polar substances being immiscible. Identifying the appropriate solvent system is crucial for optimizing the extraction of bioactive compounds from plant extracts. Sulaiman *et al.* (2011) indicated that the polarity of the solvent significantly affects the solubility of chemical constituents in the sample, impacting the extraction yield. Numerous studies have shown that extractive values are a useful method for assessing the quality of drugs or foods, as any variation in the chemical composition can alter these values (El-Wazeer, 2011; Hegazy, 2014; Abd El-Khader, 2018; Elhassaneen *et al.,* 2018; 2022 and El-Soukoty, 2021). Consequently, extractive values are important for detecting adulteration and serve as an indicator of the purity of food or medicinal products.

Table 5. Extractive value of Garden cress seeds (GCS) samples using different organic solvents and water

Extraction medium	Mean extract (%) ±SD
Ethanol (80%)	7.21 ± 0.09^{a}
Methanol (80%)	7.08 ± 0.10^{a}
Petroleum ether	4.17 ± 0.08^{c}
Chloroform	3.29 ± 0.11^{d}
Hexane	4.09 ± 0.09^{cd}
Water (Distilled)	5.98 ± 0.37^{b}

Each value represents the mean of four replicates ±SD. Values for various superscript letters in the same raw are significantly $p \leq 0.05$ different.

Bioactive compounds in Garden cress seeds (GCS) extracts:

The extracts of GCS contain bioactive substances summarized in Table (6). Total phenolics emerged as the most abundant bioactive compound across all extracts, while kaempferol was found in the smallest amounts. The quantities of other bioactive compounds varied depending on the extraction method. Among the extracts, the ethanolic extract was rich in total phenolics, flavonoids, terpenoids, tannins, and kaempferol. The methanolic extract contained significant levels of total carotenoids and polysaccharides, while the aqueous extract exhibited the highest content of total anthocyanins, triterpenoids, and alkaloids. The variation in the bioactive compound content observed in GCS extracts can be attributed to several factors, such as the extraction methods and the type of solvent used (Al-Farsi *et al.,* 2005; Khanavi *et al.,* 2009 and Ardekani *et al.,* 2010). Also, the findings of this study confirm that the presence of these bioactive compounds in different extracts is influenced by the polarity of the solvents used, in line with the principle "like dissolves like." Non-polar solvents typically dissolve non-polar compounds, while polar solvents are more effective at dissolving polar compounds. Sulaiman *et al.* (2011) noted that the polarity differences of

extraction media affect the solubility of chemical constituents and, consequently, the yield of bioactive compounds. Prior studies have shown that bioactive compounds (phenolics and flavonoids), present in GCS extracts play essential roles in the prevention and treatment of diseases like cancer, diabetes, obesity, atherosclerosis, and aging (Elhassaneen *et al.*, 2016 a,c; 2019). Polysaccharides in GCS are believed to enhance immune function, contributing to antitumor activity (Wasser, 2005), while triterpenoids may serve as precursors for the synthesis of potent antitumor agents (Ma *et al.*, 2005). Tannins, though beneficial, can negatively affect feed intake and protein digestibility in animals (Chung *et al.*, 1998). Kaempferol, a flavonoid phytoestrogen, is known to reduce the risk of chronic diseases, particularly cancer, by enhancing the body's antioxidant defenses and modulating processes like apoptosis, angiogenesis, inflammation, and metastasis (Allen and Yi, 2013).

Biological activities of Garden cress seeds (GCS) extracts:

Antioxidant activity (AA):

The AA of the GCS extracts is presented in Table (7). The data reveals noticeable variations in the antioxidant activity, ranging from 88.44% to 92.95%. The ethanolic extract (EtE) exhibited the highest antioxidant activity, likely due to its elevated content of bioactive compounds, while the aqueous extract (AqE) demonstrated substantial antioxidant activity along with a notable concentration of the assayed bioactive compounds. These findings are partially consistent with previous studies on various plant parts rich in bioactive compounds, similar to those found in GCS (Elhassaneen *et al.*, 2016b; 2019, 2021; 2023; Mashal, 2016; Sayed Ahmed, 2016; Aly *et al.*, 2017; Hallabo *et al.*, 2018; El-Nassag *et al.*, 2019; Abd Elalal *et al.*, 2021 and Gharib *et al.*, 2022). These studies have shown that various bioactive compounds, such as phenolics, carotenoids, flavonoids, anthocyanins, polysaccharides, terpenoids, triterpenoids, tannins, and alkaloids, are highly correlated with antioxidant activity. Therefore, the observed variations in the antioxidant activity of GCS extracts can be attributed to differences in their bioactive compound content.

Table 6. Bioactive and antinutritional compounds content of Garden cress seeds (GCS) extracts

Compound	MeE	EtE	AqE
Total phenolics (mg gallic acid equivalent. 100 g^{-1})	1289.12 ± 26.56 ^{a}	1348.67 ± 28.57 ^{a}	914.56 ± 11.53 ^{b}
Total carotenoids (mg catechin equivalent. 100g^{-1})	69.12 ± 2.76 ^{a}	61.52 ± 3.45 ^{a}	49.12 ± 1.78 ^{b}
Total flavonoids (mg rutin equivalent. 100 g^{-1})	529.12 ± 16.45 ^{b}	615.78 ± 19.65 ^{a}	119.56 ± 3.76 ^{c}
Total anthocyanins (mg cyanidin-3,5-diglucoside equivalent. 100 g^{-1})	132.89 ± 3.87 ^{b}	117.89 ± 10.53 ^{c}	149.90 ± 5.56 ^{a}
Polysaccharides (mg starch. g^{-1})	66.47 ± 3.67 ^{a}	54.12 ± 2.82 ^{a}	30.65 ± 2.45 ^{b}
Terpenoids (mg linalool equivalent. 100 g^{-1})	37.98 ± 1.65 ^{b}	58.67 ± 3.12 ^{a}	18.89 ± 0.98 ^{c}
Triterpenoids (mg ursolic acid equivalent. 100 g^{-1})	18.56 ± 0.97 ^{ab}	15.41 ± 1.11 ^{b}	21.15 ± 2.07 ^{a}
Tannins (mg catechine equivalent. 100 g^{-1})	38.45 ± 4.08^{b}	49.42 ± 5.09 ^{a}	19.76 ± 0.55 ^{c}
Kaempherol (mg. 100 g^{-1})	6.84 ± 0.54^{a}	8.89 ± 0.37 ^{a}	2.17 ± 0.08 ^{b}
Total alkaloids (g atropine.100g^{-1})	1.04 ± 0.08 ^{a}	1.04 ± 0.06 ^{a}	1.09 ± 0.04 ^{a}

Each value represents the mean of four replicates ±SD. Values for various superscript letters in the same raw are significantly $p \leq 0.05$ different.

Table 7. Antioxidant activity of Garden cress seeds (GCS) extracts

Extract	Antioxidant value AOX (A/h)		Antioxidant activity AA (%)		Oxidation rate ratio (ORR)		Antioxidant activity coefficient (AAC)	
EtE	0.040±	0.010 ^{b}	92.95±	1.61 ^{b}	0.070±	0.012 ^{b}	788.21±	11.67^{b}
MeE	0.048±	0.002 ^{b}	91.59±	0.98 ^{b}	0.084±	0.021 ^{b}	764.56 ±	9.90 ^{b}
AqE	0.065±	0.016 ^{a}	88.44±	0.55 ^{c}	0.115±	0.002 ^{a}	709.80±	11.65 ^{c}
BHT, 50 mg/L	0.072±	0.014 ^{a}	87.22±	0.54 ^{c}	0.127±	0.003 ^{a}	688.59±	3.98 ^{c}
BHT, 200 mg/L	0.015±	0.004 ^{c}	97.26±	0.42 ^{a}	0.027±	0.004 ^{c}	863.14±	2.65 ^{a}
α-tocopherol, 50 mg/L	0.010±	0.000 ^{c}	98.15±	0.29^{a}	0.018±	0.002 ^{c}	878.61±	1.67 ^{a}

Each value represents the mean of four replicates ±SD. Values for various superscript letters in the same raw are significantly $p \leqslant 0.05$ different.

DPPH radical scavenging activity:

The free radical scavenging activity (FRSA) of GCS extracts and butylated hydroxytoluene (BHT) is presented in Figure (2) and Table (8). The data reveal that the ethanol extract exhibited the highest scavenging activity, followed by the methanol and aqueous extracts. At a concentration of 100 µg/mL, the FRSA of the aqueous, ethanol, and methanol extracts were 80.56%, 87.88%, and 85.76%, respectively, while the BHT standard showed a scavenging activity of 92.05%. The IC50 values for the aqueous, ethanol, and methanol extracts were found to be 15.12±0.28, 11.79±0.34, and 13.12±0.31 µg/mL, in that order, with BHT having an IC50 value of 8.95±0.14 µg/mL. Thus, the FRSA of the GCS extracts and BHT standard followed the order: BHT > ethanol extract > methanol extract > aqueous extract. The DPPH assay, used to measure radical scavenging, relies on the absorption of conjugated dienes in the presence of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (Antolovich *et al.*, 2002). Numerous studies have successfully used the DPPH assay to evaluate the scavenging activity of extracts from various plant sources, including plant seeds (Aaby *et al.*, 2004; Laura *et al.*, 2010; Aminzare *et al.*, 2019; Aly *et al.*, 2017; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2021; 2023 and Fayez, 2022). The FRSA observed in date seed extracts is attributed to the presence of several bioactive antioxidant compounds, such as phenolics, carotenoids, flavonoids, anthocyanins, polysaccharides, terpenoids, kaempferol, and alkaloids. The FRSA properties are crucial for mitigating the adverse effects of free radicals in various diseases, including obesity, diabetes, cancer, anemia, neurological disorders, pulmonary diseases, nephropathy, and cardiovascular conditions (Gharib *et al.*, 2022; Elhassaneen *et al.*, 2023 and Mahran & Elhassaneen, 2023).

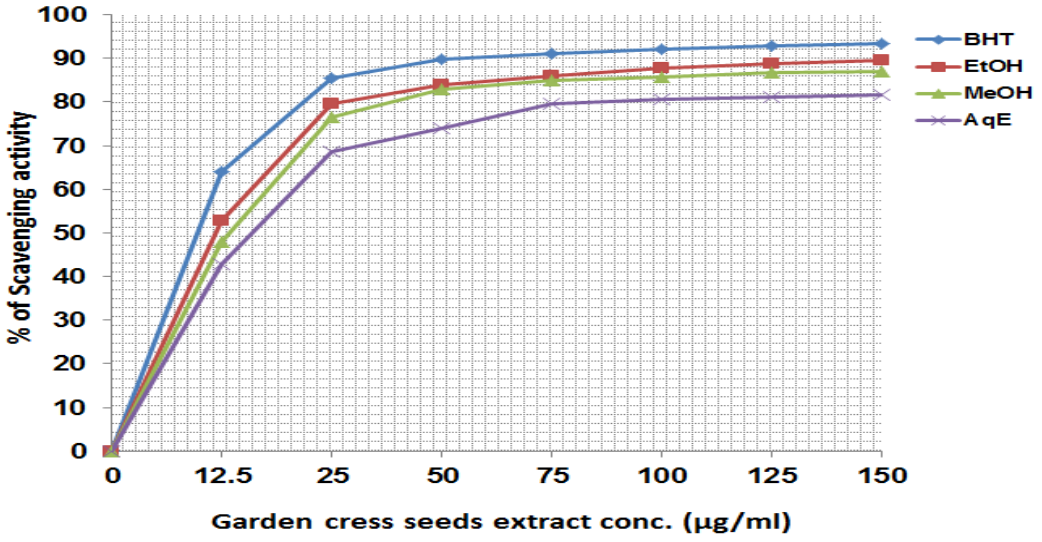

Fig. 2. DPPH radical scavenging activity (%) of Garden cress seeds (GCS) extracts and standard (BHT)
Each value represents the mean value of three replicates.

Table 8. IC_{50} (DPPH) of Garden cress seeds (GCS) extracts and BHT (Standard)

Name of sample	BHT	AqE	EtE	MeE
IC_{50} (µg/mL)	8.95± 0.14 ^{c}	15.12±0.28 ^{a}	11.79±0.34 ^{b}	13.12±0.31 ^{ab}

Each value is presented as the mean of three replicates ± standard deviation (SD). Values for various superscript letters in the same raw are significantly $p \leq 0.05$ different.

GCS applications in food technology and therapeutic nutrition:

***In vitro* Anti-Atherosclerotic Properties of GCS: Inhibition of low-density lipoprotein (LDL) oxidation:**

Fig. (3) illustrates the dose-dependent inhibition of $CuSO_4$-induced LDL oxidation in vitro by GCS extracts. The data reveal that GCS extracts effectively inhibited $CuSO_4$-induced LDL oxidation, as evidenced by the reduction in conjugated dienes production in a dose-dependent manner. A comparative analysis of the GCS extracts showed that the aqueous, ethanol, and methanol extracts exhibited the most significant protection against LDL oxidation. The effectiveness of the different GCS extracts in protecting LDL from oxidation followed this order: ethanol > methanol > aqueous extracts. Reactive oxygen species (ROS) are known to cause cellular damage by peroxidizing the lipid components of cell membranes, including those of mitochondria and lysosomes, leading to cellular dysfunction (Lien *et al.*, 2008). The present study demonstrated that GCS extracts have lipid peroxidation-inhibiting properties, suggesting that these extracts can mitigate cellular damage caused by free radicals by halting the chain reactions responsible for lipid peroxidation. In line with this, various in vivo studies have shown that phenolic compounds, the primary bioactive constituents in GCS extracts, protect LDL from oxidation by enhancing the levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in the liver and lungs, as well as inhibiting NADPH-dependent lipid peroxidation (Majid *et al.*, 1991; Elbasouny *et al.*, 2019; El-Gamal, 2020; Elhassaneen *et al.*, 2020 and Gouda *et al.*, 2024). Additionally, the inhibition of LDL oxidation has been reported in various plant extracts containing similar bioactive compounds to those found in GCS extracts (Aly *et al.*, 2017; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2021; 2023 and Fayez *et al.*, 2021). LDL oxidation in the vascular endothelium has been identified as an early event in atherosclerotic plaque formation (Poznyak *et al.*, 2021), with ROS production triggering phenotypic changes in endothelial cells. The inhibition of LDL oxidation by GCS extracts is likely due to their high content of various bioactive compounds, including antioxidants and free radical scavengers, such as phenolics, anthocyanins, polysaccharides, carotenoids, terpenoids, and tannins. Therefore, the findings of this study suggest that GCS extracts could serve as a promising agent in preventing atherosclerosis by inhibiting LDL oxidation.

The impact of GCS extracts as a natural antioxidant on the chemical and microbial quality of meatballs during storage:

Rancidity evolution:

The progression of rancidity (thiobarbituric acid content, TBA) in meatballs with various GCS extracts added during storage is depicted in Figures (4 and 5). Analysis of variance for the TBA data shows that the TBA levels in meatball samples were significantly influenced ($P \leq 0.05$) by both the type of extract treatment and the duration of storage. Initially, TBA values for all meatball samples treated with extracts were significantly lower ($P \leq 0.05$) compared to the control group.

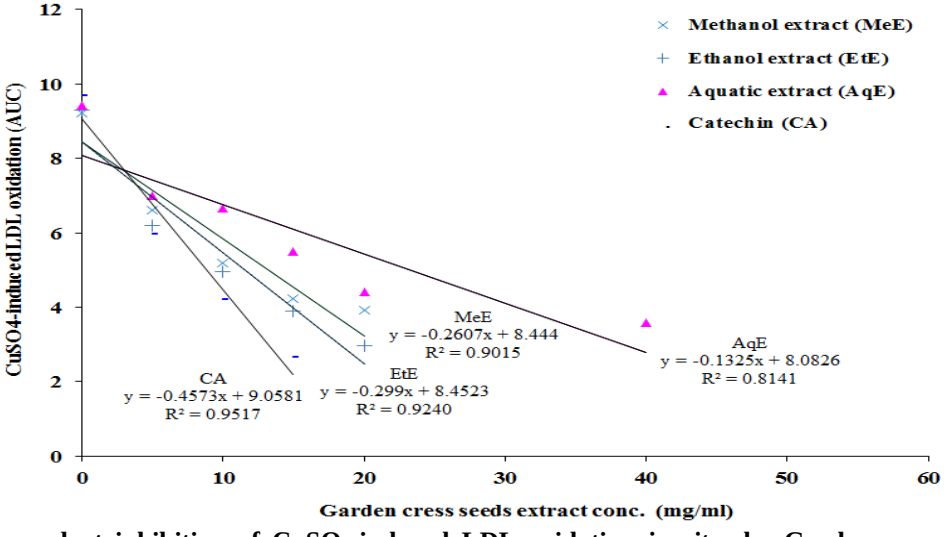

Fig. 3. Dose-dependent inhibition of $CuSO_4$-induced LDL oxidation *in vitro* by Garden cress seeds (GCS) extracts

For the control meatball samples, the TBA value started at 0.32 mg/kg and increased to 4.49 mg/kg (an increase of 1303.12%) by the end of the 12-day storage period at 4°C. All GCS extract treatments resulted in a significant ($P \leq 0.05$) reduction in TBA formation in meatball samples after storage. The most substantial reductions were observed in the samples treated with EtE, followed by MeE and AqE, respectively. Decades ago, there was growing global concern regarding the presence and harmful effects of certain toxic compounds, particularly those found in TBARs, such as MDA. This compound is produced in fresh and ready-to-eat foods, such as meats, as a result of the oxidation of polyunsaturated fatty acids during storage, cooking, and processing (Gray & Morton, 1981; El-Shafie & Elhassaneen, 1992 and Elhassaneen & Twfik, 1998). Additionally, Chen *et al.* (1984) observed that cooking releases iron from blood heme, leading to an increase in non-heme iron, which plays a key role in lipid oxidation. Sato and Hegarty (1971) also found that non-heme iron is a primary catalyst in the oxidation process in cooked meats. Many studies have documented the detrimental effects of MDA on human health, highlighting its mutagenic and carcinogenic properties (Mukia & Goldstein, 1976; Shamberger *et al.*, 1979 and Tawfik *et al.*, 2003). The results of the present study indicate that GCS extracts, as natural products, effectively slowed lipid oxidation during and immediately after cooking. These findings are consistent with those reported by several researchers who studied other natural antioxidants applied to cooked beef (Ahn *et al.*, 2002; Fernández-Giné *et al.*, 2003; Hassan, 2013; Elhassaneen & Esa, 2015; Essa, 2015 and Elhassaneen *et al.*, 2023).

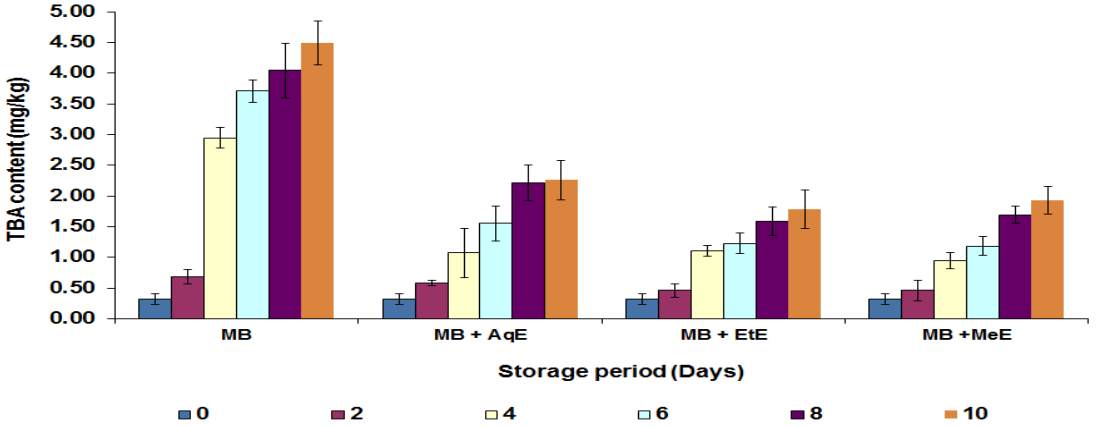

Fig.4. The effect of Garden cress seeds (GCS) extracts on thiobarbituric acid (TBA) levels (mg/kg sample) in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates ±SD.

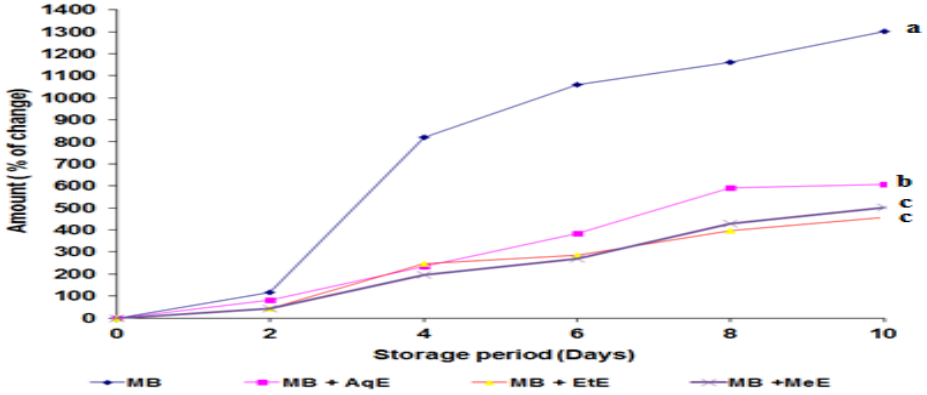

Fig.5. The effect of Garden cress seeds (GCS) extracts on thiobarbituric acid levels (TBA, as a percent of control) in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates. Means with distinct letters among the different treatments at the end of the storage period are significantly different at $p \leq 0.05$.

The current data, along with previous studies, suggest that all GCS extracts used in cooked meatballs demonstrated strong antioxidant properties. This is attributed to their high content of bioactive compounds, such as phenolics, carotenoids, anthocyanins, polysaccharides, flavonoids, terpenoids, triterpenoids, and alkaloids (Singh *et al.*, 2002; Pereira *et al.*, 2007; El-Safty, 2008 and Aber & Afa, 2011). These bioactive compounds are known for their antioxidant and free radical scavenging activities, as well as their ability to inhibit lipid oxidation (Aly *et al.*, 2017; Abd Elalal *et al.*, 2021; Gharib *et al.*, 2022; Elhassaneen *et al.*, 2024a and Gouda *et al.*, 2024).

Total volatile basic nitrogen (TVB-N) formation:

The TVB-N content in meatballs incorporated with GCS extracts throughout the storage period is illustrated in Figures (6 and 7). The data reveal that TVB-N levels in the meatball samples were significantly ($P \leq 0.05$) influenced by both the extract treatments and the storage duration. For all extract-treated meatball samples, TVB-N values were notably ($P \leq 0.05$) lower than those in the control samples. The most significant effect was observed for EtE, followed by MeE and AqE, respectively. These findings align with those reported by several researchers who have used natural antioxidants in cooked meat products (El-Dashlouty, 1978; Chatepa *et al.*, 2021 and Elhassaneen *et al.*, 2023). TVB-N refers to a group of nitrogenous compounds, including ammonia, amines, and other volatile nitrogenous substances, that form during meat spoilage due to microbial and enzymatic actions. It is commonly used as an indicator of the freshness and quality of meat. Elevated levels of TVB-N generally signify advanced spoilage and are often measured to monitor the meat's shelf life and storage conditions (Bekhit *et al.*, 2021). The importance of TVB-N lies in its reflection of the microbial breakdown of proteins in meat, which can lead to the formation of compounds, such as trimethylamine (TMA), responsible for off-putting odors and potentially harmful effects when consumed in excessive amounts.

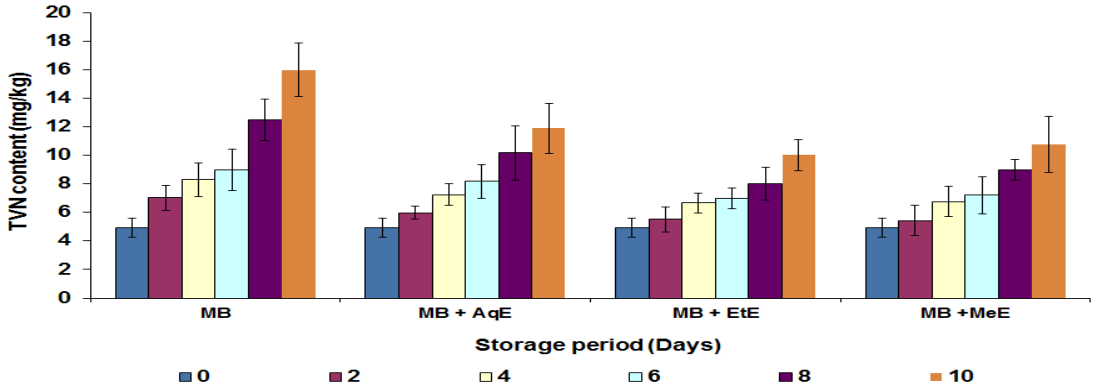

Fig.6. The effect of Garden cress seeds (GCS) extracts on total volatile basic nitrogen (TVB-N, mg/kg sample) formation in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates ±SD. (MB) Meatball; (AqE) Aqueous Extract; (EtE) Ethanol Extract; (MeE) Methanol Extract.

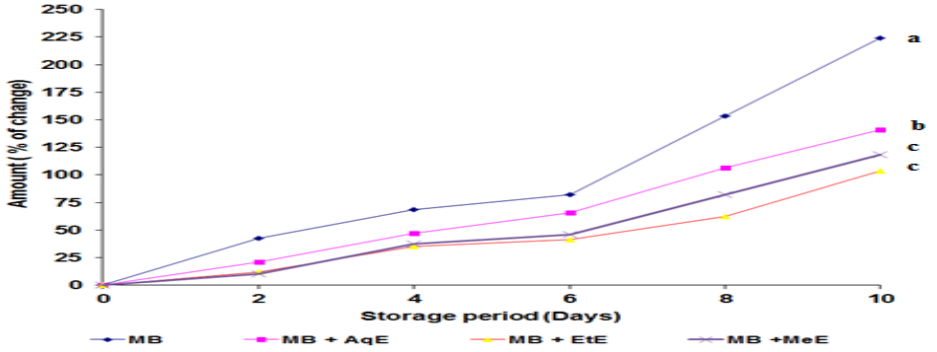

Fig.7. The effect of Garden cress seeds (GCS) extracts on total volatile basic nitrogen (TVB-N, as a percent of control) formation in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates. Means with distinct letters among the different treatments at the end of the storage period are significantly different at $p \leq 0.05$.

Research on toxicity has shown that excessive intake of TMA, a component of TVB-N, may be linked to adverse health effects, including cardiovascular diseases and other metabolic disorders (Zuo *et al.*, 2020). Thus, monitoring TVB-N levels is crucial for ensuring both food safety and product quality. Several studies have highlighted that the rate at which TVB-N forms is influenced by factors, such as microbial contamination, the type of meat, and the storage conditions and duration. The current study, in agreement with others, indicates that various technological treatments, including fermentation, the use of tenderizing agents, and the application of natural antioxidant extracts, can delay protein degradation and reduce the rate of TVB-N formation (Elhassaneen, 1990; El-Kholie, 1994; Hassan, 2013; Chatepa *et al.*, 2021 and Elhassaneen *et al.*, 2023). Additionally, our data demonstrated that all GCS extracts applied to cooked meatballs exhibited strong antioxidant activity due to their high content of bioactive compounds such as phenolics, carotenoids, total anthocyanins, polysaccharides, flavonoids, terpenoids, triterpenoids, tannins, and alkaloids. These bioactive compounds showed antioxidant and free radical scavenging properties, which may be effective in reducing TVB-N formation (Aly *et al.*, 2017; Abd Elalal *et al.*, 2021; Gharib *et al.*, 2022 and Elhassaneen et al., 2024 b, c).

Lactic acid bacteria (LAB) counts:

The LAB counts in meatballs with added GCS extracts throughout the storage period are illustrated in Figures (8 and 9). The results show that the LAB count in the meatball samples was significantly ($P \leq 0.05$) influenced by both the type of extract treatment and the length of the storage period. At the beginning of storage, LAB values in all GCS extract-treated samples were notably ($P \leq 0.05$) lower than those in the control samples. The LAB count for the control meatballs started at 1.01 log10 cfu/g and increased to 5.03 log10 cfu/g by the end of the storage period, showing an increase of 398.02%. In contrast, all GCS extract treatments resulted in a reduction of LAB counts over the storage period. At the end of storage, the greatest reductions in LAB counts were observed in the EtE-treated samples (96.04%), followed by MeE (133.06%) and AqE (194.06%), as compared to the initial counts. These findings are consistent with those reported by other researchers who applied natural antioxidants to cooked meat products (Elhassaneen & Esa, 2015; Essa, 2015 and Elhassaneen *et al.*, 2023). The results of this study, along with others, confirm that several factors affect LAB counts, including the fermentation process, storage conditions, contamination by spore-forming and heat-resistant strains, meat fat content, physical interactions within the food matrix, and water activity in the product, in addition to the application of natural antioxidant extracts (Borch *et al.*, 1996; Kuri, 1998; Davies *et al.*, 1999; Lario *et al.*, 2003; Hassan, 2013; Hegazy, 2014 and Elhassaneen *et al.*, 2023). The GCS extracts used in this study contain a variety of bioactive compounds, such as phenolics, carotenoids, flavonoids, polysaccharides, terpenoids, triterpenoids, tannins, and alkaloids. These bioactive compounds possess known antibacterial and antifungal properties, which likely inhibited LAB growth, thereby reducing the formation of TVB-N and TBARS (Abd Elalal *et al.*, 2021; Gharib *et al.*, 2022; Elhassaneen *et al.*, 2023).

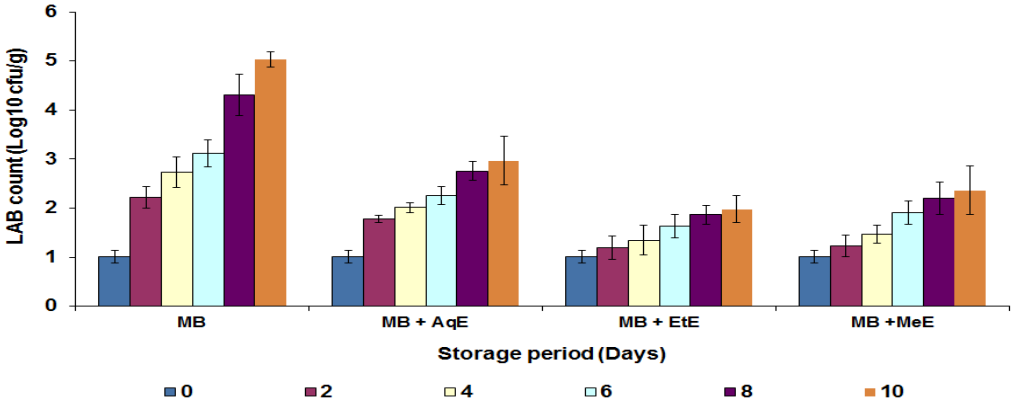

Fig .8. The effect of Garden cress seeds (GCS) extracts on lactic acid bacteria counts (LBC, log10 cfu/g) in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates ±SD.

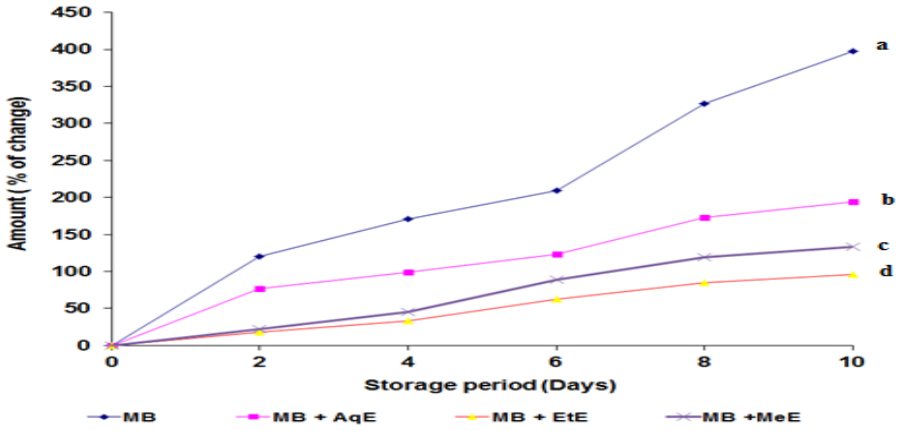

Fig. 9. The effect of Garden cress seeds (GCS) extracts on lactic acid bacteria counts (LBC, log10 cfu/g) in meatball samples stored at 4°C for 10 days

Each value denotes the mean of three replicates. Means with distinct letters among the different treatments at the end of the storage period are significantly different at p≤0.05.

CONCLUSION

The results of the current study highlight the proximate composition of Garden cress seeds (GCS), which demonstrates its potential as a nutrient-rich food, containing high levels of protein, fat, fiber, and carbohydrates. The nutritional profile of GCS powder is similar to other high-protein, energy-dense seeds, making it an ideal supplement for individuals looking to enhance their protein and energy intake. Additionally, GCS is rich in important minerals, such as Ca, K, Mg, and Fe, suggesting that it could be a valuable addition to a well-balanced diet, particularly for those seeking plant-based alternatives to essential micronutrients. Furthermore, GCS is a promising source of essential vitamins, particularly Vitamin A and Vitamin E. However, its Vitamin C and B Vitamin concentrations are relatively moderate when compared to other more common dietary sources. The bioactive compound content in GCS extracts varies depending on the solvent used for extraction. The ethanol extract consistently displayed the highest concentrations of beneficial compounds, including flavonoids, terpenoids, tannins, and kaempferol, while the aqueous extract contained higher levels of anthocyanins. These findings suggest that GCS, especially when extracted with ethanol, is a rich source of bioactive compounds with potential health benefits. The differences in compound concentrations across various extraction methods emphasize the importance of selecting the appropriate solvent for specific therapeutic or nutritional uses. In vitro studies have shown that GCS can act as a natural antioxidant, helping to slow lipid oxidation, protein degradation, and bacterial growth in various food products, particularly meat. Furthermore, GCS shows promise as an effective agent for preventing atherosclerosis by inhibiting the oxidation of LDL cholesterol.

Ethical considerations

The ethical considerations of this study were examined and approved by the Scientific Research Ethics Committee at the Faculty of Home Economics, Menoufia Uni., Shebin El-Kom, Egy. (Approval # 12-SREC-03-2024).

Conflict of interest

The authors confirm that there are no conflicts of interest regarding the publication of this paper.

Authors' Contribution

All authors made equal contributions to all stages of this research, from designing and developing the study protocol to reviewing and refining it. They were hands-on in conducting the experiments, overseeing the procedures, collecting and analyzing the data, and interpreting the results. Their involvement also extended to gathering relevant background information, drafting the manuscript, critically assessing its content, and providing final approval for its publication.

Abbreviations

(GCS) Garden cress seeds, (AA) antioxidant activity, (Abs) absorbance, (AAC) antioxidant activity coefficient, (EtE) ethanol extract, (AqE) aquatic extract, (DMSO) dimethyl sulfoxide, (FRSA) free radicals scavenging activity, (LAB) lactic acid bacteria, (LDL) low-density lipoprotein, (DPPH) 2,2-diphenyl-1-picrylhydrazyl, (MDA) malonaldehyde, (MeE) methanol extract, (ORR) oxidation rate ratio, (SD) standard deviation, (TBA) thiobarbituric acid, (TVB-N) total volatile base-nitrogen.

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الملخص العربي

المغذيات والمركبات النشطة بيولوجيًا والأنشطة المضادة للأكسدة لبذور حب الرشاد وتطبيقاتها في تكنولوجيا الأغذية والتغذية العلاجية

مروة عز الدين إبراهيم، يوسف عبد العزيز الحسانين ، أمانى أحمد عبد العزيز

تهدف الدراسة الحالية إلى استكشاف المغذيات والمركبات النشطة بيولوجيا والأنشطة المضادة للأكسدة في بذور حب الرشاد (*Lepidium sativum* L.)، كما تم أيضًا فحص التأثيرات الوقائية المحتملة لمستخلصات حب الرشاد ضد تصلب الشرايين وجودة اللحوم باستخدام الأنظمة النموذجية معمليا خارج الجسم. ولقد أظهر التركيب الكيميائي لبذور حب الرشاد عن إمكانياتها كغذاء غني بالمغذيات، حيث يحتوي على البروتين والدهون والألياف والكربوهيدرات. بالإضافة إلى ذلك، فهو مصدر ممتاز للمعادن الأساسية مثل الحديد والبوتاسيوم والكالسيوم والمغنيسيوم، كما أنها مصدر واعد للفيتامينات، وخاصة بادىء فيتامين A وفيتامين E. كما يختلف محتوى المركبات النشطة بيولوجيا في مستخلصات بذور حب الرشاد حسب المذيب المستخدم فى استخلاصها. ولقد أظهر مستخلص الإيثانول أعلى تركيزات من المركبات المفيدة، بما في ذلك الفلافونويدات، التربينويدات، التانينات، وكامبفيرول، بينما احتوى المستخلص المائي على مستويات أعلى من الأنثوسيانين. كما أظهرت جميع مستخلصات بذور حب الرشاد أنشطة قوية مضادة للأكسدة وقدرة على مسح الجذور الحرة. كما سجلت IC_{50} باستخدام اختبارالـ DPPHللمستخلص المائى (AqE) والإيثانول (EtE) والميثانول (MeE) قيما مقدارها 15.12±0.28 و 11.79±0.34 و 13.12±0.31 ميكروغرام/مل على التوالي، بينما سجل المركب القياسي BHT قيمه مقدارها 8.95±0.14 ميكروغرام/مل. وبالتالي، وتبعاً لأنشطة مسح الجذور الحرة لمستخلصات بذور حب الرشاد كان الترتيب التالى: BHT > EtE > MeE > AqE. وأخيراً أظهرت الدراسات المعملية خارج الجسم أن بذور حب الرشاد يمكن أن تعمل كمضاد أكسدة طبيعي، يساعد في منع أكسدة الدهون، وتحلل البروتين، ونمو البكتيريا في كرات اللحم. علاوة على ذلك، أظهرت بذور حب الرشاد إمكانات كعامل فعال في الوقاية من تصلب الشرايين عن طريق منع أكسدة الكوليسترول منخفض الكثافة (LDL-c).

الكلمات المفتاحية: التركيب الكيميائي، التقييم الغذائي، الفيتامينات، المعادن، القيمة الاستخراجية، DPPH، نشاط الكسح، كرات اللحم، تصلب الشرايين.