Phytochemicals, phenolic compounds and antioxidant activity of garden cress (Lepidium sativum L.) seeds

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ABSTRACT

Garden cress (*Lepidium sativum* L.) is an annual herb that is native to West Asian. Although it is now planted all over the world. It is grown for its seeds, as it is the most widely used part. The chemical composition of garden cress (GC) seed powder was examined. The results revealed that GC contains10.39% moisture,22.42% protein,25.57% fat and 22.19% carbohydrates. Moreover, the recorded ash and fiber content were 9.60% and 9.83%, respectively. Also, phytochemical analysis of GC extracts showed that plant extracts contain tannins, saponins, alkaloids, terpenoids, flavonoids, phenols, phytosterols and sterols. The highest amount of total phenolic was found in the ethanol extract, which gave 25.13 µg/ml of gallic. While the highest amount of total flavonoids was found in the methanol extract, which gave 41.30 µg/ml of rutin. The HPLC analysis was carried out on the ethanolic and methanolic extracts of garden cress. Seven phenolic acid compounds have been detected in ethanolic extract including catechol, syringenic, cinnamic, caffeic, pyrogallol, gallic and ellagic, while nine phenolic compounds have been detected in methanolic extract, including catechol, syringenic, cinnamic, caffeic, gallic, ferulic, salicylic, ellagic, and benzoic. Furthermore, the methanol extract of garden cress showed the highest DPPH scavenging activity compared to ethanol extract. It can be concluded that GC seeds and their extracts can be used in different fields such as nutritional supplements and medical fields because it is rich in antioxidants.

Keywords: Garden cress; Phytochemical; Total phenolics; Total flavonoids; Antioxidant activity.

INTRODUCTION

A member of the Cruciferae (Brassicaceae) family is *Lepidium sativum*. Its popular name is garden cress, and it is a plant that is most commonly grown in hot, and temperate areas around the world for both culinary and medicinal purposes. It is native to Southwest Asia (Shabbir *et al.,* 2018).

Garden cress seeds, leaves, and roots are all valuable commercially. The plant is primarily farmed for its seeds. Garden cress seeds are galactogogo, diuretic, thermogenic, antiseptic, bitter, expectorant, antihistamine, antispasmodic, aphrodisiac, diaphoretic, and stimulant. It is used as a treatment for various diseases such as cough with expectoration, asthma, diarrhea, leprosy, dysentery, skin diseases, and poultices for sprains, lumbago, seminal weakness, enlarged spleen, scurvy, indigestion, and runny nose, which can be treated with cress seeds. Moreover, GC seeds consist of 13-17% seed, 80-85% endosperm, 24% protein, 34-55% carbohydrates, 12-24% fat, 8% fiber (Adera *et al.,* 2022). GC seeds can be used in drinks either crushed in honey or as an infusion in hot milk. They can also be used fresh, dry, or cooked. Garden cress has numerous pharmacological properties. Leaf and seed extracts have been shown to have antihypertensive activity, while seed extracts have also shown to be hepatoprotective, hypoglycemic, and effective in the treatment of bronchial asthma. Its young leaves can be either raw or fried. Chemical analysis has revealed that seeds and leaves include flavonoids, choline ester (sinapin), ascorbic acid, vitamin A, and secondary metabolites Ait-Yahia *et al*., (2015). Chatoui *et al*., (2016) reported that the phytochemicals screened from the methanolic extract of GC showed good result for alkaloids, sterols, flavonoids, tannins, saponins, and Polyterpene. The extracts of GC seeds had a high amount of total phenolics and total flavonoids. Gallic acid was the most abundant phenolics in GC seed extract having a value of 3001.75µg/100g (El-Salam *et al*., 2019). Also, DPPH tests, the antioxidant activity of GC extract implement percentage more than 89%. Selek *et al*., (2018) found that the antioxidant content and activity of the methanol extract of *Lepidium sativum* was investigated in vitro. The extract contained high amounts of phenolic and flavonoid compounds and showed significant antioxidant activity.

Therefore, the aim of this study is to analyze the chemical composition of GC seeds,

phytochemical screening, determine the total phenolic contents, total flavonoids contents, Identification of phenolic compounds by HPLC and antioxidant activity of extracts results using different organic solvents by DPPH method.

MATERIALS AND METHODS

Materials

Garden cress (*Lepidium sativum* L.) seeds were purchased from the local market, Mansoura City, Dakahliya Governorate, Egypt. The seeds have been cleaned and made free of dust, dirt, foreign matter, and broken seeds. All chemical standards and reagents were of analytical grade and were purchased from El Gomhouria Co., Cairo Branch, Egypt.

Methods

Preparation of garden cress seeds extract

Garden cress (*Lepidium sativum* L.) seeds were used for extraction according to (Ferrigni *et al.,* 1982). The seeds were purified and ground. (80 g) was extracted with 800 ml ethanol 70% (v/v) and methanol on a rocking apparatus and soaked for 24 hrs. The samples were then filtered using Whatman No. 1 filter paper. Then the solvents were evaporated from the samples efficiently and carefully by an apparatus rotary evaporator. Then keep at 4°C until uses.

Chemical composition of garden cress seeds

Quantitative estimation of the chemical composition (Fat, moisture, protein, ash, fibers, and total carbohydrates) of the collected seeds from of the studied garden cress was achieved with the electromagnetic spectrum using a Near-infrared (NIR) Spectrometer, model DA1650, which is manufactured by FOSS Corporation (Taha *et al.,* 2016), according to (AOAC, 2010).

Carbohydrates content was calculated by difference from the following equation:

% Carbohydrates content = 100 – (% Protein + % Moisture + % Ash + % Lipids + % Fiber).

Phytochemical screening of garden cress seeds

The following phytochemicals were qualitatively determined in garden cress seeds extracts: Alkaloids, tannins and terpenoids were detected by Wagner's, Braemer's and Salkowski test, respectively according to (Sasidharan *et al.,* 2011). Flavonoids and phytosterols were detected by alkaline reagent and Salkowski test, respectively according to (Tiwari *et al.,* 2011). Phenolic was detected by ferric chloride test according to (Cai *et al.,* 2011). Saponins and steroids were detections by the froth and Salkowski test, respectively according to (Savithramma *et al.,* 2011).

Determination of total phenolic content (TPC)

Total phenol content (TPC) in each extract was determined using the Folin-Ciocalteu's method described by (Do *et al.,* 2014). Total phenolic of the plant extracts was determined by relation to that of the Gallic equivalents (μg/ml). A standard curve with the linear equation $y = 0.004x + 0.1474$ was generated as shown in Figure (1).

Determination of total flavonoids content (TFC).

Flavonoid contents were determined in each extract according to the aluminum chloride colorimetric method described by (Do *et al.,* 2014). Total flavonoids of the plant extracts were determined by relation to that of the Rutin equivalents (μg/ml). A standard curve with the linear equation $y = 0.0021x + 0.5603$ was generated as shown in Figure (2).

Identification of phenolic compounds using HPLC

Total phenolic compounds were extracted and subjected to HPLC analysis according to (Spanos *et al.,* 1990; Schieber *et al.,* 2001 & Tsao and Yang, 2003). HPLC analysis was carried out using a GBC 1100 Series HPLC system equipped with a UV detector. Phenolic were separated using C18 column (250 mm × 4.6 mm; 5μm). The mobile phase consists of 10.2% acetic acid in 2 mM sodium acetate (solvent A) and acetonitrile (solvent B). The flow rate was kept constant at 1 ml/min for a total run time of 20 min at 25°C. The system was run with an isocratic program, (70:30) (B: A) at wavelength 300 nm, The injection volume was 50 μl extract.

Data Analysis

The tests were performed in triplicate and the means were recorded, Complete Randomized Design analysis for all data obtained was carried out with three replications and differences between means were calculated using L.S.D test according to (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical Composition of GC seeds

The chemical composition of garden cress seeds is illustrated in Table (1). GC seeds powder contained 10.39% moisture, 22.42% protein, 25.57% fat and 22.19% carbohydrate. Furthermore, recorded ash and fiber contents were 9.60% and 9.83%, respectively. Based on these findings, it can be concluded that the macronutrient content is both high and suitable for human nutrition. These findings nearly agree with earlier findings by Kharkwal *et al.* (2021), who reported that the chemical of GC seeds includes 5.67% moisture, 29.06% protein, 20.55% crude fat, 6.76% crude fibre, 5.8% ash, and 38.26% carbohydrate. GC seeds have a chemical that includes 4.8% ash, 7.05% moisture, 14.18% fat, 18.79% fibres, 19.73% protein, and 35.45% carbohydrate according to El-Salam *et al.* (2019). Zia-Ul-Haq *et al.* (2012) observed the proximate chemical compositions the moisture 2.9%, crude protein 24.2%, crude fat 23.2%, carbohydrate 30.7%, crude fiber 11.9%, and ash 7.1%. It was clear from previous studies that the leftovers from pressing GC seeds might be utilized to create goods with excellent health benefits. It is advised to consume plant proteins in order to maintain excellent health and nutrition and to treat problems such as cancer, diabetes, obesity, and heart disease (Kumar *et al.,* 2021).

Phytochemical screening

Qualitative phytochemical analysis of garden cress seeds extracts illustrated in Table (2) indicated the presence of tannins, saponins, alkaloids, terpenoids, flavonoids, phenols, phytosterols and steroids in ethanol and methanol extracts. These results are consistent with Al-Snafi (2019), who mentioned that garden cress contained alkaloids, phenolic, flavonoids, saponins, sterols, tannins. Flavonoids, alkaloids, saponins, sterols, terpenoids, and tannins were found to be the phytochemical components contained in the methanolic extract, according to Chatoui *et al.* (2016). According to Ahmad *et al.* (2015), phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, and phenolic compounds. Prajapati *et al.* (2014) indicated that seeds are mainly contains alkaloids, saponins, flavonoids, and sterols. Ghante *et al.* (2011) who found that seeds contain flavonoid, triterpene, steroids, tannins, alkaloids, and saponins. Manohar *et al.* (2012) reported that seeds mainly contain alkaloids, saponins, flavonoids, sterols. Garden cress is high in proteins, carbohydrates, steroids, saponin, and triterpenoids with various pharmacological effects, according to Shah *et al.* (2021).

Total phenolic content

The amounts of total phenols in the different extracts are presented in Table (3). The total phenol content ranged from 19.34 to 25.13 μg/ml as gallic equivalents. The highest amount was found in the ethanolic extract of garden cress, which gave 25.13 μg/ml of gallic. The lowest amount was found in methanolic extract of garden cress, which gave 19.34 μg /ml of gallic. Türkoğlu *et al.* (2018) reported that garden cress contains phenolic compounds. The positive effects may be as a result of its antioxidant features due to the polyphenols and organosulphur compounds. According to the "like dissolves like" concept, the compatibility of phenolic compounds with the solvent system directly affects the extraction of phenolic compounds (Zhang *et al.,* 2007). The most prevalent phyto-antioxidants are terpenes and polyphenols. Their polarity, solubility, and molecular weight are used to make this differentiation. By preventing lipid peroxidation, phenolic groups have an impact on protein phosphorylation. The most prevalent terpenes are carotenoids, which operate as singlet oxygen quenchers, and the most prevalent polyphenols are flavonoids and stilbenes (Pandey and Rizvi, 2009). By reacting with different biomolecules, seed extracts, which are rich in flavonoid and phenolic chemicals, can cause oxidative damage while simultaneously possessing antioxidant qualities (Hoang *et al.,* 2021).

Total flavonoid content

Total flavonoid content of the seed extracts is presented in Table (3). The total flavonoid content ranged from 23.73 to 41.30 μg/ml as equivalents to rutin. The highest amount was found in the methanol extract of cress which gave 41.30 μg/ml of rutin. The lowest amount found in ethanol extract of garden cress, which gave 23.73 μg/ml. These results are consistent with those obtained by (El-Salam *et al.,* 2019), who reported that flavonoids are widely present in the plant kingdom. Cress seeds have been shown to contain at least four types of flavonoids, including rutin. Flavonoids have long been known to possess many medicinal effects e.g., anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, anticoagulant, antiviral, and anticancer activities. Phytochemicals from plants are utilized to prevent a variety of ailments, mostly those brought on by free radicals (Prakasha *et al.,* 2001). Similar research indicates that using synthetic antioxidants in excess may be harmful to your health. Antioxidants can be

employed to treat cancer. It is well known that plants produce natural antioxidant substances that might lessen the amount of oxidative stress brought on by oxygen and sunlight (Babbush *et al.,* 2020).

HPLC analysis of phenolic compounds

The HPLC analysis was carried out on the ethanolic extract of garden cress. The detailed tabulations of HPLC analysis of the extracts are given in Table (4) and Figure (3) from the analysis, seven phenolic acid compounds have been detected in ethanolic extract obtained from garden cress. Catechol (2.46 µg/ml), Syringenic (2.06 µg/ml), Cinnamic (3.04 µg/ml), Caffeic (2.14 µg/ml), Pyrogallol (0.69 µg/ml), Gallic (2.14 µg/ml) and Ellagic (3.09 µg/ml). These results are in agreement with Sethiya *et al.* (2014), who indicated that phenolic compounds are present in the ethanol extract. Gallic acid was the main phenolic compound followed by ellagic and protocatechuic. In addition, pyrogallol was found at the lowest level (Zia-Ul-Haq *et al.,* 2012; Panwar and Guha, 2014).

The HPLC analysis was carried out on the methanolic extract of garden cress. The detailed tabulations of HPLC analysis of the extracts are given in Table (5) and Figure (4). From the analysis, nine phenolic compounds have been detected in methanolic extract obtained from garden cress, including Catechol (8.98 µg/ml), Syringenic (1.66 µg/ml), Cinnamic (2.54 µg/ml), Caffeic (10.23 µg/ml), Gallic (9.87 µg/ml), Ferulic (2.55 µg/ml), Salicylic (1.42 µg/ml), Ellagic (8.06 µg/ml)), and Benzoic (1.05 µg/ml). According to HPLC analysis, the levels of each detected compound's individual phenolics increased by many fold, and new phenolic compounds appeared in the methanolic extract including ferulic, salicylic, and benzoic. These results are in agreement with (Abdel-Aty *et al.,* 2019), who indicated that phenolic compounds present in methanol extract seeds are Gallic acid and protocatechuic acid, gallic acid was the main phenolic compound followed by ellagic, protocatechuic, rutin and ferulic acid. In addition, pyrogallol was found at the lowest level.

DPPH radical scavenging activity

The plant extracts showed concentration-dependent scavenging activity by quenching DPPH roots as mentioned in Table (6). The hydrogen donation activity was demonstrated using concentrations of 100 µg/ml, 500 µg/ml, and 1000 µg/ml respectively. The highest amount was found in the methanolic extract of garden cress with a percentage of 99, 99.2 and 99.3 respectively. The ethanol extract came in the second category 87.5, 85.8 and 83.7 respectively.

Research on vegetable sources and the screening of raw materials for new antioxidants have been encouraged by the increased interest in replacing synthetic food antioxidants with natural ones. Antioxidants are widely required to prevent the deterioration of other oxidizable commodities, such as cosmetics, pharmaceuticals and foods as oxidation processes are a problem for the food business. The main plant components having antioxidant action are polyphenols (Moure *et al.,* 2001). The present findings support the findings of Chatoui *et al.* (2016), who reported that extracts of GC are effective at scavenging free radicals at all concentrations. Garden cress was used in this study as it showed a good ability to remove free radicals in all concentrations and extracts studied. The results are also in agreement with Attia *et al.* (2019), who found that the antioxidant activity of garden cress extracts has a higher potential for radical scavenging likely as a result of a higher content of antioxidant species.

CONCLUSION

Results of these study revealed that plant extracts obtained from garden cress have promising in vitro antioxidant activity against various antioxidant systems due to the presence of various phytochemicals which counteract the free radicals responsible for various health complications. We report that the seeds of garden cress contain antioxidants that enhance the immune system in the body and have beneficial effects on human health when they drink and eat continuously. The effect of various plant extracts showed a strong antioxidant effect.

Finally, this study paves the way for the development of several treatment regimens based on these extracts. Therefore studies need to be undertaken to elucidate other unidentified compounds and mechanisms of action of these compounds at the molecular level as well as in vivo studies.

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Table 1: The chemical composition of garden cress seeds powder

Components	Percentages (%)
Moisture	10.39
Protein	22.42
Fat	25.57
Ash	9.60
Fiber	9.83
Carbohydrate	22.19

Table 2: Phytochemical screening of ethanol and methanol of garden cress.

Compound	Ethanol	Methanol
Tannins	+++	++
Saponins	+	+
Alkaloids	++	++
Terpenoids	+++	++
Flavonoids	+++	++
Phenols	+++	+++
Phytosterols	++	++
Steroids	++	++

Legend: Positive (+) , Negative (-).

Table 3: Total phenolic and total flavonoid Contents of garden cress seed extracts

Extracts	Total phenolic (µg gallic /ml)	Total flavonoid (µg rutin/ml)
Ethanol	25.13 ± 0.020	23.73 ± 0.38
Methanol	19.34 ± 0.020	41.30 ± 0.22
L.S.D 0.05	0.39	0.71

Table (4): HPLC Analysis of ethanol extract of garden cress seeds

RT#	Compound	(Concentration µg/ml)
4.2	Catechol	2.46
5.1	Syringenic	2.06
7.0	Cinnamic	3.04
8.0	Caffeic	2.14
9.0	Pyrogallol	0.69
9.8	Gallic	2.14
12.8	Ellagic	3.09

Table 5: HPLC Analysis of methanol extract of garden cress seeds

RT#	Compound	(Concentration µg/ml)
4.0	Catechol	8.98
5.1	Syringenic	1.66
7.0	Cinnamic	2.54
8.0	Caffeic	10.23
9.7	Gallic	9.87
11.0	Ferulic	2.55
12.0	Salicylic	1.42
12.9	Ellagic	8.06
15.2	Benzoic	1.05

Table 6: DPPH radical-scavenging activities of garden cress seed extracts

Extracts	DPPH Radical scavenging activity (%)		
	100 µg/ml	500 µg/ml	1000 µg/ml
Ethanol	87.5 ± 0.023	85.8 ± 0.013	83.7 ± 0.036
Methanol	99 ± 0.014	99.2 ± 0.007	99.3 ± 0.004
L.S.D 0.05	0.59	0.87	0.38

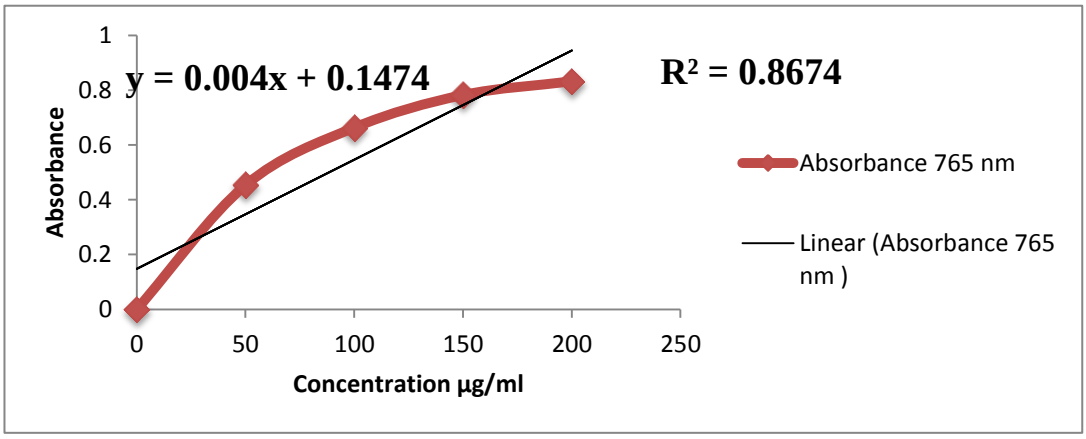

Figure 1: Gallic standard curve for determination of total phenolic

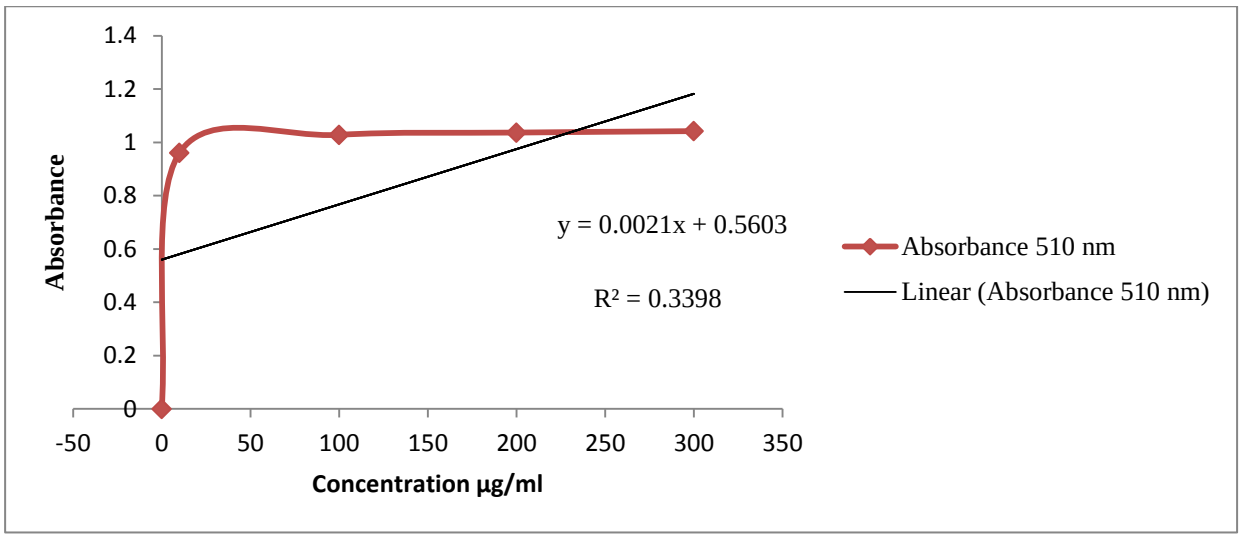

Figure 2: Rutin standard curve for determination of total flavonoid**.**

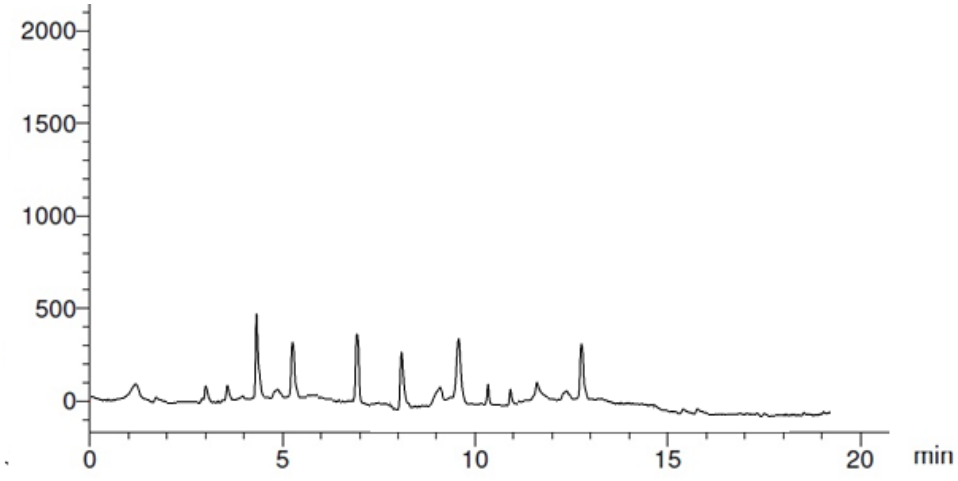

Figure 3: Identification of ethanol extract of garden cress seeds.

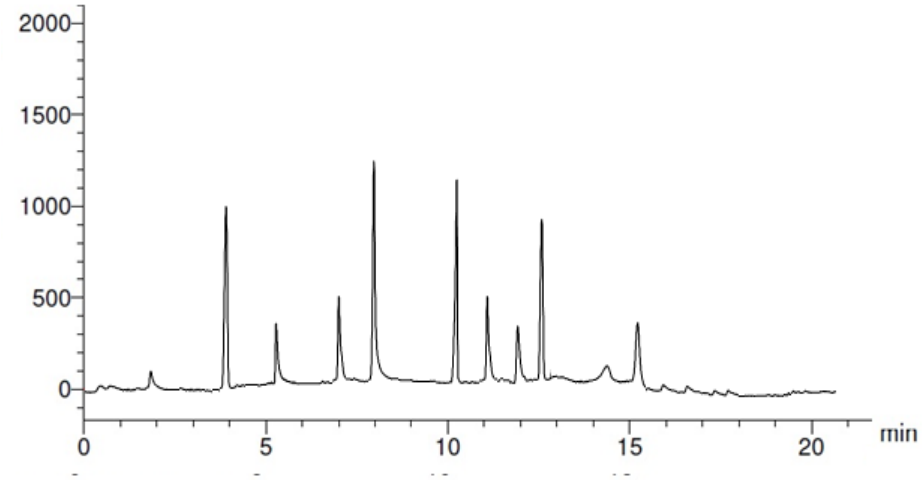

Figure 4: Identification of methanol extract of garden cress seeds.

المواد الفيتوكيميائية والمركبات الفينولية ونشاط مضادات الأكسدة لبذور حب الرشاد (.Lepidium sativum L)

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الملخص العربي

حب الرشاد هو عشب سنوي سريع النمو ينمو في إثيوبيا ومصر ودول غرب آسيا، على الرغم من أنه يُزرع الآن في جميع أنحاء العالم .يُزرع من أجل بذوره ، فهى الجزء الأكثر استخدامًا .تمت دراسة التركيب الكيميائي لمسحوق بذور حب الرشاد. أوضحت النتائج أن بذور حب الرشاد تحتوي على 10.39٪ رطوبة ، 22.42٪ بروتين خام ، 25.57٪ دهن خام ، 21.40٪ كربوهيدرات .علاوة على ذلك ، كان محتوى الرماد والألياف 9.60٪ و9.83٪ على التوالي .كما أظهر التحليل الكيميائي النباتي لمستخلصات بذور حب الرشاد أن المستخلصات النباتية تحتوي على التانينات ، والصابونين ، والقلويدات ، والتربينويدات ، والفلافونويدات ، والفينولات ، والفيتوستيرول ، والستيرولات .أعلى كمية من الفينولات الكلية وجدت في مستخلص الايثانول والتي بلغت 25.13 ميكروغرام / مل من الجاليك .بينما وجدت أعلى كمية من مركبات الفلافونويد الكلية في مستخلص الميثانول والتي بلغت 41.32 ميكروجرام / مل من الروتين .تم إجراء تحليل HPLC على المستخلصات الإيثانولية والميثانولية من بذور حب الرشاد. كشفت النتائج عن سبع مركبات فينولية في المستخلص الإيثانولي مثل كاتيكول ، سيرينجينيك ، سيناميك ، كافيك ، بيروجالول ، جاليك وإيلاجيك ، بينما كشفت النتائج عن تسع مركبات فينولية في المستخلص الميثانولي مثل كاتيكول ، سيرينجينيك ، سيناميك ، كافيك ، جاليك ، فيروليك ، ساليسيليك و إيلاجيك و البنزويك. علاوة على ذلك ، قدرت فاعلية مضادات الأكسدة باستخدام طريقة DPPH ، أوضحت النتائج ان مستخلص الميثانول من بذور حب الرشاد أعلى فعالية مقارنة بمستخلص الإيثانول . أخيرا، يمكن الاستنتاج أن بذور حب الرشاد ومستخلصاتها يمكن استخدامها في مجالات مختلفة مثل المكملات الغذائية والمجالات الطبية لأنها غنية بمضادات الأكسدة.

الكلمات الاسترشادية: رشاد الحديقة ، المواد الكيميائية النباتية ، الفينولات الكلية ، مركبات الفلافونويد الكلية ، النشاط المضاد للأكسدة.