

Article

The Definition of The Percentage of Fatty Acids In The Seeds of The Plants (Eruca Sativa - Radish - Celery) and Study Their Antibacterial Properties

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Abstract: The content of seed oil of radish, watercress and celery extracted with hexane and determination of percentages of fatty acids was investigated using TLC chromatography and GC-Mass gas chromatography. Results showed that plant seeds contain the following compounds: Eruca sativa : Palmitic acid, methyl ester, Stearic acid, methyl ester, Methyl 18-methylnonadecanoate, Erucic acid, Tricosanoic acid, methyl ester, 15-Tetracosenoic acid, methyl ester, Tetracosanoic acid, methyl ester. Radish: Palmitic acid, methyl ester, Stearic acid, methyl ester, Linolenic acid, ethyl ester, Methyl 18-methylnonadecanoate, Behenic acid, methyl ester, Ethyl 13-docosenoate, 15-Tetracosenoic acid, methyl ester, Tetracosanoic acid, methyl ester. Celery: Eudesma-4(14),11-diene, 1-Propanone, 2-chloro-1-(2,4 dimethylphenyl)-2-methyl, Phenol, 4-(2-aminoethyl), Palmitic acid, methyl ester, Stearic acid, methyl ester, Oleic acid, ethyl ester.

Keywords: Eruca sativa, Radish, Celery, seeds oil, fatty acids.

Citation: Ziad K N. The Definition of The Percentage Of Fatty Acids in The Seeds of The Plants (Eruca Sativa – Radish – Celery) and Study Their Antibacterial Properties. Central Asian Journal of Medical and Natural Science 2026, 7(1), 443-452.

Received: 25th Oct 2025
Revised: 17th Nov 2025
Accepted: 27th Nov 2025
Published: 24th Dec 2025



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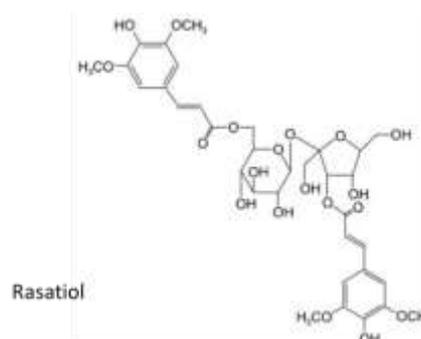
1. Introduction

Medicinal plants have become a major part of agricultural and industrial production and receive great attention from many producing countries. It is the primary source of pharmaceutical drugs (or the source of chemical substances used in drug preparation), and is considered the core of the synthesis of certain chemicals such as cortisone, sex hormones, plasma substitutes, and others. Therefore, medicinal plants are among the most important materials in the pharmaceutical industry. [1] The ancient Egyptians, since the early third millennium BC, were interested in medicinal plants, controlling and using them more than 4000 or 5000 years BC. A medicinal plant is defined as one or more of its different parts containing one or more chemical substances at low or high concentrations that have physiological efficacy against a specific disease, or at least against one of the most common symptoms of that disease. The scientist Dragandroff defined anything of plant origin used in medicine as a medicinal product. [2] Therefore, the chemistry of living organisms (phytochemistry) is a rich source that supports the global pharmaceutical industry by providing new models that possess strong and important physiological activities against many intractable and chronic diseases. [3]

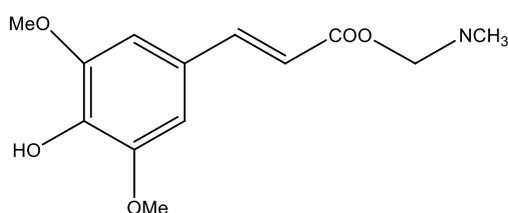
The radish plant (*Raphanus sativus*) belongs to the genus *Raphanus* and the Brassicaceae family. It is an annual or perennial herbaceous plant, both wild and cultivated, and is a vegetable crop that reproduces by seed. Biological studies have shown that radishes have several medicinal benefits, including: treating liver ailments, dissolving kidney stones and gravel, strengthening bones, treating anemia, and aiding in the treatment of asthma and chest diseases in general. [4] The seeds are used as an appetite stimulant and also for indigestion. [5] The juice of the fresh leaves has been used as a diuretic and laxative. [6] Radish seeds contain glucosinolates, which studies have shown to be beneficial in cancer prevention. [7,8] - Recent studies have also demonstrated that 4-methylthiobutanyl derivatives isolated from radish seeds possess anti-tumor properties. [9] Modern chemical studies have focused on the benefits of sulfur compounds in radishes in treating tumors [10].

2. Materials and Methods

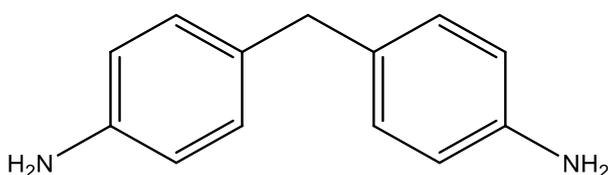
There are many previous studies on the seeds of this plant, including: - Isolating a new compound, Rasatiol, for the first time from radish seeds. Studies have shown that the compound has an anti-inflammatory effect on the growth of dermal fibroblasts. The compound has the following formula:



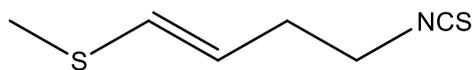
- Sinapine was isolated from radish seeds [11]. It belongs to the alkaloids and has the formula:



- 4,4'-Methylenedianiline was also isolated from the alkaloids [12].



- 4-Methylthio-3-butenyl isothiocyanate is the compound responsible for the pungent taste of radishes [13]. It has the formula:

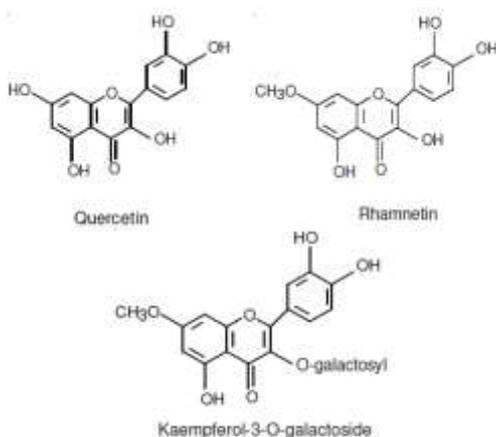


Rocket (*Eruca sativa*) belongs to the genus *Eruca* and the Brassicaceae family. It is an annual or perennial herbaceous plant. Some of its benefits include: Rocket seeds and seed oil have been used in traditional medicine since ancient times to treat various ailments, such as bronchial asthma in adults, and also as a laxative, carminative, and remedy for intestinal diseases. Rocket has been used to treat various diseases, such as dropsy, gout, scurvy, and liver diseases. It is also used as an aphrodisiac, a wound disinfectant, and a diuretic.

Recent studies have also shown that an infusion of the leaves has significant benefits for digestive system ailments, particularly indigestion. Below, we will review some of the plant's chemical studies:

- A study of the watercress plant by Salma Nassrullah Malik revealed that watercress seed extract possesses antibacterial properties against two types of bacteria: *Staphylococcus aureus* and *Bacillus cereus*.

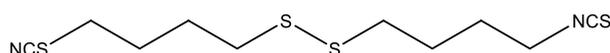
- Our reference survey of watercress seeds showed that most of their compounds are terpenes and flavonoids.



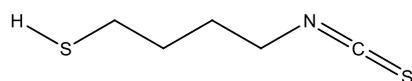
In another study, the two compounds responsible for the taste and flavor of rocket [20,21] were found to be:

1,2-bis(4-isothiocyanatobutyl)disulfane

4-mercaptobutyl isothiocyanate

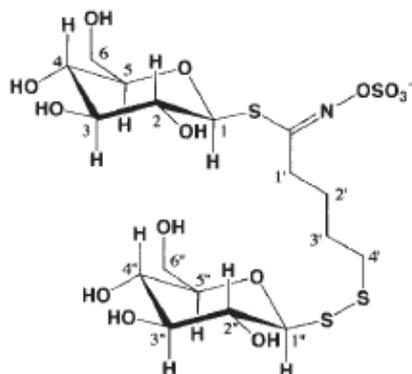


1,2-bis (4-isothiocyanatobutyl)disulfane



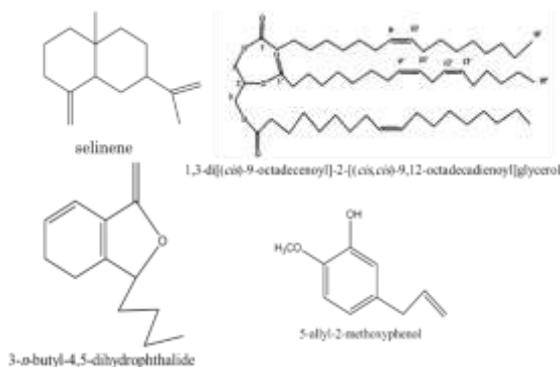
4- mercaptobutyl isothiocyanate

The compound 4-(β-D-Glucopyranosyldisulfanyl)butyl was also isolated for the first time from rocket leaves, and its antioxidant activity was demonstrated.

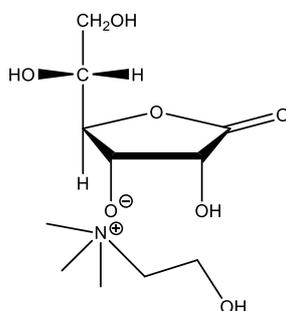


Celery (*Apium graveolens*) belongs to the genus *Apium* and the Apiaceae family. It is an annual herbaceous perennial plant. Some biological studies on this plant show that it is used to treat respiratory illnesses, coughs, and high blood pressure. It is also used as a diuretic and in cases of arthritis. Studies have also shown that it stimulates saliva production. Celery seeds have been used in traditional medicine to treat various ailments, including bronchitis, asthma, and liver and spleen diseases. Numerous chemical studies have been conducted on this plant, including:

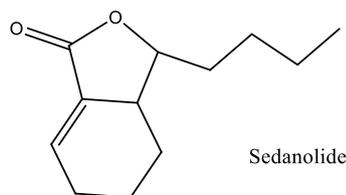
In 2000, four compounds were isolated from celery seeds and found to possess antibacterial and antifungal properties.



The compound Choline ascorbate was first isolated from the celery plant by the scientist Gulsel Uval, and this was the first time that this compound was isolated from a natural source.



- The compound Sedanolide is considered to be the compound responsible for the taste and flavor of celery and has the following formula:



The research aims to study the oil composition of the seeds of (radish, rocket, celery) in terms of fatty acids, determine their ratio, and the effect of these oils on *Escherichia coli* and *Staphylococcus aureus* bacteria.

3. Results

It Radish: Its leaves are long, rough, green (about 13 cm), arranged in a rosette. It has a taproot that can be spherical, tapered, or cylindrical [11], [12]. The root color ranges from white to pink and red, and its length ranges from 7 cm to 12 cm. Its flowers are white and appear in clusters. It blooms in spring or autumn, depending on the surrounding environment. It grows in dry and semi-dry environments and in warm regions. It has a distinctive and strong aroma and a sweet, crisp taste when young, but it becomes bitter and tough if left in the ground for a long time [13], [14], [15].

Rocket: An annual or perennial herbaceous plant that can reach a height of 80 cm. It grows wild and is also cultivated. Native to the Mediterranean basin, it is a leafy vegetable crop that reproduces by seed. The seed diameter is 1.5–2 mm, and the leaves are 10–25 cm long. It flowers in spring, summer, or autumn, depending on the environment. It thrives in dry, semi-arid, and warm climates [16].

Celery: An annual or perennial herbaceous plant, also wild and cultivated. It is a vegetable crop that reproduces by seed. It flowers in spring or summer, depending on the environment, and matures in summer or autumn, depending on the climate. It grows in semi-humid and dry environments. Its height ranges from 40 cm to 1 m. Its stem is erect, cylindrical, distinctly ribbed, glabrous, and hollow. The leaf stalk is paddle-shaped, and the leaves are dark green and glossy. The lower leaves have petioles and are divided into five toothed segments at both ends, while the upper leaves are divided into three segments. Less widespread, the flowers are small, white, and arranged in loose umbels. It has a strong, distinctive aroma and a fragrant taste. [17], [18]

Chemical Composition of the Extracted Oil:

100 g of the studied seeds were taken before drying, and the oil was extracted by hexane leaching. The extraction process lasted 3-4 days and was performed in two stages [19]. The solvent was then separated from the extracted oil using a rotary evaporator, and the sample was stored in a refrigerator in a dark place at 4°C.

The fatty acid composition of the extracted oil was determined using GC-Mass, and the results were as follows:

Table 1. The fatty acid composition of the extracted oil

%	Compound name	
47.08	Palmitic acid, methyl ester	Seed of Rocket
10.25	Stearic acid, methyl ester	
13.9	Methyl 18-methylnonadecanoate	
7.57	Erucic acid	
0.64	Tricosanoic acid, methyl ester	

8.9	15-Tetracosenoic acid, methyl ester	Seed of Radish
11.66	Tetracosanoic acid, methyl ester	
49.24	Palmitic acid, methyl ester	
10.18	Stearic acid, methyl ester	
1.41	Linolenic acid, ethyl ester	
13.62	Methyl 18-methylnonadecanoate	
5.95	Behenic acid, methyl ester	
2.46	Ethyl 13-docosenoate	
10.5	15-Tetracosenoic acid, methyl ester	
6.64	Tetracosanoic acid, methyl ester	
11.09	Eudesma-4(14),11-diene	Seed of Celery
54	1-Propanone, 2-chloro-1-(2,4-dimethylphenyl)-2-methyl-	
7.96	Phenol, 4-(2-aminoethyl)-	
23.59	Palmitic acid, methyl ester	
2.48	Stearic acid, methyl ester	
0.88	.Oleic acid, ethyl ester	

Studying the effect of oil extracted from (radish, watercress, and celery) on *Escherichia coli* and *Staphylococcus aureus* bacteria:

The following concentration series (25 mg/l, 50 mg/l, 100 mg/l, 200 mg/l, and 400 mg/l) were prepared from each plant seed extract (watercress, radish, and celery) using the following method:

One milliliter (0.8 g) of the extract was mixed with two milliliters of DMSO (dimethyl sulfoxide) to produce solution S1 with a concentration of 400 mg/l. Then, one milliliter of S1 was thoroughly mixed with one milliliter of DMSO to produce solution S2 with a concentration of 200 mg/l. Finally, one milliliter of S2 was mixed... In 1 ml of DMSO, a solution of S3 with a concentration of 100 mg/l is prepared [20], [21].

Then, (1 ml) of S3 is taken and mixed with 1 ml of DMSO, resulting in a solution of S4 with a concentration of 50 mg/l.

Then, (1 ml) of S4 is taken and mixed with 1 ml of DMSO, resulting in a solution of S5 with a concentration of 25 mg/l.

38 g of the medium powder (according to the number on the package) is suspended in (1 L) of distilled water, then mixed thoroughly. The mixture is then heated to boiling and allowed to boil for one minute to ensure complete dissolution [22], [23]. It is then sterilized with a sealed agar at 121°C for 15 minutes, and allowed to cool to 45°C. The cooled Mueller-Hinton agar is then poured into sterile Petri dishes, with a thickness of (4 mm) after solidification. Exactly

This is done by taking several bacterial colonies using a loop needle from the solid culture medium on which they were isolated (the medium varied depending on the number of times the experiment was repeated [25]; *E. coli* was sometimes taken from EMB medium and sometimes from Mac-Conkey medium) and suspending them in sterile distilled water (so that the bacterial count in the suspension is reasonable, neither too low nor too high, at a concentration of approximately 1.5×10^8 CFU/mL, visually compared

to the Macfarlane standard). 100 μ L of the bacterial suspension is taken and spread evenly (using a glass diffuser) [26], [27]. After waiting 15 minutes, wells 0.6 mm in diameter are made, and 100 μ L of each extract concentration is placed in each well. Five wells are placed in each dish, and 100 μ L of one of the same extract concentrations is placed in each well. [28], [29]

Bacterial culture and concentration series preparation were performed several times over the past 6 months, but the application method of the studied extracts to the bacteria, as well as the placement of the plates in the incubator throughout the incubation period, varied [30], [31].

Extracts were placed in wells, and the plates were placed in the incubator with the culture medium at the bottom, No bacterial growth occurred [32].

Extracts were placed in wells, and bacteria were cultured on a plate without adding any extract or solvent to the medium. All plates were incubated with the culture medium at the bottom, No growth except in the control plate (bacteria only).

Extracts were applied using two methods: well application and impregnation on blotting paper discs. Incubation was performed with the culture medium at the bottom, No bacterial growth.

Immersing the extracts onto blotting paper discs and incubating the plates upside down, No bacterial growth.

The extracts were impregnated onto blotting paper discs and incubated upside down with the culture medium on top, No bacterial growth occurred... This prompted us to proceed with the incubation conditions [33].

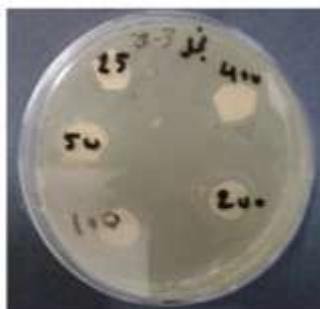
The incubator was changed so that it was connected to electricity throughout the incubation period. The extracts were impregnated onto blotting paper, and the incubation position was changed so that the medium was on the bottom. After 24 hours, there was initial bacterial growth, and the same position was maintained for 48 hours.

Seventh time: The extracts were impregnated onto blotting paper discs and incubated upside down in an incubator connected to electricity throughout the incubation period, Bacterial growth was observed with a halo of non-growth around the discs impregnated with the extracts. The results are shown in the following table 2:

Table 2. Zone of inhibition and MIC of extracts

	Amoxycilin25 μ g		Celery		Rocket		Radish	
	MIC (mg/ml)	Zone of inhibition (m.m)	MIC (mg/ml)	Zone of inhibition (m.m)	MIC (mg/ml)	Zone of inhibition (m.m)	MIC (mg/ml)	Zone of inhibition (m.m)
Staphylococcus Aures	200	36	200	2.5	50	2	400	1.5
Escershi a Coli	250	25	100	2	100	1.5	200	1

Figure 6. Celery with Staphylococcus Aures

Figure 1. Radish with *Escherichia coli*Figure 2. Celery with *Escherichia coli*Figure 3. Rocket with *Escherichia coli*Figure 4. Radish with *Staphylococcus aureus*Figure 5. Rocket with *Staphylococcus aureus*

4. Conclusion

The chemical composition of fatty acids in the oil extracted from radish, watercress, and celery seeds was determined using systemic hexane, and the percentages of these acids were determined by gas chromatography (GC-mass).

The highest percentage of palmitic acid methyl ester in all hexane extracts of the seeds was observed in watercress (47.08%), radish (49.24%), and celery (23.59%). The presence of both palmitic acid methyl ester and stearic acid methyl ester was observed in the hexane extracts of the three seeds. The effect of the extract on *Escherichia coli* and *Staphylococcus aureus* was studied.

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