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Original Article

Investigation of Antioxidant and Antibacterial Effect of Chemical Compounds *Cardamine uliginosa* Plant

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Abstract

Background & Aims: *Brassicaceae* is a medium-sized family of flowering plants. The family contains more than 327 genera. *Cardamine uliginosa* is a type of plant of this species. Chemical and biochemical findings of this genus are very limited. This study aimed to investigate the chemical compounds and antioxidant and antibacterial properties of *C. uliginosa*.

Materials and Methods: Essential oil was prepared by hydrodistillation method and microwave assisted hydrodistillation (MAHD). The chemical composition of essential oil was evaluated by GC and GC-MS methods. The antioxidant property of the hydroalcoholic extract was investigated by 2, 2-dipheny1-1-picrylhydrazyl (DPPH) solution. Finally, the antibacterial activity of *C. uliginosa* was investigated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods.

Results: Phetalic acid, Phenil, Caryophyllene, Eicosane, and other chemical compounds were found in the essential oil of *C. uliginosa*. The half maximum inhibitory concentration (IC50) of hydroalcoholic extract was 0.42 µg/mL. The MIC and the MBC against both *Escherichia coli* and *Staphylococcus aureus* were 15.62 and 31.25 respectively. The major compounds in the essential oils were 3-methyl-4-isopropylphenol (15.20%) and 1, 2-benzenedicarboxylic acid (16.75%).

Conclusion: Considering the chemical compounds of *C. uliginosa* and the finding of the antibacterial and antioxidant activity of *C. uliginosa* in vitro, capability of this plant needs to be further investigated for use in health and medical industries.

Keywords: *Cardamine uliginosa*, Antioxidants, Anti-bacterial agents, Plants

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1. Introduction

Plants are used as primary health care products and in traditional medicines, in addition to the diet [1]. The World Health Organization (WHO) also notes that a significant section of the inhabitants in underdeveloped countries continues to rely on medicinal plants as their primary sources of healthcare [2].

Plants often contain antioxidant and antibacterial compounds such as flavonoids, polyphenols, lignins, tannins, vitamins, carotenoids, tocopherols, and other bioactive substances [3,4]. Today, much information about wild plants is known. Medicinal plants are widely used among the people of the world due to fewer side effects than allopathic medicines [5]. Therefore, it is always necessary to evaluate medicinal plants to find a more potent active substance in terms of phytochemical, antioxidant, and antibacterial activity to find intermediate compounds [6]. One of these medicinal plants is *Cardamine uliginosa*, which has received less attention in scientific essays.

Cardamine uliginosa belongs to the order *Brassicaceae* and the genus *Cardamineae* and has global distribution [7]. The seed shape of *C. uliginosa* is circularis–rectangular is, and its surface ornamentation is reticulate-foveate, brown in color. The seed dimension varies between 0.91 mm and 2.20 mm in length and between 0.41 mm and 1.65 mm in width. *C. uliginosa* has raphe on seeds. Herbaceous plant, stoloniferous rhizome, having saccate, ovate, often white and purple-tinted sepals, unguiculate petals, large, oblong-elliptic, yellow anthers and a glabrous ovary with distinct style [7,8]. Some species of *Cardamine* were reported from Iran in 1968 by Hedg, then in 2005, Naqinezhd et al, reported the other *Cardamine* genus in Iran in the Hyrkan region of Mazandaran for first time [9]. So far, seven species of this genus have been identified in Iran [9]. *Cardamine uliginosa* is one of these seven species. This genus shows a great variety of phytology and morphology [8]. According to Nadiroğlu and Behçet's study [10], some parts of this plant are mainly collected in spring in order to aerial parts, stems, and leaves of this plant are used as vegetables in food preparation [1,10]. The antibacterial properties of this plant have been discussed in various studies [8]. The previous study also shows that the *C. uliginosa* plant is a rich source

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of antioxidants that remove free radicals in the test tube [11]. According to a study by Ebadollahi-Natanzi [11], *C. uliginosa* contains a type of glucosinolate called gluconasturtiin, which produces phenylethylensenevol by hydrolysis. Gluconasturtiin plays a prominent role in genotoxic responses [11]. On the other hand, Zurayk and colleagues' study [12] showed that the *C. uliginosa* plant could be bioconcentration factors species to monitor environmental pollution by some elements, especially Nickel [12]. The 24 h LC50 (The median lethal concentrations) values for *C. uliginosa* extractives against brine shrimp were calculated as 176.88 ± 4.80 µg/mL. Plant extracts with LC50 less than 1 mg/mL are potentially toxic and considered active plants [11].

Altogether, considering the different properties of this plant's extract and essential oil in different geographical regions of the world, more specialized studies are still needed on the characteristics of karyology and morphology of this plant species.

2. Aims of the study

This study investigated three practical objectives for evaluating the extract and essential oil of the plant *C. uliginosa*.

1. Assessment of essential oils' chemical composition of *C. uliginosa* by microwave-assisted and hydrodistillation extraction
2. Determination of the antioxidant activity of the hydroalcoholic extract of *C. uliginosa* by the DPPH method
3. Evaluation of the antimicrobial activity of methanolic extracts of *C. uliginosa* by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Method.

3. Materials and Methods

3.1. Plant material

Cardamine uliginosa was harvested in late May 2022 in the agricultural research farm in Mazandaran province, Iran. The plant material was first characterized by Dr. Bostan Rodi, botanist in the Damghan Branch, Islamic Azad University. For further verification, the *C. uliginosa* plant was deposited at the Research Institutes of Forest Herbarium and Rangelands (No: 16862). The image of the *C. uliginosa* plant is shown in Figure 1.

Figure 1. Image of the *Cardamine uliginosa* plant

3.2. Hydrodistillation method

Two methods, microwave-assisted, and hydrodistillation extraction, were used to investigate the active ingredients of the *C. uliginosa* plant, GC and GC-MS approaches [13].

In the hydrodistillation method, we soaked 50 g of dried *C. uliginosa* in 500 mL of distilled water for 12 hours. The essential oil was extracted using a Clevenger machine (Ashk Shishe Co., Tehran, Iran). The produced essential oil was separated with 0.5 mL of n-hexane from the Clevenger tube; it was separated and capped under nitrogen and kept in the refrigerator at -4 °C. The resulting essential oil was sent to Islamic Azad University, Esfahan branch for GC and GC-MS analysis.

3.3. Microwave-assisted hydrodistillation

In the microwave-assisted hydrodistillation (MAHD) approach, the microwave oven (Samsung, South Korea) was used. This method used 50 g of dried *C. uliginosa* plant moistened in 50 mL of distilled water at 25 °C under atmospheric pressure for 1 hour. Quantitative and qualitative evaluation of the essential oils was done using GC and GC instruments.

The microwave oven used for the MAHD at a frequency of 2450 MHz. The interior cavity of the microwave was 29 × 37 × 40 cm, and the maximum power was equal to 1000 W in this test. Aluminum foil around the microwave hole was used to avoid scattering microwave beams in the laboratory environment.

3.4. GC and GC-MS analysis

The study used GC and GC-MS instruments, including an Agilent 5975C mass detector with an electron ionization source (EI) coupled with an Agilent 7890 gas chromatography device, which consisted of a HP-5 MS column with a length of 30 m, an inner diameter of 0.25 µm and 0.32 µm film thickness. The temperature of the injection site of the gas chromatography (Inlet) and the ionization source of the mass detector and analyzer (quadrupole) was 280 °C, 150 °C, and 230 °C, respectively. The interface temperature between GC and MS should be set at 280 °C.

3.5. Preparation of hydroalcoholic extract

The *C. uliginosa* plant used in this study was prepared from Mazandaran province. 50 g of the dried plant was ground, then mixed with 85% (v/v) alcohol solution at a ratio of 15:100 (g/mL plant material to solvent). The resulting mixture was poured into an Erlenmeyer covered with aluminum foil and put on the heater for 24 hours (with the rotation of the magnet in the solution). After 24 hours, the solution was filtered through Whatman paper. Subsequently, the hydroalcoholic extract was vacuumed twice using a vacuum pump and rotary vapor (IKA, Germany) at 40 °C for 30 minutes. The obtained hydroalcoholic extract was kept at a temperature of 4 °C

in a refrigerator.

3.6. Determination of antioxidant activity by the DPPH solution

Alothman et al [14] method was used to measure the antioxidant capacity of *the C. uliginosa* plant. The extract's concentrations of 100, 200, and 400 µg/mL were prepared. Different extract concentrations were prepared with DPPH (2, 2-dipheny1-1-picrylhydrazyl) solution in a 1:1 ratio (to prepare DPPH solution: 0.002 g of DPPH combined with 50 mL of methanol). The solutions were stored in a beaker covered with aluminum foil. The optical absorption of all different extract concentrations was read with a spectrophotometer at a wavelength of 517 nm after 15 min. Methanol as blank and DPPH solution was used as a control sample; also, the antioxidant power of extract was compared with vitamin C.

The samples antiradical activity was expressed using IC50 (mg/L) [12]. The DPPH scavenging effect (%) is given by (Eq. 1)

$$\text{DPPH scavenging effect (\%)} = (1- \frac{Abs\ sample}{Abs\ control}) \times 100 \quad (1)$$

In Eq (1). $Abs_{control}$ is the absorbance of DPPH solution without extracts. Numerical IC50 values denote the sample's concentration (Concentration of substrate in µg/mL), which is required to scavenge 50% of DPPH free radicals.

3.7. Preparation of microbial strains

The strains used in this study were *Staphylococcus aureus* RTCC 1885 (gram-positive) and *E. coli* PTCC 1330 (gram-negative), which were lyophilized from the microbial collection of the Faculty of Pharmacy, Tehran University of Medical Sciences. In order to purify bacteria and standardize bacterial culture, sterile lyophilized ampoules (*S. aureus* and *E. coli*) were transferred to nutrient broth (manufactured by Merck, Berlin, Germany) and cultivated linearly in differential culture medium overnight and incubated at 37 °C for 24-48 hours. After incubation, one loop was taken from the bacterial colony and used for the rest of the experiment.

3.8. Determination of microbial susceptibility

The disc diffusion method was used to qualitatively determine the microbial strain's susceptibility to *C. uliginosa* methanolic extract [15]. 0.5-McFarland's turbidity standard microbial suspension was prepared by adding the cultured bacteria of the previous step to 0.9% normal saline. In the qualitative method, the Kirby-Bauer disc diffusion technique was used. The microbial suspension prepared from both types of bacteria, *S. aureus*, and *E. coli*, by the lawn culture or carpet culture method was cultured on Mueller-Hinton agar culture medium (prepared by the company Merck, Berlin, German).

To scrutinize the studied extract's antibacterial properties, paper blank sterilized discs (6 mm, Padtan-Teb Company, Iran), soaked in different extract concentrations for 5 minutes, were placed at a certain distance from each other and the edge of the plate. The culture media containing bacteria were incubated for 24 hours at 37 °C. The diameter of the growth inhibition zone formed around the discs was measured using a digital caliper. The results were compared with the Clinical and Laboratory Standards Institute (CLSI) table. According to the CLSI standard, discs of vancomycin (30 mg) and gentamicin (10 mg) antibiotics were used for positive control and dimethyl sulfoxide for negative control. All the tests were performed for each bacterial strain in triplicate.

3.9. Evaluation of the antibacterial activity of the Cardamine uliginosa methanolic extract by the MIC and the MBC method

From the new culture of *S. aureus* and *E. coli* in the Tryptic Soy Broth medium (TSB; Quelab, Montreal, Canada), a turbidity equal to 0.5 MacFarland was prepared. It was diluted in a ratio of 1 to 100 to obtain an opacity equal to 106. On the other hand, we prepared seven test tubes and poured 2 ml of Müller-Hinton broth medium (prepared by Merck, Berlin, German) into each of them. Concentrations of 500 to 7.8 mg/mL extract (1:1-1:64 dilution) were added to each culture medium. Finally, 20 microliters of microbial suspension were added to each test tube. The test tubes were incubated at 37°C for 24 hours (The experiment was repeated three times for each extract concentration). The positive control tube contained Müller-Hinton broth medium and bacterial suspension, and the negative control tube contained Müller-Hinton broth medium and extract). In the final stage of the MIC technique, a 5 mg/mL solution of triphenyl tetrazolium chloride was used. The tubes were incubated for one hour again. The formation of red color indicated the growth of bacteria. Tubes with green and yellow colors did not grow bacteria.

For the MBC test, all the concentrations of the extract in the MIC with yellow and green color, which indicated the inhibition of bacterial growth, were cultivated separately on Mueller-Hinton agar culture medium, and the samples were incubated at 37°C for 24 hours. The lowest concentration of the extract of *C. uliginosa*, in which the bacteria did not grow, was reported as MBC.

3.10. Statistical analysis

The results are presented as mean ± SD. Statistical analysis was reported on the data by SPSS V 20, Kruskal-Wallis and Dunn's tests. *P*-value smaller than *0.05 was considered significant.*

4. Results

4.1. Chemical constituents of the essential oils of Cardamine uliginosa

The chemical constituents of the essential oils obtained from *C. uliginosa* are shown in Table 1, and the chromatogram in Figure 2. The major compounds in the essential oils were 3-methyl-4-isopropylphenol (15.20%) and 1, 2-Benzenedicarboxylic acid (16.75%).

Table 1. Chemical compositions of essential oils of *Cardamine uliginosa* using GC/MS

No.	Compounds	Rt[a]	%
1	α-Pinene	6.600	0.69
2	d-Limonene	8.713	0.60
3	Linalool 1,6-Octadien-3-ol, 3,7	10.140	2.24
4	Borneol	11.474	0.97
5	Dodecane	12.039	2.69
6	Fenchyl acetate	12.448	2.30
7	Mint furanone	12.535	0.73
8	Naphthalene, decahydro-1,5-dimethy	12.633	2.03
9	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	12.852	1.40
10	3-Methyl-4-isopropylphenol	13.762	15.20
11	3-Methyl-4-isopropylphenol	13.952	5.55
12	Bicyclo[2.2.1]heptane	14.717	0.64
13	Tetradecane	15.500	2.16
14	Caryophyllene	15.939	1.37
15	3-Buten-2-one, 4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)-	16.946	1.15
16	2H-3,9a-Methano-1-benzoxepin, octa	17.248	0.53
17	Naphthalene, 1,2,3,4,4a,5,6,8a-oct	17.409	0.53
18	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	17.526	1.90
19	Caryophyllene oxide -5-O	18.490	1.13
20	Hexadecane	18.577	1.67
21	Guaiol	18.655	1.66
22	2-Naphthalenemethanol, 1,2,3,4,4a	18.787	0.73
23	1H-Cyclopropa[a]naphthalene, 1a,2	19.025	5.07
24	Hinesol	19.235	0.84
25	Valencene naphthalene, 1,2,3,5	19.361	1.21
26	Bicyclo[4.4.0]dec-1-ene, 2-isoprop	19.473	2.72
27	Ledene 1H-cycloprop[e]azulene	19.853	0.81
28	Octadecane	21.333	1.57
29	2-Pentadecanone, 6,10,14-trimethyl	21.922	8.20
30	1,2-Benzenedicarboxylic acid	22.239	0.61
31	1H-Cyclooctapyrazole, 4,5,6,7,8,9-	22.891	1.53
32	2-Hexadecen-1-ol, 3,7,11,15	22.988	1.60
33	1,2-Benzenedicarboxylic acid	23.188	0.60
34	Eicosane	23.431	1.86
35	Hexadecane	24.162	0.76
36	2-Hexadecen-1-ol, 3,7,11,15-tetram	24.288	3.26
37	Hahnfett	24.488	0.88
38	2.alpha-Methylpregna-3,5-diene	24.605	1.25
39	Heptadecane	24.756	1.28
40	Nonahexacontanoic acid	25.642	1.14
41	1,2-Benzenedicarboxylic acid	26.280	16.75

[a]Rt, retention time.

4.2. Antioxidant capacity

DPPH is an unstable free radical. It can receive an electron or a hydrogen radical and become stable. The DPPH solution is purple in color, and due to the presence of a single electron in the DPPH structure, this radical has a good absorption at the wavelength of 517 nm. In the presence of antioxidant compounds, the color of DPPH disappears, and the decrease of absorption in 517 nm is an expression of antioxidant activity. The amount of antioxidant activity is expressed as IC50. A smaller IC50 indicates greater antioxidant power or free radical scavenging.

The DPPH radical scavenging activities of the *C. uliginosa* and vitamin C extract in different concentrations are shown in Figure 3. IC50 of extract for *C. uliginosa* and vitamin C were 0.42 µg/mL and 0.55 µg/mL, respectively.

4.3. Antibacterial capacity

The diameter of the growth inhibitory zone of *C. uliginosa* extract against *S. aureus* and *E. coli* in the disc diffusion method at a concentration of 500 mg/mL was 23.8 and 20.4 mm, respectively. According to Table 2, all concentrations of the extract could inhibit bacterial growth against *S. aureus*, and it is noteworthy that the dilution of 1:8 (62.5 mg/mL) of the extract of the *Cardamine uliginosa* also had a more powerful effect than the vancomycin antibiotic. Therefore, the extract of the *C. uliginosa* has a much better inhibitor effect for controlling *S. aureus* bacterial infection compared to vancomycin.

Also, all concentrations of the extract had an inhibitory effect against *E. coli*. However, the gentamicin antibiotic had a more substantial effect on inhibiting the bacteria than the *C. uliginosa* extract.

Table 3 shows the MIC and MBC of *Cardamine uliginosa* compared to vancomycin and gentamicin. The MBC against *E. coli* and *S. aureus* strains was 31.25 mg/mL.

According to Table 4, the group's inhibition growth zone's mean and standard deviation were 21.81 and 4.27, respectively ($P < 0.05$). The Kruskal-Wallis test showed that *C. uliginosa* extract had antimicrobial properties equal to gentamicin and vancomycin antibiotics ($P < 0.05$).

5. Discussion

So far, many botanical, molecular, and biochemical studies have been conducted on medicinal plants [16]. *Cardamine*, one of the most prominent plant families, includes more than 200 species [17]. Due to the many similarities of different species of this family, it is difficult to identify some genus of this family [18]. So far, seven species of this family have been identified in

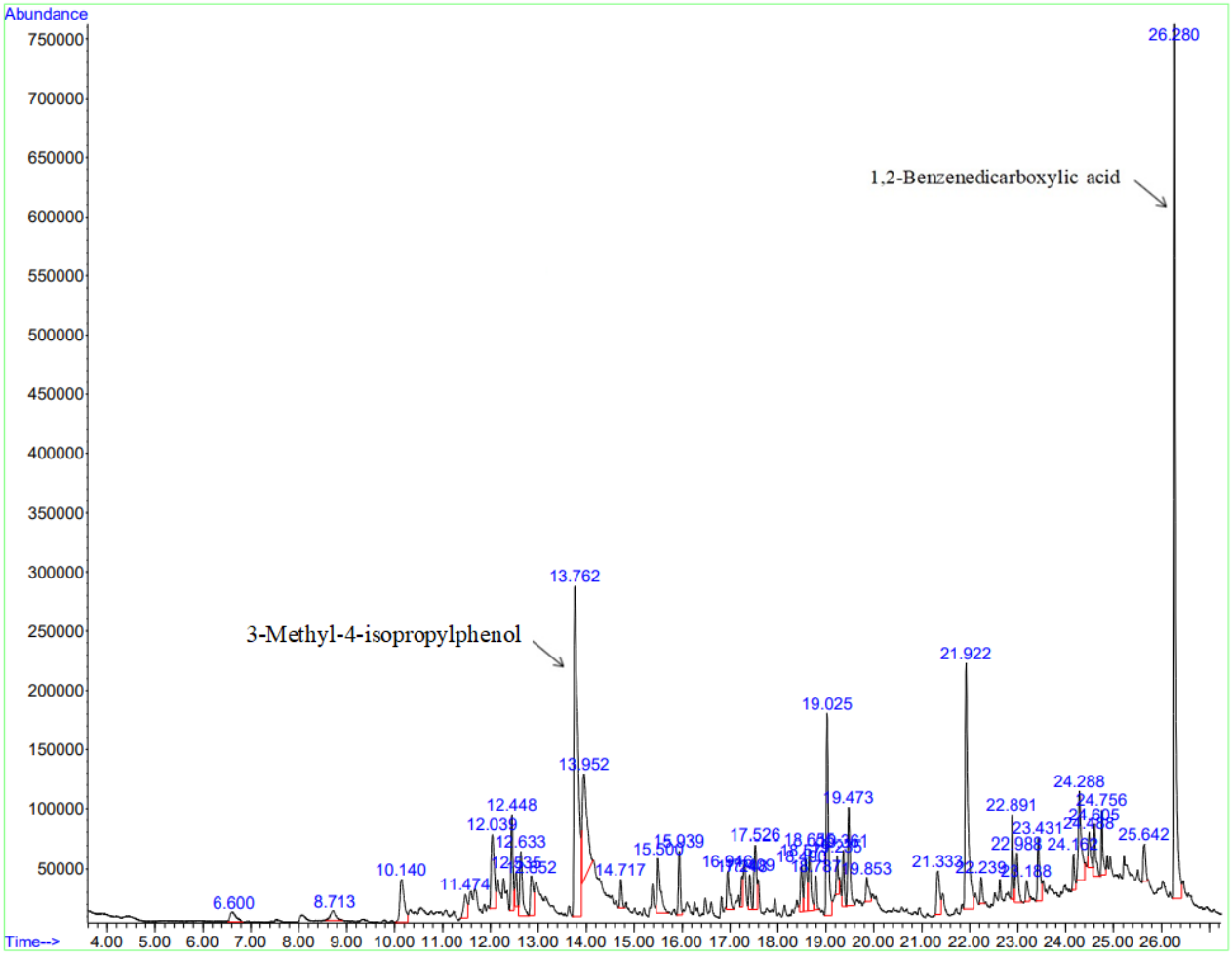

Figure 2. GC chromatogram of essential oils of *Cardamine uliginosa*

Iran [17]. Unfortunately, limited molecular tests have been performed on their chemical and biochemical properties. According to our study, the most compounds found in the essential oil of *the C. uliginosa* plant include 1, 2-benzenedicarboxylic acid (phthalic acid: 16.75%), 3-methyl-4-isopropyl phenol (phenil: 20.75%) and caryophyllene, eicosane, etc. The mentioned compounds show the antioxidant, antibacterial and antifungal ability of the essential oil of *the C. uliginosa* plant. IC50 of this plant was 0.42 µg/mL, which is a low number compared to many plants, indicating this plant's high antioxidant activity. The alcoholic extract of this plant caused the zone of inhibition growth diameter of 23.80 mm against *S. aureus* bacteria and 20.4 mm against *E. coli* bacteria, which was higher or almost similar to vancomycin and gentamicin antibiotics.

Among the other *Cardamine* species known in Iran are *C. hirsuta* and *C. flexuosa*. According to the Narzary et al (19) study, *hirsuta* is one of the native plants of India which has edible use. According to this study, the methanol extract of *hirsuta* (30 mg/mL) had zone inhibition diameters of 14 mm and 13 mm against *S. aureus* and *E. coli*, respectively. This extract had a weaker effect than amoxicillin antibiotic (30µg/mL) with a zone inhibition diameter of 24 mm [19]. Ragasa and colleagues' study (20) showed that *C. flexuosa* has different chemical compounds, including β-sitosterol, stigmasterol, dietary lutein, triglycerides, linoleic acid, and linolenic acid. These compounds express the effect of anti-cancer, reducing cardiovascular and inflammatory diseases, and reducing intestinal absorption of cholesterol in the human body [20]. Both studies showed similar results of *C. uliginosa*, a member of *Cardamine* family, in the present study.

6. Study limitations

One of the limitations of the study was the difficulty in identifying *C. uliginosa* plant samples and the need for more scientific articles about the chemical and biochemical properties of this type of plant in the world.

7. Conclusion

In general, this research has shown well that *C. uliginosa*, commonly known as aquatic weed, has compounds with antioxidant, antibacterial, and antifungal properties, including phthalic acid, phenyl, caryophyllene, and eicosane, etc. The results of present study to identify

Table 2. Mean±SEM deviation of inhibition growth zone (mg/mL) of methanolic extract of *Cardamine uliginosa* in different concentrations against *S. aureus* and *E. coli*

Different concentrations of extracts (mg/mL)	Zone of inhibition mean of methanolic extract of *Cardamine uliginosa* for Tested Bacteria (mm)	
	Staphylococcus aureus	*Escherichia coli*
500	23.80±0.28	20.40±0.43
250	21.50±0.46	18.20±0.66
125	17.90±0.29	14.36±0.44
62.5	14.80±0.26	12.33±1.02
31.25	7.93±0.32	9.08±0.31
15.62	5.25±0.14	1.78±0.37
7.8	1.41±0.42	Indeterminate
Vancomycin	14.2±0.08	0
Gentamicin	0	24.1±0.09
DMSO	0	0

DMSO, dimethyl sulfoxide.
Values are expressed as mean±SEM.

Table 3. MIC and MBC of extracts of *Cardamine uliginosa*

Methanolic extract	MIC (mg /mL)			
	Staphylococcus aureus		*Escherichia coli*	
	MIC	MBC	MIC	MBC
Cardamine uliginosa	15.62	31.25	15.62	31.25
Vancomycin (30 µg disc)	2 ≥	4-8	-	-
Gentamicin (10 µg disc)	-	-	2-4	4-8

Table 4. Mean and standard deviation of the inhibition growth zone of groups

Groups	Groups name	Mean±SEM	SD	*P* value[a]
1	Treated with *Cardamine uliginosa*	21.81	4.272	
2	Treated with vancomycin (30 µg disc)	14.2±0.08	2.3	0.025
3	Treated with gentamicin (10 µg disc)	25.1±0.09	4.69	

[a] Kruskal-Wallis test result.

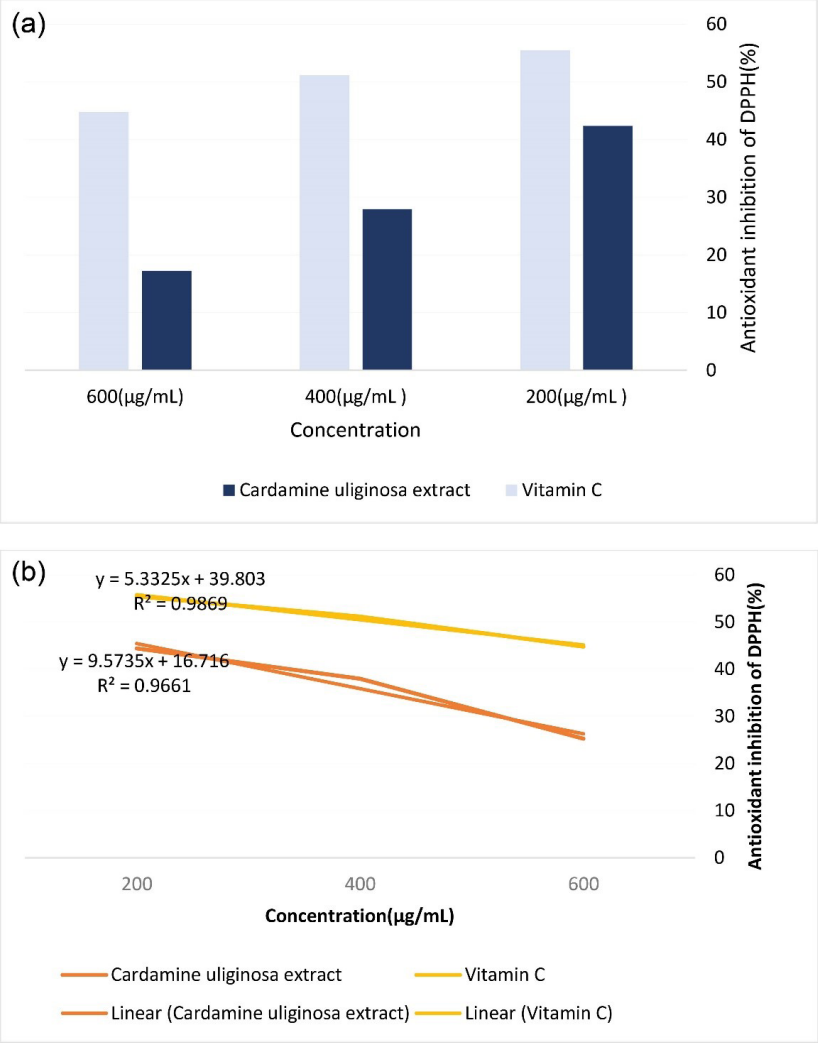

Figure 3. (a) Antioxidant activity of *Cardamine uliginosa* extract compared with vitamin C at the different concentrations (200, 400, 600 µg/mL). (b) The radical scavenging activity, represented by percentage of inhibition, of the different concentration *Cardamine uliginosa* extract compared to vitamin C

the essential oils constituents showed that the highest composition of essential oils were 3-methyl-4-isopropylphenol (15.20%) and 1,2-benzenedicarboxylic acid (16.75%).

Emphasizing the results of antioxidant and antibacterial specialized tests can be another proof of the valuable properties of this plant. It is suggested to continue the research on this plant family.

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Authors' Contribution

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Writing–review & editing: Maryam Soori, Reza Moradi.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

This research was approved at the Islamic Azad University, Damghan Branch, Damghan, Iran with thesis code 14230520922016.

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