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Determination of the Antimicrobial and Biochemical Content of Black Carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.)

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Siyah Havuç *(Daucus carota* ssp. *sativus* var. *atrorubens* Alef.*)* Bitkisinin Antimikrobiyal ve Biyokimyasal İçeriğinin Belirlenmesi

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Abstract

The aim of this study was to determine the antimicrobial activity of *Daucus carota* ssp. *sativus* var. *atrorubens* Alef. using disk diffusion and MIC methods, the antioxidant activity using the DPPH method, and the biochemical content using the GC-MS method. A total of 28 microorganisms were used, including 10 standard isolates, 1 clinical isolate, 7 food isolates, 9 multi-drug resistant strains, and 1 standard yeast. In the disk diffusion test, conducted with disks impregnated with 30 µl, 70 µl, and 150 µl concentrations, the highest results at the highest concentration were observed as 10 mm for *Staphylococcus aureus*, 16 mm for *Enterococcus faecium*, and 9 mm for *Streptococcus pneumoniae*. In the MIC test, the highest result was found to be 1.18 mg/mL for the *Enterococcus faecium* strain. The antioxidant activity was determined using the DPPH method, with the highest antioxidant activity observed at a concentration of 1 mg/mL. The GC-MS analysis identified linolelaidic acid (18.30%) as the major component. As a result, antimicrobial activity was detected against 21 out of the 28 microorganisms tested. It was concluded that *D. carota* ssp. *sativus* var. *atrorubens* is not only effective in antimicrobial and antioxidant activities but also rich in various biochemical components, such as vitamins, phenolic compounds, and flavonoids.

Keywords: *Daucus carota ssp. sativus var. atrorubens Alef.; Antimicrobial activity; Antioxidant activity; DPPH; GC-MS.*

Öz

Bu çalışmada, *Daucus carota* ssp. *sativus* var. *atrorubens* Alef. bitkisinin disk difüzyon ve MİK yöntemi ile antimikrobiyal aktivitesinin, DPPH yöntemi ile antioksidan aktivitesinin ve GC-MS yöntemi ile biyokimyasal içeriğinin belirlenmesi amaçlanmıştır. 10 standart izole, 1 klinik izole, 7 gıda izole, 9 çoklu ilaca dirençli suş ve 1 standart maya olmak üzere toplamda 28 mikroorganizma kullanılmıştır. 30 µl, 70 µl ve 150 µl miktarlarında emdirilen disklerle yapılan disk difüzyon testinde en yüksek konsantrasyonun en yüksek sonuçları *Staphylococcus aureus* suşunda 10 mm, *Enterococcus faecium* suşunda 16 mm ve *Streptococcus pneumonia* suşunda 9 mm olarak gözlemlenmiştir. MİC testinde en yüksek sonuç *Enterococcus faecium* suşunda 1.18 mg\mL olarak tespit edilmiştir. Antioksidan aktivitesi DPPH yöntemi ile belirlenmiştir ve 1 mg/mL konsantrasyonunda en yüksek antioksidan aktiviteyi göstermiştir. GC-MS analizinin sonucunda majör madde olarak Linoelaidik asit (%18.30) tespit edilmiştir. Sonuç olarak 28 mikroorganizma ile yapılan bu testte 21 mikroorganizmaya karşı antimikrobiyal etkinlik saptanmıştır. *D. carota* ssp. *sativus* var. *atrorubens'*in, antimikrobiyal ve antioksidan aktiviteye ek olarak, çeşitli vitaminler, fenolik bileşikler ve flavonoidler gibi önemli biyokimyasal bileşenler açısından zengin olduğu tespit edilmiştir.

Anahtar Kelimeler: Daucus carota ssp. sativus var. atrorubens Alef.; Antimikrobiyal aktivite; Antioksidan aktivite; DPPH; GC-MS

1. Introduction

Throughout history, people have utilized plants not only for nutrition but also for addressing health issues (Bouasla and Bouasla 2017). According to the World Health Organization (WHO), a significant portion of the global population (80%) relies on traditional herbal remedies (Oladeji et al. 2020). Turkey's rich flora hosts approximately 10,000 plant species (Bağdat 2006). Among these, plants with purple and black hues stand out for their health benefits due to their abundance of anthocyanins, vitamins, minerals, and other beneficial compounds (Akarca et al. 2006).

Anthocyanins are phenolic compounds responsible for giving color to flowers and fruits, and research has shown

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that these compounds can help reduce the risk of heart disease, as well as offer protective effects against obesity, cancer, and genetic damage (Espinosa-Acosta et al. 2018).

Daucus carota ssp. *sativus* var. *atrorubens* Alef., commonly known as black carrot, is a plant that has been cultivated for approximately 3,000 years in Turkey, the Middle East, and the Far East (Karataş et al. 2014). This plant belongs to the Umbelliferae-Apiaceae family, known for its umbrella-like flower structures, and is characterized by its distinctive color resulting from its anthocyanin content. It is used not only as a natural dye but also in various industries such as turnip production and the pharmaceutical sector (Akarca et al. 2006, Pereira-Caro et al. 2021).

Black carrot is a potent source of natural antioxidants due to its high content of vitamins C and E, carotenoids, and phenolic compounds (Algarra et al. 2014). In daily life, factors such as poor diet, sedentary lifestyle, and exposure to environmental pollutants contribute to the accumulation of harmful substances known as free radicals in the body, which can weaken the immune system. Antioxidants help neutralize these harmful effects. Scientific research has increasingly focused on the benefits of obtaining antioxidants from natural and organic sources (Faydaoğlu and Sürücüoğlu 2013, Tosun and Karadeniz 2013). Studies have shown a strong correlation between higher anthocyanin levels and increased antioxidant activity (Espinosa-Acosta et al. 2018).

Infectious diseases have posed a significant threat to humanity throughout history, and they continue to be a major health concern today (Inhorn and Brown 1990). These diseases, caused by pathogenic microorganisms, are spreading rapidly, driven by environmental and ecological factors (Gayer et al. 2007). The rising death toll from epidemic diseases indicates a need for intensified efforts to combat these issues (Yetgin et al. 2017). The antimicrobial properties of plant extracts offer a promising area for developing natural and cost-effective antimicrobial agents (Balouiri et al. 2016). According to the WHO, the use of medicinal aromatic plants in drug production yields positive results (Manandhar et al., 2019). Today, many drugs used in the treatment of various infectious diseases are derived from secondary metabolites found in plants (Yuled Çakır 2022).

The indiscriminate use of synthetic antibiotics has led to antimicrobial resistance, making microorganisms less responsive to drugs and complicating treatment (Kayiş 2019, Dadgostar 2019). The WHO reports that antimicrobial resistance is becoming increasingly common, especially among multi-drug resistant (MDR) microorganisms (Escolà-Vergé et al. 2020). This growing resistance underscores the urgent need for new, effective antimicrobial agents. Recently, research has accelerated on developing these agents from plant sources (Vaou et al. 2021).

Research has shown that *D. carota* ssp. *sativus* var. *atrorubens* exhibits antimicrobial activity against various pathogens, including those resistant to multiple drugs (Espinosa-Acosta et al., 2018; Smeriglio et al., 2018). Additionally, the amount of anthocyanins present in the plant is a key factor in determining its antioxidant activity. Compared to other fruits and vegetables, black carrot stands out due to its high anthocyanin content, highlighting its significant role in promoting health.

The aim of this study is to investigate the antimicrobial and antioxidant properties of *D. carota* ssp. *sativus* var. *atrorubens* and to examine the health impacts of its biochemical components in detail. The findings from this research are expected to contribute to the development of natural antimicrobial and antioxidant agents, thereby offering significant benefits in the field of healthcare.

2. Materials and Methods

2.1 Plant Sample

D. carota ssp. *sativus* var. *atrorubens* was collected from Kazdağı, Çanakkale, Turkey, and identified by Dr. Mustafa Eray Bozyel. The specimens were deposited at the Fauna and Flora Research and Application Center, Dokuz Eylül University, Buca, Izmir, Turkey.

2.2 Microorganisms

10 standard isolates, 1 clinical isolate, 7 food isolates, 9 multidrug resistant strains and 1 standard yeast were used.

2.3 Extraction Method

The plant material of *Daucus carota* ssp. *sativus* var. *atrorubens* was pulverized using a grinder (Ika, Germany). A 25 g portion of the powdered sample was accurately weighed and transferred into a glass Erlenmeyer flask (Isolab, Germany), followed by the addition of 200 mL of 99% ethanol (Merck, Germany). The mixture was agitated at 160 rpm for 48 hours at ambient temperature using an orbital shaker (WilkeShake, South Korea). After the extraction process, the extract was filtered through Whatman No. 1 filter paper (110 mm diameter, China) into a flask (S&H Labware, USA). Upon completion of the filtration, the flask was attached to a rotary evaporator (Buchi, Switzerland) to remove the ethanol at 40°C. A total of 1.045 grams of solid residue was obtained, and 11 mL of

extract was prepared through accurate weighing on an analytical balance (Shimadzu, Japan) (Bozyel et al. 2021).

For the preparation of the DMSO-water extract intended for use in the minimum inhibitory concentration (MIC) assay, the ethanol extract was further concentrated using rotary evaporation at 40°C. The resulting precipitate was treated with dimethyl sulfoxide (DMSO) (İron Kimya, Turkey) and subsequently diluted with ultrapure water, obtained from a distillation system (Thermo Fisher Scientific, USA), to achieve a final concentration of 1% DMSO.

2.2 Inoculum Preparation

The bacterial strains used in the study were incubated on Mueller-Hinton agar (Oxoid, UK) at 37°C for 24 hours in an incubator (Nüve, Turkey), while the yeast species were incubated at 27°C for 48 hours. The bacterial strain densities were adjusted to approximately 10^8 cfu.mL^{-1}, and the density of Candida albicans was adjusted to 10^7 cfu.mL^{-1}. To standardize the inocula of these microorganisms, the suspensions were prepared according to the 0.5 McFarland standard using a sterile 0.9% NaCl solution (Osel, Turkey) (Yetgin et al. 2017, Benek et al. 2021).

2.5 Antimicrobial Activity Test

2.5.1 Disk Diffusion Test

The antimicrobial activity of the ethanol extract of *Daucus carota* ssp. *sativus* var. *atrorubens* was evaluated using the disk diffusion method. In the initial step, Mueller-Hinton Agar (Oxoid, UK) with a thickness of 0.5 mm was poured into sterile plastic petri dishes with a diameter of 90 mm (Fıratmed, Turkey). Subsequently, the extract was impregnated onto antimicrobial susceptibility test disks (Oxoid, UK) at volumes of 30 µL (2 mg), 70 µL (6 mg), and 150 µL (14 mg). After drying the impregnated disks, microorganisms were spread onto the petri dishes containing Mueller-Hinton Agar using sterile swabs (LP Italiana SPA, Italy). Finally, the extract-impregnated disks were placed onto the inoculated petri dishes, which were transferred to the incubator for incubation. After incubation, the inhibition zones formed around the disks were measured and recorded in millimeters. In this study, blank antibiotic disks and ethanol-loaded disks were used as negative controls, while gentamicin (GEN) (BD BBL, USA) and tobramycin (TOB) (BD BBL, USA) served as positive controls. The test was performed in triplicate (Tunca-Pınarlı et al. 2023).

2.5.2 Minimum İnhibitory Concentration (MIC) Test

The aqueous extract of *Daucus carota* ssp. *sativus* var. *atrorubens* was initially filtered (GVS North America, USA) to ensure sterilization. Prepared Mueller-Hinton broth (Oxoid, UK) was transferred into a 96-well microplate (Jet Biofil, China) at 100 µL per well. Subsequently, 100 µL of the extract was added to the wells. Serial dilutions were performed by transferring 100 µL from the first well to the eighth well, with each solution being transferred sequentially to the following wells. Finally, microorganisms, standardized to a 0.5 McFarland value in isotonic water, were added to the microplate wells. The eleventh column was used as a negative control, containing only the medium and the extract, to confirm the absence of contamination and ensure that the medium itself did not have an inhibitory effect. The twelfth column, containing only the medium and the bacteria, served as a positive control. All tests were performed in triplicate (Canlı et al. 2019).

2.6 Antioxidant Activity Test (DPPH)

A total of 3.9432 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) was weighed using a precision balance and dissolved in 50 mL of ethanol. To protect the DPPH solution from light exposure, the outer surface of the glass Erlenmeyer flask was wrapped with aluminum foil. The extract obtained from *Daucus carota* ssp. *sativus* var. *atrorubens* was mixed with the DPPH solution. The mixture was then incubated at room temperature in the dark at 30-minute intervals. The absorbance of the sample was measured at 515 nanometers using a spectrophotometer (Biotek, USA). Ascorbic acid (Carlo Erba, Spain) was used as the positive control in this study (Turu et al. 2020).

2.7 Gas Chromatography-Mass Spectrophotometry Method (GC-MS)

The biochemical content analysis of *Daucus carota* ssp. *sativus* var. *atrorubens* was determined using the GC-MS method employed by Canlı et al. (2017). This method, applied for the analysis of general aromatic compounds, was used exactly as described without any modifications.

2.8 Statistics

The statistical analysis of the ethanol extract of *Daucus carota* ssp. *sativus* var. *atrorubens*, which was repeated three times, was performed using one-way analysis of variance (ANOVA), a parametric method. Pearson's correlation coefficient was determined to assess any potential relationship between antimicrobial activity and concentration. P-values less than 0.05 were considered statistically significant. This statistical analysis was conducted using R Studio.

3. Results and Discussions

3.1 Disk Diffusion Test

The results of disk diffusion are given in Table 1. The antimicrobial activity of the ethanol extract obtained from the *D. carota* ssp. *sativus* var. *atrorubens* plant was assessed against 28 strains of microorganisms using the disk diffusion method. The zones formed around the discs impregnated with the extract are shown in Table 1.

Table 1. Disk Diffusion Test (Inhibition zones in mm.).

Microorganisms	30 µl	70 µl	150 µl	GEN	TOB
Listeria monocytogenes ATCC 7644	0±0.00	8±0.00	10±0.33	28	27
Enterobacter aerogenes ATCC 13048	0±0.00	0±0.00	0±0.00	24	18
Salmonella enteritidis ATCC 13076	7±0.00	7±0.00	7±0.00	21	-
Escherichia coli ATCC 25922	7±0.00	7±0.00	7±0.00	22	20
Staphylococcus aureus ATCC 25923	8±0.00	8±0.00	10±0.00	21	14
Candida albicans DSMZ 1386	0±0.00	0±0.00	0±0.00	12	13
Bacillus subtilis DSMZ 1971	0±0.00	0±0.00	0±0.00	30	26
Staphylococcus epidermidis DSMZ 20044	7±0.00	8±0.00	9±0.00	30	26
Pseudomonas aeruginosa DSMZ 50071	0±0.00	7±0.00	9±0.00	15	22
Pseudomonas fluorescens P1	0±0.00	7±0.00	8±0.00	13	12
Salmonella typhimurium SL 1344	0±0.00	0±0.00	0±0.00	24	15
Staphylococcus aureus (CI)	0±0.00	0±0.00	9±0.00	22	18
Enterococcus faecium (FI)	9±0.00	11±0.00	16±0.33	28	15
Listeria innocua (FI)	7±0.00	8±0.00	9±0.00	13	15
Salmonella Kentucky (FI)	0±0.00	0±0.00	0±0.00	12	16
Klebsiella pneumoniae (FI)	0±0.00	0±0.00	9±0.00	19	23
Escherichia coli (FI)	0±0.00	9±0.00	0±0.00	20	-
Enterococcus durans (FI)	7±0.00	7±0.00	9±0.00	11	13
Salmonella infantis (FI)	7±0.00	7±0.00	7±0.00	17	14
Acinetobacter baumannii (MDR)	0±0.00	0±0.00	7±0.00	0	-
Streptococcus pneumoniae (MDR)	9±0.00	9±0.00	9±0.00	10	8
Serratia odorifera (MDR)	0±0.00	7±0.00	8±0.00	7	9
Escherichia coli (MDR)	0±0.00	0±0.00	0±0.00	8	9
Staphylococcus aureus MRSA + MDR (MDR)	8±0.00	9±0.00	9±0.00	22	21
Enterobacter aerogenes (MDR)	0±0.00	0±0.00	0±0.00	16	18
Staphylococcus aureus MRSA (MDR)	0±0.00	7±0.00	9±0.33	0	7
Klebsiella pneumoniae (MDR)	7±0.00	9±0.00	9±0.00	15	20
Proteus vulgaris (MDR)	0±0.00	0±0.00	7±0.00	11	11

GEN: Gentamicin, TOB: Tobramicin, Standard error (±SE)

In a disk diffusion test conducted with 28 microorganisms to determine the antimicrobial activity of the plant sample, a total of 21 inhibitory effects were observed against 21 microorganisms. These included 7 standard isolated strains (ST), 1 clinical isolated strain (CI), 6 food isolated strains (FI), and 7 multidrug-resistant strains (MDR). These microorganisms are, *E. coli* ATCC 25922 (7mm), *L. monocytogenes* ATCC 7644 (10 mm), *P. aeruginosa* DSMZ 50071 (9 mm), *P. fluorescens* P1(8 mm), *S. enteritidis* ATCC 13076 (7 mm), *S. aureus* ATCC 25923 (10 mm), *S. epidermidis* DSMZ 20044 (9 mm*), S. aureus* (9 mm), *E. durans* (9 mm), *E. faecium* (16 mm), *K. pneumoniae* (9 mm), *L. innocua* (9 mm), *S. infantis* (7 mm), *K. pneumoniae* (9 mm), *S. odorifera* (8 mm), *P. vulgaris* (7 mm), *S. pneumoniae* (9 mm), *S. aureus* MRSA (9 mm), *S. aureus* MRSA + MDR (9 mm).

3.2 Minimum Inhibitory Concentration (MIC) Test

The results of minimum inhibition concentration (MIC) test are given in Table 2. The MIC values of the water extracts obtained from the *D. carota* ssp. *sativus* var. *atrorubens* plant were determined against 21 bacterial strains, as shown in Table 2. MIC values ranged from 1.18 mg/ml to 4.75 mg/ml. The readings were performed visually.

Table 2. Minimum Inhibitory Concentration (MIC) Test

Microorganisms	MIC (mg\mL)
Escherichia coli ATCC 25922	-
Listeria monocytogenes ATCC 7644	-
Pseudomonas aeruginosa DSMZ 50071	4.75 mg\ml
Pseudomonas fluorescens P1	-
Salmonella entiritidis ATCC 13076	-
Staphylococcus aureus ATCC 25923	-
Staphylococcus epidermidis DSMZ 20044	2.37 mg\ml
Staphylococcus aureus	2.37 mg\ml
Enterococcus durans	4.75 mg\ml
Enterococcus faecium	1.18 mg\ml
Klebsiella pneumoniae	-
Listeria innocua	4.75 mg\ml
Salmonella infantis	-
Escherichia coli	2.37 mg\ml
Acinetobacter baumannii	-
Klebsiella pneumoniae	-
Serratia odorifera	-
Proteus vulgaris	-

Streptococcus pneumoniae	-
Staphylococcus aureus MRSA	4.75 mg\ml
Staphylococcus aureus MRSA + MDR	-

(-): No effect was observed.

The test was conducted in triplicate. MIC values *P. aeruginosa* DSMZ 50071 (4.75 mg/ml), *S. epidermidis* DSMZ 20044 (2.37 mg/ml), *S. aureus* (2.37 mg/ml), *E. durans* (4.75 mg/ml), *E. faecium* (1.18 mg/ml), *L. innocua* (4.75 mg/ml), *E. coli* (2.37 mg/ml), *S. aureus* was determined to be MRSA (4.75 mg/ml).

3.3 Antioxidant Activity Test (DPPH)

The results of the antioxidant activity test (DPPH) are given in Table 3. The antioxidant activity results of the *D. carota* ssp. *sativus* var. *atrorubens* extract are shown in Table 3. The positive control was achieved using ascorbic acid. It was observed that the free radical scavenging efficiency of DPPH increased proportionally with concentration. The highest scavenging efficiency of the DPPH radical was found at a concentration of 1 mg/mL. Figure 1 demonstrates the variation in radical scavenging activity with concentration.

3.4 Gas Chromatography-Mass Spectrophotometry Method (GC-MS)

The biochemical content analysis of *Daucus carota* ssp. *sativus* var. *atrorubens* plant was determined by GC-MS method and the results are given in Table 4.

4. Discussions and Conclusions

4.1 Discussion

Despite the rich content of bioactive compounds in *Daucus carota* ssp. *sativus* var. *atrorubens*, studies on its antimicrobial activity remain relatively scarce and insufficient. Therefore, our study is significant in addressing the existing gap in the literature and contributing to the development of future antimicrobial agents. The findings obtained overlap with and differ from other studies conducted on similar plant species, providing a unique perspective.

Table 3. Antioxidant Activity Test (DPPH)

Concentration µg/mL	DPPH %	Content of ascorbic acid %
1000	45.921	94.665
500	24.752	93.391
250	16.402	92.077
125	9.067	90.086
62.5	5.945	69.943
31.25	3.666	35.794
15.625	3.523	17.698
7.8125	2.258	8.739

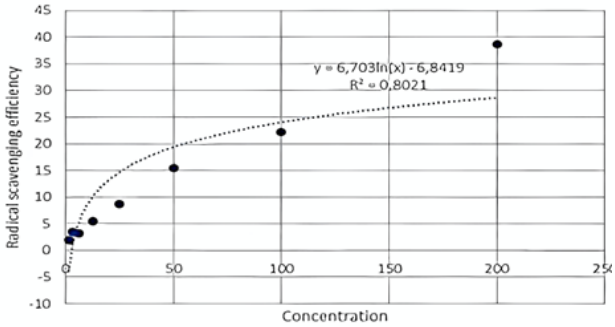

Figure 1. Radical scavenging efficiency (Graph)

Table 4. Gas Chromatography-Mass Spectrophotometry Method (GC-MS)

Retention Time	Components	Formula	Moleculer Weight	Area (%)
10.948	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12g/mol	0.79
10.948	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12g/mol	0.79
17.492	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12g/mol	1.31
21.066	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11g/mol	14.17
23.322	4-vinyl-2-methoxyphenol	$C_9H_{10}O_2$	150.17g/mol	1.46
38.544	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-	$C_{11}H_{12}O_4$	208.21g/mol	0.78
41.949	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42g/mol	8.36
42.339	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5g/mol	8.88
43.410	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	$C_{17}H_{24}O$	244.37g/mol	3.31
46.083	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4g/mol	4.83
46.374	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.5g/mol	18.30
46.507	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.5g/mol	3.08
52.661	Cyclododecasiloxane, tetracosamethyl-	$C_{24}H_{72}O_{12}Si_{12}$	889.8g/mol	2.92

For instance, Asilbekova et al. (2017) investigated the antimicrobial activities of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Candida albicans* using essential oils obtained through hydrodistillation. In their study, *B. subtilis* and *S. aureus* were found to be sensitive to the essential oil, with the highest antimicrobial activity observed against *C. albicans*, showing a 13 mm inhibition zone in the disk diffusion test. In contrast, our study identified inhibition zones of 10 mm for *S. aureus*, 16 mm for *Enterococcus faecium*, and 10 mm for *Listeria monocytogenes* using 150 µl ethanol extract. The high inhibition observed against L. monocytogenes is particularly significant, considering this pathogen's potential for rapid resistance development.

The primary differences between our study and that of Asilbekova et al. lie in the extraction methods and testing techniques used. While our study employed ethanol extract in the disk diffusion method, Asilbekova et al. examined the antimicrobial effects of essential oils using the agar diffusion method. In addition to these differences, environmental factors such as the geographical location where the plants were grown, soil composition, and the amount of sunlight exposure may have influenced the results. Furthermore, in our study, a minimum inhibitory concentration (MIC) of 1.18 mg/ml was determined for *E. faecium*, but due to limited extract concentration, MIC test results could not be obtained for *S. aureus* and *L. monocytogenes*. This limitation may explain the discrepancy where some microorganisms show inhibition in the disk diffusion test but not in the MIC test.

Moreover, gas chromatography-mass spectrometry (GC-MS) analysis identified a total of 12 main components, with linoleic acid (18.30%) being the most prominent. Linoleic acid is a polyunsaturated fatty acid reported in the literature to possess antimicrobial and antioxidant properties (Mendis et al. 2005). The presence of linoleic acid may significantly contribute to the potential biological activity of the plant.

Additionally, the compound 5-hydroxymethylfurfural (HMF), known for its antimicrobial, antioxidant, and anti-inflammatory effects and isolated from various plant sources (Zhang et al. 2010), was detected in the study. The presence of HMF may explain the potential protective effects against oxidative stress and the inhibitory impact on microorganisms.

These findings should be compared with existing studies in the literature to evaluate the contributions of the chemical constituents of *D. carota* ssp. *Sativus* var. *atrorubens* to its biological activity in a broader context. The high levels of compounds such as linoleic acid and HMF support the potential use of this plant as an antimicrobial and antioxidant agent. However, variations in the levels of these compounds across different parts of the plant or samples obtained using different extraction methods should also be considered.

In terms of antioxidant activity, the findings support a direct relationship between anthocyanin content and antioxidant capacity. For example, in the study by Demir (2010), the highest total phenolic and anthocyanin contents were determined from black carrot samples treated with microwaves. Similarly, in our study, it was shown that the initially high antioxidant activity decreased during oral digestion, highlighting the dynamic nature of antioxidant bioavailability. The DPPH radical scavenging activity test also yielded strong results, with the highest scavenging efficiency observed at a concentration of 1 mg/ml, underscoring the importance of concentration in enhancing antioxidant properties.

In light of these analyses, our comprehensive study contributes valuable insights into the biochemical properties and biological activities of *D. carota* ssp. *Sativus* var. *atrorubens*. While differences with other studies can be attributed to factors such as extraction techniques and environmental conditions, our findings suggest that this plant may serve as a potential source for the development of new antimicrobial and antioxidant agents.

4.2 Conclusion

In this study, the antimicrobial activity of *Daucus carota* ssp. *sativus* var. *atrorubens* was comprehensively evaluated using disk diffusion and minimum inhibitory concentration (MIC) tests. The findings revealed antimicrobial activity against 21 out of 28 tested microorganisms. Notably, significant antimicrobial effects were observed on multidrug-resistant microorganisms, such as *Staphylococcus aureus*, which poses a major health challenge today. These results suggest that the plant's antimicrobial potential is promising and could contribute to the development of new therapeutic options in this field. MIC tests for microorganisms exhibiting antimicrobial activity showed values ranging from 1.18 to 4.75 mg/ml, indicating that the lack of inhibition observed for some microorganisms in the MIC test may be attributed to the limited concentration of the extract.

Additionally, our study analyzed the biochemical composition of the plant using gas chromatography-mass spectrometry (GC-MS), identifying 12 major components. Linoleic acid (18.30%) was found to be the predominant component, and it has been reported in the literature to possess antimicrobial and antioxidant properties. This finding suggests that linoleic acid may significantly contribute to the plant's biological activity. Furthermore, the presence of 5-hydroxymethylfurfural (HMF), known for its antimicrobial, antioxidant, and anti-inflammatory properties, may support the plant's potential protective effect against oxidative stress.

The antioxidant activity was assessed using the DPPH radical scavenging method, and it was found that radical scavenging efficiency increased linearly with higher concentrations. These findings indicate that this plant, which is rich in anthocyanins, polyphenols, and

flavonoids, exhibits strong antioxidant activity even at low concentrations, showing meaningful biological activity, though not as potent as ascorbic acid.

In conclusion, this study supports the potential use of *D. carota* ssp. *sativus* var. *atrorubens* as a biological agent with antimicrobial and antioxidant properties. The findings contribute to the literature by providing a detailed characterization of the plant's biochemical components and biological activities, suggesting that this plant could be considered as a natural source for the development of future antimicrobial and antioxidant agents.

Declaration of Ethical Standards
The authors declare that they comply with all ethical standards.

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Credit Authorship Contribution Statement
Author-1: Conceptualization, investigation, methodology and software, visualization and writing – original draft.
Author-2: Investigation, methodology and software, Resources,
Author-3: Methodology / Study design, Resources, Data curation
Author-4: Methodology / Study design, Resources, Data curation
Author-5: Resources
Author-6: Resources
Author-7: Resources, Methodology / Study design
Author-8: Resources, Methodology / Study design, Writing – review and editing, Visualization, Supervision, Project administration

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement
All data generated or analyzed during this study are included in this published article.

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