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Research Article

Chemical Composition and Antibacterial Activity of Volatile Compounds *Genista carinalis* Plant

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ABSTRACT

Volatile compounds play a significant role in the plant chemistry. Natural products have been widely used in antiviral, antibacterial, antiparasitic, antifungal, insecticidal, medicinal and cosmetic applications since the Middle Age. Our aim in this study was to investigate the volatile components of the *Genista carinalis* Griseb. (Fabaceae) plant grown in the Trakya region by GC-MS method and to determine the antibacterial activity of the raw extracts. Volatile components of n-hexane extract from the plant *G. carinalis* were investigated using the GC-MS method. As a result of our research on the volatile components of *G. carinalis*, fifty-two bioactive components were identified. The ethyl acetate extract of *G. carinalis* was the most effective on both Gram-positive and Gram-negative bacteria tested, with higher antibacterial activity against Gram positive bacteria.

Keywords: *Genista, Genista carinalis, Fabaceae, Volatile compound, Antibacterial activity*

Genista carinalis Bitkisinin Uçucu Bileşiklerinin Kimyasal Bileşenleri ve Antibakteriyel Aktivitesi

ÖZ

Uçucu bileşikler, bitki kimyasında önemli bir rol oynamaktadır. Doğal ürünler, Orta Çağ'dan beri antiviral, antibakteriyel, antiparazitik, antifungal, böcek öldürücü, tıbbi ve kozmetik uygulamalarında yaygın olarak kullanılmaktadır. Bu çalışmadaki amacımız, Trakya bölgesinde yetişen *Genista carinalis* Griseb. (Fabaceae) bitkisinin uçucu bileşenlerini GC-MS yöntemi ile araştırmak ve ham ekstrelerinde antibakteriyel aktivitesini belirlemektir. *G. carinalis* bitkisinden elde edilen n-hekzan ekstresinin uçucu bileşenleri, GC-MS yöntemi kullanılarak araştırılmıştır. *G. carinalis*'in uçucu bileşenleri üzerine yaptığımız araştırma sonucunda elli iki adet biyoaktif bileşen tespit edilmiştir. Çalışmada denenen hem Gram-pozitif hem de Gram-negatif bakteriler üzerinde en yüksek antibakteriyel etki *G. carinalis*'in etil asetat ekstresi ile elde edilmiştir. Bu ekstrenin Gram-pozitif bakterilere etkisinin Gram-negatiflere oranla daha yüksek olduğu gözlenmiştir.

Anahtar Kelimeler: *Genista, Genista carinalis, Fabaceae, Uçucu bileşik, Antibakteriyel aktivite*

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I. INTRODUCTION

Natural products from some of the higher plants may be a new source of antimicrobial agents, which may have biological mechanisms of action [1]. Volatile compounds from aromatic plants are used in medicine and pharmacology as antimicrobial, anti-inflammatory, antioxidant, expectorant, analgesic and in the treatment of many ailments. They are also effective in defense against herbivores and pathogens [2]. The volatile compounds, which include chemical classes such as alcohols, esters, aliphatic and aromatic hydrocarbons, terpenes, nitrogen and sulphur compounds, are small molecules biosynthesized by primary and secondary metabolic pathways [3].

Plant essential oils, which may be called under different names such as volatile oil, aromatic oil or spirit, are important components of plant chemistry [4]. Essential oils, which constitute an important part of the traditional pharmacopoeia (codex), are generally obtained from various aromatic plants that grow in the geography between the warm-tropical countries and the temperate Mediterranean countries [5].

Nowadays, obtaining and evaluating medicinal plants and essential oils from such plants is very important scientifically and economically. Researches show that the essential oils of these plants have antimicrobial activities. In the antibiotic resistance era, new extracts and materials having antimicrobial activities are vital [6]. Besides the antimicrobial effects, by examining the pharmacological properties of essential oils and their components, it is stated that the possibilities of using them in medicine, cosmetics and industrial areas can be beneficial [7].

Genista species are used in the treatment of some human diseases. Of these, flowers or flowering branches of *G. tinctoria* and *G. lydia* species are used as diuretic, diaphoretic or laxative. In addition, the flowers of these plants are used to dye wool and linen fibers yellow or green [8].

The flowering time of the *G. carinalis* plant is June. It spreads around the world in Bulgaria, Greece and Türkiye. In Türkiye, it spreads in the northwest and west (Kırklareli, Istanbul, Kocaeli, Balikesir and Izmir). It is found in maquis and forest clearing areas [9].

Our aim in this study is to investigate the volatile components of n-hexane exract of the *G. carinalis* grown in the Trakya region by GC-MS method and to determine the antimicrobial activity of the raw extracts (n-hexane, chloroform, ethyl acetate and n-butanol).

II. MATERIAL AND METHODS

A. PLANT MATERIAL AND EXTRACTIONS

G. carinalis (2048.56 g) plant was collected from Trakya region (Kırklareli; Location: 41°52'47.8"N 27°34'42.9"E and 41°52'29.5"N 27°34'36.4"E), at the time of flowering in May 2018. Identification of the plant has been done and its Herbarium number (EDTU-16811) has been given by Asst. Prof. N. Guler at Trakya University. After the whole plant was collected, it was dried in shade, ground into powder and divided into small portions and extracted with methanol (Merck-1070184000) at room temperature. The extraction process was repeated every three days, a total of four times. After the methanol was evaporated in the evaporator, a small amount of water was added to the crude extract obtained and back-extracted with n-hexane (Merck-1043742500) (46.85 g), chloroform (Merck-

1070242500) (15.50 g) ethyl acetate (Merck-1007892500) (45.66 g) and n-butanol (Merck-1019902500) (434.51 g) in order of polarity. Then, the solvents were evaporated in the evaporator and crude extracts were obtained [10]. A total of four crude extracts were obtained. The n-hexane, chloroform, ethyl acetate and n-butanol extracts have been used in the antibacterial activity tests. In addition, n-hexane extract was investigated by GC-MS method for the determination of volatile compounds in the plant.

B. GC-MS ANALYSIS

The instrument Hewlett-Packard HP 6890 series GC-MS equipped with a mass selective detector was used for chromatographic analysis. HP-5MS (5% phenyl methyl siloxane, 30m x 250 µm x 0.25 mm) capillary column was used. Helium was used as carrier gas at a flow rate of 1.51 ml/min with an injection volume of 5 µl. After injection, the samples were introduced into the column, which was initially held at 50℃ for 5 minutes, then the temperature was elevated to 220℃ with a 10℃/min heating ramp. The samples were injected in the split mode (split ratio: 40:1). The working time was recorded as 50 minutes [11]. The MS scanning range was found using electron effect ionization (EI) (70 eV) and an ion source temperature of 200℃. Compound determinations were performed using Wiley 9 and NIST libraries. The proportions of discrete compounds were computed by the computer integrator based on total ion chromatography. Retention index (RI) were determined extensively under the same chromatographic conditions, using a series of n-alkanes (C_6-C_{22}) [12].

C. ANTIBACTERIAL ACTIVITY

The obtained extracts were tested on 2 Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 278523) and 2 Gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) using agar well diffusion method [13]. The bacteria were obtained from Turkish Republic General Directorate of Public Health, Ankara, Türkiye. They were first grown on blood agar (BioMerieux, France) and adjusted to be 0.5 McFarland in sterile saline solution. The bacteria were spread on Mueller Hinton agar plates (under aseptic conditions. Agar wells of 6 mm diameter were formed on agar and 100 µl of each extract dissolved in anhydrous DMSO (99.9%, Sigma Aldrich, Germany) was added in the wells. DMSO was also used as negative control, while Gentamicin sulfate (Sigma Aldrich, Germany) was used as positive control [14]. The plates were kept at 37℃ overnight. The diameter of the zones around each well were measured. The experiment was repeated twice. The effects of extracts were compared using one-way ANOVA in Minitiab 13 software.

III. RESULTS AND DISCUSSION

The GC-MS chromatography analysis of *G. carinalis* revealed that there were fifty-two peaks and compounds in the n-hexane extract. The chemicals were identified according to their retention indices (RI) and concentration (calculated by the peak area %). These characterized compounds of *G. carinalis* plant are shown in Table 1 and Table 2, the former revealed a total of 52 compounds as a result of GC-MS analysis of the n-hexane extract of *G. carinalis* plant. In particular, *G. carinalis* n-hexane extract contains Hexadecanoic acid (Palmitic acid) (23.09%), one of the saturated fatty acids, 1-hexadecene (Flax) (10.46%), which is an alkene, and fatty acid (Z, Z, Z)-9,12,15-octadecatrienoic acid (Linolenic acid) (9.20%) and the phenolic compound 2,4-bis(1,1-dimethylethyl)-phenol (8.66%) are thought to be potential sources.

Table 1. *The composition of n-hexane extract obtained from G. carinalis.*

No	RI	Compounds	Percentage (%)
1	1022	1-methyl-2-pyrrolidinone	0.96
2	1055	Nonanal	0.51
3	1095	1-Dodecene	3.53
4	1102	Decanal	0.22
5	1144	1-Methoxyethyl benzoate	0.28
6	1151	Dodecane	0.15
7	1153	N,N-dibutyl-formamide	0.11
8	1195	1-Pentadecene	9.29
9	1199	Hexadecane	0.33
10	1206	Dodecanal	0.38
11	1261	2,6,11-trimethyl-dodecane	0.13
12	1273	1-bromo-3-(2-bromoethyl)-nonane	0.31
13	1281	1-methylpropyl ester 2-propenoic acid	0.17
14	1284	(1-methyl-1,2-ethanediyl) bis[oxy(methyl-2,1-ethanediyl)] ester 2-propenoic acid	0.18
15	1297	Docosane	0.59
16	1310	Hexadecanal	0.13
17	1315	2,4-bis(1,1-dimethylethyl)- phenol	8.66
18	1341	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	0.41
19	1361	Lauric acid	0.62
20	1381	1,6-hexanediyl ester 2-propenoic acid	0.27
21	1389	Cetene	10.46
22	1396	Tridecane	0.27
23	1444	1-Dodecanol	0.21
24	1457	3-ethyl-2,7-dimethyl-octane	0.12
25	1460	(1-hydroxycyclohexyl) phenyl-methanone	1.23
26	1465	5-methyl-5-propyl-nonane	0.33
27	1467	Cyclopentadecanol	0.36
28	1493	Tetradecanoic acid	0.75
29	1498	(4-methylphenyl) phenyl- methanone	0.36
30	1564	1-Octadecene	6.87
31	1610	Isopropyl myristate	0.14
32	1617	Neophytadiene	0.12
33	1620	6,10-dimethyl-2-undecanone	1.14
34	1627	Pentadecanoic acid	0.14
35	1629	Dotriacontane	0.13
36	1633	Isobutyl phthalat	0.24
37	1634	N,N-dioctyl-1-Octanamine	0.19
38	1638	5-Methyl-2-ethoxy-3,4-dihydro-2H-pyran	0.48
39	1643	Nonadecane	0.42
40	1646	Eicosane	0.35
41	1653	3-Ethyl-5-(2'-ethylbutyl) octadecane	0.17
42	1655	Undecanal	0.28
43	1658	Methyl palmitate	1.23
44	1659	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	1.14

45	1977	Palmitic acid	23.09
46	1694	E-15-Heptadecenal	3.21
47	1734	Cetal	1.17
48	1750	Phytol	0.56
49	1759	Methyl linolelaidate	2.94
50	1763	Linolenic acid	9.20
51	1774	Stearic acid	4.28
52	1793	1-Docosene	1.22

RI: Retention Indices.

These compounds are evaluated according to their functional group; the main components in the extract are alkenes (31.37%), saturated fatty acids (24.60%), unsaturated fatty acids (13.48%) and phenolic compounds (8.66%) (Table 2).

Table 2. *The chemical class distribution of G. carinalis in n-hexane extract.*

Compounds	**Percentage (%)**
Nitrogen Containing Heterocyclic Compound	0.96
Aldehyde	4.73
Alkene	31.37
Ester	6.59
Alkane	2.99
Amide	0.11
Alkyl Halides	0.31
Phenolic Compound	8.66
Terpene	1.09
Saturated Fatty Acid	24.60
Alcohol	1.74
Ketone	2.73
Amine	0.19
Heterocyclic Compound Containing Oxygen	0.48
Unsaturated Fatty Acid	13.48
Total (%)	**100.03**

Looking at the literature; Palmitic acid value also found in GC-MS analysis of *G. numidica* (15.34%), *G. saharae* (32.32%), *G. ulicina* (18.60%) and *G. vepres* (26.40%) plants, was observed at a value close to that of our study (%23.09) [15], [16]. Additionally, Rigano et al. [17], suggested that hexadecanoic acid (20.20%) was the main fatty acid identified in the *G. sessilifolia* and (E)-β-ionone (9.10%) was the main carbonylic compound identified in the *G. tinctoria*.

The antibacterial activities of *G. carinalis* n-hexane extract on different bacteria are demonstrated in Figure 1, and examples of agar plates for the antibacterial testing of different extracts of *G. carinalis* are given in Figure 2. The selected bacteria are representatives of normal human flora which have opportunistic subspecies that cause serious nosocomial infections [18]. It is well known that the solvent DMSO has no antibacterial activity [19], [20]. The ethyl acetate extract of *G. carinalis* was the most effective extract on *E. coli* ($p=0.001$). Apart from ethyl acetate, the others did not have significant effect on *E. coli*. On the other Gram-negative bacterium *P. aeruginosa* ethyl acetate extract of the plant was the most effective ($p=0.000$), followed by chloroform and n-butanol back extracts. N-hexane extract of *G. carinalis* did not have a significant antibacterial effect on this bacterium.

The degree of the effects of the back extracts were not the same on each bacteria tested (Figure 1). Species specific characteristics of the bacteria can affect the efficacy of antibacterial agents, as suggested for metal antimicrobial agents [21]. In addition, the structural difference between Gram-positive and Gram-negative bacteria can be responsible for the different resistance levels to antibacterial agents. Nevertheless, ethyl acetate extract was also the one exerting the highest antibacterial activity on the other two bacteria *S. aureus* and *E. faecalis* (p=0.000 for both). Ethyl acetate can be used as a good solvent for the plant extracts for medicinal purposes. There are several studies showing the efficacy of plant extracts obtained in ethyl acetate [22], [23], [24]. Therefore, ethyl acetate should be the choice for the back extraction of *G. carinalis* n-hexane extracts.

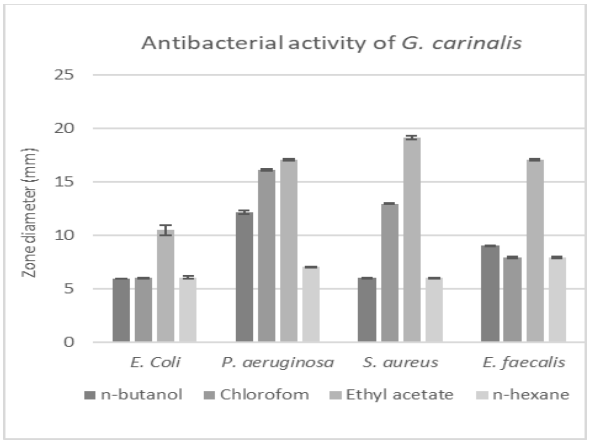

Figure 1. *The antibacterial activities of G. carinalis in different solvents.*

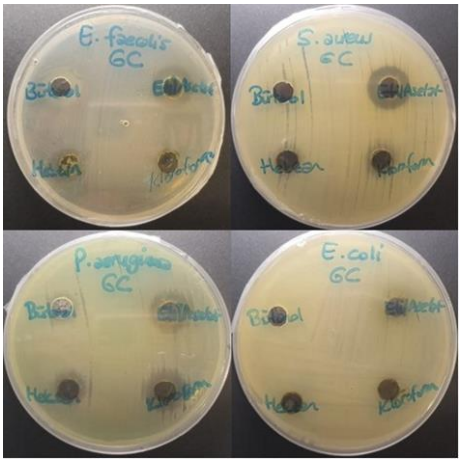

Figure 2. *The plate photos of the antibacterial testing of G. carinalis extracts on different bacteria.*

As tabulated in Table 1, one of the dominant ingredients of n-hexane extract of *G. carinalis* was palmitic acid (23.09%). Palmitic acid was found to be the major antibacterial ingredient of ethyl acetate extract of *Pentanisia prunelloides*. The extracts were reported to have antimicrobial activity against *E. coli*, *K. pneumoniae*, *B. subtilis* and *S. aureus* [25]. The second most abundant content of extract of *G. carinalis* was centere (10.46%) which was found to be an ingredient of the extract of *Loxostylis alata*, which had significant antibacterial effect against *Salmonella* species [26]. Linoleic acid and stearic acid are fatty acids known to have antibacterial activities [27] are also found in the extract of *G. carinalis* in 9.20% and 4.28%, respectively. To the best of our knowledge, there is no study investigating the antibacterial activity of *G. carinalis*, however, other species from the same

genus were studied. In one study, linoleic acid was detected in the oil obtained from *G. ulicina* and *G. vepres* in ratios of 3.1 and 11.7%. The oils obtained were also found to have antibacterial activity against the bacterial species used in our present study [16]. Another study carried out on *G. numicida* revealed that methanol extract of the aerial parts of the plant had antibacterial activity against some Gram-positive and Gram-negative bacteria. The contents of n-hexane extract of *G. carinalis* should have structured contents similar to the ones cites in the literature, therefore they can be said to have antibacterial activity against the two Gram-negative and two Gram-positive bacteria tested.

IV. CONCLUSION

The purpose of this document was to analyze the components of the *G. carinalis* n-hexane extract and to determine the antibacterial activity of the raw extracts. This research is the pioneer study on chemical composition of n-hexane extracts of *G. carinalis* together with the antibacterial activity of the raw extracts (n-hexane, chloroform, ethyl acetate and n-butanol).

The chemical classes of the n-hexane extract of *G. carinalis* were reported Table 2. Based on the administered chemical classes, the compounds were divided into fifteen classes, nitrogen containing heterocyclic compound, aldehyde, alkene, ester, alkane, amide, alkyl halides, phenolic compound, terpene, saturated fatty acid, alcohol, ketone, amine, heterocyclic compound containing oxygen and unsaturated fatty acid. On the whole, alkenes (31.37 %) constituted the main fractions of the n-hexane extract of *G. carinalis*. GC-MS analysis of hexane extracts from *G. carinalis* revealed various chemical compounds of pharmaceutical importance with different chemical structures.

The agar plates for the antibacterial testing results exhibited that the ethyl acetate extract of *G. carinalis* was the most effective extract on the bacteria tested. Therefore, ethyl acetate can be selected as the solvent to exhibit the antibacterial activity of the extracts of *G. carinalis*.

The results triggered us as a future study to antibacterial MIC testing and the isolation studies.

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