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ORIGINAL ARTICLE / ORIGINALBEITRAG

GC-MS Analysis and Evaluation of Antibacterial and Antifungal Activity of Essential Oils Extracted From Fruit Peel of *Citrus aurantium* L. (Rutaceae) Grown in the West Anatolian Area

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Abstract

In this study, chemical composition and *in vitro* antibacterial and antifungal effects of essential oils extracted from fruit peel of *Citrus aurantium* L. grown in West Anatolian ecological conditions, were investigated. Antibacterial and antifungal activities were determined with the use of Minimum Inhibitory Concentration (MIC) method. Essential oils were obtained in a clevenger apparatus and their compositions were analysed by Gas Chromatography-Mass Spectrometry (GC-MS). According to the GC-MS results, dl-limonene (72.51%) and hexasiloxane (13.28%) were the major components in the essential oil obtained from *C. aurantium* Central population. Limonene (77.27%) and hexasiloxane (6.19%) dominated in the samples originated from Germencik region. Limonene (79.77%) was found to be in the highest concentration in the essential oil obtained from *C. aurantium* Koçarlı population. Limonene (95.70%) followed by β-myrcene (0.76%) were the major components of the essential oil obtained from *Citrus aurantium* Nazilli population. MIC values ranged from 2.5 to 5 μL/mL for gram (–) bacteria and from 0.3125 to 5 μL/mL for gram (+) bacteria. Essential oil from Germencik region generally showed the best effect against fungi. Essential oil obtained from *Citrus aurantium* fruit peel could be an alternative to synthetic antimicrobial agents.

Keywords Citrus aurantium · Essential oil · Antibacterial · Antifungal · Turkey

GC-MS-Analyse und Beurteilung der bakteriziden und fungiziden Wirkungen von ätherischen Ölen, gewonnen aus Fruchtschalen von *Citrus aurantium* L. (Rutaceae), angebaut in der Provinz West-Anatolien

Schlüsselwörter Citrus aurantium · Ätherisches Öl · Antibakteriell · Pilzhemmend · Türkei

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Introductıon

Aromatic plants constitute a large part of the natural flora and are considered as an important raw material for many branches of industry such as pharmaceutical, flavour and fragrance, perfume and cosmetic (Swamy et al. 2016). Essential oils are volatile, strongly scented and oily mixtures obtained by water vapour distillation from plants or herbal drugs, and are found in the liquid form at room temperature and sometimes they freeze (Özcan et al. 2013). These mixtures may include a wide range of free radical scavenger molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids which are responsible for high antioxidant activity (Elkıran et al. 2018). The use of

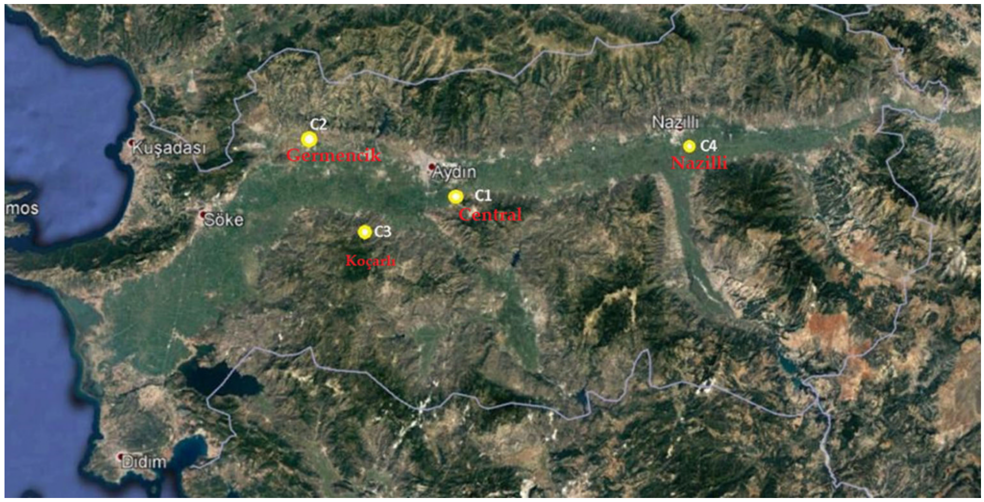

Fig. 1 Location of *Citrus aurantium* populations Aydın/Turkey (C1: Central, C2: Germencik, C3: Koçarlı, C4: Nazilli) (https://www.google.com/maps)

essential oils for the treatment of various inflammatory diseases such as rheumatism, fever and diabetes suggests that oxidative stress plays a role in human diseases and antioxidant intake may improve human health (Burits et al. 2001; Ekren et al. 2013).

Rutaceae family, which is of economic importance, includes 153 genera and approximately 1800 species in the world (Luo et al. 2010; Aktaş 2017). *Citrus* L., which is a nutritionally important genus, is a member of the Aurantioideae subfamily in the Rutaceae family and includes many fruit plants such as sour orange, sweet orange, citrons, pummelos, kumquats, lemon, mandarins and grapefruit. This genus is one of the most important horticultural crops with over 80 million tons of agricultural production worldwide (Bourgou et al. 2012; Kumar et al. 2013; Nagano et al. 2018). *Citrus* plants are indigenous to tropical Asia, but are found in all tropical and subtropical countries. It is an easily obtainable plant widely used in the treatment of various diseases (Suryawanshi 2011). *Citrus* fruits and fruit juices are sources of bioactive compounds important for human health such as ascorbic acid, flavonoids, phenolic compounds and pectins (Wali et al. 2013). *Citrus aurantium* L., which belongs to the genus *Citrus*, known as sour orange or bitter orange is traditionally grown in the Mediterranean region (Sarrou et al. 2013). The essential oils obtained from *C. aurantium* are used as an antioxidant, antimicrobial, anti-ulcerogenic, neuroprotective, anti-anxiety, anti-inflammatory, anti-cancer, anti-viral and anti-larvicidal agent (Almeida et al. 2015; Radan et al. 2018).

The aim of this study was to determine the composition of essential oils obtained from fruit peel of *Citrus aurantium* fruits growing in Aydın/Turkey and to evaluate their antimicrobial effects against some highly pathogenic bacteria and fungi.

Materials and Methods

Plant Materials

Fruits of *Citrus aurantium* plants were obtained as study materials in November and in December 2018 (Fig. 1).

Isolation of Essential Oils and GC-MS Analysis

Essential oils were obtained with a clevenger apparatus and their compositions were determined by GC-MS. The analyses were performed withan Agilent 7890A gas chromatograph coupled with the 5975C inert Mass Selective Detector. Helium was used as a carrier gas. Separation was carried out with a capillary column coated with 5% phenylmethyl polysiloxane (30.0 m × 250 µm × 0.25 µm). The temperature of injection port with split mode (split ratio 1:10) was set at 250 °C. The GC column temperature was programmed as follows: initial temperature was set at 50 °C and held for 2 min, then raised to 80 °C (held for 2 min), 100 °C (held for 1 min), 150 °C (held for 1 min), 240 °C (held for 1 min) and finally to 270 °C (held for 7 min), with heating rate of 5 °C/min (Rambla et al. 2015; Apaydın 2018). The slower heating rate is supposed to provide better separation of compounds with similar volatility. Mass spectrometer conditions were: ionization voltage of 70 eV and, molecular weight spectrum range was 100–550 Da. The recorded mass spectra were compared with the reference spectra using the computer libraries (Wiley and NIST).

Microorganisms

Four species of gram (+) and gram (–) bacteria, as well as four species of fungi were used in the assays. *Enterobacter*

Table 1 Composition of the essential oil from *C. aurantium fruit* peel

Central region			Germencik region			Koçarlı region			Nazilli region		
RT	Component	%	RT	Component	%	RT	Component	%	RT	Component	%
7.452	Gamma.-terpinene	0.26	7.452	Cyclohexene	0.39	7.452	Cyclohexene	0.39	7.452	1,3,6-octatriene	0.39
9.117	Pyridine	0.45	9.111	β-myrcene	0.98	8.671	β-pinene	0.26	8.671	β-pinene	0.28
10.461	d-limonene	72.51	10.484	d-limonene	77.27	9.117	β-myrcene	0.86	9.117	β-myrcene	0.76
13.214	α-terpınolene	0.37	24.228	Oxazole	0.30	10.479	d-limonene	79.77	10.467	d-limonene	95.70
18.672	Cis-ocimene	0.30	27.993	Octadecane	0.31	13.203	Linalool	0.33	13.220	Linalool	0.26
25.110	β-copaene	0.32	30.540	Tridecane	0.28	25.110	β-cubebene	0.51	18.673	Pyridine	0.31
43.843	Hexacosane	1.55	30.648	Tetracosane	0.72	40.554	Triacontane	0.58	25.110	α-amorphene	0.48
45.514	Heneicosane	1.70	42.241	Octacosane	0.53	42.236	Tetracosane	1.07	43.838	Eicosane	0.36
47.191	Eicosane	2.15	43.494	9-octadecenamide	3.01	43.455	Pentadecene	5.43			
50.183	Cyclopentadecanone	0.34	43.838	Triacontane	2.49	43.844	Heneicosane	1.70			
51.740	Nonadecane	1.36	45.508	Eicosane	1.19	47.185	Hexacosane	1.49			
52.249	Hexasiloxane	13.28	47.185	Docosane	1.34	48.776	Nonadecane	0.95			
			48.776	Nonadecane	0.93	50.275	Eicosane	1.06			
			52.249	Hexasiloxane	6.19						

RT Retention Time

aerogenes CCM 2531 and *Proteus vulgaris* FMC 1 were obtained from Fırat University; *Gordonia rubripertincta* (laboratory isolate), *Staphyloccocus cohnii* (laboratory isolate) and *Yersinia pseudotuberculosis* ATCC 911 were acquired from Yeditepe University; *Bacillus cereus* 702 ROMA and *Enterococcus faecalis* ATCC 29212 were acquired from Rize University; *Escherichia coli* ATCC 35218 was obtained from Giresun University; *Candida albicans* FMC 17 and *Candida tropicalis* ATCC 13803 were obtained from Fırat University; *Candida parapsilosis* ATCC 22019 was obtained from Giresun University, and *Saccharomyces cerevisiae* ATCC 9763 was obtained from Giresun Province Control Laboratory.

Antibacterial and Antifungal Activities

Antibacterial and antifungal activities of the essential oils were estimated by determination of MIC. MIC can be defined as the lowest concentration of the essential oil that prevents the growth of the test microorganisms. For MIC, a broth microdilution assay was used. Two-fold serial dilutions (in DMSO), from 0.01953 to 20 μL/mL of the *C. aurantium* essential oils, were prepared in a 96-well microplate. Into each well 95 μL of Müller Hinton Broth and 5 μL of the inoculum were added. An aliquot of 100 μL of essential oil was placed into the first well in a plate. Then, 100 μL from the serial dilutions were transferred into seven consecutive wells. The last well containing 195 μL of nutrient broth without compound and inoculum on each row was used as a negative control. Plates were incubated at 30 °C for 48 h for fungi and at 37 °C for 24 h for bacteria (Güllüce et al. 2004).

Results and Discussions

Chemical Composition of the Essential Oils

The components of essential oil from peel of *C. aurantium* fruits are gathered in Table 1 and the GC-MS chromatograms are presented in Fig. 2, 3, 4 and 5.

According to the results of the GC-MS analysis, the essential oil obtained from fruits of *C. aurantium* grown in the Central region contained the highest amount of dl-limonene (72.51%) and hexasiloxane (13.28%), whereas in the oil samples extracted from fruits picked in the Germencik region limonene (77.27%) and hexasiloxane (6.19%) were found to be in the highest concentration. In the case of the Koçarlı population of *Citrus aurantium*, essential oil contained mainly limonene (79.77%). Similarly, limonene was the main component of essential oil extracted from fruits grown in the Nazilli region and it constitutes 95.70%, whereas β-myrcene with concentration of 0.76% was the in the second place (Table 1).

In the earlier studies, chemical composition of essential oil extracted from peel, flowers and leaves of *C. aurantium* was studied. Bozkurt et al. (2017) found that d-limonene (89.29%) and linalool (3.43%) were the main components of essential oils from *C. aurantium* fruit peels purchased from the local markets in Adana/Turkey. Similar conclusions have been drawn by Radan et al. (2018) who de-

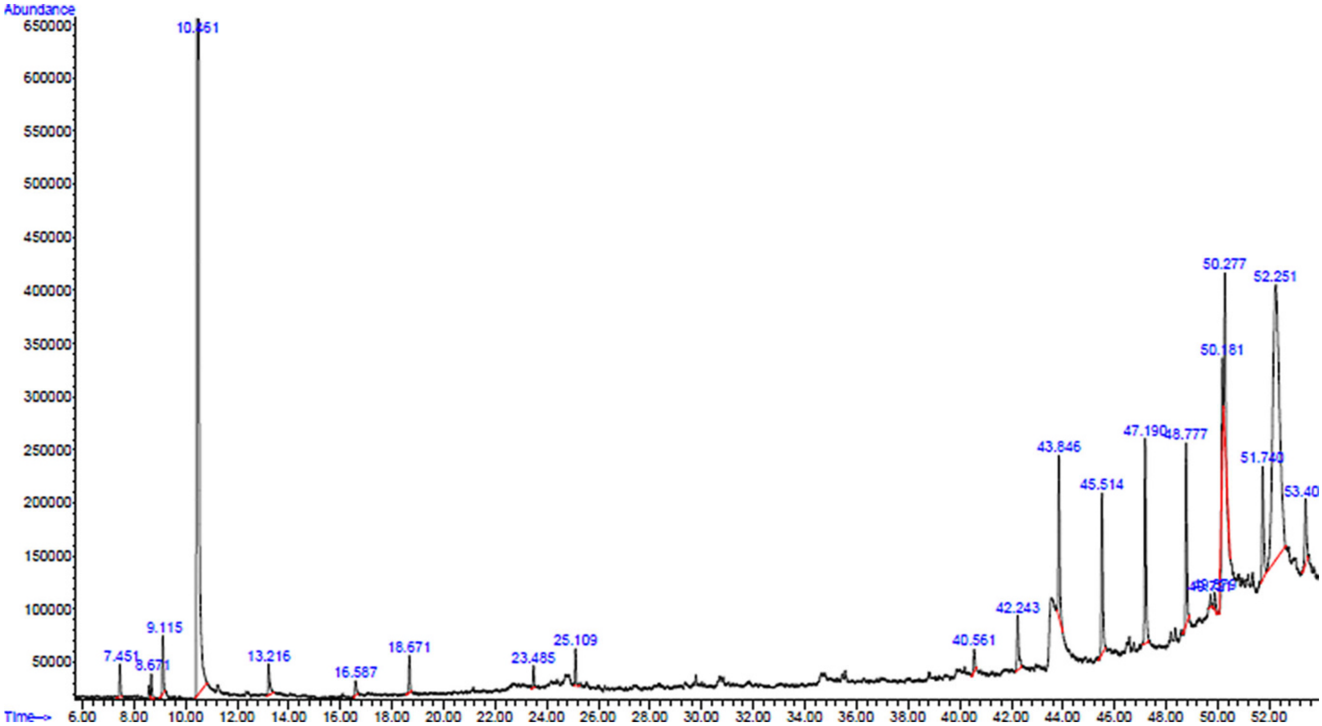

Fig. 2 Gas chromatogram of the essential oil of *C. aurantium* (Central region)

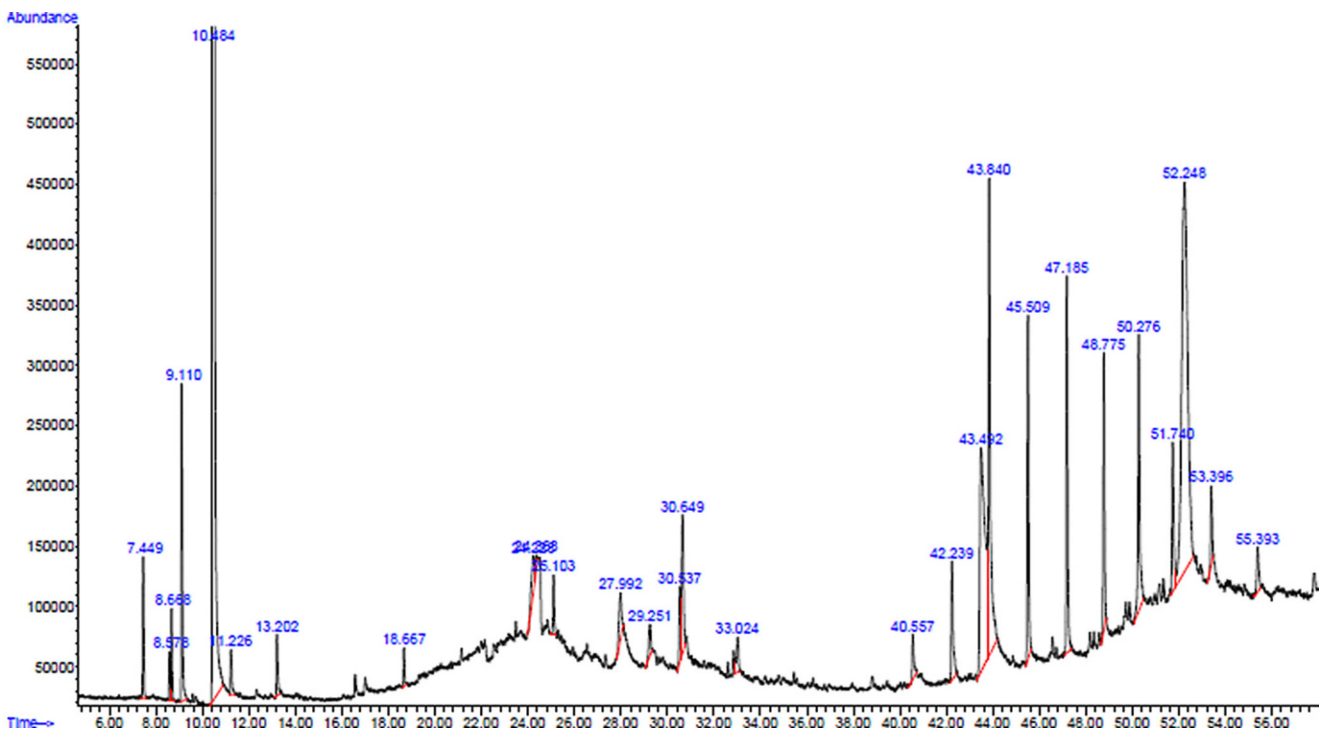

Fig. 3 Gas chromatogram of the essential oil of *C. aurantium* (Germencik region)

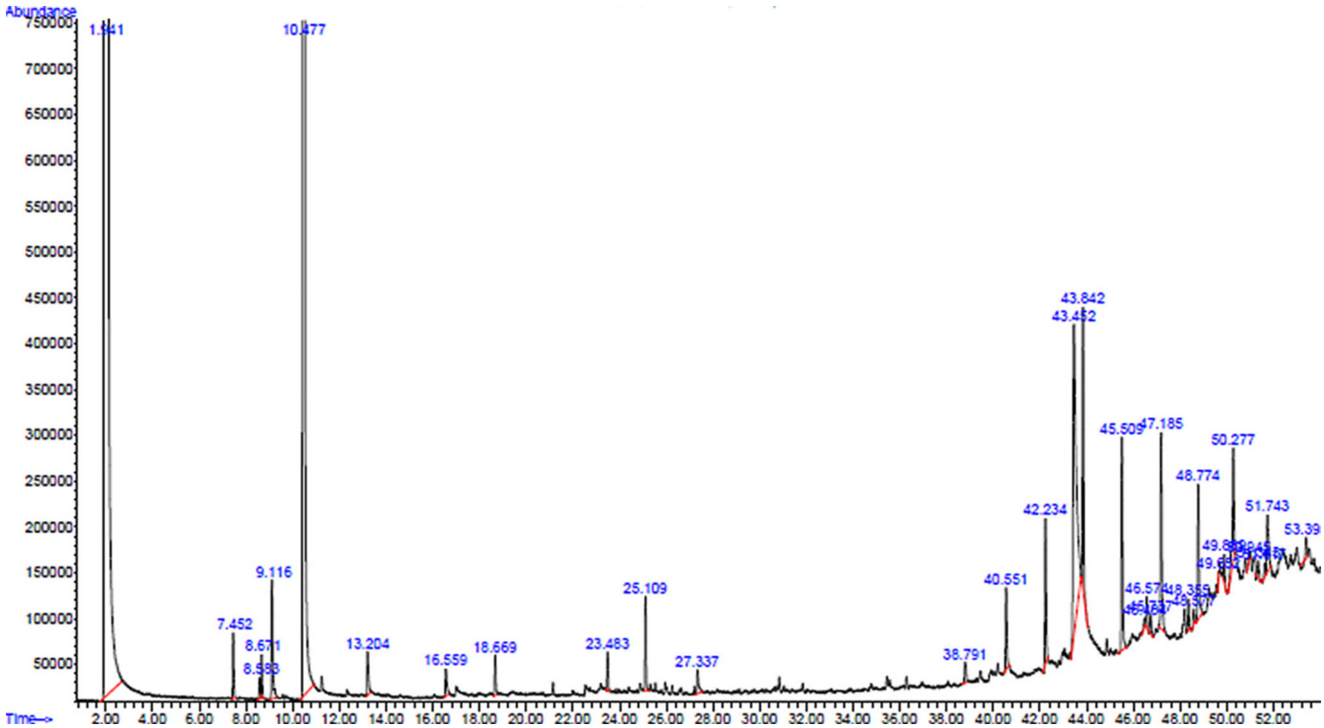

Fig. 4 Gas chromatogram of the essential oil of *C. aurantium* (Koçarlı region)

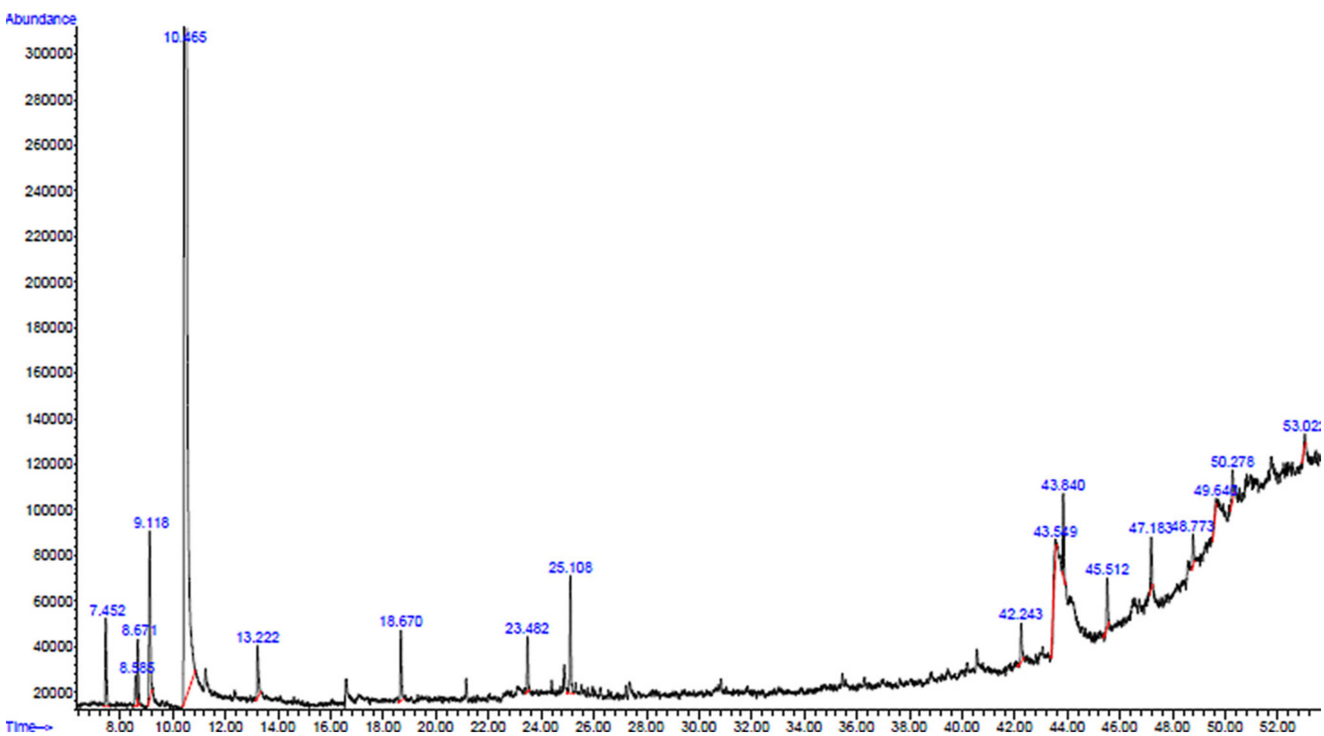

Fig. 5 Gas chromatogram of the essential oil of *C. aurantium* (Nazilli region)

termined the chemical composition of the essential oils from *C. aurantium* fruit peels (region of Split, Dalmatia) and found that limonene constituted from 51.3 to 91.1% and linalool—from 1.5 to 17.2%. Azhdarzadeh and Hojjati (2016) investigated the chemical composition of the essential oils obtained from leaves, and peels of mature and immature *C. aurantium* fruits from southwest Iran. It was found that concentrations of the following components were the highest: linalool (32.6%) and α-terpineol (17.24%) in leaves, limonene (81.6%) and linalool (2.19%)—in mature peels, and limonene (59.88%) and alloaromadendrene (5.88%)—in immature peels. Rowshan and Najafian (2015) from Fars Research Center for Agriculture and Natural Resources in Fars province (South of Iran) investigated composition of essential oil obtained from peels of *Citrus aurantium* fruits harvested in three different periods. They found that limonene (78.14%) and linalool (8.19%) were dominant components in essential oil obtained from peel of immature fruits of green colour, limonene (94.72%) and myrcene (1.87%)—from peel of semi-mature fruits of green colour, and limonene (93.47%) and myrcene (1.78%)—from peel of mature fruits of orange colour. According to Gölükçü et al. (2015) limonene (94.00–94.65%) was the most important component of the oil extracted from *Citrus* peel. Sevindik et al. (2018) studied the chemical composition of essential oil of *C. aurantium* flowers collected from Aydın province. As a result of the study, they identified nerolidol (28.07%) and linalool (14.47%) as the most important components. Sarrou et al. (2013) investigated the chemical composition of the essential oils obtained from peel, flowers and leaves of C. *aurantium* grown in Greece. In the oil from flowers they detected mainly linalool (29.14%) and β-pinene (19.08%), whereas essential oil from peel contained mainly limonene (94.67%) and myrcene (2.00%). There were differences in composition of essential oils extracted from young and old leaves. Linalool (58.21%) and linalyl acetate (12.42%) dominated in oil from young leaves, and linalool (36.03%) and linalyl acetate (23.00%) were main components of oil from old leaves. Trabelsi et al. (2016) examined the chemical composition of the essential oils obtained from flowers, leaves and fruit peel of *C. aurantium* grown in Nabeul region of Northern Tunisia. They found that linalyl acetate (53.76%) and linalool (22.11%) had the highest concentration in oil from flowers, linalool (39.74%) and linalyl acetate (25.44%) dominated in oil in leaves, whereas limonene (87.02%) and α-terpineol (6.74%) were main components in oil from fruit peel. As a result, in our study, it was found that limonene had the highest concentration among all chemical components of the essential oils obtained from *C. aurantium* fruit peel. Limonene is one of the most common essential oil components of aromatic plants, which is widely used as a flavour and fragrance additive in products such as perfumes, beverages, detergents and soaps. Another application is a component in household cleaning products (Erasto and Viljoen 2008).

Antibacterial and Antifungal Activity of the Essential Oil

MIC values of the essential oils were given in Table 2. Low MIC values indicates high antimicrobial activity (Abubakar 2009). The investigated essential oils had the similar activities against gram (–) bacteria except for that collected from Koçarlı region, which showed higher antibacterial activity against *Y. pseudotuberculosis* than other oil samples. The highest antibacterial activity was obtained for essential oil from Aydın region against gram (+) bacteria. Also essential oil extracted from fruits of *C. aurantium* grown in Germencik region showed good activity against gram (+) bacteria. MIC values for gram (–) bacteria ranged from 2.5 to 5 µL/mL, whereas those for gram (+) strains ranged from 0.3125 to 5 µL/mL The present study revealed that gram (+)

Table 2 MIC values (unit should be here) of essential oils from *C. aurantium* (µL/mL) fruit peel

Microorganisms		Germencik region	Koçarlı region	Nazilli region	Central region
E. aerogenes	Gram (–) bacteria	2.5	2.5	2.5	2.5
Y. pseudotuberculosis		5	2.5	5	5
E. coli		5	5	5	5
P. vulgaris		2.5	2.5	5	2.5
B. cereus	Gram (+) bacteria	1.25	2.5	2.5	0.625
G. rubripertincta		1.25	2.5	5	0.3125
E. faecalis		2.5	2.5	2.5	0.625
S. cohnii		2.5	5	5	1.25
S. cerevisiae	Fungi	0.625	0.625	1.25	0.625
C. tropicalis		2.5	2.5	2.5	2.5
C. albicans		1.25	2.5	2.5	2.5
C. parapsilosis		0.625	1.25	0.3125	1.25

bacteria were more susceptible to the tested essential oils compared with gram (–) bacteria species.

The different sensitivity of bacteria can be explained by differences in cell composition between gram (+) and gram (–) strains. Generally, gram (–) bacteria are more resistant than gram (+) bacteria to essential oils, as the cell wall of gram (–) bacteria is more complex (Trombetta et al. 2005; Vasconcelos et al. 2018). When antifungal effects of essential oils were compared, the best result was found for essential oil originated from Nazilli region against *C. parapsilosis* (0.3125 μL/mL). Generally the highest antifungal activity was shown by essential oil from Germencik region. The investigated essential oils have rather similar activity against *S. cerevisiae, C. tropicalis* and *C. albicans*. Bendeha et al. (2016) studied the properties of essential oils isolated from fresh peel of *C. aurantium* grown in Eastern Morocco. They reported that tested essential oils were active against *Listeria monocytogenes, Staphylococcus aureus, E. coli, Citrobacter freundii, S. cerevisiae, Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Alternaria* sp. Our results are in aggrement with this research, because we also found that *E. coli* and *S. cerevisiae* were susceptible to the essential oils from *C. aurantium* fruit peels. Hsouna et al. (2013) investigated antimicrobial activity of the essential oil extracted from fresh C. aurantium flowers cultivated in North East of Tunisia. It was found that the growth of all tested microorganisms was inhibited at varying degree. MIC values were also found 1.25 μL/mL, 0.625 μL/mL and 1.25 μL/mL for E. coli, B. cereus and E. faecalis, respectively. MIC values in our study were found 5 μL/mL, 0.625 μL/mL- 2.5 μL/mL and 0.625 μL/mL-2.5 μL/mL against *E. coli, B. cereus* and *E. fae*calis, respectively. Differences in results can be explained by different origin of plant samples. Kırbaşlar et al. (2009) investigated antimicrobial activity of essential oils from *Citrus* fruits collected from Antalya, Turkey. It was found that peel oil from bitter orange (*C. aurantium*) had activity against *E. coli, B. cereus, P. vulgaris* and *C. albicans*, which is in agreement with our results. Bozkurt et al. (2017) reported that essential oil from sour orange (*C. aurantium*) demonstrated antimicrobial activity of against *B. cereus* and *E. faecalis* but did not show any effect on *E. coli*. In contrast to this study, we found that *Citrus* peel oil inhibited the growth of *E. coli*. Our results are also consistent with those obtained by Azhdarzadeh and Hojjati (2016) who found that essential oil of unripe and ripe peel of *C. aurantium* had antimicrobial activity against *B. cereus, E. coli* and *S. cerevisiae*

GC-MS analysis of the essential oils obtained from the fruit peel of *C. aurantium* of four different populations (Central, Germencik, Koçarlı, Nazilli) revealed that limonen was the major volatile component. According to the results obtained in this study, it is possible to conclude that peel oils show antimicrobial and antifungal activities against gram (–) bacteria (*E. aerogenes, Y. pseudotuberculosis, E. coli, P. vulgaris*), gram (+) bacteria (*B. cereus, G. Rubripertincta, E. faecalis, S. cohnii)* and fungi (*S. cerevisiae, C. tropicalis, C. albicans, C. parapsilosis)*. The findings of this study can be a guide for biotechnological, pharmaceutical and medical studies on *C. aurantium* in the future.

Conflict of interest E. Sevindik, S. Aydın, M. Sujka, E. Apaydın, K. Yıldırım and G. Palas declare that they have no competing interests.

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