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Seasonal variations and antimicrobial activity of essential oil composition of *Orthurus heterocarpus* (Boiss.) Juz. from Turkey

Türkiye'de yayılış gösteren Orthurus heterocarpus (Boiss.) Juz. uçucu yağ bileşenlerinin mevsimsel değişimi ve antimikrobiyal aktivitesi

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ABSTRACT

The underground parts of *Orthurus heterocarpus* (Boiss.) Juz., belonging to Rosaceae family, are used traditionally by local people from Göksun province in Kahramanmaraş city and the seasonal variations of the essential oil of *O. heterocarpus* roots were analyzed by GC–MS/FID. Eugenol was found as a major component (95.80%-87.53%) in the roots oil but no significant seasonal variations as statically . Antimicrobial activity of essential oil was also carried out using disk diffusion method against nine bacteria *(Sarcina lutea, Enterobacter aerogenes, Bacillus subtilis, Escherichia coli, Serratia marcescens, Enterococcus faecalis, MRSA (Methicillin-resistant Staphylococcus aureus), Pseudomonas aeruginosa, Klebsiella pneumonia)* and one yeast (*Candida albicans*) strain. The results revealed that the essential oil of *O. heterocarpus* shows a high broad spectrum antimicrobial activity.

Keywords: *Orthurus heterocarpus*, Essential oil, Eugenol, Antimicrobial activity

ÖZET

Orthurus heterocarpus (Boiss.) Juz., Rosaceae familyasına ait olup, bitkinin toprak altı kısımları Kahramanmaraş İli'nin Göksun yöresinde halk arasında geleneksel olarak kullanılmaktadır. *O. heterocarpus* köklerinin uçucu yağlarının kompozisyonu GC-MS/FID ile belirlenmiştir. En önemli bileşen öjenol (95.80%-87.53%) olarak tespit edilmiştir. Uçucu yağların antimikrobiyal etkisi dokuz bakteri *(Sarcina lutea, Enterobacter aerogenes, Bacillus subtilis, Escherichia coli, Serratia marcescens, Enterococcus faecalis, MRSA* (metisilin dirençli *Staphylococcus aureus*), *Pseudomonas aeruginosa, Klebsiella pneumonia)* ve bir mayaya (*Candida albicans*) karşı disk difüzyon yöntemi kullanılarak belirlenmiştir. Sonuçlar *O. heterocarpus* uçucu yağlarının geniş spektrumlu antimikrobiyal aktiviteye sahip olduğunu göstermiştir.

Anahtar sözcükler: *Orthurus heterocarpus*, Uçucu yağ, Eugenol-öjenol, Antimikrobiyal aktivite

1. Introduction

Orthurus heterocarpus (Boiss.) Juz.(Syn. *Geum heterocarpum* Boiss.) belonging to Rosaceae family is a short rhizomatous perennial herb (1) and distrubited in S. Europe (Spain & Balkans), Cyprus, W. Syria, N. Iraq, N. & N.W. Iran, Caucasus, C. Asia, N.W. Africa in the world and West, South and Central Anatolia in Turkey (2).

The plant root is known as clove because of its aromatic similarity and Turkish name is "karanfil" and has different names as "zencefil kökü or yellice otu" among the local people (3,4). Dried roots of the plant are used by peasants to flavor tea and taken as a decoction for treatment of stomach ache and diarrhoea and used for prevention the halitosis (1). Also, the plant used to treat respiratory diseases (5). The root of the plant grinded as flour and spreaded over the wounds for treatment among the local people.

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The chemical structures (tannins, triterpenoids, essential oils, flavonoids) and biological activities (antiviral, antioxidant, anticoagulant, antimicrobial and antiinflammatory properties) of different *Orthurus* spp. were reported (6). The essential oil of *O. heterocarpus* was analysed only by Sener et al. and eugenol was found the major component (1). Eugenol (4-allyl-2-methoxy phenol), is a phenolic compound occurring as highest ratio in clove, basil, cinnamon and nutmeg (7). In clove (*Syzigium caryophyllatum*) leaf oil, eugenol was found 74.3% and 49.7 % in bud oil (8). Eugenol mixed with zinc oxide is used in dentistry as a filling material and a pulp capping agent or as a sedative agent (7). In traditional medicine, eugenol has been used in the treatment of flatulence, cholic, chronic diarrhoea and other gastrointestinal disorders (9). Eugenol improves gamma-radiation induced clastrogenic effects (10) and genotoxin-induced DNA damage (11) in vivo. Eugenol, is a natural compound isolated form the *Eugenia caryophyllata* (Myrtaceae), induced apoptosis of human cancer cells (12).

To our best knowledge, this is the first report about antimicrobial effect and seasonal variations of essential oils of *O. heterocarpus* roots.

2. Materials and Methods

2.1. Plant Material

O. heterocarpus is herb or small spiny shrub; leaves simple, digitate or pinnate; stipules present. Inflorescence a dense head, spike-like raceme or few flowered corymb or cyme. Epicalyx present. Ovary superior, surrounded by the deep hypanthium (receptacle). Fruit of 1-4 achenes, included within the dry hypanthium (2).

Roots of *Orthurus heterocarpus* were collected in 2010 from a wild population of South East Mediterranean, K. Maras, Goksun region, at nine different months (February to October). The plant material was collected 1528 meter above sea level, 38.08° latitude, 35.51° longitudes, growing under *Juniperus* trees. Plant material collected by authors and identified by taxonomist Dr. Ahmet Ilcim using Flora of Turkey and East Aegean Islands (2). In this region, the average climatic values of the research year are given in Table 1.

2.2 Essential Oil Analysis

The air dried root parts of the plants were distilled for 3 h using a Clevenger type apparatus according to the European Pharmacopiea (13). Percent yields (v/w) of the oils calculated and given in Table 2. GC-MS/FID analyses were conducted in the Plant Physiology Laboratory in Biology Dept. of Kahramanmaras Sutcu Imam University. Qualification of the oil was analyzed on Agilent GC-6890II series coupled with Agilent 5975C Mass Spectrometer. Agilent two-way microfluid splitter (G3180B) allowed for simultaneous data acquisition using two different detectors MS and FID. The GC was equipped with HP-88 capillary column (100 m ´250 mm´ 0.20 mm film thickness) coated with 88%-cyanopropyl aryl-polysiloxane. It is high polarity column; it shows better separation than DB-23. He was used carrier gas with flow rate of 1.0 mL/min. The GC oven temperature was programmed as follows: 70°C (1 min), 230°C at of 10°C/min and then kept at 230°C at 20 min. The injector temperature was 250°C. The mass spectrometer was operating in EI mode at 70 eV. Split ratio was 20:1. Mass range 35–400 *m/z*; scan speed (amu/s): 1000. 10 mikroliter of the oil was mixed 500 mL diethyl ether and 1 mikroliter of the concentrations injected into the column. The components of the oil were identified by mass spectra with those of pure authentic samples and NIST08, Willey7n.1 and HPCH 1607 libraries reference compounds. Retention indices were computed from gas chromatograms by logarithmic interpolation between *n*-alkanes. The homologous series of *n*-alkanes C7–C40, Supelco, USA were used as standard. Retention indices calculated as HP-88 capillary column. The ratios of compounds were evaluated according to MS results. All samples were repeated three times.

2.3 Microbial Studies

2.3.1. Microorganisms

Candida albicans (clinical isolate), *Sarcina lutea* ATCC9341NA, *Enterobacter aerogenes* ATCC 13048, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 39628, *Serratia marcescens* (clinical isolate), *Enterococcus feacalis* ATCC 29212, Methicillin-resistant *Staphylococcus aureus* (*MRSA* clinical isolate), *Pseudomonas aeruginosa* (clinical isolate), *Klebsiella pneumonia* (clinical isolate) were obtained from Celal Bayar University Biology

Table 1: Mean temperature and Precipitation Values from February to November 2010 Göksun/Kahramanmaraş.

	March	April	May	June	July	August	September	October	February
Temperature C°	6.60	8.80	14.20	18.20	22.70	22.50	18.40	10.80	2.20
Precipitation	29.60	86.80	33.20	8.20	0.90	0.00	0.20	75.60	49.90

Department and Kahramanmaraş Sütçü İmam University Medical Faculty Microbiology Department.

2.3.2 Preparation of Microorganism Culture

Screening of the essential oils of *O. heterocarpus* roots for antimicrobial activity was carried out by the disc diffusion method (14). Mueller Hinton and Sabouraud dextrose agar cultures of test microorganism were prepared with a standardized inoculum giving 1 x 10^8 bacteria and 1 x 10^6 yeast per mL (15). The inoculated plates with bacterial strains were incubated overnight at 36°C and the yeast plates incubated at 30°C. After incubation period, the diameters of the inhibition zones were measured and evaluated (16,17). The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) and sterilized by filtration through a 0.45 µm membrane filter. Previously sterilized disc (whatman no 1; 6 mm in diameter) were impregnated with 50 µL of different concentrations (1:1, 1:5, 1:10) of the essential oil and placed on the inoculated agar surface (14). Paper disc impregnated with aqueous DMSO and a standard antibiotic discs containing chloramphenical (30 µg/ disc), Ampicillin (10 µg/disc) and Nystatine (100U) were placed on as control.

3. Results and Discussion

Essential oil composition of *O. heterocarpus* roots collected from April is given in Table 2 and the chromatogram is given in Figure 1. As Table 2, Twenty two compounds were determined in the oils, 99.74% of total compounds were determined by GC-MS.

Main components of the oils collected from different seasons were found as eugenol and myrtenol and calculated according to FID detector results and analyzed as statistically, values are given in Table 3. As seen in the Table 3, essential oil content changed as seasonal and high essential oil content observed on October (0.34%), generally high essential oil content was obtained in autumn.

Although eugenol content of the roots collected from different seasons were not found significant as statistically, high eugenol content was obtained March collecting (95.80%) following October (94.73%) and August (93.82%) collecting, and the least was in May (87.53%). Myrtenol was second compound in the oil but there was no myrtenol in the February. Myrtenol contents also changed as seasonal and high myrtenol content was observed August collecting (3.83%).

Table 2: Percentage composition of the root oil of *Orthurus heterocarpus* in April

RT	RI	Compounds	Percentage %
11.682	1139.61	sabinen	0.03
12.092	1188.36	limonene	0.05
15.204	1471.62	nonanal	0.02
15.868	1527.54	nonyl acetate	0.07
16.069	1544.40	valencene	0.06
16.338	1566.64	β sesquiphellandrene	0.05
17.597	1672.04	pinocarveol	0.07
17.766	1686.00	campholenol	0.11
17.909	1697.71	α terpineol	0.04
18.269	1729.78	ısoborneol	0.06
18.704	1768.01	myrtenal	0.23
18.893	1784.34	myrtenol	2.50
19.358	1826.64	methyl salicylate	0.23
20.356	1922.35	guaicol	0.06
20.835	1975.03	p-mentha-1,8-dien-7-ol	0.05
21.240	2020.05	benzothiazole	0.19
21.427	2041.42	α cedrol	0.16
23.220	2254.64	eugenol	93.10
29.332	2909.69	ısoeugenol	1.76

***RT**=Retention time, **RI**=Retention index*

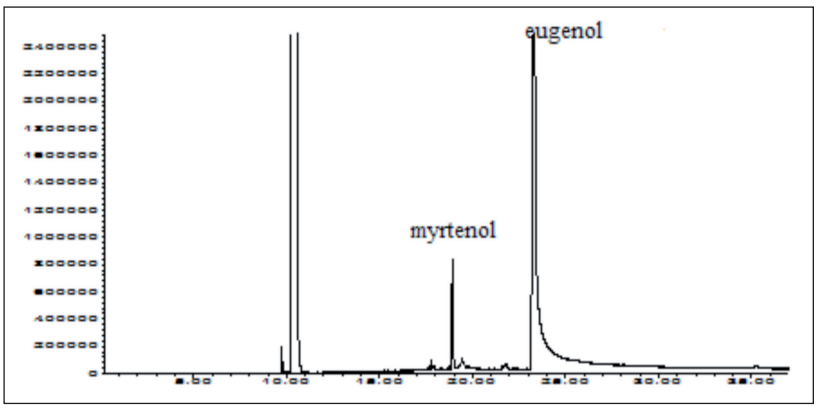

Figure 1: GC-MS chromatogram of *Orthurus heterocarpus* roots essential oil.

In the previous reports by Tanker and Sener (18) the same plant collected from Antalya, south west part of Turkey. The major components of the oil were found as eugenol (87.17%), nerol (3.99%), borneol (2.19%) and camphor (1.19%). The same authors analyzed the same material after 18 years and collected the plant material in June 1989 from same area and determined the oil composition by GC-MS. The main component was also found as eugenol (93.01%-95.49%). They reported that the oil composition was almost the same in the collected material May 1976 and June 1989 (1). Our results were similar to Sener et al. (1) findings. Azimova et al. (19) declared that *O. heterocarpus* oil has eugenol content between 80.05%-84.13% ratio. Faramarzi et al. (20), analyzed different *Orthurus* species from Uzbekistan, which separated from *O. heterocarpus* with shorter gynophore and wider sepals, has 80.09% eugenol content. In studies with plants containing high eugenol, Shahani et al. (21) analyzed the *Geum iranicum* roots extract with different chromatographic methods and one of the major component was found as eugenol. The same authors reported the chemical composition of *Geum iranicum* roots and aerial parts of essential oils in the year 2011 and eugenol was found 83.9 % and 2.8 % respectively (22). Nassar et al. (23), determined the chemical composition of *Syzigium aromaticum* buds by GC-MS and found eugenol content as 71.56%. Bhuiyan et al. (8), reported that main compounds of essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston) were eugenol in leave (74.3%) and in bud (49.7%). In the cinnamon leaf oil, eugenol was determined as 65-92% and in the different varieties of cinnamon leaves eugenol was found 75%-78% ratio. But in the root and flower oil of cinnamon, eugenol could not find as a major component (24).

It could be seen that *O. heterocarpus* roots include higher amount eugenol content than other *Orthurus* species (*O. kokanicum*, *O. iranicum*) and other known sources of eugenol (*Syzigium caryophyllatum. Syzigium caryophyllatum, Cinnamon* species).

In this research antimicrobial activity of *O. heterocarpus* essential oil was examined against four gram (+) (*Sarcina lutea, Bacillus subtilis*, *Enterococcus feacalis, MRSA*), five gram (–) (*Enterobacter aerogenes, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumonia)* bacteria and 1 yeast strains (*Candida albicans*) and the results given in Table 4. Essential oil of *O. heterocarpus* showed antimicrobial activity with varying potency. The yeast showed sensitivity higher than antibiotics. Among the tested bacteria, the essential oil was highly effective on *E. aerogenes*, *MRSA* at lower concentrations, *B.subtilis and E. coli* at higher concentrations as much as standart antibiotics. The results showed that the gram negative bacteria were more sensitive than gram positive bacteria.

Table 3: Roots oil amount and main components according to GC-FID results

Months	March	April	May	June	July	Agust	September	October	February	P
Oil amount %	0.11	0.25	0.33	0.10	0.24	0.32	0.20	0.34	0.11	**
Myrtenol	2.70	2.40	2.49	3.81	3.60	3.83	1.56	2.83	0.00	**
Eugenol	95.80	93.10	87.53	92.53	91.71	93.82	93.19	94.73	90.42	NS

** *P < 0.01* ***NS:*** *Non significant*

Table 4: Antimicrobial activities of *Orthurus heterocarpus* roots essential oil

	Concentration			Control		
Microorganisms	**25 µl**	**10 µl**	**5 µl**	**Amp**	**Chl**	**Nys**
*Candida albicans**	40	30	20	NT	NT	18
Sarcina lutea ATCC 9341NA	20	20	18	42	32	NT
Enterobacter aerogenes ATCC 13048	38	38	38	12	40	NT
Bacillus subtilis ATCC 6633	18	18	16	-	20	NT
Escherichia coli ATCC 39628	19	14	12	26	26	NT
*Serratia marcescens**	19	14	12	10	24	NT
Enterococcus feacalis ATCC 29212	18	16	15	26	28	NT
*MRSA**	16	16	14	10	24	NT
*Pseudomonas aeruginosa**	16	16	8	12	16	NT
*Klebsiella pneumonia **	16	10	10	18	20	NT

**: clinic isolate, NT: not tested, Diameter of inhibition zone (mm) including well diameter of 6 mm*

Because antimicrobial activities of *O. heterocarpus* have not been studied yet, we compared the different *Orthurus* species.

According to Faramarzi et al. (20) report, the antimicrobial activity of *Orthurus kokanikus* (Regel&Schmal.) had strong antibiotic effects against *Shigella dysenteriae, Bacillus subtilis*, *Aspergillus flavus.* Also, Shahani et al. (21) reported that eugenol extracted from *Geum iranicum* was effective against to *H. pylori.* Antimicrobial activity of eugenol was investigated by Devi et al. (25) against *Salmonella typhi* and it was showed that eugenol has distruptive effect on bacterial cytoplasmic membrane. Vazquez et al. (2001) studied the antifungal effect of eugenol and thymol on the growth and production of citrinin from *Penicillium citrinum* NRRL 2274 and NRRL 2269 in culture media and in different Spanish cheeses Arzua-Ulloa and the results showed eugenol has a stronger inhibitory effect than thymol, but both of them couldn't prevent the production of citrinin at the applied concentrations (26). Arora et al. (27) and Lopez et al. (28), reported that the clove oil showed antimicrobial activity against to some human pathogenic bacteria resistant to certain antibiotics. Hemaiswarya et al. (2009) also reported synergistic interaction of eugenol with antibiotics against five gram negative bacteria and showed that eugenol enhanced the activity of antibiotics because of its membrane damaging effect (29). Our antimicrobial assays supported that previous studies showing *O. heterocarpus* roots oil has an effective inhibitory activity against the microorganisms.

Conclusions

In conclusion this study confirmed that the essential oil of *Orthurus heterocarpus* roots are effective source about eugenol component. We suggested that roots of the plant could be collected in late of autumn for high essential oil and eugenol content. The antimicrobial results of the study revealed that the essential oil of *O. heterocarpus* showed high antimicrobial activity with varying potency. The essential oil was highly effective on *Candida albicans* and also *E.aerogenes* as much as standart antibiotics even at low concentrations. *O. heterocarpus* can be used for different medicinal purpose because of high eugenol content.

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