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Original Research Article

## Chemical constituents, quantitative analysis and insecticidal activities of plant extract and essential oil from *Origanum onites* L.

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### ABSTRACT

*Origanum*, widely used for food and pharmaceutical industries, belonging to the Lamiaceae family, is an aromatic and medicinal plant. The aerial parts of the *Origanum onites* L. were dried at shade and extracted with methanol. Rosmarinic acid, gentisic acid, 4-hydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, 4-hydroxybenzaldehyde, *p*-cumaric acid, ferullic acid, apigenin-7-glucoside, and naringenin were found in *O. onites* L. methanol extract. Quantitative analyses of these compounds were determined by LC-TOF/MS and rosmarinic acid was the main constituent (32.05 mg/100 g dried plant). The essential oil was isolated by steam distillation and the isolated compounds were identified by GC-MS analysis. The essential oil included the carvacrol (88.71%) as the main product. *p*-Cymene (3.09%) was found as the second constituting component from frequency point of view. The essential oil exhibited excellent insecticidal activity against *Sitophilus granaries* and *Sitophilus oryzae* pests. However, methanol extract revealed a moderate activity on *S. granarius* pet.

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### 1. Introduction

Plants have been used in traditional medicine for years due to their potential treatment effects (Mohammadhosseini et al., 2016; Camilo et al., 2017; Ganesan and Xu, 2017; Mohammadhosseini, 2017a, 2017b; Mohammadhosseini et al., 2017; Nunes and Miguel, 2017; Pavunraj et al., 2017). In addition, they include the fascinating bioactive compounds that have a potency for drug discovery and development process (Elmastas et al., 2004; Aksit et al., 2014; Erenler et al., 2014; Elmastas et al., 2016).

*Origanum* (Lamiaceae) consists of twenty-three species and six hybrids, fourteen of which are endemic to Turkey (Duman, 2000). *Origanum* is known as an effective stimulating, antirheumatic and antispasmodic agent, and has antibacterial effects to treat various illness such as revulsion, dyspepsia, muscle contraction,

diarrhea and infection diseases as an herbal tea (Wakim et al., 2013). Numerous reports have revealed great biological activities for *Origanum* species including antioxidant and antibacterial properties (Ozcan and Chalchat, 2009; Özcan et al., 2015; Erenler et al., 2017a), as well as antiproliferative effects (Erenler et al., 2016; Erenler et al., 2017b). *Origanum* is famous for its essential oils which have been applied in the flavoring of several foods, particularly soups, sauces, meat, fish and canned foods (Busatta et al., 2008). The main essential oils components of *Origanum* are carvacrol, caryophyllene, terpinene and thymol which have biological activities so as it gained great commercial interest (Bagci et al., 2005; Özcan et al., 2008). *O. onites* L. is grown in Aegean and Mediterranean region as natural as well cultivated. Among the other plants from the *Origanum* genus, *O. onites* L. is the most exported and commercial species (Ozkan et al., 2010).

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Insects have been known as the most significant problem for agricultural industries. They damage the crops while growing up and after harvest. Many works have been conducted to find the best solution to these issues. The synthetic chemicals used as insecticides can harm the environment and all living as well as human beings who consume chemically contaminated food (Park et al., 2002). Natural products have attracted the attention of scientists who research to find the non-toxic, and environmentally safe insecticides (Abay et al., 2012.).

*Sitophilus granaries* (L.) (Coleoptera: Curculionidae) is one of the most dangerous pests affecting stored wheat kernels. Due to the hidden infestation, nutritional and technological advancements wheat values deteriorate and an increase in hygiene problems can be observed (Piasecka-Kwiatkowska et al., 2014). *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is considered to be one of the most destructive species in stored cereal grains throughout the world. It is able to attack intact grain. The adults feed on grains and the larva grows inside the grain kernels causing both quantitative and qualitative losses of the stored grain commodities (Koutsaviti et al., 2018).

Essential oils (EOs) exhibiting a broad spectrum of biological activities are generated from various aromatic plants. Essential oils have been extensively used in food and pharmaceutical industries (Shaaban et al., 2012). In addition, due to the insecticidal (Huang et al., 2000), acaricidal (Monteiro et al., 2017) and nematocidal (Oka et al., 2000) activities, they have been employed in agricultural industries.

In our previous work, we presented an outstanding insecticidal activity of *Origanum cyriacum* essential oil against *Sitophilus oryzae* and *Rhyzopertha dominica* (Karan et al., 2018). Herein, we aimed the quantitative analysis of chemical constituents of methanol extract of *O. onites* L. using LC-TOF/MS. In addition, the goal of our research was to present the insecticidal activity of essential oil and methanol extract of this plant on *Sitophilus granarius* and *Sitophilus oryzae* pests.

## 2. Experimental

### 2.1. Material and methods

#### 2.1.1. Plant material

*O. onites* L. was collected from Denizli, Cameli, Gokceyaka village, Turkey at N37°02'55.1"-E29°16'07.73", 1461 m, in July 2015 and identified by Prof. Dr. Ozgur Eminagaoglu, Forest Engineering Department, Artvin University where a voucher specimen was deposited at the Herbarium (ARTH: 5580).

#### 2.1.2. Insects

The cultures of *S. granaries* and *S. oryzae* were

obtained from the Department of Biology, Cankiri Karatekin University. One-third of 5-L glass jars were filled with clean wheat (removal of non-wheat material), and then adult males and females were added to lay eggs to get a single aged population. The adults were removed and cultures were incubated at  $27 \pm 2$  °C and  $60 \pm 5\%$  r.h. (relative humidity) in a dark climate chamber for 48 h. The new generation of adults comes into view by 45 day and 3-4 week-old adults were used in trials (Abay et al., 2012.).

#### 2.1.3. Extractions

The air-dried *O. onites* L. sample was extracted with methanol for 24 h. After filtration, the solvent was removed by rotary evaporator to yield a solvent free extract and it was stored at the fridge (+4 °C) for further analyses.

### 2.2. Quantitative and qualitative LC-TOF/MS analysis

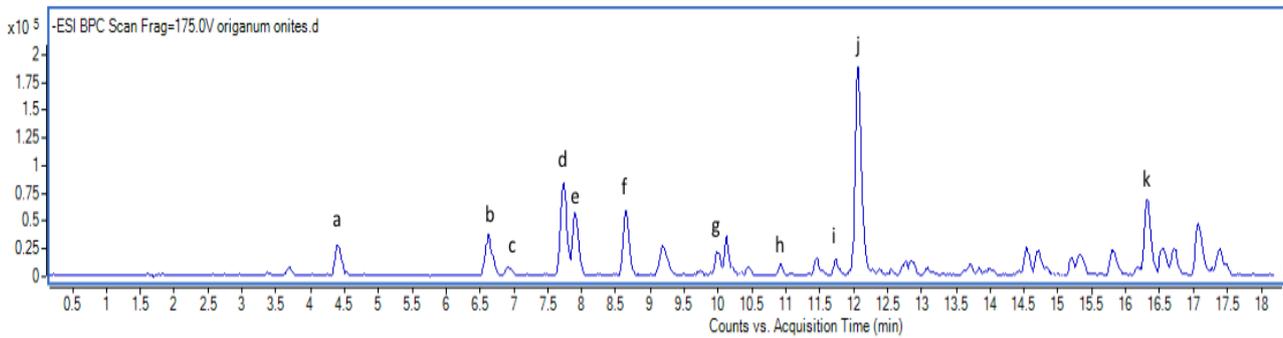
LC-TOF/MS was used for quantitative analysis. It was Agilent 6210 with column ZORBAX SB-C18, 4.6×100 mm, 3.5 μm (Agilent Technologies) with an injection volume of 10 μL. The mobile phase consisted of the eluent A, water with 0.1% formic acid and B, acetonitrile. The flow rate was 0.6 mL/min at 35 °C. The gradient program was fixed as follows: 0-1 min, 10% B; 1-20 min, 50% B; 20-23min, 80% B; 23-25 min, 10% B; 25-30 min, 10% B. Total time of evaluation was 30 min. TOF analyses were carried out in positive ion mode; gas temperature, 325 °C; drying gas flow, 10 mL/min; fragmentor voltage, 175 V (Erenler et al., 2015).

#### 2.3. Single-dose contact effects assay

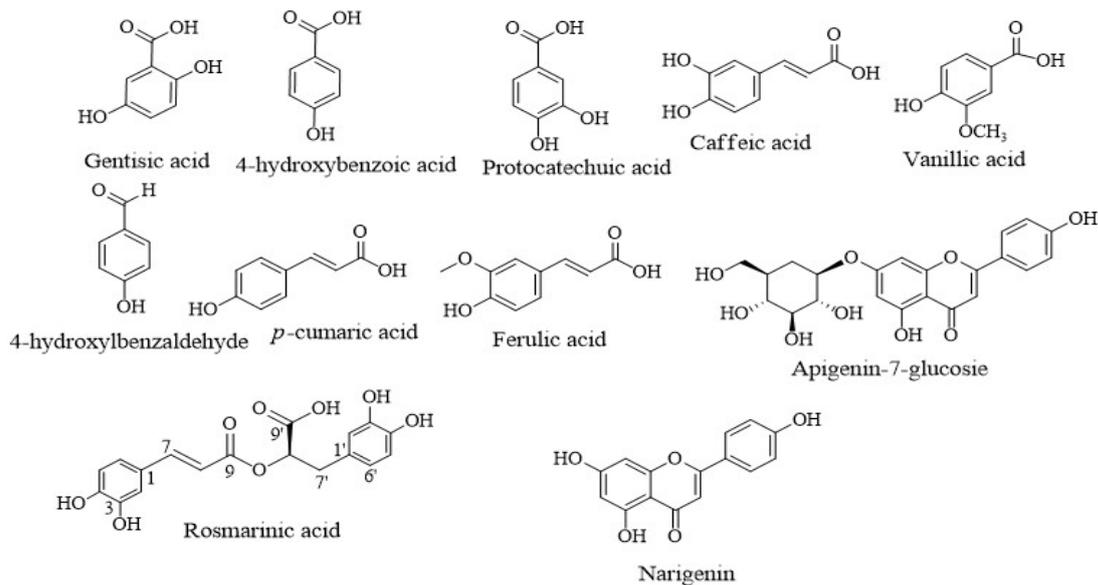
The essential oil and methanol extract of *O. onites* L. were diluted with acetone to 100 μL/mL and 100 mg/mL respectively. Diluted essential oil and extract were applied to the dorsal surface of thorax of *S. granarius* and *S. oryzae* at an amount of 1 μL/insect with 50 μL Hamilton syringe. Acetone was used as control at a dozen of 1 μL/insect. For each replication, 20 insects were used and each experiment was repeated three times. The tested insects were transferred to the 60 mm diameter clean Petri dishes filled with 5 g of wheat and incubated at  $27 \pm 2$  °C in a dark climate chamber. The number of dead insects was recorded after 24 h. A randomized block design was operated comprising treatments and blank con.

#### 2.4. Isolation of the essential oils

Aerial parts of the 25 g plant diluted with 250 mL distilled water were subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored in the freeze (4 °C) for analyses.



**Fig. 1.** LC-TOF/MS spectrum of a: gentisic acid, b: 4-hydroxybenzoic acid, c: protocatechuic acid, d: caffeic acid, e: vanillic acid, f: 4-hydroxybenzaldehyde, g: *p*-cumaric acid, h: ferulic acid, i: apigenin-7-glucoside, j: rosmarinic acid, k: narigenin.



**Fig. 2.** Compounds from the methanol extract of *Origanum onites* L.

## 2.5. GC and GC-MS analysis

GC analyses were carried out on a Perkin-Elmer Clarus 500 Series, in split mode, 50:1, equipped with a flame ionization detector (FID) and a mass spectrometer-equipped BPX-5 apolar capillary column (30 m×0.25 mm, 0.25 m i.d.). The injection temperature was fixed and FID was executed at 250 °C. The carrier gas was helium at a rate of 1.0 mL/min. The initial column oven temperature was 50 °C and was raised to 220 °C at a rate of 8 °C/minute. In the mass spectrometer, transfer line temperature was at 250 °C, ionization energy was 70 eV. Analytical standards were used for the identification of components and Kovats retention indices (RIs) were determined for all the sample components using the Van den Dool and Kratz equation according to the retention times of homologous series of *n*-alkane.

## 2.6. Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 2000,

SPSS Inc., Chicago, IL, USA).

## 3. Results and Discussion

### 3.1. Quantitative analysis

Two extraction methods were applied for *O. onites* L., one is direct extraction with methanol and the other is the ethyl acetate extraction in which, the plant materials were boiled in water then aqueous parts were extracted with ethyl acetate. Quantitative analysis of methanol extract (Table 1) was determined by LC-TOF/MS (Fig. 1) and the result revealed that methanol extract composed of rosmarinic acid (mg/100 g plant) (32.1), 4-hydroxybenzoic acid (25.5), caffeic acid (17.6) and gentisic acid (12.1) as the chief constituents (Fig. 2).

### 3.2. Essential oil

Essential oils of *O. onites* L. exhibited an excellent insecticidal activity. Carvacrol was the main compound of essential oil (Table 2). The methanol extract showed 66.7% activity on *S. granarius*. The essential oil of *O.*

**Table 1**

Quantitative analysis of *O. onites* L. methanol extract by LC-TOF/MS.

Composition (mg/100 g plant)	Methanol extract
Gallic acid	t
Genistic acid	12.11
4-Hydroxybenzoic acid	25.46
Protocatechuic acid	0.82
Caffeic acid	17.64
Vanillic acid	2.38
4-Hydroxybenzaldehyde	2.51
Rutin	trace
<i>p</i> -Coumaric acid	1.12
Chicoric acid	0.14
Ferulic acid	0.96
Hesperidin	0.32
Apigenin-7-glucoside	2.77
Rosmarinic acid	32.05
Protocatechuic acid ethyl ester	trace
Salicylic acid	trace
Quercetin	t
Naringenin	0.75
Campherol	trace
Total	99.02

**Table 2**

Chemical compositions of essential oil from of *Origanum onites* L. by GC/MS.

No	Components*	RT (min)	RI	Content (%)
1	Camphene	12.747	958	0.15
2	$\beta$ -Pinene	13.286	981	0.29
3	$\beta$ -Myrcene	14.363	990	0.47
4	$\alpha$ -Terpinene	15.019	1022	0.80
5	<i>p</i> -Cymene	15.422	1030	3.09
6	<i>cis</i> -Ocimene	15.690	1041	0.22
5	$\gamma$ -Terpinene	15.907	1064	2.65
7	$\gamma$ -Terpinene	16.852	1072	0.11
8	$\beta$ -Ocimene	17.932	1094	0.13
9	Linalool	18.146	1099	0.59
10	Borneol	20.840	1178	0.61
11	4-Terpineol	21.170	1182	0.75
12	Thymol	24.792	1293	0.24
13	Thymol acetate	25.220	1351	0.26
14	Carvacrol	27.538	1365	88.71
15	Isocaryophyllene	29.513	1440	0.35
16	2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one	31.893	1517	0.48
17	6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	34.567	1608	0.11

\*RI: Retention index determined relative to *n*-alkanes (C<sub>8</sub>-C<sub>26</sub>) on the BPX-5 apolar capillary column.

RT: Retention time (minute)

*onites* L. displayed an excellent activity on *S. granarius* (99.8%) and *S. oryzae* (98.0%) (Table 3). A research presented that *O. vulgare* essential oil could be regarded as a probable alternative hatching eggs disinfectant versus formaldehyde fumigation without adverse effect (Yildirim et al., 2003).

Carvacrol, a well-known monoterpene is the main

**Table 3**

Insecticidal activity of *Origanum onites* L. essential oil and methanol extract on *S. granarius* and *S. oryzae*.

Plant materials	% Mortality $\pm$ SD*	
	<i>S. granarius</i>	<i>S. oryzae</i>
Control	0.00 $\pm$ 0.00c <sup>1</sup>	0.00 $\pm$ 0.00c
Essential oil	99.86 $\pm$ 0.42a	97.96 $\pm$ 0.07a
Methanol extract	66.73 $\pm$ 0.28a	2.39 $\pm$ 0.17c

<sup>1</sup>Means in column followed by a different lowercase letter are significantly different (Anova  $p < 0.05$ , Tukey test). \*SD=Standard deviation.

compound of *O. onites* L. essential oil (88.7%). It was reported that carvacrol revealed antioxidant (Aeschbach et al., 1994), antimicrobial (Bagamboula et al., 2004), antifungal and phytotoxic activities (Kordali et al., 2008). Carvacrol also exhibited a dose-related inhibition of growth of the foodborne pathogen *Bacillus cereus* on rice (Ultee et al., 2000).

Generally essential oils contain many organic compounds (Bakkali et al., 2008) but interestingly, essential oils of *O. onites* L. include high rate of pure organic compound, carvacrol (88.7%) which is worthwhile in organic and pharmaceutical chemistry.

### 3.3. LC-TOF/MS analysis

Recently, extracts, essential oils, and active compounds derived from diverse plant organs have gained a great interest due to the exhibiting the significant insecticidal activity (Edde, 2012). Insects have the ability of developing resistance to the synthetic insecticides and the side effect on human health; therefore, bio-pesticides are preferred due to their non-toxic, biodegradable and environmental friendly effects (Sundararajan and Kumuthakalavalli, 2001). The plants consist of bioactive compounds such as flavonoids, alkaloids, terpenoids, organic acids and lipids which are biodegradable with non-residual effects on the ecosystem (Demirtas et al., 2013). Hence, the essential oils and extracts obtained from *O. onites* L. could be appropriate insecticide for the stored products. The observation of outstanding activity of *O. onites* L. essential oil could be attributed to the major product, carvacrol. In brief, the natural compounds in the methanol extract of *O. onites* L. were determined. In addition, rosmarinic acid was found as a major product. The essential oil constituents were presented and carvacrol was the chief compound. The essential oil displayed an excellent insecticidal activity.

### 4. Concluding remarks

Due to the exhibiting excellent insecticidal activity of *O. onites* L. essential oil, it has a potential to be a natural insecticidal agent. Pharmaceutically and medicinally valuable compound, rosmarinic acid is the main constituent of methanol extract. The other chief

constituents of *O. onites* L. were 4-hydroxybenzoic acid, caffeic acid and gentisic acid. These compounds are abundantly found in plant kingdom. The significance of *O. onites* L. for pharmaceutical and food industries could be attributed to the chemical constituents presented in this work. Rosmarinic acid has been used in food and pharmaceutical industries. Hence, *O. onites* L. may be a novel source of this compound. However, further agricultural investigations should be carried out to increase the rosmarinic acid content in *O. onites* L.. Carvacrol is the main constituent of essential oil (88.7%). The insecticidal activity of essential oil may be due to the carvacrol. Therefore, chromatographic techniques should be applied to purify the carvacrol.

### Conflict of interest

The authors declare that there is no conflict of interest.

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