



Scavenging Effect, Chemical Composition and Antispasmodic Activity of the Essential Oil of *Origanum Onites* L.

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 05/08/2019 Accepted : 21/11/2019</p> <p>Keywords: <i>Origanum Onites</i> L Antioxidant activity Antispasmodic activity Essential oils Rat ileum</p>	<p><i>Origanum onites</i> L essential oil of antispasmodic activity, chemical composition and the radical scavenging were determined in vitro. GC-MS analysis that was been carried out upon the essential oil revealed 31 different composites which equal to 94.60% in which Carvacrol (49.01%), linalool (13.3%) were found as major components. Free radical scavenging capacities of the essential oil were measured by 2,2-diphenyl-1-pikrilhidrazil (DPPH) assay and via β-carotene linoleic acid experiment were determined. It showed IC₅₀ value of <i>Origanum onites</i> essential oil was been identified as 430 mg/mL, the IC₅₀ value for BHT was 19.8 mg/mL, the β-carotene linoleic acid assay also showed the 80% inhibition with the essential oil of <i>Origanum onites</i>.it showed very strong antioxidant activity. In addition, dose dependently (0.1, 0.5, 1.0 and 2 mg/mL) inhibits the contraction induced by, it was able to inhibit the rat ileum muscle by 2 mg/mL to 100%.</p>

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Introduction

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butyl hydroquinone (TBHQ) which are the most widely used synthetic antioxidants in food stuffs. These synthetic substances may adversely affect health or cause certain diseases (Chan,1987; Barlow, 1990). In recent years for this reason, there is a growing interest in studies of natural additives as potential antioxidants as a natural additive for some plant extracts and essential oils is intended to use (Reynolds, 1996; Tepe et al., 2004; Akkuş-Çetinus et al., 2007; Tepe et al., 2016; Göze Saygın et al., 2018).

The essential oils of the plants which are widely use as alternative remedies for the treatment of many diseases and natural therapies and pharmaceutical alternative medicine have been gain great interest (Tepe et al., 2004; Akkuş-Çetinus et al 2007; Tepe et al., 2016; Göze Saygın et al., 2018).

Origanum onites L (*O. onites*) is widely used in traditional medicine and known as Turkish oregano. This is widely used as a spice and herbal tea in Turkey. It is used

for the treatment of several kinds of ailments, such as gastrointestinal disorders, treat colds, bronchitis (Tepe et al., 2016). Based on this information, there is a limited number of research about *O. Onites* (Aydın et al., 1996; Pizzale et al., 2002; Ozel and Kaymaz, 2004; Yaldiz et al., 2005; Tasdemir et al., 2006; Sarac and Ugur, 2008; Copur et al., 2010; Özkan and Erdoğan, 2011; Tepe et al., 2016; Başer and Kırimer, 2019). The essential oil composition of *O. onites* the in vitro antimicrobial and antioxidant activities have recently been reported (Aydın, 1996; Pizzale et al., 2002; Yaldiz et al., 2005; Tasdemir, 2006; Sarac et al., 2008; Copur et al., 2010; Özkan et.al., 2011; Başer et al.,2019). However, there is not any of report about antispasmodic properties of it.

The causes of abdominal pain are very varied, usually gastrointestinal system motility disorders are the cause of pain (Pascoe PJ, 1990). In animals with high economic value (horse, cow), antispasmodic drugs are not preferred because they may have toxic effects. Some side effects of atropine as antispasmodic solvents due to tachycardia,

salivary and gastric secretion prevention, long-term bowel paralysis) is not used very often today.

This study was planned with the idea that antispasmodic properties are very important in this period, especially since drugs additives in animal feeds are prohibited.

Experimental

Plant

O. onites was derived from Isparta-Sütçüler, Turkey which identified by Dr. Erol Dönmez and deposited in the Herbarium of the Department of Biology, Sivas Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: ED 10328).

Isolation of The Essential Oil

Aerial parts of *O. onites* was air-dried via water distillation (Clevenger apparatus; yield:3.6%) which dried, filtrated and stored at +4 °C.

Analysis of Gas Chromatography Mass Spectrometry (GC/MS)

The chemical composition of the *O.onites* essential oil was analysed using a Shimadzu QP-5000 gas chromatograph-mass spectrometer equipped with a GL Science capillary column TC5 (30 m × 0.25 mm i.d.,0.25 mm) and a 70 eV EI Quadrupole detector. Helium was the carrier gas, at a flow rate of 1.9 mL/min. Injector and MS transfer line temperatures were set at 250 and 280°C, respectively. The column temperature was initially at 40°C held for 2 min, then gradually increased to 125°C at a 2°C/min rate, held for 2 min, and finally increased to 250°C at 5°C/ min held for 2 min. Diluted samples (1:100 v/v, in acetone) of 1.0 µL were injected manually and split less.

Identification

The components were identified by comparison with their relative retention indices and MS (NBS75K library data of the GC-MS system) as well as the literature (Adams, 2001).

Animals

The study was carried out with approval by the Ethics Committee of Sivas Cumhuriyet University Medical School (05/08/2019-65202830/050.04.04-298). Eight male wistar rats weighted 250–300 g was used which were fed and water ad libitum.

Antioxidant Activity DPPH Free Radical Scavenging Activity

The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm appearing as a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction (Blois,1958; Burits et al., 2000; Cuendet et al., 1997). The remaining DPPH measured after a certain time corresponds concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotting inhibition percentage against extract concentration. Butylated hydroxytoluene

(BHT) was used as positive control. All tests were carried out in triplicate.

β-Carotene-linoleic Acid Assay

Antioxidant activity is a method based on the measurement of the inhibition of volatile organic compounds and conjugated diene hydroperoxides resulting from the oxidation of linoleic acid (Cuendet et al., 1997; Ozel et al.,2004) This method is based on the loss of yellow carotene as a result of its reaction with radicals formed by the oxidation of linoleic acid in the emulsion. This test is preferred in the evaluation of antioxidant activity of essential oil compared to known synthetic and natural antioxidants, BHT. The solution content was dissolved in 0.5 mg mL-carotene, 1.0 mL chloroform (high performance liquid chromatography class), then 25 mL linoleic acid and 200 mg Tween 40 were added. After the chloroform was evaporated, oxygenated (30 min, 100 mL / min) was added to 100 mL of distilled water. 2500 µL of this reaction mixture was dispersed into test tubes and 350 µL portions of the essential oil prepared in ethanol at 2 g/L concentrations was added and the emulsion system was incubated up to 48 h at room temperature. It was repeated with BHT and a blank as a positive control. After incubation, the absorbance was measured at 490 nm. The antioxidant capacity of essential oils was compared with BHT and blank at the same concentration.

Determination on Antispasmodic Activity

Male rats (250–400 g) were killed by stunning and cervical dislocation. Small segments of ileum (2 cm long) were removed and mounted vertically in organ baths containing Tyrode solution of composition (mmol/L. NaCl 136.0, KCl 5.0, MgCl₂ 0.98, CaCl₂ 2.0, NaH₂PO₄ 0.36, NaHCO₃ 11.9, glucose 5.5) bubbled with air (37° C, pH 7.4). Spontan contractions of ileum has been observed without adding any drug. Following an equilibration period of 1 h then various drugs has been added to organ bath with cumulative doses 0.1, 0.5 and 1 mg/mL and changes in amplitude and frequency of contractions have been noted. *O.onites* essential oil has been added to organ baths cumulative with concentrations of 0.1 mg/ml, 0.5 mg/ml, 1 mg/mL and 2 mg/mL .

In experiments examining the relaxation of the basal tonus of the ileum, paired segments of ileum were set up; one piece exposed to the oil and the other receiving no treatment and the second one has been used as a control. (Akkuş Çetinus et al., 2007; Goze et al., 2010; Göze Saygın et al., 2017).

Results and Conclusion

The composition of *O. onites* essential oil was analysed via GC/MS. Thirty-one compounds, representing 94.22% of the oil were identified, with carvacrol (49.01%), linalool (13.3%), geraniol (6.51%), and α terpinene (5.82%), being the major constituents (Table1). Çopur et al. (2010) %57 and Tasdemir (2006) %70 were reported ratio of carvacrol. Başer research determined the ratio of carvacrol to %80 (Başer et al., 2019). Other researchers Özkan et al. (2010), Görmez (2014), Koca (2010), Ayvaz (2010) Bostancıoğlu et al. (2012) and Orhan (2011) reported the rate of carvacrol as 60-70%. Atak et al. (2016) as %57, Sertkaya

et al. (2010) as %58, Yaylı et al. (2014) as %47 reported carvacrol rates. In this study, the carvacrol ratio is similar to those Yaylı et al. (2014). When the linalool ratio was investigated Bostancıoğlu et al. (2012) as %12, Ayvaz et al. (2015) as %12, Atak et al. (2016) reported as %8. In this study it was %13.3. It is an important variable in plant and soil characteristics and climate active substance contents.

Free radical scavenging capacities of the essential oil measured in DPPH assay and the β -carotene linoleic acid experiment were determined. While the IC₅₀ value of *O. onites* essential oil was been identified as 430 mg/mL, the IC₅₀ value for BHT was 19.8 mg/mL, the β -carotene

linoleic acid assay also showed the 80% inhibition with the essential oil of *O. onites* as shown in Table 2. *O. onites* showed very strong antioxidant activity.

Özkan and Erdoğan (2011) were evaluated antioxidant properties with 2 in vitro complementary test systems of DPPH radical scavenging activity and linoleic acid oxidation inhibition which they reported strong antioxidant activity. Pizalle et al. (2002) were also investigated the methanol extract by methods of crocin and rancimat and reported as the same strong antioxidant activity. Findings and experimental results in this study are constituent with other studies.

Table 1 Components of *Origanum onites* essential oil

CC	LRIs	RT	Compounds	%
1	930	12.51	α -thujene ^a	0.52
2	939	12.77	α -pinene ^a	0.64
3	954	13.56	camphene ^b	0.35
4	978	15.18	β -pinene ^b	0.12
5	991	16.42	myrcene ^b	1.29
6	1010	17.10	α -phellandrene ^a	0.28
7	1011	17.50	δ -3-Carene ^b	0.10
8	1015	18.00	α -terpinene ^b	5.82
9	1023	18.19	p-cymene ^b	1.18
10	1030	18.20	β -phellandrene ^a	0.17
11	1036	18.83	dl-limonene ^a	0.60
12	1040	19.52	(E)- β -Ocimene ^a	0.08
13	1043	19.82	(Z)- β -Ocimene ^b	0.14
14	1060	20.67	γ -Terpinene ^b	4.76
15	1089	21.91	terpinolene ^a	0.01
16	1097	23.81	linalool ^b	13.3
17	1139	27.81	trans-Pinocarveol ^a	0.12
18	1158	28.50	Iso-borneol ^b	0.89
19	1177	29.98	Terpinen-4-ol ^a	2.16
20	1229	35.50	carvone	0.17
21	1232	36.09	geraniol	6.51
22	1266	37.25	thymol	0.65
23	1278	38.63	carvacrol	49.01
24	1427	45.98	β -caryophyllene	2.29
25	1455	47.20	Alloaromadendren	0.41
26	1461	47.99	α -humulene	0.13
27	1482	50.38	β -guajene	0.25
28	1505	51.24	γ -bisabolene	1.86
29	1507	51.63	γ -cadinene	0.14
30	1620	53.54	(+)-Spathulenol	0.40
31	1644	55.91	γ -cadinol	0.25
			Monoterpene hydrocarbons	16.06%
			Σ Oxygenated monoterpenes	72.81%
			Σ Sesquiterpene hydrocarbons	5.08%
			Σ Oxygenated sesquiterpene	0.65%
			Total identified	94.60%

CC: Chemical Composition, RT: retention time; LRI: linear retention indices (HP-5 column); ^a Tentative identification; ^b Identification of components based on standard compounds.

In this study, the effect of direct *O. onites* essential oil on spontaneous contractions in the ileum of the rat was found to be decreased at 0.1 mg / mL while the frequency did not change. Frequency and amplitude decreased at 0.5 mg / mL, whereas spontaneous contractions were inhibited at almost 1mg/mL. spontaneous contractions completely disappeared at 2 mg/mL. dose level. The results are shown in Figure 1 and Figure 2. Tween 80 was used as solvent of essential oil. There was no inhibition effect on the contractions.

There is no report mentioning the antispasmodic activity of *O. onites* essential oil. In this study *O. onites* essential oil was found to cause a significant decrease in booth amplitude in the rat ileum and the frequency of spontaneous contractions of the ileum. The effects of *O.onites* is potent concentration dependent and fully reversible on washout. essential oil is strongly antispasmodic effective.

Table 2 Effects of *Origanum onites* essential oil and on the in vitro free radical DPPH and β -carotene-linoleic acid system.

Samples	Inhibition IC ₅₀ (μ g/ml) (DPPH)	Inhibition % (β -carotene-Linoleic acid)
<i>Origanum onites</i> L.	430	80
BHT	19.8	100

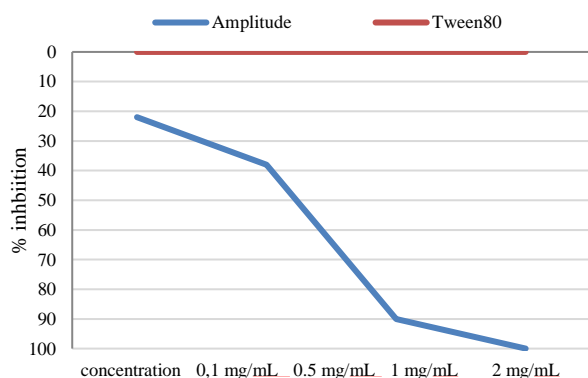


Figure 1 Inhibitory effect of *Origanum onites* L. essential oil on rat ileum spontaneous contractions (amplitude)

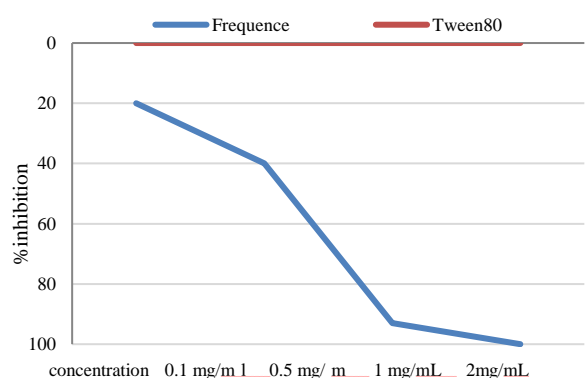


Figure 2 Inhibitory effect of *Origanum onites* essential oil on rat ileum spontaneous contractions (frequency)

It's being non-resistant or in other words its reversibility is a big advantage in terms of optimizing intestinal motility. *O. onites* is providing wide therapeutic application areas and making it a good candidate for pharmaceutical industries because of its high potency.

The mechanism of action of *O. onites* is still unclear and yet more research is needed on the antispasmodic mechanism.

Moreover, it may suggest being a good candidate for addition to animal feeds in particular as antispasmodic effect.

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