

CHEMISTRY

Chemistry of natural compounds

CHARACTERIZATION OF ESSENTIAL OILS
FROM MEDICINAL PLANTS AND ASSESSMENT
OF THEIR ANTIMUTAGENIC EFFECTS USING
AMES SALMONELLA/MICROSOMAL TEST

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Received on October 19, 2022

Presented by H. Najdenski, Corresponding Member of BAS, on January 31, 2023

Abstract

We investigated the antimutagenic activities and chemical compositions of essential oil (EO) from *Melissa officinalis*, *Lavandula angustifolia* and *Origanum onites* plants belonging to the Lamiaceae family. The chemical compositions of the plants were determined by GC/MS analysis and Ames assay was used in antimutagenic activity analysis. Citral, linalool, thymol and terpinenes were the major components in the EOs of *M. officinalis*, *L. angustifolia* and *O. onites*. In Ames assay, it was determined that the EO of *M. officinalis* had strong antimutagenic effects at all concentrations except for the highest concentrations on *Salmonella typhimurium* TA98 and 100 strains. *L. angustifolia* EO showed moderate antimutagenic activity against the Sodium azide (NaN₃) mutagen, and reduced mutant colonies with 25.40% inhibition effect in the *S. typhimurium* TA100 strain at only 0.1 µl/plate concentration. Furthermore, *L. angustifolia* EO indicated strong antimutagenic activity against both 4-Nitro-o-phenyldiamine (NPD) and NaN₃ mutagens in the *S. typhimurium* TA98 and 100 strains at 0.5 and 1 µl/plate concentrations. It was determined that the EO of *O. onites* had a moderate antimutagenic effect at the lowest concentration and a strong antimutagenic effect at all other concentrations on *S. typhimurium* TA98 strain. The 0.01, 0.05 and 0.1 µl/plate concentrations of EO of *O. onites* showed strong antimutagenic effect against *S. typhimurium* TA100 strain. The present study demonstrated that EOs of *M. officinalis*,

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DOI:10.7546/CRABS.2023.02.03

L. angustifolia and *O. onites* possesses antimutagenic activity on the *S. typhimurium* TA98 and 100 strains.

Key words: medicinal plants, antimutagenic activity, *Salmonella typhimurium*

Introduction. The Lamiaceae family is a very large plant family that is represented by about 245 genera and 7886 species that can be found in Australia, South West Asia, South America, and Mediterranean basin countries. There are 46 genera and 648 species belonging to this family widely distributed in the mountainous areas of the Mediterranean region of Turkey [1]. Many of these plant species are economically valuable and have both medicinal and aromatic properties. The phytotherapeutic properties of these plants are mostly due to the essential oils (EOs) in their content.

Melissa, *Lavender* and *Origanum* which are among the important genera of the Lamiaceae family, are widely used in food, cosmetics and pharmacology due to some biological properties. *Melissa officinalis* L. is a 40–120 cm tall, lemon-scented, perennial herbaceous species. The leaves are used both fresh and dried, in salads, sauces, soups, vegetables, meat and desserts. It is also consumed as a sweetener in some beverages and as herbal tea. *M. officinalis* is also used as an analgesic and muscle relaxant, as well as for skin rashes and inflammations, in the treatment of Alzheimer's disease, insomnia, relieving and relaxing. *Lavandula angustifolia* Mill. is a shrub-like plant that blooms with blue, purplish or red flowers. It is a perennial flowering plant, 20–60 cm tall, with grayish or lilac coloured flowers. Partially dried flowers and leaves are used in perfumery and cosmetic industry [2]. *L. angustifolia* is used to treat central nervous system disorders, such as anxiety, stress, and sleep disorders [3]. *O. onites* L. is a semicircular, evergreen, perennial herb with a height of 30–50 cm, but can reach 80–100 cm under suitable conditions. It is used as a spice in meals, various sauces and salads, cheese and sausage production. Both the essential oil and leaves of Izmir thyme are used against digestive system and upper respiratory tract disorders, indigestion, loss of appetite and cough. It also has properties such as antiseptic, sedative, gas expectorant, expectorant, cramp solver.

Natural components such as essential oils have a potential use in the treatment of many diseases, including cancer. In recent years, many researches have been conducted to document the traditional uses of EOs of *M. officinalis*, *L. angustifolia* and *O. onites* and to find new biological effects for these plants. These studies have reported a wide range of pharmacological activities including antimicrobial, antioxidant, antiprotozoal, anticancer, and genotoxic/cytotoxic effects [4]. Plants (essential oils/extracts) have the potential to synthesize numerous chemical compounds with mutagenicity/antimutagenicity properties. However, information about the potential health threat *M. officinalis*, *L. angustifolia* and *O. onites* plants pose is limited. This study aimed to investigate the EO components of

M. officinalis L., *L. angustifolia* Mill. and *O. onites* L. plants belonging to the Lamiaceae family and to investigate their antimutagenic activities using the Ames assay. In Ames assay mutant *Salmonella typhimurium* strains are used. This test is simpler, cheaper and fast method to identify mutagens/antimutagens instead of experimental animals which are very costly and time-consuming tests.

Materials and methods. Plant materials and isolation of essential oils. *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L. were collected from Düzce situated in Western Black Sea Region of Turkey (latitude: 40°49'59" N, longitude: 31°10'0" E) between 2019–2020 and were identified by Mehmet Fatih Cakir (Environment and Health Coordination, Düzce University, Düzce, Turkey). The dried plants (500 g) were weighed and crushed and placed in the flask of the Clevenger apparatus. One litre of distilled water was added to it and distillation was carried out for 3 h. The essential oils obtained were stored in closed tubes at +4 °C in the refrigerator to be used in the study.

Essential oil characterization. The chemical compounds of EOs of plants were identified by GC/MS analysis using a Agilent 7890A GC System coupled to an Agilent 5975C inert MSD with Triple Axis Detector. Agilent HP5-MS (30 m × 0.25 mm × 0.25 µm) column was used as GC column. The operating conditions of the analysis are as follows: the oven temperature was held at 40 °C for 5 min, then ramped at 5 °C/min to 100 °C for 5 min, then ramped at 20 °C/min to 225 °C and held at this temperature for 8 min. The total run time was 33.25 min. The injector temperature was fixed at 200 °C and splitless mode was used with helium carrier gas. The ion source was electron ionization and the MS source temperature was set at 230 °C. The injection volume was 1.0 µl.

Bacterial strains. The *S. typhimurium* test strains TA98 and TA100 (lyophilized) were purchased from EBPI Bio-Detection Products (Mississauga, ON, Canada). 4-Nitro-o-Phenyldiamine-NPD and Sodium Azide-NaN₃ were used as positive controls for *S. typhimurium* TA98 and TA100, respectively, in the absence of S9 mix.

Antimutagenicity assay. Two and a half millilitres of top agar supplemented with histidine-biotin was added in equal amounts to sterile tubes and then 0.1 mL of bacterial cultures was added. After three different non-cytotoxic concentrations of EOs and positive mutagens were added to the test tube and spread homogeneously on Minimal Glucose Agar plates. Plates were incubated for 48–72 h at 37 °C. Then, the returning colony numbers were determined for each sample. The inhibition effect of the essential oil of positive mutagens was evaluated between 0% and 100%. Three plates were used for each treatment in each experiment, and each experiment was repeated two times.

To quantify antimutagenic effects by EO, classification was made according to inhibition rates and according to this, antimutagenic effect is defined as < 20%, negative; 20–40%, moderate; > 40%, strong.

T a b l e 1

Major compounds of *M. officinalis*, *L. angustifolia* and *O. onites* oils obtained by GC/MS analysis

Classification	Major compounds	<i>M. officinalis</i>		<i>L. angustifolia</i>		<i>O. onites</i>	
		RT (min)	%	RT (min)	%	RT (min)	%
Monoterpene hydrocarbons	Citral	15.158	33.004	–	–	–	–
	γ -Terpinene	12.407	0.335	–	–	12.616	8.727
	α -Terpinene	–	–	–	–	11.378	6.077
Oxygenated monoterpenes	Linalool	13.268	2.184	13.529	23.354	13.389	6.006
	Thymol	–	–	–	–	15.633; 23.607	21.414

Statistical analysis. The data were analyzed by using SPSS 20 for Windows (SPSS Inc., Chicago, IL, USA) and results obtained were expressed as mean \pm standard deviation (SD).

Results. Chemical compositions of the EOs. GC-MS analysis resulted in the identification of 75, 73 and 94 chemical compounds for *M. officinalis*, *L. angustifolia* Mill. and *O. onites* L. essential oils, respectively, and the percentages and the retention times (RI) of the identified major compounds of the EOs were shown in Table 1. According to these, a total of 75 constituents, representing 99.996% of the total oil, were identified for *M. officinalis* L. The essential oil consisted of monoterpene hydrocarbons representing a fraction of 37.07%. The major constituent was found to be Citral (33.004%).

A total of 73 constituents, representing 100% of the total oil, were identified for *L. angustifolia* Mill. Oxygenated monoterpenes were the major components, accounting for 46.46% of the total oil. Linalool (23.354%) was the predominant constituent of oxygenated monoterpenes (23.354%).

A total of 94 constituents, representing 99.999% of the total oil, were identified for *O. onites* L. The essential oil was dominated by the monoterpene hydrocarbons and oxygenated monoterpenes representing fractions of 34.11 and 33.83%, respectively. The major constituent was found to be Thymol (21.414%) and Terpinenes (14.804%; γ -Terpinene 8.727% and α -Terpinene 6.077%).

Antimutagenicity assay. In the present study, four different concentrations of EOS of *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L. were used as material. The results are shown in Table 2. It was determined that the EO of *M. officinalis* L. had a strong antimutagenic effect at all concentrations on *S. typhimurium* TA98 and 100 strains except for the highest concentrations. In addition, the lowest concentration (0.1 μ l/plate) of *M. officinalis* L. had a moderate antimutagenic effect on the *S. typhimurium* TA98 strain with a 43.20% inhibition rate.

L. angustifolia Mill. EO showed moderate antimutagenic activity against the NaN₃ mutagen, and reduced mutant colonies with 25.40% inhibition effect in the

Table 2

Antimutagenic effects of different concentrations of EOs of *Melissa officinalis* L., *Lavandula angustifolia* Mill. and *Origanum onites* L. on *S. typhimurium* TA98 and TA100 strains

Groups	Conc. (μ l/plate)	Revertant colonies number			
		<i>Salmonella typhimurium</i> TA98	<i>Salmonella typhimurium</i> TA100	Mean \pm SD	Inhibition rate %
<i>Melissa officinalis</i> L.	0.1	2581.33 \pm 2133.08	43.20*	1522.66 \pm 186.93	62.95**
	0.5	163.00 \pm 110.01	97.10**	82.33 \pm 7.02	99.96**
	1	149.00 \pm 98.50	97.41**	13.66 \pm 5.50	99.57**
	5	0.00 \pm 0.00	ND	0.00 \pm 0.00	ND
<i>Lavandula angustifolia</i> Mill.	0.1	5916.00 \pm 273.20	-31.12	2944.33 \pm 68.25	25.40*
	0.5	77.66 \pm 9.29	99.00**	135.33 \pm 92.93	99.60**
	1	113.33 \pm 39.17	98.20**	133.00 \pm 149.36	99.67**
	5	0.00 \pm 0.00	ND	0.00 \pm 0.00	ND
<i>Origanum onites</i> L.	0.01	3404.33 \pm 534.67	24.86*	371.00 \pm 175.06	93.38**
	0.05	1521.00 \pm 330.97	66.83**	290.66 \pm 353.04	95.50**
	0.1	60.33 \pm 63.73	99.38**	87.33 \pm 9.71	99.83**
	0.5	129.66 \pm 84.21	97.84**	0.00 \pm 0.00	ND
Negative control		32.66 \pm 3.21		120.33 \pm 56.04	
NPD		4519.66 \pm 840.70			
NaN ₃				3905.66 \pm 206.25	

Legend:

EOs: Essential oils;

Conc: Concentrations;

*Moderate antimutagenic effect;

**Strong antimutagenic effect;

ND: not determined;

NPD: 4-Nitro-o-Phenylendiamine (0.02 mg/plate);

NaN₃: Sodium Azide(0.02 mg/plate)

S. typhimurium TA100 strain at only 0.1 µl/plate concentration. Furthermore, *L. angustifolia* Mill. EO presented strong antimutagenic activity against both 4-Nitro-o-Phenylendiamine and NaN₃ mutagen in the *S. typhimurium* TA98 and 100 strains at 0.5 and 1 µl/plate concentrations.

It was determined that the EO of *O. onites* L. had a moderate antimutagenic effect at the lowest concentration (0.01 µl/plate) and a strong antimutagenic effect at all other concentrations on *S. typhimurium* TA 98 strain. 0.01, 0.05 and 0.1 µl/plate concentrations of EO of *O. onites* L. showed strong antimutagenic effect against *S. typhimurium* TA100 strain. The inhibition rates of the EO against NaN₃ on *S. typhimurium* TA100 ranged from 93.38% to 99.83%.

The relationship between concentrations of EOs of *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L. and the percentage of inhibition was investigated by Pearson correlation test (Fig. 1). According to this, different concentrations of EOs significantly increased the inhibition percentage in *S. typhimurium* TA98 and TA100 strains and these increases are dose-dependent (in *S. typhimurium* TA98 and TA100: $R^2 = 1$, $P \leq 0.05$ for *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L.) (Fig. 1).

Discussion. Epidemiological studies have shown that essential oil components have important effects in preventing and curing many diseases, especially cancer. Therefore, the investigation of the chemical compositions of essential oils continues to arouse interest.

The chemical characterizations of essential oils of some medicinal plants from the family Lamiaceae collected from Düzce (Turkey) are comparable to those reported in the literature. According to these, a total of 75 constituents, representing 99.996% of the total oil, were identified for *M. officinalis* L. The essential oil was dominated by the monoterpene hydrocarbons representing a fraction of 37.07%. The major constituent was found to be Citral (33.004%). Citral (3,7-dimethyl-2,6-octadienal) consists of the cis-isomer geranial and the trans-isomer neral [5] and it can be used in the treatment of diseases such as oral cavity and hepatic diseases, gastrointestinal disorders [6]. Previous studies on the chemical composition of *M. officinalis* EO have shown that the citral component has different values. The citral component values in *M. officinalis* grown in Bulgaria, Iran, Jordan and Turkey are 10.6%, 10.10%, 6.7%, and 10.10–17.43%, respectively [7]. The citral value obtained in our study was higher than the values in other studies.

A total of 73 constituents, representing 100% of the total oil, were identified for *L. angustifolia* Mill. Oxygenated monoterpenes were the major components, accounting for 46.46% of the total oil. Linalool (23.354%), the dominant constituent of oxygenated monoterpenes (23.354%), is a monoterpene alcohol that is used as an intermediate in the production of vitamin E. There are studies showing that the linalool compound is neuroprotective in disease models such as Alzheimer's and can be used as an alternative tool in anxiety and social disorders [8]. There are many studies on the chemical composition of the essential oil

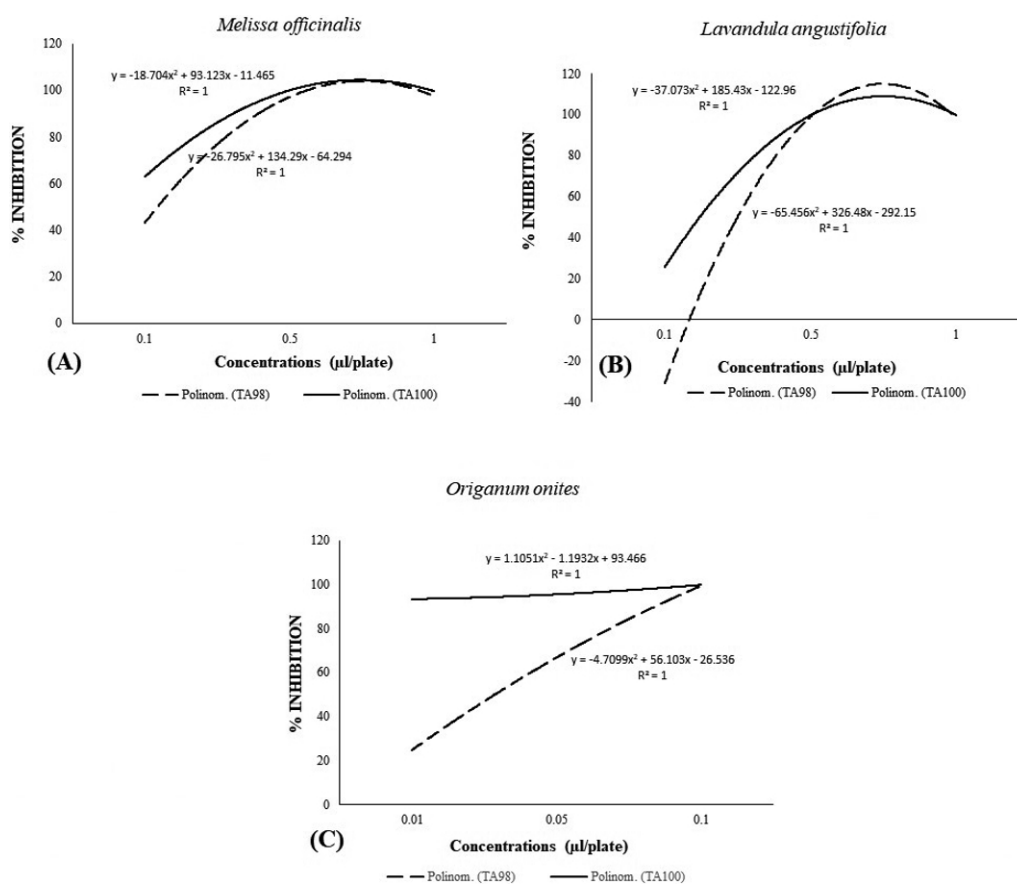


Fig. 1. Relationship between concentration and inhibition rate (%) of test bacterial strains after exposure to essential oils of medicinal plants from the Lamiaceae. A) *M. officinalis* L.; B) *L. angustifolia* Mill.; C) *O. onites* L.

of *L. angustifolia* Mill. According to some of these studies the linalool component values in EO of *L. angustifolia* Mill. grown in Italy, India, Turkey and China are 32.75%, 28.06 and 29.7%, 33.57%, and 19.71%, respectively [9]. When we compared the linalool value reported in other studies according to the European Pharmacopoeia and International Organization for Standardization [10], it was seen that the linalool value is suitable for *L. angustifolia* Mill. grown in Italy, India and Turkey.

A total of 94 constituents, representing 99.999% of the total oil, were identified for *O. onites* L. The essential oil was dominated by the monoterpene hydrocarbons and oxygenated monoterpenes representing fractions of 34.11 and 33.83%, respectively. The major constituents found were Thymol (21.414%) and Terpinenes (14.804%; γ -Terpinene 8.727% and α -Terpinene 6.077%). Thymol, chemically known as 2-isopropyl-5-methylphenol is a colourless crystalline monoterpene phe-

nol; it has a protective effect against cardiovascular, metabolic, gastrointestinal and mental disorders [11]. α -terpinene and γ -terpinene are naturally occurring cyclic monoterpenes [12]. Such monoterpene hydrocarbons have antibacterial, antioxidant, antifungal, antiparasitic and antitumour activities [13]. STEFANAKIS et al. [14] found that the thymol value in the essential oil of *O. onites* grown in Naxos island of Greece is between 20.21–34.62%. ECONOMOU et al. [15] indicated that the γ -terpinene value in the essential oil of *O. onites* grown in Ikaria island of Greece is between 1.37–6.51%. These differences may occur depending on the genetic structure, variety, type, origin, age, organ, and climate and soil conditions of the source plant.

Antimutagenicity is defined as eliminating the mutagenic or carcinogenic effects of mutagenic substances or preventing their interaction with DNA. According to their mode of action, these substances are divided into desmutagens and bioantimutagens. Desmutagens are antimutagenic substances that block the entry of mutagenic agents into the cell, that is, inactivate them without being included in the structure of DNA. Bioantimutagenic compounds are substances that reduce mutagenesis by regulating DNA replication and DNA repair mechanisms after the mutagen is incorporated into the structure of DNA [16]. Investigating the antimutagenic activities of plant extracts/essential oils is important in terms of determining the compounds that are effective in preventing many diseases caused by mutations. For this reason; in vitro antimutagenicity tests, such as the Ames test, are used as the first step to identify new anticarcinogenic components. In the present study, four different concentrations of EO of *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L. were used as material and it was determined that the EOs of these plants had strong antimutagenic effect on *S. typhimurium* TA 98 and 100 strains. The results of the present study support previous findings obtained by many other researchers. DE MARTINO et al. [17] investigated the mutagenic effects of 88, 177 and 442 μg concentrations of *M. officinalis* oil in strains *S. typhimurium* TA98 and TA100 in the presence (+S9) and absence (–S9) of the metabolic activation and reported that the EO did not have a mutagenic effect due to the combination of desmutagen components. In previous mutagenicity studies, methanolic and ethanolic extracts of *M. officinalis* were shown to have antimutagenic potential on mice using chromosomal aberration assay, micronucleus and comet assays [18]. Therefore, the antimutagenic activity of EO of *M. officinalis* L. observed here may be due to the presence of some important phytoconstituents with antimutagenic activity and possible interaction between the compounds.

EO of *L. angustifolia* Mill. indicated strong antimutagenic activity against both NPD and NaN_3 mutagens in the *S. typhimurium* TA98 and 100 strains. These results are consistent with the results of previous studies carried out on *S. typhimurium* strains. In those studies, significant decreases in the mutant colonies numbers in the TA98 strain were reported exposed to the direct mutagen 2 nitrofluorene different concentrations of EO of *L. angustifolia* showed antimu-

tagenic activity [19] and it was thought that the decreases observed in mutation might be due to protective effect of EO.

According to the results obtained from our study, it was found that the EO of *O. onites* had a strong antimutagenic effect on *S. typhimurium* TA98 and TA100 strains with an inhibition rate ranging from 66.83% to 99.83%. There is a limited number of studies investigating the antimutagenic effect of EO of *O. onites*. IPEK et al. [20] have showed that the essential oil of *O. onites* did not have any mutagenic effect; on the contrary, it had an antimutagenic effect on *S. typhimurium* TA98 and TA100 both with and without metabolic activation. The mechanism of antimutagenic activity is thought to be due to its ability to alter membrane lipids and the permeability of ion channels or antioxidant activity.

Several mechanisms have been identified on antimutagenic activity of EOs. For example, the major components may have caused antimutagenic activity due to cell permeability reduction by blocking the absorption of mutagens into the bacterial cell membrane, or due to inhibition of mutagens by chemical or enzymatic means [16].

Conclusions. In our study we demonstrated that *M. officinalis* L., *L. angustifolia* Mill. and *O. onites* L. essential oils inhibit the effects of NPD and NaN₃ mutagens through action of various bioactive molecules and have antimutagenic activity against these mutagens. However, studies with essential oil components of medicinal plants should be supported by preclinical and clinical studies due to their potential to be used as raw materials in the development of drugs.

Acknowledgements. We thank Mustapha Touray for checking the English language of the text.

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