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Original article

Phytochemistry and insecticidal effect of different parts of *Melissa officinalis* on *Tetranychus urticae*

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Abstract
Background and objectives: In recent years, biological control of parasites by essential oils (EOs) derived from plants is one of the alternatives to synthetic pesticides. *Melissa officinalis* from Lamiaceae family is distributed in many parts of Iran. It is known as an excellent source of antioxidants, antibacterial, antiviral and antifungal constituents. The present study investigated the insecticide properties of *M. officinalis* against *Tetranychus urticae* tick. **Methods:** The EO of different parts of plant was extracted and analyzed by gas chromatography and mass spectrometry (GC/MS). The ticks were placed on the filter paper in the bottom of a petri dish (9 mm), and contact toxicity assay was then performed by contacting the extract with the ticks. **Results:** The EO of leaves showed the most potent insecticidal effect while the stem EO demonstrated the weakest effect. The lowest concentration of EO from the leaves showed more considerable insecticide activity compared to the highest concentration of stem and flower EOs. **Conclusion:** *Melissa officinalis* is an effective insecticide with potent effect against *T. urticae* and it could be suggested as a natural pesticide against *T. urticae.*

Keywords: essential oil, insecticidal effect, *Melissa officinalis*, *Tetranychus urticae*

Introduction
Melissa officinalis is one of the most common and oldest medicinal herbs, which belongs to *Lamiaceae* family. It is called lemon balm because of having lemon scent [1]. *Melissa officinalis* populations could be found in all Mediterranean countries including Turkish coasts, south Alp and also different reigns of Iran [2]. It is an aromatic plant and some people have found its medicinal properties since long ago, using the species for treatment of different diseases [1]. The leaves, flower and stem of the plant have been considered as a medicine in the Iranian herbal pharmacopoeia. The extract of the plant has been used as heart tonic in the middle

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ages for treatment of different diseases [3]. In the Austrian traditional medicine, leaf of *M. officinalis* was used as tea or in the form of extract for treatment of gastrointestinal, neural, hepatic and biliary disorders [4].

Scholars have found that consuming *M. officinalis* tea results in considerable improvement of plasma level of catalase, superoxide dismutase, glutathione peroxidase and considerable decrease of myeloperoxidase and lipid peroxidation in plasma and preventing DNA damage [5]. Chopped leaves of the plant have been locally applied to repel mosquitoes [6].

Melissa officinalis has been used as tea or in the form of extract to decrease anxiety and increase relaxation [7]. The plant improves mood and mental function through affecting muscarinic acetylcholine and nicotine receptors [8]. Antibacterial properties of the plant have been proved in some studies [9]. The extract of *M. officinalis* has high antioxidant activity [10] and has been used to treat hyperthyroidism through thyroid stimulating hormone (TSH) inhibition and binding with TSH receptors [11]. This plant has been also used for treatment of different diseases such as bronchitis, asthma, coughs and fevers, menstrual problems, migraines, dizziness, skin problems, eczema, gout, insect stings and bites [12]. Monoterpenoid aldehydes, flavonoids, polyphenolic compounds especially rosmarinic acid and monoterpenes glycosides have been detected in *M. officinalis* [13]. The polyphenolic compounds of *M. officinalis* are responsible for its antivirus properties [14].

Mites are obligating and bloodsucking parasites of vertebrates especially mammals and birds, which weaken them through their bites, causing trauma, irritation, inflammation and hypersensitivity reactions [15,16]. Anemia and reduced animal production will occur as a result of severe parasitic infections. Saliva of some species of mites causes livestock intoxication and paralysis. Mites have been also known as agents which transferee rickettsia, protozoa and virus pathogens between human and animals [17].

The use of chemical toxins as anti-mite compounds has been the simplest method proposed by governments and producers of pesticides to farmers and ranchers in order to control such pests and decrease the incurred losses, whereas excessive use of these compounds cause resistance and they will remain in the environment and foodstuff [18].

Two-spotted spider mite causes damage to most crops and ornamental plants. Among 1200 spider mite species are known in the world, the *T. urticae* is one of the most polyphagous species, which has more than 1000 hosts in 100 plant families. In addition to agricultural fields, it is one of the most damaging agents in the forests and rangelands.

One of the main problems with the two-spotted spider mites is that they, soon, become resistant to the acaricides [19]. According to the findings of different studies, eliminating the mites is one of the methods that will prevent disease transmission to the animals and human and the reduction of economic losses in the livestock. To achieve this goal anti-mite bath or chemical toxicants such as phosphorus and chlorine carbamate have been used [20]. However, there is the risk of intoxication of humans and animals by these toxicants. On the other hand, these toxicants act exclusively and also their stability in the environment causes various environmental problems. The other major problem is creating the resistance to the toxicant in the fighting species [21-23].

The aim of the present study was to evaluate the effects of *M. officinalis* extract on the two-spotted spider mite.

Experimental

Plant material

Melissa officinalis was collected from East Azerbaijan Province, Iran during spring and summer (2012) and identified by the Herbarium of Institute of Medicinal Plants, Medical University of Tabriz, Iran.

The plant's EO was extracted by hydro distillation and using Clevenger type apparatus.

The EO was dried by anhydrous sodium sulfate and stored at 4 °C until further analysis.

GC/MS analysis

The essential oil was analyzed by gas chromatography, according to the method of Ehsani and Mahmoudi (2012) [24]. To confirm the results, essential oil was also analyzed by gas chromatography/mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent Technologies, Berkshire, UK) with the same capillary column and analytical conditions as above-mentioned. The MS was run in electron-ionisation mode with ionisation energy of 70 eV [24].

Toxicity tests

The contact toxicity tests of EOs were carried out using Whatman filter papers. A filter paper was placed inside a petri dish (diameter 9 cm). The EO was tested at five levels (10, 20, 30, 40 and 50 µL/cm^2). Acetone was the main solvent used in the study. Using micropipette, 1 mL of the EOs was placed on the filter paper and poured into the petri dishes. Only acetone was used in the control dishes. After 10 min when the filter paper dried a number of 10 insects, regardless of their gender, were placed on the filter paper inside the petri dishes and the petri dish's caps were fastened. Then they were placed in incubator at temperature of 28 °C, relative humidity of 70% and in a dark place. The test was conducted with three repetitions at five time intervals [25].

The number of dead insects (those which couldn't move their legs and antennae) was recorded after five h. *Phaseolus vulgaris* L. was used as host plant of two-spotted spider mites [25,26].

Statistical analysis

Statistical analyses were conducted using SPSS ver. 22 for windows (Chicago, USA). Analysis of Variance (ANOVA) was used to determine significant differences (p<0.05) between treatment groups and the contrast between means were used to assess the differences between the variables.

Results and Discussion

The main chemical compounds identified in *M. officinalis* leaves EO have been showed in the table 1. E-citral (21.45%), Z-citral (19.09%), trans-caryophyllene (15.8%), caryophyllene oxide (13.3%) were the main compounds in *M. officinalis* leaves EO measured by GC/MS. The main compounds of the stem EO have been presented in the table 2. Compounds such as 2-cyclohexanol, 5-methyl-2-(1-methylethyl) (33.48%), cyclohexanol,5-methyl-2-(1-methylethyl) (15.65%) and 1h-pyrazole-1-carboxaldehyde,4-ethyl-4,5-dihydro-5-propyl (9.78%) were the main phytochemicals in *M. officinalis* stem EO .

The main chemical compounds identified in the flower EOs have been shown table 3. Hence, compounds such as trans-carveol (28.89%) and citronellol (25.24%) were the main compounds.

Comparison of the insecticide effects of the leaves, stems and flowers EOs from *M. officinalis* plant revealed that the insecticide effects of these EOs were significantly different (p<0.01). This study indicated that the leaves EO showed the highest insecticide effect while the stem EO demonstrated the lowest effect. (figure 1). The leaves EO at concentration of 10 µL/cm^2 showed more insecticide effect compared to higher densities of the stems and flowers EO. The results showed that the insecticide power of EO significantly increased in a concentration dependent manner. The insecticidal effect of EOs from different parts of *M. officinalis* were significantly different (p<0.01) in different time intervals. The leaves EO had the highest insecticidal effect compared to the flowers and stems EO followed by the flowers EO and stems EO (figure 2).

GC/MS analysis indicated that E-citral, 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl) and trans-carveol were the main compounds in the leaf, stem and flower EOs of the *Melissa officinalis*, respectively.

Table1. The main chemical compounds of essential oil of *Melissa officinalis* leaf

No	Compound	KI	RT	(%)
1	E-citral	976	9.82	21.4
2	Z-citral	983	11.52	19.09
3	trans-Caryophyllene	1091	13.23	15.8
4	Caryophyllene oxide	1182	14.21	13.3
5	phenol,5-methyl-2- (1-methyl)	1312	15.23	4.87
6	6-methyl-5-hepten-2-one	1375	17.00	3.21
7	2,6-octadien-1-ol,3,7-dimethyl-,acetate,(E)	1565	21.25	3.1
8	Benzenea cetaldehyde	1572	23.61	2.21

KI: Kovats Index/ RT: Retention Time

Table 2. Main chemical compounds of stem oil of *Melissa officinalis*

No	Compound	KI	RT	(%)
1	2-cyclohexen-1-one,2-methyl-5-(1-methylethenyl)	993	10.52	33.48
2	Cyclohexanol,5-methyl-2-(1-methylethyl)	1031	11.58	15.65
3	1H-pyrazole-1-carboxaldehyde,4-ethyl-4,5-dihydro-5-propyl	1065	12.93	9.78
4	Cyclohexanone,5-methyl-2-(1-methylethylidene	1141	13.65	6.89
5	L-menthal one	1160	15.81	5.33
6	Borneol	1179	17.78	4.83
7	E-citral	1188	19.25	3.99
8	Bicyclo[4.1.0]heptanes,3,7,7-trimethyl	1210	21.67	3.36

KI: Kovats Index/ RT: Retention Time

Table 3. Main chemical compounds of flower oil of *Melissa officinalis*

No	Compound	KI	RT	(%)
1	trans-carveol	1025	12.90	28.89
2	Citronellol	1035	13.52	25.24
3	δ -3-cavene	1072	14.05	5.26
4	Linalool	1100	15.25	4.05
5	1-Octen-3-ol	1154	16.80	2.03
6	Geraniol	1185	17.70	2.2
7	Spathulenol	1198	18.92	2.06

KI: Kovats Index/ RT: Retention Time

Analysis of *M.officinalis* EO in Cuba showed that the major compounds were geraniol (41%) and Neril (29%) [27]. The main compounds identified of *M. officinalis* EO from Orumieh were Neral and beta-caryophyllene [28].

In the previous studies about phytochemicals of *M. officinalis* EO, citronal, neral and geraniol have been reported as the main compounds in this plant's EO [29]. The percentage of these materials in EO was slightly different depending on the climatic conditions of the plant habitat and its harvesting time. Furthermore, in another study chemical compounds of the EO obtained from aerial parts of *M. officinalis* were analyzed by GC/MS and the obtained results indicated that the highest percentage of the compounds was citronal, neral, geranioland and beta-caryophyllene [29]. The plant EO quality and quantity could be considerably influenced by different factors such as the geographical condition, agricultural operations, harvesting time and drying method of the plant [30]. A number of studies have been carried out on the plants' insecticidal properties which emphasize on using the plants as insecticidal agents [31-34]. The present study revealed that EOs from the leaves, stems and flowers of *M. officinalis* were effective for controlling tow-spotted spider mite. The EOs from other medicinal plants such as *Nepeta parnassica* have shown to be effective in repelling *Pogonomyrmex spares* ant and *Culex pipiens* mosquito [36]. The EO of *Nepeta cataria* was effective in killing and repelling *Anophelesgambiac* mosquitoes [36]. An amount of 20 mg of the *Nepeta cataria* EO has been able to repell *Stomoxy scalcitrans* and *Muscadomestica*. Catnip oil obtained from *Nepeta cataria* has been considered as a relatively safe repellant compared to the other insect repellants such as picardin, p-methane-3,8-dicl, which might cause skin irritations [37].

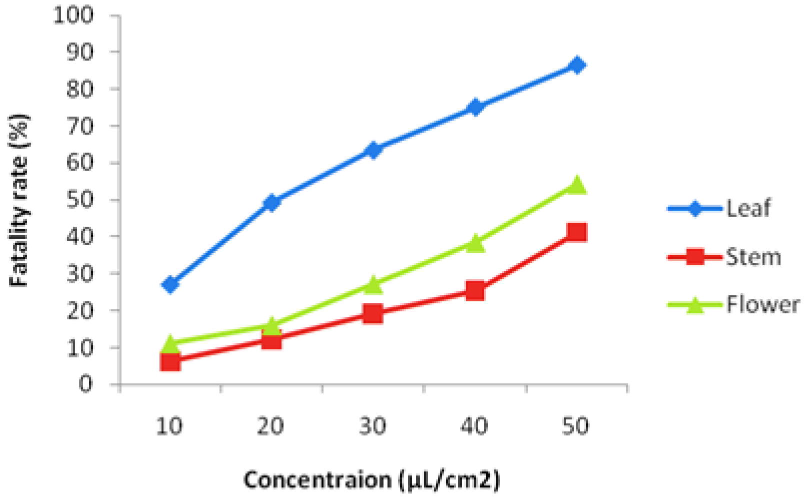

Figure 1. Insecticidal effect of the EOs from different parts of *Melissa officinalis* at different concentrations

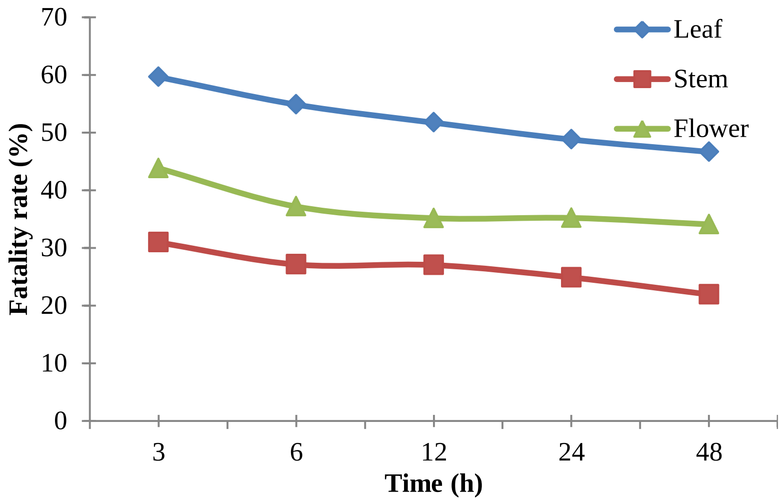

Figure 2. The insecticidal effect of different parts of *Melissa officinalis* EOs at the concentration of 40 µL/cm^2 concentration in different time intervals

Melissa officinalis leaves EO (5.0-49.21%) showed more considerable insecticide properties compared to the stems and flowers EOs. In an investigation, it has been shown that ethanol extracts of *Melia azedarach* L. and *Syzygium aromaticum* significantly disturbed spawning behavior of the two-spotted spider mites [38].In another study, it was reported that the EOs of two medicinal plants including *Nepeta grandiflora* and *Nepeta Crispa* showed considerable aphicide effect on aphid *Sito- bion avenae*. Both plants can be used as insect repellant and natural absorbent of the insects [39]. The EOs of *M. officinalis* leaves, stems and flowers were effective on tow-spotted spider mites. Compress, pomade and oil obtained from *M. officinalis* has been prescribed for repelling insects, treating their bite scar and relieving nervous tension since Avicenna time [40].

The present study revealed that EOs from the leaves and stems of *M. officinalis* showed the highest and the lowest insecticide effect, respectively. The results of the present study indicated that *M. officinalis* EO was effective on two-spotted spider mites and could be suggested as an alternative for chemical insect repellants.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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