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ORIGINAL ARTICLE

Chemical composition along with anti-leishmanial and cytotoxic activity of *Zataria multiflora*

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ABSTRACT

Context: Natural products and their compounds are some of the most interesting sources of new drugs. Reviews have reported various pharmacological properties such as antimicrobial effects of *Zataria multiflora* Boiss (Lamiaceae).

Objective: The present study investigates the chemical composition of *Z. multiflora* essential oil and evaluates its cytotoxic effects and anti-leishmanial activities against *Leishmania tropica* in an *in vitro* model.

Materials and methods: The components of *Z. multiflora* oil were identified by gas chromatography/mass spectrometry (GC/MS) analysis. Anti-leishmanial effects of the essential oil (0–100 µL/mL) and methanol extract of *Z. multiflora* (0–100 µg/mL) on promastigote forms as well as their cytotoxic activities against J774 cells were evaluated using MTT assay for 72 h. The leishmanicidal activity against amastigote forms of *L. tropica* was evaluated at the concentrations of 0–50 µg/mL in a macrophage model for 48 h.

Results: The chemical analyses demonstrated that the main components of essential oil were thymol (41.81%), carvacrol (28.85%), and *p*-cymene (8.36%). Regarding leishmanicidal activity, the IC₅₀ values for the essential oil and methanol extract were 3.2 µL/mL and 9.8 µg/mL against promastigote forms and 8.3 µL/mL and 34.6 µg/mL against amastigote forms, respectively. Essential oil (CC₅₀ 89.3 µL/mL) indicated a higher cytotoxic effect than the methanol extract (CC₅₀ 591.6 µg/mL) of *Z. multiflora*.

Conclusion: The present study revealed the chemical composition of *Z. multiflora* that might be a natural source of new anti-leishmanial agents in terms of use against cutaneous leishmaniasis.

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Introduction

Cutaneous leishmaniasis (CL) is a protozoan infection which is caused by the *Leishmania* protozoa. This disease is characterized by chronic skin lesions and leaves permanent scars through the deformation of the infected area (Desjeux, 2004). CL is a public health problem at a global level and affects 1.5 million people annually worldwide (World Health Organization [WHO], 2010). At present, the administration of pentavalent antimony organic compounds (meglumine antimoniate and sodium stibogluconate) remains as the first choice of therapy for all leishmaniasis forms (Desjeux, 2004). However, resistance and high frequency of side effects are still the problems associated with this treatment (Santos et al., 2004). Thus, these factors require the search for new effective anti-leishmanial drugs.

Historically, natural products and their compounds have been one of the most attractive sources for developing new drugs (Rojas-Silva et al., 2014). *Zataria multiflora* Boiss (Lamiaceae) commonly grows in Iran, Afghanistan, and Pakistan. *Z. multiflora* called “Avishan-e Shirazi” in Persian has been used as a flavoring agent (spice) in a variety of foodstuff in Iran (Mahboubi & Ghazian, 2010). Reviews have reported its immunostimulant, pain-relieving, antinociceptive, anti-inflammatory, antioxidant, antibacterial, antiviral, and antifungal effects (Sajed et al., 2012). Previous studies have demonstrated that the main constituents of *Z. multiflora* essential oil are phenolic compounds such as carvacrol and thymol (Basti et al., 2007; Saei-Dehkordi et al., 2010). However, some factors such as the geographical origin of the variety and harvesting season could affect the chemical composition and biological activity

of *Z. multiflora* essential oil (Yesil Celiktas et al., 2007a,b). To the best knowledge of the present authors, no studies have been conducted on the anti-leishmanial effects of *Z. multiflora*. Thus, the present work aims to evaluate the chemical composition of *Z. multiflora* essential oil and investigate its antileishmanial activity against *L. tropica* in an *in vitro* model.

Materials and methods

Chemicals

Meglumine antimoniate (MA, Glucantime[®]), used as a control drug, was purchased from Aventis, Paris, France. Penicillin and streptomycin were obtained from Alborz Pharmacy, Karaj, Iran, and stored at room temperature (25°C) until testing. MTT powder [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide], Dulbecco's Modified Eagle's Medium (DMEM), fetal calf serum (FCS), RPMI-1640 medium with L-glutamine, Griess reagent (A and B), and Tween 20 were prepared from Sigma-Aldrich, (St. Louis, MO). All the other applied chemicals and solvents had the highest commercially available purity.

Plant materials

The aerial parts of *Z. multiflora* were collected from the rural regions of Kohpayeh, Kerman district (Kerman Province, Iran) in September 2013. The plant materials were identified by Dr. Mirtajaldin, a botanist at Shahid Bahonar University of Kerman, Kerman, Iran. A voucher specimen of the plant materials was deposited at the herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Iran (KF1375).

Preparing methanol extract

The dried aerial parts of the plant (100 g) were ground and extracted by percolation method using methanol (80%) for 72 h at room temperature. The solvents were removed in a rotary evaporator and, after filtering, the extract was concentrated to dryness and stored at -20°C until testing (Mahmoudvand et al., 2014a).

Phytochemical analysis of methanol extract

The preliminary phytochemical analysis of the methanol extract was carried out to determine the presence of tannins, saponins, alkaloids, phenols, and glycosides as described elsewhere (Evans, 1998).

Isolation of the essential oil

Air-dried aerial parts of the plant were subjected to hydro-distillation for 3 h using an all-glass Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and stored in darkness at 4°C until testing (Mahmoudvand et al., 2015).

Gas chromatography/mass spectrometry (GC/MS) analysis of essential oil

GC analysis

GC analysis was carried out by Hewlett-Packard 6890 (Hewlett-Packard, Palo Alto, CA) with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 mm). The column temperature was maintained at 55°C for 3 min, programmed to 180°C in the rate of 5°C per min, and kept constant at 220°C for 5 min. Injector and interface temperatures were 220 and 290°C, respectively. The flow rate of helium as carrier gas was (1 mL/min C.F). The percentages were calculated by the electronic integration of FID peak areas without using response factor correction. Linear retention indices for all the components were determined by the co-injection of the samples with a solution containing homologous series of C8-C22 *n*-alkanes.

GC/MS analysis

GC/MS analysis was performed using a Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (30 m × 0.25 mm, film thickness 0.25 mm) coupled with a TRACE mass (CAS, Manchester, UK). Helium was used as the carrier gas with an ionization voltage of 70 eV. Ion source and interface temperatures were 220 and 290°C, respectively. Mass range was from 40 to 400 u. Temperature program of the oven was as mentioned above for the GC.

Identifying the essential oil components

The components of the essential oil were identified by comparing their relative retention time and mass spectra with the standards, Wiley 2001 library data of the GC/MS system, or those reported in the literature data (Adams, 2004).

Preparation of the dilutions

To prepare the dilutions of the *Z. multiflora* essential oil, 0.1 mL of the essential oil was dissolved in 0.97 mL of normal saline. In addition, to enhance the dispersal of the essential oil in normal saline, 0.03 mL of Tween 20 was added to the test tube. The resulting solution was

mixed adequately by a magnetic stirrer. Serial dilutions were carried out to obtain the essential oil at 3.125, 6.25, 12.5, 25, 50, and 100 $\mu\text{L}/\text{mL}$. Methanol extract (40 mg) was dissolved in 10 mL of normal saline and serial dilutions were subsequently performed to obtain methanol extract at 0.025–4 $\mu\text{g}/\text{mL}$. Selecting the dilutions of the essential oil and methanol extract of *Z. multiflora* was based on the initial experiments, which also showed that normal saline plus Tween 20 did not influence the growth of parasite.

Parasite and cell culture

Standard strain of *L. tropica* (MHOM/IR/2002/Mash2) was kindly prepared from Center for Research and Training in Skin Diseases and Leprosy (Tehran, Iran). The parasites were cultured at Novy–MacNeal–Nicolle medium (NNN) and sub-cultured in RPMI 1640 supplemented with penicillin (200 IU/mL), streptomycin (100 $\mu\text{g}/\text{mL}$), and 15% heat-inactivated FCS. Murine macrophage cells (J774-A1) were obtained from Pasteur Institute of Iran (Tehran, Iran). The cells were cultured and maintained at Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS at 37°C in 5% CO_2 .

Anti-promastigote assay

To evaluate the anti-promastigote effects of essential oil and methanol extract of *Z. multiflora* on the promastigote forms of *L. tropica*, colorimetric cell viability assay with MTT was used according to the method described by [Ezatzpour et al. \(2015\)](#). In brief, 100 μL of the promastigotes (10^6 cells/mL) harvested from logarithmic growth phase was added to a 96-well tissue culture plate. Then, 100 μL of various concentrations of the essential oil (0–25 $\mu\text{L}/\text{mL}$) and methanol extract (0–100 $\mu\text{g}/\text{mL}$) was added to each well and incubated at $25 \pm 1^\circ\text{C}$ for 48 h. After the incubation, 10 μL of the MTT solution (5 mg/mL) was added to each well and incubated at 25°C for 4 h. Promastigotes cultured at complete medium without treatment were used as positive control and complete medium without promastigotes and drugs were used as the blank. The absorbance was measured for each well at 560 nm using an ELISA reader (BioTek-ELX800, BioTek, Winooski, VT). In addition, 50% inhibitory concentrations (IC_{50} values) were measured for all the tested products by probit test in SPSS software (ver. 17.00, SPSS Inc., Chicago, IL).

Anti-amastigote assay

Assay on amastigote was carried out as described by [Mahmoudvand et al. \(2014b\)](#). Initially, 1 cm^2 cover slips

were placed in the wells of 6-chamber slides (Lab-Tek, Nalge Nunc International, Rochester, NY). In the next step, 200 μL of macrophage (10^5 cells/mL) was added and incubated at 37°C in 5% CO_2 for 2 h. Then, 200 μL ($10^6/\text{mL}$) promastigotes in stationary phase was added to the macrophage culture, which led to obtaining the *Leishmania*/macrophage proportion of 10:1 and the plate was incubated in similar conditions for additional 24 h. Free parasites were removed by washing with RPMI 1640 medium and the infected macrophages were treated with 50 μL of various concentrations of essential oil (0–50 $\mu\text{L}/\text{mL}$) and methanol extract. The plate was incubated at 37°C in 5% CO_2 for 48 h. Finally, the dried slides were fixed with methanol, stained by Giemsa, and studied under a light microscope. Also, the macrophages containing amastigotes without products and those with no parasite and treatment were considered the positive and negative controls, respectively. Activity of anti-intramacrophage amastigotes of the products was evaluated by counting the number of amastigotes in each macrophage by examining 100 macrophages (% amastigotes viability) in comparison with the positive control. In addition, IC_{50} values were measured for all the tested products by probit test in SPSS software (SPSS Inc., Chicago, IL).

Inhibition of infection in macrophage cells

To determine the inhibitory effect of the essential oil and methanol extract of *Z. multiflora* against the promastigote invasion of macrophages, the promastigotes of *L. tropica* were pre-incubated in essential oil (2 $\mu\text{L}/\text{mL}$) and methanol extract (5 $\mu\text{g}/\text{mL}$) for 2 h at room temperature. Then, the promastigotes were washed with RPMI-1640 medium and incubated by macrophage cells (J774 cells) for 4 h. After washing, the macrophages were stained by Giemsa and studied using a light microscope to evaluate the infection frequency by counting 100 macrophages ([Mahmoudvand et al., 2014c](#)).

Determining nitric oxide production

Nitric oxide (NO) release in the supernatants of macrophage culture was measured by the Griess reaction for nitrites. About 100 μL of the supernatants were collected 48 h after introducing the extracts into the culture medium. The assay was done in triplicate in a 96-well tissue culture plate. Then, 60 μL of Griess reagent A and 60 μL of Griess reagent B were added. The plates were read at 540 nm in an ELISA plate reader (BioTek-ELX800, BioTek, Winooski, VT).

Table 1. Infectivity rate of the *L. tropica* promastigotes after treatment with the essential oil and methanolic extract of *Z. multiflora*. Data are expressed as the mean \pm SD ($n = 3$).

Plant materials	Percentage of infected macrophages by non-treated promastigotes	Percentage of infected macrophages by treated promastigotes	Percentage of infectiveness reduction
Essential oil	84.1 \pm 3.05	11.3 \pm 1.15	86.5
Methanolic extract	84.1 \pm 3.05	29.6 \pm 2.15	64.8

Cytotoxic effects

Cytotoxic effects of *Z. multiflora* against J774-A1 cells were evaluated after the treatment of macrophages (5×10^5 /mL) with various concentrations of the essential oil (0–100 μ L/mL) and methanol extract (0–500 μ g/mL) in 96-well tissue culture plates at 37°C and 5% CO₂ for 48 h. Cell viability was determined by colorimetric MTT assay and the results were displayed as percentage of dead cells compared with the MA and non-treated macrophages (100% of viability). Moreover, CC₅₀ (50% cytotoxic concentration) was calculated by probit test in SPSS software (SPSS Inc., Chicago, IL) (Mahmoudvand et al., 2014c).

Statistical analysis

All the tests were performed in triplicate. Selectivity index (SI), calculated using the equation of CC₅₀ for murine macrophage/IC₅₀ for the amastigote forms of *L. tropica*, was used to compare the toxicity and activity of the essential oil and methanol extract *Z. multiflora* (Weninger et al., 2001). Data analyses were carried out by SPSS statistical package, ver.17.0 (SPSS Inc., Chicago, IL). Differences between the test and control groups were analyzed by *t*-test. In addition, $p < 0.05$ was considered statistically significant.

Results and discussion

Medicinal plants are the oldest medicines used by humans. Their increasing use in recent years provides a clear piece of evidence for the public interest in their use instead of the conventional drugs (Tapsell et al., 2007). *Z. multiflora* is a thyme-like plant that broadly grows in the central and southern parts of Iran. This plant is used in traditional medicine to treat a wide range of diseases, such as infectious ones (Mahboubi & Ghazian, 2010). The essential oil and methanol extract of *Z. multiflora* were found to have potent anti-leishmanial effects on the promastigote forms of *L. tropica* ($p < 0.05$). Findings also revealed that essential oil caused significantly high leishmanicidal effects ($p < 0.05$) on the promastigotes of *L. tropica* (3.2 μ L/mL) compared with

the methanol extract (9.8 μ g/mL). Moreover, the IC₅₀ value for the essential oil against promastigotes of *L. tropica* was significantly lower ($p < 0.05$) than that of MA (88.3 μ g/mL). We found that the essential oil and methanol extract of *Z. multiflora* significantly ($p < 0.05$) inhibited the growth rate of intra-macrophage amastigotes. The IC₅₀ values of essential oil and methanol extract were 8.3 and 34.6 μ g/mL, respectively. Results also demonstrated that the essential oil was statistically more effective ($p < 0.05$) for the amastigotes than methanol extract. This value was 41.6 μ g/mL for MA as the control drug. Our findings demonstrated that the promastigote forms of *L. tropica* without treatment were able to infect 84.1% of macrophage, while promastigotes treated with essential oil and methanol extract of *Z. multiflora* had a potency to infect only 11.3 and 29.6%, respectively (Table 1).

The results of the present study revealed the potent leishmanicidal activity of *Z. multiflora* in comparison with MA. Findings of optical density (OD) and consequently IC₅₀ values showed that *Z. multiflora*, particularly its essential oil, significantly inhibited the growth rate of promastigote forms of *L. tropica*. Likewise, it significantly reduced mean infection rate and subsequently the viability of amastigote forms in the macrophage cells in comparison with the reference drug. In agreement with our findings, Barati et al. (2010) reported that *Z. multiflora* extract (IC₅₀ value 7.4 μ g/mL) significantly inhibited the growth rate of promastigote forms of *L. major* in MTT assay. Results of the present study also exhibited that amastigotes were more susceptible to *Z. multiflora* than promastigote forms. This difference in the susceptibility of promastigote and amastigote stages might be related to structural, biochemical, and morphological features as previously shown by other researchers (Shokri et al., 2012).

The findings of primary phytochemical screening of the *Z. multiflora* methanol extract demonstrated the presence of phenols, tannins, and flavonoids; individual activities of these compounds have been proven (Cowan, 1999). In addition, these results demonstrated a high content of phenolic compounds in *Z. multiflora*, especially thymol and carvacrol, which could act on the cell

membrane microorganisms and cause damage and depletion of their contents (Moshayedi et al., 2013). The essential oil and methanol extract of *Z. multiflora* triggered the production of 18 μM of nitric oxide at low concentrations of 3.125 and 10 $\mu\text{g/mL}$, respectively, compared with 11 μM for the untreated macrophages. In contrast, essential oil ($\geq 6.25 \mu\text{g/mL}$) and methanol extract ($\geq 25 \mu\text{g/mL}$) at higher concentrations reduced the production of NO at the concentration of 6 and 9 μM , respectively, compared with the untreated macrophages (11 μM). Similar results were reported by Kavooosi et al. (2012), who demonstrated that *Z. multiflora* essential oil, thymol, and carvacrol significantly reduced nitric oxide activities, H_2O_2 production, NO synthesis, and NADH oxidase in LPS-stimulated murine. Thus, the exact mechanisms of leishmanicidal activity of *Z. multiflora* are not obvious and further studies are required to elucidate these mechanisms.

Table 2. Composition of essential oil from *Z. multiflora* identified by GC/MS.

Components	KI ^a	% Composition
3-Octanone	956	0.56
α -Pinene	973	0.36
β -Myrcene	987	0.36
3-Octanol	993	0.49
d-Carene-3	1017	0.41
1,8-Cineole	1026	0.28
Limonene	1031	0.34
p -Cymene	1035	8.36
γ -Terpinene	1060	3.98
trans-Sabinene hydrate	1072	0.23
Linalool	1097	1.75
2-Nonanol	1101	0.15
Borneole	1179	0.28
4-Terpineol	1184	1.14
α -Terpinolene	1196	1.28
Decenal-z-4	1199	0.12
Thymol methyl ether	1234	1.3
Thymol	1288	41.81
Carvacrol	1297	28.85
Thymol acetate	1354	0.46
b-Caryophyllene	1428	2.06
Aromadendrene	1448	0.86
a-Selinene	1498	0.54
Apofarensol	1581	0.96
Caryophyllene oxide	1587	0.85
Total		99.25

^aKovats index on non-polar DB-5 ms column in reference to *n*-alkanes

In the present study, yellow-colored essential oil (yield 3.1% v/w) was obtained by hydro-distillation method and analyzed using GC/MS. Table 2 indicates the results of *Z. multiflora* essential oil obtained by GC/MS analysis. Twenty-five compounds were identified, which represented 99.78% of the total oil. The main components were thymol (41.81%), carvacrol (28.85%), and *p*-cymene (8.36%). Composition of *Z. multiflora* essential oil, which has been previously analyzed by other research groups, was shown to depend on species, climate, collection time, and growth stage (Daferera et al., 2000), thereby altering the studied biological activities (Vardar-Unlu et al., 2003). In the previous studies, the main components of the essential oil have been only reported to be thymol (5–56%) and carvacrol (5–78%), both at high percentage and with few other compounds. However, in the present study, the main components were found to be thymol (41.81%), carvacrol (28.85%), and *p*-cymene (8.36%). Thus, the plant analyzed in this research could be a different chemotype of *Z. multiflora*. Regarding the leishmanicidal effects of thymol and carvacrol, de Melo et al. (2013) indicated that thymol could exhibit the IC_{50} value of 9.8 $\mu\text{g/mL}$ and carvacrol of 2.3 $\mu\text{g/mL}$ for *L. chagasi* promastigotes. The IC_{50} value for carvacrol was close to the IC_{50} value for amphotericin B (0.51 $\mu\text{g/mL}$), one of the commercial drugs used for leishmaniasis treatment. In addition, in the study conducted by Monzote et al. (2014), carvacrol showed a significant leishmanicidal activity with the IC_{50} value of 15.3 for promastigotes and 13.6 $\mu\text{g/mL}$ for the amastigote forms of *L. amazonensis*.

Cytotoxicity of *Z. multiflora* was investigated in J774-A1 cells. The *in vitro* assay demonstrated no significant cytotoxicity in the cells. However, the essential oil indicated a higher cytotoxic effect ($p < 0.05$) on murine macrophages than the methanol extract. Table 3 shows the CC_{50} value for the essential oil (89.3 $\mu\text{L/mL}$), methanol extract (591.6 $\mu\text{g/mL}$), and subsequently their SI values. The obtained results demonstrated that essential oil and methanol extract of *Z. multiflora* had no significant cytotoxic effects on J774-A1 cells. In addition, $\text{SI}_s \geq 10$ of the extracts showed their safety to

Table 3. The IC_{50} , CC_{50} values, and selectivity index (SI) determined for the essential oil, methanolic extract of *Z. multiflora* and control drug (MA) against intra-macrophage amastigote forms of *L. tropica*.

Tested material	IC_{50}		CC_{50}^b	SI ^c
	Promastigote	Amastigote		
Essential oil ($\mu\text{L/mL}$)	3.2 \pm 0.15	8.3 \pm 0.6	89.3 \pm 3.5	10.7
Methanolic extract ($\mu\text{g/mL}$)	9.8 \pm 0.52	34.6 \pm 2.15	591.6 \pm 3.6	17
MA ^a	88.3 \pm 3.05	41.6 \pm 2.51	1012.6 \pm 13.6	24.3

^aMeglumine antimoniate.

^b CC_{50} (cytotoxic concentration for 50% of cells).

^cSelectivity index ($\text{CC}_{50}/\text{IC}_{50}$ value of amastigote forms).

the macrophages and specificity to the parasite (Weninger et al., 2001). In line with these findings, Malekinejad et al. (2012) demonstrated that *Z. multiflora* had no toxicity on Chinook salmon (*Oncorhynchus tshawytscha*) embryo (CHSE-214) cells. Therefore, the *Z. multiflora* could be safe for mammalian cells.

In conclusion, the findings of the present study revealed the new chemical composition of *Z. multiflora* that might be a natural source in terms of searching for new anti-leishmanial agents to explore against cutaneous leishmaniasis. However, further pre-clinical studies are required to evaluate the exact biological activity of *Z. multiflora* in animal models.

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Declaration of interest

The authors report that they have no conflict of interest.

References

- Adams RP. (2004). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream, IL: Allured Publishing Corporation.
- Barati M, Sharifi I, Sharififar F. (2010). *In vitro* evaluation of anti-leishmanial activities of *Zataria multiflora* Boiss, *Peganum harmala* and *Myrtus communis* by colorimetric assay. *J Kerman Univ Med Sci* 17:32–41.
- Basti AA, Misahgi A, Khaschabi D. (2007). Growth response and modelling of the effects of *Zataria multiflora* Boiss. Essential oil, pH and temperature on *Salmonella typhimurium* and *Staphylococcus aureus*. *LWT* 40:973–81.
- Cowan MM. (1999). Plant products as antimicrobial agents. *Clin Microb Rev* 12:564–82.
- Daferera DJ, Ziogas BN, Polissiou MG. (2000). GC-MS analysis of essential oils from Greek aromatic plants and their fungi toxicity on *Penicillium digitatum*. *J Agric Food Chem* 48:2576–81.
- de Melo JO, Bitencourt TA, Fachin AL, et al. (2013). Antidermatophytic and antileishmanial activities of essential oils from *Lippia gracilis* Schauer genotypes. *Acta Trop* 128:110–15.
- Desjeux P. (2004). Leishmaniasis: Current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 128:110–15.
- Evans WC. (1998). *Trease and Evans Pharmacognosy*. 14th ed. Edinburgh: WB Saunders Company Limited, 15–6.
- Ezatpour B, Saedi Dezaki E, Mahmoudvand H, et al. (2015). *In vitro* and *in vivo* antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. *Evid Based Complement Alternat Med* 2015:149707.
- Kavoosi G, Teixeira da Silva JA, Saharkhiz MJ. (2012). Inhibitory effects of *Z. multiflora* essential oil and its main components on nitric oxide and hydrogen peroxide production in lipopolysaccharide-stimulated macrophages. *J Pharm Pharmacol* 64:1492–500.
- Mahboubi M, GhazianBidgoli F. (2010). Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. *Phytomedicine* 17:548–50.
- Mahmoudvand H, Asadi A, Harandi MF, et al. (2014a). *In vitro* lethal effects of various extracts of *Nigella sativa* seed on hydatid cyst protoscoleces. *Iran J Basic Med Sci* 17:1001–6.
- Mahmoudvand H, Ezzatkah F, Sharififar F, et al. (2015). Antileishmanial and cytotoxic effects of essential oil and methanol extract of *Myrtus communis* L. *Korean J Parasitol* 53:21–7.
- Mahmoudvand H, Sharififar F, Sharifi I, et al. (2014b). *In vitro* inhibitory effect of *Berberis vulgaris* (Berberidaceae) and its main component, Berberine against different *Leishmania* species. *Iran J Parasitol* 9:28–36.
- Mahmoudvand H, Tavakoli R, Sharififar F, et al. (2014c). Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. *Pharm Biol* 53:1052–7.
- Malekinejad H, Bazargani-Gilani B, Tukmechi A, et al. (2012). A cytotoxicity and comparative antibacterial study on the effect of *Zataria multiflora* Boiss, *Trachyspermum copticum* essential oils, and enrofloxacin on *Aeromonas hydrophila*. *Avicenna J Phytomed* 2:188–95.
- Monzote L, García M, Pastor J, et al. (2014). Essential oil from *Chenopodium ambrosioides* and main components: Activity against *Leishmania*, their mitochondria and other microorganisms. *Exp Parasitol* 136:20–6.
- Moshayedi SH, Shahraz F, Schaffner DW, et al. (2013). *In vitro* control of *Enterococcus faecalis* by *Zataria multiflora* Boiss, *Origanum vulgare* L. and *Mentha pulegium* essential oils. *J Food Saf* 3:1745–65.
- Rojas-Silva P, Graziöse R, Vesely B, et al. (2014). Leishmanicidal activity of a daucane sesquiterpene isolated from *Eryngium foetidum*. *Pharm Biol* 52:398–401.
- Saei-Dehkordi SS, Tajik H, Moradi M, et al. (2010). Chemical composition of essential oils in *Zataria multiflora* Boiss. From different parts of Iran and their radical scavenging and antimicrobial activity. *Food Chem Toxicol* 48:1562–7.
- Sajed H, Sahebkar A, Iranshahi M. (2013). *Zataria multiflora* Boiss. (Shirazi thyme) – An ancient condiment with modern pharmaceutical uses. *J Ethnopharmacol* 145:686–98.
- Santos DO, Coutinho CE, Madeira MF, et al. (2004). Leishmaniasis treatment – A challenge that remains: A review. *Parasitol Res* 103:1–10.
- Shokri A, Sharifi I, Khamesipour A, et al. (2012). The effect of verapamil on *in vitro* susceptibility of promastigote and amastigote stages of *Leishmania tropica* to meglumine antimoniate. *Parasitol Res* 110:1113–17.
- Tapsell LC, Hemphill I, Cobiac L, et al. (2006). Health benefits of herbs and spices: The past, the present, the future. *Med J Aust* 185:14–24.
- Vardar-Unlu G, Candan F, Sokmen A, et al. (2003). Antibacterial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. Et Mey var. *pectinatus* (Lamiaceae). *J Agric Food Chem* 51:63–7.
- Weninger B, Robledo S, Arango GJ, et al. (2001). Antiprotozoal activities of Colombian plants. *J Ethnopharmacol* 78:193–200.

- World Health Organization. (2010). *Control of the Leishmaniasis*. Geneva: WHO (Technical Report Series) 949: 5–12.
- Yesil Celiktas O, Girgin G, Orhan H, et al. (2007a). Screening of free radical scavenging capacity and antioxidant activities of *Rosmarinus officinalis* extracts with focus on location and harvesting times. *Eur Food Res Tech* 224:443–51.
- Yesil Celiktas O, Hames Kocabas EE, Bedir E, et al. (2007b). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem* 100:553–9.