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In Vitro Effect of Some Medicinal Plants on *Leishmania major* Strain MRHO/IR/75/ER

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ABSTRACT

Background and objectives: Leishmaniasis is a tropical disease caused by protozoan parasites from the genus *Leishmania*. In this study, we aimed at investigating the in vitro anti-leishmanial effect of essential oils of *Rosmarinus officinalis*, *Mentha pulegium*, *Foeniculum vulgare*, *Lippia citriodora* and *Pelargonium graveolens*.

Methods: The essential oils were prepared from freshly dried and powdered plants with steam-distilled water. Iranian strain of *Leishmania* promastigotes was cultured in RPMI medium and the inhibitory effects of different concentrations (25, 32, 62.5, 125, 250, 500 and 1000 µg/ml) of the essential oils were investigated at 24, 48 and 72 hours. The number of live parasites before and after treatment with the essential oils was counted by trypan blue 10% staining and using neobar lam.

Results: The essential oils significantly decreased the number of promastigotes in a dose-dependent manner ($P<0.05$). However, the inhibitory effects of *F. vulgare* and *R. officinalis* essential oils were more profound compared to other essential oils. Moreover, concentrations of 500 and 1000 µg/ml of these two essential oils exerted equal and more anti-leishmanial potency compared to glucantime, the first-line drug used for treatment of leishmaniasis.

Conclusion: Based on the results, it is recommended to evaluate the in vivo anti-leishmanial effects of the tested essential oils, particularly *F. vulgare* and *R. officinalis*.

Keywords: Rosmarinus officinalis, Mentha pulegium, *Foeniculum vulgare*, Lippia citriodora, Pelargonium graveolens, Leishmania

INTRODUCTION

Leishmaniasis is a common, tropical disease caused by the protozoan *Leishmania* parasites , which mainly affects developing countries (1-3). Leishmaniasis is transmitted through the bite of *Phlebotomus papatasi* and *Lutzomyia* sandflies (4). The disease is clinically classified into visceral, coetaneous (the most common) and mucocutaneous forms (5). Iran has the highest prevalence of coetaneous leishmaniasis and is ranked fourth in terms visceral leishmaniasis in the Middle East (6). Despite national and international investments against this disease, not only the disease has not been eradicated, but new hotspots have emerged in Iran (7).

Recent evidence suggests the emergence of resistance to pentavalent antimonials, which are commonly used for treatment of leishmaniasis (8). In addition, the high cost and lack of access to medications, particularly in rural areas, have further complicated the treatment of this disease. Therefore, researchers have been seeking suitable, effective alternatives to these drugs for treatment of leishmaniasis (9). Given the disadvantages of chemical medications including high-cost, adverse effects and risk of maladaptation, the use of medicinal plants has received a lot of attention (10). Some studies have reported moderate-strong anti-leishmanial activity of different plant essential oils (11-14).

Foeniculum vulgare is a herbaceous, aromatic plant from the parsley family, which looks like a dill with yellow umbelliferous flowers. The most active ingredients of this plant's essential oil include anethole, fenchone and phellandrene (15). *Rose geranium* (*Pelargonium graveolens*) is a slow-growing, perennial herb with long stems, rounded leaves and a rose-like flower (16). *Lemon verbena* (*Lippia citriodora*) is a shrub that growth 1.5 to 2 meter high and has simple leaves (diameter of 7 to 10 cm) (17).

According to a previous study, the constituents of essential oil of *Mentha pulegium* include alpha-pinene, beta-pinene, limonene, 3-octanol, paracymene, 3-octyl acetate, menton, isomenton, pulegone, isopulegone, pyrethrin, cis-pulegone oxide, trans-pulegone oxide, caryophyllene, lauric acid, myristic acid, palmitoleic acid, salisylaldehyde and hesperidine (11).

Rosmarinus officinalis L. is a plant species that mainly originates from the Mediterranean region and Southern Asia. A limited number of studies have investigated the anti-parasitic effects of the above mentioned plants. Therefore, we aimed to evaluate the in vitro anti-leishmanial effects of essential oils of *R. officinalis*, *M. pulegium*, *F. vulgare*, *L. Citriodora* and *P. graveolens*.

MATERIALS AND METHODS

The essential oils were prepared by distillation with water. First, 100 g of *F. vulgare* (seeds), *P. graveolens*, *L. citriodora*, *M. pulegium* and *R. officinalis* leaves were powdered using a small electric mill and then distilled with 900 ml water for two hours in a Clevenger type apparatus. The obtained essential oils were kept in colored glass containers at 4 ^{o}C.

After isolation, the Iranian strain of *Leishmania major* (MRHO/IR/75/ER) was cultured in RPMI-1640 containing fetal calf serum (30%) at the School of Public Health and Health Research, University of Tehran. Then, the culture suspension containing 20×10^{6} parasites/ml was treated with different concentrations (31.25, 62.5, 125, 250, 500 and 1000 µg/ml) of the prepared essential oils. Inhibitory effects of the essential oils on the parasites were assessed after 24, 48 and 72 hours by MTT assay.

Since, 100 µl of culture medium containing *L. major* promastigotes (with 2×10^{5} parasites per ml) were added to wells of a 96-well plate in duplicate. Subsequently, 10 µl of different concentrations of the essential oils and glucantime (positive control) were added to the wells. In each plate, a well containing only the medium was considered as negative control. After 24, 48 and 72 hours of incubation, 10 µl of MTT solution were added to each well. The plates were then incubated at 25 ± 1 °C in the dark for 4 hours. After incubation, 100 µl of dimethyl sulfoxide were added to each well to stop the reaction. Viability was assessed by reading absorbance at 570 nm using an ELISA reader. All experiments were repeated three times.

The mean absorbance was compared using two-way ANOVA. Data analysis was done using SPSS software (version 20) at significance of 0.05.

RESULTS

The major components of the essential oils are listed in table 1. As shown in figure 1, all tested essential oils could inhibit the growth of *L. major* promastigotes in a dose-dependent manner. The highest inhibitory effect was recorded at concentrations of 500 and 1000 µg/ml. In addition, 1000 µg/ml of *R. officinalis* and *F. vu lgare* had significantly higher inhibitory effect on *L. major* promastigotes compared to glucantime (Figure 2). Based on the results, 1000 µg/ml of *P. graveolens* essential oil and 500 µg/ml of *R. officinalis*, *F. vulgare* and *L. citriodora* essential oils had no significant difference with glucantime in terms of anti-leishmanial effect.

Table 1. Major components of the essential oils using GC/MS analysis

Essential oil	Major components	KI	Percent
Foeniculum vulgare	E-anethole	1303	**67.06**
	Fenchone	1097	**10.98**
	α-phellandrene	1010	**5.22**
Pelargonium graveolens	Citronellol	1236	**31.33**
	Geraniol	1260	**15.22**
	Cytronellyl formate	1279	**8.07**
Aloysia citrodora	Limonene	1035	**53.19**
	α-Terpincol	1206	**7.14**
Rosemarinus officinalis	Pinene –α	934	**32.44**
	1,8 Cineole	1037	**25.04**
	Verbenone	1220	**4.15**
Mentha pulegium	δ-terpinene	1052	**32.16**
	α-terpinene	1012	**18.84**

Figure 1. Inhibitory effects of the essential oils on promastigotes of *L. major* using the MTT assay

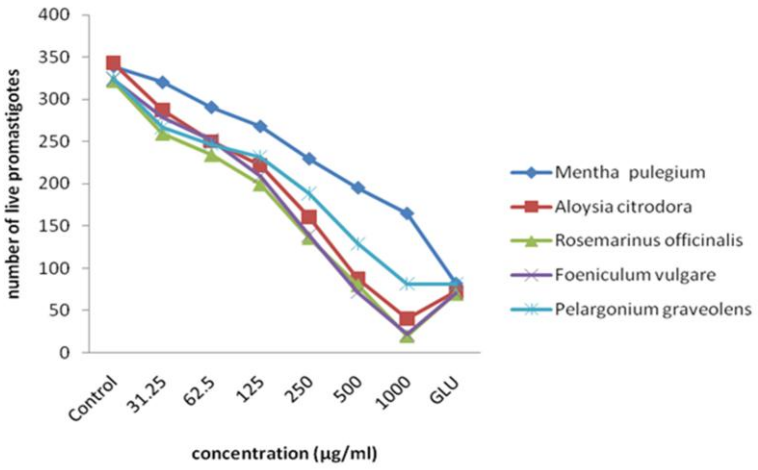
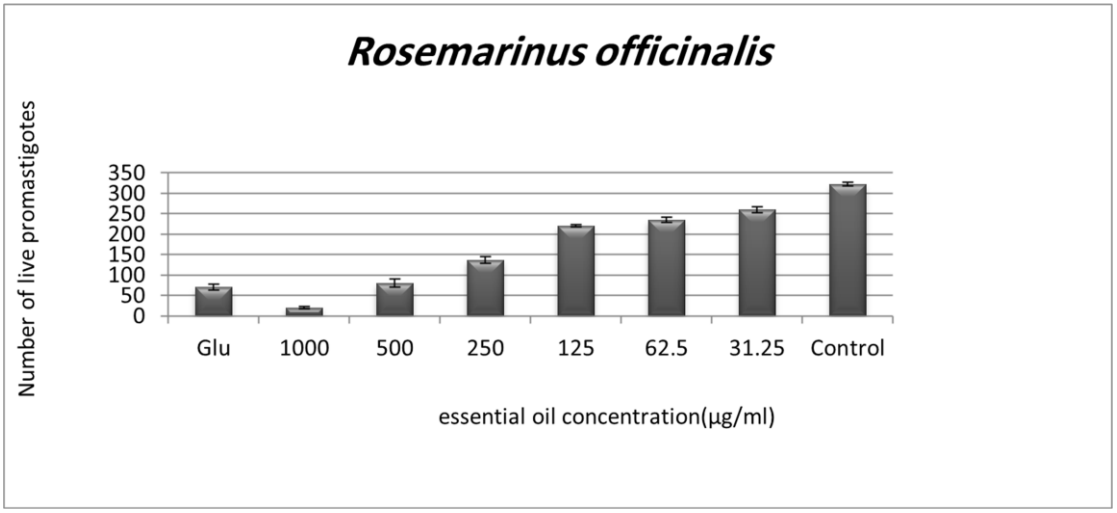


Figure 1. Inhibitory effects of the essential oils on promastigotes of *L. major* using the MTT assay

Figure 2. Anti-leishmanial effect of the essential oils of *R. officinalis* (a), *F. vulgare* (b), *P. graveolens* (c), *A. citrodora* (d) and *M. pulegium* (e)

a



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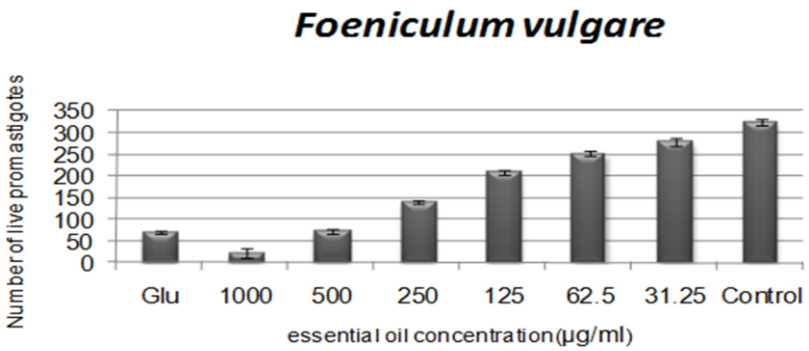
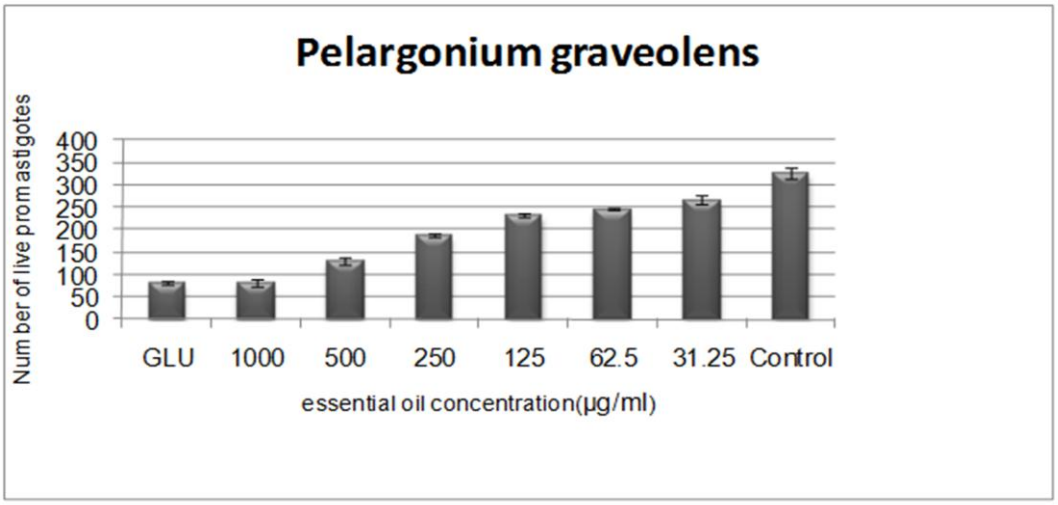
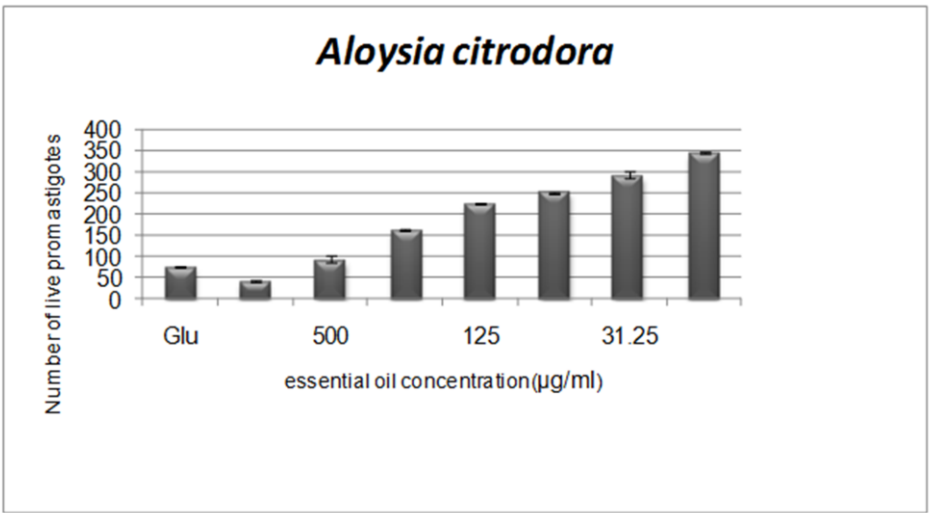


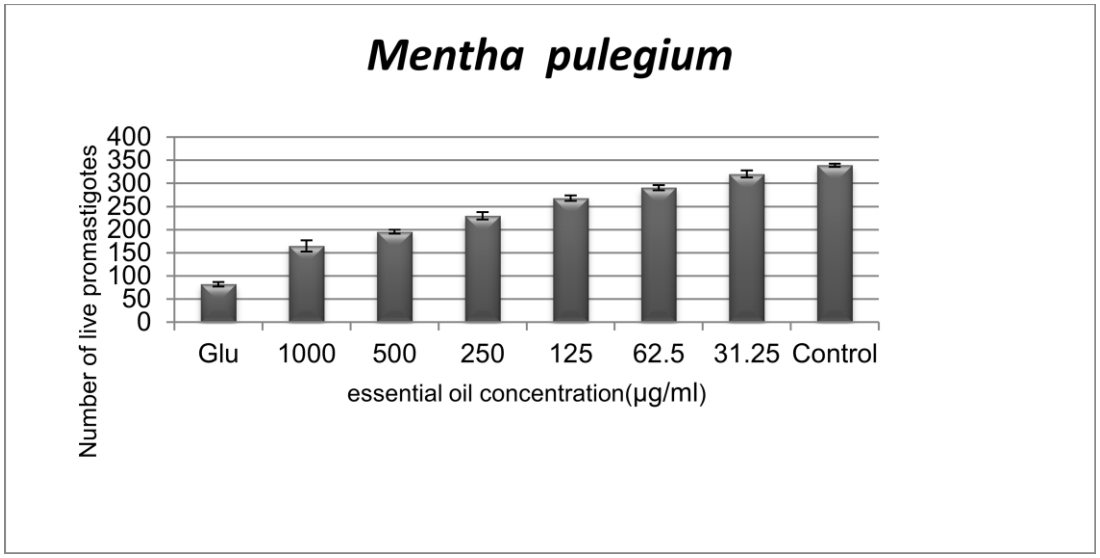
c



d



e



DISCUSSION

Several factors such as parasite, vector, host and environment may affect the pidemiology and symptoms of leishmaniasis (18). Given the lack of a vaccine and recent emergence of resistance to pentavalent antimonials, we investigated the anti-leishmanial effect of essential oils of *R. officinalis*, *M. pulegium*, *F. vulgare*, *L. Citriodora* and *P. graveolens*, which are native to Iran.

The main constituents of *F. vulgare* essential oil include E-anethole, fenchone and α-phellandrene (19). The main components of *P. graveolens* essential oil were citronellol, geraniol and cytronellyl formate. The major components of *L. citriodora* essential oil were lemonene and α-terpineol. Inconsistent with our findings, Hanna et al. identified 43 compounds in the essential oil *of L. citriodora* and reported that the most effective compounds are citral (14.21%), β-caryophyllene (10.71%), 1, 8-cineole (9.1%) and citronellol (8.87%). (20)The main components of *R. officinalis* essential oil were α-pinene, 1,8 cineole and verbenone. Two other studies identified 20-25 compounds in *R. officinalis* essential oil and reported oxygenated mono-terpenes (camphor) and hydrocarbon terpenes (pinene) as the main constituents. The major components of *M. pulegium* essential oil were δ-terpinene and α-terpinene. In a study conducted by Gulluce et al., pyrethrin epoxide and pulegone were found as the major components of *M. pulegium* essential oil, which is inconsistent with our findings. The difference in chemical composition and antimicrobial activity of essential oils can be attributed to the differences in planting season, weather, geographic area, method and duration of essential oil extraction and tested microbial species (21-24).

Several studies have evaluated the effectiveness of Iranian medicinal plants against various diseases and infections such

as leishmaniasis. Jedi et al. reported that the extract of *Achilles* spp. can have significant inhibitory effect on *L. major* in a time-dependent manner (26). In a study by Jorjani et al., the essential oil of *Eugenia caryophyllata* and *Cinnamomum zeylanicum* significantly decreased the number of promastigotes compared with a control group (25).

In the present study, all tested essential oils exerted anti-leishmanial effects in a dose-dependent manner. However, the essential oils of fennel and rosemary showed the highest inhibitory effects at concentrations of 500 and 1000 μg/ml. In another study, Albakhit and Doudi reported the significant inhibitory effects ofmethanolic and aqueous extracts of *Zizyphus spina-Christi* against *L. major* (MHOM/IR/75/ER) promastigotes (26). In another study, the essential oil of *P. angustifolium* was introduced as a promising alternative for treatment of visceral leishmaniasis (14).

Ogeto et al. reported that the aqueous and methanolic extracts of *Aloe secundiflora* have the highest inhibitory effect on *L. major* promastigotes in vitro (27).

Plant essential oils can easily cross the cytoplasmic membrane and various cell layers due to their lipophilic structure and cause cell lysis. They may also interrupt with biosynthesis of lipids and proteins, increase the production of nitric oxide in damaged cells or stimulate de-polymerization of mitochondrial membrane, thereby causing cell death (28). The effect of essential oils on mitochondria of promastigotes and amastigotes have been demonstrated (29). De Medeiros et al. also reported that treatment with some plant extracts significantly alters the morphology of parasites in a dose-dependent manner (30).

CONCLUSION

All tested essential oils, particularly the essential oils of fennel and rosemary, are capable of eliminating *L. major* promastigotes with equal or higher potency than glucantime, the first-line drug for treating leishmaniasis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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