OriginalArticle

The Antileishmanial Activity of Essential Oils from Some Traditionally Used Medicinal Plants in Iran

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Abstract

Background and Aim: Cutaneous leishmaniasis (CL) is the most common type of leishmaniasis affecting 1.5 million people through the world annually. Treatment of CL by pentavalent antimony compounds remains a challenge because of adverse side effects. The present study was designed to evaluate the *in vitro* antileishmanial properties of six essential oils from selected spices and herbs ethno-medicinally used in Iran against *Leishmania tropica* and *L. major* promastigotes.

Materials and Methods: The *in vitro* antileishmanial effects of selected medicinal plants against *L. tropica* and *L. major* promastigotes were evaluated by colorimetric cell viability (MTT) assay. The IC_{50} values were also calculated by probit test using SPSS software.

Results: The findings demonstrated that all the tested essential oils had inhibitory effects on promastigote growth of *L. tropica* with IC_{50} values ranging from 3.2µg/mL to 19.3µg/mL and 2.7µg/mL to 18.8µg/mL for *L. tropica* and *L. major*, respectively. *Zataria multiflora* Boiss essential oil significantly ($P<0.05$) was much more effective than essential oils of the other tested plants and control drugs once they demonstrated lower IC_{50} values promastigote form.

Conclusions: The findings of present study indicated antileishmanial effects of some Iranian medicinal plants particularly *Z. multiflora* Boiss. However, further studies, on the animal models as well as volunteer human, are needed to confirm these results.

Keywords: Promastigote, *Leishmania tropica*, *Leishmania major*, Medicinal plants, cutaneous leishmaniasis

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Introduction

Leishmaniasis is caused by parasitic protozoa of the genus of *Leishmania* transmitted by bites of phlebotomine sand flies. Cutaneous leishmaniasis (CL) is the main ordinary form of leishmaniasis affecting every twelve months 1.5 million people globaly (1). The disease is identified through chronic skin lesions, leaving permanent scars with bend of the infected region (2). In Iran, there are exist both epidemiological forms of this skin disease: anthroponotic CL (ACL) and zoonotic CL (ZCL)

caused by *Leishmania tropica* and *Leishmania major*, respectively (3). Right now, management of leishmaniasis by chemotherapy, pentavalent antimony compounds such as meglumine antimoniate and sodium stibogluconate, leftovers a challenge as a result of limited efficacy, toxic side effects and drug conflict (4). These factors deliver the progress of new useful antileishmanial drugs as a necessity. Latest studies have revealed that plant extracts, by reason of having smaller amount side effects, low cost and high accessibility, are important sources which are frequently used to care for a wide series of disease conditions including leishmaniasis (5). Spices and herbs are a element of the daily food in various regions of the world, encompass the most significant products used for flavoring foods and play a major role in concepts of sickness and medicinal. As well importance for general health, they are fairly often parts of habitual formulae (6, 7). Furthermore diets loaded in bioactive phytochemicals decrease the risk of degenerative disorders such as cancer, diabetes, cardiovascular disease and oxidative dysfunction (7-9). In Iran, the use of spices and other aromatic plants as food flavoring is an essential part of dietary manners for centuries. To the best of our knowledge, few studies have investigated the effects of Iranian medical spices and herbs against leishmaniasis. Therefore, the present study was designed to evaluate the *in vitro* antileishmanial properties of six essential oils of selected spices and herbs ethno-medicinally used in Iran against *L. tropica* promastigotes.

Materials and Methods

Parasite strain

Standard strains of *L.tropica* (MHOM/IR/2002/Mash2) and *L. major* (MRHO/IR/75/ER) were kindly obtained from the Leishmaniasis laboratory, department of medical parasitology, Tehran University of Medical Sciences (Tehran, Iran).The parasite was cultured in NNN medium, subcultured in RPMI-1640, supplemented with penicillin (100IU/mL), streptomycin (100µg/mL), and 15% heat-inactivated fetal calf serum (FCS).

Plant collection

The plant materials of three spices (*Zataria multiflora* Boiss, *Elettaria cardamomum* L., and *Heracleum persicum* Boiss) and three herbs (*Artemisia absinthium L.*, *Calendula officinalis L.*, and *Salvia officinalis L.*) were collected from rural regions of Kerman and Lorestan provinces, (Iran) from March to September 2013. The identities were confirmed by the botanist at the botany department of Shahid Bahonar University, Kerman, Iran.

Preparation of essential oils

Air dried plant materials (100g) were subjected to hydro-distillation for 4h using an all-glass Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and stored in darkness at 4°C in airtight glass vials closed under nitrogen gas until testing. The essential oils were dissolved in normal saline plus Tween 20. The selection of dilutions of the essential oils was based on initial experiments, which also showed that normal saline plus Tween 20 have no effect on the growth of promastigotes.

GC analysis

GC analysis was carried out by a Hewlett-Packard 6890 with a HP-5MS column (30m × 0.25 mm, film thickness 0.25mm). The column temperature was maintained at 55°C for 3 min and programmed to 180°C at a rate of 5°C per min, and kept constant at 220°C for 5 min. Injector and interface temperatures were 220°C and 290°C, respectively. The flow rate of Helium as carrier gas was 1mL/min C.F. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by coinjection 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 (30m × 0.25 mm, film thickness 0.25mm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 220°C and 290°C, respectively. Mass range was from 40to 400u. Oven temperature program was the same given above for the GC.

Identification of the essential oil components

The constituents of the essential oil were determined using assessment of their relative retention time and mass spectra with standards Wiley 2001 library data of the GC/MS system or with those of reported in the literature data (10).

Antileishmanial effects against promastigote form

In order to evaluate antileishmanial effect of essential oils from selected Iranian medicinal plants, colorimetric cell viability (MTT) assay was used according to the methods described by elsewhere (11). At first, 100µL of the promastigotes (10^6cells/mL) both species harvested from logarithmic growth phase was added to a 96-well microtiter plate. Then, 100µL of different concentrations (0-50 µg/mL) of each plant essential oil was added to each well and incubated at 25°C±1°C for 72 hours. After incubation, 10µL of MTT solution (5mg/mL) was added to each well and incubated at 25°C for 4 hours. The promastigotes were cultured in the complete medium with no drug used as positive control and with no promastigotes and drugs as blank. Finally, absorbance was measured by an ELISA reader (BioTek-ELX800) at 490nm. 50% inhibitory concentrations (IC_{50} values) were also calculated by probit test in SPSS.

Statistical analyses

All the experiments were repeated in triplicate. We used SPSS software, ver. 17, (SPSS Inc., Chicago). Data entry and statistical analysis and differences between the groups were determined using one-way analysis of variance (ANOVA) test. Moreover, to compare IC_{50} values of the groups, *t*-test was performed. Pvalue of less than 0.05 was considered statistically significant.

Results

Antileishmanial effects

Antileishmanial effects of essential oils from six selected Iranian medicinal plants against promastigote forms of *L. tropica* and *L. major* were determined by MTT assay. The findings demonstrated that all the tested essential oils were effective in inhibiting promastigote growth of *L. tropica* and *L. major* based on a dose-dependent manner. *Z. multiflora* Boiss essential oil significantly ($P<0.05$) was much more effective than essential oils of the other tested plants and control drugs once they demonstrated lower IC_{50} values for promastigote forms of both species. The measured IC_{50} values forsix selected medicinal plants and MA as control drug against promastigote forms of *L. tropica* are shown in Table 1.

GC/MS analysis of essential oil

In the present study, yellow-colored essential oil (yield 3.1% v/w) of *Z. multiflora* Boiss (the most active essential oil) was analyzed using GC/MS. As shown in

Table 1: Comparison of the mean IC_{50} values of the six essential oils from selected Iranian medicinal plants and meglumine antimoniate (MA) as control drug against *L. tropica* and *L. major* promastigotes.

No.	Scientific Name	Family	Voucher No.	Common Name (Persian)	Part used	IC_{50}(µg/mL)	
						L. tropica	***L. major***
1	*Artemisia absinthium* L.	Asteraceae	KF 1186	Efesentine	**Flower**	14.6	13.9
2	*Calendulaofficinalis* L.	Asteraceae	KF 1367	Hamishe Bahar	**Flower**	8.9	6.6
3	*Elettariacardamomum* L.	Zingiberaceae	KF 1246	Hel	Seed	19.3	18.8
4	*Heracleum persicum* Boiss	Apiaceae	KF 1143	Golpar	Leaf	15.6	16.2
5	*Salvia officinalis* L.	Lamiaceae	KF 1432	Maryam Goli	**Flower**	11.4	11.6
6	*Zataria multiflora* Boiss	Lamiaceae	KF1375	Avishan e Shirazi	Leaf	3.2	2.7
7.	MA [a]	-		-	-	12.4	11.9

[a]Meglumine antimoniate

Table 2, twenty-five compounds were identified, representing 99.78% of the total oil. The main components were thymol (41.81%), carvacrol (28.85%), and *p*-cymene (8.36%).

Discussion

In the past decades the advent of synthetic antimicrobials drugs caused reluctance in plants as a rich resource for antimicrobial agents (12). However, in the recent years' emergence of some limitations in the use of these drugs caused changes in situation and interest in field of ethnobotanical researches (13). Thus, the present study was aimed to investigate the *in vitro* antileishmanial activities of eight essential oils from spices and herbs ethno-medicinally used in Iran against promastigotesof *L. tropica* and *L. major*. Each of the essential oil tested in the present study demonstrated remarkable leishmanicidal activity on promastigotes of both species. However, differences were observed between antileishmanial activities as most of the tested plant essential oils. These variations in antileishmanial activity could be due to the differences in the chemical composition of these plants as the secondary metabolites of plants have many effects including antimicrobial properties (12). Our findings revealed that essential oil of *Z. multiflora* Boiss significantly inhibited the growth rate of promastigotes, while other essential oils demonstrated weak to moderate antileishmanial activities. The results of present study are in line with previous studies reporting that the commonly used herbs and spices have antimicrobial properties that, in some cases, can be used therapeutically (14). It has been formerly proven that *Z. multiflora* Boiss, due to having higher content phenolic compounds especially thymol and carvacrol, acts on the cell membrane microorganisms and causes damage and depletion of the contents of the cells (15). Regarding the leishmanicidal effects of thymol and carvacrol, de Melo *et al*. (2013) indicated that thymol exhibited IC_{50} of 9.8µg/mL and carvacrol 2.3µg/mL for *L. chagasi*

Table 2: Essential oil composition of *Z. multiflora* identified by GC/MS.

No	Components	KI[a]	% Composition
1.	3-Octanone	956	0.56
2.	α-Pinene	973	0.36
3.	β-Myrcene	987	0.36
4.	3-Octanol	993	0.49
5.	d-Carene-3	1017	0.41
6.	1,8-Cineole	1026	0.28
7.	Limonene	1031	0.34
8.	*ρ*-Cymene	1035	8.36
9.	γ-Terpinene	1060	3.98
10.	trans-Sabinene hydrate	1072	0.23
11.	Linalool	1097	1.75
12.	2-Nonanol	1101	0.15
13.	Borneole	1179	0.28
14.	4-Terpineol	1184	1.14
15.	α-Terpinolene	1196	1.28
16.	Decenal-z-4	1199	0.12
17.	Thymol methyl ether	1234	1.3
18.	Thymol	1288	41.81
19.	Carvacrol	1297	28.85
20.	Thymol acetate	1354	0.46
21.	b-Caryophyllene	1428	2.06
22.	Aromadendrene	1448	0.86
23.	a-Selinene	1498	0.54
24.	Apofarensol	1581	096
25.	Caryophyllene oxide	1587	0.85
	Total		99.25

[a] Kovats index on non-polar DB-5 ms column in reference to n-alkanes

promastigotes. The IC_{50} for carvacrol was similar to the IC_{50} for amphotericin B (0.51 µg/mL), one of the commercial drugs used for leishmaniasis treatment (16). In addition, in the study conducted by Monzote *et al*. (2014), carvacrol and caryophyllene oxide showed a significant leishmanicidal activity with IC_{50} of 15.3µg/mL and 4.9µg/mL for promastigotes and 13.6µg/mL and 4.4µg/mL for amastigote forms of *L. amazonensis* (17). In the present study, the main components of *Z. multiflora* essential oil were found to be thymol (41.81%), carvacrol (28.85%), and *p*-cymene (8.36%). However, it has previously proven that composition of *Z. multiflora* essential oil was depend on species, climate, and time of collection along with growth stage, thereby altering the biological activities studied (18).

Conclusion

The findings of the present study demonstrated the antileishmanial effects of some Iranian medical plants particularly *Z. multiflora* Boiss. However, further studies are needed to confirm these results by checking in the animal models as well as volunteer human.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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