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THE EFFECT OF *STACHYS LAVANDULIFOLIA VAHL*. AND *MESPILUS GERMANICA L.* LEAVES HYDROALCOHOLIC EXTRACTS ON *LEISHMANIA MAJOR* (MRHO/IR/75/ER) I*N VITRO*

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Abstract

Cutaneous leishmaniasis is endemic in 88 different countries. There are an estimated 1.5 million new cases each year, with over 90% occurring in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, and Syria and in Brazil and Peru. This study is the first one for evaluating the effect of hydroalcoholic extracts of *Stachys lavandulifolia Vahl.* and *Mespilus germanica L.* leaves against *L. major* (MRHO/IR/75/ER) promastigotes. The results of this study showed that, extracts of *S. Lavandulifolia* and *M. germanica* leaves are effective on activity against the proliferation of promastigotes of *L. major* suggest that these extracts might be a promising approach for developing new anti-leishmanial drugs.

Keywords:

Leishmania major, Stachys lavandulifolia Vahl, Mespilus germanica, in vitro.

Introduction

Leishmaniasis caused by the obligate intracellular protozoan parasite of the genus *Leishmania* is still considered a major health problem in the rural areas of the Middle East, Africa, Asia, Europe and Central and South America. The disease is widely distributed, affecting approximately 12 million people worldwide, causing a wide spectrum of clinical menifestantions. Control measures of leishmaniasis must focus on rapid detection, vaccination and effective treatment of the disease. Numerous attempts have been made on developing a successful vaccine against leishmaniasis(1). Zoonotic cutaneous leishmaniasis (CL) caused by *Leishmania major* is common in many rural areas of Iran (2). Antimonial compounds particularly meglumine antimoniate (Glucantime) are the first line drugs for the treatment of all forms of leishmaniasis in Iran (3). Recent circumstantial evidences are suggesting that an increasing number of Iranian patients with cutaneous leishmaniasis are unresponsive to meglumine antimoniate (4). Systemic treatment with antimonial compounds is expensive and relatively ineffective besides unacceptable side effects. An effective topical preparation would be of great value as it would permit an unsupervised and safely treatment with a reasonable cost (5, 6).

Stachys lavandulifolia Vahl has been largely used for its action on insomnia and anxiety. There are about 300 species of genus *Stachys* wide spread throughout the world (7).

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In Iran, 34 species of this genus are present among which, 13 are endemic (8). *Stachys* species are traditionally used in different conditions: headache, neuralgia, nervous conditions, as tonic at dyspepsia and for treating wounds and skin inflammation (9) as astringent and antidiarrheal (10). In pharmacological studies *Stachys* species showed variety of effects: anti-inflammatory (11, 12) antibacterial (13, 14) antinephritic (15-17) and anxiolitic(18).

The Medlar ('*Mespilus germanica'*, *Turkish:* töngel) is a large shrub or small tree, and the name of the fruit of this tree. Despite its Latin name, which means German or Germanic Medlar, it is indigenous to southwest Asia and also southeastern Europe, mostly the Black Sea coasts of modern Turkey, and was introduced to Germany by the Romans. Common Medlar leaves are dark green and elliptic, 8–15 cm long and 3–4 cm wide. The leaves turn a spectacular red in autumn before falling. In Iranian folklore medicine, leaf sodden of *Mespilus germanica* is claimed to be used as treatment of children diarrhea, pharyngitis and Cutaneous leishmaniasis(8).

In this study, we aimed to clarify assessing the effectiveness of *Stachys lavandulifolia* and *Mespilus germanica* extracts against *L. major* (Iranian strain) *in vitro* condition.

Materials and methods

Plant Materials

Aerial parts of *S. lavandulifolia Vahl.(Lamiaceae)* were collected from Chaharmahal and Bakhtiyari Province and leaves of *M. germanica L.(Rosaceae)* collected from Mazandaran Provinces of Iran in spring. The plants were identified at the Botany Department of the Faculty of Sciences, Shahrekord University of Medical Science. A voucher specimen of the plant has been deposited in herbarium of the Faculty of Pharmacy Shahrekord University of Medical Sciences, Chaharmahal and Bakhtiyari, Iran. Voucher specimen number for *Stachy lavandolifolia* is 204 and for *Mespilus germanica* is 203.3.

Hydroalcoholic Extract

Air-dried and powdered aerial parts of the *S. Lavandulifolia* and leaves of *M. germanica* were macerated at room temperature with 1L of ethanol: water (75:25) for 24 hours. The extractions continued two times and then were concentrated in a rotary evaporator under reduced pressure to give 0.33 of primary substance.

L. major promastigotes(MRHO/IR/75/ ER) were obtained from Shiraz University of Medical Sciences of Iran using NNN medium. Promastigotes transferred to RPMI 1640 medium supplemented with 15% Fetal calf serum for mass cultivation. Test flasks were seeded with medium containing 1×10^6 promastigotes per ml. Extracts diluted with DMSO transferred to the culture flasks to obtain final concentrations of 50, 100, 250, 500 and 1000 µg/ml. The highest concentration of DMSO and a normal culture flask were used as controls. Antileishmanicidal activity was studied after 24, 48 and 72 hours incubation at 25 °C. Promastigotes were counted after adding 10% formaldehyde. Experiments were performed three times.

The inhibitory effect on parasite population caused by each of the compounds was calculated with respect to the 'control' set. Data in Table 1 were evaluated statistically with analysis of variance (ANOVA) followed by Tukey's test.

Results

The *in vitro* inhibitory effects of *S. lavandulifolia* and *M. germanica* against *Leishmania major* promastigotes are presented in Table 1. The effects of these extracts were found to be concentration

Table 1: Parasitological results of different concentration of hydroalcoholic extracts of *S. Lavandulifolia* and *M. germanica* leaves on the proliferation of promastigotes of *L.major*

Plant Extract	Number of promastigotes population ($\times 10^5$) in different concentrations of plant extract					
	Control	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000µg/ml
S. Lavandulifolia	54.0±3.5	43.5±4.5	42.0±2.0	40.0±4.0	39.0±2.0	23.5±3.5
M. germanica	54.0±3.5	46.5±4.5	44.0±6.0	41.0±5.0	37.0±4.0	17.0±2.0

dependent. Analysis of variance test showed that significant difference was existed between the different concentrations ($p<0.05$).

The mean difference was significant between 1000 and 50 (µg/ml) groups ($p<0.05$). There was no significant difference between other groups.

The mean difference in 50 (µg/ml) group ($p<0.05$) and 100(µg/ml) group ($p<0.05$) had a significant comparison with 1000 (µg/ml) group. There was no significant difference between other groups.

Discussion

The perspectives for the cure of leishmaniasis are still uncertain. There is a strong need to easy synthesized and low cost therapeutic agents especially in undeveloped and developing countries. Many natural products have already provided valuable clues for potentially antiparasitic compounds (19). However, no treatment has proved to be completely satisfactory. Pharmaceutical research in natural products represents a major strategy for discovering and developing new drugs. The use of medicinal plants for the treatment of parasitic diseases is well known and has been documented since ancient times by the use of *Cinchona succiruba* (Rubiaceae) as an antimalarial (20). Our study is providing useful data in order to find new active products against *L. major* parasite. Hydroalcoholic extracts of *S. lavandulifolia* and *M. germanica* leaves have the strong antileishmanial activities.

Stachys lavandulifolia and *Mespilus germanica* metabolites includes: flavonoids, polyphenols, tannins and others. Many studies have proven antioxidant and free radical scavenging activity of various polyphenols. Polyphenols also possess many biological effects and these are generally attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (21-23).

Several reports have indicated polyphenols as the leishmanicidal constituents of some plants (24). With respect to preliminary phytochemical screening which indicates the presence of flavonoids and tannins, the leishmanicidal activity could be attributed to one these classes of compounds.

Several reports. (24)) have indicated that ethanolic extracts of some plants have favorable antileishmanial activity and kill the *L. major* promastigotes in a dose-dependent manner.

In summary, our results indicate that extracts of *S. lavandulifolia* and *M. germanica* leaves showed *in vitro* antileishmanial activity against *L. major*. Therefore, the present study provided biological evidence that these extracts are a promising source of natural compounds for the development of new chemotherapeutic agents to treat leishmaniasis. Moreover, further biological and phytochemical investigations aiming to identify the active compounds of these extracts against Leishmania species should be undertaken

to fully investigate its potential for the treatment of this serious illness.

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