Evaluation of leishmanicidal effect of *Perovskia abrotanoides* Karel. root extract by in vitro leishmanicidal assay using promastigotes of *Leishmania major*

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**Running title:** Antileishmanicidal effect of *Perovskia abrotanoides*

**ABSTRACT**

*P. abrotanoides* dried root has been successfully used for the treatment of Cutaneous leishmaniasis in Iranian traditional medicine as a poultice. To provide a scientific reason for ethnomedicinal use of *P. abrotanoides*, in this study the leishmanicidal effect of *Perovskia abrotanoides* extracts was evaluated on promastigotes of *L. major* in vitro.

In this study, the antileishmanicidal effect of different extract of *Perovskia abrotanoides* root was evaluated on the promastigotes of *Leishmania major* in vitro. The dried and ground root of the plant was extracted using either maceration in 80% ethanol or Soxhlet in methanol. Then, 5 different concentrations (0.06, 0.12, 0.25, 0.5 and 1 mg/ml) of each extract, one positive control (Amphotericin B, 0.5 mg/ml), one negative control (culture medium), and one solvent control (DMSO) were prepared and were placed in a 24-well plates containing 50,000 parasites/well. The plates were incubated at 25°C for six days and the number of parasites in each well was determined on days 2, 4, and 6 of experiment microscopically using Neubauer chamber. It was observed that amphotericin B and both macerated and Soxhlet extracts at concentration of 1 mg/ml killed all the parasites. Lower doses exhibited a dose-dependent antileishmanial activity. The average of IC50 for macerated extract in DMSO was 4.03 × 10⁻² mg/ml and for Soxhlet extract in DMSO was 7.33 × 10⁻² mg/ml. The control solvents had no significant effect on the *L. major* promastigotes. These results indicated that both macerated and Soxhlet extracts of *Perovskia abrotanoides* have favorable leishmanicidal activity.

**Key words:** *Perovskia abrotanoides*; Antileishmanicidal activity; Cutaneous leishmaniasis; *Leishmania major*

**INTRODUCTION**

Leishmaniasis is a protozoal disease of man that occurs in most parts of the world. This disease affect approximately 12 million people world wide with 1.5-2 million new cases occurring each year [1]. Cutaneous leishmaniasis (CL), which caused by the different species of *Leishmania*, produces a skin ulcer that heals spontaneously in most cases, leaving an unsightly scar [2]. Control strategies are not always effective and available drugs for the treatment of leishmaniasis are either toxic, have limited efficacy or both and emerging drug resistance is a significant concern. The effects of available topical antileishmanial products are
also minimal [3]. Therefore, there is a great and urgent need for the development of new, effective and safe drugs for the treatment of leishmaniasis [4]. One strategy to discover new drug leads is to investigate natural products from medicinally used plants. Most people in areas where leishmaniasis is endemic depend largely on traditional medicine. *Perovskia* genus, Lamiaceae family, has seven species such as *P. abrotanoides*, *P. atriplicifolia* and *P. hybrida*. *Perovskia abrotanoides* with vernacular name of "Berazamble", "Domou" and "Gevereh" is a perennial herb growing wild in Iran, Afghanistan, Pakistan and Turkmenistan [5]. There are few scientific reports about *P. abrotanoides*. It has some pharmacological effects such as leishmanicidal (some constituents of plant), antiplasmodial and cytotoxic activity [6], as well as antinociceptive and anti-inflammatory effects [7-8].

*P. abrotanoides* dried root has been successfully used for the treatment of CL in Iranian traditional medicine as a poultice [6]. To provide a scientific reason for ethnomedicinal use of *P. abrotanoides*, in this study the leishmanicidal effect of ethanolic macerated and methanolic Soxhlet extracts of *P. abrotanoides* was evaluated on promastigotes of *L. major* in vitro.

**MATERIALS AND METHODS**

**Plant material**

*Perovskia abrotanoides* was collected from near Chenaran (Mashhad Province, Iran). The root of the plant was dried and powdered. It was identified in the Herbarium of Ferdowsi University and voucher samples were preserved for reference at the Herbarium of the Mashhad School of Pharmacy with reference number of (152-120-1002).

**Preparation of extract**

Soxhlet methanolic extract: The plant powder (50 g) was extracted with methanol (200 ml) for 12 hours using Soxhlet apparatus. The methanol was removed under reduced pressure and dried. The extract was kept in refrigerator until use.

**RESULTS**

Antileishmanial activity of macerated ethanolic extract of *Perovskia abrotanoides* in DMSO
Amphotericin B (0.5 mg/ml) and macerated ethanolic extract of *Perovskia abrotanoides* (1 mg/ml) in DMSO killed all of the *L. major* promastigotes (Figure 1, 2, 3) and lower doses of macerated ethanolic extract of *Perovskia abrotanoides* in DMSO killed *L. major* promastigotes dose-dependently while DMSO did not have any effect on the *L. major* promastigotes. The IC₅₀ for macerated ethanolic extract of *Perovskia abrotanoides* in DMSO was 0.213, 0.652 and 0.343 mg/ml after 2, 4 and 6 days of incubation, respectively.

**Figure 1.** Effect of different concentrations of *P. abrotanoides* macerated extracts against *L. major* promastigotes after 2 days of incubation. Each bar represents the mean + S.E.M. of the number of promastigotes in 4 wells. **p<0.01, ***p<0.001, Tukey-Kramer test.

**Figure 2.** Effect of different concentrations of *P. abrotanoides* macerated extracts against *L. major* promastigotes after 4 days of incubation. Each bar represents the mean + S.E.M. of the number of promastigotes in 4 wells. **p<0.01, ***p<0.001, Tukey-Kramer test.

**Figure 3.** Effect of different concentrations of *P. abrotanoides* macerated extracts against *L. major* promastigotes after 6 days of incubation. Each bar represents the mean + S.E.M. of the number of promastigotes in 4 wells. **p<0.01, ***p<0.001, Tukey-Kramer test.

**Antileishmanial activity of Soxhlet methanolic extract of *Perovskia abrotanoides* in DMSO**

Different concentrations of Soxhlet methanolic extract of *Perovskia abrotanoides* in DMSO killed parasites dose-dependently (Figure 4, 5, 6). The IC₅₀ for Soxhlet methanolic extract of *Perovskia abrotanoides* in DMSO was 0.926, 0.723 and 0.550 mg/ml after 2, 4 and 6 days of incubation, respectively.

**Figure 4.** Effect of different concentrations of *P. abrotanoides* soxhlet extracts against *L. major* promastigotes after 2 days of incubation. Each bar represents the mean + S.E.M. of the number of promastigotes in 4 wells. **p<0.01, ***p<0.001, Tukey-Kramer test.
promastigotes in 4 wells. **p<0.01, ***p<0.001, Tukey-Kramer test.

![Figure 5](image)

**DISCUSSION**

People customarily use the plant(s)/plant-derived preparations and consider them to be efficacious against cutaneous leishmaniasis without any scientific base to explain the action of such plants. Since cutaneous leishmaniasis has become one of the major health issues in Iran and chemotherapy is somewhat ineffective and painful, people are using medicinal plants sold on the local market as a remedy to cure their wounds. *P. abrotanoides* root has been used successfully in the treatment of cutaneous leishmaniasis in different parts of Iran. Traditionally, the dried root of this plant is crushed and grinded. Then it is mixed with water, sesame oil and wax in a proper formula. The final product is like a paste. Then this remedy is applied on the lesions of the cutaneous leishmaniasis. The current study was therefore carried out on *L. major* promastigotes to evaluate its acclaimed efficacy using in vitro assay based toxicity. *L. major* and *L. tropica* are the major causes of cutaneous leishmaniasis in Iran [10-11]. Both macerated and Soxhlet extracts of the root of *P. abrotanoides* were prepared and tested against promastigotes of *Leishmania major*. All tested concentrations of both extracts exhibited antileishmanicidal activity after 2, 4, and 6 days of incubation. The average of IC50 for macerated extract in DMSO was $4.03 \times 10^{-2}$ mg/ml and for Soxhlet extract in DMSO was $7.33 \times 10^{-2}$ mg/ml. This difference could be due to the effect of heat on the constituent of extract during the Soxhlet process.

Recently the root of *P. abrotanoides* has been extracted by ethyl acetate and four active compounds cryptotanshione, 1β-hydroxycryptotanshione, 1-oxocryptotanshione and 1-oxomiltirone has been isolated [6]. These compounds are all diterpenes and constitute 0.8, 0.67, 0.01 and 0.0018% of the extract. The IC50 of these compounds against *L. major* promastigotes have been $5.45 \times 10^{-3}$,
14.6 × 10^{-3}, 7.9 × 10^{-3} and 5.28 × 10^{-3} mg/ml, respectively. These IC_{50} approximately are 10 times less than the IC_{50} of total extract of \textit{P. abrotanoides} that we acquired in our studies. Therefore, the leishmanicidal effect of total extract not only depends to these compounds, but also in the extract could be other active components that might have leishmanicidal effect in very low concentration. Further fractionation of the \textit{P. abrotanoides} is required to characterize other antileishmanial constituents.

The phytochemical screening of aerial parts of \textit{P. abrotanoides} has shown the presence of high content of monoterpenes and sesquiterpenes like 1, 8-Cineolo, myrcene, pinene, camphor, caryophyllene, humulene, camphene and bisabolol [11-12]. Beside the diterpenoids that present in the root of \textit{P. abrotanoides} [6], these monoterpenes and sesquiterpenes might be also in the root of \textit{P. abrotanoides} and involved in the leishmanicidal activity of the extracts of \textit{Perovskia abrotanoides} root. Antileishmanial activity of terpenoides has also been reported by others [13].

These results indicate that the ethanolic macerated and methanolic Soxhlet extracts of \textit{Perovskia abrotanoides} root have favorable leishmanicidal activity and kill the \textit{L. major} promastigotes in a dose-dependent manner. Furthermore, \textit{Perovskia abrotanoides} exratx posses antinociceptive and anti-inflammatory effects [7-8]. Therefore, \textit{Perovskia abrotanoides} root exratx could be suitable topical treatment candidate for the treatment of cutaneous leishmaniasis.

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REFERENCES