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Evaluation of leishmanicidal effect of *Euphorbia erythadenia* extract by *in vitro* leshmanicidal assay using promastigotes of *Leishmania major*

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ABSTRACT

Objective: To evaluate leishmanicidal effects of *Euphorbia erythadenia* plant extract.
Methods: Extraction was done using methanolic Soxhlet of dried and ground aerial parts of the plant. Then, five different extract concentrations, in addition of positive, negative and solvent controls were prepared and added to a 24-well plate containing 40 000 parasites/well. The extract concentrations were 1, 0.5, 0.25, 0.125 and 0.062 5 mg/mL. Amphotricin B (0.5 mg/mL) was used as positive control while negative control contained only culture medium. After 3 d incubation at 25 °C the amount of parasites in each well was determined on each day of experiment microscopially using Neubar chamber.
Results: Soxhlet extract as well as amphotricin B killed all parasites at concentration of 1 mg/mL. The leshmanicidal activity of lower doses of extract was dose-dependent. The EC_{50} for Soxhlet extracts in dimethylsulfoxide was 0.30 mg/mL. The EC_{50} for Soxhlet extracts in methanol was 0.23 mg/mL. No obvious effects from the control solvent on the *Leishmania major* promastigotes were observed.
Conclusions: The Soxhlet extract of *Euphorbia erythadenia* showed suitable leishmanicidal activity, especially in higher concentration fractions.

1. Introduction

Leishmaniasis is a protozoa disease which has been observed in many parts of the world. World Health Organizeation (WHO) reported near 12 million infected patients, while there is a risk of infection by leishmania parasites for 350 million other people. The annual incidence of new cases is about 2 million out of which 1.2 million have cutaneous leshmaniasis (CL)[1].

CL, is one of the three clearly distinguishable clinical manifestations of leishmaniasis which leads to a chronic, non-contagious infection with skin ulcer that heals spontaneously in most cases, leaving an unsightly scar[2].

Due to intracellular location of the parasite it is difficult to treat leishmaniasis. Current leishmaniasis treatment protocols are based on antimony compounds which have unwanted side effects and sometimes are inefficient. The effects of available topical leishmanicidal products are minimal. Therefore, it is necessary to find and develop new therapeutic components with high efficacy and safety in order to replace or supplement those currently used drugs[3,4].

Medicinal plants are potential sources of natural products which could be considered as alternatives to conventional drugs[5]. Traditional medicine is a popular therapeautic method in areas where leishmaniasis is endemic[4,6]. Euphorbiaceae family is one of the largest families of the phylum Anthophyta. In this family, the genus *Euphorbia* is the largest, which comprises over 2 000 species, grows in the forms of laticiferous, shrubs, herbs and small trees, living in different parts of the world such as the temperate and tropical zones of Asia and other[7].

Euphorbia species have been successfully used for the treatment of CL by local people in Mashhad suburb, Iran, where growing number of cases are reported recently[8,9].

The goal of this study was to find the scientific evidence for the leishmanicidal effect of methanolic Soxhlet extract

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of *Euphorbia erythadenia* (*E. erythadenia*) on promastigotes of *Leishmania major* (*L. major*) *in vitro*.

2. Materials and methods

2.1. Plant material

E. erythadenia was collected from near Kerman (Kerman Province, Iran). The aerial part of the plant was dried in shade and powdered.

The plant material was identified in the Herbarium of Ferdowsi University of Mashhad (Mashhad, Iran) and voucher sample was preserved for reference at the Herbarium of Mashhad School of Pharmacy (Mashhad, Iran) with reference number 301.

2.2. Preparation of extract

The plant powder (50 g) was extracted with 200 mL methanol for 24 h using Soxhlet apparatus. Then the extract was dried and solvent was removed by rotary evaporator and kept in refrigerator until use.

2.3. Leishmania parasites

Promastigotes of *L. major* strain MRHO/IR/75/ER, were obtained by isolation of the amastigotes from the lesions of infected BALB/c mice followed by transformation into promastigotes on Novy-MacNeal-Nicolle medium. Then the promastigotes were subcultured in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 100 IU/mL of penicillin and 100 mg/mL of stereptomycin sulfate (RPMI-FCS) at 25 °C. To perform leishmanicidal assays, stationary-phase promastigotes were used.

2.4. Assay of leishmanicidal activity

The assay was performed according to the previously reported method[10]. Breifly, *L. major* promastigotes were seeded at 40 000 parasites/well in 24-well plate in 400 μL RPMI-FCS. The extract were dissolved in dimethylsulfoxide (DMSO) and added further 400 μL/well to give final concentrations of 1 mg/mL. Then serial two-fold dilutions were prepared. After treatment, promastigotes were incubated over a period of 3 d at 25 °C and the amount of the parasites in each well was determined on days 1, 2 and 3 of experiment using Neubar chamber under a microscope. Positive and negative controls including amphotricin B (0.5 mg/mL) and culture media were used, respectively. DMSO and methanol alone were also used as solvent control.

2.5. Statistical analysis

Statistical analysis was carried out using One-way ANOVA and multiple comparison Tukey-Kramer test was used to compare the mean of different treatment groups. The EC_{50} was determined by Litchfoeld and Wilcoxon method.

3. Results

To give an exploratory motivation to ethnomedicinal utilization of *E. erythadenia*, in this study the leishmanicidal effect of methanolic Soxhlet extract of *E. erythadenia* was assessed on promastigotes of *L. major in vitro*.

Soxhlet extract of the aerial part of *E. erythadenia* were prepared and tried against promastigotes of *L. major*.

Both DMSO and methanol diluted extracts of *E. erythadenia* showed leishmaniacidal activity. Parasites were killed by

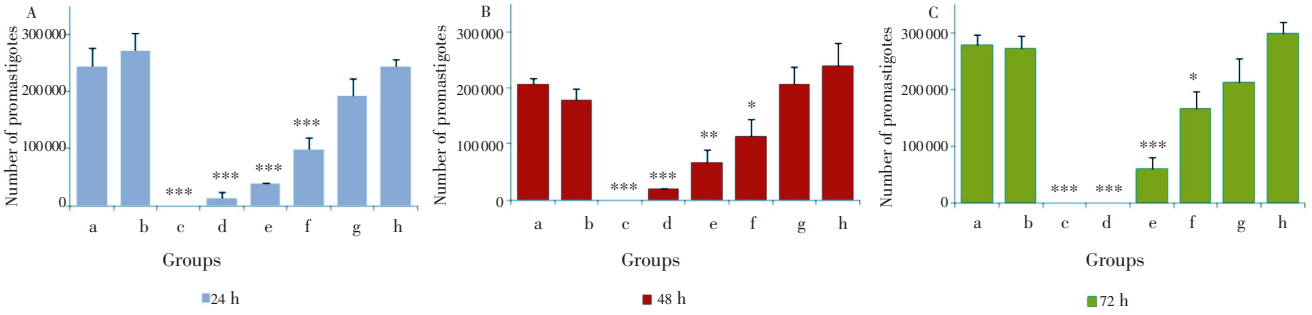

Figure 1. Effect of different concentrations of *E. erythadenia* Soxhlet extract in DMSO against *L. major* promastigotes.

A: 24 h, B: 48 h and C: 72 h after incubation. a: Control; b: DMSO; c: Amphotricin B; d: Extract (1 mg/mL); e: Extract (0.5 mg/mL); f: Extract (0.25 mg/mL); g: Extract (0.125 mg/mL); h: Extract (0.0625 mg/mL). Each bar represents the mean±SEM of the number of promastigotes in 12 wells. ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$ Tukey-Kramer test.

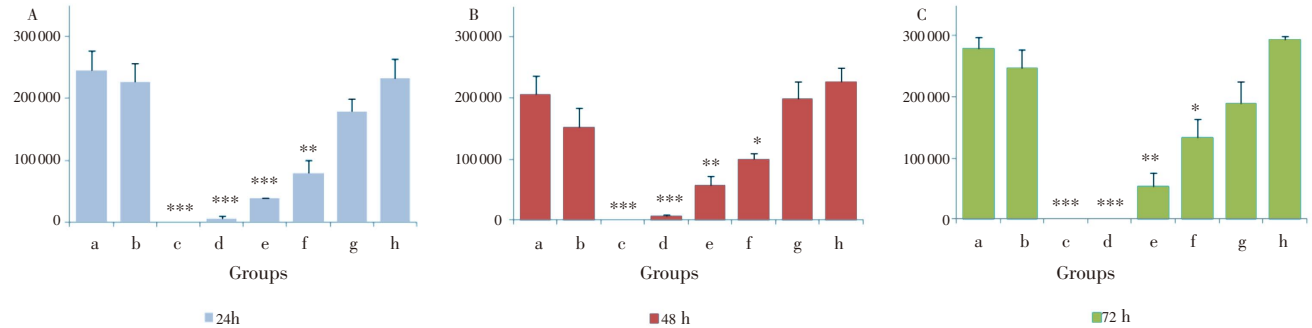

Figure 2. Effect of different concentration of *E. erythadenia* Soxhlet extract in methanol against *L. major* promastigotes.

A: 24 h, B: 48 h and C: 72 h after incubation. a: Control; b: Methanol; c: Amphotricin B; d: Extract (1 mg/mL); e: Extract (0.5 mg/mL); f: Extract (0.25 mg/mL); g: Extract (0.125 mg/mL); h: Extract (0.0625 mg/mL). Each bar represents the mean±SEM of the number of promastigotes in 12 wells. ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$ Tukey-Kramer test.

different concentrations of Soxhlet methanolic extract of *E. erythadenia* in DMSO, in a dose-dependent manner (Figure 1). The EC_{50} for soxhlet methanolic extract of *E. erythadenia* in DMSO was 0.3 mg/mL after 1 d of incubation. DMSO did not have any effect on the *L. major* promastigotes.

Also, different concentrations of Soxhlet methanolic extract of *E. erythadenia* in methanol killed parasites dose-dependently (Figure 2). The EC_{50} for Soxhlet methanolic extract of *E. erythadenia* in methanol was 0.23 mg/mL after 1 d of incubation. Methanol did not showed signifigant effect on the *L. major* promastigotes.

All tested concentration of extract exhibited leishmanicidal activity after 1, 2 and 3 d of incubation. Although the number of live promastigotes after 3 d of incubation was lower than those of 1 and 2 d of incubation, no significant differences between these groups was observed.

4. Discussion

Individuals in developing countries generally utilize the plant(s)-derived preparations and consider them to be effective against CL without any experimental base to demonstrate the activity of such plants. In the absence of an effective vaccine and difficulties of chemicals, (for example, invasive administration), people are using medicinal plants sold on the nearby market as a solution for cure their wounds. Topical use of the *E. erythadenia* latex is one of those cures for the treatment of CL.

The present study was done on promastigotes to assess its claimed efficacy using an *in vitro* assay based toxicity.

The leishmanicidal activity of the *E. erythadenia* could be attributed to different constituent of extract. Jaafari *et al*. in their study on *Euphorbia myrsinites*, reported that the methanolic extract at a concentration of 1 mg/mL had suitable leishmanicidal activity which killed the *L. major* promastigotes in a dose-dependent manner, with an EC_{50} value between 0.25 mg/mL[7,8]. A study on *Euphorbia lagascae* extract compounds, reported moderate anti-leishmanial activity of stilbenes against promastigotes[11]. Also taxane type diterpenoids, which are structurally similar to myrisinane type diterpenoids, present in the *Euphorbia* genera had shown anti-leishmanial activity[12]. In another study, triterpene derived from two *Euphorbia* species were evaluated for their antileishmanial and antitrypanosomal activity on *Leishmania infantum* and *Trypanosoma cruzi* and showed 76% and 64% antiparasitic for the test compounds, respectively[13]. The chloroform fraction of *Euphorbia aellenii* (1 mg/mL) in DMSO, also showed a growth inhibitory effect on *L. major* promastigotes, with an IC_{50} value of (140±24) μg/mL compared with amphotericin B [(0.29 ±0.05) μg/mL] and pentamidine [(5.09±0.04) μg/mL][6].

In a study on the herbal formulation composed of *Euphorbia mysinites*, *Alkanna tinctoria* and *Peganum harmala*, it has been reported that herbal combination in comparison with glucantime is more effective on experimental leishmaniasis induced in BALB/c mice; hence it could be considered as an alternative of glucantime in the treatment of leishmaniasis[14].

The results of our study showed that Soxhlet extract of *E.erythadenia* have suitable leishmanicidal activity, especially in higher concentration fractions. Further fractionation of the *E. erythadenia* is required to pinpoint leishmanicidal constituent(s).

Conflict of interest statement

We declare that we have no conflict of interest.

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