An *in vitro* study of antileishmanial effect of *Portulaca oleracea* extract

Elham Gharirvand Eskandari, Monir Doudi & Saeid Abedi

*Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

**ABSTRACT**

*Background & objectives:* Leishmaniasis is caused by protozoa of *Leishmania* genus and is considered as a zoonotic disease. It is a major public health problem worldwide, with high endemicity in developing countries like Iran. Various chemical drugs are used for leishmaniasis treatment, but their side-effects and the emergence of drug resistance have led to look for new effective compounds. The aim of this study was to introduce purslane (*Portulaca oleracea*) as a traditional and medicinal herb which might act as a valuable source for designing new pharmaceutical drug/lead against *Leishmania* sp.

*Methods:* This study was conducted in the laboratory of Seddigheh Tahereh Infectious Disease Research Center, Isfahan, Iran during the spring of 2015. The essence from the purslane plant was prepared through water distillation and the alcoholic extract was prepared through maceration method. The essence was dried, and diluted with DMSO (5%). *Leishmania major* promastigotes were cultured in 25 ± 2°C temperature in the stationary phase of RPMI-1640 medium, enriched with 10% fetal calf serum and penicillin-streptomycin to yield higher quantity. The biological activity of herb essence was evaluated on *L. major* promastigotes and compared to glucantime reference drug using methylthiazole tetrazolium (MTT) colorometric assay. The optical density absorbance was measured with Eliza reader set, and the IC50 value was calculated at different time intervals. All tests were repeated thrice. Results were analyzed by using Tukey test and t-test.

*Results:* The IC50 values after 48 h, for glucantime against standard parasite promastigotes and clinical strains were equal to 12 and 19 µg/ml, respectively, whereas for purslane herb leaves and stems essence; it was equal to 360 and 680 µg/ml, respectively. Although, the glucantime pharmaceutical drug was more efficient compared to the investigated herb essence, the essence had significant effect on *L. major* promastigotes with increasing density (*p* <0.05). The ingredients of the herb leaves and stem essence were—Phytol, squalene, palmitic acid, ethyl-linoleate, ferulic acid, linolenic acid, scopoletin, linoleic acid, rhein, apigenin, and bergapten.

*Interpretation & conclusion:* The study showed that essence of purslane has considerable antileishmanial effects and can stop the growth of parasites in the laboratory compared to glucantime. More experiments are necessary to investigate its effect on *Leishmania* parasite in animal model.

**Key words** *Leishmania major; Leishmaniasis; MTT; Portulaca oleracea; purslane*

**INTRODUCTION**

Leishmaniasis is an infectious disease caused by various species of the parasitic genus *Leishmania*. This disease affects many people in different countries, especially in developing countries. Leishmaniasis can be clinically divided into four forms: Cutaneous, cutaneous mucosal, diffuse cutaneous and visceral leishmaniasis, among which the cutaneous form is more prevalent and is frequently reported in developing countries like Iran1.

Different species of *Leishmania* are transmitted through sandfly bite, especially the bite of *Phlebotomus papatasi* and some other species of the *Phlebotomus* and *Lutzomyia* genera. No suitable vaccine or drug has been developed so far for controlling these parasites, and no effective chemical control method has been introduced yet either for patients. The pentavalent antimonial drugs sodium stibogluconate (pentostam) and meglumine antimoniate (glucantime) are among the main drugs used for treating this disease in recent decades. Amphotericin B deoxycholate is effective but relatively toxic, and no completely satisfactory treatment has been suggested so far. Use of medicinal plants for treating parasitic diseases dates back to ancient times when *Cinchona succirubra* (Rubiaceae) was used as an antimalarial drug1–2.

Sawadogo et al3 selected five plant species from the central and western parts of Burkina Faso (that were traditionally used to treat parasitic diseases and cancer, and
their antioxidant and antiproliferative activities had been studied before), and using colorimetric and spectrophotometric methods, tested their alcoholic extracts for possible antileishmanial and antitrypanosomal activities. They concluded that extracts of Lantana ukambensis had antileishmanial activity with IC$_{50}$ of 9.6 µg/ml.

Ogeto et al$^{10}$ studied antileishmanial activity of Aloe secundiflora extract against promastigotes of L. major under laboratory conditions. Among extracts of this plant, the aqueous and methanolic ones inhibited the growth of the promastigotes the most, and their IC$_{50}$ values were 279.48 and 42.82 µg/ml, respectively.

Sharif et al$^5$ investigated the effects of methanolic extracts of tarragon and chamomile against L. major under laboratory conditions and found that their various concentrations had considerable antileishmanial activities.

Mirzaei et al$^6$ compared the antileishmanial activities of the extract obtained from harmel (Peganum harmala) and of a trivalent antimonial drug named potassium antimonyl tartrate against growth of promastigotes of L. major using the MTT assay method under laboratory conditions. Both the extract and the antimonial drug controlled the growth of the parasite in culture media. In fact, they had almost identical inhibitory effects against the parasite, and these effects increased at higher concentrations. The IC$_{50}$ values were 1832.65 ± 89.72 and 17.87 ± 2.05 µg/ml for the extract and the drug, respectively. Therefore, considering different complications caused by the antimonial drugs, the extract of this plant can be used as an agent against L. major under laboratory conditions.

Asadi et al$^7$ studied the effects of hydroalcoholic extracts of mountain (Stachys lavandulifolia) and medlar (Mespilus germanica) on promastigotes of L. major under laboratory conditions. Their results indicated decline in the number of promastigotes with increase in the concentration of the extracts of these two plant species. Eskandari et al$^8$–$^9$ carried out different studies on anti-leishmanial effects of black medick (Medicago lupulina) alcoholic leaf extract on promastigotes of the L. major strains under laboratory conditions. The studies indicated that they have relatively good antileishmanial effects.

**Purslane (Portulaca oleracea)**

The active ingredients of the herb comprises of—oxalic acid, cinnamic acid, caffeic acid, maleic acid, citric acid, coumarins, flavonoids, alamine, tannin, alphalinolenic acid, saponins, steroids, phenols, substances such as gum substance, jelly-like, oil, fats and menotropin glycoside; and it has been reported that alkaloids are the most important chemicals of the herb$^{10}$. Purslane is a rich source of antioxidants such as vitamins A, B1, B2, C, E, β-carotene and other essential amino acids. It is also a rich source of minerals such as calcium, iron, phosphorus, copper and potassium, and has a significant amount of necessary linoleic acid (omega-3). Linoleic acid is an essential fatty acid that the body is unable to synthesise and it should always be ingested with food$^{11}$. More recently a flavonoid called apigenin has been derived from the herb. Studies have shown that flavonoids have antitumor features$^{10–12}$.

Dhole et al$^{10}$ have demonstrated the antimicrobial effect of methanol and water extracts of leaves and roots of purslane on gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), gram-negative bacteria (Pseudomonas aeruginosa) bacteria and Aspergillus niger. Agar diffusion method was used in that test. Methanol and water extracts of the herb root at a concentration of 750 µg/ml had formed inhibition haloes with large diam. In spite the methanol and water extracts of leaves of the herb were less successful compared to roots, they had formed inhibition haloes with more significant diameters.

In a test conducted by Nayaka et al$^{12}$, the antimicrobial effect of chloroform extract of the herb on bacteria such as Escherichia coli, S. aureus, Klebsiella pneumoniae, B. cereus, A. niger, A. fumigatus and Neurospora crassa was proved. In this study, the antileishmanial effect of aerial parts (stems and leaves) essence of the purslane herb on promastigotes of L. major parasite (MRHO/IR/75/ER) and a number of clinical strains of the parasite have been evaluated in vitro by MTT assay.

**MATERIAL & METHODS**

The study experiment was conducted in the laboratory of Sedigheh Tahereh Infectious Disease Research Center, Isfahan, Iran for analysing and evaluating the antileishmanial effect of purslane stems and leaves on promastigotes of L. major (MRHO/IR/75/ER) and a number of clinical strains of the parasite using MTT assay.

Purslane (Portulaca oleracea) with herbarium code of 001/001/151 was collected from arable lands of Ahvaz county (capital of Khuzestan province), Iran in early March 2015. According to the provisions contained in investigated articles regarding antiulcer and antimicrobial effects of leaves and stems of the plant, these parts were harvested before flowering stage (Fig. 1).

Stems and leaves of the plant were collected in sterile conditions and rinsed with distilled water, then dried at room temperature (20–25°C) by an electric fan in shade.
Essence extraction

Extraction was done by distillation using Clevenger apparatus. About 100 g of dried purslane powder was extracted for 2 h. In total, 300 g of the plant dried powder was used as the extraction was performed three times. At the end of each extraction, the essence formed as a separate layer on the water. Finally, the essence was transferred to small sterilized jars and covered with aluminum foil to protect from the sun light. The essence was kept at the temperature of 4°C until usage time.

Essential oil extract was used for antileishmanial effect.

Alcoholic extraction

Alcoholic extraction was also done in accordance to maceration method. To perform this procedure, chopped plant parts were put in a glass container, and 50 ml of 80% ethanol was poured on it. The procedure was conducted away from the sun light in order to be safe against chemical changes caused by sun biochemical interactions; also the extraction container lid was tightly closed in order to prevent from solvent evaporation. Alcoholic extract was used only to determine the composition.

The extracts were treated for five days on a shaker maintaining the balance between the concentration of solvent substances and the plant tissues. The resulting extract was purified by a syringe filter and the plant residue was pressed by a mangle. Finally, the extracts were mixed and kept at the temperature < 15°C for five days so that the sediments and turbidities settle down, which were then purified with caution by solvent evaporation method

Gas chromatography-mass spectrometry (GC-MS)

For determining the composition of alcoholic extracts, gas chromatography-mass spectrometry (GC-MS) device was used which includes Agilent 5975 C mass detectors with electron ionization source (EI) coupled with Agilent 7890 gas chromatography composed of HP-5MS column with length 30 m, diam of 0.25 mm, and film thickness of 0.25 mm. The temperatures of gas chromatography device injection site, C mass detector ionization, and analyzers (quadruple) were 280, 150 and 230°C, respectively, and the growth medium temperature between MS and GC was adjusted to 280°C. Alcoholic extract ingredients of purslane leaves and stems are presented in Table 1.

The study of antileishmanial effect of purslane leaves and stems essence

The standard strain of *L. major* parasite (MRHO/IR/75/ER) obtained from the parasitology laboratory of Medical Science Faculty of Isfahan University, Iran and a small amount was transferred to a flask containing 3 cc Schneider growth medium, 10% fetal bovine serum and 15 ml stock Penicillin streptomycin in the laboratory, and placed for three days at 25°C. Schneider growth medium plays the auxiliary role, and provides the parasite with physiological life conditions.

For preparing the clinical sample, first the lesion (wet wound) of a patient (courtesy of middle-aged woman) was disinfected with 70% ethanol, and then a small cut was created on the leading edge of wound by a disposable surgical blade. Some amount of tissue along with cerussite was removed from the lesion and expansion was made on glass slide. Smears prepared on glass slides were used for antileishmanial effect.

![Fig. 1: Leaves and stems of purslane (*Portulaca oleracea*) plant.](image)

Table 1. Ethanol extracts compounds of purslane leaves and stems obtained after gas chromatography-mass spectrometry

<table>
<thead>
<tr>
<th>Compounds extracted</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytol</td>
<td>29.54</td>
</tr>
<tr>
<td>Squalene</td>
<td>18.91</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>17.06</td>
</tr>
<tr>
<td>Ethyl linoleate</td>
<td>16.04</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>13.10</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>10.72</td>
</tr>
<tr>
<td>Scopoletin</td>
<td>8.70</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>7.66</td>
</tr>
<tr>
<td>Rhein</td>
<td>5.23</td>
</tr>
<tr>
<td>Apigenin</td>
<td>5.08</td>
</tr>
<tr>
<td>Bergapten</td>
<td>2.71</td>
</tr>
</tbody>
</table>
dried in air, fixed with methanol for a few minutes and stained with Giemsa for 20–30 min. The slides were studied preliminary under the microscope with lens of 40x, then tested with oil lens of 100x to observe amastigote forms of *Leishmania*. Direct examination of amastigotes was positive. In this way, the samples were taken from the leading edge of the wound using the scalpel, and transferred to physiological saline; and the clinical samples were transferred to biphasic Navy-Nicolle-Neal (NNN) growth medium, a few hours later. However, to ensure the full growth of the parasites, a part of NNN growth medium and physiological saline were transferred to Schneider growth medium containing 10% fetal bovine sera on.

Initially, the *L. major* parasites (standard strain and clinical isolates) were kept in the Schneider growth medium within the thermos for three days. After that, slides were prepared to see the parasite growth rate. Then the animated promastigotes were observed in low light using a light microscope. Visible promastigotes suggested that the parasites were growing. As the parasites could produce toxin if remained in the growth medium for long-term, and food level would also decline, they were transferred to another flask containing 3 cc Schneider growth medium, 10% fetal bovine serum and 15 ml of stoke Pn-Strap, kept at the temperature of 25°C. The clinical strain was taken again after three days and the animated promastigotes were observed. Passage and slide making continued every three days until rosette bodies were found. The observance of rosette under a microscope indicates that the parasite has reached to log growth phase. After that, promastigotes were transferred to the RPMI-1640 growth medium for the mass cultivation.

The dried essence was diluted at room temperature using 200 ml DMSO. The DMSO was used as emulsifier. Then in order to evaluate the impact of essence on the parasite, dilutions of 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml were prepared through serial dilution using RPMI-1640 environment. According to Fig. 2, IC50 for purslane herb leaves and stems essence against the clinical *L. major* promastigotes in 10 different concentrations (1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) on standard *L. major* promastigotes were presented in Fig. 2. According to Fig. 2, IC50 for purslane herb leaves and stems essence against the standard *L. major* promastigotes in vitro after 24, 48 and 72 h, was calculated as 950, 360 and 130 µg/ml, respectively.

The results of the antileishmanial effect leaves and stems essence of purslane herb in 10 different concentrations (1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) on standard *L. major* promastigotes were presented in Fig. 2. According to Fig. 2, IC50 for purslane herb leaves and stems essence against the standard *L. major* promastigotes in vitro after 24, 48 and 72 h, was calculated as 950, 360 and 130 µg/ml, respectively.

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effectiveness of leaves and stems essence of purslane herb on the clinical and standard \textit{L. major} promastigotes \textit{in vitro} by MTT assay after 24, 48 and 72 h, and the results are presented in Figs. 4 and 5. The IC$_{50}$ for glucantime drug against the standard \textit{L. major} promastigotes obtained \textit{in vitro} after 24, 48, and 72 h was equal to 27, 12, and 8 \( \mu \text{g/ml} \) (Fig. 4) while against the clinical \textit{L. major} promastigotes it was equal to 26, 19 and 11 \( \mu \text{g/ml} \) respectively (Fig. 5).

At this level, the clinical and standard \textit{L. major} parasites cells were faced with a concentration of glucantime and purslane herb essence (which was the IC$_{50}$ after 48 h), and were observed at 100x magnification at 24, 48 and 72 h which resulted in some morphological changes. These changes begun after treatment with glucantime and herb essence, which included cell shrinkage, more circu-
lation, cytoplasm densifying, and cells size decrease (Fig. 6). The number of transformed promastigotes for the standard *L. major* was more than the clinical strain. No change was observed in control cell parasites. The percentage of transformed promastigotes is presented in Table 2. Alcoholic extract ingredients of purslane leaves and stems are presented in Table 1.

**DISCUSSION**

According to the World Health Organization, leishmaniasis is one of the most important infectious diseases and the currently chemical drugs used against it have many side-effects. Hence medicinal plants have attracted the researchers’ attention for generation of possible leads/drug candidates. The use of medicinal plants in the treatment of parasitic diseases dates back to ancient times when *Cin-*
chona succirruba was used as an antimalarial drug\(^2\). For studying the impact of antileishmanial chemical drugs and herbal medicines, use of haemocytometer for studying slides over the time has become inappropriate\(^16\).

Colorimetric methods now used to check the growth of cells have many benefits, in addition to being non-radioactive and repeatable\(^17\). MTT colorimetric method was used in this study to determine the antileishmanial effect of plant parts, and it has been used also in earlier studies\(^11, 18\).

In a study which examined the antileishmanial effect of \textit{Thymus vulgaris} and \textit{Peganum harmala} by MTT, the IC\(_{50}\) values calculated were equal to 4.7 and 2.7 µg/ml, respectively\(^18\) while in another study that examined the antileishmanial effect of \textit{Aloe vera}, the IC\(_{50}\) values were calculated as 100 to 180 µg/ml\(^11\).

In this study, IC\(_{50}\) values for glucantime against standard parasite and clinical strain after 48 h were equal to 12 and 9 µg/ml respectively, whereas for purslane herb leaves and stem essence it was equal to 360 and 680 µg/ml, respectively. Although, the glucantime pharmaceutical drug was more efficient compared to the investigated herb essence, the essence had significant effect on \textit{L. major} promastigotes at higher concentration (\(p <0.05\)).

Earlier studies have also analysed the active ingredients of the purslane plant parts such as alkaloids and flavonoids, which showed antibacterial, antifungal and antitumor activities\(^10, 12\). In the study, the existence of substances such as palmitic acid, ethyl linoleate, linolenic acid, phytol, squalene, apigenin, renin, bergapten, ferulic acid, linoleic acid and scopoletin in alcoholic extract of purslane parts was proved using GC-MS as shown in Table 1. In this study, in addition to the RPMI-1640 culture medium, NNN and Schneider were also used while in other studies only RPMI-1640 and NNN media have been used. The parasite in Schneider culture emerged in two weeks\(^2–10\). The leaves of purslane against amastigote form of the \textit{L. major} parasite and wound seeker were not investigated in the study which are shortcomings of this study.

According to the results presented in Tables 2 and 3, it can be concluded that in comparison to glucantime, the purslane aerial parts essence caused lesser modification in the promastigote cells. Also on comparing the clinical and standard strains \textit{L. major} promastigotes, it can be said that in all the cases, the numbers of transformed cells in the standard strains were more than the clinical ones.

**CONCLUSION**

In this study, the antileishmanial effect of purslane stems and leaves extract was evaluated in \textit{in vitro} and it was observed this has relatively good antileishmanial effects. Although, there are differences between IC\(_{50}\) of extract and glucantime, the plant extract might be preferred over the harmful effects of chemical drugs. Also due to presence of substances like alkaloids and flavonoids, it can be considered for the treatment of leishmaniasis scars.

However, the need for further tests to assess them on \textit{Leishmania} parasite in animal models and human volunteers is felt, and this was the biggest limitation of the study as the main form of pathogenic parasites is intracellular (amastigote) one, and these studies are required to be done in order to confirm purslane stems and leaves as antileishmanial substances.

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Correspondence to: Dr Monir Doudi, Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran. E-mail: monirdoudi@yahoo.com; doudi@iaufala.ac.ir

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