Cytotoxic and antileishmanial effects of various extracts of *Capparis spinosa* L.

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**ABSTRACT**

**Background:** Nowadays, cutaneous leishmaniasis (CL) is one of the most important infections in the world in which human being infected by the protozoa of the genus *Leishmania*. Today, the common and selective drugs for the treatment of CL are antimonial compounds which have some limitations in use. This study aims to investigate the cytotoxic and antileishmanial effect of various extracts of *Capparis spinosa* L. on *in vitro* model.

**Methods:** The primary phytochemical analysis of the *C. spinosa* extracts was done to assess the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and glycosides. Here, the *in vitro* cytotoxic and antileishmanial activity of *C. spinosa* extracts on *Leishmania tropica* promastigote is evaluated as well as J774-A1 cells by colorimetric cell viability (MTT) assay.

**Results:** In this study, the findings of primary phytochemical screening of the *C. spinosa* extracts demonstrated the existence of flavonoids, tannins, terpenoids, glycosides and alkaloids in this plant. The findings indicated that the aqueous and methanolic extracts of *C. spinosa* have high potency to inhibit the growth of *L. tropica* promastigotes with IC₅₀ (inhibitory concentration=50 %) values 28.5 and 44.6 µg/ml, respectively. Based on the obtained results, *C. spinosa* extracts did not display considerable cytotoxicity on J774-A1 macrophage cells.

**Conclusion:** The obtained findings exhibited remarkable antileishmanial effects of *C. spinosa* extracts on *L. tropica*; indicating the ability of *C. spinosa* as a natural cause for to create a new antileishmanial drug. Nevertheless, supplementary investigations will be obligatory to reach these findings especially in human subjects.

**Keywords:** Herbal medicines, in vitro, *Leishmania tropica*, macrophage, promastigote

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INTRODUCTION
Today, one of the most important and parasitic infections in the world is cutaneous leishmaniasis (CL); whereas human being infected by the protozoa of the genus *Leishmania*. The most important features of this disease are chronic and prolonged ulcers that their scars remain after the recovery [1]. Every year about 1.5 million people become infected with this infection, so it can be considered as a main health and economic problem [2]. Previous studies demonstrated that in Iran, there are both common types of CL including anthroponotic CL (*L. tropica*) and zoonotic CL (*L. major*) [3].

Today, the common and selective drugs for the treatment of CL are antimonial compounds including meglumine antimoniate and sodium stibogluconate; but recent studies suggest some restrictions about the use of these drugs such as excessive side effects, and parasite resistance to these agents [4, 5]. Therefore, it is highly felt that the discovery of a new drug with same efficacy to current agents and even higher than them as well as lower toxicity can be a priority for researchers.

From centuries ago, the use of natural compounds has been considered as the main resources for treatment of several diseases such as infectious ones [6, 7]. *Capparis spinosa* L. from the family of Capparidaceae which is called “Kabar” in Persian broadly grows in various parts of the world especially in Iran. Past studies have shown that various parts of this plant have a wide range of biological and medicinal effects including antimicrobial, antioxidant, and anticancer activities [8]. Therefore, we decided to investigate the cytotoxic and antileishmanial effects of various extracts of *C. spinosa* on the in vitro model.

MATERIALS AND METHODS

**Parasite strain**
Here we obtained the *L. tropica* (MHOM/IR/2002/Mash2) strain from the Leishmaniasis Research Center (Kerman, Iran). The promastigotes were cultured in NNN medium, and then subcultured in RPMI-1640, complemented with penicillin (200 IU/ml), streptomycin (100 μg/ml), and 15 % heat-inactivated fetal calf serum (FCS).

**Collection of plant materials**
We collected the aerial parts of *C. spinosa* from the mountains of Lorestan Province, Iran. The materials were then recognized by a botanist at Razi Herbal Medicines Research Center, Khorramabad, Iran. A voucher specimen was deposited at the herbarium of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

**Preparation of extracts**
After chopping the fruits into smaller portions and drying them in the shade, the fruits were powdered. Then powdered materials were extracted using the technique of percolation with methanol, and water for three days at 21 C°. The obtained extracts were allowed to pass through a filter paper to take away the excess particles. Lastly, by means of a rotary evaporator (Heidolph, Germany) the extracts were vacuum concentrated at 50 C°, and kept at -20 C° until testing [9-11].

**Phytochemical Analysis**
The primary phytochemical analysis of the both *C. spinosa* extracts were done to assess the presence of tannins, alkaloids, flavonoids, saponins, terpenoids and glycosides via following
reagents and chemicals [12]: alkaloids with Mayer and Dragendorff’s reagents, flavonoids with the use of Mg and HCl, tannin with 1% gelatin and 10% NaCl solutions, terpenoids with chloroform and conc. sulphuric acid, glycosides with FeCl₂ and H₂SO₄, and saponin with the ability of producing suds.

**Antileishmanial effects of C. spinosa extracts**

To determine the antileishmanial effects of *C. spinosa* extracts we used colorimetric cell viability (MTT) method as explained by some researchers [13-15]. After adjusting the promastigotes from logarithmic growth phase to 10⁶ cells per each ml, 0.1 ml of suspension of promastigotes was put in a 96-well plate. In the next step, promastigotes were treated with the various concentrations of each plant extract (0–200 µg/ml) at 25 °C ± 1 °C for three days. After finishing exposure time, 0.01 ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (5 mg/ml) was poured to wells and again incubated at 25 °C for 4 h. Next, to solve the formazan crystals and subsequently generate the purple color, 0.1 ml isopropanol was added to wells. At the end, an ELISA reader (BioTek-ELX800) was used and the absorbance level of wells was determined at 490 nm. The complete medium containing promastigote and no extract was considered as positive control; while complete medium with no parasite and extract considered as negative control (blank).

**Cytotoxic Effects**

To do this, J774-A1 cells cultured at Dulbecco's modified eagle's medium (DMEM) were adjusted at 5 × 10⁵ cell per ml. Then, they were treated in 96-well plates with different concentrations of each extract (0-5000 µg/mL) at 37°C in 5% CO₂ for 48 h. Finally, cytotoxic effects of extracts were measured by colorimetric MTT assay as mentioned above [15-17].

**Statistical Analysis**

Here we performed experiments in in triplicate. Analysis of collected data was done by means of SPSS software version 22.0. Moreover, CC₅₀ (cytotoxic concentration for 50% of macrophages) and IC₅₀ (50% inhibitory concentrations for promastigotes) was measured by linear regression method. Furthermore, the selectivity index (SI) was measured as the equation of CC₅₀ for J774-A4 /IC₅₀ for promastigotes to assess the toxicity and activity of *C. spinosa* extracts. Also one-way analysis of variance (ANOVA) test was applied to assess the variations among the test and control groups. Furthermore, *P* < 0.05 was considered to be significant, statistically.

**RESULTS**

**Phytochemical Analysis**

In this study, the findings referred to primary phytochemical screening of the *C. spinosa* methanolic and aqueous extracts demonstrated the presence of tannins, flavonoids, terpenoids, glycosides and alkaloids in this plant.

**Antileishmanial effects of C. spinosa extracts**

Figure 1 shows the antileishmanial effects of different extracts of *C. spinosa* on *L. tropica* promastigote. The obtained findings demonstrated that different extracts of *C. spinosa* mostly methanolic extract displayed effective antileishmanial effects on *L. tropica* promastigote as depended on dose (*P* < 0.05). The obtained IC₅₀ values of aqueous and methanolic extracts were 28.5 and 44.6 µg/ml on *L. tropica* promastigote, respectively. Meglumine antimoniate (MA) also as control drug revealed effective antileishmanial effects with IC₅₀ value of 35.7 µg/ml on *L. tropica* promastigotes.

**Cytotoxic activity**
Based on the obtained results, *C. spinosa* extracts did not display considerable cytotoxicity on J774-A1 macrophage cells. As shows in Table 1, the CC50 values of aqueous and methanolic extracts of *C. spinosa* on J774-A1 macrophage cells were 261.3 and 373.6 µg/ml, respectively. Table 1 also demonstrated the SI values of different extracts of *C. spinosa*.

**DISCUSSION**

Since long ago, herbal medicines have been recognized as one of the most important therapeutic factors around the world. In recent years, the therapeutic and preventive use of medicinal plants has attracted more attention, due to having the low post-consumption complications and the various biological properties of them [18-20].

So far, reviews have reported demonstrate antileishmanial effects of a broad spectrum of medicinal herbs such as black cumin, garlic, savory, pistacia, berberis, myrtle, periwinkle, black beans, *etc.* on CL [21]. Although previous investigations have been reported a number of pharmacological benefits of *C. spinosa* such as antioxidant, anticancer and antibacterial activities; however, there is no documented study regarding the anti-parasitic effects of this plant. Thus, we decided to evaluate the antileishmanial and cytotoxic effects of different extracts of *C. spinosa* in vitro. The results revealed that different extracts of *C. spinosa* mostly methanolic extract displayed effective antileishmanial effects on *L. tropica* promastigote as dose-dependent mode (*P* < 0.05). The obtained IC50 values for aqueous and methanolic extracts were 28.5 and 44.6 µg/ml on *L. tropica* promastigote, respectively.

In this study, the results of the primary phytochemical analysis of the *C. spinosa* extracts indicated the existence of tannins, flavonoids, terpenoids, glycosides and alkaloids in this plant. Based on the previous studies on phytochemical analysis *C. spinosa*, it has been proven that this plant containing the high amount of bioactive components, such as alkaloids, flavonoids, steroids, terpenoids and tocopherols [8]. Moreover, in a study conducted by Tlili et al (2011) on the phytochemical analysis of *C. spinosa*, the results showed that aerial parts of this plants are rich in quaternary ammonium compounds, alkaloids, phenolic compounds, as well as glycosides such as glucosinolates; that indicated various pharmacological properties in modern medicine (22).

Regarding the antileishmanial effects of polyphenolic compounds, Achiaa Antwi et al (2015) demonstrated that rosmarinic acid (as a phenolic compound) had anti-leishmanial effect through iron chelation that results in morphological changes and cell cycle arrest against promastigote and intracellular amastigote forms of *Leishmania donovani* (23). In the other study done by Monzote et al (2016), the potent antileishmanial activity of ten phenolic compounds including cinnamic acid, coumaric acid isomers, gallic acid, sinapic acid, gentisic acid, morin, rutin, ellagic acid, and vanillic acid against intracellular amastigotes as well as experimental cutaneous leishmaniasis in BALB/c mice infected with *L. amazonensis* (24). Regarding antileishmanial activity of alkaloids, Delorenzi et al (2001) showed that indole alkaloid coronaridine have considerable antileishmanial effects, which led to the growth of promastigote and amastigote forms. Through change in their mitochondrial functions (25). Tasdemir et al (2006) also demonstrated that some flavonoid compounds have potent antileishmanial and antitrypanosomal effects against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani* in vitro and in vivo (26). Arruda et al demonstrated that nerolidol as a sesquiterpene (terpenoids) prevented the growth of *L. amazonensis*, *L. braziliensis*, and *L. chagasi* promastigotes and *L. amazonensis* amastigotes with IC50 of 85, 74, 75, and 67 µM, respectively; whereas reduction of lesion sizes in *L.
amazonensis-infected BALB/c mice treated with nerolidol (27). Considering the mechanisms of antimicrobial action of polyphenolic compounds; some studies have shown that antimicrobial mechanisms polyphenolic compounds are associated to their lipophilia as well as their effects on protein synthesis [28-31]. On the other hand, Puupponen-Pimiä et al (2001) have demonstrated that polyphenolic compounds through their disruptive action on the external membrane can inhibit the growth of bacteria [32-34]. Therefore, although the accurate antileishmanial mechanisms of C. spinosa is not clear; however, we can suggest that anti-parasitic effects of this plant is referred to the existence of polyphenolic compounds in it. Here we found that, C. spinosa extracts did not display considerable cytotoxicity on J774-A1 macrophage cells; moreover, SI values above 10 of methanolic and aqueous extracts of C. spinosa revealed their immunity to the macrophages and specificity to the parasite according to Weninger et al. [35, 36].

CONCLUSION
The obtained findings exhibited remarkable antileishmanial effects C. spinosa extracts on L. tropica; indicating the ability of C. spinosa as a natural cause to create a new antileishmanial drug. Nevertheless, supplementary investigations will be obligatory to reach these findings especially in human subjects.

Interest conflict
The author declared no interest conflict in the present study.

REFERENCES

<table>
<thead>
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<th>Drug</th>
<th>IC₅₀ (µg/ml)</th>
<th>CC₅₀ (µg/ml)</th>
<th>SI</th>
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<tr>
<td>Methanolic extract</td>
<td>28.5</td>
<td>261.3</td>
<td>9.1</td>
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<td>Aqueous extract</td>
<td>44.6</td>
<td>373.6</td>
<td>8.4</td>
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<td>Meglumine antimoniate</td>
<td>35.7</td>
<td>261.3</td>
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</table>

Table 1. CC₅₀ values of various extracts of C. spinosa on J774-A1 macrophage cells as well as their IC₅₀ and selectivity index (SI) values on L. tropica promastigotes
Figure 1. Antileishmanial effects of various extracts of *C. spinosa* on the viability rate of *L. tropica* promastigote. Data are expressed as the mean ± SD (n = 3)