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ORIGINAL ARTICLE

# Cytotoxic and Antileishmanial Effects of Various Extracts of *Capparis spinosa* L.

## *Capparis spinosa* L.'nin Farklı Ekstrelerinin Sitotoksik ve Antileishmanial Etkileri

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## ABSTRACT

**Objectives:** Cutaneous leishmaniasis (CL) is considered as one of the most critical infections worldwide, in which the protozoa of the genus *Leishmania* infects a person. Today, the common and selective drugs for the treatment of CL are antimonial compounds present some limitations to their usage. The objective of this study is to investigate the cytotoxic and antileishmanial effects of various extracts of *Capparis spinosa* L. on the *in vitro* model.

**Materials and Methods:** The primary phytochemical analysis of the *C. spinosa* extracts was performed to assess the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, and glycosides. Furthermore, the *in vitro* cytotoxic and antileishmanial effects of *C. spinosa* extracts on *Leishmania tropica* promastigote were evaluated. Additionally, these effects on the J774-A1 macrophage cells by colorimetric cell viability 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay were also assessed.

**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. Importantly, the findings indicated that the aqueous and methanolic extracts of *C. spinosa* exhibit a high potency to inhibit the growth of *L. tropica* promastigotes with inhibitory concentration 50 values of aqueous and methanolic extracts being 28.5 and 44.6 µg/mL, respectively. Based on the obtained results, *C. spinosa* extracts did not display a considerable cytotoxicity on the J774-A1 macrophage cells.

**Conclusion:** The obtained findings exhibited remarkable antileishmanial effects of *C. spinosa* extracts on *L. tropica*, thereby indicating the ability of *C. spinosa* as a herbal product to be developed as a new antileishmanial drug. Nevertheless, supplementary investigations will be obligatory to achieve these findings, especially in human subjects.

**Key words:** Herbal medicines, *in vitro, Leishmania tropic*a, macrophage, promastigote

## ÖZ

**Amaç:** *Leishmania* protozoa türünün insanı enfekte etmesiyle görülen kutanöz leishmania (CL) dünya genelindeki en kritik enfeksiyonlardan biri kabul edilmektedir. Bugün, CL'nin tedavisi için sıklıkla kullanılan ve seçilmiş ilaçlar olan antimonial bileşiklerin kullanımlarında bazı kısıtlamalar vardır. Bu çalışmanın amacı of *Capparis spinosa* L.'nin farklı ekstrelerinin *in vitro* modelde sitotoksik ve antileishmanial etkilerinin incelenmesidir.

**Gereç ve Yöntemler:** *C. spinosa* ekstrelerinin primer fitokimyasal analizi tannen, alkaloid, saponin, flavonoid, terpenoid ve glikosidlerinin değerlendirmek için yapılmıştır. Ayrıca, *C. spinosa* ekstrelerinin *Leishmania tropica* promastigotu üzerindeki *in vitro* sitotoksik ve antileishmanial etkileri değerlendirilmiştir. Ek olarak, bu etkiler kolorimetrik hücre canlılığı 3-(4,5-dimetil-tiyazolil-2,5-)difeniltetrazolyum bromid yöntemiyle J774-A1 makrofaj hücrelerinde de belirlenmiştir.

**Bulgular:** Bu çalışmada, *C. spinosa* ekstrelerinin primer fiotokimyasal izlemlenmesi bu bitkide tannen, alkaloid, saponin, flavonoid, terpenoid ve glikosidlerinin varlığını göstermiştir. Önemli olarak, bulgular *C. spinosa*'nın sulu ve metanolik ekstrelerinin sırasıyla 28,5 ve 44,6 µg/mL inhibitor konsantrasyon 50 değerleriyle *L. tropica* promastigotunun büyümesini yüksek bir potens ile inhibe ettiğini göstermiştir. Elde edilen verilere göre, *C. spinosa* ekstreleri J774-A1 makrofaj hücrelerinde belirgin bir toksisite göstermemiştir.

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**Sonuç:** Elde edilen bulgular *C. spinosa* ekstrelerinin *L. tropica* üzerinde belirgin antileishmanial etkiler gösterdiğini ve *C. spinosa*'nın bir herbal ürün olarak yeni antileishmanial ilaç için geliştirilmesi için kullanılabileceğini göstermiştir. Bununla birlikte, özellikle insanlarda bu verilerin ek araştırmalarla doğrulanması gerekmektedir.

**Anahtar kelimeler:** Herbal ilaç, *in vitro, Leishmania tropica*, macrofaj, promastigot

## INTRODUCTION

In present times, cutaneous leishmaniasis (CL) is one of the main parasitic infections worldwide, in which human beings are infected by the *Leishmania* protozoan parasites. The most important characteristics of this disease are chronic and prolonged ulcers that leave scars even after recovery.[1] Every year, about 1.5 million people become infected with this disease; hence, it can be considered as a main health and economic challenge.[2] Previous studies have demonstrated that in Iran, the common types of CL are anthroponotic CL (*Leishmania tropica*) and zoonotic CL (*L. major*).[3]

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

From centuries ago, the use of natural compounds has been considered for the treatment of several diseases such as infectious ones.[6,7] *Capparis spinosa* L. from the family of *Capparidaceae*, which is called "Kabar" in Persian, widely grows in the various parts of the world, especially in Iran. Previous studies have shown that various parts of this plant represent some biological and medicinal effects such as antimicrobial, antioxidant, and anticancer activities.[8] Therefore, the objective of this study is to investigate the *in vitro* cytotoxic and the leishmanicidal activities of extracts of *C. spinosa*.

## 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 the 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.

*Collection of plant materials*

We collected the aerial parts of *C. spinosa* from the mountains of Lorestan Province, Iran. The materials were recognized by a botanist, and 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 shade, the fruits were powdered. Afterward, the powdered materials were extracted using the technique of percolation with methanol and water for 3 days at 21°C. The obtained extracts were allowed to pass through a filter paper to remove the excess particles. Finally, 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 was conducted 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 by using Mg and HCl, tannin with 1% gelatin and 10% NaCl solutions, terpenoids with chloroform, and concentrated sulfuric acid, glycosides with $FeCl_2$ and $H_2SO_4$, 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 the colorimetric cell viability 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method as explained by some researchers.[13-15] After adjusting the promastigotes from the logarithmic growth phase to $10^6$ 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 3 days. After finishing the exposure time, 0.01 ml of MTT solution (5 mg/mL) was poured into wells and again incubated at 25°C for 4 hours. Next, to solve the formazan crystals and subsequently generate the purple color, 0.1 mL of isopropanol was added into 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 a positive control, whereas a complete medium with no parasite and extract considered as a negative control (blank).

*Cytotoxic effects*

To assess the cytotoxic effects, the J774-A1 cells cultured at Dulbecco's modified eagle's medium were adjusted at $5x10^5$ cell per mL. Then, they were treated in 96-well plates with different concentrations of each extract (0-5.000 µg/mL) at 37°C in 5% $CO_2$ for 48 hours. Finally, the cytotoxic effects of extracts were measured by the colorimetric MTT assay as mentioned above.[15-17]

*Statistical analysis*

We performed experiments in triplicates. The collected data were analyzed by SPSS software version 22.0. Moreover,

[cytotoxic concentration for 50% ($CC_{50}$)of macrophages] and inhibitory concentration 50 ($IC_{50}$) (50% ICs for promastigotes) were measured by the linear regression method. Furthermore, the selectivity index (SI) was measured as the equation of $CC_{50}$ for J774-A1/$IC_{50}$ for promastigotes to assess the toxicity and activity of *C. spinosa* extracts. Additionally, One-Way ANOVA test was applied to assess the variations among the test and control groups. Furthermore, $p<0.05$ was considered to be statistically significant for this study.

## RESULTS

*Phytochemical analysis*

In this study, the findings referred to the 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 showed that different extracts of *C. spinosa*, mostly methanolic extract, displayed effective antileishmanial effects on *L. tropica* promastigote in a dose-dependent manner ($p<0.05$). The obtained $IC_{50}$ values of aqueous and methanolic extracts on *L. tropica* promastigote were 28.5 and 44.6 µg/mL, respectively. Meglumine antimoniate also as control drug revealed effective antileishmanial effects with the $IC_{50}$ 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 the J774-A1 macrophage cells. As shown in Table 1, the $CC_{50}$ 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 presents the SI values of different extracts of *C. spinosa*.

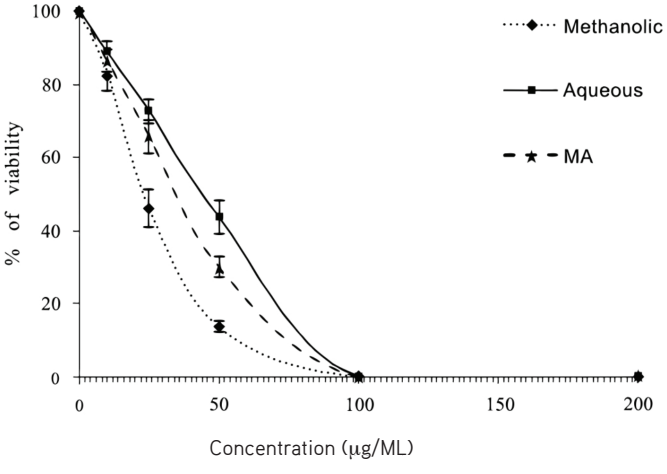


**Figure 1.** Antileishmanial effects of various extracts of *Capparis spinosa* on the viability rate of *Leishmania tropica* promastigote. Data are expressed as mean ± SD (n=3)

SD: Standard deviation

## DISCUSSION

Since long ago, herbal medicines have been recognized as one of the main therapeutic agents worldwide. In recent years, the therapeutic and preventive use of medicinal plants has attracted increased attention because of low post-consumption complications and the various biological properties.[18-20]

So far, researchers have demonstrated the antileishmanial effects of a broad spectrum of medicinal herbs, such as black cumin, garlic, savory, pistacia, berberis, myrtle, periwinkle, black beans, and others, on CL.[21] Although previous investigations have reported a number of pharmacological benefits of *C. spinosa* such as antioxidant, anticancer, and antibacterial activities, there is no documented study regarding the antiparasitic effects of this plant. Thus, we decided to investigate the *in vitro* antileishmanicidal and cytotoxic activities of *C. spinosa* extracts. The results revealed that different extracts of *C. spinosa*, mostly methanolic extract, displayed effective antileishmanial effects on *L. tropica* promastigote in a dose-dependent manner ($p<0.05$). The obtained $IC_{50}$ values for methanolic and aqueous extracts on *L. tropica* promastigote were 28.5 and 44.6 µg/mL, 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. Previous studies on phytochemical analysis *C. spinosa* have proven that this plant contains high amounts of bioactive components, such as alkaloids, flavonoids, steroids, terpenoids, and tocopherols.[8] Moreover, a study conducted by Tlili et al.[22] on the phytochemical analysis of *C. spinosa* showed that aerial parts of this plants are rich in quaternary ammonium compounds, alkaloids, phenolic compounds, and glycosides, such as glucosinolates, further indicating various pharmacological properties useful in modern medicine.

Regarding the antileishmanial effects of polyphenolic compounds, Antwi et al.[23] demonstrated that rosmarinic acid (as a phenolic compound) exerted antileishmanial effect through iron chelation that results in the morphological changes and cell cycle arrest against the promastigote and intracellular amastigote forms of *L. donovani.* Monzote et al.[24] demonstrated the potent antileishmanial activity of ten phenolic compounds including cinnamic acid, coumaric acid isomers, gallic acid, sinapic acid, gentisic acid, morin, rutin extrasynthese, and ellagic acid, vanillic acid against intracellular amastigotes

**Table 1. $CC_{50}$ values of various extracts of *Capparis spinosa* on the J774-A1 macrophage cells as well as their $IC_{50}$ and selectivity index values on *Leishmania tropica* promastigotes**

| Drug | $IC_{50}$ (µg/mL) | $CC_{50}$ (µg/mL) | SI |
|---|---|---|---|
| Methanolic extract | 28.5 | 261.3 | 9.1 |
| Aqueous extract | 44.6 | 373.6 | 8.4 |
| Meglumine antimoniate | 35.7 | 261.3 | 7.3 |

SI: Selectivity index, $IC_{50}$: Inhibitory concentration 50, $CC_{50}$: Cytotoxic concentration 50%

as well as experimental CL in BALB/c mice infected with *L. amazonensis*.

Regarding antileishmanial activity of alkaloids, Delorenzi et al.[25] showed that indole alkaloid coronaridine have shown considerable antileishmanial effects, which led to the growth of promastigote and amastigote forms. Through change in their mitochondrial functions. Tasdemir et al.[26] also demonstrated that some flavonoid compounds exert potent antileishmanial and antitrypanosomal effects against *Trypanosoma brucei rhodesiense, Trypanosoma cruzi*, and *L. donovani in vitro* and *in vivo.*

Arruda et al.[27] demonstrated that nerolidol as a sesquiterpene (terpenoids) prevented the growth of *L. amazonensis, L. braziliensis*, *L. chagasi* promastigotes, and *L. amazonensis* amastigotes with $IC_{50}$ values of 85, 74, 75, and 67 μM, respectively; whereas a reduction of lesion sizes was observed in *L. amazonensis*-infected BALB/c mice treated with nerolidol. Considering the mechanisms of the antimicrobial action of polyphenolic compounds, some studies have shown that antimicrobial mechanisms of polyphenolic compounds are associated with their lipophilia as well as their effects on protein synthesis.[28-31] Previously, Puupponen-Pimiä et al.[32] have shown 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 unclear, we can suggest that antiparasitic 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 the J774-A1 macrophage cells; moreover, the SI values above ten of methanolic and aqueous extracts of *C. spinosa* revealed their immunity against the macrophages and specificity to the parasite, according to Weninger et al.[35,36]

## CONCLUSION

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

*Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.*

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