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## ***In vitro* antileishmanial activity of Khinjuk essential oil against *Leishmania major* promastigotes**

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### **ABSTRACT**

The present study aims to assess the *in vitro* antileishmanial activity of Myrtle (*Pistacia khinjuk*) essential oil against *Leishmania major* promastigotes. The *in vitro* antileishmanial effects of Khinjuk essential oil against *L. major* promastigotes were performed using MTT method. The  $IC_{50}$  values were also calculated by Probit test in SPSS software. The obtained results showed that myrtle extract was significantly inhibited promastigote growth of *L. major* based on a dose and time dependent manner. The measured  $IC_{50}$  values for Khinjuk essential oil and MA as control drug against promastigote forms of *L. major* were 8.9  $\mu$ L/mL and 44.6  $\mu$ g/mL, respectively. The obtained results showed antileishmanial effects of Khinjuk against promastigote forms of *L. major*. However, further studies will be needed to confirm these results by checking in the animal models as well as volunteer human.

**Key words:** Promastigote, *Leishmania major*; Medicinal plants; Cutaneous leishmaniasis, *Myrtus communis*

### **INTRODUCTION**

Cutaneous leishmaniasis (CL) is one of the most common types of leishmaniasis, which caused by parasitic protozoa of the genus of *Leishmania* transmitted by bites of phlebotomine sand flies. The disease affects nearly 1.5 million people annually around the world [1, 2]. At present first choice treatment for CL is chemotherapy with pentavalent antimony components including Glucantime and Pantostame [3, 4]. However, the current studies have demonstrated that this drugs due to some problems including different efficacy, adverse side effects and emergence of resistance have limitations in using [4, 5]. Human from last centuries have been used natural products and plant extracts for treatment of a wide spectrum of diseases due to having less side effects, low cost and high availability [6]. One of this interesting plants which widely used in Iranian traditional medicine is Khinjuk (*Pistacia khinjuk* Stocks [7]. Reviews have demonstrated that various parts of Khinjuk have different pharmacological properties in folk and modern medicine including antiinflammatory, antioxidant, antitumor, antiasthmatic, and antimicrobial ones (7-9). The present study aims to investigate the *in vitro* antileishmanial effect of *Khinjuk* essential oil and to compare its efficacy with Glucantime against *L. major* promastigotes.

### **MATERIALS AND METHODS**

#### *Chemicals*

Meglumine antimoniate (MA, Glucantime) as a control drug was purchased from Aventis, France. Penicillin and streptomycin were obtained from Alborz Pharmacy, Karaj, Iran, and were stored at room temperature (25°C) until testing. MTT powder [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], fetal calf serum (FCS) and RPMI-1640 medium with L-glutamine were prepared from Sigma-Aldrich, (St. Louis, MO, USA). All the other chemicals and solvents were of analytical grade.

**Parasite strain**

Standard strain of *L. major* (MRHO/IR/75/ER) was kindly obtained from the Laboratory for Leishmaniasis, Department of Medical Parasitology, Tehran University of Medical Sciences (Tehran, Iran), and then were cultured in RPMI-1640, supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL), and 15% heat-inactivated fetal calf serum (FCS) (10, 11).

**Collection of plant materials**

The Khinjuk fruits were collected from rural regions of Baft district of Kerman Province, southeastern Iran, in September 2014. The identity was confirmed by a botanist at the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran (KF1356).

**Preparing the methanolic extract**

The plant materials were extracted by methanol (80%) for 72 h at room temperature using the percolation method. Then, the extract was passed via filter paper (Whatman No.3, Sigma, Germany) to delete plant debris. Finally the isolated extract concentrated in vacuum at 50 °C using a rotary evaporator (Heidolph, Germany) and stored at -20 °C, until use [12-15].

**Antileishmanial effects against promastigote form**

We used MTT method to assess the antileishmanial effect of Khinjuk essential oil [16, 17]. At first, 100 µL of the promastigotes ( $10^6$  cells/mL) both species harvested from logarithmic growth phase was added to a 96-well microtiter plate. Then, 100 µL of different concentrations (0-50 µg/mL) of each plant essential oil was added to each well and incubated at  $25^\circ\text{C} \pm 1^\circ\text{C}$  for 72 hours. After incubation, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated at  $25^\circ\text{C}$  for 4 hours. The promastigotes were cultured in the complete medium with no drug used as positive control and with no promastigotes and drugs as blank. Finally, absorbance was measured by an ELISA reader (BioTek-ELX800) at 490 nm. 50% inhibitory concentrations ( $\text{IC}_{50}$  values) were also calculated by Probit test in SPSS software.

**Statistical analyses**

All the experiments were repeated in triplicate. We used SPSS software, ver. 17, (SPSS Inc., Chicago) for data entry and statistical analysis and differences between the groups were determined using one-way analysis of variance (ANOVA) test. Moreover, to compare  $\text{IC}_{50}$  values of the groups, *t*-test was performed. *P*-value of less than 0.05 was considered statistically significant [18-20].

**RESULTS****Antileishmanial effects**

Antileishmanial effects of Khinjuk essential oil against promastigote forms of *L. major* was evaluated using MTT assay. The obtained results showed that myrtle extract was significantly inhibited promastigote growth of *L. major* based on a dose and time dependent manner. So that, with increasing of time and concentration, Khinjuk essential oil revealed higher leishmanicidal activity in comparison with control group. The measured  $\text{IC}_{50}$  values for Myrtle methanolic extract and MA as control drug against promastigote forms of *L. major* were 8.9 µl/mL and 44.6 µg/mL, respectively.

**DISCUSSION**

Since last centuries, plants have been widely used to remedy a wide spectrum of illness and diseases conditions such as infectious ones [4, 21]. Studies have demonstrated various properties of Khinjuk in traditional and modern medicines such as antimicrobial, anti-inflammatory, antinociceptive, antioxidant, anti-hepatic ischemia, neuro-protective [7,8]. Here we found that Khinjuk essential oil was significantly inhibited promastigote growth of *L. major* based on a dose and time dependent manner. The measured  $\text{IC}_{50}$  values for Khinjuk essential oil and MA as control drug against promastigote forms of *L. major* were 8.9 µl/mL and 44.6 µg/mL, respectively. Similarly, Ezatpour et al (2015) have reported that Khinjuk, particularly its essential oil, significantly ( $P < 0.05$ ) inhibited the growth rate of promastigote and amastigote forms of *L. major* based on a dose-dependent response; whereas the  $\text{IC}_{50}$  value was 58.6 µg/ml against promastigotes [8].

Previous investigations revealed that the presence of terpenoid, flavonoids, tannins, and phenols in the phytochemical screenings of the Khinjuk plant [22, 28]. Reviews have reported various antimicrobial activity of these compounds particularly terpenoid components such as antibacterial, antifungal, and antiparasitic activities [23-35]. Thus, we can conclude that these components in Khinjuk essential oil could be responsible for its

antileishmanial activity; while their exact mechanism of action is fully understood. However, Sikkema *et al.* and also other researchers [36-47] have reported that some terpenoid compounds such as monoterpenes, can diffuse into pathogens and damage cell membrane structures.

Regarding cytotoxicity effects of Khinjuk essential oil, Ezatpour *et al* (2015) demonstrated that Khinjuk had no significant cytotoxicity in J774 cells on *in vitro* [8].

### CONCLUSION

The obtained results showed antileishmanial effects of Khinjuk essential oil against promastigote forms of *L. major*. However, further studies will be needed to confirm these results by checking in the animal models as well as volunteer human.

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