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Original Article

Antileishmanial Activity of Myrtle Methanolic Extract against *Leishmania major*: an In Vitro Study

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Abstract

Background and Aim: In this study we assessed the *in vitro* antileishmanial activity of myrtle *(Myrtus communis* L.) methanolic extract against *Leishmania major*.

Materials and Methods: The *in vitro* antileishmanial effects of myrtle methanolic extract against *L. major* promastigote and amastigotes were determined by colorimetric cell viability (MTT) assay and macrophage model, respectively. The IC_{50} values were also calculated by probit test in SPSS software.

Results: 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 myrtle methanolic extract and MA as control drug against promastigote forms of *L. major* were 23.6µg/mL and 88.3µg/mL, respectively. The obtained IC_{50} values were 13.8*µ*g/mL and 44.6*µ*g/mL for the myrtle essential oil and MA, respectively.

Conclusion: This investigation showed antileishmanial effect of myrtle against promastigote and amastigote forms of *L. major*. However, further studies are needed to confirm these results by checking in the animal models and volunteer human.

Keywords: Promastigote, Amastigote, *Leishmania major*; Medicinal plants; Cutaneous leishmaniasis, Myrtus communis

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Introduction

Cutaneous leishmaniasis (CL), which is caused by parasitic protozoa of the genus of *Leishmania*, is one of the most frequent types of leishmaniasis (1). Currently, the best treatment for CL is the chemotherapy with pentavalent antimony compounds such as meglumine antimoniate (MA, Glucantime) and sodium stibogluconate; however, this treatment has some limitations due to some side effects as well as the emergence of drug resistance (2-4). It has now been proven that plant-derived components, because of possessing minimum side effects, low cost and high availability, are the reliable sources for treatment of a

large number of diseasesincluding leishmaniasis (5). Several studies showed that different parts of myrtle *(Myrtus communis* L.) have been used widely as a folk remedy to treat various diseases such as infectious ones (6). Moreover, reviews have revealed some of the medical features of this plant such as anti-inflammatory, antinociceptive, antioxidant, anti-hepatic ischemia, neuro-protective and antimicrobial ones(6, 7).

The present study aims to evaluate the *in vitro* antileishmanial properties of myrtle methanolic extract against promastigote and amastigote forms of *Leishmania major*.

Materials and Methods

Parasite strain

L. major (MRHO/IR/75/ER) obtained from the Laboratory of Leishmaniasis, Kerman University of Medical Sciences (Kerman, Iran), were cultured in RPMI-1640, supplemented with penicillin (100IU/mL), streptomycin (100µg/mL), and 15% heat-inactivated fetal calf serum (FCS).

Plant materials and extraction

The myrtle leaves were acquired from rural regions of Baft city (Kerman Province, southeastern Iran), in September 2014.

By the percolation method, the dried aerial parts of the plant (100g) were extracted by methanol (80%) for three days at 21°C. Subsequently, the obtained extract was passed from a filter paper (Whatman No.3, Sigma, Germany) for the elimination of wastes, and then it was concentrated in vacuum at 50°C by means of a rotary evaporator (Heidolph, Germany) and kept at -20°C, until testing (8)

Antileishmanial effects against promastigote form

Antileishmanial effect of myrtle extract was performed by colorimetric cell viability ([3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide)], MTT) assay according to the methods by Mahmoudv et al (9). Furthermore, we calculated the 50% inhibitory concentrations (IC_{50} values for each of tested drugs.

Anti-amastigote effects

In this study, antiamastigote activity of myrtle extract was evaluated using the methods described by Mahmoudv et al. (3). Activity of anti-intramacrophage amastigotes of the extracts was evaluated by counting the number of amastigotes in each macrophage via examining 100 macrophages (% amastigotes viability) in comparison with those obtained by positive control.

Statistical analyses

All the experiments were repeated in triplicate. We used SPSS software, ver. 17, (SPSS Inc., Chicago) for data entry and statistical analysis. P value of less than 0.05 was considered statistically significant.

Results and Discussion

Anti-promastigote effects

The obtained results showed that myrtle extract was significantly inhibited promastigote growth of *L. major* based on a dose and time dependent mode. Meanwhile, with increasing of time and concentration, methanolic extract of Myrtle revealed higher leishmanicidal activity in comparison with control group. The measured IC_{50} values for Myrtle methanolic extract and MA as control drug against promastigote forms of *L. major* were 23.6µg/mL and 88.3µg/mL, respectively (Table 1).

Anti-amastigote effects

According to the obtained findings, myrtle extract significantly ($P<0.05$) inhibited the growth rate of intramacrophage amastigotes as a dose-dependent response. The obtained IC_{50} values were 13.8μg/mL and 44.6 μg/mL for the myrtle essential oil and MA, respectively (Table 1).

Studies have shown various medical features of myrtle in traditional and modern medicines such as antimicrobial, anti-inflammatory, antinociceptive, antioxidant, anti-hepatic ischemia, and neuro-protective properties (6, 7). We found that myrtle extract significantly inhibited promastigote and amastigote growth of *L. major* based on a dose and

Table 1: IC_{50} values of Myrtle extract against promastigote and amastigote forms of L. major.

Tested drug	IC_{50} value (µg/ml)	
	Promastigote	Amastigote
Myrtle extract	23.6	13.3
Glucantime	88.3	44.6

time dependent manner. The measured IC_{50} values for myrtle methanolic extract and MA as control drug against promastigote forms of *L. major* were 23.6µg/mL and 88.3µg/mL respectively. The obtained IC_{50} values against amastigote forms of *L. major* were 13.8*µ*g/mL and 44.6*µ*g/mL for the myrtle essential oil and MA.

Similarly, Mahmoudv et al. (2015) have reported that myrtle, particularly its essential oil, significantly ($P<0.05$) inhibited the growth rate of promastigote and amastigote forms of *L. tropica* based on a dose-dependent response; whereas the IC_{50} values for essential oil and methanolic extract were 8.4 and 28.9 µg/ml against promastigotes respectively (11).

Based on the previous investigations, terpenoid, flavonoids, tannins, and phenols are the main components in the phytochemical analysis of the myrtle extract (6). Recent studies have indicated the antimicrobial properties of these compounds, particularly terpenoid components (12-16). Thus, we can conclude that these components in myrtle could be responsible for its antileishmanial activity; while their accurate mechanism of action is not completely clear. However, some researchers have demonstrated that some terpenoid compounds, such as monoterpens, can spread into pathogens and break cell membrane structures (17-19).

In relation to the cytotoxic effects of myrtle, Mahmoudv et al. demonstrated that myrtle extract had no considerable cytotoxicity in J774 cells; whereas its essential oil indicated a more cytotoxic effect as compared with the methanolic extract of myrtle (11).

Conclusion

The obtained results showed antileishmanial effects of myrtle against promastigote forms of *L. major*. However, further studies are needed to confirm these results by checking in the animal models as well as volunteer humans.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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