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# Evaluation of the Antileishmanial Properties of *Ixora brachiata* Roxb on *Leishmania major* and *Leishmania infantum* by Colorimetric MTT Assay

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**Abstract**

**Background:** Leishmaniasis is a major global health problem which affects millions of people, especially in the developing countries. The incidence of leishmaniasis has increased and there is no vaccination for *Leishmania* infections and standard drugs for treatment of the disease have many side effects; therefore, it is necessary to find new effective alternatives.

**Objectives:** The purpose of this study was to evaluate the *in vitro* antileishmanial activity of *Ixora brachiata* root extract against *Leishmania major* and *Leishmania infantum* promastigotes.

**Materials and Methods:** Different doses of the selected plant extract was tested against *L. major* and *L. infantum* promastigotes using colorimetric MTT [3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. Glucantime was used as the positive control.

**Results:** Anti-parasitic activity was revealed for *I. brachiata* root on *L. major* and *L. infantum* with 50% inhibitory concentration ($IC_{50}$) values of 0.91 and 2.63 µg/mL, respectively compared to the standard drugs, glucantime, which had an $IC_{50}$ value of 40.2 µg/mL for *L. major* and 18.5 µg/mL for *L. infantum* after 72 hours.

**Conclusion:** The results of this study created a new background on the development of drug against leishmania parasite.

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## Background

Leishmaniasis, a parasitic disease caused by parasites of the genus *Leishmania*, is a major worldwide health problem that menaces about 350 million people, with almost 2 million new cases reported annually.[1] It is endemic in 80 countries. The disease has 3 manifestations: visceral, mucocutaneous or cutaneous leishmaniasis; the most severe form is the visceral leishmaniasis caused by *Leishmania donovani* and *Leishmania infantum* with approximately 500 000 new cases reported annually.[1] Cutaneous leishmaniasis is a parasitic disease that has affected 98 countries and regions around the world and 90 % of cutaneous leishmaniasis cases occur in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria.[1,2] Herbal medicine is a beneficial remedy as an alternative drug for treatment of infectious diseases, due to lower costs and scanty side effects. Therefore, a number of studies have been done to investigate the anti-leishmanial activity of medicinal herbs.[3-6] A few researchers reported that herbal medicine can be used as an adjuvant in vaccination against *Leishmania* spp.[7] Recent studies showed that the extracts of *Peganum harmala* and *Hedera helix* have potent antileishmanial activity.[8,9] Moreover, the anti-leishmanial activity of *Juglans regia* and *Lawsonia inermis* were shown in another study.[10] *Ixora brachiata* Roxb. is a genus of the flowering plants from the family Rubiaceae, consisting of tropical evergreens and shrubs. It is a small evergreen tree, about 6-10 feet in height. It has elliptic-oblong leaves which are about 20 cm long and 7.5 cm wide, which is usually used in India for skin diseases by traditional medicine practitioners.[11] Flowers are white (Figure 1) and found to be growing in high rainfall locality.[12] *Ixora* plant and its various parts like flowers, leaves, roots, barks, and fruits have been used by various racial groups of Africa, Asia and Europ.[13] In folklore medicine, *Ixora* is recognized to be useful for many illnesses such as cancer, hepatic disorder, pain, microbial infection, antioxidant, inflammation, and so on. The genus *Ixora* has been reported to possess different compounds like triterpenoids (lupeol, ursolic

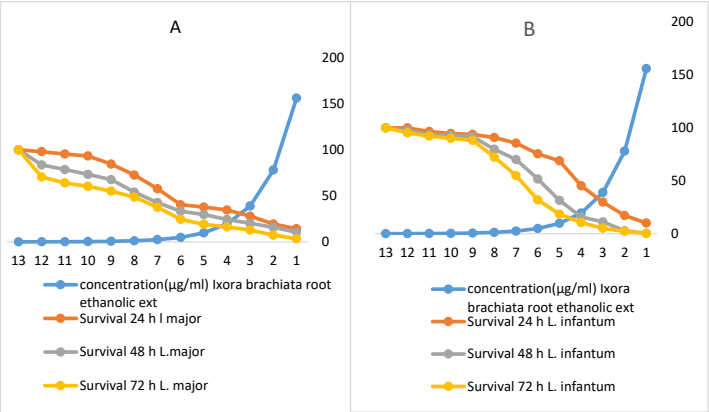


**Figure 1.** The Viability of *Leishmania major* and *Leishmania infantum* Promastigotes in Various Concentrations of *Ixora brachiata* Root Extract After 24, 48, and 72 Hours.

acid, oleanolic acid, betunolic acid, amyrins, etc), aromatic acrid oils, tannins, saponins, carbohydrate, fatty acids, flavonoids (rutin, formononetin, β-sitosterol, quercetin and kaempferol), and sterols.[14] Previous researches reported the antileishmanicidal activity of the flavonoids from *Consolida oliveriana* and the efficacy of the carbohydrates in the treatment of leishmaniasis. In addition, tannins isolated from *Anogeissus leiocarpus* was shown to possess a good activity against *Leishmania.*[15] In the last decades, sesquiterpene lactone (STL) and triterpenes isolated from Asteraceae. This family have been reported as having trypanocidal and leishmanicidal activities.[16] Therefore, natural products are effective for the treatment of important tropical diseases caused by protozoans such as leishmaniasis. The novelty of this study is that the anti-parasitic activities of this plant have been studied for the first time.

## Materials and Methods

### Plant Material

*Ixora brachiata* was collected from Ratnagiri, Maharashtra State, India. The plant parts were identified by Research Center for Botanical Sciences in India (RCBSI) and voucher specimens were deposited in the herbarium of Botany University of Pune, India, with voucher numbers of RCBSI-PU-007.

### Extract Preparation

For preparation of hydroalcoholic extracts, 10 g of shade-dried and powdered plant material was macerated in 100 ml ethanol (85%) solution on a rotary shaker for 72 hours. The plant extracts were filtered through Whatman No. 1 and kept at room temperature until evaporation.[17] Dried extracts were obtained and stored at 4°C for further assays.

### Antileishmanial and Cytotoxicity Assays

#### *Parasite Culture*

*Leishmania infantum* (MCAN/IR/96/LONDON 49) and *Leishmania major* (MRHO/IR/75/ER) promastigotes strains were obtained from School of Health, Tehran University of Medical Sciences, Iran. They were cultured in RPMI-1640 (Sigma, Chemical Company) medium and supplemented with 10% fetal calf serum (Sigma), 100 IU/mL penicillin, and 100 µg/mL streptomycin. All promastigotes ($10^6$ parasites/mL) were incubated at 26°C for 24, 48, and 72 hours in fresh RPMI-1640.

### The Cell Proliferation Elisa by Colorimetric MTT Assay

MTT [3-(4, 5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay measured conversion of MTT dye (tetrazolium) into formazan by mitochondrial enzymes in viable cells. The test was done according to the procedure described previously.[18-20] Briefly, 100 µL aliquots of promastigotes ($1\times10^6$) at logarithmic phase were grown in various concentrations of *Ixora brachiata* root extract (0.61-156 mg $mL^{-1}$ w/v) in 96-well micro-plate. Then, 20 µL MTT reagent (Sigma Chemical Company) was added to a final concentration of 400 µg $mL^{-1}$ per well, and the micro-plate was incubated for 3 hours at 24°C. The cells were centrifuged for 10 minutes and 100 µL DMSO (dimethyl sulfoxide, Sigma Co.) was added to micro-plate and incubated for 15 minutes. The optical density (OD) at 540 nm was measured using an ELISA plate reader (Convergys EL-Reader, Convergent Technologies, Germany). The results were represented as the concentration of different extracts that inhibited parasite growth by 50% ($IC_{50}$).

### Statistical Analysis

The data were analyzed by SPSS version 22.0 using one-way ANOVA test. The results were defined as the mean values of at least three experiments ± SD by linear regression. *P* values less than 0.05 were considered significant.

## Results

In the present study, anti-parasitic activity of the root extract of *I. brachiata* was determined using MTT assay. Fifty percent inhibitory concentration ($IC_{50}$) values for the root extract of *Ixora brachiata* at 24, 48, and 72 hours for *L. major* were 3.1, 1.61, and 0.91 µg/mL and

for *L. infantum*, the values were 17.5, 4.32, and 2.63 µg/mL, respectively, whereas these values for glucantime at 24, 48, and 72 hours for *L. major* were reported to be 104.45, 61.4, and 40.2 µg/mL and for *L. infantum*, the values were 99.7, 45.6, and 18.5 µg/mL, respectively. The findings demonstrated that the root extract of *Ixora brachiata* had potent antileishmanial activity against *L. major* and *L. infantum* promastigotes after 24, 48, and 72 hours of incubation ($P<0.05$). These results also revealed that the root extract of *Ixora brachiata* had significantly ($P<0.05$) higher leishmanicidal effect on the promastigotes of *L. major* and *L. infantum* in comparison with glucantime since it exhibited lower $IC_{50}$ values for the tested promastigotes. The effect of the root extract of *Ixora brachiata* in different concentrations on the parasite has been shown in Tables 1, 2 and Figure 1. Additionally, glucantime revealed $IC_{50}$ values of 40.2 and 18.5 µg/mL against promastigotes of *L. major and L. infantum* after 72 hours of incubation.

**Table 1.** $IC_{50}$ of *Ixora Brachiata* Root Extract and Glucantime Against *Leishmania major* and *Leishmania infantum* Promastigotes After 24, 48, and 72 Hours of Incubation

| Compounds | $IC_{50}$ (µg/mL) | | |
|---|---|---|---|
| | 24 h | 48 h | 72 h |
| *I. brachiata* on *L. major* | 3.1 | 1.61 | 0.91 |
| *I. brachiata* on *L. infantum* | 17.5 | 4. 32 | 2.63 |
| Glucantime on *L. major* | 104.45 | 61.4 | 40.2 |
| Glucantime on *L. infantum* | 99.7 | 45.6 | 18.5 |

**Table 2.** The Results of Comparison of Paired Samples *t*-test for *I. brachiata* Root and Drug Against the Tested Promastigotes after 72 Hours

| Plant and Drug | Mean±SD | Significant (2-tailed) |
|---|---|---|
| *I. brachiate* root-*L. major* & *L. infantum* | 41.90731±34.70750 | 0.001 |
| Glucantime - *L. major* & *L. infantum* | 27.85000±16.05132 | 0.246 |

## Discussion

Antimony compounds are still the first choice for treatment of leishmaniasis; however, due to their toxic effects, long duration of therapy and progression of drug resistance, they have been considered unsatisfactory.[21] On the other hand, herbal medicines are rich sources of antileishmanial drugs with a shorter treatment duration. They are safe, cheaper and partly available in endemic countries.[22-25] Therefore, the research on the antileishmanial effects of medicinal plants is urgently essential. In this study, the antileishmanial effects of the ethanolic extracts of *Ixora brachiata* root on *L. major* and *L. infantum* promastigotes were evaluated by MTT assay. The results of this study revealed that *I. brachiata* root extract possess antileishmanial properties against *L. major* and *L. infantum* Promastigotes. Previous research reported that *I. brachiata* root extract revealed the best activity against *L. major* Promastigotes with $IC_{100}$ value of 2.5 mg/mL and $IC_{50}$ value of 0.078 mg/mL.[17] Another researcher investigated the antileishmanial activity of another species of Ixora such as *Ixora coccinea* leaf against *L. donovani* promastigotes by *in vitro* promastigote cell toxicity assay by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] and reported that this plant inhibited the growth of *L. donovani* promastigotes.[26] Moreover, other researchers reported that the bark extract of *I. brachiata* inhibited the growth of *Staphylococcus*[11] and the root extract of *I. brachiata* showed antidermatophytic activity.[27] Antimicrobial activity of the methanolic extract of Ixora species against bacteria such as *Bacillus cereus, B. megaterium, B. subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae, Vibrio mimicus,* and *V. parahaemolyticus* and fungi like *Candida albicans, Aspergillus niger,* and *Saccharomyces cerevisiae* showed a broad-spectrum of antibacterial and anti-yeast activities.[28] *I. brachiata* root extract gave positive test results for triterpenes and coumarin by HPTLC (high-performance thin-layer chromatography) in another study.[27] Moreover, the presence of compounds such as tannins, flavonoids, saponin carbohydrates, coumarins, and triterpenes reported by previous studies,[17] may be responsible for leishmanicidal activity in this plant. These results are in line with the observations of previous researchers,[29-30] who have shown the anti-leishmanicidal activity of the flavonoids isolated from *Consolida oliveriana* and the efficacy of the carbohydrates in treatment of leishmaniasis. In addition, tannins isolated from *Anogeissus leiocarpus* was shown to possess a good activity against *Leishmani.*[15] Other compounds such as terpenoids (sesquiterpene lactones) have been reported to be active against *Leishmania.*[16] The toxicological activity of this extract should also be investigated to ensure the safety of this herbal medicine in the future.

## Conclusion

The evaluation of side effects of *I. brachiata* root extract on host cells in animals and humans is necessary.

### Authors' Contributions

Study concept and design: SK; performing all laboratory tests and data collection: AE; writhing manuscript, statistical analysis, and interpretation of data: BSN; selection and extraction of the selected herbal medicine: KK.

### Ethical Approval

This study was approved by the Ethics Committee of Abadan School of Medical Sciences, Abadan, Iran (IR.SBMURECH.1395: No. 95U-1100).

### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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