RESEARCH ARTICLE

Antileishmanial effect of *Ixora coccinea* leaf extracts on the *in vitro* growth of *Leishmania donovani* promastigotes

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ABSTRACT

The objective of the present study was to evaluate *in vitro* antileishmanial activity of leaves form *Ixora coccinea* L. (Rubiaceae). In the present study, the *in vitro* antileishmanial activity of ethyl acetate and methanol extracts from *I. coccinea* leaf was evaluated against *Leishmania donovani* (strain AG 83) promastigotes by *in vitro* promastigote cell toxicity assay by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide]. Here, both the extracts markedly inhibited the growth of *L. donovani* promastigotes *in vitro* in a concentration dependent manner. The methanol extract was the most active followed by ethyl acetate extract. Therefore, from the present study it can be inferred that *I. coccinea* leaf exhibited remarkable antileishmanial activity against *Leishmania donovani* promastigotes *in vitro.*

Keyword: Antileishmanial, promastigotes, *Leishmania donovani, Ixora coccinea.*

INTRODUCTION

Leishmaniasis is a wide spread life-threatening disease caused by protozoa of genus Leishmania transmitted by sandflies. According to available estimates of World Health Organization (WHO), the disease is spread across 88 countries causing serious health problems especially in developing countries with 350 million at risk of contracting the disease and with approximately 2 million new cases being reported each year. The three main manifestations of disease are visceral, cutaneous and muco-cutaneous leishmaniasis. Visceral leishmaniasis (VL), also known as *kala-azar* is caused by *L. donovani*. More than 90% of world's cases of VL are reported in India, Bangladesh, Nepal, Sudan, Brazil and Ethiopia. In India, most of the leishmaniasis cases are reported in Bihar, Orissa and Uttar Pradesh states. Cutaneous and muco-cutaneous leishmaniases are more prevalent in Afganistan, Saudi Arabia and some Latin American countries. [1-4]

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Proven therapies against human leishmaniasis include pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, and paromomycin[5, 6]. The mentioned drugs have the disadvantages of high cost, lack of oral formulation (e. g., amphotericin B can be used only intravenously), or serious side effects that require close monitoring of the patients. [6] Also, rapid development of resistance by the parasites has been reported, [7-9] so that new therapies are needed to supplement or replace currently available therapies. More recently, emergence of co-infection of leishmaniasis with HIV has made the treatment even more challenging. [10]

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Ixora coccinea L. (Rubiaceae), commonly known as jungle geranium and jungle flame in English, *Rangan* in Bengali, is an evergreen shrub found throughout India. The flowers, leaves, roots, and the stem are used to treat various ailments in the Indian traditional system of medicine, and also in various folk medicines. The fruits, when fully ripe, are used as a dietary source. Pharmacological studies report that the plant

possesses antioxidative, antibacterial, gastroprotective, hepatoprotective, antidiarrhoeal, antinociceptive, antimutagenic, antineoplastic and chemopreventive effects. [11, 12] However, the antileishmanial effect has not been reported still now. In the present study, therefore, we have aimed to evaluate the *in vitro* antileishmanial activity of *I. coccinea* leaf extracts against *Leishmania donovani* promastigotes.

MATERIALS AND METHODS

Plant material

The mature leaves of *Ixora coccinea* L. (Rubiaceae), were collected during November 2012 from Kalyani, Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/4/2013/Tech. II/962] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of extracts

The dried powdered plant material (350 g) was first defatted with petroleum ether (60-80 °C) and the defatted powdered material thus obtained was further extracted successively with ethyl acetate and methanol for 72 hrs in a cone shaped percolator. The extracts were filtered and their solvents were distilled off in reduced pressure and resulting semisolid masses were vacuum dried using rotary flash evaporator to yield the solid chloroform and methanol extracts (yields: 1.54% and 10.35% respectively). The preliminary phytochemical analysis was performed on these three extracts to identify the phytoconstituents present in the extracts. [13]

Reagents and chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially. Doubled distilled water from all-glass still was used throughout the study.

Parasite culture and antileishmanial evaluation

In vitro promastigote cell toxicity assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] cell proliferation assay was used to assess the antileishmanial activity *in vitro* as per reported methods. [14, 15] Briefly, *Leishmania donovani* strain AG 83 was collected and maintained in golden hamsters by sequential passage. After 2 months, the hamster was sacrificed and its spleen was isolated and macronized. The spleenic culture was made in Medium-199 (L-glutamine with HEPES buffer without $NaHCO_3$) supplemented with 10% fetal bovine serum of pH 7.2. The logarithm phases of promastigotes (2×10^6 cells/ml) were incubated with or without the test agents along with Medium-199 at 22 °C. The two test extracts were dissolved in 0.2% dimethyl sulphoxide (DMSO), and added to the culture in graded concentrations of 3, 5, 10, 15 and 30 µg/ml. After 2 hrs of treatment, the tubes were centrifuged at 8000 *g* for about 10 min. The supernatant was decanted and the pellets were washed with 20 mM phosphate buffer saline (PBS). Each pellet was dissolved in 100 µl (2 mg/ml) of MTT [3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide] solution, and the tubes were incubated at 22°C for 4 hrs and then centrifuged at 8000 *g* for 10 min. The resulting pellets were dissolved in 500 µl of 0.2% DMSO and the absorbance was measured spectrophotometrically at 570 nm. Lysis of promastigotes (%) by the extracts was calculated by the formula as shown below.

Lysis % = 100 – {(test – positive control)/(control – positive control)} × 100

All the tests were carried out in triplicate and the results averaged. The IC_{50} value (50% inhibitory concentration) was determined by linear regression analysis using Graph Pad Prism 3 software.

RESULTS

Preliminary phytochemical screening on the test extracts revealed the presence of triterpenoids and steroids in ethyl acetate extract; and alkaloids, tannins, glycosides and carbohydrates in methanol extract of *I. coccinea* leaf.

In the present antileishmanial evaluation, both of the extracts of *I. coccinea* leaf significantly and concentration dependently inhibited the *in vitro* growth of the promastigote forms of *L. donovani* (strain AG 83) under the experimental conditions. The results are summarized in the Tables 1 and 2. From the determined IC_{50} values it became evident that methanol extract was the most active followed by the ethyl acetate extract of *I. coccinea* leaf.

DISCUSSION

Parasites of the genus *Leishmania* are transmitted by sandflies that ingest the parasite in the amastigote stage resident within macrophages, and then inoculate the promastigote stage into other hosts. There is a general lack of effective and inexpensive chemotherapeutic agents for the treatment of leishmaniasis. Although trivalent antimonials [Sb(III)] like potassium antimonyl tartrate and pentavalent antimonial drugs are the first-line treatment for this disease, with amphotericin B and pentamidine being used as alternative drugs, all of these have serious side effects and resistance has become a severe problem. Therefore, new drugs are urgently required. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans. [16]

The *in vivo* efficiencies of drugs have been reported to be under the control of different parameters, such as pharmacokinetic parameters, [17] so that for various reasons, including simplicity in *in vitro* culture maintenance, routine screenings of antileishmanial chemotherapeutic agents are often based on promastigote susceptibility assays. [18] In the present study, a relevant viability test (MTT) was used to investigate the inhibitory effect of the test extracts on the *in vitro* growth of *Leishmania donovani* promastigotes. The test extracts of *I. coccinea* leaf significantly and concentration dependently inhibited the growth of *L. donovani* (strain AG 83) promastigotes *in vitro.* The methanol extract was the most active followed by ethyl acetate extract.

Therapeutic evaluations for medicinal plants are essential because of the growing interest in alternative therapies and the therapeutic use of natural products. Natural products can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development, and the discovery of new therapeutic properties not yet attributed to known compounds. [19] Natural products have made, and are continuing to make, an important contribution to this area of therapeutics. Perhaps their future potential will be even greater. In this study we report the inhibitory effect of *I. coccinea* leaf extracts on the *in vitro* growth of *Leishmania donovani* promastigotes. This activity represents an exciting advance in the search for novel antileishmanial agents from natural sources, since a significant and important effect against the promastigote form of the protozoan was demonstrated in the present study.

From the present preliminary investigation, it can be concluded that *I. coccinea* leaf extracts demonstrated remarkable *in vitro* antileishmanial activity against *Leishmania donovani* promastigotes. To the best of our knowledge, this is the first experimental report of the antileishmanial activity of *Ixora coccinea* leaf. However, further phytochemical and *in vivo* studies and are necessary in this context, in pursuit of a new effective antileishmanial agent from the plant kingdom.

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Table 1: Effect of Ethyl acetate extract of *I. coccinea* leaf against *Leishmania* promastigotes culture (2×10^6 cells/ml).

Ethyl acetate extract (µg/ml)	Percentage lysis of promastigotes with respect to control (0.2% DMSO)*	IC_{50} value (µg/ml)
3	49.13	7.89
5	54.24	
10	63.33	
15	72.29	
30	83.67	

*Mean of three replicates

Table 2: Effect of methanol extract of *I. coccinea* leaf against *Leishmania* promastigotes culture (2×10^6 cells/ml).

Methanol extract (µg/ml)	Percentage lysis of promastigotes with respect to control (0.2% DMSO)*	IC_{50} value (µg/ml)
3	52.29	7.33
5	60.19	
10	68.18	
15	73.27	
30	89.72	

*Mean of three replicates.

REFERENCES

1. Anonymous. Report of the Consultative Meeting on Cutaneous Leishmaniasis. Geneva: World Health Organization; 2011.
2. Desjeux P. The increase in the risk factors for leishmaniasis worldwide. Trans. Royal Soc. Trop. Med. Hyg. 2010; 95: 239.
3. Handman E. Leishmaniasis: current status of vaccine development. Clin. Microbiol. Rev. 2009; 14: 229-243.
4. Chappuis F., Sundar S., Hailu A., Ghalib H., Rijal S., Peeling R.W., Alvar J, Visceral M.B. leishmaniasis: what are the needs for diagnosis, treatment and control. Nature Rev. Microbiol. 2008; 5: 873.
5. Berman J.D. Treatment of new world cutaneous and mucosal leishmaniases. Clin. Dermatol. 1996; 14: 519-522.
6. Berman J.D. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin. Infect. Dis. 2008; 24: 684-703.
7. Ephros M., Waldman E., Zilberstein D. Pentostam induces resistance to antimony and the preservative chlorocresol in *Leishmania donovani* promastigotes and axenically grown amastigotes. Antimicrob. Agents Chemother. 1997; 41: 1064-1068.
8. Lira R., Sundar S., Makharia A., Kenney R., Gam A., Saraiva E., Sacks D. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. J. Infect. Dis. 2009; 180: 564-567.
9. Boelaert M., Leray D., Vander S.P. How better drugs could change kala-azar control. Lessons from a cost-effectiveness analysis. Trop. Med. Int. Health. 2009; 7: 955-959.
10. Laguna F. Treatment of leishmaniasis in HIV-positive patients. Ann. Trop. Med. Parasitol. 2008; 97: S135.
11. Baliga M.S., Kurian P.J. *Ixora coccinea* Linn.: traditional uses, phytochemistry and pharmacology. Chin. J. Integr. Med. 2012; 18: 72-79.
12. Elumalai A., Eswaraiah C., Venkatesh Y., Kumar B.S., Narendar C. Phytochemical and pharmacological profile of *Ixora coccinea* Linn. Int. J. Pharm. Life Sci. 2012; 3: 1563-1567.
13. Harborne J.B. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis. New Delhi: Springer (India) Pvt. Ltd.; 2010.
14. Pal D., Bhattacharya S., Baidya P., De B.K., Pandey J.N., Biswas M. Antileishmanial activity of Polyalthia longifolia leaf extract on the *in vitro* growth of *Leishmania donovani* promastigotes. Global J. Pharmacol., 2011; 5: 97-100.
15. Bhattacharya S., Biswas M., Haldar P.K. The triterpenoid fraction from *Trichosanthes dioica* root exhibits *in vitro* antileishmanial effect against *Leishmania donovani* promastigotes. Pharmacogn. Res. 2013; 5: 109-112.
16. Wright C.W., Phillipson J.D. Natural products and the development of selective antiprotozoal drugs. Phytother. Res. 1990; 4: 127-139.
17. Sereno D., Lemesre L.L. Axenically cultured amastigote forms as an *in vitro* model for investigation of antileishmanial agents. Antimicrob. Agents Chemother. 1997; 41: 972-976.
18. Cowan M.M. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12: 564-582.
19. Hamburger M., Hostettmann K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry. 1991; 30: 3864-3874.

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