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Antileishmanial activity of *Mangifera indica* leaf extracts on the *in vitro* growth of *Leishmania donovani* promastigotes

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ABSTRACT

Mangifera indica L. (Anacardiaceae), commonly known as Mango, is a large evergreen tree indigenous to Asia and found throughout the Indian subcontinent. In the present study, the in vitro antileishmanial activity of petroleum ether, chloroform and methanol extracts from M. indica 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, all the test extracts markedly inhibited the growth of L. donovani promastigotes in vitro in a dose dependent manner. The methanol extract was found to be the most active followed by the chloroform and petroleum ether extracts respectively. Therefore, from the present study it can be inferred that M. indica leaf extracts exhibited remarkable antileishmanial activity against Leishmania donovani promastigotes in vitro.

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].

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.

Mangifera indica L. (Anacardiaceae), commonly known as Mango in English is a large evergreen tree indigenous to tropical Asia including India and now completely naturalized in many parts of tropics and subtropics. In the Indian subcontinent it is commercially cultivated for its highly palatable fruits. Traditionally this plant has been used for several medicinal purposes. In all the regions of *M. indica* distribution, one of main parts used medicinally is the bark; its medicinal uses throughout the world are reported [11, 12]. However, reports on the experimental studies on its leaf are comparatively scanty. In the present study, therefore, we have aimed to evaluate *in vitro* antileishmanial activity of *M. indica* leaf extracts against *Leishmania donovani* promastigotes.

Materials and methods

Plant material

The mature leaves of *Mangifera indica* L. (Anacardiaceae), were collected during August 2011 from Krishnanagar, 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/94/2011/Tech II/590] 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.

Extraction

The dried powdered plant material was defatted with petroleum ether (60-80°C), the percentage extractive value was 1.06 % w/w. The defatted powdered material thus obtained was further extracted successively with chloroform and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 3.59 % w/w and 4.70 % w/w respectively.

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The preliminary phytochemical analysis was performed for all 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.

Parasite culture and antileishmanial activity

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 compounds along with Medium-199 at 22 °C. The 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 MEPL 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 and 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 *M. indica* leaf extracts on the *in vitro* growth of *Leishmania donovani* promastigotes. Here, all the test extracts significantly and dose dependently inhibited the growth of the promastigote forms of *L. donovani* (strain AG 83) *in vitro* (Tables 1-3). From the IC_{50} values it became evident that the methanol extract was the most active followed by the chloroform and petroleum ether extracts respectively.

Prelimiary phytochemical analysis showed the presence of triterpenoids and steroids in the petroleum ether extract, triterpenoids and alkaloids in the chloroform extract and triterpenoids, alkaloids, polyphenolics, saponins, glycosides and carbohydrates in the methanol extract. Among them alkaloids and polyphenolics are the most reported phytoconstituents showing antimicrobial property both *in vitro* and *in vivo* [19]. The presence of maximum constituents including alkaloids and/or polyphenols may be responsible for the most promising antileishmanial activity of the methanol 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 [20]. 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 *M. indica* leaf 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 investigation, it can be concluded that *Mangifera indica* 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 *M. indica* leaf. However, further phytochemical and *in vivo* studies 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 petroleum ether extract of *M. indica* leaf against *Leishmania* promastigotes culture (2×10^6 cells/ml)

Pet ether extract (µg/ml)	Percentage lysis of promastigotes with respect to control (0.2% DMSO)*	IC_{50} value (µg/ml)
3	50.14	2.99
5	57.17	
10	65.26	
15	72.18	
30	82.91	

*Mean of three replicates.

Table 2. Effect of chloroform extract of *M. indica* leaf against *Leishmania* promastigotes culture (2×10^6 cells/ml)

Chloroform extract (µg/ml)	Percentage lysis of promastigotes with respect to control (0.2% DMSO)*	IC_{50} value (µg/ml)
3	51.50	2.91
5	58.27	
10	68.37	
15	76.28	
30	89.87	

*Mean of three replicates.

Table 3. Effect of methanol extract of *M. indica* 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	54.59	2.74
5	60.29	
10	69.16	
15	78.18	
30	91.71	

*Mean of three replicates.