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of plants used in Indian traditional
medicine**

Evaluation of antileishmanial activity of plants used in Indian traditional medicine

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Abstract

The present study was aimed at *in vitro* antileishmanial screening of 10 plants used in the traditional medicine in India. MTT method was used to evaluate the cell death after application of 100, 250, 350 and 500 µg/mL of the methanolic extracts followed by incubation for 24 hours at 25°C. Methanolic leaf extracts of *Acorus calamus*, *Alstonia scholaris* and *Berberis aristata* showed significant antileishmanial activity at a concentration of 500 µg/mL. In order to identify the antileishmanial compounds present in the active extracts of the screened plants, an LC-MS analysis of the tested extracts was carried out. The active extracts revealed the presence of some natural products with known antileishmanial activity along with other compounds. The present study suggests that the active plant extracts may be processed to isolate the compounds that may further be screened for their antileishmanial potential.

Introduction

Leishmaniasis is a current public health concern and is among the five parasitic diseases of high social impact world-wide (de Albuquerque Melo et al., 2014). Despite the existence of several antileishmanial drugs including pentavalent antimonials, amphotericin B and pentamidine, none is fully effective due to toxicity, variable efficacy, long-term parenteral administration and emergence of drug resistance (Camacho et al., 2003; Croft et al., 2006). The drugs which are effective to some extent are very expensive and are usually unavailable in the endemic areas of the disease. People in rural areas of developing countries have been using traditional medicinal plants as oral decoctions for the treatment of visceral leishmaniasis and the paste form for topical application for curing skin infections like cutaneous leishmaniasis (Chan-Bacab and Pena-Rodriguez 2001). In the present study, ten of the traditionally used medicinal plants were selected on the basis of their ethno-botanical reports and were screened for the anti-leishmanial potential against *L. donovani* promastigotes.

Materials and Methods

Plant material and preparation of extracts

Authenticated plants were obtained from Y.S. Parmar University of Horticulture and Forestry, Nauni, H.P., India. Plant parts included in the study were dried under shade and pulverized to yield coarse powder. The powder of each plant was then extracted in methanol using hot soxhlet extraction for 24 hours. The extracts were concentrated under reduced pressure using rotary evaporator and the concentrated extracts were further dried in a desiccator using calcium chloride as desiccant. Dried extracts were weighed to obtain the percentage yield and stored in air tight bottles at 4°C until use.

Parasite stock culture

Axenic culture of *L. donovani* (LdMIPL-1) was maintained at 25°C in RPMI 1640 (Himedia, India) medium supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) (Himedia, India), streptomycin (150 µg/mL), penicillin G (100 µg/mL) and gentamycin



(150 µg/mL) at pH 7.2.

Antileishmanial assay

For antileishmanial activity, pro-mastigotes of *L. donovani* were sub-cultured in Schneider's Insect Medium (Himedia, India) supplemented with 10% heat inactivated FBS, streptomycin (150 µg/mL), penicillin G (100 µg/mL) and gentamycin (150 µg/mL). The antileishmanial screening was performed in 96-well flat bottom tissue culture plates (Corning Life Sciences, USA). One hundred microliters of cell suspension containing 2×10^6 to 3×10^6 cells/mL was poured in each well of the plate. Four different concentrations of the methanolic extracts i.e. 100, 250, 350 and 500 µg/mL, dissolved in dimethyl sulfoxide (<0.025% v/v), were added to the culture. The plates were then incubated at 25°C for 24 hours. Amphotericin B and sodium stibugluconate were used as positive controls and cell suspension with 0.02% DMSO was used as a negative control. Inhibition of the promastigotes was assessed by measuring the cleavage of 10 mg/mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Mossman, 1983). The absorbance was measured by using ELISA plate reader (BioTek, USA) at 595 nm. Percent growth inhibition was calculated by the following formula:

$$\% \text{ of inhibition} = \frac{\text{OD control} - \text{OD treated}}{\text{OD control}} \times 100$$

LC-MS analysis of the active extracts

The LC-MS analysis was carried out on X-Bridge C18 (2.1 × 50 mm, 3.5 µm) column fitted to an LC-MS 6320 Ion Trap instrument (Agilent Technologies). A gradient of 50 to 100% acetonitrile in 25 mM ammonium acetate buffer was used as a mobile phase and the flow rate was set at 0.4 mL/min. Injection volume was 5 µL.

Statistical analysis

All the assays were performed in triplicate with at least

two replicates of each concentration tested. The results were expressed as mean ± standard error of the mean. The statistical analysis of the differences between mean values obtained was done by means of one-way ANOVA using GraphPad Prism 5.02 software. A value of $p < 0.05$ was considered significant.

Results

A total of ten methanolic extracts of medicinal plants used in traditional medicine in India were evaluated for their antileishmanial potential using MTT reduction assay (Table I). Methanolic extracts of *Acorus calamus* leaves, *Alstonia scholaris* leaves and *Berberis aristata* leaves showed significant leishmanicidal activity against *L. donovani* promastigotes. The most active extract was *A. scholaris* at a concentration of 500 µg/mL. It inhibited 40.3% of *L. donovani* promastigotes within 24 hours of incubation. The inhibition was concentration dependent as the inhibition increased with the increase in concentration of the extract. Leaf extract of *B. aristata* at a concentration of 500 µg/mL showed 36.2% inhibition whereas *A. calamus* showed 35.6% inhibition (Figure 1).

The three active extracts viz. *Acorus calamus*, *Alstonia scholaris* and *Berberis aristata* were subjected to LC-MS analysis so as to identify the antileishmanial compounds present in the extracts. In the case of *A. calamus*, the major chromatographic peak eluted at 5.7 min and presented a base peak at m/z 210 [M+2]⁺ in the mass spectrum. The molecular ion peak, however, was detected at m/z 208 [M]⁺. This molecular weight matched with that of the three major phenyl propanoids namely α -, β - and γ -asarones (**1-3**) reported from the plant (Figure 2). The chromatogram of *A. scholaris* revealed the presence of several overlapping peaks attributed to alkaloids. The MS analysis indicated the presence of picrinine (eluted at 6.0 min, m/z 337 [M-1]⁺, **4**) and nareline and/or tetrahydroalstonine (eluted at

Table I

Details of the plants used for antileishmanial activity evaluation

Botanical name	Family	Local Name	Part used	Extractive value (%)
<i>Acorus calamus</i>	Acoraceae	Boiye	Leaf	7.1
<i>Alstonia scholaris</i>	Apocynaceae	Chitvan	Leaf	9.4
<i>Andrographis paniculata</i>	Acanthaceae	Kalmegh	Stem	11.2
<i>Berberis aristata</i>	Berberidaceae	Kashmal	Leaf	10.8
<i>Butea monosperma</i>	Fabaceae	Palash	Flower	18.2
<i>Eclipta prostrata</i>	Asteraceae	Bhringraja	Whole plant	9.4
<i>Gloriosa superba</i>	Colchicaceae	Kalihari	Tuber	2.5
<i>Juglans regia</i>	Juglandaceae	Akhrot	Bark	10.9
<i>Mesua ferrea</i>	Calophyllaceae	Nagkesara	Flower bud	14.7
<i>Tinospora cordifolia</i>	Menispermaceae	Guduchi	Stem	6.4

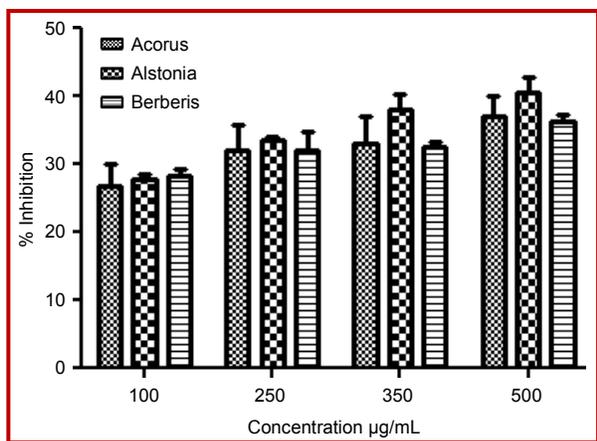


Figure 1: Percentage inhibition of *L. donovani* promastigotes in the presence of methanolic leaf extracts of *Acorus calamus*, *Alstonia scholaris* and *Berberis aristata* (100-500 µg/mL). Each data value represents mean \pm SEM of at least three experiments performed in duplicate

6.6 min, m/z 351 $[M-1]^+$, **5a/b**). The major peak in the chromatogram of *B. aristata* extract eluted at 4.6 min and presented an $[M]^+$ peak at m/z 336. This molecular weight corresponds to berberine (**6**), a major compound reported from this plant. Epiberberine (**7**), another compound with similar molecular structure, also corresponds to this molecular weight. A small peak in the chromatogram, eluting at 5.4 min, gave an $[M]^+$ ion at m/z 352 in the mass spectrum. This molecular ion could be attributed to berberastine (**8**). Figure 2 presents the structures of compounds **1-8**.

Discussion

Out of the ten crude methanolic extracts, three have

shown significant leishmanicidal activity by inhibiting 35-40% promastigotes within 24 hours of application. These active extracts were further analysed by LC-MS to determine the presence of various components. The major compounds present in the extract of *Acorus calamus* were isomeric phenyl propanoids (**1-3**). This is the first report of the antileishmanial activity of this plant. The ethanolic extract of the stem of *Alstonia scholaris* has been previously screened for its antileishmanial activity against some strains of *L. donovani* (Rocha et al., 2005). However, no study so far has reported the antileishmanial activity of the compounds detected in the extract *viz.* picrinine (**4**) nareline (**5a**) and tetrahydro-alstonine (**5b**). In the case of *B. aristata*, the chief alkaloid *i.e.* berberine (**6**) is used in folk remedies for the treatment of cutaneous leishmaniasis. Other structurally related alkaloids have also been found active against *Leishmania* (Chan-Bacab and Pena-Rodriguez, 2001). Further studies are required to establish the antileishmanial potential of these compounds.

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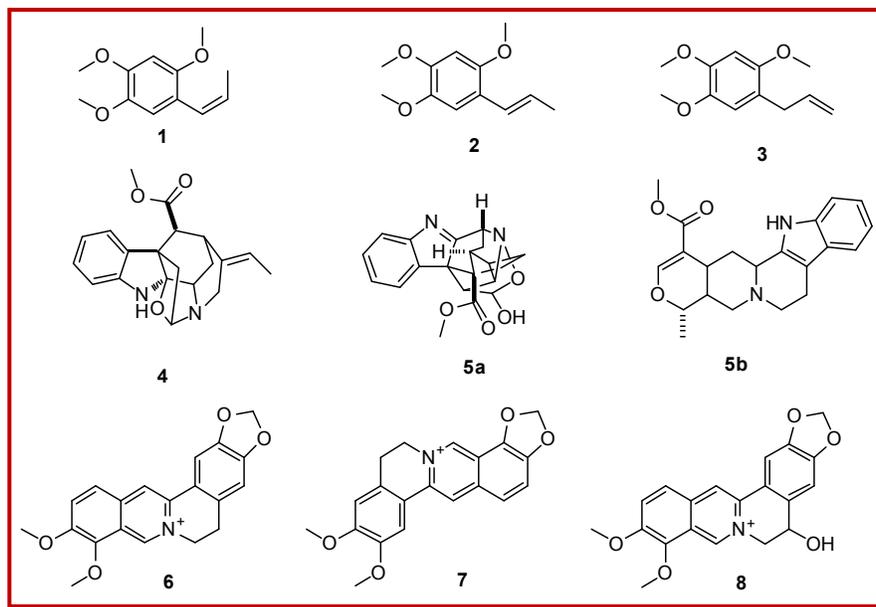


Figure 2: The structures of the compounds present in the active extracts as indicated by LC-MS analysis

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