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Evaluation of antileishmanial activity of South Indian medicinal plants against *Leishmania donovani*

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HIGHLIGHTS

- Medicinal plant extracts were evaluated against *Leishmania donovani*.
- In this study, we assessed 10 extracts on *L. donovani* promastigote viability.
- The extracts from *Anisomeles malabarica* and *Ricinus communis* were found effective.
- IC_{50} value of *A. malabarica* and *R. communis* showed 126 and 184 μg/mL.

GRAPHICAL ABSTRACT

Antileishmanial activity of acetone and methanol leaf extracts of *Anisomeles malabarica*, *Ocimum basilicum*, flower of *Gloriosa superba*, leaf and seed of *Ricinus communis* were evaluated against *Leishmania donovani*. Results demonstrated that leaf methanol extracts of *A. malabarica*, and *R. communis* showed good antileishmanial activity and could play an important role in herbal formulations for the treatment of visceral leishmaniasis.

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ABSTRACT

Infections due to protozoa of the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries. The aim of this study was to evaluate the *in vitro* antileishmanial activity of the acetone and methanol leaf extracts of *Anisomeles malabarica*, flower of *Gloriosa superba*, leaf of *Ocimum basilicum*, leaf and seed of *Ricinus communis* against promastigotes form of *Leishmania donovani*. Antiparasitic evaluations of different plant crude extracts were performed on 96 well plates at 37 °C for 24–48 h. Out of the 10 experimental plant extracts tested, the leaf methanol extracts of *A. malabarica*, and *R. communis* showed good antileishmanial activity (IC_{50} = 126 ± 19.70 and 184 ± 39.33 μg/mL), respectively against promastigotes. Effective antileishmanial activity was observed making these plants as good candidates for isolation of antiprotozoal compounds which could serve as new lead structures for drug development.

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1. Introduction

Leishmaniasis comprises a group of diseases caused by several species of *Leishmania* and expresses a variety of clinical symptoms. In addition, this group of diseases is the third largest among infectious diseases transmitted by vectors, behind malaria and filariasis (Solano-Gallego et al., 2009). World Health Organization (WHO) classified leishmaniasis as a category 1 disease, i.e. emerging and uncontrollable disease (Murray et al., 2005). Leishmaniasis occurs mainly in three clinical forms, of which visceral leishmaniasis (VL) or kala-azar caused by *Leishmania donovani* is the most severe form. The estimated annual incidence of VL is around 500,000 in 61 countries with 90% of these cases confined to 5 countries namely India, Bangladesh, Nepal, Sudan and North Eastern Brazil (World Health Organization, 2007). Therefore, there is an urgent need for new, less toxic, safe, effective and economically feasible drugs for the treatment of leishmaniasis. The researchers therefore have diverted their attention towards plant kingdom, which are eco-friendly and cost effective. The use of secondary metabolites from certain plants were effective in *in vitro* studies on different forms of *Leishmania* sp., demonstrating the feasibility of obtaining new combating compounds against the parasite (Chen et al., 1993; Schinor et al., 2007; Soares et al., 2007).

Anisomeles malabarica is distributed in major parts of India and especially in South India as a traditional medicinal plant commonly known as Peymarutti. The herb is reported to possess anti-spasmodic, anti-periodic properties and used in rheumatoid arthritis, it is used in the tradional treatment of snakebite as antidote (Perumalswamy et al., 2008). In India, *Gloriosa supberba* is used as an embolic in labor, purgative, anthelminthic and cure against leprosy, colic, chronic ulcers, hemorrhoids, skin parasites, head lice and tumors (Geetha et al., 2007; Jagtap et al., 2006). Previous studies showed that the fragrant leaves of *Ocimum basilicum* (basil) is used as an antiseptic, preservative, sedative, digestive regulator and diuretic (Ji-Wen et al., 2009). Basil has been reported as an insect repellent, and the essential oil was used for anti-viral, anti-microbial, anti-oxidant, and anti-cancer activity (Bozin et al., 2006; Chiang et al., 2005). *Ricinus communis* is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world (Ivan, 1998). The plant is documented to possess antihelmintic, antifertile, diuretic and many other medicinal properties (Nath et al., 2011).

As far as our literature survey could ascertain, no information was available on the antileishmanial activity of the experimental plant species given here. Therefore, the aim of this study was to investigate the leishmanicidal activity of the acetone and methanol extracts of four plant species from Tamil Nadu, India. This is the first report on *in vitro* activity on *L. donovani* of selected plants. The effective extracts will be further explored extensively with a view to isolate the most active fraction and also to extract a pure compound that may be responsible for activity.

2. Materials and methods

2.1. Collection of materials

The leaves of *A. malabarica*, flower of *G. superba*, leaves of *O. basilicum*, and leaves and seed of *R. communis* were collected in and around Melvisharam, Vellore district, Tamil Nadu, India. The taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Govt. Arts College for Women, Walajapet, Vellore, India. The voucher specimens were numbered and kept in the Laboratory of Zoology, C. Abdul Hakeem College, Melvisharam, South India. All chemicals were obtained from Sigma–Aldrich (St. Louis, Missouri, USA) except phenazine methosulphate (PMS) (Sisco Research Laboratories, Mumbai, India), MTS or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (Promega, Madison, Wisconsin, USA), and RPMI 1640 (Gibco-BRL).

2.2. Preparation of plant extracts

Plant materials (leaves, seed, and flower) were air-dried for 10–25 days in the shade at the environmental temperatures (27–37 °C). The dried plant parts were powdered mechanically using commercial electrical stainless steel blender and were extracted with acetone (2000 mL) and methanol (1700 mL) (Qualigens) using soxhlet apparatus (boiling point range 60–80 °C) for 8 h. The extracts were concentrated under reduced pressure of 22–26 mm Hg at 45 °C and the residue obtained were stored at 4 °C (Rahuman and Venkatesan, 2008). One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 500 μg/mL was prepared with dechlorinated tap water. Dimethyl sulfoxide (DMSO) was used as an emulsifier at the concentration of 0.04% in the final test solution.

2.3. Parasite culture

Promastigotes of *L. donovani* strain MHOM/IN/83/AG83 were routinely cultured at 24 °C in medium 199 (M199) supplemented with 10% fetal bovine serum (FBS), penicillin G (50 IU/mL) and streptomycin (50 μg/mL). Log phase promastigotes were sub cultured every 72–96 h, inoculum being 1×10^6/mL (Saha et al., 2009).

2.4. In vitro evaluation of anti-promastigote activity

The antileishmanial activity of experimental plant extracts established in promastigotes, and cell viability was measured using the modified MTS–PMS assay (Ganguly et al., 2006). Briefly, log phase promastigotes (1×10^5 cells/200 μL of M199 medium/well) were incubated with supplied extracts (0–500 μg/mL) for 24–48 h and parasite viability was measured. MTS [3-(4,5 dimethylthiazol-2-yl)5-(3-carboxymethoxyphenyl)-2-(-4-sulfonyl)-2Htetrazolium, inner salt] (2.0 mg/mL) and PMS (Phenazine methosulphate) (0.92 mg/mL) were added in a ratio of 5:1 (20 μL per well) and the plates were incubated in dark for 3 h at 37 °C, resultant absorbance measured at 490 nm using ELISA reader. Accordingly, the specific absorbance that represented formazan production was calculated by subtracting of background absorbance from total absorbance. The mean % viability was calculated as follows:

$$\frac{\text{Mean specific absorbance of treated parasites}}{\text{Mean specific absorbance of untreated parasites}} \times 100$$

2.5. Statistical analysis of data

Each experiment was performed at least thrice in duplicates and results expressed as mean ± standard error of the mean (SEM). The IC_{50} values were calculated using sigmoid dose–response curves in Graph Pad Prism 5.0 software (Grecco et al., 2012).

3. Results and discussion

The acetone and methanol leaf extracts of *A. malabarica*, flower of *G. superba*, leaf of *O. basilicum*, leaf and seed of *R. communis* were selected based upon their medicinal uses (Table 1). In the present study, we have examined the concentration-dependent inhibitory effect of different plant extracts on *L. donovani* to ascertain their