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In vitro Antileishmanial Activity of *Pleumeria pudica* Leaf Extracts on *Leishmania donovani* Promastigotes

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Abstract: The objective of the present study was to evaluate *in vitro* antileishmanial activity of leaves form *Plumeria pudica* Jacq. (Apocynaceae). In the present study, the *in vitro* antileishmanial activity of petroleum ether, chloroform and methanol extracts from *P. pudica* 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 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 chloroform and petroleum ether extracts. Therefore, from the present study it can be inferred that *P. pudica* leaf exhibited remarkable antileishmanial activity against *Leishmania donovani* promastigotes *in vitro*.

Key words: Antileishmanial · Promastigotes · *Leishmania donovan* · *Plumeria pudica*

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.

Plumeria pudica Jacq. (Apocynaceae), is indigenous to Panama, Colombia and Venezuela and grows well in subtropical countries like India. It is an evergreen to semi-deciduous shrub with white flowers that continue to develop whenever the plant is in active growth. This profuse bloomer has unusual spoon-shaped leaves and its flowers are white with a yellow centre [11]. It is commonly called wild plumeria, bridal bouquet, gilded

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spoon etc in English and *Brindabon Champa* in Bengali. It is grown throughout the plains of India as ornamental plant for its attractive flowers. The plant looks attractive even when it is not flowering, because of its beautiful leaves. However, reports on the experimental pharmacological studies on this plant are practically not found. In the present study, therefore, we have aimed to evaluate the *in vitro* antileishmanial activity of *P. pudica* leaf extracts against *Leishmania donovani* promastigotes.

MATERIALS AND METHODS

Plant Material: The mature leaves of *Plumeria pudica* Jacq. (Apocynaceae), were collected during November 2011 from Howrah, 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/86/ 2011/ Tech. II/584] 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 (350 g) was first defatted with petroleum ether (60-80°C) and the petroleum ether extract was obtained (yield: 3.2%). The defatted powdered material thus obtained was further extracted successively with chloroform 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: 10% and 12.4% respectively). The preliminary phytochemical analysis was performed on these three extracts to identify the phytoconstituents present in the extracts [12].

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 [13, 14]. 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 three 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.

$$\text{Lysis \%} = 100 - \{(\text{test} - \text{positive control})/(\text{control} - \text{positive control})\} \times 100$$

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 petroleum ether extract, triterpenoids in chloroform extract; and triterpenoids, saponins, glycosides and carbohydrates in methanol extract of *P. pudica* leaf.

In the present antileishmanial evaluation, each of the three extracts of *P. pudica* 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 1-3. From the determined IC_{50} values it became evident that the methanol extract was the most active followed by chloroform and petroleum ether extracts of *P. pudica* leaf.

Table 1: Effect of petroleum ether extract 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	49.24	3.04
5	53.18	
10	61.36	
15	69.19	
30	70.21	

*Mean of three replicates.

Table 2: Effect of chloroform extract 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	50.53	2.98
5	54.29	
10	63.27	
15	72.18	
30	83.81	

*Mean of three replicates.

Table 3: Effect of methanol extract 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	51.19	2.93
5	59.24	
10	61.18	
15	73.78	
30	87.79	

*Mean of three replicates.

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 [15].

The *in vivo* efficiencies of drugs have been reported to be under the control of different parameters, such as pharmacokinetic parameters [16], 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 [17]. 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 *P. pudica* 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 chloroform and petroleum ether extracts.

Preliminary phytochemical studies revealed the presence of triterpenoids, steroids saponins, glycosides and carbohydrates in *P. pudica* leaf. The observed antileishmanial activity may be due to the presence of triterpenopids in all three test extracts [18].

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 *P. pudica* 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 *Plumeria pudica* 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 *Plumeria pudica* 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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REFERENCES

1. Anonymous, 2011. Report of the Consultative Meeting on Cutaneous Leishmaniasis. Geneva: World Health Organization.
2. Desjeux, P., 2010. The increase in the risk factors for leishmaniasis worldwide. Transactions of the Royal Society of Tropical Medicine and Hygiene, 95: 239.
3. Handman, E., 2009. Leishmaniasis: current status of vaccine development. Clinical Microbiology Reviews, 14: 229-243.
4. Chappuis, F., S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar and M. Boelaert Visceral, 2008 leishmaniasis: what are the needs for diagnosis, treatment and control. Nature Reviews Microbiology, 5: 873.
5. Berman, J.D., 1996. Treatment of new world cutaneous and mucosal leishmaniases. Clinical Dermatology, 14: 519-522.
6. Berman, J.D., 2008. Human leishmaniasis: clinical, diagnostic and chemotherapeutic developments in the last 10 years. Clinical Infectious Diseases, 24: 684-703.
7. Ephros, M., E. Waldman and D. Zilberstein, 1997. Pentostam induces resistance to antimony and the preservative chlorocresol in *Leishmania donovani* promastigotes and axenically grown amastigotes. Antimicrobial Agents Chemotherapy, 41: 1064-1068.
8. Lira, R.,S. Sundar, A. Makharia, R. Kenney, A. Gam, E. Saraiva and D. Sacks, 2009. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani.* The Journal of Infectious Diseases, 180: 564-567.
9. Boelaert, M., D. Leray and S.P. Vander, 2009. How better drugs could change kala-azar control. Lessons from a cost-effectiveness analysis. Tropical Medicine and International Health, 7: 955-959.
10. Laguna, F., 2008. Treatment of leishmaniasis in HIV-positive patients. Annals of Tropical Medicine and Parasitology, 97: S135.
11. Anonymous. 2007. Master Gardener Newsletter, March.
12. Harborne, J.B., 2010. Phytochemical methods, a Guide to Modern Techniques of Plant Analysis. New Delhi: Springer (India) Pvt. Ltd.
13. Pal, D., S. Bhattacharya, P. Baidya, B.K. De, J.N. Pandey and M. Biswas, 2011. Antileishmanial activity of Polyalthia longifolia leaf extract on the *in vitro* growth of Leishmania donovani promastigotes. Global Journal of Pharmacology, 5: 97-100.
14. Bhattacharya, S., M. Biswas and P.K. Haldar, 2013. The triterpenoid fraction from *Trichosanthes dioica* root exhibits *in vitro* antileishmanial effect against Leishmania donovani promastigotes. Pharmacognosy Research, 5: 109-112.
15. Wright, C.W. and J.D. Phillipson, 1990. Natural products and the development of selective antiprotozoal drugs. Phytotherapy Research, 4: 127-39.
16. Sereno, D. and L.L. Lemesre, 1997. Axenically cultured amastigote forms as an *in vitro* model for investigation of antileishmanial agents. Antimicrobial Agents Chemotherapy, 41: 972-976.
17. Gupta, N., N. Goyal and A.K. Rastogi, 2010. *In vitro* cultivation and characterization of axenic amastigotes of Leishmania. Trends in Parasitology, 17: 150-153.
18. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12: 564-582.
19. Hamburger, M. and K. Hostettmann, 1991. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry, 30: 3864-3874.