

In-Vitro Evaluation of the Anti-Leishmanial Activity of *Euphorbia Helioscopia* Stem Extract In Comparison With Synthetic Drug Amphotericin B

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ABSTRACT

Behind malaria and filariasis, leishmaniasis is the third largest infectious diseases transmitted by vectors. Aim of this research work was to evaluate the anti leishmanial activity of *Euphorbia helioscopia*. This research work was done in the Department of Animal Sciences, Parasitology Laboratory, Quaid-i-azam University, Islamabad, Pakistan. The experiment was performed according to the protocol described previously with a slight modification. The results show that the *E. helioscopia* has anti leishmanial activity with LC₅₀ value ≤ 10 ug/ml. The standard error for the test plant extract and positive control was calculated with 95% confidence interval having significance value of 0.00. Chi square test and Phrobit analysis were done using the SPSS version 21.

Keywords: Antileishmanial activities, Leishmaniasis, *Euphorbia helioscopia*, Amphotericin B

INTRODUCTION

Behind malaria and filariasis, leishmaniasis is the third largest infectious diseases transmitted by vectors. Leishmaniasis is a term referred to a number of clinical symptoms caused by several species of *Leishmania*, a protozoan (Zahir *et al.*, 2012). These parasites belong to the genus *Leishmania* and are transmitted by the bite of a female *phlebotomine* sand fly (Bero *et al.*, 2011). It is one of the major health problems causing significant morbidity and mortality in Asia, Africa and Latin America (Tahir *et al.*, 1998). There are three clinical forms of leishmaniasis; Cutaneous leishmaniasis (CL), Mucocutaneous leishmaniasis, visceral leishmaniasis (VL), among which Cutaneous leishmaniasis (CL), is the most common form and Visceral leishmaniasis (VL) is the most fatal one with an estimated annual incidence of 500,000 in 61 countries. Several drugs are used to treat leishmaniasis but due to drug resistant strains and side effect cause the search for effective natural drug (Jaffary, 2012; Ullah *et al.*, 2013). Due to variable efficacy, resistant strains and species, the search of new traditional plants having antileishmanial activity is led (Bero *et al.*, 2011).

Some chemical compounds extracted from *Leguminosae* members showed anti leishmanial activity against *Leishmania amazonensis* (Araujo *et al.*, 1998). Extracts of *Annona muricata* were found to be effective against *L. Braziliensis* and *L. panamensis* (Jaramillo *et al.*, 2000). Some Diterpenes and triterpenes isolated from Lamiaceae family member *Salvia cilicica* was reported to have appreciable in vitro antileishmanial activity against *L. donovani* and *L. major* (Tan *et al.*, 2002). *Rhazya stricta* Decne leaves extracts were reported to have Antileishmanial activity against *L. major* (Khan *et al.*, 2012). Moreover sterols isolated from

the roots of *Pentalinon andrieuxii* are found to have anti leishmanial activity (Pan *et al.*, 2012).

The genus *Euphorbia* is the largest in Euphorbiaceae or spurge family with more than 2000 species growing in the form of laticiferous herbs, shrubs and trees, in tropical and temperate zones Asia and other parts of the world (Majid *et al.*, 2010). *Euphorbia* species having biological activities like anti-tumor and anti-cancer (Chem, 2006). For decades *Euphorbia* plant materials have been known poisonous to humans and animals. However pharmacological investigation of the genus revealed that its latex has antiviral, anti-cancer, anti-bacterial, cytotoxic and anti-leishmanial properties (Majid *et al.*, 2010).

MATERIALS AND METHOD

Aim of the current study was to explore the anti leishmanial activity of the stem extract of *Euphorbia helioscopia*. The present research was done in the Department of Animal Sciences, Parasitology Laboratory, Quaid-i-azam University, Islamabad, Pakistan.

Plant Collection

Euphorbia helioscopia (Sun spurge) plants were collected from the vicinity of Quaid-i-azam University, Islamabad, Pakistan. These plants were washed with running water and were identified by Department of Plant Sciences, Quaid-i-azam University, Islamabad. A voucher specimen (QAU/PS/AS256) was kept for future research. Leaves, stem, flowers and roots of the plants were separated and were dried in shade for about three weeks. The stem part was then powdered by electric blender.

Preparation of Plant Extract

Thirty grams fine powder of the *Euphorbia helioscopia* stem part was used for extraction in 300 ml of water for 6 hours using Soxhlet apparatus. Following extraction, solvent was removed using Rotary evaporator at lowest temperature of 37 °C. Crude extract obtained was collected and stored at 4 °C.

Anti-Leishmanial Assay Procedure

In vitro antileishmanial activity of experimental plant was performed according to the protocol described by Nabi *et al.* (2012) with a slight modification.

Parasite Culture

Promastigotes of *L. tropica* (KWH 23) strains were used for the assay. The strains were incubated at 24°C at standard laboratory condition till a culture of 1×10^6 /ml promastigotes was achieved.

Preparation of Stock Solution and Dilutions

A stock solution of 10,000 ppm was prepared from the crude extract by dissolving 1mg of the crude extract in 1 ml Dimethyl sulfoxide (DMSO). The first row of the plate contains 196 µl of M199 media while the remaining rows contain 180 µl of media. About 4 µl of compounds was added to the first row and serially diluted by 10 % and discard 20 µl from the last row.

Evaluation of Anti-Promastigotes Activity

The concentration of DMSO was observed not to exceed >5% so that it has no effect on *Leishmania* parasite morphology (1). For maintaining positive and negative control Amphotericin B and DMSO were used respectively. The plate was then incubated at 24°C for 72 hrs. After three days, the mortality was recorded by counting the live promastigotes in neubar counting chamber.

Statistical Analysis

Experiment was carried in triplicates. The average mortality was subjected to probit analysis for Calculating LC_{50} at 95 % confidence limit using SPSS version 21.

RESULTS

Different concentration of the plant stem extract was taken in comparison with the amphotericin B as a positive control and 5% DMSO as a negative control. The concentration of the *E. helioscopia* and amphotericin B were kept same i.e. 0.05, 0.5, 5, 50, and 500 μ g/ml. The percent mortality recorded were 4.66 ± 0.76 , 10.96 ± 1.05 , 29.62 ± 0.86 , 60.47 ± 1.44 and 100 ± 0.00 for the aqueous stem extract of *E. helioscopia* and 30.27 ± 0.54 , 45.12 ± 1.10 , 80.87 ± 1.78 , 100 ± 0.00 and 100 ± 0.00 for amphotericin B respectively. The mortality in the negative control was zero. The LC_{50} and LC_{90} for *E. helioscopia* (9.94 μ g/ml, 226.54 μ g/ml) and amphotericin B (0.35 μ g/ml, 9.49 μ g/ml) were also calculated. The chi square (R^2) value for the test plant and positive control show negligible differences having value of 0.927 and 0.922, as shown in table 1.

The standard error for the test plant extract and positive control was calculated with 95% confidence interval having significance value of 0.00, and represented with standard error bars in figure 1. The comparative percent mortality of *E. helioscopia* and amphotericin B were also shown with different colors along with the trend lines.

Table 1. In vitro anti leishmanial activity of *E. helioscopia* stem extract, showing average, LC_{50} , LC_{90} values

Concen. in (μ g/ml)	Anti leishmanial activity of <i>Euphorbia helioscopia</i>					LC_{50}	LC_{90}	Df	X^2	R^2
	0.05 (μ g/ml)	0.5 (μ g/ml)	5 (μ g/ml)	50 (μ g/ml)	500 (μ g/ml)					
E. helioscopia	4.66 ± 0.76	10.96 ± 1.05	29.63 ± 0.86	60.47 ± 1.44	100 ± 0	9.94	226.54	3	41.71	0.927
Amp B (Positive control)	30.27 ± 0.54	45.12 ± 1.10	80.87 ± 1.78	100 ± 0	100 ± 0	0.35	9.49	3	13.34	0.922
Negative Control	100	100	100	100	100	--	--	--	--	--

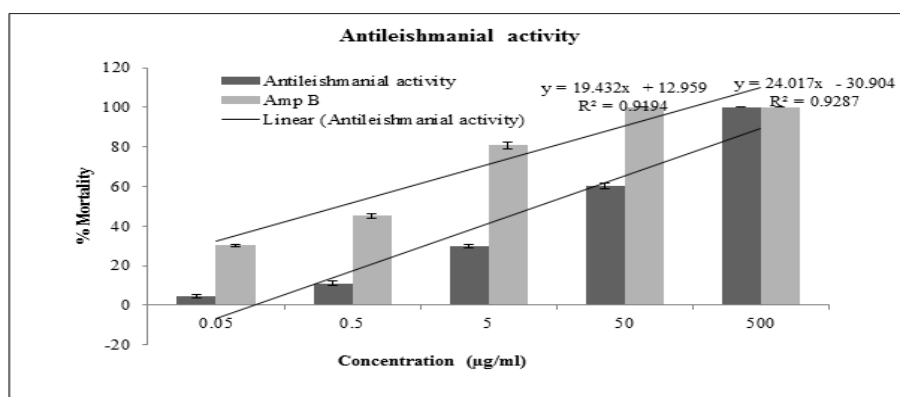


Figure 1. In-vitro anti leishmanial activity of *E. helioscopia* stem extract

DISCUSSION

Over 100 plants have been reported to be active against various forms of leishmanial parasites (Rocha *et al.*, 2005). The other studies showed that the *Ixora coccinea* leaf extract having anti leishmanial activity against the promastigotes of *L. donovani* (Naskar *et al.*, 2013). The root extract of *Perovskia abrotanoides* shows anti leishmanial activities against the *L. major* (Jaafari *et al.*, 2007). The pharmacological screening of methanolic extract of *Aloe vera* leaf and *Tamarix aphylla* bark were assessed to investigate the in vitro anti leishmanial activity of the medicinal plants against cutaneous leishmaniasis by Iqbal *et al.*, (2012).

Their finding show that, *Aloe vera* and *Tamarix aphylla* had a significant dose dependant anti promastigote activity against *L. tropica* as that suggest promising phytotherapeutic agents for cutaneous leishmaniasis. The present study showed the anti leishmanial activity of the aqueous extract of *E. helioscopia* on the promastigotes of *L. tropica* (KWH 23) strains. Our finding also revealed that, extract of *E. helioscopia* has anti promastigote activity against *L. tropica*. Both studies show that, the different plants used in the research having anti leishmanial activities.

Other members of Euphorbiaceae family are reported to have anti leishmanial, antioxidant, larvicidal and insecticidal activities (Zahir *et al.*, 2012). In vivo anti leishmanial effects of traditional herbal extracts against Cutaneous Leishmaniasis was studied by Mohammad, 2011. It is also found that members of the genus *Euphorbia* have anticancer, anti-proliferative, antimicrobial, anti-inflammatory, anti-helminthic, cytotoxic and antioxidant properties (Serkan Ozbelgin, 2012). The current study showed the anti leishmanial activity of the aqueous extract of *E. helioscopia* on KWH strain (LC₅₀= 9.94 ug/ml).

CONCLUSION

The current study revealed that the stem extract of *E. helioscopia* have anti leishmanial activity. The current investigation reveals that *E. helioscopia* extract contain secondary metabolites and other members are used traditionally for the ailment of different diseases, posses' activity against *Leishmania tropica*. As crude form of *E. helioscopia* extract showed promising antileishmanial results. *E. helioscopia* and other members need further investigation so that the pure bioactive antileishmanial compounds should be isolated with promising results, less side effects and cost effective.

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REFERENCES

- [1] Ali et al. (2011). Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* Curt. *BMC Complementary and Alternative Medicine*, 11:99.
- [2] Araujo et al. (1998). Antileishmanial activity of compounds extracted and characterized from *Centropogon sclerophyllum*. *Phytochemistry*, 49(3), 751–754. doi:10.1016/S0031-9422(97)00976-X
- [3] Bero et al. (2011). In vitro antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. *Journal of ethnopharmacology*, 137(2), 998–1002. doi:10.1016/j.jep.2011.07.022
- [4] Chem, T. J. (2006). Secondary Metabolites from *Euphorbia helioscopia* and Their Vasodepressor Activity. *Turk J Chem.*, 30, 325–332.
- [5] Iqbal et al. (2012). Comparative efficacy of *Aloe vera* and *Tamarix aphylla* against cutaneous leishmaniasis. *International Journal of Basic Medical Sciences and Pharmacy*, 2(2), 42-45.
- [6] Jaramillo et al. (2000). Cytotoxicity and antileishmanial activity of *Annona muricata* pericarp. *Fitoterapia*, 71(2), 183–6. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10727816>
- [7] Jaafari et al. (2007). Evaluation of leishmanicidal effect of *Perovskia abrotanoides* Karel. root extract by in vitro leishmanicidal assay using promastigotes of *Leishmania major*. *Pharmacologyonline*, 1, 299–303.
- [8] Kaur et al. (2010). Antileishmanial effect of cisplatin against murine visceral leishmaniasis. *Parasitology International*, 59(1), 62–9. doi:10.1016/j.parint.2009.10.006
- [9] Khan et al. (2012). Antileishmanial, cytotoxic, antioxidant activities and phytochemical analysis of *Rhazya stricta* Decne leaves extracts and its fractions. *Asian Journal of Plant Science and Research*, 2(5), 593-598.
- [10] Majid et al. (2010). Biological Screening of *Euphorbia Aellenii*. *Iran J Pharm Res.*, 9(4), 429–436.
- [11] Mohammad, B. A. (2011). Antileishmanial effects of traditional herbal extracts against cutaneous leishmaniasis in vivo. *Advances in Environmental Biology*, 5(10), 3188-3195
- [12] Nabi et al. (2012). In vitro Antileishmanial, Antitumor Activities and phytochemical Studies of Methanolic Extract and its Fractions of *Juniperus Excelsa* Berries. *World Applied Sciences Journal*, 19(10), 1495–1500.
- [13] Naskar et al. (2013). Antileishmanial effect of *Ixora coccinea* leaf extracts on the in vitro growth of *Leishmania donovani* promastigotes. *Journal of Advanced Pharmacy Education & Research*, 3(4), 471-474.
- [14] Pan et al. (2012). Sterols with antileishmanial activity isolated from the roots of *Pentalinon andrieuxii*. *Phytochemistry*, 82, 128–35. doi:10.1016/j.phytochem.2012.06.012

- [15] Özbilgin, S., Citoğlu, G. S. T (2012). Review: Uses of Some Euphorbia Species in Traditional Medicine in Turkey and their Biological Activities. *Turk J. Pharm. Sci.*, 9(2), 241–255.
- [16] Rocha et al. (2005). A review of natural products with antileishmanial activity . *Phytomedicine*, 12, 514-535.
- [17] Tahir et al. (1998). The Potential Antileishmanial Activity of some Sudanese Medicinal Plants, *Phytotherapy Research*, 12(June), 576–579.
- [18] Tan et al. (2002). Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. *Phytochemistry*, 61(8), 881–4. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12453510>
- [19] Ullah et al. (2013). Prevalence of *Cutaneous leishmaniasis* in afghan refugees in Upper Dir district Pakistan. *Golden Research Thoughts*, 3(6), 1-6.
- [20] Uniyal et al. (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. *Journal of Ethnobiology & Ethnomedicine*, 2(14) 1-14.
- [21] Zahir et al. (2012). Evaluation of antileishmanial activity of South Indian medicinal plants against *Leishmania donovani*. *Experimental parasitology*, 132(2), 180–184. doi:10.1016/j.exppara.2012.06.012