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Full Length Research Paper

In vitro screening of selected Saudi medicinal plants for potential leishmanicidal activity

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Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania*. This major world health problem ranks among the six most important tropical infectious diseases by the World Health Organization. The current study was undertaken to exploit the antileishmanic activity of three medicinally active plants of Saudi flora, *Calotropis procera*, *Caralluma sinaica* and *Cordia sinensis* using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. The highest antileishmanial activity against *Leishmania major* was exhibited at 200 µg/ml by *n*-hexane fraction of *C. sinaica* (81.44% inhibition). Meanwhile, approximately 60% inhibition was shown by chloroform, ethyl acetate, *n*-butanol and aqueous extracts of this plant. *C. procera* extracts showed lower inhibition potency ranging from 47 to 56% of inhibition, compared to *C. sinaica* at similar concentration.

Key words: *Leishmania*, *Cordia*, *Caralluma, Calotropis*.

INTRODUCTION

Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*. In the vertebrate host, *Leishmania* survives and divides in tissue macrophages as intracellular amastigote, whereas in the invertebrate host (sand fly) they are transformed into free living flagellates called promastigotes (El-On et al., 2009). Several forms of the disease exist: visceral leishmaniasis (VL), mucocutaneous (MCL), diffuse cutaneous leishmaniasis (DCL) and cutaneous leishmaniasis (CL) (Martín-Quintal et al., 2009). The World Health Organization (WHO) estimates the worldwide prevalence to be approximately 12 million cases, with approximately 2 million new cases per year, 500, 000 of which are VL and 1, 500,000 CL. *Leishmania tropica* and *Leishmania major* are the causative parasites of CL in the Middle East, and 90% of the estimated cases occur in Saudi Arabia, Iran Syria, Afghanistan, Brazil and Peru (WHO, 2007).

Treatment options are severely limited and there is no acceptable vaccine against this disease. Chemotherapy with pentavalent antimonials, either sodium stibogluconate or meglumine antimonite, remain the drugs of choice in the treatment of leishmaniasis (Alavi-Naini, 2008). However, these compounds are expensive, not very effective and may have severe side effects, and patients require prolonged treatments.

The second line drugs, such as amphotericin B and pentamidine, may be even more toxic (Martín-Quintal et al., 2009; Kigondu et al., 2009). Miltefosine is the first effective oral treatment for *Leishmaniasis* (Khademvatan et al., 2011b). In laboratory studies, it has been proven to be effective in treating visceral leishmaniasis (Sundar et al., 2002); however, data on miltefosine for the treatment of cutaneous leishmaniasis are limited and it is undergoing clinical trials in several countries (Richard and Werbovetz, 2010).

Given the limitations of the current treatments, there is an impelling need for new leishmanicidal drugs. Plant extracts or plant-derived compounds are likely to provide a valuable source of new medicinal agents.

The current study was undertaken to exploit three medicinally active plants of Saudi flora, *Calotropis procera* (Ait). R. Br. (Asclepiadaceae) *Caralluma sinaica*

(Asclepiadaceae) and *Cordia sinensis* (Boraginaceae).

MATERIALS AND METHODS

Collection and processing of plant material

The plants material used in this study were collected in March 2008. *C. procera*, was collected from Riyadh, meanwhile, *C. sinensis* and *C. sinaica* were collected from Albaha and Altaif, respectively. They were identified by taxonomist at the Department of Pharmacognosy, College of Pharmacy, King Saud University, and voucher specimens were kept at the herbarium of Research Center for Medicinal, Aromatic and Poisonous plants of the same college.

Extraction

The aerial parts of the plants were air-dried under shade and then ground to a coarse powder using a Condux Mill. Ground plants materials (200 g each) were percolated with ethanol. After filtration, the solvent was evaporated to dryness under reduced pressure at a temperature lower than 40°C. The crude ethanolic extract was redissolved in water and further fractionated by sequential partitioning against organic solvents of increasing polarity (Abdel-Sattar et al., 2010). The dried extracts were stored at -20°C until used.

Preparation of test sample

Stock solutions of the extracts were made by dissolving the sample in the minimal volume of dimethyl sulfoxide (DMSO) and diluted with the culture medium for the antileishmanial assay to <1% DMSO (Dorin et al., 2010). The stock solutions were re-sterilized by passing them through 0.22 µM micro-filters under sterile conditions in a laminar flow hood, stored at -20°C and retrieved only during use (Kigondu et al., 2009).

Leishmania culture

L. major was cultured at 26°C in Schneider's insect medium (Sigma Chemical company, St. Louis, MO. USA) supplemented with 10% fetal bovine serum (FBS), and penicillin and streptomycin added at a concentration of 100 IU/ml and 100 µg/ml, respectively. All incubations were carried on at 26°C.

Promastigotes extracts susceptibility assay

Promastigotes susceptibility testing was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) colorimetric assay purchased from Promega, Madison WI, USA (Cory et al., 1991), and a cell concentration of 10^6 cell/ml (100 µL per well) using Roswell Park Memorial Institute medium (RPMI) as culture media. Promastigotes were seeded in 96-well plates and extract/fraction was added in triplicates at concentrations 50, 100 and 200 µg/ml. After 72 h of incubation, the MTS dye was added (20 µL/well), and the plate was re-incubated for 4 h. The absorbance was read at 490 nm wavelength according to manufacturer's instructions. Amphotericin B at a concentration of 50 µM was used in the test protocol as a control. Percentage of inhibition was calculated by comparing the percent viability with untreated control.

RESULTS

In vitro sensitivity of *L. major* promastigotes toward different extracts of *C. procera*, *C. sinensis* and *C. sinaica* was investigated using MTS assay and the results are shown in Table 1. In this preliminary study, the extracts exhibiting >40% inhibition at the tested concentrations were considered to have high antileishmanial potential, and are considered to be tested further on the *L. major* macrophage infection assay (Kigondu et al., 2009).

DISCUSSION

Plants offer novel possibilities to obtain new compounds that are active against *Leishmania*. For example, *in vitro* studies on the anti-leishmanial activity of aqueous garlic extract (*Allium sativum)* on *L. major* promastigotes and amastigotes using the MTT assay were reported. *A. sativum* showed a dose-dependent cytotoxic effect on *L. major* with an IC_{50} of 37 µg/ml (Khademvatan et al., 2011a) and was reported to be effective on *L. major* infected-macrophage with most potent dose of 37 mg/ml for 48 h (Gharavi et al., 2011). In the current study, the activity of three selected medicinal plants growing in Saudi Arabia against a promastigote form of *L. major* was evaluated using MTS colorimetric assay.

C. procera is locally known as Osher and is used by the folk medicine practitioners of Saudi Arabia as a purgative, anti-rheumatic, diaphoretic, expectorant, antidysentric and for treatment of bronchial asthma (Al-Yahya et al., 1990). Moreover, in Iran, related species *C. gigantean* is used traditionally to treat CL and its methanol extract exhibited 100% inhibition of *L. major* parasites at a concentration of 1 mg/1 ml (Ramezani et al., 2006). The methanolic extract of *C. procera* growing in Oman was shown to be of significant activity against *Leishmania donovani* promastigotes with IC_{50} 16.22 µg/ml compared to its aqueous extract (IC_{50} 76.19 µg/ml) (Camacho et al., 2003). These facts stimulated the interest to screen the effect of various extracts of *C. procera* growing in Saudi Arabia on *Leishmania*, and subsequently specify the extract with the highest activity for further investigation. The current study showed significant activity of ethanol, hexane, chloroform and ethyl acetate extracts at 200 µg/ml (46.83 ± 0.042, 49.04 ± 0.046, 48.04 ± 0.098 and 55.75 ± 0.044, respectively). The effect of ethyl acetate extract effect was not significantly lowered by decreasing the dose to its half level (51.41 ± 0.028, 100 µg/ml).

The genus *Cordia* has a wide range of uses in traditional medicine. Members of this genus are used to treat rheumatism, painful menstruation, bladder diseases and gastric ulcers, infectious diseases and wound healing (Do Vale et al., 2012; Okusa et al., 2012). Traditionally, *C. sinensis* is used for treatment of gastric disorders, malaria (Orwa et al., 2009). In 2008, Kanami et al. reported significant activity of the wood of *Cordia*

Table 1. Effect of selected plants preparations on growth of *L. major* promastigotes.

Botanical Name	Preparation	% Inhibition ± SD		
		50 (µg/ml)	100 (µg/ml)	200 (µg/ml)
Calotropis procera	Ethanol extract	NI	5.53 ± 0.016	46.83 ± 0.042
	n-Hexane fraction	NI	35.46 ± 0.081	49.04 ± 0.046
	Chloroform fraction	NI	40.7 ± 0.088	48.04 ± 0.098
	Ethyl acetate fraction	NI	51.41 ± 0.028	55.75 ± 0.044
	n-Butanol fraction	NI	12.42 ± 0.003	16.34 ± 0.016
	Aqueous fraction	NI	NI	NI
Cordia sinensis	Ethanol extract	3.00 ± 0.160	2.38 ± 0.081	-
	n-Hexane fraction	NI	8.19 ± 0.009	15.07 ± 0.090
	Ethyl acetate fraction	NI	NI	NI
	n-Butanol fraction	NI	NI	NI
Caralluma sinaica	*n*-Hexane fraction		52.18 ± 0.019	81.44 ± 0.002
	Chloroform fraction		50.915 ± 0.044	60.93 ± 0.036
	Ethyl acetate fraction		54.64 ± 0.038	60.18 ± 0.002
	n-Butanol fraction		55.59 ± 0.088	59.40 ± 0.043
	Aqueous fraction		60.07 ± 0.101	59.40 ± 0.026

Parasites were seeded in complete medium containing indicated concentration of each preparation and the viability of cells was estimated using MTS assay. NI, No inhibition.

fragrantissima against *L. major* promastigotes with a minimum lethal concentration of 25 µg/ml (Mori et al., 2008). However, in current study, *C. sinensis* leaves were devoid of antileishmanial activity.

Many species of *Caralluma* are commonly used as traditional medicine for the treatment of rheumatism, diabetes, leprosy, paralysis, and inflammation and have antimalarial, anti-trypanosomal, anti-ulcer, antioxidant, antinociceptive, and anti-proliferative activities (Dutt et al., 2012). In Saudi Arabia, *C. sinaica* is used traditionally to lower glucose level (Habibuddin et al., 2008). To the best of our knowledge, no previous report was available regarding the evaluation of *C. sinaica* antileishmanial activity against *L. major*. *n*-Hexane extract of *C. sinaica* exhibited the highest inhibition percentage of *L. major* promastigotes (81.44 ± 0.002) at 200 µg/ml and (52.18 ± 0.019) at half this concentration. Additionally, chloroform, ethyl acetate, *n*-butanol and aqueous extracts of *C. sinaica* showed inhibition of approximately 60% at 200 µg/ml, and there was 5 to 10% reduction of the inhibition percentage at half this concentration, but no reduction was associated with the aqueous extract. All selected plants extracts were shown inactive or not significantly active at the lowest tested concentration (50 µg/ml).

Conclusion

Based on this study, *C. sinaica* and *C. procera* could be considered as having high potential of leishmanicidal activity against *L. major*. The active extract will be further tested against *L. major* amastigotes and will be subjected to further studies to identify the leading compounds.

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