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In vitro Assessment of Anti - Cutaneous Leishmaniasis Activity of Some Sudanese Plants

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SUMMARY: Examination of crude methanol extracts of four Sudanese plants (*Azadirachta indica, Acacia nilotica, Balanites aegyptiaca* and *Allium sativa*) revealed that only three species had a considerable in-vitro anti-leishmanial activity on *Leishmania major* promastigotes. The plants *Azadrachta indica*, *Allium sativa*, and *Acacia nilotica* gave a LC50 of 10.2, 4.94, and 89.38 µg/ml, respectively. Extracts of *Balanites aegyptiaca* had a moderate biological activity on *L major* promastigotes.

Key words: Leishmaniasis, cutaneous, herbal therapy, *in vitro*

Sudan'da Yetişen Bazı Bitkilerin Anti-kutanöz Leishmaniasis Aktivitesinin *in vitro* Olarak Değerlendirilmesi

ÖZET: Sudan'da yetişen dört bitkinin (*Azadirachta indica, Acacia nilotica, Balanites aegyptiaca* and *Allium sativa*) ham metanol ekstraktlarının incelenmesi sonucunda, sadece üç tanesinin *Leishmania major* promastigotlarına karşı *in vitro* olarak etkisi olduğu görülmüştür. *Azadrachta indica*, *Allium sativa*, and *Acacia nilotica* bitkilerinin ekstrakları için %50 öldürücü konsantrasyonun (LC50) sırasıyla 10.2, 4.94, 89.38 µg/mL olduğu, *Balanites aegyptiaca* ekstraktı ise *L major* promastigotları üzerine orta derecede etkiye sahip olduğu saptanmıştır.

Anahtar Sözcükler: Leishmaniasis, kutanöz, bitkisel tedavi, *in vitro*

INTRODUCTION

Leishmaniasis is a group of disease, caused by *Leishmania* species. The disease is considered as a major public health problem in 82 countries in the world causing morbidity and mortality (14).

Leishmaniasis is an endemic disease spread in variable directions in Sudan. The visceral leishmaniasis hyper-endemic foci are mainly in east and south while cutaneous type is shoed sporadic pattern meanly in the west and central parts of Sudan (1-3).

Different modes of treatment are used in the treatment of cutaneous leishmaniasis. Pentavalent antimonial compounds are the first line treatment but generally are toxic so several significant advances in the chemotherapy of the leishmaniasis have occurred in the last 10 years (6).

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Perhaps 80% of the world populations rely solely upon medicinal plants as the source of remedies for treatment of the disease. In the vast areas of the world, modern drugs are simply not available, or if they are available they often prove to be too expensive. The majority of drugs active against infectious agents are in fact derived from natural products (9).

Different plants of medicinal value are used traditionally worldwide for the treatment of leishmaniasis. This study therefore aims to investigate the potential of anti leishmanial activity of some Sudanese plants of medicinal value.

MATERIALS AND METHODS

Plant preparation: Four plants species (Table 1) of Sudanese plants used in traditional medicine were collected, samples of each plant were dried, coarsely powdered (except garlic, which used fresh). 100 g of each sample soaked in 80% methanol over night with continuous shaking at 37 ^{0}C then filtered, and kept at 4 ^{0}C for *Leishmania* test.

Culture: 0.1 ml of the blood was taken from the edge of cutaneous leishmaniasis lesion. Under aseptic condition the sample was mixed with normal saline, then it was inoculated in the prepared media NNN. The culture was incubated at 24 ^{o}C, and then it was examined for promastigotes growth after 4-5 days. Sub cultures were made from the positive culture using complete RPMI 1640 media with 10% fetal calf serum.

According to El-Tahir *et al* method (7) the counted promastigotes were harvested on day 4-5 of subculture in RPMI 1640 media and used for evaluation of anti leishmanial activity of plant methanol extracts The stock plant methanol extracts (100 mg/ml) were diluted with culture medium for working concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml).

Using micropipette, volumes of 100 µL of each prepared agents (0.2 mg/ml) was added to the first row of cells of 96-well Nunc microtiter plate containing 100 µL fresh complete RPMI medium. The suspension was well mixed & 100 µL from each well was transferred to the next second well of each row. This process continued in the same manner till the 6th row of the well. Control wells receive only 100 µL of fresh RPMI medium. Then 100 µL of cultured medium enriched with promastigotes (1.7X 10^{6} /ml) were added to all control and test agents. The parasites were allowed to multiply at 26^{o} C. Promastigotes were counted after 24 hrs & 72 hrs in each well using haemocytometer.

Table 1. Plants used in the present study

Plant name	Part used	Family	Varnacular name
Azadirachta indica	Leaves	Meliaceae	Neem tree
Acacia nilotica	Fruits	-	Acacia
Balantis aegyptiaca	Seeds	Balanitaceae	Hajleeg
Allium sativa	Bulb	Liliaceae	Garlic

RESULTS

Table 2 and figure 1 shows the mean growth inhibition of parasite *in vitro* using different agents after 24 hours and 72 hours. From the table pentostam (reference) showed increase in growth inhibition with the reduction in the concentration till 250 µg/ml then decrease in growth inhibition with the more reduction in concentration. All other agents showed the decrease of growth inhibition with the reduction in concentration. Garlic showed higher growth inhibition at different concentration compared to other agents. Garad and laloob had the lowest effect on parasite in different concentrations.

Table 2. The mean of growth inhibition of anti leishmanial agents in all time (24 & 72 hrs) at different concentrations

Agents	Concentrations					
	1000	**500**	**250**	**125**	**62.5**	**31.25**
Pentostam	51	51	54	48	45	40.5
Neem	51	48.5	45	41.5	42	36.5
Gard	41.5	36.5	35	32	31	26.5
Laloob	40.5	35.5	34.5	31	30.5	25.5
Garlic	52	50	48.5	45.5	43.5	40.5

Table 3. The correlation between pentostam and other agents

Pentostam vs	Mean difference	S.D	S.E	T-value	Sig.
Neem	4.0833	4.03	1.1642	3.507	.005
Garad	12.75	3.10	.8972	14.211	.000
Laloob	15.25	3.22	.9303	16.392	.000
Garlic	1.5	2.90	.8394	1.787	.10

Table 4. The LC 50 of antileishmanial agents & pentostam index (Pi)

Medicinal agents	LC50 value	Pi
Pentostam	3.077	1.0
Leishmanol	45.46	14.77
Neem	10.21	3.31
Garad	89.38	29.04
Laloob	191.17	62.12
Garlic	4.94	1.60

(**LC50**: the concentration, that which caused 50% inhibition of parasite growth; **Pi**: pentostam index, the ratio of studied agents and pentostam).

DISCUSSION

The different statistical procedures using the standard agents (pentostam) proved that neem and garlic are the more effective agents against *L. major* promastigotes compared to other agents. They showed 44.04 & 46.667 mean of growth inhibition respectively after 72 hours. Also they showed the lowest mean differences with the standard 4.08 & 1.5 respectively.

Garlic the only agent that showed insignificance difference with standard agent (pentostam) (P=0.10) this means that garlic can be considered to have powerful anti-leishmanial activity. Garlic also showed LC50 at concentration 4.94 µg/ml, (the standard showed LC50 equal 3.07µg/ml). This finding had been supported by Atta-Ur-Rahaman *et al*, (4) whose work on leishmancidal natural product had led to the identification of ajoene (one of the garlic compounds) as a potent leishmancidal substance. He reported LC50 of garlic

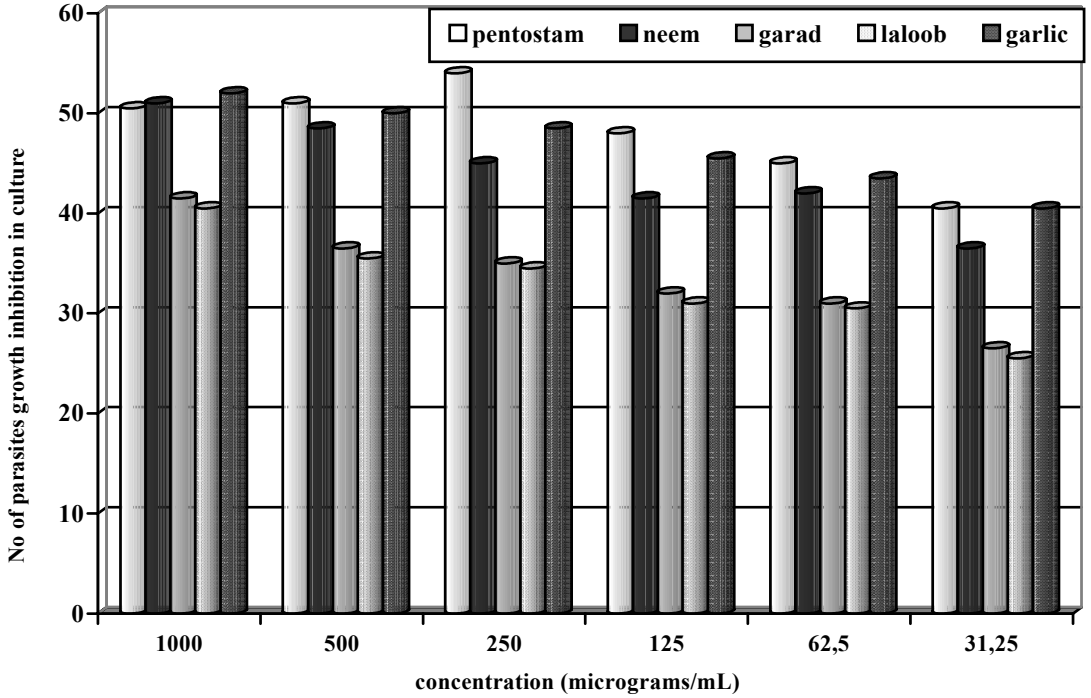

Figure 1. No of parasites growth inhibition after 24hrs and 72 hrs

compound <0.39 µg/ml. Nok *et al* (11) reported that garlic-induced death of protozoans with a dose of 5.0 mg/ml. The extract of garlic pulp completely suppressed the ability of *Trypanosoma brucei brucei* to cause trypanosomiasis in mice.

On the other hand many researches and laboratory studies demonstrated that garlic had antibiotic like effects. They investigated the ability of garlic to inhibit antibiotic resistant strains of bacteria, Singh *et al* (12) found that garlic was more effective than many antibiotics against 9 clinical strains of bacteria.

Webber *et al*, (13) also illustrated that garlic had antiviral activity.

Neem had LC50 equal 10.2 µg/ml. This indicates its assessable *in vitro* as anti-leishmanial agents. El-Tahir *et al* (7) study determined the activity of neem on promastigotes of *L major* and showed that neem had LC50 equal 11.2 µg/ml. Also the result of our study had been indirectly supported by the results of Bray *et al*, (5) and Khalid *et al* (10), which postulated the potential activity of extract from neem in the treatment of some parasitic diseases like malaria.

Garad methanol extract had LC50 equal 89.5 µg/ml. on *L. major* promastigote. This result showed low activity of garad compared to garlic and neem. In Sudan garad had been used as anti-parasitic agent. Garad ethyl acetate extract possessed high activity (LC50 1.5 µg/ml) against *Plasmodium falciprum* (8).

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