

Screening of *Aloe otallensis* Exudate and Its Effect on *Leishmania aethiopica*

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Abstract

Background and objectives: The different Aloaceae plant extracts have been tested and found to contain components with anti leishmanial activity. Our study was conducted to assure anti leishmanial activity of the methanol extract of *Aloe otallensis* on the promastigot stage of *Leishmania aethiopica* as compared to currently used drugs and also tried to screen its phytochemical constituents.

Methods: *Aloe otallensis* leaf exudate was extracted by using methanol solvent and phytochemical screening was done using the method mentioned by Trease and Evans as well as the extract was evaluated for *in vitro* anti leishmanial activity against *Leishmania aethiopica* which was taken from the Black Lion Specialized Hospital parasitology unit. The result was compared with currently used drug like; Sodium stibogluconate, milfostin and paramomycin.

Result: The extract has a good anti leishmanial activity with an IC₅₀ of 0.041 µg/mL on *L. aethiopica* (LDC/134). The experimental result shows that the extract has better anti leishmanial activity on *L. aethiopica* than paramomycin and milfostin but less activity than sodium stibogluconate. The data analyses was done by pad graph prison version 5 software after it was read by ELISA reader at the wave length of 650 nm. The phytochemical screening of *aloe otallensis* exudate showed the presence of phenol, alkaloid and saponin.

Conclusion: *Aloe otallensis* exudate methanol extract was found to have a good anti leishmaniasis activity against *L. Aethiopica* and this may be attributed to phenol, alkaloid and saponin present in the plant. Even though this is our conclusion it needs further investigations to confirm which constituent(s) is/are responsible for such effect and at how many concentrations.

Keywords: Anti leishmaniasis; *Aloe otallensis*; *Leishmania aethiopica*; IC₅₀

Introduction

Leishmaniasis is a group of diseases caused by protozoan parasites of the genus *Leishmania* [1]. The disease is an intracellular protozoan parasite of vertebrates that infect various mammalian species, including humans and transmitted by sand fly vectors [2]. The bite of infected sand flies, genus *Phlebotomushuman* pathogens, transmits the diseases [3]. The diseases clinical manifestations range from local cutaneous or mucocutaneous lesions to life-threatening visceral disease [4,5]. The disease is distributed worldwide which appear to be far more abundant and a public health problem. The overall worldwide prevalence of leishmaniasis is above 12 million cases [6]. Current estimates shows that over 0.2-0.4 million cases of visceral leishmaniasis and 0.7-1.2 million cases of cutaneous leishmaniasis occur each year in 98 countries [7]. During the last decade, the geographic region of *Leishmania* as well as incidence of infection significantly increased mainly due to increased urban development and immunosuppressive illnesses such as HIV/AIDs [8]. Because of these, the World Health Organization listed leishmaniasis as the third most important vector born disease next to malaria and sleeping sickness [9]. East Africa is

one of the world's main *Leishmania* endemic areas and the disease occurs mainly in Eritrea, Ethiopia, Kenya, Somalia, Sudan and Uganda [10]. Cutaneous leishmaniasis in Ethiopia is mainly due to *Leishmania aethiopica* (*L. Aethiopica*), but rarely by *Leishmania tropica* and *Leishmania major*. *L. Aethiopica* causes cutaneous leishmaniasis in the high lands of Ethiopia whereas the latter two species causes the disease in the lowland regions of the country [11-13]. The disease presents in three clinical forms: Localized cutaneous leishmaniasis (LCL), mucocutaneous cutaneous leishmaniasis (MCL) and diffused cutaneous leishmaniasis (DCL) [11-14]. Localized cutaneous leishmaniasis (LCL) lesions are often benign and self-healing; occasionally resulting in severe and persistent lesions. Persistent/severe LCL, MCL and DCL lesions are disfiguring and often require protracted treatment schedules [14-16]. In the case of DCL, definite cure is hardly ever achieved, since relapse is common [17].

The number of treatment options for *Leishmania* has increased in the past few years [18]. Some of the drugs used for the treatment are pentavalent antimonials such as sodium stibogluconate (SSG), amphotericin B, paromomycin (PM), miltefosine (MLT) and meglumine antimoniate (glucantime) [19]. But each treatment still has many drawbacks. Mostly they are difficult and lengthy to administer, toxic, expensive and resistance is a major problem. Currently due to

these problems, researches were carried out to investigate historically claimed plants for their *in vitro* anti-leishmanial activity against *Leishmania* parasites [20]. The Aloaceae family are group of succulent perennial plants varying from small herbs to large woody trees. *Aloe otallensis* (*A. otallensis*) which is one species of Aloaceae is an Ethiopian endogenous plant forming small clumps. Their leaves are a rosette, erect and slightly recovered. They have grey-green color and they are sometimes very finely spotted. The marginal teeth are 8-14 per 10 cm with reddish brown color [21]. The different species in the genus *Aloe* contains variety classes of secondary metabolites as it is approved by their extraction using different solvents. For instance, water extraction of *Aloe vera* (*A. vera*) has been screened for tannins, saponins, anthraquinones, flavonoids, alkaloids and phenols [22]. Methanol extraction of *A. vera* also shows that tannins, flavones, alkaloids and quinones are positive [23-33].

Materials and Methods

Plant material

The exudate of *Aloe otallensis* were collected during November 2010 from Hammer district, in region of southern nation nationality of Ethiopia. Authentication and Botanical identification was done using standard identification keys by Addis Ababa University Department of Biology, Herbarium Unit; after that the exude of the leaves was taken and drayed at room temperature to make ready for extraction.

Extraction

Ten grams of powdered *Aleo otallensis* exudate was macerated by 80% V/V methanol alcohol for 6 h with continuous shaking using shaker machine. The resulting supernatant solution was filtered using Whatman filter paper No. 1. The filtrate was concentrated in Buchi Rota Vapor and dried in an oven at a temperature of 40°C to remove the solvent (methanol) and kept in the refrigerator.

Preparation of stock solutions of plant extracts

The plant extracts were solubilized by dimethyl sulfoxide (DMSO) to final stock concentration of 10 mg/mL. Stock solution of standard drug (milfosin, sodium stibogluconat and paramomycine) was used as positive control and the parasite itself used as negative control.

Parasite isolation

Clinical isolate strain LDC/134 and AM 563 of *L. Aethiopica* were used for evaluation of anti-leishmanial activity of the methanol extract of the plant. This was taken from the *Leishmania* Research and Diagnostic Laboratory located in Black Lion Specialized Hospital, Addis Ababa University, Ethiopia.

Preliminary phytochemical screening

The phytochemical screening was performed according to Trace and Evince (1989). Based on these identification tests for Tannins, Saponins, Anthraquinones, Flavonoids, Alkaloids, Phenols, and quinine were conducted. For Tannin test 200 mg of plant extract was mixed with 10 mL of distilled water and filtrated. The 2 mL of filtrate was mixed with 2 mL of FeCl₃. The formation of blue-black precipitate indicates the presence of tannin. Similar amount of plant extract was mixed and filtrated then 2 mL of the filtrate and three drops of 1% of

HCl heated in steam. From the hot mixture, 1 mL was mixed with 6 mL of Mayer's reagent/winger reagent/dragendrof. The formation of cream/brown/red/orange/precipitate indicates the presence of alkaloids. For saponnin test 0.5 mL of extract and 5 mL of distilled water mixed together and shake well. The formations of persistence frothing indicate the presence of saponnin. For flavonoid, 200 mg of plant extract was mixed with 10 mL of ethanol and filtrated. 2 mL of the filtrate, concentrated HCl and magnesium ribbon are mixed together. The formation of Pink, tomato or red color indicate the presence of flavonoid, glycoside. Finally for phenol, 1 mL of the extract was mixed with three drops of FeCl₃ and then 1 mL of K₂Fe(CN₆) added to the mixture. The formations of green blue color indicate the presence of phenol [27].

In vitro antipromastigot assay

The effect of the crude extract of the plants was evaluated in 96 well micro titer plate using *Leishmania aethiopica* promastigot. Ninety-six well micro titer plates were filled with 100 mL complete RPMI media containing 3×10^6 promastigotes and 100 mL of each extracts with different concentrations with three folds of 10 mg/mL of first concentrations of six divisions. Each division of extracts were evaluated by three replicate and repeated two times. In all tests medium alone used as negative control while the reference drugs was used as positive control. These reference drugs were Miltefosine (50 mg/mL), sodiumstibo gluconate (80 mg/mL) and paramomycin 20 mL. The plates were maintained at 26°C under 5% CO₂ incubator for 24 h. After examination of the culture media for contamination and for estimating morphological and motility changes of drug treated parasites, 20 mL of Resazurin, prepared as 12.5 mg with 100 mL of distilled water added. Then optical density of each plate was measured after 24 h using Wallac ELISA reader flour metrically with 450 nm as an excitation wavelength and 630 nm as an emission wavelength. The experimental results were analyzed by using graph pad prism version 5 software and expressed as concentration at which an extracts induces 50% reduction in parasite proliferation (IC₅₀) by comparing with the controls. The data was analyzed by measuring optical density of resazurin. Resazurin (alamar blue) is an indicator which is added in the 96 well plates after the drug and extract was dispensed on it. The indicator used for checking how many of the parasite was survived. Those parasites which were not dead were interacting with the almar blue and change the color from pink to yellowish. The intensity of the color changed depends to the concentration of the live parasite survived. Based on these optical density measured the change in color using Elisa reader that is used to calculate IC₅₀ by the software.

Results

From qualitative and preliminary Phytochemical screening of flavonoid, alkaloid tannin, saponnin, phenol and anthraquinone positive result was seen only on phenol, alkaloid and saponnin (Table 1).

The *Aloe otallensis* extract and drug inhibited the growth of promastigote forms of *L. aethiopica in vitro* after 24 h, 48 h, 72 h of incubation, and a 50% inhibitory concentration (IC₅₀) was also done. *Aloe otallensis* extract had a good anti *Leishmaniasis* activity with an IC₅₀ value approximately 0.041 mc/mL for *L. aethiopica*. Details of the *in vitro* inhibitory effect of different concentrations of *Aloe otallensis* extracts and drugs against Leishmanial major promastigotes are presented in the following Table 2.

Secondary metabolite	Phytochemical screen result
Tannin	–
Saponin	+
Alkaloids	+
Flavonoid	–
Antraquinon	–
Phenol group	+

Table 1: Phytochemical screen of *Aloe otallensis*.

Serial dilution	Survived <i>L. aethiopica</i> promastigots concentration relative to almar blue color change intensity from pink to yellowish			
	<i>Aloe otallensis</i> Extract	Milfostin	Paromomycin	Sodium stibogluconat
10.00 µg/mL	0.147666667	0.164000000	0.165000000	0.015333333
3.333 µg/mL	0.161333333	0.183000000	0.164000000	0.082666667
1.111 µg/mL	0.165000000	0.182666667	0.207333333	0.160000000
0.370 µg/mL	0.189000000	0.210333333	0.196000000	0.167666667
0.123 µg/mL	0.193000000	0.211666667	0.206666667	0.171333333
0.041 µg/mL	0.203000000	0.251666667	0.207666667	0.190666667
0.013 µg/mL	0.209666667	0.285666667	0.198333333	0.201333333
0.004 µg/mL	0.236333333	0.386000000	0.346333333	0.222000000

Table 2: Optical density measurement for *Leishmaniasis aethiopica* and anti promastigot activity assay.

Comparison IC₅₀ of extracts and drugs also shown in Table 3. When we see potency of the extract comparing to IC₅₀ of the standard drugs *Aloe otallensis* extracts is more potent than milfostin and

paramomycin while less potent than sodium stibogluconat (SSG). IC₅₀ value of standard drugs used as positive control and the plant in the study.

Drug used for the test	IC ₅₀ concentration in mg/mL on <i>L. aethiopica</i>
<i>Aloe otallensis</i> extract	0.041
Melifostin	2.41
Paramomicin	1.143
Sodium stibogluconate	0.0704

Table 3: IC₅₀ data for the drug and plant extract.

Discussion

Over 100 plants have been reported to be active against various forms of leishmanial parasites [21]. The other studies showed that the *Ixora coccinea* leaf extract having anti leishmanial activity against the promastigotes of *L. aethiopica*. The root extract of *Perovskia abrotanoides* shows anti leishmanial activities against the *L. major* [30]. 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. [31].

Their finding show that, *Aloe vera* and *Tamarix aphylla* had a significant dose dependant anti promastigote activity against *L. Aethiopica* as that suggest promising phototherapeutic agents for cutaneous leishmaniasis. The present study showed the anti leishmanial activity of the aqueous extract of *E. helioscopia* on the promastigotes of *L. Aethiopica* (KWH 23) strains. Our finding also revealed that, extract of *E. helioscopia* has anti promastigote activity against *L. Aethiopica*. Both studies show that, the different plants used in the research having anti leishmanial activities. Other members of Euphorbiacea family are reported to have anti leishmanial, antioxidant, larvicidal and insecticidal activities [32]. *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. The current study showed the anti leishmanial activity of the aqueous extract of *E. helioscopia* on KWH strain ($LC_{50}=9.94$ ug/mL).

Conclusion

The results of this study reveal antileishmanial activity against *L. Aethiopia* by exudate of *Aloe otallensis* and suggest that these methanolic extracts have the potential to be used as anti leishmanial drug against the promastigotes of *L. aethiopia*. So, further studies will be necessary to investigate the effect of these active plant extracts on *L. aethiopia*. This would help us in obtaining a novel drug that could potentially be less toxic and more therapeutically effective against the *Leishmania* parasites.

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