Phytochemical screening of the exudate of *Aloe otallensis* and its effect on *Leishmania donovani*

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**ABSTRACT**

**Objective:** To evaluate the antileishmanial activity of methanolic extract of *Aloe otallensis* (*A. otallensis*) on the promastigote stage of *Leishmania donovani* (*L. donovani*) as compared to standard drugs and to screen its phytochemical constituents.

**Methods:** Phytochemical screening was done by using the method mentioned by Evans and Trease on methanolic extract of the exudates of *Aloe otallensis* leaves. The extract was also evaluated for *in vitro* antileishmanial activity against *L. donovani* which is found from the Parasitology Unit of Black Lion Hospital. The result was compared to standard drugs of sodium stibogluconate, milfostin and paramomycin.

**Results:** The extract has a good antileishmanial activity with an IC50 of 0.1230 μg/mL on *L. donovani* (AM 563). The experimental data showed that relatively it had better activity than paramomycin and milfostin but less activity than sodium stibogluconate. The data analyses were done by GraphPad Prism version 5 software after it was read by ELISA reader at the wave length of 650 nm. The phytochemical screening of the exudates of *A. otallensis* showed the presence of phenol, alkaloid and saponin.

**Conclusions:** The methanol extract of the exudates of *A. otallensis* has a good anti-leishmaniasis activity and this may be attributed to phenol, alkaloid and saponin present in the plant. But it needs further analysis for the conformation of which constituent presents in high concentration to know which one has the strongest effect.

1. Introduction

Leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania*[1]. The bite of infected sand flies, genus *Phlebotomus* human pathogens, transmits the diseases[2]. This is characterized by a spectrum of clinical manifestations: cutaneous, mucocutaneous, and visceral leishmaniasis[3]. They are distributed worldwide and appear to be far more abundant and a public health problem. The overall prevalence of leishmaniasis is 12 million cases and an approximate occurrence of 0.5 million cases of visceral leishmaniasis (VL) and 1.5 million cases of cutaneous leishmaniasis (CL) is reported[4]. Because of this, the World Health Organization listed leishmaniasis as the third most important vector born disease next to malaria and sleeping sickness[5]. Eastern Africa is one of the world’s main *Leishmania* endemic areas, and the disease occurs mainly in Eritrea, Ethiopia, Kenya, Somalia, Sudan and Uganda[6]. Leishmaniasis in Ethiopia is mainly due to *Leishmania donovani* (*L. donovani*) which cause VL. In few cases, *Leishmania tropica* and *Leishmania major* (*L. major*) can cause CL. The most affected age groups were from 11–20. VL was discovered in Ethiopia in around 1942 and since then it has been recognized as an endemic disease in most lowlands and arid areas of the country[7], such as Segen, Weyto and Omo valley in the southern part of the rift valley, Ocholo in southwest and Metema, Humera lowland in the northwes[8]. The female phlebotomine sand flies require a blood meal to provide nutrition for the development
of eggs except plant material (nectar). To satisfy their need, they bite mammals including human being during the dark time. Almost exclusively, the transmission of leishmaniasis is through the bite of an infected sand fly but congenital and venereal infected sharing needles are reported to transmit the disease[9].

The number of treatment options has increased in the past decade. 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)[10]. But each treatment still has many drawbacks. Mostly they are difficult and lengthy to administer, toxic, expensive, and resistance is a major problem. Due to these, the patients should be treated by admitting in the hospital. Currently due to these problems, researches were carried out to investigate historically claimed plants for their in vitro anti-leishmanial activity against Leishmania parasites[11].

Aloaceae is a succulent perennial varying from small herbs to large woody trees. The family of Aloaceae, in general, have 7 genera and 650 species mostly resited to Southern Africa with only exceeding into tropical Africa and Arabia. Aloe otallensis (A. otallensis) is one of the Ethiopian endogenous plant forming small clamps. 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[12].

The species in the genus Aloe contain different classes of secondary metabolites which are made from their extraction using different solvents. For instance, water extraction of Aloe vera (A. vera) has been screened for tannins, saponins, anthraquinones, flavonoids, alkaloids, phenols, and quinones were conducted.

For tannin test, 200 mg of the plant extract was mixed with 10 mL of distilled water and filtrated. Two milliliters of the filtrate was mixed with 2 mL of FeCl3 and then 1 mL of K2Fe(CN)6 was added to the mixture. The formations of green blue color indicated the presence of phenols[17].

2. Materials and methods

2.1. Plant materials

The exudates of Aloe otallensis were collected in November 2010 in Hammer district of Southern Ethiopia. Authentication and botanical identification were done using standard identification keys by Herbarium Unit, Department of Biology, Addis Ababa University. After that, the exudate of the leaves was taken and dried at room temperature for extraction.

2.2. Extraction

Ten grams powdered exudate of the plant was macerated by using 80% methanol for 6 h with a continuous shaking of the mixture using a shaker machine. The existed supernatant solution was filtrated using Whatman filter paper No. 1. The filtrate was concentrated in Buchi Rotavapor and dried in an oven at 40 °C to remove the solvent labeled and kept in the refrigerator[15].

2.3. Preparation of stock solutions of the plant extracts

The plant extracts were solubilized by dimethylsulfoxide for a final stock concentration of 10 mg/mL. The stock solution of the standard drug (MLT, SSG and PM) was used as positive control and the parasite itself was used as negative control.

2.4. Parasite isolation

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

2.5. Preliminary phytochemical screening

The phytochemical screening was performed according to Trease and Evans[16]. Based on these, identification tests for tannins, saponins, anthraquinones, flavonoids, alkaloids, phenols, and quinones were conducted.

For tannin test, 200 mg of the plant extract was mixed with 10 mL of distilled water and filtrated. Two milliliters of the filtrate was mixed with 2 mL of FeCl3. The formation of blue-black precipitate indicated the presence of tannins. Similar amount of the plant extract was mixed and filtrated, then 2 mL of the filtrate and three drops of 1% HCL were heated in steam. From the hot mixture, 1 mL was mixed with 6 mL of Mayer’s reagent/Wanger’s reagent/ Dragendorff’s reagent. The formation of cream/brown/red/orange precipitates indicated the presence of alkaloids. For saponin test, 0.5 mL of the extract and 5 mL of distilled water were mixed together and shaken well. The formation of persistent froth indicated the presence of saponins. For flavonoids, 200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two milliliters of the filtrate, concentrated HCl and magnesium ribbon were mixed together. The formation of pink, tomato or red color indicated the presence of flavonoids and glycosides. Finally, for phenols, 1 mL of the extract was mixed with three drops of FeCl3 and then 1 mL of K2Fe(CN)6 was added to the mixture. The formations of green blue color indicated the presence of phenols[17].

2.6. In-vitro antipromastigote assay

The effect of the crude extract of the plants was evaluated in 96-well micro titer plates using L. donovani promastigote. The 96-well micro titer plates were filled with 100 μL complete Roswell
Park Memorial Institute media containing $3 \times 10^6$ promastigotes and 100 μL of each extract with different concentrations with three folds of 10 μg/mL of first concentrations of six divisions. Each division of the extracts was evaluated by three replicates and repeated two times. In all tests, medium alone was used as a negative control while the reference drugs were used as the positive control. These reference drugs were MLT (50 μg/mL), SSG (80 μg/mL) and PM (20 mmol). The plates were maintained at 26 °C under 5% CO₂ incubator for 24 h. After examination of the culture media for contamination and for estimation of morphological and motility changes of drug treated parasites, 20 μL of resazurin prepared as 12.5 mg with 100 mL of distilled water was added. Then optical density of each plate was measured after 24 h using ELISA reader at 450 nm as an excitation wavelength and 630 nm as an emission wavelength. The experimental results were analyzed by using GraphPad Prism version 5 software and expressed as concentrations at which an extract induced 50% reduction in parasite proliferation (IC₅₀) by comparing with the controls. The data was analyzed by measuring the optical density of resazurin. Resazurin (alamar blue) is an indicator which is added in the media for contamination and for estimation of morphological and motility changes of drug treated parasites, 20 μL of resazurin prepared as 12.5 mg with 100 mL of distilled water was added. Then optical density of each plate was measured after 24 h using ELISA reader at 450 nm as an excitation wavelength and 630 nm as an emission wavelength. The experimental results were analyzed by using GraphPad Prism version 5 software and expressed as concentrations at which an extract induced 50% reduction in parasite proliferation (IC₅₀) by comparing with the controls. The data was analyzed by measuring the optical density of resazurin. Resazurin (alamar blue) is an indicator which is added in the 96-well plates after the drug and extract were dispensed on it. The indicator was used for checking how many of the parasites survived. Those parasites which were not dead were interacting with the alamar blue and changed the color from pink to yellowish. The intensity of the color changed depending on the concentration of the survived parasite. Based on these measured optical density, the change in color using ELISA reader is used to calculate IC₅₀ by the software.

### 3. Results

From qualitative and preliminary phytochemical screening of flavonoids, alkaloids, tannins, saponins, phenols and anthraquinones, positive results were seen only on phenols, alkaloids and saponins (Table 1).

**Table 1**

| Secondary metabolites | Phytochemical screening result |
|-----------------------|-------------------------------|
| Tannins               | -                             |
| Saponins              | +                             |
| Alkaloids             | +                             |
| Flavonoids            | -                             |
| Anthraquinones        | -                             |
| Phenols               | +                             |

The *A. otallensis* extracts and drugs inhibited the growth of the promastigote forms of *L. donovani* in vitro after 24 h, 48 h and 72 h of incubation, and the IC₅₀ was also done. *A. otallensis* extract had a good anti-leishmaniosis activity with an IC₅₀ value of approximately 0.1230 μg/mL for *L. donovani*. Details of the *in vitro* inhibitory effect of different concentrations of *A. otallensis* extracts and drugs against *L. donovani* were presented in Table 2. Comparison between IC₅₀ of extracts and drugs was shown in Table 3. When we saw potency of the extract, as compared to IC₅₀ of the standard drugs, *A. otallensis* extracts were more potent than MLT and PM but less potent than SSG.

**Table 2**

| Serial dilution (μg/mL) | *A. otallensis* extract | MLT | PM | SSG |
|-------------------------|-------------------------|-----|----|-----|
| 10.000                  | 0.147666667             | 0.1640000000 | 0.1650000000 | 0.015333333 |
| 3.333                   | 0.161333333             | 0.1830000000 | 0.1640000000 | 0.082666667 |
| 1.111                   | 0.1650000000            | 0.182666667 | 0.207333333 | 0.160000000 |
| 0.370                   | 0.1890000000            | 0.210333333 | 0.196000000 | 0.167666667 |
| 0.123                   | 0.1930000000            | 0.211666667 | 0.206666667 | 0.171333333 |
| 0.041                   | 0.2030000000            | 0.251666667 | 0.207666667 | 0.190666667 |
| 0.013                   | 0.209666667             | 0.285666667 | 0.198333333 | 0.201333333 |
| 0.004                   | 0.236333333             | 0.3860000000 | 0.346333333 | 0.222000000 |

IC₅₀ value of standard drugs used as the positive control and the plant extract as treatment group in the study. Survived *L. donovani* promastigotes concentration is relative to alamar blue color change intensity from pink to yellowish.

**Table 3**

| Drugs used for the test | IC₅₀ concentrations in μg/mL on *L. donovani* |
|-------------------------|---------------------------------------------|
| *A. otallensis* extract | 0.1230                                      |
| MLT                     | 2.4100                                      |
| PM                      | 1.1430                                      |
| SSG                     | 0.0704                                      |

3. **Discussion**

Over 100 plants have been reported to be active against various forms of leishmanial parasites[18]. The other studies showed that the *Ixora coccinea* leaf extract had an antileishmanial activity against the promastigotes of *L. donovani*[19]. The root extract of *Perovskia abrotanoides* shows antileishmanial activities against the *L. major*[20]. The pharmacological screening of methanolic extract of *A. vera* leaf and *Tamarix aphylla* bark was assessed to investigate the *in vitro* antileishmanial activity of the medicinal plants against CL by Iqbal et al.[21]. Their finding showed that, *A. vera* and *Tamarix aphylla* had a significant dose-dependent antipromastigote activity against *L. donovani*, which suggested promising phototherapeutic agents for CL. The present study showed the antileishmanial activity of the aqueous extract of *A. otallensis* on the promastigotes of *L. donovani* (AM 563) strains. Our finding also revealed that the extract of *A. otallensis* has antipromastigote activity against *L. donovani*. Both studies showed that the different plants used in the research had antileishmanial activities. Other members of Euphorbiacea family are reported to have antileishmanial, antioxidant, larvicidal and insecticidal activities[22]. *In vitro* antileishmanial effects of traditional herbal extracts against CL were studied by Iqbal et al.[21]. It was also found that members of the genus *Euphorbia* had
antitumor, anti-inflammatory, anti-helminthic, cytotoxic and antioxidant properties[23]. The current study showed the antileishmanial activity of the aqueous extract of *A. otallensis* on *L. donovani* (AM 565) strains (IC₅₀ = 0.1230 µg/mL).

The results of this study reveal an antileishmanial activity against *L. donovani* by exudates of *A. otallensis* and suggest that these methanolic extracts have the potential to be used as antileishmanial drugs against the promastigote forms of *L. donovani*. But it needs further analysis for the conformation of which constituent presents in high concentration and to know which one has the highest effect of this active plant extract. This would help us in obtaining a novel drug that could potentially be less toxic and more cost-effective against the Leishmania parasites.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**References**

[1] Kuru T, Jirata D, Genetu A, Barr S, Mengistu Y, Aseffa A, et al. *Leishmania ethiopica*: identification and characterization of cathepsin L-like cysteine protease genes. Exp Parasitol 2007; 115(3): 283-90.

[2] Alexander B, Maroli M. Control of phlebotomine sandflies. Med Vet Entomol 2003; 17(1): 1-18.

[3] Garg R, Dube A. Animal models for vaccine studies for visceral leishmaniasis. Indian J Med Res 2006; 123(3): 439-54.

[4] Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 2004; 27(5): 305-18.

[5] Mengistu G, Ayele B. Visceral leishmaniasis and HIV co-infection in patients admitted to Gondor University hospital, northwest Ethiopia. Ethiop J Health Dev 2007; 21(1): 53-60.

[6] Reithinger R, Brooker S, Kolaczinski JH. Visceral leishmaniasis in eastern Africa - current status. Trans R Soc Trop Med Hyg 2007; 101(12): 1169-70.

[7] Edessa N. Investigation of cutaneous leishmaniasis using conventional and molecular methods in Silit Woreda, Ethiopia [dissertation]. Addis Ababa: Addis Ababa University; 2007.

[8] Balkew M, Gebre-Michael T, Berhe N, Ali A, Haitu A. Leishmaniasis in the middle course of the Ethiopian Rift Valley: II. Entomological observations. Ethiop Med J 2002; 40(3): 271-82.

[9] Bari AU, Rahman SB. Cutaneous leishmaniasis: an overview of parasitological and host-parasite-vector interrelationship. J Pak Assoc Dermatol 2008; 18(1): 42-8.

[10] Malaria Consortium. Leishmaniasis control in eastern Africa: past and present efforts and future needs. Situation and gap analysis. London: Malaria Consortium; 2010. [Online] Available from: http://www.malarial consortium.org/userfiles/file/NTD%20Resources/VI%20EA%20Situation%20Analysis%20Fina_Janl.pdf [Accessed on 8th February, 2016]

[11] Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. A review of natural products with antileishmanial activity. Phytomedicine 2005; 12(6-7): 514-35.

[12] Edwards S, Demissew S, Hedberg I. *Flora of Ethiopia and Eritrea*. Addis Ababa: The National Herbarium; 1997.

[13] Nwoaguikpe RN, Braide W, Ezejiofor TI. The effect of Aloe vera plant (*Aloe barbadensis*) extracts on sickle cell blood (HbSS). Afr J Food Sci Technol 2010; 1(3): 58-63.

[14] García M, Scull R, Cuesta O, Boulet G, Maes L, Cos P, et al. Bioassay-guided in vitro study of the antileishmanial and cytotoxic properties of *Bixa orellana* seed extract. J Coast Life Med 2014; 2(6): 484-9.

[15] Taye B, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pac J Trop Biomed 2011; 1(5): 370-5.

[16] Trese GE, Evans WC. A textbook of pharmacognosy. 13th ed. London: Bailliere Tindall; 1989.

[17] Debela A. *Manual for phytochemical screening of medicinal plants*, Addis Ababa: Ethiopian Health and Nutrition Research Institute; 2002.

[18] Rocha FJ, Schleicher U, Mattner J, Alber G, Bogdan C. Cytokines, signaling pathways, and effector molecules required for the control of *Leishmania* (*Viannia*) *braziliensis* in mice. Infect Immun 2007; 75(8): 3823-32.

[19] Mukherjee B, Mukhopadhyay R, Bannerjee B, Chowdhury S, Mukherjee S, Naskar K, et al. Antimony-resistant but not antimony-sensitive *Leishmania donovani* up-regulates host IL-10 to overexpress multidrug-resistant protein 1. Proc Natl Acad Sci U S A 2013; 110(7): E575-82.

[20] Jaffari MR, Hooshmand S, Samiei A, Hosseinzadeh H. Evaluation of leishmanicidal effect of *Perovskia abrotanoides* Karel, root extract by *in vitro* leishmanicidal assay using promastigotes of *Leishmania major*. Pharmacologyonline 2007; 1: 299-303.

[21] Iqbal H, Khattak B, Ayaz S, Rehman A, Ishfaq M, Abbas MN, et al. Comparative efficacy of *Aloe vera* and *Tamarix aphylla* against cutaneous leishmaniasis. Int J Basic Med Sci Pharm 2012; 2(2): 42-5.

[22] Zahir AA, Rahaman AA, Pakrashi S, Ghosh D, Bagavan A, Kamaraj C, et al. Evaluation of antileishmanial activity of South Indian medicinal plants against *Leishmania donovani*. Exp Parasitol 2012; 132(2): 180-4.

[23] Qureshi NA, Rahman JU, Ali A, Ullah N, Shah SAA, Khan I. *In-vitro* evaluation of the anti-leishmanial activity of *Euphorbia helioscopia* stem extract in comparison with synthetic drug amphotericin B. Asian J Nat Appl Sci 2014; 3(3): 12-7.