Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article doi:

©2016 by the Journal of Coastal Life Medicine. All rights reserved.

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

Zerihun Tesfaye Nigusse[1], Woldea Abebe Wondifraw[2], Sefinew Migbaru Abate[3*]

[1]*Department of Pharmacy, Tikur Anbessa Specialized Hospital, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*

[2]*Outpatient Pharmacy Case Team, Debre Markos Referral Hospital, East Gojjame Zone, Amhara Regional State, P.O. Box 37, Debre Markos, Ethiopia*

[3]*Oncology Pharmacy Case Team, Tikur Anbessa Specialized Hospital, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*

ARTICLE INFO

Article history:
Received 12 Feb 2016
Received in revised form 29 Feb 2016
Accepted 25 Mar 2016
Available online 20 May 2016

Keywords:
Anti-leishmaniasis
Aloe otallensis
Leishmania donovani
IC_{50}

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. donavani* 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 IC_{50} 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 northwest[8]. The female phlebotomine sand flies require a blood meal to provide nutrition for the development

*Corresponding author: Sefinew Migbaru Abate, Oncology Pharmacy Case Team, Tikur Anbessa Specialized Hospital, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.
Tel: 251913893037
E-mail: sefinew.migbaru@aau.edu.et

The journal implements double-blind peer review practiced by specially invited international editorial board members.

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 and phenols. The results are all positive[13]. Methanol extraction of *A. vera* shows that tannins, flavones, alkaloids and quinones are positive[14].

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 Leishmania 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 $FeCl_3$. 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 $FeCl_3$ and then 1 mL of K_2Fe (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. donovan* promastigote. The 96-well micro titer plates were filled with 100 μL complete Roswell

Park Memorial Institute media containing 3×10^6 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 ℃ under 5% CO_2 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 (IC_{50}) 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 IC_{50} 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
Phytochemical screening of *A. otallensis*.

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 IC_{50} was also done. *A. otallensis* extract had a good anti-leishmaniosis activity with an IC_{50} 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 IC_{50} of extracts and drugs was shown in Table 3. When we saw potency of the extract, as compared to IC_{50} of the standard drugs, *A. otallensis* extracts were more potent than MLT and PM but less potent than SSG.

Table 2
Optical density measurement for *L. donovani* inhibition activity assay.

Serial dilution (µg/mL)	*A. otallensis* extract	MLT	PM	SSG
10.000	0.147 666 667	0.164 000 000	0.165 000 000	0.015 333 333
3.333	0.161 333 333	0.183 000 000	0.164 000 000	0.082 666 667
1.111	0.165 000 000	0.182 666 667	0.207 333 333	0.160 000 000
0.370	0.189 000 000	0.210 333 333	0.196 000 000	0.167 666 667
0.123	0.193 000 000	0.211 666 667	0.206 666 667	0.171 333 333
0.041	0.203 000 000	0.251 666 667	0.207 666 667	0.190 666 667
0.013	0.209 666 667	0.285 666 667	0.198 333 333	0.201 333 333
0.004	0.236 333 333	0.386 000 000	0.346 333 333	0.222 000 000

IC_{50} 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
IC_{50} data for the drugs and plant extracts.

Drugs used for the test	IC_{50} concentrations in µg/mL on *L. donovani*
A. otallensis extract	0.1230
MLT	2.4100
PM	1.1430
SSG	0.0704

4. 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

anticancer, anti-proliferative, antimicrobial, 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 563) strains (IC_{50} = 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.

Acknowledgments

The authors would like to thank the staffs of Tikur Anbessa Specialized Hospital for their cooperation during collection of the data.

References

[1] Kuru T, Jirata D, Genetu A, Barr S, Mengistu Y, Aseffa A, et al. *Leishmania*a *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 Silti Woreda, Ethiopia [dissertation]. Addis Ababa: Addis Ababa University; 2007.

[8] Balkew M, Gebre-Michael T, Berhe N, Ali A, Hailu 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.malariaconsortium.org/userfiles/file/NTD%20Resources/VL%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] Nwaoguikpe 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] Trease 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] Jaafari MR, Hooshmand S, Samiei A, Hossainzadeh 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, Rahuman 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 HU, 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.