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RESEARCH ARTICLE

In vitro Antileishmanial Effect of the Plant Extracts from *Aloe vera* (L.) Burm.f. and *Hypericum perforatum* L. Leaves [1]

Mahmut ÜLGER [1,a] Seda TEZCAN ÜLGER [2,b (*)] Efdal OKTAY GÜLTEKİN [2,c] Erdal YABALAK [3,d]
Harun GÜLBUDAK [2,e] Nuran DELİALİOĞLU [2,f] Ahmet Murat GİZİR [3,g] Gönül ASLAN [2,h]

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[1] Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, TR-33169 Mersin - TURKEY
[2] Mersin University, Faculty of Medicine, Department of Medical Microbiology, TR-33343 Mersin - TURKEY
[3] Mersin University, Faculty of Arts and Science, Department of Chemistry, TR-33343 Mersin - TURKEY
ORCIDs: [a] 0000-0001-6649-4195; [b] 0000-0002-0823-3680; [c] 0000-0002-0962-152X; [d] 0000-0002-4009-4174; [e] 0000-0003-3199-3132
[f] 0000-0001-8535-3291; [g] 0000-0002-9781-446X; [h] 0000-0002-1221-7907

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Abstract

The activity of currently available antiparasitic drugs has been threatened by the occurrence of drug-resistant parasite populations, toxic effects, and high cost. Therefore, the discovery of more potent antiparasitic drugs coming from medicinal plants is seen as a significant approach to overcome the problem. This study aimed to evaluate the *in vitro* efficiency of plant extracts of *Aloe vera* (L.) Burm.f. and *Hypericum perforatum* L. leaves against promastigote forms of *Leishmania tropica*. The antileishmanial activity of the plant extracts was determined using *in vitro* microdilution method. Decreasing concentrations (25 to 0.01 mg/mL) of extracts were tested on *Leishmania* promastigotes. The effect of plant extracts on the viability of promastigotes of *L. tropica* was evaluated by counting viable or motile forms in a Neubauer hemocytometer. The data assessed as % growth in comparison to the controls. The 50% inhibitory concentration (IC_{50}) values of the plant extracts were determined using The Quest Graph™ IC_{50} Calculator by logistic regression analysis. *A. vera* (L.) Burm.f. showed leishmanicidal activity at high concentrations of 25, 12.5, and 6.25 mg/ml with 100% growth inhibition of *L. tropica* promastigotes, while *H. perforatum* L. was found to be effective at the concentration range of 25 to 1.56 mg/mL. The IC_{50} of *H. perforatum* L. was determined as 0.23 mg/mL, and IC_{50} of *A. vera* (L.) Burm.f was determined as 1.91 mg/mL. Our study showed that *A. vera* (L.) Burm.f. and *H. perforatum* L. leaves can be a potential medicinal alternatives for the treatment of Leishmaniasis. The antiparasitic efficiency of these plant extracts can be considered a significant improvement in the specification of antileishmanial agents and should be supported by further *in vivo* studies.

Keywords: *In vitro antileishmanial effect, Aloe vera (L.) Burm.f., Hypericum perforatum L.*

Aloe vera (L.) Burm.f. ve *Hypericum perforatum* L. Yapraklarından Elde Edilen Bitki Ekstraktlarının *İn vitro* Antileishmanial Etkisi

Öz

Günümüzde mevcut antiparazitik ilaçların etkinliği, ilaca dirençli parazit popülasyonlarının ortaya çıkması, toksik etkiler ve yüksek maliyet nedeniyle tehdit altındadır. Bu nedenle, tedavi edici bitkilerden parazitlere karşı daha etkili ilaçların ortaya çıkarılması, bu sorunun üstesinden gelmek için önemli bir yaklaşım olarak görülmektedir. Çalışmada, *Aloe vera* (L.) Burm.f. and *Hypericum perforatum* L. bitki ekstraktlarının *Leishmania tropica*'nın promastigot formlarına karşı *in vitro* etkinliğinin değerlendirilmesi amaçlandı. Bitki ekstraktlarının *in vitro* antileishmanial aktivitesi mikrodilüsyon yöntemi kullanılarak belirlendi. Ekstraktların azalan konsantrasyonları (25 ila 0.01 mg/mL) *Leishmania* promastigotları üzerinde test edildi. Bitki ekstraktlarının *L. tropica* promastigotlarının canlılığı üzerindeki etkisi, Neubauer hemositometrede canlı veya hareketli formlar sayılarak değerlendirildi ve veriler kontrollere kıyasla % büyüme olarak değerlendirildi. Her ekstrakt için %50 inhibitör konsantrasyon (IC_{50}) değerleri, Quest Graph™ IC_{50} Hesaplayıcı kullanılarak lojistik regresyon analizi ile belirlendi. *A. vera* (L.) Burm.f., *L. tropica* promastigotları üzerinde %100 büyüme inhibisyonu ile 25, 12.5 ve 6.25 mg/mL'lik yüksek konsantrasyonlarda *Leishmania* parazitlerini öldürücü aktivite gösterirken, *H. perforatum* L.'un 25 ila 1.56 mg/mL konsantrasyon aralığında etkili olduğu bulundu. *H. perforatum* L.'nin IC_{50}'si 0.23 mg/mL ve *A. vera* (L.) Burm.f'nin IC_{50}'si 1.91 mg/mL olarak belirlendi. Çalışmamız, *A. vera* (L.) Burm.f. ve *H. perforatum* L. yapraklarının, leishmaniasis tedavisi için potansiyel tıbbi alternatif olabileceğini gösterdi. Bu bitki ekstraktlarının antiparazitik etkinliği, antileishmanial ajanlarının belirlenmesinde büyük bir gelişme olarak düşünülebilir ve daha ileri *in vivo* çalışmalarla desteklenmelidir.

Anahtar sözcükler: *İn vitro antileishmanial etki, Aloe vera (L.) Burm.f., Hypericum perforatum L.*

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(*) Corresponding Author

Tel: +90 324 341 2815-1576 Cellular phone: +90 532 761 0723 Fax: +90 324 341 2312
E-mail: tezcanseda@yahoo.com, tezcanseda@mersin.edu.tr (S. Tezcan Ülger)

Introduction

Leishmaniasis is a vector-borne tropical/subtropical disease caused by the *Leishmania* genus obligate intracellular parasite and is still one of the globally major health problems, particularly in developing countries [1]. The transmission of *Leishmania* to humans occurs through the bite of the *Phlebotomus* genus sandfly or the *Lutzomyia* species in the Old World and the New World, respectively [1,2]. *L. major*, *L. tropica*, *L. infantum*, *L. donovani,* and *L. aethiopica* are Old World leishmaniasis agents [3]. The disease has three clinical manifestations called Visceral, Mucocutaneous and Cutaneous Leishmaniasis (CL). The visceral form is the severest caused mostly by *L. donovani* and *L. infantum* and manifested by an infection of the liver and spleen. The predominantly CL agents are *L. tropica*, *L. major,* and *L. aethiopica*, and CL skin lesions, in most cases, heal on their own and leave permanent scars. Mucocutaneous Leishmaniasis which is caused by *L. braziliensis* and *L. amazonensis* is characterized by destruction and/or obstruction of the nose, pharynx, larynx, and generation of painful mucosal lesions [4].

The treatment of Leishmaniasis is very difficult because the amastigotes, one of the developmental forms of the parasite, are reside in the macrophages of the host [5]. The drug-resistance developed by parasites is the main determinant of treatment failure, although other factors also lead to this event, including immune-deficiency in patients and malnourishment prevent from elimination of the parasites by a natural defense mechanism [5,6]. The pentavalent antimonials have been the first-choice drugs for the treatment of Leishmaniasis, but high cardiotoxicity and development of resistance are the main reasons for treatment failure [7]. Amphotericin B and its lipid formulations, paromomycin, pentamidine, and miltefosine have been alternative drugs for antileishmanial chemotherapy, however, they have restricted use owing to their severe side effects, high cost, or high potential for resistance [6,8]. The toxic effects of the drugs used in the treatment of Leishmaniasis, the expensive treatment, and the resistance of the parasite to the drug have led to the research of alternative treatment methods [9,10].

In search of a better and cheaper leishmanicidal agent, plant extracts and plant-derived bioactive compounds are probably to be a new source of medicinal agents [11]. Moreover, the results obtained from a search about natural products with antileishmanial activity raise the interest in synthetic compounds as potential therapeutic candidates [2,12]. According to the World Health Organization (WHO), up to 80% of developing country populations rely on traditional medicine because of cultural customs or no other choice [13]. Due to the urgent need for alternative treatments in the treatment of Leishmaniasis, it has led researchers screen the activities of natural products for potential use.

A. vera (L.) Burm.f. is a widely used multifunctional medicinal plant belonging to the *Liliaceae* family and has been recognized as an excellent source of home remedies in Asia and the world [14]. The different fractions of *A. vera* leaf are well documented for their different potential activities, including cytotoxic, antimicrobial, anti-leishmanial, antioxidant, and wound healing [14-16]. These features are mainly related to the found of more than 200 different biologically active substances in the inner gel of the leaves [17].

H. perforatum L., belongs to the *Hypericaceae* family, and the olive oil macerate of the flowering plants is a popular home-remedy especially used for quick recovery of cuts and burns in Turkish folk medicine [18]. Moreover, therapeutic effects of this plant have been confirmed for numerous medicinal purposes such as wound healing, treatment of myalgia, antioxidant, anti-inflammatory, anti-cancer, antimicrobial effects, and the antidepressant in different parts of the world [19-21].

The current problems with anti-leishmania drugs have led to the growing need for new drug alternatives. The plants such as *A. vera* (L.) Burm.f. and *H. perforatum* L., which have the potential to be used for drug development against *Leishmania* parasites, may lead to the development of new medicinal substances with more study. The present study was intended to evaluate the *in vitro* efficiency of plant extracts of the *A. vera* (L.) Burm.f. and *H. perforatum* L. leaves obtained by Soxhlet and ultrasonic-assisted extraction (UAE) methods against *L. tropica* promastigotes.

Material and Methods

Preparation of Plant Extract

The *A. vera* (L.) Burm.f. and *H. perforatum* L. leaves were obtained from local people during summer 2018 in the Mersin Province of Southern Turkey.

Soxhlet apparatus was used for Soxhlet extraction, and Bandelin Sonopuls HD 3200 (Berlin, Germany) ultrasonic apparatus (20 kHz, 200 W) with a probe (KE-76) were used for sonication in the UAE method. Ethanol was supplied from JT Baker. Rotary evaporator (Hei-VAP, Heidolph Instruments) was used to concentrate the plant extracts.

Soxhlet Extraction Method

In this study, the most commonly used soxhlet extraction method was used in plant extraction, and ethanol was used as a solvent. 10 g of air-dried and grounded *H. perforatum* L. and *A. vera* (L.) Burm.f. samples were extracted with 300 mL of ethanol for 3.5 h under reflux in each experiment. The densities of the obtained extracts of *H. perforatum* L. and *A. vera* (L.) Burm.f. after concentrating by rotary evaporator were found as 0.068 g/mL and 0.0424 g/mL, respectively.

Ultrasonic Assisted Extraction Method

Ultrasonic-assisted extraction is the other method used in the extraction of *A. vera* (L.) Burm.f. and *H. perforatum* L. samples. 8.3 g of air-dried and grounded samples were placed into a glass beaker following by the addition of 250 mL of ethanol in each experiment. After immersing the probe of the ultrasonic system into the beaker, the UAE process was initialized at 36% amplitude value. The process was carried out under atmospheric pressure for an hour at a fixed temperature of 333 K of temperature. The densities of the concentrated extracts of *A. vera* (L.) Burm.f. and *H. perforatum* L. were found as 0.0295 g/mL and 0.0439 g/mL, respectively.

Preparation of the Dilution of Plant Extracts

The concentrations of *A. vera* (L.) Burm.f. and *H. perforatum* L. plants extract obtained by Soxhlet and UAE methods were adjusted to 25 mg/mL with diluted RPMI 1640 medium (Sigma-Aldrich). The stock solutions were sterilized by filtration through a 0.22 µm pore diameter membrane filter in a laminar cabinet. The extract was utilized fresh and also prepared at different concentrations to evaluate its antileishmanial activity.

Parasite Cultures

L. tropica patient isolate was kindly provided by Professor Gulnaz Culha (Mustafa Kemal University, Faculty of Medicine, Parasitology Department). *L. tropica* promastigotes were cultured in Novy-MacNal-Nicol (NNN) medium overlaid with consolidation fluid, supplemented with 20% heat-inactivated fetal bovine serum (FBS, Biological Industries, USA), 100 IU/mL penicillin-G/0.1 mg/mL streptomycin (Pen Strep, Gibco Thermo Fisher Scientific, USA). Mid-log phase promastigotes were maintained in T25 sterile disposable culture flasks (25 cm^2) by weekly passages at 26°C in RPMI-1640 medium with L-glutamine at pH 6.9 supplemented with 10% FBS, and 100 IU/mL penicillin/0.1 mg/mL streptomycin. The culture is observed daily for parasitic density by using light inverted microscopy and Neubauer haemocytometer. Promastigotes were kept at densities ranging between 1-5x10^5 promastigotes/mL growth.

Promastigote counts: The appearance and motility of promastigotes were monitored microscopic examinations and evaluated by counting the parasites on a Neubauer hemocytometer. Parasite culture samples mixed with vortex were mixed with an equal volume of 0.01 M phosphate-buffered saline (PBS), pH 7.2 containing 2% formaldehyde (Riedel-de Hain, Germany) for immobilization of promastigotes. The promastigote concentration was determined after counting fixed parasites in a Neubauer hemocytometer (Marienfeld SuperiorTM, Germany) at 400x magnification, followed by sufficient dilution in PBS. Parasites were adjusted at densities 5x10^5 promastigotes/mL growth.

In vitro Antileishmanial Activity Assay

In this study, the antileishmanial activity of plant extracts obtained by two different methods was investigated in 96-well microplates by the microdilution method. Promastigotes of *L. tropica* (5x10^5 on growth concentration) were subjected to decreasing concentrations (25 to 0.01 mg/mL) of plant extracts in RPMI-1640 medium supplemented with 20% heat-inactivated FBS on 96 well microtiter plates at 26°C. As a control antileishmanial drug, Amphotericin B (Biological Industries, USA) was used at 4 µg/mL concentration (100% of mortality). After 4 days of cultivation at 26°C, parasites viability was evaluated by counting viable or motile forms in a Neubauer hemocytometer to determine the number of live parasites per well. All experiments were performed in duplicate; after calculating the means, the parasites counted in each dilution well were evaluated as % growth compared to controls [22].

Statistical Analysis

The statistical analysis was determined by Mann Whitney U test and statistical differences were considered significant at p-values less than 0.05. The 50% inhibitory concentration (IC_{50}) values of the plant extracts were determined using The Quest Graph™ IC_{50} Calculator by logistic regression analysis [23]. Drug concentration-parasite inhibition curves were was determined by graphical extrapolation.

Results

After 4 days of incubation at +26°C, the antileishmanial activity of *A. vera* (L.) Burm.f. and *H. perforatum* L. extracts (25 to 0.01 mg/mL) with both of Soxhlet and UAE method resulted in dose-dependent parasite killing by microdilution method. The results were shown as % growth inhibition calculated after counting motile or viable promastigotes for all dilutions of both plant extracts.

A. vera (L.) Burm.f. extracts obtained by Soxhlet extraction method at concentrations of 25, 12.5, and 6.25 mg/mL showed 100% growth inhibition of *L. tropica* promastigotes and showed high to moderate leishmanicidal activity at the concentration range of 3.12 to 0.01 mg/mL by reducing the parasite viability in the range of 95% to 3.4%. *H. perforatum* L. extracts obtained by Soxhlet extraction method at the concentration range of 25 to 1.56 mg/mL showed 100% growth inhibition of promastigotes and showed high to moderate leishmanicidal activity at the concentration range of 0.78 to 0.01 mg/mL by reducing the parasite viability in the range of 96.2% to 3.8% *(Table 1; Fig. 1)*.

At the end of incubation, IC_{50} of the *A. vera* (L.) Burm.f. against *L. tropica* promastigotes was determined as 1.91 mg/mL and IC_{50} of *H. perforatum* L. as 0.23 mg/mL with Soxhlet extraction method.

A. vera (L.) Burm.f. extracts obtained by UAE at concentrations of 25, 12.5, and 6.25 mg/mL showed 100% growth inhibition

Table 1. *Antileishmanial activity of A. vera (L.) Burm.f. and H. perforatum L. plant extracts obtained by Soxhlet extraction method*

Concentration (mg/mL)	*A. vera* (L.) Burm.f.		*H. perforatum* L.	
	Growth Rate (%)	Inhibition Rate (%)	Growth Rate (%)	Inhibition Rate (%)
25	0	100	0	100
12.5	0	100	0	100
6.25	0	100	0	100
3.12	5	95	0	100
1.56	71.8	28.2	0	100
0.78	86.8	13.2	3.8	96.2
0.39	92.3	7.7	11.1	88.9
0.19	94	6	67.2	32.8
0.09	95.2	4.8	87.4	12.6
0.04	96	4	93.6	6.4
0.02	96.2	3.8	95.1	4.9
0.01	96.6	3.4	96.2	3.8

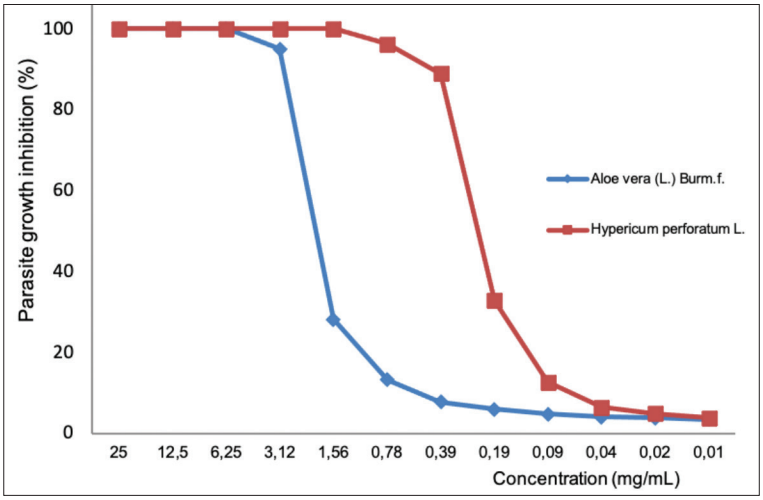

Fig 1. Inhibition of *L. tropica* promastigote growth by *A. vera* (L.) Burm.f. (diamonds) and *H. perforatum* L. (squares) extracts by Soxhlet extraction method

and showed high to moderate leishmanicidal activity at the concentration range of 3.12 to 0.01 mg/mL by reducing the parasite viability in the range of 95.3 to 2.8%. *H. perforatum* L. extracts obtained by UAE at the concentration range of 25 to 1.56 mg/mL showed 100% growth inhibition and showed high to moderate leishmanicidal activity at the concentration range of 0.78 to 0.01 mg/mL by reducing the parasite viability in the range of 98.6% to 3.9% *(Table 2; Fig. 2)*.

There was no statistically significant difference between the effects of extracts of *A. vera* and *H. perforatum* L. plants obtained by Soxhlet extraction method and UAE method on Leishmania promastigotes (P= 0.887).

The IC_{50} of the *A. vera* (L.) Burm.f. against *L. tropica* promastigotes was determined as 2.08 mg/mL and IC_{50} of *H. perforatum* L. as 0.23 mg/mL with UAE method. Amphotericin B was found 100% active at 4 µg/mL against *L. tropica* promastigotes.

Discussion

Leishmaniasis is recognized as a major public health problem by the WHO. It is reported that approximately 350 million people worldwide are at risk of becoming infected with Leishmaniasis, and approximately 0.7-1.2 million of CL and 0.2-0.4 million of Visceral Leishmaniasis cases occur annually [3]. The most common clinical form of Leishmaniasis is CL, and it generally affects poor and developing countries such as the Mediterranean Basin, Asia, the Middle East, Africa, and South America [2,3]. CL is endemic, particularly in the Southeastern and Mediterranean Regions in Turkey [24]. Because of the civil war in Syria, the southern or the southeastern part of Turkey have received great migration, and this is the cause of the complicated epidemiological status of Leishmaniasis [25]. In Turkey, *L. tropica* is responsible for the CL cases, and meglumine antimonate is widely used in the treatment of patients with CL. It has been experimentally shown that

Table 2. *Antileishmanial activity of A. vera (L.) Burm.f. and H. perforatum L. plant extracts obtained by UAE method*

Concentration (mg/mL)	*A. vera* (L.) Burm.f.		*H. perforatum* L.	
	Growth Rate (%)	Inhibition Rate (%)	Growth Rate (%)	Inhibition Rate (%)
25	0	100	0	100
12.5	0	100	0	100
6.25	0	100	0	100
3.12	4.7	95.3	0	100
1.56	83.8	16.2	0	100
0.78	89.1	10.9	1.4	98.6
0.39	92.1	7.9	3.6	96.4
0.19	93.2	6.8	74	26
0.09	94.4	5.6	91.1	8.9
0.04	94.7	5.3	93.1	6.9
0.02	96.1	3.9	94.4	5.6
0.01	97.2	2.8	96.1	3.9

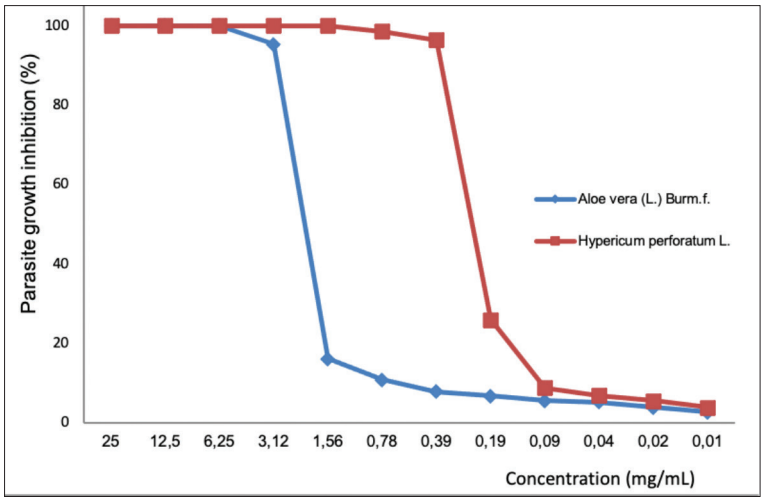

Fig 2. Inhibition of *L. tropica* promastigote growth by *A.vera* (L.) Burm.f. (diamonds) and *H. perforatum* L. (squares) extracts by UAE method

L. tropica isolates acquire resistance against meglumine antimoniate in a very short time in our country and stated that in case of inadequate and incomplete treatment of CL patients, the number of resistant cases might increase rapidly, and resistant leishmaniasis foci might occur [26].

Due to the drug resistance, toxic effects, and high cost of the available drugs for the current treatment of Leishmaniasis [12], various studies have focused on *in vitro/vivo* efficiency of various plant extracts against *Leishmania* species to detect a new antileishmanial component. A range of plant extracts have been shown to exhibit *in vitro* antileishmanial activity and have been approved for use in folk medicine [27].

This is the first study reporting the leishmanicidal activity for locally grown *A. vera* (L.) Burm.f. and *H. perforatum* (L.) species on *L. tropica* in our region, Mersin Province of Southern Turkey. Here, it was shown that, the ethanolic extracts of *A. vera* (L.) Burm.f. and *H. perforatum* (L.) were able to kill promastigote forms of *L. tropica* with the dose-dependent manner.

A. vera (L.) Burm.f. has widespread use in health products, and studies can be performed on the whole herbs, inner gel, and leaf exudate. In several studies, it was identified that the *Aloe* plant extracts had a direct leishmanicidal activity on promastigotes. In a study aimed at evaluating the *in vitro* efficiency of *A. vera* leaf exudate (AVL) against Leishmaniasis, promastigotes were found as susceptible to AVL, and their IC_{50} ranged from 100 to 180 µg/mL [28]. The efficiency of *A. nyeriensis* extracts used in the treatment of parasitic diseases by rural indigenous communities against *L. major* promastigotes was shown to exhibit 68.4±6.30% mortality at 1000 g/mL [9]. In a study by De Queiroz et al.[29] for the first time to confirm the ethno-pharmacological use of traditional medicinal plants including *A. vera* from the Brazilian flora for the treatment of Leishmaniasis, it has been shown that an extract of the *A. vera* plant exhibits direct activity against promastigote

forms at 100 µg/mL and inhibits growth by 82.9%. The IC_{50} values of methanol fraction of *A. vera*, on promastigotes of *L. infantum* were reported as 1.54 µg/mL. The inhibitory effect was determined in *L. infantum* promastigotes, but the low efficiency on amastigote forms and high cytotoxicity in the *in vivo* tests were attributed to secondary metabolites belonging to the quinone group abundant in this plant [30]. However, we found a relatively low efficiency at higher concentrations. In our study, it was observed that ethanolic extracts of *A. vera* (L.) Burm.f. totally inhibited the *in vitro* growth of promastigote forms of *L. tropica* at concentrations of 25, 12.5, and 6.25 mg/mL, by both Soxhlet and UAE methods. The IC_{50} of the *A. vera* (L.) Burm.f. against *L. tropica* promastigotes was determined as 1.91 mg/mL with Soxhlet extraction method and 2.08 mg/mL with UAE method. The low effect of this plant can be attributed to the different distribution or amounts of active substances that mediate the antileishmanial effect.

It has been reported that different extracts of *Aloe* genus plants show activity against protozoa such as *Toxoplasma gondii*, *Plasmodium falciparum*, *Babesia* sp. [31-33], confirming this potential. Therefore, we think that with more studies to be conducted, *A. vera* (L.) Burm.f. may have the potential to develop drugs against *Leishmania* parasites.

Based on our research, although biological activity has been discovered from *H. perforatum*, we did not attain sufficient regional studies on antileishmanial activity. Several reports have confirmed the therapeutical potential of the *Hypericum* genus plants for many medicinal purposes, but there is still insufficient evidence on the efficiency of *H. perforatum* L. against the *Leishmania* parasites. Promising results have been reported showing that lipophilic extracts of *Hypericum* plants are contain useful bioactive compounds for treating leishmaniasis. It was shown that *H. carinatum*, *H. polyanthemum* and *H. linoides* could kill the *Leishmania* parasites depending on the dose, and *H. polyanthemum* exhibited significant IC_{50} leishmanicidal activity at concentration of 36.1 µg/mL [34]. Here we demonstrate antileishmanial activity obtained from extracts of *H. perforatum* L. against promastigote forms of the *L. tropica* parasite. It was observed that ethanolic extracts of *H. perforatum* L. totally inhibited the *in vitro* growth of promastigote forms of *L. tropica* at concentrations of 25 to 1.56 mg/mL, by both of Soxhlet and UAE methods in our study. The IC_{50} of the *H. perforatum* L. against *L. tropica* promastigotes was determined as 0.23 mg/mL with both of Soxhlet and UAE methods.

Studies have shown that plant extracts or isolated compounds belonging to the *Hypericum* genus exhibit antiprotozoal activity against *Trichomonas vaginalis*, *P. falciparum*, *T. gondii*, and inhibition on *Entamoeba* encystation [35-38]. In one study, it was reported that *H. perforatum* olive oil macerate showed mild inhibitory activity against *Trypanosoma brucei rhodesiense* (IC_{50} of 15.9-64.5 µg/mL) [39]. These results demonstrated that *Hypericum* species is a candidate to treat other parasitic diseases, the antiparasitic properties of *H. perforatum* should be revealed by further *in vitro* and *in vivo* studies.

The applied extraction method and the solvent used are among the most important factors in the extraction process from various matrices [40,41]. In our study, for the extraction of antileishmanial agents from plants preferred to use the Soxhlet extraction method and UAE methods using ethanol as a solvent. The methods based on ultrasonic irradiation are new and effective as well as they are environmentally friendly [42]. Nowadays, ultrasonic irradiation-based methods are used in many processes such as oxidation, extraction, etc. [42,43]. UAE method has advantages over conventional methods such as high extraction yield, short extraction time and low energy consumption [44]. At the end of our study, it was determined that there was no difference in the antileishmanial activities of the plant extracts obtained by both methods.

In conclusion, the results of the present study showed that both Soxhlet and ultrasonic-assisted extracts of *A. vera* (L.) Burm.f. and H. *perforatum* L. leaves exhibited antileishmanial activity against *L. tropica* promastigotes *in vitro*, which seems to be promising their use in folk medicine. The antiparasitic efficiency of these plant extracts can be considered as a major improvement in the specification of antileishmanial agents and should be supported by further *in vivo* studies.

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Conflict of Interests

The authors declare that there is no conflict of interest.

Author Contributions

MÜ, STÜ, and GA designed the project. EY, EOG, and AMG performed the extraction of plants. MÜ, STÜ, and EOG performed the experiment and analyzed the data. HG and ND maintained of parasite culture. MÜ and STÜ wrote the manuscript. All authors reviewed and approved the final manuscript.

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