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In vitro efficacy of *Hypericum perforatum* and *Urtica dioica* on *Leishmania tropica*

Hypericum perforatum ve *Urtica dioica*'nın *Leishmania tropica* üzerindeki in vitro etkinliği

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ABSTRACT

Objective: *Leishmania* parasites cause a wide range of human diseases from localized self-healing cutaneous lesions to fatal visceral disease. Failure of the drugs used in the treatment of leishmaniasis, side effects, and drug resistance has increased the need for new drugs. As an alternative to treatment under these requirements, the use of herbal extract comes up. The aim of this study is to research the anti-leishmanial effect of herbs that *Urtica dioica* and *Hypericum perforatum*.

Methods: Our study was designed ın vitro efficacy of herbal products. In this study, the promastigotes these are growth and passaged RPMI-1640 medium with L-glutamine buffered and supplemented with 10% Fetal calf serum (FCS) are inoculated in mixed solutions prepared with different herbal drug extract concentrations and medium. Parasites were allowed to incubate at + 26 °C for 72 hours. End of time, the parasites were incubated in the plate are counted on the Thoma slide. The 50% inhibitory concentrations are calculated.

ÖZET

Amaç: *Leishmania* cinsi parazitler, kendiliğinden iyileşebilen lokalize cilt lezyonlarından, ölümcül visseral hastalıklara kadar bir grup insan hastalığına neden olabilir. Leishmaniasis tedavisinde kullanılan ilaçların yetersizliği, yan etkileri ve direnç sorunu yeni ilaçlara olan gereksinimi arttırmıştır. Bu gereksinimler doğrultusunda tedaviye alternatif olarak bitkisel ekstraktların kullanımı gündeme gelmektedir. Bu çalışmanın amacı *Urtica dioica* ve *Hypericum perforatum* bitkilerinin anti-leishmanial etkinliklerini araştırmaktır.

Yöntem: Yaptığımız çalışmada, %10 Fetal calf serum (FCS) eklenmiş L-glutaminli RPMI-1640 besiyerinde çoğaltılan ve pasajlanan promastigotlar, değişik konsantrasyonlarda hazırlanan bitkisel ilaç ekstraktı-besiyeri karışımına inoküle edildi. 72 saat boyunca +26°C'de inkübasyona bırakıldı. Bu süre sonunda plakta inkübe edilen parazitler thoma lamında sayıldı ve %50 inhibitör konsantrasyon değerleri (IC50) hesaplandı.

Bulgular: *U. dioica* kök tentürünün 0,07 mg/ml konsantrasyonunda promastigotların üremesini inhibe etmediği, 9,37 mg/ml konsantrasyonunda üremeyi

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Results: It was observed that concentration of *U. dioica* root tincture 0.07 mg/ml doesn't inhibit promastigote proliferation, in case 9.37 mg/ml inhibits growth completely, the concentration of *U. dioica* leaf fluid extract 250 mg/ml inhibitors growth completely The concentration of *H. perforatum* essential oil 0.02 mg/ml don't inhibit promastigote proliferation, in case 3.12 mg/ml inhibits growth completely. The 50% inhibitory concentration (IC50) values for root and leaves of *U. dioica* were calculated that respectively 579.93 µg/ml, 244.16 µg/ml, and *H. perforatum* essential oil was calculated that 189.88 µg/ml.

Conclusion: It was found that *U. dioica* and *H. perforatum* had inhibitory effects on *Leishmania tropica* promastigotes *in vitro*. *H. perforatum* essential oil has the best antileishmanial activity of the drugs used in this study. The high effect of the *H. perforatum* essential oil, *U. dioica* root, and leaf extracts, against *Leishmania* promastigotes has been reported with this study, so this issue will lead to other studies. It was thought that both of them can be used as an alternative treatment option in the future because they are less toxic than many drugs in routine.

Key Words: Leishmania, *Hypericum perforatum*, *Urtica dioica*, antileishmanial effect, herbal treatment

tamamen inhibe ettiği, *U. dioica* yaprak ekstraktının 250 mg/ml konsantrasyonda üremeyi tamamen inhibe ettiği görüldü. *H. perforatum* esansiyel yağının ise 0,02 mg/ml konsantrasyonda promastigotların üremesini inhibe etmezken, 3,12 mg/ml konsantrasyonda üremeyi tamamen inhibe ettiği görüldü. *U. dioica* kök ve yaprakları için IC50 değerleri sırasıyla 579,93 µg/ml, 244,16 µg/ml olarak hesaplanmıştır. *H. perforatum* esansiyel yağı için ise IC50 değeri 189,88 µg/ml olarak hesaplanmıştır.

Sonuç: *U. dioica* ve *H. perforatum*'un *Leishmania tropica* promastigotlarına karşı *in vitro* ortamda anti-leishmanial aktivitelerinin varlığı tespit edilmiştir. Çalışmamızda kullandığımız ilaçlar arasında *Hypericum perforatum* esansiyel yağı en etkili anti-leishmanial aktivitesi olan ilaçtır. *H. perforatum* esansiyel yağının, *U. dioica* kök ve yaprak ekstraktlarının, *Leishmania* promastigotlarına karşı yüksek düzeyde etki gösterdiğinin ilk defa bu çalışma ile rapor edilmiş olması, bu konuda yapılacak diğer çalışmalara öncülük edecektir. Bu ekstraktlarn rutin kullanımdaki birçok ilaçtan daha az toksik olmaları nedeniyle ileride alternatif tedavi seçeneği olabilecekleri düşünülmektedir.

Anahtar Kelimeler: Leishmania, *Hypericum perforatum*, *Urtica dioica*, antileishmanial etki, bitkisel tedavi

INTRODUCTION

Leishmaniasis is a vector-borne infectious disease caused by the *Leishmania* species that can lead to different clinical conditions (1). More than twenty-one species of *Leishmania* transmitted by the sand fly vector can cause leishmaniasis (2). Leishmaniosis, whose two main clinical forms are visceral leishmaniasis and cutaneous leishmaniasis, is seen in approximately 100 endemic countries, with 0.7-1 million new cases reported annually (3). In endemic regions where the poorest segments of the global population reside, the risk of infection increases due to poor housing conditions, insufficient environmental cleanliness, a lack of personal protective measures, work, and migration, depending on the economy (4). World Health Organization (WHO) defines leishmaniosis as a neglected tropical disease due

to the difficulty and cost of diagnosis, treatment, and follow-up, lack of control tools, insufficient investment in research, and the fact that the affected people live in rural areas where access to treatment is inadequate (5).

Pentavalent antimonials, amphotericin B, paromomycin, and miltefosine are used as first-line therapies in the treatment of leishmaniosis and these medicine may lead to adverse events such as renal failure, cardiotoxicity, pancreatitis, and impaired liver functions. However, increased parasite resistance against these chemotherapeutics can be observed (6). There is no proven vaccine against the disease in humans, and less costly immunotherapy approaches that have limited side effects and do not create parasite resistance are emphasised (1). Given the increased demand for herbal products, for which there is insufficient data on their efficacy and safety profile, studies have been conducted on their use in the treatment of leishmaniosis (7). Most studies have been performed using promastigote forms of *Leishmania* in vitro. Further studies need to be conducted in vivo and in host macrophages (8).

Among natural products, essential fatty acids showed significant activity against *Leishmania* promastigotes (9). With their hydrophobic structure, they can penetrate cells and show a broad spectrum of biological activity (10). The chemical composition and the pharmacologically antidepressant, antinociceptive, and inhibition of monoamine oxidase, antiviral, antibacterial, antifungal, and anti-proliferative activity of *Hypericum perforatum* (*H. perforatum*) , known as Saint John's Wart, is a genus of Hyperceae (11,12). *H. perforatum*, which grows in Europe, North America, North Africa, and East Asia, and has more than 450 species, is the most important of the *Hyperium* species due to its pharmacological activity (13) (Figure 1-2).

Figure 1. *Hypericum perforatum* flowers

Figure 2. *Hypericum perforatum* leaf

Stinging Nettle (*Urtica dioica* (*U. dioica*)), is known to be in the *Urticaceae* plant family, can be seen in different parts of the world, such as India, Iran, Malaysia, and the United States. It has long been known as a fibre and medicinal plant and has been used for the control of hypertension, the regulation of blood sugar, prostatic hyperplasia, and anti-inflammatory treatment (14,15) (Figure 3).

In our study, we aimed to show the in vitro anti-leishmanial effect of *H. perforatum* essential oil and anti-leishmanial effect of *U. dioica* root and leaf extract due to their inhibitory effects on *Leishmania* promastigotes.

Figure 3. *Urtica dioica*

MATERIAL and METHOD

Preparation of the Medium

Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chemical Co, USA) broth containing L-glutamine and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was used as the medium for sensitivity and passages in the production of *Leishmania*. The PH value of the medium was adjusted to 7.3-7.4. To prevent contamination, 80 µg/ml of gentamicin was added to the medium to be used for passage. The medium mixture was sterilised by filtering it into sterile bottles with 0.45 µm filters and stored at +4°C until use. Before use, the medium was brought to room temperature, and 10% inactivated fetal calf serum (FCS) (Pan Biotech, Germany) was added.

Obtaining *Leishmania tropica* Promastigotes

The study was conducted at the İstanbul Aydın University Faculty of Medicine Medical Microbiology Research Laboratory. In the study, *L. tropica* promastigotes, which were isolated from a cutaneous leishmaniasis patient and produced by passage into RPMI 1640 medium with HEPES containing 10% inactivated FCS and gentamicin in a culture flask, were used.

The culture medium was checked with an inverted microscope to look for promastigotes.

Herbal Medicine Solutions

As an active ingredient, *H. perforatum* essential oil (Bristol Botanicals, UK), *U. dioica* leaf liquid extract (Bristol Botanicals, UK), and *Urtica dioica* root tincture (Bristol Botanicals, UK) were used. *H. perforatum* essential oil (1,000 mg/ml) was dissolved in 10% dimethylsulfoxide (DMSO, 10% solution in PBS) at a ratio of 1/10, and subsequent dilutions were made with RPMI medium at a ratio of 1:2 each time.

Urtica dioica leaf extract (1,000 mg/ml, 25% ethanol) and root tincture (300 mg/ml, 25% ethanol) were prepared in an RPMI medium in 1/2-1/4096 dilutions.

Inoculation of Parasites in Herbal Medicine Solutions

Parasite cultures in an RPMI 1640 medium and 2% formol-PBS solution were taken in equal amounts and mixed in a micro-test tube, and the promastigotes were immobilised. Parasites were counted on Thoma slides. Serial dilutions of herbal medicine extracts were prepared in

96-well plates. After the dilutions were prepared, promastigotes were inoculated into each well, the number of which was determined by counting on the Thoma slide.

A total of 200 µl of parasite-herbal medicine in dilutions of 500-0.244 mg/ml for the *U. dioica* leaf extract, 150-0.07 mg/ml for the *U. dioica* root tincture, and 50-0.02 mg/ml for *H. perforatum* essential oil extract mixtures were obtained.

Evaluation of Incubation and Growth

The inoculated plate was incubated at 26-26.5 ºC for 72 hours. At the end of the incubation, the contents of the wells in the plate were transferred to the Thoma slide, and a parasite count was performed. Tables were created by determining the inhibition and growth rates for each concentration, and herbal medicine extract-promastigote growth inhibition curves were drawn. Concentration values (IC50) inhibiting 50% of promastigotes were calculated online (16).

Experiments were run at least three times for each herbal medicine extract. The drug concentrations causing 50% inhibition (IC50) are shown in the graph drawn with the % inhibition values calculated against the herbal drug extract concentrations.

RESULTS

When the growth and inhibition rates were determined by counting the parasites on Thoma slides in the cultivations made on the media, after 72 hours of incubation at +26°C, the *U. dioica* root tincture did not inhibit the growth of promastigotes at concentrations of 0.07 mg/ml but it was observed that it inhibited reproduction by 100% at concentrations of 9.37 mg/ml (Table 1).

Table 1. Promastigote growth rates and inhibition rates for *Urtica dioica* root tincture

U. dioica root tincture Dilutions (mg/ml)	Growth rate (%)	İnhibition rate (%)
Control	100	0
0,07	100	0
0,14	77	23
0,29	61	39
0,58	42	58
1,17	23	77
2,34	13	87
4,68	3	97
9,37	0	100*
18,75	0	100
37,5	0	100
75	0	100
150	0	100

**U. dioica* root tincture inhibited reproduction of 100% at concentrations of 9.37 mg/ml

The IC50 value for the *U. dioica* root tincture was calculated as 579.93 µg/ml (Figure 4). Dilutions were prolonged because inhibition continued even in the last dilution with the *U. dioica* leaf extract. It was observed that the *U. dioica* leaf extract inhibited the growth of promastigotes by 100% at a concentration of 250 mg/ml (Table 2).

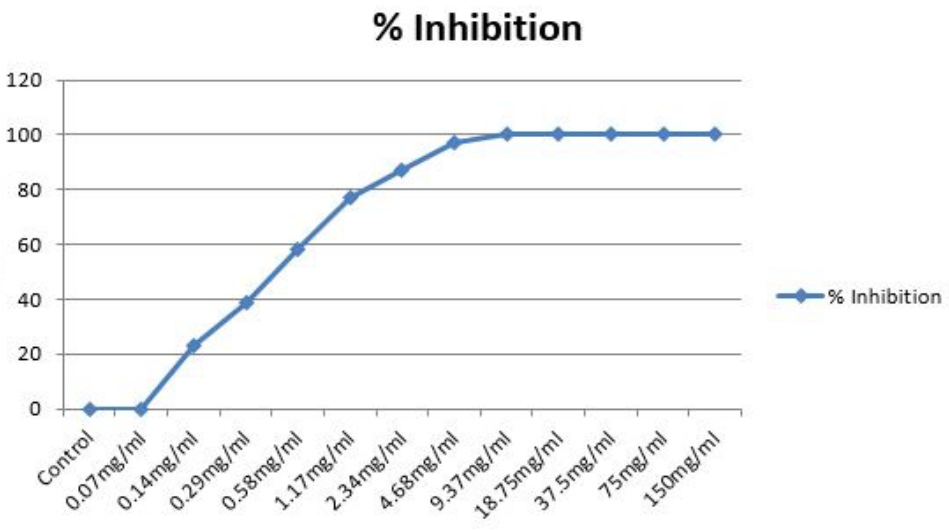

Figure 4. Effect of *U. dioica* root tincture on the growth of *Leishmania tropica* promastigotes

Table 2. Promastigote growth rates and inhibition rates for *Urtica dioica* leaf extract

U. dioica leaf extract dilution (mg/ml)	Growth rate (%)	Inhibition rate (%)
Control	100	0
0,003	92	8
0,007	72	28
0,015	67	33
0,030	77	23
0,061	62	38
0,122	43	57
0,244	48	52
0,488	29	71
0,976	25	75
1,953	24	76
3,906	24	76
7,812	24	76
15,625	18	82
31,25	18	82
62,5	8	92
125	3	97
250	0	100*
500	0	100

* *U. dioica* leaf extract inhibited the growth of promastigotes of 100% at a concentration of 250 mg/ml

The IC50 value for the leaf extract of the *U. dioica* was calculated as 244.16 µg/ml (Figure 5). It was observed that *H. perforatum* essential oil did not inhibit the growth of promastigotes at a concentration of 0.02 mg/mL and inhibited growth by 100% at a concentration of 3.12 mg/ml (Table 3). The IC50 value for *H. perforatum* essential oil was calculated as 189.88 µg/ml (Figure 6).

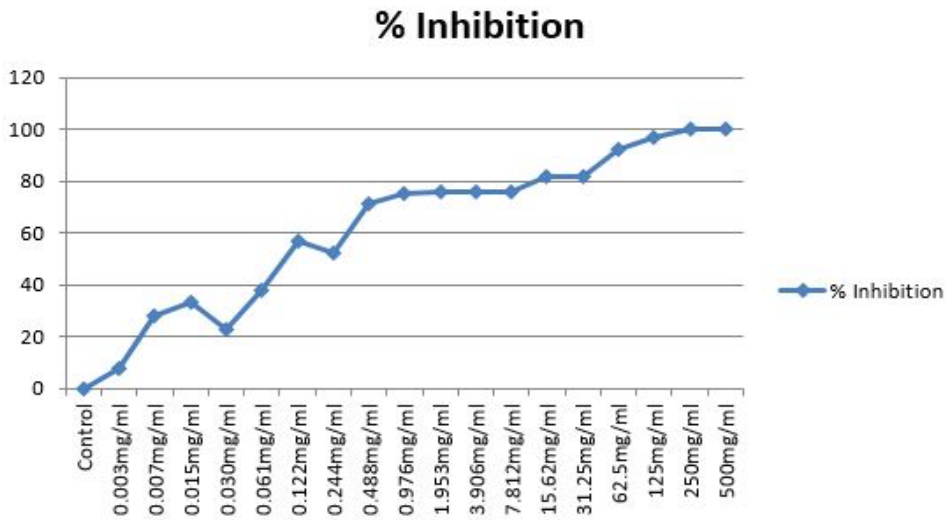

Figure 5. Effect of *U. dioica* leaf extract on the growth of *Leishmania tropica* promastigotes

Table 3. Promastigote growth rates and inhibition rates for *Hypericum perforatum* essential oil

H. perforatum essential oil dilution (mg/ml)	Growth rates (%)	Inhibiton rates (%)
CONTROL	100	0
0,02	100	0
0,04	84	16
0,09	82	18
0,19	47	53
0,39	37	63
0,78	18	82
1,56	5	95
3,12	0	100*
6,25	0	100
12,5	0	100
25	0	100
50	0	100

* *Hypericum perforatum* essential oil inhibited the growth of promastigotes at a concentration of 3.12 mg/ml

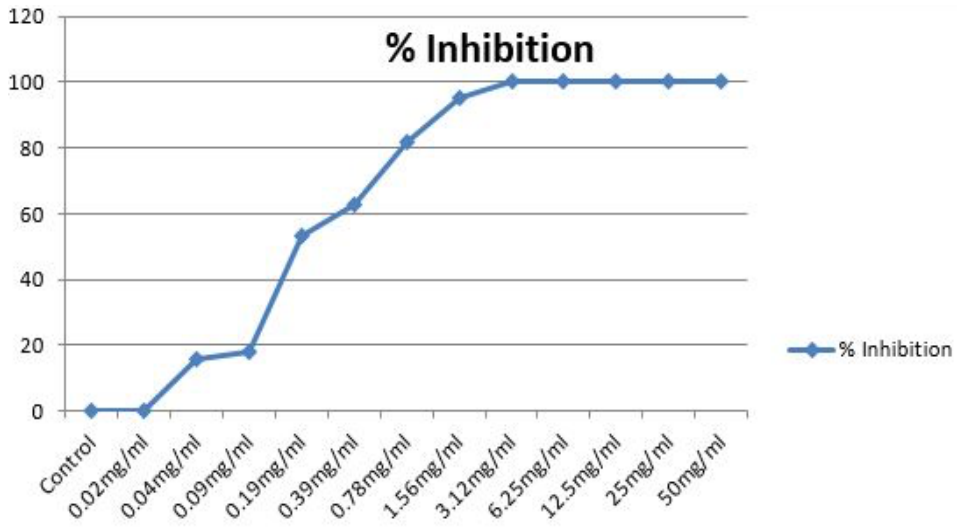

Figure 4. Effect of *H. perforatum* essential oil on the growth of *Leishmania tropica* promastigotes

Amphotericin B was used as a positive control. It inhibited growth at a concentration of 2µg/ml. The contribution of 25% ethanol in the content of the *U. dioica* plant solutions to the inhibitory effect on promastigotes was investigated. It was determined that the inhibitory effect detected in the dilutions made with *U. dioica* did not persist in the dilutions made with ethanol. The contribution of 10% DMSO, which we used to dissolve the essential oil, to the inhibitory effect on promastigotes was also investigated. No inhibitory effect of DMSO on promastigotes was detected.

DISCUSSION

In our study, the presence of the anti-leishmanial activities of *H. perforatum* and *U. dioica* against *L. tropica* promastigotes was determined. The IC50 value was the lowest for the *H. perforatum* essential oil, which was found to be more effective than the others.

Leishmaniasis is a disease caused by the obligate intracellular parasite *Leishmania* and *Leishmania* exists in two forms in its life cycle: as promastigote in a vector and amastigote in the mammalian host (17,18). Leishmaniasis can occur in various clinical forms, ranging from self-healing skin lesions to severe fatal diseases with organ involvement (19). Meglumine antimoniate and pentamidine, which are used in the treatment of leishmaniosis because of without effective vaccine, require long injections. New drugs are needed because of the toxic effects of amphotericin B and pentamidine (20). A total of 65% of the 15 antiparasitic drugs approved between 1981 and 2006 consisted of natural products or the derivatives of natural products (21). In the treatment of leishmaniosis, many plants with different medicinal effects collected from the tropical region are used (18). Chincinella-Carmona et al. investigated the anti-Leishmanial effect of 67 fresh or dried extracts of plants measuring % 50 inhibitory concentration (IC50) in their study and found that 16 plants were more effective than others (22). Montesino et al. investigated the in vitro activities of 58 herbal extracts against *Leishmania*, *Trypanosoma*, and *Plasmodium* and found that 16 extracts were effective (23).

Previous studies have noted the effectiveness of *U. dioica* root tincture in allergic rhinitis, arthritis, cardiovascular diseases, and prostate diseases. Badirzadeh et al. conducted the only study in the literature on anti-Leishmania activity (15). They investigated the in vivo mice infected with *Leishmania* major and in vitro activity of *U. dioica* aqueous extract against *Leishmania* and determined that *U. dioica* was effective against the *Leishmania* parasite.

In our study, besides determining the in vitro activity of the *U. dioica* root tincture against *Leishmania* promastigotes, we used the *U. dioica* leaf extract for *Leishmania* activity for the first time. When their efficacy was evaluated according to IC50 values, we found that the *U. dioica* leaf extract was more effective against *Leishmania* than the *U. dioica* root tincture.

Hypericum perforatum, which grows in many parts of the world and is accepted as a nutritional supplement, is an invasive species, especially in Asia and Europe. It has antidepressive, anxiolytic effects and is used in the treatment of depression, postmenopausal symptoms, obsessive-compulsive disorder, behavioural disorders, and psoriasis (24). Some bioactive molecule derived from Hypericum species is studied for leischmanicidal activity by measurement of IC50. For example a study by Dagnino et al. indicated that substances obtained from the *Hypericum* species may be high leischmanicidal activity against promastigot by inducing mitochondria and reactive oxygen compounds in *Leishmania* promastigotes (25). In their other study, Dagnino et al. found that the lipophilic extracts of four *Hypericum* species were effective against *L. amazonensis* (26). In this study most effective types are *H. carinatum, H. linoides* and *H. polyanthemum*. Studies have shown that caffeic acid, ferulic acid, syringic acid, 4-hydroxybenzoic acid, and chemicals found in herbal extracts exhibit parasiticidal effects against *L. tropica* (27). Studies have also shown that herbal extracts, such as *Zatara multiflora* oil, *Pistacia vera* essential oil, and *Myrrutus communis* oil, are effective against *L. tropica*, which is the causative agent of cutaneous *Leishmania* (28,30). *H. perforatum* has not previously been used in the treatment of *L. tropica*. Thus, our study was the first to establish that *H. perforatum* was effective against *L. tropica*.

In recent years, herbal products have been used to treat a wide range of infectious diseases as well as other diseases. We detected the presence of the antileishmanial activities of *H. perforatum* and *U. dioica*, which are less toxic than many drugs routinely used against *L. tropica* promastigotes. We think that examining the substance or substances responsible for the antiLeishmanial effect of the plant solutions we used in our study, conducting toxicity studies, and developing the results of these studies with in vivo models may be useful for future clinical studies.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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