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RESEARCH ARTICLE

Assessment of *In-vitro* Antileishmanial Activities of *Cynara scolymus* Extracts Against *Leishmania tropica* [1]

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Abstract

It was aimed to investigate *in vitro* antileishmanial activities of the receptacle, bractea, and stem leaves extracts of *Cynara scolymus* (artichoke) against *Leishmania tropica*. The *Leishmania* isolate, isolated from a cutaneous leishmaniasis patient from Manisa province, Turkey and stored in liquid nitrogen, was identified as *L. tropica* (MHOM/TR/2012/CBCL-LT) by genotyping. *In vitro* antileishmanial activities of *C. scolymus* plant extracts were examined by CelltTiter-glo and hemocytometry, and cytotoxic activities by MTT. IC_{50} values of receptacle water (WRC), aqueous ethanol (ARC) and ethanol (ERC), bractea leaf water (WBC), aqueous ethanol (ABC) and ethanol (EBC), and stem leaf water (WSC), aqueous ethanol (ASC) and ethanol (ESC) extracts were determined as 2.45 mg/mL, 1.52 mg/mL, 1.66 mg/mL, 3.45 mg/mL, 1.46 mg/mL and 0.58 mg/mL, 0.24 mg/mL, 0.21 mg/mL and 0.08 mg/mL, respectively. When these results are compared with the drug-free control group, it was determined that stem leaf aqueous ethanol (SI: 7.98), ethanol (SI: 4.96) and water (SI: 2.71) extracts with the highest selectivity index (SI) values showed antileishmanial activity ($P<0.05$). Extracts of *C. scolymus* did not show cytotoxic activity except for WBC, WRC and ARC. In conclusion, the data presented in the current study indicated that *C. scolymus* stem leaf extracts (ESC, ASC and WSC) present effective antileishmanial activity. Future studies could focus on the identification and purification of the antileishmanial compounds within these extracts for analysis of their *in vivo* antileishmanial activity.

Keywords: *Antileishmanial activity, Artichoke, Cutaneous leishmaniasis, Cynara scolymus, Leishmania tropica*

Leishmania tropica'ya Karşı *Cynara scolymus* Ekstrelerinin *In-vitro* Antileishmanial Etkinliğinin Değerlendirilmesi

Öz

Çalışmamızda *Cynara scolymus* (enginar) bitkisinin reseptakulum, brakte ve gövde yaprağı kısımlarından hazırlanmış ekstrelerinin *Leishmania tropica*'ya karşı *in vitro* antileishmanial aktivitelerinin araştırılması amaçlanmıştır. Türkiye'nin Manisa ilinden kutanöz leishmaniasis hastasından izole edilen ve sıvı azotta saklanan bir *Leishmania* izolatı, yapılan tür tayini sonucunda *L. tropica* (MHOM/TR/2012/CBCL-LT) olarak saptanmıştır. *Cynara scolymus* bitki ekstrelerinin *in vitro* antileishmanial aktiviteleri CelltTiter-glo ve hemositometri yöntemleriyle, sitotoksik aktiviteleri ise MTT yöntemiyle incelenmiştir. Reseptakulum su, sulu etanol ve etanol, brakte yaprağı su, sulu etanol ve etanol ve gövde yaprağı su, sulu etanol ve etanol ekstrelerinin IC_{50} değerleri sırasıyla 2.45 mg/mL, 1.52 mg/mL, 1.66 mg/mL, 3.45 mg/mL, 1.46 mg/mL ve 0.58 mg/mL, 0.24 mg/mL, 0.21 mg/mL ve 0.08 mg/mL olarak saptanmıştır. Bu sonuçlar ilaçsız kontrol grubuyla karşılaştırıldığında; en yüksek seçicilik indeksi (SI) değerlerine sahip olan gövde yaprağı sulu etanol (SI: 7.98), etanol (SI: 4.96) ve su (SI: 2.71) ekstrelerinin antileishmanial etkinlik gösterdiği saptanmıştır ($P<0.05$). *Cynara scolymus* bitkisinin brakte yaprağı su, reseptakulum su ve reseptakulum sulu etanol ekstreleri dışındaki ekstreler sitotoksik aktivite göstermemiştir. Sonuç olarak elde edilen verilere göre *C. scolymus* gövde yaprağı sulu etanol, etanol ve su ekstrelerinin etkili antileishmanial aktivite gösterdiği saptanmıştır. İleriki çalışmalarda, bu ekstrelerin içeriğindeki antileishmanial aktiviteden sorumlu bileşiklerinin tanımlanması, saflaştırılması ve *in vivo* antileishmanial aktivitelerinin analizine çalışılacaktır.

Anahtar sözcükler: *Antileishmanial aktivite, Cynara scolymus, Enginar, Kutanöz leishmaniasis, Leishmania tropica*

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Introduction

Leishmaniasis is a zoonotic/anthroponotic parasitic infectious disease caused by obligate intracellular *Leishmania* protozoan parasites and transmitted by the sand fly vector *(Phlebotomus* spp.). Leishmaniasis, which ranks second among the neglected parasitic diseases in the world after malaria that causes the most common death, is seen in a wide geographical area including Turkey, especially in developing countries (Middle East, South America, Africa, Central Asia and Mediterranean Basin) [1]. According to the World Health Organization (WHO), more than 1 billion people in 92 countries and 83 regions, worldwide in 2018, were living at risk of infection in areas endemic for leishmaniasis, and an estimated 30.000 new cases of visceral leishmaniasis (VL) and more than 1 million new cases of cutaneous leishmaniasis (CL) are reported each year [2].

The clinical manifestation of the disease varies depending on the species of parasite and isoenzyme structure. Accordingly, in addition to the CL and VL forms of leishmaniasis, there are different clinical forms such as mucocutaneous leishmaniasis (MKL), post kala azar dermal leishmaniasis (PKDL) and diffuse cutaneous leishmaniasis (DCL). The cases usually recover spontaneously without treatment by leaving a scar in CL, which is the clinically mildest form of the disease. *Leishmania tropica*, *L. major*, *L. aethiopica* and *L. infantum* are causative agents of CL in the Old World include. The causative agents of CL in Turkey are *L. tropica* and *L. infantum*. However, recently, *L. major* and *L. donovani* were identified by molecular methods using clinical samples of patients with CL from Şanlıurfa and Adana provinces in the southern part of Turkey [3]. There is a decrease in the incidence of leishmaniasis due to malaria eradication studies that have been implemented in Turkey since the 1950s. However, since 1980, it has come up again as a public health problem in certain regions, especially in Cukurova and Southeast Anatolia. Because of the migration to Turkey from countries and regions, where leishmaniasis is endemic, the increasing number of cases has been confirmed in recent studies [4,5].

Pentavalent antimonial compounds are used as the first line treatment of leishmaniasis. In addition, drugs such as paramomycin, miltefosine, fluconazole and liposomal amphotericin b are beneficial as an alternative therapy. For reasons such as development of resistance to drugs in use, the toxic side effects of some drugs and their high cost, studies on alternative treatment methods and vaccine development carry on. Investigating the use of herbal products for medical purposes in the development of new drugs with low cost and few side effects take an important place in the development of alternative treatment methods [1].

In this study, it was aimed to investigate *in vitro* antileishmanial activity of *C. scolymus* (artichoke) plant extracts, prepared from the receptacle, bractea leaves and stem leaves, by using water, aqueous ethanol and ethanol solvents, against *L. tropica* in order to investigate new active substances and new drug candidates that can be used in the treatment of leishmaniasis.

Material and Methods

Ethical Statement

This study was approved by the Ethics Committee of Clinical Research at Medical School of Manisa Celal Bayar University (Approval no: 21/05/2019-E.42582).

Preparation of Cynara scolymus Plant Extracts

Cynara scolymus aerial parts were gathered from the region belonging to 38°25'24.0"N 26°35'05.0"E coordinates from İzmir province *(Fig. 1)*. The receptacle, bractea leaves and stem leaves of the gathered *C. scolymus* plant were separated and air dried under appropriate conditions and pulverized in the grinder. The water extracts were prepared by 2% infusion of plant materials. The ethanol and 50% aqueous ethanol extracts were prepared by maceration under stirring whereas the solvent/plant material ratio was 15/1. The water extract of bractea leaf (WBC), aqueous ethanol extract of bractea leaf (ABC), ethanol extract of bractea leaf (EBC), water extract of receptacle (WRC), aqueous ethanol extract of receptacle (ARC), ethanol extract of receptacle (ERC), water extract of stem leaf (WSC), aqueous ethanol extract of stem leaf (ASC), ethanol extract of stem leaf (ESC) of *C. scolymus*, nine extracts in total, were filtered through Whatman no. 1 filter paper and evaporated under reduced pressure to dryness. The dried residues were lyophylisied and stored in screw capped vials at -20°C until analysis [6,7].

In Vitro Culture of Leishmania spp. Isolate

The *Leishmania* isolate, obtained from Turkey and stored in liquid nitrogen at the Parasite Bank of Medical School of Manisa Celal Bayar University, was removed from the liquid nitrogen tank under proper conditions and cultured in NNN medium after viability control. The NNN medium

Fig 1. *Cynara scolymus* plant

was placed in an incubator at 26°C and incubated. The medium was checked for growth on consecutive days after inoculation. Promastigotes, obtained from NNN medium detected parasite growth, were inoculated into 5 mL RPMI-1640 medium (10% FCS) in cell culture flask on the fifth day of incubation. The flasks were then incubated in an incubator (Panasonic®, Japan) at 26°C. On consecutive days, the medium was controlled for promastigote growth. *Leishmania* promastigotes, grew logarithmically ($1x10^6$ promastigotes/mL), were used in the current study [8].

Genotyping of Leishmania spp. Isolate

Genetic material was obtained to determine the genotype of the *Leishmania* isolate and DNA isolation was performed in accordance with the High Pure PCR Template Preparation Kit (Qiagen®, Germany) procedure. Real-time PCR method was applied using probes specific to the internal transcribed spacer 1 (ITS1) gene region in the current study. Ribosomal ITS1 region, separating the genes encoded SSU rRNA and 5.8S rRNA of *Leishmania* parasites, was amplified using the specific primers, the QuantiTect Probe PCR Kit Master (Qiagen®, Germany) mix and the specific probes [9,10]. A total of 25 µL reaction mixture, prepared for real-time PCR analysis, contained 4.5 µL H_20, 1 µL forward primer, 1 µL reverse primer, 0.5 µL probe1, 0.5 µL probe 2, 12.5 µL QuantiTect Probe PCR Kit Master mix (Qiagen®, Germany) and 5 µL gDNA. The *Leishmania* isolate was genotyped with the results obtained by melting analysis in the Rotor-Gene.

Cytotoxic Activity (CC_{50})

L-929 fibroblast cell line was cultured to evaluate the cytotoxic activity of nine different extracts prepared from receptacle, bractea leaves and stem leaves samples of *C. scolymus*. The cell line was cultivated in cell culture flasks under appropriate conditions (37°C, 5% CO_2 and 95% humidity/incubator (Thermo Fisher Scientific® USA) using RPMI-1640 medium (10% FCS). By preparing different final concentrations from plant extracts, their cytotoxic activities were determined colorimetrically using the MTT (Biomatik®, Germany) method and the cytotoxic concentration (CC_{50}) values, killing 50% of the cells, were determined statistically [11].

In Vitro Antileishmanial Activity (IC_{50})

Concentrations of each plant extracts (WBC, ABC, EBC, WRC, ARC, ERC, WSC, ASC, ESC) (0.01 mg/mL - 125 mg/mL) were prepared for *in vitro* experiments. The extracts were dissolved and diluted in fresh RPMI-1640 medium containing 10% FCS. The final volume was adjusted to 200 µL with fresh RPMI-1640 medium for each well of a 96-well flat-bottom cell culture plate (Ratiolab®, Germany). Promastigotes were suspended to yield 1×10^6 promastigotes/mL in each well by haemocytometry. Cell culture plates were incubated at 26°C for 48 h. At the end of the incubation process, *in vitro* antileishmanial activities of *C. scolymus* plant extracts were evaluated by hemocytometry and CellTiter-Glo methods, and inhibitor concentration (IC_{50}) values, killed 50% of the cells, were determined. Otherwise, Giemsa-stained slides were prepared and examined under the light microscope to evaluate the changes in promastigote morphology due to the efficiency of *C. scolymus* plant extracts after 48 h of incubation. As a reference drug, amphotericin b was prepared in sterile DMSO. In all experiments, in order not to affect parasite growth rate, mobility or morphology, the final concentration of DMSO was not higher than 0.5% (v/v). Cell culture plates were then read in luminoscan ascent (Thermo Fisher Scientific®, USA), viability and the antileishmanial activity values (IC_{50}) were calculated according to the absorbance values obtained, and all the tests were carried out in triplicate in the current study [8,12].

Selectivity Index (SI)

The selectivity index (SI) is a comprehensive parameter used to express *in vitro* efficacy of an herbal compound in parasite proliferation inhibition and the ratio of the cytotoxic activity value (CC_{50}) to the antileishmanial activity value (IC_{50}). SI values were determined for each plant extract of *C. Scolymus* [11].

Statistical Analysis

Statistical analysis of the data, obtained by applying Sidak's multiple comparisons test with GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA), was performed.

Results

Genotyping the Leishmania Isolate

The *Leishmania* strain, isolated from CL patient in Turkey and stored in liquid nitrogen at Parasite Bank of Medical School of Manisa Celal Bayar University, was matched to *L. tropica* (MHOM/TR/2012/CBCL-LT) as a result of genotyping with the RT-PCR method.

Cytotoxic Activity (CC_{50})

Cytotoxic activities (CC_{50}) of *C. scolymus* plant extracts, water, aqueous ethanol and ethanol extracts prepared from the receptacle, bractea and stem leaves, were determined colorimetrically by MTT method and were statistically evaluated. While WBC, ARC and WRC plant extracts of *C. scolymus* exhibited cytotoxic activity and CC_{50} values were determined as 1.61 mg/mL, 0.58 mg/mL and 1.71 mg/mL, respectively, all other extracts didn't display cytotoxic activity.

Antileishmanial Activity (IC_{50})

Antileishmanial activity (IC_{50}) of different concentrations of *C. scolymus* plant extracts, water, aqueous ethanol and ethanol extracts prepared from the receptacle, bractea

leaves and stem leaves, on *L. tropica* promastigotes were evaluated by haemocytometry and CellTiter-glo® methods after 48 h of incubation. In the evaluation of antileishmanial activity, the IC_{50} value of AmB as a control drug was evaluated as 0.05 µM. When the antileishmanial effects of water, aqueous ethanol and ethanol extracts prepared from the receptacle, bractea leaves and stem leaves of *C. scolymus* were evaluated, IC_{50} values of ESC (0.08 mg/mL), ASC (0.21 mg/mL), WSC (0.24 mg/mL), EBC (0.58 mg/mL), ABC (1.46 mg/mL), ARC (1.52 mg/mL), ERC (1.66 mg/mL), BRC (2.45 mg/mL) and WBC (3.45 mg/mL) plant extracts were determined. When these results are compared with parasite control (drug-free) results; ESC (IC_{50}=0.08 mg/mL), ASC (IC_{50}=0.21 mg/mL), WSC (IC_{50}=0.24 mg/mL) and EBC (IC_{50}=0.58 mg/mL) extracts exhibited antileishmanial effect ($P<0.05$). However, other extracts did not display antileishmanial effect ($P>0.05$). It is seen that the ESC (IC_{50}=0.08 mg/mL) extract has the highest inhibition degree among the extracts with antileishmanial activity *(Fig. 2)*. In the wake of light microscopic examination of Giemsa-stained slides, prepared after 48 h of incubation, nuclear and cytoplasmic changes of promastigotes weren't detected in promastigotes at concentrations below the IC_{50} values of *C. scolymus* extracts in terms of promastigote morphology. On the other hand, significant pathological changes such as rounding, granulation, shortening or extinction of flagellum, and expansion of nucleus were observed at IC_{50} values and higher concentrations of *C. scolymus* plant extracts *(Fig. 3-A,B)*.

Selectivity Index (SI)

The selectivity index (SI) is the ratio of the cytotoxic activity value (CC_{50}) to the antileishmanial activity value (IC_{50}). Selectivity index values were determined for each plant extract of *C. scolymus*, ESC (4.96), ASC (7.98), WSC (2.71), EBC (1.65), ABC (1.10), WBC (0.46), ERC (1.64), ARC (0.38), WRC (0.69), and control drug (AmB) (277.2) *(Table 1)*.

Discussion

Meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) are used as the first preference in the treatment of CL. When long-term use of these drugs is required, many disadvantages such as toxicity, cost and drug resistance are encountered. In recent years, resistance development against these drugs has been reported, notably in the northern regions of India [13]. AmB is highly costly and preferred secondary option in the treatment of leishmaniasis, and has been reported to

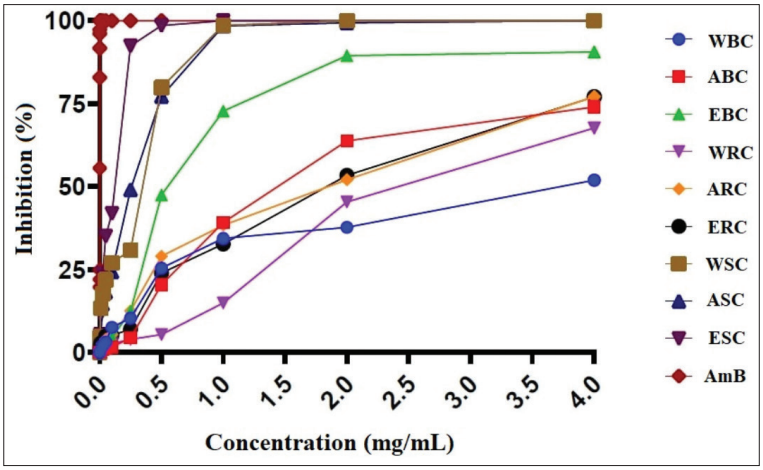

Fig 2. Antileishmanial activities of receptacle, bractea leaf and stem leaf extracts of *Cynara scolymus*. WRC: Water extract of receptacle, ARC: Aqueous ethanol extract of receptacle, ERC: Ethanol extract of receptacle, WBC: Water extract of bractea leaf, ABC: Aqueous ethanol extract of bractea leaf, EBC: Ethanol extract of bractea leaf, WSC: Water extract of stem leaf, ASC: Aqueous ethanol extract of stem leaf, ESC: Ethanol extract of stem leaf, AmB: Amphotericin B

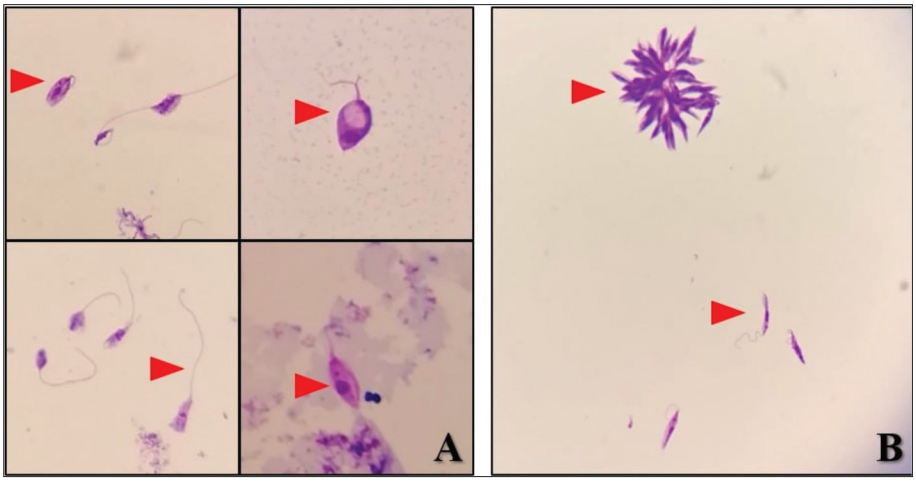

Fig 3. Morphological changes of *Leishmania tropica* promastigotes. A: Cytopathological changes due to *Cynara scolymus* stem leaf extracts on *Leishmania tropica* promastigotes, B: *Leishmania tropica* promastigotes with normal morphology (1.000× magnification)

have toxic effects on the kidneys. In addition, miltefosine, a new drug perorally used, has been reported to have teratogenic effects [14]. There is no vaccine currently in use for leishmaniasis prophylaxis. Thus, there is a need for safe, effective and cheap drugs. WHO encourages the search for new drugs and the use of natural products for the treatment of leishmaniasis due to the limitations of current treatment regimens [15]. It is believed that the plants used to treat tropical diseases can be obtained by establishing the new generation of antiparasitic drugs or the necessary infrastructure for their synthesis [16]. Therefore, new treatment options for leishmaniasis are being investigated. Especially, new drugs are defined on the basis of pharmacological concepts related to the activity of active molecules of medicinal plants or compounds with similar structures [17]. In order for chemical compounds obtained from natural sources or synthesized within the scope of pre-clinical evaluation to be used in clinical practice, toxicity and screening tests must be performed in the process of development and evaluation of new antileishmanial drugs. Although drug screening tests can mostly be performed in an *in vitro* experimental model, *in vivo* experimental models are suitable for studies investigating metabolic rates, biokinetic properties of the active substance and interaction between organs. The compounds, found effective as a result of drug screening tests, are subjected to toxicity tests in order to examine their histopathological, biochemical and functional toxic effects. It has been reported that phase I, phase II, phase III and phase IV steps should be implemented within the scope of clinical evaluation [18].

It is reported that natural plant extracts, which have the potential to be used in the treatment of leishmaniasis, can be used in the treatment by determining their biological activities with drug screening tests or by making synthetic forms of some natural compounds. In a study, conducted by Costa et al.[19] in 2021, which evaluated the antileishmanial activity of the synthetic Rip-E compound of riparin, which is an alkamide found in the immature fruits of *Aniba riparia*, $IC_{50}^{promastigote}$=4.7 µg/mL, $IC_{50}^{amastigote}$=1.3 µg/mL, CC_{50}=50.6 µg/mL and SI=38.9 values were attained and the synthetic Rip-E compound of riparin was found to show antileishmanial activity against *L. amazonensis*.

Table 1. *In vitro antileishmanial activity, cytotoxicity and selectivity index values of water, aqueous ethanol and ethanol extracts prepared from the receptacle, bractea leaf and stem leaf of Cynara scolymus*

Extracts	IC_{50} (mg/mL)	CC_{50} (mg/mL)	SI (CC_{50}/IC_{50})
ESC	0.08	0.40	4.96
ASC	0.21	1.68	7.98
WSC	0.24	0.65	2.71
EBC	0.58	0.96	1.65
ABC	1.46	1.61	1.10
WBC	3.45	1.61	0.46
ERC	1.66	2.73	1.64
ARC	1.52	0.58	0.38
WRC	2.45	1.71	0.69
AmB (Control drug)	0.0005	0.14	277.2

Cynara scolymus, researched in the current study in terms of antileishmanial activity, is a medicinal plant with a wide range of different study areas. Artichoke leaf extract (ALE) has been reported to show antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer activity. When the efficacy of C. scolymus plant extracts on parasites was researched, a limited number of references were found. The antiparasitic and hepato-protective properties of ALE on mice, experimentally infected with *Schistosoma mansoni,* were examined and compared with praziquantel in a study conducted by Sharaf EL-Deen et al.[20] in 2017. In order to evaluate the antischistosomial features of ALE, parasite burden, number of eggs, number and diameter of granuloma were measured. Although ALE did not have a significant effect on egg load and number of granulomas, it was ultimately observed that it caused a significant decrease in diameter of granuloma and improvement in liver functions and liver fibrosis [20]. In the current study, the antileishmanial effects of water, aqueous ethanol and ethanol extracts prepared from the receptacle, bracktea leaves and stem leaves of *C. scolymus* plant extracts were evaluated and compared with AmB, and it was observed that EBC, ESC, ASC and WSC extracts exhibited antileishmanial effect and were statistically significant.

Antifungal activities of chloroform, ethanol and ethyl acetate extracts of leaves, heads and stems of *C. scolymus* plant were investigated using agar-well diffusion technique in a study conducted by Zhu et al.[21] in 2005. All structures of the *C. scolymus* (leaf, head and stem) showed activity against the organisms tested. At the end of the study, it was concluded that leaf extracts and ethanol fractions were the most effective extracts against all organisms tested. It was reported that *C. scolymus* leaves could be a new potential agent in the treatment of fungal infections in the study [21]. Also, the antileishmanial activity of the receptacle, brachtea leaves and stem leaves of the *C. scolymus* plant extracts was investigated and it was concluded that the leaf extracts were superior to other extracts in the current study.

The effects of chloroform, ethyl acetate and n-butanol extracts of *C. scolymus* leaf extracts on seven bacteria, four yeast and four mold species were investigated by antimicrobial disc method in a study conducted by Zhu et al.[22] in 2004. Eight phenolic compounds were isolated from artichoke leaf n-butanol extracts and when examined for their antimicrobial activities, all of them were reported to be effective against the studied organisms. Among

these compounds, chlorogenic acid, cynarin, luteolin-7-rutinoside and sinoroside have been reported to show higher activity than other compounds, are more effective against fungi compared to bacteria, and the minimum inhibitory concentration (MIC) of these compounds is 50-200 µg/mL. Among these compounds, chlorogenic acid, cynarin, luteolin-7-rutinoside and sinoroside have been reported to show higher activity than other compounds and be more effective against fungi compared to bacteria, and the minimum inhibitory concentrations (MIC) of these compounds are 50-200 µg/mL [22].

When the cytotoxic concentrations (CC_{50}) of the water, aqueous ethanol and ethanol extracts, prepared from the receptacle, bractea leaves and stem leaves of the *C. scolymus*, were evaluated, ESC, ASC, WSC, EBC, ABC and ERC extracts did not show cytotoxic activity. However, EBC, ARC ve ERC extracts of *C. scolymus* showed cytotoxic activity.

When the efficacy of the water extract obtained from *Physalis angulata* roots against *Leishmania amazonensis* was evaluated, morphological changes, observed in promastigotes at concentrations of IC_{50} and above, were associated with antileishmanial activity by Da-Silva et al.[23] in 2015. Similarly, significant pathological changes such as rounding of promastigotes, granulation, shortening or disappearance of flagellum length, widening of the nucleus were also described at IC_{50} and higher concentrations following incubation with *C. scolymus* plant extracts in the current study.

The selectivity index (SI) is an important indicator of whether a plant compound is effective or not. The selectivity index is used in *in vitro* efficacy studies of herbal compounds that inhibit parasite reproduction and is widely accepted as an important parameter. The SI value is calculated by the ratio of the cytotoxic concentration (CC_{50}) value to the inhibitor concentration (IC_{50}) value [24]. Cytotoxic concentration (CC_{50}) values and SI values of water, aqueous ethanol and ethanol extracts, prepared from the receptacle, bracktea leaves and stem leaves of *C. scolymus*, are between 0.40-2.73 mg/mL and 0.38-7.98, respectively. Various criteria have been stated in the studies to define the selective antiparasitic activities of medicinal plant extracts. For instance; selective antiparasitic activity has been reported for medicinal plant extracts when the SI value is greater than 1 by Tempone et al.[25], greater than 2 by Arevalo-Lopez et al.[26], and greater than 3 by Joshi et al.[27]. In addition, researchers working on synthetic derivatives recommend SI>6 as a convenient criterion [28]. It was accepted that *C. scolymus* plant extracts with a SI value greater than 2 showed antileishmanial activity in the current study. Compared with AmB SI value (277.2), ESC, ASC and WSC extracts with the SI values of 4.96, 7.98 and 2.21, respectively, showed high antileishmanial activity against *L. tropica* promastigotes. Therefore, the antileishmanial activity of ESC, ASC and WSC extracts whose SI values are greater than two is considered to be an important and promising development in the detection of antileishmanial agents, which is the aim of the current study.

In conclusion, according to IC50, CC50 and SI data, the lack of cytotoxic activity of stem leaf extracts (ESC, ASC and WSC) of C. scolymus and the detection of antileishmanial activity on promastigotes of L. tropica, which is the predominant etiologic agent of CL in Turkey, is considered to be an important and promising development for screening antileishmanial agents, which is the aim of the project.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

ICB, AY and TA designed the project. HK obtained plant extracts. AY, TA, SSC and ET carried experiments. AY, TA and SSC performed statistical analysis of data. The manuscript was written by TA and AY.

References

1. Özbel Y, Töz SO: Leishmaniasis. **In,** Özcel MA, Özbel Y AM (Eds): Özcel'in Tıbbi Parazit Hastalıkları. 197-244, Türkiye Parazitoloji Derneği, İzmir, 2007.

2. WHO: Leishmaniasis. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis; *Accessed:* 20.01.2021.

3. Özbilgin A, Çulha G, Uzun S, Harman M, Topal SG, Okudan F, Zeyrek F, Gündüz C, Östan İ, Karakuş M, Töz S, Kurt Ö, Akyar I, Erat A, Güngör D, Kayabaşı Ç, Çavuş İ, Bastien P, Pratlong F, Kocagöz T, Özbel Y: Leishmaniasis in Turkey: First clinical isolation of *Leishmania major* from 18 autochthonous cases of cutaneous leishmaniasis in four geographical regions. *Trop Med Int Health*, 21 (6): 783-791, 2016. DOI: 10.1111/tmi.12698

4. Ok UZ, Balcioglu IC, Taylan Ozkan A, Ozensoy S, Ozbel Y: Leishmaniasis in Turkey. *Acta Trop*, 84 (1): 43-48, 2002. DOI: 10.1016/S0001-706X(02)00134-1

5. Toz SO, Nasereddin A, Ozbel Y, Ertabaklar H, Culha G, Sevil N, Alkan MZ, Jaffe CL: Leishmaniasis in Turkey: Molecular characterization of *Leishmania* from human and canine clinical samples. *Trop Med Int Health,* 14 (11): 1401-1406, 2009. DOI: 10.1111/j.1365-3156.2009.02384.x

6. Ural IO, Kayalar H, Durmuskahya C, Cavus I, Özbilgin A: *In vivo* antimalarial activity of methanol and water extracts of *Eryngium thorifolium* Boiss (Apiaceae Family) against *P. berghei* in infected mice. *Trop J Pharm Res*, 13 (8): 1313-1317, 2014. DOI: 10.4314/tjpr.v13i8.16

7. Ozbilgin A, Durmuskahya C, Kayalar H, Ostan I: Assessment of *in vivo* antimalarial activities of some selected medicinal plants from Turkey. *Parasitol Res*, 113 (1): 165-173, 2014. DOI: 10.1007/s00436-013-3639-1

8. Ozbilgin A, Cavuş I, Yıldırım A, Kaya T, Ertabaklar H: Evaluation of

in vitro and *in vivo* drug efficacy over *Leishmania tropica*: A pilot study. *Turkiye Parazitol Derg*, 42 (1):11-19, 2018. DOI: 10.5152/tpd.2018.5554

9. Toz SO, Culha G, Zeyrek FY, Ertabaklar H, Alkan MZ, Vardarlı AT, Gunduz C, Ozbel Y: A real-time ITS1-PCR based method in the diagnosis and species identification of *Leishmania* parasite from human and dog clinical samples in Turkey. *PLoS Negl Trop Dis*, 7(5):e2205, 2013. DOI: 10.1371/journal.pntd.0002205

10. An I, Harman M, Cavus I, Ozbilgin A: The diagnostic value of lesional skin smears performed by experienced specialist in cutaneous leishmaniasis and routine microbiology laboratory. *Turk J Dermatol*, 13 (Suppl. 1): 1-5, 2019. DOI: 10.4274/tdd.galenos.2018.3812

11. Badirz adeh A, Heidari-Kharaji M, Fallah-Omrani V, Dabiri H, Araghi A, Salimi Chirani A: Antileishmanial activity of *Urtica dioica* extract against zoonotic cutaneous leishmaniasis. *PLoS Negl Trop Dis*, 14 (1): e0007843, 2020. DOI: 10.1371/journal.pntd.0007843

12. Ostan I, Saglam H, Limoncu ME, Ertabaklar H, Toz SO, Ozbel Y, Ozbilgin A: *In vitro* and *in vivo* activities of *Haplophyllum myrtifolium* against *Leishmania tropica*. *New Microbiol*, 30 (4): 439-445, 2007.

13. Croft SL, Yardley V: Chemotherapy of Leishmaniasis. *Curr Pharm Des*, 8 (4): 319-342, 2002. DOI: 10.2174/1381612023396258

14. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, Pountain AW, Mwenechanya R, Papadopoulou B: Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. *PLoS Negl Trop Dis*, 11 (12): e0006052, 2017. DOI: 10.1371/journal.pntd.0006052

15. WHO: Status of endemicity of cutaneous leishmaniasis: 2019. https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html; *Accessed:* 20.01.2021.

16. Newman DJ, Cragg GM: Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*, 75 (3): 311-335, 2012. DOI: 10.1021/np200906s

17. Araújo IAC, Paula RC, Alves CL, Faria KF, Oliveira MM, Mendes GG, Dias EMFA, Ribeiro RR, Oliveira AB, Silva SM: Efficacy of lapachol on treatment of cutaneous and visceral leishmaniasis. *Exp Parasitol*, 199, 67-73, 2019. DOI: 10.1016/j.exppara.2019.02.013

18. Kayaalp SO: Klinik öncesi değerlendirmesi. **In,** Klinik Farmakolojinin Esasları ve Temel Düzenlemeler. 4th ed., 29-46, Faryal Matbaacılık, Ankara, 2008.

19. Costa LM, Alves MMM, Brito LM, Abi-Chacra EA, Barbosa-Filho JM, Gutierrez SJC, Barreto HM, Carvalho FAA: *In vitro* antileishmanial and immunomodulatory activities of the synthetic analogue riparin E. *Chem Biol Interact*, 336:109389, 2021. DOI: 10.1016/j.cbi.2021.109389

20. Sharaf EL-Deen SA, Brakat RM, Mohamed ASED: Artichoke leaf extract protects liver of *Schistosoma mansoni* infected mice through modulation of hepatic stellate cells recruitment. *Exp Parasitol*, 178, 51-59, 2017. DOI: 10.1016/j.exppara.2017.05.005

21. Zhu XF, Zhang HX, Lo R: Antifungal activity of *Cynara scolymus* L. extracts. *Fitoterapia*, 76 (1): 108-111, 2005. DOI: 10.1016/j.fitote.2004.1 0.016

22. Zhu X, Zhang H, Lo R: Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *J Agric Food Chem*, 52 (24): 7272-7278, 2004. DOI: 10.1021/jf0490192

23. Meira CS, Guimarães ET, Dos Santos JAF, Moreira DRM, Nogueira RC, Tomassini TCB, Ribeiro IM, Campos de Souza CV, Dos Santos RR, Soares MBP: *In vitro* and *in vivo* antiparasitic activity of *Physalis angulata* L. concentrated ethanolic extract against *Trypanosoma cruzi*. *Phytomedicine*, 22 (11): 969-974, 2015. DOI: 10.1016/j.phymed.2015.07.004

24. Badirzadeh A, Heidari-Kharaji M, Fallah-Omrani V, Dabiri H, Araghi A, Chirani AS: Antileishmanial activity of *Urtica dioica* extract against zoonotic cutaneous leishmaniasis. *PLoS Negl Trop Dis*, 14 (1): e0007843, 2020. DOI: 10.1371/journal.pntd.0007843

25. Tempone AG, Martins De Oliveira C, Berlinck RGS: Current approaches to discover marine antileishmanial natural products. *Planta Med*, 77 (6): 572-585, 2011. DOI: 10.1055/s-0030-1250663

26. Arévalo-Lopéz D, Nina N, Ticona JC, Limachi I, Salamanca E, Udaeta E, Paredes C, Espinoza B, Serato A, Garnica D, Limachi A, Coaquira D, Salazar S, Flores N, Sterner O, Giménez A: Leishmanicidal and cytotoxic activity from plants used in Tacana traditional medicine (Bolivia). *J Ethnopharmacol*, 216, 120-133, 2018. DOI: 10.1016/j.jep.2018.01.023

27. Joshi B, Hendrickx S, Magar LB, Parajuli N, Dorny P, Maes L: *In vitro* antileishmanial and antimalarial activity of selected plants of Nepal. *J Intercult Ethnopharmacol*, 5 (4): 383-389, 2016. DOI: 10.5455/jice.20160728031236

28. Leal SM, Amado DF, Kouznetsov VV, Escobar P: *In Vitro* antileishmanial, trypanocidal, and mammalian cell activities of diverse N,N′-dihetaryl substituted diamines and related compounds. *Sci Pharm*, 81 (1): 43-55, 2013. DOI: 10.3797/scipharm.1205-14