Turkiye Parazitol Derg 2022;46(2):91-6
DOI: 10.4274/tpd.galenos.2021.53825

The *in vitro* anti-*Leishmania* Effect of *Zingiber officinale* Extract on Promastigotes and Amastigotes of *Leishmania major* and *Leishmania tropica*

Zingiber officinale Özütünün *Leishmania major* ve *Leishmania tropica*'nın Promastigotları ve Amastigotları Üzerindeki *in vitro* anti-*Leishmania* Etkisi

Jasem Saki[1], Elaheh Biranvand[2], Reza Arjmand[1]

[1]Cellular and Molecular Research Center, Medical Basic Sciencec Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran

Cite this article as: Saki J, Biranvand E, Arjmand R. The *in vitro* anti-*Leishmania* Effect of *Zingiber officinale* Extract on Promastigotes and Amastigotes of *Leishmania major* and *Leishmania Tropica*. Turkiye Parazitol Derg 2022;46(2):91-6.

ABSTRACT

Objective: Recently, the use of pentavalent antimony compounds for *Leishmaniasis* treatment has been associated with disease recurrence, drug resistance, and severe side effects. Therefore, there is a need to develop alternative treatment strategies. This study investigates the *in vitro* effects of *Zingiber officinale* on promastigotes and amastigotes of *Leishmania major* and *Leishmania tropica*.

Methods: Promastigotes and amastigotes of *Leishmania major* and *Leishmania tropica* were cultured and mass-produced in an RPMI1640 medium enriched with other necessary compounds. The MTT colorimetric method and calculating the IC50 value were used to evaluate the anti-leishmania activity of hydroalcoholic extract of *Zingiber officinale*.

Results: The hydroalcoholic extract of *Zingiber officinale* inhibited the growth of *Leishmania major* and *Leishmania tropica* promastigotes in 24, 48, and 72 hours after *in vitro* incubation. The IC50 of hydroalcoholic extract of *Zingiber officinale* was 56 µg/mL for *Leishmania major* and 275 µg/mL for *Leishmania tropica* promastigotes after 72 hours. The IC50 of hydroalcoholic extract of *Zingiber officinale* was 75 µg/mL for *Leishmania major* and 325 µg/mL for *Leishmania tropica amastigotes* after 72 hours.

Conclusion: The results showed that hydroalcoholic extract of *Zingiber officinale* has cytotoxicity properties, and *Leishmania tropica* has a higher resistance to hydroalcoholic extract of *Zingiber officinale* than *Leishmania major*. Further research is recommended.

Keywords: *Zingiber officinale*, *Z. officinale*, gingers, *Leishmania major*, *Leishmania tropica*

ÖZ

Amaç: Son zamanlarda, *Leishmaniasis* tedavisinde beş değerlikli antimon bileşiklerinin kullanımı, hastalığın tekrarlaması, ilaç direnci ve ciddi yan etkiler ile ilişkilendirilmiştir. Bu nedenle alternatif tedavi stratejilerinin geliştirilmesine ihtiyaç vardır. Bu çalışmanın amacı, *Zingiber officinale*'nin *Leishmania major* ve *Leishmania tropica*'nın promastigotları ve amastigotları üzerindeki laboratuvar etkilerini araştırmaktır.

Yöntemler: *Leishmania major* ve *Leishmania tropica* promastigotları ve amastigotları, diğer temel bileşiklerle zenginleştirilmiş RPMI1640 ortamında kültürlenerek toplu olarak üretilmiştir. Zencefilin hidroalkolik ekstraktının *anti-Leishmania* aktivitesini değerlendirmek için MTT kolorimetrik yöntem ve IC50 değeri kullanılmıştır.

Bulgular: *Zingiber officinale*'nin hidroalkolik özütü, *in vitro* inkübasyondan 24, 48 ve 72 saat sonra *Leishmania major* ve *Leishmania tropica* promastigotlarının büyümesini engellemiştir. *Zingiber officinale*'nin hidroalkolik ekstraktının IC50'si 72 saat sonra *Leishmania major* için 56 µg/mL ve *Leishmania tropica* promastigotlar için 275 µg/mL olarak tespit edilmiştir. *Zingiber officinale*'nin hidroalkolik ekstraktının IC50'si 72 saat sonra *Leishmania major* için 75 µg/mL ve *Leishmania tropica* amastigotes için 325 µg/mL olarak sonuçlanmıştır.

Received/Geliş Tarihi: 10.04.2021 **Accepted/Kabul Tarihi:** 17.12.2021

Address for Correspondence/Yazar Adresi: Reza Arjmand, Cellular and Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Department of Parasitology, Ahvaz, Iran
Phone/Tel: +989161135448 **E-mail/E-Posta:** arjmand.reza@yahoo.com **ORCID ID:** orcid.org/0000-0003-4990-6586

Türkiye PARAZİTOLOJİ Dergisi

Sonuç: Sonuçlar, *Zingiber officinale*'nin hidroalkolik özütünün sitotoksisite özelliklerine sahip olduğunu göstermektedir. Ayrıca, *Leishmania tropica*, *Zingiber officinale*'nin hidroalkolik özüne karşı *Leishmania major*'dan daha yüksek bir dirence sahiptir. Konu ile ilgili daha fazla araştırmaya ihtiyaç vardır.
Anahtar Kelimeler: *Zingiber officinale, Z. officinale,* zencefil, *Leishmania major, Leishmania tropica*

INTRODUCTION

The protozoan parasite of Kinetoplastida called *Leishmania* causes *Leishmaniasis*, which has different forms of clinical presentation, including skin and mucosal lesions and visceral signs (1,2). *Leishmania* is an intracellular parasite that lives in the phagolysosome of vertebrate phagocytic cells. Phlebotomine sand flies play the role of the vector of this parasite (3-5). This tropical infection, commonly seen and ignored in developing countries, is an emerging public health problem in about 2 to 4 million new cases and almost leads to 70,000 deaths each year (6). According to the World Health Organization (WHO) report, around 13 million people have been affected, 350 million others are at the risk of exposure to this disease, and 2 million new patients are added to this number annually (7-10). Iran is one of 10 countries where 75% of global cutaneous leishmaniasis cases have been reported. In addition, it ranks first place in the Middle East in terms of reported cases of cutaneous leishmaniasis (7).

Today, with the increase in the number of patients with defective immune systems, opportunistic infections such as leishmaniasis are increasing. The treatment with pentavalent antimony drugs, which is the first preference for treating leishmaniasis, is limited due to its numerous side effects and drug resistance (11). Pentavalent antimony compounds, including meglumine antimoniate (glucantime), and sodium stibogluconate (pentostam), remain the first-line treatment for all clinical forms of Leishmaniasis. These compounds inhibit adenosine triphosphate production by interrupting the parasite phosphodiesterase enzyme (12-14). However, due to inherent toxicity and frequent infections, these drugs have side effects such as liver and heart disorders and biochemical changes (15). Considering the drug resistance and tremendous side effects of these compounds, researchers seek alternative forms of treatment that are more effective with fewer side effects (16).

It is essential to study medicinal plants to find a suitable drug against the *Leishmania* parasite and Leishmaniasis (17). Several herbal compounds have been used so far for the treatment of cutaneous leishmaniasis lesions. Various researchers have recently evaluated the *in vivo* and *in vitro* effects of the parasite's essential oils and extracts of some local and native plants. One of these plants is *Zingiber officinale*, that antifungal, antibacterial, and even antiparasitic properties have been proven (17-21). *Zingiber officinale* is a yellow plant with purple petioles. Its main constituents include sugars (50-70%), fats (3-18%), oleoresins (4-5.5%) and spicy compounds (1-3%). The ingredients of *Zingiber officinale* include *gingerol shogaols, zingerone*, *Zingiberene*, and *Zabolin*, which make the odor and flavor of *Zingiber officinale* (22,23).

This study, among the MTT colorimetric, aims to determine the *in vitro* effects of the extract of *Zingiber officinale* on promastigotes and amastigotes of *Leishmania major* (MRHO/IR/75/ER) and *Leishmania tropica* (MHOM/IR/02/Mash 10).

METHODS

Cultivation of Promastigotes

The standard promastigotes of *L. tropica* (MHOM/IR/02/Mash 10) and *L. major* (MHOM/IR/75/ER) were prepared from Ahvaz Jundishapur University of Medical Sciences. Parasites were culture in RPMI1640 enriched with 10% fetal calf serum (FCS) and antibiotics (100 IU/mL penicillin and 100 IU/mL streptomycin) at 25±2 °C. The optimal 1×10^6 per mL parasite was obtained by counting promastigotes by a hemocytometer slide.

Cultivation of Amastigotes

To culture, the amastigotes forms of *L. major* and *L. tropica*, in the first step, the enriched RPMI1640 medium's acidity with 10% FCS containing 1×10^6 mL of the parasite was reduced to pH=5-5.5. Culture flasks were kept at an incubator (25 °C & 5% CO_2). The temperature was increased by 2-3 °C daily until the optimum temperature of 32°C was obtained. Within four days, the parasites were transformed from promastigotes to amastigotes (24).

Preparation of *Zingiber officinale* Extract

The *Zingiber officinale* rhizome was washed with water and dried, and then completely ground. Extraction was carried out using the 80% methanol percolation method and then evaporated at 40 °C and a low pressure using a rotary evaporator device, and the extract was condensed. The condensed extract was stored at 60 °C in a hot, dry oven and then was kept at 4 °C until use. The *Zingiber officinale* extracts were prepared at 600, 300, 150, 75, 37.5, 18.75, and 9.375 µg/mL concentrations.

Promastigotes were exposed to different concentrations of *Zingiber officinale* extract. 100 µL of the RPMI1640 medium enriched with 10% FCS containing at least 1×10^5 promastigotes were placed in 96-well plates, and 100 µL of the hydroalcoholic solution of *Zingiber officinale* extract was added to the wells at concentrations of 600, 300, 150, 75, 37.5, 18.75, and 9.375 µg/mL. The promastigotes were incubated for three days at 25±2°C. The RPMI1640 culture medium containing parasites without *Zingiber officinale* extract was used as a control. All tests were performed in triplicate**.** Amastigotes were exposed to different concentrations of *Zingiber officinale* extract. 100 µL of the RPMI1640 medium enriched with 10% FCS containing at least 1×10^5 amastigotes was placed in 96-well plates, and 100 µL of the *Zingiber officinale* extract solution was added to the wells at concentrations of 1.200, 600, 300, 150. 75, 37.5, and 18.75 µg/mL. The amastigotes were incubated for three days at 32 °C. The RPMI1640 culture medium containing parasites without *Zingiber officinale* extract was used as a control.

The parasites incubated with *Zingiber officinale* extract were evaluated using the methyl thiazolyl tetrazolium assay (MTT) method after 24, 48, and 72 hours. The MTT powder was prepared at a concentration of 5 mg/mL in PBS (Phosphate Buffered Saline), and 20 µL of it was added to each well so that the final concentration of MTT would reach 0.5 mg/mL. The plates were incubated at 25±2 °C for 2-5 hours. Subsequently, 100 µL of 1% dimethyl sulfoxide was added to each well and mixed

well to help solve the insoluble crystalline formazan formed due to the reduction of tetrazolium by the succinate dehydrogenase enzyme. After 20 minutes of incubation, the wells' optical density (OD) was read with The ELISA reader device at 570 nm. The cell viability percentage was calculated using the Excel software and the formula: [Viable cell %= (AT-AB/(AC-AB) ×100], where AB represents the optical density of the blank well, AC represents the optical density of the control well, and AT represents the optical density of the drug-treated cell. The results were calculated in an IC50 measurement by linear regression test (25).

Statistical Analysis

IC 50 values of promastigotes and intracellular amastigotes were calculated for the mean and standard deviation. We performed all tests in triplicate. The mean and standard deviation of the three trials were registered. The data were analyzed using SPSS 19. Statistical analysis of the differences among mean values gotten for the experimental groups was done by analyzing variance (ANOVA). Values less than 0.05 were considered significant.

RESULTS

The IC50 values of the *Zingiber officinale* extract for *L. major* after 24, 48, and 72 hours were 112, 90, and 56 µg/mL, respectively, while the IC50 values for *L. tropica* at those times were 600, 390, and 275 µg/mL respectively.

The IC50 values of the *Zingiber officinale* extract for amastigotes of *L. major* after 24, 48, and 72 hours were 130, 105, and 75 µg/mL, respectively, while the IC50 values for *L. tropica* at those times were 720, 430, and 325 µg/mL respectively. According to these results, *Zingiber officinale* extract showed a concentration and time-dependent cytotoxicity activity against promastigotes and amastigotes of *L. major* and *L. tropica*. However, the value of time for inhibitory effect was observed less than an increase in *Zingiber* concentrations. Over time, the cell viability increased but was still not significant for *L. tropica* promastigotes, *L. major* amastigotes, and *L. tropica* amastigotes between 24 and 48 hours. At the same time, cell viability was substantial between 24 and 72 hours (Table 1, Figures 1-4).

DISCUSSION

Leishmaniasis is a public health concern throughout the world, especially in tropical and subtropical countries (9). There are various drugs for the treatment of Leishmaniasis, but their toxicity, side effects, and drug resistance are problems associated with their use (14,26). According to the WHO, almost 80% of people use traditional medicines to treat their illnesses (27). Today, herbal drugs for treating parasitic diseases, especially Leishmaniasis, have special significance, and essential oils and extracts of various herbs have been used in recent years to treat cutaneous Leishmaniasis. Since herbal medicines are more cost-effective and have fewer side effects than chemical medicines, they can be an excellent alternative. Many studies have been conducted on the effect of medicinal herbs such as Thyme, Yarrow, Propolis, Sideritis, Medlar tree leaf, and many other herbs on Leishmaniasis. These studies indicate that the medicinal herb extract has an inhibitory effect on parasite growth in some cases. The inhibitory effect of *Zingiber officinale* extract on many microorganisms has been reported in recent years (28-30).

Table 1. The cytotoxicity activity of *Zingiber officinale* extracts on *L. major* and *L. tropica*

Parasite	Cytotoxicity µg/mL (IC50)		
	24 h	48 h	72 h
L. major (promastigotes)	112±9.03	90±6.6	156±10*
L. tropica (promastigotes)	600±17.1	390±14.2**	275±8.9***
L. major (amastigotes)	130±8	105±6.3*	75±8.4**
L. tropica (amastigotes)	720±39.04	430±22.8**	325±15.4**

Data expressed as mean ± standard deviation. *p<0.05, **p<0.01, ***p<0.001 compared to first time (24 h)

A study conducted by Shoaie et al. (20) in 2012 on clinical species of *Candida albicans* showed that *Zingiber officinale* has an inhibitory effect on the growth of it. The minimum inhibitory concentration of *Zingiber* officinale extracts on *Candida albicans* was 62.25 µg/mL (20). Feizi et al. (31) investigated the effects of methanol extract of *Zingiber officinale* on protoscoleces at three concentrations of 25, 50, and 100 mg/mL at different times. This study showed that the ethanolic extract of *Zingiber officinale* in concentrations of 50, 100, and 150 mg/mL killed 100% of the protoscoleces after 60, 90, and 120 minutes (31). Duarte et al. (32), investigated the effect of *Zingiber officinale* extract and F10 fraction on promastigotes of *L. amazonasis* and obtained the IC50 values of 125.5 µg/mL for aqueous extract of *Zingiber* officinale and 49.8 µg/mL for the F10 fraction of *Zingiber officinale* F10.

In some studies, nanoemulsion preparation based on plant extracts for anti-Leishmania use has been very promising (33). One of these anti-Leishmania extracts is derived from *Artemisia dracunculus* (*Tarragon*). Some active ingredients of which are flavonoids, phenolic acids, coumarins, and Alkamides (33,34). Another herbal medicine that has been attributed to the anti-amastigote forms of *L. tropica* properties is *Zataria multiflora* (35), however, there are limited studies on *L. tropica* in the literature. Further, the analyses confirmed that the main components of essential oil were *thymol* (*monoterpenoid phenol*), *carvacrol* (*phenolic monoterpenoid*), and *p-cymene* (*monoterpene*), that the presence of all these active ingredients in *Zingiber officinale* has also been reported (35-37). The therapeutic activities of *Zingiber* are mainly attributed to its active plant compounds *6-gingerol*, *6-shogaol*, *zingerone*, and other phenolics and flavonoids. *Gingerol* has been reported as the most abundant bioactive compound in *Zingiber* with various medicinal effects, including antioxidant, analgesic, anti-inflammatory, and antipyretic properties (38,39). A study evaluating *6-gingerol* alone in combination with amphotericin B on the *L-major* stage using experimental and *in vivo* rat models stated that the binding affinity of *6-gingerol* and *IFN-γ* (interferon-gamma) was the basis of the docking conformations (38).

This study investigated the inhibitory effects of *Zingiber officinale* extract on both promastigotes and amastigotes of *L. major* and *L. tropica*. The study showed an increase in the extract concentration and an increase in incubation time, decreasing the survival percentage of the parasite's two strains. Besides, the *Zingiber officinale* extract has a more significant inhibitory effect on *L. major* than *L. tropica*. As a result, higher concentrations of *Zingiber officinale* are needed to kill the *L. tropica* parasite, and the *Zingiber officinale* extract had a more significant effect on the promastigotes of the two strains than on their amastigotes. However, the value of time for inhibitory effect in this study was less observed than an increase in *Zingiber* concentrations.

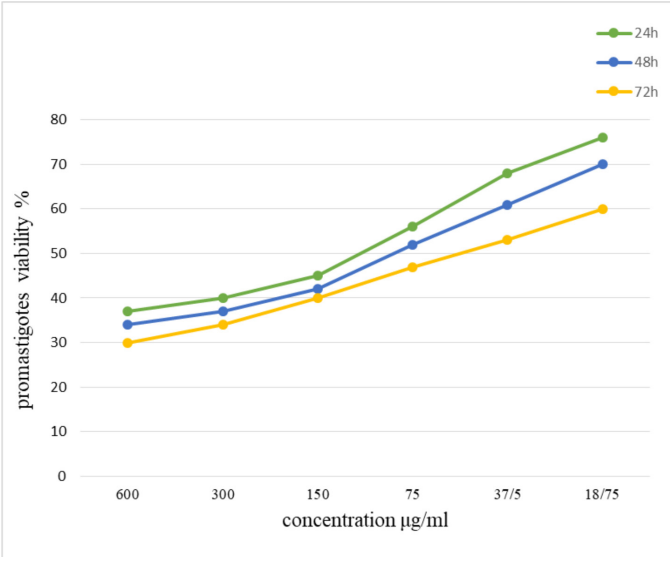

Figure 1. The cell viability of *L. major* promastigotes at different concentrations of *Zingiber officinale* extract after 24, 48, and 72 hours of incubation

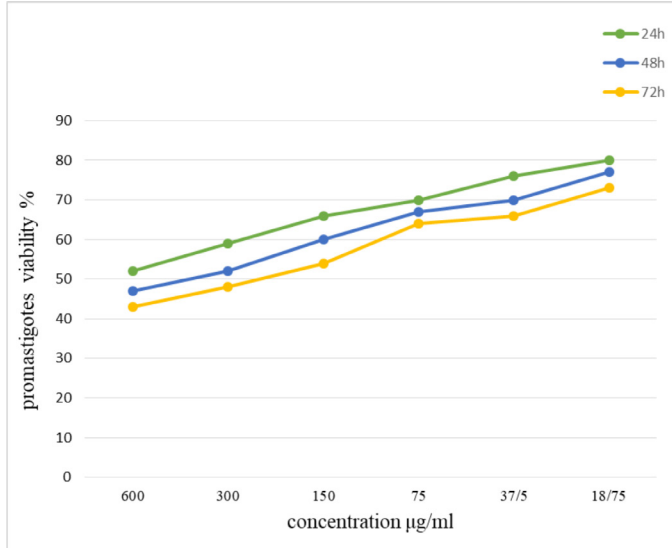

Figure 2. The cell viability of *L. tropica* promastigotes at different concentrations of *Zingiber officinale* extract after 24, 48, and 72 hours of incubation

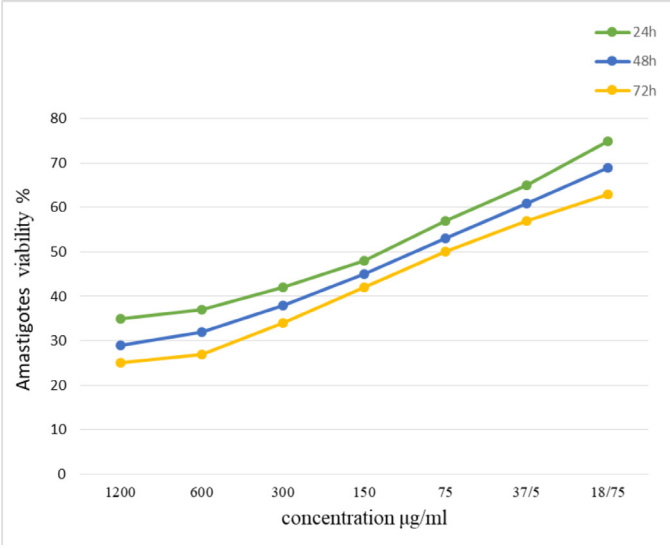

Figure 3. The cell viability of *L. major* amastigotes at varying concentrations of *Zingiber officinale* extract after 24, 48, and 72 hours of incubation

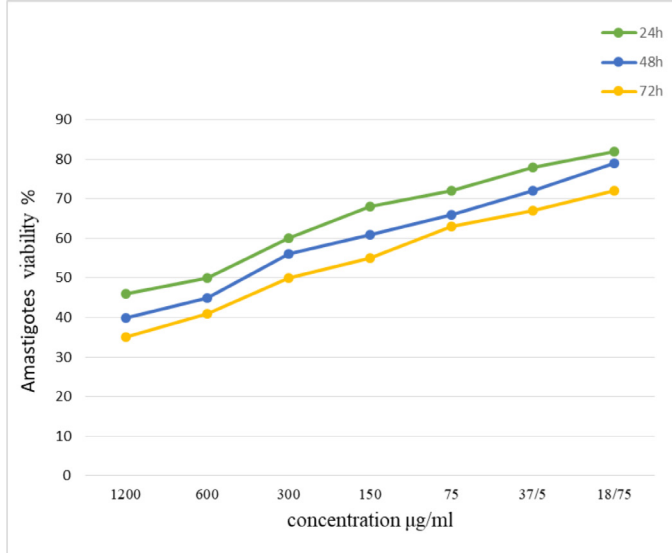

Figure 4. The cell viability of *L. tropica* amastigotes at varying concentrations of *Zingiber officinale* extract after 24, 48, and 72 hours of incubation

CONCLUSION

This study confirms the *in vitro* inhibitory effects of *Zingiber officinale* on the promastigotes and amastigotes of both *L. major* and *L. tropica*. Since the pathogens form of this parasite is intracellular, further experiments are necessary to evaluate the effect of the mentioned extract on the *Leishmania* parasite in animal models and human volunteers in later stages.

ACKNOWLEDGMENT

This study is financially supported by Grant (no: OG-94112) from the Research Deputy of Ahvaz Jundishapur University of Medical Sciences, Iran. The authors would like to kindly appreciate the cooperation of the parasitology department staff for the period of conduction. The Ethics Committee of Ahvaz Jundishapur University of Medical Sciences approved this experiment, and ethical approval no: IR.AJUMS.REC.1394.151.

*Ethics

Ethics Committee Approval: The Ethics Committee of Ahvaz Jundishapur University of Medical Sciences approved this experiment, and ethical approval no: IR.AJUMS.REC.1394.151.

Informed Consent: Given that this study was performed in vitro, there was no need for informed consent.

Peer-review: Externally and internally peer-reviewed.

*Authorship Contributions

Surgical and Medical Practices: J.S., E.B., R.A., Concept: J.S., R.A., Design: J.S., R.A., Data Collection or Processing: E.B., R.A., Analysis or Interpretation: J.S., R.A., Literature Search: J.S., E.B., R.A., Writing: J.S., E.B., R.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study is financially supported by Grant (no: OG-94112) from the Research Deputy of Ahvaz Jundishapur University of Medical Sciences, Iran.

REFERENCES

1. Babaeekhou L, Mohebali M, Lahiji N, Mehrabitavana A. The therapeutic effects of Eucalyptus, Myrtus, Ferula, Aretmisia, Allium and Urtica extracts against cutaneous leishmaniasis caused by Leishmanaia major in small white mice (out- bred). Hakim Research Journal 2007; 10: 21-7.
2. Gürel MS, Yesilova Y, Ölgen MK, Özbel Y. [Cutaneous leishmaniasis in Turkey]. Turkiye Parazitol Derg 2012; 36: 121-9.
3. Saha P, Mukhopadhyay D, Chatterjee M. Immunomodulation by chemotherapeutics against Leishmaniasis. Int Immunopharmacol 2011; 11: 1668-79.
4. Taran M, Mohebali M, Esmaeli J. In vivo efficacy of gum obtained pistacia atlantica in experimental treatment of cutaneous leishmaniasis. Iran J Public Healt 1970; 39: 36-41.
5. Van Assche T, Deschacht M, Inocêncio da Luz R, Maes L, Cos P. Leishmania-macrophage interactions: Insights into the redox biology. Free Radic Biol Med 2011; 51: 337-51.
6. Direkel Ş, Ünver Y, Akdemir C. Antileishmanial Antileishmanial Activity of New Synthesized Schiff and Mannich (Morpholine) Base Compounds. Turkiye Parazitol Derg 2020; 44: 216-20.
7. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS One 2012; 7: e35671.
8. Sinha PK, Pandey K, Bhattacharya SK. Diagnosis & management of leishmania/HIV co-infection. The Indian J Med 2005; 121: 407-14.
9. WHO Expert Committee on the Control of the Leishmaniases & World Health Organization. Control of the leishmaniases: report of a meeting of the WHO Expert Commitee on the Control of Leishmaniases, Geneva, 22-26 March 2010. Geneva: World Health Organization; 2010.
10. Shakila A, Bilqees FM, Salim A, Moinuddin M. Geographical distribution of cutaneous leishmaniasis and sand flies in Pakistan. Turkiye Parazitol Derg 2006; 30: 1-6.
11. Limoncu ME, Eraç B, Gürpinar T, Özbilgin A, Balcıoğlu IC, Hoşgör-Limoncu M. Investigation of in vitro antileishmanial activity of moxifloxacin, linezolid and caspofungin on Leishmania tropica promastigotes. Turkiye Parazitol Derg 2013; 37: 1-3.
12. Khademvatan S, Gharavi MJ, Rahim F, Saki J. Miltefosine-induced apoptotic cell death on Leishmania major and L. tropica strains. Korean J Parasitol 2011; 49: 17-23.
13. Croft SL, Yardley V. Chemotherapy of leishmaniasis. Curr Pharm Des 2002; 8: 319-42.
14. Croft SL, Sundar S, Fairlamb AH. Drug Resistance in Leishmaniasis. Clin Microbiol Rev 2006; 19: 111-26.
15. Kazemi E, Talari S, Hooshyar H. The effect of an alcoholic extract of Berberis Vulgaris on Cutaneous leishmaniasis (L. major) in BALB/c mice. Journal of School of Public Health and Institute of Public Health Research 2007; 5: 35-42.
16. Kobets T, Grekov I, Lipoldova M. Leishmaniasis: prevention, parasite detection and treatment. Curr Med Chem 2012; 19: 1443-74.
17. Luize PS, Tiuman TS, Morello LG, Maza PK, Ueda-Nakamura T, Dias Filho BP, et al. Effects of medicinal plant extracts on growth of *Leishmania (L.) amazonensis* and *Trypanosoma cruzi*. Brazilian Journal of Pharmaceutical Sciences 2005; 41: 85-94.
18. Martín T, Villaescusa L, Gasquet M, Delmas F, Bartolomé C, Díaz-Lanza A, et al. Screening for protozoocidal activity of Spanish plants. Pharmaceutical Biology 1998; 36: 56-62.
19. Arbabi M, Delavari M, Kashan ZF, Taghizadeh M, Hooshyar H. Ginger (Zingiber officinale) induces apoptosis in Trichomonas vaginalis in vitro. Int J Reprod Biomed 2016; 14: 691-8.
20. Shoaie N, Mohammadi P, Roudbar Mohammadi S. Antifungal effect of Teucrium polium and Zingiber officinale extracts on clinical isolates of Candida species. Armaghane-Danesh 2012; 17: 416-22.
21. Feyzi F, Moradkhani S, Matini M, Parandin F, Roshan A, Fallah M. In vitro Scolicidal effects of methanolic extract of artemisia (Artemisia aucheri) and ginger (Zingiber officinale) on live protoscoleces of hydatid cyst. J Arak Uni Med Sci 2015; 18: 45-52.
22. Momeni L, Zamanzad B. The antibacterial properties of Allium cepa (onion) and Zingiber officinale (ginger) extracts on Staphylococcus aureus Pseudomonas aeruginosa Escherichia coli and Candida albicans isolated from vaginal specimens. J Shahrekord Univ Med Sci 2010; 11: 81-7.
23. Chen JC, Huang LJ, Wu SL, Kuo SC, Ho TY, Hsiang CY. Ginger and its bioactive component inhibit enterotoxigenic Escherichia coli heat-labile enterotoxin-induced diarrhea in mice. J Agric Food Chem 2007; 55: 8390-7.
24. Nasereddin A, Schweynoch C, Schonian G, Jaffe CL. Characterization of Leishmania (Leishmania) tropica axenic amastigotes. Acta Trop 2010; 113: 72-9.
25. Yousefi E, Eskandari A, Javad Gharavi M, Khademvatan S. In vitro activity and cytotoxicity of Crocus sativus extract against Leihmania major (MRHO/IR/75/ER). Infect Disord Drug Targets 2014; 14: 56-60.
26. Croft SL, Seifert K, Yardley V. Current scenario of drug development for leishmaniasis. Indian J Med Res 2006; 123: 399-410.
27. Calixto J. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000; 33: 179-89.
28. Asadi M, Bahrami S, Ansari Samani R, Pakniat N. Effect of hydroalcoholic extracts of Stachys lavandulifolia Vahl and Mespilus germanica leaves on Leishmania major. Hormozgan Medical Journal 2012; 15: 279-84.
29. Barati M, Sharifi I, Sharififar F. In vitro Evaluation of Anti-Leishmanial Activities of Zataria Multiflora Boiss, Peganum Harmala and Myrtus Communis by Colorimetric Assay. JKMU 2010; 16: 32-42.
30. Shirani-Bidabadi L, Mahmoudi M, Saberi S, Zolfaghari-Baghbaderani A, Nilforoushzadeh M, Abdoli H, et al. The effectiveness of mix extracts of Thyme, Yarrow and Propolis on Cutaneous Leishmaniasis: a comparative study in animal model (Balb/c). Tehran Univ Med J 2009; 66: 785-90.
31. Feizi F, Moradkhani S, Matini M, Parandin F, Roushan A, Fallah M. To study the solicidal effects of the extracts of Ginger (Zingiber officinale) and Artemisia (Artemisia aucheri) on protoscoleces of Hydratid *Cyst in vitro*. AMUJ 2015; 18: 45-52.
32. Duarte MC, Tavares GS, Valadares DG, Lage DP, Ribeiro TG, Lage LM, et al. Antileishmanial activity and mechanism of action from a purified fraction of Zingiber officinalis Roscoe against Leishmania amazonensis. Exp Parasitol 2016; 166: 21-8.
33. Ghanbariasad A, Azadi S, Agholi M, Osanloo M. The nanoemulsion-based nanogel of Artemisia dracunculus essential oil with proper activity against Leishmania tropica and Leishmania major. Nanomedicine Research Journal 2021; 6: 89-95.
34. Mumivand H, Babalar M, Tabrizi L, Craker LE, Shokrpour M, Hadian J. Antioxidant properties and principal phenolic phytochemicals of Iranian tarragon (*Artemisia dracunculus L.)* accessions. Hortic Environ Biotechnol 2017; 58: 414-22.
35. Saedi Dezaki E, Mahmoudvand H, Sharififar F, Fallahi S, Monzote L, Ezatkhah F. Chemical composition along with anti-leishmanial and cytotoxic activity of Zataria multiflora. Pharm Biol 2016; 54: 752-8.
36. Shokri A, Saeedi M, Fakhar M, Morteza-Semnani K, Keighobadi M, Teshnizi SH, et al. Antileishmanial activity of *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils and nano-emulsions on *Leishmania major* (MRHO/IR/75/ER). Iran J Parasitol 2017; 12: 622-31.
37. Gupta R, Singh PK, Singh R, Singh RL. Pharmacological activities of Zingiber officinale (ginger) and its active ingredients: A review. IJSIR 2016; 4: 1-18.
38. Keyhani A, Sharifi I, Salarkia E, Khosravi A, Tavakoli Oliaee R, Babaei Z, et al. In vitro and in vivo therapeutic potentials of 6-gingerol in combination with amphotericin B for treatment of Leishmania major infection:

Powerful synergistic and multifunctional effects. Int Immunopharmacol 2021; 101: 108274.

39. de Lima RMT, Dos Reis AC, de Menezes AAPM, Santos JVdO, Filho JWGdO, Ferreira JRdO, et al. Protective and therapeutic potential of ginger (Zingiber officinale) extract and [6]-gingerol in cancer: A comprehensive review. Phytother Res 2018; 32: 1885-907.