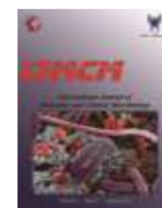
IJMCM 13(1) (2023) 1802-1809 Doi: 10.22034/IJMCM.2023.703699

International Journal of
Molecular and Clinical Microbiology



Research Article

Anti-leishmaniasis activity of *Rhamnus cathatica* on amastigote stages of *Leishmania major* standard strain invitro

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ARTICLE INFO

Article history:
Received 05 September 2022
Accepted 20 June 2023
Available online 1 July 2023

Keywords:
Leishmania major,
Rhamnus cathartica,
Cutaneous leishmaniasis,
amastigote

ABSTRACT

Leishmaniasis is one of the most important parasitic diseases in the world. Due to the increasing prevalence of this disease and the increasing reports of glucantime resistance, it seems necessary to study and find a safe and natural alternative drug. The aim of this study was to evaluate the anti-leishmanial effects of methanolic, hydroalcoholic and chloroform extracts of *Rhamnus cathartica* (*R. cathartica*) on *Leishmania major* (*L. major*) parasite in vitro. The L. *major* parasite was prepared from Leishmaniasis research center from Tehran University of Medical Sciences and cultured in RPMI-1640. The effect of different concentrations on Leishmania parasite was evaluated after 12, 24 and 48 hours using viability staining and MTT assay. In this study, methanolic, hydroalcoholic and chloroform extracts of *R. cathartica* in all concentrations had acceptable antiparasitic effects against *L. major* parasite and among different extracts, chloroform extract was the most effective treatment. The concentration of 800 µg / ml in all extracts after 24 and 48 hours showed a better effect than glucantime. Considering the lethality of chloroform extract of *R. cathartica* plant on *L. major* parasite and better effect than positive controls, it can be concluded that the above compounds can be a suitable candidate for the treatment of leishmaniasis, of course, after supplementary studies.

1. Introduction

Leishmaniasis is one of the most important parasitic diseases transmitted through a vector, which is associated with different clinical manifestations in humans, including cutaneous (CL), Mucocutaneous (MCL), and visceral (VL) forms(Rahimi Esboei et al., 2018). This disease is caused by a parasitic protozoan from the genus Leishmania and order Kintoplastida. This protozoan, in addition to being transmitted through the bite of an infected mosquito, through the infected syringe of drug addicts, blood and placenta can also be transmitted to the fetus (Dong et al., 2022, Wioland et al. 2022). CL first starts with a small nodule and gradually appears as a diffuse ulcer. It's occurs in two forms: wet CL or rural leishmaniasis and dry CL or urban leishmaniasis, which are caused by *L. major* and *L. tropica*, respectively (Mousavi et al., 2022).

In CL the promastigotes are released into the skin by the bite of an infected mosquito and the parasite enters the host's body. By connecting

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the surface molecules of the parasite with surface receptors of macrophages, endothelium of capillaries and other small blood vessels, it turns into amastigote and phagocytosis occurs (Cordeiro-da-Silva et al., 2022). The lysis of amastigotes takes place following the activation of macrophages by sensitized lymphocytes (Landfear., 2022). A granulomatous reaction leads to the formation of a local nodule, which becomes injured after the local blood flow decreases due to damage caused by the parasite. Wounds of *L. major* are moist, it may take several years to heal and it can be infected with secondary infections (Frasca et al., 2021; Semegn et al., 2022). CL is widespread in most parts of the world, and the occurrence of this disease is more prominent in the northern region of Iran (Azerbaijan, Khorasan, Mazandaran) and military areas, and 28 people are infected with CL per 1000 people. *L. major* as a cause of rural CL, infects about 1.5 million people a year. CL showed problems in the field of health, and treatment standards have often faced problems such as cost, drug resistance, toxicity, and low efficacy (Hide et al., 2007; Pagheh et al., 2013; Mahdavi et al., 2020).

The traditional treatment is with antimony, the disadvantages of which are the necessary equipment for intramuscular or intravenous injection daily for 20-28 days, and cases of poisoning and drug resistance have been observed (Rojas et al., 2006; Bekhit et al., 2018). Currently, extensive studies have been conducted in most parts of the world to measure the anti-leishmanial ability of synthetic, natural and herbal compounds.

The *Rhamnus cathartica* (*R. cathartica*) plant belongs to the Rhamnaceae family, which is native to Europe, North Africa, East Asia, and is found in Iran at altitudes above 1300 meters above sea level (Chabra et al., 2019). The leaves and seeds of *R. cathartica* are used as laxatives and detergents. In ancient Greece, the infusion of its fruit was used as an antiseptic for wounds (Heneghan et al., 2006; Knight et al., 2007). The bitter taste of its fruit is due to the presence of anthraquinone derivatives such as frangolin and glucofrangolin. Also, flavonoid compounds such as catharcin, kaempferol, quercetin have been extracted from this plant. Anthraquinone and flavonoid compounds have antimicrobial effects that have been proven in various studies (Chabra et al., 2019). Since an effective and active combination against CL has not been introduced so far and due to the little research done on the antiparasitic properties of *R. cathartica*, therefore, in this study, methanolic, hydroalcoholic and chlorformic extracts were prepared and tested at different concentrations in vitro *L. major* amastigotes.

2. Materials and Methods

2.1. Plant and extraction

The *R. cathartica* plant was collected from the mountainous and semi-arid areas of Mazandaran province (at an altitude of 200 to 800 meters) in the spring and was transported in special plastic bags to the medicinal plant laboratory located in the Faculty of Pharmacy of Mazandaran University of Medical Sciences. The scientific name was determined with the help of the pharmacognosy department of the Faculty of Pharmacy of Mazandaran University of Medical Sciences. After determining the scientific name of the plant, the complete identification card of the plant, including the scientific name, the name of the place of collection of the plant, the date of collection, was prepared and attached to the plant sample. Separate the skin part of the plant and spread it on the paper to dry in air flow and normal heat, away from direct light. After a few days, the plant dries completely and its volume decreases. After drying, the plant was crushed by an electric mill. Extraction was done by maceration method. In this method, 500 grams of the plant is soaked in 2.5 liters of the desired solvent. In this study, methanol, chloroform and hydroalcohol (distilled water + methanol 50:50) solvents were used. So that the solution obtained from the plant powder and the solvent were placed at the temperature and laboratory conditions for 48 hours, and after this time, the resulting solution is filtered using a 40X sieve, and the plant debris is removed, and the solvent is filtered using a rotary device. It evaporates and the remaining extract is separated from the bottom of the container. A standard stock solution was prepared from the powder obtained from methanolic, chloroform and hydroalcoholic extracts in a suitable proportion of normal saline (45 degrees Celsius). Concentrations of 100, 200, 400 and 800 µg/ml were prepared from each of the standard stock solutions (Chen et al., 2020; Nayyeri et al., 2020).

2.2. Preparation of the L. major and culture

The standard strain of *L. major* was prepared from the Leishmaniasis Reference Laboratory located in the Faculty of Health, University of Tehran, and in order to strengthen the pathogenicity, it was first injected into the peritoneum of the rat, and after 5 days, a sample was taken from the peritoneum of the mouse, and the new sample was cultured in RPMI- 1640 was cultivated. Continuous testing and monitoring of culture mediums was done once every 2-3 days, and in this study, penicillin was used at the rate of 100 I.U/ml to eliminate the microbial contamination of the culture medium. When motile promastigotes were seen, 2-3 drops of the upper phase of the culture medium were passed into the new culture medium, and if the number of promastigotes was high, a little of the new culture medium was added to the previous medium (Esboei et al., 2018; Akhtari et al., 2019).

2.3. Preparation and culture of Human macrophages and amastigote

Macrophage cell strain J774 was purchased from Pasteur Institute of Karaj and cultured in RPMI-1640 culture medium at 37°C with 10% Javi FCS and antibiotics and Co2. 200 µl of macrophage-containing cell suspension containing 2x105 cells were taken and added in 24-well plates. Then we close the lid of the plate and place the plates in an incubator at 37°C for 2-3 hours with 5% CO2 in a suitable humidity. During this time, macrophages stick to the bottom of the plate (Luo et al., 2021).

The parasites propagated in RPMI-1640 medium, which are in their stable growth stage, were centrifuged at 3200 rpm for 15 minutes, and the resulting sediment containing parasite promastigotes was washed twice using sterile PBS with pH=7.4 and the final sediment RPMI-1640 culture medium containing 10% fetal bovine serum is diluted at 56°C in such a way that there are 5 million parasites /ml (Zilberstein., 2020).

2.4. Infection of the macrophage Cells with the L. major promastigotes

To infect the macrophage cells by *L. major* promastigotes, 200 µl of the culture medium containing one million promastigotes parasites in the infective metacyclic stage (long cylindrical forms, ~ 5-6 day old culture) were add it to the plates containing macrophages so that the ratio of parasites to macrophages is 5:1. The plates incubated at 37°C for 24 hours. After the incubation time, the medium containing the parasite not entered into the macrophages were replaced by the fresh RPMI-1640 medium containing 10% FCS (Najm et al., 2021).

2.5. Treatment of Infected Macrophages with Test Drugs/Compound

To assess the anti-amastigote efficacy, at first, the culture plates and chamber slides infected with L. major promastigotes were washed at least 5 times with serum-free, RPMI-1640 medium to ensure complete removal of non-internalized promastigotes. 100 µl of treatments in all concentrations were added to each well. One well was incubated by glucantime and one well was treated with PBS as positive and negative controls, respectively. The plates were incubated at 37 °C, 5% CO_2 for 48 h. After incubation time, three thin smear were prepared from each well and fixed by methanol. All smears were stained by Giemsa staining for 15 minutes and microscopically evaluated at 1000X under light microscope (Badirzadeh et al., 2022).

2.6. Statistical analysis

Analysis of variance (ANOVA) using SPSS version 23 software (SPSS Inc., Chicago, IL, USA) was applied to assess the differences between mean values of the experimental groups and a value of $P < 0.05$ was considered statistically significant. All parts of the current experiments were done in triplicate.

3. Results

In this study, the antiparasitic effect of methanolic, hydroalcholic and chloroform extract of *R. cathartica* on *L. major* amastigotes after 12, 24 and 48 hours was investigated by two microscopic and MTT techniques.

3.1. Anti-parasitic effect of R. cathartica methanolic extract on L. major amastigotes

The results of the microscopic test revealed that the number of amastigotes in the cytoplasm

of macrophages decreased with a significant difference ($P<0.05$) by increase in incubation time and drug concentrations. Also, the results of this study showed that the difference between 12 and 24 and 12 and 72 hours is statistically significant ($P>0.05$), while the difference between 24 and 48 hours is not statistically significant ($P<0.05$). The results of the plant extracts were compared with glucantime as a positive control and with PBS as a negative control. According to Table 1-4, the concentrations of 100, 200 and 400 μg/ml have a significantly lower effectiveness than the positive control, but the concentration of 800 μg/ml showed similar efficacy to the positive control ($P>0.05$). After 12 hours of incubation, the results have shown that the antiparasitic effect of the methanolic extract of *R. cathartica* at concentrations of 100, 200 and 400 μg/ml was significantly lower than the positive control ($P<0.05$), but At the concentration of 800 μg/ml, the antiparasitic effect was similar to the positive control group ($P=0.074$). After 24 hours, the concentrations of 200, 400 and 800 μg/ml and after 48 hours, the concentrations of 400 and 800 μg/ml showed similar antiparasitic effect to the positive control ($P>0.05$).

3.2. Anti-parasitic effect of R. cathartica chloroformic extract on L. major amastigotes

The results of the extract showed that with the increase of the incubation time and the concentrations, the number of amastigotes in the cytoplasm of macrophages were decreased with a significant difference ($P=0.003$). Also, the results of this study revealed that the difference between 12 and 24 hours is statistically significant ($P=0.061$), while the difference between 24 and 48 hours is not statistically significant ($P=0.026$). The results of the effectiveness of plant extracts were compared with glucantime as a positive control and with PBS as a negative control. According to Table 1, the concentrations of 100 and 200 μg/ml were less effective than the positive control, but no significant difference was reported between the effectiveness of the concentration of 400 μg/ml and the positive control. The concentration of 800 μg/ml indicated better effectiveness than the positive control at all times. Also, from the comparison of methanolic and chloroform extracts, it has been found that the amount of antiparasitic effect in chloroform extract is significantly higher than methanolic extract ($P=0.013$).

3.3. Anti-parasitic effect of R. cathartica hydroalcoholic extract on L. major amastigotes

The results of the extract showed that with the increase of the incubation time and the concentration, the number of amastigotes in the cytoplasm of macrophages has decreased with a significant difference ($P<0.05$) and the difference between 12 and 24 hours is statistically significant ($P>0.05$), while the difference between 24 and 48 hours is not statistically significant ($P<0.05$). By comparison to the control groups, the concentrations of 100, 200 and 400 μ/ml were less effective than the positive control but, the concentration of 800 μg/ml showed bettereffectiveness than the positive control at all times.

3.4. Investigating the anti-parasitic effect of MTT colorimetric and biochemical methods

In this study, in addition to microscopic examination, the MTT colorimetric and biochemical method was also used to check the anti-parasitic effect and the results were completely similar to the microscopic method. In the MTT method, the chloroform extract had a better anti-parasitic effect than the methanolic and hydroalcoholic extracts. The results of the microscopic method and the MTT test showed that the two mentioned methods are completely consistent with each other and the correlation coefficient or kappa coefficient between the examined tests was 0.09. In both methods, it has been proven that the R. cathartica had a good anti-parasitic effect and the chloroform extract had a better effect than the methanolic extract in all concentrations.

4. Discussion

In current study, the antiparasitic effect of methanolic, hydroalcoholic and chloroform extracts of *R. cathartica* on *L. major* amastigotes after 12, 24 and 48 hours was investigated. The anti-parasitic effect was investigated first by microscopy and amastigote counting, and then it was evaluated using the MTT technique, and acceptable results have been reported.

Table 1. The anti-parasitic effect of R. cathartica methanolic, chloroformic and hydroalcholic extracts on L. major amastigotes after 12, 24 and 48 hours.

Treatments		12 h	24 h	48 h	P-value	P-value*
Methanolic Extract	100 µg/ml	15.33	9.66	7	.001	.001
	200 µg/ml	11.66	5.66	3.66	.005	
	400 µg/ml	10.66	3.33	1.66	.003	
	800 µg/ml	5.33	3.33	0.66	.027	
Chloroformic extracts	100 µg/ml	12.66	5	2.33	.005	.001
	200 µg/ml	10	4.66	2	.006	
	400 µg/ml	5.66	1.33	0.66	.036	
	800 µg/ml	3.33	0	0	.022	
Hydroalcholic Extract	100 µg/ml	15.66	6.6	4	.002	.001
	200 µg/ml	12.6	5.6	3.3	.015	
	400 µg/ml	8	4.6	2	.029	
	800 µg/ml	5.3	3.33	0	.035	
PBS		19.66	>25	>25	.004	-
Glucantime	100 mg/ml	5	0.66	0	.002	-

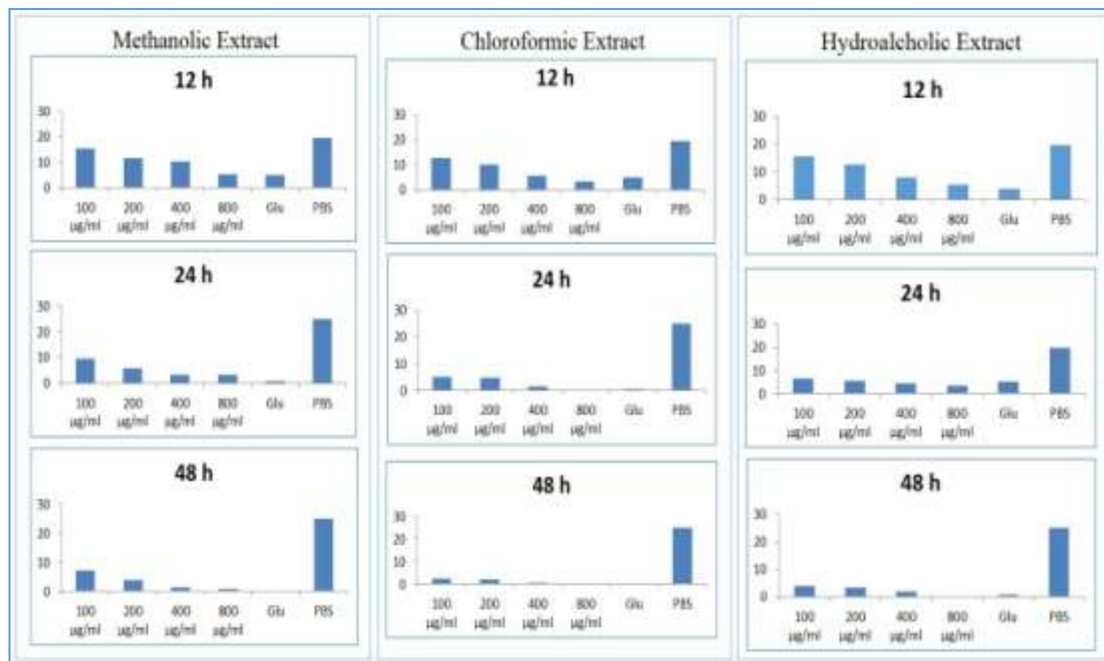

Figure 1. The anti-parasitic effect of *R. cathartica* methanolic, chloroformic and hydroalcholic extracts on *L. major* amastigotes after 12, 24 and 48 hours.

The results of this study showed that the number of amastigotes in the cytoplasm of macrophages decreased with a significant difference with the increase of the incubation time and the concentration of the examined drugs. Chabra et al., (2020) revealed that the qualitative analysis of the chemical compounds in the *R cathartica* plant showed that the stem of this plant contains anthraquinone, flavonoid, synoside and tannic compounds and does not contain cardiac glycosidic derivatives, alkaloids and aloin. The amount of flavoid present in the extract of the stem bark of *R. cathartica* according to the standard diagram of quercetin, the amount of total phenol and total tannin according to the standard diagram of gallic acid, the amount of anthraquinone based on glucophrangolin 23.15±1.355, 66.4±850,

respectively 351/351, 221.34±2.65 mg/g and 3.478 in 100 g was calculated. Also, according to the DPPH antioxidant test, it was found that the IC50 of the methanolic extract of the stem bark of *R. cathartica* is equal to 74.46±1.89 µg/ml; while the IC50 of vitamin C as a standard was equal to 57.92±2.21 µg/ml (Nayyeri et al., 2020). In the study of Carranza et al. in 2015, it was found that the methanolic extract of the stem bark of *R. californica* has a group of alkaloid, flavonoid, tannin, saponin and steroid compounds that are similar to cathartica species (Carranza et al., 2015). In the study of Kosalec et al. in 2013, it was found that the amount of total phenol and (flavonoid) present in the methanol extract of the stem bark of *R.alaternus*, *R.fallax*, *R.pumila* and *R.intermedia* is 38.4±1.56, respectively. 33.6±1.50), 139.66±7.88 (93.9±2.32), 29±0.19 (13.1±1.05) and 123±0.644 (64.9±1.79) (Kosalec et al., 2013). In Boussahel's study, it was also found that the amount of phenol and (flavonoid) of *R.alaternus* leaves in aqueous and methanol extracts were 8.549±0.553 and 33.65±2.503. The results of studies show that the amount of flavonoids in *R. cathartica* species is average compared to other species, but the amount of phenol in this species is much higher than other species (Boussahel et al., 2013). Anthraquninones, anthrones, flavonoides, anthracene derivatives, geshoidin, sorigenin glycoside, chrysophanol, physcion, musizin, emodin, polyphenolic, procyanidin glycoside and essential oil are previously isolated from different *Rhamnus* species (Chen et al., 2020, Nigussie et al., 2021). According to the compounds that have been reported from this plant, it is expected that the extracts obtained from this plant have strong antimicrobial effects. Leishmaniasis as one of the most important vector borne disease is endemic in 102 countries especially in tropical and sub-tropical areas of the world (Nayyeri et al., 2020).

Up to now, there are many reports from the used chemical and natural remedies for evaluation their anti-leishmanial effects and antimonate (glucantime), pentavalent antimonials, meglumine pentamidine, amphotericin B, sodium stibogluconate (pentostam), miltefosine, sitamaquine and paromomycin are the conventional treatment for all kinds of leishmaniasis (Hussain et al., 2014). Unfortunately, several reports revealed some inevitable side effects that limited the use of these drugs in newborns, child, pregnant women and other high risk population and also in recent years; unavoidable resistance hasdeveloped in *leishmania* parasites to these drugs (Li et al., 2015).

Natural products containing active agents such as alkaloids, flavonoids, chalcones, isoflavones, coumarins, xanthones, benzophenones, iridoids, sesquiterpenes, diterpenes, triterpenes and saponins are the most effective antleishmanial drugs (Atanasov et al., 2021).

In this study, we used *R. cathartica*, which is rich in alkaloids and flavonoids compounds and had a very good anti-leishmanial effect. Based on different study, anticancer, antioxidant, antimicrobial, insecticidal and free radical-scavenging activity were reported from different species of *Rhamnus* spp and certainly these effects are due to the effective compounds in the plant. One of the important features of our study is the investigation of the anti-parasitic effect on the amastigote stage of *Leishmania* parasite, which few studies have been done in this regard. As you know, most of the in vitro studies against *Leishmania* are performed on the promastigote stage. In the event that the promastigote stage is seen in vector and in culture medium, and only the amastigote form exists in the human body and other main hosts. So it is better to conduct in vitro studies on this stage of the parasite. Considering that we reported a good anti-parasitic effect in this study, it can be assumed that we will have good anti-leishmanial effects in the internal environment, although the internal system of the body also has complications that may violate our theory.

Acknowledgements

The authors wish to express their sincere thanks to the department of Pharmacy, Ayatollah Amoli branch, Islamic Azad University.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Refereces

Akhtari, J., Faridnia, R., Kalani, H., Bastani, R., Fakhar, M., Rezvan, H., et al. (2019). Potent in vitro antileishmanial activity

of a nanoformulation of cisplatin with carbon nanotubes against Leishmania major. Journal of global antimicrobial resistance.16:11-6.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Supuran, C.T. (2021). Natural products in drug discovery: advances and opportunities. Nature reviews Drug discovery. 20(3):200-16.

Badirzadeh, A., Alipour, M., Najm, M., Vosoogh, A., Vosoogh, M., Samadian, H., et al. (2022). Potential therapeutic effects of curcumin coated silver nanoparticle in the treatment of cutaneous leishmaniasis due to Leishmania major in-vitro and in a murine model. Journal of Drug Delivery Science and Technology. 74:103576.

Banuls, A-L, Hide, M., Prugnolle, F. (2007). Leishmania and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. Advances in parasitology. 64:1-458.

Bekhit, A.A., El-Agroudy, E., Helmy, A., Ibrahim, T.M., Shavandi, A., Bekhit, AE-DA. (2018). Leishmania treatment and prevention: Natural and synthesized drugs. European journal of medicinal chemistry. 160: 229-44.

Boussahel, S., Dahamna, S., Ruberto, G., Siracusa, L., Harzallah, D. 2023. Phytochemical Study and Antioxidant Activities of Leaves Extracts from Rhamnus alaternus L. Pharmacognosy Communications. 3(1):46.

Carranza, M.G., Sevigny, M.B., Banerjee, D., Fox-Cubley, L. (2015). Antibacterial activity of native California medicinal plant extracts isolated from Rhamnus californica and Umbellularia californica. Annals of clinical microbiology and antimicrobials. 14(1):29.

Cecílio, P., Cordeiro-da-Silva, A., Oliveira, F. (2022). Sand flies: Basic information on the vectors of leishmaniasis and their interactions with Leishmania parasites. Communications biology. 5(1):1-12.

Chabra, A., Rahimi-Esboei, B., Habibi, E., Monadi, T., Azadbakht, M., Elmi, T., et al. (2019). Effects of some natural products from fungal and herbal sources on Giardia lamblia in vivo. Parasitology. 146(9):1188-98.

Chen, G-L, Munyao Mutie, F., Xu, Y-B., Saleri, FD., Hu, G-W., Guo, M-Q. (2020). Antioxidant, anti-inflammatory activities and polyphenol profile of Rhamnus prinoides. Pharmaceuticals. 13(4):55.

Douanne, N., Dong, G., Amin, A., Bernardo, L., Blanchette, M., Langlais, D., et al. (2022) Leishmania parasites exchange drug-resistance genes through extracellular vesicles. Cell Reports. 40(3):111121.

Esboei, B.R., Mohebali, M., Mousavi, P., Fakhar, M., Akhoundi, B. (2018). Potent antileishmanial activity of chitosan against Iranian strain of Leishmania major (MRHO/IR/75/ER): In vitro and in vivo assay. Journal of vector borne diseases. 55(2):111.

Heneghan, L., Fatemi, F., Umek, L., Grady, K., Fagen, K., Workman, M. (2006). The invasive shrub European buckthorn (Rhamnus cathartica, L.) alters soil properties in Midwestern US woodlands. Applied Soil Ecology. 32(1):142-8.

Hussain, H., Al-Harrasi, A., Al-Rawahi, A., Green, I.R., Gibbons, S. (2014). Fruitful decade for antileishmanial compounds from 2002 to late 2011. Chemical reviews.114(20):10369-428.

Knight, K.S., Kurylo, J.S., Endress, A.G., Stewart, J.R., Reich, P.B. (2007). Ecology and ecosystem impacts of common buckthorn (Rhamnus cathartica): a review. Biological Invasions.9(8): 925-37.

Kosalec, I., Kremer, D., Locatelli, M., Epifano, F., Genovese, S., Carlucci, G., et al. (2013). Anthraquinone profile, antioxidant and antimicrobial activity of bark extracts of Rhamnus alaternus, R. fallax, R. intermedia and R. pumila. Food chemistry. 136(2):335-41.

Kotila, T., Wioland, H., Selvaraj, M., Kogan, K., Antenucci, L., Jégou, A., et al. (2022). Structural basis of rapid actin dynamics in the evolutionarily divergent Leishmania parasite. Nature communications. 13(1):1-18.

Landfear, S.M. (2022). New Vistas in the Biology of the Flagellum—Leishmania Parasites. Pathogens. 11(4):447.

Li, W., Rong, P., Yang, K., Huang, P., Sun, K., Chen, X. (2015). Semimetal nanomaterials of antimony as highly efficient agent for photoacoustic imaging and photothermal therapy. Biomaterials. 45:18-26.

Luo, M., Lai, W., He, Z., Wu, L. (2021). Development of an Optimized Culture System for Generating Mouse Alveolar Macrophage–like Cells. The Journal of Immunology. 207(6):1683-93.

Mahdavi, S.A., Javadian, B., Barzegari, S., Rahimi Esboei, B., Moosazadeh, M. (2020). Epidemiological study and geographical distribution of cutaneous leishmaniasis in golestan province, 2014-2016. Journal of Mazandaran University of Medical Sciences. 29(181):125-30.

Mann, S., Frasca, K., Scherrer, S., Henao-Martínez, A.F., Newman, S., Ramanan, P., et al. (2021). A review of leishmaniasis: current knowledge and future directions. Current tropical medicine reports. 8(2):121-32.

Mousavi, P., Rahimi Esboei, B., Pourhajibagher, M., Fakhar, M., Shahmoradi, Z., Hejazi, S.H., et al., (2022). Anti-leishmanial effects of resveratrol and resveratrol nanoemulsion on Leishmania major. Bmc Microbiology. 22(1):1-14.

Najm, M., Hadighi, R., Heidari-Kharaji, M., Alipour, M., Hajizadeh, M., Rafiei-Sefiddashti, R., et al. (2021). Anti-Leishmanial Activity of Artemisia persica, A. spicigera, and A. fragrance against Leishmania major. Iranian Journal of Parasitology. 16(3):464.

Nayyeri, S., Azadbakht, M., Chabra, A., Asgarirad, H., Akbari, J., Davoodi, A., et al. (2020). Antibacterial Activities of Gel Containing 5% Hydroalcoholic Extract of Rhamnus cathartica L. bark. Journal of Mazandaran University of Medical Sciences. 29(182):106-10.

Nigussie, G., Alemu, M., Ibrahim, F., Werede, Y., Tegegn, M., Neway, S., et al. (2021). Phytochemistry, Ethnomedicinal Uses and Pharmacological Properties of Rhamnus prinoides: A Review. International Journal of Secondary Metabolite. 8(2):136-51.

Pagheh, A.S., Fakhar, M., Mesgarian, F., Rahimi-Esboei, B., Badiee, F. (2013). Incidence trend of rural cutaneous leishmaniasis in Gonbad-e-Qabus city,(Golestan, Iran) during 2009-2012. Journal of Mazandaran University of Medical Sciences. 23(104):27-33.

Rojas, R., Valderrama, L., Valderrama, M., Varona, M.X., Ouellette, M., Saravia, N.G. (2006). Resistance to antimony and treatment failure in human Leishmania (Viannia) infection. The Journal of infectious diseases. 193(10):1375-83.

Shita, E.Y., Semegn, E.N., Wubetu, G.Y., Abitew, A.M., Andualem, B.G., Alemneh, M.G. (2022). Prevalence of Leishmania RNA virus in Leishmania parasites in patients with tegumentary leishmaniasis: A systematic review and meta-analysis. PLOS Neglected Tropical Diseases. 16(6):e0010427.

Zilberstein, D. (2020). In vitro culture for differentiation simulation of Leishmania spp. Trypanosomatids: Springer; 2020. 39-47.