

Original Research Article

## Anti-leishmanial effect of the hydroalcoholic extract of the leaves, roots and seeds of *Arctium lappa*

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### Abstract

**Objective:** Zoonotic cutaneous leishmaniasis is a common including endemic infectious disease in many parts of the world and Iran. Due to *Arctium lappa* wide therapeutic applications, the anti-leishmanial effect of the hydroalcoholic extract of its leaves (L), roots (R) and seeds (S) has been investigated in this research.

**Materials and Methods:** The leaves, seeds and roots of the greater burdock plant were extracted. In the *in vitro* phase, its cytotoxic and anti-leishmanial effects on promastigote and amastigote forms of *Leishmania major*(*L.major*) were investigated. In the *in vivo* stage, the leishmaniasis mouse model was dosed with concentrations of 50, 100, and 200 mg/kg and the liver and spleen parasite burden was checked.

**Results:** The results of this research in the *in vitro* phase showed that the antileishmanial effect of the hydroalcoholic extract of leaves, roots and seeds on the promastigote and amastigote forms of *L. major* has a significant relationship with the increase in the concentration of the extract (for all  $p \leq 0.001$ ). Also, exposure time and interaction effect of concentration and exposure time were significant. In the *in vivo* phase the significant effect of the increase in concentration (L:  $p \leq 0.001$ , R:  $p = 0.02$ , S:  $p = 0.03$ ), exposure time (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.01$ ) and the interaction effect of these two factors (L:  $p = 0.002$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ) on reducing the size of the wound was shown.

**Conclusion:** The investigation established that hydroalcoholic extract of the leaves, roots, and seeds of the greater burdock in high concentration exhibited beneficial inhibitory effects on the leishmanial lesions.

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## Introduction

Leishmaniasis is a zoonotic disease transmitted by a flagellated protozoan called *Leishmania* from the Trypanosomatidae family. This disease has spread in all parts of the world, including tropical and semi-tropical regions, and in 98 countries throughout Asia, Europe, Africa. According to the report of the World Health Organization, about 1.5 to 2 million people are infected with various types of *Leishmania* every year, of which 70,000 people die. (Saini et al., 2022; WHO, 2023). This disease is hyper-endemic in some areas of Iran and 95% of cases are related to cutaneous leishmaniasis with an incidence rate of 31 cases per thousand people (Shirzadi et al., 2015). The pentavalent antimonials including glucantime (meglumine antimoniate) and pentostam (sodium stibogluconate), are the main treatments and are administered by intramuscular or intralesional injection. The use of these medicines is associated with side effects including heart, liver and kidney disorders and other difficulties such as importation and scarcity and therefore lack of access for patients in rural areas. Recurrence due to incomplete or insufficient treatment and, the emergence of resistant strains due to mutation and drug resistance has been previously noted (Aronson et al., 2017; Ponte-Sucre et al., 2017). On the other hand, the medicinal properties of floras have always been of interest to researchers and many studies have shown the beneficial effects of their various naturally occurring compounds. For example, the anti-leishmanial effects of plant extracts such as thyme, yarrow, henna and garlic as well as *Ganoderma lucidum* fungi have been investigated to deduce the potential efficacy on the improvement of cutaneous leishmaniasis (Hejazi et al., 2009; Akbari et al., 2019). Also, in other studies, the anti-leishmanial effect of the fruit and flower of *Punica granatum* plant (Mardani et al., 2020), and the phenolic fractions isolated from plants such as olive, bay

leaves and black alfalfa have been mentioned (Gharirvand et al., 2020).

One herbaceous plant with great therapeutic potential is *Arctium lappa*, or the greater burdock, which has long been associated with unique properties in traditional Chinese medicine. This is an herbaceous plant from the family of chicory and marguerite daisy (Compositae (Asteraceae)), grows wild in most parts of the world and has been used as a traditional herbal medicine in Asian as well as western countries for centuries. In Iran, it has been seen in Arak, Tabriz, Mazandaran, Alborz and Gorgan provinces (Boldizsár et al., 2010; Esmaeili et al., 2022). The active compounds described within the plant include flavonoids, arctiopicrin, protein, carbohydrates, inulin, polystyrene compounds, caffeic acid, chlorogenic acid, arctin/arctigenin, cynarin and vitamin C which its potential therapeutic properties on the immune system, anti-malignant effects, antiviral, antibacterial and antifungal, the treatment of skin diseases such as acne and skin pimples is attributed to these compounds (Boldizsár et al., 2010; Sohn et al., 2011). Various biologically active compounds, such as terpenoids (beta-eudesmol, C<sub>15</sub>H<sub>24</sub>O, present in the fruit), sterols (sitosterol-beta-d-glucopyranoside, C<sub>35</sub>H<sub>60</sub>O<sub>6</sub>, contained in the root), lignans, polyphenols, and fructans, are found in the plant but each part of the burdock plant has a different composition, especially in terms of bioactive compounds. The roots of burdock contain arctiin, luteolin, quercetin rhamnoside, and it is a remarkable source of proteins, potassium, calcium, folate and is rich in phytochemicals. Burdock possesses antioxidant and antiinflammatory properties and is reportedly pharmacologically active as an anti-diabetic agent, which improves blood lipid profiles, hypoglycemia, and hyperinsulinemia (Ha et al., 2021). In addition to antioxidant properties, other studies have indicated antimicrobial effects (Gram-negative and Gram-positive

bacteria) and antiviral activity against herpes virus (HSV-1 and HSV-2), HIV and adenovirus, which can be attributed to the presence of compounds such as caffeic acid, rutin, hydrobenzoic acid, chlorogenic acid and p-coumaric acid in burdock extracts (Parham *et al.*, 2020). Burdock leaves contain chelating metals and elements such as sodium, magnesium, selenium and zinc as well as effective compounds such as arctiol, fukinone, fukinanolide,  $\beta$ -eudesmol, petastilone, eremophilone, taraxasterol, polyphenol, chlorophyll, carotenoid, tannin, arctigenin and glycoside arctin which have a variety of biological activities, such as anti-cancer, anti-HIV, antioxidant and antimicrobial effects. The chemical compounds in the burdock seeds include a bitter glucoside called arctin, chlorogenic acid, lapaul A and B and germacranolide (Jafari Shiran *et al.*, 2022; Mir *et al.*, 2022). Some studies have shown that the different organs of the *Arctium lappa* such as roots, leaves, seeds and fruits may have different therapeutic uses. The roots and seeds are indicated as diuretics and blood cleansing while the leaves are indicated for the treatment of burns, ulcers and skin healing (Ferracane *et al.*, 2010; Luquis *et al.*, 2021). Few studies have investigated the anti-parasitic properties of *Arctium lappa* plant, among which Farghi Yamchi *et al.* study evaluated the anti-leishmanial properties of the methanol extract of plant's root *in vitro* (Farghi Yamchi *et al.*, 2016). Considering the extensive properties of this plant, this study was designed to investigate the anti-leishmanial properties of the leaves, seeds and roots of the native and Iranian species of *Arctium lappa* *in vitro* and in a laboratory animal model.

## Materials and Methods

### *In vitro* stage

#### Preparation of hydroalcoholic extract of leaves, roots and seeds of *Arctium lappa*

Burdock leaves, roots and seeds were purchased from a medicinal plant company

between May and July 2021 and were approved by the Pharmacognosy Department of the Faculty of Pharmacy and Medicinal Plants, Isfahan University of Medical Sciences, with the herbarium code 2084. Hydroalcoholic extracts were prepared using the maceration method and 64.5 g of seeds, 345 g of roots and 539 g of plant leaves were mixed with 1000 ml of 70% ethanol separately. After 48 hr, the mixture was filtered using Whatman paper and a vacuum and subsequently underwent distillation. In this way, the yields of extraction for leaves, roots and seeds of the plant were 3.7%, 2.9% and 7.6%, respectively.

### Cultivation of Promastigote

The standard strain of Leishmania parasite (MRHO/IR/75/IR) was provided by the Department of Parasitology, Faculty of Medicine, Isfahan University of Medical Sciences. The parasite was cultured in NNN medium (Novy Mac Neal-Nicolle) and subsequently transferred to RPMI-1640 medium (Roswell Park Memorial Institute) enriched with fetal bovine serum (FBS 10%) and antibiotics (streptomycin 100 mg/ml and penicillin 100 unit/ml).

### Examining the effect of the extracts on promastigote form by MTT method

In this part, 100  $\mu$ l of culture medium containing  $1 \times 10^5$  promastigotes in logarithmic phase was added to the wells of a 96-well plate. Then, 100  $\mu$ l of the extracts of leaves(L), roots(R) and seeds(S) of burdock plant in concentrations of 12.5, 25, 50, 100, 200, and 400  $\mu$ g/ml were added to each well (n=2). The positive control wells contained Glucantime as well as amphotericin B and the negative control contained only parasite promastigotes. In order to control the effect of the solvent of the extracts, which was dimethyl sulfoxide (DMSO), and also for the blank (culture medium without promastigotes and active compounds), two wells were considered

for each. The plates were incubated at 24°C for 24 and 48 hr. Then 20 µl of 5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution was added to each well and placed in aluminum foil in an incubator for 4 hr. In the next step, 100 µl of DMSO was added to each well and incubated for 15 min. Then, the optical absorption of the plates was read at a wavelength of 570 nm (Neubig et al., 2003). The survival percentage was calculated using the following formula:

$(AT-AB) / (AC-AB) \times 100 = \text{survival percentage of promastigotes (\%)}$

AT: optical absorption of treated cells

AC: optical absorbance of untreated cells

AB: Blank optical absorption:

Determination of half-maximal inhibitory concentration (IC<sub>50</sub>) was carried out using nonlinear regression based on the following formula (Oliveira et al. 2018):

$\text{Log (IC}_{50}) = \log(x1) + [y1-y0/2] / (y1-y2) [\log(x2)-\log(x1)]$

### **Investigating the effect of extracts on the promastigote form by counting with a hemocytometer**

In this part, 100 µl culture medium containing  $77 \times 10^4$  parasites in logarithmic phase were transferred to the wells of 96-well plates. Then 100 µl of six concentrations of 12.5, 25, 50, 100, 200, and 400 µg/ml of the extract of leaves, roots and seeds of the plant was added and after 24 and 48 hr, the number of live and mobile parasites was counted and these steps were repeated twice. Two wells with promastigote without extract were utilized as negative control, whereas Glucantime (50 µg/ml) as well as amphotericin B (0.6 µg/ml) was applied as a positive control. The samples were counted using a hemocytometer and to calculate the number of viable cells/mL, the following formula was used:

The average cell count in 5 RBC chamber  $\times 10^4 \times 25 \times \text{dilution factor}$

### **Preparing the amastigote-macrophage model and investigating the effect of the extracts on the intracellular form of the parasit**

Since macrophage is the best cell for creating the amastigote form of the parasite and investigating the effect of compounds or drugs on it, and usually J774 murine macrophage cell line is used in many researches therefore in this research J774A1 cell line was purchased from Tehran Pasteur Institute and used to preparation the amastigote-macrophage model (Santiago et al., 2022; Sherafati et al., 2023). After the growth of macrophages, they were separated from the bottom of the flask using a cell scraper, washed, centrifuged and counted. A cell count of  $15 \times 10^4$  macrophage /ml enriched RPMI 1640 (20% FBS and antibiotics penicillin and streptomycin with concentrations of 100 IU/ml and 100 µg/ml, respectively) was poured into the each well of the 6-well plate, whose bottoms were previously covered with a sterile slide (22×22mm) and placed in an incubator with conditions of 37°C and 5% CO<sub>2</sub>. After 24 hr and the adhesion of macrophages to the lamellae, 250 µl containing  $15 \times 10^5$  metacyclic promastigotes (10 parasites / macrophage) were added to the wells and transferred to the incubator again. After 6 hr, the wells were washed with sterile phosphate-buffered saline PBS, and fresh culture medium was added to the plates. After 24 hr, concentrations of 12.5, 25, 50, 100, 200, and 400 µg/ml of each of leaves, root and seed extracts were added to the wells. Plates without any treatment served as negative control whereas plates administered with Glucantime as the positive control. After 24 and 48 hr, the sterile coverslips that were placed in the bottom of the plate were removed and, first fixed with methanol and subsequently stained with Giemsa dye. The number of amastigotes was determined per 100 macrophage using light microscopy and IC<sub>50</sub> values were determined.

### Determination of cytotoxicity of extracts by MTT method

In this part, 100  $\mu$ l of  $5 \times 10^4$  macrophages was transferred to a 96-well plate. Then, 24 hr later, 100  $\mu$ l of the extracts prepared with concentrations of 12.5, 25, 50, 100, 200, and 400  $\mu$ g/ml was added. After 24 and 48 hr, the survival of macrophages was calculated using the MTT method mentioned above and  $CC_{50}$  values were determined.

### *In vivo* phase

#### Investigation of the effect of prepared extracts on the animal model of cutaneous leishmaniasis

At this stage, 5, 10 and 20% topical creams were prepared using 2% carbomer gel (Saeedi *et al.*, 2018). To prepare an animal model of cutaneous leishmaniasis, 5-6 week old female BALB/c mice were used, and 0.1 ml containing  $1 \times 10^6$  metacyclic promastigote prepared in the previous part was injected intradermally at the base of the tail by insulin syringe. After four weeks, cutaneous leishmaniasis lesions appeared at the injection site. A total of 55 BALB/c mice were separated into 11 test groups consisting of 5 mice each, in which 9 groups were treated with hydroalcoholic extracts of leaves, roots, and seeds with concentrations of 50, 100, and 200 mg/kg. The 10th group treated with Glucantime and the 11th group without any treatment were the positive and negative controls respectively. The treatment with the extracts was done twice a day with a time interval of 10-12 hr and using a sterile swab for up to 4 weeks. Before and at the end of each week of treatment, the wounds were measured and recorded using vernier calliper, and the percentage of wound reduction was calculated using the following formula:

$$\text{Percentage reduction in wound size} = \frac{(\text{The size of the wound on day 0} - \text{the size of the wound on the day n})}{\text{The size of the wound on day 0}} \times 100.$$

Finally, the liver and spleen of the infected mice were removed and the

parasite load was calculated using the following formula (Grant *et al.*, 2004):

$$(\text{body weight in grams}) \times \text{number of amastigotes per 2000 nucleated cell}$$

### Statistical analysis

Statistical analysis was done by SPSS 20 software.  $IC_{50}$  and  $CC_{50}$  were calculated from the nonlinear regression curve from the log of the inhibitor concentration versus the normalized response. Also selectivity index (SI) which is a ratio  $CC_{50}:IC_{50}$  were calculated. All data was first checked for normality by the Kolmogorov-Smirnov test. The data were compared among experimental groups using Two-way ANOVA and Tukey's post hoc and a  $p < 0.05$  was considered to be statistically significant. All analyses were performed with GraphPad Prism 6 software (GraphPad Software Inc.).

## Results

The results of this research are shown in Figures 1-5 and Tables 1 and 2. From the results of the effects of the extracts on the promastigote form of the parasite by two counting and MTT methods, due to the almost identical results, the viability percentage of the promastigote by the MTT method is presented in Table 1 and the analysis of this results shows the effect of concentration (L:  $p \leq 0.001$  (S:  $p \leq 0.001$  R:  $p \leq 0.001$ ), exposure time (L:  $p = 0.002$  S:  $p \leq 0.001$  R:  $p = 0.004$ ) and the interaction effect of both factors (L:  $p \leq 0.001$  S:  $p \leq 0.001$  R:  $p \leq 0.001$ ) is significant on reducing the number of promastigotes. Also, by performing Tukey's post hoc test, it was found that there is a significant relationship between the different concentrations of the hydroalcoholic extract of the leaves, roots and seeds of the burdock plant with each other and the negative control. There was no significant relationship between the concentrations of 50, 25, and 12.5  $\mu$ g/ml of the leaves, roots, and seeds with the positive control (Glucantime) ( $p > 0.05$ ) whereas a

## Anti-leishmanial effect of *Arctium lappa*

significant relationship was observed between the higher concentrations of 100, 200, and 400  $\mu\text{g/ml}$  with the positive control ( $p \leq 0.05$ ). On the other hand,

amphotericin B with high lethality and very low percentage of live parasites exhibited a significant difference across the concentration gradient ( $p \leq 0.05$ ).

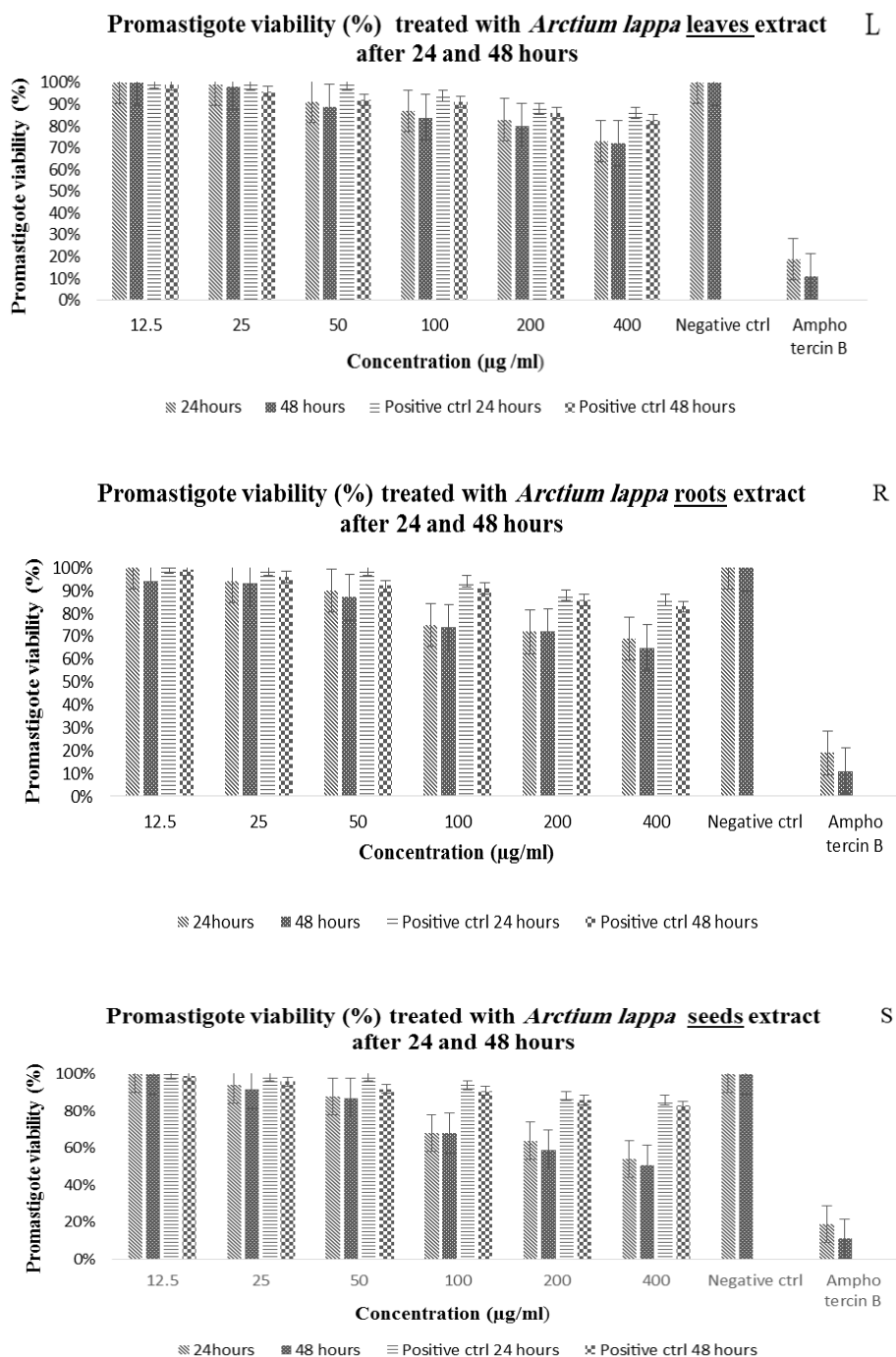


Figure 1. The viability% of promastigotes after exposure to different concentrations of leaves, roots and seeds of *Arctium lappa* after 24 and 48 hours using MTT method. L,leaves; R, roots; S,seeds. Data analysis with two-way ANOVA method showed the significant effect of the concentration of each extract (L:  $p \leq 0.001$  R:  $p \leq 0.001$  (S:  $p \leq 0.001$ ), exposure time (L:  $p = 0.002$ , R:  $p = 0.004$ , S:  $p \leq 0.001$ ) and the interaction effect of these two factors (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ) on the viability of promastigote form. The results illustrated that the highest inhibitory effect on promastigotes of *L. major* is respectively related to the seed (IC<sub>50</sub> = 480 and 420  $\mu\text{g/ml}$ ) then the root (IC<sub>50</sub> = 983 and 771  $\mu\text{g/ml}$ ) and finally the leaf (IC<sub>50</sub> = 1025 and 920  $\mu\text{g/ml}$ ). Positive control (Positive ctrl), Negative control (Negative ctrl).

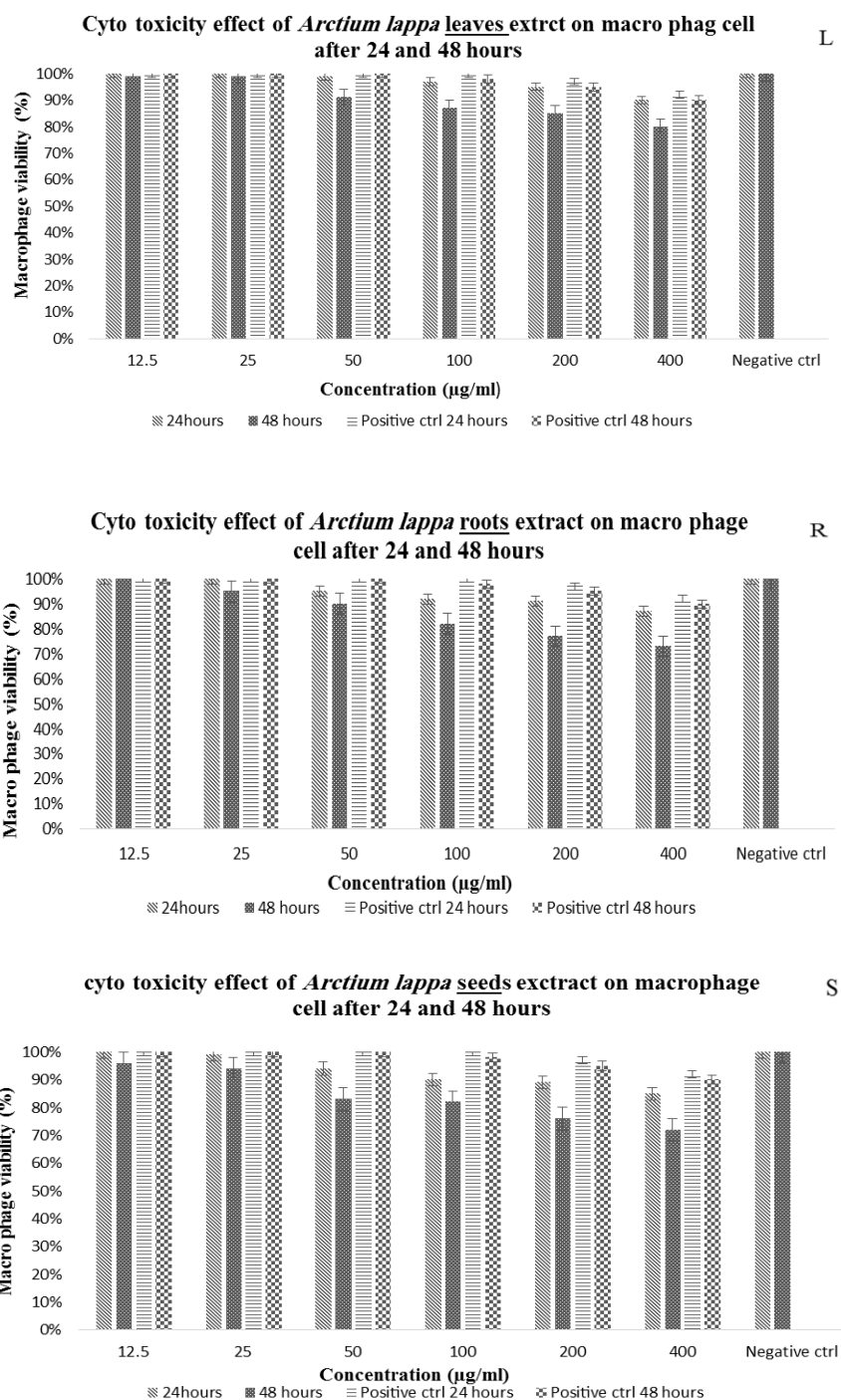


Figure 2. The viability (%) of macrophage cells after exposure to different concentrations of leaves, roots and seeds of *Arctium lappa* after 24 and 48 hours. L,leaves; R, roots; S,seeds. The results of the data analysis by two-way ANOVA statistical tests showed the significant effect of the concentration of the extracts (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ), the exposure time (L:  $p \leq 0.001$ , R:  $p = 0.004$ , S:  $p \leq 0.001$ ) and the interaction effect of these two factors (L:  $p = 0.002$ , R:  $p = 0.019$ , S:  $p = 0.024$ ) on the viability of macrophages. Also, by performing Tukey's post hoc test, it was found that there is a significant relationship between the different concentrations of the hydroalcoholic extract of the leaves, roots and seeds of the *Arctium lappa* with each other and with the negative control ( $p < 0.05$ ). In the period of 48 hours, there was a significant difference between high concentrations (100, 200, and 400 µg/ml) and the positive control ( $p < 0.05$ ). Positive control (Positive ctrl), Negative control (Negative ctrl).

## Anti-leishmanial effect of *Arctium lappa*

The results of cytotoxicity of different concentrations of *Arctium lappa* on living macrophage cells are shown in Figure 2. Two-way ANOVA statistical test showed the significant effect of the concentration of the extracts (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ), the exposure time (L:  $p \leq 0.001$ , R:  $p = 0.004$ , S:  $p \leq 0.001$ ) and the interaction effect of these two factors (L:  $p = 0.002$ , R:  $p = 0.019$ , S:  $p = 0.024$ ) on the viability of macrophages. Also the significant relationship was found between the different concentrations of the hydroalcoholic extract of the leaves, roots and seeds of the *Arctium lappa* with each other and with the negative control ( $p \leq 0.05$ ).

According to the results shown in Table 1, the highest inhibitory effect of the prepared extracts on *L. major* amastigote was in the order of leaves ( $IC_{50}^{24hr} = 155$  and  $IC_{50}^{48hr} = 25 \mu\text{g/ml}$ ) then the roots ( $IC_{50}^{24hr} = 210$  and  $IC_{50}^{48hr} = 50 \mu\text{g/ml}$ ) and finally the seeds ( $IC_{50}^{24hr} = 470$  and  $IC_{50}^{48hr} = 181 \mu\text{g/ml}$ ). Regarding the results of the promastigote form of the parasite, the highest inhibitory effect was related to the seeds ( $IC_{50}^{24hr} = 470$  and  $IC_{50}^{48hr} = 420 \mu\text{g/ml}$ ) then the roots ( $IC_{50}^{24hr} = 983$  and  $IC_{50}^{48hr} = 771 \mu\text{g/ml}$ ) and finally the leaves extract of *Arctium lappa*

( $IC_{50}^{24hr} = 1025$  and  $IC_{50}^{48hr} = 920 \mu\text{g/ml}$ ).

The viability % of the intracellular form of the parasite, which was evaluated by the amastigote-macrophage model, and the significant effect of extract concentration, treatment time and the interaction effect of these two factors on the intracellular form of the parasite are shown in Figure 3.

The results of the *in vivo* phase and the effect of the hydroalcoholic extract of the leaves, roots, and seeds of the *Arctium lappa* on wound healing caused by *Leishmania major* in BALB/c laboratory mice are shown in Figure 4. The analysis of the results of this step determined the effect of concentration (L:  $p \leq 0.001$  S:  $p = 0.03$  R:  $p = 0.02$ ), exposure time (L:  $p \leq 0.001$  S:  $p \leq 0.01$  R:  $p \leq 0.001$ ) and the interaction effect of concentration and exposure time (L:  $p = 0.002$  S:  $p \leq 0.001$  R:  $p \leq 0.001$ ) is significant and showed a greater decrease in lesion size in the groups treated with higher concentrations and in the third and fourth weeks. It was also found out there is no significant difference between the first and second week after starting the treatment ( $p > 0.05$ ), but at the end of the third and fourth week, this difference was significant ( $p \leq 0.05$ ). The results are shown in Figure 4.

Table 1.  $IC_{50}$  and  $CC_{50}$  values and SI indices of the three extracts of leaves, roots and seeds of *Arctium lappa*

Compound	Time (hour)	Anti-promastigote activity $IC_{50}$ ( $\mu\text{g/ml}$ )	Anti-amastigote activity $IC_{50}$ ( $\mu\text{g/ml}$ )	Toxicity $CC_{50}$ ( $\mu\text{g/ml}$ )	Selectivity index (SI)
Seeds	24	480 $\pm$ 3.5	470 $\pm$ 7.07	1800 $\pm$ 12.8	3.8 $\pm$ 2.5
	48	420 $\pm$ 1.2	181 $\pm$ 8.84	1550 $\pm$ 13.94	8.5 $\pm$ 0.3
Leaves	24	1025 $\pm$ 3.78	155 $\pm$ 4.82	2000 $\pm$ 10	12.9 $\pm$ 2.92
	48	920 $\pm$ 9.3	25 $\pm$ 7.80	1600 $\pm$ 15.2	64 $\pm$ 1.87
Roots	24	983 $\pm$ 5.96	210 $\pm$ 3.62	1700 $\pm$ 14.3	8 $\pm$ 1.9
	48	771 $\pm$ 9.35	50 $\pm$ 3.2	1500 $\pm$ 18.2	30 $\pm$ 2.2
	24				
Glucontime (ctrl pos)	48	1720 $\pm$ 6.87	349 $\pm$ 2.78	2550 $\pm$ 14.5	8.5 $\pm$ 0.5
	24	1660 $\pm$ 7.87	53 $\pm$ 4.78	2040 $\pm$ 22.2	24 $\pm$ 1.4
Amphotericine B	48	0.06			



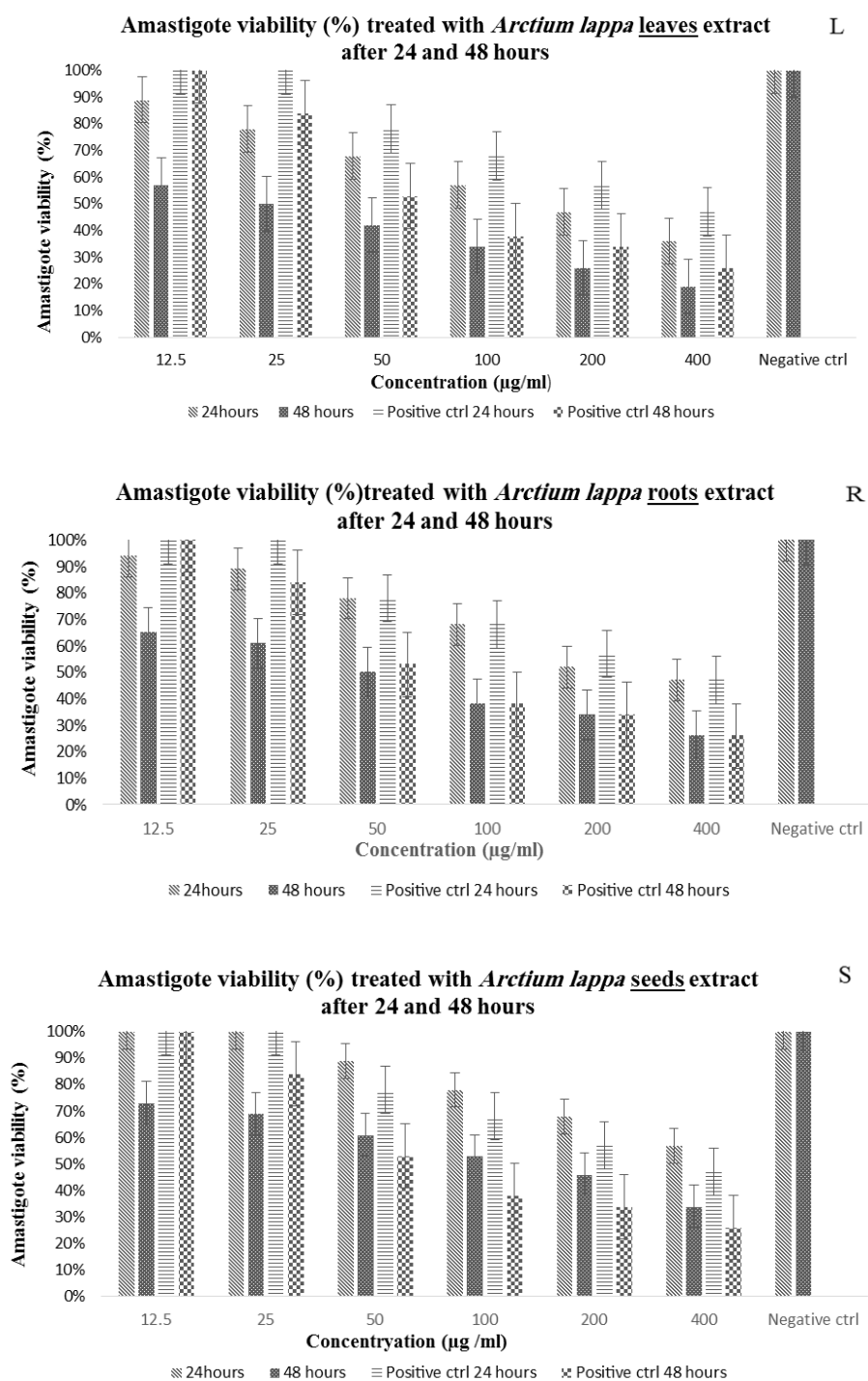


Figure 3. Viability % of amastigotes after exposure to different concentrations of the three types of extracts in 24 and 48 hr. L,leaves; R, roots; S,seeds. Data analysis with two-way ANOVA method showed the significant effect of the increasing concentration (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ), increasing contact time (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.01$ ) and the interaction effect of concentration and exposure time (L:  $p = 0.002$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ) on reducing the intracellular form of the parasite. Also, by performing Tukey's post-hoc test, it was found that there was a significant relationship between different concentrations of hydroalcoholic extract of leaves, roots and seeds of *Arctium lappa* and the positive and negative controls ( $p < 0.05$ ). Positive control (Positive ctrl), Negative control (Negative ctrl).

## Anti-leishmanial effect of *Arctium lappa*

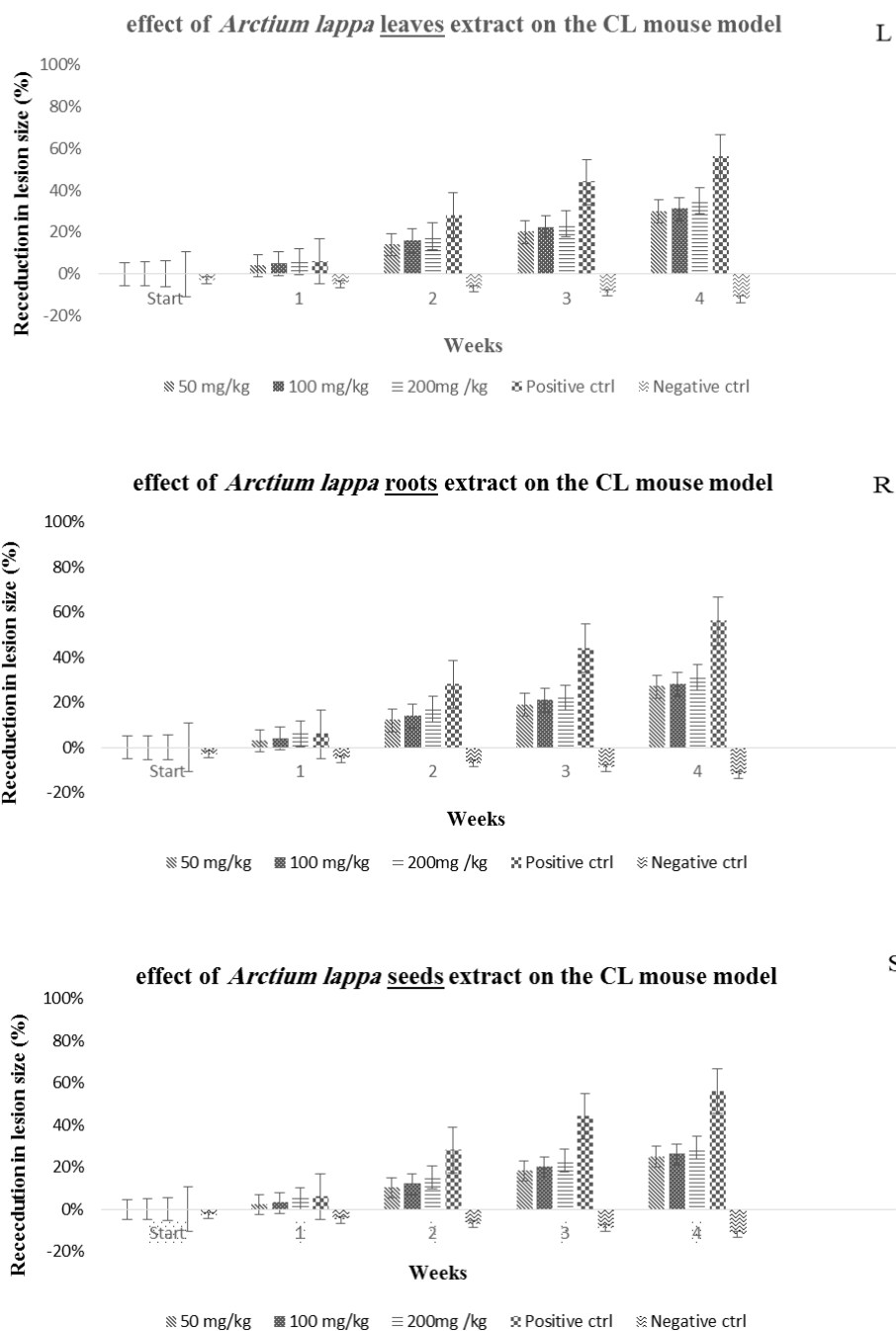


Figure 4. percentage of wound reduction in the groups treated with hydroalcoholic extracts of leaves, roots, and seeds of the *Arctium lappa*. L,leaves; R, roots; S,seeds. By using the two-way ANOVA statistical method, the significant effect of the increase in concentration (L:  $p \leq 0.001$ , R:  $p = 0.02$ , S:  $p = 0.03$ ), the increase in exposure time to the extract (L:  $p \leq 0.001$ , R:  $p \leq 0.001$ , S:  $p \leq 0.01$ ) and the interaction effect of these two factors (L:  $p = 0.002$ , R:  $p \leq 0.001$ , S:  $p \leq 0.001$ ) on reducing the size of the wound was determined. According to the obtained results and the comparison of the average lesions, it could be suggested that greatest effect is related to Glucantime, then the concentration of 200 mg/kg of leaves, roots and seeds respectively. Also, the statistical analysis of Variance test showed that in each group of L, R and S extracts, the effect of the extract increases with the increase of concentration and exposure time ( $p \leq 0.001$ ). Positive control (Positive ctrl), Negative control (Negative ctrl).

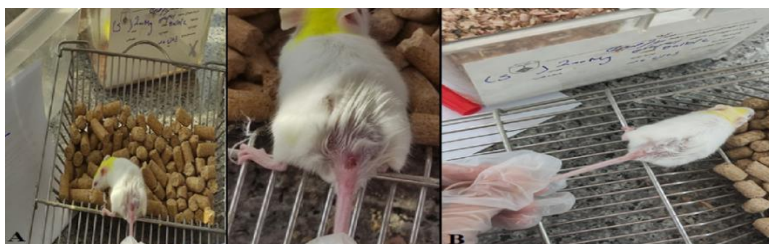
The effect of extracts of leaves, roots and seeds of *Arctium lappa* in mouse model of Cutaneous Leishmaniasis (CL) before and after treatment and negative control is shown in Figures 5. The selectivity index (SI) of each extract of the leaves, roots and seeds of *Arctium lappa*, which is defined based on the ratio of the toxic concentration of a sample to its effective bioactive concentration

( $CC_{50}/IC_{50}$ ), is shown in Table 1. The results showed that the values of SI for all concentrations of three types of extracts were greater than one.

The comparison of the lesion size reduction (%) and the spleen and liver parasite burden in CL mouse model in the groups treated with extracts is summarized in Table 2.



The effect of *Arctium lappa* leaves extract on the CL mouse model



The effect of *Arctium lappa* seeds extract on the CL mouse model



The effect of *Arctium lappa* roots extract on the CL mouse model



Negative Control on the CL mouse model

Figure 5. The effect of *Arctium lappa* leaves, roots and seeds extract (the concentration of 200 mg/kg) on the CL mouse model before (A) and after (B) treatment compared to the negative control. CL, Cutaneous Leishmaniasis.

## Anti-leishmanial effect of *Arctium lappa*

Table 2. Percentage of lesion size reduction and parasite burden among the different groups of CL mouse model exposed to different concentrations of extracts (leaves, roots and seeds of *Arctium lappa*).

Type of extract	Concentrations (µg/ml)	Reduction in Lesion size (%)	Spleen parasite burden (LU)	Liver parasite burden (LU)
Seeds	50	25%	260±3.5	252±4
	100	26%	245±2.78	245±2.9
	200	29%	230±4.2	220±3.48
Leaves	50	30%	195±5	182±2.42
	100	31%	190±1.72	180±3.42
	200	35%	185±2.28	170±5.2
Roots	50	27%	235±2.25	210±3
	100	28%	220±6.78	190±8.92
	200	31%	195±3	180±2.78

**CL**, Cutaneous Leishmaniasis. Note: The highest reduction in lesion size (%) and parasite burden in CL mouse model was respectively related to groups treated with Glucantime, extracts of leaves, roots and finally seeds of *Arctium lappa*. (in Table each point represents the mean±SD of at least three experiments).

## Discussion

Today, leishmaniasis is not only a disease, but in a broader and more realistic view, it is considered a very important and challenging health problem in a wide range of human societies (Tiuman et al., 2011). At present, pentavalent antimony compounds including sodium stibogluconate (Pentostam) and meglumine antimony (Glucantime) are the most important drugs of choice used in the treatment of leishmaniasis (Brito et al., 2017). Research has shown that these drugs have disadvantages such as toxicity, side effects, recurrence rate and high cost, long treatment period and the resistance that the parasite has recently shown to these drugs, which have prompted researchers to look for medicinal compounds with more economical and effective characteristics, with fewer side effects (Milani et al., 2011). Since herbal medicines are suggested to present less harmful side effects, and are more readily accessible than chemical medicines, as a result, it can be said that the use of native plants of any region can be a source of medicinal agents, including anti-leishmania, due to their effective compounds. One of these plants is *Arctium lappa* and many researches point to its

medicinal properties such as antibacterial, antiviral, antifungal, anti-allergic, immune system strengthening, anti-tumor, anti-inflammatory, sugar-reducing and blood pressure reducing effects. (Janačković et al., 2004; Rocha et al., 2005). *Arctium lappa* contains effective biological compounds such as caffeic acid, rutin, cynarin, quercetin, luteolin, diarchetigenin and arctigenin. The compounds in the plant seeds include chlorogenic acid, cynarin, arctin, arctigenin, etc. The seed consists of linoleic acid and oleic acid, and these fatty acids have anti-inflammatory and antioxidant properties (Almoradie et al., 2018). The compounds found in the roots of the plant include chlorogenic acid, inulin, cynarin, quercetin, arctin and luteolin (Wang et al., 2019). In the study conducted by Hanoon et al. the effect of aqueous and alcoholic extract of *Arctium lappa* has been investigated only on the promastigote form and it was shown that the concentration of 1.5 mg/ml had the greatest effect, whereas the hydroalcoholic extract had a better inhibitory effect than the aqueous extract (Hanoon et al., 2022). In the study of Farghi Yamchi et al., where the methanol extract of the root was investigating,  $IC_{50} = 131.25 \mu\text{g/ml}$  after 24 hr was reported, which was higher in our

study. Also the average number of amastigotes in macrophages after 24 hr at concentrations of 500 and 1000  $\mu\text{g/ml}$  was reported 3.52 and 2.02, respectively, and in our study with a lower concentration (400  $\mu\text{g/ml}$ ) it was 4.5. (Farghi Yamchi et al., 2016). The results of our study also confirm the anti-leishmanial effect of this plant. The growth inhibition of promastigotes in the presence of different concentrations of hydroalcoholic extracts of roots, seeds and leaves after 24 and 48 hours compared to the negative control was significant and dependent on the concentration and exposure time ( $p \leq 0.001$ ). The results showed that among the investigated extracts, the seed extract ( $\text{IC}_{50} = 480$  and  $420 \mu\text{g/ml}$ ) had a greater effect on inhibiting promastigote growth than the roots ( $\text{IC}_{50} = 983$  and  $771 \mu\text{g/ml}$ ) and leaves ( $\text{IC}_{50} = 1025$  and  $920 \mu\text{g/ml}$ ), and consequently the  $\text{IC}_{50}$  value reported in the time intervals of 24 and 48 hr was the lowest for the seed extract and the highest for the leaves extract (Figure 1). If in the amastigote stage, the greatest effect was related to the leaves extract ( $\text{IC}_{50} = 155$  and  $25 \mu\text{g/ml}$ ), then the roots ( $\text{IC}_{50} = 210$  and  $50 \mu\text{g/ml}$ ) and finally the seeds ( $\text{IC}_{50} = 470$  and  $181 \mu\text{g/ml}$ ) (Figure 3).

On the other hand, the results of the effect of the hydroalcoholic extract of the leaves, roots and seeds of the *Arctium lappa* plant in different concentrations on the viability of macrophages (cell line J774 after 24 and 48 hr) show that the highest  $\text{CC}_{50}$  value was related to the roots then the seeds and finally the leaves (Figure 2). Although by calculating the SI index (the ratio of  $\text{CC}_{50}$  to  $\text{IC}_{50}$ ) for all the extracts, it was found that the prepared extracts did not have significant toxicity, but based on these results, the toxicity of the leaves and seeds was lower than that of the roots. These results are consistent with studies such as Dias and colleagues who investigated the anti-schistosomal and antiviral activities of *Arctium lappa* against *Schistosoma mansoni* and herpes simplex virus-1 in laboratory conditions

and showed that *Arctium lappa* in high concentrations such as 400  $\mu\text{g/ml}$  had no cytotoxic effect on Vero cells (Dias et al., 2017; Koriem et al., 2016). In the in vivo phase of this study, the effect of three concentrations of 50, 100, and 200 mg/kg hydroalcoholic extracts of the leaves, roots, and seeds of *Arctium lappa* on the wounds caused by *Leishmania major* in BALB/c mice was investigated for a period of 4 weeks. The findings regarding the examination of the wound diameter in the treatment groups under study show that in the fourth week after the challenge, the average diameter of the wounds in all groups has decreased compared to the negative control group. Also, the results showed that the highest percentage of wounds diameter reduction at the end of the treatment period in different treatment groups compared to the control group was related to Glucantime, followed by leaves, roots and seeds extracts. In addition, there was a significant difference between similar concentrations of the extracts together, and between different concentrations ( $p \leq 0.05$ ). The concentration of 200 mg/kg of each type of extract had a greater effect than other analyzed concentrations. In fact, the results show that in each group of the desired extracts, the effect of the desired extract increases with the increase in concentration and the passage of time ( $p \leq 0.001$ ). In terms of the status of the parasite load in the spleen of the mice under study, the greatest decrease in the parasite load was related to the hydroalcoholic extract of the leaves compared to other groups. It seems that the anti-leishmanial effect of this plant is related to the compounds present in the different organs of the plant, including the biologically active lignans arctigenin (a dietary phytoestrogen) and its glycoside, arctin (lignanoides) which are usually present in the seeds, roots, fruits and leaves of *Arctium lappa* (Wang et al., 2019). Arctigenin extracted from *Arctium lappa* shows interesting activity due to its ability to reduce the levels of nitric oxide

(NO) and pro-inflammatory cytokines (Knipping et al., 2008; Pospíšil et al., 2021). Also, due to the presence of beta-sitosterol ( $\beta$ -sitosterol) in this plant and its effect in the treatment of leishmaniasis lesions caused by *L.tropica* compared to ketoconazole and mupirocin in other studies, the healing effect of the lesion can be attributed to the presence of this substance (Pramanik et al., 2020).

Among the polyphenols of this plant, caffeic acid has anti-promastigote activity with an apoptosis-like process and anti-amastigote activity with the production of TNF- $\alpha$ /ROS/NO and reducing the availability of iron. Studies have shown that caffeic acid, in addition to increasing NO production by macrophages infected with *Leishmania* parasites, can inhibit arginase and improve visceral leishmaniasis (Qian et al., 2016; Bortoleti et al., 2019; Alson et al., 2018).

Another polyphenol of this plant is chlorogenic acid, studies have shown that this compound inhibits the cell cycle of *Leishmania donovani* and modulates cytokines and nitric oxide in laboratory conditions thereby exerting potential anti-leishmanial effects (Majumder et al., 2020; Manjolin et al., 2013). Luteolin is another polyphenol isolated from burdock plant that inhibits arginase, the central enzyme in *Leishmania (Leishmania amazonensis)* infection. This non-toxic polyphenol seems to be a strong candidate for anti-leishmanial drug design. (Majumder et al., 2020; Manjolin et al., 2013). Also, quercetin is one of the other polyphenols isolated from this plant, which inhibits the proliferation and migration of fibroblasts, whilst also playing a role in the inhibition of inflammation and increase the expression of growth factors in mice through Wnt/ $\beta$ -catenin and telomerase reverse signaling pathways via transcriptase (TERT) and thus promote wound healing (Abdeyazdan et al., 2022). This study showed the anti-leishmanial effects of the ethanol extract of *Arctium lappa* leaves, roots and seeds *in vivo* and *in*

*vitro* conditions. Due to its diverse and effective compounds, this plant has the capability of more specific research to separate its fractions and investigate their anti-leishmanial effects in order to find an effective herbal compound for the treatment of cutaneous leishmaniasis.

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### Conflicts of interest

The authors have declared that there is no conflict of interest.

### References

- Abdeyazdan S, Mohajeri M, Saberi S, Mirzaei M., Ayatollahi S. A, Saghaei L, Ghanadian M. 2022. Sb (V) Kaempferol and Quercetin Derivative Complexes: Synthesis, Characterization and Antileishmanial Activities. *Iran J Pharm Res*, 21: 128379.
- Akbari S, Chabavizadeh J, Abtahi SM, Yegdaneh A, Namdar F, Saberi S. 2019. Evaluation of antileishmanial effect of hydroalcoholic extract of *Ganoderma Leucidum* on *Leishmania major* *in vitro*. *Journal of Isfahan Medical School*, 36: 1628-1634.
- Almoradie AM, Angeles RJ, Beltran EV, Ugali M, Valles NS, Los Banos ZD, Mahboob T, Barusrux S, Nissapatorn V. 2018. Cryptosporicidal activity of plant extracts against *Cryptosporidium parvum* and *Cryptosporidium hominis*. *Asian J Pharmacogn*, 2: 22-31.
- Alson SG, Jansen O, Cieckiewicz E, Rakotoarimanana H, Rafatro H, Degotte G, Francotte P, Frederich M. 2018. In-vitro and in-vivo antimalarial activity of caffeic acid and some of its derivatives. *J Pharm Pharmacol*, 70: 1349-1356.
- Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho E,

- Ephros M, Jeronimo S, Magill A. 2017. Diagnosis and treatment of Leishmaniasis: Clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Am J Trop Med Hyg*, 96: 24-45.
- Boldizsár I, Füzfa Z, Tóth F, Sedlák E, Borsodi L, Molnár-Perl I. 2010. Mass fragmentation study of the trimethylsilyl derivatives of arctiin, matairesinoside, arctigenin, phylligenin, matairesinol, pinoresinol and methylarctigenin: their gas and liquid chromatographic analysis in plant extracts. *J Chromatogr A*, 1217: 1674-1682.
- Bortoleti BTDS, Tomiotto-Pellissier F, Gonçalves MD, Miranda-Sapla MM, Assolini JP, Carloto AC, Lima DM, Silveira GF, Almeida RS, Costa IN, Conchon-Costa I, Pavanelli WR. 2019. Caffeic acid has antipromastigote activity by apoptosis-like process; and anti-amastigote by TNF- $\alpha$ /ROS/NO production and decreased of iron availability. *Phytomedicine*, 57: 262-270.
- Castelano BN, Rabello A, Cota GF. 2017. Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: A systematic review. *PloS One*, 12: 9.
- Dias MM, Zuza O, Riani LR, de Faria Pinto P, Pinto PLS, Silva MP, de Moraes J, Ataíde ACZ, de Oliveira Silva F, Cecílio AB, Da Silva Filho AA. 2017. In vitro schistosomicidal and antiviral activities of *Arctium lappa* L.(Asteraceae) against *Schistosoma mansoni* and Herpes simplex virus-1. *Biomed Pharmacother*, 94: 489-498.
- Esmaeili F, Hashemiravan M, Eshaghi MR, Gandomi H. 2022. Encapsulation of aqueous extract of *Arctium lappa* L. root and study its effect on physicochemical and functional properties of Synbiotic orange-carrot juice. *IFSTRJ*, 18: 657-679.
- Farghi-Yamchi A, Dabirzadeh M, Miri A. 2016. Effects of methanolic extract of *Arctium lappa* root on promastigotes and amastigotes of *Leishmania major* (in vitro). *Med Lab J*, 10: 45-50.
- Ferracane R, Graziani G, Gallo M, Fogliano V, Ritieni A. 2010. Metabolic profile of the bioactive compounds of burdock (*Arctium lappa*) seeds, roots and leaves. *J Pharm Biomed Anal*, 51: 399-404.
- Gharirvand Eskandari E, Setorki M, Douidi M. 2020. Medicinal plants with antileishmanial properties: A review study. *Pharm Biomed Res*, 6: 1-16.
- Grant KM, Dunion MH, Yardley V, Skaltsounis AL, Marko D, Eisenbrand G, Croft SL, Meijer L, Mottram JC. 2004. Inhibitors of *Leishmania mexicana* CRK3 cyclin-dependent kinase: chemical library screen and antileishmanial activity. *Antimicrob Agents Chemother*, 48: 3033-3042.
- Ha MS, Yook JS, Lee M, Suwabe K, Jeong WM, Kwak JJ, Soya H. 2021. Exercise training and burdock root (*Arctium lappa* L.) extract independently improve abdominal obesity and sex hormones in elderly women with metabolic syndrome. *Sci Rep*, 11: 5175.
- Hanoon MT, Mohammed AS, Ateyaa GF. 2022. Effect of aqueous and alcoholic extract of *Arctium Lappa* to the growth of *Leishmania cutaneous* in Samara City. *HIV Nursing*, 22: 2462-2465.
- Hejazi S, Shirani - Bidabadi L, Zolfaghari - Baghbaderani A, Saberi S, Nilforoushzadeh MA, Moradi SH, Mahmoudi M, Khosravi SH, Ataei A. 2009. Comparison effectiveness of extracts of Thyme, Yarrow, Henna and Garlic on Cutaneous Leishmaniasis caused by *L. major* in animal model (Balb/c). *J Med Plants*, 8: 129-136.
- Jafari Shiran M, Naseri S, Sadeghian-Rizi T, Khani S, Shoormij M, Dakhilpour SS. 2022. Therapeutic and preventive effects of aqueous extracts of *Arctium lappa* L. and *Cichorium intybus* L. against fatty liver in rats. *Jorjani Biomed J*, 10: 53-61.
- Janackovic P, Tesevic V, Milosavljevic S, Vajs V, Marin PD. 2004. Sesquiterpene lactones lignans and flavones of *Centaurea affinis*. *Biochem Syst Ecol*, 32: 355-357.
- Koriem KM, Idris ZH, Haron HF, Omar NA, Lazain HS. 2016. Therapeutic effect of *Arctium lappa* in *Schistosoma haematobium* associated kidney disturbance: biochemical and molecular effects. *J Parasit Dis*, 40: 1246-1254.
- Knipping K, van Esch EC, Wijering SC, van der Heide S, Dubois AE, Garssen J. 2008. In vitro and in vivo anti-allergic effects of *Arctium lappa* L. *Exp Biol Med*

## Anti-leishmanial effect of *Arctium lappa*

- (Maywood), 233: 1469-1477.
- Luquis F, Silva AAO, Wadt NSY, Hi EMB, Martins AMCC, Bach EE. 2021. *Arctium lappa* L.: Chemical composition, antioxidants, phytochemical compounds and use for healing activity. *SFJD*, 2: 2063-2071.
- Majumder N, Ganguly S, Ghosh AK, Kundu S, Banerjee A, Saha S. 2020. Chlorogenic acid acts upon *Leishmania donovani* arresting cell cycle and modulating cytokines and nitric oxide in vitro. *Parasite Immunol*, 42: 12719.
- Manjolin LC, dos Reis MBG, do Carmo Maquiaveli C, Santos-Filho OA, da Silva ER. 2013. Dietary flavonoids fisetin, luteolin and their derived compounds inhibit arginase, a central enzyme in *Leishmania (Leishmania) amazonensis* infection. *Food Chem*, 141: 2253-2262.
- Mardani H, Khalili B, Saberi S, Lorigooini Z, Ghaderi M, Abdizadeh R. 2020. Effect of hydroalcoholic extracts of flower and fruit peel of *Punica granatum* on *Leishmania major* promastigotes in vitro. *Adv Herb Med*, 6: 32-48.
- Milani E, Koocheki A, Golimovahhed A. 2011. Extraction of inulin from Burdock root (*Arctium lappa*) using high intensity ultrasound. *IJFST*, 46: 1699-1704.
- Mir SA, Dar LA, Ali T, Kareem O, Rashid R, Khan NA, Chashoo IA, Bader G N. 2022. *Arctium lappa*: A review on its phytochemistry and pharmacology. In: Masoodi M.H, Rehman MU, (Eds) *Edible Plants in Health and Diseases*, pp: 327-348, Singapore, Springer.
- Neubig RR, Spedding M, Kenakin T, Christopoulos A. 2003. International union of pharmacology committee on receptor nomenclature and drug classification on receptor nomenclature and symbols in quantitative pharmacology. *Pharmacol Rev*, 55: 597-606.
- Oliveira IDSDS, Moragas Tellis CJ, Chagas MDSDS, Behrens MD, Calabrese KDS, Abreu-Silva AL, Almeida-Souza F. 2018. Carapa guianensis Aublet (Andiroba) seed oil: Chemical composition and antileishmanial activity of limonoid-rich fractions. *Biomed Res Int*, 2018: 5032816.
- Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, RamaKrishna S, Berto F. 2020. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants (Basel)*, 21: 1309.
- Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, Pountain AW, Mwenechanya R, Papadopoulou B. 2017. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. *PLoS Negl Trop Dis*, 11: 0006052.
- Pospíšil J, Konrádová D, Strnad M. 2021. Antileishmanial activity of lignans, neolignans, and other plant phenols. *Prog Chem Org Nat Prod*, 15: 115-176.
- Pramanik PK, Chakraborti S, Bagchi A, Chakraborti T. 2020. Bioassay-based *Corchorus capsularis* L. leaf-derived  $\beta$ -sitosterol exerts antileishmanial effects against *Leishmania donovani* by targeting trypanothione reductase. *Sci Rep*, 10: 20440.
- Qian XJ, Jin YS, Chen HS, Xu QQ, Ren H, Zhu SY, Tang HL, Wang Y, Zhao P, Qi ZT, Zhu YZ. 2016. Trachelogenin, a novel inhibitor of hepatitis C virus entry through CD81. *J Gen Virol*, 97: 1134-1144.
- Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. 2005. A review of natural products with antileishmanial activity. *Phytomedicine*, 12: 514-535.
- Saeedi M, Akbari J, Morteza-semnani K, Rostamkalaei S, Fathalinejad F, Khoshvishkaie E. 2018. Evaluation of Carbomer, CMC and HPMC on dissolution and bioadhesion of buccoadhesive Cinnamaldehyde tablets. *J Mazandaran Univ Med Sci*, 27: 70-82.
- Saini I, Joshi J, Kaur SU. 2022. Unwelcome prevalence of leishmaniasis with several other infectious diseases. *Int Immunopharmacol*, 110: 109059.
- Santiago C, Ortega-Tenezaca B, Barbolla I, Fundora-Ortiz B, Arrasate S, Dea-Ayuela MA, González-Díaz H, Sotomayor N, Lete E. 2022. Prediction of antileishmanial compounds: General model, preparation, and evaluation of 2-acylpyrrole derivatives. *J Chem Inf Model*, 22: 3928-3940.
- Sherafati J, Dayer MS, Ghaffarifar F, Akbarzadeh K, Pirestani M. 2023. Evaluating leishmanicidal effects of *Lucilia sericata* products in combination with *Apis mellifera* honey using an in vitro model. *PLoS One*, 18: 0283355.
- Shirzadi MR, Esfahania SB, Mohebalia M, Ershadia MR, Gharachorlo F, Razavia MR,



- Postigo JA. 2015. Epidemiological status of leishmaniasis in the Islamic Republic of Iran, 1983-2012. *East Mediterr Health J*, 21: 736-742.
- Sohn EH, Jang SA, Joo H, Park S, Kang SC, Lee CH, Kim SY. 2011. Anti-allergic and anti-inflammatory effects of butanol extract from *Arctium Lappa* L. *Clin Mol Allergy*, 9: 4.
- Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BP, Nakamura CV. 2011. Recent advances in leishmaniasis treatment. *Int J Infect Dis*, 15: 525-532.
- Wang D, Bădărau AS, Swamy MK, Shaw S, Maggi F, da Silva LE, López V, Yeung AWK, Mocan A, Atanasov AG. 2019. *Arctium* species secondary metabolites chemodiversity and bioactivities. *Front Plant Sci*, 10: 834.
- World Health Organization (WHO), 2023. Leishmaniasis. [<http://www.who.int/leishmaniasis/en/>]