Review

Medicinal plants with promising antileishmanial activity in Iran: a systematic review and meta-analysis

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HIGHLIGHTS

• We systematically reviewed all published papers regarding herbal medicine with antileishmanial activity in Iran among nine databases from 1999 to April 2015.
• Overall 68 articles including 140 in vitro and 48 in vivo, met our eligibility criteria. Also, 98 types of plants were examined against three genera of Leishmania spp.
• Our study shows, the most Iranian plants with anti-leishmanial activity were Artemisia species, Allium sativum, Achilleamille folium, Peganum harmala and Thymus vulgaris.

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ABSTRACT

Background: Leishmaniasis is a major public health problem worldwide. The aim of the present study was to investigate medicinal plants with anti-Leishmania activity which used in Iran.

Methods: Data were systematically gathered from five English databases including Ebsco, Science Direct, PubMed, Google Scholar and Scopus, four Persian databases including Magiran, Iran doc, Iran medex and the Scientific Information Database (SID) from 1999 to April 2015. Information obtained included plant family, extraction method, concentrations of extracts, animal models and parasite strains.

Results: A total of 68 articles including 188 experiments (140 in vitro and 48 in vivo) between 1999 and 2015, met our eligibility criteria. Thoroughly, 98 types of plants were examined against three genera of Leishmania spp. For the heterogeneity study conducted, it was showed that there was a great deal of variation among studies. Based on random effect, meta-analysis pooled mean of IC50 was obtained 456.64 (95% CI: 396.15, 517.12).

Conclusion: The most Iranian plants used as anti-leishmanial activity were Artemisia species, Allium sativum, Achilleamille folium, Peganum harmala and Thymus vulgaris. The present systematic and meta-analysis review provide valuable information about natural products with anti-Leishmania activity, which would be examined in the future experimental and clinical trials and herbal combination therapy.

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1. Introduction

Leishmaniasis is a parasitic disease caused by an obligate intracellular parasite of genus *Leishmania*, which is transmitted to human by the bite of a female sand fly [1]. The disease has wide clinical spectrums from self-limiting cutaneous to fatal visceral form which depends on both host immune response and the species of *Leishmania* parasite. The World Health Organization (WHO) emphasizes on leishmaniasis as one of the seven important infections [2]. Approximately, 350 million people in 98 countries are at the risk of infection. It is estimated that 12 million people are affected with the disease and about 1.5 million new cases of cutaneous leishmaniasis (CL) are reported annually. Approximately, 90% of the CL cases occur in eight countries of Afghanistan, Saudi Arabia, Syria, Iran, Algeria, Iraq, Brazil and Peru [3,4]. Pentavalent antimony is conventionally used from 1959 for leishmaniasis but it is toxic with side effects, which requires prolong injections. The efficacy of pentavalents has been decreased and the emergence of resistance limits their usage [5,6]. The first line drugs in leishmaniasis including meglumine antimoniate (Glucantime), pentamidine (Pentacarinat), and sodium stibogluconate (Pentostam) are not effective orally and require prolonged injections. The second line drugs such as amphotericin B and pentamidine are very toxic [5]. In the absence of an effective vaccine, there is an urgent need for new and more effective drugs to replace or supplement those in current use. Plant derivatives or plant extracts are likely to provide a valuable source of new medicinal agents. The urgent need for substituting treatments has led to a program for screening natural products in leishmaniasis. Actually, the WHO recommended the use of traditional medicine in societies with poor health services. Moreover, the data obtained from reviewing would lead to the emergence of natural products with anti-leishmanial activity and would be the way for the production of new effective synthetic compounds. It has been estimated that there are about 250,000 medicinal plant species in the world. Nevertheless, the biological activities of only about 6% of them have been evaluated. Besides, only around less than 1% of medicinal plant compounds have been assessed in clinical trials [6,7].

About 35% of approved drugs belong to natural products or semi synthetic derivatives, while 30% are synthetic molecules based on natural products or pharmacophore developed from natural compounds. It is noteworthy, out of 15 antiparasitic medications that have been approved by health authorities between January 1981 and June 2006, 65% are natural products or derivatives [8].

Medicinal plants are an effective source of pharmaceutical products in Iran [9,10]. A critical evaluation of the clinical data due to the adverse effects has shown that herbal medicine is generally accepted better than synthetic medications. However, potentially, serious adverse events including herbal drug interactions have been described. This suggests the need to be attentive when using herbal therapies, mainly in specific situations such as throughout pregnancy and in the children age group [11]. About 820 forms of herbal drugs are produced in Iran [12,13]. However, in different cultures and countries, indigenous medicinal plants are used to treat parasitic diseases such as leishmaniasis. Hence, clinical trials and empirical studies have been carried out about medicinal plants in different parts of the world especially in Asian countries including Iran [9,14]. Our study attempts to provide an overview on the native medicinal plants, which was investigated against *Leishmania* parasite in Iran.

2. Methods

2.1. Search method

An exclusive search was performed through all scientific databases from April 1999 to August 2015 including five English databases (Science Direct, Scopus, Ebsco, Pub Med and Google Scholar), four Persian databases (Iran medix, Magiran, Iran doc) and the Scientific Information Database (SID). All articles which related to the medicinal plants and leishmaniasis were chosen (Fig. 1). Additionally, reference lists of all articles were reviewed for prevention of missing relevant data. The search terms were: “Leishmania,” “plant extract,” “herbal extract,” “medicinal plants,” “traditional medicine,” and “herbal medicine” alone or in combination together. Furthermore, the synonyms of herbal medicines were considered as follow: herbal preparations, herbal medications, herbal products, herbal remedies, medicinal herbs and phytopharmaceuticals. Other relevant topics such as *Leishmania* parasite were also reviewed and included if the appropriate outcomes were retrieved. The search was performed both in English and Persian languages.

2.2. Paper selection

Papers selected for inclusion were studied carefully: repetitive papers, studies out of Iran and papers with poor methodology were excluded. (See Fig. 1). The following information was extracted: the year of publication, the first author, parasite species, herbal plant, type of extract, part of plant used for extraction, concentrations, exposure time, animal models, diameter of lesions and outcomes. Two reviewers independently screened studies identified for inclusion and determined study eligibility (Kapp index showed an agreement 89% between two reviewers). Disagreements were
resolved by the third opinion.

2.3. Statistical analysis

In this meta-analysis, the mean and 95% confidence intervals of the half-maximal inhibitory concentration (IC50) values were calculated for each individual study in order to estimate the pooled mean of herbal extract effect on Leishmania spp. in Iran. The results were reported using a random-effect model with 95% confidence interval (CI). Heterogeneity among studies was evaluated by the Q-Cochran test (p < 0.1 indicate heterogeneity) and I-square statistic [low (25%–49%), moderate (50%–74%) and high (>75%) [12]. Subgroup analysis was performed to investigate potential sources of heterogeneity [14]. Publication bias was evaluated using the funnel plots and Egger test [15]. Statistical software Stata 11 (Stata Corp, College Station, TX, USA) was used to data analysis.

3. Results

Out of 7500 articles of literature searched from 1999 to 2015, 68 articles with 188 experiments (140 in vitro and 48 in vivo), met our eligibility criteria and included the current systematic review and meta-analysis. Unpublished data, duplicated papers, congresses proceeding abstracts were excluded from our systematic review and meta-analysis. Totally, data extracted comprised of 98 types of plants, their families, extraction methods, animal models IC50 and meta-analysis. Totally, data extracted comprised of 98 types of plants, their families, extraction methods, animal models IC50 and meta-analysis. Unpublished data, duplicated papers, congresses proceeding abstracts were excluded from our systematic review and meta-analysis. 5559 articles out of Iran excluded. 1750 irrelevant articles removed by title search. 55 repeated articles removed. 35 excluded, because of poor methodology after full text screening. 33 conferences abstracts excluded, because of unavailable full text. Totally, 68 articles included in the current systematic review.

Figure 1. Flowchart describing the study design process.

4. Discussion

Leishmaniasis is an important parasitic disease all around the world. For reducing the resistance in endemic areas, alternative strategies including the use of herbal plants are considered [84-85]. The present study showed a wide range of plant extracts with antileishmanial properties in vitro and in vivo experiments. Among all medicinal plants, the genus Artemisia (Asteraceae) is a large, heterogeneous, and widely dispersed genus all over the world. These species are small shrubs biennial and perennial or annual herbs. The genus Artemisia has 30 species in Iran out of which two are endemic [86]. Artemisia plants contain chemical components such as sesquiterpenes, monoterpenes lactones, flavonoids, coumarins, sterols and polyacetylenes [86]. Artemisia species has cytotoxic and anti-inflammatory activity [86-88]. The results of a study carried out by Nikoofarzadeh et al. (2008), showed that hydroalcoholic extracts of propolis Thymus vulgaris and Achillea millefolium were significantly more effective than systemic glucantime or alcoholic extract for the treatment of dermal leishmaniasis in Balb/c mice. The highest efficacy was observed for propolis, followed by Achillea millefolium and then Thymus vulgaris [65]. The efficacy of ethanol extract of the root leaves and stem of Berberis vulgaris were topically used on experimental dermal lesions of Balb/c mice. The result after two weeks statistically revealed a significant reduction of ulcer size in mice [89]. Doroogdar et al. (2008) reported the effect of various concentrations of Artemisia essence in Balb/c mice. They showed that cutaneous lesions in mice inoculated by L. major were enlarged after the application of higher concentration of the Artemisia essence. As a result, the lesions did not heal, and their size increased. In addition, parasitologic examination also remained positive [67]. The result showed that the size of lesion in mice received 40, 60, and 80% of Rubia. tinctorum extracts revealed no significant difference in comparison with the lesion size in control group [66]. Seidlitizia rosmarinus (S. ros- marinus) has been traditionally used in Mashhad and its suburbs for the treatment of CL. Despite little available data about the possible efficacy of this plant against leishmaniasis, the efficacy of herbal extracts of S. rosmarinus against cutaneous leishmaniasis in Balb/c mice was examined in this study. The natives in Khorasan Province used pure dried leaves’ powder of S rosmarinus leaves on their cutaneous lesions. Therefore, alcoholic extract of stem and leaves, which is almost similar to the pure powder, was used in this study. In this study, Eucerein was used as a base for the extracts; however, the results could be different if the researcher used vaseline or lanoline as a base for transdermal delivery of herbal extract [61]. The ulcer size in Balb/c mice received Eucerein alone was significantly increased more than other groups which approved the
| Family and botanical name | Preparation | Organism (strain) tested | Stem bark | Concentration | Exposure time | Result | Reference |
|---------------------------|-------------|-------------------------|-----------|---------------|--------------|--------|-----------|
| Allium hirtifolium        | Hydro alcoholic | L. infantum            | Fruit     | 0.01, 0.05, 0.1 and 0.2 mg/mL | For 7 days | Parasite growth at all concentrations was stopped after 3 days | [16] |
| A. aucheri                | Methanolic   | L. major                | Aerial parts | 150, 300, 450, 600, and 750 µg/mL | 24, 48, and 72 h | 750 µg/mL methanolic extract of A. aucheri was able to kill about 25% of both developmental stages of the parasite after 72 h | [17] |
| Camellia sinensis         | Methanolic   | L. major                | Green leaves | 150, 300, 450, 600, and 750 µg/mL | 24, 48 and 72 h | Methanolic extract of C. sinensis inhibited the parasite multiplication Concentration of 1000 and 500 µg/mL suppressed multiplication of promastigotes but at a concentration of 100 µg/mL it accelerated growth of promastigotes. | [18] |
| Mimosa tenuiflora         | Methanolic   | L. tropica              | NR        | 20,000, 1000 and 2000 µg/mL | 72 h | IC50 = 926, 723 and 550 µg/mL after 2, 4 and 6 days of incubation | [19] |
| Perovskia abrotanoides Karel | Methanolic | L. major (MRHO/IR/75/ER) | Root      | 0.06, 0.12, 0.25, 0.5 and 1 mg/mL | 2, 4 and 6 days incubation | IC50 = 213, 652 and 343 µg/mL after 2, 4 and 6 days of incubation, IC50’ = 1832.65 ± 89.72 µg/mL | [20] |
| P. abrotanoides Karel     | Ethanolic    | L. major (MRHO/IR/75/ER) | Root      | 0.06, 0.12, 0.25, 0.5 and 1 mg/mL | 2, 4 and 6 days incubation | IC50 = 4.9 µg/mL | [21] |
| Peganum harmala           | Unknown      | L. major (MRHO/SU/59/P) | Seed      | 5000-20000 µg/mL and 62.5 – 500 µg/mL | 72 h | IC50 P. harmala after 60 h = 0.7 µg/mL IC50 A. tinctoria after 60 h = 0.6 µg/mL IC50 combination of two extracts after 60 h = 0.6 µg/mL | [22] |
| Ferula szowitsiana        | Unknown      | L. major (MRHO/IR/75/ER) | Root      | 10,100, 500 and 1000 µg/mL | 48 h | LD50 for the promastigotes were determined as 22,300, 16,700, 16,600, 19,800 and 1230 µg/mL at 8, 16, 24, 48 and 72 h respectively. 125,000 and 50,000 µg/mL concentrations of this extract were able to kill 100% of the parasite after 48 h | [24] |
| Peganum harmala           | Aqueous      | L. major (MRHO/IR/75/ER) | Seeds     | 0.06, 0.12, 0.25, 0.5 and 1 mg/mL | 2, 4 and 6 days incubation | IC50 = 0.6 µg/mL | [25] |
| Alkanna tincturia         | Chloroformic | L. major (MRHO/IR/75/ER) | Stems and roots | 20,40,100 and 200 µg/mL | 24, 48 and 60 h | IC50 = 37 µg/mL | [26] |
| A. aucheri                | Methanolic   | L. major (MRHO/IR/75/ER) | Aerial parts | 31.25, 62.5, 125, 250, 500 and 5000 µg/mL | NR | IC50 = 7.5 µg/mL | [23] |
| F. asafoetida             | Methanolic   | L. major (MRHO/IR/75/ER) | Gum       | 31.25, 62.5, 125, 250, 500 and 5000 µg/mL | NR | IC50 = 3.6 µg/mL (better effect) | [27] |
| Gossypium hirsutum        | Methanolic   | L. major (MRHO/IR/75/ER) | Boll      | 31.25, 62.5, 125, 250, 500 and 5000 µg/mL | NR | IC50 = 5.9 µg/mL | [23] |
| Echinacea purpurea        | Ethanolic    | L. major (MRHO/IR/75/ER) | Root      | 0.5, 2.5, 50 and 125 mg/mL | 8, 16, 24, 48 and 72 h | IC50 combination of two extracts after 60 h = 0.6 µg/mL | [22] |
| Calendula officinalis     | Aqueous      | L. major (MRHO/IR/75/ER) | Flowers   | 500, 250, 125 and 62.5 µg/ml | 24,48,72 h | LD5050 for the promastigotes were determined as 22,300, 16,700, 16,600, 19,800 and 1230 µg/mL at 8, 16, 24, 48 and 72 h respectively. 125,000 and 50,000 µg/mL concentrations of this extract were able to kill 100% of the parasite after 48 h | [24] |
| C. officinalis            | Ethanolic    | L. major (MRHO/IR/75/ER) | Flowers   | 500, 250, 125 and 62.5 µg/ml | 24,48,72 h | IC50 was calculated for ethanolic & watery C. officinalis; 170 µg/ml, 215µg/ml after 24 h respectively. The extract at concentration of 500 µg/ml was found to kill all the parasites. | [25] |
| A. sativum                | Aqueous      | L. major (MRHO/IR/75/ER) | Small pieces | 0, 10, 20, 40, 60, 80, 100 µg/mL | 72 h | IC50 = 37 µg/mL | [26] |
| Satureja khuzestanica     | Ethanolic    | L. major (MRHO/IR/75/ER) | Aerial parts | 0.07–19.9 mg/ml | after 24 h | Cytotoxic effect in L. major with almost 100% death at a concentration of 93 µg/ml | [27] |
| Plant Extract | Method | Leishmania Species | Part | Concentration (mg/mL) | IC50 (μg/mL) |
|---------------|--------|--------------------|------|-----------------------|--------------|
| S. khuzestanica | Methanolic | L. major (MRHO/IR/75/ER) | Aerial parts | 0.07–19.9 mg/mL | after 24 h |
| A. sativum | Aqueous | L. major (MRHO/IR/75/ER) | Bulbs | (9.25, 18.5, 37, 74, 148 mg/mL) | 18, 24 and 48 h |
| A. euchroma | Alcoholic | L. major (MRHO/IR/75/ER) | Root | 0.78, 1.5, 3.2, 6.5 and 12.5 mg/mL | 0, 24, 48, 72 and 96 h |
| Achillea millefolium | Alcoholic | L. major (MRHO/IR/75/ER) | Root | 0.78, 1.5, 3.2, 6.5 and 12.5 mg/mL | 0, 24, 48, 72 and 96 h |
| Green tea | Ethanolic | L. major (MRHO/IR/75/ER) | Leaves | 3, 6, 12, 24, 48 and 96 mg/mL | 24, 48, 72 h |
| A. millefolium | Alcoholic | L. major (MRHO/IR/75/ER) | Leaves | 3, 6, 12, 24, 48 and 96 mg/mL | 0.24, 48, 72 h |
| Wormwood | Alcoholic | L. major (MRHO/IR/75/ER) | Flowers | 25 mg/mL | 0.24, 48, 72 h |
| Walnut leaves | Alcoholic | L. major (MRHO/IR/75/ER) | Leaves | 25 mg/mL | 0.24, 48, 72 h |
| Verbascum thapsus | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Caparis spinosa | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| A. barbatus | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Sesamum indicum | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| A. dracunculoides | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Tribulus terrestris | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Ficus bengalensis | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Prospis juliflora | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| A. dracunculoides | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Paliurus spina Christi | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Rhamnus persica boiss | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Caesalpinia gilliesii | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Acacia farnesiana | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Satureja hortensis | Hydro alcoholic | L. major (MRHO/IR/75/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h |
| Family and botanical name | Preparation | Organism (strain) tested | Stem bark | Concentration | Exposure time | Result       | Reference |
|--------------------------|-------------|--------------------------|-----------|---------------|---------------|--------------|-----------|
| *Carum copticum heirm*   | Hydro alcoholic | *L. major* (MRHO/IR/76/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h | IC50 = 15.625 ± 3.76 μg/mL |          |
| *Thymus migricus*        | Hydro alcoholic | *L. major* (MRHO/IR/76/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h | IC50 = 31.25 ± 15.44 μg/mL |          |
| *A. vulgar*              | Hydro alcoholic | *L. major* (MRHO/IR/76/ER) | Leaves or twigs | 5 to 500 μg/mL | After 28–30 h | IC50 > 625 μg/mL |          |
| *Stachys lavandulifolia* | Hydro alcoholic | *L. major* (MRHO/75/IR) | Aerial part | 50, 100, 250, 500 and 1000 μg/mL | NR | With increasing concentrations of *S. lavandulifolia* and leaves *M. germanica* extract reduced the number promastigotes. Efficacy of the two extract were not significant difference and almost have same effect on the average number of *Leishmania* promastigotes | [33] |
| *Mesplius germanica*     | Hydro alcoholic | *L. major* (MRHO/75/IR) | Leaves | 50, 100, 250, 500 and 1000 μg/mL | NR | | |
| *Calotropis gigantea*    | Methanolic | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 96.3 μg/mL | [34] |
| *C. gigantea*            | Ethyl acetate | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 92.5 μg/mL |          |
| *C. gigantea*            | Dichloromethane | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 11.3 μg/mL |          |
| *Artemisia annua*        | Ethanolic | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 38.7 μg/mL |          |
| *A. annua*               | Ethyl acetate | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 68.16 μg/mL |          |
| *A. annua*               | Dichloromethane | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 50.7 μg/mL |          |
| *A. biennis*             | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 1050 ± 2.0 μg/mL |          |
| *A. biennis*             | Ethyl acetate | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. ciniformis*          | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. ciniformis*          | Ethyl acetate | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. ciniformis*          | Dichloromethane | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. sieberi*             | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. sieberi*             | Ethyl acetate | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. kulbadica*           | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. santolina*           | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |
| *A. turanica*            | Ethanol | *L. major* | Aerial parts | 0.12, 0.25, 0.50 and 1.0 mg/mL | 24, 48, 72 h | IC50 = 25 ± 0.4 μg/mL |          |

The ethanol extracts of *A. ciniformis* has one of the most potent leishmanicidal activity.
A. turanica  
Ethyl acetate  
L. major  
Aerial parts  
NR  
IC50 = 675 ± 2.1 µg/mL

A. turanica  
Dichloromethane  
L. major  
Aerial parts  
NR  
IC50 = 425 ± 0.9 µg/mL

A. fragrans  
Hexane  
L. major  
Aerial parts  
NR  
IC50 = 1120 ± 2.5 µg/mL

A. absinthium  
Ethanol  
L. major  
Aerial parts  
NR  
IC50 = 500 ± 0.6 µg/mL

A. absinthium  
Dichloromethane  
L. major  
Aerial parts  
NR  
IC50 = 425 ± 1.3 µg/mL

A. absinthium  
Hexane  
L. major  
Aerial parts  
NR  
IC50 = 1050 ± 2.5 µg/mL

A. fragrans  
Ethanol  
L. major  
Aerial parts  
NR  
IC50 = 1000 ± 2.0 µg/mL

A. absinthium  
Dichloromethane  
L. major  
Aerial parts  
NR  
IC50 = 1375 ± 2.2 µg/mL

A. absinthium  
Hexane  
L. major  
Aerial parts  
NR  
IC50 = 1150 ± 2.2 µg/mL

A. fragrans  
Ethanol  
L. major  
Aerial parts  
NR  
IC50 = 475 ± 0.7 µg/mL

A. fragrans  
Dichloromethane  
L. major  
Aerial parts  
NR  
IC50 = 445 ± 0.5 µg/mL

A. fragrans  
Hexane  
L. major  
Aerial parts  
NR  
IC50 = 925 ± 1.6 µg/mL

A. seiberi  
Aqueous  
L. major (MRHO/IR/75/ER)  
Aerial parts and roots  
5, 10, 25, 50 and 100 µg/mL  
24, 48 and 72 h  
A. seiberi has a higher growth inhibitory effect on promastigotes but the cytotoxic effect of seven concentrations of Artemisia seiberi on uninfected splenic macrophages of Balb/c mice has very low cytotoxic effect on uninfected and healthy macrophages. [38]

Scrophularia striata  
Aqueous  
L. major (MRHO/IR/75/ER)  
Aerial parts & root  
1, 5, 10, 20 and 25%  
24, 48 and 72 h

Indium curcumin  
Unknown  
L. major (MRHO/IR/75/ER)  
Turmeric plant extracts  
NR  
IC50 = 26 µg/mL

Diacethyle curcumin  
Unknown  
L. major (MRHO/IR/75/ER)  
Turmeric plant extracts  
NR  
IC50 = 52 µg/mL

Gallium curcumin  
Unknown  
L. major (MRHO/IR/75/ER)  
Turmeric plant extracts  
NR  
IC50 = 32 µg/mL

Alkanna frigida  
Ethyl acetate  
L. major  
Root limb  
62.5, 125, 250 and 500 µg/mL  
24, 48, and 72 h  
The inhibitory effects = 46% IC50 = 106 µg/mL

A. frigida  
Ethanol  
L. major  
Root limb  
62.5, 125, 250 and 500 µg/mL  
24, 48, and 72 h  
The inhibitory effects = 45% IC50 = 86 µg/mL

(continued on next page)
| Family and botanical name | Preparation | Organism (strain) tested | Stem bark | Concentration | Exposure time | Result | Reference |
|---------------------------|-------------|--------------------------|-----------|---------------|---------------|--------|-----------|
| *A. frigida*              | Chloroformic| *L. major*               | Root limb | 62.5, 125, 250 and 500 μg/mL | 24, 48, and 72 h | The inhibitory effects = 13% IC50 value after 48 and 72 h = 330 and 68 μg/mL | | |
| *A. frigida*              | Hexane      | *L. major*               | Root limb | 62.5, 125, 250 and 500 μg/mL | 24, 48, and 72 h | The inhibitory effects = 15% IC50 value = (384 μg/mL for 48 h) and (98 μg/mL 72 h). | | |
| Nerium oleander,* ricinus communis, capsicum, almond powder | Unknown     | *L. major*               | Leaves and stems | 1/10, 1/100, 1/1000 and 1/10,000 | For 7 weeks | In terms of quantity, the number of promastigotes of *Leishmania* in the face of the herbal combination reduced compared with the control group IC 50 = 25 μg/mL after 24 h | [41] |
| Artemether                | Unknown     | *L. infantum (MHOM/TN/80/IPI1)* | Ointment and injection | 0, 10, 25, 50, and 100 μg/mL | 72 h | IC50 = 52.79 μg/mL | [42] |
| aloe-emodin               | Unknown     | *L. major*               | Powder    | 40, 80, 120 and 160 μg/mL | 24, 48 and 72 h | In treatment of promastigotes of *L. major* with *S. striata* extract at the concentration of 25%, the parasites were killed at the day three | [43] |
| Scrophularia striata      | Aqueous     | *L. major*               | Aerial parts and root | 1, 5, 10,20 and 25% | 72 h | There was a significant difference in reducing parasites on groups receiving *Satureia hortensis* and *N. sativa* with Glucantime | [44] |
| *Nigella sativa*          | Essential oil| *L. major (MRHO/IR/75/ ER)* | Aerial parts | 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2% | 24, 48 and 72 h | The viability of the *L. major* promastigotes in the concentration of 312 μg/mL aqueous onion extracts was 80% and in the same concentration, 20% of the *L. major* promastigotes were unmovable. At the concentration of 2500 μg/mL aqueous *A. cepa* extracts, 70% of the *L. major* promastigotes were unmovable and the viability of promastigotes was 30%. Moreover, in the concentration of 5000 μg/mL of aqueous *A. cepa* extracts, 100% of the *L. major* promastigotes were unmovable and the viability of the *L. major* promastigotes in this concentration was 0%. IC50 = 1250 μg/mL IC100 = 5000 μg/mL | [45] |
| *S. hortensis*            | Essential oil| *L. major (MRHO/IR/75/ ER)* | Aerial parts | 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, and 2% | 24, 48 and 72 h | The viability of the *L. major* promastigotes in the concentration of 312 μg/mL aqueous onion extracts was 80% and in the same concentration, 20% of the *L. major* promastigotes were unmovable. At the concentration of 2500 μg/mL aqueous *A. cepa* extracts, 70% of the *L. major* promastigotes were unmovable and the viability of promastigotes was 30%. Moreover, in the concentration of 5000 μg/mL of aqueous *A. cepa* extracts, 100% of the *L. major* promastigotes were unmovable and the viability of the *L. major* promastigotes in this concentration was 0%. IC50 = 1250 μg/mL IC100 = 5000 μg/mL | [46] |
| *A. cepa*                 | Aqueous     | *L. major*               | Root      | 0.312, 2.5 and 5 mg/mL | 24 and 48 h | The viability of the *L. major* promastigotes in the concentration of 312 μg/mL aqueous onion extracts was 80% and in the same concentration, 20% of the *L. major* promastigotes were unmovable. At the concentration of 2500 μg/mL aqueous *A. cepa* extracts, 70% of the *L. major* promastigotes were unmovable and the viability of promastigotes was 30%. Moreover, in the concentration of 5000 μg/mL of aqueous *A. cepa* extracts, 100% of the *L. major* promastigotes were unmovable and the viability of the *L. major* promastigotes in this concentration was 0%. IC50 = 1250 μg/mL IC100 = 5000 μg/mL | [47] |
| *Ixora brachiata*         | Ethanolic   | *L. major*               | Root      | 0.312, 2.5 and 5 mg/mL | 24 and 48 h | The viability of the *L. major* promastigotes in the concentration of 312 μg/mL aqueous onion extracts was 80% and in the same concentration, 20% of the *L. major* promastigotes were unmovable. At the concentration of 2500 μg/mL aqueous *A. cepa* extracts, 70% of the *L. major* promastigotes were unmovable and the viability of promastigotes was 30%. Moreover, in the concentration of 5000 μg/mL of aqueous *A. cepa* extracts, 100% of the *L. major* promastigotes were unmovable and the viability of the *L. major* promastigotes in this concentration was 0%. IC50 = 1250 μg/mL IC100 = 5000 μg/mL | [47] |
| Hyssopus officinalis       | Alcoholic   | *L. major*               | Leaves    | 0, 0.5, 0.1, 0.2, 0.4 and 1 μg/mL | 24, 48 and 72 h | That extract was effective | [48] |
| Plant                     | Extract Type | Plant (Code)                        | Part                | Concentrations                  | Duration (h) | Results |
|--------------------------|--------------|-------------------------------------|---------------------|----------------------------------|--------------|---------|
| L. major (MRHO/IR/75/ER) | Alcoholic    | Tussilago farfara                   | Leaves              | 0, 05, 0.1, 0.2, 0.4 and 1 μg/mL | 24, 48 and 72 | That extract was effective |
| Carum copticum           | Alcoholic    | L. major (MRHO/IR/75/ER)            | Seed                | 0, 05, 0.1, 0.2, 0.4 and 1 μg/mL | 24, 48 and 72 | That extract was effective |
| B. vulgaris              | Methanolic   | L. tropica (MHOM/IR/-2002/Mash2)    | Aerial parts        | Between 5 and 100 μg/mL and 1 μg/mL for 48 h at 37°C −10 μg/mL | That extract was effective |
| B. vulgaris              | Aqueous      | L. tropica (MHOM/IR/-2002/Mash2)    | Aerial parts        | Between 5 and 100 μg/mL and 1 μg/mL for 48 h at 37°C −10 μg/mL | That extract was effective |
| B. vulgaris              | Methanolic   | L. infantum (MCAN/IR/07/Moheb-gh)   | Aerial parts        | Between 5 and 100 μg/mL and 1 μg/mL for 48 h at 37°C −10 μg/mL | That extract was effective |
| B. vulgaris              | Aqueous      | L. infantum (MCAN/IR/07/Moheb-gh)   | Aerial parts        | Between 5 and 100 μg/mL and 1 μg/mL for 48 h at 37°C −10 μg/mL | That extract was effective |
| B. vulgaris              | Methanolic   | L. major (MRHO/IR/75/ER)            | Leaves and stems    | 0,150, 300, 450, 600, 750 μg/mL | 0,24,48,72 and 96 | That extract was effective |
| A. sativum               | Methanolic   | L. tropica (MHOM/IR/-2002/Mash2)    | Bulbs               | 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 16.1 μg/mL. Inhibitory effects against amastigote forms IC50 = 39.4 μg/mL. Inhibitory effects against promastigote forms IC50 = 52.8 μg/mL. Inhibitory effects against amastigote forms IC50 = 59.2 μg/mL. Inhibitory effects against promastigote forms IC50 = 3.9 μg/mL. Inhibitory effects against amastigote forms IC50 = 11.6 μg/mL. Inhibitory effects against promastigote forms IC50 = 26.6 μg/mL. Inhibitory effects against amastigote forms IC50 = 40.8 μg/mL. |
| A. sativum               | Aqueous      | L. tropica (MHOM/IR/-2002/Mash2)    | Bulbs               | 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 12.3 μg/mL. Inhibitory effects against amastigote forms IC50 = 19.2 μg/mL. Inhibitory effects against promastigote forms IC50 = 34.8 μg/mL. Inhibitory effects against amastigote forms IC50 = 21.4 μg/mL. |
| Myrtus communis          | Essential oil| L. tropica (MHOM/IR/-2002/Mash2)    | Leases              | 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 9.3 μg/mL. Inhibitory effects against amastigote forms IC50 = 21.4 μg/mL. |
| M. communis              | Methanolic   | L. tropica (MHOM/IR/-2002/Mash2)    | Leases              | 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 9.3 μg/mL. Inhibitory effects against amastigote forms IC50 = 21.4 μg/mL. |
| N. sativa                | Essential oil| L. tropica                          | Aerial parts        | 0–200 μg/mL                      | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 9.3 μg/mL. Inhibitory effects against amastigote forms IC50 = 21.4 μg/mL. |
| Vinca major              | Chloroformic | L. major                            | Leaves and stems    | 0,150, 300, 450, 600, 750 μg/mL | 0,24,48,72 and 96 | That extract was effective |
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(continued on next page)
| Family and botanical name       | Preparation          | Organism (strain) tested | Stem bark                  | Concentration | Exposure time | Result                                                                                     | Reference |
|--------------------------------|----------------------|--------------------------|----------------------------|---------------|---------------|-------------------------------------------------------------------------------------------|-----------|
| N. sativa                      | Methanolic           | L. tropica               | Aerial parts               | 0–200 µg/mL   | 72 h incubation | Inhibitory effects against promastigote forms IC50 = 14.8 µg/mL                           |           |
|                                |                      |                          |                            |               |               | Inhibitory effects against amastigote forms IC50 = 30.8 µg/mL                            |           |
|                                |                      |                          |                            |               |               | Inhibitory effects against promastigote forms IC50 = 11.7 µg/mL                           |           |
|                                |                      |                          |                            |               |               | Inhibitory effects against amastigote forms IC50 = 26.3 µg/mL                            |           |
|                                |                      |                          |                            |               |               | Inhibitory effects against amastigote forms IC50 = 15.7 µg/mL                            |           |
|                                |                      |                          |                            |               |               | Inhibitory effects against amastigote forms IC50 = 34.6 µg/mL                            |           |
|                                |                      |                          |                            |               |               | Higher concentrations (35.25 and 50 µL) had a stronger effect on promastigotes, causing total mortality. IC50 = 26,000 µg/mL | [53]      |
|                                |                      |                          |                            |               |               | IC50 = 35,000 µg/mL                                                                          |           |
|                                |                      |                          |                            |               |               | It was determined that anti-protozoal activity of Caparis extract (900 µg/mL) was able to kill 97.8% of promastigotes after 72 h | [55]      |
|                                |                      |                          |                            |               |               | Promastigote: IC50 = 58.6 ± 3.15 µg/mL                                                      |           |
|                                |                      |                          |                            |               |               | Amastigote: IC50 = 37.3 ± 2.51 µg/mL                                                        |           |
|                                |                      |                          |                            |               |               | IC50 = 118.6 ± 5.19 µg/mL                                                                 |           |
|                                |                      |                          |                            |               |               | IC50 = 586.2 ± 47.6 µg/mL                                                                  |           |
|                                |                      |                          |                            |               |               | Methanolic extract was more effective than aqueous extract. This extract was less effective as compared to the control drug |           |
|                                |                      |                          |                            |               |               | IC50 = 159.45 µg/mL                                                                          |           |
|                                |                      |                          |                            |               |               | Toxicity for macrophage cell line – 10% d                                                  |           |
|                                |                      |                          |                            |               |               | IC50 = 224.45 µg/mL                                                                          |           |
|                                |                      |                          |                            |               |               | Toxicity for macrophage cell line – 8%                                                     |           |
|                                |                      |                          |                            |               |               | IC50 = 171.1 µg/mL                                                                           |           |

IC50: concentration of drug that causes 50% growth inhibition of amastigote or promastigote forms of *Leishmania*.
IC100: concentration of drug that causes 100% growth inhibition of amastigote or promastigote forms of *Leishmania*.
CC50: as the Cytotoxic concentration of the extracts to cause death to 50% of viable cells in the host.
LD50: (Lethal Dose, 50%) It is the amount of the substance required (usually per body weight) to kill 50% of the test population.
NR: Not reported.
Table 2
Included publications of survey on the efficacy and activity of herbal medicines used against leishmaniasis in vivo in Iran.

| Family and botanical name | Preparation | Organism tested | Stem bark | Animals kind | Concentration | Result | Reference |
|---------------------------|-------------|----------------|-----------|--------------|---------------|--------|-----------|
| Z-HE                      | Crude extract | L. major        | -         | Human        | Topical       | In the group treated with Z-HE (group A), complete cure was observed in 74.4% (Figs 1 and 2), partial cure in 11.6%, and failure in 14.0%. In the group treated with meglumine antimoniate (Glucantime) (group B), complete cure was observed in 24.1%, partial cure in 14.1%, and failure in 58.8% | [59] |
| A. sativum                | Aqueous     | L. major        | Bulbs     | Mice (Balb/c) | 300,000 promastigotes. lesion was measured on days 1, 10, 20, 30 and 45 | The diameter of lesion was reduced by aqueous extract of garlic within 30 days of treatment. However, the maximum reduction was induced when mice were subjected to vitamin A for 10 days before the administration of the aqueous extract for 30 days. A significant correlation between healing and the amount of NO release was also found. | [60] |
| Berberis vulgaris         | Alcoholic   | L. major        | Leaves, stems and roots | Mice (Balb/c) | 2.5, 4.0, 5.5 and 7.0% | The results showed that after 2 weeks, a statistically significant decrease of ulcer size of treated mice observed, while in the control group the lesion growth continued. The examinations showed that using higher concentration of the extract caused more decrease in surface area of CL lesions on day 15 and negative direct smear on day 20. Alcoholic extract of B. vulgaris root was more effective than leaves and stem extract. | [61] |
| Eucalyptus globulus       | Essence     | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Essence 10% | The essences reduced the diameter of lesions or caused small lesions to disappear completely. | [62] |
| Myrtus communis           | Essence     | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Essence 10%, 20% | No change was noticed in the size of the lesions or the number of parasites. | |
| Ferula gumosa             | Essence     | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Essence 10%, 20% | No change was noticed in the size of the lesions or the number of parasites. | |
| A. herbaalba              | Essence     | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Essence 10%, 20% | No change was noticed in the size of the lesions or the number of parasites. | |
| A. sativum                | Tincture    | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Tentor 50% & 100% | No change was noticed in the size of the lesions or the number of parasites. | |
| Urtica dioica             | Crude extract | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Pure extract | No change was noticed in the size of the lesions or the number of parasites. | |
| A. dracunculus            | Essence     | L. major (MRHO/IR/76/ER) | NR | Mice (Balb/c) | Essence 10% | The essences of A. dracunculus reduced the diameter of lesions or caused small lesions to disappear completely. | |
| Cassia Fistula            | Concentrated boiled | L. major | Fruits | Human | - | Mean healing time was 4.6 ± 3.7 weeks | [63] |
| C. Fistula                | Hydro alcoholic | L. major | Fruits | Human | - | Mean healing time was 4.9 ± 3.8 weeks. There was no significant difference between the efficacy of concentrated boiled extract and that of the hydro alcoholic extract of the Cassia Fistula | |
| Berberis vulgaris         | Alcoholic   | L. major        | Stem skin | Mice (Balb/c) | 20, 40, 80% for 30 days | With the 20% preparation: by the end of the treatment period, the mean diameter of the lesions had decreased, with complete healing in 5 mice (27.7%), (p < 0.001). By the time of the decrease in diameter, the mean weight of the animals had increased and the number of parasites in the lesions had declined (80%). Total elimination of the parasites was observed in 12 animals (p < 0.001). At a concentration of 40%: mean ulcer diameter decreased, with complete healing in 2 mice (11.1%, p < 0.001). By the time of the decrease in diameter, the mean weight of the mice had increased (p < 0.05). The mean number of parasites in lesions decreased (64.3%), with total elimination in 9 animals (p< 0.05). Mean of ulcer size reduction – 36.0% that T. vulgaris, hydro alcoholic extracts were significantly more effective in reduction of ulcer size as compared with Glucantim | |
| Thymus vulgaris           | Hydro alcoholic | L. major (MRHO/IR/75/ER) | NR | Mice (Balb/c) | NR | |
| Achillea millefolium      | Hydro alcoholic | L. major (MRHO/IR/75/ER) | NR | Mice (Balb/c) | NR | |

(continued on next page)
| Family and botanical name | Preparation    | Organism tested          | Stem bark | Animals kind | Concentration | Result                                                                                          | Reference |
|---------------------------|----------------|--------------------------|-----------|--------------|---------------|-----------------------------------------------------------------------------------------------|-----------|
| propolis                  | Hydro alcoholic| L. major (MRHO/IR/75/ER) | NR        | Mice (Balb/c) | NR            | Mean of ulcer size reduction = 43.29% that A. millefolium hydro alcoholic extracts were significantly more effective in reduction of ulcer size as compared with Glucantim. | [65]      |
| A. millefolium            | Hydro alcoholic| L. major (MHOM/64/IR/ER75) | NR        | Mice (Balb/c) | NR            | The mean weight of the mice that received 40, 60 and 80% concentrations of A. millefolium extracts were significantly more effective in reduction of ulcer size as compared with Glucantim. | [66]      |
| A. sieberi                | Hydro alcoholic| L. major (MHOM/64/IR/ER75) | NR        | Mice (Balb/c) | 1.35% after 30 days | At the end of the 30 day treatment period with concentrations of A. sieberi, any of the mice treated with complete remission were observed. And microscopic examination of samples taken from the animals tested were positive. | [67]      |
| Thyme                     | Hydro alcoholic| L. major (MRHO/IR/75/ER) | NR        | Mice (Balb/c) | NR            | Observed significant difference between mean of lesion diameter before and after treatment in control, Yarrow and Thyme groups showed no significant difference between mean of lesion diameter after treatment between treatment and Glucantime groups. | [68]      |
| A. millefolium            | Hydro alcoholic| L. major (MHOM/64/IR/ER75) | NR        | Mice (Balb/c) | NR            | Ulcer diameter before treatment = 4/35 ± 0/5 mm Ulcer diameter after treatment = 3/4 ± 0/67 mm The mean ulcer size in the group receiving thyme has been good impact on preventing the process of development of wound Ulcer diameter before treatment = 5/39 ± 0/41 mm Ulcer diameter after treatment = 4 ± 0/34 mm The mean ulcer size in the group receiving yarrow has been good impact on preventing the process of development of wound Ulcer diameter before treatment = 5.77 ± 0/30 mm Ulcer diameter after treatment = 7/11 ± 0/56 mm Were did not showen statistical signifi cant difference between mean diameter of lesions after treatment with the treated group with Plant extracts and treated with Glucantime. | [69]      |
| Henna                     | Hydro alcoholic| L. major (MHOM/64/IR/ER75) | NR        | Mice (Balb/c) | NR            | Ulcer diameter before treatment = 5.18 ± 0/47 mm Ulcer diameter after treatment = 7/06 ± 1/09 mm Were did not showen statistical significant difference between mean diameter of lesions after treatment with the treated group with Plant extracts and treated with Glucantime. | [70]      |
| Pistacia Atlantica        | Unknown        | L. major (MHROM/IR/75/ER) | Gum obtained of trunk and branches | Mice (Balb/c) | 0.4,8 week | Gum daily for 28 days decreased skin lesion size in the mice infected with L. major compared with that in the control. Treatment Balb/c mice with gum obtained P. atlantica var. kurdica and Glucantime causes decrease number of parasitologically positive mice. | [70]      |
| E. camaldulensis          | Methanolic      | L. major (MRHO/IR/75/ER) | NR        | Mice (Balb/c) | NR            | Amastigote number into the lesions, were significantly decreased, nanogold solutions were also decreased mortality rate in the mice Lesions’ size in SKEO treated groups was restrained but not significantly different from the control group the mortality rate in treated groups was clearly less than the control. | [71]      |
| Satureja khuzestanica     | Essential oil   | L. major MRHO/IR/75/ER    | Aerial parts | Mice (Balb/c) | 0.01, 0.001, 0.0001% for 7 week | The results showed that R10 had good therapeutically efficacy in treatment of lesions in mice (P < 0.05) that this efficacy was significant in sixth, seventh and eighth weeks after the treatment. | [71]      |
| A. sativum                | Aqueous         | L. major                  | Bulbs     | Mice (Balb/c & Suri) | Promastigote injection and evaluated For 8 week | The mean of lesion size in each group of mice were compared and analyzed. No significant differences in the lesions size were found between the three mice groups. Therefore, E. purpurea extract was not effective against L. major based on the findings of this study. | [72]      |
| Echinacea purpurea        | Hydro alcoholic | L. major                  | Aerial parts | Mice (Balb/c) | 40, 60 and 80% | | | | | |
| Mespilus germanica        | Ethanol         | L. major                  | Leaves    | Mice (Balb/c) | 40, 60 and 80% | | | | |
Extract of *M. germanica* has the highest effectiveness in concentration of 40%, causing greater reductions in both ulcer diameter and the number of parasites in the lesions compared with other prepared concentrations.

The results indicated that herbal extract was able to affect on lesion size, its performance and to prevent visceralization of the parasite. This is the first report indicating visceralization caused by the cutaneous form of *L. major* in the Balb/c mice.

This skin lesion at the base of the tail of mice under investigation also indicate a significant effect on the composition of the herbal form wound and skin nodule at the base of the tail of mice treated with the control group.

In vivo experiments indicated that oral artemether treatment of mice, during 3 days and every 6 h (0.625 mg/kg) was more significant than parenteral (0.625 mg/kg IP) treatment.

Mean diameter of lesion in the infected group treated with ointment of artemether decreased from 1.294 to 0.214 cm mean diameter of lesion in the infected group treated with artemether injection decreased from 0.913 to 0.256 cm.

Increased the level of IFN-γ and lowered the parasite burden in the proximal lymph nodes and prevented the necrosis of the footpad as compared with the untreated infected mice.

Significant increase in the lesion size of treated mice compared with reference group except for treated group by 15% extract.

Injection was very effective by the prevention of ulcers caused by *Leishmania major* in Balb/c mice compared to untreated control.

In the aqueous extract group only 10% of mice healed.

In the ethanolic extract group only 40% of mice healed. Results showed that ethanol extract of *P. harmala* had good therapeutic efficacy in treatment of lesions in mice.

Extract concentration (25%) at the concentration at the ethanol extract led to a decrease in the parasite rate to 36 amastigotes. For all of the concentrations (10%, 20%, 25%) we observed completely elimination of amastigotes on the third day.

Injection was very effective by the prevention of ulcers caused by *Leishmania major* in Balb/c mice compared to untreated control.

Extract concentration (25%) at the concentration at the third day, the extract led to a decrease in the parasite rate to 36 amastigotes. For all of the concentrations (10%, 20%, 25%) we observed completely elimination of amastigotes.

Study showed that the main lesion size did not decrease significantly, or the small lesions did not completely disappear after treatment by *H. helix* alcoholic extract. Amastigotes counts (mean ± SD) of the skin lesions decreased in control A and 20% concentration groups, but in negative control and 70% concentration groups the number of parasites did not reduce.

In vivo – after 30 days of treatment, 75 and 87.5% recovery were observed in the infected mice treated with 30% extract and meglumine antimoniate, respectively, while *P. kinjuk* extract at the concentration of 20% recovered 50% of the infected mice.

It was determined that 700 μg/mL and 900 g/mL *Caparis* root extract concentrations were more effective than other concentrations on the skin amastigotes of *Leshmania* in ulcers. The results were suggestive that *Caparis* root extract had significantly similar effect in reduction of ulcer size as compared to Glucantim.

The comparison of these three groups revealed that wound healing in group one and group two were 58.3% and 80% respectively, which was significant whereas no healing was seen in the control group

* A. absinthium extract was statistically significant

The lesion size in different groups mice after 30 day = 9.9 ± 2.4 μg/mL

The lesion size in different groups mice after 30 day = 13.1 ± 2.8 μg/mL

A. absinthium extract was statistically significant

The lesion size in different groups mice after 30 day = 15.3 ± 2.6 μg/mL

a Mixture of Althaea rosa, Althaea officinalis, and Pharmacology, Pathology, and members of the families Leguminosae, Faliaceae, Malvaceae, and Lythraceae.
The administration form of a drug is also important. In the present study, the extracts were topically used as an ointment, but the results would be different if the extracts were administered intravenously. Recent studies have shown that nanoparticles of anti-leishmanial drugs are highly effective to treat CL. The important advantages of such drugs are low dosage and minimum adverse reactions [90].

In the present systematic review and meta-analysis, the Begg’s test showed no publication bias among all studies (t = 1.25, p = 0.215). In addition, subgroup analysis revealed that there was a significant difference in extracting preparation including hexane, dichloromethane, hydroalcoholic and ethyl acetate with higher IC50 values, and aqueous or methanolic with lower IC50 values. (p < 0.001) (Table 3).

However, several studies have demonstrated that the hexane and ether acetate extracts present low or no toxicity to host cells at the effective concentrations [91,92]. Ribeiro et al. (2014) evaluated and ether acetate extracts present low or no toxicity to host cells at 16 Brazilian plant species against anti-leishmania activity of 44 extracts and fractions derived from L. amazonensis. Among them, the most potent extracts were the hexanic extract [92].

In general, the ethanolic extracts were less effective and more toxic than the hexanoic extracts and buthanolic, dichloromethane ethyl acetate and hexanic fractions in the mammalian cells [93]. Thus, the application of the hexanic extract against Leishmania parasites as a potent fraction is recommended in the in vivo experiments.

In the study of Hooshyar et al. (2014), a significant decrease was shown in the main lesion size, or the small lesions were not completely disappeared after treatment by Hedera helix (H. helix) alcoholic extract. Their results disagreed with those of Talari et al. who used, 100 and 50 mg/mL of H. helix extract and observed that all promastigotes of L. major were killed in vitro [94]. This difference of findings may be due to different preparation methods and concentration of the plant extract was used in two studies. Different extract was gathered from eleven Iranian Artemisia species. Their leishmanicidal activities against the growth of L. major showed that ethanol extracts especially those taken from A. ciniformis, A. santolina and A. kalbadica had the strongest effects [86]. In the present study, they demonstrated the inhibitory effect of different extracts from eleven Artemisia species on the growth of L. major promastigotes in vitro. It was previously reported that the aqueous extract and essential oil of A. herbaalba had antileishmanial activity against L. tropica and L. major promastigotes [37]. In addition, the aqueous extract of leaves of A. indica exhibited leishmanicidal activity (IC50 = 430 μg/mL) [95]. Here, some of tested Artemisia spp showed most strong antileishmanial activities. In this study, all tested extracts exhibited antileishmanial activity after incubation, however, ethanol extracts from A. ulbidaca and A. cinformis showed the stronger leishmanicidal activity at value of (IC50 = 25 μg/mL). Growth inhibitory effect of ethanol extract of other plants such as Haplo phyllum myrtifolium against L. tropica promastigotes were previously reported (IC50 = 10.9 μg/mL) [96]. Comparing the antileishmanial effect of non-polar extracts revealed that ethyl acetate extract of A. fragrans had less antileishmanial activity against L. major promastigotes. Ethyl acetate extracts of studied Artemisia species (except for A. turanica and A. fragrans) were also more active in comparison with their dichloromethane extract.

In vitro antileishmanial activity of ethyl acetate and dichloromethane extracts of Irricinia spinosula (IC50 = 16.09, 47.38 μg/mL) were reported against L. major promastigotes [97]. The lethal dose (LD50) of dichloromethane extract and hexane extract of Calophyllum brasiliense on L. amazonensis promastigotes were 40 mg/mL and 20 mg/mL, respectively [98]. In comparison with other extracts, Artemisia species hexane extracts (except for A. fragrans) were less active than L. major. Hexane extracts of A. biennis, A. annua, A. turanica, A. fragrans and A. absinthium were less effective than other species. Other investigators have also reported lower activity of hexane extracts of plants than Leishmania species in comparison with other extracts. For example, ethanol extracts of Arbutus unedo significantly decreased L. tropica promastigotes counts [99].

Leishmanicidal activity of Allium sativum (garlic extract) has been established against infection with L. major, so that it can induce a Th1-type response, stimulate INF-γ and NO production in macrophage and thus prevent the progression of the infection [73,28]. To improve the therapeutic efficacy and reduce toxicity, above mentioned natural molecules can be applied as either scaffold for producing and exploring new immune drugs or natural immunomodulators in synergy and in combination with existing drugs [100,101]. Targeting anti-leishmanial drugs to macrophages with drug delivery systems reflects a hopeful strategy overcoming the problems associated with the current treatment protocols.

Another important issue is the safety of natural remedies. Although natural immune therapy in different generations has been tested and approved, it is necessary to prove the overall pharmacological safety of the correction. Chemical agents in Iranian drug market have disadvantages such as high cost and side

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**Table 3**  
Results of subgroup meta-analysis for the mean of IC50 separately characteristics.

| Characteristics          | n | IC50   | 95% CI     | I-squared | P     |
|--------------------------|---|--------|------------|-----------|-------|
|                          |   |        | Lower      | Upper     |       |
| Stem bark                |   |        |            |           |       |
| Aerial parts             | 44| 553.10 | 470.09     | 636.11    | 100.00% | P<0.001|
| Root limb                | 8 | 123.43 | 23.18      | 247.07    | 98.7%  |       |
| Bulbs                    | 3 | 22.83  | 4.00       | 41.66     | 100%   |       |
| Leaves or twigs          | 3 | 422.36 | 393.00     | 451.72    | 99.8%  |       |
| Preparation              |   |        |            |           |       |
| Ethanol                  | 9 | 251.33 | 137.64     | 365.03    | 89.32% | P<0.001|
| Ethyl acetate            | 11| 448.00 | 337.96     | 558.04    | 97.01% |       |
| Dichloromethane          | 10| 531.82 | 457.96     | 605.68    | 100.00%|       |
| Hexane                   | 11| 910.92 | 781.92     | 1019.93   | 99.30% |       |
| Methanic                 | 3 | 59.75  | 16.12      | 103.38    | 93.28% |       |
| Aqueous                  | 4 | 23.89  | 5.88       | 41.90     | 89.36% |       |
| Chloroformic             | 3 | 132.90 | 12.99      | 252.81    | 100.00%|       |
| Hydroalcoholic           | 3 | 510.00 | 490.77     | 529.23    | 100.00%|       |
| Botanic name             |   |        |            |           |       |
| Artemisia spp            | 49| 8.0    | 7.4        | 8.6       | 97.1%  | P<0.001|
| Allium spp               | 4 | 14.2   | 13.7       | 14.6      | 99.7%  |       |
| Allkanna spp             | 6 | 9.4    | 8.3        | 10.6      | 98.5   |       |

n: sample size.
effects. Considering the effectiveness of these plants would make them as a source of natural and safe agents for the treatment of leishmaniasis. However, anti-leishmanial drugs or natural compounds are safe when their selectivity index is more than 10 [50].

5. Conclusion

In conclusion, the present review showed that a range of plant extracts had effects on promastigote stage of *Leishmania* and interesting anti-leishmanial properties exhibited in vitro and in vivo. Therefore, it might be possible to use the extracts instead of chemical drugs. However, almost all of the authors claimed successful results about their investigated plants, but their studies really had limitations which affected with the accuracy of their results. Some of defects included in these studies are described in detail as lacking of randomized double blind clinical trials in all of human based studies. Also some of investigations were performed in vitro and were not performed in vivo [102,103]. The period of exposure of extracts was not enough in some of the studies [104] and at last in one study, the toxicity level of the plant investigated was very high for testing in volunteer patients [105]. Most of data published were obtained from animal model and were not tested on human [106]. According to all documented data, phytotherapy has provided a large and hopeful vision to new, safe, and effective leishmaniacal agents. Nevertheless, it needs to generalize all results obtained from in vitro and in vivo studies on the efficacy of plant extracts, metabolites or formulations against different *Leishmania* species to validate their activities. We
concluded that the mechanism of action was enhancing the hosts’ cellular immunity.

The present systematic investigation on anti-leishmanial activity of the medicinal plants together with their toxicity, mechanism of action and chemical properties for improvement is the most favorable formulation urgently required to confirm their efficacy in the treatment of leishmaniasis.

As a whole, the present systematic review provide valuable information about the natural products with anti-leishmanial activity which would be very favorable for experimental and clinical trials and herbal combination therapy studies. Consequently, further clinical researches are needed to establish the effective and safe medicinal plants therapy. It is necessary to find their active components, and potential toxic effects would lead to producing the well-tolerated and safe drugs for leishmaniasis.

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Author contribution

Data collection: Masoud Soosaraei. Study concept: Mahdi Fakhar. Data interpretation: Masoud Soosaraei, Mahdi Fakhar, Saeed Hosseinieh, Hajer Ziaei Hezarjaribi.

Writing the paper: Masoud Soosaraei, Mahdi Fakhar.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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