



# In vitro anti-leishmanial effects of garlic extract on *Leishmania major*: A Systematic Review

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## Article History

Received: 5 December 2022  
Received in revised form: 27 May 2023  
Accepted: 31 July 2023  
Published online: 10 May 2024  
DOI: [10.29252/mlj.18.3.21](https://doi.org/10.29252/mlj.18.3.21)

## Keywords

Garlic  
Leishmania Major  
Systematic Review

Article Type: Systematic Review



## Abstract

**Background:** Leishmaniasis is a vector-borne parasitic disease caused by an obligate intracellular protozoan. Despite the significant frequency of cutaneous leishmaniasis, there is still no appropriate prevention, control and treatment. In recent years, a few studies have been performed on the effects of garlic extract for the treatment of cutaneous leishmaniasis. Current drugs, such as glucantime, have several side effects and lead to direct cellular damage. If an herbal-based drug reduces *Leishmania* by augmenting the immune system, it has the potential to possess a wider margin of safety. This study aimed to investigate the effects of *in vitro* systematic review of the effects of garlic (*Allium sativum*) and its compounds on *Leishmania major*.

**Methods:** Five English databases (PubMed, Google Scholar, Embase, Scopus, and Web of Science) were searched until the end of December 2022. The syntax and specific tags have been used for each database. The studies with poor methodology, inadequate information, inappropriate analysis, and confusing presentation were excluded from the current study. The quality of articles was assessed by eleven questions developed by the authors and the data were extracted from the selected articles and discussed.

**Results:** In this study, 198 articles were selected in the search step, of which five eligible articles were included for examination. The most commonly used solvent for the preparation of garlic extract was distilled water (60%), followed by methanol (40%). In these studies, the bulb of garlic is mostly used (80%). Cell type of J774 was used in only one study (20%), and murine peritoneal macrophages were used in the other studies (80%). The effect of garlic on *Leishmania major* was strong (80%). Studies have shown that garlic extract or its active compounds can increase cellular immune responses, which play an important role in inhibiting the *Leishmania* parasite. This is associated with the activation of macrophages and increased IFN- $\gamma$  levels and NO production.

**Conclusion:** According to the studies, the effect of different combinations of garlic on the *Leishmania* parasite has been shown. However, the exact mechanism of the anti-Leishmanial effect of garlic has not been determined. Thus, this issue needs further investigation.

## Introduction

Leishmaniasis is a vector-borne parasitic disease caused by an obligate intracellular protozoan. It is a public health problem in many tropical and subtropical countries. Annually, the frequency of leishmaniasis is evaluated to be 0.7-1.3 million cases globally, though the real number of infected cases is estimated to be 6- to 10-fold higher than that reported (1). The disease includes a variety of clinical syndromes, including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). CL is the most common form of leishmaniasis. It is caused by over 15 different species of the protozoan parasite *Leishmania*. This microorganism is transmitted to humans by infected female sandflies (2). CL can have disturbing effects on local communities. It causes scarring skin lesions that can leave life-long scars and lead to severe social dishonor, especially for women and children (3).

Currently, the treatment of cutaneous leishmaniasis is based on intralosomal injection or oral medication, along with cryotherapy, topical controlled heat, CO<sub>2</sub> laser, and photodynamic therapy. Antimonial drugs have been widely used for CL treatment (4). Despite the high prevalence of CL, there is still a lack of appropriate prevention, control, and treatment methods. In various cultures and countries, local medicinal plants are utilized for the treatment of diseases, particularly leishmaniasis. Currently, extensive research is being conducted on treatment methods for leishmaniasis. Medicinal plants have been traditionally used in medicine to treat or reduce the severity of CL lesions (5).

Garlic belongs to the Liliaceae family and its scientific name is *Allium sativum* (6). The antibiotic properties of garlic have been recognized for a long time. Several studies have been done on the antimycotic properties of garlic (7). In recent years, a few studies have been performed on the effects of garlic extract for the treatment of cutaneous leishmaniasis. Current drugs, such as glucantime, have several side effects and lead to direct cellular damage. If an herbal-based drug reduces *Leishmania* by augmenting the immune system, it has the potential to possess a wider margin of safety (8). This study aimed to investigate the effects of *in vitro* systematic review of the effects of garlic (*Allium sativum*) and its compounds on *Leishmania major*.

## Methods

### Information sources and search strategy

The current study was conducted according to the PRISMA guideline (9). Five English databases (PubMed, Google Scholar, Embase, Scopus, and Web of Science) were searched until the end of December 2022. No further search (e.g., from reference lists of the papers) was performed. The search was carried out in the English language. The syntax of all databases has been presented in Table 1. The articles were included in this study in which the effect of garlic extract or at least one of its compounds was examined on *Leishmania* parasites. The studies with poor methodology, inadequate information, inappropriate analysis, and confusing presentation were excluded from the current study. The search strategy flowchart is presented in Figure 1.

Table 1. The syntax for each of the databases used in the current systematic review

Database	Syntax
PubMed	(garlic[tiab] OR "allium sativum"[tiab] OR alliaceous[tiab] OR allicin[tiab] OR ajoene[tiab] OR "diallyl sulfide"[tiab] OR alliin[tiab] OR "diallyl disulfide"[tiab] OR "diallyl trisulfide"[tiab] OR "S-allyl-cysteine"[tiab]) AND (protozoa*[tiab] OR leishmani*[tiab])
Scopus	TITLE-ABS (garlic OR "allium sativum" OR alliaceous OR allicin OR ajoene OR "diallyl sulfide" OR alliin OR "diallyl disulfide" OR "diallyl trisulfide" OR "S-allyl-cysteine") AND TITLE-ABS(protozoa* OR leishmani*)
EMBASE	(garlic/ OR ('allium sativum' OR alliaceous OR allicin OR ajoene OR 'diallyl sulfide' OR alliin OR 'diallyl disulfide' OR 'diallyl trisulfide' OR 'S-allyl-cysteine':ti,ab) AND (protozoa*/ OR (leishmani*:ti,ab)
Web of Science	TS=garlic OR "allium sativum" OR alliaceous OR allicin OR ajoene OR "diallyl sulfide" OR alliin OR "diallyl disulfide" OR "diallyl trisulfide" OR "S-allyl-cysteine") AND (TS=protozoa* OR leishmani*)
Google Scholar	("garlic" OR "allium sativum" OR "alliaceous" OR "allicin" OR "ajoene" OR "diallyl sulfide" OR alliin OR "diallyl disulfide" OR "diallyl trisulfide" OR "S-allyl-cysteine") AND ("protozoa*" OR "leishmani*")

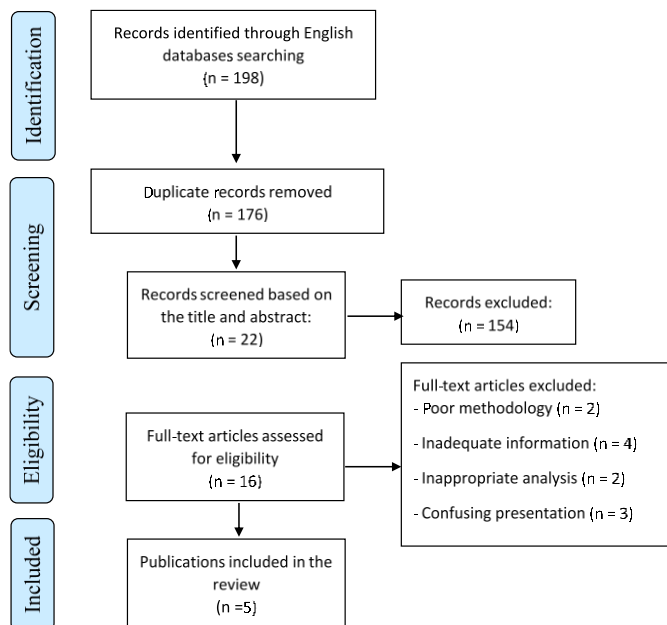


Figure 1. Flow diagram of the selected articles.

**Quality assessment**

The quality assessment tool was developed by the authors specifically in which eleven questions were answered as follows: 1) Is the use of garlic extract or the name of its active compound mentioned in the study? 2) Is the method of obtaining the extract or its active composition clearly stated? 3) Is it mentioned

that the parasite was isolated from the patient or is it a standard strain? 4) Is the method of passage and maintenance of the parasite mentioned? 5) Is the name of the parasite species mentioned? 6) Are the dilutions in a wide range used to determine the IC<sub>50</sub>, or are a few given dilutions used? 7) Is there a formula or software for determining IC<sub>50</sub>? 8) Is the name of the cell line for transforming the parasite into amastigote form stated? 9) Is the method of transforming the parasite into amastigote form clearly stated? 10) Is cell cytotoxicity 50 (IC<sub>50</sub>) and subsequent selectivity index (SI) calculated? 11) Is there an emphasis on a particular result, or the results are magnified? Each question is answered with “Yes”, “Unclear”, and “No”, and 1, 0.5, and 0 scores are given to each question, respectively. The articles that have scores ≥8 are evaluated for good quality and are included in the study.

**Data extraction**

Data were extracted from each of the selected articles by two independent authors (HK and RF). The interrater reliability was 100% and no divergence was observed. The extracted data were: the extracted data were as follows: author, garlic parts/components, solvent, cell type, treatment concentrations, examination times, measured objects (Examination method), positive control (Dose), important results, cytotoxicity for host cell, and effectiveness of garlic alone.

**Results**

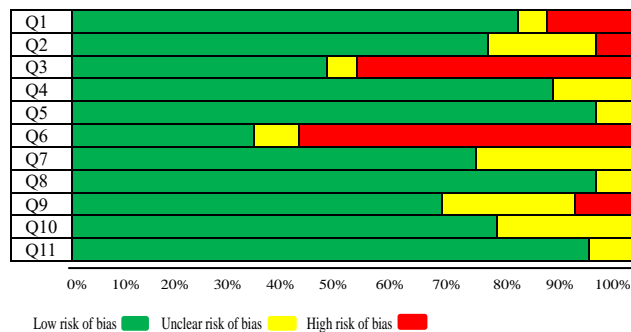
The selected studies are shown in Figure 1. Accordingly, 198 articles were selected in the search step, of which five eligible articles were included for examination. The most used solvent for the preparation of garlic extract was distilled water (60%, n = 3), followed by methanol (40%, n = 2). In these studies, the bulb of garlic is mostly used (80%, n = 4). Cell type of J774 was used in only one study (20%, n = 1), and murine peritoneal macrophages were used in the other studies (80%, n = 4). The effect of garlic on *Leishmania major* was strong (80%, n = 4), and had the greatest effect. More details are presented in Table 2.

Among the reviewed articles, five articles were evaluated as good based on quality, and the answers to the questions for all the articles are pooled and presented in Figure 2.

Table 2. In vitro *Leishmania major* effects of garlic (*Allium sativum*) or its compounds in the reviewed studies

Author	Garlic parts/Components	Solvent	Cell type	Treatment concentrations	Examination times	Measured objects (Examination method)	Positive control (Dose)	Important results <sup>*,**</sup>	Cytotoxicity for host cell	Effectiveness of garlic alone	Reference
Ahmadi-Renani. K (2015)	Garlic powder	Distilled water	Murine peritoneal macrophages	N/A	Hour 24	Nitric oxide (Griess assay)	Glucantime (Not specified)	**Aqueous extract of garlic reduced lesion size within 30 days; however, it was less effective than Glucantime.	Not evaluated	Weak	(10)
Kinuthia. GK (2014)	Bulb	Methanol	Murine peritoneal macrophages	125, 62.5, 31.25, 15.63 µg/ml	Day 5	Infection rate and multiplication index (Giemsa stain)	-Pentostam (50, 25, 12.5, 6.25) -Liposomal amphotericin B (50, 25, 12.5, 6.25)	**Methanolic extract of garlic was effective in inhibiting the survival of amastigote in peritoneal macrophages.	Low toxicity as compared to Pentostam and Liposomal amphotericin B	Strong	(11)
Gharavi. M (2011)	Bulb	Distilled water	J774	9.25, 18.5, 37, 74, 148 mg/ml	Hours 18, 24, and 48	- Amastigote mortality rate (MTT assay) - IL-10, IL-12 (ELISA)	Not specified	*The best antileishmanial effect was observed for 37 mg/ml concentration at hour 48. Additionally, an increase in IL-12 level was significant statistically.	Not evaluated	Strong	(12)
Wabwoba. BW (2010)	Bulb	Methanol	Macrophages	25,50,100µg/ml	Day 5	-Infection rate and multiplication index (Giemsa stain) - Nitric oxide (Griess assay)	-Pentostam (25, 50, 100 µg/ml) -Amphotericin B (25, 50, 100 µg/ml)	**The difference in infection rates between the extract and the standard drugs for <i>L. donovani</i> and <i>L. major</i> amastigotes was not statistically significant.	Very low toxicity as compared to Pentostam and Amphotericin B	Strong	(13)
Ghazanfari. T (2006)	Bulb	Distilled water	Murine peritoneal cells	N/A	Day 1 and 7	Infection rate and multiplication index (Giemsa stain)	Not specified	*The reduction of amastigote numbers in murine peritoneal macrophages after 7 days post-infection.	Not evaluated	Strong	(14)

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ELISA = Enzyme-Linked Immunosorbent Assay; NO = Nitric Oxide; N/A = Not Applicable



**Figure 2.** The pooled quality assessment of the five selected studies. Q1) Is the use of garlic extract or the name of its active compound mentioned in the study? Q2) Is the method of obtaining the extract or its active composition clearly stated? Q3) Is it mentioned that the parasite was isolated from the patient or is it a standard strain? Q4) Is the method of passage and maintenance of the parasite mentioned? Q5) Is the name of the parasite species mentioned? Q6) Are the dilutions in a wide range used to determine the IC50, or are a few given dilutions used? Q7) Is there a formula or software for determining IC50? Q8) Is the name of the cell line for transforming the parasite into amastigote form stated? Q9) Is the method of transforming the parasite into amastigote form clearly stated? Q10) Is cell cytotoxicity 50 (IC50) and subsequent selectivity index (SI) calculated? Q11) Is there an emphasis on a particular result, or are the results magnified?

## Discussion

Garlic belongs to the Liliaceae family and is systematically named *Allium sativum*. For a long time, the antibiotic properties of garlic have been recognized (15). The antibacterial effect of garlic on Gram-positive and negative bacteria has been well established for a long time. Several studies have been conducted on the antifungal properties of garlic (16).

In recent years, a few studies have been done on the effects of garlic extract on the treatment of cutaneous leishmaniasis. It has been proposed that garlic reduces the advancement of leishmaniasis by promoting the growth of the immune system. Identifying the specific component of garlic extract, that is effective in treating CL, is important for approving a cure protocol. Current drugs, such as glucantime, have several side effects and lead to direct cellular damage. If an herbal-based drug can eliminate *Leishmania* by enhancing the development of the immune system (17). So far, there is no appropriate vaccine or drug to prevent the parasite. Studies have shown that garlic extract or its active compounds can increase cellular immune responses, which play an important role in inhibiting the *Leishmania* parasite. This is associated with the activation of macrophages and increased interferon-gamma (IFN- $\gamma$ ) levels and nitric oxide (NO) production (18). NO plays an important role in controlling the *Leishmania* parasite, so the amount of NO produced has an inverse relationship with the size of the lesion caused by the *Leishmania* parasite, and hence it plays an important role in healing the *Leishmania* lesion (10). In addition to increasing the production of cytokines, garlic extract increases phagocytosis by macrophages, which is very important for exposing antigens to the immune system (14). Additionally, in line with recent studies, it appears that garlic extract can increase the IgG2a-to-IgG1 ratio, highlighting the increase in responses related to the cellular immune system. This system is capable of controlling infection when challenged with an ulcer-causing strain of *Leishmania* (19). The most effective response from the cellular immune system was observed at a concentration of 37 mg/mL of garlic extract within 48 hours (19). This concentration of garlic extract has a toxic effect on the parasite, which this is associated with an increase in the expression of IFN- $\gamma$  levels and inducible nitric oxide synthase (iNOS) genes (8). The results of another study showed that this concentration of garlic extract is significantly able to induce apoptosis of the parasite in the *in vitro* situation (20), and this is probably the mechanism of anti-Leishmanial action of garlic extract.

## Conclusion

According to the studies, the effect of different combinations of garlic on the *Leishmania* parasite has been shown; however, the exact mechanism of the anti-leishmanial effect of garlic has not been determined; thus, this issue needs further investigation. Further studies are necessary to investigate the combined effect of garlic extract and glucantime, which in case of acceptable results can be used to reduce the dose of glucantime and its side effects.

## Acknowledgement

None.

## Funding sources

The Vice-chancellor of Research at Golestan University of Medical Sciences funded this study (Grant No. 112524).

## Ethical statement

Not applicable.

## Conflicts of interest

The authors declare that there is no conflict of interest.

## Author contributions

Faramarz Koohsar designed the study and read title and abstract of the articles; Hamed Kalani searched the literature; Fatemeh Livani read title and abstract of the articles and extracted the data; Oghol Niaz Jorjani wrote the draft of the manuscript; Ganesh Yadagiri performed the quality assessment. Alireza Ahmadi selected the included articles; Roghiyeh Faridnia read the selected articles, performed the quality assessment, and finalized the manuscript. All authors read and approved the final manuscript.

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**How to Cite:**

Koohsar F, Kalani H, Livani F, Jorjani ON, Yadagiri G, Ahmadi A, et al. In vitro anti-leishmanial effects of garlic extract on *Leishmania major*: A Systematic Review. *Med Lab J.* 2024;18(3):21-4.