



زانكۆی سه‌لاحه‌دین-هه‌ولنیر

Salahaddin university-Erbil

# ***Allium sativum* (Garlic) effects upon *Entamoeba histolytica* parasite**

## **Research project**

**Submitted to the department of (Biology) in partial fulfillment  
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## Summary

*Entamoeba histolytica*, a protozoan parasite which came in second among parasitic infections that result in death after malaria, and is most commonly known for its symptoms of acute diarrhea, dysentery, amebic colitis, and amebic liver abscesses. Following infection, *E. histolytica* settles in the large intestine, causing intestinal amebiasis. primarily affects children in impoverished nations and is spread by contaminated food and water. The use of conventional antiparasitic drugs is often inhibited due to limited availability, side effects or parasite resistance. Medicinal plants can be used as alternatives or adjuncts to current antiparasitic therapies. In the present study underscores garlic's potential as a potent natural remedy for combating *Entamoeba histolytica* infections. It highlights the remarkable antiparasitic properties of allicin, which is one of the active principles of freshly crushed garlic effectively inhibits the growth and proliferation of *Entamoeba histolytica* trophozoites. And this underscores garlic's promising role in the development of alternative or adjunct therapies for addressing such parasitic infections.

## Introduction

Medicinal plants have served as man's basic health care for treating several diseases since ancient times. These plants are used in complementary and traditional medicine for the prevention, management, and treatment of several diseases of man (Yuan, H et al., 2016) One of the medicinal plants commonly exploited for its ethnomedicinal uses and diverse pharmacological health benefits and importance to man is *Allium sativum* commonly known as garlic which has generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Garlic is a perennial and herbal plant with bulbs containing different fibers divided and enclosed in a whitish skin. It is characterized by a strong pungent smell, and the bulbs are used for medicinal activities that contain active ingredients such as allicin and alliin used for various purposes, the Allicin is one of the active principles of freshly crushed garlic that have impressive growth inhibitory effect on *Entamoeba histolytica* (Okoroa et al., 2023)

Genus *Entamoeba* is divided into some species that have been described in humans. Among these, *Entamoeba histolytica* is the only pathogenic species (Lebbad M., 2010). *E. histolytica* is a protozoa cause Amoebiasis that is an infectious parasitic disease in humans and one of the deadliest parasites in the world with a strong pathogenic possibility. (Betanzos, Bañuelos and Orozco, 2019). Millions of people are infected with *E. histolytica*, and more than 55,000 people die each year globally due to amoebic colitis, a leading cause of diarrhea (Shirley D-AT et al., 2018). The life cycle of *E. histolytica* begins when infection cysts are ingested by swallowing polluted water or food and travel through the digestive system until they reach the large intestine, Infection occurs indirectly there are multiple stages and mechanisms for the onset of the disease (Kantor et al., 2018). *E. histolytica* can invade and destroy human tissues, using many molecules and biological properties related to virulence. The amoeba utilizes three main virulence factors: Gal/GalNAC lectin, ameba pore, and proteases (Babuta et al., 2020). There are two main types of amebiasis. First is Intestinal amebiasis which is the most common form of amebiasis and is characterized by symptoms such as diarrhea, abdominal pain, and weight loss. Second type is Extraintestinal amebiasis (Nasrallah et al., 2022). This occurs when the parasite spreads from the intestine to other parts of the body, such as the liver, lungs, or brain. It can cause symptoms such as fever, weight loss, and pain in the affected area. It's important to note that some individuals may be infected with the parasite but show no symptoms, making it possible to spread the infection to others without realizing it (Kantor et al., 2018).

### Life Cycle (Fig 1):

*E. histolytica* has a simple life cycle that comprises a resistant infectious cyst stage and a mobile disease-causing trophozoite (Lebbad M., 2010) passes its life cycle in one host, humans. The infection is transmitted by swallowing cysts in contaminated food or water, which pass through the stomach without damage due to the cyst wall, is resistant to gastric juice. it reaches the ileum where its excystation occurs, the cyst wall shattered due of the alkaline medium of the caecum or lower part of the ileum. This results in the metacystic where the mitotic divided produces it causes the nucleus division first, followed by the cytoplasm then there will be eight small amoebae. Each amoeba can evolve into trophozoites. There are small size trophozoites that feed on bacteria. The large ones, feed on erythrocytes and attack the epithelial cells of the mucosal epithelium of the large intestine, causing amoebiasis. Some develop into cysts in the bowel lumen that passed into the feces to repeat the cycle (Al-Ahzzaa, G.R. et al., 2022). *E. histolytica* can be transmitted by asymptomatic hosts infected with cysts and patients with intestinal amoebiasis, who are the natural transmitters.

The Entamoeba cysts are resistant to desiccation in soil and can survive in humid environments and food or water for several weeks, and susceptible hosts exposed to the infection sources ingest the cysts, which then undergo excystation during their pass through the gastrointestinal tract (Morán, P et al., 2023). *E. histolytica* causes significant damage to the intestine, primarily characterized by mucosal destruction, inflammation, and the development of dysentery (Shirley et al., 2018). Upon infection, the trophozoites of *E. histolytica* adhere to the intestinal mucosa, where they release proteolytic enzymes and toxins (Nasrallah et al., 2022). These enzymes degrade the intestinal lining, leading to tissue damage and the formation of ulcers, particularly in the colon (Al-Ahzzaa, 2022). This invasion triggers an inflammatory response, resulting in inflammation and swelling of the intestinal mucosa. The inflammatory process, combined with tissue damage, leads to symptoms such as bloody diarrhea, abdominal pain, and cramping, which are characteristic of dysentery (Azadbakht et al., 2020).

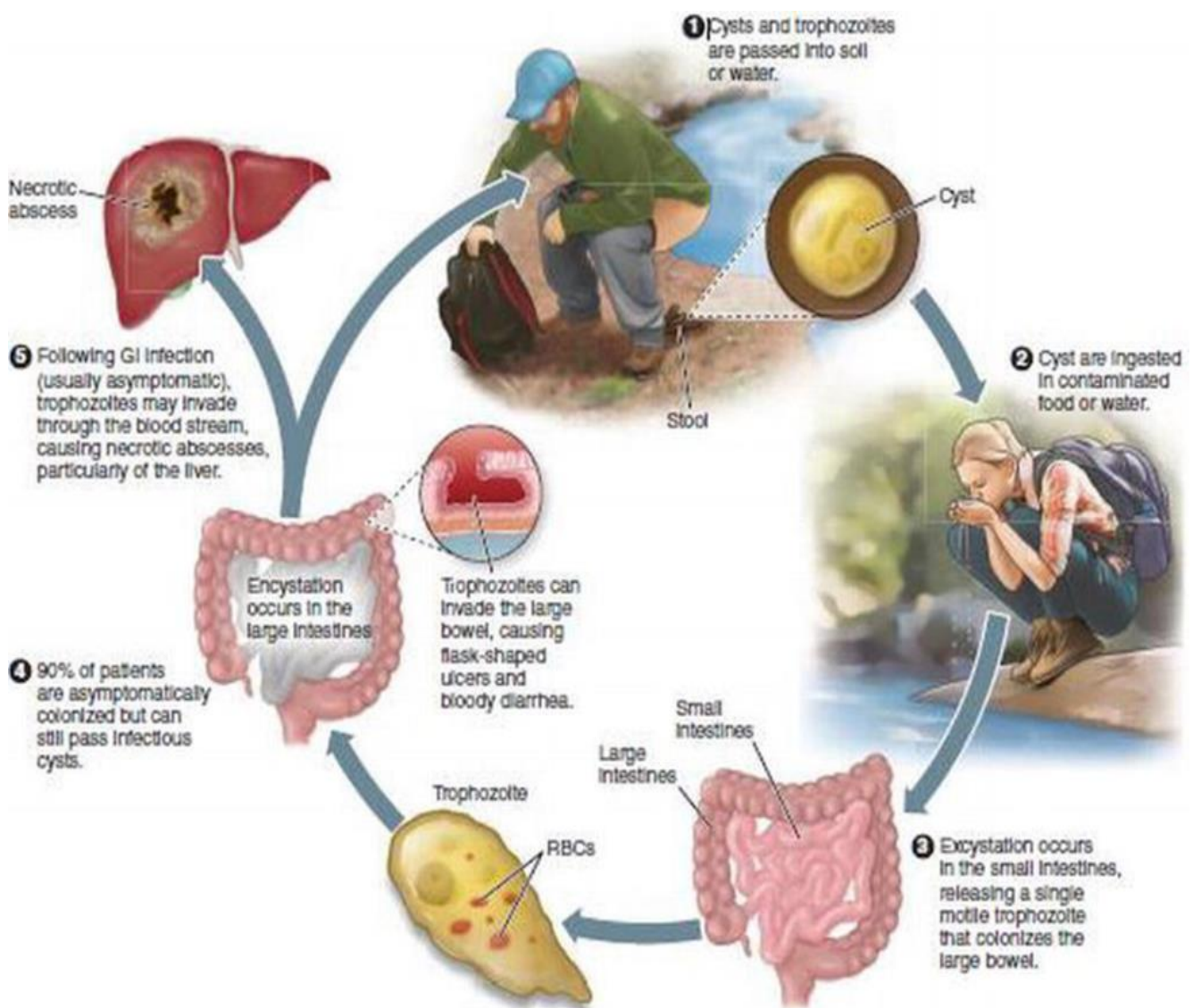


Fig1: The life cycle of Entamoeba histolytica.

## Treatments

some studies have been done about Garlic that has been widely recognized for its antimicrobial properties such as antibacterial, antifungal, antiviral, and antiparasitic effects, and several studies have shown that the extract of garlic is effective toward the host of Protozoa including, *E. histolytica* that cause amebiasis is an intestinal illness (Dissanayake, K.G.C et al., 2021). Researchers are investigating at plants and natural alternatives that can be used for the treatment of this disease due to the occurrence of bad side effects and increasing resistance to the synthetic pharmaceuticals that have been recommended for the disease. According to the early studies, garlic can be used as a treatment for this disease (Harris, J.C., et al., 2001).

The mechanism by which garlic and its constituents exert their antiparasitic effects against *Entamoeba histolytica* likely involves multiple pathways, including:

### 1. Inhibition of *Entamoeba histolytica* Growth

Garlic contains bioactive compounds such as allicin and ajoene, which exhibit potent antiparasitic properties. Studies (Azadbakht et al., 2020; Ranasinghe et al., 2023) have shown that garlic extracts inhibit the growth and proliferation of *Entamoeba histolytica* trophozoites. Allicin, in particular, has been identified as a key component responsible for this inhibitory effect. Allicin interferes with essential metabolic pathways within the parasite, disrupting its cellular functions and leading to growth suppression and eventual elimination.

➤ **Primary Compound:** Allicin is a sulfur-containing compound in garlic that is formed when garlic is crushed or chopped, coming from the precursor compound alliin. This compound has been widely studied for its antimicrobial properties.

**Action:** Allicin exerts its antiparasitic effect on *Entamoeba histolytica* by penetrating the cell membrane of the amoeba and interacting with its internal components, leading to disruption of vital processes. It is known to inhibit various enzymes crucial for the survival of the parasites, impairing their ability to metabolize and reproduce (Azadbakht et al., 2020; Ranasinghe et al., 2023).

➤ **Secondary Compound:** Ajoene is another sulfur-containing compound found in garlic, known for its antiparasitic, antifungal, and antibacterial properties.

**Mechanism:** Ajoene interferes with the biochemical pathways of *E. histolytica*, particularly those involved in the formation of the cyst wall, a crucial factor for its survival and infectivity. By disrupting these pathways, ajoene limits the parasite's ability to form protective cysts, making it more vulnerable to the host's immune system (Ohanu and Inyang-Etoh, 2015).

## 2. Disruption of Parasitic Enzymes

### ➤ Allicin's Role in Enzyme Inhibition

**Allicin Mechanism:** Allicin is believed to interact directly with the thiol groups of enzymes, which are essential for the amoeba's metabolism and survival. This interaction leads to the inactivation of these enzymes, disrupting processes like energy production, nutrient uptake, and detoxification within the parasite.

**Enzymatic Targets:** Key enzymatic systems within *E. histolytica*, such as those involved in glycolysis and cysteine protease activity, are particularly vulnerable to allicin's action. By inhibiting these enzymes, allicin starves the amoeba of essential nutrients and impairs its ability to invade host tissues (Azadbakht et al., 2020; Ranasinghe et al., 2023).

## 3. Immunomodulatory Effects

Garlic exerts immunomodulatory effects that enhance the host's immune response against *Entamoeba histolytica* infection. Studies (Mohamed Fathy Abouel-Nour et al., 2017; Ohanu and Inyang-Etoh, 2015) suggest that garlic stimulates the activity of immune cells, such as macrophages and natural killer cells, and enhances cytokine production. This immune activation promotes the clearance of the parasite and contributes to the resolution of inflammation associated with amoebic dysentery.

## 4. Anti-inflammatory Properties

In addition to its direct antiparasitic and immunomodulatory effects, garlic possesses anti-inflammatory properties (Hussein et al., 2023; Dissanayake et al., 2020). By reducing inflammation in the gut, garlic may alleviate symptoms such as abdominal pain and diarrhea, enhancing overall therapeutic outcomes in patients with amoebic dysentery. Garlic's ability to modulate inflammatory pathways contributes to its efficacy in managing the symptoms of the infection.

## 5. Antioxidant Properties and Enzymatic Protection

**Oxidative Stress Induction:** Garlic's antioxidant properties might paradoxically induce oxidative stress within *E. histolytica* by depleting its antioxidant defenses, leading to damage to its own enzymes and structural components. This oxidative stress can inhibit the parasite's growth and viability (Delshad et al., 2019).

To harness the antimicrobial benefits of garlic, the recommended daily intake varies based on individual preferences and health considerations, there is no standardized daily intake for garlic in the treatment of amebic dysentery, some studies suggest a range of dosages based on its antimicrobial potency:

1. **Fresh Garlic:** Consuming fresh garlic cloves is a common way to harness its antimicrobial benefits. Eating 2 to 3 fresh garlic cloves per day may provide health benefits for some individuals. However, it's essential to consider personal taste preferences and digestive tolerance, as raw garlic can be strong and may cause gastrointestinal discomfort in some people (Ranasinghe et al., 2023)
2. **Garlic Supplements:** Garlic supplements, such as garlic capsules or tablets, are available and often standardized for allicin content. Dosages may vary depending on the concentration of active compounds in the supplement. It's advisable to follow the manufacturer's instructions or consult with a healthcare professional for personalized recommendations (Ohanu and Inyang-Etoh, 2015).
3. **Cooked Garlic:** Cooking garlic can help to mellow its flavor while retaining some of its antimicrobial properties. Incorporating cooked garlic into meals, such as stir-fries, soups, or roasted vegetables, is a flavorful way to enjoy its benefits (Mohamed Fathy Abouel-Nour et al., 2017)
4. **Garlic Extracts:** Liquid garlic extracts (Allicin) are also available and may be used as a dietary supplement. recommended a daily intake of 600 to 1200 milligrams of garlic extract for its antimicrobial effects against *E. histolytica* (Hussein et al., 2023).

**Scientific Evidence** on the Efficacy of Garlic against *E. histolytica* Several studies have investigated the effects of garlic on *Entamoeba histolytica* and its potential to treat amoebic dysentery such as:

- In vitro research conducted by (Ranasinghe et al., 2023) and published in the *Journal of Ethnopharmacology* revealed the significant amoebicidal activity of garlic extract, particularly allicin, the active component of garlic, against *E. histolytica*. This suggests that garlic compounds have the capability to directly eliminate the parasite or hinder its growth."
- **Animal Studies:** In a comprehensive exploration featured in the *Veterinary Science and Development* journal by (Ohanu and Inyang-Etoh, 2015) garlic was found not only reduced the number of parasites within the intestines of infected animals but also to alleviate the symptoms of amoebiasis, including mitigating intestinal damage.
- **Clinical Relevance:** An investigation in the *Journal of Pharmacognosy* by (Azadbakht et al., 2020) highlighted the potential for garlic to be used as a supplementary treatment alongside conventional amoebicidal drugs, potentially enhancing their effectiveness and reducing side effects.

## **Conclusion**

The examination of garlic (*Allium sativum*) and its effects on *Entamoeba histolytica* has illuminated a promising avenue for the treatment of amoebiasis, underscoring the potential of natural remedies in addressing parasitic infections. The active compound in garlic, allicin, emerges as a potent antiparasitic agent, offering a multifaceted approach to inhibiting the growth and viability of *E. histolytica*. The mechanisms through which garlic exerts its effects are believed to involve the disruption of essential biochemical pathways within the parasite, induction of oxidative stress, and impairment of the parasite's ability to invade host tissues. As research evolves, the potential of garlic in combating parasitic infections increasingly becomes evident, potentially leading to safer, more effective treatments for amoebic dysentery and other parasitic diseases.



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