



## Review Article

### Plant Extracts as Therapeutic Mediators Against the Infections of *Entamoeba histolytica*: A Review

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#### ABSTRACT

*Entamoeba histolytica* is a protozoan parasite that causes amoebiasis, a disease that continues to pose a global health challenge, particularly in developing countries. Although metronidazole and related nitroimidazole drugs remain the standard treatment, their adverse effects, incomplete cyst eradication, and emerging resistance have driven the search for alternative therapies. Recently, plant-derived compounds have received attention for their accessibility, safety, and broad pharmacological activities. This review summarizes current evidence on the anti-amoebic potential of several medicinal plants, including *Tamarindus indica*, *Camellia sinensis*, *Punica granatum*, *Aloe vera*, *Nigella sativa*, *Ruta graveolens*, and others. These plants exhibit diverse mechanisms of action including disruption of parasite membranes, inhibition of protein synthesis, interference with DNA replication, and regulation of oxidative stress. Both studies (in vitro and in vivo) demonstrated that plant extracts not only reduce the viability of *E. histolytica* trophozoites and cyst viability but also aid tissue repair and enhance antioxidant defense. Collectively, the reviewed evidence supports the promising role of phytochemicals as natural, cost-effective, and complementary therapies for amoebiasis, warranting further experimental and clinical validation.

**Keywords:** *Entamoeba histolytica*, Herbal medicine, Medicinal plants and Plant extracts.

## INTRODUCTION

*Entamoeba histolytica* is an anaerobic, protozoan parasite that causes either amebic dysentery or amoebiasis. The majority of infections do not cause any symptoms; however, invasive intestinal disease can occur and cause cramping, abdominal pain, bloody or watery diarrhea, and weeks-long weight loss (Lucius, *et al.*, 2017). Disseminated extraintestinal main infestations have been documented, including liver abscess, pneumonia, purulent pericarditis, and even cerebral amoebiasis (Haque *et al.*, 2003). Up to 50 million people are thought to be infected with *E. histolytica* globally, mostly in underdeveloped nations, and every year, the pathogen kills over 100,000 individuals worldwide (Ximénez *et al.*, 2010).

Typically, transmission happens when contaminated food or water is consumed (due to cysts being passed in the stool) and can even occur through fecal-oral transmission within families and during homosexual intercourse (Salit *et al.*, 2009).

Current treatment for *E. histolytica* infection mainly relies on nitroimidazole derivatives such as metronidazole and tinidazole. Despite their effectiveness, drugs have several challenges: - side effects (nausea, metallic taste, and neurotoxicity), which limit patient compliance; requiring additional luminal agents; and emerging reports of drug resistance that threaten long-term efficacy (Hernandez *et al.*, 2019). Due to the parasite's significance in medicine, efforts have been made to gain a deeper understanding of the parasite, its disease and treatment. Plant extracts are becoming increasingly popular as treatments for parasitic diseases, as some extracts contain compounds that can inhibit parasite growth and aid in the removal and treatment of intestinal ulcers (Swiderski *et al.*, 2007). The parasite's life cycle consists of several successive stages of *Entamoeba histolytica*. These stages include the trophozoite, precyst, cyst, post cyst, and postcyst trophozoite stages (El-Dib, 2017).

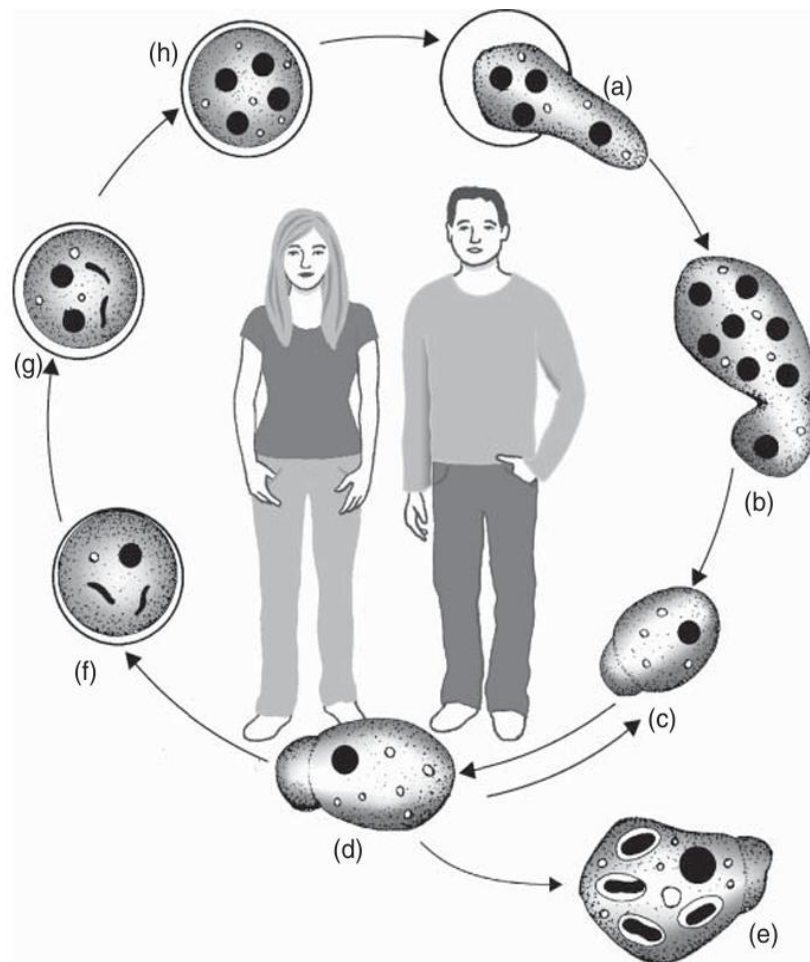
The trophozoite and cyst stages are described below and are considered the life cycle's main phases:

### 1) Stage of trophozoites

They are in an actively developing stage referred as vegetative cells (Gunn and Pitt, 2012). The trophozoites can survive for only 20 minutes outside the organism, making it impossible for them to spread from one host to another. *Entamoeba histolytica* trophozoites are also extremely sensitive to pH shifts and dehydration (Wells and Arguedas, 2004). They inhabit the rectum and large intestine of humans and other primates, as well as occasionally the lower small intestine (Bogitsh *et al.*, 2013).

### 2) Stage of Cyst:

Infection usually occurs through ingestion of food contaminated with tetranucleated cysts and less often through direct contact. Each cyst releases eight motile trophozoites after excystation in the small intestine. Cysts can survive outside the body for several weeks and cause infection. Thus, cysts excreted in the stool can transmit amoebiasis to others (Stanley, 2003). Infected individuals are usually asymptomatic or experience only mild symptoms of *E. histolytica* as shown in Fig. (1). The infection can mimic inflammatory bowel disease and include symptoms such as abdominal cramping, diarrhea, constipation, and weight loss. Amebic dysentery, amebic colitis, and amebic liver abscesses can result from severe infections (Mathison and Pritt, 2022).

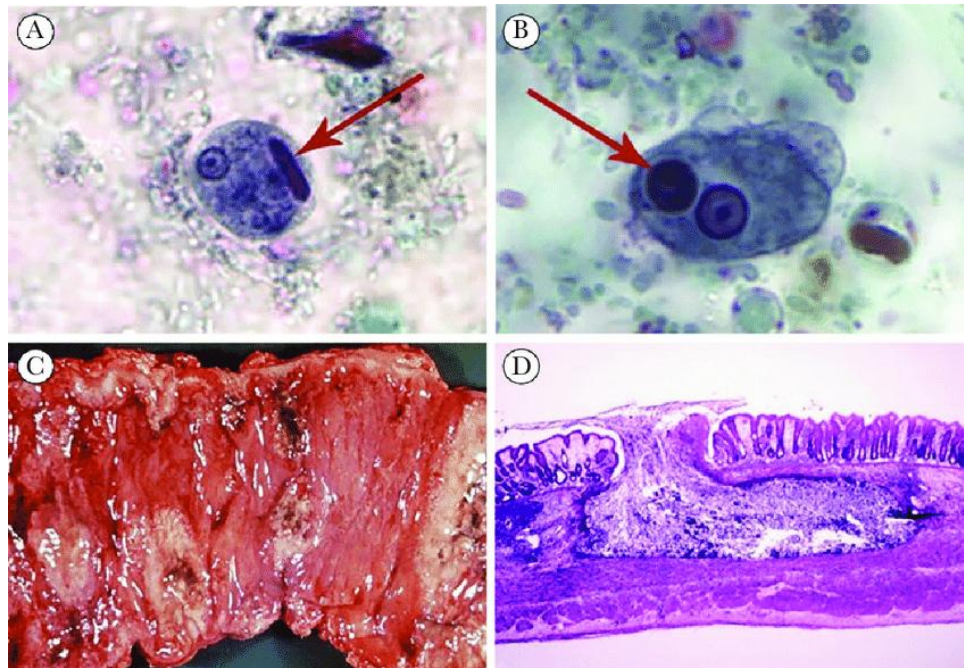


**Fig. 1: Life cycle of *E. histolytica*: (a) Hatching of an extranuclear amoeba. (b) Octa nuclear amoeba with an amoeba being pinched off. (c) Amoebae. (d) Trophozoite. (e) Tissue-invading trophozoite with ingested erythrocytes. (f) Mononuclear cyst. (g) Binuclear cyst. (h) Tetranuclear cyst. (Based on several authors (Lucius *et al.*, 2017)).**

The Centers for Disease Control and Prevention (CDC) estimates that between 10% and 20% of individuals who contract *E. histolytica* develop illness. In the United States, amebic colitis is more common than amebic liver abscesses and amebiasis, which are observed primarily in immigrants and individuals who travel to and from endemic areas. Symptoms of amebic colitis can include abdominal cramps, pain, and bloody diarrhea. Peritonitis and intestinal perforation may occur when *Entamoeba histolytica* trophozoites cause ulcers by invading the gut mucosa (Fleming *et al.*, 2015).

Amebic liver abscesses may arise from trophozoites that penetrate the intestinal wall and spread into the bloodstream as reported in approximately 5% of patients with intestinal amebiasis. Patients may experience right upper quadrant pain and fever. In rare cases, Amebic abscesses can spread to the lungs, brain, skin, and other parts of the body (Mathison *et al.*, 2022).

A diagnosis of intestinal amebiasis can be confirmed by identifying the pathogen in stool samples, as shown in Fig. (2). However, trophozoites found outside the intestine or those containing erythrocytes indicate invasive infection with *E. histolytica* (Shirley *et al.*, 2018).



**Fig. 2: The parasite forms: A: cyst of *E. histolytica* isolated from stool sample and stained with Gimza. B: trophozoite of *E. histolytica* isolated from stool sample and stained with Gimza. C: a gross visible of amebic ulcer. D: Cross section of amebic ulcer stained with Gimza (Shirley *et al.*, 2018).**

Metronidazole, a common antiparasitic and anti aerobic medication, is the traditional treatment for *E. histolytica* infections. It works well against trophozoites; however, it is often insufficient to eliminate intestinal cysts (Freeman *et al.*, 1997). Like all drugs, nausea, diarrhea, appetite loss and a metallic taste in the mouth is among the side effects of metronidazole (Hernandez *et al.*, 2019)

Traditionally, *E. histolytica* is treated with drugs such as metronidazole and tinidazole, however, these medications are often associated with undesirable side effects, including nausea, neurotoxicity, and the potential development of drug resistance. This drug has sparked growing interest in natural alternatives such as plant extracts, which are rich in bioactive compounds with anti-amoebic properties (Shirley *et al.*, 2018). Due to their low cost and perceived safety, plants are a popular option in underdeveloped nations. Over 30% of global medicine sales are plant-based products, and the medicinal qualities of whole plants and their active components have been crucial in maintaining health (Carvalho *et al.*, 2019).

These plants produce a wide variety of secondary metabolites that have been used to address various diseases and pains (Shahrajabian *et al.*, 2019). One of the most explored areas and the subject of numerous thorough screening procedures is the use of the biological activity of various medicinal plants or their active ingredients to treat illness (Barati *et al.*, 2017). Pharmaceutical companies are increasingly using natural substances to combat infectious diseases caused by protozoan parasites (Newman and Cragg, 2016).

Plant extracts used for the treatment of infections caused by *E. histolytica* include *Tamarindus indica*, which has been used in trials reported by Mehdi *et al.*, (2019) they demonstrated that rats infected with *E. histolytica* when treated with *T. indica* extract (both ethanolic and aqueous) the *E. histolytica* can be killed by *T. indica* because it includes a variety of vacuable substances, including flavonoids, alkaloids, tannins, and phenols. The *E. histolytica* cell membrane was disrupted by the plant extracts alkaloids, resulting in the expulsion of the cell's contents as lipids and proteins, additionally, the *T. indica* extract interferes with *E. histolytica*'s DNA. *E. histolytica* dies as a result of alkaloids' actions. Also, the transfer of proteins and enzymes across cell membranes is inhibited

by tannins, another constituent of *T. indica* extract. By working together, tannins and phenols block enzymes, leading to protein precipitation by forming hydrogen bonds between proteins, hydroxyl groups and nitrogen molecules, an in vivo study showed that treatment of rats with 500 mg/kg of the extract results in fewer *E. histolytica*. In addition, the extract aids in intestinal tissue restoration, this phenomenon may be observed in the histological section of the rats' colon, where administration of *T. indica* extracts leads to a slight rise in the number of opacities, which show a diffuse increase in the follicle. There was also a greater increase in goblet cells associated with a more immune mucosa and production of antimicrobial antibodies in the colon. These two incidents established that extract is a reliable indicator of successful recovery from an *E. histolytica* infection (Mehdi *et al.*, 2019).

Another herbal compound used to fight *E. histolytica* is *Camellia sinensis*. Shaker *et al.*, (2018) found that the extract of *C. sinensis* can have anti-*E. histolytica* properties. Some of its beneficial constituents are resins, glycosides, flavonoids, phenols, tannins, alkaloids, and saponins, all of which are toxic *E. histolytica*. All these secondary compounds are capable of effectively combating *E. histolytica*.

High-flavonoid plants, including cacao, grapes, and *Geranium mexicanum* are known to have anti-amebic properties. These include the effects of alterations in DNA replication, enzymes and cytoskeletal proteins associated with *E. histolytica*'s glycolytic metabolism, which can serve as targets for molecules. These changes suggest the loss of factors that make the amoeba virulent (Bolaños *et al.*, 2014; Bolaños *et al.*, 2015).

It is well recognized that the inflammatory response may promote the growth of intestinal and hepatic amoebas. High levels of oxidative stress, ROS, and hypochlorous acid are present in this environment. These oxidant metabolites all damage tissue. Flavonoids can modulate the antioxidant enzymes CAT, SOD, and GPx, which activate inflammation. Flavonoids can also eliminate these free radicals by acting as scavengers (Rahimova, 2021). It is difficult to rule out the possibility that flavonoids kill amoebas by direct interaction given the numerous advantages and potential for treating of *E. histolytica* infection. Examining these natural substances in basic research to establish their doses is essential. Also routes of administration, bioavailability, and (Glycosidation, glucuronidation, sulfation, and O-methylation) are examples of metabolic biotransformation. With all of these points in mind, flavonoids make excellent substitutes (Martínez-Castillo *et al.*, 2018).

Resveratrol, a polyphenol, demonstrated anti-amebic activity in vivo and in vitro. It induces apoptosis in *E. histolytica* in vitro by stimulating cell growth arrest, ROS generation, membrane lipid damage, elevated intracellular  $Ca^{2+}$ , and decreased superoxide dismutase activity. Furthermore, in hamsters with ALA, oral gavage of 30 mg/kg resveratrol for 2 or 10 days prevented trophozoite-induced liver damage, while animals that were not pretreated developed severe ALA (80% of the entire liver) (Martínez-Castillo *et al.*, 2018).

Histopathological examination demonstrated a gross difference between untreated hamsters, which showed liver necrosis, and that were treated with resveratrol. It revealed a retraction of the granulomatous reaction in the healthy liver parenchyma and no liver necrosis. In light of these findings, the authors proposed resveratrol as an alternative treatment for amebiasis (Pais-Morales *et al.*, 2016). All of resveratrol is a polyphenol. However, further studies are required to demonstrate its possible anti-amebic efficacy. In addition to flavonoids, they could be used for supplementation or as adjunct treatments (Martínez-Castillo *et al.*, 2018).

One such species is the pomegranate, pericarpium granatum (*Punica granatum* L.), a member of the family Punicaceae. This fruit is the oldest cultivated species, which has been known to humans for a long time and is cited in the Holy Quran and other religious scriptures (Heber *et al.*, 2006).

*P. granatum* also has a variety of vital medicinal properties and nutrients, including alkaloids present in the fruit peel, tree bark, and roots.

*Pomegranate* seeds and juice contain poly phenols with antioxidant properties. In addition to the aforementioned, hydroxybenzoic acid is the main available form in seeds of pomegranate and as for flesh, it is a good source of potassium, vitamin K and vitamin C, among other substances, sterols, tocopherols, triterpenes, isoflavones, phenyl aliphatic glycosides, conjugated and non-conjugated

fatty acids, and lignins (Prakash and Prakash, 2011). *P. granatum* can be used in the treatment of kidney stones, painful urination, diarrhea, dysentery, anemia, hemorrhoids, and other conditions (Daabo, 2022). The extract of *P. granatum* prevent the formation of biofilms in foodborne pathogenic bacteria (Jam *et al.*, 2022).

As cited by Dahham *et al.*, (2010), pomegranates possess antibacterial and antifungal properties. To study the anti-protozoan activity of extracts of various plant species. In an in vitro study of 26 plant species commonly used in traditional medicine for digestive disorders, *P. granatum* was one of the plants tested, and exhibited significant activity against *E. histolytica*.

Anti-amoebic potential and a synergistic effect with metronidazole of *P. granatum* oils and juice and anti-amoebic effect of alcoholic extract of *P. granatum* pulp against *E. histolytica* (Nagappan, 2012). It is a putative medicinal herb, and traditional healers worldwide have extensively explored its therapeutic potential. These herbs have been traditionally used by the Kipsigis people in Kenya to treat diarrhea and malaria (Nagappan, 2012). Ahmed *et al.*, (2009) examined the effects of *Hibiscus sabdariffa* and *Glycyrrhiza glabra*, two aqueous plant extracts on the development of the isolated parasite *E. histolytica* from stool samples grown in vitro in two media. Liver infusion agar medium (LIAM) and Locke-egg medium (LEM). Aqueous extracts from both *H. sabdariffa* and *G. glabra* were able to reduce parasite size in the LEM medium. The rate of *G. glabra* reproduction in the two-culture media did not differ significantly, while the third concentration of *H. sabdariffa* (19.71 mg/ml) was successful in lowering the reproduction rate in LEM and LIAM media to 57.6 and 83.6%, respectively. *Ruta graveolens* L., commonly known by most as Rue (Jinous and Roghaieh, 2012), is a Mediterranean herb belonging to the *Rutaceae* family used as a spice in Jordan (Oran, 2014). In traditional medicine, *R. graveolens* is used to cure a wide range of conditions, such as ulcers, parasite infections, menstrual issues, reproductive illnesses and injuries as well as promoting conception.

According to Carvalho *et al.*, (2019), *R. graveolens* has schistosomicidal and anticancer properties. Extracts and essential oils from *R. graveolens* have been shown to have antibacterial and antifungal properties (Amabye, 2015). When various doses of *R. graveolens* ethanolic extract were employed, there was no detrimental impact on liver structure, according to Al Qaisi *et al.*, (2021).

*Peganum harmala* L., also known as Harmal or Harjal belongs to the Zygophyllaceae family and is used in traditional medical treatments. (Alfarrayeh *et al.*, 2022).

Phytochemicals isolated from *P. harmala* have been discovered to have anticancer, antifungal, insecticidal, antibacterial, and anti-leishmanial effects (Sohrabi *et al.*, 2018). Another plant in the Cucurbitaceae family is *Citrullus colocynthis* L. (Oran, 2014), which has been used as an insecticide, anti-diabetic, and anti-parasitic medication. It is distinguished by diverse elemental composition, including mucilage, polysaccharides, gums, phenols, and tannins (Al Qaisi *et al.*, 2022). The results of the Alzamilie and Alkhanaq (2024) study showed that the alcoholic and aqueous extracts of *Aloe vera* decreased the number of *E. histolytica* cysts with the alcoholic extract outperforming the aqueous extract in terms of *A. vera* effectiveness at a concentration of 50 mg/L. The biological effects of *A. vera* can be attributed to its secondary metabolites, which include flavonoids, anthraquinones, tannins, coumarins, terpenes, saponins, and alkaloids. Additionally, phenolic compounds found in *A. vera* exhibit anti-inflammatory, anti-oxidant, and anti-parasitic properties (Pegu and Sharma, 2019).

The oil of *Nigella sativa* efficacy in treating amoebic dysentery compared to the medication metronidazole. *N. sativa* oil contains various alkaloids that prevent *E. histolytica* trophozoite protein synthesis, which may affect the trophozoite. In addition, *N. sativa* seeds contain phenolic compounds, which are wellknown for their various physiological properties, such as anti-inflammatory, anti-parasitic, and anti-carcinogenic effects (Ma and Kinner, 2002). The oil capacity of *N. sativa* to eradicate *E. histolytica* trophozoites cultured in media negatively affect bacteria in amoeba xeric culture, as *E. histolytica* exhibits greater virulence when it associates with appropriate bacterial cells (Al-jubory, 2005). The findings of this investigation agree with those of Adagu *et al.*, (2002), who found that metronidazole killed the *E. histolytica* trophozoite by altering the amoeba's protoplasmic organelles and inhibitory protein.

Some edible plants used as antiamebic agents include Pumpkin (*Cucurbeita pepo*), Garlic (*Allium sativum*), Pawpaw (*Carica papaya*) and Guava (*Psidium guajava*) (Kaiatyet *et al.*, 2021).

Methanolic extract of *Argemone mexicana* leaves showed growth inhibitory activity against trophozoites of *E. histolytica* *in vitro*. HPLC examination of the extract's fractions revealed increased amoebicidal activity, suggesting the presence of jatrorrhizine and berberine alkaloids (Elizondo-Luevano *et al.*, 2018).

Watery or bloody diarrhea caused by an *E. histolytica* infection is known as amoebiasis diarrhea (Mohammed, 2016). *E. histolytica* causes an increase in levels of the liver enzymes ALT and AST, as well as various tissue alterations, which were discovered by Khaleel *et al.*, (2024), who showed that 90% of patients with dysentery and an *E. histolytica* infection had elevated liver enzyme levels.

## CONCLUSIONS

This review shows that plant extracts are a safe and effective alternative to conventional anti-amoebic drugs. Many of these medicinal plants contain important anti-parasitic, antioxidant and anti-inflammatory agents whose actions can inhibit the growth of *Entamoeba histolytica* and promote the recovery of intestinal tissues. These effects are primarily mediated by compounds such as flavonoids, alkaloids, tannins, and phenolics. Since the emergence of drug resistance and adverse side effects associated with conventional medicines is at an alarming stage, herbal medicines can potentially play an important role as a sustainable source for new therapeutic molecules. Nevertheless, clinical trials and standardized *in vivo* research are required to elucidate appropriate dosing, toxicity profiles, and modes of action of these plant-derived agents. In summary, our findings suggest that the addition of phytotherapy to amoebiasis treatment may enable an effective, safe and inexpensive treatment for amoebiasis which can be developed in the future.

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## المستخلصات النباتية كوسائط علاجية ضد عدوى الاميبا الحالة للنسيج: مقالة معالجة

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### الملخص

الاميبا الحالة للنسيج هو طفيلي ابتدائي مسؤول عن احداث داء الاميبا، وهو مرض لا يزال يُشكل تحديًا صحيًا عالميًا كبيرًا، لا سيما في الدول النامية. على الرغم من أن الميترونيدازول وأدوية النيتروإيميدازول المشابهة له لا تزال تُمثل العلاج المُعتمد، إلا أن آثاره الجانبية، وعدم قدرته على القضاء على طور الكيس، وظهور مقاومة لها، دفعت إلى البحث عن علاجات بديلة. في الآونة الأخيرة، حظيت المركبات المشتقة من النباتات باهتمام كبير نظرًا لسهولة الحصول عليها وسلامتها وفعاليتها الدوائية الواسعة. تلخص هذه المقالة الأدلة الحالية على قدرة الاميبا المضادة للعديد من النباتات الطبية، بما في ذلك التمر الهندي، والكاميليا الصينية، والرمان، والصبار، وحب البركة، والسذاب، وغيرها. تتميز هذه النباتات بآليات تأثير متنوعة مثل إتلاف أغشية الطفيليات، وتثبيط تخليق البروتين، والتدخل في تضاعف الحامض النووي، وتحويل الإجهاد التأكسدي. لقد أثبتت الدراسات التي أُجريت داخل وخارج الجسم الحي أن المستخلصات النباتية لا تقلل من حيوية الاميبا الحالة للنسيج بشكلها طور الكيس والطور الفعال وإنما تساعد أيضًا في إصلاح الأنسجة وتعزيز دفاعات مضادات الأكسدة. وتدعم الأدلة التي تمت مراجعتها، مجتمعةً، الدور الواعد للمواد الكيميائية النباتية كعلاجات طبيعية وفعالة من حيث التكلفة وعلاجات مكمل لداء الأميبا، مما يستدعي المزيد من التحقق التجريبي والسريري.

**الكلمات الدالة:** الاميبا الحالة للنسيج، المستخلصات النباتية، الطب الشعبي والنباتات الطبية.