

Mobilization of Natural Substances Against *Blastocystis* sp. With a Focus on Medicinal Plants: A Mini-Review



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Abstract

Blastocystis is known as a mysterious polymorphic single-celled protozoan that can infect humans and various animals. Despite its worldwide isolation from people with gastrointestinal complications, its exact pathogenicity is in an aura of ambiguity. However, drug treatments, and the most prominent of them, metronidazole (MTZ), are prescribed as the first line of treatment. Various clinical complications, such as neurotoxic symptoms, kidney and heart failure, and the like, have been defined for MTZ. A large body of research has been performed to find a safer alternative with a preference for materials of a natural nature and is still ongoing. Recently, interest has grown in the potential use of plant derivatives for antiparasitic purposes. Among them, some plants have shown significant effects on different species and subtypes of *Blastocystis* in *in vitro* and *in vivo* conditions. The present mini-review has attempted to draw a more comprehensive picture of different forms of anti-*Blastocystis* plants from previous studies and provide an idea-generating content for future studies.

Keywords: *Blastocystis* sp., Anti-parasitic, Plants, Natural substances, Review

Background

Blastocystis sp. is a polymorphic, single-celled parasitic protozoan that can be colonized in the human colon, similar to many other animal hosts.¹ Different subtypes have been defined for *Blastocystis* (1–38), of which 1–9 and 12 have been isolated from humans, and the most abundant subtypes are ST1 and ST3; of the 14 subtypes, which have been reported in humans, ST1 to ST4 are the most common, while reports of ST5–ST10, ST12, ST14, ST16, and ST23 in humans range from relatively uncommon to rare.² *Blastocystis* sp. is a mysterious parasite; the pathogenicity of which, despite its worldwide distribution, is still not well understood, although in many cases, the frequency of *Blastocystis* sp. isolated from the stool of people with gastrointestinal complications was higher than that of asymptomatic individuals.³ However, the precise mechanism for *Blastocystis* pathogenicity has not been defined, and *in vitro* designed studies have shown that *Blastocystis* sp. can stick to gut mucin and secrete cysteine proteases that contribute to pathogenesis through the degradation of secretory immunoglobulin A, Rho-associated coiled-coil kinase (Rho/ROCK)-mediated tight junction compromise, nuclear factor kappa B-mediated secretion of inflammatory cytokines, and host cell apoptosis.⁴

Acute and/or chronic diarrhea, abdominal pain and bloating, constipation, nausea, irritable bowel syndrome, inflammation, and the like are attributed to *Blastocystis* infection; in addition, loss of appetite, weight loss, fatigue, fever, and depression are among the other symptoms of the disease, especially among infected children.^{5–7}

Importantly, the gastrointestinal complications mentioned in immunocompromised people can worsen the host's health condition and are considered life-threatening in some cases.⁸ The association of this thousand-faced parasitic organism with many disorders of unknown etiology, most notably with irritable bowel syndrome, has been investigated repeatedly, and the findings have been contradictory.⁹ Today, with the increase in the population of susceptible hosts with relatively naive immune systems, infections can be more dangerous than before. Although many of the mentioned symptoms are self-limiting in people with an adequate immune system, in some cases, drug treatment is necessary.¹⁰ The choice drug for symptomatic cases, metronidazole (MTZ), has been introduced; however, it should be kept in mind that serious clinical complications of MTZ, such as leukopenia, neutropenia, neuropathy, depression, trouble sleeping, feeling irritable, and the like, have been proven.¹¹ On the other hand, confirmed reports of resistance to MTZ are available in some cases, which has led to increased concerns.¹² Hence, many studies have been conducted to find a safer alternative to MTZ, preferring substances of natural nature, and are still ongoing.¹² Among them, medicinal plants/herbal medicines have been the focus of attention. Various experimental trials have been performed in *in vitro* and *in vivo* settings, particularly for common *Blastocystis* subtypes (ST1 and ST3), and in some cases, the outcomes have been promising.¹³ The current mini-review is limited to providing a snapshot of various studies on the cytotoxic effects of certain natural (herbal) substances on *Blastocystis* species and their results.



Once upon a time, humans used natural substances (mostly herbal) to treat some disorders, diseases, and infections; these materials, which were generally native to that region, were known and used over the years through trial and error and according to experimental evidence.¹⁴ Parasitic infections were not excluded from these traditional treatments. Today, human society is attempting to revive traditional treatments with the aim of optimizing and improving them without clinical complications and consequences such as drug resistance.¹⁵ Aqueous and alcoholic extracts of plants are highly popular for extracting and applying the maximum effect of active substances on infectious agents (here *Blastocystis* sp.), although until now, there has been no alternative formulated with a better effect than MTZ (lower dose and cytotoxicity). Some key studies in this field have been mentioned in the present research.

Eurycoma longifolia

Despite growing in a limited geographical area, the *Eurycoma longifolia* plant is world-renowned for its many therapeutic properties (i.e., wound healing, anti-cancer, anti-bacterial, and anti-parasitic properties), *Blastocystis* sp. was one of the target parasites.¹⁶

Girish et al conducted an *in vitro* study on the coexistence of *Blastocystis* species with aqueous and alcoholic extracts of the *E. longifolia* (Tongkat Ali) plant, as a result of which, surprisingly, the aqueous and alcoholic extracts of the *E. longifolia* plant showed a significant effect on subtypes 1, 3, and 5 at low concentrations and within the range of MTZ.¹⁷ This study first investigated the anti-protozoal properties of Tongkat Ali against the isolates of *Blastocystis* sp. so that the ethyl acetate fraction of this

plant showed the highest activity against *Blastocystis* sp. Tongkat Ali also demonstrated more uniform sensitivity across subtypes in comparison to allopathic drugs. As it is known, MTZ is still used as the first-line treatment, followed by second-line drugs such as trimethoprim-sulfamethoxazole, nitazoxanide, and ketoconazole in clinical cases. A more recent study by Girish et al revealed that the extract of the *E. longifolia* plant is capable of inducing apoptosis in different *Blastocystis* sp. subtypes.¹⁸

Salvadora persica

It is a plant with a long history that has been utilized for centuries in oral health, food seasoning, and traditional medicine. The growth of this plant is limited to African and Middle Eastern regions.¹⁹ Similar to the previous one, the antimicrobial properties and pharmacological activities of this plant have also been proven, according to research. El-Bali et al compared the aqueous extract of the root of this plant with virulent *Blastocystis* subtypes (1, 3, and 5) isolated from symptomatic patients, and the results of this research indicated that at a concentration of 2.5 µg/mL, this extract has significant lethality compared to the control group.²⁰ In addition, the findings of this study demonstrated that heat-stable components are among the main anti-parasitic agents and can cause significant parasite growth-inhibiting activity. Based on the results (Table 1), the highest anti-parasitic effect of the toothbrush plant was at a concentration of 20 µg/mL. However, more studies are needed to evaluate the effect of this plant as accurately as possible.

Ferula asafoetida

Asafoetida contains a compound called oleo-gum-resin,

Table 1. Characteristics of Included Studies With *In Vitro* Findings

Author Name/Publication Year	Study Design	The Studied Plants	Concentrations	Findings
Girish et al (2015) ¹⁷	<i>In vitro</i>	<i>Eurycoma longifolia</i>	0.1 mg/mL and 1.0 mg/mL	It exhibited the highest anti-protozoal activity at 1.0 mg/mL. The ethyl acetate fraction demonstrated a slightly higher percentage of anti-protozoal activity at 1.0 mg/mL across subtypes.
El-Bali et al (2020) ²⁰	<i>In vitro</i>	<i>Salvadora persica</i>	2.5–40 µL/mL	The maximum anti-parasitic effect of the extract was at a concentration of 20 µL/mL, and this plant prevented the growth and survival of the parasite in the culture medium.
El Deeb et al (2012) ²¹	<i>In vitro</i>	<i>Ferula asafoetida</i> (Ap)* <i>Ferula asafoetida</i> (Ao)**	2, 4, 8, 16, and 20 mg/mL 5, 10, 25, 40, and 50 mg/mL	Asafoetida, either as Ap or Ao, decreased the counts and viability of all tested isolates of <i>Blastocystis</i> sp. subtype 3.
Sawangjaroen and Sawangjaroen (2005) ²²	<i>In vitro</i>	<i>Brucea javanica</i> seed	62.5–2000 µg/mL	At a concentration of 2000 µg/mL, the dichloromethane and methanol extracts were able to kill <i>Blastocystis hominis</i> by 82% and 75% and inhibit 94% and 100% of the tested samples, respectively.
Sawangjaroen and Sawangjaroen (2005) ²²	<i>In vitro</i>	<i>Quercus infectoria</i> nut gall	62.5–2000 µg/mL	At a concentration of 2000 µg/mL, the methanol extract killed 67% and inhibited 76% of <i>Blastocystis hominis</i> samples.
Kordestani Shargh et al (2020) ²³	<i>In vitro</i>	CM11	0.75, 1.5, 3, 6, 12, and 24 µg/mL	The highest toxicity effect of CM11 was observed at a concentration of 24 µg/mL, leading to 28.7% and 25% viable parasites after 24 hours and 48 hours of incubation, respectively. Interestingly, disruption of the <i>Blastocystis</i> cell membrane could be observed in the treated parasites.

Note. * Powder-form; ** Oil-form.

which is obtained from many species of *Ferula* and has been a main component of traditional medicine, especially in the Middle East. The growth of this plant has often been observed in the mountainous regions of Iran and parts of Afghanistan.²⁴ In addition to the anti-diabetic, anti-tumor, and anti-fungal properties of the active ingredient of this plant, anti-parasitic properties have also been defined for it. In an *in vitro* experimental study, Deeb et al examined the effects of both powder and oil forms on blastocyst sp. subtypes and found that both forms derived from this plant can reduce the viability rate (in number) in *Blastocystis* subtype, ST3.²¹

A majority of spices can improve the activity of pancreatic lipase and amylase when they directly influence the enzyme; therefore, the use of these traditional herbs as a spice or seasoning, along with food, can improve the digestive condition and help strengthen the gut microbiota in the anti-*Blastocystis* direction.

Artemisia judaica

Recently, scattered trials with *in vivo* and *ex vivo* anti-parasitic designs of *Artemisia judaica* plant extract have been conducted on various parasites such as *Plasmodium* spp., *Cryptosporidium* spp., and *Blastocystis* sp. As in previous cases, *Artemisia* is one of the plants that has many uses in traditional medicine in the Middle East, especially in Egypt.^{12,25} In this regard, Mukhtar et al experimentally compared the ethanolic extract of *Artemisia* with common *Blastocystis* subtypes.³ Several evaluations have been performed regarding the antiparasitic effects of this plant(s) species on the common subtypes of *Blastocystis* (ST1 and ST3). Amid these, significant growth inhibition of *Blastocystis* was detected when exposed to both *A. judaica* (99.3%) and *A. fragrantissima* (95.6%) with a minimal inhibitory concentration (MIC90) of 2000 µg/mL. Changes in *Blastocystis* morphology were noted under the effect of the extracts, with the complete destruction of *Blastocystis* forms after 72 hours with a dose of 4000 µg/mL. Different subtypes displayed various responses to the tested herbal extracts. The findings of this study demonstrated that solvent-portioned fractions [dichloromethane (DCM), ethyl acetate (EtOAc), and n-hexane] in *A. judaica* were introduced as active fractions against both prevalent subtypes of *Blastocystis* (ST1 and ST3). Interestingly, different fractions derived from plants can have a wide range of effects, which should be considered in future studies for the optimization and non-use of raw materials.

Tunceli garlic (*Allium tuncelianum*)

It is an endemic plant species found only in Tunceli province, especially in the foothills of Munzur mountains in Ovacık, Pülümür, Hozat, and Pertek districts.²⁶ *Allium tuncelianum* has single-clove bulbs and small formations (e.g., small bulbs) and can also produce fertile flowers and

seeds; with this feature, it is different from other garlics.²⁶ The anti-cancer, anti-diabetic, and anti-infectious properties of this plant with a long history have been studied many times. In this regard, Aykur et al, in an *in vitro* design study, investigated the inhibitory impacts of *Tunceli garlic* (*A. tuncelianum*) ethyl alcohol extracts on the *Blastocystis* ST3 subtype growth²⁷. Based on their findings, the MIC90 of *A. tuncelianum* was 115 mg/mL after three days (72 hours), while the MIC90 of MTZ was 1 mg/mL after three days (94.96%). The parasite growth inhibitory action of the *A. tuncelianum* extract increased gradually in all doses and time points. In this study, the obtained findings showed the potential inhibitory effect of the *A. tuncelianum* plant extract against *Blastocystis* ST3. Consequently, it may be suggested that the complete *A. tuncelianum* be consumed as a food supplement. The *A. tuncelianum* extract could be considered an alternative for the potential treatment of a parasitic *Blastocystis* infection.

Quercus infectoria Nut Gall

Quercus infectoria is a small tree about 2.5 m high with 4-6 cm long leaves and acorn fruits that are narrow, scaly, and cylindrical.²⁸ This plant is one of the native plants of different regions of the world, especially Asia (East Asia and the heights of the Iranian plateau), and has a long history of use in traditional medicine.²⁸ The anti-bacterial and anti-parasitic properties of different parts of this plant, including the root, fruit, and the like, have been repeatedly studied, and satisfactory results have been obtained in this respect.²² Regarding the anti-parasitic properties of this plant, the effects of inhibiting the growth of amoebae in *in vitro* and *in vivo* environments have been emphasized.²⁹ In mice with cecal amoebiasis, the *Q. infectoria* extract at concentrations of 500 and 250 mg/kg/d cured 26% and 13% of mice, respectively. In this regard, different concentrations of the methanol extract of oak have been tested against *Blastocystis* by Sawangjaroen et al. The results showed that concentrations of this plant's extract killed the parasite and inhibited its growth.³⁰ It seems that different parts of this plant have good potential for further trials in the field of anti-*Blastocystis*.

Brucea javanica

Brucea javanica is one of the other plants with a strong history in traditional medicine, especially in East Asia³¹. Experimental evidence has shown its anti-tumor, anti-inflammatory, anti-malarial, and anti-blastocystic properties²². Pharmacological research has reported that tetracyclic triterpene quassinoids are the main antitumor and anti-microbial components.³² However, most of these active components have poor water solubility and low bioavailability, which greatly limits their clinical application. Therefore, nanoparticle delivery systems are used to improve the bioavailability of *Brucea javanica*.³¹

According to the study of Sawangjaroen and Sawangjaroen in line with the anti-blastocystic properties of different extracts of this plant, it was found that at a concentration of 2000 µg/mL, the dichloromethane and methanol extracts could kill *Blastocystis hominis* 82% and 75% and inhibit 94% and 100% of tested samples, respectively.²² In another study with an *in vitro* design that evaluated the responses of *Blastocystis* against different extracts of traditional Chinese medicinal plants, Yang et al found that the *B. javanica* extract could induce the properties of inhibiting the growth of the parasite.³³ According to the obtained evidence, it seems that more promising anti-parasitic results will be obtained by further optimizing the different parts of this plant.

CM11

As a naturally synthesized short cationic antimicrobial peptide with amino acid sequences, CM11 is known as an antimicrobial peptide whose antimicrobial effects have been tested and proven. This peptide is used in a pure or modified form in studies that have employed both forms in *Blastocystis* species. In the study of Kordestani Shargh et al, the CM11 peptide was tested on different subtypes of *Blastocystis* and compared with MTZ.²³ In this *in vitro* experiment, gastrointestinal cell lines were cultured, as a result of which, different percentages of inhibition of parasite growth and anti-parasitism were observed after 24 hours and 48 hours. Therefore, CM11 can be suggested as a potential treatment for *Blastocystis*-infected patients after further *in vitro* and *in vivo* assessments. However, more studies are needed to optimize this peptide and its antiparasitic effect.

Discussion

Regarding the possible mechanisms of the pathogenesis of *Blastocystis* species, as mentioned earlier, studies in the *in vitro* environment showed that *Blastocystis* sp. can stick to intestinal mucin and secrete cysteine proteases that contribute to pathogenesis through the degradation of secretory immunoglobulin A, Rho/ROCK-mediated tight junction compromise, nuclear factor kappa B-mediated secretion of inflammatory cytokines, and host cell apoptosis.³⁴ Nevertheless, it is presently unidentified whether this occurs in *in vivo* conditions. Furthermost gut microbiota investigations that include *Blastocystis* report that *Blastocystis* is a common constituent of the healthy gut microbiota and is associated with higher bacterial richness, and that long-term asymptomatic carriage is common. In contrast, a couple of recent studies have proposed that *Blastocystis* decreases beneficial gut bacteria, leading to a dysbiotic state.³⁵

Many forms of the mentioned plants have food and seasoning uses; as it is known, spices strengthen the flow of saliva and the secretion of gastric juice and support the digestion process due to their enzymatic participation in

digestion.³⁶ Some common spices or active principles have been evaluated for their possible effects on pancreatic digestive enzymes in animal models (*in vivo* conditions). Therefore, the use of these plants with an old background as food supplements (spices and seasonings), in addition to helping the digestion process, facilitating digestion, and improving the condition of the intestinal microbiota, can also have anti-parasitic properties similar to what is defined for probiotics.³⁷ Studies have shown that different forms of plant derivatives (e.g., water-based extracts, alcoholic and oily and powder forms, and the like) have different effects on parasites (*Blastocystis* sp.), helping in optimizing plants and natural derivatives, and designing future studies should be taken into consideration.

The difference in the impressionability of parasite subtypes has been emphasized in various studies.³⁸ Studies at the electron microscopy and molecular levels indicate structural differences in dissimilar subtypes of the parasite, and this difference in characteristics causes the different effectiveness of the studied drugs or extracts on diverse subtypes, therefore, identifying and determining the subtypes of parasitic isolates seem necessary and useful at the initial stages of the study.³⁹ In this regard, *Blastocystis* subtype 3 has more sensitivity than subtype 1 when exposed to the CM11 peptide.

Each geographical region has its own vegetation according to the weather conditions and geophysical location, which are considered valuable natural reserves. Native plants can be reused against a variety of parasitic agents, especially virulent species and subtypes, referring to their traditional use history. The methods of using the maximum properties of medicinal substances have been optimized with the advancement of biotechnology. Nowadays, various techniques are applied to improve the properties and increase the effectiveness of the studied extracts; combining the active ingredient of the extracts with nano-sized particles for better and more targeted delivery is one of these approaches.⁴⁰ Similar to what happens in pharmacology studies, optimizing the extraction method (e.g., the use of ultrasound/microwaves and the like) for better shelf life by maintaining maximum heat-labile active ingredients is highly practical.⁴¹ Combining with some other natural compounds to increase the synergism of substances extracted from plants has also resulted in satisfactory results. Additionally, evidence has shown that modifying peptide or polysaccharide sequences and making slight changes in their structure have improved their effectiveness.⁴²

Conclusion

Overall, the use of plant extracts with a medicinal background against parasites, especially *Blastocystis* species, has been satisfactory; however, they still cannot be introduced as an alternative to chemical drugs. Therefore, it seems necessary to optimize and identify

effective ingredients/effective fractions of plants to improve their effectiveness, which should be considered for future studies. The use of new tools such as the *in silico* environment that investigates the interaction of plant components with membranes or cell components is suggested for future studies; different forms of extracts (powder, solution, and the like) should be studied in extracorporeal and intracorporeal environments. It is recommended that the native plants of each region that have a history of use in traditional medicine also be evaluated against *Blastocystis* sp.

Authors' Contribution

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Investigation: Zahra Ghazi.

Methodology: Zahra Ghazi, Majid Pirestani.

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Competing Interests

The authors declare no competing interests.

Consent for Publication

Not applicable.

Ethical Approval

Not applicable.

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