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MINI-REVIEW ARTICLE

Medicinal Plants as Natural Anti-Parasitic Agents Against *Blastocystis* Species

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Abstract: ***Background*:** *Blastocystis* species (sp.) are enteric parasites that live in both humans' and animals' gastrointestinal tracts. *Blastocystis hominis* (*B. hominis*) is the recognizable human isolates in clinical and diagnostic specimens. Human infection occurs *via* the oro-fecal route, particularly in developing areas due to the lack of sanitation and hygienic facilities. *B. hominis* can exist in the large intestine for weeks to years until treated appropriately. Metronidazole is the drug of choice for the treatment of *Blastocystis* infection. However, it induces intolerable side effects and has been shown to have teratogenic and carcinogenic potential. Several medicinal plant extracts have been experimentally tested against *Blastocystis* infection in comparison to currently available treatments.

***Objective*:** Based on *in vitro* and *in vivo* studies, this article reviewed anti-*Blastocystis* activity of some medicinal plants.

***Methods*:** To conduct the research for this review, Google Scholar and PubMed were the primary search engines used to find relevant literature. A total of 19 published *in vitro* and *in vivo* studies were evaluated to identify the anti-*Blastocystis* effects of various medicinal plants

***Results*:** Multiplication of *Blastocystis* parasites as well as nucleic acids and protein synthesis, all be inhibited by extracts from different medicinal plants. These natural agents have been shown to be both safe and effective when compared to the existing treatment options.

***Conclusion*:** Different medicinal plants can combat *Blastocystis* infection and could be a good substitute for metronidazole and other synthetic treatments.

Keywords: *Blastocystis* infection, treatment, metronidazole, medicinal plants, *in vitro*, *in vivo* studies.

1. INTRODUCTION

Human blastocystosis is a parasitic disease caused by *Blastocystis hominis* (*B. hominis*) which is an enteric protozoan parasite inhibiting the human large intestine and is the most frequent eukaryotic organism detected in human feces. Humans acquire infection *via* ingestion of *Blastocystis* cysts with contaminated raw food or water. The prevalence of *Blastocystis* infection ranges from 10% to 60% in developed and developing countries, respectively [1]. Differences in hygiene levels, waste management, ingestion of parasite-contaminated raw vegetables or water, and animal contact could all contribute to the variance in the prevalence of this infection. *Blastocystis*' pathogenic potential is still debated, as it is detected in many individuals without causing any clinical signs [2]. The pathogenicity of *Blastocystis* infection is influenced by a number of factors, such as the number of parasitic forms, genetic subtype,

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developmental stage, host genetics and immunological status, therapeutic interventions, and associated infection with other parasites [3].

In human stool samples, many morphological forms of *B. hominis* were found, including vacuolar, granular, multivacuolar, avacuolar, cyst, and ameboid forms. The vacuolar form is the most common type, which is spherical and has a big central vacuole that occupies up to 90% of the cell. According to animal experimental studies, the parasite's transmissible stage was water and environmental-resistant infective cysts. After ingesting cysts, the parasite undergoes excystation in the large intestine and converts into vacuolar forms. The vacuolar forms divide *via* binary fission and may transform into granular and amoeboid forms. Then, the cyst may encyst while passing through the colon before being excreted in the stool [4].

According to a small subunit rRNA gene study, *Blastocystis* has a genetic diversity. There are more than 13 different *Blastocystis* subtypes (STs) in both humans and animals. Humans have been found to have nine of these STs (ST1-9), the most frequent of which is ST1-4, which accounts for almost 90% of all humans surveyed. Other STs (5-9) identified occasionally in humans were more frequently found in non-human hosts suggesting zoonotic transmission: ST5 is found in livestock, ST6 and ST7 are found in birds, and ST8 is found in non-human primate species [5]. There are variances in virulence between *Blastocystis* STs that have been identified in different studies. STs 1 and 3 were detected in symptomatic cases [6, 7], however, ST2 was found in asymptomatic carriages [8]. According to other investigators, STs 1, 4, and 7 are the most pathogenic, whereas STs 2 and 3 are nonpathogenic [4].

Blastocystosis is frequently asymptomatic or may cause nonspecific gastrointestinal symptoms, such as diarrhea, abdominal discomfort, flatulence, nausea, anorexia, bloating, vomiting, constipation, dehydration, insomnia, and loss of weight or exhaustion [4]. Increased epithelial permeability, induction of apoptosis in host intestinal epithelial cells, and disruption of epithelial barrier function are among *B. hominis*' pathogenic mechanisms [9], which also include immune modulation, cytokine release from colonic epithelial cells, and oxidative damage [10]. The genetic diversity of *Blastocystis* accounts for the variability in clinical presentations [4, 9, 11].

Irritable bowel syndrome (IBS), a gastrointestinal illness marked by abdominal pain and discomfort, as well as changes in bowel habits, has been linked to *Blastocystis* infection. *Blastocystis* growth may be aided by IBS-induced alterations in the gut environment, and continued antigenic exposure may result in low-grade inflammation. The isolates ST1 and ST3 of *Blastocystis* sp. were found to be significantly associated with IBS patients [2, 11, 12].

Blastocystis infection has also been linked to a variety of other clinical disorders including arthritis [13] and cutaneous disorders such as acute or chronic urticaria [14, 15]. *Blastocystis* sp. belonging to the ST2 and ST3 were identified in the patient's stools with these disorders. There was also a link detected between urticaria and the ST3 isolate's amoeboid form. It's thought that the amoeboid form attaches well to the intestinal epithelium, disrupting gut immune homeostasis and triggering an inflammatory reaction toward *Blastocystis*, resulting in urticaria [16].

Blastocystis infection is commonly diagnosed microscopically through direct inspection of stool, concentration techniques, or *in vitro* culture. The diagnostic yield is increased by examining multiple stool samples. However, the parasite's polymorphic character and variation in its size and number all contribute to variables that make microscope identification difficult. As a result, alternative detection approaches such as stool immunoassays [17] and molecular techniques including PCR have been developed [6]. The maximum diagnostic usefulness in defining stool specimens is achieved when these approaches are used with short-term axenic *in vitro* culture. Surprisingly, many laboratories do not report *Blastocystis* since some in the medical community have long believed that it is always nonpathogenic.

2. CURRENT THERAPEUTIC AGENTS AGAINST *BLASTOCYSTIS* INFECTION

Some researchers believe that if high numbers of *Blastocystis* organisms are found in a stool sample, therapy should be considered even if no other bacterial, viral, or parasite infection is present [4, 18]. Metronidazole (MTZ), trimethoprim-

sulfamethoxazole (TMP-SMX), nitazoxanide (NTZ), paromomycin, iodoquinol, ketoconazole, secnidazole, emetine, tinidazole, and the probiotic *Saccharomyces boulardii* have all been used to treat *Blastocystis* infection. However, each one has yielded substantially disparate percentages of clinical cure and parasite elimination from stool samples [19]. The remission of symptoms in conjunction with the total eradication of the organisms indicates successful treatment. *Blastocystis* sp. genome study and the use of current techniques like microsatellites, microarrays, and differential display, in combination with proteonomics and bioinformatics analysis, are usually able to reveal treatment response [20].

The most common treatment for *Blastocystis* infection is metronidazole. According to Nasirudeen *et al.* [21], metronidazole causes programmed cell death in *Blastocystis*, and apoptosis-like features can be seen in growing axenic cultures. Metronidazole is given in a variety of dosages, ranging from 250 to 750 mg three times daily for ten days. After treatment with metronidazole at a dose of 1.5 g/day for ten days, diarrhea improved and parasites were cleared from the stools within one month. However, extending the follow-up period to six months resulted in a higher rate of parasitological relapses. As a result, metronidazole may fail to achieve total eradication of *Blastocystis* organisms due to the development of resistance. Only 80% of metronidazole-treated individuals achieve resolution [19].

According to several research, human isolates from various geographical areas exhibit differing degrees of resistance to metronidazole. The patients who aren't responding could be infected with *Blastocystis* resistant subtypes [19, 22]. Subtypes 4 and 7 were shown to be resistant to metronidazole and to have cross-resistance to tinidazole [23]. *Blastocystis* cystic forms are genetically varied and resistant to the drug's cytotoxic effect [4, 24]. Metallic taste, headache, dry mouth, nausea, glossitis, urticaria, pruritus, and dark-coloured urine have all been recorded as adverse effects of metronidazole. There have also been reports of therapeutic failures, as well as carcinogenic, teratogenic, and embryogenic effects [24]. It's possible that the treatment's apparent failure is related to patient noncompliance, drug pharmacokinetic differences, and drug inactivation by the natural bacterial flora [22]. As a result, new anti-*Blastocystis* drugs that are both safe and efficacious must be developed.

Trimethoprim-sulfamethoxazole (TMP-SMX) improves the cure rate and clinical symptoms in *Blastocystis*-infected patients. It is recommended as an alternative to metronidazole if symptoms continue following treatment. Ok *et al.* [25] found that patients who were treated with TMP-SMX recovered completely. In another study, 97.3 percent of cases demonstrated clinical improvement after 7 days of treatment with TMP-SMX [26]. Moghaddam *et al.* [27], on the other hand, found that TMP-SMX treatment resulted in 22 percent parasite eradication. TMP-SMX is also associated with a lower *Blastocystis* detection rate in patients infected with the human immunodeficiency virus [28].

The broad-spectrum anti-parasitic nitazoxanide has been proven to have strong efficacy against *Blastocystis* [19, 29]. It's well-tolerated and hasn't caused any adverse reactions [29]. Treatment failures with metronidazole in *Blastocystis* infection may react effectively to nitazoxanide. Subtype 7 of *Blastocystis* was found to be substantially more sensitive to nitazoxanide *in vitro* than subtype 4 [23]. In a placebo-controlled trial, the use of nitazoxanide 500 mg twice a day for three days resulted in clinical and parasitological cures. Children can attain clearance rates of 97-100 percent when given this medication [29].

Paromomycin is a broad-spectrum aminoglycoside antibiotic that has been shown to be beneficial in treating *Blastocystis*-related cutaneous disorders, especially urticaria [30]. It has been demonstrated to be more effective than metronidazole in various studies [31]. A preliminary *in vitro* study found that paromomycin had no effect on the parasite. Because it is bactericidal, it may work by destroying the gut bacterial flora, which is necessary for *Blastocystis* viability [4]. According to Mirza *et al.* [23], paromomycin does not affect nonhuman *Blastocystis* subtypes.

Tinidazole, ornidazole, secnidazole, ketoconazole, pentamidine, furazolidone, quinine, iodoquinol, iodochlorhydroxyquin, and emetine are some of the other medications that have been reported to have varying degrees of activity against *Blastocystis* [19, 23]. It was revealed that a combination of secnidazole, nitazoxanide, and furazoli-

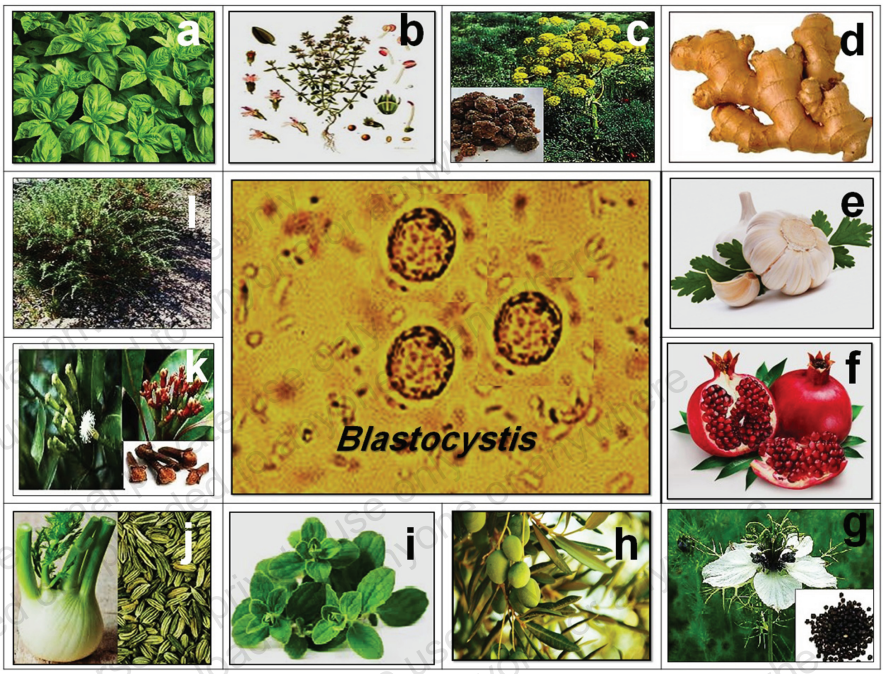

Fig. (1). Some Egyptian medicinal plants have anti-*Blastocystis* activity: (**a**) *Ocimum basilicum*, (**b**) *Thymus vulgaris*, (**c**) *Ferula asafoetida*, (**d**) *Zingiber officinale*, (**e**) garlic, (**f**) *Punica granatum*, (**g**) *Nigella sativa*, (**h**) olive leaf, (**i**) *Origanum majorana*, (**j**) *Foeniculum vulgare*, (**k**) *Syzygium aromaticum*, (**l**) *Artemisia judaica*. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

done is effective in eliminating the parasite. However, in many countries, all three drugs are currently unavailable [19].

Blastocystis cysteine proteases play a vital role in the cell cycle and pathophysiology, causing pro-inflammatory cytokines to be upregulated. As a result, cysteine proteases inhibitors could be used to treat *Blastocystis* isolates that are resistant to traditional therapy [23].

Saccharomyces boulardii, non-pathogenic yeast, has been shown to be efficient against *Blastocystis* infection. It maintains microbial homeostasis in the intestine, inhibits pathogens' ability to colonize and infect the mucosa, modifies local and systemic immune responses, stabilizes gastrointestinal barrier functions, and produces enzymatic activity that promotes absorption and nutrition [32].

3. ANTI- *BLASTOCYSTIS* EFFECT OF SOME MEDICINAL PLANTS

As an alternative to presently used synthetic drugs for the treatment of *Blastocystis* infection, many researchers studied a variety of medicinal plants as novel treatment options and concluded that these new agents have high anti-*Blastocystis* activity while posing no or minimal toxic effect. *Ocimum basilicum* (sweet basil) and *Thymus vulgaris* (thyme) [33], *Ferula asafoetida* [34], *Zingiber officinale*, garlic, onion, turmeric [35, 36], *Punica granatum* [37], *Nigella sativa* [38], olive leaf and bee pollen compound [39], *Origanum majorana* (marjoram), *Foeniculum vulgare* (fennel) [40], *Syzygium aromaticum* [41], *Artemisia judaica* and *Achillea fragrantissima* [42] are among the Egyptian medicinal herbs that have been demonstrated to have anti-parasitic effects against *Blastocystis* sp. (Fig. **1**). The medicinal herbs used were chosen based on their anti-microbial properties and chemical compositions. Anti-*Blastocystis* efficiency of the medicinal plants extracts can be attributed to their phytochemical compositions including flavonoids, alkaloids, tannins, saponins and glycosides which are responsible for varying levels of inhibition against pathogenic organisms, as well as their antioxidant activities and free radical scavenging characteristics [43]. According to El-Sayed [33] and Wink [44], disrupting parasite membrane integrity, inhibiting infectious agent reproduction, inhibiting DNA/RNA synthesis, and interfering with aromatic amino acid metabolism

are all effects of these extracts against *Blastocystis*.

In addition, other studies from different countries have demonstrated anti-*Blastocystis* activity of different medicinal plants. In a semi-quantitative *in vitro* experiment using 20 crude extracts of traditional Chinese medicines, Yang *et al.* [45] demonstrated that aqueous extracts of *Coptis chinensis* (100 μg/mL) and *Brucea javanica* (500 μg/mL) substantially suppressed axenic *Blastocystis* cell lines.

According to Vital and Rivera [46], both *Chromolaena odorata* leaves ethanol extract and *Uncaria perrottetii* stem bark ethyl acetate extract efficiently suppressed the growth of *Blastocystis* and reduced cell counts at dosage of 0.5 and 1% respectively. Interestingly, daily administration of 600 mg of emulsified Mediterranean oregano (*Origanum vulgare*) oil for 6 weeks resulted in 100% eradication of *B. hominis* from the stools of infected patients and improved gastrointestinal symptoms [47].

According to Özbílgín *et al.* [48], methanol extract of *Achillea millefolium* was found to be effective against *Blastocystis* STs 1, 2, and 3 and could be employed as an antiprotozoal agent. In addition, *Mallotus oppositifolius*, *Vemonia colorata*, *Zanthoxylum zanthoxyloides*, *Clausena anisata* and *Eythrina senegalensis* are among medicinal plants from Ghana that demonstrated high activity against *Blastocystis* [49].

In an *in vitro* study conducted in Thailand, the anti-*Blastocystis* activities of n-hexane, dichloromethane, and methanol extracts from five anti-diarrheic medicinal plants, *Acacia catechu* resin, *Amaranthus spinosus* whole plant, *Brucea javanica* seed, *Piper longum* fruit, and *Quercus infectoria* nutgall, were evaluated by comparing to metronidazole. Different isolates of *B. hominis* were treated with these extracts for 48 hours at doses ranging from 62.5 to 2000 μg/mL. The most effective extracts were dichloromethane and methanol extracts from *Brucea javanica* seed and methanol extract from *Quercus infectoria* nutgall. *B. hominis* was destroyed by 82, 75, and 67 percent in tested isolates at a concentration of 2000 μg/mL of these three extracts, respectively. In comparison, metronidazole was able to kill 97 percent of *B. hominis* isolates at a dosage of 40 μg/mL [50].

Grabensteiner *et al.* [51] observed that *in vitro* activities of ethanol extracts of saw palmetto, thyme, and pumpkin fruit, as well as aqueous and ethanol extracts of grape seed, effectively suppressed *Blastocystis* growth after 24 and 48 hours when given at a dose of 5 mg/mL.

3.1. Anti-*Blastocystis* Activity of *Thymus vulgaris*

Thymus vulgaris, also recognized as Zaatar, has analgesic, expectorant, antitussive, antispasmodic, antibroncholitic, anti-inflammatory, carminative, anthelmintic, and diuretic effects and is widely used in traditional medicine. According to El-Sayed [33], ethanol extract of *Thymus vulgaris* leaves inhibited the growth of *B. hominis* cysts significantly. The inhibition appears to be dose and time-dependent. *Thymus vulgaris* extract at the highest dose (4 mg/mL) had the best results, inhibiting *B. hominis* cyst growth after 24 hours with no significant difference in inhibitory efficacy between it and metronidazole. Also at lower doses, 2 mg/mL inhibited *B. hominis* cyst growth but required a longer incubation period (48 hours) than the highest dose, whereas the doses of 0.5 and 1 mg/mL reduced growth by 84.8 and 95 percent after 96 hours, respectively. *Thymus vulgaris*' anti-*Blastocystis* activity is thought to be due to its active components, thymol and carvacrol, which have the ability to alter cell membrane permeability, as well as membrane organization and surface electrostatics, resulting in the release of membrane-associated materials from cells to the external medium and the fragmentation of microorganisms [52].

3.2. Anti-*Blastocystis* Activity of *Ocimum basilicum* (Sweet Basil)

The aromatic plant *Ocimum basilicum* has been used as a flavoring agent in the food and pharmaceutical industries. *Ocimum basilicum* has antibacterial, antioxidant, antiviral, antifungal, and antiprotozoal properties, according to many researches. In a study from Egypt, *Ocimum basilicum* ethanol extract suppressed the growth of *B. hominis* cysts with doses of 2 mg/mL after 24 hours and 1.5 mg/mL after 72 hours, with no significant difference compared to metronidazole. After 96 hours, the doses of 0.5 and 1 mg/mL, on the other hand, didn't completely suppress the growth, but they reduced it by 79.7 & 86.3 percent, respective-

ly [33]. Linalool (30-40%) and eugenol (8-30%) oils are the most prevalent phenolic components of *Ocimum basilicum*. The ability of these constituents to alter microbial cell permeability, allowing the loss of macromolecules from the interior and interact with membrane proteins, causing deformation in its structure and function, and also related to enzymatic inhibition of cysteine protease, resulting in parasitic organism destruction, could be the mechanism of action on microbial growth [53].

3.3. Anti-*Blastocystis* Activity of *Ferula asafoetida*

Many *Ferula* plants produce *Asafoetida*, which is an oleo gum resin. *Ferula asafoetida* is a traditional remedy for a range of diseases and also a flavoring additive in food. The effectiveness of *Ferula asafoetida* either in powder and oil forms on the *in vitro* growth of *Blastocystis* sp. subtype 3 was evaluated in comparison to metronidazole by El Deeb *et al.* [34]. *Ferula asafoetida*, in both powder and oil form, reduced the numbers and survivability of all *Blastocystis* ST3 isolates tested, which was validated by microscopy. The viable vacuolar forms that were commonly visible before incubation with *Ferula asafoetida* were replaced by many other granular forms, which by the time had lost survivability and appeared wrinkled. The inhibitory effect was proportional to the concentration, form, and duration of incubation with *Ferula asafoetida* extracts.

3.4. Anti-*Blastocystis* activity of Green Tea Extract

Anti-*Blastocystis* effect of green tea extract (GTE) on the growth and viability of three *B. hominis* symptomatic genotype subtypes (I, III, and IV; four isolates per genotype/subtype) was tested *in vitro* and *in vivo*. All tested isolates were susceptible to GTE throughout the *in vitro* experiment at concentrations ranged 20-35 mg/L after 24 h. In addition, the ultra-structures of GTE-treated *B. hominis* by transmission electron microscopy demonstrated an increase in size, cytoplasmic membrane injury and disposition of vacuolated nonhomogeneous particles in the cytoplasm. Moreover, the nucleus shifted periphery, expulsion of the cytoplasmic content and then cell rupture were detected suggesting necrotic changes. *In vivo* experiment revealed that the number of *B. hominis* cysts in stool samples of GTE-treated rats at a dose of 20 g green tea leaves/L of water, was lower than the number of cysts excreted in stools of infected non-treated rats [54]. Green tea polyphenols and epigallocatechin gallate, which have several effects including inhibition of proteases and proteasome function, anti-inflammatory, cell cycle modulation, and reduction of oxygen-derived free radicals, may be attributable to the eradication of *B. hominis* observed after GTE administration to infected rats [55]. Moreover, green tea has an anti-adhesive ability against a wide range of microorganisms [56]. GTE also increases IgA synthesis, which can reduce contact-independent apoptosis [57] and impair the protective barrier induced by *Blastocystis* [58].

3.5. Anti-*Blastocystis* Activity of *Eurycoma longifolia* (Tongkat Ali)

Eurycoma longifolia (Tongkat Ali) is one of the most widely used traditional folk remedies in Malaysia. The medicinal characteristics of this plant include antioxidant, anti-inflammatory, gastroprotective, and antibacterial effects. Tongkat Ali extract was used to treat *Blastocystis* isolates from human stool samples at doses of 0.1 mg/mL and 1.0 mg/mL and *Blastocystis* viability was determined after 72 hours of treatment. At dose 1.0 mg/mL, Tongkat Ali had the best anti-*Blastocystis* effect against *Blastocystis* isolate subtype 3 (95.1%), then ST1 (94.9%), and ST5 (94.3%), compared to metronidazole, which had the strongest anti-*Blastocystis* effect against *Blastocystis* isolate ST1 (95.8%), followed by ST3 (93.4%), and ST5 (90.8%) [59]. According to pharmacological analyses, Tongkat Ali has a considerable number of bioactive components, mainly alkaloids and quassinoids, which are linked to its antiparasitic activity [60].

3.6. Anti-*Blastocystis* Activity of *Zingiber officinale* (ginger)

The perennial herb *Zingiber officinale* has a wide range of therapeutic properties. Numerous studies have demonstrated that *Zingiber officinale* (ginger) has anti-parasitic effects. Ginger's anti-*Blastocystis* activity was evaluated by detecting *Blastocystis* excreted in the feces of infected mice as well as histopathological alterations of their intestines. The levels of malondialdehyde (MDA) and nitric oxide (NO) production were also meas-

ured to determine its antioxidant activity. In comparison to the infected untreated group, ginger treatment reduced cyst shedding, alleviated pathological lesions, and significantly lowered high NO and MDA levels in *Blastocystis*-infected mice [35]. MDA and NO downregulation are major mechanisms of ginger's antiparasitic activities. Also, Abdel-Hafeez *et al.* [36] studied *B. hominis* growth pattern and *in vitro* sensitivity to nitazoxanide, garlic, ginger, onion, and turmeric on *Blastocystis* culture after 24 and 48 hours. After 48 hours of ginger treatment, *Blastocystis* number reduced by 92.98 percent. On the other hand, treatments with onions and turmeric had just a minor effect on parasite number. Interestingly, Yakoob *et al.* [61] found that ginger had no effect on *Blastocystis* STs 1 and 3 in comparison to garlic and metronidazole.

Anti-*Blastocystis* effect of *Zingiber officinale* could be related to its phytochemical contents, which include flavonoids, alkaloids, tannins, saponins, and glycosides, which have been linked to various inhibitory effects against parasitic organisms such as decreasing parasite viability, inhibiting parasite DNA synthesis and reproduction, affecting parasite cell membrane permeability leading to parasite disintegration, as well as affecting parasite infectivity [62].

3.7. Anti-*Blastocystis* Activity of *Allium sativum* (Garlic)

Allium sativum, also known as garlic, is a perennial bulb-forming plant with nutritional and therapeutic effects. In comparison to metronidazole, Yakoob *et al.* [61] found that garlic extract exhibits a significant efficacy against *Blastocystis* genotype-1. According to Abdel-Hafeez *et al.* [35], garlic administration to *Blastocystis*-infected mice (20 mg/kg/day for 3 days) significantly reduced *Blastocystis* cyst counts in stool samples by 92.44 percent after 48 hours and significantly reduced malondialdehyde production when compared to nitazoxanide. Garlic consists of several thiosulfinates (such as allicin), which are responsible for its antimicrobial effects. Allicin in garlic works by completely suppressing parasite RNA synthesis but only partially disrupting DNA and protein synthesis. Allicin also functions as an antioxidant by scavenging reactive oxygen species from the body, avoiding lipid oxidation and the formation of pro-inflammatory mediators [61].

3.8. Anti-*Blastocystis* Activity of Pomegranate (*Punica granatum*)

In vivo anti-*Blastocystis* effect of pomegranate (*Punica granatum*) peel extract on infected rats was studied by Abdel-Hafeez *et al.* [37]. It was demonstrated that daily administration of 500 µl from *Punica granatum* dose of 3 g/kg body weight by gastric tubes for 3 days, caused complete eradication of cyst shedding by day 10 post-infection in a comparable way to nitazoxanide therapy and also, generated an anti-lipid peroxidation effect indicated by the malondialdehyde levels as well as improved histopathological changes in the intestine caused by *Blastocystis* infection. The effect of *Punica granatum* treatment may relate to the existence of metabolic toxins that induce a direct inhibitory effect on parasite growth, the sexual phase's generation, as well as the formation of cysts. Additionally, *Punica granatum* peel has several phenolic constituents, like organic acids, which have been shown to lower parasite viability [63]. Furthermore, the hydroxyl group of *Punica granatum's* phenolic compounds can boost toxicity against microorganisms [64].

3.9. Anti-*Blastocystis* Activity of *Nigella sativa*

Nigella sativa (black seed) has a number of pharmacological properties, including antiprotozoal efficacy. Eida *et al.* [38] compared the therapeutic potential of *Nigella sativa* aqueous extract at various doses to metronidazole on *B. hominis* isolates *in vitro* and *in vivo*. In *in vitro* experiment, *Nigella sativa* aqueous extracts at doses of 100 and 500 µg/mL had a possibly fatal effect on *B. hominis* isolates. Furthermore, in *in vivo* experiment, the caecal tissue of *Blastocystis* infected mice treated with two doses of *Nigella sativa* (250 & 500 mg/kg/d) demonstrated regression of pathological abnormalities, particularly with the larger dose. These improvements were probably due to the ability of *Nigella sativa* to enhance the immune system of infected mice as well as its antioxidant properties.

3.10. Anti-*Blastocystis* Activity of Olive Leaf Extract

El-Sayed *et al.* [39] tested the *in vitro* and *in vivo* efficacy of *Olea europaea*, olive leaf extract, and bee pollen compound, against subtype 3 *Blastocystis* isolated from infected patients. Both natural medicinal compounds with a graded concentra-

tion of 500,1000 µg/mL inhibited *Blastocystis* growth *in vitro* in a concentration-dependent manner and significantly reduced the number of *Blastocystis* in stool samples and intestinal contents of treated mice for seven consecutive days after the establishment of infection, which was higher than the effect of metronidazole, as well as induced apoptotic-like death and programmed cell death in *Blastocystis*. Bee pollen compound reduced tissue pathology severity more effectively than olive leaf extracts and exhibited an immunostimulatory effect on intestinal cells by increasing IgA secretory level in the intestinal mucosa. In the host defense against *Blastocystis* infection, these IgA secretory cells perform a major and dominant role. Anti-*Blastocystis* effect of olive leaf extract is related to oleuropein, the main component of olives which has a variety of biological activities including an antiparasitic effect [65, 66]. These results brought attention to the potential therapeutic effects of olive leaf extract and bee pollen compound as effective and safe natural alternatives to *Blastocystis* infection.

3.11. Anti-*Blastocystis* Activity of *Origanum majorana* and *Foeniculum vulgare*

Origanum majorana (marjoram) and *Foeniculum vulgare* (fennel), two extensively used herbs in Egypt, have been used to treat a range of diseases, including parasite infections. Méabed *et al.* [40] compared the efficiency of various doses of both plant aqueous extracts on *Blastocystis* sp. cysts for different incubation periods (24, 48, and 72 hours) *versus* nitazoxanide as a pharmaceutical control. At a dose of 400 µg/mL, *Origanum majorana* extract had high efficacy rates of 96 percent and 100 percent against *Blastocystis* parasite which was nearly identical to the impact of nitazoxanide after 48-72 hours, respectively. However, the effectiveness rate of *Foeniculum vulgare* at a dose of 250 µg/mL was 56.4 percent after 48 hours and improved to 70.7 percent after 72 hours. These herbs are abundant in phenolic and flavonoid compounds, which function as antioxidants and free radical scavengers [67, 68]. Anti-*Blastocystis* activity of *Origanum majorana* and *Foeniculum vulgare* extracts can be related to their phytochemical constituents, which are concerned with various levels of microorganisms' inhibition.

3.12. Anti-*Blastocystis* Activity of *Artemisia Judaica*

Artemisia judaica, *Achillea fragrantissima* and *Echinops spinosus L*. are Egyptian herbs that have been reported to have a variety of therapeutic characteristics, including anti-parasitic effects. The effectiveness of ethanol extracts from these plants against two *Blastocystis* subtypes (ST1 and ST3) was assessed and compared to metronidazole by Mokhtar *et al.*, [42]. The most potent extracts against *Blastocystis* were *Artemisia judaica* and *Achillea fragrantissima*. At a minimum inhibitory concentration (MIC_{90}) of 2000 µg/mL, *Blastocystis* growth was considerably suppressed by 99.3% after exposure to *Artemisia judaica* and 95.6 percent after exposure to *Achillea fragrantissima*. While at a dose of 4000 µg/mL, these extracts elicited morphological alterations in *Blastocystis* with complete destruction. Regarding *Blastocystis* subtypes, the response of *Blastocystis* ST1 to the herbal extracts was considerably different from those of ST3. According to phytochemical analysis, *Artemisia judaica* has a high amount of flavonoid and phenolic components [69], which are responsible for its anti-*Blastocystis* efficacy.

3.13. Anti-*Blastocystis* Activity of *Syzygium aromaticum*

Syzygium aromaticum (clove) is an aromatic plant, rich in volatile compounds and antioxidants like eugenol. The essential oil of *Syzygium aromaticum* possesses a wide range of medicinal properties including antimicrobial and antioxidant. In comparison to metronidazole, Ezz Eldin [41] evaluated the effect of various doses of *Syzygium aromaticum* essential oil on the vitality of *Blastocystis* sp. With minimal lethal concentrations of 300, 200, 100, and 50 µg/mL, *Syzygium aromaticum* treated *Blastocystis* cultures showed a complete inhibitory effect on *Blastocystis* growth after 24, 48, 72, and 96 hours, respectively. In contrast, metronidazole at a dosage of 1 mg/mL failed to cause 100% inhibition till the completion of the research. Furthermore, *Syzygium aromaticum* induced significant morphological changes in the size of *Blastocystis* compared to the control group. These results highly suggest that *Syzygium aromaticum* essential oil may be a promising and safe agent for the treatment of blastocystosis.

Table 1. Anti-*Blastocystis* effect of various medicinal plants detected in some studies from different countries.

Country	Type of Study	Used Medicinal Plant	Part Used	Extract Type	Effective Concentration/ Dosage	References
China	*In vitro*	*Coptis chinensis* *Brucea javanica*	Dried crude herbs	Aqueous extract	100 µg/mL 500 µg/mL	[45]
Philippines	*In vitro*	*Chromolaena odorata* *Uncaria perrottetii*	Leaves Stem bark	Ethanol extract Ethyl acetate extract	0.5 & 1.0% concentra-tions	[46]
USA	*In vivo* (Patients)	*Origanum vulgare*	*ND	Oil	600 mg/daily for 6 weeks	[47]
Turkey	*In vitro*	*Achillea millefolium* *Quercus infectoria*	Aerial parts Nut galls	Methanol extract *n*-hexane extract Methanol extract	500 µg/mL at 48 h 4000 µg/mL at 48 h 4000 µg/mL at 48 h	[48]
Ghana	*In vitro*	*Mallotus oppositifolius* *Vemonia colorata* *Zanthoxylum zanthoxy-loides* *Clausena anisata* *Eythrina senegalensis*	Herba Radix Cortex & Radix Radix Cortex	Ethanol extract	27.8 µg/mL at 24 h 117.9 µg/mL at 24 h 255.6 µg/mL at 24 h 335.7 µg/mL at 24 h 314.0 µg/mL at 24 h 527.6 µg/mL at 24 h	[49]
Thailand	*In vitro*	*Acacia catechu* *Amaranthus spinosus* *Brucea javanica* *Piper longum* *Quercus infectoria*	Resin Whole plant Seeds Fruit Nut gall	*n*-hexane, Dichloro-methane & methanol extracts	62.5 to 2000 µg/mL	[50]
Egypt	*In vitro*	*Thymus vulgaris* *Ocimum basilicum*	Leaves Leaves	Ethanol extract Ethanol extract	4 mg/mL at 24 h 4 mg/mL at 48 h 1.5 mg/mL at 72 h 2 mg/mL at 24 h	[33]
Egypt	*In vitro*	*Ferula asafoetida*	Oleo-gum-resin	Powder & oil form extracts	16 mg/mL 40 mg/mL	[34]
Saudi Arabia	*In vitro* & *In vivo* (Rats)	Green tea	Leaves	Aqueous extract	20-35 mg/L at 24 h 20 g/L	[54]
Malaysia	*In vitro*	*Eurycoma longifolia* (Tongkat Ali)	Roots	Ethyl acetate fraction of crude Aqueous extract	1.0 mg/mL at 72 h	[59]
Egypt	*In vitro* & *In vivo* (Mice)	*Zingiber officinale* *Allium sativum (garlic)*	Rhizomes Bulb	Aqueous extract	0.1mg/mL at 48 h 20 mg/kg/day for 3 days	[35] [36]
Egypt	*In vivo* (Rats)	*Punica granatum* (Pomegranate)	Peels	Aqueous extract	500 µl from *Punica granatum* dose of 3 g/kg body weight for 3 days	[37]

(Table 1) Contd…

Country	Type of Study	Used Medicinal Plant	Part Used	Extract Type	Effective Concentration/ Dosage	References
Egypt	*In vitro* & *In vivo* (Mice)	*Nigella sativa*	Seeds	Aqueous extract	100, 500 µg/mL 250, 500 mg/kg/day	[38]
Egypt	*In vitro* & *In vivo* (Mice)	*Olea europaea* (Olive)	Leaves	Aqueous extract	500,1000 µg/mL	[39]
Egypt	*In vitro*	*Origanum majorana,* *Foeniculum vulgare*	Leaves Seeds	Aqueous extract	400 µg/mL at 72 h 250 µg/mL at 72 h	[40]
Egypt	*In vitro*	*Artemisia judaica,* *Achillea fragrantissima*	Aerial parts Aerial parts	Ethanol extract	4000 µg/mL at 72 h	[42]
Egypt	*In vitro*	*Syzygium aromaticum*	Cloves	Essential oil	300 µg/mL at 24 h, 200 µg/mL at 48 h, 100 µg/mL at 72 h & 50 µg/mL at 96 h	[41]
Iraq	*In vitro*	*Cucurbita pepo* (pumpkin seed)	Seeds	Aqueous extract	200, 400 µg/mL	[74]

Note: *ND: Not detected.

The ability of eugenol to suppress cysteine protease activity in *Blastocystis* sp. could explain anti-*Blastocystis* effect of *Syzygium aromaticum* essential oil [70]. The potential of *Syzygium aromaticum*-eugenol-rich essential oil to break the cell wall, induce cell leaks, promote cell permeability, and cause cell shrinking [71] could also explain the effect of *Syzygium aromaticum* on *Blastocystis* growth and viability.

3.14. Anti-*Blastocystis* Activity of Amygdalin and *Cucurbita pepo*

Amygdalin, also known as vitamin B17, is a naturally occurring compound found in the seeds of apricots, almonds, cherries, peaches, and plums. It has a number of medical properties, including the ability to protect the digestive tract from microbe-caused infections [72]. *Cucurbita pepo* (pumpkin seed), one of the most flavorful plants, has medicinal characteristics due to its Phyto-constituents, which include alkaloids, flavonoids, fatty acids, and various amino acids as palmitic, oleic, and linoleic acids. Pumpkin seed has long been used as a traditional medicine to treat gastrointestinal parasites [73]. Pumpkin seed and Amygdalin could be used as natural therapies for *Blastocystis* STI infections based on their possible therapeutic properties. Salman and Ardalan [74] evaluated the *in vitro* effectiveness of amygdalin (200, 400 µg/mL) and pumpkin seeds (200, 400 µg/mL) against clinical isolates of *Blastocystis* subtype 1 from symptomatic patients at different incubation times compared to metronidazole. Both amygdalin and pumpkin seed demonstrated significant suppression in *Blastocystis* growth in all incubation durations (1, 2, 24, 48 hours), depending on the concentration employed and the highest effect came from amygdalin. After 48 hours, the highest dose of amygdalin (400 µg/mL) resulted in complete growth inhibition of *Blastocystis*, which was comparable to metronidazole at 150 µg/mL.

As shown in the previous studies, using such medicinal plants enhances the chances of discovering innovative drugs with high efficacy against *Blastocystis* sp. and the potential to reduce the side effects caused by chemotherapy agents (Table **1**).

CONCLUSION

Treatment of *Blastocystis* infection exacerbates the problem of anti-*Blastocystis* therapeutic drug adverse effects, as well as the possibility for resistant strains to emerge. According to the results of *in vitro* and *in vivo* experiments, medicinal herbs may be effective agents in the treatment of

Blastocystis infection. As a result, several research works will be required to evaluate, and standardize the doses of these natural compounds, recognize the involved mechanisms, and toxic effects on animal models and establish the efficacy of their biological effects. Clinical trials will also be conducted to determine the safety and efficacy of these plants in the prevention and treatment of blastocystosis.

LIST OF ABBREVIATIONS

GTE = Green Tea Extract

IBS = Irritable Bowel Syndrome

MTZ = Metronidazole

STs = Subtypes

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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