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Medicinal plants as a source of antiparasitics: an overview of experimental studies

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ABSTRACT

Despite advances in modern human and veterinary medicine, gastrointestinal (GI) parasitic infections remain a significant health issue worldwide, mainly in developing countries. Increasing evidence of the multi-drug resistance of these parasites and the side effects of currently available synthetic drugs have led to increased research on alternative medicines to treat parasitic infections. The exploration of potential botanical antiparasitics, which are inexpensive and abundant, may be a promising alternative in this context. This study summarizes the *in vitro*/*in vivo* antiparasitic efficacy of different medicinal plants and their components against GI parasites. Published literature from 1990–2020 was retrieved from Google Scholar, Web of Science, PubMed and Scopus. A total of 68 plant species belonging to 32 families have been evaluated as antiparasitic agents against GI parasites worldwide. The majority of studies (70%) were conducted *in vitro*. Most plants were from the Fabaceae family (53%, $n = 18$). Methanol (37%, $n = 35$) was the most used solvent. Leaf (22%, $n = 16$) was the most used plant part, followed by seed and rhizome (each 12%, $n = 9$). These studies suggest that herbal medicines hold a great scope for new drug discoveries against parasitic diseases and that the derivatives of these plants are useful structures for drug synthesis and bioactivity optimization.

KEYWORDS

Antiparasitic; gastrointestinal parasites; natural medicine; parasitic drug resistance; plant compounds

1. Introduction

Gastrointestinal (GI) parasitic diseases have become a significant health problem affecting billions of humans and livestock worldwide, particularly in developing countries. An estimated 3.5 billion people are affected globally, while 450 million are symptomatic, and more than 200,000 annual deaths are reported [1]. Diseases caused by GI parasites in animals also create severe disease burdens and significant economic losses associated with food production in many regions of the globe [2,3]. For example, the estimated annual cost of GI parasitic diseases in the Australian sheep industry is AUD 436 million [4], and in the European ruminant livestock industry is more than €1.8 billion [5].

The most important GI parasites in humans consist of both helminths and protozoans. The main helminth infections are those transmitted through contaminated soil (soil-transmitted helminths or geohelminths). They include *Ascaris lumbricoides* (commonly called roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale* (old world hookworm), and *Necator americanus* (new world hookworm) [6]. Approximately 1.5 billion people (24% of the world's population) are infected with at least one of these helminths during their life span; they are among the most common infectious disease agents reported in humans [7]. The estimated number of *A. lumbricoides* (roundworm) infections exceeds a billion, while *T. trichiura* and *N. americanus* account for 795 million and 740 million infections, respectively [8].

Protozoan parasites infect about one-third of the human population worldwide [9]. *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*), *Entamoeba histolytica*, and *Cryptosporidium* spp. are the most common GI protozoan parasites reported in humans [10]. Given the high prevalence in people mainly in resource-poor areas, both *G. duodenalis* and *Cryptosporidium* spp. were included in the WHO Neglected Diseases in 2004 [11,12]. Further, the rising incidence of giardiasis in developed nations has led to its designation as a reemerging infectious disease [13]. As shown in epidemiological studies, the prevalence of *G. duodenalis* varies from 2–5% in developed countries to 20–30% in developing countries [14,15]. The World Health Organisation annually notifies approximately 50 million cases of *E. histolytica* with invasive diseases such as amoebic liver abscesses [16]. Other accounts claim that *E. histolytica* causes 55,000 deaths annually, making it the second most common cause of death from infectious parasitic diseases [17]. Of

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the 46 *Cryptosporidium* spp. characterized, *C. hominis* and *C. parvum* are the most frequently detected species in humans and are responsible for approximately a million deaths yearly [18]. The approximate overall *Cryptosporidium* prevalence in HIV/AIDS patients is 8.69% [19].

Each of these enteric parasites has a direct life cycle. Human-to-human transmission mainly occurs through the fecal-oral route, during which cysts/oocysts or eggs are shed in human feces. Lack of environmental sanitation, contaminated food and water, poor public and personal hygiene standards, and poor socio-economic and demographic conditions promote the spread of such parasites [20], [21].

Gastrointestinal parasites can severely affect the health and nutritional status of the host and cause a range of adverse health problems [22,23]. For instance, soil-transmitted helminths cause a broad spectrum of illnesses, including maldigestion and malabsorption, impaired growth, and anemia [24,25]. Anemia and micronutrient deficiencies, including iron, vitamins, and folate, can lead to reduced work capacity, poor cognitive function, and pregnancy disorders [26,27]. Tissue migration of the larval stages of roundworms and hookworms leads to acute dermatitis or eosinophilic pneumonitis. Colonic bleeding and severe dysentery-like syndrome can occur due to trichuriasis [28]. In addition to these morbidities, deaths have also been reported due to acute infections with soil-transmitted helminths [29].

Giardia duodenalis and *E. histolytica* infections usually cause diarrhea or dysentery and a complex of symptoms such as stomach ache, cramps, bloating, tenderness, nausea, and weight loss [30]. *Giardia duodenalis* generally affects the small intestine (mainly the duodenum), leading to nutritional deficiencies and significant morbidity and mortality, especially among children, the elderly, travelers in developing countries and immunocompromised patients [31,32]. *Entamoeba histolytica* infects the large intestine, and amoebic abscesses from the large intestine can spread to the liver, pleura, pericardium, and brain [33].

Infection with *Cryptosporidium* spp. is usually self-limiting in immunocompetent people; however, it can be devastating to immunocompromised individuals. In HIV-positive patients, symptoms may include chronic or protracted diarrhea that can become life-threatening. These patients may develop extraintestinal manifestations, spreading to other sites, including the gall bladder, biliary tract, pancreas, and pulmonary system [34,35]. In developing countries, malnutrition in children can lead to significantly higher rates of infection [36], and a single episode of *C. parvum* infection during the first two years of life can result in growth deficits [36,37].

Gastrointestinal helminths are a significant cause of mortality and morbidity of ruminants and are responsible for high costs and production losses throughout the world [38]. For example, the annual cost of GI nematode infections in Europe is estimated to be € 686 million [5]. One of the most important GI helminth parasites of livestock is *Haemonchus contortus*, a highly virulent parasite, primarily of small ruminants in tropical, subtropical, and warm temperate regions [39,40]. It is a frequent cause of mortalities in sheep, goats, and other ruminants due to its blood-feeding behavior, which can result in ascites, weight loss, anemia, and death [3,41,42]. The estimated annual global economic loss caused by *H. contortus* to the livestock industry is $30–300 million [3,43] and makes *H. contortus* one of the most economically important parasitic nematodes in its main endemic zones [38,44,45].

Protozoan parasites are also of concern to the livestock industry, with species of *Cryptosporidium*, *Giardia* and *Eimeria* perhaps the most important. *Cryptosporidium* infection affects many animal hosts worldwide, including the main livestock animals such as cattle, sheep, goats, and pigs, causing significant growth and production losses [46]. Neonatal (<6 weeks old) calves are very vulnerable to cryptosporidiosis [47]. Infected animals may develop clinical signs including watery diarrhea, reluctance to feed and dehydration, which in severe cases, can result in death [47,48]. The gut takes a few weeks to recover from the infection and regain its ability to absorb nutrients effectively [48].

Giardia duodenalis infections are commonly reported in livestock worldwide [49]. Young animals have higher prevalence rates than adults due to underdeveloped immune systems [50]. In livestock, giardiasis can cause diarrhea, growth retardation, weight loss, reduced productivity and even death, resulting in significant production losses [50,51]. Subclinical infections can also lead to poor growth and productivity losses in these animals [50].

Coccidiosis, caused by *Eimeria* spp., is an important and widespread enteric disease in livestock ruminants and poultry worldwide, with high morbidities and significant mortalities. Young animals are more vulnerable to infection, and symptoms include diarrhea, dysentery, anemia, dehydration, weakness, anorexia, and emaciation [52]. The worldwide annual economic consequences of coccidiosis in cattle and bison were estimated to be $400 million in 1995 [53]. The recent estimates suggest a total nominal financial cost of £99.23 million per annum, a 2.6–fold increase from the last calculation in 1995 [54]. The cost of coccidiosis to the global poultry industry has been estimated to exceed US$ 3 billion per annum [55].

1.1 Conventional management of gastrointestinal parasites

Chemotherapy remains the mainstay for the control of all GI parasites. However, the use of conventional

antiparasitic drugs is often inhibited due to limitations in availability, side effects of treatments, or parasite resistance.

1.1.1. Limitations in availability

Controlling GI helminths is achieved by periodic deworming with one of several drug compounds. Benzimidazoles (mebendazole and albendazole) along with pyrantel pamoate and levamisole, are the most frequently used anthelmintics for soil-transmitted helminths [56]. However, the global coverage of periodic deworming is still not sufficiently meeting target levels in all endemic countries. In 2020, only 42% of children requiring treatment for GI helminths had access to available anthelmintic medicines [57].

Metronidazole, a 5-nitroimidazole derivative, is the gold standard for protozoan parasites such as *E. histolytica* and *G. duodenalis*. Following oral administration, metronidazole is quickly absorbed and penetrates body tissues and secretions such as saliva, breast milk, vaginal secretions, and semen [58]. Therefore, it should be avoided in the first trimester of pregnancy.

There is no effective chemotherapeutic intervention for *Cryptosporidium* species, even though several compounds have been tested for their anti-cryptosporidial effects [59]. Currently, nitazoxanide, the only drug recommended by the United States Food and Drug Administration (FDA), has some promise in immunocompetent people [60]. However, it does not benefit malnourished children and immunocompromised patients with cryptosporidiosis [61,62]. Paromomycin and azithromycin are other alternatives that have been used. However, these drugs have not been approved for cryptosporidiosis.

Managing livestock helminthic infections is based mainly on the preventive or curative use of chemotherapeutics. Several anthelmintic groups with distinct mechanisms, such as benzimidazoles, imidazothiazoles and macrocyclic lactones, are available for blood-feeding parasites like *H. contortus* [63].

However, no licensed therapeutics are currently available to prevent cryptosporidiosis in livestock [64]. Similarly, there are no approved drugs for *G. duodenalis* in livestock, although anthelmintic drugs, such as albendazole and fenbendazole, have been used effectively [50]. Numerous anticoccidial drugs have been developed, tested, and used to prevent or reduce ruminant production losses, but none are 100% effective.

1.1.2 Side effects of treatments

Long-term use and sometimes high-dose treatment regimens can be toxic to the host. For instance, high doses of mebendazole induce alopecia, allergic skin reactions, hepatitis, headache, vertigo, and oligospermia. Reversible bone marrow suppression with neutropenia has also been reported [65].

The prolonged use and high doses of metronidazole can lead to side effects such as a metallic taste in the mouth, dry mouth, headache, glossitis, and nausea [30,66]. Rare adverse effects caused by the drug include thrush, vertigo, neutropenia, seizures, and encephalopathies due to the toxic effects on the central nervous system. Metronidazole has shown mutagenic action in bacteria. Its carcinogenic activity has been evident in mice and rats at high doses over prolonged periods [67,68]. However, its mutagenicity has never been reported in humans [69].

There are several examples of a reduction in the efficacy of drug treatments over time. Low cure (7.6%) and egg count reduction (52.1%) rates were observed in school children treated with mebendazole in Pemba Island, Zanzibar [70] compared to the results before exposure to treatment, in which mebendazole had a comparatively high cure rate (22.4%) and egg reduction rate (82.4%) [71]. No reduction in the number of *N. americanus* eggs was observed after treatment with a single dose of mebendazole (500) mg in patients in Mali [72]. The failure of pyrantel (10 mg/kg) in treating human *A. duodenale* infections was reported in Australia [73]. Treatment of *T. trichiura* with single oral doses of current anthelmintics is unsatisfactory. Poor efficacy of single-dose albendazole against *T. trichiura* was reported in school children in Jimma Town, Ethiopia [74]. Reduced single-dose albendazole (400 mg) efficacy against *A. lumbricoides* is reported in school children in Rwanda [75].

Both paromomycin and azithromycin were reported to have partial efficacy in *C. parvum* infections in HIV/AIDS patients [76,77]. Nitazoxanide, the FDA-approved drug for human use, did not show a therapeutic effect in immunocompromised patients [61]. The efficacy of metronidazole is variable; with evidence, metronidazole alone is often insufficient to eliminate amoebic cysts from the colonic lumen [78]. *Giardia duodenalis* trophozoites within cysts are less affected by nitroimidazoles, probably due to the poor penetration of the drug through the cyst wall [79].

These examples of treatment failure may be due to poor patient compliance, incorrect dosage, or inappropriate administration procedures. Still, they may also indicate the development of drug resistance in the parasite population.

1.1.3 Parasite drug resistance

Drug resistance occurs through a genetic change in a parasite population in response to selection by an antiparasitic drug that impairs the treatment and control of parasitic infection [80]. In contrast to human GI parasites, drug resistance is now a well-established fact in parasites of livestock. The often excessive and frequent use of the same drug compounds for controlling parasites in livestock has led to high resistance levels,

threatening the sustainability of livestock industries [81].

Anthelmintic resistance is an escalating problem in sheep, goats, and horses in industrial livestock systems worldwide [82,83]. *Haemonchus contortus*, for instance, has shown a remarkable ability to develop resistance to all major anthelmintic drug classes worldwide [63,84]. The resistance has appeared in most cases less than ten years of the introduction of each drug group. Resistance of *H. concortus* to benzimidazole was reported as early as 1964 in the U.S.A [85]. Resistant of *H. contortus* to moxidectin, a broad-spectrum antiparasitic, in small ruminants was reported in Europe [86]. Ivermectin and benzimidazole-resistant *Haemonchus spp*. were detected in the sheep flocks in Ontario, Canada [87]. Moxidectin-resistant *H. contortus* populations were identified in sheep farms in Queensland, Australia [88]. Resistance in *H. contortus* to the most recently introduced amino-acetonitrile derivates (monepantel) has also been reported [89]. Ivermectin resistance in horse nematodes was reported in an *in vivo* study in Lithuania [90].

Multiple anthelmintic resistance of sheep and cattle is also a significant concern [91]. Multiple anthelmintic resistance of *H. contortus* to milbemycins and avermectins, moxidectin and doramectin, and fenbendazole has been evident in a sheep flock in Europe [82]. Cattle nematodes resistant to multiple anthelmintic classes were also reported in New Zealand and Argentina [92,93]. Broad-spectrum anthelmintic resistance of *H. contortus* was evident in sheep in Northern New South Wales, Australia [94].

The resistance of GI protozoa, particularly *G. duodenalis*, has been reported in several *in vitro* studies. High *in vitro* susceptibility of *G. duodenalis* isolates to albendazole was reported compared to metronidazole [95]. A laboratory-induced metronidazole-resistant line of *G. duodenalis* was reported, which grows in low, sub-lethal concentrations of the drug [96]. Subsequently, laboratory-induced metronidazole and furazolidone-resistant lines of *G. duodenalis* were established in concentrations of drug lethal to the parent stock [97]. *Giardia duodenalis* cell lines isolated from refractory patients proved metronidazole and albendazole resistant in a mouse model [98]. Resistance of *C. parvum* to the methionyl-tRNA (CpMetRS) inhibitor 2093 was reported in the neonatal dairy calf model after four days of drug exposure [99].

The need for new drug compounds against GI parasites which are safe, effective, and cheap is, therefore, imperative if we are to effectively deal with the current global burdens of parasitic disease in people and livestock. Screening of naturally occurring antiparasitic substances, such as those found in plants, which are inexpensive and abundant, appears as a promising alternative in this context. Plants have been a rich source of bioactive compounds and have been used in traditional medicine for centuries.

1.2. The use of plants in traditional medical systems

Plants have been the basis of many traditional medical systems across the world, such as Indian (Ayurveda), Chinese, and Arabic (Unani) [100]. The therapies of these traditional medical systems are based on the empirical findings of thousands of years, and in some medical systems, these findings have been comprehensively documented [101,102]. These ancient systems of medicine use a mixture of plants or extracts, which comprise hundreds of different constituents with widely differing physiochemical properties.

Ayurveda, the Science of Life, is a comprehensive medical system originating approximately 5000 years ago in India and is considered the most ancient of all medicinal traditions. Its primary emphasis is on the prevention of disease and health promotion. The focus of Ayurveda is to achieve optimal health and well-being through a holistic approach that considers each individual's physical, emotional, mental and spiritual aspects [103]. Several thousand medicinal plants are employed in Ayurveda medicine, many of which have known antiparasitic properties. Some of the most popular plants in the Ayurveda system are *Allium sativum* (garlic), *Alstonia scholaris* (blackboard tree), *Artemisia annua* (sweet wormwood), *Azadirachta indica* (neem), *Centella asiatica* (Gotu kola), *Curcuma longa* (turmeric), *Justicia adhatoda* (Malabar nut) and *Moringa oleifera* (drumstick).

Traditional Chinese Medicine (TCM) is an ancient healing system practised for about 2000 years in China. This medical system is based on two fundamental theories (*Yin-yang*) that govern good health and longevity and five elements (*Wuxing*) that together explain all natural phenomena in the universe, including human beings [104]. These natural phenomena are applied in the diagnosis and treatment of diseases. Medicine is used to restore or maintain the balance between these elements and to grant vital energy [105]. Common plants used in TCM include *Angelica polymorpha* (female ginseng), *Ephedra sinica* (Chinese ephedra), *Ginkgo biloba* (ginkgo), *Paeonia lactiflora* (Chinese peony), *Panax ginseng* (ginseng), *Rheum palmatum* (Chinese rhubarb), *Glycyrrhiza glabra* (licorice), and *Zingiber officinale* (ginger).

The traditional Arabic medical system is also known as Unani or Islamic medicine. The term Unani means 'Greek'. The Unani medical system originated in Greece, and it is believed that this system is based on the experiences of the Greek physicians Hippocrates and Galen [106]. The traditional Unani healers widely used plant, mineral, and animal-origin drugs and have formulated many polyherbal-mineral recipes [106].

Table 1. Traditional uses of medicinal plants to treat veterinary ailments.

Plant scientific name (Common name)	Family	Parts used	Disease target	Country
Azadirachta indica (Neem)	Meliaceae	Leaves	Indigestion, tick infestation and toxaemia	India, Pakistan
Acokanthera oppositifolia (Bushman's poison)	Apocynaceae.	Leaves	Gastrointestinal and external parasites	Africa
Allium cepa (Onion)	Amaryllidaceae	Cloves	Gastrointestinal parasites, foot and mouth disease, poisoning	India
Allium sativum (Garlic)	Amaryllidaceae	Cloves	Gastrointestinal parasites	India
Cannabis sativa (Hemp)	Cannabaceae	Resin	Abdominal pain	India
Datura metel (Thornapple)	Solanaceae	Fresh fruits	Gastrointestinal parasites	China
Mentha spicata (Common mint)	Lamiaceae	Whole plant	Gastrointestinal parasites	
Musa paradisiaca (Banana)	Musaceae	Leaves	Gastrointestinal parasites	Pakistan
Nicotiana tabacum (Tobacco)	Solanaceae	Leaves	Ectoparasites	
Piper nigrum (Black pepper)	Piperaceae	Seeds	Indigestion, diarrhea, flatulence, and poisoning	India
Senna italica (Italian senna)	Fabaceae	Whole plant	Gastrointestinal parasites	Africa
Trachyspermum ammi (Ajwain)	Apiaceae	Seeds	Bloat	India
Trianthema portulacastrum (Black pigweed)	Aizoaceae	Whole plant	Gastrointestinal parasites	Pakistan
Vernonia anthelmintica (Purple fleabane)	Asteraceae	Seeds	Gastrointestinal parasites	India

Allium cepa (onion), *Carum carvi* (caraway), *Crocus sativus* (saffron), *Ferula asafoetida* (asafoetida), *Papaver somniferum* (opium poppy), *Ricinus communis* (castor), *Trachyspermum ammi* (ajwain) are among popular Unani plants.

1.2.1. Ethnoveterinary uses of plants

It is important to highlight that plants are not only used in traditional medicine for human treatment but also used in veterinary medicine to treat a range of ailments in animals. Ethnoveterinary medicine is crucial in many rural areas with limited access to modern drugs. People living in these remote areas rely on traditional therapies to treat domestic animals. Table 1 shows the traditional use of plants to treat veterinary ailments.

1.3 Plant-derived synthetic medicines

The traditional use of plants has led to the isolation of promising lead molecules from plant products and the synthesis of new drugs [107,108]. Isolation of morphine from the plant opium was reported in 1803 and was the first pure, naturally derived medicine [109]. Subsequently, many well-known plant compounds have been identified and synthesized for medicinal use (Table 2). More than 50% of the currently available synthetic drugs are based on plant products [110]. These successful inventions have undoubtedly revolutionized medicine. However, they have undergone a complex, time-consuming, and expensive process. The estimated time from discovering a new drug to reaching the clinic is 12 years, involving more than $1 billion investment in today's context.

1.4 Plants in the antiparasitic drug discovery pipeline

Although medicinal plants have been used to prevent and treat GI parasitic infections in humans and animals for centuries, many such plants lack systematic scientific evaluation for their efficacy, mode of action and active chemicals. More recently, however, an increase in controlled experimental studies has been reported aiming to validate and quantify the antiparasitic activities of plants and plant products used in traditional

Table 2. Plants and their compounds that have led to synthetic medicines.

Botanical source (common name)	Family	Lead compound	Chemical class	Disease target
Artemisia annua (sweet wormwood)	Asteraceae	Artemisinin	Alkaloids	Malaria
Atropa belladonna (belladonna)	Solanaceae	Atropine	Alkaloids	Bradycardia (low heart rate), pesticide poisoning
Catharanthus roseus (rose periwinkle)	Apocynaceae	Vinblastine, Vincristine	Alkaloids	Cancer
Cephaelis ipecacuanha (ipecac)	Rubiaceae	Emetine	Alkaloids	Amoebiasis
Cinchona ledgeriana (cinchona)	Rubiaceae	Quinine	Alkaloids	Malaria
Coffea arabica (Arabian coffee)	Rubiaceae	Caffeine	Alkaloids	Fatigue, drowsiness, and migraine
Colchicum autumnale (autumn crocus)	Colchicaceae	Colchicine	Alkaloids	Gout
Digitalis purpurea (common foxglove)	Plantaginaceae	Digoxin	Phytosterols	Heart failure and cardiac arrhythmias (abnormal heart rhythms)
Ginkgo biloba (ginkgo)	Ginkgoaceae	Ginkgolides	Terpenoids	Ischemic strokes
Justicia adhatoda (Malabar nut)	Acanthaceae	Vasicine	Alkaloids	Bronchitis and cough
Mucuna pruriens (monkey tamarind)	Fabaceae	Levodopa	Amino acid	Parkinsonism
Papaver somniferum (opium poppy)	Papaveraceae	Codeine, morphine	Alkaloids	Moderate to severe pain
Prunus africana (African cherry)	Rosaceae	Sitosterol	Phytosterols	Benign prostate hyperplasia
Salix spp. (willows)	Salicaceae	Aspirin	Alkaloids	Clot-related strokes and cardiovascular disease

medicine [111–113]. In addition, advances in instrumental technology have led to the identification, fingerprinting and chemical characterization of individual compounds in plant extracts [114,115]. For example, separation techniques such as gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been used to separate phytochemicals in crude extracts to identify each specific component [116]. Spectroscopic detection technologies, such as mass spectrometry (MS), infrared spectroscopy (IR), and nuclear magnetic resonance (NMR), have also been used to analyze molecular structures [117]. The combination of these techniques has led to the expansion of medicinal plant analytics. Furthermore, with the rapid development of nanomedicine, nanoparticles have attracted researchers' attention for antiparasitic drug discovery.

This review was undertaken to synthesize the available literature on the efficacy of different plants and active compounds and their potential mode of action against common human GI parasites and parasitic models. An extensive literature search was performed using electronic databases, including Google Scholar, Web of Science, PubMed and Scopus, for published experimental studies that aimed to validate and quantify such antiparasitic plant activities between 1990 and 2020. The following keywords and MESH headings were used. Ayurved* TCM OR Plant* OR Herb* OR Medic* OR 'Plant extracts' OR 'Plants Medicinal' AND Parasit* OR Gastrointestinal Protozoa OR Helminth* AND '*In vitro*' OR '*In vivo*'. All peer-reviewed full-text articles of *in vitro* and *in vivo* studies investigating the antiparasitic effects of a plant or plant compound on any GI parasite capable of infecting humans or appropriate model parasites were included. Plant names, families, plant parts used, solvents used for extraction, parasites targeted, *in vitro* and *in vivo* evaluation methods and outcomes were extracted and summarized.

2. Results and Discussion

This review included 68 research articles published between 1990 and 2020. Overall, 87 plant species belonging to 34 families were screened for their antiparasitic effect against GI parasites. The majority (70%, $n = 50$) were *in vitro* studies. Most plants were from the Fabaceae family (53%, $n = 18$). Methanol (37%, $n = 35$) was the most used solvent. Leaf (22%, $n = 16$) was the most used plant part, followed by seed and rhizome (each 12%, $n = 9$).

2.1. Experimental approaches

2.1.1 *In vitro* and *in vivo* susceptibility assays

Several *in vitro* and *in vivo* screening assays for antiprotozoals and anthelmintics have been developed to evaluate the viability and motility of parasites [118]. Most of the research evidence found in the literature is focused on *in vitro* rather than *in vivo* evaluation of plants against GI parasites. This is mainly due to low cost, higher throughput, quick turnover of results, and ethical approaches to reducing the use of animals [119]. Moreover, *in vitro* studies allow screening against the different stages of parasite life cycles. For example, Hussain et al. [120] tested the *in vitro* anthelmintic activity of the crude aqueous methanolic extract of the plants *Musa paradisiaca* (banana) and *Trianthema portulacastrum* (black pigweed) against both mature female *H. contortus* and their eggs.

Compared to *in vitro* studies, *in vivo* studies are more realistic; however, they are more time-consuming, costly, and challenging to reproduce. *In vivo* studies are designed to evaluate the safety, toxicity, and efficacy of a drug candidate in the host of interest or a model organism. However, most of the *in vivo* studies found for this review have focused on the efficacy of the candidate. Only a few studies have evaluated the acute toxicity of the drug candidate on the host using toxicity assays [113,121,122].

Successful results from *in vitro* tests are advantageous before *in vivo* assays; however, compounds that have shown efficacy *in vitro* may or may not be effective *in vivo* to the same extent. For example, *Thymus vulgaris* (thyme) essential oil was ineffective in reducing the egg production of female *H. contortus* in *in vivo* tests. However, it was effective against the three main life stages of the parasite *in vitro* [112]. Similarly, despite the significant *in vitro* activity, the ethyl acetate extract of husk fiber of *Cocos nucifera* (coconut) showed no activity against *H. contortus* *in vivo* [122]. In contrast, some plants have shown more potency within *in vivo* studies than *in vitro*. For example, *in vivo* efficacy of the aqueous extract of the seeds of *Coriandrum sativum* (coriander) was greater than its *in vitro* efficacy against *H. contortus* [123].

2.1.2 Experimental parasitic models

Some parasites of humans and livestock can be successfully cultured and studied in the laboratory. For example, advanced *in vitro* models have been established to cultivate protozoan parasites in the laboratory. Air-liquid interface culture derived from stem cells [124], 2D cell culture platform using human esophagus squamous cell carcinoma (COLO-680N) cell line [125], and 3D organoid culture using hollow fiber technology [126] have been used to facilitate complete life cycle development and long-term growth of *C. parvum*. *Giardia duodenalis* trophozoites are primarily cultured axenically in a medium supplemented with bile due to the infeasibility of co-culturing parasitic trophozoites and intestinal epithelial cells. A co-culture model of trophozoites and human colorectal adenocarcinoma (Caco-2) cells has been introduced, mimicking the

small intestinal epithelium [127]. This model has provided a viable system for the long-term culture of *G. duodenalis* and helps explore host-parasite interactions [127].

Similarly, the infective L3 stage of *H. contortus* is easy to establish in experimental conditions and can be stored at 4 °C for prolonged periods [128]. Adults of *H. contortus* are relatively large, highly fecund and can stay viable in culture for 2–3 days [128,129]. The adult nematode can be used as a small animal model to screen compounds before assessing them in infected sheep. Most *in vitro* studies in this search have used *H. contortus* to screen anthelmintic drugs.

However, many important GI parasites, especially helminths, cannot be easily cultured in the laboratory and model species have been used for *in vitro* studies of anthelmintics.

2.1.2.1 Non-parasitic models for human and animal parasites.

Free-living worms such as *Pheretima posthuma* and *Caenorhabditis elegans* are often exploited as models of parasitic nematodes of medical and veterinary importance because of their anatomical and physiological similarities [130]. These worms are abundant and can be easily maintained in the laboratory. However, not being parasitic species, they lack the process essential for parasitic life cycles.

Pheretima posthuma (Phylum Annelida; Class Oligochaeta), commonly known as the Indian earthworm, is found mainly in parts of Southern Asia, where it lives in burrows made in moist soil [131]. This worm is considered a model with significant experimental advantages in cellular and molecular biology experiments because of its short life cycle and ability to generate transgenic strains [132].

Caenorhabditis elegans (Phylum Nematoda; Class Chromadorea) is naturally found in temperate soil environments [133] and can be easily maintained in the laboratory on agar plates [134]. *Caenorhabditis elegans* has a short life cycle and can be frozen and stored in liquid nitrogen or at −70 °C, simplifying stock-keeping. Despite its morphological simplicity, *C. elegans* comprises a diversity of complex organs and tissues like muscles, intestine, hypodermis, and a well-established nervous system present in more complex organisms. Because of the complexity of the nervous system, *C. elegans* has been used to study a large variety of behaviors and responses [134,135]. Due to its small genome size, *C. elegans* has widely been used in genetic and molecular research [134]. Its genome has been comprehensively studied and mapped. A recent study revealed that the *C. elegans* genome possesses homologs of about two-thirds of all human disease genes [136].

2.1.2.2 Veterinary parasites as models for human parasites.

As the major helminthic parasites of humans are relatively host-specific and do not efficiently infect laboratory animals [137], helminths of veterinary importance are sometimes used as models in experimental studies of human parasitic diseases. The rodent parasites *Heligmosomoides bakeri* (also known as *H. polygyrus* or *H. polygyrus bakeri*) and the ruminant parasite *H. contortus* are among this group.

Heligmosomoides bakeri is a nematode that has been used as a laboratory model for intestinal nematode infections for over half a century [138]. This nematode is a convenient and manipulable model for hookworm infections in humans and the economically important trichostrongyloid nematodes of sheep and cattle because of similarities to these parasites in its life cycle [138–140]. Unlike other rodent models, *H. bakeri* causes chronic infections and provides a useful paradigm for exploring the host-protective mechanisms in the intestinal mucosa [140]. *Heligmosomoides bakeri* is also helpful in exploring the genetic basis of resistance and immunological aspects of chronic intestinal nematode infections [138].

2.2. Experimental evidence of plant antiparasitic activities

2.2.1 The importance of solvents in the preparation of plant extracts

The discovery of possible plant antiparasitic activities typically begins with extractions containing the active molecule(s). Different solvents are used to separate medicinally active molecule(s) from various plant materials [141]. Generally, the solvents are chosen based on the polarity of the solute of interest; a solvent with similar polarity to the solute will properly dissolve the solute [142]. In the literature, a diverse range of solvents and solvent mixtures such as water (H_2O), methanol (MeOH), ethanol (EtOH), hexane (HEX), dichloromethane (DCM), ethyl acetate (EtOAc), chloroform (CF), petroleum ether (PET), and tetrahydrofuran (THF) have been used to obtain crude extracts. Methanol is the most widely used solvent and is highly effective at extracting high polar phytochemicals (e.g. phenolics and alkaloids), resulting in high extraction yields [143].

The bioactivities of plant extracts depend on the solubility of active molecules in the solvent. In some studies, alcohol or other organic extracts were more potent than aqueous extracts of the same concentration. For instance, Eguale et al. [123] found that hydro-alcoholic extract of *C. sativum* seeds showed better *in vitro* activity against adult parasites of *H. contortus* than its aqueous extract. Similarly, among the hot water, cold water and ethanol extracts of *Cucurbita pepo* (pumpkin) seeds on model nematodes such as *C. elegans* and *H. bakeri*, ethanol extracts demonstrated the maximum diversity of active components and the most significant inhibitory effect on egg-hatching

[144]. In contrast, the aqueous extracts of the plant *Iris kashmiriana* (Kashmir iris) exhibited greater anthelmintic activity than its methanolic extracts under both *in vitro* and *in vivo* conditions against *H. contortus* [145]. This study suggested that favorable results were due to water-soluble active ingredients in the plant extract.

In some studies, different solvents have demonstrated variations in phytochemical profiles of the same part of the plant. For example, flavonoids and tannins were the identified phytochemicals from the ethanolic extracts of the stem of *Piper betle* (betel betle), while only steroids were found in the aqueous extract [146]. The presence of phytochemicals can also vary according to the plant part where they are deposited. Methanolic extracts of *A. indica* seeds were found to contain azadirachtin M and azadirachtin N [147], while methanolic extracts of its flowers have shown the presence of prenylated flavonoids [148].

2.2.2 Plants with potential antiparasitic activities

Several medicinal plants and isolated secondary metabolites have been screened for antiparasitic activity. A complete list of the plants screened against GI parasites *in vitro* and *in vivo* can be found in the supplementary material (Supplementary material Table S1 and S2). In most studies, authors have opted for a serial concentration of plant extracts to test against parasites. Table 3 lists the *in vitro* and *in vivo* measures used to evaluate plant compounds' efficacy against parasites. Some plants that have demonstrated antiparasitic activity in experimental studies are described below.

Allium sativum has been shown to possess potential antiparasitic activity against many pathogens, including *G. duodenalis*, *E. histolytica*, and *H. contortus*. The freeze-dried whole *A. sativum* extract (dissolved in sterile media) has shown inhibitory actions on the *in vitro* growth of *G. duodenalis* (IC_{50} = 0.3 mg/mL) [149]. A clinical study performed on patients suffering from giardiasis showed a rapid reduction of clinical symptoms in the patients treated with *A. sativum* extract compared to metronidazole [150]. A methanolic extract of *A. sativum* has shown moderate *in vitro* antiprotozoal activity against *E. histolytica* (IC_{50} = 61.8 µg/mL) [151]. In another *in vitro* study, the same extract killed 100% of test *H. contortus* at six hours post-exposure [152].

Zingiber officinale has also exhibited antiparasitic activity against both helminths and protozoa. Methanolic extract of its rhizome killed 100% of *H. contortus* at two hours post-exposure in an *in vitro* test [152]. Furthermore, the *in vitro* worm paralysis rate of its methanolic extract on *Ascaridia galli* (86% at 100 mg/mL) was similar to that of the control drug, albendazole (87% at 7.5 mg/mL) [153]. The hydro-alcoholic (70% ethanol) extract of *Z. officinale* led to significantly greater mortality of *P. posthuma* at 50 mg/mL compared to the control drug, piperazine citrate [154]. The reduction of fecal *G. duodenalis* cyst counts and trophozoite counts was significantly more significant in the dichloromethane *Z. officinale* extract-treated albino rats compared to the infected nontreated group [155].

Punica granatum (pomegranate) has demonstrated antiparasitic potential against a wide range of pathogens, including *A. galli* [156], *C. parvum* [157], and *E. histolytica* [151]. The worm mortality of *A. galli* at 25 and 50 mg/mL of *P. granatum* peel ethanol extract was significantly different from the control drug, fenbendazole (at 5 mg/mL) [156]. *Cryptosporidium parvum* infected mice treated with *P. granatum* peel suspension displayed improvement in all parameters tested [157]. A reduction of oocyst shedding was significantly less in *P. granatum* treated mice compared to control (untreated) mice by day 14 post-inoculation (pi) ($P <$ 0.5), and fecal oocyst shedding was undetectable by day 28 pi [157]. A methanolic extract of *P. granatum* was one of the most effective *in vitro* on *E. histolytica* among other tested plants with IC_{50}<30 µg/mL [151].

The expected antiparasitic activity of *A. indica* was investigated by feeding fresh leaves to sheep infected with *H. contortus* [158]. The total worm count in the control group (n = 3242) was significantly higher than in the treated group (n = 1004). However, fecal egg counts (FEC) showed no significant difference between the control and treated groups (p = 0.081). The study

Table 3. *In vitro* and *in vivo* measures for the efficacy of plant compounds against parasites.

Outcome measure	Definition
IC_{50} (Half maximal inhibitory concentration) *	The concentration of drug required for 50% inhibition of the treated population
LC_{50} (Median lethal concentration) *	The concentration of a drug that will lead to the deaths of 50% of the dosed population
EC_{50} (Median effective concentration) *	The concentration of a drug or toxicant which induces a response halfway between the baseline and maximum response
LC_{90} (Maximum lethal concentration) *	The concentration of a drug required to kill 90% of the parasite population
TC_{50} (Median toxic concentration) *	The concentration of a drug which induces 50% cell mortality
ED_{50} (Median effective dose) **	Dosage of a drug that produces a therapeutic effect in 50% of subjects
LD_{50} (Median lethal dose) **	Dosage of a drug required to kill 50% of the parasite population
LD_{90} (Maximum lethal dose) **	Dosage of a drug required to kill 90% of the parasite population

*In vitro.
**In vivo.

suggested that this result could be due to the high initial egg counts and worm burdens of the animals in the control group [158].

The hydro-alcoholic (70% ethanol) leaf extracts of *Anacardium occidentale* (cashew tree), *Psidium guajava* (common guava), *Chenopodium ambrosioides* (Mexican tea), *Stachytarpheta cayennensis* (blue snakeweed), and *Passiflora edulis* (passion fruit) all showed inhibitory effects on the *in vitro* growth of *G. duodenalis* trophozoites [159]. The exhibited *in vitro* giardicidal activity was moderate for *A. occidentale* and *P. guajava* ($250 \leq IC_{50} \leq 500$ µg/mL), high for *C. ambrosioides* and *S. cayennensis* ($100 \leq IC_{50} \leq 250$ µg/mL); and very high for *P. edulis* ($IC_{50} \leq 100$ µg/mL) [159]. A methanolic extract of *C. ambrosioides* had a similar *in vitro* giardicidal activity in a different study ($IC_{50} = 116.10$ µg/mL) [151].

Methanolic extracts from *Acalypha phleoides* (copperleaf), *Cnidoscolus tehuacanensis* (bad woman), *Geranium niveum* (geranium), *Helianthella quinquenervis* (little sunflower) and *Teloxys graveolens* (fetid goosefoot) were also found to possess *in vitro* antiprotozoal activity against *G. duodenalis* protozoa with IC_{50} values ranging from 2.6 to 20.6 µg/mL [160]. The same plants have shown IC_{50} values of 4.6 to 13.7 µg/mL against *E. histolytica* [160].

Calzada et al. [151] tested the *in vitro* effectiveness of several plants used in traditional Mexican medicine against *G. duodenalis* and *E. histolytica*. Methanolic crude extracts of *Chiranthodendron pentadactylon* (monkey's hand tree) and *Annona cherimola* (custard apple) were active against *E. histolytica* with $IC_{50} < 30$ µg/mL. The study included two positive controls, i.e. metronidazole and emetine, and the potency of *C. pentadactylon* extract (IC_{50} 2.5 µg/mL) was close to emetine but far less than metronidazole. Methanolic extracts of *Dorstenia contrajerva* (snakewort), *Senna villosa* (senna), and *Ruta chalepensis* (fringed rue) were the most active plants against *G. duodenalis* trophozoites with $IC_{50} < 38$ µg/mL. The methanolic extracts of *C. nucifera*, *T. vulgaris*, and *Ocimum basilicum* (basil) demonstrated moderate *in vitro* activity with IC_{50} values ranging from 44.1 to 99.8 µg/mL for *G. duodenalis* and from 41.7 to 96.4 µg/mL for *E. histolytica* [151].

Crude aqueous methanolic extracts of *T. portulacastrum* and *M. paradisiaca* have shown significant dose and time-dependent anthelmintic activity *in vitro* and *in vivo* [120]. Methanolic *M. paradisiaca* extract ($LC_{50} = 2.13$ µg/mL) was found to be more potent than *T. portulacastrum* extract ($LC_{50} = 2.41$ µg/mL) in the egg hatch test. Both plant extracts exhibited strong *in vivo* anthelmintic activity compared to the untreated control. However, the *in vivo* maximum fecal egg count reduction of the treated groups was not significantly different from the levamisole treated group [120].

The *in vitro* and *in vivo* anthelmintic activity of the ethanolic extract of *C. pepo* seeds on *A. galli* increased with the dose and treatment time. However, its anthelmintic activity was not significantly different from fenbendazole at all concentrations [156].

The *in vitro* anthelmintic activity of the crude extracts of aerial parts of *Cissus quadrangularis* (veldt grape) and leaves of *Schinus molle* (Peruvian pepper) against *H. contortus* was also dose and time-dependent, with the wormicidal activity of the plant extracts at their highest concentration (10 mg/mL) were 95% and 100%, respectively [161].

2.2.3 Plant combinations for added therapeutic efficacy

In traditional medical systems, plant extracts are often used in combinations. However, the literature has limited evidence for analyzing plant extracts' combined antiparasitic effect. A study that examined combinations of the plant extracts of *Picria fel-terrae* (curanja), *Linariantha bicolor* (true baby-stars) and *Lansium domesticum* (langsat) against *C. elegans in vitro* revealed that most combinations significantly reduced the viability and survival of adult worms showing higher nematocidal and anthelminthic activities compared to the control drugs and the individual plant extracts [162]. The combination of hexanic extract of *A. sativum* and acetone extract of *Tagetes erecta* (Mexican marigold) caused higher mortality of *H. contortus* larvae *in vitro* and a higher reduction of parasite burden in *H. contortus* infected gerbils than the individual plant extracts [163]. While limited, these studies have shown that combining different plant extracts may be more effective than a sole plant extract.

2.2.4 Plant essential oils exhibited antiparasitic activities

Plant essential oils are biomolecules with biotechnological and pharmaceutical importance, particularly anthelmintic activities [164,165]. These are aromatic, highly volatile, hydrophobic liquids produced by different parts of plants as secondary metabolites [166].

Plant-extracted essential oils from *Cymbopogon martinii* (palm rose), *C. schoenanthus* (camel grass), *Mentha piperita* (mint), *P. betle*, *Syzygium aromaticum* (clove), and *T. vulgaris* have exhibited *in vitro* effects against different GI parasites (Table 4).

2.2.5 Plant-derived compounds against parasites

In some cases, researchers have isolated the active biomedical compounds of plants that have antiparasitic effects (Table 5). The identified metabolites are chemically diverse, comprising different chemical families. Phytochemicals are classified as polyphenols, terpenoids, alkaloids, phytosterols, and organosulfur compounds. Alkaloids, terpenoids, and polyphenols are the major biological compounds with medicinal properties [178]. Matrine, oxymatrine, steroids,

Table 4. *In vitro* screening studies of essential oils against gastrointestinal parasites.

Source of the essential oil	Family	Parasite tested	Outcome of the Study	Reference
Cymbopogon martinii (palm rose)	Poaceae	*Haemonchus contortus*	EHA LC_{50} = 0.13 mg/mL[2] LDA LC_{50} = 0.15 mg/mL[2]	[167]
Cymbopogon martinii (palm rose)	Poaceae	*Caenorhabditis elegans*	ED_{50} = 125.4 µg/mL[2]	[168]
Cymbopogon schoenanthus (camel grass)	Poaceae	*Haemonchus contortus*	EHA LC_{50} = 0.045 mg/mL[2] LDA LC_{50} = 0.063 mg/mL[2]	[167]
Mentha piperita (mint)	Lamiaceae	*Haemonchus contortus*	EHA LC_{50} = 0.26 mg/mL[1] LDA LC_{50} = 0.26 mg/mL[1]	[167]
Piper betle (betel pepper)	Piperaceae	*Taenia solium*	Time taken for Paralysis at 0.4% = 11 min[2] Time taken for Death at 0.4% = 19 min[2]	[169]
Piper betle (betel pepper)	Piperaceae	*Bunostomum trigonocephalum*	Time taken for Paralysis at 0.4% = 13 min[2] Time taken for Death at 0.4% = 29 min[2]	[169]
Syzygium aromaticum (clove)	Myrtaceae	*Giardia duodenalis*	IC_{50} = 134 µg/mL[3]	[170]
Thymus vulgaris (thyme)	Lamiaceae	*Haemonchus contortus*	EHA IC_{50} = 0.436 mg/mL[2] LDA IC_{50} = 0.0131 mg/mL[2]	[112]
Thymus vulgaris (thyme)	Lamiaceae	*Entamoeba histolytica*	MIC after 24 h = 0.7 mg/mL[3]	[171]

1= No significant effect; 2 = significant reduction in parasite survival compared to controls; 3 = significant reduction in parasite population growth compared to controls.
EHA= Egg hatch assay; LDA= Larval development assay; MIC= Minimal inhibitory concentration.

polysteroids, withanolides, saponins and sophocarpine are among the alkaloids isolated from plants, while flavonoids, tannin, catechins and phenols are polyphenols, and geraniol and cedrelone are terpenoids.

2.2.6 Nanoparticles as antiparasitic treatments

Nanotechnology has opened up novel dimensions in the field of drug discovery research. This technology involves the synthesis and application of materials that range between 1 to 100 nanometers in size [179]. Nanoparticles (NP) are emerging novel drug carriers which, because of their substantial specific surface area and strong adhesion capacity [180], may overcome shortcomings of the current applications of many antiparasitic drugs; low bioavailability, poor cellular permeability, nonspecific distribution and rapid elimination from the body [181,182].

Table 5. *In vitro* screening studies of plant-derived compounds against gastrointestinal parasites.

Compound	Chemical group	Source of the compound (Common name)	Family	Parasite tested	Outcome of the study	Reference
Hydrolysable tannins	Polyphenols	*Acer rubrum* (Canadian maple)	Sapindaceae	*Caenorhabditis elegans*	LC_{50} = 1.03 mg/mL[2]	[172]
Hydrolysable tannins	Polyphenols	*Quercus alba* (white oak)	Fabaceae		LC_{50} = 0.75 mg/mL[2]	
Hydrolysable tannins	Polyphenols	*Rosa multiflora* (multiflora rose)	*Rosaceae*		LC_{50} = 2.14 mg/mL[2]	
Allyl alcohol	Polyphenols	*Allium sativum* (garlic)	Amaryllidaceae	*Giardia duodenalis*	IC_{50} = 7 µg/mL[3]	[149]
Allyl mercaptan	Organosulfur	*Allium sativum* (garlic)	Amaryllidaceae	*Giardia duodenalis*	IC_{50} = 37 µg/mL[3]	[149]
Bromelain	Polyphenols	*Ananas comosus* (pineapple)	Bromeliaceae	*Haemonchus contortus*	LC_{50} = 0.50 mg/mL[3]	[173]
Gallic acid	Polyphenols	*Anogeissus leiocarpus* (African birch)	Combretaceae	*Caenorhabditis elegans*	LC_{50} = 3.22 mM[2]	[113]
Gentisic acid	Polyphenols				LC_{50} = 6.12 mM[1]	
Ellagic acid	Polyphenols				LC_{50} = 0.085 mM[2]	
Deoxysappanone B 7,4	Polyphenols	*Biancaea sappan* (sappan wood)	Fabaceae	*Cryptosporidium parvum*	EC_{50} = 0.734 µM[1] TC_{50} = 64.9 µM[1]	[174]
Geraniol	Terpenoids	*Cymbopogon martinii* (palm rose)	Poaceae	*Caenorhabditis elegans*	ED_{50} = 66.7 µg/mL[2]	[168]
(–)-Epicatechin	Polyphenols	*Geranium mexicanum* (geranium)	Geraniaceae	*Entamoeba histolytica*	IC_{50} = 1.9 µg/mL[3]	[111]
				Giardia duodenalis	IC_{50} = 1.6 µg/mL[3]	
(+)-Catechin	Catechin	*Geranium mexicanum* (geranium)	Geraniaceae	*Entamoeba histolytica*	IC_{50} = 65.6 µg/mL	[111]
				Giardia duodenalis	IC_{50} = 33.9 µg/mL[1]	
Plumbagin	Polyphenols	*Plumbago* Spp.	Plumbaginaceae	*Caenorhabditis elegans*	LC_{50} = 220 µM for L4; 156 µM for L1[2]	[175]
Condensed tannins	Polyphenols	*Robinia pseudoacacia* (false acacia)	Fabaceae	*Caenorhabditis elegans*	LC_{50} = 0.65 mg/mL[2]	[172]
Seed alkaloids	Alkaloids	*Sophora moorcroftiana* (pea-flowered tree)	Fabaceae	*Caenorhabditis elegans*	LC_{50} = 14.29 mg/mL[2]	[176]
Eugenol	Polyphenols	*Syzygium aromaticum* (clove)	Myrtaceae	*Giardia duodenalis*	IC_{50} = 101 µg/mL[3]	[170]
Thymol	Polyphenols	*Thymus vulgaris* (thyme)	Lamiaceae	*Haemonchus contortus*	IC_{50} = 0.442 mg/mL[2]	[112]
Cedrelone	Terpenoids	*Toona ciliata* (red cedar)	Meliaceae	*Cryptosporidium parvum*	EC_{50} = 0.267 µM[3]	[174]
Asarini	Polyphenols	*Zanthoxylum liebmannianum* (prickly ash)	Rutaceae	*Entamoeba histolytica*	IC_{50} = 19.86 µg/mL[3]	[177]
				Giardia duodenalis	IC_{50} = 35.45 µg/mL[3]	

1= no significant effect; 2 = significant parasite killing effect compared to controls; 3 = significant reduction in parasite population growth compared to controls.

A wide range of plant species has been successfully used in making NPs. Bioactive molecules present in plant extracts, such as alkaloids, phenolic compounds and terpenoids, may act both as reducing agents and stabilizing agents in the synthesis of NPs [183]. Silver (Ag) and gold (Au) NPs have been a specific focus in plant-based syntheses. However, studies evaluating the efficacy of plant-based nanoparticles against intestinal parasites are scarce. Said et al. [184] assessed the antiparasitic potential of curcumin extracted from *C. longa* and silver, chitosan and curcumin NPs against *G. duodenalis in vitro*. Curcumin extract had the least trophozoite reduction rate (13.1%), while curcumin NPs had 54.6% trophozoite reduction. A combination of silver, chitosan, curcumin nanoforms gave 100% reduction of trophozoites [184]. Plant-synthesized NPs show great potential to explore in future research.

2.3. Mode of actions of antiparasitic plants

A detailed examination of their mechanism of action is imperative for the potential clinical application of plants or plant-derived products. Studies exploring the mode of action can be complex as they need to assess every stage of the parasite life cycle to understand the therapeutic potential. Although many studies have focused on the efficacy of medicinal plants and their phytochemistry, evaluation of the mode of action has largely been neglected.

Morphological or motility changes may be used to identify the mode of action of plant products [185]. The tegument/cuticle of helminth parasites has been recognized as one of the main target sites for antiparasitic drugs [186,187]. Morphological changes of *G. duodenalis* trophozoites, including loss of flagellar movement and cell motility, detachment of organisms from the reaction vessel, cell swelling and collapse of the electrochemical membrane potential, were observed after treatment with whole *A. sativum* extract [149]. Similarly, the essential oil of *S. aromaticum* induced several morphological changes, including irregular ventral and dorsal surfaces, precipitates and large vacuoles in the cytoplasm, and intracellular and nuclear clearing in *G. duodenalis* trophozoites [170]. The *in vitro* use of condensed tannin reduced the survival and development of sheep nematode larvae [119]. Similar findings have been observed when the GI nematode larvae are treated with condensed tannin [188].

The mode of action of plant extracts toward human or animal cells is generally explained through the act of secondary metabolites. These metabolites often modulate a corresponding molecular target in cells, such as proteins, biomembranes or nucleic acids [189], leading to disrupted membrane permeability, neurotoxic activity, or antioxidant activity (Figure 1.).

2.3.1 Disrupted membrane permeability

The suggested antiparasitic activity of *A. sativum* may occur because *A. sativum* extracts are rapidly permeable through biological membranes and enhance the membrane permeability to small molecules [190]. *Allium sativum* also can increase intracellular nitric oxide (NO) synthase activity [191]. Nitric oxide has shown cytotoxic effects on *G. duodenalis*, inhibiting growth and encystation of trophozoites and excystation of cysts [192].

Compounds in essential oils like terpenoids (e.g. thymol and geraniol) or flavonoids may also interfere with membrane permeability by disturbing cellular balance, pH, and inorganic ion balance [193,194]. Likewise, it has been reported that the essential oil geraniol found in *O. basilicum*, *C. sativum* and *T. vulgaris* could damage cell membranes, membrane-bound protein activity, and intracellular signaling pathways [195]. Eugenol-rich essential oil of *Ocimum gratissimum* (clove basil) has been found to induce swelling of parasite mitochondrial cell membranes with a significant increase in the number of folds in its inner membrane [196].

2.3.2. Neurotoxic activity

Alkaloid compounds in almost all plant families are known neurotoxic agents and act as agonists or antagonists at neuroreceptors and ion channels [197,198]. Alkaloids can inhibit the acetylcholine receptors (AChR) of multicellular parasites leading to muscular paralysis [198]. As a result, parasitic worms attached to the intestinal walls cannot stick to the walls and can be easily removed from the gut [198].

2.3.3 Antioxidant activity

It has been suggested that the nematocidal activity of phenolic compounds such as thymol is possibly derived from their inherent antioxidant properties [199]. Phenolic compounds are classified as primary antioxidants, and they could form a phenoxy radical upon donating a hydrogen atom that results in antioxidant properties through the radical scavenging mechanism [200]. It has been recently demonstrated that the action of thymol against *H. contortus* is equivalent to that of the anthelmintic drug class macrocyclic lactones [201]. These drugs cause cell hyperpolarisation by enhancing chloride ion reflux, leading to the inhibition of parasite development and larval and adult motility [202,203].

2.4. Limitations of the use of plant-based medicines

Plant-based medicines have promising potential in the prevention and treatment of GI parasitic diseases. However, their application may be limited by bioavailability. The major phytochemicals, including glycosides,

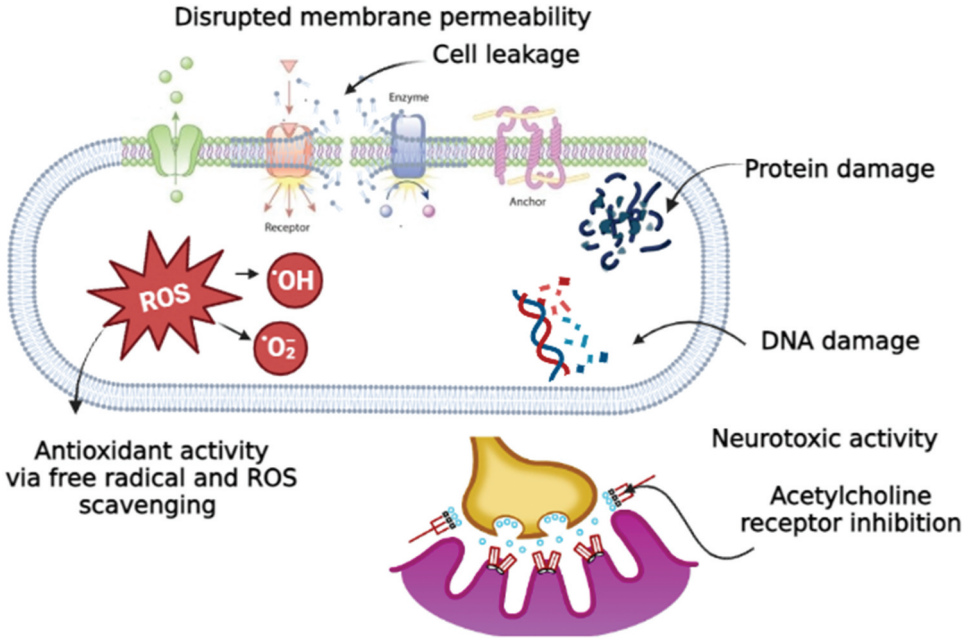

Figure 1. Schematic representation of the mechanism of action and different pharmacological targets of plant extracts/compounds.

tannins and flavonoids, have poor water and lipid solubility, which limits their ability to cross biological membranes, resulting in poor absorption [204]. Additionally, the pharmacokinetics of these compounds can be further modified by the highly acidic gastric pH [205]. In addition, plants are subjected to different procedures such as extraction, distillation, purification, concentration or fermentation to obtain bioactive compounds. During these processes, active components are exposed to oxidation and hydrolysis, raising concerns about their stability [206]. Furthermore, plant products are often prone to deterioration, particularly during storage, leading to the loss of active components and the production of metabolites with no activity [207].

With the increasing global use of plant-based medicines, concerns related to their safety are also increasingly identified. Even though plants have promising potential and are extensively used, many of them remain unproven for their safety or toxicity. This leads to limited knowledge of their potential adverse effects and difficulties in the identification of the safe and most effective therapies [208].

3. Conclusion and future perspectives

This review focused on studies that have evaluated plants and plant derivatives as antiparasitic agents in searching for novel drugs and lead compounds. Plants or their isolates have been tested against almost all common GI parasites, although only a few studies are available on *Cryptosporidium* species. Overall, the results of these experimental studies present valuable information on bioassays which can help design future studies in terms of methods, doses, and experimental models. This review found plants and plant-derived compounds with significant *in vitro* and *in vivo* effects on GI protozoan and helminth parasites. Some plant extracts have shown similar effects to broad-spectrum antiparasitic drugs. The traditional use of plants provides crucial evidence for identifying and developing synergistic drugs; however, this aspect needs to be explored further.

The studies reviewed here encourage further investigation of plants or plant derivatives as potential origins for novel therapies for GI parasitic infections. Most importantly, searching for natural alternatives is critical for parasites like *Cryptosporidium* spp., which do not have effective chemotherapeutics. However, these studies also point to several areas requiring further information. Most of the studies in the literature invite future research to determine the optimal dose to maximize the effectiveness of the examined plants. Conversion of the *in vitro* research results into *in vivo* trials is also essential. Moreover, clinical trials on successful animal experiments are required using the new compounds alone or with established antiparasitic drugs to prove their efficacy and safety. Future research should also focus on exploring the combined effect of plant extracts against parasites. More in-depth studies are required to evaluate the molecular mechanisms of these plant extracts and their bioactive components.

Plant products inspire synthesizing analogues with enhanced pharmacological properties, leading to new drug candidates in the development pipeline. However, many plants with claimed antiparasitic properties have not been reproduced under experimental conditions. Many such unexamined plants may be a source of valuable pharmacologically active substances against parasites and invite future research.

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