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REVIEW ARTICLE

A Review on the Use of Phytochemicals for the Control of Zoonotic Giardiasis

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ABSTRACT

Giardiasis is among the most prevalent protozoan infections around the globe infecting various species of mammals, birds, reptiles, amphibians, and humans. Among all the species of Giardia, only *Giardia lamblia* (assemblages A and B) have high zoonotic importance. It is an enteric disease marked by dysentery, abdominal cramps, perfused diarrhea, and anorexia. The giardiasis is majorly controlled by metronidazole, which plays a primary role in the control. The reports of resistance, ecotoxicity, and side effects of this drug necessitate the need for an alternative for the control of this disease; among all the alternatives, phytochemicals are the most promising substance to be used for future anti-giardiasis drug development. Plant preparations containing simple phenolics, alkaloids, saponins, flavonoids, and vitamins have been recently used by researchers. These drugs have been proven effective because of several direct and indirect mechanisms. Simple phenolics easily penetrate the cell of Giardia and disturb energy synthesis, flavonoids destroy the enzymatic process, and alkaloids disturb glycolysis. The vitamins alter the cell energy primarily. Because of these actions, they can be used for control of giardiasis. However, their pharmacological interactions and clinical toxicity studies are needed for their future use.

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INTRODUCTION

Giardiasis is a parasitic disease caused by multiple species of the genus Giardia (Ma'ayeh and Svärd, 2024). This flagellate protozoan is known for its typical two nuclei in the trophozoite stage of the cell (Ryan *et al.*, 2021; Suo *et al.*, 2021). The life cycle of Giardia consists of two stages i.e., cyst and trophozoite, requiring no intermediate hosts (Silva and Sabogal-Paz, 2021; Benchimol *et al.*, 2022b) (Fig. 1). Multiple species of this genus remain infecting the vertebrate hosts. Giardia is among the parasites that have the most diverse host spectrum (Moratal *et al.*, 2020; Jones and Tardieu, 2021). Although several species of Giardia have been reported in animals and humans (Aliyi and Yusuf, 2023), mainly *Giardia lamblia* (*G. duodenalis*; *G. intestinalis*) is the most important species of zoonotic importance (Adam, 2021; Wielinga *et al.*, 2023). *G. lamblia* is subdivided into 8 subgroups (Table 1) called assemblages A, B, C, D, E, F, G, and H (Agresti *et al.*, 2021; Fantinatti *et al.*, 2023). All the assemblages have been reported in humans (Mahmoudi *et al.*, 2020), but assemblages A and B mainly infect humans and have a variety of hosts making them the most threatening among all the hosts (Zajaczkowski *et al.*, 2021). Both assemblages A and B have been subdivided into multiple sub-assemblages, but they have a mixed trend in humans (Iwashita *et al.*, 2021). *G. lamblia* is found globally in people of all ages, in multiple species and all the assemblages of this species have zoonotic importance (Bahramdoost *et al.*, 2021). This disease is mainly found in poorly developed areas, rural areas, and areas of low sanitation and living quality (Hajare *et al.*, 2022). Although rarely fatal, this disease can pose significant complications in humans and animals (Allain and Buret, 2020). Its complications may be lifelong, and the risks of zoonosis make it a disease to be controlled on a priority basis.

Giardiasis is a highly prevalent disease, so its treatment is the priority of practitioners (Rivero *et al.*, 2020). Metronidazole is the most used antibiotic drug for the control of giardiasis around the globe (Loderstädt and Frickmann, 2021). Although multiple other drugs including tinidazole, nitazoxanide, etc. are being practiced, metronidazole is among the most preferred drug. Metronidazole is still the drug of choice and stands as the first line of defense against giardiasis (Hu *et al.*,

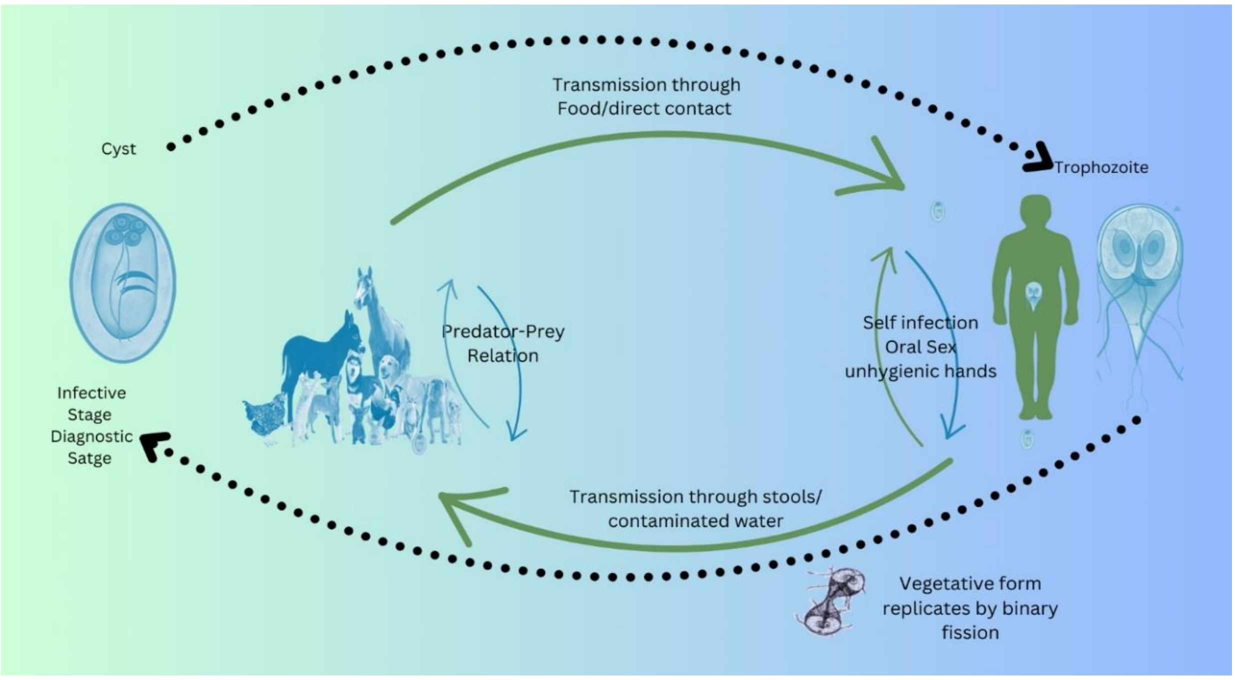

Fig.1: The zoonotic transmission pattern and life cycle of *Giardia.*

Table 1: Species and assemblages of *Giardia* with zoonotic potential with their common hosts.

Sr. No	Species	Assemblages	Host	References
1.	*G. lamblia*	A (AI, AII, AIII, AIV)	Primate humans, canines, felines ruminants	(Alasadiy *et al.*, 2022)
2.		B (BIII, BIV)	Primate humans, canines, felines ruminants	(Fantinatti *et al.*, 2020)
3.		C	Canines	(Wielinga *et al.*, 2023)
4.		D	Canines	(Alasadiy *et al.*, 2022)
5.		E	Ruminants	(Fantinatti *et al.*, 2023)
6.		F	Felines	(Peng *et al.*, 2020)
7.		G	Rats and mice	(Eslamirad *et al.*, 2022)
8.		H	Fishes and other water vertebrates	(Pacheco *et al.*, 2020)
9.	*G. ardeae*		Birds	(Adam, 2021)
10.	*G. muris*		Rodents	(Sursal and Yildiz, 2020)
11.	*G. agilis*		Amphibians	(Lyu *et al.*, 2020)
12.	*G. psittaci*		Birds	(Reuschel *et al.*, 2020)
13.	*G. microti*		Rodents	(De Liberato *et al.*, 2021)

Table 2: Phytochemicals and their mechanisms of action to control giardiasis in various experiments.

Sr. No	Plant	Compounds	Experiment mode	Species of *Giardia*	Stage		Mechanism of action	Results	References
1.		Quercetins	*In vivo*	*G. lamblia*	Trophozoite	Rats	Direct antiparasitic activity and oxidative stress management	Giardiasis was controlled	(Albogami, 2023b)
2.		Kaempferol	*In silico*	*G. lamblia*	Trophozite model	--	Interrupts with synthesis of DNA and induces death via apoptotic mechanisms	Kaempferol can control giardiasis by its direct antiprotozoal mechanisms	(Argüello-García *et al.*, 2020a)
3.	*Cymbopogon citratus*	Flavonoids	*In vivo*	*G. lamblia*	Trophozoite	Rabbits	Cell permeability disturbance and disturbance with energy production mechanisms	Flavonoids have more potency than metronidazole and efficacy increase when added with gold nanoparticles	(Al-Badry *et al.*, 2023)
4.	*Psidium guajava*	Quercetine	*In vivo*	*G. lamblia*	Trophozoite	Swiss albino rats	Damage to the internal structure of Giardia, reduction in oxidative stress and reduction in inflammation	Reduction in the signs of giardiasis	(Khedr *et al.*, 2021)
5.	*Cryptolepis sanguinolenta*	Isocryptolepine	*In vitro*	*G. duodenalis*	Cyst and trophozoite	-	Glycerophospholipid metabolism was involved	Isocryptolepine has potent antigardail activity	(Popruk *et al.*, 2024)
6.	-	Eucalyptol, 1,8 cineol	*In vitro* and *in vivo*	*G. lamblia*	Trophozoite	Mice	Increased permeability of cell membrane and reduction in expression of infectivity-related genes	Eucalyptol and 1,8 cineol were effective against *G. lamblia* trophozoite	(Masoori *et al.*, 2024)
7.	*Curcuma zeodaria*	Curzerenone, 1,8 cineol, Terpenoids, Essential oils	*In vivo* and *in vitro*	*G. lamblia*	Cyst and trophozoite	BALB/c Mice (Male)	Effected the growth rate of trophozoites, and stopped excystation from cysts	The essential oil was effective in treating giardiasis	(Albalawi and Alanazi, 2023)

2024), but some problems are demanding research to find suitable alternatives. Giardia is reported to have developed several mechanisms of resistance against metronidazole (Krakovka *et al.*, 2022). Resistance poses a great threat to the future use of metronidazole and other drugs used for the treatment of giardiasis frequently (Jain *et al.*, 2023). Researchers are frequently reporting resistance against metronidazole in several hosts (Krakovka *et al.*, 2022). Besides the resistance, another ported problem is the side effects. Metronidazole is reported to induce abdominal cramps, nausea, etc. (Karrar *et al.*, 2021; Starrs and Yenigun, 2021). Moreover, ecotoxicity is also a major problem for its use because its residues are severely ecotoxic and cause lethal effects on several symbionts (Li *et al.*, 2023). Metronidazole is also threatening for normal microflora of the intestine and causes several problems in the intestine (Shah *et al.*, 2021). Vaccination of Giardia spp. is being tried but the diversity of species and variation in assemblages make it technically difficult to be implemented (Klotz *et al.*, 2023). This situation is focused on the need for alternative control measures for giardiasis.

The researchers are focusing on the use of various drug candidates for the control of giardiasis including organic acids, botanicals, etc. (Praseetha *et al.*, 2023). Phytochemicals are among the most suitable candidates for the treatment of giardiasis (Ullah *et al.*, 2020). Phytochemical compounds of various classes are being used as therapeutic agents for various infectious diseases including protozoan diseases (Batiha *et al.*, 2020; Saeed and Alkheraije, 2023). Phytochemicals have safe mechanisms of action and have reported ecofriendly effects (Ahmad *et al.*, 2020). The phytochemicals are present in the various parts of plants and can be extracted easily (Abbas *et al.*, 2023; Bitwell *et al.*, 2023). Recently multiple researchers have suggested the use of phenolics, flavonoids, vitamins, and other compounds for the control of giardiasis in humans and animals (Bhattacharyya, 2021). These phytochemicals have several direct and indirect mechanisms and have diverse mechanisms of action so they can perform effective control because of this diversity of activities (Wink, 2022). This review highlights the pathogenicity of giardiasis, mechanisms to control giardiasis, and the potential of phytochemicals to control giardiasis.

Life Cycle and Pathogenesis of Giardia: The life cycle of *G. lamblia* begins with the ingestion of cysts of the parasite (De Liberato *et al.*, 2021). The cyst of Giardia is an oval structure that has thick walls (Benchimol, 2021). There are four nuclei in each oocyst of Giardia, flagella in retracted forms, and axonemes (Hardin *et al.*, 2022). The cyst contains median bodies that possess a bar-like appearance and are considered precursor forms of the ventral disc (Gadelha *et al.*, 2020). A dense cytoplasm is also present in the cyst of Giardia (Benchimol *et al.*, 2022b). The cyst is ingested with contaminated water, raw or uncooked food, or other routes of oral transmission (Gabriël *et al.*, 2022). The excystation process is mainly triggered by the actions of gastric acids (Lagunas-Rangel *et al.*, 2021; Klimczak *et al.*, 2024). In the small intestine, bile salts and pancreatic secretions, especially trypsin, play a vital role in the excystation of its trophozoites and internally some proteases are also involved which leads to the completion of the excystation process (Fekete *et al.*, 2022). Meanwhile, genetic changes also occur, and the trophozoite-associated genes replace cyst-associated genes (Heller *et al.*, 2020; Chen *et al.*, 2021). The cell wall of the cyst is removed, and water efflux is facilitated which helps the formation of trophozoite (Rojas *et al.*, 2022). Each cyst gives rise to two trophozoites, each having two nuclei and a set of flagella (Lagunas-Rangel *et al.*, 2021).

The trophozoite of *G. lamblia* is free swimming protozoa having 8 flagella, making it easy to swim in the intestinal environment (Fink *et al.*, 2020). The flagella are attached in pairs on the anterior, posterior, and ventral sides (Gadelha *et al.*, 2020). It is a unicellular organism that can be easily identified by the presence of its two nuclei giving a typical smiling face shape to the organism (Ranjbarian *et al.*, 2023). Its characteristic structure is its ventral adhesive disc which helps assist the adhesion of giardia trophozoite to the intestinal epithelium (Gadelha *et al.*, 2022). The energy production of the *G. lamblia* is done by specialized structures called mitosomes instead of mitochondria which are normally present in the eukaryotic cells (Benchimol *et al.*, 2022a). The additional structures that are present are axonemes (cytoplasmic extensions of flagella) (Hagen *et al.*, 2020) and median bodies (bar-shaped structures responsible for the ventral disk evolution) (Verdan *et al.*, 2024). The ventral disc of the Giardia plays a critical role in the pathology as it counters the defense mechanisms of the host.

G. lamblia remain free swimming in the epithelium and their attachment is facilitated by the ventral disk (Nosala *et al.*, 2020). The ventral disk is a cup-like structure which acts like a suction pump and creates a physical suction between the parasite and the host (Gunaratnam *et al.*, 2024). The main pathogenesis is its attachment to the epithelial cells of the intestine which replaces normal microflora of the intestine (Dysbiosis), causes injury, and leads to the induction of apoptosis, creating an anaerobic environment (Adam, 2021). *G. lamblia* has no specific toxins that may be responsible for any extra toxicities; however, some secretions are present that contribute to its pathogenesis. These are surface glycoproteins (development of fluidity in the intestine) (Elias *et al.*, 2020), lectins and proteinases (Direct injury induction to epithelial cells) (Martínez-Ocaña *et al.*, 2020), and enterotoxins (they lead to depletion of Chloride ions from the intestinal lumen) (Barroeta-Echegaray *et al.*, 2022). Because of these mechanisms they lead to damage of intestinal villi, induce inflammation, and deprivation of nutrients (Solaymani-Mohammadi, 2022). *G. lamblia* replicates rapidly, causing intense inflammation and decreasing the host symbiotic organisms, leading to malabsorption, epithelial sloughing, and intestinal problems (Ruwandeepika *et al.*, 2023). These mechanisms need to be controlled to control the impacts of giardiasis.

The trophozoites of Giardia multiply by binary fission consisting of mitotic divisions repeatedly (Li, 2022). They remain multiplying to an unpredictable number of cycles and in unsuitable environments, the encystation process is started. The cysts of Giardia are released into the environment, where they can reside for a long time (Silva and Sabogal-Paz, 2021). They can be ingested by any species and remain multiplying either or without producing the signs of disease depending upon the ingesting organism and types of *G. lamblia* (Smith, 2020). They release excess to humans through food or water, and the cycle continues.

Phytochemicals for the control of Giardiasis: Phytochemicals are the organic compounds present naturally in plants (Awuchi, 2020). These chemicals are produced in the plants either for defense purposes or as the natural component of their products (Twaij and Hasan, 2022). The phytochemicals show various medicinal properties because of their ability to interact with the body easily (El-Beltagi *et al.*, 2022). Compounds of various groups from the plant have been used for the control of giardiasis in the *in vivo* and *in vitro* experiments (Calzada and Bautista, 2020; Alnomasy *et al.*, 2021). In this section, we will review various classes of Phytochemicals present in the plant that can control Giardia and have potential anti-Giardia mechanisms of action. Various important classes and their mechanisms are discussed below.

Simple Phenolics: Phenolics are the most found chemical containing phenolic rings in their structure present in various parts of plants around the globe (Kumar *et al.*, 2020). Phenolics are among the major groups that have prominent medicinal properties (Pinto *et al.*, 2021). Simple phenolic compounds are those compounds that contain a single phenolic ring in their structure (Hu *et al.*, 2022). They show high medicinal efficacies because of their low molecular weight and rapid actions and diffusion in the cells. Phenolics can control giardiasis by several direct or indirect mechanisms (Palomo-Ligas *et al.*, 2022).

Phenolics affect the trophozoite stage of Giardia and show direct activity because of several mechanisms (Garza-Ontiveros *et al.*, 2024). Multiple compounds belonging to simple phenols, i.e., coumarins, ferulic acids, kaempferols, etc. have a high affinity to attack the cell membrane of various microbes, including Giardia. They interact with lipid bilayers and get attached to them either physically or chemically, altering their structure, thus leading to abnormal functioning (Xavier *et al.*, 2022). The cell permeability is lost, and the vital functions of the cell are lost because of disturbed nutrient intake and loss of vital ions from the cytosol. Moreover, they interact with the proteins present in the cell membrane and disturb their transportability and inhibit their physiological role inside the body (Palomo-Ligas *et al.*, 2023). Phenolics can also interact with multiple other processes in which they can control giardiasis.

Energy synthesis mechanisms are the main functions that are associated with the viability of the cells of microbes, especially Giardia. The simple phenolics attack various portions of the energy production of Giardia and control the ATP production within the trophozoite (Adetunji and Oyeyemi, 2022). Energy deficiency leads to the induction of apoptosis, leading to the death of the cell (Raj *et al.*, 2015). Simple phenolics also can disturb the ion chemistry of the Giardia and protein synthesis. These activities lead to the killing of the trophozoite thus facilitating the direct control of Giardia infection.

The simple phenolics can also show indirect activities that can help the control of giardiasis in the body. The basic phenolics have immunomodulatory and anti-inflammatory properties (Ghiringhelli *et al.*, 2012; Sobhani *et al.*, 2021). These properties help the immune system to control the proliferation of Giardia and reduction of the inflammatory damage in the body. In this way, they help assist in the control of giardiasis infection. Several researchers have conducted experiments to prove that simple phenolics are effective in the control of giardiasis (Machado *et al.*, 2010; Davoodi and Abbasi-Maleki, 2018; Calzada and Bautista, 2020; Pintong *et al.*, 2020; Bhattacharyya, 2021).

Flavonoids: Flavonoids are polyphenols that have more than one phenolic ring inside the body (Jayusman *et al.*, 2022). The flavonoids are a diverse group which are categorized into various subgroups. They have proven medicinal actions such as antibacterials, antivirals, anti-inflammatory and antiprotozoal agents (Zulhendri *et al.*, 2021). Flavonoids have several mechanisms which can help them control giardiasis (Ticona *et al.*, 2022). They can target the cell membrane of the Giardia by a set of actions and reduce the functionality of the cell membrane (Biharee *et al.*, 2020). They destroy the Giardia cell structure by disturbing the integrity of the cell membrane by several mechanisms i.e. interaction with the protein channels and denaturing the phospholipids present in the cell membrane, altering the fluidity of the cell membrane, and altering the osmotic pathways and ions of the cells. Flavonoids are known to disturb several cellular processes by disturbing the enzymes of various parts (Ghasemian Yadegari *et al.*, 2022). They disturb the enzymes involved in fatty acid synthesis, ATP production, and several other enzymes involved in the energy uptake mechanisms (Hussain *et al.*, 2020). Because of these properties, flavonoids, especially quercetins, have been searched for control of giardiasis (Al-Badry *et al.*, 2023; Albogami, 2023a).

Essential oils and terpenes: Essential oils are fatty fractions of plants that contain terpenes (lipophilic hydrocarbons) and their derivatives (Al-Snafi, 2020; Siddiqui *et al.*, 2024). They have well-known antiprotozoal, anti-inflammatory, and antioxidant properties (Dias *et al.*, 2021). They can arrest the division of trophozoite. In this sense, they stop the reproduction of Giardia, controlling the pathogens (Abdelmaksoud *et al.*, 2022; Grüttner *et al.*, 2023). They can also act on the protein and DNA synthesis of the Giardia spp. limiting the central dogma of the cell (Menezes and Tasca, 2023). In addition to these activities, they have well-known anti-inflammatory and immunomodulatory activities that can control giardiasis (Calzada *et al.*, 2017; Sandner *et al.*, 2020).

Alkaloids: Alkaloids are basic, nitrogen-containing compounds that are complex, bitter-tasting compounds having no color typically (Zuluaga, 2024). They have similar effects against giardiasis as simple phenolics as the alkaloids and can disturb energy production mechanisms, and cell permeability by acting on cell membrane functionality (Rahman *et al.*, 2021). Moreover, they have strong immunomodulatory and anti-inflammatory activities and help the body to control giardiasis indirectly. Limited research has proven that alkaloids e.g. kaempferol can control giardiasis because of these mechanisms (Argüello-García *et al.*, 2020b)

Pharmacological interactions: The phytochemicals having anti-Giardia properties are highly reactive compounds, so they show unpredictable reactivity in the aqueous environment (Efferth and Koch, 2011; Engwa, 2018). They react unpredictably so they may act as synergists or antagonists to each other (Ji *et al.*, 2009; Panossian *et al.*, 2024). Although the studies show that the different classes of phytochemicals show a synergistic effect (Ahamed *et al.*, 2021; Mitra *et al.*, 2023), the exact mechanisms of actions need to be understood that how they will interact in the body. These reactions are dependent on the atmosphere and compound type. In giardiasis, there is an alteration in the intestinal environment or there may be an alteration in the environment of the cells (Allain and Buret, 2020; Klimczak *et al.*, 2024). This alteration in the environment and comparison with the normal environment must be studied to predict the behavior of these compounds within the body (Pant *et al.*, 2021; Rai *et al.*, 2023).

Conclusions: Phytochemicals are among the widely searched compounds for the control of various infectious diseases, including giardiasis. Multiple compounds e.g. flavonoids, and essential oils have shown anti-Giardia activities. Understanding the mechanisms of action and pharmacological interactions will help approach the clinical preparation of drugs. Moreover, specifying the compounds via *in vivo, in vitro,* and *in silico* approaches is necessary so that the most suitable compound can be discovered.

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