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RESEARCH ARTICLE

Antiprotozoal Activity of Plant Extracts and their Bioactive Compounds against *Cryptosporidium* of Zoonotic Concern

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ABSTRACT

Cryptosporidiosis caused by *Cryptosporidium* protozoa is a widespread intestinal disease that affects both humans and animals globally. Direct contact or contaminated food and water can spread infectious parasitic oocysts, which are excreted in the feces of infected individuals and can live in harsh environments. It is challenging to remove the parasite from polluted surroundings because of the oocyst's small size, flexibility, persistence, and resistance to standard disinfectants. Both the inactivation of oocysts and treatment of infected individuals are required to achieve adequate control. However, few medications are used to treat cryptosporidiosis in animals and several medications are frequently used to treat disease in humans. Unfortunately, none of them fully addresses the parasitological and clinical response. Therefore, control of cryptosporidiosis remains a global challenge in both veterinary and human medicine. New alternative compounds are needed to treat cryptosporidiosis because existing chemotherapeutic treatments are not very effective. Plant products are considered efficient sources for their treatment as they are environment-friendly, non-toxic, and have wide therapeutic potential. The current review will focus on plant-based extracts with their minimum side effects and multifaceted bioactivity, representing a suitable alternative in combating cryptosporidiosis. Plant acts through different mechanisms and several studies are summarized here.

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INTRODUCTION

Cryptosporidium is an obligate intracellular apicomplexan protozoon that infects intestinal and respiratory epitheliums of various species including reptiles, birds, ruminants, felines, canines, and humans (Scorza and Lappin, 2021; Abbas *et al*., 2022; Antonio *et al*., 2023). Cryptosporidiosis is a diarrhea-causing disease in humans and animals (Zhang *et al*., 2000; Helmy and Hafez, 2022; Golomazou *et al*., 2024). A severe infection damages the villi, enlarges the crypts, and causes plasma cells and lymphocytes to gather in the lamina propria. Life-threatening watery diarrhea and dehydration are due to electrolyte imbalance and increased permeability of chloride ions through the membrane (Chen *et al*., 2002; Leitch and He, 2011; Khalil *et al*., 2018). Additional mild symptoms include fever, nausea, vomiting, thirst, abdominal cramping, anorexia, and stunted growth. Symptoms appear in the first week after infection and resolve in two to three weeks in healthy individuals with better immune status (Namazi and Razavi, 2024). In immunocompromised (HIV-infected) individuals, four clinical symptoms including chronic diarrhea, recurring diarrhea, transient diarrhea, and cholera-like conditions have been reported (Helmy and Hafez, 2022; Zuo *et al*., 2023). The highest global prevalence of cryptosporidiosis ranging from 11-78% was reported in claves (Hatam-Nahavandi *et al*., 2019) and the causative agent was *C. parvum* in cattle manure.

The control of *Cryptosporidium* is very important due to its global outbreaks and the severity of infections. For this reason, various chemical drugs with known mechanisms of action have been used over the years to control cryptosporidiosis (Ali *et al*., 2024; Lenière *et al*., 2024). The frequent and continuous use of these synthetic chemical anti-*Cryptosporidium* drugs has led to the development of parasitic resistance. Some modified drugs such as nitazoxanide and paromomycin are being used globally in immunocompetent patients. However, in immunocompromised (AIDS) patients where immunity is

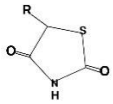
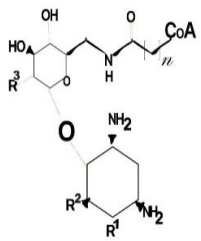
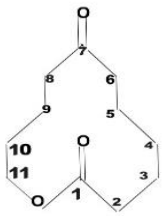
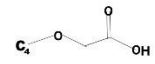
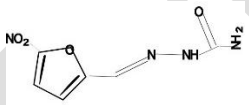
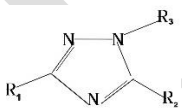
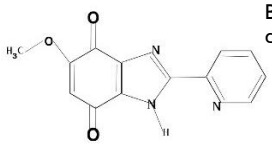
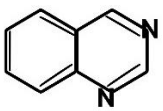
too weak to fully eliminate the parasite, they can only be effective in improving clinical manifestations. Both these drugs are target-specific but are not effective for all life stages of the *Cryptosporidium* parasite (Ali *et al*., 2024). Additionally, *Cryptosporidium* species have developed some natural resistance against these drugs (Zhu *et al.*, 2021) because of their unique location in the host intestine, variation in biochemical pathways, and the existence of specific proteins that are responsible for the transport of drugs inside and outside of the cell (Hasan *et al*., 2021; Ali *et al*., 2024). The genomic study revealed that some of the *Cryptosporidium* species particularly *C. parvum* have a close resemblance with gregarine parasites and separate from other parasites of apicomplexans (Khan *et al*., 2018). Furthermore, this parasite shows variation in its protein structure and lacks a plastid genome responsible for coding for ribosomal proteins and amplification of the products (Baptista *et al*., 2021). As a result, the activity of various drugs (clindamycin and other macrolides) has been greatly reduced. Moreover, this parasite possesses a different enzymatic genome compared to another apicomplexan. For example, the genomic structure of the dihydrofolate reductase (DHFR) enzyme of *Cryptosporidium* is quite different from the DHFR of *Plasmodium* (Bhagat *et al*., 2022). This change in the sequence of a gene enables the *Cryptosporidium* to resist 2, 4-aminopyrimidine inhibitors (Chaianantakul *et al*., 2020). Furthermore, the existence of multi-drug resistant (MDR) transporters in *Cryptosporidium* could aid in resistance (Knight, 2024). Other than resistance some other problems related to the side effects of drugs have also been observed for example the prolonged use of a major drug named nitazoxanide leads to abdominal pain, nausea, vomiting, headache, and loss of appetite (El Saftawy *et al*., 2024). Ecotoxicological effects were observed when paromomycin and azithromycin were used (Tagliazucchi *et al*., 2024). These drugs are poorly metabolized and excreted in urine and feces, contaminate the aquatic environment, and disturb the nitrogen cycle and ecological niche (Stanley, 2024). These drugs also disturb the microflora of the soil hence causing the decomposition of nutrient and microbial imbalance. The other anti-*Cryptosporidium* drugs also disturb the microflora of the intestine in humans and animals and cause intestinal ulcers and some other severe infections (Thakur *et al*., 2024). Similarly, vaccination against *Cryptosporidium* has also been tried but not implemented yet due to the complex life cycle, diversity of parasitic strains, antigenic variation, unique intracellular location, and immunomodulation (Du, 2021; Hasan and Mia, 2022; Palomo-Ligas *et al*., 2023). No doubt the development of the vaccine is in progress, but it is projected to be expensive (Jumani *et al*., 2021). Table 1 summarizes various chemical drugs used to treat cryptosporidiosis including their mode of action, targeted hosts, and associated limitations.

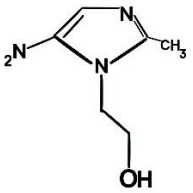
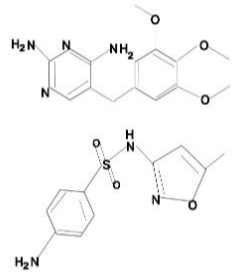
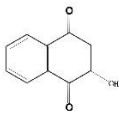
Because of the drug resistance, ecotoxicity, side effects, and high costs, there is a dire need to generate some alternatives including botanicals, essential oils, nanoparticles, and probiotics (Ahmad *et al*., 2024; Abbas *et al*., 2025; Ambrose *et al*., 2025). Nowadays, scientists and researchers are moving towards more reliable alternatives called botanicals and their active components (Munir *et al*., 2023; Gholamine *et al*., 2024). The reason for selecting plants and their components is that they are locally sourced, biodegradable and eco-friendly, broad-spectrum activity, less toxic, cost-effective, and target specific to control intestinal *Cryptosporidium* (Akinnubi, 2024; Maji *et al*., 2024; Moreno-Mesonero *et al*., 2024).

Many plant extracts and their bioactive components are broadly investigated to determine their efficacy against *Cryptosporidium*, and their targeted mechanism of action (El-Shewehy *et al*., 2023; Namazi and Razavi, 2024;). These research findings have revealed the antiprotozoal action of plant extracts and their active components (El-Shewehy *et al*., 2023; Ranasinghe *et al*., 2023). The plant components are very unique antioxidants in targeting the acetylcholine receptors of the protozoa and on the other hand, they cause excystation of the oocyst of the *Cryptosporidium* species (Palomo-Ligas *et al*., 2023). By considering their importance and their medicinal and therapeutic potentials, this review study discusses various plant extracts, their chemical composition, and mode of action against *Cryptosporidium* species, limitations, and future challenges.

Life Cycle and Zoonotic Transmission of *Cryptosporidium:* *Cryptosporidium* has a monoxenous complex life cycle consisting of various developmental stages including asexual multiplication and sexual reproduction (Jamil *et al*., 2023). The cycle begins when mature, thick-walled sporulated oocysts, each containing four sporozoites, are ingested by the host's digestive tract (Abdullah and Dyary, 2023). Stimulating factors and the microenvironment of the intestine such as temperature, pH, bile salts, carbon dioxide, gastric secretions, and pancreatic enzymes cause the excystation of mature oocytes that result in the release of sporozoites (Kato *et al*., 2001; Mayerberger *et al*., 2023). Moreover, the excystation also depends on sporozoite-associated aminopeptidases, cysteine and serine proteases, phospholipases, and heat shock proteins (Okhuysen *et al*., 1994; O'Hara and Chen, 2011). Glycoproteins attached to intestinal epithelium aid sporozoites in actively penetrating the host cell membrane forming an extra cytoplasmic parasitophorous vacuole that acts as a niche for the replication and development of sporozoites (Mayerberger *et al*., 2023). Sporozoites are transformed into trophozoites inside the vacuole which then go through the asexual growth phase and produce meronts of type 1 (6 to 8 merozoites) and type 2 (4 merozoites (Bandyopadhyay *et al*., 2022; Bertuccini *et al*., 2024). When released these merozoites start asexual multiplication by infecting other host cells and produce further type 1 and type 2 meronts (Tandel *et al*., 2019). The sexual phase starts when Type 2 meronts produce micro and macrogamonts which undergo fertilization and produce thick and thin-walled oocysts (Lamont, 2024). The thin-walled oocysts remain inside the host body, where they rupture and cause autoinfection while thick-walled shed through feces and infect other susceptible hosts (Balendran *et al*., 2024). The quantity of oocysts that an infected individual excretes can vary significantly. Calves infected with 10^5 oocysts often expel 10^9 to 10^{10} oocysts over 7-10 days (English *et al*., 2022).

Table 1: Use of various chemical drugs against *Cryptosporidium* parasite, their mode of action, efficacy, and limitations

Drug class	Structure	Drug name	Species	Mode of action	Efficacy of the drug	Limitation	References
Thiazolide Derivatives		Nitazoxanide	Humans, cats, dogs	Targets ferredoxin oxidoreductase and interferes with the electron transport chain	Reduced egg shedding, improved diarrhea	Does not affect oocysts and is not distributed globally	(Rossignol *et al*., 2001; Diptyanusa and Sari, 2021; Sykes, 2022)
		Aminoxanide	Humans, dogs, cats, calves, lambs bucks, goats, and sheep	Interferes with ferredoxin oxidoreductase and inhibit its activity	Reduced egg shedding, improved clinical presentation	Does not affect oocysts and is not distributed globally	(Widmer *et al*., 2020; François *et al*., 2021)
Aminoglycosides		Paromomycin	Humans, calves, kids, lambs, goats and bucks	Binds with 30S ribosomal subunit, inhibit the synthesis of mRNA	Reduced egg shedding, improved diarrhea	Drug resistance, poor penetration, and nephrotoxicity	(Lin *et al*., 2018; (Diptyanusa and Sari, 2021; François *et al*., 2021)
Macrolide		Azithromycin	Humans, cats, dogs, foals, calves, goats and lambs	Inhibits peptidyl transferase activity and inhibit protein synthesis	Reduced egg shedding, improved clinical conditions	Minimum parasite clearance, and diarrhea, showed better results when used in combination with Spiramycin, drug resistance	(Kadappu *et al*., 2002; Sykes, 2022; Namazi and Razavi, 2024)
		Spiramycin	Humans, cats, dogs, calves, goats and lambs	Blocks peptide elongation and inhibit translation	Reduced egg shedding, improved clinical presentations	Diarrhea, abdominal cramps, and minimum parasite clearance showed better results when used in combination with azithromycin, drug resistance, ototoxicity	(FarahatAllam *et al*., 2020; Al-Dulaimi *et al*., 2021)
Rifamycin class of antibiotic		Rifaximin	Humans	Attached is the beta subunit of RNA polymerase, which inhibits transcription	Reduced egg shedding, improved clinical signs,	Action is limited and indirect, not a primary line treatment	(Amenta *et al*., 1999; Gathe *et al*., 2008)
Nitrofurazone derivatives		Furazolidone	Humans, cats, dogs, calves, lamb, goats	Production of hydrogen peroxide and hydroxyl radical. Also inhibits the activity of glutathione reductase of *C. parvum*	Reduced oocyst shedding, kill trophozoites, improved diarrhea	Non-targeted drug, drug resistance, ecotoxic	(Randhawa *et al*., 2012; Sumbria and Singla, 2019)
Triazole derivatives		Itraconazole	Humans	The exact mechanism is unknown but good anti-inflammatory	Reduced egg shedding	Not target specific for *Cryptosporidium* but it is an antifungal	(Patel *et al*., 2023; Vaillant and Naik, 2023)
Heterocyclic aromatic compounds		Benzimidazoles	Humans, calves, lambs, goats	Inhibits tubulin polymerization, deformed the cytoskeleton, and decreased glucose uptake	Reduced egg shedding, improved clinical presentations	Non-effective in rodents, Drug resistance, ecotoxicity,	(MacDonald *et al*., 2004; Kirubakaran *et al*., 2012; Zhang *et al*., 2012)
Quinazolinone alkaloids		Halofuginone	Dogs and calves	Inhibits prolyl-tRNA-synthetase, inhibit the production of proline (used to synthesize sporozoites and merozoites)	Reduced egg shedding	Drug resistance, prohibited in diarrhea, and it is also non-licensed	(Silverlås *et al*., 2009 Brainard *et al*., 2021; Namazi and Razavi, 2024)
Nitroimidazole antibiotics		Metronidazole	Humans	Production of nitrosol compounds damages DNA and Nucleic acid	Reduced egg shedding, improved	Drug resistance, toxicity, abdominal	(Masood *et al*., 2013; Abouel-Nour *et al*., 2016;

		Tinidazole	Humans	Production of nitrogen reactive species and induced oxidative stress	clinical presentations Reduced egg shedding, improved clinical presentations	cramps, nausea, vomiting Drug resistance, toxicity, abdominal cramps, nausea, vomiting	Sn and Al-Khashab, 2022) (Mejia, 2016)
Co-trimoxazole		Trimethoprim-Sulfamethoxazole	Dogs and humans	Inhibitsbiosynthesis of folate synthesis necessary for DNA and nucleotide synthesis	Reduced egg shedding, improved clinical signs	Antibiotic, non-targeted drug, drug resistance, toxicity, dizziness and diarrhea	(Nelson *et al*., 2001; Ordoobadi, 2024)
Hydroxy-1,4-naphthoquinone derivatives		Atovaquone and buparvaquone	Humans, calves	Targets mitochondrion and reduced oocyst shedding	Reduced egg shedding, improved signs	Expensive, specific for malaria, non-targeted to *Cryptosporidium*	(Giacometti *et al*., 1996; Güney and Şentürk, 2023)
Hyperimmune bovine colostrum			Human and claves	Production of IgG antibodies enables oocyst not to invade microvilli	Reduced diarrhea	Not effective in immunocompromised patients	(Gamsjäger *et al*., 2023)

Transmissions of *Cryptosporidium* protozoa happen from animal to animal, animal to human (zoonosis), human to animal (reverse zoonosis), and human to human (Hussain *et al*., 2021; Javed and Alkheraije, 2023; Utami, 2024). Zoonotic transmission mostly takes fecal-oral routes, contact with the manure of infected animals, and contaminated water and food (Robertson and Woolsey, 2023). Since the 1980s, it has been believed that cattle and cattle manure are a significant source of zoonotic cryptosporidiosis. The estimated annual global *Cryptosporidium* load in livestock manure is 3.2×10^{23} oocysts (Polley *et al*., 2022). Humans, particularly farmers, veterinarians, and researchers, get infections through the ingestion of mature thick-walled oocysts excreted by infected animals (Vermeulen *et al*., 2019). The midwestern states of the United States where the livestock and dairy sector was most prevalent, had the highest incidence of cryptosporidiosis (Yoder *et al*., 2007). Similarly in the United Kingdom, *Cryptosporidium* infections are higher in manure-rich landfill areas (Lake *et al*., 2007). Conversely, a small number of epidemiologic investigations have linked sheep to human cryptosporidiosis. There is minimal evidence linking companion animals to the spread of human cryptosporidiosis. The idea that dogs may be a major source of human cryptosporidiosis has been around for a while. However, a misunderstanding that *C. parvum* causes cryptosporidiosis in all mammals and the finding of direct transmission of the parasite from calves to humans served as the main foundation for this (Shukla *et al*., 2006). In England, there was no evidence that contact with dogs or cats increased the risk of contracting cryptosporidiosis (Goh *et al*., 2004).

Mature oocysts are very stable, resistant to intense environmental conditions, and survive during disinfection and chlorination of water (Lefebvre *et al*., 2021). These enduring parasites constitute the largest disease hazard to the water sector and are accountable for the majority of the worldwide protozoal water outbreaks (Gharpure *et al*., 2019). Additionally, *Cryptosporidium* is acknowledged as a significant foodborne pathogen, responsible for about 8 million foodborne illness cases per year and over 40 major outbreaks to date (Zahedi, 2018). Food contaminations occur during direct contact with utensils, infected food handlers, contaminated surfaces, or exposure to *Cryptosporidium*-contaminated water. Raw salad and unpasteurized milk may also be the source of foodborne outbreaks of cryptosporidiosis (Zahedi and Ryan, 2020). Fig. 1 shows the life cycle of *Cryptosporidium* and its zoonotic transmission from humans to animals.

Plant extracts: Plant extracts are complex substances that are extracted from plants using a variety of techniques including maceration, soxhlet, hydro distillation, ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, pressurized liquid extraction, cold press extraction, liquid-liquid extraction, chromatography, and fermentation-assisted extraction (Bitwell *et al*., 2023). Every plant has its own composition, and it varies due to differences in extraction solvents, techniques, temperature, duration, and drying methods (Heinrich *et al*., 2022; Nurzyńska-Wierdak, 2023). Additional causes include additional processing and procedures used to concentrate or eliminate specific elements or groups of constituents (Wen *et al*., 2023). Genetic, climatic, and agricultural factors can cause further diversity in the composition of botanical extracts produced from the same plant species and plant part as starting materials (Palit and Mandal, 2021). Using standardized extraction techniques and controlling the inherent variability in the starting material can help produce extracts with a constant composition. Furthermore, the chemical composition of plant extract from the same plant varies at different growing periods of the plant as reported previously in the *Mentha piperita* plant (Abdi and Karami, 2020; Hudz *et al*., 2023).

Plants can be extracted using a variety of techniques, but the most straightforward and significant economic way is hydro distillation, which is employed in laboratories (Katekar *et al*., 2023). Plants and plant extracts have been used since ancient times as home remedies (Azam *et al*., 2020; Islam *et al*., 2021; Sebo *et al*., 2024). People use them because of their therapeutic and pharmacological effects. They have been used as anti-bacterial (Seukep *et al*., 2023; Abdallah *et al*., 2024), antivirals (Mohammed *et al*., 2023), antifungals (Zhou *et al*., 2023), antiparasitic (Benlarbi *et al*., 2023), and antiprotozoals (Namazi and Razavi, 2024).

Chemical composition of plant extracts: Plant extracts are different in co mposition and contain hundreds of active chemical components (Mengjie *et al*., 2023). Mostly two to three components are higher in each plant. For example, the extract of *Camellia sinensis* commonly known as green tea is rich in polyphenols (30-40%) and alkaloids (2-4%) while extract obtained from the rhizome of *Curcuma longa* has 2-8% curcuminoids in it (Hondale *et al*., 2024; Wu *et al*., 2024). Plant extracts are primarily composed of different classes i.e. polyphenols, terpenoids, alkaloids, and other nitrogenous-based compounds (Elshafie *et al*., 2023). Polyphenols and terpenoids are the most important in them. Depending on phenol number, polyphenols are further classified into flavonoids, non-flavonoids, and phenolic acid while terpenoids are classified as carotenoids, non-carotenoids, and thiols based on their isoprenoid unit (Min *et al*., 2023; Zagoskina *et al*., 2023;). Flavonoids and phenolic acids are more important in them. Fig. 2 gives the general classification of plant extracts and their chemical compounds with their general structures.

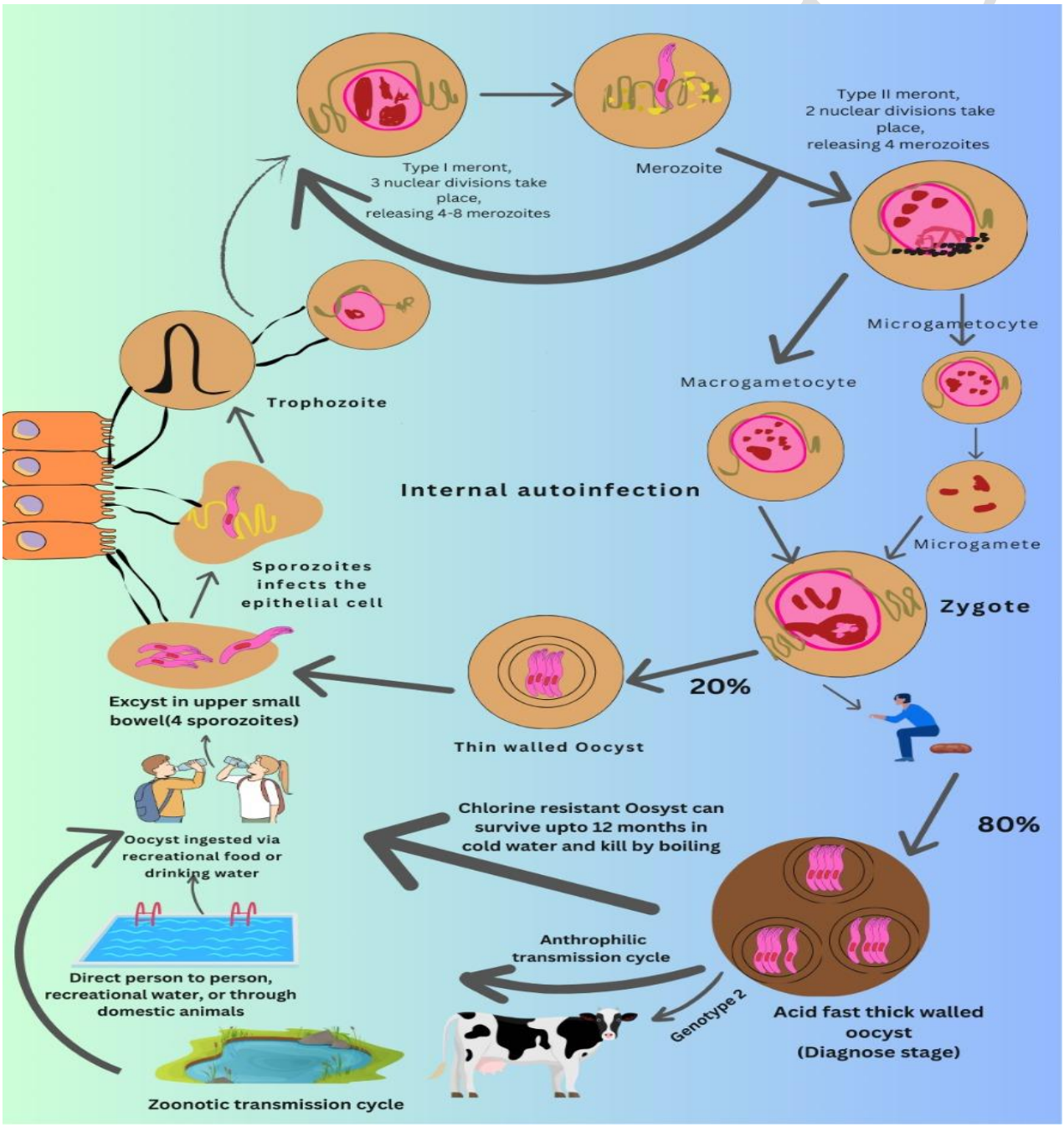


Fig. 1: Life cycle of *Cryptosporidium* and its zoonotic transmission (www.canva.com).

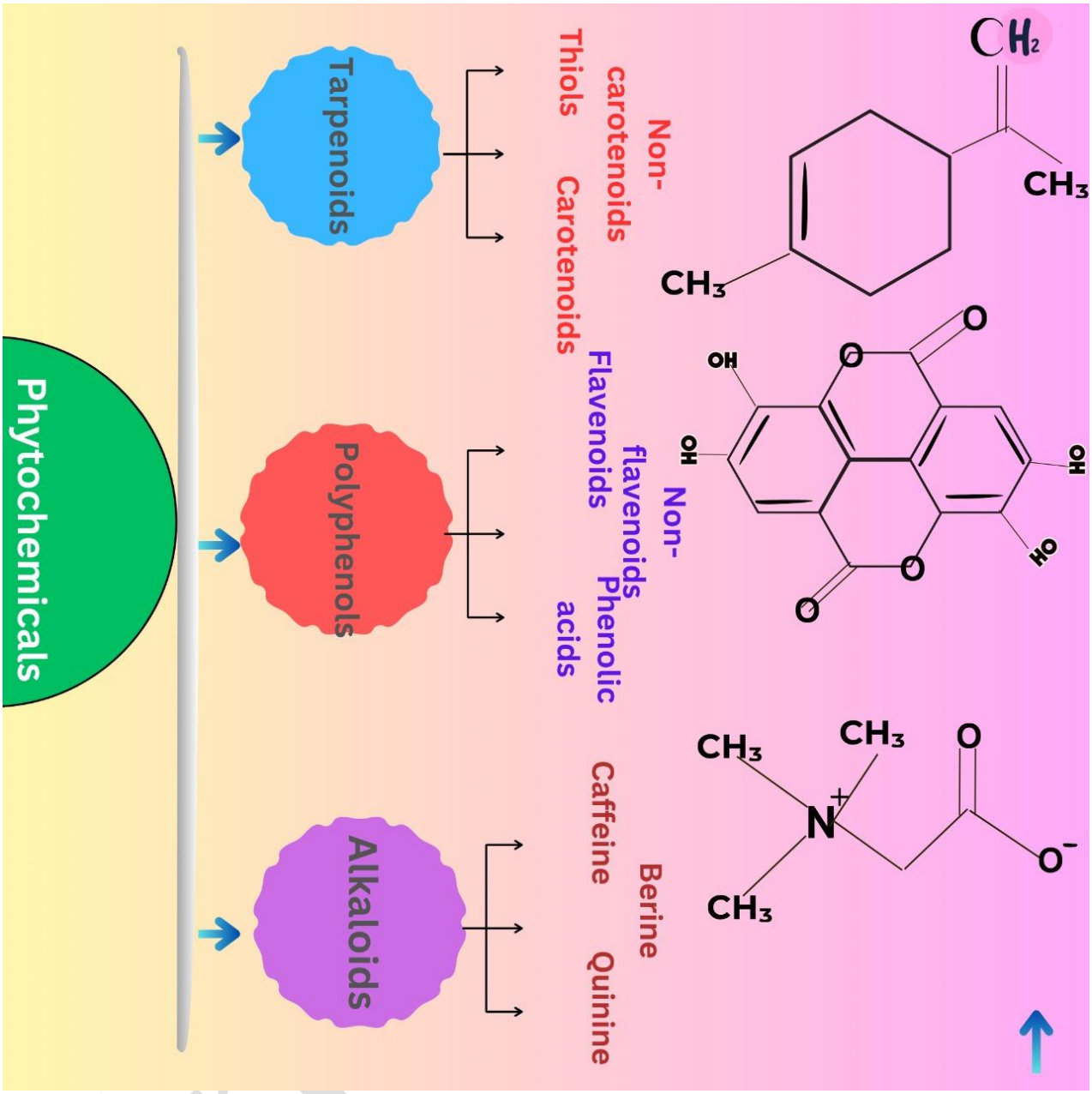


Fig. 2: Classification of plant-derived active compounds (www.canva.com).

Important plants and their bioactive components: Various plant species have been used for centuries in treating protozoal infections and they have shown promising results against them (Woolsey *et al*., 2019). Some of the plant species which are very effective and studied against cryptosporidiosis include *Allium cepa* (onion), *Zygophallum fabago* (Syrian bean caper), *Zingiber officinale* (ginger), *Viscum album* (mistletoe), *Vaccinium myrtillus* (blueberries), *Thymus vulgarus* (thyme), *Syzygium aromaticum* (clove), *Silybum marianum* (thistle), *Salvia officinalis* (sage), *Panax ginseng* (ginseng), *Punica granatum* (pomegranate), *Origanum vulgare* (origanum), *Olea europae* (olive), *Nigella sativa* (black cumin), *Moringa oleifera* (drum stick), *Mangifera indica* (Mango), *Mentha piperita* (peppermint), *Matricaria chamomilla* (chamomile), *Ficus carica* (common figs), *Ferula asafoetida* (ferula), *Echinacea purpurea* (echinacea), *Cinnamomum verum* (cinnamon), *Curcuma longa* (turmeric), *Commiphor molmol* (mirazid), *Artemisia spicigera* (spiked wormwood), *Artemisia herba alba* (white wormwood), *Aloe vera* (aloe vera), *Allium sativum* (garlic) etc. (Ojuromi and Ashafa, 2020; Silva dos Santos *et al*., 2021; Ranasinghe *et al*., 2023; Namazi and Razavi, 2024). These plant species have various bioactive molecules that have shown therapeutic action against cryptosporidiosis-causing parasites. Some important plants and their major active compounds used against the *Cryptosporidium* parasite are shown in Fig. 3.

Mode of action of plant extracts: The antiprotozoal action of plant extracts is strongly associated with the purified compounds and active biomolecules in them (Ranasinghe *et al*., 2023). Since there are so many active biomolecules, plant extracts do not seem to have any specific mechanism of action (Khursheed *et al*., 2022). It has also been studied that plant extract as a whole has shown better results and efficacy as compared to its components because of their

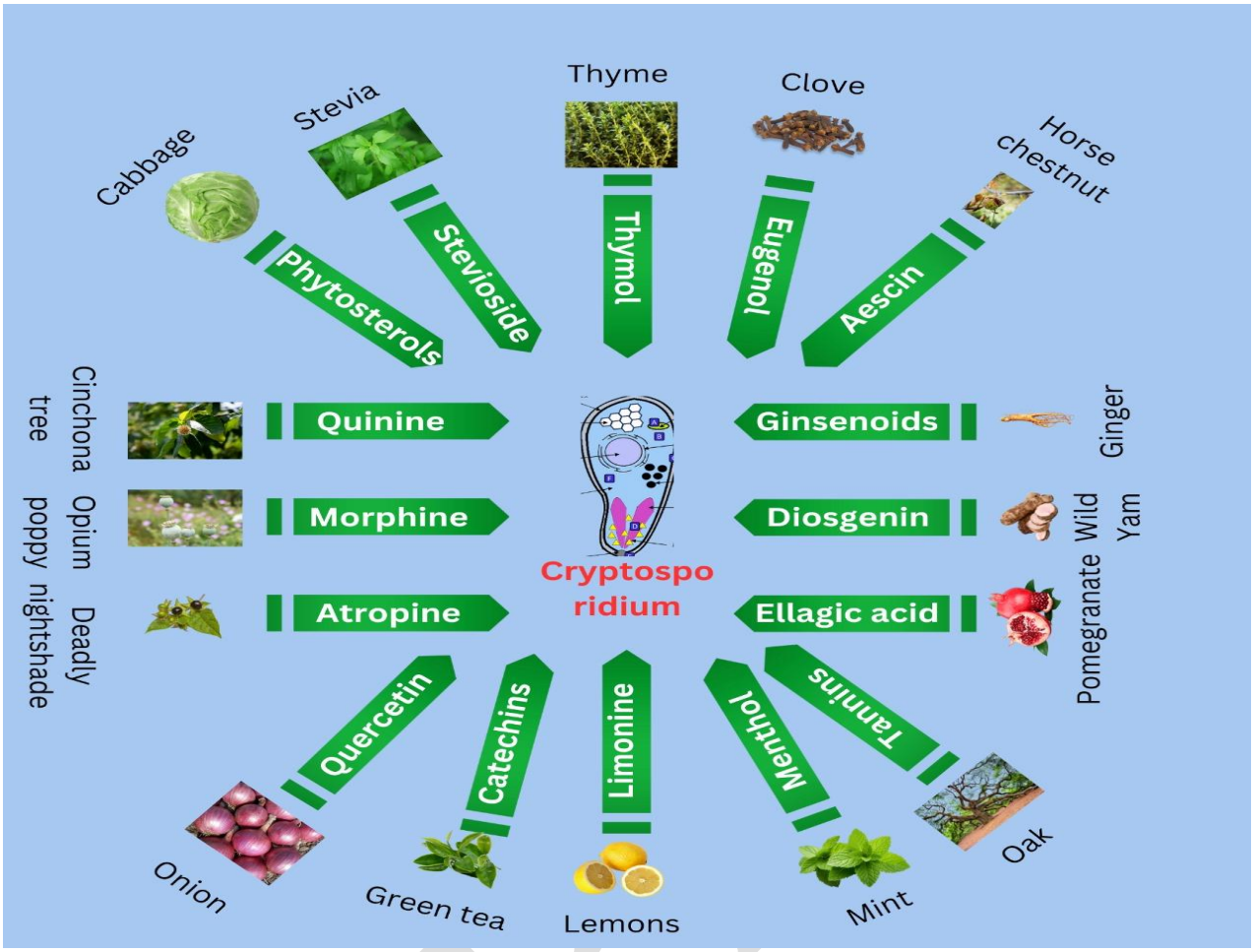


Fig. 3: Some important plants and their active components (www.canva.com).

synergistic mode of action in terms of better absorption and neutralization of toxic metals, increased solubility and permeability, multitarget interaction, and decreased degradation (Chen *et al*., 2022; Vaou *et al*., 2022; Jeong *et al*., 2023; Khan *et al*., 2023). Furthermore, a single biomolecule can accumulate in the cell cause toxicity to the cellular organelles, and interfere with the energy metabolism of the host cell (Xu *et al*., 2020). For example, a comparison of the entire turmeric extract and isolated curcumin (*Curcuma longa*) revealed that the extract has higher antioxidant activity because of the synergistic effects of polysaccharides and volatile oils (Ballester *et al*., 2023). Plant extracts and their components showed various mode actions against *Cryptosporidium* (Namazi and Razavi, 2024). They act as antioxidants, neurotoxic, disrupt membrane permeability, inhibit protein synthesis, and damage to nucleus and DNA of *Cryptosporidium* (Ranasinghe *et al*., 2023; Kumar *et al*., 2023).

Plant extracts as antioxidants against cryptosporidiosis: Antioxidants are those substances that support the cell to reduce oxidative stress generated by reactive oxygen species (superoxide ion, hydrogen peroxide, hydroxyl radical, and free oxygen) and reactive nitrogen species (RNS) (nitric oxide and peroxynitrite) (Jaffri, 2023; Jomova *et al*., 2023). The detrimental effects of ROS and RNS in biological systems can be countered or inhibited by plant extracts and their bioactive constituents (Bouyahya *et al*., 2024). Phenolic chemicals, which are categorized as major antioxidants among plant extracts, can donate a hydrogen atom to produce a phenoxy radical, which confers antioxidant capabilities via the radical scavenging process (Santos-Sánchez *et al*., 2019; Atrooz *et al*., 2024). Plant extracts and their components are useful in antiparasitic therapy because they frequently show selective toxicity against parasites while protecting host cells (El-Seedi *et al*., 2023). The *Artemisia* plant extracts have been found effective against various genus protozoans including the genus *Giardia*, *Plasmodium*, *Trypanosoma*, and *Blastocystis* (Mokhtar *et al*., 2019; Ojuromi and Ashafa, 2020; Saqlain *et al*., 2024). *Olea europaea* and *Fiscus carica* extracts have been shown to have *in vivo* anti-*Cryptosporidium* properties, raising plasma levels of glutathione reduced form, superoxide dismutase, and catalase (Abd El-Hamed *et al*., 2021). The oocysts of *Cryptosporidium* are very resistant to harsh environmental conditions and synthetic chemical drugs and can survive up to 6 months to one year (Rousseau *et al*., 2018). Based on the above statement, the antioxidant effect of ethanolic extract of *Artemisia Judaica* extract and its phenolic (ArPh) and terpenoids (ArT) components have been investigated and found effective against the resistant oocysts of *C. parvum*. ArPh and ArT not only reduced the oocyst number but also changed the morphology of the oocysts of *C. parvum* (Ahmed *et al*., 2023). Another study showed that six polyphenolic compounds have anti-*C. parvum* activity, suggesting that these compounds could be used either by

themselves or in combination to increase their effectiveness (Ali *et al*., 2024). Similarly, when *C. parvum-infected* mice were treated with *P. granatum* peel suspension, the mice showed improvement in terms of reduced oocyst count, and intestinal morphology was changed (Al-Mathal and Alsalem, 2012).

Membrane Disruption and Ion Imbalance: All *Cryptosporidium* protozoans have double membrane-bounded parasites with a specialized structure called a pellicle for protection (host immune response) and structural support (Tomazic *et al*., 2018). Essential intracellular substances leak out when the integrity of the membrane is compromised. It has been demonstrated that a number of plant extracts can interfere with the cell membrane of *Cryptosporidium* and change its permeability, causing cytoplasmic leakage and parasite death (Ullah *et al*., 2020). Extracts from plants, particularly those high in lipophilic substances like flavonoids, terpenoids, and saponins, interact with the lipid bilayer of the parasite (Ramdani *et al*., 2023). These substances can form pores in the membrane of the oocyst, resulting in ion imbalance and the leaking of essential cell components, integrate into the membrane and increase its fluidity and ultimately death of the *Cryptosporidium* oocyst (Al-Mathal and Alsalem, 2013*)*. For example, saponins obtained from *Quillaja Saponaria* combine with lipid membranes and destabilize them. This results in decreased parasite viability and the leaking of cellular contents (Böttcher, 2017). Numerous substances derived from plants interfere with membrane proteins, inhibit ion channels and membrane transporters, and impair the parasite's capacity to absorb nutrients and eliminate waste (Gorlenko *et al*., 2020; Kocyigit *et al*., 2023). On the other hand, they change the membrane's protein composition, which causes dysfunction and destabilization. For example, a berberine alkaloid obtained from *Berberis vulgaris* binds with membrane-bound enzymes and transport systems, reducing the parasite's ability to survive by altering its membrane function (Qian *et al*., 2023). Ahmad *et al*. (2023) verified the anti-oocyst activity of ArPh obtained from *Artemisia Judaica* against *C. parvum*. The study confirmed that phenolics bind with the outer surface of the oocyst of *C. parvum* and produce morphological alterations by increasing folds in the inner membrane that result in lysis and expulsion of their contents. Another study confirmed that naringenin and genistein obtained from *Citrus sinensis* and *Glycina max* respectively were effective against *C. parvum.* They bind with the parasitic membrane and block the ion transport channel (Bose *et al*., 2022).

Neurotoxic activity: *Cryptosporidium* needs neurotransmitters for its parasitic motility and cellular growth. Plant extracts and their active components such as flavonoids, saponins, and alkaloids can block neurotransmitters, thus reducing their invasion into the host cell and stopping intracellular growth (Borges *et al*., 2016). For example, quercetin and kaempferol obtained from flavonoids inhibit the dependent process. Similarly, an alkaloid berberine has neurotoxic effects, disrupting intracellular signals by blocking acetylcholine neurotransmitters. This causes the parasite not to stick to the intestinal wall and is easily removed from the gastrointestinal tract (Silva *et al*., 2021). Similarly, oregano and carvacrol block the calcium-dependent protein kinase 1 (CDPK1) and affect the Ca^{2+} mediated signaling of *C. parvum*, which is required for invasion, differentiation, and regulation of other vital functions (Mohanty and Murhekar, 2023). The hydrophobicity and presence of hydroxyl groups in carvacrol and thymol may allow the phenols to penetrate the cell membrane and reduce parasitic infection by modulating cytoplasmic metabolic pathways such as ATP synthesis (Ali *et al*., 2024). In an *in vivo* study, the 100% inhibitory effect of ethanolic extract of leaves of *Curcuma longa* has been observed. Potential crypto sporicidal effects have also been observed for *Vaccinium myrtillus* with its polyphenolic compounds, *Cinnamomum verum* with its phenolic compounds, *Allium cepa* with its flavonoids and sulfide compounds, *Allium sativum* with its allicin, *Mangifera indica* with its mangiferin, *Olea europaea* with its oleuropein, and *Punica granatum* with its polyphenols and tannins especially against *Cryptosporidium parvum* and *Cryptosporidium hominis* (Anthony *et al*., 2007; Al-Mathal and Alsalem, 2013; Almoradie *et al*., 2018; Ali *et al*., 2024). All these plant extracts and their components not only reduced the oocyst shedding but also improved the morphology of the damaged intestinal tissues and increased the interferon level in *C. parvum*-infected mice. Furthermore, a study reported that *A. sativum* disrupts the normal physiological functions of parasite mobility, food absorption, and reproduction (Anthony *et al*., 2007)

Nucleus and DNA damage: Plant extracts and their derivatives such as polyphenols and terpenoids produce ROS (hydrogen peroxide, hydroxyl ion, and superoxide ions) and RNS (nitric oxide) inside the parasitic cell that destroys the nucleotides and DNA strands (Chaves *et al*., 2020). The DNA accumulates inside the parasitic cell and prevents transcription and translation. For example, a chemical component of curcumin obtained from *Curcuma longa* neutralizes ROS that causes the DNA to break into fragments and form new cross-links leading to the denaturation of the genetic material (Aljedaie and Al-Malki, 2020). Similarly, the plant alkaloids and other bioactive components inhibit DNA polymerase and topoisomerase enzymes (Bhambhani *et al*., 2021). Interference with DNA replication renders parasitic reproduction and induces cell death. Certain plant compounds such as quinones attach to the DNA molecule through covalent bonds or alkylation that leads to the insertion between the DNA strands and prevents gene expression and replication in *Cryptosporidium*. Ahmed *et al*. (2023) studied early and late apoptosis by using trypan blue staining, DNA fragmentation by Comet assay, and high ROS-mediated DNA fragmentation and confirmed that increased doses of ArPh did not induce any infection in mice infected with *Cryptosporidium*. Similar results were reported about the anti-*Cryptosporidium* activity of *A. spicigera* (Shahbazi *et al*., 2021). In another study, the methanol extract of Asafoetida reduced *Cryptosporidium* infection in experimentally infected mice and improved the histological alterations of small intestinal villi (Abdelmaksoud *et al*., 2020). In contrast, neither water

nor ethanol extracts of propolis could eliminate the infection, but they did lower oocyst shedding and affected sexual-stage development (Asfaram *et al*., 2021).

Inhibit Protein Synthesis in *Cryptosporidium*: Protein synthesis is very important for cell integrity and survival due to its structural importance in every organelle of the *Cryptosporidium*. Plant extracts such as alkaloids and flavonoids interfere with the ribosomes by binding with 40S and 60S subunits and inhibit translation (Lim-Sylianco and Shier, 2020). Some plant components such as quercetine interfere with tRNA by binding with aminoacyl-tRNA, thus inhibiting translation (Mohammed *et al*., 2024). Epigallocatechin gallate obtained from green tea has the same mode of action in inhibiting protein synthesis. Some plant components such as curcumin obtained from *Curcuma longa* inhibit RNA polymerase which in return inhibits the synthesis of mRNA necessary for protein synthesis (Lee *et al*., 2021). Certain plant compounds inhibit enzymes involved in modifying proteins after synthesis, such as kinases and phospholipases (Corona-España *et al*., 2024). The parasite expends energy attempting protein synthesis, leading to metabolic stress and cell death. Plant extracts often selectively target parasite-specific pathways, sparing host cells (Anthony *et al*., 2007; Asfaram *et al*., 2021; Ballester *et al*., 2023). Fig. 4 illustrates the mechanism of the plant extracts which outlines their physiological and biochemical pathways. As represented, the plant extracts primarily act as antioxidants, disrupt membrane permeability, and cause neurotoxicity. They also target DNA and nucleotides and inhibit protein synthesis. These mechanisms are further supported by the data presented in Table 2, which provides a brief overview of each plant extract with its extraction method and accurate dose with better efficacy against *Cryptosporidium* parasite.

Limitations: Cryptosporidiosis may be avoided with the help of plant-based medications (Ullah *et al*., 2020). However, variability in composition and bioavailability can restrict their use (Shi *et al*., 2022). The main phytochemicals, such as flavonoids, glycosides, and tannins, are poorly soluble in water and lipids, which restrict their capacity to pass through biological membranes and cause inadequate absorption (Suteu *et al*., 2020). Furthermore, the extremely acidic pH of the stomach and carbonated environment can further alter the pharmacokinetics of these substances (Mueed *et al*., 2024). To get bioactive components, plants are also put through a variety of processes, including fermentation, distillation, purification, concentration, and extraction. The stability of active ingredients is questioned because they are subjected to oxidation and hydrolysis during these procedures (Finotti *et al*., 2024). Additionally, plant products frequently deteriorate, especially when stored, which results in the loss of active ingredients and the generation of inactive metabolites (Ansari *et al*., 2024). Concerns about the safety of plant-based medications are becoming more prevalent as their use grows worldwide. Despite their widespread use and appealing potential, many plants have not yet been confirmed safe or poisonous (Vilas-Boas *et al*., 2021). This results in a lack of awareness regarding their possible side effects and makes it challenging to determine the safest and most efficient treatments.

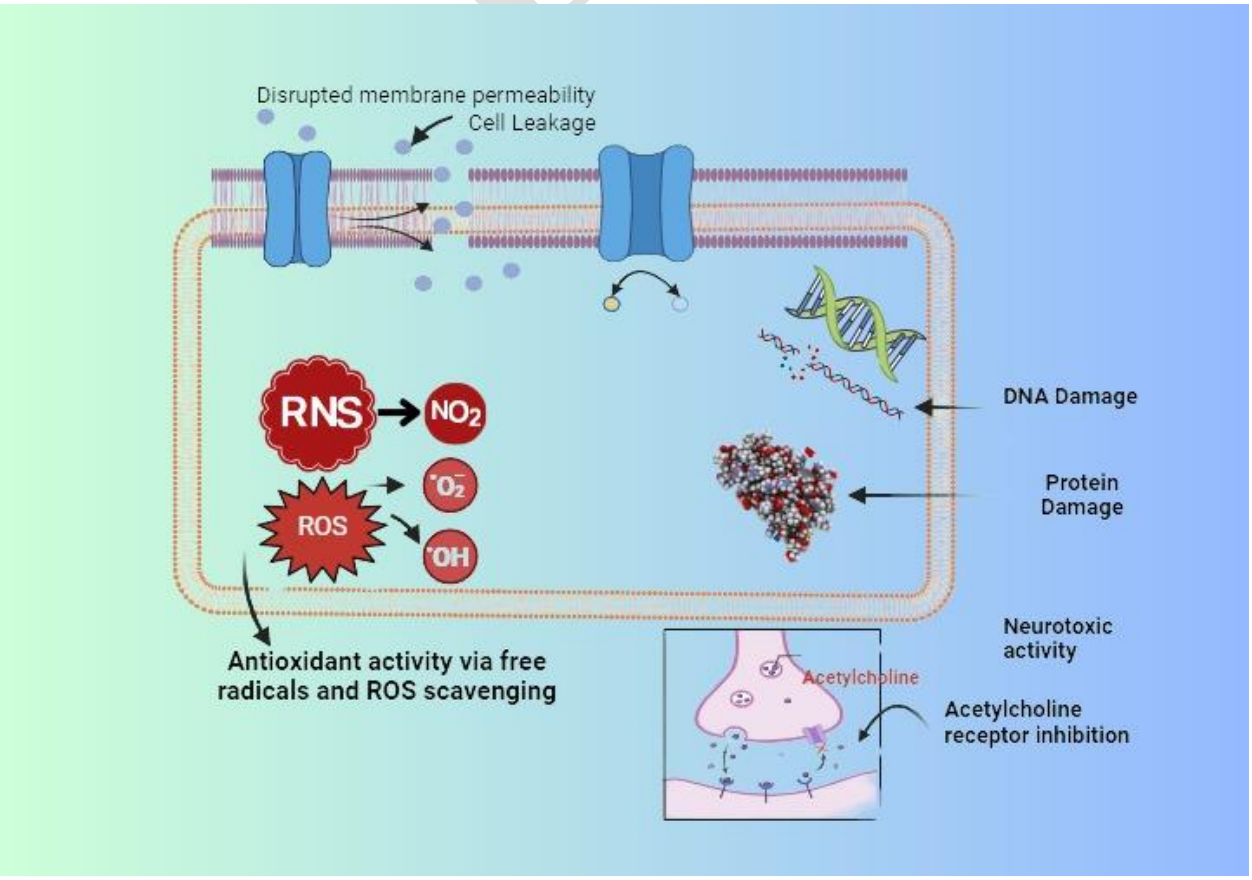


Fig. 4: Mode of action of various plant extracts against *Cryptosporidium* (www.canva.com).

Table 2: Use of various plants and their active bioactive molecules with their specific mode of action against *Cryptosporidium* species.

Plant name (scientific name)	Common names	family name	Major compounds	Method of extraction	Solvent used	Dose (per kg body weight)	Methodology	Animal model	Mode of action	Efficacy of the plants	Parasitic species	References
Allium cepa	Onion	Amaryllidaceae	Flavonoid and sulphoid compounds	Hydro distillation	Water	1mL/g	*In vitro* and *in vivo*	Mice	Antioxidant	Marked reduction in oocyst shedding	*C. parvum*	(El Ezz *et al*., 2011)
Allium sativum	Garlic	Amaryllidaceae	Allicin, diallyl disulfide	Maceration, Hydrodistillation	Water	50mg/L	*In vitro* and *in vivo*	Cattle, buffalo, Mice	Antioxidant	82% reduction of oocyst shedding	*C. parvum*	(Farid *et al*., 2022)
Aloe vera	Aloe vera	Asphodelaceae	Acemannan, glucomannan, pectins	Hydrodistillation	Water	250mg/L	*In vitro and in vitro*	Mice	Antioxidants, immunomodulators, anti-inflammatory	100% reduction of infection		(Farid *et al*., 2021)
Artemisia herba alba	White wormwood	Asteraceae	Artemisinin, quercetin	Hydrodistillation	Ethanol	500mg/day	*In vivo*	Mice	Antioxidant, immunomodulatory, and anti-inflammatory	50.50% reduction in oocyst count	*C. parvum*	(Elbahaie *et al*., 2023)
Anethum graveolens	Dill	Apiaceae	Coumarins, Flavonoids, Tannins	Hydrodistillation	Water	20µL	*In vitro* and *in vivo*	Mice	Anti-oxidant, Anti-secretory	95% Reduction in oocyst shedding, increased level of interferons	*C. parvum*	(Gaber *et al*., 2022)
Artemisia spicigera	Spiked wormwood	Asteraceae	Phenols and flavonoids	Hydrodistillation	Ethanol	0.2-20mg/mL	*In vivo*	Mice	Antioxidant	Marked reduction in oocyst shedding	*C. parvum*	(Shahbazi *et al*., 2021)
Commiphor myrrha	Mirazid	Burseaceae	Phenolics and flavonoids	Hydrodistillation	Water	10mg/kg/day	*In vivo*	Mice	Antioxidant and immunomodulatory	Marked reduction in oocyst shedding, increased IL-5 and IFN-γ in the infected host, increased humoral response	*C. parvum*	(Abouel-Nour *et al*., 2016)
Commiphor molmol		Burseaceae	Phenolics, (camphoric acid)	Hydrodistillation, maceration	Water	500mg/kg/day	*In vivo*	Mice	Antioxidant, immunomodulatory	70.15% reduction in oocyst shedding and intestinal trophozoites	*C. parvum*	(Fahmy *et al*., 2021)
Coriander sativum	Coriander	Apiaceae	Phenolics	Hydrodistillation	Aqueous and ethanol	750-1000mg/kg/day	*In vivo*	Mice	Antioxidant	41% reduction in oocyst shedding	*C. parvum*	(Obiad *et al*., 2012)
Curcuma longa	Turmeric	Zingiberaceae	Phenols (Curcumin)	Soxhlet	Ethanol	4.33mg/kg/day and 3.125–200 µM	*In vitro* and *in vivo*	Mice	Antioxidant, anti-inflammatory	Inhibit phospholipase A2, oxidative damage, oocyst shedding reduced	*C. parvum*	(Ganai *et al*., 2023)
Citrus sinensis	Orange	Rutaceae	Hesperidin, coumarins poly ethoxy flavones	Soxhlet	Ethanol	3g/kg	*In vivo*	Mice	Interfere with lectin receptors, immunomodulatory	Reduced oocyst shedding, reduced trophozoites, improved intestinal morphology	*C. parvum*	(Abd El Wahab *et al*., 2022)
Citrus maxima	Pomelo	Rutaceae	Phenols, Flavonoids, Alkaloids	Soxhlet and hydro distillation	Aqueous	50 and 100mg/kg	*In vivo*	Mice	Interfere with lectin receptors, immunomodulatory	Reduced oocyst shedding, improved morphology, and increased IFN-γ in infected host	*C. parvum*	(Hafez and Hamed, 2021)
Cichorium intybus	Chicory	Asteraceae	Coumarins, flavonoids	Hydrodistillation	Dimethyl sulphoxide. methanol	9.375-300 µg/mL	Parasite growth inhibitory assay, trophozoite invasion	Human	Antioxidant, anti-inflammatory	Inhibition of *C. parvum* adult and its trophozoite stage	*C. parvum*	(Woolsey *et al*., 2019)

							inhibition assay (*In vitro*)					
Cinnamomum verum	Cinnamon	Lauraceae	Flavonoids and sulphoid compounds	Hydrodistillation	Methanol	500g/kg/day	*In vivo*	Mice	Antioxidant	Reduced parasitic growth and oocyst shedding, reduced trophozoite development	*C. parvum*	(Woolsey *et al*., 2019)
Echinacea purpurea	Echinacea	Asteraceae	Alkaloids, caffeic acid derivatives, polysaccharides	Hydrodistillation	Aqueous	100mg/kg/day	*In vivo*	Mice	Antioxidant and anti-inflammatory	Reduced oocyst shedding and improved intestinal morphology, reduced IL-17 and COX-2 in the intestinal epithelium	*C. parvum*	(Marwa *et al*., 2018)
Ferula asafoetida	Ferula	Umbelliferae	Phenolics, terpenes, coumarins	Hydrodistillation	Methanol	5%	*In vivo*	Mice	Antioxidant	Marked reduction in oocyst shedding	*C. parvum*	(Abdelmaksoud *et al*., 2020)
Ficus carica	common figs	Moraceae	Flavonoids, phenols, tannins	Hydrodistillation	Methanol	200mg/kg/day	*In vivo*	Mice	Free radical scavenging and antioxidant properties	Marked reduction in oocyst shedding, increased plasma level of glutathione peroxidase, catalase, and superoxide dismutase	*C. parvum*	(Abd El-Hamed *et al*., 2021)
Matricaria chamomilla	Chamomile	Asteraceae	Organic acids, flavonoids, coumarins	Hydrodistillation	Aqueous	1000mg/kg/day	*In vivo*	Mice	Antioxidant and anti-inflammatory	67.2% reduction in oocyst shedding	*C. parvum*	(Taha *et al*., 2023)
Mentha piperita	Peppermint	Lamiaceae	Menthol, menthone, and iso menthone	Hydrodistillation	Aqueous	20mg/kg/day	*In vivo*	Mice	Antioxidant	74.7 reduction in oocyst shedding, reduction in malondialdehyde, increase in superoxide dismutase	*C. parvum*	(Taha *et al*., 2023)
Mangifera indica	Mango	Anacardiaceae	Mangiferin, Rutin, epicatechin, organic acids, vitamins, phenols	Hydrodistillation	Aqueous	40µg/100mL	*In vitro*	Mice	Antioxidant, immunomodulatory properties	Marked reduction in oocyst shedding	*C. parvum*	(Tarantino *et al*., 2004)
Moringa oleifera	Drum stick	Moringaceae	Flavonoids, alkaloids, steroids, tannins	Hydrodistillation, Soxhlet	Methanol	300mg/kg/day	*In vitro* and *in vivo*	Mice	Interfere with lectin receptors, antioxidants, immunomodulators	91.8% reduction in oocyst shedding, increased interferon level in infected mice,	*C. parvum*	(El-Sayed and Fathy, 2019)
Nigella sativa	Black cumin	Ranunculaceae	Phenols, thymoquinone	Hydrodistillation	Methanol	1.25mg/kg/day	*In vivo*	Mice	Antioxidant and anti-inflammatory	Marked reduction in oocyst shedding, improvement and histological changes in ileum	*C. parvum*	(Sadek *et al*., 2020)
Ocimum basilicum	Basil	Lamiaceae	Eugenol, rosamarinic acid,	Hydrodistillation	Aqueous	500mg/kg/day	*In vivo*	Mice	Antioxidant and anti-inflammatory	68.2% reduction in oocysts shedding, improved intestinal morphology	*C. parvum*	(Taha *et al*., 2023)

Olea europaea	Olive	Oleaceae	Flavonoids, phenols, tannins	Hydrodistillation	Methanol	200mg/kg/day	*In vivo*	Mice	Free radical scavenging and antioxidant properties	Reduction in oocyst shedding, increased plasma level of glutathione peroxidase, catalase, and superoxide dismutase	*C. parvum*	(Abd El-Hamed *et al*., 2021)
Origanum vulgare	Origanum	Lamiaceae	Phenols (carvacrol), tannins, terpenoids	Hydrodistillation	Aqueous	7-1000 µg/mL and 30mg/kg/day	*In vitro, in vivo*	Humans, mice	Antioxidant and anti-inflammatory	Alter ion channel and enzyme actions, reduced oocyst shedding	*C. parvum, C. hominis*	(Almoradie *et al*., 2018)
Punica granatum	Pomegranate	Lythraceae	Phenols (anthocyanins), flavonoids and tannins	Hydrodistillation	Methanol	40µg and 50-100mg/kg	*In vitro* and *in vivo*	Mice	Antioxidant, immunomodulatory properties	Reduced oocyst shedding, alteration in villus morphology	*C. parvum*	(Weyl-Feinstein *et al*., 2014)
Panax ginseng	Ginseng	Araliaceae	Phenols	Hydrodistillation	Methanol	100mg/kg/day	*In vivo*	Mice	Interacts with glycoproteins of epithelium and alters them	93% reduction in oocyst shedding	*C. parvum*	(Abouelsoued *et al*., 2020)
Salvia officinalis	Sage	Lamiaceae	Oleic acid, flavonoids, chlorogenic acid	Hydrodistillation	Methanol	50-100mg/kg/day	*In vivo*	Mice	Antioxidant and anti-inflammatory	91.8% reduction in oocyst shedding	*C. parvum*	(Abouelsoued *et al*., 2020)
Silybum marianum	Thistle	Asteraceae	Silymarin	Hydrodistillation	Aqueous	50mg/L	*In vivo*	Mice	Antioxidant and anti-inflammatory	Marked reduction in oocyst shedding	*C. parvum*	(Namazi and Razavi, 2024)
Syzygium aromaticum	Clove	Myrtaceae	Phenols (carvacrol)	Hydrodistillation	Aqueous	33mg/kg	*In vivo*	Mice	Antioxidant and anti-inflammatory	74.65% reduction in oocyst shedding	*C. parvum*	(Gaber *et al*., 2022)
Thymus vulgarus	Thyme	Lamiaceae	Thymol, *p*-cymene, carvacrol	Hydrodistillation	-	15µg/kg/day	*In vivo* and *in vitro*	Humans and mice	Antioxidant and anti-inflammatory	67.2% reduction in oocyst shedding and parasitic colonization, improved intestinal morphology	*C. parvum*	(Taha *et al*., 2023)
Vaccinium myrtillus	Blueberries	Ericaceae	polyphenols (anthocyanins)	Solid phase extraction	Aqueous	167 and 213µg	Oocyst excystation assay	Laboratory	Antioxidant	Reduced oocyst and trophozoite colonization	*C. parvum*	(Almoradie *et al*., 2018)
Viscum album	Mistletoe	Santalaceae	Phenolics and terpenes	Hydrodistillation	Water, ethanolic	750-1000mg/kg/day	*In vivo*	mice	Antioxidant	50% reduction in oocyst shedding	*C. parvum*	(Obiad *et al*., 2012)
Zingiber officinale	Ginger	Zingiberaceae	Phenols, gingerol, terpenes, zingiberene	Hydroditillation	Ethane	100mg/kg/day	*In vivo*	Mice	Antioxidants and anti-inflammatory	93.8% reduction in oocyst shedding	*C. parvum*	(Abouelsoued *et al*., 2020)
Zygophallum fabago	Syrian bean caper	Zygophallaceae	Phenols, alkaloids, glycosides	Hydrodistillation	Aqueous	1.5-5mg/mL	*In vivo*	Sheep, goat, cow, chicken	Antioxidants, alter ion channels and enzyme actions	Marked reduction in oocyst shedding. Improved intestinal morphology	*C. parvum*	(Namazi and Razavi, 2024)

Conclusion and future perspectives: To find new medications and lead compounds, this study concentrated on research that assessed plants and plant derivatives as anti-cryptosporidiosis medicines. The development of targeted formulations, including oral, injectable, and nanoparticle-based delivery systems, holds great promise for increasing the efficacy of plant extracts against *Cryptosporidium*. Innovative nanoparticle-based drug delivery can enhance bioavailability, stability, and targeted action while minimizing the required dosage and potential side effects. However, the importance of clinical trials and safety validation cannot be compromised. Preclinical and clinical studies are very necessary to ensure the efficacy, optimal dosage, and safety of plant-based therapeutics. Additionally, regulatory challenges remain a significant hurdle because standardization,

quality control, and approval processes for plant-derived treatments are complex and vary across the globe.

Overall, the findings of these experiments provide insightful data about bioassays that can guide the development of new research projects concerning procedures, dosages, and experimental setups. According to this review, plants and chemicals derived from plants have a major impact on protozoans, especially *Cryptosporidium*, both *in vitro* and *in vivo*. Broad-spectrum antiparasitic medications and several plant extracts have demonstrated comparable benefits. Although this component needs more research, the traditional use of plants offers vital evidence for finding and creating synergistic medications.

Exploration of plants and derivatives of plants as potential candidates for novel treatment of *Cryptosporidium* infection are encouraged in the studies reviewed here. *In vitro*, research results should be converted into *in vitro* trials for more optimal and authentic results. To prove efficacy and safety, trials on successful animals, with the newly studied compounds separately and with the already proven anti-parasitic drugs are required. The combined effects of plant extracts against parasites should also be taken into consideration in future research studies. A study on the molecular mechanism of these plant extracts and their bioactive compounds is required.

Plant products motivate synthesizing equivalents with boosted pharmacological properties, leading to new drug contenders in the development pipeline. Many plants with proven anti-*Cryptosporidium* properties have not yet been considered for experimental conditions. Several such unexamined plants may be potential candidates for valuable pharmacologically active substances against parasites and bid for future research.

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