CHAPTER – 1

INTRODUCTION

1.1. INTRODUCTION

Helminths, or parasitic worms, are multicellular eukaryotic organisms that have a significant impact on human, animal, and plant health, making them one of the most prevalent groups of parasites worldwide. These organisms are typically large enough to be visible to the naked eye in their adult form and can infect a wide variety of hosts, including humans, other animals, and plants. While some helminths live freely in the environment, others have evolved to rely entirely on hosts for survival. These parasitic helminths possess remarkable adaptations that enable them to survive and thrive within their hosts. One key strategy is their ability to modulate or suppress the host's immune system, which allows them to evade detection and establish chronic, sometimes lifelong, infections. Though they cannot multiply in their adult form within humans, helminths propagate by releasing eggs or larvae into the environment, which can infect new hosts when ingested or otherwise encountered. These parasites are classified into three major groups based on their morphology, lifecycle, and biological characteristics: cestodes, trematodes, and nematodes (Ali et al., 2020; WHO, 2020; CDC, 2024).

Cestodes (tapeworms) are flat, ribbon-like worms that are divided into segments called proglottids, each of which contains both male and female reproductive organs, allowing the tapeworm to reproduce within its host. Cestodes lack a functional digestive system; instead, they absorb nutrients directly from the host's small intestine through their outer surface. The lifecycle of cestodes begins when their cyst-like larvae, called metacestodes, form in the tissues or organs of intermediate hosts, such as cattle or pigs. When a definitive host, such as a human, consumes undercooked meat containing these cysts, the larvae develop into adult tapeworms in the intestines. Cestodes can infect the intestines, liver, lungs, and even the brain. Some common examples include *Taenia solium* (pork tapeworm) and *Taenia saginata* (beef tapeworm), both of which can cause significant health problems, ranging from digestive issues to more severe neurological complications if the larvae migrate to the brain (Heyneman, 1996; Craig and Ito, 2007; Tang et al., 2024).

Trematodes, also known as flukes, are flatworms with leaf-shaped or oval bodies. These worms possess a bilaterally flattened body that contains a simple digestive tract, rudimentary excretory and nervous systems, and reproductive organs. Trematodes are typically hermaphroditic, meaning they have both male and female reproductive organs, which allows them to produce eggs on their own or with a mate. Most trematodes require one or more intermediate hosts, typically snails or other aquatic organisms, to complete their lifecycle. When humans consume contaminated water, fish, or other intermediate hosts, the mature flukes migrate to various organs, such as the intestines, liver, lungs, or blood vessels. Trematodes are responsible for a range of diseases, including schistosomiasis, caused by *Schistosoma mansoni*, which infects the blood vessels and liver, and fascioliasis, caused by *Fasciola hepatica*, which infects the liver. Other examples include *Fasciola gigantica*, and *Schistosoma japonicum*. These infections can lead to organ damage, chronic inflammation, and complications such as cirrhosis or cancer, particularly in cases where the infection is left untreated over long periods (Galaktionov, 2013; Bogitsh et al., 2019; Fried, 2024).

Nematodes, or roundworms, are cylindrical worms that have a bilaterally symmetrical, unsegmented body. These worms are often thread-like and may be transparent. Nematodes are pseudocoelomate, meaning they possess a body cavity that is not completely lined with tissue, and they are triploblastic, with three germ layers. Nematodes are incredibly diverse, with species ranging in size from microscopic to several meters long. They inhabit various body systems, including the skin, intestines, lungs, bloodstream, and tissues. Nematodes exhibit a range of feeding behaviors, from absorbing nutrients directly from the host to feeding on blood or tissue. The larvae of some nematodes, such as *Ascaris lumbricoides* (the giant roundworm), mature in the intestines and can cause blockages and malnutrition, while others, like *Wuchereria bancrofti*, cause lymphatic filariasis, a disease that leads to severe swelling and deformities in the limbs. *Trichuris trichiura*, the whipworm, infects the large intestine and can lead to abdominal pain, diarrhea, and in severe cases, rectal prolapse. Nematodes are found in a wide variety of environments and have evolved to infect nearly every organ system in their hosts, making them one of the most versatile and widespread groups of helminths (Lee, 2002; Lacey, 2012; Samuel and Dines, 2023).

The impact of helminth infections on public health is significant, particularly in areas with poor sanitation and limited access to healthcare. These parasites contribute to malnutrition, impaired growth, and cognitive development, especially in children. Infections can also lead to chronic diseases and complications that burden healthcare systems and hinder economic development. The ability of helminths to manipulate the immune system, evade host defenses, and persist for years or even decades in their hosts makes them particularly difficult to eliminate. Global health organizations, such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention

(CDC), emphasize the need for comprehensive control strategies, which include improving sanitation, promoting hygiene education, and providing targeted treatments to reduce helminth prevalence. Mass drug administration programs, deworming initiatives, and public health campaigns have been successful in reducing the burden of helminth infections, but continued efforts are essential to prevent their resurgence, particularly in resource-limited regions where these parasites remain a major health threat (Ali et al., 2020; Craig and Ito, 2007; Lacey, 2012; Bogitsh et al., 2019; Tang et al., 2024).

Paramphistomum species are flatworms that belong to the genus *Paramphistomum*, within the trematode (fluke) class. These parasitic flatworms are primarily known to infect a wide range of hosts, particularly livestock such as cattle, sheep, goats, and various species of wild ruminants. *Paramphistomum* species are responsible for a disease known as paramphistomosis, which affects the gastrointestinal system of domesticated animals. This parasitic disease is characterized by the infestation of the rumen, the first compartment of the stomach, where the parasites typically reside. Paramphistomosis can lead to significant digestive disturbances, impacting the health and productivity of infected livestock. Common species of *Paramphistomum* include *Paramphistomum cervi*, *Paramphistomum microbothrioides*, and *Paramphistomum suiferae*, among others. The impact of these parasites is a concern in animal husbandry, particularly in regions where livestock are raised in environments conducive to the development and transmission of trematode infections (Chaoudhary et al., 2015; Hotessa and Kanko, 2020).

1.2. ZOONOTIC LIFE CYCLE OF HELMINTH PARASITES

The zoonotic life cycle of helminth parasites is a complex and intricate process, involving multiple hosts, developmental stages, and diverse transmission routes, which collectively facilitate the survival and propagation of these parasites. Most helminths follow a dual-host system in their life cycle. The primary host, typically a vertebrate animal such as a human or livestock, is where the parasite reaches its adult form, matures, and reproduces. In contrast, the secondary host, which may be another animal species, serves as a temporary reservoir for the immature forms of the parasite, often in the form of larvae or cysts. This system allows the parasite to spread across species and environments, ensuring its persistence in nature (Castro, 1996; O'Dempsey, 2010; Benesh et al., 2021).

In the case of trematodes (flukes), their life cycle is particularly dependent on an intermediate host, usually a mollusk like a snail, where the larvae undergo crucial stages of development before they can infect the definitive host. After leaving the snail, the larvae infect various organs of the definitive host, such as the liver, lungs, blood vessels, or even the intestines. These worms are capable of causing diseases like schistosomiasis, where the blood flukes live in the blood vessels of the host (Poulin and Cribb, 2002; Galaktionov, 2013; Chai and Jung, 2024). Cestodes (tapeworms), on the other hand, primarily reside in the intestinal lumen of their definitive hosts, where they absorb nutrients directly from the host. The larvae of these parasites, such as in the case of *Taenia solium* (pork tapeworm), can form cysts in the tissues of intermediate hosts (e.g., pigs), and when humans consume undercooked meat containing these cysts, they can develop into adult tapeworms in the intestines (Georgiev et al., 2006; Del Brutto et al., 2014). Nematodes (roundworms) exhibit even more variability in their life cycles, as they can inhabit a wide range of sites within the host's body. These worms can be found in intestinal tissues as well as extraintestinal sites such as the lungs, blood vessels, and even muscle or nervous tissues. For example, the roundworm *Ascaris lumbricoides* infects the intestines, while *Wuchereria bancrofti* causes lymphatic filariasis, leading to swelling in the limbs, while some nematodes, like *Trichinella spiralis*, can infect muscle tissues and cause trichinosis when undercooked meat is consumed (Morand, 2006; Scott, 2008; Bodri, 2022).

The life cyle of Paramphistomum species involves an indirect development process with freshwater snails serving as intermediate hosts. The cycle begins with adult flukes residing in the rumen and reticulum of the definitive host, where they lay eggs and are excreted in the host's feces. Development in snail- After approximately 2 weeks, miracidia hatches from the eggs and infect freshwater snails. Then within the snail, the miracidia develop into sporocysts and then to rediae. The rediae then produce cercariae, which are released from the snail. Infection in the final host- The cercariae encyst on vegetation, forming metacercariae. Ruminats ingest the metacercariae while grazing in contaminated pastures. The metacercariae excyst in the small intestine and the young flukes attach to the intestinal mucosa, mature, and eventually develop into adult flukes in the rumen and reticulum. This life cycle highlights the complex relationship between Paramphistomum, its intermediate snail host, and its definitive ruminant host as shown in the Photo plate 1 (Dinnik and Dinnik, 1954; Hotessa and Kanko, 2020).

Helminths can be classified based on their specific habitat in the definitive host, which influences the type of disease they cause. They are categorized into blood flukes, lung flukes, intestinal flukes, throat flukes, and pancreatic flukes, based on the organs or tissues they primarily inhabit. For example, *Schistosoma mansoni* (a blood fluke) resides in the blood vessels of the intestines, while *Fasciola hepatica* (a liver fluke) infects the liver of various mammals, including humans. These distinctions are important for understanding the pathogenesis, transmission, and control measures for each type of helminth infection (Castro, 1996; Parker et al., 2003; Blasco-Costa and Poulin, 2017; Chai and Jung, 2019). Helminths exhibit remarkable diversity in their transmission routes. They can be endoparasitic, inhabiting the internal environment of the host, or ectoparasitic, living temporarily on the external surface of the host. The transmission of helminths to humans occurs through several routes, each posing distinct risks to public health. The most common routes include fecal-oral contamination, where eggs or larvae in contaminated food, water, or hands are ingested, as well as zoonotic transmission, where humans are infected after coming into contact with intermediate hosts such as animals or insects. Person-to-person contact can also contribute to the spread of some helminths, especially in overcrowded or unsanitary living conditions. Additionally, certain species, like hookworms, enter the host by skin penetration when individuals walk barefoot on contaminated soil (Bowman et al., 2021; Milgroom, 2023; Pal et al., 2023).

One of the most concerning routes of transmission is through the consumption of contaminated food, particularly raw or undercooked meat containing encysted larvae (Ortiz et al., 2024). This is especially true for parasitic worms like tapeworms and Trichinella, which can infect the host’s gastrointestinal system and then spread to systemic tissues, causing more severe complications such as neurological disorders and muscle damage (Darwish, 2022; Møller et al., 2022). These diseases can have a chronic course, leading to long-term health issues if left untreated. The range of pathophysiological complications caused by helminth infections is broad and varies depending on the species involved and the organs affected. In some cases, the presence of the parasite can lead to digestive disturbances such as nausea, vomiting, abdominal pain, and diarrhea. In more severe cases, particularly with chronic infections, helminths can lead to organ damage, malnutrition, anemia, and in some cases, death (Hailu et al., 2020; Weinstock, 2022; Wu et al., 2022). In addition to gastrointestinal damage, some helminths like Schistosoma can cause inflammation of the liver or kidney, while lung flukes can lead to respiratory issues. Blood flukes, such as *Schistosoma mansoni*, can also cause severe cirrhosis and increase the risk of developing cancer in the affected organs (Barsoum, 2003; Colley et al., 2014; Anderson, 2020).

Understanding the complex life cycles, transmission routes, and the pathogenesis of helminths is crucial in designing effective prevention, treatment, and control strategies.

Global health organizations like the WHO and CDC emphasize the importance of improving sanitation, hygiene practices, and access to medical care, along with public health education campaigns (Wescott, 1982; Prichard et al., 2012; Blair, 2015). Mass Drug Administration programs and deworming initiatives are essential to reducing the burden of helminth infections, particularly in regions with poor sanitation and limited healthcare infrastructure. Additionally, efforts to educate people about the risks of consuming undercooked meat and the importance of proper food handling can significantly reduce zoonotic transmission (Humphries 2012; Farrell et al., 2017; Hailu et al., 2020).

1.3. HELMINTHS AND THEIR INFECTIONS

Helminth infections can lead to various diseases in humans, with symptoms ranging from mild to severe. Some people infected with helminths may not show symptoms, but in more serious cases, they can cause abdominal issues, including diarrhea, vomiting, and stomach pain. If left untreated, especially in children, these infections can affect growth and development, leading to malnutrition and developmental delays (WHO, 2023). One common helminth is *Ascaris lumbricoides,* which causes ascariasis. This infection can be mild and asymptomatic, but it often leads to growth retardation and malnutrition. More severe symptoms include nausea, bloating, and abdominal pain. The infection occurs when people ingest eggs from contaminated soil. The larvae hatch in the intestines, move through the liver, heart, and lungs, causing damage to the lungs before maturing in the air sacs (de Lima Corvino and Horrall, 2022; Marie and Petri, 2022). Hookworm infection, or ancylostomiasis, is caused by the worms *Ancylostoma duodenale* and *Necator americanus*. The infection occurs when the larvae penetrate the skin as shown in the Figure 1(a) and travel through the body to the intestines. *Necator americanus* is transmitted through skin penetration, while *Ancylostoma duodenale* can also be transmitted through oral, skin, or placental routes. Hookworm infections can lead to Wakana disease as shown in Figure 1(b), which causes symptoms like vomiting, nausea, cough, and stomach discomfort (Loukas et al., 2016; Hailu et al., 2020).

Whipworm infection, caused by *Trichuris trichiura*, affects the large intestine as shown in the Figure 1 (c) and often causes more severe symptoms than ascariasis. It leads to painful bowel movements, diarrhea with blood or mucus, and frequent trips to the bathroom. If untreated, it can cause rectal prolapse, anemia, and growth problems (CDC, 2020). Schistosomiasis, also known as bilharzias as shown in Figure 1(e), is caused by

Schistosoma parasites that live in the blood vessels. These parasites produce large amounts of eggs, which can either exit the body through waste or become trapped in tissues. This causes an immune response that leads to inflammation as shown in Figure 1(d) and damage to organs like the liver and bladder. The long-term effects of schistosomiasis include anemia, growth issues, and cognitive problems (Colley et al., 2014). Ocular toxocariasis is caused by a roundworm, *Toxocara canis* which infects the which causes a serious eye infection as shown in the Figure 1(f). It can cause in both children and adult (Ahn et al., 2014). These infections show how helminth parasites can affect various organs and lead to both short-term and long-term health problems. Effective prevention, treatment, and control measures are crucial to reduce the impact of these infections, especially in vulnerable populations.

a. Ancylostomiasis b. Hookworm infection c. Trichurasis

d. Schistosomiasis e. Bilharzia f. Toxocariasis

Figure 1. Images of some of infection caused by helminth parasites https://app.lecturio.com/#/article/3398; ajol-file-journals_556_articles_271005_664d9fd1c3ef7.pdf; https://www.nejm.org/doi/abs/10.1056/NEJMicm240662

1.4. GLOBAL EPIDEMIOLOGY OF HELMINTHIASIS

Helminthiases are diseases caused by helminth parasites that affect millions of people world-wide, especially those living in developing countries of the world. It causes morbidity and mortality to the host organisms by compromising their nutritional status, affects cognitive processes, induces tissue reactions, and causes intestinal obstruction or rectal prolepses (Nalule et al., 2013). WHO has proclaimed that poor social infrastructure, unhygienic livelihood conditions, and global climate changes are the key factors of the high prevalence of helminth infection in tropical and sub-tropical countries (WHO, 2017). Intestinal cestodes mainly, *Taenia saginata* has a high endemicity in middle east Asia, central Asia, Africa and Latin America whereas it has moderate endemicity in Europe, South Asia, Japan, and Philippines (Eichenberger et al., 2020). Intestinal nematode infections are most prevalent in Asia, with roughly 67% of cases according to an analysis by WHO (Pisarski, 2019).

Globally, there are more than 91 species belonging to 46 genera that are digenetic trematodes infecting humans. About 1/3rd of the human population living in developing countries is infected with one or more helminth parasites (Khan et al., 2019). Globally, helmitnhs infects nearly two billion people (Liu et al., 2020). It is estimated that about 30-100 million persons worldwide have strongyloidiasis. The infection is caused by a nematode, *S. stercoralis*, the infection is more common in tropical countries with poor sanitation, especially in countries of Southeast Asia and parts of Africa (Ahmadpour et al., 2019). It is estimated that about 187 million people in Sub-Saharan Africa, India, China, East Asia, and America suffer from Schistosomiasis (McManus et al., 2018). It has been reported that lymphatic filariasis affects more than 120 million people globally with about 40 million disFigured and incapacitated by the disease (Lupenza et al., 2022). Globally, about 819 million people are infected with *Ascaris lumbricoides*, 464 million with *Trichuris trichuria*, and 438 million with hookworms (Pullan et al., 2014). More than a quarter of the world's population is at risk of infection with the soil-transmitted helminths *As. lumbricoides, Ancylostoma duodenale, Necator americanus, Trichuris trichiura,* and *Strongyloides stercoralis* (Jourdan et al., 2018).

1.5. ANTHELMINTIC DRUGS AND THEIR MODE OF ACTION

Helminth infections are a widespread public health issue, affecting large segments of the global population, particularly in developing countries. These infections are some of the most common diseases in humans, and anthelmintic drugs are frequently used to treat these parasitic infections (Yadav and Singh, 2011). These drugs work by targeting the parasites' neuromuscular systems, disrupting their normal functions and often leading to paralysis and elimination of the parasite. Certain anthelmintic drugs act quickly and selectively by targeting neuromuscular junctions in nematodes (roundworms), which leads to spastic paralysis (Martin, 1997; Martin and Robertson, 2010). Drugs like levamisole, pyrantel, and morantel function as agonists at nicotinic acetylcholine receptors, stimulating the receptors in the muscle of the parasite and causing its paralysis. Other drugs like dichlorvos and haloxon inhibit cholinesterase, an enzyme crucial for neurotransmission, effectively paralyzing the parasite (Harrow and Gration, 1985; Martin, 1997; Singla, 2021).

One widely used broad-spectrum anthelmintic drug is mebendazole, which is primarily used to treat gastrointestinal parasitic infections. It has been approved by the FDA for treating infections caused by hookworms (*Necator americanus*, *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*), pinworms (*Enterobius vermicularis*), and whipworms (*Trichuris trichiura*). The drug works by inhibiting microtubule formation in the parasite, disrupting glucose uptake and cellular functions, ultimately leading to parasite death (Chai et al., 2021; Eskandari et al., 2022; Karunarathna et al., 2024). Albendazole is another commonly prescribed drug for intestinal nematode infections such as ascariasis, hookworm infections, trichuriasis, strongyloidiasis, and enterobiasis, as well as intestinal tapeworm infections like taeniasis and hymenolepiasis. Albendazole also plays a key role in treating various filarial diseases like lymphatic filariasis, onchocerciasis, loiasis, mansonellosis, and dirofilariasis, often in combination with other drugs like ivermectin or diethylcarbamazine (Horton, 2000; Chai et al., 2021; Chakraborty et al., 2022). For schistosomiasis, praziquantel (PZQ) is considered the most effective treatment, particularly for adult schistosome worms. It is also effective for treating other trematode and cestode infections, such as taeniasis and cysticercosis (Nogueira et al., 2022). Ivermectin is another potent anthelmintic drug, effective against a wide range of parasitic nematodes, including those in the gastrointestinal tract and lungs. It works by activating glutamate-gated chloride channels in the parasite's muscles, causing hyperpolarization and paralysis of the muscles, thereby killing the parasite (Dourmishev, 2005). Overall, these anthelmintic drugs play a critical role in managing and treating helminth infections, offering effective solutions for both intestinal and systemic parasitic diseases. However, the effectiveness of these drugs depends on factors like the type of parasite, the severity of infection, and the host's immune system, highlighting the importance of accurate diagnosis and timely treatment (Yadav and Singh, 2011; Dyary, 2016).

1.6. BIOINFORMATICS IN DRUG DISCOVERY AND DEVELOPMENT

In-silico drug design is an advanced, computer-based methodology that harnesses the power of computational modeling and simulation to aid in the discovery of new drug candidates. By utilizing sophisticated algorithms and bioinformatics tools, in-silico techniques allow researchers to simulate the interactions between molecules, predict their behaviors, and identify potential therapeutic compounds. This approach has become integral to modern drug discovery, as it accelerates the process by predicting drug efficacy and optimizing compounds before they are synthesized and tested in the laboratory, thereby saving time, labor, and resources (Kapetanovic et al., 2004; Shaker et al., 2021; Niazi and Mariam, 2023).

The drug discovery process typically begins with the identification of a suitable biological target—usually a protein, enzyme, or receptor—followed by the validation of the drug's potential for therapeutic intervention (Terstappen and Reggiani, 2001). The ultimate goal is to identify molecules that can be developed into effective therapies for various diseases, including those caused by genetic mutations (endogenous) or infectious agents like bacteria, viruses, or parasites (exogenous). Drug design in its simplest form involves creating small molecules that are chemically and structurally complementary to their target biomolecule, enabling optimal binding and the desired therapeutic effect (Rao and Srinivas, 2011; Ferreira et al., 2015; Wu et al., 2023). Bioinformatics tools, such as molecular docking and molecular dynamics (MD) simulations, play an essential role in drug discovery. These techniques enable researchers to predict how potential drug compounds interact with their protein targets, thereby facilitating the screening and optimization of promising compounds. Molecular docking is a computational method that simulates the binding of ligand molecules to receptors, predicting binding orientations, strengths, and affinities. By providing insight into how compounds bind to specific targets, molecular docking helps identify candidates with the highest potential for therapeutic efficacy (Agarwal and Mehrotra, 2016; Hollingsworth et al., 2018; Singh et al., 2022).

Combining molecular docking with molecular dynamics simulations further enhances drug discovery. MD simulations allow researchers to predict the binding modes, affinities, and stability of protein-ligand complexes by modeling the dynamic behavior of molecules over time. This combination of docking and simulation enables more accurate predictions about how compounds will behave in real biological environments, streamlining the process of discovering and developing novel therapeutics (Naqvi et al., 2018). Molecular dynamics simulations have proven particularly valuable in exploring the mechanisms underlying protein misfolding diseases, such as Alzheimer's or Parkinson's, and in conducting virtual screening of large compound libraries to identify potential drug candidates. They are also used to study drug resistance mechanisms that arise due to mutations in target proteins, offering insights that can inform the development of drugs that overcome such resistance (Liu et al., 2018). In summary, in-silico drug design, through techniques like molecular docking and molecular dynamics simulations, has revolutionized the drug discovery process, allowing researchers to screen and optimize compounds with greater speed and accuracy. These computational methods play a vital role in the development of new therapies for a wide range of diseases, making drug development more efficient and cost-effective (Śledź et al., 2018; Brogi et al., 2020; Singh et al., 2022).

1.7. URGE FOR NOVEL ANTHELMINTIC AGENTS

The use of synthetic drugs such as albendazole, benzimidazole, levamisole, mebendazole, praziquantel, etc. is the most common practice of controlling helminth infections (Prichard et al., 2008; Moser et al., 2017). However, adaptation and evolutionary changes of helminth parasites due to climatic changes as well as worldwide resistance capacity against commercial drugs have minimized the effective controlling of helminthiasis (Hotez et al., 2016; Rashid et al., 2018). The helminth parasites get adapted through various mechanisms. One of the mechanisms is the ability of dendritic cells which acts as a mediator between innate and adaptive immunity interpret helminth-inherent signals and induce T-helper cell Type 2 responses play a crucial role in resistance to helminths. Dendritic cells (DCs) play a central role in activating CD4 T (T helper, Th) cells. As a component of their response to pathogen-associated stimuli, DCs produce cytokines and express surface molecules that provide important cues to modulate the effector functions of responding Th cells (Carvalho et al., 2009; Motran et al., 2018). Helminths are very successful parasites because of their ability to modulate the host's immune response. They have evolved a spectrum of immunomodulatory molecules that are now defined and heralding a molecular revolution in parasite immunology. The ability of parasites to go against the host immunity reflects their powerful manipulation of the immune system, which is achieved through the release of a spectrum of finely tuned and highly evolved modifications in immuno-modulatory factors like Cytokine & Innate Defense Homologs and Growth Factors (eg- alarmin release inhibitor, asparaginyl-tRNA synthetase, Granulin-like growth factor-1, etc.), modification in TLR Signaling (fatty acid binding protein, lysophosphatidylserine, mucin-like polypeptide), Intracellular Signaling and Gene Expression (some of them are abundant larval transcript, ATP-diphosphohydrolase, Cathepsin L peptidases), they also cause modification in enzymes and inhibitors (Anti-inflammatory protein-1, Kunitz-type serine protease inhibitor, tissue inhibitor of metalloprotease), lipid or lipid-binding (Protein of *Ascaris suum*-1, Prostaglandin-2) and extracellular vesicles (Maizels et al., 2018). One of the important characteristics of helminths is that they are macro pathogens that prevent them from being ingested by phagocytic cells. Thus, during infection by helminths, the products secreted by these parasites play a fundamental role as modulators of phagocyte activation by modifying the microenvironment in which these cells participate in the induction and instruction of the innate and adaptive immune responses (Motran et al., 2018). Helminths exploit the microRNAs (miRNAs) which are a class of noncoding RNAs that contribute to a broad range of biological processes through post-transcriptional regulation of gene expression. In this way, they can modulate the host immune system (Donnelly and Tran, 2021). As an alternative to the growing incidence of anthelmintic resistance, there has been considerable interest in the search for effective and safe dewormers in the form of medicinal plants which has its root in the traditional ethnomedicine system (Buza et al., 2020, Kumsa et al., 2020).

1.8. EXPLORING ANTHELMINTIC AGENTS FROM PLANTS

Plants have been an essential source of medicinal remedies for thousands of years, serving as natural healers for both human and animal diseases (Singh et al., 2020; Sureshbabu et al., 2020; Radha et al., 2021). India, with its rich cultural heritage, has a long-standing tradition of using plants in healing practices, with systems like Ayurveda, Unani, Siddha, and folk medicine being integral to its indigenous knowledge. These systems have significantly influenced the approach to wellness and healing in India, where about 70% of the population still relies on traditional medicine (Mukherjee, 2020). Medicinal plants contain a wide range of bioactive compounds such as alkaloids, terpenoids, tannins, saponins, and phenolic compounds, which contribute to their therapeutic properties, including anti-inflammatory, antimicrobial, anthelmintic, antifungal, antiviral, and antioxidant activities (Swargiary et al., 2016; Alagbe et al., 2020; Biswas et al., 2020; Darzuli et al., 2021). With over 391,000 vascular plant species recognized, around 31,128 of these plants have documented medicinal uses (Dhyani, 2020). These secondary metabolites, found in plants, play a crucial role in defense mechanisms against diseases and contribute to immunity against both acute and chronic illnesses (Nandagoapalan et al., 2016). Antioxidants, such as phenolic and flavonoid compounds, are vital in neutralizing harmful free radicals that damage cells, offering protection against various metabolic diseases (Swargiary et al., 2017; Liu et al., 2018). Many medicinal plants with antioxidant properties also demonstrate strong anthelmintic activity, and studies have shown a positive correlation between antioxidant content and the effectiveness of plants against parasitic infections (Adnan et al., 2019; Akkol et al., 2022). With their high nutritional content and antioxidant properties, medicinal plants hold promise as valuable sources of phytomedicine for combating diseases, including parasitic infections (Swargiary et al., 2017).

The use of plants and plant-based herbal products as an effective and sustainable strategy for the control of helminth infestations has been practiced for a long in many parts of the world (Hoste and Torres Acosta, 2011; Tandon et al., 2011). Many studies throughout the world have established the presence of anthelmintic properties in many plants (Giovanelli et al., 2018; Esteban-Ballesteros et al., 2019; Biswal et al., 2022). In India, many researchers have investigated the anthelmintic efficacy of traditionally used medicinal plants and also the isolated active compounds showing considerable anthelmintic properties (Gogoi et al, 2021; Majumdar and Kar, 2023). The northeastern part of India is rich in natural flora and fauna. Accordingly, many healthcare practices in this part of India are based on traditional methods of zoo-and phytotherapy mode of treatment. Several medicinal plants that are consumed by local people as deworming medicines are scientifically and experimentally well-established (Roy and Tandon, 1996; Roy et al., 2009 (a); Swargiary et al., 2016; Hazarika et al., 2022).

Hypericum japonicum Thunb., belonging to the Hypericaceae family, is a valuable medicinal plant that has been used in traditional medicine for centuries, particularly for its anthelmintic properties (Swargiary et al., 2019). It is widely recognized in various cultures for its diverse therapeutic applications, including its ability to treat helminth infections, as well as its broader pharmacological potential. *H. japonicum* is reported to have a range of medicinal benefits, including hepatoprotective, anti-tumor, antibacterial, antiviral, and antioxidant activities (Huang et al., 2018; Anoopkumar et al., 2021; Li et al., 2023). These effects are attributed to the diverse phytochemicals found in the plant, such as alkaloids, flavonoids, and other bioactive compounds, which contribute to its ability to protect against liver damage, fight infections, and neutralize harmful free radicals in the body. *H. japonicum* is a small annual herb that typically grows to a height of 5-35 cm, characterized by its small, diffuse branches and bright yellow flowers (Figure 2). This plant is predominantly found across South-East Asia, with significant populations in India and other regions where traditional healing practices have long utilized its medicinal properties. In the rural areas of Assam, particularly within the Bodo community, *H. japonicum* is consumed as a traditional remedy. The community uses the raw juice of the plant, consumed on an empty stomach, to treat helminthic infections, which suggests its historical importance as an anthelmintic agent. The traditional use of the plant reflects its deep integration into local healing practices and its potential effectiveness in combating parasitic worm infections (Swargiary et al., 2019).

Despite the plant's long history of use in traditional medicine, there has been limited scientific research on its pharmacological properties, particularly in terms of its anthelmintic activity and the specific bioactive compounds responsible for its therapeutic effects. This gap in research has prompted the present study, which aims to explore the phytochemical content of *H. japonicum* in greater detail, as well as its antioxidant properties. By analyzing the plant's chemical composition, the study intends to identify the specific compounds that contribute to its healing effects and assess their potential for therapeutic applications. A critical aspect of the research is the isolation, purification, and characterization of the bioactive compound(s) that exhibit anthelmintic activity. The focus is to identify natural compounds that could be developed into anthelmintic agents, potentially providing an alternative to synthetic drugs currently used to treat parasitic worm infections. This could be particularly valuable in regions where helminthic diseases remain prevalent, and there is a need for cost-effective, natural remedies that can be easily incorporated into traditional healthcare systems.

Furthermore, the antioxidant properties of plants are also of significant interest. Antioxidants play a crucial role in neutralizing free radicals and reducing oxidative stress, which is linked to a variety of chronic diseases. By evaluating the antioxidant potential of *H. japonicum*, the study aims to determine its ability to protect cells from damage caused by reactive oxygen species (ROS), which are involved in the development of conditions such as cancer, cardiovascular disease, and neurodegenerative disorders (Huang et al., 2018; Anoopkumar et al., 2021; Li et al., 2023). Understanding the antioxidant capacity of *H. japonicum* could further enhance its medicinal value, highlighting its potential as a broad-spectrum therapeutic agent. In conclusion, *Hypericum japonicum* holds significant promise as a medicinal plant, with a rich history of use in traditional medicine, particularly for treating helminthic infections. By investigating its phytochemical profile, antioxidant activity, and anthelmintic potential, the current study aims to bridge the gap between traditional knowledge and modern pharmacological science, uncovering new possibilities for the development of plant-based therapeutics for parasitic diseases and oxidative stress-related conditions.

Taxonomic classification of plant

Kingdom: Plantae
Phylum: Tracheophyta
Class: Equisetopsida
Order: Malpighiales
Family: Hypericaceae
Genus: *Hypericum*
Species: *H. japonicum* Thunb.

Figure 2: *Hypercium japonicum*

OBJECTIVES

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The present study aims to evaluate the anthelmintic potential of *Hypericum japonicum* by assessing its bioactive compounds and their effectiveness against helminths. The objectives of the study include:

1. Study of phytochemical content, antioxidant and anthelmintic activity of *Hypericum japonicum* crude extracts and solvent fractions.
2. Isolation, purification, and characterization of bioactive compound(s) from *Hypericum japonicum* and its anthelmintic study.
3. Study of histological, ultrastructural, and biochemical alterations in helminth parasites treated with bioactive compound(s).
4. Evaluation of molecular interaction and docking of bioactive compound(s) with enzymes using bioinformatics tools.