CHAPTER - 2

REVIEW OF LITERATURE

2.1. INTERNATIONAL

Medicinal plants have long been an integral part of traditional medicine and continue to offer a promising source of natural compounds for combating diseases. Their role is particularly important in addressing the challenges posed by drug resistance in parasitic worms, such as *Haemonchus contortus*, and the high cost of synthetic anthelmintic drugs. Research highlights the therapeutic potential of various plants for their antioxidant and anthelmintic activities. Eguale and Griday (2009) demonstrated the significant anthelmintic properties of crude aqueous and hydroalcoholic extracts from *Chenopodium ambrosioides* and *Jatropha curcas*, indicating their potential to disrupt parasite development. Similarly, Aremu et al. (2010) reported the remarkable nematicidal activity of organic solvent extracts from South African plants such as *Cyathea dregei*, *Felicia erigeroides*, *Hypoxis colchicifolia*, and *Senna petersiana*, with *Acokanthera oppositifolia* additionally showing COX-inhibitory, anti-inflammatory effects. Antioxidant-rich plants have also been extensively studied, Komes et al. (2010) revealed the high antioxidant capacities of *Melissa officinalis*, *Thymus serpyllum*, *Lavandula officinalis*, *Rubus fructicosus*, and *Olea europaea*, which are attributed to their polyphenols and flavonoids. Eguale et al. (2011) expanded on this by showing the potent anthelmintic effects of *Senna occidentalis*, *Leonotis ocymifolia*, *Rumex abyssinicus*, and *Albizia schimperiana* against parasite eggs and larvae, highlighting their stage-specific activity against *H. contortus*. Li et al. (2011) further emphasized the strong antioxidant properties of *Pouzolzia zeylanica*, with its acetone and ethyl acetate extracts demonstrating remarkable free radical scavenging ability. Gatachew et al. (2012) reported that alcoholic extracts of plants such as *Myrsine africana*, *Rhus glabra*, *Foeniculum vulgare*, and *Jasminum abyssinicum* were particularly effective against nematode parasites, outperforming aqueous extracts in both egg-hatch and larval development inhibition tests. These studies collectively highlight the importance of solvent selection, as organic solvents like ethanol, methanol, and acetone are more effective in extracting bioactive compounds compared to water. Furthermore, the dual functionality of many medicinal plants, combining antioxidant and anthelmintic properties, underscores their versatility and potential for treating both parasitic infections and oxidative stress-related diseases.

Medicinal plants have been widely recognized as valuable sources of natural antioxidants and anthelmintic agents, offering effective and affordable alternatives to

synthetic drugs, particularly in addressing parasitic infections and oxidative stress-related disorders. For example, Keser et al. (2012) highlighted the excellent antioxidant properties of the aqueous and ethanol extracts of *Crataegus monogyna*, which can help combat free radical damage. Similarly, Ahmed et al. (2012) reported significant anthelmintic activity in ethanolic extracts of 25 plants tested against *Haemonchus contortus*, demonstrating the broad therapeutic potential of plant-based treatments. In another study, Bazh and Bahy (2013) compared the efficacy of ginger and curcumin on *Ascaridia galli* and found ginger to be more potent, killing 61.7% of worms at 50 mg after 24 hours, compared to 56.7% for curcumin. Furthermore, Soumia et al. (2014) emphasized the antioxidant and antimicrobial properties of medicinal plants, where *Hypericum japonicum* was shown to contain secondary metabolites such as flavonoids, phloroglucinols, and xanthones, which contribute to its hepatoprotective, antibacterial, antiviral, antioxidant, and immunomodulatory activities. Moreover, Mazimba et al. (2015) demonstrated the antioxidant and antimicrobial activity of *Cinnamomum verum*, suggesting its potential in addressing both infections and oxidative stress. Bazh and EL-Bahy (2015) expanded their research by studying the anthelmintic activity of ginger, curcumin, and praziquantel against *Raillietina cesticillus*, finding that ginger and curcumin were more effective in vitro than in vivo. Similarly, Williams et al. (2016) identified *Claisena anisata*, *Zanthoxylum zanthoxyloides*, and *Punica granatum* as potent anthelmintic agents, while Wangchuk et al. (2016) found that compounds derived from the Bhutanese daisy, *Anjania nubigena* exhibited strong activity against *Schistosoma mansoni* and *Trichuris muris*. Additionally, Ngouateu Teufack et al. (2017) reported that *Annona senegalensis* and *Nauclea latifolia* had synergistic ovicidal and larvicidal effects, highlighting the potential of combining plant extracts for enhanced efficacy. In the same vein, Zabre et al. (2017) demonstrated that *Acacia* species possess ovicidal and larvicidal activities against *H. contortus* and adulticidal effects against *Caenorhabditis elegans*. Furthermore, Ondua et al. (2018) revealed that the acetone extracts of *Typha capensis* significantly inhibited egg hatching and larval development of *H. contortus*. These studies highlight the immense pharmacological potential of medicinal plants, particularly their ability to provide natural compounds with antioxidant, anthelmintic, and antimicrobial properties.

Similarly, Akande et al. (2018) showed that the essential oils of the plant *Albizia adiantifolia* possess strong anthelmintic activity against adult earthworms. Paul et al. (2018) examined the anthelmintic activity of *Piper sylvaticum*: In-vitro and in-silico studies. They confirmed the potential anthelmintic activity of *Piper sylvaticum* stem extract

and all compounds were found to be effective in computer-aided drug design models. For the in-silico study, three compounds isolated from *Piper sylvaticum* stem were selected for molecular docking study, which showed that piperine has the best docking score against beta-tubulin and the binding energy was found to be −6.22 kcal/mol. The results were compared to that of the standard drug of Levamisole which gives a docking score −6.527 kcal/mol. The drug-likeliness activity of the ligand molecule was classified using ADME properties SwissADME Analysis and Moleinspiration Chemoinformatics software. The ADME properties (absorption, distribution, metabolism, and elimination) of the piperine, piperlonguminine and N-isobutyl deca-trans-2-trans-4-dienamide were evaluated. The selected properties are well-known to influence cell permeation, bioavailability, and metabolism. Hence, the predicted properties of all compounds were in the range to satisfy Lipinski's rule of five to be recognized as a drug like potential. Gonzales et al. (2019) examined the anthelminthic potential of the *Ficus insipida* latex on monogeneans of *Colossoma macropomum*, a medicinal plant from the Amazon, and found that it showed good results against parasites. Chy et al. (2019) studied the antibacterial, anthelmintic, and analgesic activities of *Piper sylvaticum* leaves and in-silico molecular docking and PASS prediction studies of its isolated compounds showing significant antibacterial, anthelmintic, and analgesic activities.

In-vitro anthelmintic activity of seven medicinal plants used to control livestock internal parasites in chief Albert Luthuli municipality, South Africa concluded that four of the selected plants used by livestock farmers in the study area are good candidates for use in controlling internal parasites (Chitura et al., 2019). The thin-layer chromatography fractions of the leaves of the herbal plant *Dalbergiella welwitschii* were found to possess profound anthelminthic activity against earthworms (*Pheretima posthuma*) and liver flukes (*Fasciola hepatica*) even better than the standard drug and did not provoke toxic effects (Ofodile et al., 2019). A study conducted by Dkhil et al. (2020) to evaluate the anthelmintic activity of *Indigofera oblongifolia* leaf extract against the earthworm, *Allolobophora caliginosa* showed that *Indigofera oblongifolia* leaf extract is an effective anthelmintic agent. A study was done on the anthelmintic potentials of medicinal plant extracts and an isolated compound (rutin) from *Terminalia catappa* against *Gastrothylax crumenifer* showed that the solvent crude extracts of medicinal plants exhibited moderate anthelmintic activity on *G. crumenifer*. Nevertheless, the highest parasite mortality was observed in n-butanol extract of *T. catappa*. In addition, rutin is isolated from *T. catappa* and had the anthelmintic effect against *G. crumenifer*. Therefore,

rutin has the potential to be an efficacious anthelmintic agent for the treatment of paramphistomosis (Minsakon et al., 2021). Similarly, anthelmintic activity LC_{50} values for n-hexane, chloroform, and acetone extracts from *Sarcocephalus latifolius* ŀeaf were 47.85, 35.76, and 5.72 (mg/mL), respectively. Chromatographic separation of acetone extract afforded two bioactive epimers, identified as vincosamide (LC_{50}, 14.7 mg/mL) and strictosamide (LC_{50}, 12.8 mg/mL) (Aderibigbe et al., 2021). A study was done to assess the ovicidal and larvicidal activity of a hydroalcoholic extract and their fractions (aqueous, Aq-F, and organic, EtOAc-F) from *Guazuma ulmifolia* leaves using *H. contortus* as a biological model, concluded that *G. ulmifolia* leaves could be potential candidates for the control of *H. contortus* or other gastrointestinal parasitic nematodes (Reséndiz-González et al., 2022). The studies of total phenolic and flavonoid content were quantified for all parts of the plant *Physalis minima.* The results suggested that *P. minima* extracts could be a promising alternative to the commercially available anthelmintics for the treatment of gastrointestinal nematodes in cattle (Ahmed et al., 2022). The study done by Ocampos et al. (2023) demonstrated that *Cassia occidentalis, Artemisia absinthium,* and *Kyllinga odorata* parts that have been traditionally used as medicinal plants are a valuable source of active compounds with anthelmintic activity against gastrointestinal nematodes. Anthelmintic efficacy of aqueous methanolic and ethyl acetate extract of *Haloxylon salicornicum* was investigated against *H. contortus.* The result showed the promising anthelmintic potential of *H. salicornicum* leaves against haemonchosis (Al-Saeed et al., 2023).

Hypericum japonicum Thunb. ex Murray, a plant species extensively used in traditional medicine, is characterized by its broad geographical distribution across Asia, Oceania, and North America, and its abundance of valuable secondary metabolites, including flavonoids, phloroglucinols, and xanthones (Liu et al., 2014). A study done in *Hypericum japonicum* for the characterization of the plant found that five novel terpenoid polymethylated acylphloroglucinols (TPAPs) with unusual architectures, namely Hyperjapones A–E (Yang et al., 2016). Similarly, a study was done aiming for the characterization of a methanolic extract of *H. japonicum* (HJME) collected from the Northern region of Nepal by LC–MS^n and UPLC-QTOF found that fifty-seven phytoconstituents were identified, being mainly flavonoids, phloroglucinols, phenolic acids and xanthones. Although compounds characteristic of *H. japonicum* were detected (quercetin, quercetin-7-O-α-l-rhamnoside, quercitrin and hyperoside), several others are here reported for the first time in this species (Peron et al., 2019). Thirteen phloroglucinols

including three new ones were isolated from *H. japonicum* as ferroptosis inhibitors (Peng et al., 2021). Four undescribed meroterpenoids along with seven known analogues were isolated from *H. japonicum* showing significant anti-inflammatory effect through suppressing NF-κB signaling pathway (Deng et al., 2022). Two new phenolics were also isolated from *H. japonicum* plant which showed effective anti-inflammatory activity (Li et al., 2023). The inhibitory effect of *H. japonicum* on H9N2 avian influenza virus was also studied and found that the S06-60% extract, obtained through resin column separation using 60% ethanol, demonstrated dose-dependent inhibition of H9N2 avian influenza virus replication in Madin-Darby canine kidney cells (Hu et al., 2024).

2.2. NATIONAL

In India, traditional medicine systems such as Ayurveda, Unani, and Siddha have been pivotal in the treatment and prevention of diseases for centuries. Herbal medicines form the cornerstone of these practices, offering remedies for a wide array of physiological and pathological conditions. Recognizing the growing need for scientific validation of traditional practices, numerous studies have been undertaken to evaluate the pharmacological properties of medicinal plants, including their anthelmintic activities. One such study by Kosalge and Fursule (2009) investigated the anthelmintic potential of *Thespesia lampas* roots through aqueous extraction. The study evaluated its efficacy against *Pheretima posthuma* (Indian earthworm), *Rallietina spiralis* (a tapeworm species), and *Ascardia galli* (a roundworm). Their findings demonstrated that the aqueous root extract exhibited significant anthelmintic activity, validating its traditional use in treating helminth infections. Similarly, *Tamarind indica* juice extracts were examined for their anthelmintic properties by Sanghavi et al. (2009). Using *P. posthuma* as the test organism, they concluded that the juice derived from the leaves showed notable anthelmintic effects, highlighting its potential as a natural remedy for helminthic infestations. Further, Jain and Jain (2010) explored the ethanol extract of *Myrica esculenta* bark for its anthelmintic activity. Their study targeted adult *P. posthuma*, a commonly used model for evaluating anthelmintic potential due to its physiological similarity to intestinal parasites. The results indicated significant activity, suggesting that *Myrica esculenta* could be a valuable resource for developing natural anthelmintic agents.

In another noteworthy study, Patel et al. (2010) assessed successive ethanolic and hydroalcoholic extracts of the whole plant *Eupatorium odoratum*. Their research

demonstrated that these extracts were highly effective against *P. posthuma*, further reinforcing the therapeutic potential of this plant in traditional and modern medicine. Parida et al. (2010) conducted a comprehensive evaluation of *Pterospermum acerifolium*, focusing on crude extracts derived from its leaves, bark, and flowers. Their findings revealed strong anthelmintic activity against tapeworms, roundworms, and flatworms, indicating the plant's broad-spectrum efficacy against various helminth species. Further, Suman et al. (2011) conducted a preliminary phytochemical investigation of *Acacia suma* bark and tested its anthelmintic activity on *P. posthuma*. The study confirmed the anthelmintic properties of the extract, attributing its activity to bioactive compounds present in the bark. Thomas and Devi (2013) by the hydroalcoholic extract of *Cortus pictus* confirmed anthelmintic activity for the treatment of helminthiasis. An ethnobotanical study by Sindhu and Manorama (2014) to investigate the preliminary phytochemicals in the petroleum ether, acetone and ethanol extracts of leaves, stem and rhizome of *Costus mexicanus* revealed the presence of alkaloid, flavanoid, terepenoid in the *C. mexicanus*. Similarly, Soni and Chauhan (2015) studied six medicinal plants like, *Aeglem armelos, Vernonia anthelmintica, Zingber officinalis, Tinospora cordifolia,* and *Phyllanthus acidus* of the Gujarat region, and reported the presence of different phytochemicals as well as strong antioxidant activity. Roopan et al. (2016) studied the ethanolic extract of the vegetable crop *Lagenaria siceraria* showed high anthelmintic properties. The study of the antioxidant activity of *Ocimum lamiifolium* and *O. basilium* by Nair et al. (2016) confirmed the presence of good antioxidant properties in the aqueous leaf extracts of both plants. Similarly, Shelu et al. (2017) from the plant *Boerhaaria diffusa* root extract on helminths like *Monezia expansa, H. contortus,* and Amphistomes showed that the root has high anthelmintic properties.

Mishra and Varma (2018) by studying leaves extract of the plant *Rhizophora mucronata* found that the plant has high anthelmintic activity against *P. posthuma.* George (2018) from the alcoholic extract of the sun-dried fruits of *Piper nigrum* concluded that the extract possesses dose-dependent anthelmintic activity and is more effective than the albendazole against *P. posthuma.* Davuluri et al. (2020) evaluated the anthelmintic activity of *Anacardium occidentale* shell, *Illicium verum* fruit, and *Artocarpus heterophyllus* seed to substantiate their traditional use against helminth parasites. A study was aimed for the in-vitro anthelmintic activity of ethanolic leaf extract of *Gossypium herbaceum* against the Indian earthworm *P. posthuma*. Three concentrations (25, 50, and 75 mg/ml) were tested and results were expressed in terms of time for paralysis and time of death of worms. In the

study, albendazole was used as a standard drug. Ethanolic leaf extract of *Gossypium herbaceum* showed more significant activity at higher concentrations when compared to the standard group (albendazole). The methanolic and ethyl acetate extracts of *Malvastrum coromandelianum* leaves were investigated for their anthelmintic activity against *P. posthuma* and *Ascardia galli*. For the experiment purpose, various concentrations were used in the bioassay, which involved paralysis and death time of the worms. Both extracts showed significant anthelmintic activity (Yadav et al., 2020).

An in-vitro experiment was conducted to determine the possible anthelmintic effects of crude aqueous and alcoholic extracts of the resins of *Boswellia serrata* and leaves of *Aloe barbadensis* on adult Indian earthworm (*P. posthuma*) and the study proved that the combination of *B. serrata* and *A. barbadensis* possess significant anthelmintic activity against *P. posthuma* (Shelke et al., 2020). A study aimed to reveal the phytochemical compositions, anthelmintic activity, and antioxidant and antimicrobial potential of *Salvadora persica* leaves extract in different extraction solvent systems. From the study, it was concluded that leaves of *Salvadora persica* have an anthelmintic effect, and good antioxidant and antimicrobial potential, the consumption of which may exert a beneficial effect on human and animal health as well (Kumar and Sharma, 2021). Similarly, in another study, the anthelmintic activity of benzene extract from *Calotropis procera* leaves was evaluated, and it was confirmed that the plant extract showed anthelmintic activity at concentration-dependent manner (10, 20, and 50 mg/ml) (Tejaswini et al., 2021). Anthelmintic activity of aqueous and alcoholic extract of leaves of *Psidium guajava* was evaluated separately with different concentrations on adult earthworms and compared with the standard drug piperazine citrate. The result found that the extract exhibited dose-dependent action, inhibition of spontaneous motility (paralysis), and death of earthworm (Biswal et al., 2022). Similarly, the phytotherapy, anthelmintic, and antimicrobial efficacy of *Dodonaea viscosa* leaf extracts were examined. The research revealed that *D. viscosa* has significant anthelmintic and antibacterial activities and it can be used for further elucidation and characterization (Pandiyan et al., 2022). In-vitro anthelmintic activity of crude methanolic extracts of the leaves *Talinum portulacifolium* on the Indian adult earthworms *P. posthuma* was investigated. The study found that *T. portulacifolium* leaf extracts exhibited considerable anthelminthic action in a dose-dependent reduction of earthworms' spontaneous death (Nazmi, 2023). Another plant *Pisonia alba* plant was selected for testing anthelmintic activity. The plant possesses significant anthelmintic activity at 60 mg/mL concentration measured by the time taken to paralyze or death of the

earthworms. The investigation concluded that the leaves of *Pisonia alba* have potent anthelmintic activity and might be efficacious against other species of helminths (Sangameswaran et al., 2023).

LCMS and HPLC are the liquid chromatography study which helps in isolation and identification of the compounds present in the medicinal plants. A study done in *Rauvolfia* species with the help of LCMS and HPLC found the presence of reserpine, which is a bioactive indole alkaloid. The presence of reserpine was shown in six pant specices of *Rauvolfia* species (Bindu et al., 2014). Another study conducted ovicidal and larvicidal effects of extracts from leaves of *Andrographis paniculata* against *Ancylostoma duodenale*, observed the presence of Andrographolide, a bioactive compound which was confirmed with the help of HPLC. They concluded that andrographolide is one of the main phytochemical responsible for significant ovicidal and larvicidal activity against *A. duodenale* (Banerjee et al., 2019). From the LCMS study, two compounds were identified notoginsenoside with mass of m/z 770.98 and another compound 1,6-Digalloyl glucose with mass of m/z 448.36 from ethyl acetate extract of *Chenopodium album* which in turn were responsible for the anthelmintic activity. In the same study, in-silico molecular docking was also carried out and found that both the bioactive compound identified were showing strong binding affinity towards the GABA receptor in compared to the reference drug (Choudhary et al., 2021). Similarly, in another study a LCMS study was done for the methanolic crude extract of *Lindernia crustacea*, they have identified five compounds from the LCMS study, they have also concluded that the leaves extract of the plant showing strong anthelmintic activity may be because of the presence of the bioactive compounds present in the plant (Ghori et al., 2021). A noteworthly study done on phytochemical profiling of *Manilkara zapota* extract, found significant compounds, (1Z,2E)-1-({[(2R,3S,4S,5R,6S)-6-{[2-(3,4-dihydroxyphenyl)-7-hydroxy-5-oxo-5H-chromen-3-yl]oxy}-3,4-dihydroxy-5{[(2S,3R,4S,5R)-3,4,5 trihydroxy tetrahydro-2H-pyran (HP), with a mass of m/z 768.2105, α-Tocotrienol with a mass of m/z 389.3209, benzyl β-primeveroside with a mass of m/z 403.1606, δ-Tocotrienol with a mass of m/z 419.2922. Furthermore, docking study was carried out and the phytochemicals HP, δ-Tocotrienol, and α-Tocotrienol exhibited significant binding and showed better binding affinity compared to the standard drug albendazole on the GABA receptor. The docking scores revealed that the compounds from *M. zapota* have a potential to become an anthelminthic agent (Goswami et al., 2024).

Ultrastructural study are important to see the morphological changes occurred in the tegument, body and also the organs and other parts of the helminth when treated with the plant extract or the bioactive compound and compare with the control helminth parasite. A study done on the ultrastructure study with the help of SEM on *Paramphistomum explanatum* when treated with methanol extract of *Bombax malabaricum* leaves observed that in the control untreated parasite the acetabulum was circular, bounded by a muscular rim around the mouth opening along with scattered papillae. The mouth situated at the posterior end which was transversely elongated oval in shape with a muscular boundary, featuring a prominent groove. The surrounding tegument displayed numerous papillae, elevated and arranged in transverse rows, separated by distinct grooves. Whreeas, in the treated parasite no much changes was observed in the acetabulum but wrinkles were observed surrounding the mouth. It also showed irregular stretched configuration. Although, the genital pore was unaffected, but the adjacent portion was under strain. Again, in the albendazole-treated parasites, minor damage was seen in the acetabulum with modification of shape and also papillae were distorted. The shape of the mouth also was changed, and denaturized tegument was also observed. Thus, with the ultrastructural study it was confirmed that the plant extract has shown anthelmintic effect (Hossain et al., 2012a). Again similar kind of study was done on anthelmintic effect of a methanol extract of leaves of *Dregea volubilis* on *Paramphistomum explanatum*, found that in the treated parasites minor damage occured in the acetabulum. The shape got modified and also papillae were distorted. Sharpness of the mouth was found changed and the groove was completely absent. The tegument of the parasite was also found damaged with lost of papillae and the texture of the tegument was fully disrupted wheres in the control untreated parasite no change was observed (Hossain et al., 2012b). Another study done on the anthelmintic efficacy of *Holarrhena pubescens* against *Raillietina spp.*, under the SEM it was observed that in the control parasites the entire surface of the scolex and proglottids shows a smooth appearance even the suckers were found smooth with distinct hooks appearance. Whereas in the treated parasites noticeable distortions were observed in the head region, folding of the tegument, outward protrusion of the suckers, and detachment of the encircling hooks. Furthermore, the proglottids exhibited a severely compromised tegument, displaying extensive folding and cracking in the tegument (Saha et al., 2023).

The development of novel, plant-based anthelmintic drugs is particularly significant in light of the growing resistance of parasitic worms to synthetic anthelmintic agents. These studies collectively underscore the rich potential of medicinal plants in India for combating

helminthic infections. By validating the traditional use of these plants through scientific methodologies, they pave the way for the development of novel, plant-based anthelmintic drugs. The diversity of plant species investigated and the variety of extraction methods employed highlight the importance of ongoing research in this field to uncover more bioactive compounds with therapeutic applications. The ongoing exploration of India's rich medicinal plant heritage holds immense promise for addressing global health challenges. Continued research in this field can pave the way for the development of safer, cost-effective, and eco-friendly anthelmintic therapies, ultimately contributing to improved healthcare outcomes and the preservation of biodiversity.

2.3. REGIONAL

Northeast India is rich in medicinal plants and is unique in the diversity and traditional utilization of these medicinal plants. People of this region have been using medicinal plants as a source of medicine since ancient times. The shoot of plant, comprising of leaves, stems and branches have several medicinal properties. Roy and Swargiary (2009) in their study of the ethanolic shoot extract of *Alpinia nigra* found that the extract showed an anthelmintic effect on *Fasciolopsis buski*, a giant intestinal trematode. The roots of the plants are also known for containing high medicinal compounds offering a wide range of health benefits (Basak et al., 2010). Roy et al. (2010) showed that the ethanolic root peel extract of *Potentilla fulgens* has anthelmintic activity against the cestode parasite, *Raillietina echinobothrida,* and the trematode parasite, *Gastrothylax crumenifer*. Phyochmeical analysis and in vitro amthelmintic activity of *Imperata cylindrica* underground parts found that the roots extract effectively killed and also caused detrimental effect on the roundworms and tapeworm (Lalthanpuii and Lalchhandama, 2020). Seeds also contain high medicinal value with wide range of properties which deals with several health issues (Kichu et al., 2015). Tandon et al. (2011) studied that the crude aqueous extract and hydro-alcoholic extracts of the seeds of *Croton macrostachys* contain anthelmintic activity. In an investigation done by Kamal et al. (2015) to evaluate anthelmintic activity of methanolic extract of seeds of sesame (*Sesamum indicum*) showed that alcoholic extract exhibited significant anthelmintic activity at highest concentration of 10 mg/mL as compared with levamisole (1 mg/mL).

The most commonly used parts among all the plant's part are the leaves. It contains various medicinal compounds which in turn offer a wide range of health benefits. Mahato

et al. (2014) studied the methanolic extract of the leaves of *Heliotropium indicum* and showed that the extract has strong anthelmintic activity against the adult Indian earthworm, *P. posthuma*. Similarly, Swargiary et al. (2016) studied the phytochemical content, antioxidant, and anthelmintic properties of some edible wild plants of lower Assam. The plants were *Clerodendrum viscosum, Eryngium foetidum, Lippia javanica,* and *Murraya koenigii*. They found that the methanolic extracts of the plants showed as a natural source of free radical scavengers and plausible veterinary uses. In a similar study, Bora et al. (2017) investigated thirteen Ayurvedic plants published by various researchers and found that the plants have anthelmintic properties. Moreover, Elija (2018) by studying the methanolic extract of the leaves of *Citrus limon* which is commonly found in Assam showed that the plant contains anthelmintic properties. Ikbal et al. (2020) studied that the traditional healers of Barak Valley region of Assam used traditional medicine for the treatment of various diseases including helminths infection. Assessment of phytochemical and anthelmintic activity of some selected ethnomedicinal plants namely *Justicia adhatoda, Vernonia amygdalina, Mikania micrantha* and *Momordica charantia* from Barak Valley Region of Assam was done and found that the phytochemical study demonstrated the presence of flavonoids, tannins, phenolic compounds, alkaloids and glycosides and it also also showed significant anthelmintic activity by inducing mortality of earthworm *Pheritima posthuman*. The Santhal tribe in Assam, India utilizes *Sesbania sesban, Cyperus compressus* and *Asparagus racemosus* to treat intestinal-worm infections. A study was done on the in-vitro anthelmintic efficacy of these plants against *Gastrothylax crumenifer*. The study showed considerably high nematicidal efficacy of the methanolic leaf extract of *S. sesban* provided scientific evidence in support of its use in traditional medicine (Soren and Yadav, 2021). A study was undertaken to investigate the anthelmintic activity of crude extracts prepared from leaves of *Nyctanthes arbortristis* in water, ethanol, and hydro-ethanol against *Ascaridia galli*. The experiment concluded that crude extracts of *N. arbortristis* leave prepared in water, ethanol, and hydro-ethanol exhibited promising anthelmintic activity against *A. galli* in an in-vitro study. The anthelmintic activity of aqueous extract was time-dependent while hydro-ethanolic and ethanolic extracts were both concentration-dependent and time-dependent. The hydroethanolic extract was better than the ethanolic extract alone and was equivalent to that of the standard drug piperazine (Hazarika et al., 2022). In-vitro cestocidal activity of *Persicaria hydropiper*, a traditionally used anthelmintic plant in India was investigated. The result indicated that *P.*

hydropiper leaves possess noteworthy anthelmintic efficacy and justified their use in traditional medicine against intestinal worms (Nagi et al., 2023).

Medicinal plants are known for the presence of secondary metabolites which in turn possess high antioxidant property. Paul et al. (2010) in their study of in-vitro antioxidant activity of methanolic extract from leaves of four plants viz. *Clerodendrum colobrookianum, Gnetum gnemon, Sarcochlamys pulcherrima,* and *Garcinia lancifolia* showed that the plants possess high antioxidant activity. Mudoi et al. (2012) also investigated the antioxidant properties of the dried pulp of *Garcinia pedunculata* showed potential antioxidant activity. Similar studies were found to be done, where one of the study where antioxidant activities and total phenolic and flavonoid contents in three indigenous medicinal vegetables in three solvent extracts (hexane, ethyl acetate and methanol) was done and found that the methanol extracts of all the species showed the highest antioxidant activities and high values for total phenolic and flavonoid contents. They have also concluded that there is a strong correlation between the antioxidant activities and the total phenolic content of the plants (Handique et al., 2012). Basumatary et al. (2015) investigated the antioxidant activity of methanolic fruit of *Hodgsonia heteroclita* and found that the fruit extract exhibits free radical scavenging and reducing properties which might constitute an important source of natural antioxidants. Also, Handique and Gogoi (2016) studied the antioxidant activity of eight medicinal plants of Assam and the results indicated that all the tested plants showed potential antioxidant activity. An antioxidant study done on six wild edible plants which is consumed by Bodo people of North-east India found that among the six pants studied *M. perpusilla* possessed highest antioxidant property (Basumatary and Narzary, 2017). Interestingly, a study done on the essential oil of *Curcuma caesia Roxb,* found that the leaf essential oil of *C. caesia* has chemical composition of diverse biological importance. The study also confirmed that the leaf has a high amount of antioxidant property, which is even more than some of the standards used during their experiment (Borah et al., 2019).

The external surface of helminth parasites is called tegument which serve various biological function especially nutrient absoption, locomotion, excretion and regulations of electrochemical and osmoregulation. Acid phosphatase, alkaline phosphatase and 5'-adenosine triphospatase enzymes are present in the teguments of the helminths. All these enzymes which are involved in various metabolic processes, are also believed to be involved in absorption and/or digestion in the parasite (Roy and Swargiary, 2009). Roy et al. (2012) studied the crude alcoholic extract of a plant named *Alpinia nigra* which contains

flavonoids, terpenoids, and kavalacetones that causes destruction and degeneration of the surface architecture of tegument and also inhibit energy metabolism-related enzymes. Glycolytic enzymes are very crucial for survival of helminth. It helps in energy metabolism and also help helminth for their respiration. Some of the glycolytic enzymes found in the helminth are pyruvate kinase, phosphoenolpyruvate carboxykinase (PEPCK), malate dehydrogenase, lactate dehydrogenase (Swargiary et al., 2013). A study done on alteration in the activities of some glycolytic enzymes in the cestode, *Raillietina echinobothrida* when treated with *Flemingia vestita* discovered that the activities of hexokinase (HK), phosphofructokinase (PFK), PEPCK, MDH, malic enzyme and LDH increased their activity. Whereas, the activitity of pyruvate kinase decreased (Das et al., 2004). Swargiary et al. (2013) studied the anthelmintic efficacy of the shoot extract of *Alpinia nigra*, root peel of *Potentilla fulgens,* and root tuber of *Carex baccans* against glycolytic enzymes, like phosphoenolpyruvate carboxykinase, pyruvate kinase, lactate dehydrogenase and malate dehydrogenase of the zoonotic fluke *Fasciolopsis buski* and found that the plants cause anthelmintic stress and inhibition of enzyme activities in paralyzed helminths. Neurotrasmitter enzyme plays a very crucial role in helimth. Some of the neurotransmitter enzyme found in the helminths are Acetylcholine esterase, glutamate decaboxylase. A study conducted to explore the inhibitory potential of phytochemicals on neurotransmitters and their associated enzymes in the helminth parasite *R. echinobothrida* found that the parasites showed a significant decrease in the activity of acetylcholinesterase and nitric oxide synthase when compared with the control parasites (Giri and Roy, 2015). Another study done in *R. echinobothrida* when treated with the bioactive compound extracted from *Lysimachia ramosa,*found that the activities of the AchE and NO altered significantly when compared with the untreated parasites (Dey et al., 2021).

Plants are known to contain several bioactive compounds which in turn help the plant to fight against several health issues. Many studies have revealed how bioactive compounds found in the medicinal plants act against anthelmintics. A bioactive compound astragalin isolated from the plant *Alpina nigra* against *Fasciolopis buski* showed that the compound as a strong anthelmentic agent (Swargiary and Roy, 2015). Tandon and Das (2018) showed that the plant *Flemingia vestita* and its active compound, Genistein which is a multifarious botanical also shows anthelmintic properties along with anti-diabetic, anti-cancer, and antioxidant properties. In another study, using the bioassay-guided method, 13 compounds have been isolated from *Cinnamomum bejolghota* leaves. Among the 13 compounds, linalool, b-sitosterol, cinnamic acid, and fridelin was isolated from the plant

and exhibited significant angiotensin-converting enzyme (ACE) inhibitory activity and might be the lead compounds to develop ACE inhibitor from this plant to control hypertension. In a separate study, twenty-two compounds were identified from the plant extract *Imperata cylindrical*, out of which fatty acids were found the predominant compounds. Among them, Palmitic acid was the most abundant. Again, bioactive phytosterols such as campesterol and stigmasterol were also detected. The plant extract was significantly effective on the helminths and showed dose-dependent anthelmintic activity as that of albendazole (Lalthanpuii and Lalchhandama, 2020). A kaempferol derivative was isolated from *Lysimachia ramosa* showed significant anthelmintic activity against *R. echinobothrida* and also showed significant alteration in enzymetic activities (Dey et al., 2021). Further, compounds geranial, linoleic acid, cinnamic acid, and fridelin showed promising results and affected the growth of nematode, disrupting the ultrastructure of eggs and preventing the hatching of the eggs to develop into the larvae stage. These results justified the traditional application of the plant as an antihypertensive and anthelmintic agent (Gogoi et al., 2021).

Histological study and also ultrastructural studies are done to see the structural changes that have occurred on the helminth parasite when they get treated with the plant extracts or the bioactive compounds. Several studies have demonstrated how the study in important. The ultrastructural study done by Roy et al. (2012) found that the changes that have occurred included mitochondrial swelling, nuclear enlargement, an increase in number and predominance of nucleoli when compared with the control parasites. Another study where histological and ultrastructural activity was done on *R. echinobothrida* found destruction throughout the general topography of the body, the scolex appeared greatly distorted with suckers extensively shrunken and the spines round the suckers were also sharply crooked when observed under Scanning Electron Microscopy (SEM). Scars and pits were evident throughout the tegument surface when observed under Transmission Electron Microscopy (TEM) (Challam et al., 2012). Ultrastructural study done on the cestode revealed irrevocable destruction of general topography of the body and the scolex appeared greatly distorted with suckers extensively shrunken and the spines round the suckers were sharply crooked when observed under SEM. Whereas, under TEM, it revealed that the tegument along with the basal lamina and the subtegumental cyton beneath was distorted at many places (Giri and Roy, 2014). The tapeworm treated with *Imperata cylindrica* showed deformities on the suckers, clumping of the spines, tegumental folds and erosion of microtriches. Extensive damage was also seen on the roundworm

including cuticular shrinkage, collapse of the lips, and formation of warty surface throughout the body (Lalthanpuii and Lalchhandama, 2020). The leaves of *Caesalpinia bonducella* and *Croton joufra* are traditionally used anthelmintics by the Missing tribe in Assam, India. The study evaluated their effects on the tegument of *Hymenolepis diminuta* (Cestoda) and the cuticle of *Syphacia obvelata* (Nematoda) using scanning electron microscopy. The result suggested that adult and juvenile *H. diminuta* exposed to extracts of both the test plants showed damaged scolex, suckers, and altered tegument. *S. obvelata* treated with *C. bonducella* showed a damaged apical region, closed mouth, and a damaged cuticle. Worms exposed to *C. joufra* showed deformations in the apical region with a closed mouth, loss of cephalic papillae, distorted lips, and damaged cuticle (Gogoi et al., 2022).

In-silico studies have been a essential for the drug discovery and also to target new drug from medicinal plants. Studies have revealed how in-silico study plays significance roles in identification of bioactive compound and also study the relation and interaction between the protein and the ligand. In a molecular docking study on adult Indian earthworm to study the potential of *Grewia bilamellata* found that molecular docking with β-tubulin revealed that 15 compounds exhibited superior binding energy (-8.3 to -6.3 kcal/mol) compared to anthelmintic drug albendazole (-6.1 kcal/mol) (Gurivelli and katta, 2023). A molecular docking study on *Chenopodium album* against *Haemonchus contortus* showed that the compounds isolariciresinol 4'-O-beta-D-glucoside, (1S,2S,4R)-1,8-epoxy-p-menthan-2-ol glucoside and linalool had binding energies of -7.8, -7.5, and -7.1 kcal/mol, respectively, with the Nu-class GST protein. Moreover, the ADMET properties of these compounds also showed ideal behavior to known drug molecules (Islam et al., 2023).

It is observed from the review of literature that various plant parts have been used for phytochemical, antioxidant, and anthelmintic studies, with leaves and aerial parts being the most commonly utilized. Methanolic crude extracts are generally preferred for plant extractions. The dose for the helminth treatment used by several authors were ranging from 5 mg/mL to 25 mg/mL. There are several bioactive compounds isolated from the plants which showed direct effect against the helminthes. GC-MS, LC-MS and HPLC are mostly used for identification and isolation of bioactive compounds. In-silico studies such as molecular docking and MD simulation found to be effective in screening of drugs and analyzing the prediction of protein-ligand interaction. Molecular Dynamics Simulation is effectively used computational tool in drug discovery processes.