ABSTRACT

Helminthiasis, parasitic worm infections affecting billions globally, poses a significant threat to human and animal health, particularly in regions with poor sanitation and limited access to healthcare. These infections contribute to a range of health problems, from malnutrition and anemia to impaired cognitive development in humans, and cause substantial economic losses in livestock industries. The growing problem of drug resistance among helminths, especially *Paraphistomum* species that cause paraphistomosis in livestock, makes the search for new and effective treatments a critical priority. Traditional medicinal plants offer a rich source of potential new drugs. This study focuses on *Hypericum japonicum Thunb.*, a plant used in Northeast Indian traditional medicine to treat worm infections. We aim to thoroughly investigate this plant's potential. Crucially, we will explore the *mechanisms* by which *H. japonicum* works against these parasites. This involves examining how the plant's chemicals interact with essential enzymes within the worms, such as those involved in muscle function and energy production. We will also use microscopy to observe the structural effects of the plant extracts on the worms themselves. By combining traditional knowledge with modern scientific methods, this study aims to validate the traditional use of *H. japonicum* and determine if it can be used to develop new, safe, and effective treatments for worm infections in both humans and animals.

The present study aimed to systematically investigate the anthelmintic properties of *Hypericum japonicum* through several key objectives. First, we sought to determine the phytochemical composition and antioxidant potential of *H. japonicum* by analyzing four solvent fractions—hexane, diethyl ether, ethyl acetate, and methanol—using qualitative and quantitative techniques. Second, we aimed to evaluate the in vitro anthelmintic activity of these solvent extracts against parasitic worms to identify the most effective fraction. Third, we focused on isolating, identifying, and characterizing the specific bioactive compound(s) responsible for the observed anthelmintic activity using chromatographic and spectroscopic methods. Fourth, to further validate the potential anthelmintic properties, we employed both in vitro assays and in silico molecular docking studies to assess the compound's interaction with key parasite targets. Finally, to provide comprehensive mechanistic insights, we conducted histological examinations of treated worms, ultrastructural analysis of parasite cells

using electron microscopy, and biochemical enzyme assays to evaluate the impact of the bioactive compound on essential enzymatic pathways within the parasite. Through this multi-faceted approach, we aimed to bridge traditional medicinal knowledge with modern scientific validation, potentially paving the way for new anthelmintic drug development.

The medicinal plant *Hypericum japonicum* was collected from the Kokrajhar area, Assam, and taxonomically identified by a botanical expert with the aid of a herbarium sheet and photographic documentation. To investigate its phytochemical composition and biological activities, qualitative phytochemical screening involved the identification of 15 different phytocompounds, while quantitative estimations were conducted to determine the total content of key biomolecules, including proteins, carbohydrates, phenolics, and flavonoids. To assess the antioxidant potential of *H. japonicum*, multiple antioxidant assays were performed. These included the Total Antioxidant Capacity (TAC) Assay, which measures the cumulative antioxidant activity of the extracts, and the Ferric Reducing Antioxidant Power (FRAP) Assay, which evaluates the ability of the extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Additionally, the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay was conducted to determine the free radical scavenging potential of the extracts. The Lipid Peroxidation Inhibition Assay was performed to evaluate the extract's ability to prevent oxidative damage to lipids, while the 2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonate) (ABTS) Assay was used to quantify the extract's ability to neutralize ABTS radicals. Heavy metal analysis was carried out to determine the concentration of essential and toxic elements in the plant extracts. Using spectroscopic methods, the presence of two essential elements, Copper (Cu) and Zinc (Zn), was quantified, along with three toxic heavy metals—Chromium (Cr), Lead (Pb), and Cadmium (Cd)—to ensure safety and assess potential toxicity.

The anthelmintic activity of *H. japonicum* was evaluated against *Paramphistomum* sp. The experiment was conducted using an in vitro assay and plant extract was administered to the parasites, and the time taken for paralysis and death was recorded as an indicator of efficacy.

To isolate and characterize the bioactive compound(s) responsible for the observed anthelmintic activity, chromatographic techniques were employed. Thin Layer

Chromatography (TLC) was used for preliminary separation of the bioactive components, while advanced analytical techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS) and High-Performance Liquid Chromatography (HPLC) were utilized for precise identification and purification of the active constituents. Biochemical assays were conducted to determine the effects of the bioactive compound on key enzymatic pathways in the parasites. Five essential enzymes—alkaline phosphatase (ALP), acid phosphatase (ACP), malate dehydrogenase, lactate dehydrogenase, and acetylcholinesterase—were analyzed using standard enzymatic assay protocols to evaluate the potential mechanism of action of the plant extract at the molecular level. Histological and ultrastructural analyses were performed to further assess the impact of the bioactive compound on the parasites. Additionally, Scanning Electron Microscopy (SEM) was conducted to visualize structural and morphological changes in the parasite's tegument, which could provide insights into the mode of action of the plant extract. For *in silico* investigations, molecular docking studies were conducted to evaluate the interaction of the isolated bioactive compound with the five key enzymes using AutoDock Vina. Drug-likeness properties, as well as pharmacokinetic and toxicological predictions, were assessed using SwissADME and ADMETlab databases. To further validate the stability and binding interactions of the compound with its target enzymes, Molecular Dynamics (MD) simulations were carried out using GROMACS software, allowing for an in-depth computational assessment of the bioactive compound's potential as a drug candidate.

The study revealed the presence of most bioactive compounds, except anthocyanins and glycosides across all extracts. Among the solvent extracts, ethyl acetate exhibited the highest protein content and total phenolic content. Hexane extract had the highest carbohydrate content, whereas diethyl ether showed the highest total flavonoid content.

Heavy metal analysis demonstrated that the plant contained only negligible amounts of toxic metals, with Cr at 0.436 ppm, Cu at 0.182 ppm, Zn at 1.430 ppm, Cd at 0.010 ppm, and lead at 0.081 ppm. Antioxidant activity assays confirmed strong radical scavenging and reducing potential. The diethyl ether extract exhibited the highest total antioxidant capacity, while the ethyl acetate extract demonstrated the strongest ferric reducing antioxidant power. In free radical scavenging assays, ethyl acetate extract showed the strongest activity for

DPPH, TRABS and ABTS. In contrast, the hexane extract displayed the weakest antioxidant activity.

The anthelmintic assay against *Paramphistomum* sp. demonstrated that the diethyl ether extract exhibited the strongest anthelmintic activity, with a death time of 3:49 ± 0:21 h:min, outperforming the reference drug albendazole. Further analysis using Thin Layer Chromatography indicated that fraction A of the diethyl ether extract showed the most potent anthelmintic effect among the four fractions (A, B, C, and D). Enzymatic assays revealed that treatment with diethyl ether extract inhibited all five key parasite enzymes: ACP, ALP, AchE, MDH and LDH. ALP exhibited the highest inhibition, while ACP showed the lowest inhibition. The bioactive compound responsible for the observed anthelmintic activity was identified as quercetin using LC-MS and HPLC. Treatment of parasites with isolated quercetin confirmed strong anthelmintic activity. Histological and ultrastructural analysis further demonstrated significant damage to the parasite's tegument, including surface roughness, shrinkage, and tegumental breakage, indicative of a potent antiparasitic effect. Further enzymatic analysis after quercetin treatment showed a more pronounced reduction in enzyme activity compared to the crude extract. ALP exhibited the highest inhibition while ACP showed the least inhibition.

Molecular docking analysis revealed that quercetin had the strongest binding affinity with AChE among the five enzymes. Consequently, molecular dynamics (MD) simulations were performed on the AChE-quercetin complex to evaluate binding stability over time. Key parameters such as RMSD, RMSF, hydrogen bonding, Rg, and total energy analysis confirmed that the complex remained stable throughout the simulation, further supporting its strong binding interaction. Additionally, the MM/PBSA study showed a negative delta value, reinforcing the strong binding affinity of quercetin with AChE. Drug-likeness analysis of quercetin indicated compliance with Lipinski's Rule of Five, suggesting good oral bioavailability. ADMET profiling further supported its favorable pharmacokinetic properties, indicating that quercetin holds promise as a potential anthelmintic drug candidate.

The present study highlights the therapeutic potential of *Hypericum japonicum* as an anthelmintic agent. Its rich phytochemical content, antioxidant properties, and ability to inhibit key enzymes involved in parasitic functions make it a promising candidate for new

drug development. The identification of quercetin as the key active compound strengthens its role as a lead for future drug discovery. In vivo studies are needed to test the safety and effectiveness of these compounds in treating helminth infections. Additionally, expanding molecular docking studies to explore other potential targets in the parasite could help identify new drug targets. Large-scale clinical trials will be necessary to evaluate the plant's effectiveness in real-world applications. Developing standardized formulations and improving drug delivery systems could also enhance its therapeutic value. In conclusion, *Hypericum japonicum* and its active compounds, especially quercetin, offer great potential for developing new anthelmintic treatments, especially in addressing drug resistance in parasitic worms.