CHAPTER – 6
CONCLUSION

6. CONCLUSION

The medicinal potential of *Hypericum japonicum* has long been recognized in traditional practices, particularly for treating helminth infections. This plant, used in folk medicine, has been thoroughly studied in the current research, which highlights its various pharmacological properties that contribute to its potential as an anthelmintic agent. The study provides an in-depth look into its chemical composition, bioactivity, enzymatic interactions, and structural effects on parasites, shedding light on the mechanisms that could lead to the development of novel and effective treatments for helminthiasis. The preliminary phytochemical analysis revealed that *Hypericum japonicum* contains a rich array of bioactive compounds. This diverse range of phytochemicals, including alkaloids, flavonoids, tannins, and phenolics, is likely responsible for the observed therapeutic effects. Ethyl acetate extract was identified as the one with the highest crude protein content and phenolic content, both of which are known to have potent antioxidant and therapeutic properties. Tannins and flavonoids, which were found in high concentrations in some extracts, have been shown to exhibit anti-inflammatory, antimicrobial, and anthelmintic properties, contributing to the overall bioactivity of the plant. In contrast, hexane extracts showed the highest carbohydrate content, which is crucial as carbohydrates play a role in metabolic processes, potentially affecting the parasite’s energy requirements. Diethyl ether extract exhibited the highest flavonoid content, and flavonoids are well-documented for their bioactivity, particularly in modulating immune responses and inhibiting parasitic growth. This complex chemical composition suggests that the plant’s therapeutic effects may arise from the synergistic action of multiple compounds. The presence of flavonoids and phenolic compounds could help neutralize oxidative stress in the parasites, contributing to their mortality by disrupting cellular integrity and physiological functions.

The antioxidant potential of *H. japonicum* was one of the highlights of the study. Ethyl acetate extract exhibited the strongest antioxidant properties, which is important because oxidative stress plays a central role in many parasitic diseases. The ability of the plant to scavenge free radicals could help mitigate oxidative damage in host tissues while also compromising the parasitic organisms. The study's findings reinforce the idea that plants with high antioxidant potential may contribute to the protection of host tissues while targeting the parasite's survival mechanisms. The plant’s ability to induce significant

anthelmintic activity, particularly through the diethyl ether extract, sets it apart as a promising candidate for future drug development. In the study, the diethyl ether extract exhibited better activity than the reference drug, albendazole. This result is significant because it suggests that *H. japonicum* extracts, especially diethyl ether, may not only match but potentially exceed the efficacy of currently used anthelmintic drugs. Additionally, fraction **A** of the diethyl ether extract showed the most promising results in inhibiting parasitic growth, underscoring the importance of identifying and isolating specific active fractions from medicinal plants. This approach could enhance the specificity and potency of treatment while reducing possible side effects.

Enzymatic inhibition was a central finding in the present study, with five critical enzymes—ACP, ALP, AchE, MDH, and LDH—showing reduced activity when treated with *H. japonicum* extracts. These enzymes are involved in crucial metabolic processes in parasites, including nutrient uptake, energy production, and neuromuscular activity. The inhibition of these enzymes disrupts the parasite's ability to carry out essential functions, leading to paralysis and death. ALP plays an essential role in protein transport, cell signaling, and maintaining the pH environment of the parasite. Inhibition of ALP, which showed the highest reduction in activity, disrupts these physiological processes, leading to an impaired ability of the parasite to maintain cellular integrity and growth. ACP also plays a role in maintaining the pH balance within the parasite's internal environment. While ACP showed the lowest inhibition (16.21%), the reduced activity still suggests an impact on the parasite's ability to survive in its host. AchE is a neurotransmitter enzyme that is critical for neuromuscular communication. Inhibition of AchE (33.83% reduction) results in the disruption of neuromuscular activity, leading to paralysis and death. Quercetin, a major bioactive compound identified in *H. japonicum*, showed a strong inhibitory effect on AchE, indicating its central role in the plant's anthelmintic activity. Similarly, glycolytic enzymes are involved in energy production, particularly during anaerobic respiration. Inhibition of MDH and LDH further interferes with the parasite's metabolic processes, limiting its energy production, which is essential for survival. The overall inhibition of these enzymes by *H. japonicum* extracts points to a multi-target mechanism of action that significantly impairs the physiological and metabolic functions of the parasites, ultimately leading to their death.

Quercetin, identified as the primary bioactive compound through LCMS and HPLC, is of particular interest due to its strong anthelmintic activity. The molecular docking studies revealed that quercetin exhibited the highest binding affinity to AchE, suggesting

that it may directly interfere with neuromuscular signaling and contribute to paralysis in helminths. Furthermore, the molecular dynamics simulation provided additional insights into the stability and interaction of the AchE-quercetin complex, showing favorable bonding and compactness. This not only confirmed quercetin's inhibitory action but also highlighted its potential as a lead compound for developing novel anthelmintic drugs. The successful inhibition of AchE and other enzymes by quercetin strengthens its role as a potent therapeutic agent in treating helminth infections. Additionally, the ability of quercetin to bind strongly to its target protein underscores its potential to be developed into an effective drug, particularly when compared to traditional treatments.

The histological and ultrastructural studies provided significant insights into how *H. japonicum* extracts and quercetin affect the structural integrity of the parasites. Scanning electron microscopy (SEM) revealed extensive damage to the tegument, the outer protective layer of the parasites, which is crucial for survival. The observed rupture, roughness, and shrinkage of the tegument suggest that the extracts and quercetin compromise the parasite's structural integrity, making it more vulnerable to the host immune system and ultimately causing its death. The tegument is responsible for vital functions such as nutrient uptake, waste excretion, and protection from the host's immune system. Disruption of the tegument compromises these functions, leading to the parasite's inability to maintain homeostasis and survive. The histological alterations observed in this study reinforce the conclusion that *H. japonicum* extracts, particularly those enriched with quercetin, could be used to design drugs that target the parasite's tegument, making it an effective strategy for parasitic control.

The molecular docking results showed that quercetin had a strong binding affinity for AchE, with a binding energy of -7.6 Kcal/mol. This interaction was further supported by molecular dynamics simulations, which demonstrated the stability of the quercetin-AchE complex throughout the simulation period. The parameters studied, such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), hydrogen bonding, radius of gyration (Rg), and total energy, indicated that the complex remained stable, further confirming the strength of the binding interaction. From the ADMET and drug-likeness study, it was also confirmed that the bioactive compound Quercetin followed the Lipinski's Ro5, and no violation was found and also showed good pharmacokinetic properties, suggesting good drug-likeness property and also orally active. The MM/PBSA analysis, which showed a negative delta value, further suggested a strong and favorable

binding between quercetin and AchE, reinforcing quercetin’s potential as a therapeutic agent.

The findings from this study collectively suggest that *Hypericum japonicum* has a high therapeutic potential as an anthelmintic agent. The plant's rich phytochemical composition, its potent antioxidant properties, and its ability to inhibit key enzymes involved in parasite metabolism and neuromuscular function make it a promising candidate for the development of new anthelmintic drugs. The identification of quercetin as a key bioactive compound responsible for the plant’s therapeutic effects further strengthens its value as a lead compound for drug development. Future research should focus on isolating and characterizing other active compounds within *H. japonicum* to enhance the understanding of its mechanisms of action. Additionally, in vivo studies are needed to assess the safety, efficacy, and potential side effects of these extracts and compounds in the treatment of helminth infections. Molecular docking and dynamics studies can also be expanded to other enzymes and proteins involved in parasitic survival to discover novel drug targets. Overall, *Hypericum japonicum* and its bioactive compounds, particularly quercetin, represent a promising area for future anthelmintic drug discovery and could offer an alternative or adjunct to existing treatments for helminthiasis.