Pharmacogn. Res., 2025; 17(1):283-294.
https://www.phcogres.com

Original Article

# Anthelmintic Activity of *Hypericum japonicum* Thunb.: An *in vitro* and *in silico* Studies

**Mritunjoy Kumar Roy, Ananta Swargiary***

Department of Zoology, Pharmacology and Bioinformatics Laboratory, Bodoland University, Kokrajhar, Assam, INDIA.

## ABSTRACT

**Background:** *Hypericum japonicum* Thunb. is a vital medicinal plant in Northeast India, traditionally used by tribal communities to treat helminth infections with leaf extracts. **Objectives:** The present study investigates the *in vitro* and *in silico* studies of anthelmintic activity of *H. japonicum*. **Materials and Methods:** The anthelmintic activity was tested on *Paramphistomum* sp. with a test dose of 5 mg/mL to see the paralysis and death times. Phytocompounds were identified using GC-MS technique. 5 key enzymes-Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), malate-and lactate dehydrogenase, and acetylcholinesterases were assayed using standard protocol. Furthermore, identified compounds were studied for their binding activity with the enzymes. **Results:** Diethyl ether extract of *H. japonicum* showed the most potent anthelmintic activity against *Paramphistomum* sp. in the present study. GC-MS analysis identified 12 compounds in the diethyl ether extract. Of the five enzymes studied, ALP showed highest reduction (42.59%) and the least was found in ACP (16.21%) compared to control. Molecular docking observed strongest binding affinity between compound-2 and AchE (-6.73 kcal/mol) followed by ALP, ACP, and MDH enzymes. **Conclusion:** The findings suggest that *Hypericum japonicum* could be a potential source of anthelmintic agents, warranting further studies to elucidate its exact mechanism of action.

**Keywords:** Anthelmintic, GC-MS, *Hypericum japonicum*, Molecular docking, Molecular dynamics.

**Correspondence:**
**Dr. Ananta Swargiary**
Department of Zoology, Pharmacology and Bioinformatics Laboratory, Bodoland University, Kokrajhar-783370, Assam, INDIA.
Email: ananbuzoo101@gmail.com

**Received:** 18-11-2024;
**Revised:** 20-12-2024;
**Accepted:** 09-01-2025.

## INTRODUCTION

Plants have been used in traditional medicine since ancient times. Like many other diseases, plants and plant-derived products have been used to control helminth infestation in many parts of the world.[1] Many studies have established the anthelmintic properties of several plants.[2-4] Helminthiases are diseases caused by helminth parasites affecting millions worldwide, especially those living in developing countries.[5,6] It causes delayed mental and physical development in children, and complications during pregnancy, directly affecting the educational and economic conditions of a country.[7] Gastrointestinal nematodes are a major limiting factor for the success of livestock production worldwide.[8,9] Poor social infrastructure, unhygienic livelihood, and climate changes are the critical factors for the high prevalence of helminthiases.[10] The use of commercial drugs such as benzimidazole, levamisole, mebendazole, albendazole, praziquantel, etc., are the most common control strategy of helminthiases.[11,12] However, there are reports of anthelmintic drug resistance from different parts of the world decreasing the productivity of livestock and also threatening the success of treatment in humans.[13,14] However, through continuous drug administration, the helminth parasites resist those particular drugs.[15] As an alternative to the growing incidence of anthelmintic resistance, there has been considerable interest in searching for effective and safe dewormers in the form of medicinal plants that have their roots in the traditional ethnomedicine system. Many authors documented several medicinal plants against helminthiasis in various parts of the world.[16-18]

North-east India is rich in flora and fauna covering approximately 43% of the total plant species of India.[19] Tribal communities of this region have been practicing ethnomedicine for several diseases.[20-22] Recent studies have found that several medicinal plants are consumed by tribal communities of Assam to treat helminth infections.[4,16,23] *Hypericum japonicum* Thunb. (Family Hypericaceae) is one such medicinal plant having rich ethnomedicinal properties, including anthelmintic activity.[24] *H. japonicum* is an annual herb growing 5-35 cm in height and having small diffused branching. The plant is mainly distributed throughout South East Asia, including India (https://indiabiodiversity.org/). With a rich source of phytochemicals, the plant has been reported to contain hepatoprotective, antitumor, antibacterial, antiviral, and antioxidant activities.[25,26] Rural Assam, especially the Bodo community, consume the raw juice

**DOI:** 10.5530/pres.20252026

of plant (on an empty stomach) to expel helminth infestation. A preliminary study has shown that the crude extract of *H. japonicum* possesses better anthelmintic activity compared to the reference drug, albendazole.[27] The present study investigated the anthelmintic activity of different crude extracts of *H. japonicum* and also explored the alterations in enzymatic activities in response to plant extract treatments.

## MATERIALS AND METHODS

### Collection and identification plant

The sample plant was collected from the Tinali area of Kokrajhar district of Assam and was identified with the help of botanical taxonomist. The plant was confirmed as *Hypericum japonicum* Thunb. (BUBH2000129).

### Preparation of plant crude extract and solvent fractions

Fresh leaves of *H. japonicum* were collected from Kokrajhar area and processed for crude extract preparation following the method of Seidel.[28] Leaves were washed with distilled water and dried entirely in an oven at 45±2°C. Dry leaves were powdered using a grinder machine and macerated with 80% methanol (1:5, w/v) for 72 hr. Next, with Whatman filter paper no. 1, the solutions were filtered, and the filtrate obtained was dried in a rotary evaporator. The solid material was collected as a Methanolic Crude Extract of *H. japonicum* (MCEHJ). MCEHJ was further processed for successive solvent extraction using different solvents such as n-hexane, diethyl ether, and ethyl acetate following the liquid-liquid partitioning method. The solvents were dried in a rotary evaporator, and the solid materials obtained were kept at -20°C till further study.

### *In vitro* Anthelmintic study

The *in vitro* anthelmintic study for different plant solvent extracts followed the methods outlined by Eguale and Giday[29] and Belemlilga *et al.*[30] Paramphistomum sp. was collected from cow's rumen in 1xPBS (pH 7.4). The parasites were acclimatized at 37°C for 30 min before treatment. Albendazole and plant extracts were dissolved in 100 µL DMSO, then made up to 25 mL with PBS. After washing in PBS, 10-15 adult parasites were incubated at 37°C with a test dose of 5 mg/mL of plant extracts. Albendazole (5 mg/mL) was used as the reference drug. Control parasites were incubated at 37°C in PBS only. Each treatment set included three replicates, with records taken for the time taken for paralysis and death of the parasites.[31]

### Biochemical study

#### *Preparation of tissue homogenate*

A 5% tissue homogenate (w/v) was prepared in an ice-cold buffer solution using a tissue homogenizer. Following homogenization, the mixture was centrifuged at 15000 rpm for 10 min at 4°C. The resulting supernatant were used as enzyme source and was stored at -20°C until further use.

### Enzyme assay

#### *Acid Phosphatase (ACP)*

The ACP activity was assessed by measuring p-nitrophenol formation following the method outlined by Plummer[32] with little modifications as described by Swargiary *et al.*[33] The ACP activity was determined using a standard graph of p-nitrophenol ($y=0.0143x+0.203$, $R^2=0.981$).

### Alkaline Phosphatase (ALP)

The estimation of ALP activity was done by estimating the p-nitrophenol formation which was followed by Plummer[32] with little modifications as described by Swargiary *et al.*[33] The color formed was observed at 410 nm with the help of a spectrophotometer.

### Malate Dehydrogenase (MDH)

The estimation of MDH activity was done following Bergmeyer *et al.*[34] 850 µL sodium phosphate buffer (100 mM, pH 7.4), 50 µL of 10 mM oxaloacetic acid, 50 µL of 10 mM NADH, and 50 µL tissue supernatant was added to make an assay mixture of 1 mL. The assay mixture was warmed up without NADH at 37±1°C for 1 min. Then the reaction was started by adding NADH. The absorbance was monitored for 5 min at 340 nm. For the preparation of the blank, NaOH was added before adding tissue supernatant and all other steps were the same as the main protocol.

### Lactate Dehydrogenase (LDH)

The estimation of LDH activity was done following Bergmeyer *et al.*[35] 910 µL of phosphate buffer (0.1 M, pH 7.4), 20 µL of 50 mM

**Table 1: Anthelmintic activity of different solvent fractions of *Hypericum japonicum*.**

| Plants | Solvent Fractions | Paralysis time (h: min) | Death Time (h: min) |
|---|---|---|---|
| *Hypericum japonicum* | Hexane | 3:49±0:11 | 3:55±0:12 |
| | Diethyl ether | 3:22±0:17 | 3:49±0:21 |
| | Ethyl acetate | 13:20±0:37 | 14:30±0:24 |
| | Methanol | 9:26±0:38 | 9:54±0:48 |
| Reference chemical | Albendazole | 3:50±0:15 | 4:21±0:19 |

Control parasite lived up to 73:21+0:33 min. Values are expressed as mean±SD, *n*=3.

pyruvic acid, 20 µL of 50 mM NADH and 50 µL tissue supernatant was added to make an assay mixture of 1 mL. The assay mixture was warmed up without NADH at 37±1°C for 1 min. Then the reaction was started by adding NADH. The absorbance was monitored for 5 min at 340 nm. For the blank solution, NADH was replaced with phosphate buffer.

## Acetylcholinesterase (AchE)

The estimation of AchE enzyme activity was done by Ellman *et al.*[36] 30 µL of tissue supernatant was added to 430 µL phosphate buffer (0.1 M, pH 8.0) in a 1 mL assay mixture. The mixture was then incubated for 5 min. After this, 20 µL of 10 mM Ellman's reagent was added, and then 20 µL of 75 mM acetylcholine iodide was also added. The addition of AchI increased absorbance. The absorbance was then observed at 405 nm with the help of a spectrophotometer. Changes in the absorbance were read at 30-sec intervals at 37±1°C for 5 min.

## GC-MS analysis

The most potent diethyl ether extract of *H. japonicum* underwent GC-MS analysis using a Perkin Elmer system comprising a Clarus 680 GC and Clarus 600 GC MS with TurboMass Ver. 5.4.2 software by following Swargiary *et al.*[33]

## Identification of Peaks

The database software of the National Institute Standard and Technology-2008 (NIST-2008) was used for the interpretation of the peaks. The peaks that appeared in the GC-Chromatogram were made by library search of the mass spectrum of the corresponding peaks. The compounds were identified by name, empirical formula, and molecular weight.

## Protein modeling and active site prediction

NCBI database (https://www.ncbi.nlm.nih.gov/) was used for retrieving amino acid sequences. The amino acid sequences were ACP (GenBank: THD26168.1), ALP (GenBank: THD21487.1), MDH (GenBank: TPP64788.1), LDH (GenBank: TPP62930.1), and AchE (GenBank: KAA0195891.1). The sequences were

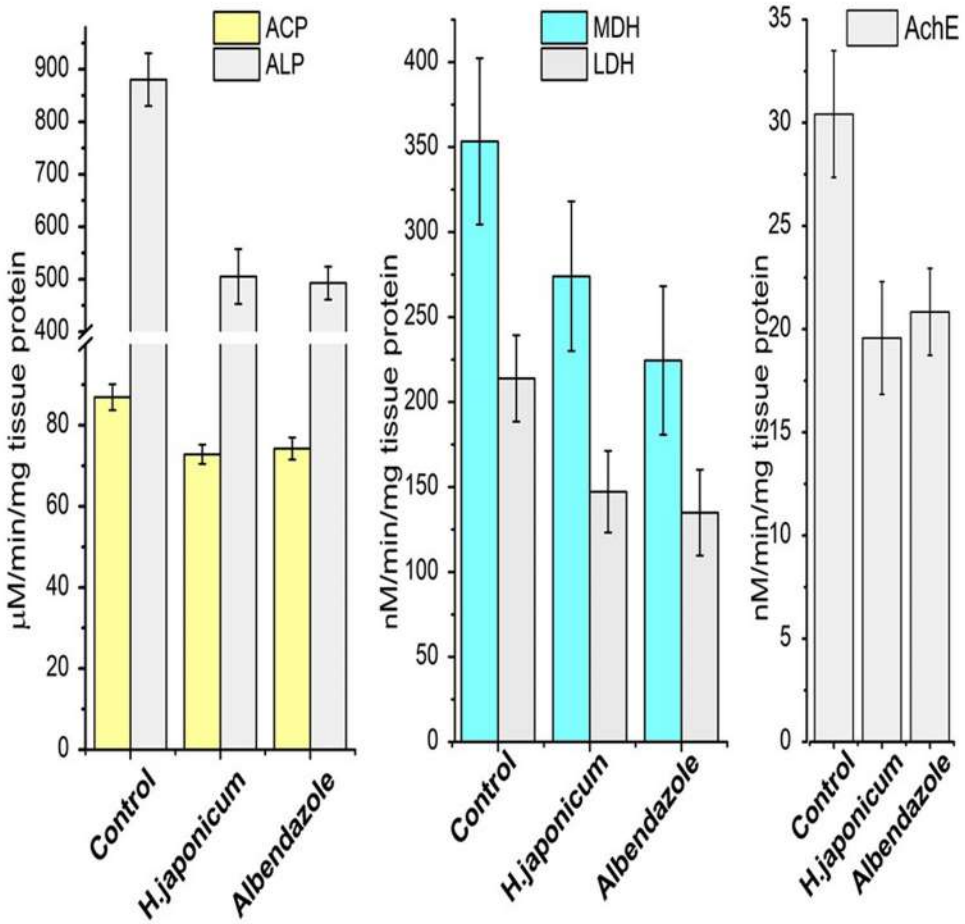


**Figure 1:** Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Malate Dehydrogenase (MDH) and Acetylcholinesterase (AchE) enzyme, activity of control and plant extract treated parasite. All the enzyme activities showed significant difference between control and plant extract-treated parasites at $p$=0.05 level, except AchE enzyme.

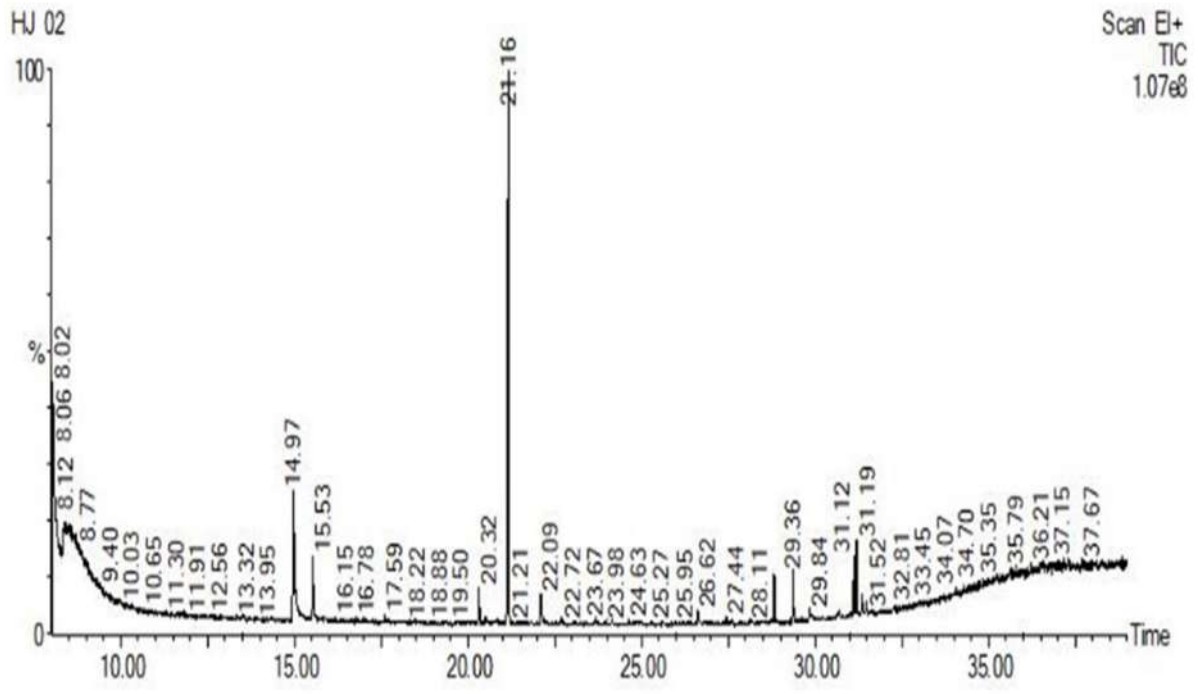


**Figure 2:** Complete GC-MS spectra profile of diethyl extract of *Hypericum japonicum*.

**Table 2: GC-MS identified compounds from the diethyl ether extracts of *Hypericum japonicum*.**

| Sl. No. | Name of the compounds | RT | PC id | Area (%) | MW (g/mol) | MF |
|---|---|---|---|---|---|---|
| 1. | Benzene, (ethenyloxy)- **[C1]** | 15.531 | 69840 | 1.212 | 120.15 | $C_8H_8O$ |
| 2. | 1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester **[C2]** | 29.362 | 6782 | 0.703 | 274.34 | $C_{16}H_{22}O_4$ |
| 3. | Methyl 12,13-Octadecadienoate **[C3]** | 31.118 | 91694366 | 0.692 | 294.5 | $C_{19}H_{34}O_2$ |
| 4. | Z,Z,Z-8,9-Epoxyeicosa-5,11,14-Trienoic Acid, Methyl Ester **[C4]** | 31.193 | 71684441 | 0.982 | 334.5 | $C_{21}H_{34}O_3$ |
| 5. | Octadecanoic acid, 11-methyl-, methyl ester **[C5]** | 28.817 | 554143 | 0.707 | 312.5 | $C_{20}H_{40}O_2$ |
| 6. | Catechol **[C6]** | 14.971 | 289 | 3.607 | 289 | $C_6H_6O_2$ |
| 7. | Bicyclo[3.1.0]Hexan-2-ONE, 1,5-Bis(1,1-Dimethylethyl)-3,3-Dimethyl- **[C7]** | 20.318 | 29561 | 0.492 | 236.39 | $C_6H_{28}O$ |
| 8. | Butylated Hydroxytoluene **[C8]** | 21.159 | 31404 | 7.422 | 220.35 | $C_{15}H_{24}O$ |
| 9. | 1R,2c,3t,4t-Tetramethyl-Cyclohexane **[C9]** | 22.104 | 94277 | 0.645 | 140.27 | $C_{10}H_{20}$ |
| 10. | 1-Hexyl-2-Nitrocyclohexane **[C10]** | 26.631 | 544017 | 0.336 | 213.32 | $C_{12}H_{23}NO_2$ |
| 11. | Z,Z-6,28-Heptatriactontadien-2-One **[C11]** | 31.353 | 5365964 | 0.417 | 530.9 | $C_{37}H_{70}O$ |
| 12. | Heptacosanoic Acid, 25-Methyl-, Methyl Ester **[C12]** | 28.817 | 554101 | 0.707 | 438.8 | $C_{29}H_{58}O_2$ |

**Table 3: Binding energies (kcal/mol) of *Hypericum japonicum* phytocompounds with different enzymes.**

| Compounds | AchE | ACP | ALP | LDH | MDH |
|---|---|---|---|---|---|
| C1 | -5.46 | -4.66 | -4.50 | -4.66 | -4.10 |
| C2 | -6.73 | -6.53 | -6.00 | -5.33 | -5.53 |
| C3 | -6.06 | -4.80 | -5.23 | -4.43 | -4.46 |
| C4 | -5.86 | -5.83 | -5.63 | -4.83 | -5.30 |
| C5 | -5.03 | -5.63 | -4.93 | -4.40 | -4.20 |
| C6 | -5.50 | -4.80 | -4.70 | -4.60 | -4.13 |
| C7 | -6.26 | -5.63 | -5.60 | -5.86 | -5.03 |
| C8 | -6.33 | -6.30 | -5.96 | -5.53 | -5.00 |
| C9 | -5.76 | -5.03 | -4.86 | -4.50 | -4.80 |
| C10 | -6.63 | -5.73 | -5.43 | -5.20 | -5.20 |
| C11 | -5.90 | -4.86 | -5.06 | -4.56 | -3.73 |
| C12 | -5.16 | -4.90 | -4.73 | -4.23 | -4.30 |
| Albendazole | -6.60 | -2.70 | -6.46 | -5.56 | -5.46 |

submitted to the Swiss-Model server (https://swissmodel.expasy.org/) for protein modelling. Enzyme active sites were generated by submitting the model proteins to CASTp 3.0 server.[37]

## Docking study

PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to download the 3D structures of phytocompounds. The 3D protein structures were downloaded from the PDB database (https://www.rcsb.org/). Molecular docking was carried out in AutoDock vina.[38] The grid parameters were set as x, y, z size-coordinate and grid box center-coordinate i.e., 25.511, 10.566, 16.228 and 74, 74, 62 for AchE, 50.440, 65.413, 24.461, and 54, 106, 78 for ACP, 33.323, -9.164 and 44, 44, 50 for ALP, 17.925, -14.018, 27.237 and 62, 48, 54 for LDH and 35.028, 144.289, 42.579 and 52, 44, 46 for MDH. Biovia Discovery Studio was used for the output visualisation. Three replicates were carried out for docking for all the phytocompounds and proteins.

## Molecular Dynamics Simulation Study

For Molecular Dynamic (MD) simulations study GROMACS was used for All-atom MD simulations of ligand-protein complexes.[39,40]

## Molecular Mechanics/Poisson Boltzmann Surface Area (MMPBSA) analysis

The free energies of all protein-ligand complexes were analysed by the MMPBSA package.[39,40] The major energy components such as binding energy (kJ/mol), electrostatic energy, van der Waals energy, polar solvation energy, non-polar solvation energy, and total energy were determined which contributed together to understanding the binding affinity of ligand-protein complexes. The MM/PBSA method–based binding free energy of the protein-ligand systems was calculated using the following equation:

$$\Delta G\ MMPBSA=\{Gcomplex-Gprotein-Gligand\}$$

where Gcomplex represents the total free energy of the docking complex, and Gprotein and Gligand depict the total free energies of the isolated protein and ligand in the solvent, respectively. Solvent Accessible Surface Area (SASA) value was calculated from the non-polar component as follows:

$$Gnon\text{-}polar=\gamma*SASA$$

Where, $\gamma=0.0301$ kJ/mol/A2

## Statistical analysis

Microsoft Excel was used for all the statistical calculations. OriginPro-8.5 software was used for the test of significance and correlation study. All the experiments were carried out in triplicates ($n$=3), and the results were represented as mean±Standard Deviation (SD).

# RESULTS

## Anthelmintic activity

Table 1 shows the anthelmintic activity of the solvent fractions with a fixed test dose of 5 mg/mL. Compared to the reference drug, diethyl ether extract of *H. japonicum* showed better anthelmintic activity. Among the four extracts, diethyl ether showed the strongest activity with time 3:49±0:21 hr:min. Hexane extract also showed almost similar activity compared to diethyl ether extract. Ethyl acetate extract showed the weakest activity taking about 14:30±0:24 hr:min.

## Biochemical Enzyme Assay

Two important tegumental enzymes, namely, acid-and alkaline phosphatase, two glycolytic enzymes malate-and

**Table 4: Total binding free energies of protein complexes with albendazole. All values are presented in kJ/mol.**

| Parameters | Ache-C2 | Ache-albendazole |
|---|---|---|
| VDWAALS | -2537.69±34.4 | -3080.12±16.1 |
| EEL | -24609.22±75.84 | -24619.6±50.09 |
| EPB | -6730.28±59.61 | -6676.16±17.29 |
| ENPOLAR | 95.26±0.64 | 94.51±0.3 |
| Total | -9908.26±38.24 | -9925.59±71.66 |

VDWAALS-Van der Waals Energy, ELL-electrostatic energy, EPB-Polar solvation energy, ENPOLAR -Non-polar Solvation Energy, All values are represented as ±standard deviation.

**Table 5: Free energy changes (Delta values) of protein complexes with albendazole. All values are in kJ/mol.**

| Parameters | Ache-C2 | Ache-albendazole |
|---|---|---|
| ΔVDWAALS | -37.41±1.74 | -29.48±0.2 |
| ΔEEL | -5.23±1.77 | -30.75±0.83 |
| ΔEPB | -24.88±0.67 | 41.67±0.59 |
| ΔENPOLAR | -3.94±0.07 | -2.9±0.01 |
| Total | -21.7±0.26 | -21.46±1.04 |

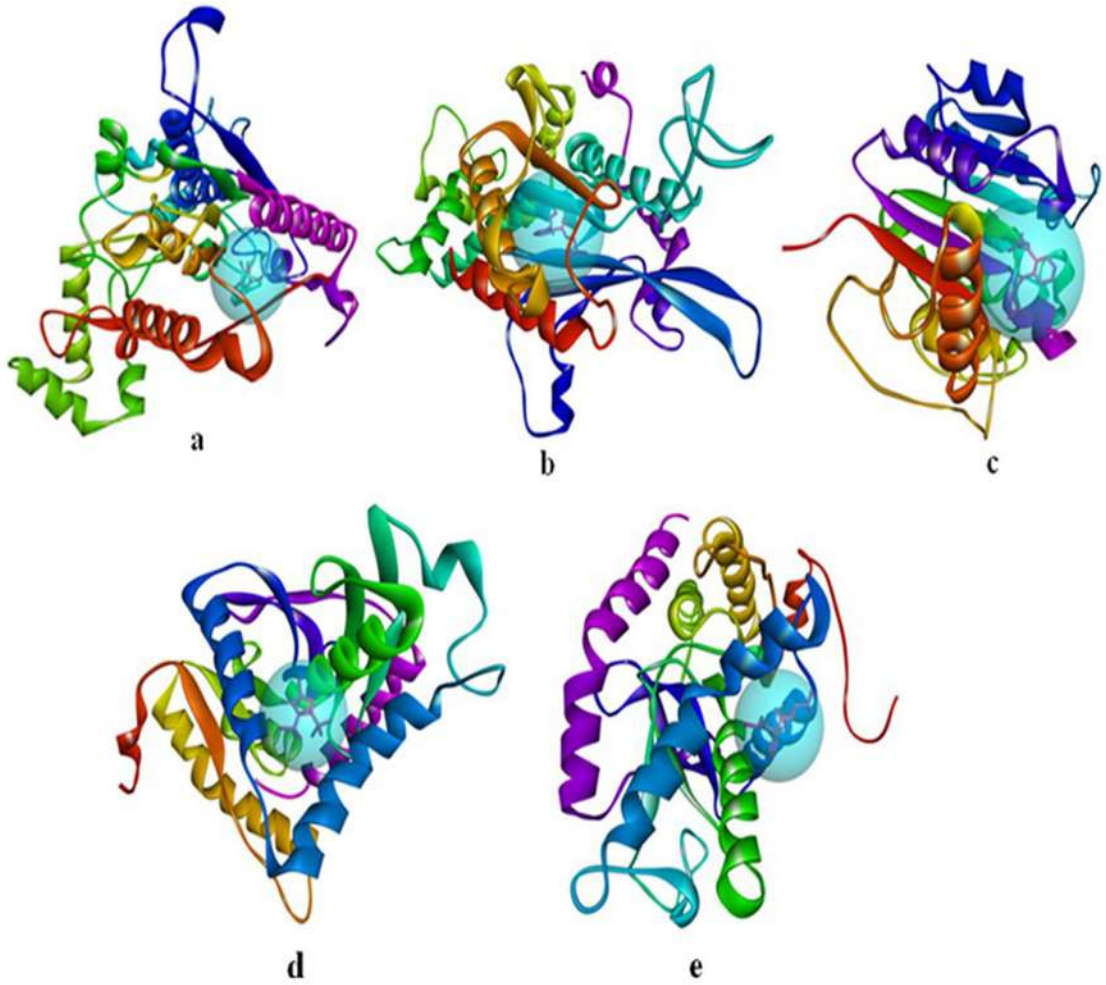


**Figure 3:** Molecular docking and 3D binding affinities of enzymes and phytocompounds. The circle colour indicates the superimpose binding site between the receptor and the ligand and with the reference drug. (a) AchE with albendezole and C2, (b) ACP with albendezole and C2, (c) ALP with albendezole and C2, (d) LDH with albendezole and C7, and (e) MDH with albendezole and C2.

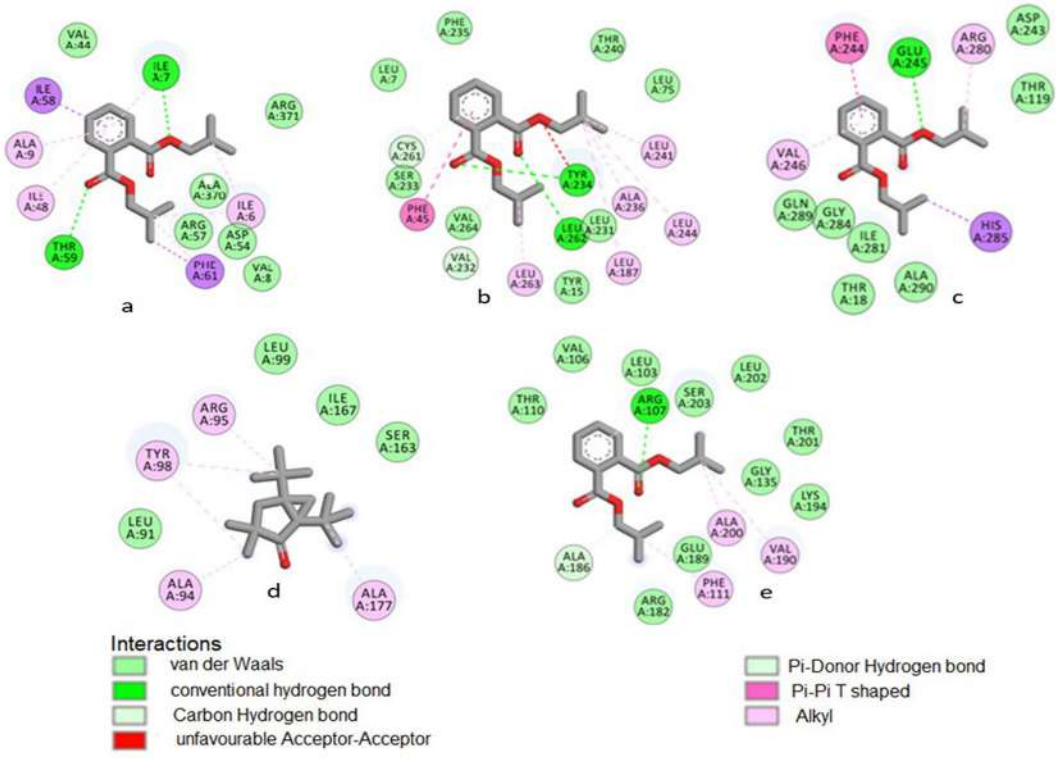


**Figure 4:** 2D binding affinities of enzymes and phytocompounds. (a) AchE and C2, (b) ACP and C2, (c) ALP and C2, (d) LDH and C7, and (d) MDH and C2.

lactate dehydrogenase, and one neurotransmitter enzyme, acetylcholinesterase have been investigated in the present study (Figure 1). Paralyzed parasites after plant extract treatment showed a decrease in ACP, while control, untreated parasites showed 86.94±3.17 µM/min/mg protein, 880.33±50.19 µM/min/mg protein, and 30.43±8.07 nM/min/mg protein for ACP, ALP, and AchE enzymes, respectively. Like tegumental enzymes, glycolytic enzymes, MDH, and LDH also showed reduced activity in plant extract and albendazole-treated parasites. The control parasite showed 353.39±38.87 and 213.41±25.41, nM/min/mg tissue protein for MDH and LDH, respectively. In ALP, LDH, and MDH, the diethyl extract of the plant showed good activity compared to albendazole. Whereas in ACP and AchE, the plant extract showed less activity compared to albendazole. The enzyme activities show significant differences at $p$=0.05 with the control, except AchE. The highest percentage of inhibition of plant extract was found in ALP (42.59%), and the least was found in ACP (16.21%) compared to control.

## GC-MS analysis of phytocompounds

The GC-MS study of the diethyl ether fraction of *H. japonicum* observed 12 probable phytocompounds from the plant. The images of the phytocompounds are provided as Supplementary File 1. The names of the compounds with the GC-MS profile are shown in Table 3. The spectra analyzed of the compounds are shown in Figure 2. The highest percentage was shown in peak structure with Retention Time (RT) 21.16. The identified phytocompounds with the RT, molecular weight, etc., are presented in Table 2.

## Protein modeling and Molecular docking

Protein modeling was carried out using the Swiss-Model server. The templates used were 2hpa.1.A, 3mk1.1.A, 4l4s.1.A, 2dfd.1.A, and 5fpq.2.A ACP, ALP, LDH, MDH, and AchE enzymes, respectively. All 12 identified compounds were docked with the model proteins. The binding affinities of compounds with all five enzymes are shown in Table 3. The compound 1,2-Benzenedicarboxylic Acid, Bis (2-Methylpropyl) Ester (C2) showed the best binding affinity with AchE (-6.73 kcal/mol), ACP (-6.53 kcal/mol), ALP (-6 kcal/mol), and MDH (-5.53 kcal/mol). Similarly, Bicyclo[3.1.0]Hexan-2-ONE, 1,5-Bis (1,1-Dimethylethyl)-3,3-Dimethyl- (C7) showed the best binding with LDH (-5.83 kcal/mol). Overall, phytocompounds showed the best binding affinity with AchE followed by ACP, ALP, LDH, and MDH. Figure 3 showed the 3D binding interactions of proteins and compounds with the reference drug (albendazole). Figure 4 showed the 2D binding interactions of proteins and compounds. Albendazole showed the best binding affinity with AchE. A total of 18 residues of ACP interacted with the C2, including 2H-bonds with Ala-234 and Leu A:262 residue. Eight residues formed a van der Waals interaction. MDH involved 15 residues, including a single H-bond with Arg A:245 residue and ten residues of van der Waals interaction. AchE with 13 residues where 2H bond and 6 Van der Waals interaction. ALP has 12 residues with 1 H bond

and 7 van der Waals, and LDH interacted with C7, including 8 residues with four van der Waals and no H bond. Albendazole showed the best binding affinity with AchE.

## Molecular Dynamics Simulation Study

The 3D structural conformation of AchE protein with surrounding solvent molecules during the period of MD simulation was shown in Figure. AchE-C2 complex and AchE-Alb complex simulation showed nearly similar backbone RMSD with similar solvent influences in the entire 100 ns simulation, while the apo-protein without ligand showed deviation as shown in Figure 5a. The simulation has shown several structural changes in the protein after ligand binding. However, the protein showed stability throughout the simulation period. When backbone conformation was observed with ligand, RMSDs of two complexes were seen (Figure 5b).

In AchE-C2, the structure showed higher RMSD from 45$^{th}$ ns to 100$^{th}$ ns compared to AchE-Alb. In other words, the AchE-C2 showed stronger binding affinity compared to AchE-Alb. From the RMSF study, it revealed that AchE-C2 showed higher fluctuation in the amino acid residues, while AchE-Alb showed slightly lower fluctuation. Figure 5d shows the radius of gyration of proteins. RG is studied to measure the elastic stability of a protein. RG analysis revealed that all the proteins showed instability in the elasticity of the proteins.

Figure 6a presents an analysis of hydrogen bonds involving AchE-C2, AchE-Alb, and the apo-protein in conjunction with solvent. Both AchE-C2 and AchE-Alb exhibited a range of 700 to 850 hydrogen bonds with the surrounding solvent, whereas the apo-protein displayed a higher count, ranging from over 800 to nearly 900 hydrogen bonds. This indicates that AchE-C2 and AchE-Alb had fewer hydrogen bonds compared to the

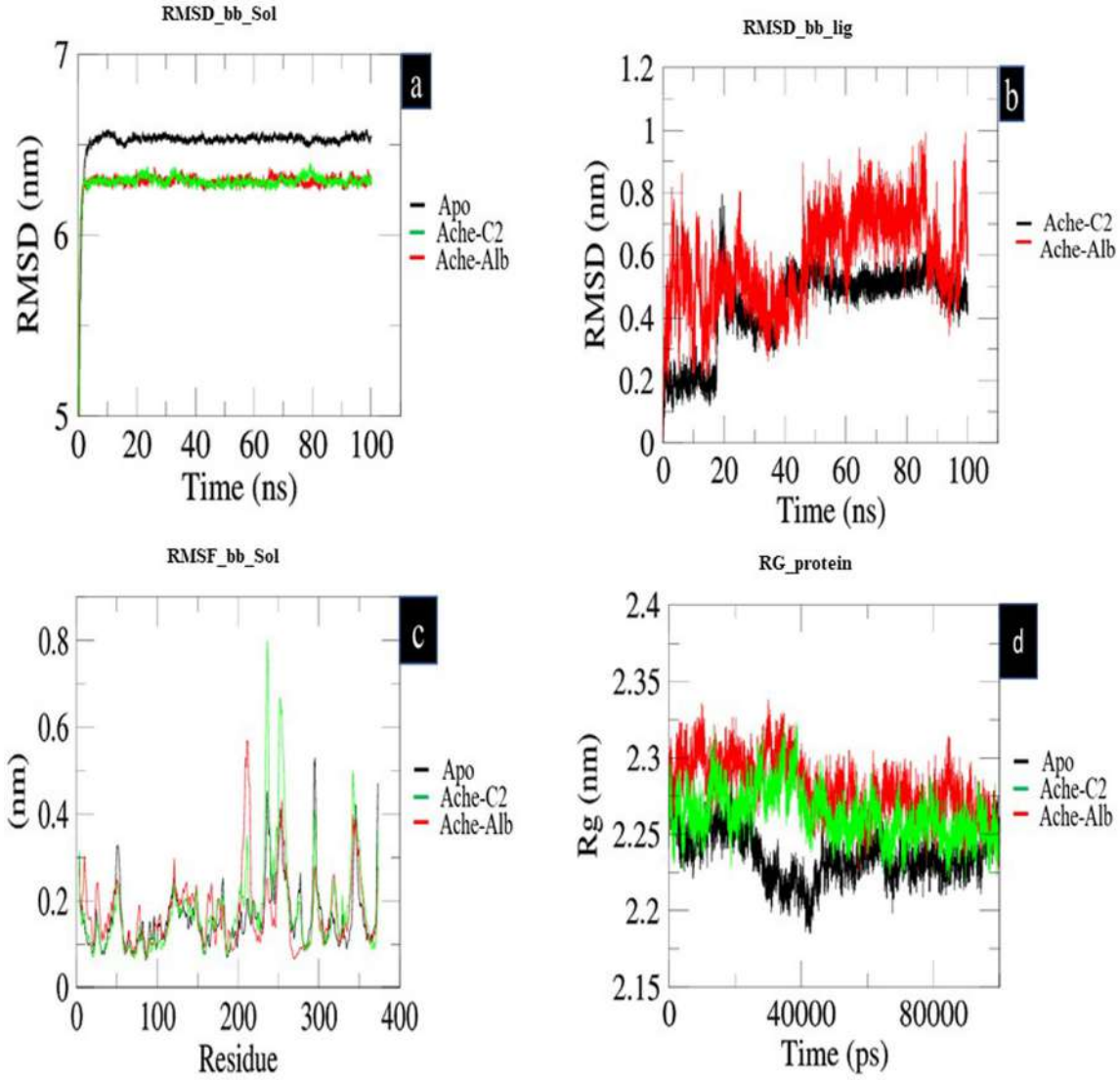


**Figure 5:** Molecular dynamics simulation (a) RMSD of apo protein (AchE), AchE-C2 and AchE-Alb with solvent, (b) RMSD with ligand (c) RMS Fluctuation of amino acid residues, and (d) Radius of gyration.

apo-protein. The assessment of H-bonds formed between ligands and proteins, depicted in Figure 6c, revealed that AchE-C2 had a greater number of H-bonds than AchE-Alb, with up to 4 and 3 H-bonds detected, respectively, during the simulation period. These bonds persisted in both AchE-Alb and AchE-C2 until the 100th ns. Notably, compound C2 exhibited stronger hydrogen bonding with the ligand than Alb. Additionally, Figure 6b illustrates the protein's total energy throughout the 100 ns simulation time, with the AchE-C2 and AchE-Alb complex displaying nearly identical energy levels, while the apo-protein exhibited lower energy levels compared to the complexes.

## MMPBSA Analysis

The total binding energies and binding free energy changes of the complexes are displayed in Tables 4 and 5, respectively. The thermodynamics property of the complexes has been shown. The contribution of the proteins (receptors) showing binding energies compared to the contribution of the ligands. The binding energy is mainly contributed by Van der Waals Energy, electrostatic energy, polar solvation energy and non-polar Solvation Energy. The total binding free energy of the Alb was found almost slightly higher with value -9925.59±71.66 kcal/mol than compound C2 with value -9908.26±38.24 kcal/mol. All the interaction has shown almost similar binding except for van der Waals force, which has shown higher in Alb with -3080.12±16.1 kcal/mol whereas -2537.69±34.4 kcal/mol in C2.

The free energy changes that is the delta value has shown almost similar result in both the complexes, except for ΔEEL value with -5.23±1.77 kcal/mol in Ache-C2 complex and -30.75±0.83 kcal/mol in Ache-Alb complex and also in ΔEPB value where Ache-C2

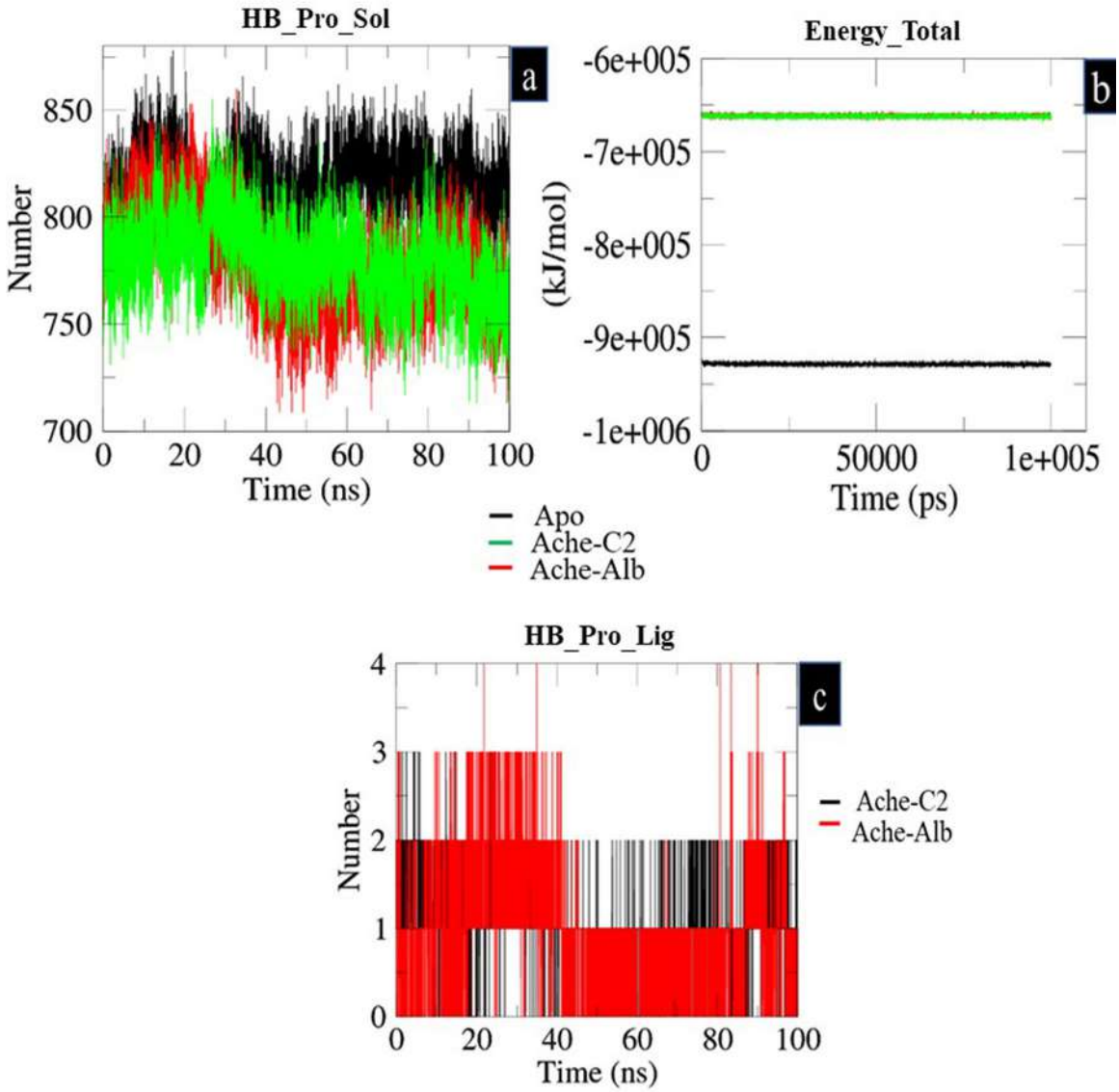


**Figure 6:** Molecular dynamics simulation (a) total number of H-Bonds (HB) between protein and solvent during the simulation period, (b) total number of H-Bonds (HB) between protein and ligand during the simulation period, and (c) Total energy (-kJ/mol) of proteins during the simulation period.

value was found as -24.88±0.67 kcal/mol and -41.67±0.59 kcal/mol for Ache-Alb. The values of total free energy for both the complexes was found almost similar as shown in the Table 5.

## DISCUSSION

Parasites can cause substantial morbidity and mortality in livestock animals resulting considerable productivity losses, mainly to farmers.[41] Due to helminth infection millions of people are affected worldwide, especially in developing countries. The biochemical analysis and the *in vitro* bioassay exhibited the presence of considerable anthelmintic properties of *H. japonicum*. In the current study, four solvent extracts of *H. japonicum* were prepared, and anthelmintic activity was analyzed. Diethyl ether extract showed the strongest activity, hexane extract also showed almost similar activity against the helminth parasite. The diethyl ether extract of *H. japonicum* extract also showed better anthelmintic activity than albendazole. A study done on the ovicidal and larvicidal activity of *Melia azedarach* extracts found that ethanol extract showed better anthelmintic activity than hexane.[42] In another study, it was investigated that the anthelmintic property of different solvent extracts of *C. asiatica, G. superba, P. daemia,* and *P. emblica* extracts showed better activity in methanolic extracts than other solvent extracts.[43] Similar to our study, a study reported dose-dependent anthelmintic activity of ethanolic extracts of *Platycladus orientalis* leaves.[44] Our earlier study on *P. strigosa* on different solvent extracts showed a slightly different result than our present study, where ethyl acetate has shown the strongest activity with a death time of 7:52±0:24 hr.[45]

Metabolic pathways have become a focal point for developing novel drugs targeting checkpoint enzymes, offering promise in combating various infectious diseases.[46] Within helminth parasites, tegumental enzymes play a pivotal role by mediating crucial reactions such as phosphorylation and dephosphorylation, integral to cell signaling and gene regulation. Acid and alkaline phosphatase, among these enzymes, are vital for functions such as protection, absorption, and secretion within the parasite's outer covering, known as the tegument. Our study unveiled a reduction in tegumental enzyme activity in parasites treated with both plant extract and albendazole. Similar trends were observed in investigations on *Potentilla fulgens* and *Amomum maximum* Roxb, highlighting a noteworthy decline in enzyme activity.[47,48] Moreover, glycolytic enzymes like malate dehydrogenase and lactate dehydrogenase, crucial for aerobic and anaerobic respiration, displayed decreased activity in parasites treated with *H. japonicum* extract. Similar outcomes were reported in studies on *Lysimachia ramosa* Wall. Ex Duby and *Punica granatum* ethanol extract, where lowered enzyme activity influenced glycogen metabolism.[49,50] Acetylcholinesterase, a crucial neurotransmitter enzyme, plays a pivotal role in helminths' neuromuscular activity. Hindering its activity can lead to paralysis and subsequent death of the parasites. Essential oils of *Origanum* have also exhibited anthelmintic effects by inhibiting acetylcholinesterase activity against Anisakis simplex L3 larvae.[51] Twelve potential phytocompounds were identified in the diethyl ether extract of *H. japonicum*, some of which have been reported in other plants for their antimicrobial and antioxidative properties.[52-54] Molecular dynamics simulations of AchE protein with compound C2 and albendazole suggested similar binding affinity between C2 and the reference drug. Despite structural changes post-ligand binding, protein stability was maintained throughout the simulation. Analysis of radius of gyration indicated protein instability. Apo protein exhibited more hydrogen bonding than C2 and albendazole, suggesting slightly lower binding affinity in the complexes. However, total energy analysis showed comparable energy levels between C2, albendazole, and apo protein.[55-57] MMPBS analysis revealed negative delta energy, indicating spontaneous and stable binding, essential for predicting biomolecular complex affinities.

## CONCLUSION

The present study enlightened *H. japonicum* to be a natural source of antioxidants and a potential anthelmintic agent. The ethyl acetate extract of the plant showed a good source of antioxidant properties. The diethyl ether extract of the plant enlightened to be a strong source of anthelmintic property. Treated parasites showed a significant decrease in enzyme activity. Compound-C2 showed better binding affinity with enzymes compared to other compounds. The highest percentage inhibition of plant extract was found in ALP and the least was found in ACP. Biochemical enzyme assays and *in silico* docking studies suggested significant enzyme inhibitory properties of the plant extracts along with the phytocompounds. MD simulation showed protein stability throughout the simulation period. However, further investigation, including phytochemical isolation and characterization, needs to be done to find out the accurate mode of action.

## ACKNOWLEDGEMENT

Authors would like to thank the GC-MS facility of Biotech Park, IIT, Guwahati, for the sample analysis. Authors would also like to thank the traditional healer and older people for providing ethnomedicinal information. We also acknowledge Dr. Sanjib Baruah, Department of Botany, for helping in the scientific validation of the plant. Authors also acknowledge the infrastructural facilities provided by the Department of Zoology, Bodoland University.

## FUNDING

Authors would like to acknowledge the Department of Science and Technology, INSPIRE. (DST, Inspire), Govt of India, Ministry of Science and Technology for providing financial assistance to MKR in the form of INSPIRE FELLOWSHIP (IF 190366).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

MKR carried out the anthelmintic study, biochemical analysis, docking study and writing of manuscript. AS involved in designing the study, statistical calculations, molecular dynamics and writing of the manuscript.

## ABBREVIATIONS

***H. japonicum*:** *Hypericum japonicum*; **MCEHJ:** Methanolic crude extract of *H. japonicum*; **PBS:** Phosphate buffer saline; **ACP:** Acid phosphatase; **ALP:** Alkaline phosphatase; **MDH:** Malate dehydrogenase; **LDH:** Lactate dehydrogenase; **AchE:** Acetylcholinesterase**; SD:** Standard deviation; **MD:** Molecular Dynamics; **Alb:** Albendazole; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuation; **RG:** Radius of gyration; **MMPBSA:** Molecular Mechanics Poisson-Boltzmann Surface Area.

## SUMMARY

Plants and plant-derived products have been used to control helminth infestation in many parts of the world. *Hypericum japonicum* Thunb. is a vital medicinal plant in Northeast India, traditionally used by tribal communities to treat helminth infections with leaf extracts. The findings suggest that *Hypericum japonicum* could be a potential source of anthelmintic agents, which also showed high reduction in alkaline phosphataste, warranting further studies to elucidate its exact mechanism of action.

## REFERENCES

1. Tandon V, Yadav AK, Das Roy B. Phytochemicals as cure of worm infections in traditional medicine systems. Emerg Trends Zool. 2011:351-78.
2. Mali RG, Mehta AA. A review on anthelmintic plants. Indian J Nat Prod Resour. 2008;7(5).
3. Giovanelli F, Mattellini M, Fichi G, Flamini G, Perrucci S. *In vitro* Anthelmintic Activity of Four Plant-Derived Compounds against Sheep Gastrointestinal Nematodes. Vet Sci. 2018;5(3):78. doi: 10.3390/vetsci5030078, PMID 30201869.
4. Swargiary A, Daimari A, Daimari M, Basumatary N, Narzary E. Phytochemicals, antioxidant, and anthelmintic activity of selected traditional wild edible plants of lower Assam. Indian J Pharmacol. 2016;48(4):418-23. doi: 10.4103/0253-7613.186212, PMID 27756954.
5. Nalule AS, Mbaria JM, Kimenju JW. *In vitro* anthelmintic potential of *Vernonia amygdalina* and Secamone africana on gastrointestinal nematodes. Agric Biol J North Am. 2013;4(1):54-66. doi: 10.5251/abjna.2013.4.1.54.66.
6. Liu M, Panda SK, Luyten W. Plant-based natural products for the discovery and development of novel anthelmintics against nematodes. Biomolecules. 2020;10(3):426. doi: 10.3390/biom10030426, PMID 32182910.
7. Romero-Benavides JC, Ruano AL, Silva-Rivas R, Castillo-Veintimilla P, Vivanco-Jaramillo S, Bailon-Moscoso N. Medicinal plants used as anthelmintics: ethnomedical, pharmacological, and phytochemical studies. Eur J Med Chem. 2017;129:209-17. doi: 10.1016/j.ejmech.2017.02.005, PMID 28231520.
8. Szewc M, De Waal T, Zintl A. Biological methods for the control of gastrointestinal nematodes. Vet J. 2021;268:105602. doi: 10.1016/j.tvjl.2020.105602, PMID 33468301.
9. Ahmed MU, Arise RO, Umaru IJ, Mohammed A. Antidiarheal activity of catechol and ethyl 5, 8, 11, 14, 17-icosapentanoate-rich fraction of *Annona senegalensis* stem bark. J Tradit Complement Med. 2022;12(2):190-4. doi: 10.1016/j.jtcme.2021.07.007, PMID 35528478.
10. WHO. Soil-transmitted helminth infections, WHO. World Health Organization; 2017. [cited Apr 04, 2019] Available from: http://www.who.int/mediacentre/factsheets/fs366/en/.
11. Tolochko KV, Vyshnevska LI. Scientific justification of anthelmintic medicines based on medicinal plant material. Int J Green Pharm. 2017;11(3):154-9.
12. Rashid MH, Vaughan JL, Stevenson MA, Campbell AJ, Beveridge I, Jabbar A. Anthelmintic resistance in gastrointestinal nematodes of alpacas (*Vicugna pacos*) in Australia. Parasit Vectors. 2018;11(1):388. doi: 10.1186/s13071-018-2949-7, PMID 29973276.
13. James CE, Hudson AL, Davey MW. Drug resistance mechanisms in helminths: is it survival of the fittest? Trends Parasitol. 2009;25(7):328-35. doi: 10.1016/j.pt.2009.04.004, PMID 19541539.
14. Wondimu A, Bayu Y. Anthelmintic drug resistance of gastrointestinal nematodes of naturally infected goats in Haramaya, Ethiopia. J Parasitol Res. 2022;2022:Article ID 4025902. doi: 10.1155/2022/4025902, PMID 35083085.
15. Mphahlele M, Molefe N, Tsotetsi-Khambul A, Oriel T. Anthelmintic resistance in livestock. In: Helminthiasis. 2019. IntechOpen; 2019. p. 112. doi: 10.5772/intechopen.87124.
16. Swargiary A, Roy MK, Daimari M. Survey and documentation of putative anthelmintic plants used in ethnomedicinal systems of tribal communities of Baksa District of Assam. Med plants. Int J Phytomed. 2019;11(4):368-79.
17. Raza A, Muhammad F, Bashir S, Aslam B, Anwar MI, Naseer MU. *In vitro* and *in vivo* anthelmintic potential of different medicinal plants against *Ascaridia galli* infection in poultry birds. Worlds Poult Sci J. 2016;72(1):115-24. doi: 10.1017/S0043933915002615.
18. Williams AR, Soelberg J, Jäger AK. Anthelmintic properties of traditional African and Caribbean medicinal plants: identification of extracts with potent activity against *Ascaris suum in vitro*. Parasite. 2016;23:24. doi: 10.1051/parasite/2016024, PMID 27301442.
19. Roy A, Das SK, Tripathi AK, Singh NU, Barman HK. Biodiversity in North East India and their conservation. Prog Agric. 2015;15(2):182-9. doi: 10.5958/0976-4615.2015.00005.8.
20. Gogoi B, Tamuli KJ, Bordoloi M, Sharma HK. Isolation and characterization of chemical constituents with *in vitro* antihypertensive and anthelmintic activities of *Cinnamomum bejolghota* (Buch.-Ham.) sweet leaves: an ethnomedicinal plant of North East India. India. S Afr J Bot. 2021;140:161-6.
21. Swargiary AN, Nath PU, Basumatary B, Brahma D. Phytochemical, antioxidant, and trace element analysis of anthelmintic plants of north-east India. Int J Pharm Pharm Sci. 2017;9(9):228-32. doi: 10.22159/ijpps.2017v9i9.20668.
22. Daimari M, Roy MK, Swargiary A, Baruah S, Basumatary S. An ethnobotanical survey of antidiabetic medicinal plants used by the Bodo tribe of Kokrajhar district, Assam. Indian J Tradit Knowl. 2019;18(3):421-9.
23. Swargiary A, Daimari M, Roy MK. Survey and documentation of anthelmintic plants used in traditional medicine system of tribal communities of Udalguri district of Assam, India. J app pharm sci. 2020;10(1):46-54. doi: 10.7324/JAPS.2020.101006.
24. Swargiary A, Roy MK, Daimari M. Survey and documentation of ethnobotanicals used in the traditional medicines system of tribal communities of Chirang District of Assam against helminthiasis. Biomed Pharmacol J. 2019;12(4):1923-35. doi: 10.13005/bpj/1824.
25. Huang ZQ, Chen P, Su WW, Wang YG, Wu H, Peng W, *et al.* Antioxidant activity and hepatoprotective potential of quercetin 7-rhamnoside *in vitro* and *in vivo*. Molecules. 2018;23(5):1188. doi: 10.3390/molecules23051188, PMID 29772655.
26. Anoopkumar AN, Prasad MS, Rebello S, Sini Francis CF, Aneesh EM. An assessment of ITS rDNA PCR-based molecular identification, and characterization of fungal endophytes isolated from *Hypericum japonicum*. Plant Biosyst. 2021:1-4.
27. Swargiary A, Verma AK, Singh S, Roy MK, Daimari M. Antioxidant and antiproliferative activity of selected medicinal plants of lower Assam, India: an *in vitro* and *in silico* study. Anti Cancer Agents Med Chem. 2021;21(2):267-77. doi: 10.2174/1871520620666200719000449, PMID 32682384.
28. Seidel V. Initial and bulk extract. In: Satyajit D, Sarker SD, Latif Z, Gray AI, editors. Natural product research. 2nd ed. Totowa, NJ: Humana Press; 2005. p. 29-36.
29. Eguale T, Giday M. *In vitro* anthelmintic activity of three medicinal plants against *Haemonchus contortus*. Int J Green Pharm. 2009;3(1):29-34. doi: 10.4103/0973-8258.49371.
30. Belemlilga MB, Traoré A, Ouédraogo S, Kaboré A, Tamboura HH, Guissou IP. Anthelmintic activity of *Saba senegalensis* (A.DC.) Pichon (*Apocynaceae*) extract against adult worms and eggs of *Haemonchus contortus*. Asian Pac J Trop Biomed. 2016;6(11):945-9. doi: 10.1016/j.apjtb.2016.07.015.
31. Roy B, Tandon V. Fluckicidal activity of *Alpinia nigra* (*Zingiberaceae*) against the trematode Fasciolopsis buski. Biol Med [lett.]. 1999;60:23-9.
32. Plummer DT. An introduction to practical biochemistry. 3rd ed, Tata McGraw-Hill Publishing Comp Ltd. New Delhi; 1988. p. 236-8.
33. Swargiary A, Roy MK, Boro H. *Persicaria strigosa* (R. Br.) Nakai: a natural anthelmintic? Parasitol Res. 2021;120(9):3215-27. doi: 10.1007/s00436-021-07249-x, PMID 34337681.
34. Bergmeyer HU. Malate dehydrogenase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. FL: Verlag Chemie International; 1974. p. 485.
35. Bergmeyer HU, Bernt E. Lactate dehydrogenase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. FL: Verlag Chemie International; 1981. p. 574-9.

36. Ellman GL, Courtney KD, Andres V, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95. doi: 10.1016/0006-2952(61)90145-9, PMID 13726518.
37. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res. 2018;46(W1):W363-7. doi: 10.1093/nar/gky473, PMID 29860391.
38. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31(2):455-61, doi: 10.1002/jcc.21334, PMID 19499576.
39. Swargiary A, Daimari M, Swargiary A, Biswas A. Brahma D, Singha H. Identification of phytocompounds as potent inhibitors of sodium/glucose cotransporter-2 leading to diabetes treatment. J Biomol Struct Dyn. 2024:1-4.
40. Swargiary A, Boro H. Brahma D. Tubulin-gene mutation in drug resistance in helminth parasite: docking and Molecular Dynamics simulation study. Curr Chem Biol. 2023;17(4):249-59.
41. Kumarasingha R, Preston S, Yeo TC, Lim DS, Tu CL, Palombo EA, *et al.* Anthelmintic activity of selected ethnomedicinal plant extracts on parasitic stages of *Haemonchus contortus*. Parasit Vectors. 2016;9:187. doi: 10.1186/s13071-016-1458-9, PMID 27036205.
42. Maciel MV, Morais SM, Bevilaqua CM, Camurça-Vasconcelos AL, Costa CT, Castro CM. Ovicidal and larvicidal activity of *Melia azedarach* extracts on *Haemonchus contortus*. Vet Parasitol. 2006;140(1-2):98-104. doi: 10.1016/j.vetpar.2006.03.007, PMID 16621294.
43. Bagavan A, Kamaraj C, Elango G, Abduz Zahir A, Abdul Rahuman A. Adulticidal and larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. Vet Parasitol. 2009;166(3-4):286-92. doi: 10.1016/j.vetpar.2009.09.007, PMID 19819626.
44. Sutar N, Garai R, Sharma US, Sharma UK, Jaiswal A. Anthelmintic activity of *Platycladus orientalis* leaves extract. Int J Parasitol Res. 2010;2(2):1-3. doi: 10.9735/0975-3702.2.2.1-3.
45. Swargiary A, Roy MK, Boro H. *Persicaria strigosa* (R. Br.) Nakai: a natural anthelmintic? Parasitol Res. 2021;120(9):3215-27. doi: 10.1007/s00436-021-07249-x, PMID 34337681.
46. Tyagi R, Elfawal MA, Wildman SA, Helander J, Bulman CA, Sakanari J, *et al.* Identification of small molecule enzyme inhibitors as broad-spectrum anthelmintics. Sci Rep. 2019;9(1):9085. doi: 10.1038/s41598-019-45548-7, PMID 31235822.
47. Roy B, Swargiary A, Syiem D, Tandon V. *Potentilla fulgens* (Family *Rosaceae*), a medicinal plant of north-east India: a natural anthelmintic? J Parasit Dis. 2010;34(2):83-8. doi: 10.1007/s12639-010-0018-z, PMID 21966126.
48. Chetia M, Giri BR, Swargiary A, Ronghang B, Roy B. Amomum maximum Roxb (*Zingiberaceae*), a Medicinal Plant of Tripura, India: A Natural Anthelmintic? J Adv Microsc Res. 2014;9(2):148-53. doi: 10.1166/jamr.2014.1206.
49. Veerakumari L, Lalhmingchhuanmawii K, Ashwini R. Effect of *Punica granatum* ethanol extract on the carbohydrate metabolism of Cotylophoron cotylophorum. Int J Biol Sci. 2014;1:1-5.
50. Joshi AK, Nagaraju R, Rajini PS. Involvement of acetylcholinesterase inhibition in paralyzing effects of monocrotophos in *Caenorhabditis elegans*. JoBAZ. 2018;79(1):33. doi: 10.1186/s41936-018-0047-1.
51. López V, Cascella M, Benelli G, Maggi F, Gómez-Rincón C. Green drugs in the fight against Anisakis simplex-larvicidal activity and acetylcholinesterase inhibition of *Origanum compactum* essential oil. Parasitol Res. 2018;117(3):861-7. doi: 10.1007/s00436-018-5764-3, PMID 29368038.
52. Anandan A, Eswaran R, Doss A, Sangeetha G, Anand SP. Chemical compounds investigation of *Lucas aspera* leaves-a potential folklore medicinal plant. Asian J Pharm Clin Res. 2014;5(1):86-8.
53. Mohammed NH, Ahmed MH, Hussien MO. Qualitative analysis of the essential oil of *Syzygium aromaticum* (L.) (Clove) using gas chromatography-mass spectrometry (GC-MS). Int J Res Pharm. 2015;5(2):350-4.
54. Debi C, Parkash V. Influence of microbial bioinoculants on the accumulation of new phytocompounds in *Oroxylum indicum* (L.) Benth. ex Kurz. GSC Biol PharmSci. 2020;13(3):228-43. doi: 10.30574/gscbps.2020.13.3.0413.
55. Karplus M, Petsko GA. Molecular dynamics simulations in biology. Nature. 1990;347(6294):631-9. doi: 10.1038/347631a0, PMID 2215695.
56. Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. Neuron. 2018;99(6):1129-43. doi: 10.1016/j.neuron.2018.08.011, PMID 30236283.
57. Mobley DL, Gilson MK. Predicting binding free energies: frontiers and benchmarks. Annu Rev Biophys. 2017;46:531-58. doi: 10.1146/annurev-biophys-070816-033654, PMID 28399632.


**Cite this article:** Roy MK, Swargiary A. Anthelmintic Activity of *Hypericum japonicum* Thunb.: An *in vitro* and *in silico* Studies. Pharmacog Res. 2025;17(1):283-94.