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ANNEXURE

Publications from PhD work

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Original Article

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

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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.

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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

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Pharmacogn. Res., 2023; 15(2):338-346.
https://www.phcogres.com

Original Article

Phytochemical, Antioxidant and Trace Element Analysis of *Hypericum japonicum* Thunb.

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ABSTRACT

Background: Plants possess various bioactive compounds with many biological activities. Antioxidant plays vital roles in initiating deleterious free scavenging radical reactions. **Objectives:** The present study was done to determine the presence of phytochemical properties of the plant along with its antioxidant analysis of four solvent fractions (hexane, diethyl ether, ethyl acetate and methanol) from *Hypericum japonicum*. Furthermore, five heavy metals- Zn, Cu, Cd, Pb and Cr were analyzed. **Materials and Methods:** All the experiments for phytochemical and antioxidants of different solvent extracts were investigated following standard protocols. The heavy metal content was analyzed using spectroscopy method. **Results:** Qualitative analysis revealed that almost all the phytochemicals were present on one or other solvents. The carbohydrate and protein content was highest in hexane and ethyl acetate extract. Ethyl acetate showed highest phenol content while diethyl ether showed highest in flavonoid content. Similarly, ethyl acetate extract showed strongest antioxidant activity. Heavy metals were found in very negligible amounts. **Conclusion:** The present study suggests that *H. japonicum* might have the potential to be a good source of phytochemicals and antioxidants. Further studies are required to analyze the pharmacological properties of the plant.

Keywords: Antioxidant, Heavy metal, *Hypericum japonicum*, Medicinal plants, Phytochemical.

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Received: 12-12-2022 ;
Revised: 28-12-2022 ;
Accepted: 11-01-2023.

INTRODUCTION

Plants are a good source of medicine and have been used in well-established medicinal form since primitive days. Medicinal plants are used worldwide to treat different diseases.[1] Plants possess numerous bioactive compounds having several biological activities such as anthelmintic, antifungal, anti-inflammatory, antiviral, antioxidant, antimicrobial, etc.[2-6] Today, an estimated 3,91,000 species of vascular plants are known to science, of which about 3,69,000 are flowering plants, and at least 31,128 plant species have a documented use.[7] Alkaloids, terpenoids, tannins, saponins and phenolic compounds are the most promising bioactive compounds in plants. These chemical compounds are called secondary metabolites. They work as a medicine against various conditions like diseases and stress.[8] The various phytochemicals are known to exhibit physiological activity along with medicinal properties.[9] Antioxidant has the property which can act against toxic as well as molecules which can cause damage to living organism. These compounds are generated during the different metabolic reactions in the body.[10,11] The phenolic and flavonoid compounds are known as sources of antioxidants and can clear reactive oxygen species (ROS).[12]

Heavy metals are one of the major environmental contaminants. Plants exposed to high metal concentrations illustrate down-regulated growth and development.[13] Soil contamination due to heavy metals can occur naturally or through various artificial activities like agriculture, burning fossil fuel, mining, etc. The plants are highly affected due to heavy metals present in the soil, which can cause various complications like oxidative stress resulting in damage to RNA and DNA, degradation of several proteins, and inhibits the functions of several enzymes.[14] Mostly in developing and developed countries, human activities have caused the pollution of water, air, and soil with toxic heavy metals. Therefore, these polluted environment results in declining the phytochemical content of the secondary metabolites of the medicinal plants.[15] The food quality of the plants is also affected by the presence of heavy metals in the soil.[16]

Assam is a place which is among the eight North-eastern states of India and has been known for its richness in vegetation as well as wildlife. Many studies have documented several plants for their phytochemical, antioxidant and medicinal properties.[17-19] *Hypericum japonicum* Thunb. of the family Hypericaceae is an important plant of western Assam known for its medicinal value, commonly used to treat helminth infections by

DOI: 10.5530/pres.15.2.036

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J Parasit Dis
https://doi.org/10.1007/s12639-021-01410-0

ORIGINAL ARTICLE

In vitro study of the antioxidant, antiproliferative, and anthelmintic properties of some medicinal plants of Kokrajhar district, India

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Received: 7 April 2021 / Accepted: 1 June 2021
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Abstract *Alstonia scholaris*, *Cardiospermum halicacabum*, *Hydrocotyle sibthorpioides*, and *Hypericum japonicum* are important folk medicinal plants used by tribal communities of Bodoland region of Assam to treat helminth infections. Because of their ethnomedicinal values, the present study was designed to investigate the antioxidant, antiproliferative, and anthelmintic activities of the plants. The antioxidant activity was measured by total antioxidant capacity, total phenolics (TPC), total flavonoid (TFC), FRAP, DPPH, ABTS, and TBARS assay. Antiproliferative and apoptosis-inducing activities of plants were conducted in Dalton's lymphoma (DL) cells. Cells were treated for 24 h with different doses (25–200 mg/mL) of plant extracts. Anthelmintic study was conducted by treating the *Paramphistomum* sp. at different doses of plant extracts. Phytochemical and antioxidant studies showed rich TPC, TFC, and free radical scavenging activity in *H. japonicum* and *H. sibthorpioides*. Both the antiproliferative and anthelmintic bioassays showed a dose-dependent efficacy in all plants. *H. japonicum* showed the strongest anthelmintic activity (LC_{50} 0.21 mg/mL) followed by *H. sibthorpioides* (5.36 mg/mL), *C. halicacabum* (13.40 mg/mL), and *A. scholaris* (18.40 mg/mL). Evidently, *H. sibthorpioides* showed the strongest antiproliferative and apoptosis-inducing activities among all the plants. The study observed a positive correlation between the antioxidant properties and antiproliferative and anthelmintic activities of the plants. We, therefore, conclude that the phytocompounds present in the crude extracts along with antioxidant molecules may have combined effects contributing to the antiproliferative and anthelmintic activities of the plants.

Keywords Medicinal plant · Antioxidant · Antiproliferative · Apoptosis · Anthelmintic · *Paramphistomum* sp.

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Introduction

Helminths are a group of eukaryotic organisms that affect millions of people worldwide and cause severe economic loss (Qamar et al. 2011). There are about 3.5 lakh species of endo-parasites of vertebrates many of which can complete their life cycle either in humans or in other animals (Carlson et al. 2020). Among the major helminthiases, soil-transmitted helminthiases and schistosomiasis account for more than 1 billion infections worldwide, mainly in economically poor countries (James et al. 2018). Helminth infections put children at high risk of stunted growth, impaired cognitive development, and life-threatening complications (Dixon et al. 2019). They can also lead to epilepsy, liver and bladder cancer leading to chronic health and economic burden (Mosthafa et al. 1999). Helminth infections of ruminants are a major constraint on efficient livestock production. It has been estimated that the mass deworming programs of helminth infection cost about US$300 million annually, while the cost of treatment by screening programs would likely be US$2 billion annually (UCNTD 2014). Since several decades ago, the use of commercial anthelmintics such as benzimidazoles, macrocyclic lactones, praziquantel, etc. was the most common

Published online: 15 June 2021

Seminar Presentation

Seminar presentation on **Phytochemical and antioxidant analysis of different solvent fractions of *Hypericum japonicum* Thunb.** at NATIONAL CONFERENCE ON SCIENCE & TECHNOLOGY FOR SUSTAINABLE DEVELOPMENT (STSD-2022**)** jointly organized by SCIENCE COLLEGE, KOKRAJHAR, BTR, Assam & VIJNANA BHARATI, NESM, in collaboration with INSTITUTE OF ADVANCED STUDY IN SCIENCE AND TECHNOLOGY (IASST) Guwahati, India & NORTH EAST CENTRE FOR TECHNOLOGY APPLICATION AND REACH (NECTAR) Shillong, India.


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