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Editorial

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Bamboo for Future

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Abstract

Bamboo, celebrated for its rapid growth and versatility, is emerging as a crucial resource in addressing key global challenges such as climate change, biodiversity loss, and economic inequality. Its high carbon sequestration capacity positions bamboo as a powerful tool for climate change mitigation, with certain species absorbing up to 62 tonnes of CO_2 per hectare annually. Bamboo's role in ecosystem restoration is equally significant, offering benefits like soil stabilization, water regulation, and habitat creation. Additionally, bamboo supports rural economies through sustainable industries such as construction, biofuels, and handicrafts. However, challenges remain, including gaps in research, a lack of standardized methodologies for carbon accounting, and the need for effective management to prevent ecological disruptions. Future efforts should focus on expanding research, developing global standards, and integrating bamboo into international climate policies to fully unlock its potential for environmental and economic sustainability.

Keywords: Bamboo, climate change, carbon sequestration, biodiversity conservation, sustainable development, ecosystem restoration, rural economies, environmental policy.

Bamboo, renowned for its rapid growth and versatility, is increasingly recognized as a critical tool for addressing global challenges such as climate change, biodiversity loss, and economic inequality. Its exceptional carbon sequestration ability, with some species absorbing up to 62 tonnes of CO_2 per hectare annually, positions bamboo as a cornerstone in climate change mitigation. As a renewable resource, bamboo serves as an eco-friendly alternative to traditional timber, helping to reduce deforestation pressures and enhance global carbon management strategies. Its adaptability to diverse environments, including degraded lands, further highlights its role in restoring ecosystems, stabilizing soils, and improving water regulation, making it a resilient and sustainable choice for environmental restoration.

Bamboo forests are vital for biodiversity conservation and ecosystem services. They act as habitats for numerous species, providing food and shelter while promoting genetic diversity by serving as biological corridors in fragmented landscapes. The plant's extensive root systems effectively combat soil erosion, reducing degradation by up to 75% and contributing to land stabilization. Additionally, bamboo enhances soil health through nutrient cycling, enriches it with organic matter, and improves water retention, which benefits surrounding ecosystems. These characteristics make bamboo an essential component of efforts to restore ecosystems and enhance ecological resilience[1].

From an economic perspective, bamboo offers significant potential for sustainable development. It supports industries ranging from construction and biofuel to handicrafts and agroforestry, creating jobs and stimulating local economies, particularly in rural areas. Bamboo cultivation and processing sustain millions of livelihoods in regions such as Asia and Africa, playing a crucial role in rural development. Strategic management and improved harvesting practices can enhance bamboo yields, significantly increasing its economic value. The sector's global potential is immense, with projections suggesting that bamboo-based activities could generate billions of dollars in economic opportunities. Furthermore, bamboo's cultural significance fosters conservation awareness and strengthens the relationship between communities and their environment, encouraging sustainable practices[2].

However, challenges remain in fully integrating bamboo into global climate strategies. A lack of standardized methodologies for carbon accounting, coupled with debates over its classification, limits its role in international policies. Research on bamboo's ecological and economic contributions is concentrated primarily in specific regions and species, particularly in Asia, leaving other areas underexplored. Addressing these gaps through comprehensive studies on bamboo biomass, carbon storage, and socio-economic impacts is vital for optimizing its potential in climate action and sustainable development. Developing international standards and expanding research efforts will be critical to unlocking bamboo's full capacity as a nature-based solution.

Effective management is crucial to ensure that bamboo delivers its maximum benefits without causing unintended ecological disruptions, such as invasiveness in certain regions. Careful species selection and proper management practices are essential to prevent ecological imbalances and ensure that bamboo supports local ecosystems sustainably[3].

Future efforts must prioritize addressing research gaps, developing global standards, and promoting bamboo's inclusion in international climate policies. Policymakers, researchers, and industries need to collaborate to ensure bamboo's sustainable management and integration into restoration projects, agroforestry systems, and economic frameworks. By prioritizing bamboo in global environmental strategies, the world can harness its transformative potential to create a more resilient and sustainable future[4].

In summary, bamboo offers unparalleled benefits as a sustainable and renewable resource, playing a pivotal role in climate change mitigation, biodiversity conservation, and economic development. Its unique attributes—including rapid growth, high carbon sequestration capacity, and ecological versatility—make it a critical tool for global sustainability efforts. While challenges such as research gaps and a lack of standardization remain, bamboo's immense potential makes it a valuable resource for achieving environmental and economic goals. Through innovative policies, focused research, and sustainable management, bamboo can contribute significantly to building a healthier and more equitable planet for future generations.

Conflicts of interest

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Review Article

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Exploring the Potential of Bryophytes in Cancer Research

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Abstract

Bryophytes, a diverse group of non-vascular plants, have emerged as an intriguing source of bioactive compounds with potential therapeutic properties in cancer research. Despite their modest size and simplicity, bryophytes possess unique chemical constituents, including alkaloids, flavonoids, terpenoids, and polysaccharides, that exhibit cytotoxic, anti-inflammatory, and anticancer effects. Recent studies have highlighted their ability to inhibit tumor growth, induce apoptosis in cancer cells, and modulate various signaling pathways involved in cancer progression. This review aims to explore the utility of bryophytes in cancer research, focusing on the bioactive compounds derived from these plants and their mechanisms of action against various cancer types. We also discuss the challenges and opportunities in translating bryophyte-based compounds into therapeutic agents. Given the growing interest in natural product-based drug discovery, bryophytes hold significant promise as a novel source for anticancer drug development.

Keywords: Bryophytes, cancer research, bioactive compounds, anticancer activity, phytochemicals

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1. Introduction

The plant kingdom exhibits a remarkable diversity, encompassing both primitive and advanced forms of life. In 1883, August Wilhelm Eichler divided the Plant Kingdom into two broad groups: Cryptogams and Phanerogams. Phanerogams, also known as seed-bearing plants, represent the more evolved members of the plant kingdom. Cryptogams, a term derived from the Greek words “kryptos” (hidden) and “gametes” (gametes), refer to primitive, non-flowering plants that do not produce seeds. Within Cryptogams, plants are further classified into three divisions: Thallophyta, Bryophyta, and Pteridophyta. Among these, Bryophytes occupy a significant place in the study of plant biology due to their unique characteristics and ecological importance.

1.1 Bryophytes

The term Bryophyta was first introduced by Robert Brown in 1864, derived from the Greek words “Bryon” (moss) and “Phyton” (plant). The group was later formally defined by Schimper in 1879. Bryophytes are among the earliest forms of terrestrial plants and are often referred to as the "amphibians of the plant kingdom" because of their reliance on water for reproduction.

Fossilized propagules and gamete-producing structures resembling modern bryophytes date back more than 400 million years, suggesting that they were among the first plants to adapt to land habitats. However, due to incomplete fossil records, the exact origin of bryophytes remains uncertain[1]

Bryophytes exhibit a unique alternation of generations, with a gametophyte stage and a sporophyte stage. The gametophyte, which is independent and self-sustaining, produces gametes, while the sporophyte produces spores and is typically dependent on the gametophyte for nutrition. This contrasts with vascular plants, where the sporophyte is the dominant, independent phase of the life cycle. Bryophytes thrive in damp environments as they require water for fertilization. The gametophyte relies on rhizoids for anchorage and absorbs water and nutrients through simple diffusion, as it lacks complex vascular tissues.

Bryophytes, with approximately 17,900 known species[2, 3], are classified into three distinct groups: Mosses (Bryophyta), Liverworts (Marchantiophyta), and Hornworts (Anthocerotophyta)[4]. These groups differ in morphology, molecular structure, and phytochemistry, yet they share common features that distinguish them from other plants, particularly their non-vascular nature.

1.1.1 Mosses

Mosses are the largest group of bryophytes, comprising around 14,000 species[5]. These plants are commonly found in moist environments, such as wet soils, rocks, and the shaded areas of buildings. Mosses typically exhibit a gametophyte structure with leaves arranged in multiple rows. Their reproductive organs, gametangia, are often protected by leaf sheaths[6]. The sporophyte of mosses is characterized by a sporangium that releases spores into the air. Mosses are also known for their ability to produce secondary metabolites, including compounds beneficial in medicine. However, the oil bodies present in mosses, which are responsible for the production of bioactive compounds, are not found in all species[7, 8].

1.1.2 Liverworts

Liverworts are another important class of bryophytes, with around 9,500 species. These plants are typically found in moist, tropical, and temperate regions. Liverworts can have leafy or thalloid forms and are characterized by a unique arrangement of cells in their leaves. The reproductive organs are exposed and protected by specialized structures[3, 6].

Liverworts produce oil bodies containing aromatic terpenoids and other secondary metabolites. They are used in traditional medicine, with species like *Marchantia polymorpha* being employed to treat liver disorders and pulmonary tuberculosis[7, 8]. Other species, such as *Riccia* sp., are used for treating skin diseases in folk medicine.

1.1.3 Hornworts

Hornworts are a smaller group of bryophytes, with only about 300 species. These plants feature a thalloid gametophyte, with a distinct horn-like sporophyte. The sporophyte of hornworts is capable of continuous spore production under favorable conditions[6]. Hornworts, though less studied in terms of their medicinal properties, hold potential for future research due to their unique biological characteristics.

2. Methodology

This review compiles findings from research on bryophytes and their potential anticancer properties. The data were sourced from a variety of scientific databases, including PubMed, Elsevier, Google Scholar, and Springer, alongside relevant academic books and publications.

3. Ethnobotany and Bryophytes

Ethnobotany, the study of how indigenous people use plants for food, medicine, and other purposes, has gained significant attention in recent years. Bryophytes have long been part of traditional medicine systems in various cultures, particularly in China, India, and Native America. The term "ethnobryology" was coined by Seville Flowers in 1957 to describe the use of bryophytes by the Gosiute people in Utah, highlighting the role of these plants in indigenous knowledge systems[9].

Bryophytes have been employed in traditional medicine for centuries, with many species exhibiting bioactive properties. Research on bryophytes' chemical composition reveals a rich variety of compounds, including polysaccharides, oligosaccharides, terpenoids, and phenolic compounds, which contribute to their medicinal value. Notable examples include the use of liverworts like *Marchantia polymorpha* for liver disorders and Riccia sp. for skin diseases[10].

3.1 Importance of Ethno-Bryology

Bryophytes have been used in herbal medicine for a wide range of ailments. For instance, *Marchantia polymorpha* is used in traditional medicine to treat liver disorders, while *Riccia* sp. is used to treat ringworms and other skin diseases. The therapeutic uses of bryophytes extend to conditions such as scabies, head lice, and inflammation. In addition, liverworts are recognized for their anti-inflammatory properties[11, 12, 13].

3.2 Importance in Modern Medicine

Modern pharmacological studies have revealed that bryophytes are an important source of novel natural products with potential biological activities[14-17]. Mosses and liverworts contain alkaloids, flavonoids, and polyphenolic acids, which have been shown to exhibit antimicrobial, anti-inflammatory, and anticancer properties. Over 400 novel compounds have been identified from bryophytes, demonstrating their potential for use in biotechnological and pharmaceutical applications[13].

Hence, bryophytes play a critical role in both traditional and modern medicine. Their diverse chemical composition, combined with their unique biological features, makes them an invaluable resource for future research in pharmacology and biotechnology. As the exploration of bryophytes continues, it is expected that more species will be identified for their medicinal properties, contributing to the development of new treatments and therapies[18].

Bryophytes play a vital role in traditional medicine, with around 3.2% of mosses and 8.8% of liverworts have been studied in terms of their chemical composition. Various species are used to treat health issues, including cardiovascular diseases and inflammation, with over 30 species available in Chinese pharmacies[19]. Notable examples include *Polytrichum commune* for its antipyretic and anti-inflammatory effects[20]. and *Rhodobryum giganteum* for cardiovascular concerns[21]. Mosses have fewer secondary metabolites due to the absence of oil bodies, but various compounds have been isolated from them, including terpenoids, steroids, fatty acids, plant hormones, and pheophytins. These include substances like monoterpenoids, carotenoids, and adenine, among others[22]. About 73 flavonoids and their glycosides have been identified in mosses. Benzonaphthoxanthenones, a unique flavonoid group, are found only in mosses[8, 23]. Recent findings highlight their rich chemical diversity and potential in the biopharmaceutical field (Tables 1, 2).

4. Cancer Overview

Cancer is characterized by abnormal cell growth that can occur in any tissue of the body. Tumors can be benign, localized to one area, or malignant, where they have the potential to spread through the bloodstream and lymphatic system. Cancer is typically categorized into three main types: carcinomas (affecting epithelial tissues), sarcomas (arising in connective tissues), and leukemias/lymphomas (originating from blood-forming cells). The classification of cancer depends on its origin and the type of cells affected. Lung, colorectal, and breast cancers are the most prevalent causes of cancer-related deaths, with environmental factors and genetic mutations being primary contributors to their onset[38].

Genes, which are located in the DNA of chromosomes within the nucleus, encode proteins that perform specific cellular functions. These proteins are produced when genes are activated. Mutations in genes can alter the quantity or functionality of the proteins produced, contributing to the development of cancer.

Although only a small fraction of the total genetic material is involved in these processes, certain classes of genes play pivotal roles in cancer development[39].

5. Bryophytes and Their Medicinal Potential in Cancer Research

Bryophytes, comprising over 24,000 species, are small, non-vascular, spore-producing plants that are often overlooked in chemical and medicinal research due to their low biomass and small size. However, bryophytes have demonstrated a diverse range of bioactivities, including cytotoxic, antimicrobial, antiviral, and nematocidal effects. These plants are also recognized for their ability to produce unique natural compounds such as lipids, polysaccharides, amino acids, terpenoids, phenylpropanoids, and quinones. These compounds make bryophytes a promising source of novel therapeutic agents. Despite their potential, bryophytes remain underutilized in ethnomedicine, although they are gaining attention for their various biological effects, including anti-cancer properties[40].

Bryophytes, which include mosses, liverworts, and hornworts, are traditionally found in moist environments and have been utilized for medicinal purposes by indigenous cultures. Though less studied in modern scientific literature, several species have demonstrated pharmacological properties and contain bioactive substances with potential therapeutic benefits, including anti-cancer effects[41]. Several key studies in cancer research have explored the anticancer potential of bryophytes, as summarized below:

Spjut et al.[42] reviewed the National Cancer Institute's screening program for biologically active compounds in bryophytes, which led to the discovery of cytotoxic effects in extracts from *Polytrichum ohioense*, a species of moss. Between 1980 and 1981, large-scale collection and screening of bryophyte species were conducted, revealing that extracts from 43 species exhibited significant biological activity, while 75 species had toxic properties. Families such as Thuidiaceae, Mniaceae, and Neckeraceae were highlighted for their active compounds, suggesting that bryophytes could become a valuable source of biologically active substances.

Sakai et al.[43] isolated four maytansinoids, including a novel compound, 15-methoxyansamitocin P-3, from the mosses *Isothecium subdiversiforme* and *Thamnobryum sandei*. These mosses exhibited potent cytotoxic activity against mouse P-388 lymphocytic leukemia cells in vitro, confirming their potential for anticancer drug development.

Efforts have been made to examine the medicinal properties of bryophytes, noting that they contain a variety of bioactive compounds, including terpenoids, phenols, and glycosides, which exhibit a range of biological effects. These include antifungal, cytotoxic, and antitumor activities. Bryophytes are also considered a promising source for developing natural compounds with anti-cancer potential, especially against diseases like AIDS, where liverwort bibenzyles have shown potential benefits.

Scher et al. [37] investigated the cytotoxic properties of *Schistochila glaucescens*, a liverwort species from New Zealand. Their research revealed that glaucescenolide 1, a novel sesquiterpene lactone, exhibited significant cytotoxicity against P-388 leukemia cells with an IC_{50} value of 2.3 mg/mL. The study identified additional compounds with cytotoxic effects, highlighting the therapeutic potential of this liverwort species.

Xiao et al. [44] studied the cytotoxicity of extracts from *Marchantia convoluta*, a plant used in traditional medicine. The ethyl acetate extract from this species exhibited significant cytotoxicity against human non-small cell lung carcinoma (H1299) and liver carcinoma (HepG2) cell lines, with IC_{50} values of 100 μg/mL and 30 μg/mL, respectively. Compounds such as caryophyllene, found in *Marchantia convoluta*, demonstrated anti-inflammatory properties, suggesting its potential for use in cancer therapy.

Shi et al.[45] focused on the effects of marchantin C, a compound derived from *Marchantia polymorpha*. Their study demonstrated that marchantin C induces apoptosis in human tumor cells by disrupting microtubules, causing G2/M phase cell cycle arrest, and promoting mitochondrial-mediated intrinsic apoptosis. The compound's ability to suppress tumor growth in human cervical cancer xenografts suggests its potential as a microtubule-targeting anticancer agent, providing an alternative to traditional therapies like paclitaxel and colchicine.

Huang et al.[46] identified marchantin A, a cyclic bisbibenzyl ether from *Marchantia emarginata*, which inhibited the growth of MCF-7 breast cancer cells by inducing apoptosis. This compound increased cleaved PARP and caspase levels while downregulating cyclins, demonstrating its anticancer potential as the first known apoptosis inducer from liverworts.

Mercader and Pomilio[47] reviewed biflavonoids, dimeric flavonoids found in bryophytes, noting their broad biological activities, including anticancer effects. These compounds induce apoptosis by various mechanisms, such as inhibiting enzymes and transcription factors, and have potential as cancer therapeutics with minimal impact on normal cells. Lin et al.[48] studied *Heteroscyphus tener*, a Chinese liverwort, revealing that its diterpenoid compounds, particularly compound 5, had selective cytotoxic effects against prostate cancer cells. This compound induced reactive oxygen species production, DNA damage, and cell cycle arrest, suggesting its potential for prostate cancer treatment. Dey and Mukherjee[49] reviewed the medicinal potential of bryophytes, particularly their anticancer properties. Bryophytes contain bioactive compounds like phenolic bibenzyls and terpenoids, which induce apoptosis and could be explored for cancer therapies, though their mechanisms are not fully understood.

Yuan et al.[50] investigated *Polytrichum commune*, a traditional herb, and its ethyl acetate fraction's cytotoxic effects on leukemia cells. The study demonstrated that this fraction induces apoptosis through calcium-dependent mitochondrial dysfunction, suggesting its therapeutic value in leukemia treatment. Klavina et al.[51] explored the chemical composition of moss extracts, finding a variety of secondary metabolites (e.g., terpenoids and polyphenols) with strong antibacterial and anticancer activity. Their research highlights the untapped potential of mosses for developing new bioactive compounds. Abay et al.[52] investigated *Dicranum scoparium* and found that its dichloromethane extract exhibited significant antiproliferative effects on HeLa cells. Active fractions, particularly Fr-19, enhanced antiproliferative activity and increased unsaturated fatty acid content. In contrast, the hexane extract, rich in saturated fatty acids, showed proliferative properties, suggesting that fatty acid composition plays a key role in the anticancer effects of bryophytes.

Chandra et al.[53] reviewed 50 medicinal bryophytes, highlighting their traditional uses for various ailments, including cancer. Specific species like *Polytrichum commune* and *Marchantia* species showed promising anticancer properties, with low toxicity, making bryophytes a valuable source for cancer therapies with minimal side effects. Vollár et al.[54] evaluated 108 bryophyte extracts for antiproliferative activity against human gynecological cancer cell lines. The study found 99 extracts with significant anticancer effects, particularly from species in the Amblystegiaceae and Brachytheciaceae families, with *Plagiomnium cuspidatum* being the most active. These findings underline bryophytes' potential as sources of novel anticancer agents. Yayıntaş et al.[55] studied bryophytes from Mount Ida, Turkey, and found that species like *Marchantia polymorpha* exhibited antiproliferative activity against HeLa and A549 lung cancer cells. This research highlights the potential of bryophytes for developing anticancer agents with applications in pharmaceuticals.

Abu-Izneid et al.[56] reviewed sesquiterpenes, a class of secondary metabolites found in bryophytes, which have shown effectiveness against various cancers, including breast, colon, and liver cancers. Despite their health benefits, the awareness of sesquiterpenes' anticancer properties remains low, and further research is needed to promote their consumption. Özerkan et al.[57] explored bryophytes for their potential to combat drug-resistant colorectal cancer cells. Their study found that bryophytes exhibited cytotoxic effects, suggesting that they could be incorporated into treatment regimens for colorectal cancer, particularly to prevent recurrence.

Zhou et al.[58] focused on *Marchantia polymorpha* and its ethanol extract's ability to induce apoptosis in hepatocellular carcinoma (HCC) cells. The extract triggered apoptosis through mitochondrial and endoplasmic reticulum stress pathways, demonstrating it's potential as a treatment for HCC. Ivković et al.[59] studied bisbibenzyl compounds from *Pellia endiviifolia*, finding significant cytotoxicity against various cancer cell lines, especially leukemia cells. This study underscores the anticancer potential of bisbibenzyls and their future applications in cancer treatment.

Cianciullo et al.[60] discussed the broad medicinal uses of bryophytes, particularly their secondary metabolites with antitumor properties. While many compounds have shown anticancer effects in vitro, further research is needed to assess their in vivo toxicity, bioavailability, and potential as treatments for multidrug-resistant cancers. Li et al.[61] explored the essential oil of *Plagiomnium acutum*, traditionally used in Chinese medicine for cancer treatment. The study analyzed the chemical profile of the oil, which contained alcohols, sesquiterpenes, and diterpenes. Using various cellular assays, they confirmed the anticancer potential of *P. acutum*, suggesting its future applications in pharmaceuticals. Sharma et al.[62] emphasized bryophytes' unique chemical compositions, including aromatic compounds, phenolics, and fatty acids, which contribute to their cancer-fighting properties. Liverworts, in particular, have shown immunostimulant effects and contain over 700 terpenoids, further establishing their medicinal value.

Bailly[63] focused on the sesquiterpenes, particularly plagiochilins, isolated from liverworts of the *Plagiochila* genus. These compounds exhibit significant antiproliferative effects, with plagiochilin A showing potential for targeting cancer cell cytokinesis, specifically for prostate cancer, offering promising avenues for anticancer therapy development.

Singh et al.[64] investigated the cytotoxic properties of *Bryum javanica*, a bryophyte, and found that methanolic extracts showed higher levels of bioactive compounds, demonstrating antiproliferative effects against colorectal cancer cells. These findings support the potential of bryophytes in cancer treatment and warrant further research. Sharma et al. [65] explored the phytochemical profile of *Riccia billardieri*, a liverwort from Rajasthan, revealing a range of bioactive compounds with potential therapeutic benefits, including anticancer properties. This study highlights the untapped potential of liverworts in pharmaceutical applications, particularly for colorectal cancer. Jain et al.[41] noted that terpenoids and bibenzyl compounds, abundant in liverworts, are among the most potent anticancer agents identified in bryophytes, both in vitro and in vivo. This finding underscores liverworts' significance as a source of bioactive compounds for cancer treatment.

Recently, Fernandes et al.[66] (2024) investigated two Amazonian moss species, *Leucobryum martianum* and *Leucobryum laevifolium*, finding that they contain polyphenols with strong anticancer and anti-inflammatory properties. The extracts exhibited cytotoxic effects against various cancer cell lines, with *L. martianum* showing particular promise for liver cancer treatment due to its strong activity against HepG2 cells. Pandey et al.[67] explore the therapeutic potential of bryophytes, a group of terrestrial plants known for their unique morphology and physiology. Traditionally used in medicine, bryophytes exhibit a range of pharmacological effects, including anti-inflammatory, analgesic, antimicrobial, antifungal, anticancer, antioxidant, and immunomodulatory properties. Extracts and isolated metabolites from bryophytes have shown lethal activity against various cancer cell lines, with some compounds selectively targeting cancer cells. Their high antioxidant content may also offer protective benefits against diseases linked to oxidative stress, such as cancer, diabetes, and cardiovascular diseases.

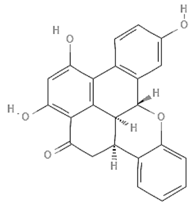
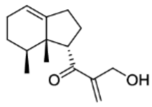
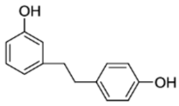
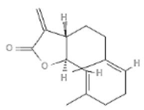
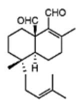
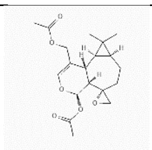
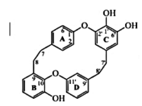
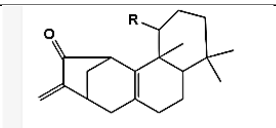
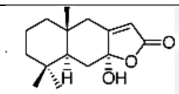
Chemically, bryophytes are diverse, containing compounds like terpenoids, phenolics, biflavonoids, and stilbenoid bibenzyls[70]. This review highlights the anticancer phytochemicals found in bryophytes, focusing on their structures, functional properties, and SMILES formats, and examines the field of ethnobryology and the pharmacological attributes of these compounds[68, 69]. Despite the promising findings, many phytocompounds in bryophytes remain underutilized in medical applications. This review emphasizes the need for improved extraction, analysis, and validation methods to fully explore their therapeutic potential. Future drug discovery efforts should integrate *in silico* drug design techniques and investigate novel bibenzyl compounds to advance complementary medicine and the development of nutraceuticals.

6. Discussion

Bryophytes, the second-largest and most diverse group of terrestrial plants[70], are often referred to as the "amphibians of the plant kingdom." This group is divided into three main categories: mosses, liverworts, and hornworts. Throughout history, plants have played a crucial role in providing food, shelter, and medicine, with bryophytes being particularly significant in traditional medicine in regions such as China, India, and Native America. This has led to the emergence of ethnobryology, which focuses on studying the medicinal properties of bryophytes. These plants are rich in bioactive compounds, including oligosaccharides, polysaccharides, amino acids, and phenolic compounds[71]. Unlike mosses and hornworts, liverworts contain oil bodies, resulting in a greater variety of secondary metabolites[72, 73]. Bryophytes, particularly liverworts, are emerging as a promising source of novel natural products such as alkaloids, flavonoids, and polyphenolic acids, with potential applications in biotechnology and pharmaceuticals. They have shown efficacy in treating a variety of health conditions, including cardiovascular diseases, inflammation, and fever. With the growing global burden of cancer, bryophytes are gaining significant attention for their potential in cancer treatment[74]. Many bryophyte species demonstrate anticancer, cytotoxic, and antitumor properties, with bioactive compounds that induce apoptosis and target various cancer cell lines, offering hope for new therapeutic options. However, the full potential of bryophytes remains underexplored due to challenges like limited availability, the need for more in-depth investigation of their compounds, and unclear mechanisms of action.

Table 1: Bryophytes with healing properties

Sr. No.	Species	Family	Applications	References
Mosses				
1.	*Polytrichum commune* Hedw.	Polytrichaceae	Used as hair oil by ancient women	12
2.	*Sphagnum teres* (Schimp.) Angstrom	Sphagnaceae	For eye diseases treatment	12, 20
3.	*Barbula indica* (Hook.) Spreng.	Pottiaceae	For menstrual pain relief an intermittent fever	24
4.	*Funaria hygrometrica* Hedw.	Funariaceae	For wound healing	11
5.	*Cratoneuron filicinum* (Hedw.) Spuce	Amblystegiaceae	Heart disease treatment	11, 13
6.	*Philonotis fontana* (Hedw.) Brid.	Bartramiaceae	For burn relief	9, 11, 13
7.	*Leucobryum bowringii* Mitt.	Dicranaceae	Leaf tip paste mixed with *Phoenix sylvestris* during body pain	24
Liverworts				
8.	*Marchantia polymorpha* L.	Marchantiaceae	Cure hepatic disorders	25
9.	*Plagiochasma appendiculatum* Lehm. & Lindenb.	Aytoniaceae	Skin diseases treatment	26
10.	*Targionia hypohylla* L.	Targioniaceae	For skin ailments	27, 28
11.	*Frullania ericoides* (Nees) Mont.	Jubulaceae	For hair issues	27, 28
12.	*Plagiochila* (Dum.) Dum.	Plagiochilaceae	For anti-leukemic and antimicrobial use	13, 29
13.	*Riccia* L.	Ricciaceae	Made into medicinal paste for ringworm affected children	30
14.	*Pallavicinia* Gray	Pallaviniaceae	As an antimicrobial agent	31

Table 2: Structure and function of anticancer compounds of bryophytes					
S. No.	**Bryophytes**	**Bioactive compound**	**Structure**	**Medicinal uses**	**References**
1	*Polytrichum* sp.	Ohioensin-A		Cytotoxic activity	32
2	*Chilosyphus rivularis*	13-hydroxychiloscyphone		Cytotoxic activity	33
3	*Dumortiera Hirsute* (Sw.) Nees	Lunularin		Cytotoxic activity	5
4	*Frullania nisqualensis*	Costunolide		Cytotoxic activity	34
5	*Porella perrottetiana*	Perrottetianal A		Cytotoxic activity	35
6	*Plagiochila* spp.	Plagiochilin A		Cytotoxic activity	14
7	*Marchantia polymorpha* L.	Marchantin A		5-lipoxygenase inhibitory activity	5
8	*Jungermannia faurian*	Jungermannenone A and B		Cell cycle arrest	36
9	*Schistochila glaucescens*	Glaucescenolide		Cytotoxic activity	37

Sources:https://azoresbioportal.uac.pt/imagensEspecies/1/1/8/1/9/l/polytrichum-commune-t00376-131.jpg; https://bryophyteportal.org/imglib/bryophytes/TENN/TENN-B-0105/TENN-B-0105776_c.jpg; https://cdn.britannica.com/07/140407-050-30CD8CA1/Broom-moss.jpg; https://inaturalist.nz/photos/113443756; https://ohiomosslichen.org/moss-polytrichastrum-ohioense/; http://www.societequebecoisedebryologie.org/mousses/Polytrichum_pallidisetum.html

7. Conclusion

Bryophytes hold immense promise in cancer research due to their rich chemical diversity and the ability to produce biologically active substances such as terpenoids, phenols, glycosides, fatty acids, and distinctive aromatic compounds. These plants, with a long history in folk medicine and ethnobryology, offer a unique source of natural products that exhibit a broad spectrum of biological effects, including cytotoxic, antitumor, and cardiotonic properties. The medicinal potential of bryophytes is increasingly recognized, and their active compounds are being explored using modern analytical techniques such as HPLC, GC-MS, and LC-TOF-MS. These methods allow for the isolation and characterization of bioactive compounds, which are critical for drug development, particularly for cancers like colorectal and prostate cancer. While promising cytotoxic effects have been observed, the mechanisms underlying the activity of these compounds are not yet fully understood, highlighting the need for further investigation into their genotoxicity, hepatotoxicity, and toxicity in animal models and cell lines. Additionally, the poor solubility and bioavailability of many secondary metabolites present challenges for large-scale production and drug manufacturing. Future research should focus on addressing these limitations and exploring bryophytes' potential in developing anti-MDR (multidrug-resistant) drugs. Investigating underexplored genera like *Plagiochila* and hornworts may uncover new bioactive compounds with applications in treating drug-resistant cancers. *In silico* drug design techniques, along with bio-guided fractionation analysis, could further optimize the therapeutic potential of bryophytes. With continued exploration and clinical trials, bryophytes offer a promising avenue for the development of novel anticancer therapies.

Authors Contributions

NR collected and compiled the data and TS finalized the text.

Conflicts of Interest

Authors declare no conflict of interest.

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Research Article

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First Record of *Macromitrium hamatum* Dix. from the Nilgiri Hills, South India

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Abstract

Macromitrium hamatum Dix. is an endemic taxon of India and has previously been reported from Meghalaya and Tamil Nadu. In Tamil Nadu, it was reported from the Tirunelveli district by Daniels. This species is now being reported for the first time from the Nilgiri Hills. It is characterized by a pleurocarpous habit, erectopotent stems, and hamate, hooked, and lanceolate leaves that are densely arranged along the stem. The leaf cells are multi-papillose. This species differs from *Macromitrium nepalense* in having hamate, lanceolate leaves and multi-papillose mid-laminar cells.

Keywords: Orthotrichaceae, *Macromitrium hamatum* Dix., corticolous, Nilgiri Hills, South India.

1. Introduction

The genus *Macromitrium* Brid., belongs to the family Orthotrichaceae and is represented by 22 species in India. These species have been reported from various bryogeographical regions, including the Western Himalaya, Eastern Himalaya, and South India by various bryologists[1-17]. In South India, *Macromitrium* is represented by 17 species, which have been reported from Tamil Nadu, Kerala, Karnataka, and Maharashtra [2, 7, 8, 14-17].

Macromitrium hamatum Dix. is an endemic species to India and has previously been reported from Meghalaya and Tamil Nadu [1, 9]. In Tamil Nadu, it was reported from the Tirunelveli district by Daniels[9]. During a survey of bryophyte specimens in the year 2000, a corticolous population of this species was observed in the Nilgiri Hills, South India. This population has been critically examined and is described here.

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2. Materials and Methods

The present study is based on dried herbarium specimens housed at the Lucknow University Hepatic Herbarium, Department of Botany, University of Lucknow, Lucknow (LWU). To facilitate investigation, the plant specimens were soaked in water for 10-20 minutes to rehydrate them and restore their original shape. The external morphology of the plants was examined under a Stereoscopic Zoom Binocular Microscope (Carl Zeiss, M140, Germany), while cellular details and microscopic structures were observed using a Compound Microscope (Olympus OIC 71078) and a Leica Binocular Compound Microscope (LEICA S6D 1044339). Temporary slides were prepared in 70% aqueous glycerin for dissected plant parts and for anatomical studies using free-hand sections. Line drawings were made using a NIKON Camera Lucida at appropriate magnifications. Measurements were recorded with the aid of a stage micrometer and an oculometer.

1.1. Taxonomic description

Macromitrium hamatum Dixon

Macromitrium hamatum Dixon, J. Bombay Nat. Hist. Soc. 39:777.1937. **(Plate 1; Figs. 1-9)**

Plants pleurocarpous, epiphytic, dark brown in colour; main stem creeping, 2-4 cm long and 3-5 mm wide with leaves, irregularly branched; branches 1-2 cm long, cross-section of stem circular, 0.1-0.2 mm in diameter, 3-4 rows of outer cortical cells thick walled, small, brown in color, inner cortical cells thin walled, large, 11-19 x 7-11 µm, central strand absent; leaves densely arranged on stem, erect, hamate, lanceolate, 2.5-3.4 x 0.3-0.5 mm, margin entire; costa single, strong and percurrent; leaf-cells thick walled, rounded-quadrate, papillose, apical cells 6-11 x 4-8 µm, middle cells multipapillose, 6-11 x 4-11 µm, basal cells rectangular, thick walled, 38-57 x 4-11 µm with single large papilla. Plants are vegetative.

1.2. Habitat: The plants are epiphytic, growing on bark in association with *Claopodium assurgens* and *Pogonatum microstomum*.

1.3. Range: Endemic to India[1].

1.4. Distribution in India: Eastern Himalaya: Meghalaya: Khasia Hills. South India: Tamil Nadu: Tirunelveli & Nilgiri hills: Ootacamund, Mukuruthy & Avalanche[1, 9].

Specimens examined: South India: Tamil Nadu, Nilgiri hills, Upper Bhawani: on way to Avalanche, alt. ca. 2000 m, S. C. Srivastava & Party, 9 October, 2000, 12526/2000 (LWU), Mukuruthy: Parthimund Reserve Forest, alt. ca. 2250 m, P. K. Verma, 24 May, 2005, 18062/05 (LWU), Ootacamund: Parson's Valley, alt. ca. 2250 m, P. K. Verma, 25 May, 2005, 18125/05, 18133/05(LWU) and Doddabetta, alt. ca. 2600 m, S. C. Srivastava & Party, 7 January, 2006, 18586/2006 (LWU).

2. Discussion

Macromitrium hamatum is an endemic species to India and has previously been reported from Meghalaya and Tamil Nadu[1, 9]. In Tamil Nadu, it was reported from the Tirunelveli district by Daniels[9]. This species is now reported for the first time from the Nilgiri Hills. It is characterized by a pleurocarpous habit, erectopotent stems, and hamate, hooked, and lanceolate leaves that are densely arranged on the stem. The leaf cells are multi-papillose. The species differs from *Macromitrium nepalense* in having hamate, lanceolate leaves (Plate 1: Figs. 5-6) and multi-papillose mid-laminar cells (Plate 1: Fig. 8).

3. Conclusion

In conclusion, *Macromitrium hamatum* is an endemic species to India, with previous reports from Meghalaya and Tamil Nadu, particularly from the Tirunelveli district. This study presents the first record of the species from the Nilgiri Hills. Characterized by its pleurocarpous habit, erectopotent stems, and densely arranged hamate, hooked, and lanceolate leaves, the species is distinct from *Macromitrium nepalense* due to its unique leaf morphology and multi-papillose mid-laminar cells. The new record from the Nilgiri Hills adds valuable information to the distribution of this species, highlighting its presence in South India.

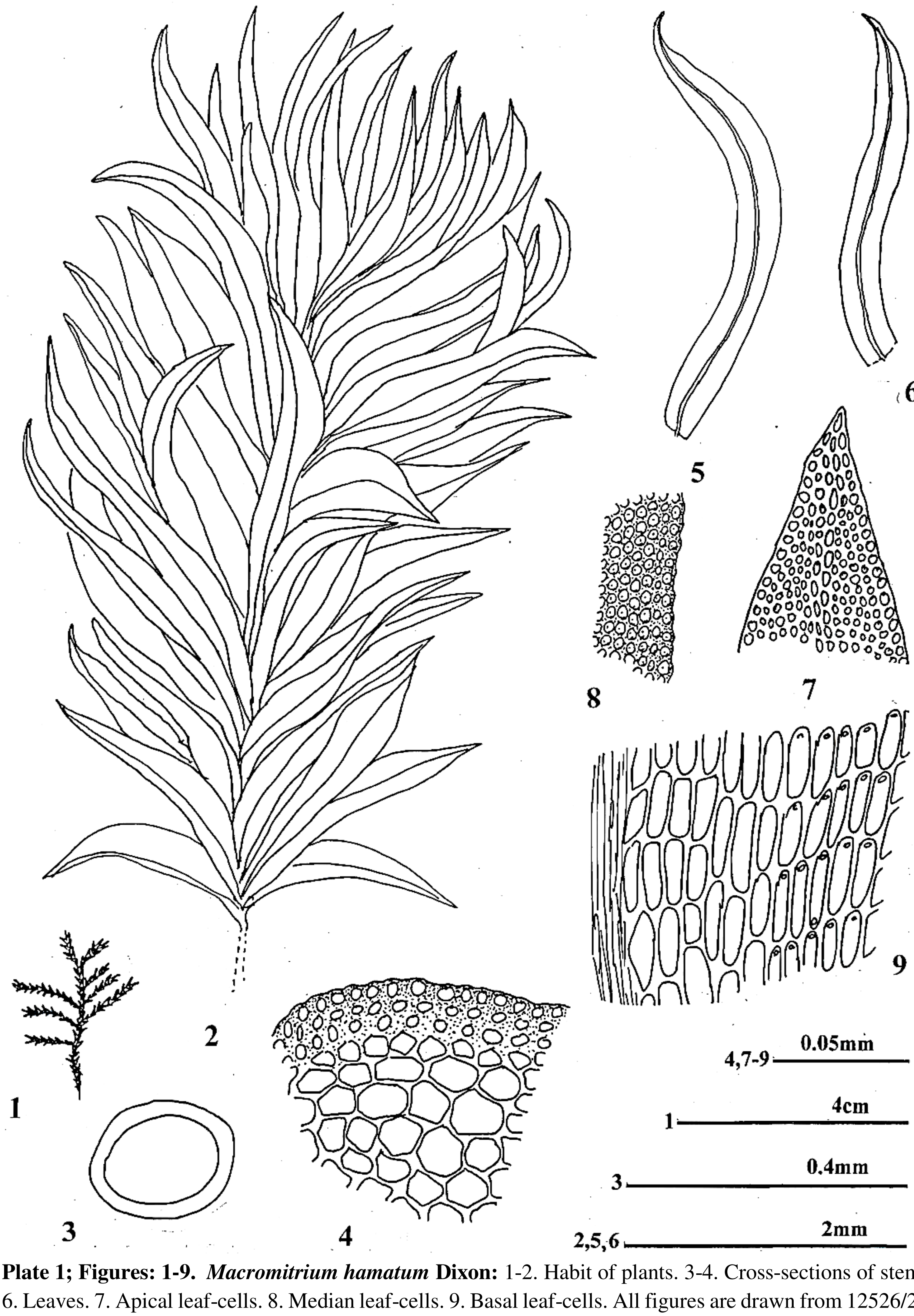

Plate 1; Figures: 1-9. ***Macromitrium hamatum*** **Dixon:** 1-2. Habit of plants. 3-4. Cross-sections of stem. 5-6. Leaves. 7. Apical leaf-cells. 8. Median leaf-cells. 9. Basal leaf-cells. All figures are drawn from 12526/2000 (LWU).

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Conflicts of interest

Not Applicable.

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Review Article

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Role of Bryophytes in Phytoremediation: A Review

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Abstract

Bryophytes, including mosses, liverworts, and hornworts, are non-vascular plants that are increasingly recognized for their potential in environmental remediation, particularly in the field of phytoremediation. These plants possess unique biological characteristics, such as high surface-area-to-volume ratios, tolerance to diverse environmental conditions, and efficient absorption mechanisms, which enable them to accumulate or degrade various pollutants from air, water, and soil. Bryophytes have demonstrated significant potential in the remediation of heavy metals, organic contaminants, and radioactive substances, as well as in the restoration of ecosystems affected by pollution. This review explores the mechanisms by which bryophytes contribute to phytoremediation, including absorption, bioaccumulation, and the breakdown of contaminants, and examines their application in different environmental contexts. The article also addresses the challenges and future prospects of using bryophytes in phytoremediation practices, highlighting the need for further research to enhance their efficiency and applicability.

Keywords: Bryophytes, Phytoremediation, Heavy metal removal, Environmental restoration, Bioaccumulation.

1. Introduction

Bryophytes, a class of non-vascular plants, consist of around 15,000 species and include hornworts, mosses, and liverworts[1]. Recent phylogenomic studies suggest that these groups form a monophyletic clade, closely related to tracheophytes[2]. Bryophytes are small, with simple structures and a dominant haploid life cycle, reproducing via spores or vegetative propagation[3]. They thrive in various habitats like soil, tree trunks, and rocks, absorbing nutrients directly from their moist surroundings. Due to their lack of epidermal cuticles and their high surface-to-volume ratio, bryophytes are highly sensitive to environmental changes. Their ability to exchange ions and chelate metals, along with their widespread distribution, makes them excellent bioindicators of potentially toxic element (PTE) pollution[4, 5].
Increased industrialization and urbanization have led to higher levels of non-degradable PTEs in natural habitats, with mosses and liverworts being widely used to monitor PTE pollution in terrestrial and aquatic environments[6, 7].

These plants can accumulate substantial amounts of heavy metals (HMs) from both the atmosphere and substrate without noticeable harm. Bryophytes show a strong correlation between metal accumulation and environmental PTE levels, further supporting their role as biomonitors [8, 9]. Different bryophyte species and habitats exhibit varying metal accumulation patterns. For example, the moss *Funaria hygrometrica* accumulates large amounts of lead (Pb)[12], while the liverwort *Marchantia polymorpha* stores high levels of copper (Cu), zinc (Zn), and cadmium (Cd)[11, 13]. Bryophytes have developed various mechanisms to tolerate elemental stress. One strategy is the formation of cell wall barriers that prevent metal ions from entering the cell. For instance, the moss *Scorpiurum circinatum* immobilizes toxic metals in its cell walls[14], while *Pohilia drummondii* blocks excess zinc through its cell wall and plasma membrane[15]. Further research is needed to better understand how cell wall composition contributes to PTE tolerance. Additionally, bryophytes differ in their cation exchange capacities and transporter activities, which influence their tolerance to metals. Inside the cells, the chelation of ions by molecules like glutathione (GSH) and phytochelatins (PCs) helps detoxify PTEs[16, 17]. These compounds are synthesized, accumulated, and sequestered into vacuoles, offering protection against metal toxicity. For example, in *Leptodictyum riparium*, GSH primarily drives cadmium (Cd) detoxification, while in *Marchantia polymorpha*, PCs play a major role in detoxifying metals like Cu, Zn, and Cd[18]. Bryophytes also utilize an antioxidant defense system to manage oxidative stress caused by PTEs. This system includes both enzymatic components, such as catalases (CAT) and peroxidases (POD), and non-enzymatic molecules. Monitoring intracellular reactive oxygen species (ROS) and antioxidant activity in bryophytes provides insight into their redox state and ability to cope with PTE-induced stress[19, 20].

Cryptogams play a significant ecological and evolutionary role, contributing greatly to global biodiversity and providing essential habitats and food sources for various organisms. They are crucial in ecosystems, aiding in nutrient cycling, soil formation, and water retention[21]. Bryophytes, in particular, are vital for maintaining moisture in ecosystems. Many cryptogams, especially lichens and mosses, serve as pioneer species that colonize bare substrates, facilitating soil development and ecological succession[22]. Additionally, certain cryptogams, particularly mosses, contribute to carbon sequestration, storing carbon in ecosystems and influencing global carbon cycles through their biomass accumulation[23, 24].

Bryophytes, the most ancient and smallest terrestrial plants, are the second-largest group of green plants in India, following angiosperms. Often referred to as the "amphibians of the plant world," they form miniature, forest-like structures, though they lack colorful flowers and remain small in size. Despite their size, bryophytes exhibit unique ecological characteristics that make them fascinating and important in various environmental contexts[25].

Habitat

Bryophytes, with around 23,000 species, are the most diverse group of terrestrial plants, thriving in harsh environments like the Alpine, Arctic, and Antarctic regions[26]. As non-vascular plants, they absorb water and nutrients through their leaf surfaces and can grow on a variety of surfaces, including rocks, walls, and tree trunks. Their growth forms, influenced by habitat conditions, include short turf, mats, dendroid shapes, and thalloid mats[27, 28].

Commonly found in temperate and tropical forests, bryophytes retain moisture and provide habitats for microfauna[29]. In wetlands, especially bogs and swamps, sphagnum mosses play a key role in water retention and carbon storage[30]. They also stabilize soil and reduce erosion along freshwater bodies and act as pioneers in disturbed areas, contributing to soil formation. As poikilohydric organisms, bryophytes depend on water for growth and reproduction, thriving in humid environments[31]. They also adapt to urban settings, growing on walls, pavements, and rooftops, enhancing biodiversity and providing ecosystem services[26].

Ecological Importance of Bryophytes

Bryophytes, despite their small size and simple structure, play a crucial role in ecosystems by aiding soil formation, nutrient cycling, carbon sequestration, and providing habitats. They absorb water and nutrients directly from the atmosphere, helping to colonize sterile soils and create conditions for new plant growth[25, 32].

Carbon Sequestration: Bryophytes, particularly peat-forming species like *Sphagnum*, store carbon in their biomass and substrates, contributing significantly to carbon dynamics. Peatlands, which cover just 3% of Earth's surface, store about one-third of global soil carbon[30, 33]. Bryophytes in forests also enhance soil organic carbon stocks, acting as long-term carbon reservoirs[34].

Nutrient Cycling: Bryophytes influence nitrogen and phosphorus cycling, absorb nutrients from precipitation, and support nitrogen-fixing bacteria, enriching soil and promoting vascular plant growth [35, 36]. They also aid ecological restoration by helping recover nutrient cycling in degraded habitats[37, 38].

Habitat Provision: Bryophytes, as ecosystem engineers, create microhabitats for various organisms, including invertebrates, fungi, and microorganisms. Species like *Frullania* and *Herbertus* provide shelter for nematodes, rotifers, and algae, while aquatic bryophytes like *Fontinalis* offer habitat for fish and amphibians[26].

Pollution Indicators: Due to their sensitivity to pollutants, bryophytes act as bio-indicators, monitoring air, soil, and water quality. Mosses, in particular, are effective in phytoremediation, absorbing heavy metals like Pb, Cd, and As, with high efficiency in removing contaminants [39, 40, 41].

Natural Pesticides: Some liverworts, such as those in the Marchantiophyta division, produce phytochemicals with nematicidal, antimicrobial, and antifungal properties[42]. *Sphagnum* moss has also been used as an antifungal agent in art conservation[43].

Pioneer Colonizers: In arid environments, bryophytes are pioneer species that establish organic layers on barren land, facilitating soil development and the growth of subsequent vegetation. They also contribute to ecological succession by forming colonies on tree trunks and creating new moist microhabitats [22, 26].

2. Importance of addressing soil and water contamination

Soil and water contamination, driven by human activities like industrial processes, agriculture, and urbanization, poses significant risks to public health, ecosystems, and agriculture. Agricultural runoff, including excess nutrients and agrochemicals, contributes to water pollution and eutrophication[44]. Industrial activities, such as mining, introduce heavy metals into soil and water, while urban runoff and improper waste disposal further degrade water quality [45, 46].

Contaminated water can cause health issues like gastrointestinal diseases, neurological disorders, and reproductive problems, with heavy metals such as mercury and lead posing severe risks[47, 48]. Contaminated soils reduce crop yields, fertility, and food safety, with long-term economic implications for agriculture and public health[49]. Pollutants also disrupt ecosystems, harming biodiversity and ecosystem services such as pollination and nutrient cycling [50]. To address contamination, a multi-pronged approach is necessary. Remediation techniques like bioremediation, phytoremediation, and soil washing are effective in cleaning polluted environments[51, 52]. Preventive measures, such as precision farming and green infrastructure, can minimize environmental impact[53, 54]. Policy interventions, including regulatory frameworks like the Clean Water Act and international agreements on hazardous substances, are crucial for effective pollution control [55, 56].

Heavy Metals and Their Sources

Heavy metals are naturally present in the Earth's crust but can accumulate in the environment due to human activities. Sources include fuel combustion (increasing sulfur), vehicle emissions (raising zinc and lead levels), industrial effluents and mining (copper), smelting industries and fertilizers (cadmium), and coal burning (cobalt).

These metals can disrupt biogeochemical cycles and accumulate in food webs, impacting both ecosystems and human health[57, 58, 59]. Besides metals, pollutants like persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) also pose environmental and health risks, making bryophytes valuable as cosmopolitan plants in pollution monitoring[60].

Phytoremediation: Phytoremediation, coined in 1983 by R.L. Chaney, is an in-situ technique that uses plants to absorb or break down contaminants. It is seen as a more cost-effective, eco-friendly alternative to traditional methods like soil excavation[61, 62]. Bryophytes play a key role in several phytoremediation mechanisms:

Phytoextraction: Bryophytes absorb metals and radioactive contaminants through their rhizoids[37].

Phytofiltration: Bryophytes filter water contaminants, despite lacking true roots[63].

Phytovolatilization: Plants release pollutants from their leaves[52].

Phytodegradation: Microbial communities in bryophytes break down organic pollutants like pesticides and PAHs[64, 65].

Phytostabilization: Bryophytes stabilize contaminated soils to prevent leaching[37].

Phytostimulation: Plants enhance microbial degradation of organic pollutants[66]

These mechanisms are cost-effective and ecologically beneficial[40], with ongoing research exploring their potential in non-bryophytic plants as well[67].

Notable Characteristics of Bryophytes for Phytoremediation

Bryophytes possess several unique morphological and physiological traits that enhance their adaptability to various environmental conditions, making them particularly effective in phytoremediation. One key feature is their dominant haploid phase in their life cycle, which allows for faster growth and quicker responses to environmental stress compared to the diploid phase dominant in higher plants [68, 69]. Despite their smaller size and simpler structure, lacking true roots, stems, and leaves, bryophytes exhibit distinct physiological processes that set them apart from vascular plants[32]. Instead of true roots, they have rhizoids, which anchor them to substrates and enable direct absorption of water and nutrients through their surfaces[70].

As non-vascular plants, bryophytes lack xylem and phloem, relying on diffusion for water and nutrient transport. This simplicity limits their size and habitat range, confining them primarily to moist environments[71]. However, their high surface area to volume ratio allows for efficient gas exchange and moisture retention, making them highly effective in absorbing and accumulating pollutants, including heavy metals[72] (Fig. 1).

Bryophytes are increasingly being integrated into combined remediation strategies, working alongside other plants, fungi, and bacteria to enhance the efficiency of pollution mitigation in complex scenarios[26]. Due to their sensitivity to environmental pollution, they also serve as effective bioindicators and biomonitors of heavy metals and other pollutants in air and water[39, 73, 74]. One remarkable feature of bryophytes is their desiccation tolerance, the ability to lose almost all intracellular water and then rehydrate and resume normal function. This tolerance allows them to quickly equilibrate with surrounding water potential, making them either fully hydrated or desiccated, with metabolic activity ceasing during desiccation. Recovery from desiccation depends on the duration and intensity of dryness [75, 76, 77]. Additionally, bryophytes can tolerate heavy metal toxicity through mechanisms like bioaccumulation and sequestration of metals in their tissues[78].

Although moisture-dependent, bryophytes are found in diverse damp habitats, such as forests, wetlands, and streams, where they contribute to soil formation, moisture retention, and nutrient cycling[31]. They also play a significant role in stabilizing soil, reducing erosion, and promoting plant biodiversity[35]. These ecological benefits highlight bryophytes' value in phytoremediation efforts, providing a low-cost, effective solution to environmental pollution[40].

Additionally, bryophytes are sensitive to climate change, which makes them useful indicators of environmental shifts. As they colonize new habitats in response to climate change, bryophytes help absorb CO_2, reducing atmospheric levels of this greenhouse gas[79]. Their role in carbon sequestration and ecosystem stabilization underscores their importance in combating the effects of global warming.

Mechanisms of Phytoremediation in Bryophytes

Recent research has renewed interest in using bryophytes for phytoremediation, particularly for alleviating heavy metal toxicity, recovering metals for recycling, and employing biomaterials in bio-sorbent filtration. Bryophytes were first proposed as effective phytoremediators by Frahm and da S. Barbosa and Carvalho in 2003[40]. These plants employ several mechanisms to facilitate phytoremediation, including bioaccumulation, adsorption, and degradation of contaminants (Fig. 2).Bioaccumulation refers to the uptake of contaminants from the environment into plant tissues, a process especially prevalent in mosses[80]. Studies have shown that species like *Sphagnum* demonstrate superior bioaccumulation and tolerance to heavy metals such as lead, cadmium, and zinc, compared to other bryophytes like *Marchantia*. These species also show increased growth during the monsoon season. Bioaccumulation occurs through both passive absorption from the substrate and active transport mechanisms, enabling bryophytes to thrive in polluted environments [81, 82]. Adsorption is another key mechanism, where bryophytes bind contaminants to their surfaces. Their high surface area-to-volume ratio enhances the adsorption of heavy metals and organic pollutants[72]. For example, *Hylocomium splendens* has demonstrated an effective ability to adsorb various heavy metals[83]. Additionally, the extracellular polysaccharides produced by bryophytes improve their capacity to adsorb pollutants, making them highly suitable for bioremediation applications[84].

Although degradation of contaminants is less common in bryophytes than in higher plants, some species can metabolize organic pollutants like polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs)[60].

Furthermore, microbial symbiosis in bryophyte habitats can enhance degradation processes, as associated microbial communities are capable of breaking down complex organic compounds, further boosting the phytoremediation potential of bryophytes[26].

Effectiveness of Specific Bryophyte Species

Mosses

Mosses are highly effective in phytoremediation due to their unique morphology and ability to absorb pollutants from both aqueous and aerial environments. They lack true roots, stems, and leaves but rely on structures that perform similar functions, allowing them to absorb water, nutrients, and contaminants efficiently. Mosses, including *Physcomitrium cyathicarpum* and *Barbula constricta*, have been shown to accumulate high levels of heavy metals like Fe, Ni, Cu, Cr, Cd, Pb, and Hg from polluted areas, with industrial and vehicular sources being the primary contributors[85]. Mosses such as *Leptodictyum riparium* and *Scorpiurum circinatum* can tolerate heavy metal stress without significant damage, making them reliable indicators of metal accumulation[86,87].

Similarly, *Polytrichum commune* accumulates metals like zinc, chromium, and copper in different parts of the plant, demonstrating its adaptability to harsh conditions[88]. Studies in Mexico showed that *Fabriona ciliaris* has a higher capacity for accumulating heavy metals like Cr, Zn, Cd, and Pb, with significant concentrations linked to anthropogenic activities[80]. *Funaria hygrometrica* can accumulate up to 74% of its dry weight in lead, utilizing multiple retention mechanisms[89], while *Warnstorfia fluitans* is effective in phytofiltration of arsenic near mine tailings[90]. Bryophytes such as *Pleurochaete squarrosa* and *Timmiella barbuloides* demonstrate sensitivity to heavy metals, with antioxidant mechanisms helping them tolerate accumulated toxicity[95].

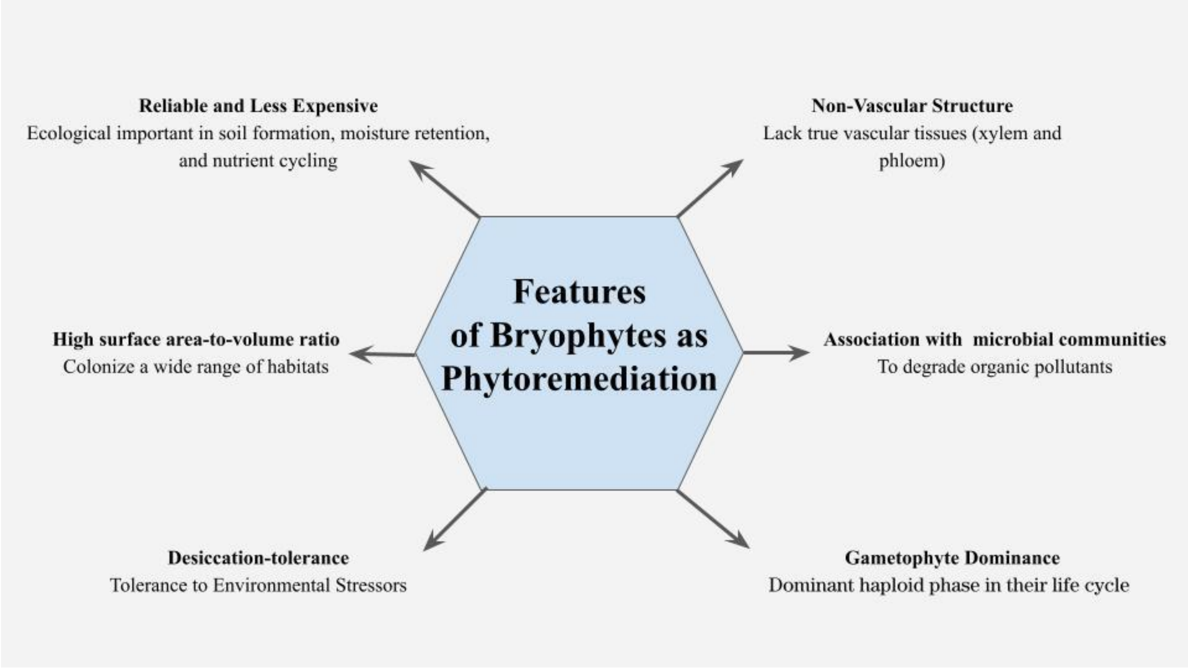

Figure 1: Characteristic features of Bryophytes as Phytoremediation

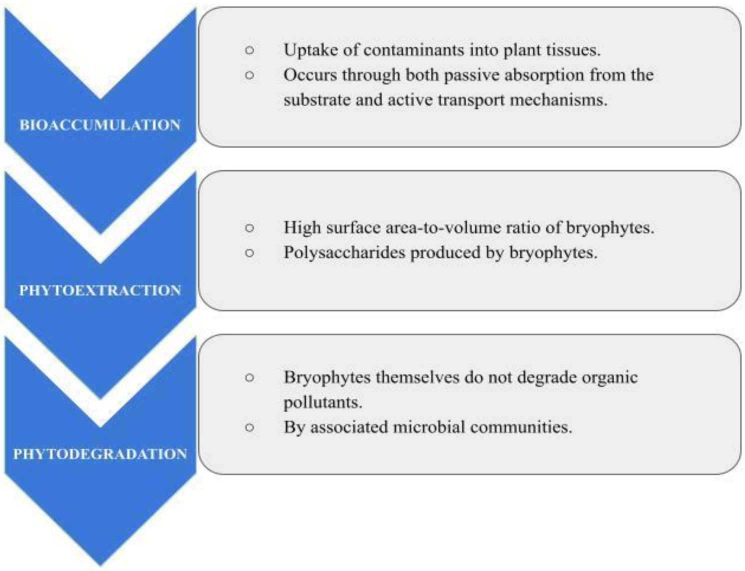

Figure 2: Mechanisms of Phytoremediation in Bryophytes; namely: Bioaccumulation, Phytoextraction and Phytodegradation.

Table 1: Various Mosses with Phytoremediation Properties

Bryophyte Species	Pollutants Targeted	Remediation Mechanism	Effectiveness	References
Barbula constricta	Heavy metals (Fe, Cu, and Pb)	Accumulation and uptake in tissue	Industrial belts and automobiles.	85
Physcomitrium cyathicarpum	Heavy metals (Fe, Cu, and Pb)	Accumulation and uptake in tissue	Industrial belts and automobiles.	85
Leptodictyum riparium	Heavy metals (Pb, Cd, Cu, Zn)	Bioaccumulation and indicator	Tolerate heavy metals stress	86
Scorpiurum circinatum	Heavy metals (Cd, Cu, Pb and Zn)	Bioaccumulation	Urban environments and disturbed sites	87
Polytrichum commune	Nutrients (N, P), Heavy metals	Uptake and nutrient cycling	Enhances soil fertility in disturbed habitats	88
Fabriona ciliaris	Heavy metals (Zn, Cd ,Pb)	Accumulation	Remediation	80
Funaria hygrometrica	Heavy metals (Cd, Zn, Pb)	Bioaccumulation	Contaminated soils with high metal levels	89
Warnstorfia fluitans	Arsenic (As)	Phytofiltration	Aquatic ecosystems and wetland restoration	90
Pleurochaete squarrosa	Heavy metals (Pb, Ni, Cu and Cr)	Bioaccumulation	Contaminated soils with high metal levels	91
Timmiella barbuloides	Heavy metals (Pb, Ni, Cu and Cr)	Bioaccumulation	Contaminated soils with high metal levels	91
Hylocomium splendens	Heavy metals (Pb, Cd, Ni and As)	Absorption	Maximum heavy metal absorption of Pb	83
Taxiphyllum barbieri	Heavy metals (Pb, Cd, Zn, Cu, As, and Cr)	Bioindication and environmental monitoring	Act as live filtering material	92
Scopelophila cataractae	Heavy metals (Cd or Cu)	Bioaccumulation and indicator	Urban soil remediation and bioindication	93; 94
Bryum coronatum	Heavy metals; (Zn,Cu, Pb, As)	Accumulation, Absorption, leaching prevention	Urban soil remediation and bioindication	95; 96
Philonotis thwaitessii	Heavy metals; Cadmium (Cd)	Phytoremediation and Biomonitoring	Contaminated soils with high metal levels	100
Fissidens involutus	Heavy metals (Cd and Zn)	Accumulation and Bioindicator	Contaminated soils with high metal levels	100
Splachnobryum oorschotii	Heavy metals; Cadmium (Cd)	Accumulation and Phytoremediation	Contaminated soils with high metal levels	100
Sphagnum spp.	Heavy metals (Hg, Pb, Zn)	Accumulation, nutrient absorption	Acidic and waterlogged conditions	97; 98
Mnium hornum	Organic pollutants (PAHs)	Uptake and degradation via microbial associations	Effective for degrading complex organic compounds	99
Vesicularia montagnei	Heavy metals; Zinc (Zn) and Cadmium (Cd)	Hyperaccumulation	Contaminated soils with high metal levels	100

Table 2: Various Liverworts with Phytoremediation Properties				
Bryophyte Species	**Pollutants Targeted**	**Remediation Mechanism**	**Effectiveness**	**References**
Asterella angusta	Heavy metals (Cu and Pb)	Bioaccumulation and indicator	Traffic areas	101
Cyathodium tuberosum	Heavy metals (Cu and Pb)	Bioaccumulation and indicator	Traffic areas	101
Riccia billardieri	Heavy metals (Co, Cu, Zn and Cd)	Accumulation and nutrient uptake	Soil purifier	38
Marchantia polymorpha	Heavy metals (Cd, Cu, Pb and Zn)	Bioaccumulation	Contaminated water	82

These species highlight the broad potential of mosses in phytoremediation and pollutant bioaccumulation.

Heavy metal absorption by *Hylocomium splendens* (also known as moss bags) was monitored at 142 stations in Romania, where the levels of Pb, Cd, Ni, and As were measured using inductively coupled plasma mass spectrometry. The highest absorption was observed for Pb, indicating significant pollution, especially due to the presence of arsenic[83].

Taxiphyllum barbieri, an aquatic moss, acts as a live filter for pollutants, including Pb, Cd, Zn, Cu, As, and Cr. In addition to pollutant removal, this moss can be used for environmental monitoring and bioindication[92]. The metallophyte moss *Scopelophila cataractae* exposed to Cd and Cu exhibited no genetic differentiation but showed limited changes in DNA methylation, highlighting its potential for metal tolerance [93, 94].

In a study, *Bryum coronatum* was found to accumulate Zn, while *Philonotis thwaitessii* accumulated Cd. Both species, along with *Fissidens involutus*, can be used as bioindicators in Cd and Zn co-contaminated areas. *Notothylas javanica* and *Splachnobryum oorschotii* are promising candidates for Cd phytoremediation and biomonitoring. Further research on *Bryum coronatum* has shown that Cd exposure reduces chlorophyll A content and decreases the number of chloroplasts, demonstrating its capacity for metal accumulation[95, 96].

Sphagnum mosses, known for their high water retention and nutrient absorption abilities, have shown great potential in removing heavy metals like Zn and Cd from contaminated water. Sorption capacity varies by species, with the following order of effectiveness: *Sphagnum* sp. > *Pleurozium schreberi* ≥ *Dicranum scoparium* ≥ *Thuidium tamtariscifolium* > *Eurhynchium praelongum* > *Leucobryum glaucum* > *Polytrichum commune*[97]. For example, *Sphagnum perichaetiale* biomass has been used as a biosorbent to remove crystal violet from water[98], while *Sphagnum squarrosum* demonstrates bioaccumulation and metal toxicity[81].

Mnium hornum is a bioindicator species that accumulates heavy metals in the following order: Fe > Zn > Mn > Cu > Pb > Ni > Co > Cr > Cd[99]. *Vesicularia montagnei,* an aquatic and terrestrial moss also known as Christmas moss, exhibits hyperaccumulation of Cd and Zn. Zinc helps mitigate the toxic effects of Cd and supports moss growth and biomass development[100] (Table 1).

Marchantiophyta (Liverworts): Liverworts can be classified as thalloid (flat, ribbon-like, or lobed) or leafy. Thalloid liverworts have a flattened body that grows directly on substrates like soil, rocks, or tree bark, while leafy liverworts possess small, leaf-like structures. Like mosses, liverworts lack vascular tissue and absorb water and nutrients directly through their surface, making them highly sensitive to environmental pollution but also effective at absorbing contaminants. Liverworts reproduce both sexually, via gametangia, and asexually, through gemmae. Asexual reproduction allows them to rapidly colonize new areas, especially in contaminated environments.

Liverworts thrive in moist, shady habitats, which often overlap with locations prone to pollution, such as industrial sites or areas affected by acid rain—conditions where other plants may struggle. For instance, a study on two bryophyte species, *Asterella angusta* and *Cyathodium tuberosum*, in the Koyana Wildlife Sanctuary found that these liverworts can serve as indicators of metal pollution. Using atomic absorption spectroscopy, the study identified heavy metals like Cr, Ni, Pb, Zn, Cd, and Cu, with pollution being most prevalent in areas impacted by traffic, which contributed high levels of Cu and Pb[101].

In another study, the thalloid liverwort *Riccia billardieri* was found to accumulate large amounts of sulfur, indicating its potential as a soil purifier. The concentration of elements in its tissues was highest for sulfur, followed by Zn, Pb, Cu, Cd, and Co[41]. Additionally, liverworts like *Marchantia polymorpha* have been studied for their ability to accumulate and tolerate heavy metals such as Cu, Cd, Pb, and Zn. These liverworts have demonstrated the ability to significantly reduce the concentrations of cadmium, zinc, and copper in contaminated water[82] (Table 2).

Anthocerotophyta (Hornworts): Hornworts are distinguished by their unique, long, horn-like sporophyte that grows out of the main thallus, giving them their characteristic appearance. The green, photosynthetic sporophyte can grow several centimeters tall. Like mosses and liverworts, hornworts lack vascular tissues and absorb water and nutrients directly through their thallus. They reproduce sexually through spores produced in sporangia on the sporophyte.

A notable feature of hornworts is their symbiotic relationship with cyanobacteria, which help fix nitrogen in their tissues. This partnership enables hornworts to thrive in nutrient-poor soils and contributes to nutrient cycling, potentially improving soil quality in polluted or degraded environments. Hornworts are also relatively resistant to environmental stresses, including pollution and fluctuations in water availability, making them well-suited for phytoremediation in polluted wetlands or water bodies [26, 34, 71, 102].

For example, *Anthoceros fusiformis* and *A. punctatus* have been used as biomonitors of atmospheric pollution, though further research is needed to explore their potential in accumulating harmful metals from soil and water for phytoremediation purposes[5].

Historical Background Phytoremediation Using Bryophytes

The studies in relevance to the application of mosses in phytoremediation found 174 and more publications using Web of Science platform, studies were developed from 2005 onwards, total 17 researches in year 2020 with the highest number of studies of using bryophytes as phytoremediation[60].

Moreover, in a review by Pandey and Alam[103] emphasizes the characteristic features of the Peat moss, a well-known hyper-sorbent, is now a days gaining more acceptance for its utilization in oil-spill clean-up techniques due to its inexpensive nature, biodegradability and relatively high oil absorption capacities due to high porosity and large surface area which, making it an efficient natural sorbent material for clearing up oil spills.

Discussion

Despite having many potential, there are some challenges that hinder the application of bryophytes as phytoremediation: (1) Slow Growth Rates: Bryophytes generally exhibit slow growth and reproduction rates, which can limit their effectiveness in rapidly remediating contaminated sites. This slow recovery may necessitate longer remediation periods compared to higher plants, therefore optimization of growth conditions and species selection are critical for maximizing their phytoremediation potential; (2)Limited Mobility: Bryophytes have limitations inherent in them to spread and colonize new areas. Their propagation typically relies on spores or vegetative fragments, which can restrict their use in larger contaminated areas unless actively managed; (3) Environmental Specificity: The effectiveness of different bryophyte species can vary significantly based on environmental conditions and pollutant types. This necessitates careful site assessments to identify the most suitable species for specific remediation tasks; (4) Limited Translocation: Bryophytes exhibit limited translocation capabilities compared to vascular plants. This limitation may hinder the movement of contaminants from the substrate to the aboveground tissues, affecting overall remediation efficiency; (5) Species-Specific Responses: The effectiveness of bryophytes in phytoremediation can vary significantly among species. This variability necessitates careful selection and assessment of species for specific remediation projects; (6) Long-term viability: While bryophytes can effectively accumulate heavy metals, there is a risk of metal toxicity to the plants themselves, which can limit their long-term viability in heavily contaminated environments [40, 52, 60].

Further research is needed to be optimized to overcome the limitations of bryophytes in phytoremediation by employing a number of tools, such as, natural and chemical amendments, genetic engineering and natural microbial stimulation and enhance the efficacy of bryophytes in phytoremediation[52]. Areas of focus includes, Genetic Engineering, to introduce genes that confer resistance to heavy metals or enhance metabolic pathways for pollutant degradation, by modifying heavy metal transporter genes and their uptake systems, by increasing heavy metal ligand synthesis and by converting heavy metals into less hazardous and volatile forms [48, 104].

Nanobioremediation is a new emerging technique for remediation of pollutants using biosynthetic nanoparticles from plants, bacteria, yeast and fungi which are emerging as nanofactories and potential application in environmental clean-up[105].

Transgenic approaches have been employed to enhance the expression of metal-binding proteins, such as metallothioneins and phytochelatins, in bryophytes, resulting in increased metal accumulation and tolerance[106].

Role of soil microbial communities can influence the overall health of plants, including bryophytes, through the application of microbial inoculants or organic amendments can enhance the degradation of pollutants and promote plant growth. Furthermore, the establishment of mycorrhizal associations, shown to improve nutrient uptake and stress tolerance[26]. This suggests that integrating microbial stimulation strategies with bryophyte could leads to more effective and sustainable remediation practices.

Conclusion

Soil and water contamination is a significant issue affecting human health, environmental sustainability, and economic stability. It is caused by various factors such as agricultural practices, industrial activities, and urbanization. To combat this, a comprehensive approach including remediation, prevention, and policy interventions is needed. Bryophytes, a valuable resource in phytoremediation, offer unique advantages as cost-effectiveness, enhancement of soil quality, and potential for promoting biodiversity for treating contaminated environments. Research shows that bryophytes can significantly influence soil moisture retention and carbon storage, affecting climate dynamics. They are essential components of terrestrial ecosystems, with unique adaptations that enable them to thrive in moist environments. Their ecological roles, including carbon sequestration, nutrient cycling, and habitat provision, are crucial for biodiversity conservation. Various species, such as *Sphagnum, Polytrichum*, and *Marchantia*, have demonstrated potential in remediating heavy metals and organic pollutants. However, they face numerous threats from environmental changes and human activities, necessitating urgent conservation and management efforts, and challenges related to growth rates and contaminant tolerance must be addressed. Future research should continue to explore the ecological roles of bryophytes, particularly in the context of global change, to inform effective conservation strategies, exploring bryophyte-microbe interactions to enhance phytoremediation efficacy and ensure the sustainability of these vital organisms.

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Conflicts of interest

Not Applicable.

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Isolation, purification and identification of cyanobacterium *Tolypothrix* sp. KJE1

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Abstract

A cyanobacterial strain, KJE1, was isolated from rice fields of Banaras Hindu University, Varanasi, during the monsoon of 2019. Morphological analysis and 16S rRNA phylogenetic studies identified it as *Tolypothrix* sp., known for nitrogen fixation. The partial 16S rRNA gene sequence (1283 bps) was submitted to NCBI (Accession Number OP353555). This study underscores *Tolypothrix*'s role in sustainable agriculture, contributing to soil fertility and reducing dependency on synthetic fertilizers, with potential applications in biofertilization.

Keywords: Rice field, Cyanobacteria, Strain, Light Microscope, Heterocyst, Hormogonia, BLAST.

1. Introduction

Cyanobacteria are Gram-negative, oxygen-producing, and widespread prokaryotes that have existed on Earth for over two billion years[1]. Approximately 2.8 billion years ago, they were the primary contributors to an oxygenic atmosphere, thriving in fossilized forms like stromatolites and oncolites[2]. These organisms exhibit diverse life forms, ranging from free-living species to those in symbiotic relationships with plants, fungi, and animals, adapted to various environments and showing significant morphological variation[3, 4, 5]. Cyanobacteria play a crucial role in maintaining soil health by enhancing nutrient availability, soil porosity, pH balance, water retention, and reducing soil salinity. Additionally, they contribute to essential plant physiological and biochemical processes. Paddy fields, in particular, serve as a key ecological habitat for cyanobacteria, where they perform vital functions such as nitrogen fixation and photosynthesis, supporting various physicochemical processes[6, 7].

Rice, an annual grass belong to the Poaceae family, includes 20 wild species and two cultivated varieties. Of these, *Oryza sativa* is the most widely cultivated, while *Oryza glaberrima* is primarily restricted to West Africa[8, 9].

The cultivation of rice is highly dependent on climatic conditions and seasonal factors. As a staple food, it provides a significant source of calories for over 60% of the global population. In India, around 45 million hectares of arable land are dedicated to rice cultivation, relying heavily on chemical fertilizers[10].

Currently, the world faces two critical challenges: the changing climate and rapid population growth. Recent estimates suggest that the global population could exceed 9 billion by 2050, putting increased pressure on food production with limited land resources.

Isolating, purifying, identifying, and screening cyanobacterial isolates is a vital step in leveraging their potential for bio-fertilizer production. The isolation process involves separating cyanobacterial species from their natural habitats, using techniques like serial dilution and streak plating to obtain individual species from various sources. Studying mat-forming cyanobacteria is crucial due to their ecological and biotechnological importance, providing insights into their survival strategies and potential applications across different fields. These cyanobacteria typically thrive in environments with fluctuating water availability, such as terrestrial soils, biological crusts, and specific aquatic settings. Therefore, understanding the processes of isolation, identification, purification, and screening of mat-forming cyanobacteria is essential for uncovering the mechanisms that enable their resilience in challenging environmental conditions. This research work aims to systematically investigate mat-formation in one or more strains of cyanobacteria using a comprehensive approach.

The isolation process begins with collecting samples from agricultural fields. Identification techniques, including microscopic examination and molecular methods, facilitate accurate taxonomic classification and confirmation of the isolated cyanobacterial strains. Purification steps refine these cultures to obtain pure isolates for further analysis. Additionally, the screening process evaluates the characteristics of mat-forming cyanobacteria. These screening efforts not only enhance our understanding of cyanobacterial mat formation but also hold potential for identifying unique candidates with beneficial mat-forming traits for biotechnological applications, such as soil enhancement and environmental restoration.

2. Materials and Methods

2.1. Sampling of cyanobacterial strain

Cyanobacteria are widely distributed across diverse ecosystems globally. Rippka et al.[11] classified these organisms into five subsections based on their morphology and cell differentiation characteristics. In the present study, we have focused on collecting heterocystous cyanobacteria from subsection IV. These cyanobacteria are crucial for nitrogen management due to the presence of heterocysts, specialized cells capable of nitrogen fixation.

The robustness of this cyanobacterial system is evidenced by the formation of akinetes under nutrient stress and the production of hormogonia, which act as reproductive filaments. Samples were collected in pre-sterilized Falcon tubes from the rice fields of BHU, Varanasi, India (25º15′50″N 82º59′13″E and 25º15′20″N 82º59′25″E). Sampling took place between August and October 2019, when cyanobacterial colonies are most abundant, thriving in temperatures of 25-35°C[12, 13]. These specific areas within BHU's agricultural fields were chosen for their consistent water availability and minimal exposure to prolonged desiccation.

2.2. Growth medium for cyanobacteria

Axenic cultures of cyanobacterial strains were carefully maintained in 120 ml of nitrate-free basal growth medium (BG-11N^{-}) within 250 ml borosilicate flasks, which lacked any combined nitrogen source[11]. The culture conditions were regulated at a pH of 7.5 and a temperature of 25 ± 2ºC, with 16 h light and 8 h dark cycle at an intensity of around 55 µmol photons $m^{-2}s^{-1}$. The specific composition of the culture medium is outlined below:

Table 1. Composition of BG-11 medium[11]

Macronutrients	gL^{-1}	Micronutrients	gL^{-1}
$K_2HPO_4.3H_2O$**	40.0	$MnCl_2.4H_2O$	1.810
$MgSO_4.7H_2O$	75.0	$ZnSO_4.7H_2O$	0.222
$CaCl_2.2H_2O$	36.0	$Na_2MoO_4.2H_2O$	0.390
Na_2CO_3	20.0	$CuSO_4.2H_2O$	0.079
Na_2-Citrate	6.0	$CoCl_2.2H_2O$	0.0494
Fe(III)$(NH_4)_3$citrate**	6.0		
EDTA	1.0		

$NaNO_3$ was not added to the medium.

** Fe $(NH_4)_3$ citrate and $K_2HPO_4.3H_2O$ were autoclaved separately and added to the precooled sterilized liquid medium to avoid precipitation. The pH of the medium was maintained to 7.5 with 0.1 N NaOH and 0.1 N HCl.

2.3. Isolation, identification and maintenance of the cyanobacterial strain

Samples were collected from the campus of Banaras Hindu University campus in Varanasi, Uttar Pradesh, India. The dilution plate method[14] was used to isolate the *Tolypothrix* from rice field soil. A 1 mL aliquot of soil suspension was spread on solidified (1.5%) BG-11N$^-$ medium. The plates were incubated at 25 ± 2°C with a 16-hour light: 8-hour dark[11] photoperiod under white fluorescent light with an intensity of 55 μmol photons $m^{-2}s^{-1}$. Morphological characteristics such as cell shape, length, and breadth of intercalary cells and heterocysts were observed for taxonomic identification based on cell or colony morphology[15]. Visible cyanobacterial colonies growing on the agar surface were aseptically picked and transferred to 100 mL of BG-11N$^-$ medium in flasks. Cyanobacterial suspensions were prepared in BG-11N$^-$ medium and spread on petri-plates. Purified colonies were isolated and transferred to fresh BG-11N$^-$ plates. Bacterial and fungal contamination, as well as cyanobacterial growth, was monitored using a light microscope. Repeated transfers between surface colonies and suspension cultures were conducted to establish axenic cultures. This iterative process continued until consistently purified cyanobacterial colonies were obtained. The cultures were shaken three times daily to enhance growth. Purified colonies were then transferred to 100 mL of BG-11N$^-$ broth, regularly checked for contamination, and maintained for further experimentation. These samples were used for mass cultivation of isolates and for analyzing growth behavior, biomass production, and generation time.

The axenic nature of the cultures were evaluated using several methods, including phase-contrast microscopic examination and plating samples on caseinate-glucose agar. This specific agar was prepared with a nutrient solution containing casamino acids (0.05%), glucose (0.5%), and agar-agar (1.2%). To check for potential bacterial contamination, a properly diluted suspension of the cyanobacterial culture was streaked onto the nutrient agar. After a week of incubation, bacteria-free micro-colonies were identified, marked with a sterile toothpick, excised along with the agar, and transferred to a 10 ml nutrient solution. The medium was sterilized, and agar slants were prepared in several sterilized tubes. The sample was inoculated into these tubes, with some incubated under light and others in darkness. After 14 days, the cultures were microscopically examined, confirming the absence of bacteria. Bacteria-free cyanobacterial colonies were selected and maintained on different agar slants, with the purity of the suspension regularly checked. Liquid cultures were manually shaken three times daily. Further investigations were postponed until a pure clonal culture of various cyanobacterial strains was successfully established and verified using taxonomic keys by Geitler[16], Desikachary[15], and Rippka et al.[11].

2.4. Identification of cyanobacterial strain

Identification of isolates by light microscope

The isolates were initially examined under light microscope by preparing temporary slides. During taxonomic characterization, various features were considered, including the shape, size, and color of the thallus, the width and length of the trichomes, the presence and location of heterocysts, filament branching, as well as the presence of hormogonia and akinetes[16, 17]

2.5. Genomic DNA Isolation and PCR Conditions

To further identify the cyanobacterial strains, partial sequencing of the 16S rRNA gene was performed. Genomic DNA was extracted from the cyanobacterial isolate using the conventional xanthogenate method[18]. The 16S rRNA gene was partially amplified with a forward primer (359F, 5'-GGG GAA TYT TCC GCA ATG GG-3') and a reverse primer (781R, 5'-GAC TAC TGG GGT ATC TAA TCC CAT T-3') (Nübel et al., 1997). The PCR reaction was carried out in 25 µl volumes, containing 30-50 µg of DNA template, 200 µM dNTPs, 0.4 µM of each primer, 1 U/µl Taq Polymerase, and 1.5 µM MgCl2 using a BioRad DNA Engine Peltier Thermal Cycler. The thermal cycler was used to amplify the DNA[19], The PCR products were sequenced using Sanger's method[20], and the resulting sequence was compared to the NCBI database using the BLAST tool.

2.6. Nucleotide sequence analysis

The partial 16S rRNA sequences obtained from DNA sequencing were thoroughly analyzed using the NCBI sequence database. The nucleotide Basic Local Alignment Search Tool (BLAST) available at http://blast.ncbi.nlm.nih.gov/Blast.cgi was used for comparison, aligning these sequences with already existing gene sequences from various cyanobacterial strains found in the database. Additionally, the partial 16S rRNA sequences of the test cyanobacterium were formally submitted to the NCBI database.

3. Results and discussion

3.1. Collection, isolation and purification of cyanobacterial isolate

A cyanobacterial strain was collected from the agricultural fields of Banaras Hindu University (BHU) in Varanasi, Uttar Pradesh, India, as a part of an extensive study aimed at isolating and identifying naturally occurring cyanobacteria from local rice fields. Cyanobacteria, known for their ability to fix atmospheric nitrogen and thrive in a variety of environments, are crucial in agricultural settings like rice paddies, where they contribute to soil fertility. The collection was carried out between mid-August and October 2019, a period chosen strategically as the monsoon season leads to optimal growth conditions for cyanobacteria in waterlogged environments like rice fields. Specific rice fields targeted for this study were chosen for their historical use in traditional farming practices, as cyanobacteria tend to proliferate in such environments. Rice paddies, with their semi-aquatic conditions and nutrient-rich soil, provide an ideal habitat for cyanobacterial growth. The water stagnation and nutrient availability create conditions in which cyanobacteria flourish, making them excellent candidates for studies on agricultural microbiomes and their potential in biofertilization.

One such strain, exhibiting heterocystous filamentous cyanobacteria, was designated as KJE1. This strain was isolated and identified based on its distinct morphological features observed under a light microscope. These features included the filamentous arrangement of cells, the presence of heterocysts at regular intervals along the filaments, and a characteristic structure that helped differentiate it from other cyanobacterial strains. To further support the identification of strain KJE1, detailed cross-referencing with classical taxonomic literature, particularly the monograph of Desikachary[15], was performed. This monograph is a key reference in the study of cyanobacteria, providing detailed descriptions of various genera and species. The morphological features of the strain KJE1 matched closely with those described for filamentous heterocystous cyanobacteria, leading to the confirmation of its taxonomic identity. Figure 1 visually represents the site of collection, providing a geographical context for the study.

Figure 1. Collection of cyanbacterial isolates from different agricultural fields at Banaras Hindu University.

3.2. Purification of cyanobacterial isolates

When we work with motile filamentous cyanobacteria, such as those that exhibit gliding motility, a slightly different approach is required. Filamentous cyanobacteria possess the ability to move across surfaces by gliding, a process facilitated by specialized structures called trichomes or hormogonia. These motile cells can be difficult to purify using standard methods, as their movement may cause them to intermingle with other microorganisms, complicating the isolation process. To address this challenge, the purification of motile filamentous cyanobacteria involves transferring individual gliding cells to fresh agar plates. During this process, a single motile filament or trichome was carefully transferred onto a new agar plate. The transferred cells were given the opportunity to glide across the fresh surface, which helped them move away from potential contaminants. As the cyanobacterial cells glided, they effectively separated themselves from unwanted microorganisms, allowing for a more precise purification of the strain. This technique was repeated multiple times, each time transferring only the desired motile cells to fresh plates, ensuring the gradual isolation of a pure, uncontaminated cyanobacterial culture (Figure 2).

3.3. Morphological identification

Morphological analysis of the cyanobacterial isolates was conducted using a light microscope to closely examine their distinctive structural features and differentiate between the various strains. This process involved a careful observation of key morphological traits, such as the shape and size of cells, the arrangement of filaments, and the presence of specialized structures known as heterocysts—cells that play a vital role in nitrogen fixation in certain cyanobacteria. These characteristics were scrutinized to help in the identification of the strains. In addition to direct microscopic observation, the morphological features were cross-referenced with the seminal monograph of Desikachary[15], a comprehensive taxonomic guide widely regarded as one of the most authoritative resources on cyanobacterial taxonomy. This monograph includes detailed descriptions and illustrations of a wide variety of cyanobacterial genera and species, making it an essential tool for accurately identifying strains based on their morphology. The process of comparison with Desikachary's work ensured that the identification was not only thorough but also scientifically grounded in established classification systems. One of the critical aspects observed in the study was the presence of heterocysts.

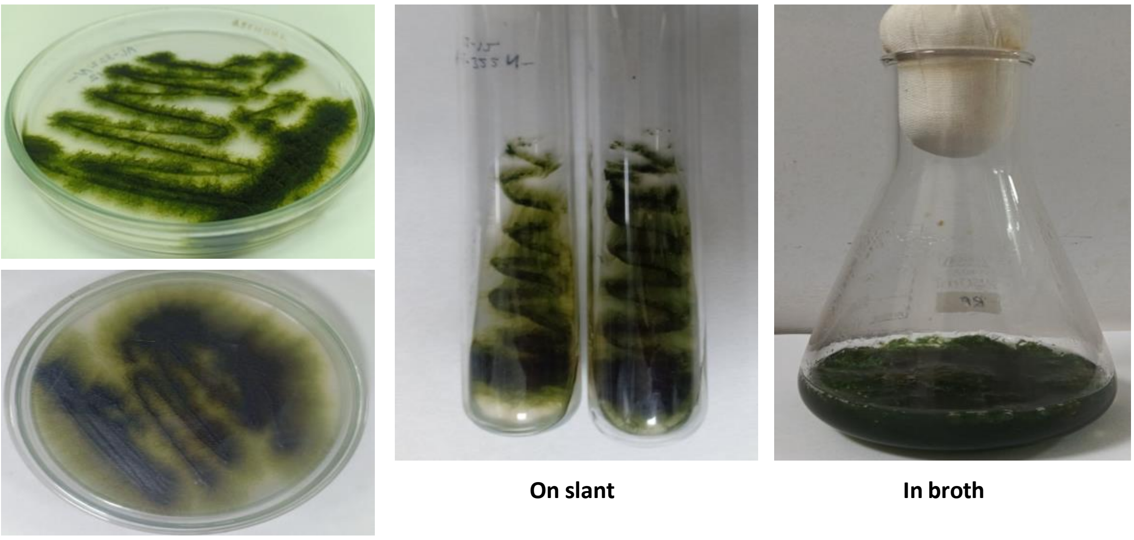

Figure 2. Pure cyanobacterial isolate KJE1 on agar plate, slant and in broth $BG^{-}11$ N^{-} media

The arrangement and frequency of heterocysts along with the filamentous structure provided important clues to the taxonomic identity of the cyanobacteria. These filaments, along with other key features such as cell dimensions, the nature of branching, and the surface texture, were meticulously compared with the descriptions provided in the monograph. The detailed examination and taxonomic matching led to the conclusive identification of the cyanobacterial strain as belonging to the *Tolypothrix* genus. *Tolypothrix* species are particularly significant in agricultural settings due to their nitrogen-fixing capacity, which contributes to soil fertility and can reduce the need for synthetic fertilizers. This makes them valuable from an ecological and agricultural standpoint, especially in regions where sustainable farming practices are being explored. The identification of this strain adds to the growing knowledge regarding cyanobacterial diversity in agricultural fields.

Figure 3, provides a visual representation of the *Tolypothrix* sp. strain under the microscope, highlighting its distinct structural features. The image serves as an important reference, visually confirming the morphological traits that were essential in its classification. These traits include the filamentous structure, the positioning of heterocysts, and the overall morphology that matches the descriptions outlined in the taxonomic literature. Through this detailed morphological analysis and taxonomic validation, the *Tolypothrix* sp. has been accurately identified, providing a foundation for further studies on its potential applications in biofertilization and sustainable agriculture.

3.4. Molecular and phylogenetic analysis of cyanobacterial isolates

Modern molecular techniques have become essential tools in studying cyanobacterial phylogenies and exploring population-level dynamics. These techniques provide insight into the evolutionary relationships and genetic diversity within cyanobacteria. Among the molecular markers used for phylogenetic studies, the 16S ribosomal RNA (16S rRNA) gene is one of the most reliable and informative markers. It is a highly conserved gene found universally across bacteria and cyanobacteria, making it an ideal target for taxonomic and phylogenetic analyses. The 16S rRNA gene contains both highly conserved regions, which are useful for broad taxonomic classification, and variable regions, which can distinguish closely related species. In the context of cyanobacterial phylogenetics, the 16S rRNA gene has been extensively utilized to assess evolutionary relationships and resolve taxonomic uncertainties[21, 22].

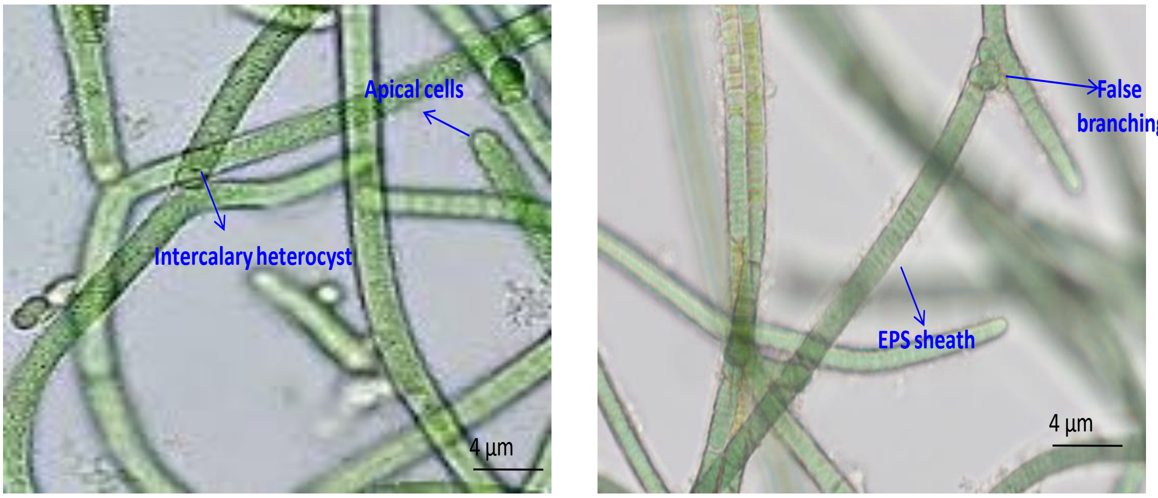

Figure 3. Light microscopic image of isolated cyanobacterial at 40X magnification *Tolypothrix* sp. KJE1

In this study, maximum likelihood phylogenetic analysis was conducted using the 16S rRNA gene sequence to determine the closest phylogenetic relatives of the cyanobacterial isolate KJE1. The results revealed that the closest species to strain KJE1 was *Tolypothrix* sp., with a nucleotide similarity of 96.72% (Figure 4.). This high level of sequence similarity confirms the close relationship between KJE1 and *Tolypothrix* sp. The partial sequence of the 16S rRNA gene for *Tolypothrix* sp. strain KJE1, consisting of 1283 base pairs (bps), was submitted to the National Center for Biotechnology Information (NCBI) gene bank database to make the sequence publicly accessible for future research and comparative studies. The deposited DNA sequence was assigned with a Gene Bank Accession Number OP353555, under the name *Tolypothrix* sp. KJE1. Cyanobacterial *Tolypothrix* sp. KJE1nucleotide sequence (16S rRNA gene) has been given below:

ACTTGCTTACCATGCAAGTCGAACGGTCTCTT
CGGAGATAGTGGCGGACGGGTGAGTAACGCG
TGAGAATCTAGCTTCAGGTCGGGGACAACCAC
TGGAAACGGTGGCTAATACCGGATGTGCCGAA
AGGTGAAAGATTTATTGCCTGAAGATGAGCTC
GCGTCTGATTAGCTAGTAGGTGTGGTAAGAGC
GCACCTAGGCGACGATCAGTAGCTGGTCTGAG
AGGATGATCAGCCACACTGGGACTGAGACAC
GGCCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATTTTCCGCAATGGGCGAAAGCCTGACGGA
GCAATACCGCGTGAGGGAGGAAGGCTCTTGGT
TGTAAACCTCTTTTCTCAGGGAAGAAAAAAAT
GACGGTACCTGAGGAATAAGCATCGGCTAACT
CCGTGCCAGCAGCCGCGGTAATACGGAGGAT
GCAAGCGTTATCCGGAATGATTGGGCGTAAAG
CGTCCGCAGGTGGCTATGTAAGTCTGCTGTTA
AAGAGTGAGGCTCAACCTCATAAGAGCAGTG
GAAACTACACAGCTAGAGTGCGTTCGGGGCA
GAGGGAATTCCTGGTGTAGCGGTGAAATGCGT
AGAGATCAGGAAGAACACCGGTGGCGAAAGC
GCTCTGCTAGGCCGCAACTGACACTGAGGGAC
GAAAGCTAGGGGAGCGAATGGGATTAGATAC
CCCAGTAGTCCTAGCCGTAAACGATGGATACT
AGGCGTGGCTTGTATCGACCCGAGCCGTGCCG
TAGCTAACGCGTTAAGTATCCCGCCTGGGGAG
TACGCACGCAAGTGTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGTATGTG
GTTTAATTCGATGCAACGCGAAGAACCTTACC
AAGACTTGACATGTCGCGAATCTTTTTGAAAG
GAAAGAGTGCCTTCGGGAGCGCGAACACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTCGTTTTATTTGCCAGCATTAAGTTGGGCAC
TCTAAAGAAACTGCCGGTGACAACCGGAGGA
AGGTGGGATGACGTCAGTCACATGCCCTTACG
CTTGGGCTACCACGTACTACATGCTACGACAA
GGGCACGAGCTACCATACAGCAATTTCTTAAC
CGGGCTCATTCAAATCCAGGTGCAATCCCCTG
CTGAAGGAGAATCCTAGATTGCGGCCCCTATT
GCGGAATTCTCCGCCTTTCCGGCGGAGGAGGA
AAAAAAAAAAG

Sequences producing significant alignments — Download — Select columns — Show 10

select all 10 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Tolypothrix sp. KJE1 16S ribosomal RNA gene, partial sequence	Tolypothrix sp. ...	2370	2370	100%	0.0	100.00%	1283	OP353555.1
Tolypothrix sp. 9k 16S ribosomal RNA gene, partial sequence	Tolypothrix sp. 9k	2054	2054	95%	0.0	96.72%	1401	KU668914.1
Calothrix sp. NIES-2099 gene for 16S ribosomal RNA, partial sequence	Calothrix sp. NI...	2036	2036	95%	0.0	96.47%	1486	LC455619.1
Tolypothrix tenuis PCC 7101 DNA, nearly complete genome	Tolypothrix tenu...	2036	10180	95%	0.0	96.47%	8700819	AP018248.1
Tolypothrix tenuis PCC 7101 gene for 16S rRNA, partial sequence	Tolypothrix tenu...	2036	2036	95%	0.0	96.47%	1444	AB325535.1
Aulosira sp. CENA272 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic s...	Aulosira sp. CE...	2030	2030	95%	0.0	96.40%	2104	MN551913.1
Aulosira sp. strain SG5-PS 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, pa...	Aulosira sp.	2030	2030	95%	0.0	96.39%	1818	PP165366.1
Calothrix membranacea SAG 1410-1 16S ribosomal RNA gene, partial sequence	Calothrix memb...	2025	2025	95%	0.0	96.32%	1459	KM019924.1
Nostoc carneum IAM M-35 gene for 16S rRNA, partial sequence	Nostoc carneu...	2021	2021	95%	0.0	96.24%	1444	AB325906.1
Tolypothrix sp. PCC 7910 chromosome, complete genome	Tolypothrix sp. ...	2019	10097	95%	0.0	96.24%	8479123	CP050440.1

Figure 4. BLAST analysis of 16s r-RNA sequence of cyanobacterial isolate KJE1.

The phylogenetic analysis of isolate KJE1 was constructed using maximum likelihood methods, which are considered a robust approach for inferring evolutionary relationships. This analysis allowed for a detailed understanding of the position of KJE1 within the broader cyanobacterial phylogeny. The high sequence similarity to *Tolypothrix* suggests that isolate KJE1 shares many genetic and possibly ecological characteristics with this genus. These findings highlight the utility of the 16S rRNA gene in resolving cyanobacterial relationships and provide a foundation for further investigation into the ecological roles and metabolic potential of isolate KJE1.

4. Conclusion

The isolation and identification of the cyanobacterial strain KJE1 from rice fields of Banaras Hindu University (BHU) in Varanasi, India, revealed its close association with the genus *Tolypothrix* species. Detailed morphological analyses, complemented by comparisons with Desikachary's monograph (1959), confirmed its filamentous structure, presence of heterocysts, and other characteristic features. These findings were further substantiated by molecular phylogenetic analysis using the 16S rRNA gene sequence, which showed a 96.72% similarity to *Tolypothrix* species. The partial 16S rRNA sequence (1283 bp) was submitted to the NCBI GenBank with Accession Number OP353555.

This study highlights the ecological and agricultural significance of *Tolypothrix* due to its nitrogen-fixing abilities, which contribute to soil fertility and promote sustainable farming practices. The identification of strain KJE1 adds to the growing body of knowledge on cyanobacterial diversity in agricultural ecosystems and provides a basis for further research into its biofertilizer potential. These findings underscore the importance of combining traditional taxonomic methods with modern molecular tools for comprehensive microbial studies.

5. Acknowledgement

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6. Authors Contribution

Research Scholar Jalaluddin is responsible for conducting fieldwork, collecting cyanobacterial samples, performing morphological and molecular analyses, and drafting the manuscript. Prof. Rajan Kumar Gupta, as the supervisor, provided guidance throughout the study, reviewed the results, and contributed to the finalization of the manuscript.

All authors have read and approved the final version of the research article.

Conflicts of interest

Not Applicable.

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Research Article

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Impact of Exogenous Sugars on the Potency of Selected Secondary Metabolites in Non-Starchy Amaranth (*Amaranthus hybridus* L.)

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Abstract

The biosynthesis of secondary metabolites plays a significant role in determining the value of medicinal herbs, with sugar metabolism frequently influencing overall metabolic processes. To investigate the regulatory mechanisms, exogenous sugars (sucrose, glucose, and fructose) were applied to the leaves of *Amaranthus hybridus* L., a highly valued and multifunctional vegetable plant, both individually and in combination (sucrose + glucose + fructose), along with a control (water). Our findings revealed that exogenous sugars enhanced the accumulation of starch and soluble sugars, while also increasing enzyme activities associated with carbohydrate assimilation. Additionally, plant biomass was significantly boosted by combined exogenous sugars, sucrose alone enhanced the photosynthetic rate, and the combined sugars accelerated the accumulation of phenols and flavonoids. Metabolomic analysis further confirmed that exogenous sugars increased the levels of phenolic and flavonoid compounds. The levels of UDP-glucose pyrophosphorylase (UGP) and hexokinase (HKX) were elevated by exogenous sugars and showed a strong correlation with their metabolic activities, which in turn stimulated the synthesis of specific secondary metabolites. These results provide valuable insights into the key factors contributing to the value formation of *A. hybridus* and suggest a potential approach to enhancing its quality.

Keywords: enzymatic activity, *Amaranthus hybridus* L., plant biomass, starch–sugar metabolism, flavonoids, phenols.

1. Introduction

The African continent is home to a wide variety of crop species that significantly contribute to both local and national wealth[1]. Fruits and vegetables are extensively cultivated and consumed in Nigeria, positively impacting the economic status of local populations. These crops thrive in agricultural settlements and are grown globally for food, medicine, and sale[2]. Amaranth is widely consumed in southwestern Nigeria compared to other edible leafy vegetables, due to its easy cultivation and consistent availability in markets. The vegetable Amaranth—particularly *Amaranthus hybridus* L.—is valued by locals for its medicinal properties. It is known to contain essential minerals, proteins, vitamins, and metabolites[3], making it a preferred choice among locals and farmers. *Amaranthus hybridus*, also known as smooth amaranth, green amaranth, or slim amaranth, belongs to the Amaranthaceae family. It is a plant with wide leaves and high nutritional content[4, 5]. *A. hybridus* is edible, possesses excellent nutritional value, and is easy to harvest. The grains of amaranth are an important dietary source, providing vital nutrients to local populations and consumers[5].

The seeds are a rich source of protein (13–19%)[6], higher than that of other grains such as maize (11–18%)[7], rice (10–18%)[8, 9], and wheat (13–16%)[10]. Additionally, amaranth contains secondary metabolites and bioactive compounds, such as antioxidants, that help protect the body from diseases[11]. The accumulation and synthesis of metabolites like flavonoids and phenols are particularly important due to their medicinal properties. However, biotic and abiotic environmental factors—such as solar radiation, soil mineral nutrient availability, microbial infections, developmental period, and growing season—affect the biosynthesis and accumulation of flavonoids and phenols in *A. hybridus*[12-14]. Secondary metabolites are typically synthesized when environmental stress factors surpass those affecting photosynthetic rates, leading to the accumulation of carbon reserves in the plant[15]. Photosynthesis is the primary process for carbohydrate production, and its accumulation is largely dependent on shoot growth. Sucrose and starch uptake is a complex process that requires the synergistic action of various enzymes[17]. Among sugars, sucrose is the primary carbon source and is transported from photosynthetically active shoots to non-photosynthetic tissues via the phloem[16]. Sucrose and starch play key roles as energy sources during stem development, potentially affecting plant structure, such as height and biomass[16]. Dong et al.[18] reported that sugar accumulation in citrus fruits is often associated with the biosynthesis of anthocyanins during fruit development. However, the relationship between carbon-based metabolites, sugar synthesis, and environmental stress factors across various plant species remains insufficiently explored. Sugars and their derivatives not only provide energy for plant cellular activities but also serve as substrates for organic compounds like lipids, proteins, and secondary metabolites, and can act as signaling molecules to regulate metabolic processes and gene expression[19-21]. Several studies have shown that sugars induce the accumulation of flavonoids and phenols. For instance, flavonoid build-up and enzyme activity increased in the root suspension cultures of *Morinda citrifolia*, correlating with increased sucrose levels[22]. Dai et al.[23] also found that sucrose, glucose, and fructose can induce anthocyanin uptake in grape plants, with sucrose levels leading to early anthocyanin accumulation. Moreover, monosaccharides and disaccharides not only contribute to carbon skeleton formation but also influence long-term artemisinin production in *Artemisia annua* plantlets[24]. These studies indicate that sugars can enhance the synthesis of plant secondary metabolites, thereby regulating plant growth and development. However, there is limited information on the regulatory role of sugar in the biosynthesis of flavonoids and phenols. To better understand the interplay between secondary metabolites and carbohydrate metabolism in *A. hybridus*, the regulation of sugar signaling on flavonoid and phenol biosynthesis was investigated through the application of individual and combined exogenous sugars. The findings aim to enhance the nutritional value of *A. hybridus* and support the breeding of vegetable plants.

2. Materials and Methods

2.1. Planting Design and Treatment

The seeds of *A. hybridus* were sourced from a variety documented as (NG/AO/11/08/075) from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria, and were planted in a screen house at the Federal University Oye Ekiti, Nigeria. The seeds were grown under controlled conditions with a 12-hour photoperiod at 25/15°C and 300 µmol m^2 s^{-1} light intensity. An initial experiment was carried out to evaluate the impact of individual exogenous sugars on *A. hybridus* and to determine the threshold at which each sugar can influence the growth of *A. hybridus*. The seeds were sown in 12 cm plastic pots and grown for 14 days, exposed to a control (C) and single exogenous sugars (sucrose, glucose, and fructose) for five days (see Fig. 1(a)).

Furthermore, seedlings were grown for 30 days and then exposed to three different treatment groups for 8 days: 1) control group sprayed with water (C), 2) exogenous sucrose (50 mmol L^{-1}) spray group (S), and 3) exogenous sucrose, glucose, and fructose (150 mmol L^{-1} each) spray group (S+G+F) [see Fig. 1(a)]. The experimental setup was organized in a completely randomized design with four replicates for each treatment. The sugar solutions were sprayed at 1-day intervals on the leaf surface of *A. hybridus*, with 10 sprays applied during the initial experiment. After the final spray, root and leaf samples were collected at 9:00 a.m. to investigate physiological and biochemical parameters, such as starch, soluble sugar contents, and secondary metabolites. The plant samples were stored in liquid nitrogen and preserved at -80°C for further analysis.

2.2. Shoot Height, Net Growth, and Fresh Weight

The shoot height, fresh weight, and net growth of all seedlings were measured before and after the spraying treatment (initial and final values). The average of these measurements was used to calculate the net growth[25]. The fresh weight of each treatment group represents the sum of the weights of its various plant organs.

2.3. Photosynthetic Parameters

After 30 days of growth and spraying treatment, the photosynthetic parameters were measured on a sunny day between 9:00 and 11:00 a.m. Three seedlings were selected for each replication, and three fully grown leaflets were chosen randomly from the fourth and fifth compound leaves beneath the apex of each selected seedling. Photosynthetic parameters, including stomatal conductance (Gs), transpiration rate (E), and net photosynthesis rate (Pn), were measured using the LI-6400XT photosynthetic system (LI-COR, Inc.).

2.4. Soluble Sugar and Starch Contents

The soluble sugar and starch contents were determined using the method described by Grechi et al.[26]. First, 50 mg of dry leaf samples were combined with 4 mL of 80% ethanol solution and heated in a water bath at 80°C for 30 minutes. The mixture was centrifuged at 1998 g for five minutes and the supernatant was collected. Activated carbon was added to the extract to decolorize it at 80°C for 30 minutes. After filtering, 5 mL of anthrone solution (100 mg anthrone in 100 mL of 76% sulfuric acid) was added to the sample for color development. The concentration of soluble sugar was determined spectrophotometrically at 625 nm.

To determine starch content, the residue from the previous extraction was treated with 3 mL of distilled water and boiled in a water bath for 15 minutes. The starch was extracted using 5 mL of water and 2 mL of 9.2 mol L^{-1} perchloric acid, followed by centrifugation. This extraction process was repeated twice. Finally, 5 mL of anthrone solution and 1 mL of the starch extract were combined, and the mixture was heated in a water bath for 10 minutes to develop color. The starch content was measured using an ultraviolet spectrophotometer at 620 nm.

2.5. Total Flavonoid and Phenol Content

To remove fat-soluble components, approximately 0.3 g of dried sample was placed in a 250 mL Soxhlet extractor and decontaminated with petroleum ether for 4 hours in a water bath at 80°C. Phenols and flavonoids were then extracted using ultrasonic-assisted extraction with 20 mL of 70% ethanol solution. The total flavonoid content was determined using a colorimetric method[27] with rutin (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) as an external standard. The total flavonoid content was expressed as milligrams of rutin equivalent per gram of dry weight (mg g^{-1}).

Polyphenols were extracted from 0.3 g of frozen leaf samples using 2 mL of 80% methanol. The mixture was centrifuged at 10,000 g for 10 minutes at 4°C. The total phenolic concentration was measured using Singleton's method[28] with minor modifications. To the 100 µL Folin-Ciocalteu reagent, 1.58 mL of deionized water and 20 µL of the sample were added. After shaking, 300 µL of a 2% sodium carbonate solution was added. The absorbance at 765 nm was measured after a two-hour incubation at room temperature in the dark. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE g^{-1} FW) using a calibration curve with gallic acid.

2.6. Metabolome Analysis of Individual Flavonoids and Phenols

For metabolome analysis, fresh leaves from exogenously sugar-treated and control *A. hybridus* plants were freeze-dried using a vacuum freeze-dryer (Scientz-100F, Scientz)[29]. A UPLC-ESI-MS/MS system was used for further analysis. Differential metabolites were identified by looking for metabolites with absolute log2 fold change ≥1 and VIP (variable importance in projection) ≥1, indicating significant variation.

2.7. Enzyme Activity

Hexokinase (HKX) and UDP-glucose pyrophosphorylase (UGP), key enzymes involved in carbohydrate metabolism, were measured using the appropriate enzyme activity assay kit supplied by Suzhou Mengxi Biological Medicine Technology Co. Ltd. The enzymes were extracted from 0.1 g of fresh material, homogenized at 4°C in an ice bath, and centrifuged at 8000 rpm for 10 minutes (UGP was measured at 10,000 rpm). The absorbance of HKX and UGP was measured at 340 nm. The activity of HKX and UGP was expressed as the amount of 1 nmol NADPH generated per 1.0 g of fresh material per minute at room temperature[30].

2.8. Data Analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS version 16.0 (SPSS Inc.). Turkey's post-hoc test was conducted to assess significant differences between treatments. A 95% confidence level was used for all statistical analyses.

3. Results

3.1. Impacts of Exogenous Sugars on Preliminary Growth Attributes of A. hybridus

The plants of *A. hybridus* were treated with single 50 mmol L^{-1} sucrose (S) solution, 50 mmol L^{-1} glucose (G), and 50 mmol L^{-1} fructose (F) mixture solution, and compared with their control group. Plant biomass growth in the exogenous sucrose (S) group was improved compared to the glucose (G) and fructose (F) spray groups, and the control group (Fig. 1a-b). The experiment revealed that sucrose (S) progressively increased, showing an 87% and 68% increase compared to the control (Fig. 1a). The glucose concentration decreased gradually, while fructose concentration showed a 42% increase (Fig. 1a, b). However, these changes in sugar concentrations were associated with modifications in the activities of sugar-metabolizing enzymes.

3.2. Effects of Exogenous Sugars on Growth and Net Photosynthetic Rate

The seedlings of *A. hybridus* were treated with water, 50 mmol L^{-1} sucrose (S) solution, and a 50 mmol L^{-1} mixture of sucrose, glucose, and fructose (S+G+F), and compared with control plants. Growth indices, such as plant biomass, were significantly enhanced in plants sprayed with the exogenous S+G+F mixture, while no significant difference was recorded between the sucrose (S) spray and the control plants (Fig. 2a). However, plant height and biomass were higher in the control plants, with a decrease in growth rate observed in the S+G+F group (Fig. 2b). Shoot weight did not show any significant differences among treatments (Fig. 2b). To determine the impact of sucrose (S) and the sucrose, glucose, and fructose mixture (S+G+F) on photosynthetic attributes in the leaves of *A. hybridus*, the results revealed that the photosynthetic rate of control plants was lower than that of seedlings treated with sucrose (S), but there was no significant difference compared to the S+G+F group (Fig. 2c). The application of exogenous sucrose (S) caused a pronounced and continuous decrease in the CO_2 assimilation (N) rate, reaching a reduction of 57% (Fig. 2d), compared to the S+G+F group with a decrease of 63%. The rate of transpiration (E) showed lower values, with a 41% decrease in S-treated plants and a 58% decrease in S+G+F-treated plants compared with the control (Fig. 2e). Therefore, these results suggest that the effects of exogenous sucrose (S) and S+G+F on *A. hybridus* photosynthesis are related to stomatal and photochemical efficiency.

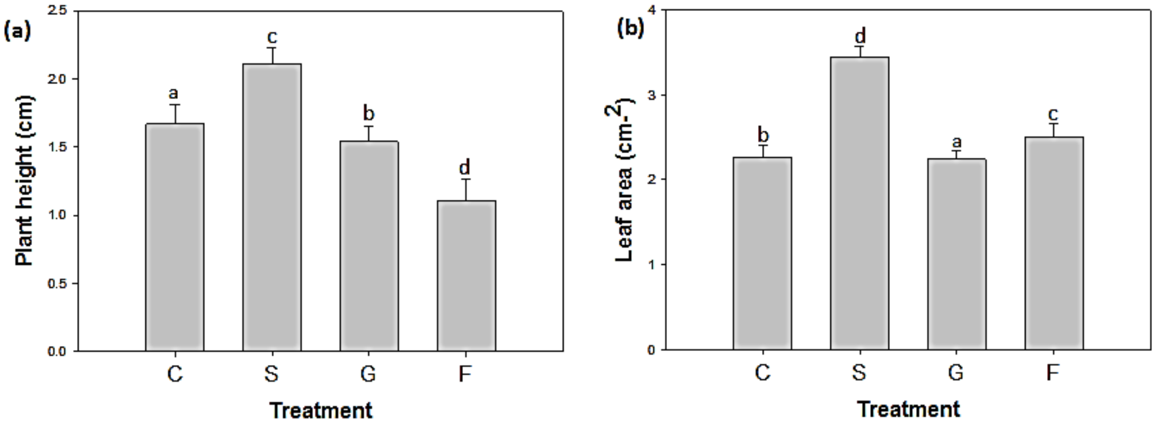

Figure 1. (*a*) plant height and (*b*). leaf area of *A. hybridus* plants exposed to single sucrose (S), glucose (G) and fructose (F) during a preliminary experiment. Non-similar small alphabet letters shows significant differences between the treatments ($P < 0.05$).

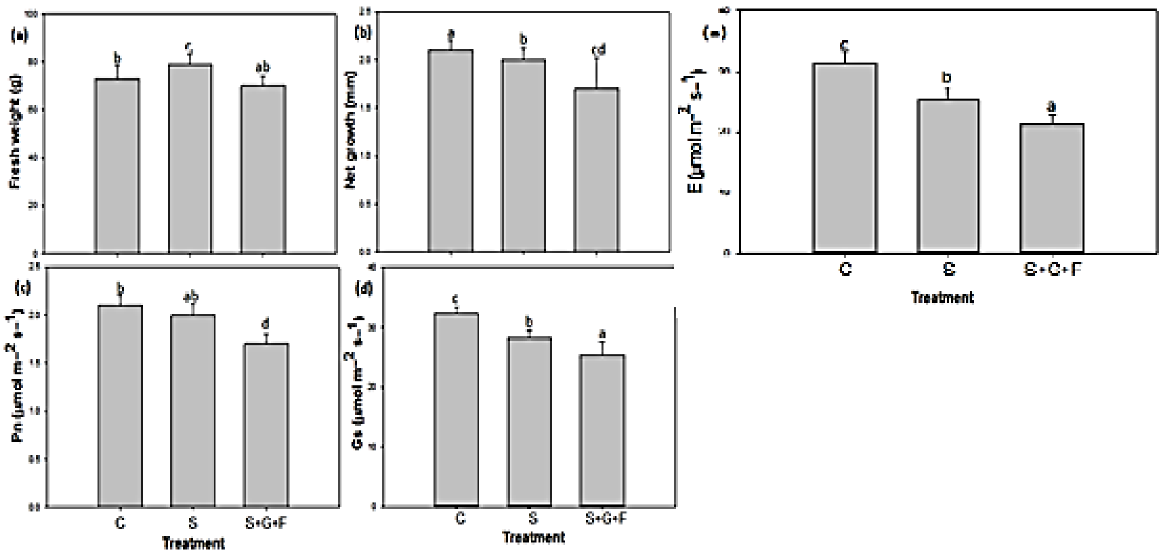

Figure 2. Morphological index and photosynthetic attributes of *A. hybridus* plants exposed to sucrose (S) and sucrose (S), glucose (G) and fructose (F) S+G+F (*a*) fresh weight, (*b*) net growth, (*c*) photosynthetic rate , (*d*) CO_2 assimilation rate and (*e*) transpiration rate. Non-similar small alphabet letters shows significant differences between the treatments ($P < 0.05$).

Table 1. Soluble sugar and starch in tissues of *A. hybridus* exposed to sucrose (S), glucose (G) and fructose (F) S+G+F

	Soluble sugar (mg g-1 DW)		Soluble starch (% w/w)	
Treatment	Leaf	Root	Leaf	Root
C	62.5+2.06^{b}	61.9+2.43^{a}	58.4+2.64^{d}	57.2+1.75^{b}
S	65.3+1.42^{c}	62.3+2.11^{c}	62.9+1.96^{b}	61.7+2.01^{c}
S+G+F	67.2+2.11^{a}	63.9+1.72^{a}	64.6+2.10^{b}	62.8+2.12^{d}

Non-similar small alphabet letters shows significant differences between the treatments ($P < 0.05$).

Table 2. Hexokinase (HKX) and UDP - glucosepyrophosphosphprylase (UGP) activity exposed to sucrose (S), glucose (G) and fructose (F) S+G+F

Treatment	HKK mmol.min-1.1	UGP mmol.min-1.1
C	9.16+0.89^{b}	19.4+1.36^{b}
S	27.2+2.14^{e}	21.6+3.10^{b}
S+G+F	16.8+1.27^{c}	49.5+2.41^{f}

Non-similar small alphabet letters shows significant differences between the treatments ($P < 0.05$).

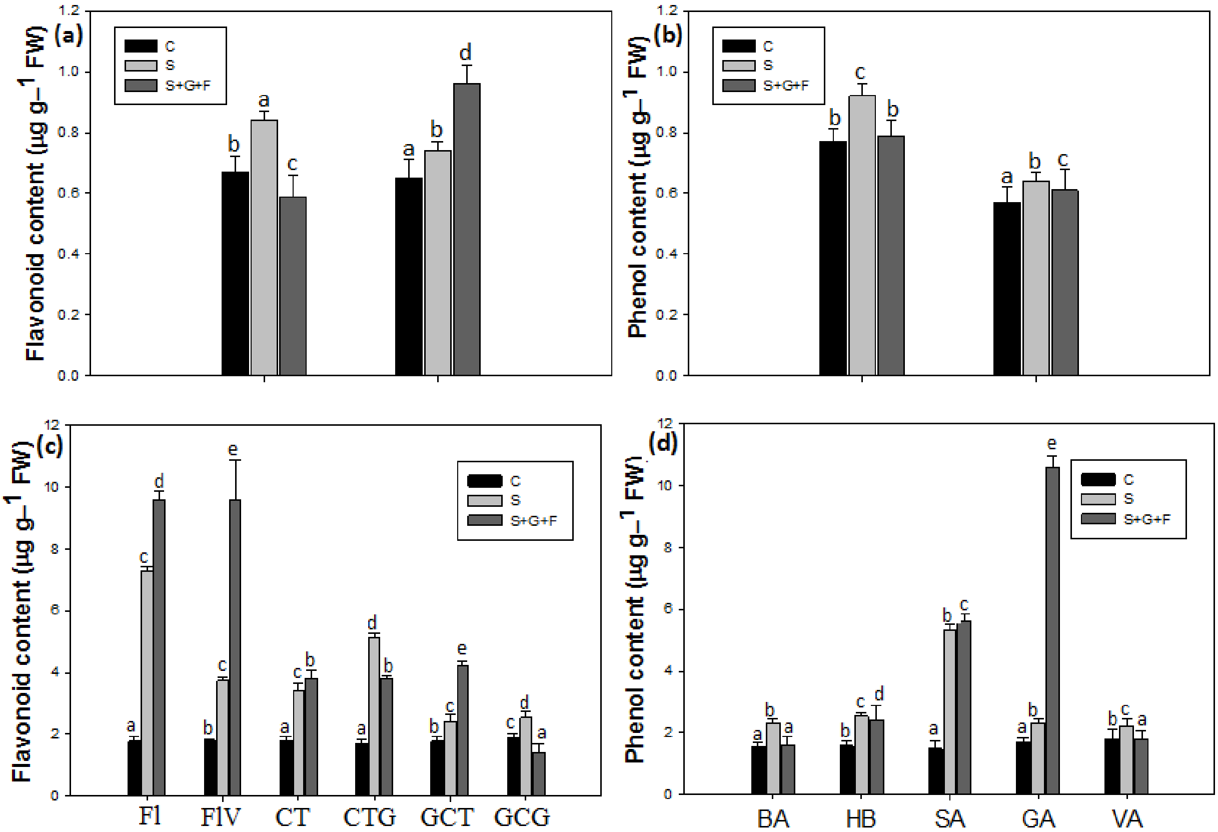

Figure 3. Secondary metabolite content in *A. hybridus* plants exposed to sucrose (S) and sucrose (S), glucose (G) and fructose (F) S+G+F. (a). Flavonoid content (b). Phenol content (c). flavonoid derivatives (flavanols FI, flavonols FIV, catechin CT, catechin-galate CTG, gallocatechin GCT, gallocatechin-galate GCG) and (d). Phenol derivatives (benzoic acid BA, hydroxybenzoic acid HA, salicylic acid SA, gallic acid GA, vanillic acid VA). Non-similar small alphabet letters shows significant differences between the treatments ($P < 0.05$).

3.3. Effects of Exogenous Sugars on Starch and Its Metabolism-Associated Key Enzyme Activity

The starch and soluble sugar content in the leaves and roots of *A. hybridus* exposed to varying exogenous treatments were analyzed (Table 1). Starch and soluble sugars were predominantly accumulated in the roots and leaves, particularly in the plants treated with exogenous sucrose (S) and the S+G+F mixture. Generally, exogenous sugar treatment enhanced the accumulation of soluble sugars. The sucrose (S) group stimulated the content of soluble sugar in the roots of *A. hybridus* by 33.4% compared to the control seedlings. No significant difference was observed in starch content after the application of the exogenous sugar treatments (Table 1). UDP-glucose pyrophosphorylase (UGP) and hexokinase (HKX) are key enzymes involved in glucose metabolism. UGP plays a significant role in the conversion process between various sugar components, while HKX is a crucial receptor for sugar signal transduction (Table 2). As shown in Table 2, the activities of UGP and HKX in the sucrose (S) and S+G+F exogenous sugar treatment groups were significantly higher than those in the control plants. The results indicated that the activities of UGP and HKX were influenced by the spraying of exogenous sugars, which was consistent with the significant increase in soluble sugar accumulation. There was a significant increase in the activities of UGP (47%) and HKX (59%) after exposure to sucrose (S) and S+G+F treatments (Table 2). Therefore, A. hybridus leaf tissues have been shown to accumulate sucrose, which helps induce metabolic changes involving both synthesizing and hydrolyzing enzyme activities.

3.4. Sugar Signaling Regulates the Synthesis of Flavonoid and Phenol Classes

In this research, the leaves were identified as the primary accumulation site for flavonoids and phenols in *A. hybridus*. In comparison to their control seedlings, exogenous sucrose (S) enhanced the total content of flavonoids in both roots and leaves (Fig. 3a). Exogenous S+G+F treatment clearly increased the total flavonoid content of the roots by 23.7%. The sucrose (S) treatment increased the total phenol content in the leaves by 33.4%, while the S+G+F treatment induced a significant increase in total phenol content (Fig. 3b). Overall, exogenous sucrose treatment, particularly sucrose (S), positively influenced the accumulation of total phenols and flavonoids. Furthermore, the effect of exogenous sugars on the synthesis of metabolites was analyzed using metabolomics to assess the differences in individual secondary metabolites contained in the leaves of A. hybridus under sucrose (S) and S+G+F treatments. Among the different expressed metabolites, flavonoids were identified, including flavanols, flavonols, flavonoids, catechin, catechin-gallate, gallocatechin, and gallocatechin-gallate (Fig. 3c, d). Additionally, five polyphenol compounds were identified: gallic acid, benzoic acid, p-hydroxy-benzoic acid, salicylic acid, and vanillic acid. The activities of these flavonoids and polyphenols, except for flavanols, were increased after exogenous sucrose (S) spray. The influence of the mixed sugars S+G+F treatment on the accumulation of flavonoids and polyphenols was similar to that of sucrose (S) treatment (Fig. 3c). Catechin-gallate and gallocatechin-gallate showed the most significant increases after treatment with exogenous sucrose (S) and mixed S+G+F treatments, with 5.9- and 8.3-fold increases compared to their controls. Additionally, exogenous sugars successfully increased the contents of phenols, except for p-hydroxy-benzoic acid, including benzoic acid, gallic acid, salicylic acid, and vanillic acid (Fig. 3d). Overall, our results indicate that exogenous sugars enhanced the associated biochemical pathways to stimulate the synthesis of phenols and flavonoids through sugar signaling.

4. Discussion

Sugars are known to provide energy for plant development and carbon for all metabolic processes, including secondary metabolism, and act as signaling molecules to control various physiological and metabolic processes[31]. Our study shows that exogenous sugars sprayed on the leaves of A. hybridus revealed that sucrose (S) and S+G+F treatments had positive impacts on growth, net photosynthesis rate, and accumulation of soluble sugars. The glucose (G) concentration slightly decreased, but sucrose (S) and fructose (F) accumulated after spraying on A. hybridus leaves. Patrick et al.[32] reported that sugar metabolism in leaves is rapid and regulated in different positions, suggesting that the sugar profile obtained in our study may be temporary. Rolland et al.[33] noted that sucrose hydrolysis and transport play a significant role in generating sugar signaling. Saksena et al.[34] described glucose as a major monosaccharide involved in metabolic signal transduction and playing a significant role in plant development and yield. Our study revealed that exogenous sucrose (S) and S+G+F treatments enhanced photosynthetic performance and chlorophyll buildup, leading to increased soluble sugar and plant biomass. However, starch content showed a different pattern, which may be due to the breakdown and modification of starch into sugars. Another theory suggests that sugar accumulation in sink tissues might influence photosynthesis through a source-sink process[35]. Previous studies[36, 37] indicated that mild exogenous sugars can enhance plant biomass and carbohydrate content, increasing antioxidant activities and improving the quality of *Citrullus lanatus*. Starch functions as a vital carbohydrate store in shoots, whereas sucrose serves as the functional soluble sugar form responsible for initiating sugar signaling and carbon source distribution[38]. Starch levels increased during the entire developmental phase, revealing that sucrose and starch maintain energy equilibrium through coordinated translation during plant development[16]. HKX plays a crucial role in influencing sugar metabolism by affecting signal sensitivity and activity, as well as regulating hexose accumulation[19]. The increase in HKX activity was beneficial for sugar conversion and breakdown, generating more carbon and ATP for anabolic processes. Exogenous sugars have also been shown to induce the activity of HKX and UGP, further stimulating the synthesis of volatile linalool and β-ocimene in *Lilium*[39]. Presented results comprehensively show that sucrose assimilation in A. hybridus leaves significantly increased HKX and UGP activities. Furthermore, our results suggest that sugar signaling enhances the biosynthesis of secondary metabolites, inducing the synthesis of major phenols and flavonoids.

Lloyd and Zakhleniuk[40] reported that confirming the involvement of sugar metabolism in higher plants is challenging, particularly in relation to whether the decrease in photosynthetic efficiency was specifically enhanced by sugars or sucrose. Most plant hormones participate in synthesizing bioactive compounds and may cross-talk with sugar signals. For instance, Lin et al.[17] suggested that sugar sensing relies on ABA signaling to control plant growth and activate defense responses. A study by Chen et al.[41] found that jasmonic acid induces the synthesis of quinones and terpenoids. The array of secondary metabolites, including flavonoids, catechin-gallate, gallocatechin, and gallocatechin-gallate, was increased in our study. These results align with previous studies, where ABA regulated flavonoid synthesis, stimulating anthocyanin accumulation in the skin of grape plants[42]. Furthermore, studies[43] indicated that gibberellin and jasmonic acid play significant roles in sucrose-induced anthocyanin synthesis in *Arabidopsis thaliana*. Future studies are required to determine whether the synthesis of secondary metabolites by sugar signaling depends on changes in these phytohormone signals. Sugar signaling plays a vital role in cell division processes, such as apical dominance[44], which is influenced by phytohormone modulation. The application of sugars in crop improvement should consider whether crop yield is source- or sink-limited, a factor that appears to be species-dependent. Future increases in crop yields are expected to result from improvements in transporter roles in source and sink processes.

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Manuscript writing, laboratory work, and data analysis were conducted by the corresponding author.

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Data Availability

The data used in this work are available upon written request from the corresponding author.

Conflicts of Interest

Not Applicable

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