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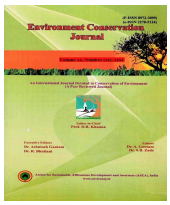

Natural extracts from *Marchantia polymorpha* against plant pathogens growth inhibition

Shivom Singh ✉
Department of Environmental Science, ITM University Gwalior (M.P.), India
Shivani Gore
Department of Biotechnology, ITM University Gwalior (M.P.), India
Sheetanshu Gupta
BSSCOP, Mohanlalganj, Lucknow, U.P, India
Gourav Kumar Singh
Department of Environmental Science, ITM University Gwalior (M.P.), India

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ABSTRACT

The health of plants, animals, and humans is seriously threatened by the production of toxins by bacteria and fungi. The aim of the current work is to find a dependable and eco-friendly microorganism biocontrol technique to alleviate this concern. In this work, the antibacterial abilities of *Marchantia polymorpha* (liverwort) extracts were examined. These extracts were collected from several altitudinal ranges in the Kumaon region in Uttarakhand province, India, in the western Himalayas. Using microbroth dilution methods, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the crude extracts were determined. The results demonstrated that the *Marchantia polymorpha* extracts exhibited potent antifungal activity against *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Colletotrichum acutatum*, as well as antibacterial activity against *Pseudomonas syringae*, *Xanthomonas campestris*, *Staphylococcus aureus*, and *Bacillus subtilis*. According to the study results, *Marchantia polymorpha* extracts may have applications as natural antimicrobials in a number of sectors, including medications, agriculture, and preserving food. The research we performed demonstrates the potential of liverwort extracts as a promising biocontrol agent against bacterial and fungal diseases and as an inducer of plant disease resistance, providing a safer and more environmentally friendly alternative to synthetic chemicals that is beneficial to both human health and the environment.

Introduction

It is commonly known that plant medicines can be used to treat a variety of ailments. In a similar manner, plant-derived pesticides were widely used before the discovery of synthetic pesticides. Tesfahun *et al.* (2000), revealed that farmers in the Welo region use a combination of physical, cultural, and chemical methods to control pests and diseases in their crops. Natural pesticides including *Phytolacca dodecandra*, *Euphorbia tirucalli*, *Croton macrostachys*, and *Aloe spp*. to keep crops free from pest and infections. Chemical pesticides are utilized in the majority of plant disease treatments. These temporary strategies may provide short-term protection, but they ultimately make farming operations susceptible to harmful chemicals and increase their potential for environmental contamination.Therefore it is suggested that bryophytes extracts be further researched as potential bioagents for plant development and disease resistance. Bryophytes, which include hornworts, liverworts, and mosses, are the second biggest macro-group of terrestrial plants and have considerable biotechnological applications in pharmaceuticals, agriculture, and

Corresponding author E-mail: *shivomsingh@itmuniversity.ac.in*
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healthcare (Nikolajeva *et al.,* 2012). Bryophytes, a group of non-vascular plants, encompass approximately 25,000 to 28,000 species (Chavhan, 2017). These plants have developed mechanisms to control their growth in the face of challenging environmental conditions, including both abiotic stressors such as temperature and ultraviolet radiation, as well as biotic stressors like irregular water supply, predation, and infectious attacks, which persist as an evolutionary force that tests their adaptability and defensive strategies (Commisso *et al.,* 2021). Sunsequently, it was reported that numerous bryophytes, including *Bazzania*, *Conocephalum*, *Diplophyllum*, *Dumortiera*, *Marchantia*, *Metzgeria*, *Lunularia*, *Pellia*, *Plagiochila*, *Porella*, *Radula*, and *Riccardia*, exhibited antimicrobial properties (Vollar *et al.,* 2018).

Marchantia polymorpha, a liverwort, has been established as a model plant for studying morphological and physiological responses to various environmental factors for over two centuries, and has recently emerged as a valuable model plant for investigating plant-microorganism interactions (Poveda, 2020). Additionally, it has been used in traditional medicine and pharmaceuticals in China, North America, and India to treat various ailments, such as diuretic activity, hepatitis, open wounds and burns, fractures, snake bites, convulsions, uropathy, pneumonia, and neurasthenia (Ludwiczuk and Asakawa, 2019; Tran *et al.,* 2020). Phytochemical research has revealed that *M. polymorpha* contains polyphenols, long-chain polyunsaturated fatty acids, and terpenoids, among which bis-bibenzyls have exhibited significant antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Cai *et al.,* 2022). However, therapeutic studies of bryophytes have been limited, with less than 10% of species studied to date (Rao, 2021).

Due to their microbicidal properties, liverworts, including *M. polymorpha*, can be used to control plant diseases, and the Himalayan region boasts the greatest diversity of bryophytes. Although *M. polymorpha* is one of the most extensively studied liverwort species, aspects of its bioactivity against plant microbes remain poorly understood. Therefore in this study, we investigated the antifungal and antibacterial efficacy of *M. polymorpha* extracts obtained using different solvents on plant pathogenic bacteria and fungi. Our primary objective was to identify and gather information on the traditional uses of *M. polymorpha* for managing plant infections. Our aim was to evaluate the potential of *M. polymorpha* extracts as a source of novel antimicrobial compounds against significant plant bacteria and fungi, offering a sustainable and environmentally friendly approach to disease control.

Material and Methods

Marchantia polymorpha, a type of liverwort, was collected from various substrates including soil, rocks, walls, and the trunks and leaves of vascular plants in the Kumaon region of the Western Himalaya (213-2100 m), Uttarakhand, India. The collection was conducted from two different altitudinal ranges, namely Artola (29º23.711'N 79º28.000'E, Alt. 6790 ft.) Uttarakhand, India. The selection of the sampling area was based on the fact that liverworts usually grow in humid locations where they form mats and cushions over soil and rocks (Ludwiczuk and Asakawa, 2019). The collected *M. polymorpha* samples were stored in sterilized polythene bags and transported to the Laboratory of Environmental Science, ITM University Gwalior, India.

Sample Preparation

For analysis, *M. polymorpha* were rinsed with distilled water to remove soil and plant residues. Further cleaning involved multiple rinses (2-3 times) with distilled water. Finally, the liverworts were dried on blotting paper in the shade at room temperature. This ensures that the liverworts are free from extraneous materials and provides accurate and reliable data for further investigation.

Extraction of *Marchantia polymorpha* for Antimicrobial Activity

The liverworts were first dried in room temperature and then subjected to an electric grinder to obtain a fine powder. The powder was then extracted using a hot Soxhlet extraction method with 80 percent solvent, including petroleum ether, chloroform, acetone, ethanol, methanol, and water. By using multiple solvents for the extraction, a diverse range of bioactive compounds from the *M. polymorpha* liverworts can be extracted and studied which will be conducted in further research. To optimize the extraction process, 10g of *M. polymorpha* powder was combined with 100mL of solvent. After the

extraction, the samples were filtered using muslin and obtained crude extracts were then concentrated using a rotary evaporator (Biogen) to produce extracts at various concentrations ranging from 1000, 500, 250 and 125 µg/ mL.

Preparation of Extract solution

To prepare the extract solution for testing, the mother extract was first prepared by dissolving the extract in a separate solvent at a concentration of 1000 µg/mL. This was achieved by mixing the extract and solvent in a 1:2 ratio, and thoroughly stirring until the extract was completely dissolved.

Once the mother extract was prepared, three additional doses were made by diluting the solution with the same solvent. These doses were 125 µg/ mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL respectively. These dilutions were tested for their antimicrobial activity against phytogenic fungal and bacterial strains.

Phytopathogenic Bacterial strain and Fungal strain

The bacterial Strain *Pseudomonas syringae* (MTCC No- 1604), *Xanthomonas campestris* (ITCC BU0001), *Staphylococcus aureus* (MTCC No-737), *Bacillus subtilis* (MTCC 441) and fungal strain *Macrophomina phaseolina* (ITCC 7209), *Fusarium oxysporum* (ITCC 4998), *Rhizoctonia solani* (MTCC 2356), *Colletotrichum acutatum* (ITCC 4214), were obtained from reputable sources such as Institute of Microbial Technology (IMTECH), Chandigarh India and The Indian Council of Agricultural Research (ICAR), New Delhi, India for the present study. These strains have a pathogenic relationship with many plants, making them ideal candidates for testing the antimicrobial activity of the extract. Each bacterial isolate was grown in tryptic soy agar (TSA) (Merck, Germany) for 24 h at 37℃, and stored in Luria-Bertani (LB) broth containing 25% glycerol at -70℃ (Changa and Fang, 2007; Gu *et al.,* 2011). The fungal strains were maintained on Potato dextrose agar (PDA) (Himedia M096) and Sabouraud Dextrose Agar (SDA) (Himedia M063) at 27 ± 2 °C.

Antibacterial and Antifungal Activity of *Marchantia polymorpha*

Bioassay for fungus:

In order to determine the antifungal activity of the organic extracts of *M. polymorpha*, 48-hour-old phytopathogenic fungal culture discs were placed on a agar plate. This plate was impregnated with varying concentrations of *M. ploymorpha* extract (ranging from 1000, 500, 250 and 125 µg/ mL) for treatment. In order to evaluate the efficacy of the liverwort extract in inhibiting the growth of fungal pathogens, the agar plates were incubated at a specific temperature of 27 ± 2 °C and monitored closely for 24, 48, and 72 hours, then compared the colony diameter of the poisoned plate (with plant extract or positive control) to the non-poisoned plates (solvent) to estimate the percentage of mycelial growth inhibition. Nystatin was used as positive control. Through this meticulous observation, we were able to determine the potency of the *M. polymorpha* extract in inhibiting the growth of fungal pathogens.

The inhibitory effect was worked out by using following formula:

$$\textbf{Percent inhibition} = \frac{C-T}{C} \textbf{ X 100}$$

"The equation compares the control and treatment data to determine the percent blockage of a specific process or activity."

"C" represents the colony diameter of the non-poisoned plate (control).

"T" represents the colony diameter of the poisoned plate (with plant extract or positive control).

Bioassay for Bacteria

The method used is the Disc diffusion method, which involves placing four discs, two of which are treated with plant extract (T) and two control discs (C), on solid agar plates. These plates were then incubated with 1 mL of bacterial culture. After 24 hours, the inhibition zone was measured in mm, to determine the effectiveness of the plant extracts on the bacterial strains tested. To ensure accurate results, two positive control antibiotics - Tetracycline and Streptomycin were included.

Determination of MIC (Minimum Inhibitory Concentration)

The quantities of the extracts' inhibitory and bactericidal/fungicidal properties were measured using a micro broth dilution assay. Diluents included freshly made potato dextrose broth for fungi and nutrition broth for bacteria. Freshly revived cultures of the test microorganisms were

multiplied by 100 in the broth (100 µl of microbes in 10 mL broth) to assure accuracy.

Using an optical density measurement at 620 nm using a UV-visible spectrophotometer, the CFU was calculated and was found to be 1X 10^6 CFU/mL for bacteria and 1X 10^9 CFU/mL for fungi (Genesys). In a two-fold dilution series, plant extract at progressively lower concentrations (1000 to 125 µg/mL) were introduced to test tubes containing live microbe cultures. All tubes containing bacterial and fungal species underwent a 24-hour and 72-hour incubation period at 37 °C and 28 °C, respectively. Using a UV visible spectrophotometer, the visible turbidity and optical density of cultures were assessed at 620 nm.

MIC was determined as the lowest concentration that significantly inhibited the growth of test organisms and MBC was defined as the lowest concentration that had no effect on microbial growth. These findings are significant as they provide critical information on the effectiveness of organic extracts in inhibiting bacterial and fungal growth.

Results and Discussion

In this study, the antibacterial and antifungal potential of five different organic extracts of *M. polymorpha* was evaluated against eight common microorganisms, including four bacteria (*Pseudomonas syringae*, *Xanthomonas campestris*, *Staphylococcus aureus*, and *Bacillus subtilis*) and four fungi (*Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Colletotrichum acutatum)*. The results showed that the methanol extract of *M. polymorpha* exhibited the highest antibacterial and antifungal activity among the tested extracts. The observed concentration-dependent (1000 to 125 µg/mL) growth inhibition further supports the antimicrobial potential of the plant extract. Additionally, the extract's MIC and MBC values (Table 1) are important metrics for assessing the strength of antimicrobial agents were measured in relation to the microorganisms. The MBC/MFC values were greater than the MIC values for most of the extracts, indicating that the methanol extract demonstrated the highest antibacterial activity against *X. campestris* with a zone of inhibition (ZI) of 14.4 ± 0.20 mm, MIC of 1.25 µg/mL, and MBC of 1.75 µg/mL at the maximum used concentration of 1000 µg/mL (Tables 1 & 3). The zone of inhibition for each bacterial strain was measured, and the results were compared to the positive control antibiotics, tetracycline and streptomycin. The findings indicated that the methanol extract had a significantly higher zone of inhibition against all four bacterial strains, with values of ZI= 12.40 ± 0.35 mm (*P. syringae*), ZI=14.4 ± 0.20 mm (*X. campestris*), ZI= 55.06 ± 0.5 (*S. aureus*) and ZI= 13.4 ± 0.21 mm (*B. subtilis*) (Table 2-5). However, the zone of inhibition for the methanol extract was not significantly higher than that of the positive control antibiotics. These results suggest that the methanol extract may have potential as a natural antibacterial agent against a broad range of bacterial strains. Similarly, the methanol extract demonstrated the highest antifungal activity against *M. phaseolina* with a ZI of 65.65 ± 0.11 mm, MIC of 2.50 µg mL^{-1}, and MBC of 3.00 µg mL^{-1} at the maximum used concentration of 1000 µg mL^{-1} (Table 1 & 6). Moreover, the zone of inhibition of the methanol extract against *M. phaseolina* (ZI= 65.65 ± 0.11 mm), *F. oxysporum* (ZI=47.45 ± 0.46) and *R. solani* (ZI= 55.06 ± 0.5) and the zone of inhibition of the chloroform extract against C. acutatum (ZI= 34.12 ± 0.77) were significantly higher than that of the positive control, nystatin (39.36 ± 0.14, 26.41 ± 0.37, 19.52 ± 0.24 and 28.46 ± 0.45 respectively) (Tables 6-9). The results indicate that *M. polymorpha's* methanol and chloroform extracts possess greater antifungal potency than the commonly used antifungal agent, nystatin. However, acetone extract had no effect on fungal populations. Since fungal infections pose a significant threat to plants, causing stress and serious diseases, these extracts may prevent infection and minimize the risk of fungal contamination from soil, seeds, crop debris, weeds, and nearby crops. Therefore, the study's findings suggest a strong link between the plant's traditional use in plant disease management and its antibacterial effects in vitro.

While all of the extracts showed some degree of activity against each of the tested fungi and bacteria, the methanolic extract was determined to be the most effective. Both gram-positive and gram-negative bacteria were killed by petroleum ether extracts of Barbula and Timmiella species, as

Table 1:Minimum Inhibitory Concentrations (MIC), Minimum Fungicidal Concentrations (MFC), and Minimum Bactericidal Concentrations (MBC) of different extract of *Marchantia polymorpha* against different plant pathogens (µg/ml)

Pathogen	Petroleum ether		Methanol		Chloroform		Ethanol		Acetone		STANDARDS*
Bacteria	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC / MFC**	**MIC**	**MBC/ MFC**	**MIC (MBC/MFC)**
P. s.	1.50	2.25	2.00	5.00	1.50	1.75	1.75	2.50	-	-	0.50 (0.80)
X. c.	1.75	2.50	5.00	6.00	0.75	2.50	2.50	3.00	-	-	1.00 (1.25)
S. a.	2.00	4.50	1.25	1.75	0.75	2.00	0.25	0.75	0.50	1.50	0.50 (0.60)
B. s.	0.75	1.50	1.00	2.00	1.25	2.50	1.25	1.75	1.50	2.25	0.50 (0.60)
Fungi											
M. p.	-	-	2.50	4.50	-	-	1.50	2.50	-	-	0.50 (0.70)
F. o.	3.0	4.0	2.50	2.75	0.25	0.75	1.75	2.50	-	-	0.25 (0.25)
R. s.	1.75	2.50	0.50	1.50	1.25	1.75	2.00	4.50	-	-	0.65 (0.80)
C. a.	0.75	1.50	0.50	1.25	1.50	2.25	0.75	1.50	-	-	0.65 (0.75)

P.s.=*Pseudomonas syringae,* X.c.= *Xanthomonas campestris*, S.a.=*Staphylococcus aureus,* B.s.=*Bacillus subtilis,* M.p.=*Macrophomina phaseolina,* F.o.= *Fusarium oxysporum*, R.s.= *Rhizoctonia solani,* C.a.=. *Colletotrichum acutatum*

***Nystatin, Tetracycline and Streptomycin are used as standards for fungi and bacteria respectively.**

Table 2:Antibacterial activity (expressed as zone of inhibition in mm) of *Pseudomonas syringae* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg ml^{-1})*Values are represented as mean ± SD.			
	1000	**500**	**250**	**125**
Petroleum ether	6.00 ± 0.15	2.50 ± 0.15	1.25 ± 0.10	0.0
Methanol	12.40 ± 0.35	10.70 ± 0.15	9.40 ± 0.21	7.70 ± 0.12
Chloroform	8.20 ± 0.10	6.20 ± 0.15	5.40 ± 0.20	4.40 ± 0.26
Ethanol	11.40 ± 0.17	10.50 ± 0.10	9.50 ± 0.10	8.30 ± 0.26
Acetone	0.0	0.0	0.0	0.0
Tetracycline	22.90 ± 0.36	22.50 ± 0.15	22.10 ± 0.52	21.10 ± 0.47

Table 3:Antibacterial activity (expressed as zone of inhibition in mm) of *Xanthomonas campestris* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg ml^{-1})*Values are represented as mean ± SD.			
	1000	**500**	**250**	**125**
Petroleum ether	6.20 ± 0.10	5.20 ± 0.15	3.40 ± 0.20	1.40 ± 0.26
Methanol	14.4 ± 0.20	12.4 ± 0.21	10.4 ± 0.20	8.4 ± 0.12
Chloroform	8.4 ± 0.21	6.6 ± 0.26	5.5 ± 0.15	4.6 ± 0.21
Ethanol	11.5 ± 0.31	10.5 ± 0.12	8.6 ± 0.10	6.7 ± 0.10
Acetone	0.0	0.0	0.0	0.0
Tetracycline	20.1 ± 0.40	20.2 ± 0.46	20.2 ± 0.32	20.0 ± 0.21

Table 4:Antibacterial activity (expressed as zone of inhibition in mm) of *Staphylococcus aureus* with different extract of *Marchantia polymorpha.*

Nature of extract	Concentration (µg ml^{-1})*Values are represented as mean ± SD			
	1000	**500**	**250**	**125**
Petroleum ether	8.25 ± 0.21	6.25 ± 0.26	5.50 ± 0.15	3.15 ± 0.21
Methanol	13.4 ± 0.21	11.5 ± 0.26	9.5 ± 0.15	7.6 ± 0.21
Chloroform	10.6 ± 0.15	8.6 ± 0.15	6.4 ± 0.10	4.0 ± 0.12
Ethanol	11.5 ± 0.26	10.4 ± 0.10	8.4 ± 0.15	6.6 ± 0.30
Acetone	0.0	0.0	0.0	0.0
Streptomycin	25.6 ± 0.75	24.6 ± 0.17	25.6 ± 0.25	26.0 ± 0.20

discovered by Gupta and Singh (1971). The chemical composition of different plant species varies, and this can be affected by factors such as where the plants were grown and when they were harvested (Burt 2004). The findings of this research are consistent with prior studies that have revealed antibacterial efficacy of bryophyte extracts against pathogenic bacteria and fungi.

Mewari and Kumar (2011) examined the antifungal activity with methanolic extracts of *Marchantia polymorpha*, *Dryopteris filix-mas*, and *Ephedra foliate*. *Marchantia polymorpha* methanolic extract had the most powerful antifungal activity against all fungal infections of the three plant species that were tested. In another study, Mewari *et al.* (2008) evaluated the crude methanol and flavanoid extracts of *M. polymorpha* and discovered that the methanolic extract was the most efficient, demonstrating the best antibacterial activity against three bacterial strains (*E. coli*, *P. mirabilis*, and *S. aureus)*, and four fungal strains (*A. flavus, A. niger, C. albicans, and T. mentagrophytes*). According to the findings of Tadesse *et al.* (2003), the ethyl acetate extract of *Marchantia polymorpha* had the highest antifungal activity against *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Rhizoctonia solani* and inhibiting the growth of the fungi at low concentrations. Several bryophyte extracts were found to have strong antifungal activity against the plant pathogenic fungi examined by Tadesse *et al.* (2003), with some extracts exhibiting equivalent or greater activity than the commercial fungicide carbendazim. The authors also discovered terpenoids and phenolic chemicals, both of which have antibacterial capabilities, in the most active extracts.

A review of the literature on several research demonstrating *Marchantia polymorpha's* antifungal activity and its application in plant protection is provided by Dey & De (2011). According to the authors, *Marchantia polymorpha* extract can be utilized to create antifungal pharmaceuticals that can treat human fungal infections. Additionally, the use of *Marchantia polymorpha* extract as a biocontrol agent in agriculture has the potential to lower the usage of chemical fungicides, which have negative effects on both the environment as well as human health. *M. polymorpha* was shown to be active against *S. aureus*, *S. pyogens*, and the majority of the Gram-negative bacteria when Kamory *et al.* (1995) isolated Marchantin from *M. polymorpha* and investigated its antimicrobial activity against five Gram-positive and five Gram-negative bacteria. In another study Bodade *et al.* (2008) conducted invitro screening for antibacterial activity using extracts from six bryophytes species. They discovered that *Polytrichum commune* and *Sphagnum* spp. had the strongest effects on fungus and both Gram-positive and Gram-negative bacteria. Additionally, the authors noted differences in the antibacterial efficacy of the various extracts from each species, suggesting the potential existence of several bioactive substances. In contrast to conventional anti-snail therapies, bryophyte extract is just as effective, according to the research of Frahm (2004). Extracts from bryophytes are non-lethal alternatives to poisons for controlling snails and slugs. Similarly, Chen *et al.* (2021) studied the induced defense response of two bryophyte species, *Hypnum plumaeforme* and *Thuidium tamariscinum*, to snail herbivory. The results demonstrated that both species produced secondary metabolites that reduced herbivory and favorably benefited plant growth and reproduction.

Currently, it seems that there is a lack of scientific evidence supporting the use of bryophyte extracts in crop protection for their ability to prevent disease in vivo. However, several studies have revealed that higher plant extracts have direct protective effects. In contrast to untreated plants, Abo-Zaid, Matar, and Abdelkhalek *et al*. (2020) showed that *Streptomyces cellulosae* isolated Actino 48 significantly decreased disease symptoms and TMV accumulation levels in tomato tissues. Additionally, compared to untreated plants, tomato plants exhibited greater growth. The study by Otero-Blanca *et al.* (2021) advances knowledge of the complex interactions between fungi and bryophytes and sheds light on the morphological and molecular alterations that take place in *P. patens* after *C. gloeosporioides* infection. In order to manage fungal diseases in bryophytes and other plants, approaches to management can be developed using findings from the research. Gimenez-Ibanez *et al.* (2019) conducted studies to find out more about the immunological mechanisms underlying interactions between M. polymorpha and the plant pathogenic bacterium Pseudomonas syringae. According to the authors, when P. syringae was present on the liverwort, it triggered an immunological response,

which included effector activity within the liverwort cells. The form and degree of this response varied among various strains of *Pseudomonas syringae*, demonstrating a unique interaction between the bacterium and the plant.

Results of the study indicate that bryophytes might be a useful source of antibacterial chemicals. However, more investigation is required to pinpoint the precise chemical components responsible for the observed bioactivity and to examine the possible applications of bryophytes in areas including agriculture, medicine, and environmental remediation. The study emphasizes the necessity of researching bryophyte's bioprospecting potential as a means of uncovering novel sources of antibacterial compounds.

Table 5:Antibacterial activity (expressed as zone of inhibition in mm) of *Bacillus subtilis* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg/ml)*Values are represented as mean ± SD.			
	1000	500	250	125
Petroleum ether	6.4 ± 0.21	4.5 ± 0.25	3.4 ± 0.15	1.3 ± 0.15
Methanol	13.4 ± 0.10	11.5 ± 0.25	10.5 ± 0.10	9.1 ± 0.10
Chloroform	10.4 ± 0.20	8.4 ± 0.20	6.3 ± 0.42	5.5 ± 0.31
Ethanol	11.3 ± 0.21	10.1 ± 0.15	8.2 ± 0.21	6.2 ± 0.15
Acetone	9.4 ± 0.21	7.5 ± 0.25	6.4 ± 0.15	4.3 ± 0.15
Tetracycline	19.9 ± 0.36	19.6 ± 0.38	19.9 ± 0.21	19.4 ± 0.50

Table 6: Percent inhibition in the growth of *Macrophominaphaseolina*with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg ml-1) *Values are represented as mean ± SD.											
	Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	65.65 ± .11	59.72 ± .38	47.65 ± 0.6	28.64 ± .16	27.16 ± .71	25.10 ± .36	21.60 ± .35	20.50 ± .67	18.07 ±0.34	8.81 ± .53	8.85 ±0.27	8.45 ±0.48
Chloroform	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol	45.33 ±0.54	38.36 ±0.6	26.08 ±0.55	39.99 ±0.83	31.82 ±0.4	20.81 ±0.42	21.27 ±0.72	16.95 ±0.54	12.29 ±0.42	8.67 ±0.74	8.89 ±0.64	8.02 ±0.30
Acetone	0.0	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	46.52 ±0.30	39.36 ±0.14	38.40 ±0.48	31.48 ±0.48	28.56 ±0.35	25.49 ±0.42	15.48 ±0.40	14.41 ±0.41	13.61 ±0.32	14.48 ±0.40	12.48 ±0.46	11.68 ±0.37

Table 7:Percent inhibition in the growth of *Fusarium oxysporum* with different extract *of Marchantia polymorpha*

Nature of extract	Concentration (µg/ml) *Values are represented as mean ± SD.											
	Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	45.65 ±0.36	43.60 ±0.34	40.65 ±0.37	34.50 ±0.48	33.50 ±0.47	30.60 ±0.41	29.42 ±0.50	28.43 ±0.38	24.46 ±0.48	27.20 ±0.06	25.59 ±0.50	24.45 ±0.48
Methanol	47.45 ±0.46	46.55 ±0.48	45.61 ±0.37	46.58 ±0.50	45.30 ±0.49	43.52 ±0.44	43.57 ±0.49	42.53 ±0.49	40.74 ±0.33	41.37 ±0.51	39.54 ±0.46	38.55 ±0.36
Chloroform	33.44 ±0.49	32.35 ±0.33	30.55 ±0.49	30.42 ±0.50	28.44 ±0.49	25.43 ±0.50	25.65 ±0.26	23.34 ±0.44	20.48 ±0.34	22.44 ±0.41	19.45 ±0.48	15.44 ±0.26
Ethanol	38.45 ±0.43	36.38 ±0.40	32.53 ±0.45	33.49 ±0.43	32.60 ±0.31	30.46 ±0.42	28.42 ±0.38	26.40 ±0.41	25.65 ±0.39	18.36 ±0.51	16.59 ±0.36	14.48 ±0.40
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	26.41 ±0.37	25.66 ±0.37	21.55 ±0.46	24.62 ±0.42	23.38 ±0.42	21.59 ±0.15	22.55 ±0.41	20.69 ±0.19	18.38 ±0.41	19.61 ±0.32	16.38 ±0.42	13.34 ±0.35

Table 8: Percent inhibition in the growth of *Rhizoctonia solani* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg/ml) *Values are represented as mean ± SD. Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	27.31 ±0.35	25.90 ±0.67	24.0 ±0.17	26.83 ±0.27	25.14 ±0.26	22.0 ±0.57	23.21 ±0.33	20.07 ±0.39	18.0 ±0.12	19.22 ±0.25	13.56 ±0.46	12.0 ±0.07
Methanol	55.06 ±0.50	52.89 ±0.85	50.03 ±0.62	45.69 ±0.57	44.70 ±0.46	43.91 ±0.51	40.0 ±0.45	38.0 ±0.35	35.0 ±0.25	30.0 ±0.26	28.0 ±0.15	26.0 ±0.60
Chloroform	14.55 ±0.50	12.97 ±0.48	10.0 ±0.48	12.50 ±0.43	13.23 ±0.50	10.0 ±0.48	11.43 ±0.32	10.0 ±0.48	10.0 ±0.48	8.74 ±0.58	6.0 ±0.48	0.0
Ethanol	49.85 ±0.48	45.01 ±0.84	44.94 ±0.27	40.20 ±0.50	38.12 ±0.62	32.44 ±0.50	29.13 ±0.45	24.52 ±0.24	23.01 ±0.65	20.35 ±0.46	16.21 ±0.03	15.42 ±0.54
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	19.52 ±0.24	14.58 ±0.49	12.57 ±0.48	16.37 ±.42	17.44 ±0.41	14.50 ±0.25	15.48 ±0.31	13.61 ±0.32	11.63 ±0.33	14.36 ±0.43	13.64 ±0.35	12.33 ±0.45

Table 9: Percent inhibition in the growth of *Colletotrichum acutatum* with different extract of *Marchantia polymorpha*

Nature of extract	Concentration (µg/ml) *Values are represented as mean ± SD. Time (hrs.)											
	1000			500			250			125		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum Ether	26.23 ±0.20	24.05 ±0.39	22.52 ±0.41	23.16 ±0.16	20.49 ±0.17	17.68 ±0.54	20.36 ±0.22	16.94 ±0.54	16.30 ±0.39	15.25 ±0.05	12.01 ±0.63	11.34 ±0.36
Methanol	25.13 ±0.20	23.24 ±0.39	22.45 ±0.43	23.29 ±0.42	22.66 ±0.54	20.27 ±0.12	20.49 ±0.34	18.45 ±0.38	15.42 ±0.38	18.58 ±0.28	15.64 ±0.36	12.53 ±0.44
Chloroform	34.12 ±0.77	30.49 ±0.42	28.73 ±0.32	26.76 ±0.38	25.66 ±0.30	23.76 ±0.15	25.31 ±0.35	23.53 ±0.19	20.33 ±0.16	13.29 ±0.32	10.52 ±0.37	9.30 ±0.38
Ethanol	23.80 ±0.48	25.56 ±0.37	22.51 ±0.49	26.93 ±0.31	23.41 ±0.39	21.70 ±0.58	24.41 ±0.38	19.94 ±0.25	18.52 ±0.47	15.24 ±0.12	12.87 ±0.27	11.77 ±0.54
Acetone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nystatin	28.46 ±0.45	25.59 ±0.51	23.47 ±0.38	22.41 ±0.37	20.46 ±0.28	24.34 ±0.44	21.49 ±0.46	17.40 ±0.41	19.46 ±0.40	20.49 ±0.42	16.37 ±0.42	14.57 ±0.30

Conclusion

In conclusion, our research lends credence to the use of bryophytes for conventional plant disease management and to their in vitro antibacterial activities. The observed differences in antibacterial and antifungal potential between different bryophyte extracts can be attributed to variations in their chemical content. Our results are consistent with earlier investigations that have demonstrated the efficacy of bryophyte extracts against pathogenic bacteria and fungi. These findings suggest that bryophytes may be a promising new source of natural antibacterial agents, with potential applications in both the pharmaceutical and agricultural industries. Further research is needed to identify the specific chemical constituentsresponsible for the observed antibacterial activity and explore their potential use in antimicrobial applications or treatment formulations derived from natural sources. Overall, our study contributes to the development of innovative and effective strategies for combating bacterial infections and highlights the importance of harnessing the therapeutic potential of natural products.

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Conflict of interest

The authors declare that they have no conflict of interest.

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