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Original Article

Deciphering the *in vitro* Antimicrobial Potential of Some Macrolichens from Burachapori Wildlife Sanctuary, Assam, India

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ABSTRACT

Background and Aim: Lichens are reported to possess antimicrobial activities. Therefore, this study was carried out to screen the macro lichens collected from Burachapori wildlife sanctuary such as *Ramalina hossei* Vain., *Ramalina nervulosa* (Müll.Arg.) Abbayes, *Parmotrema praesorediosum* (Nyl.) Hale and *Parmotrema tinctorum* (Despr. ex Nyl.) Hale against 3 g positive 3 g negative pathogenic bacterial and three fungal strains. **Materials and Methods:** Acetone, Chloroform and methanol extracts of the sample were prepared with 2 concentrations (1 mg, 2 mg) in triplicates and Agar well Diffusion method were employed for both the antibacterial and antifungal tests. Ampicillin was used as positive control in antibacterial screening in the concentration of 0.1 mg/mL. Fluconazole used in amount 100 µL as positive control in screening the antifungal activity of the lichen samples. **Results:** All the activities showed dose dependent results. Zones are found to be higher at 2 mg/mL when compared to 1 mg/mL. For the antibacterial activity, the range of zone of inhibition was 16.7±0.17 to 28.2±0.15 (in m.m.) and for antifungal activity it was in the range of 17.7±0.55 to 26.4±0.46 (in m.m.). Also it is observed that acetone extracts of all the samples have shown excellent inhibition, whereas methanol extracts have showed moderate effect followed by chloroform extracts. **Conclusion:** These results confirm the presence of antimicrobial compounds in the samples and further characterization of these potent antimicrobial compounds can be used as lead molecules against disease causing pathogenic microorganisms.

Keywords: Antimicrobial Activities, Lichens, Agar Well Diffusion Method, Inhibition Zone.

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INTRODUCTION

Lichens are complex organisms consisting of two microorganisms, an algae and a fungus, as well as several kinds of bacteria, fungi, and non-obligate viruses that form a microecosystem. Lichenicolous fungi are those that grow on the lichen's exterior, and endolichenic fungi are those that reside inside the lichen (Pant *et al* 2025). These are fascinating biological systems that result from a symbiotic relationship, usually between an algal or cyanobacterial photobiont and a fungal mycobiont. Their distinct biology enables them to synthesize a wide range of secondary metabolites that are unique in other species and to colonize harsh environments, such as exposed rock faces in deserts and polar tundra (Gopal 2024). There are over 3,200 distinct species of lichens in India alone, out of the approximately 20,000 species that are known to exist worldwide (Huneck, 1999). These microorganisms produce a variety of potent secondary metabolites including antibiotics. The different bioactive substances that may be isolated from lichens show promise for biopharmaceutical uses for developing novel formulations or methods that include cytotoxic, antioxidant, and antimicrobial agents (Pradhan 2023). The antimicrobial active components were found to be primarily concentrated in the organic solvent extract phases, particularly in the solvent extract phases such as methanol, ethanol, ethyl acetate, and acetone, while investigating the antibacterial potential of lichen extract (Tian *et al*, 2025). Several workers had investigated the antimicrobial effects of lichen species against significant pathogenic microorganisms in recent days showing the antibiotic potential of the lichen species (Prashith *et al*, 2015,Yadav *et al*, 2021, Sahoo *et al*, 2021, Essadki *et al*, 2024, Piñeiro, 2025). Matić *et al.* discovered noteworthy results when they examined the antibacterial and antioxidant activities of the methanol extracts of the lichens *Cladonia rangiferina*

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and *Lobaria pulmonaria* against *Staphylococcus aureus in vitro*. Natural endolichenic fungal products are a substantial source of new metabolites with potential uses in agriculture and medicine (Mansour *et al*, 2025).In traditional medicine, several lichens have been used to cure a variety of illnesses, such as diabetes, stomach problems, coughs, pulmonary TB, wound healing, and skin ailments (Srivastava, 2013). In today's era, the fundamental problem of persistent antibiotic resistance in microorganisms is posing a threat to public health around the world. This constant concern has made the development of new antimicrobial agents and drugs to fight infectious diseases necessary and the lichens being the interesting organisms possessing bioactive compounds are being thoroughly screened for antimicrobial agents and properties.

MATERIALS AND METHODS

Lichen collection and identification

The lichen samples used in the study were collected from Burachapori Wildlife Sanctuary of Sonitpur district, Assam (India) during the field work from October 2021 to May 2023.They were identified at Lichenology Laboratory of CSIR-NBRI,Lucknow. The voucher specimens of the identified and used lichen samples in this study- *Ramalina hossei* no. 65470(LWG), Vain. *Ramalina nervulosa* (Müll.Arg.) Abbayes no. 65471(LWG), *Parmotrema praesorediosum (*Nyl.) Hale no. 65456(LWG) and *Parmotrema tinctorum* (Despr. ex Nyl.) Hale no. 65455(LWG) (as seen in the Figure 1) were deposited in the herbarium LWG of CSIR-National Botanical Research Institute, Lucknow, India.

Preparation of lichen extracts

The dried samples were finely ground using an electric grinder and pulverized material (10 g) was extracted twice by soaking with 100 mL of acetone for 48 hr at room temperature. Further the separated extracts were filtered through Whatman No. 1 filter paper and the acetone filtrate was condensed to dryness using rotary evaporator at 40˚C. The same method was followed separately with methanol and chloroform. The extracts prepared in this way were used for antimicrobial studies (Tian 2025).

Test microorganisms

The bioprospection was performed by taking three gram positive three gram negative pathogenic bacterial and three fungal strains were procured as the test organisms from recognized collection centre in India. They are the selected gram-positive bacterial strains such as *Enterococcus faecalis* MTCC-439, *Bacillus cereus* MTCC-5521, *Clostridium botulinum MTCC-1349 and gram-negative* strains *Salmonella enteritidis MTCC-734, Pseudomonas aeruginosa* MTCC-1688, *Acinetobacter baumannii* MTCC-1425.Whereas *Aspergillus clavatus* MTCC-1344, *Aspergillus fumigatus* and *Candida glabrata* MTCC-6507 are the fungal specimens.

Determination of Antimicrobial Activity

Preparation of Acetone, Methanol and Chloroform extracts done separately and performed the Antimicrobial studies using agar well diffusion methods.

Agar Well Diffusion method for antibacterial studies

The agar well diffusion method was employed to determine the antibacterial activity of the lichen samples against the selected gram-positive bacterial strains such as *Enterococcus faecalis* MTCC-439, *Bacillus cereus* MTCC-5521, *Clostridium botulinum* MTCC-1349 *and* gram-negative strains *Salmonella enteritidis* MTCC-734, *Pseudomonas aeruginosa* MTCC-1688, *Acinetobacter baumannii* MTCC-1425. A subculture of each bacterial strain at a volume of 200 µL, equivalent to 10^6 CFU/mL, was uniformly spread onto the surface of a Petri dish containing 20mL of nutrient agar, using a sterile cotton swab and wells were punched using a sterile gel borer. On the Nutrient agar, five wells with a diameter of 8 mm each were created for the bacterial strains. The first well was designated as the negative control and was loaded with 100 µL of DMSO, using which all the extracts were dissolved in the concentrations of 1mg/mL and 2mg/mL. While second well served as the positive control and contained 100 µL of Ampicillin (an antibiotic). Rest of the wells in all the plates contained 100 µL of test drug concentration 1mg/mL. Another same set of petri dishes were prepared for drug concentration 2mg/mL. The Petri dishes were prepared in triplicates and incubated at 22°C for 3-4 days, following which the measurement of the zone of inhibition surrounding the wells was performed after the incubation period. The concentration of positive control used was Ampicillin (0.1 mg/mL). The antibacterial activity of each compound was analysed by calculating the diameter of the inhibition zone and the potency of each compound was correlated with Ampicillin.

Agar Well Diffusion method for antifungal studies

The agar well diffusion method was employed to determine the antifungal activity of lichen samples against the selected fungal strains *Aspergillus clavatus* MTCC-1344, *Aspergillus fumigatus* MTCC-343 and *Candida glabrata* MTCC-6507. A standardized suspension of the test fungi were inoculated and incubated for 16-18 hr. at 37°C to obtain fresh cultures. This was uniformly spread onto the surface of a Petri dish containing 20mL of potato dextrose agar, using a sterile cotton swab and wells were punched using a sterile gel borer. On the agar, five wells with a diameter of 8 mm each were created for the fungal strains. The extracts were dissolved in solvent DMSO with different concentrations such as 1mg and 2 mg/mL. The second well was designated as the negative control and was loaded with 100 µL of DMSO, using which all the extracts were dissolved in the concentrations of 1mg/mL and 2mg/mL. While second well served as the positive control and contained 100 µL of Fluconazole (an antifungal tablet). Rest of the wells in all the plates contained 100 µL of test drug concentration 1mg/mL. Another same set of petri

dishes were prepared for drug concentration 2mg/mL. The Petri dishes were prepared in triplicates and incubated at 22°C for 3-4 days. The antifungal activity of each compound was analysed by calculating the diameter of the inhibition zone and the potency of each compound was correlated with Fluconazole (Zambare 2012).

RESULTS

Each of the four lichen samples that were examined showed a dose-dependent inhibitory ability against the tested organisms. When comparison to 1 mg/mL, zones are observed to be higher at 2 mg/mL which can be seen in Table 1. Additionally, it is noted that whereas methanol extracts had a moderate effect, acetone extracts of every sample showed excellent inhibition. Chloroform extracts follow next. Every triple experiment showed identical findings.

Anti-bacterial studies

Antifungal studies

The screening of the lichen species against fungal specimens can be observed in Table 2.

DISCUSSION

Antibacterial activity

All the 4 lichen samples has shown to be effective against 6 tested organisms having dose dependent inhibitory capacity. Zones are found to be higher at 2 mg/mL when compared to 1mg/mL. The zone of inhibition range from 16.7±0.17 to 28.2±0.15 (in m.m.). The lowest was recorded for Chloroform extract of *Parmotrema praesorediosum* against *Acinetobacter baumannii* in 1 mg concentration. Whereas the highest zone of inhibition was recorded for Acetone extract of *Ramalina nervulosa* against *Enterococcus faecalis* in 2 mg concentration. Furthermore, it is

Table 1: Showing zone of inhibition shown by extracts obtained against 6 bacterial strains (both gram positive and gram negative).

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
1	Ampicillin	*Enterococcus faecalis*	32.2±0.45	+++
		Bacillus cereus	31.4±0.80	+++
		Clostridium botulinum	31.6±0.7	+++
		Salmonella enteritidis	33.6±0.73	+++
		Pseudomonas aeruginosa	34.6±0.75	+++
		Acinetobacter baumannii	32.6±0.62	+++
2	RHAE -1mg	*Enterococcus faecalis*	26.1±0.25	+++
		Bacillus cereus	25.5±0.35	+++
		Clostridium botulinum	21.4±0.35	++
		Salmonella enteritidis	22.1±0.37	++
		Pseudomonas aeruginosa	21.4±0.58	++
		Acinetobacter baumannii	25.4±0.41	+++
3	RHAE -2mg	*Enterococcus faecalis*	28.1±0.15	+++
		Bacillus cereus	23.5±0.4	++
		Clostridium botulinum	26.5±0.35	+++
		Salmonella enteritidis	23.4±0.85	++
		Pseudomonas aeruginosa	27.6±0.32	+++
		Acinetobacter baumannii	27.1±0.35	+++
4	RHME-1mg	*Enterococcus faecalis*	25.1±0.35	++
		Bacillus cereus	22.7±0.90	++
		Clostridium botulinum	19.3±0.49	+
		Salmonella enteritidis	20.5±0.80	++
		Pseudomonas aeruginosa	21.3±1.17	++
		Acinetobacter baumannii	23.8±0.55	++

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
5	RHME-2mg	*Enterococcus faecalis*	27.1±0.20	+++
		Bacillus Cereus	23.1±0.66	++
		Clostridium botulinum	24.8±0.86	++
		Salmonella enteritidis	22.5±0.75	++
		Pseudomonas aeruginosa	25.6±0.41	+++
		Acinetobacter baumannii	26.1±0.25	+++
6	RHCE-1mg	*Enterococcus faecalis*	23.1±0.3	++
		Bacillus cereus	18.7±0.35	+
		Clostridium botulinum	18.4±0.49	+
		Salmonella enteritidis	20.8±0.70	++
		Pseudomonas aeruginosa	21.5±0.26	++
		Acinetobacter baumannii	18.4±0.25	+
7	RHCE-2mg	*Enterococcus faecalis*	24.7±0.87	+++
		Bacillus cereus	19.6±0.26	++
		Clostridium botulinum	19.1±0.15	++
		Salmonella enteritidis	23.7±0.15	+++
		Pseudomonas aeruginosa	21.5±0.46	++
		Acinetobacter baumannii	19.6±0.35	++
8	RNAE-1mg	*Enterococcus faecalis*	26.1±0.76	+++
		Bacillus cereus	24.8±0.55	++
		Clostridium botulinum	22.1±0.38	+++
		Salmonella enteritidis	21.3±0.35	++
		Pseudomonas aeruginosa	21.1±0.31	++
		Acinetobacter baumannii	24.1±0.15	++
9	RNAE-2mg	*Enterococcus faecalis*	28.2±0.15	+++
		Bacillus cereus	23.5±0.32	++
		Clostridium botulinum	26.5±0.36	+++
		Salmonella enteritidis	23.4±0.95	++
		Pseudomonas aeruginosa	27.3±0.44	+++
		Acinetobacter baumannii	23.2±0.36	++
10	RNME-1mg	*Enterococcus faecalis*	24.5±0.35	++
		Bacillus cereus	21.9±0.46	++
		Clostridium botulinum	19.6±0.46	++
		Salmonella enteritidis	20.9±0.66	++
		Pseudomonas aeruginosa	21.5±1.06	++
		Acinetobacter baumannii	23.6±0.67	++
11	RNME-2mg	*Enterococcus faecalis*	27.1±0.21	+++
		Bacillus cereus	23.3±0.46	++
		Clostridium botulinum	24.9±0.30	+++
		Salmonella enteritidis	22.6±0.64	++
		Pseudomonas aeruginosa	23.3±0.42	++
		Acinetobacter baumannii	26.1±0.15	+++

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
12	RNCE-1mg	*Enterococcus faecalis*	22.5±0.57	++
		Bacillus cereus	18.9±0.30	+
		Clostridium botulinum	18.6±0.40	+
		Salmonella enteritidis	20.8±0.60	++
		Pseudomonas aeruginosa	21.1±0.40	++
		Acinetobacter baumannii	17.4±0.38	+
13	RNCE-2 mg	*Enterococcus faecalis*	22.9±1.01	++
		Bacillus cereus	19.3±0.67	++
		Clostridium botulinum	20.2±0.53	++
		Salmonella enteritidis	21.2±0.36	++
		Pseudomonas aeruginosa	21.4±0.67	++
		Acinetobacter baumannii	18.2±0.36	+
14	PPAE-1 mg	*Enterococcus faecalis*	25.7±1.40	+++
		Bacillus cereus	23.6±1.14	++
		Clostridium botulinum	21.6±0.40	++
		Salmonella enteritidis	21.1±0.35	++
		Pseudomonas aeruginosa	20.4±0.35	++
		Acinetobacter baumannii	22.1±0.21	++
15	PPAE-2 mg	*Enterococcus faecalis*	27.8±0.59	+++
		Bacillus cereus	23.1±0.26	++
		Clostridium botulinum	26.2±0.17	+++
		Salmonella enteritidis	23.1±0.87	++
		Pseudomonas aeruginosa	27.4±0.25	+++
		Acinetobacter baumannii	23.1±0.25	++
16	PPME-1 mg	*Enterococcus faecalis*	24.2±0.49	++
		Bacillus cereus	19.2±0.21	+
		Clostridium botulinum	19.1±0.10	+
		Salmonella enteritidis	20.5±0.40	++
		Pseudomonas aeruginosa	21±0.70	++
		Acinetobacter baumannii	23.1±0.87	++
17	PPME-2 mg	*Enterococcus faecalis*	26.5±0.36	+++
		Bacillus cereus	22.3±0.42	++
		Clostridium botulinum	22.8±0.35	++
		Salmonella enteritidis	22.7±0.50	++
		Pseudomonas aeruginosa	22.5±0.49	++
		Acinetobacter baumannii	25.5±0.49	+++
18	PPCE-1 mg	*Enterococcus faecalis*	20.9±0.21	++
		Bacillus cereus	18.7±0.36	+
		Clostridium botulinum	18.6±0.49	+
		Salmonella enteritidis	20.6±0.44	++
		Pseudomonas aeruginosa	19.9±0.71	++
		Acinetobacter baumannii	16.7±0.17	+

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
19	PPCE-2 mg	*Enterococcus faecalis*	22.9±0.85	++
		Bacillus cereus	18.3±0.55	+
		Clostridium botulinum	20.1±0.15	++
		Salmonella enteritidis	21±0.20	++
		Pseudomonas aeruginosa	20.6±0.38	++
		Acinetobacter baumannii	16.7±0.66	+
20	PTAE-1 mg	*Enterococcus faecalis*	26.3±0.46	+++
		Bacillus cereus	24.4±0.50	++
		Clostridium botulinum	21.3±0.46	++
		Salmonella enteritidis	21.5±0.46	++
		Pseudomonas aeruginosa	20.8±0.76	++
		Acinetobacter baumannii	23.8±0.44	++
21	PTAE-2 mg	*Enterococcus faecalis*	27.7±0.55	+++
		Bacillus cereus	22.9±0.21	++
		Clostridium botulinum	26.1±0.25	+++
		Salmonella enteritidis	22.8±0.78	++
		Pseudomonas aeruginosa	26.8±0.38	+++
		Acinetobacter baumannii	22.8±0.26	++
22	PTME-1 mg	*Enterococcus faecalis*	24±0.10	++
		Bacillus cereus	20.9±0.46	++
		Clostridium botulinum	19.6±0.55	+
		Salmonella enteritidis	20.8±0.26	++
		Pseudomonas aeruginosa	20.6±0.46	++
		Acinetobacter baumannii	22.5±0.56	++
23	PTME-2 mg	*Enterococcus faecalis*	25.8±0.61	+++
		Bacillus cereus	22.6±0.49	++
		Clostridium botulinum	24.6±0.57	++
		Salmonella enteritidis	22.1±0.61	++
		Pseudomonas aeruginosa	22.1±0.75	++
		Acinetobacter baumannii	25.6±0.42	+++
24	PTCE-1 mg	*Enterococcus faecalis*	21.2±0.17	++
		Bacillus cereus	18.5±0.46	+
		Clostridium botulinum	24.6±0.57	+
		Salmonella enteritidis	22.1±0.61	++
		Pseudomonas aeruginosa	20.6±0.59	++
		Acinetobacter baumannii	17.4±0.35	+

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
25	PTCE-2 mg	*Enterococcus faecalis*	22.4±0.44	++
		Bacillus cereus	19.2±0.47	+
		Clostridium botulinum	20.2±0.42	+
		Salmonella enteritidis	21.3±0.50	++
		Pseudomonas aeruginosa	20.6±0.30	++
		Acinetobacter baumannii	18.3±0.50	+

+++ is excellent, ++ is good, + is moderate.

RHAE – Acetone extract of *Ramalina hossei*, RHME – Methanol extract of *Ramalina hossei*.

RHCE – Chloroform extract of *Ramalina hossei*.

RNAE- Acetone extract of *Ramalina nervulosa*, RNME- Methanol extract of *Ramalina nervulosa*, RNCE- Chloroform extract of *Ramalina nervulosa*.

PPAE- Acetone extract of *Parmotrema praesorediosum*, PPME- Methanol extract of *Parmotrema praesorediosum*, PPCE - Chloroform extract of *Parmotrema praesorediosum*.

PTAE - Acetone extract of *Parmotrema tinctorum*, PTME- Methanol extract of *Parmotrema tinctorum*, PTCE- Chloroform extract of *Parmotrema tinctorum*.

Table 2: Showing zone of inhibition shown by extracts obtained against 3 fungal strains.

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
1	Fluconazole	*Aspergillus clavatus*	30.4±0.35	+++
2		*Aspergillus fumigatus*	31.5±0.31	+++
3		*Candida glabrata*	30.5±0.35	+++
4	RHAE -1 mg	*Aspergillus clavatus*	23.7±0.47	++
5		*Aspergillus fumigatus*	23.1±1	++
6		*Candida glabrata*	22.9±0.29	++
7	RHAE -2 mg	*Aspergillus clavatus*	26.1±0.25	+++
8		*Aspergillus fumigatus*	25.5±0.40	+++
9		*Candida glabrata*	26.1±0.31	+++
10	RHME-1 mg	*Aspergillus clavatus*	20.8±0.66	++
11		*Aspergillus fumigatus*	19.3±0.46	+
12		*Candida glabrata*	20.1±0.25	+
13	RHME-2 mg	*Aspergillus clavatus*	21.6±0.99	++
14		*Aspergillus fumigatus*	20.1±0.55	+
15		*Candida glabrata*	20.5±0.36	++
16	RHCE-1 mg	*Aspergillus clavatus*	18.4±0.26	+
17		*Aspergillus fumigatus*	18.1±0.68	+
18		*Candida glabrata*	17.8±0.23	+
19	RHCE-2 mg	*Aspergillus clavatus*	20.7±0.84	++
20		*Aspergillus fumigatus*	19.1±0.85	+
21		*Candida glabrata*	19.8±0.81	+
22	RNAE-1 mg	*Aspergillus clavatus*	23.8±0.85	++
23		*Aspergillus fumigatus*	22.7±0.40	++
24		*Candida glabrata*	22.7±0.35	++
25	RNAE-2 mg	*Aspergillus clavatus*	25.8±0.38	+++
26		*Aspergillus fumigatus*	25.4±0.57	+++
27		*Candida glabrata*	26.2±0.61	+++

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
28	RNME-1 mg	*Aspergillus clavatus*	20.9±0.56	++
29		*Aspergillus fumigatus*	19.2±0.40	+
30		*Candida glabrata*	20.1±0.38	+
31	RNME-2 mg	*Aspergillus clavatus*	21.5±0.81	++
32		*Aspergillus fumigatus*	19.8±0.56	+
33		*Candida glabrata*	20.6±0.31	++
34	RNCE-1 mg	*Aspergillus clavatus*	18.5±0.32	+
35		*Aspergillus fumigatus*	17.8±0.75	+
36		*Candida glabrata*	18.1±0.35	+
37	RNCE-2 mg	*Aspergillus clavatus*	20.9±0.74	++
38		*Aspergillus fumigatus*	19.1±0.40	+
39		*Candida glabrata*	20.2±0.50	+
40	PPAE-1 mg	*Aspergillus clavatus*	24.8±0.56	++
41		*Aspergillus fumigatus*	23.1±1.40	++
42		*Candida glabrata*	22.1±0.47	++
43	PPAE-2 mg	*Aspergillus clavatus*	26.4±0.46	+++
44		*Aspergillus fumigatus*	25.8±0.55	+++
45		*Candida glabrata*	25.4±0.40	+++
46	PPME-1 mg	*Aspergillus clavatus*	21.1±0.49	++
47		*Aspergillus fumigatus*	19.2±0.55	+
48		*Candida glabrata*	20.1±0.42	+
49	PPME-2 mg	*Aspergillus clavatus*	21.1±0.95	++
50		*Aspergillus fumigatus*	19.3±0.45	+
51		*Candida glabrata*	20.4±0.30	++
52	PPCE-1 mg	*Aspergillus clavatus*	18.6±0.44	+
53		*Aspergillus fumigatus*	17.7±0.55	+
54		*Candida glabrata*	18.6±0.12	+
55	PPCE-2 mg	*Aspergillus clavatus*	20.8±0.30	++
56		*Aspergillus fumigatus*	19.1±0.85	+
57		*Candida glabrata*	20.2±0.47	+
58	PTAE-1 mg	*Aspergillus clavatus*	24.3±0.51	++
59		*Aspergillus fumigatus*	22.6±0.59	++
60		*Candida glabrata*	22.2±0.47	++
61	PTAE-2 mg	*Aspergillus clavatus*	25.9±0.15	+++
62		*Aspergillus fumigatus*	26.1±0.40	+++
63		*Candida glabrata*	25.4±0.26	+++
64	PTME-1 mg	*Aspergillus clavatus*	20.8±0.36	++
65		*Aspergillus fumigatus*	19.2±0.62	+
66		*Candida glabrata*	20.1±0.45	+

Sl. No.	Sample	Organisms	Zone of inhibition (in mm) Avg.	Efficiency
67	PTME-2 mg	*Aspergillus clavatus*	20.8±0.60	++
68		*Aspergillus fumigatus*	19.3±0.56	+
69		*Candida glabrata*	20.4±0.40	++
70	PTCE-1 mg	*Aspergillus clavatus*	18.9±0.30	+
71		*Aspergillus fumigatus*	17.9±0.64	+
72		*Candida glabrata*	18.6±0.36	+
73	PTCE-2 mg	*Aspergillus clavatus*	20.7±0.21	++
74		*Aspergillus fumigatus*	19.2±0.45	+
75		*Candida glabrata*	20.3±0.61	+

+++ is excellent, ++ is good, + is moderate.
RHAE – Acetone extract of *Ramalina hossei*, RHME – Methanol extract of *Ramalina hossei*.
RHCE – Chloroform extract of *Ramalina hossei*.
RNAE- Acetone extract of *Ramalina nervulosa*, RNME- Methanol extract of Ramalina nervulosa, RNCE- Chloroform extract of *Ramalina nervulosa*.
PPAE- Acetone extract of *Parmotrema praesorediosum*, PPME- Methanol extract of *Parmotrema praesorediosum*, PPCE - Chloroform extract of *Parmotrema praesorediosum*.
PTAE - Acetone extract of *Parmotrema tinctorum*, PTME- Methanol extract of *Parmotrema tinctorum*, PTCE- Chloroform extract of *Parmotrema tinctorum*.

noted that whereas methanol extracts had a moderate effect, acetone extracts of every sample shown excellent inhibition and least effect was shown by the Chloroform extracts. Every triple experiment yielded identical findings.

Antifungal activity

In case of antifungal activity also all the 4 lichen samples tested has shown to be effective against 3 tested organisms having the dose dependent inhibitory capacity. Zones are found to be higher at 2 mg/mL when compared to 1 mg/mL. The zones of inhibition were seen in the range 17.7±0.55 to 26.4±0.46 (in m.m.). Chloroform extract of *Parmotrema praesorediosum* has shown the lowest zone of inhibition against *Aspergillus fumigatus* in 1mg concentration. On the other hand Acetone extract of *Parmotrema praesorediosum* has shown the highest zone of inhibition against *Aspergillus clavatus* in 2 mg concentration. Also it is observed that acetone extracts of all the samples have shown excellent inhibition, whereas methanol extracts have showed moderate effect followed by the chloroform extracts. The results obtained are same in all the triplicate experiments.

CONCLUSION

From the experiment it was confirmed that lichens are interesting organisms having the antibacterial and antifungal activities. The pathogenic microorganisms were screened *in vitro* and found to have antimicrobial potential with zones of inhibition of various ranges. This study is really helpful to identify, isolate and characterize the principal compounds exhibiting the antimicrobial activity from the potential and medicinally important lichen species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

TB: Tuberculosis; **CSIR-NBRI:** CSIR-National Botanical Research Institute; **LWG:** Herbarium of the National Botanical Research Institute; **MTCC:** Microbial Type Culture Collection; **CFU/mL:** Colony Forming Units per milliliter; **DMSO:** Dimethyl sulfoxide; **RHAE:** Acetone extract of *Ramalina hossei*; **RHME:** Methanol extract of *Ramalina hossei*; **RHCE:** Chloroform extract of *Ramalina hossei*; **RNAE:** Acetone extract of *Ramalina nervulosa*; **RNME:** Methanol extract of *Ramalina nervulosa*; **RNCE:** Chloroform extract of *Ramalina nervulosa*; **PPAE:** Acetone extract of *Parmotrema praesorediosum*; **PPME:** Methanol extract of *Parmotrema praesorediosum*; **PPCE:** Chloroform extract of *Parmotrema praesorediosum*; **PTAE:** Acetone extract of *Parmotrema tinctorum*; **PTME:** Methanol extract of Parmotrema tinctorum; **PTCE:** Chloroform extract of *Parmotrema tinctorum*; **PCCF:** Principal Chief Conservator of Forests.

(A)*Ramalina hossei*

(B)*Ramalina nervulosa*

(C) *Parmotrema praesorediosum*

(D)*Parmotrema tinctorum*

Figure 1: (Original own photographs) Lichen species screened for antibacterial and antifungal activities.

AUTHOR CONTRIBUTIONS

DT- Conceptualization, designing the analysis, Experimentation and draft manuscript preparation, MS-Draft finalization and supervision of the experiment, SN – Finalization of the paper and providing the critical feedback.

SUMMARY

This work presents the screening procedure and results of antibacterial and antifungal potential of some selected lichens from Burachapori Wildlife Sanctuary, an unexplored protected area of Assam, India. It has been reported that lichens have antibacterial properties. In order to screen the macro lichens from the Burachapori wildlife sanctuary, including *Parmotrema praesorediosum* (Nyl.) Hale, *Parmotrema tinctorum* (Despr. ex Nyl.) Hale, *Ramalina nervulosa* (Müll.Arg.) Abbayes and *Ramalina hossei* Vain., against 3 g positive, 3g negative pathogenic bacterial, and three fungal strains, this study was conducted. For the antibacterial and antifungal tests, acetone, chloroform, and methanol extracts of the sample were made in triplicate at two concentrations (1 mg and 2 mg), and the Agar well diffusion method was used. In antibacterial screening, ampicillin was utilized as a positive control at a dosage of 0.1 mg/mL. The antifungal activity of the lichen samples was screened using 100 µL of fluconazole as a positive control. Every activity displayed dose-dependent outcomes. When comparison to 1 mg/mL, zones are observed to be higher at 2 mg/mL. The zone of inhibition ranged from 16.7±0.17 to 28.2±0.15 (in m.m.) for antibacterial activity and from 17.7±0.55 to 26.4±0.46 (in m.m.) for antifungal activity. Additionally, it is noted that all of the samples' acetone extracts had exceptional inhibition, whereas methanol and chloroform extracts had a moderate impact. These findings validate the presence of antimicrobial compounds in the samples, and additional characterisation of these powerful antimicrobial compounds may serve as lead molecules against pathogenic microbes that cause disease.

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