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Phytochemical Profiling and Antibacterial Efficiency of *Hypotrachyna cirrhata* and *Parmotrema tinctorum* Lichen Extracts from Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand

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ABSTRACT

Lichens, symbiotic associations of fungi and algae, have long been utilized in traditional medicine and various industrial applications due to their rich bioactive compound diversity. In this study, we investigated the phytochemical profiles and antibacterial activities of extracts from two lichen species, *Hypotrachyna cirrhata* and *Parmotrema tinctorum*, collected from the Madhyamaheshwar valley, Uttarakhand, India. Phytochemical screening revealed distinct secondary metabolite compositions in chloroform, ethyl acetate, ethanol and methanol extracts of both lichen species. The extracts of *Hypotrachyna cirrhata* contained flavonoids, saponins, steroids, tannins and phenols across different solvents. Among these, the ethanol and methanol extracts exhibited the highest diversity of constituents and demonstrated the most effective antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. *Parmotrema tinctorum* extracts similarly showed a range of bioactive compounds, including flavonoids, glycosides, saponins, tannins and phenols, with ethanolic extracts displaying potent antibacterial activity against both bacterial strains. All lichen extracts demonstrated significant antibacterial potential. These findings underscore the importance of solvent selection in extracting bioactive compounds from lichens, influencing their phytochemical composition and antibacterial efficacy. Further exploration of specific active compounds and their mechanisms could enhance the therapeutic applications of these lichen species as natural antibacterial agents.

Keywords Lichens, Phytochemicals, Antibacterial activity, *Hypotrachyna cirrhata, Parmotrema tinctorum,* Madhyamaheshwar valley, Garhwal Himalaya.

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INTRODUCTION

The close association of two organisms, fungus and its photosynthetic companion algae, is known as lichens (Crockett *et al.* 2003). Lichens have been used in traditional cuisine and medicine for thousands of years, and they also serve important functions in ecosystem dynamics and human well-being (Crawford 2019). According to the World Health Organization (WHO), almost 80% of the global population now relies on traditional medicine as their main source of primary healthcare. Indigenous traditional medicine has significant economic advantages and may be used for the treatment of many ailments (Azaizeh *et al.* 2003). Although lichens comprise approximately 8% of the terrestrial ecosystem and are identified in over 20,000 species worldwide, their biological activities and biologically active compounds are still largely unexplored (Toma *et al.* 2001). Lichens are regarded as valuable plant resources that are employed over many different kinds of purposes, including the production of medicines, food, forage, dyes, perfumes and spices (Hegnauer 1962). Approximately 800 secondary metabolites have been identified from lichens, and these are unique compared to those produced by higher plants (Huneck and Yoshimura 1996). The various bioactive compounds extracted from lichens exhibit promising avenues for biopharmaceutical applications in developing new formulations or technologies, encompassing antimicrobial, antioxidant and cytotoxic agents (Zambare and Christopher 2012). Certain lichens have been utilized in traditional medicine to treat several kinds of diseases including stomach disorders, diabetes, coughs, pulmonary tuberculosis, wound healing and dermatological conditions (Huneck 1999). Lichens have several beneficial properties, such as antibacterial, antiviral, anticancer, analgesic and antipyretic properties (Vartia 1973, Crittenden and Porter 1991, Gollapudi *et al.* 1994, Müller 2001).

MATERIALS AND METHODS

Collection of lichen samples

Two different fresh lichen species were collected from Madhyamaheshwar valley (Figs.1-2). *Hypotrachyna cirrhata* was collected from Koon-chatti (latitude 30°62'34" N, longitude 79°21'13" E, elevation 2957 m above sea level), while *Parmotrema tinctorum* was collected from Budha Madhyamaheshwar (latitude 30°63'65" N, longitude 79°21'61" E, elevation 3484 m above sea level) (Table 1). The samples were gathered from the bark of trees (*Hypotrachyna cirrhata*) and surface of the rock (*Parmotrema tinctorum*). Lichen specimens were washed with tap water. After air-drying at room temperature, the samples were identified based on their morphological, anatomical and chemical characteristics, using available literature (Awasthi 2007). Identification was carried out in the Forest Ecology Laboratory at the Department of Botany & Microbiology, HNB. Garhwal University, and confirmed and authenticated at the Lichenology Laboratory of the CSIR - National Botanical Research Institute, Lucknow. The following accession numbers were assigned to the lichen samples: *Hypotrachyna cirrhata* (LWG – 62400) and *Parmotrema tinctorum* (LWG-61623).

Preparation of sample extracts

For extraction, air-dried lichen samples were grinded into a fine powder, and 10 g amount of the powder lichen samples was weighed and put in an individual

Fig. 1. Selected species of lichens for phytochemical analysis and antibacterial activity.

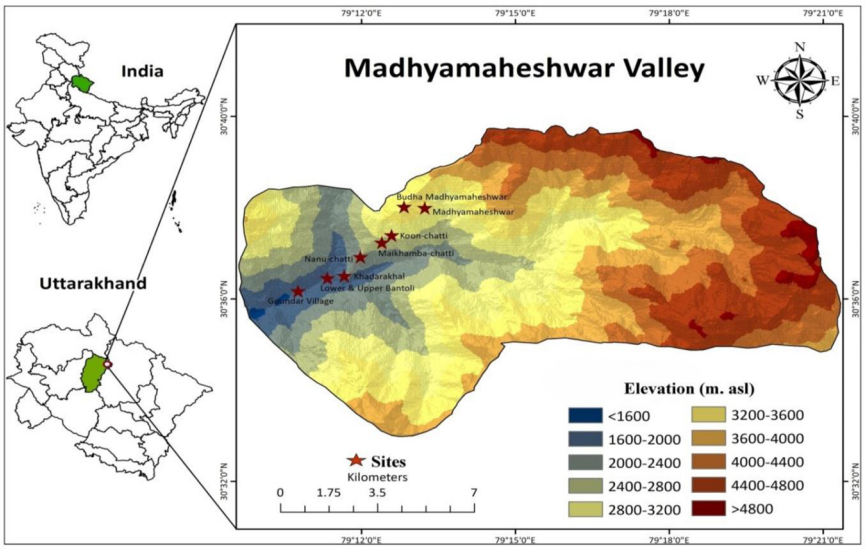


Fig. 2. Geographical map of the study area.

thimble. 10g/100ml sample in four different extract with different polarity solvents (chloroform, ethyl acetate, ethanol and methanol) were prepared for extraction using soxhlet apparatus. The respective extracts were filtered with Whatman No.1 filter paper. The filtrates were evaporated to obtain crude extracts of chloroform, ethyl acetate, ethanol and methanol, respectively. These crude extracts were subsequently dissolved again in their respective solvents and anti-bacterial activity was assessed.

Phytochemical screening

All extracts of the selected lichen samples underwent analysis using a preliminary phytochemical analysis to determine secondary metabolites, following a specific procedure (Rashmi and Rajkumar 2014).

Antibacterial activity

Microorganisms

Two different gram positive and gram-negative (*Staphylococcus aureus* MTCC 1144 and *Escherichia coli* MTCC 68) human pathogenic bacteria were obtained from microbiological research lab from the Department of Botany and Microbiology, HNB Garhwal University (A Central University), Srinagar, Garhwal Uttarakhand.

Agar well diffusion assay

Antibacterial screening of selected microorganisms was done using agar well diffusion method (Perez 1990). Mueller Hinton Agar plates were carefully poured into the sterile petri dishes. After solidifying, 100µl of the fresh culture of test bacteria (0.5 McFarland standards) was carefully spread onto each plate then a sterile cork borer (7 mm diameter) was used for well preparation. 100 µl of lichen extract at two different concentrations (5 mg and 15 mg/well) were loaded in each well. 15 mg of erythromycin was used as a standard positive control and their respective

Table 1. Lichen species and sampling locations with geographical coordinates.

Sl. No.	Lichen species	Accession number	Sampling locations	Latitude	Longitude	Elevation
1	*Hypotrachyna cirrhata*	(LWG-62400)	Koon-chatti	30°62'34" N	79°21'13" E	2957 m asl
2	*Parmotrema tinctorum*	(LWG-61623)	Budha Madhyamaheshwar	30°63'65" N	79°21'61" E	3484 m asl

solvent was used as a negative control. The plates were incubated for 24 hours at 37 °C; the inhibitory zones were measured in mm.

RESULTS

Phytochemical activity

In this investigation, we conducted a detailed phytochemical evaluation of extracts from two distinct lichen species collected in the Madhyamaheshwar Valley. The objective of our analysis was to systematically identify and characterized the phytochemical constituents in these extracts.

The preliminary phytochemical investigation revealed that the chloroform extracts of *Hypotrachyna cirrhata* contain only steroids, with no other compounds detected. Flavonoids were present in the ethyl acetate extracts, and the remaining components were absent. The ethanol extracts of *Hypotrachyna cirrhata* show the presence of flavonoids, saponins, tannins and phenols and the remaining constituents were absent. The methanol extracts revealed the presence of flavonoids, saponins, tannins and phenols, while the remaining constituents were absent. The extracts of *Parmotrema tinctorum* contained a variety of phytochemical constituents. Chloroform extracts contained only flavonoids while the remaining constituents were absent. Ethyl acetate extracts included flavonoids and glycosides, but absence of

Table 2. Preliminary phytochemical constituents of *Hypotrachyna cirrhata* and *Parmotrema tinctorum* with various solvent extracts.

Sl. No.	Phytochemicals	Phytochemical test	Solvent	*Hypotrachyna cirrhata*	*Parmotrema tinctorum*
1	Flavonoids	NaOH solution test	Chloroform	-	+
			Ethyl acetate	+	+
			Ethanol	+	+
			Methanol	+	+
2	Glycosides	Keller-Kiliani test	Chloroform	-	-
			Ethyl acetate	-	+
			Ethanol	-	+
			Methanol	-	+
3	Saponins	Foaming test	Chloroform	-	-
			Ethyl acetate	-	-
			Ethanol	+	+
			Methanol	+	-
4	Steroids	Liebermann-Burchard test	Chloroform	+	-
			Ethyl acetate	-	-
			Ethanol	-	-
			Methanol	-	-
5	Tannins	Ferric chloride test	Chloroform	-	-
			Ethyl acetate	-	-
			Ethanol	+	+
			Methanol	+	+
6	Phenols	Ferric chloride test	Chloroform	-	-
			Ethyl acetate	-	-
			Ethanol	+	+
			Methanol	+	+
7	Terpenoids	Salkowski test	Chloroform	-	-
			Ethyl acetate	-	-
			Ethanol	-	-
			Methanol	-	-
8	Alkaloids	Dragondroff's test	Chloroform	-	-
			Ethyl acetate	-	-
			Ethanol	-	-
			Methanol	-	-

[+] Presence of compound, [-] Absence of compound.

other components. Ethanolic extracts of *Parmotrema tinctorum* contained flavonoids, glycosides, saponins, tannins and phenols, while steroids, terpenoids and alkaloids were absent. The methanolic extracts contained flavonoids, glycosides, tannins and phenols, while all other constituents were absent. Specifically, terpenoids and alkaloids were not found in the both lichen extracts. Ethanolic and methanolic extracts were more active and demonstrated the maximum number of constituents in both lichen samples (Table 2).

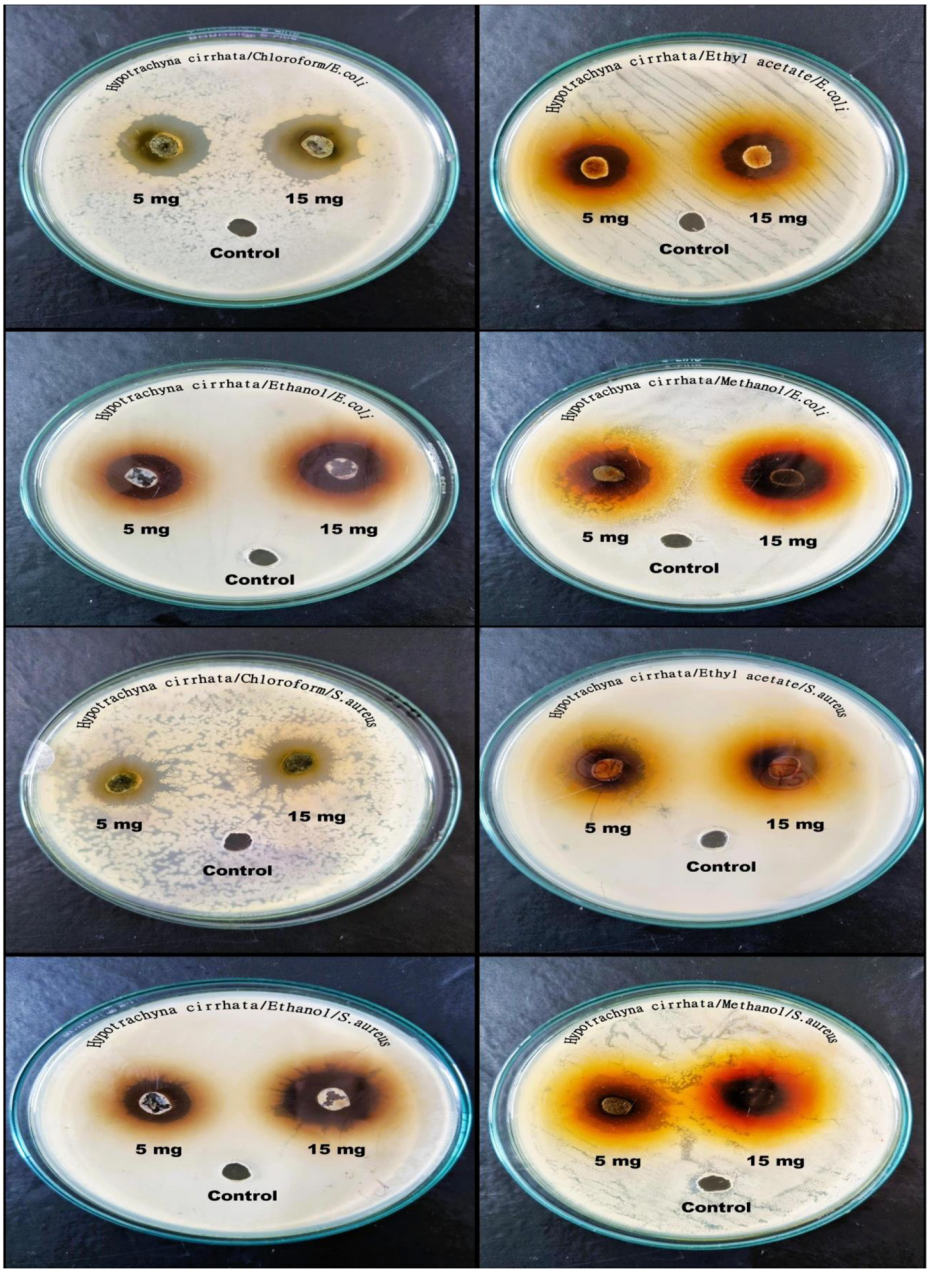
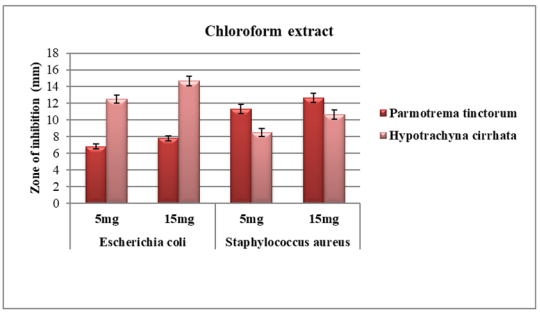
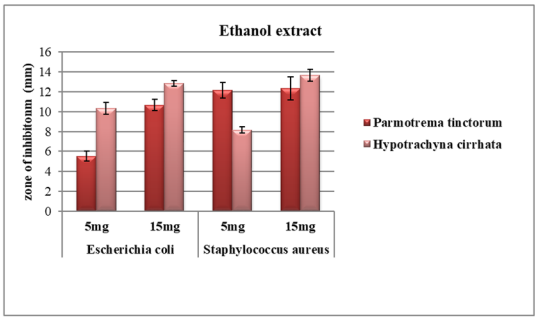


Fig. 3. Antibacterial activity of *Hypotrachyna cirrhata* lichen extracts against human pathogens *Escherichia coli* and S*taphylococcus aureus* using well diffusion method.



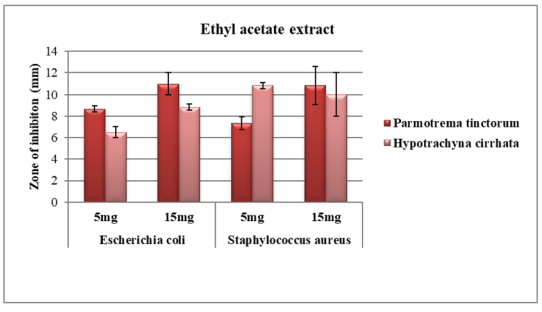
Graph 1. Antibacterial activity of *Parmotrema tinctorum* and *Hypotrachyna cirrhata* chloroform extract against *Escherichia coli* and *Staphylococcus aureus* bacterial strain.



Graph 3. Antibacterial activity of *Parmotrema tinctorum* and *Hypotrachyna cirrhata* ethanol extract against *Escherichia coli* and *Staphylococcus aureus* bacterial strain.

Antibacterial activity of *Hypotrachyna cirrhata* extracts

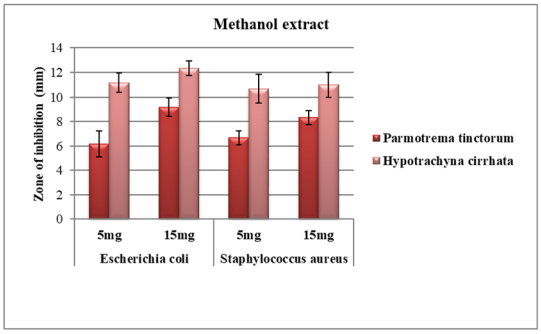
Hypotrachyna cirrhata extracts exhibited notable antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, as detailed in (Fig. 3). Tested at concentrations of 5 mg/well and 15 mg/well using chloroform, ethyl acetate, ethanol and methanol solvents, the extracts demonstrated varying degrees of effectiveness. The chloroform extract against *Escherichia coli* showed activity with a zone of inhibition (ZOI) ranging from 12.5 mm to 14.6 mm, and against *Staphylococcus aureus* with a ZOI ranging from 8.5 mm to 10.6 mm (Graph 1). Ethyl acetate extracts exhibited ZOI values ranging from 6.5 mm to 8.8 mm against *Escherichia coli* and from 10.8 mm to 10.0 mm against *Staphylococcus aureus* (Graph 2). Ethanol extracts displayed activity with ZOI values ranging from 10.3 mm to 12.8 mm against *Escherichia coli* and from 8.1 mm to 13.6 mm against *Staphylococcus aureus* (Graph 3). Subsequently, methanol extracts demonstrated activity with ZOI values ranging from 11.1 mm to 12.3 mm against *Escherichia coli* and from 10.6 mm to 11.0 mm against *Staphylococcus aureus* (Graph 4). All extracts exhibited significant antibacterial activity compared to the negative control (ZOI = 0 mm) for both bacterial strains, although the positive control (erythromycin, ZOI = 24.5 mm) demonstrated greater effectiveness against *Staphylococcus aureus.*



Graph 2. Antibacterial activity of *Parmotrema tinctorum* and *Hypotrachyna cirrhata* ethyl acetate extract against *Escherichia coli* and *Staphylococcus aureus* bacterial strain.

Antibacterial activity of *Parmotrema tinctorum* extracts

Parmotrema tinctorum extracts exhibited varying degrees of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus,* as detailed in (Fig. 4). Tested at concentrations of 5 mg and 15 mg using chloroform, ethyl acetate, ethanol and methanol as solvents, the extracts demonstrated distinct levels of



Graph 4. Antibacterial activity of *Parmotrema tinctorum* and *Hypotrachyna cirrhata* methanol extract against *Escherichia coli* and *Staphylococcus aureus* bacterial strain.

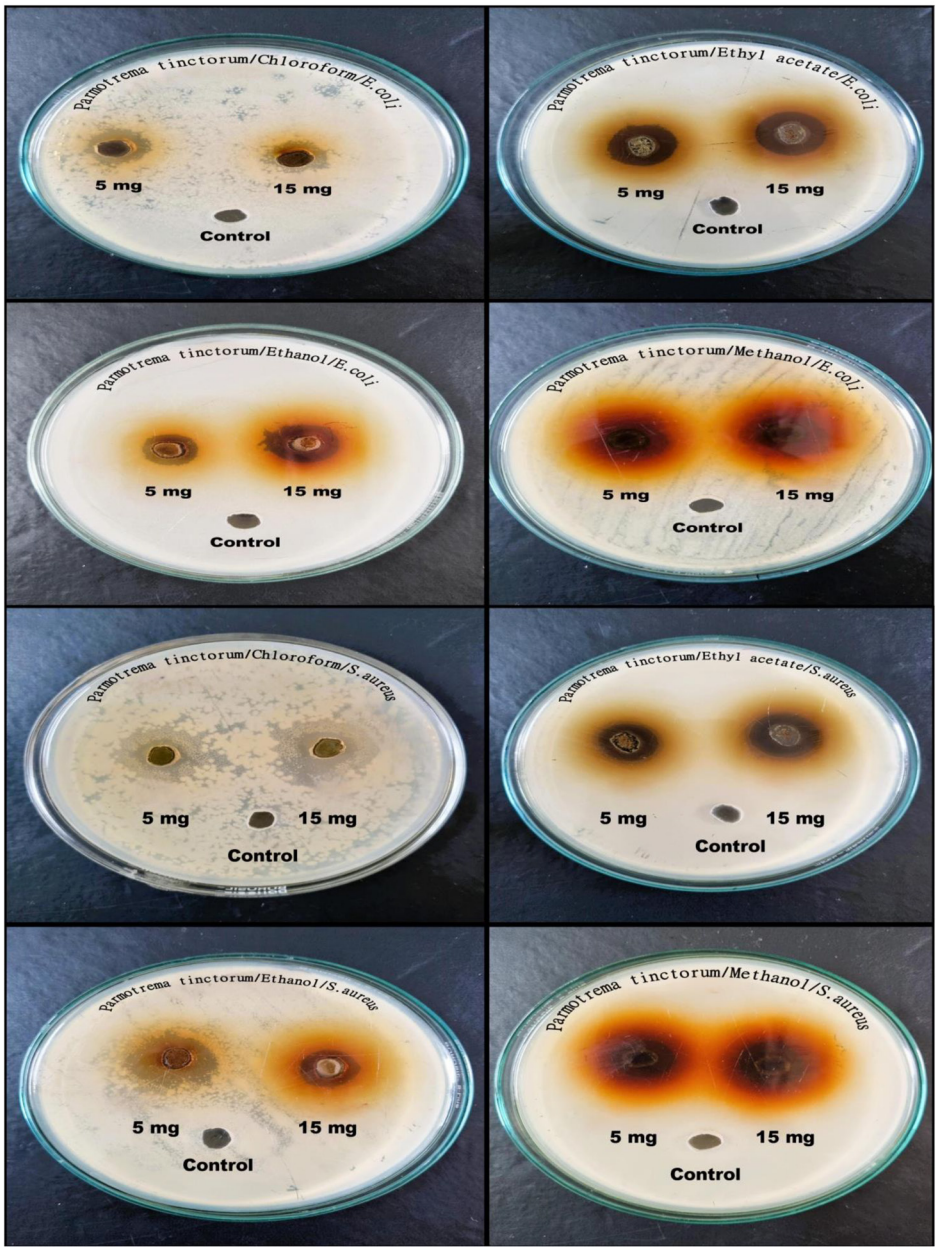


Fig. 4. Antibacterial activity of *Parmotrema tinctorum* lichen extracts against human pathogens *Escherichia coli* and *Staphylococcus aureus* using well diffusion method.

effectiveness. The chloroform extract showed activity against *Escherichia coli*, with zones of inhibition (ZOI) ranging from 6.8 mm to 7.8 mm, and against *Staphylococcus aureus*, with ZOI ranging from 11.3 mm to 12.6 mm (Graph 1). The ethyl acetate extract exhibited variable activity, with ZOI ranging from 8.6 mm to 11.0 mm against *Escherichia coli* and from 7.3 mm to 14.1 mm against *Staphylococcus aureus* (Graph 2). The ethanol extract demonstrated activity levels ranging from 5.5 mm to 10.6 mm against

Escherichia coli and ZOI ranging from 12.1 mm to 12.3 mm against *Staphylococcus aureus* (Graph 3). The methanol extract showed activity ranging from 6.1 mm to 9.1 mm against *Escherichia coli* and ZOI ranging from 6.6 mm to 8.3 mm against *Staphylococcus aureus* (Graph 4). All extracts demonstrated significant antibacterial activity against *Staphylococcus aureus* compared to the negative control (ZOI = 0 mm), although their efficacy was generally less than that of the positive control, erythromycin (ZOI = 24.5 mm). These findings underscore the potential of *Parmotrema tinctorum* extracts as sources of antibacterial agents, highlighting their varying effectiveness depending on the solvent and concentration used in the study.

The results of phytochemical analysis and antibacterial activities of *Hypotrachyna cirrhata* and *Parmotrema tinctorum* extract both exhibit significant potential as antibacterial agents. *Hypotrachyna cirrhata* extracts, characterized by their varying phytochemical profiles across different solvents, showed notable activity against both *Escherichia coli* and *Staphylococcus aureus*. The presence of flavonoids, saponins, tannins and phenols in ethanol and methanol extracts correlates with their observed antibacterial effectiveness, with methanol showing slightly higher activity against *Escherichia coli.*

Parmotrema tinctorum extracts demonstrated varying degrees of antibacterial activity, with ethyl acetate and ethanol extracts displaying the broadest spectrum of effectiveness. The presence of flavonoids, glycosides, saponins, tannins, phenols and terpenoids in ethanolic extracts contributes to their efficacy against both tested bacterial strains.

DISCUSSION

The findings of the previous research by (Swathi *et al.* 2010) detected alkaloids, saponins, tannins, and terpenoids in *Everniastrum cirrhatum*. A subsequent study by (Singh *et al.* 2024) also reported flavonoids, glycosides, saponins and tannins in *Everniastrum cirrhatum.* However, the present study finds flavonoids, saponins, steroids, tannins and phenols in *Hypotrachyna cirrhata.* For *Parmotrema tinctorum,* (Rashmi and Rajkumar 2014) revealed the existence of tannins, alkaloids, proteins, triterpenoids and carbohydrates. Similarly, a research conducted by (Reddy *et al.* 2017) revealed the existence of saponins, glycosides and flavonoids in *Parmotrema tinctorum.* However, the current study reported the presence of flavonoids, glycosides, saponins, tannins and phenols in *Parmotrema tinctorum.* In a study by (Pius and Sequeira 2022), was reported that chloroform extracts of *Hypotrachyna cirrhata* demonstrated a zone of inhibition (ZOI) of 1.7 cm against *Escherichia coli* and 1.2 cm against *Staphylococcus aureus*. Ethanol extracts showed a ZOI of 0.3 cm against *Escherichia coli* and 0.5 cm against *Staphylococcus aureus*, while methanolic extracts exhibited a ZOI of 0.5 cm against *Escherichia coli* and 0.4 cm against *Staphylococcus aureus*. In contrast, (Srivastava *et al.* 2013) reported that ethanolic and methanolic extracts of *Hypotrachyna cirrhata* showed no activity against *Escherichia coli*. However, ethanolic extracts produced a ZOI of 20.2 ± 1.1 mm against *Staphylococcus aureus*, and methanolic extracts demonstrated a ZOI of 12.2 ± 1.6 mm against *Staphylococcus aureus.*

In the present investigation reported chloroform extract exhibited activity against *Escherichia coli*, with ZOIs ranging from 6.8 mm to 7.8 mm, and against *Staphylococcus aureus*, with ZOIs ranging from 11.3 mm to 12.6 mm. The ethyl acetate extract displayed variable activity, with ZOIs ranging from 8.6 mm to 11.0 mm against *Escherichia coli* and from 7.3 mm to 14.1 mm against *Staphylococcus aureus.* The ethanol extracts demonstrated activity with ZOIs ranging from 5.5 mm to 10.6 mm against *Escherichia coli* and from 12.1 mm to 12.3 mm against *Staphylococcus aureus*. The methanol extract showed activity with ZOIs ranging from 6.1 mm to 9.1 mm against *Escherichia coli* and from 6.6 mm to 8.3 mm against *Staphylococcus aureus.*

Previous research by (Kalidoss *et al.* 2020) reported that chloroform extracts of *Parmotrema tinctorum* demonstrated a zone of inhibition (ZOI) ranging from 6.5 mm to 12.3 mm against *E. coli* and from 9.0 mm to 16.0 mm against *S. aureus*. In contrast, studies by (Ganesan *et al.* 2015) and (Rajan *et al.* 2015), found that methanolic extracts of *Parmotrema tinctorum* showed no activity against *E. coli* and *S. aureus.* The present study reported chloroform

extract against *Escherichia coli* showed activity with a zone of inhibition (ZOI) ranging from 12.5 mm to 14.6 mm, and against *Staphylococcus aureus* with a ZOI ranging from 8.5 mm to 10.6 mm. Ethyl acetate extracts exhibited ZOI values ranging from 6.5 mm to 8.8 mm against *Escherichia coli* and from 10.8 mm to 10.0 mm against *Staphylococcus aureus.* Ethanol extracts displayed activity with ZOI values ranging from 10.3 mm to 12.8 mm against *Escherichia coli* and from 8.1 mm to 13.6 mm against *Staphylococcus aureus*. Subsequently, methanol extracts demonstrated activity with ZOI values ranging from 11.1 mm to 12.3 mm against *Escherichia coli* and from 10.6 mm to 11.0 mm against *Staphylococcus aureus.*

CONCLUSION

The findings of the phytochemical analysis and antibacterial activity for both *Hypotrachyna cirrhata* and *Parmotrema tinctorum* extracts have promising potential as antibacterial agents.

Different phytochemicals, like flavonoids, saponins, steroids, tannins and phenols, were reported in *Hypotrachyna cirrhata* extracts, depending on the solvent that was used. These extracts demonstrated notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureu*s, with varying degrees of effectiveness across different solvents. *Parmotrema tinctorum* extracts also displayed significant antibacterial activity against both bacterial strains. The presence of flavonoids, glycosides, saponins, tannins and phenols in the extracts contributed to their efficacy. Although all extracts showed significant activity against *Escherichia coli* and *Staphylococcus aureus,* overall, these results show that both types of lichen could be useful sources of antibacterial agents. Further studies exploring optimal extraction conditions and additional biological activities could enhance their therapeutic applications.

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