

# Assessment of antibacterial potential of different solvent extract of foliose lichens against human pathogenic bacteria

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## ABSTRACT

*In vitro* antibacterial activity of acetone, methanol, petroleum ether, and diethyl ether extracts of three foliose lichen species *Dirinaria picta*, *Dirinaria Papillulifera*, and *Dirinaria applanata* have been investigated against two clinically significant human pathogenic bacteria (Gram-positive *Staphylococcus aureus*, MTCC 737 and Gram-negative *Escherichia coli*, MTCC 443) using agar cup diffusion method. Commercial drug streptomycin (1 mg/ml) was taken as a standard positive and 5% dimethyl sulphoxide (DMSO) as a negative control. All the extracts were found effective by showing the solvent dependent zone of inhibition. Largest zone of inhibition against *E. coli* by *D. picta*, *D. papillulifera*, and *D. applanata* have been recorded for acetone ( $37.1 \pm 0.2$  mm), methanol ( $23.5 \pm 0.6$  mm), and acetone ( $30.6 \pm 0.4$  mm) extracts, respectively. On the other hand, *S. aureus* was inhibited with maximum zone by methanol ( $23.6 \pm 0.4$  mm), diethyl ether ( $24.2 \pm 0.4$  mm), and acetone ( $21.6 \pm 0.5$  mm) extracts for *D. picta*, *D. papillulifera*, and *D. applanata*, respectively. The principal component analysis concluded that negative control (5% DMSO) does not affect the growth of test pathogens and most of the lichen extracts were more effective than the positive control.

## INTRODUCTION

Despite notable achievement in drug discovery by human being infectious diseases caused by pathogenic microorganisms pose a serious threat to health. Among them, pathogenic bacteria are the causal organism for a variety of diseases. The century-old practice of antibiotics has led to the rise of numerous drug-resistant pathogenic strains. Due to this reason, the achievement of biologically active compounds has been paid more attention by scientist all over the World. Such compounds obtained from plants have been the source of pharmaceutical science for a long time (Chauhan and Abraham, 2013).

Since ancient times lichens and their products have been conventionally used as a remedy for various disorders throughout the world. Up until now, many investigators have evaluated the bioactivity of lichen secondary metabolites against serious infectious organisms (Fournet *et al.*, 1997). Such metabolites

unique to lichens possess manifold biological activities, including antiviral, antibiotic, antitumor, and antiherbivore abilities (Dayan and Romagni, 2001; Huneck, 2001; Müller, 2001).

India, the world's 12th mega-diversity region, comprises great numbers of flora and fauna (Ali and Adithyakumari, 2015). Although numerous taxonomic works followed by Awasthi (2007) on lichen has been carried out in different states of India, studies on lichen metabolites are very few, particularly from the state of Assam. Therefore, the current study was designed to explore the *in vitro* analysis of the antibacterial potential of three foliose lichen species against two clinically significant human pathogenic bacteria.

## MATERIALS AND METHODS

### Collection and identification of lichen samples

The lichen samples for this study were collected from Chapanalla region of Nagaon district of Assam State, India which is located 25 km in the direction of the east from District headquarters Nagaon. It lies between  $26^{\circ}19'9.22''$  and  $26^{\circ}19'45.12''$  North latitude and  $92^{\circ}54'29.80''$  to  $92^{\circ}54'32.48''$  east longitude in the foothills of Karbi Anglong and the elevation of the region ranges between 87

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and 136 m. The samples were collected from the bark of trees. The morphological and anatomical identification was done using Leica S9i stereomicroscope and Leica DM 2500 optical microscope. For chemical identification color spot test (Nylander, 1866) and microcrystallography (Asahina, 1938; Orange *et al.*, 2001) was used. The specimens were identified with the help of relevant literatures (Awasthi, 2007) and confirmed by comparing them to the specimens available in the lichen herbarium of Nowgong College, Assam, authenticated by CSIR-National Botanical Research Institute, Lucknow (LWG). The voucher specimens were submitted at the herbarium of Nowgong College, Assam, India.

### Extraction of lichen samples in different solvent

Carefully cleaned lichen thalli were washed and dried by spreading on the paper sheet at room temperature in the laboratory. The dried lichen specimens were powdered using an electric grinder. Four different solvent systems, viz., acetone, methanol, petroleum ether, and diethyl ether were used for the extraction. Around 10 g of powdered sample wrapped in Whatman No. 1 filter paper was extracted in 100 ml of solvent using Soxhlet extractor (Soxhlet, 1879). Furthermore, the metabolites were obtained through the removal of solvents using Rotary evaporator. The crude extract was subsequently dissolved in dimethyl sulphoxide (DMSO) for antimicrobial bioassay. The extraction process was executed by maintaining the boiling temperature of the solvents (acetone 56°C, methanol 65°C, Petroleum ether 62°C, and Diethyl ether 35°C).

### Test pathogens and media

From the Institute of Microbial Technology, Chandigarh, India, clinically significant human pathogenic bacterial strains have been obtained. The test pathogens include one Gram-positive bacterium: *Staphylococcus aureus* (MTCC 737) and one Gram-negative bacterium: *Escherichia coli* (MTCC 443). Bacterial cultures were maintained on Nutrient Agar (NA) Media.

### Screening of antibacterial potential

Antibacterial potential of test pathogens was carried out using the agar cup diffusion method (Tayung *et al.*, 2011). Nutrient agar was poured into the Petri plates. Inoculation of 12–14 hours broth was done by streaking the plates with a sterile cotton swab. Even distribution of inoculum was ensured by rotating the plates during swabbing. The plates were allowed to dry for 15 minutes in Bio-safety cabinet after inoculation. Wells were made in agar plates using a cork borer (7 mm diameter) and 100 µl of the extract was added into each well. The plates were incubated at 37°C and after 24 hours the zone of inhibition was recorded. The diameter of the inhibition zones was measured in millimeter and expressed as

the mean value. Streptomycin (1 mg/ml) was taken as a standard positive and 5% DMSO as a negative control (Prabhu and Sudha, 2015; Tomović *et al.*, 2017). All the experiments were carried out in triplicates.

### Data analysis

Dimensionality-reduction method, principal component analysis (PCA) was used to evaluate the effect of four solvent extracts of *Dirinaria picta* (Sw.) Schaer. ex Clem, *Dirinaria papillulifera* (Nyl.) D.D. Awasthi, and *Dirinaria applanata* (Fée) D.D. Awasthi on test pathogen in comparison to controls (positive: streptomycin and negative: 5% DMSO). Correlation matrix in multivariate option was used to generate PCA biplots. PCA was done in PAST 4.02. (Hammer *et al.*, 2001; Tiwari *et al.*, 2011).

## RESULTS AND DISCUSSION

### Identified lichen species

In the current investigation, the collected lichen species were identified as *D. picta*, *D. papillulifera*, and *D. applanata* (Fig. 1).

### Antibacterial potential of lichen extracts

In the present study, the antibacterial activity of acetone, methanol, petroleum ether, and diethyl ether extracts of three foliose lichen species *D. picta*, *D. papillulifera*, and *D. applanata* have been investigated against two clinically significant human pathogenic bacteria using agar cup diffusion method (Ganesan *et al.*, 2017; Joshi and Sati, 2011; Pavithra *et al.*, 2013; Plaza *et al.*, 2018). It has been found that most of the lichen species in all solvent system showed potent antibacterial activities in terms of inhibition zone as compared to commercial drug streptomycin (Tables 1–3) which was similar to results obtained by (Srivastava

**Table 1.** Antibacterial activity of solvent extract of *D. picta* against the test pathogen.

Extracts	Diameter of inhibition zone (mm) <sup>a</sup>	
	MTCC-443	MTCC-737
Acetone	37.1 ± 0.2	23.3 ± 0.1
Methanol	27.5 ± 0.4	23.6 ± 0.4
Petroleum ether	27.2 ± 0.5	16.8 ± 0.6
Diethyl ether	20.1 ± 0.2	22.4 ± 0.2
DMSO	–	–
Streptomycin	19.2 ± 0.2	19.2 ± 0.4

<sup>a</sup>Values are in Arithmetic mean ± Standard error ( $n = 3$ ); – indicates no activity; MTCC-443 = *E. coli* and MTCC-737 = *S. aureus*.



**Figure 1.** Identified lichen species. (A) *D. picta*, (B) *D. papillulifera*, and (C) *D. applanata*.

**Table 2.** Antibacterial activity of solvent extract of *D. papillulifera* against the test pathogen.

Extracts	Diameter of inhibition zone (mm) <sup>a</sup>	
	MTCC-443	MTCC-737
Acetone	22.2 ± 0.3	23.3 ± 0.2
Methanol	23.5 ± 0.6	22.7 ± 0.6
Petroleum ether	13.1 ± 0.5	15.2 ± 0.4
Diethyl ether	22.4 ± 0.2	24.2 ± 0.4
DMSO	–	–
Streptomycin	19.2 ± 0.2	19.2 ± 0.4

<sup>a</sup>Values are in Arithmetic mean ± Standard error ( $n = 3$ ); – indicates no activity; MTCC-443 = *E. coli* and MTCC-737 = *S. aureus*.

**Table 3.** Antibacterial activity of solvent extract of *D. applanata* against the test pathogen.

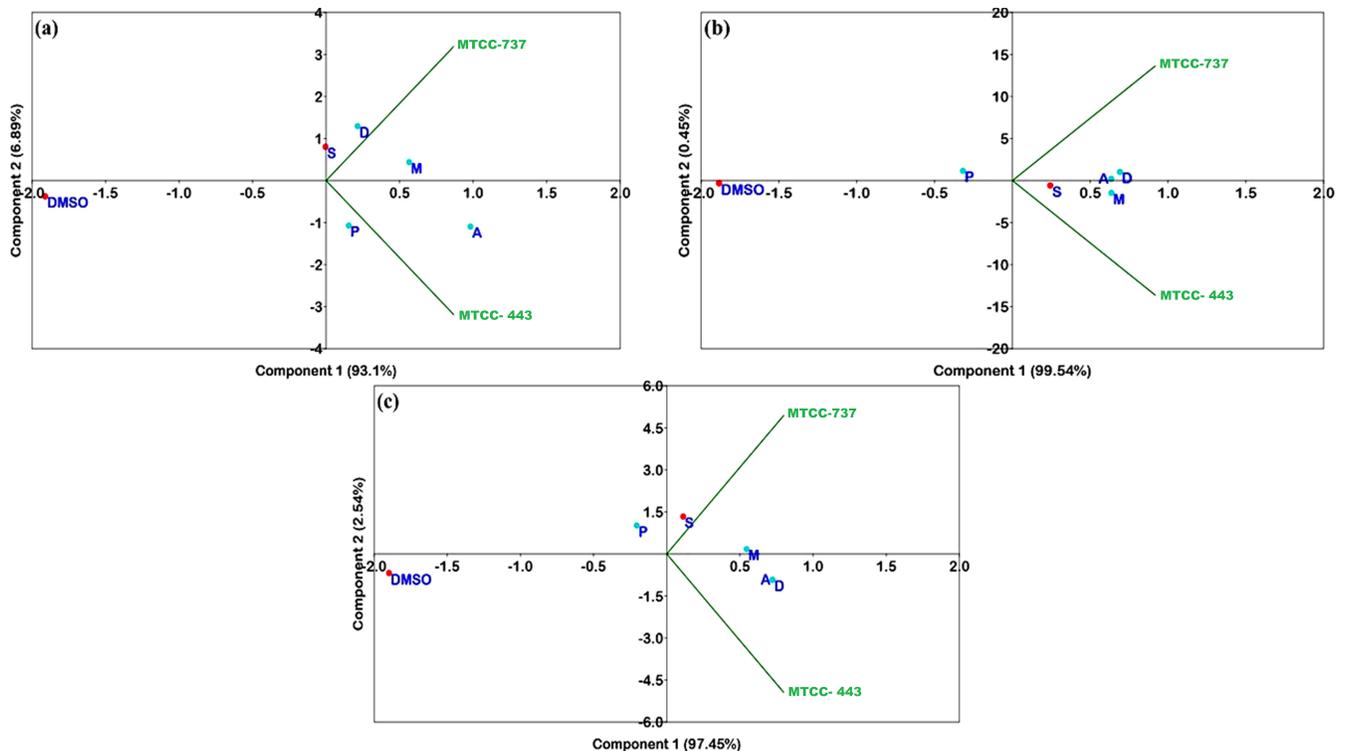
Extracts	Diameter of inhibition zone (mm) <sup>a</sup>	
	MTCC-443	MTCC-737
Acetone	30.6 ± 0.4	21.6 ± 0.5
Methanol	26.2 ± 0.3	21.4 ± 0.3
Petroleum ether	16.3 ± 0.4	16.3 ± 0.6
Diethyl ether	30.2 ± 0.5	21.1 ± 0.8
DMSO	–	–
Streptomycin	19.2 ± 0.2	19.2 ± 0.4

<sup>a</sup>Values are in Arithmetic mean ± Standard error ( $n = 3$ ); – indicates no activity; MTCC-443 = *E. coli* and MTCC-737 = *S. aureus*.

*et al.*, 2013). Largest zone of inhibition against *E. coli* by *D. picta*, *D. papillulifera*, and *D. applanata* have been recorded for acetone ( $37.1 \pm 0.2$  mm), methanol ( $23.5 \pm 0.6$  mm), and acetone ( $30.6 \pm 0.4$  mm) extracts, respectively. On the other hand, *S. aureus* was inhibited with maximum zone by methanol ( $23.6 \pm 0.4$  mm), diethyl ether ( $24.2 \pm 0.4$  mm), and acetone ( $21.6 \pm 0.5$  mm) extracts for the same. Out of these the effectiveness of diethyl ether extracts against *S. aureus* is similar to the work of Özyigitöglu *et al.* (2017). The inhibition zone formed by positive control (Streptomycin) against *E. coli* and *S. aureus* are  $19.2 \pm 0.2$  mm and  $19.2 \pm 0.4$  mm, respectively. The negative control (5% DMSO) does not affect the growth of the test pathogens (Fig. 2) which is similar to work carried out by Gunasekaran *et al.* (2016), Pavithra *et al.* (2013), and Ranković *et al.* (2011).

### Principal component analysis

The PCA biplots revealed that two extracts (methanol and diethyl ether) of *D. picta* and the positive control streptomycin were more effective against *S. aureus*, while the two other extracts (acetone and petroleum ether) were more effective against *E. coli*. For the species of *D. papillulifera*, three extracts (acetone, diethyl ether, and petroleum ether) were more effective against *S. aureus* as compared to *E. coli*, which is highly inhibited by methanol extract and the positive control streptomycin. On the other hand, biplot for *D. applanata* showed high activity of two extracts (methanol and petroleum ether) along with the positive control against *S. aureus*, while the remaining two extracts



**Figure 2.** Analysis of principal component of selected lichen species (a—*D. picta*, b—*D. papillulifera*, c—*D. applanata*,) extracts (A—Acetone, M—Methanol, P—Petroleum Ether, D—Diethyl Ether, DMSO—Dimethyl Sulphoxide) and commercially available drug Streptomycin (S) against human test pathogens *E. coli*, MTCC-443 and *S. aureus*, MTCC-737.

(acetone and diethyl ether) were more effective against *E. coli* with almost similar potency. In both the cases of *D. papillulifera* and *D. applanata*, the extract of petroleum ether was found to be less effective than the positive control. The negative control (DMSO) was unable to show any antagonistic activity against the human test pathogens. Thus, PCA biplots concluded that all the lichen extracts were effective against the pathogens in a solvent dependent manner and most of the extracts were more effective than the commercial drug streptomycin (Fig. 2). The same result to evaluate the effectiveness of lichen extracts has been carried out by Ganesan *et al.* (2015).

The discovery of different antibiotics has revolutionized the human history of medicine. However, multidrug-resistant pathogens have been emerging out continuously due to rapid uses of antibiotics discovered so far. Presently, scientists all over the world pay attention to lichen secondary metabolites due to their promising effectiveness over traditionally used compounds (Huneck, 1999). Similar to the present work acetone, chloroform, diethyl ether, methanol, and petroleum ether extracts of *Parmelia sulcata* comprising salazinic acid showed potent antibacterial activity against *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Proteus vulgaris*, *Yersinia enterocolitica*, *S. aureus*, *Streptococcus faecalis*, *Candida albicans*, and *Candida glabrata* (Candan *et al.*, 2007). Diethyl ether, acetone, and ethanol extracts of *Cetraria aculeate* comprising protolichesterinic acid have been investigated for antibacterial activity against different bacterial strains belonging to Gram-positive and Gram-negative groups which are also similar to the present investigation (Türk *et al.*, 2003). The literature revealed that antibacterial activity of lichen extracts was mostly tested on *Bacillus*, *Pseudomonas*, *E. coli*, *S. aureus*, *Klebsiella*, *Salmonella*, *Yersinia*, and *Proteus* sp. (Ingólfssdóttir *et al.*, 1985; Karagöz *et al.*, 2009; Karthikaidevi *et al.*, 2009; Manojlovic *et al.*, 2010; Martins *et al.*, 2010; Yilmaz *et al.*, 2004).

## CONCLUSION

The present bioassay of comparative effectiveness of acetone, methanol, petroleum ether, and diethyl ether extracts of *D. picta*, *D. papillulifera*, and *D. applanata* against Gram-positive and Gram-negative bacteria show solvent dependent inhibition activity. Except few instances, most of the crude extracts showed far better inhibition activity than commercial streptomycin. Therefore, it can be concluded that lichen extracts can be used as an alternative to commercially available antibacterial drugs. Thus, further investigations on purification, characterization, and identification of antimicrobial compound from lichen is needed.

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## CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

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