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(RESEARCH ARTICLE)

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Evaluation of antibacterial activity of liverwort species of Bagh Azad Jammu and Kashmir (Western Himalaya) against pathogenic bacteria

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Abstract

This research work aims to assess antibacterial potential of five liverwort species that have been collected from District Bagh Azad Jammu and Kashmir. For this purpose, two bacterial strains including *Achromobacter xylosoxidans* (Gram-negative) and *Bacillus licheniformis* (Gram- positive) were used by following disc diffusion method. The extract of the species was prepared in ethanol, methanol and water. The results showed that ethanol and methanol extracts of all liverworts have stronger and broader spectrum for the zone of inhibition against both bacteria. Among all investigated species, *Marchantia emarginata* and *Pellia epiphylla* showed most promising results, whereas lowest activity was shown by *Plagiochasma rupestre*. In aqueous extract, only *Marchantia paleacea* and *Plageochasma rupestre* showed positive results. In general, resistance shown by Gram-positive strains was superior to Gram-negative strains. This study proved that the investigated liverwort species possess strong defensive mechanism against bacteria and can be used to prepare novel antibiotics.

Keywords: Western Himalaya; Liverworts; Antibacterial activity; Disc Diffusion method; Antibiotics

1. Introduction

The ongoing and rapid increase in antibiotic resistance among pathogens has become a major global concern in clinical trials, resulting in lethal infections. Therefore, it possess a serious challenge for the researchers to discover efficient antimicrobials and potential drugs in order to cure numerous nosocomial infections [1]. Medicinal plants are being served as potential antibacterial agent from centuries because of the presence of various secondary metabolites [2]. In recent years, plentiful efforts have been made with medicinal plants to discover novel antimicrobial compounds. The development of advance techniques have played a crucial role in finding new resources of raw material for pharmaceutical industries [3]. Bryophyte is a general term used for lower non- vascular plants that are categorized in to three major groups i.e. Bryophyta (Mosses), Anthocerophyta (hornworts) and Marchantiophyta (liverworts) [4]. They are sensitive plants that lack anatomical structures to cope with biotic and abiotic stresses. Therefore, they possess diverse bioactive compounds that provide defense against pathogenic microorganisms [5]. Many researchers find out the presence of unique phytochemical compounds in bryophytes due to which they have antibacterial, antifungal, antitumor, cytotoxic and insecticidal properties [6-8]. The word "Liverwort" is used for the plant body having liver shaped structure [9]. One of the distinguishing feature of liverworts is the presence of oil bodies that show variation in morphology. These oil bodies are the source of numerous phytochemical compounds like sesquiterpenes, bis-benzyls, naphthalenes, isocoumarins, benzoates, cinnamates, etc. These compounds provide defensive mechanism against various pathogens [10]. The main objective of the present study is to investigate the possible inhibitory activity of different extracts of liverworts against identified bacterial strains.

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2. Material and methods

2.1. Study Area

District Bagh, as designated by its name (garden) is a mountainous resort that lies in Western Himalayan range and acts as a virgin for scientific exploration. It is located at a distance of 80 Km from Muzaffarabad (the capital of Azad Jammu and Kashmir) and covers 10% area of the whole state. Climate of the area is sub- tropical to moist temperate with 55% area under forest cover. The altitude of the area is between 1000- 2500 m. Average annual temperature is 21°C and precipitation is 1500 mm [11]. Present study was conducted in five villages/sites of moist temperate region of District Bagh Azad Jammu and Kashmir. Several field surveys were conducted for the collection of liverworts (Figure 1).

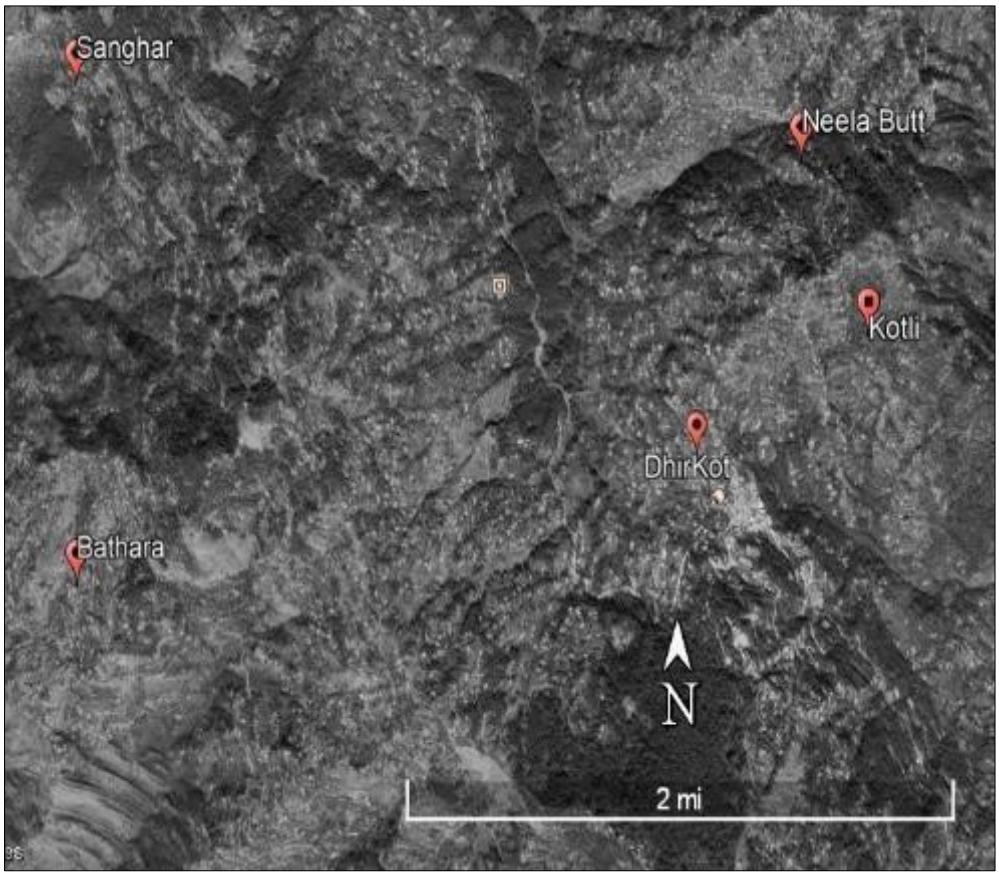


Figure 1 Google- earth map of sampling sites

2.2. Sample Collection and Processing

Samples were collected by following transect method for each sampling site [12]. The necessary field data including; altitude, locality, habitat and global positioning system coordinates were recorded on the spot. The specimens were collected with iron bar and knife, dried in air and then placed in labeled plastic zipper bags. Samples were brought to the laboratory and roughly cleaned by removing stones, mud and unnecessary vegetation. Separate specimens were properly processed by following standard herbarium techniques for the herbarium deposition. Species were identified by reviewing morphological features of various parts including; sporophyte and gametophyte. Accession number was allotted to each specimen and then deposited in the (AKASH Herbarium), Department of Botany, University of Azad Jammu and Kashmir as a future reference.

2.3. Sample Preparation

Samples were gently washed with distilled water and then allowed to dry in shade. After one week, each sample was grounded into fine powder and then stored for further processing.

2.4. Extract Preparation

Powdered sample was dissolved in respective solvent (methanol, ethanol and water) in 1:10 ratio. Maceration was done for three days and then filtered solution was allowed to evaporate. Crude extract was then stored at 4°C for further use [13].

2.5. Antibacterial Activity

The antibacterial assessment of the species was carried out by using disc diffusion method [14]. For this purpose, two bacterial strains *A. xylosoxidans* and *B. licheniformis* were used. These strains were collected from microbiology laboratory of Quaid- i- Azam University, Islamabad. Fresh culture of the strains was prepared. Crude extract was then re-dissolved in respective solvent. Culture plates including agar and bacterial suspensions were prepared and allowed to solidify for 20 minutes. 6 mm sterile filter paper discs were then carefully placed to their marked position in the petri plate and incubated at 37°C for 24 hours. Also, ampicillin served as a positive control. The zone of inhibition was measured in millimeter with standard ruler. Each step was repeated three times and the average was taken.

2.6. Statistical Analysis

Data obtained was statistically analyzed and values were expressed in terms of mean and standard error of mean of triplicate by using Microsoft excel.

3. Results

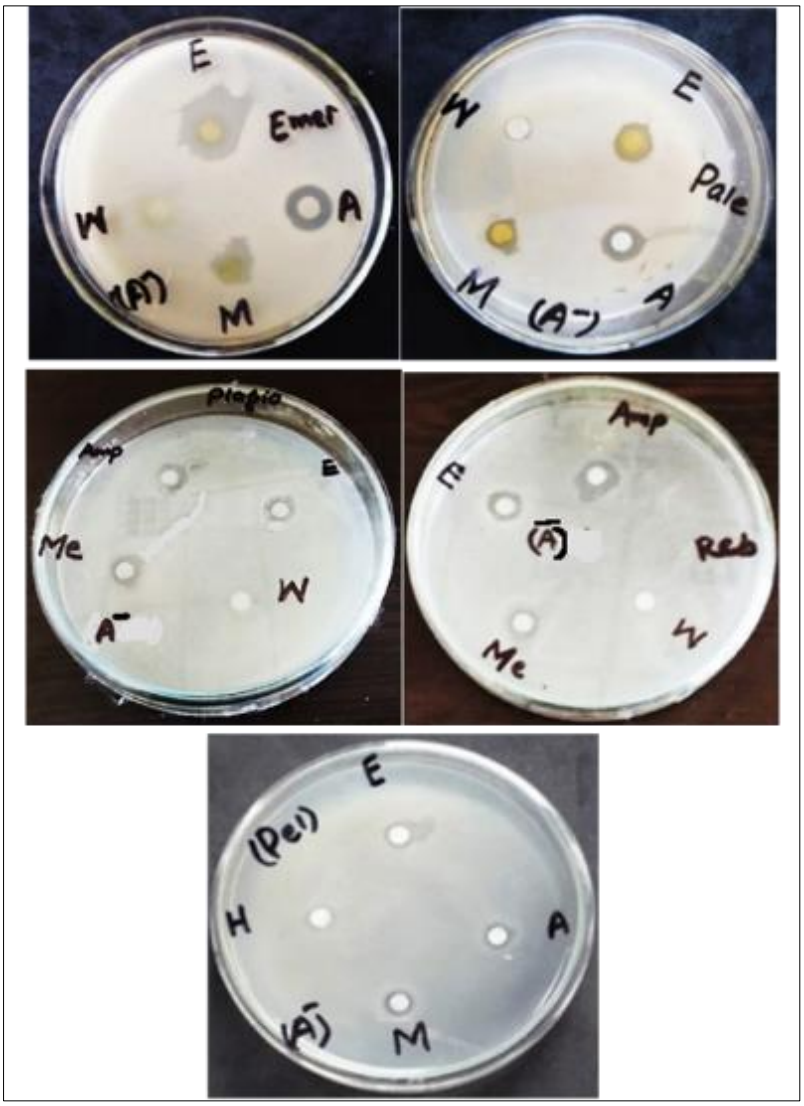
In the current research study, five liverwort species were assessed for antibacterial activity. The results are given in table 1 below.

Table 1 Antibacterial Activity of Liverwort Species

Species Name	Bacterial Strains					
	A. xylosoxidans			***B. licheniformis***		
	Ethanol	**Methanol**	**Water**	**Ethanol**	**Methanol**	**Water**
Marchantia emarginata	18 ±0.33	10 ±1.15	Nil	08 ±0.33	11 ±0.33	Nil
Marchantia paleacea	09 ±0.66	08 ±0.67	07 ±1.15	10 ±0.88	10 ±0.58	09 ±1.15
Plageochasma rupestre	08 ±0.33	07 ±1.15	Nil	07 ±0.33	08 ±0.67	Nil
Reboulia hemisphaerica	09 ±0.88	08 ±0.67	Nil	12 ±0.33	08 ±0.88	Nil
Pellia epiphylla	10 ±1.15	07 ±0.67	Nil	14 ±1.15	07 ±0.33	07 ±0.67
Ampicillin (Control)	23 ±0.33			18 ±1.15		

3.1. Antibacterial Activity of Liverworts against *A. xylosoxidans*

In case of gram- negative bacteria, ethanolic extract of *M. emarginata* showed the maximum and *P. rupestre* showed the minimum zone of inhibition. In methanolic extract, *M. emarginata* showed the highest value and *P. epiphylla* showed the lowest value. In aqueous extract, only *M. paleacea* showed resistance against gram- negative bacteria (Figure 2).

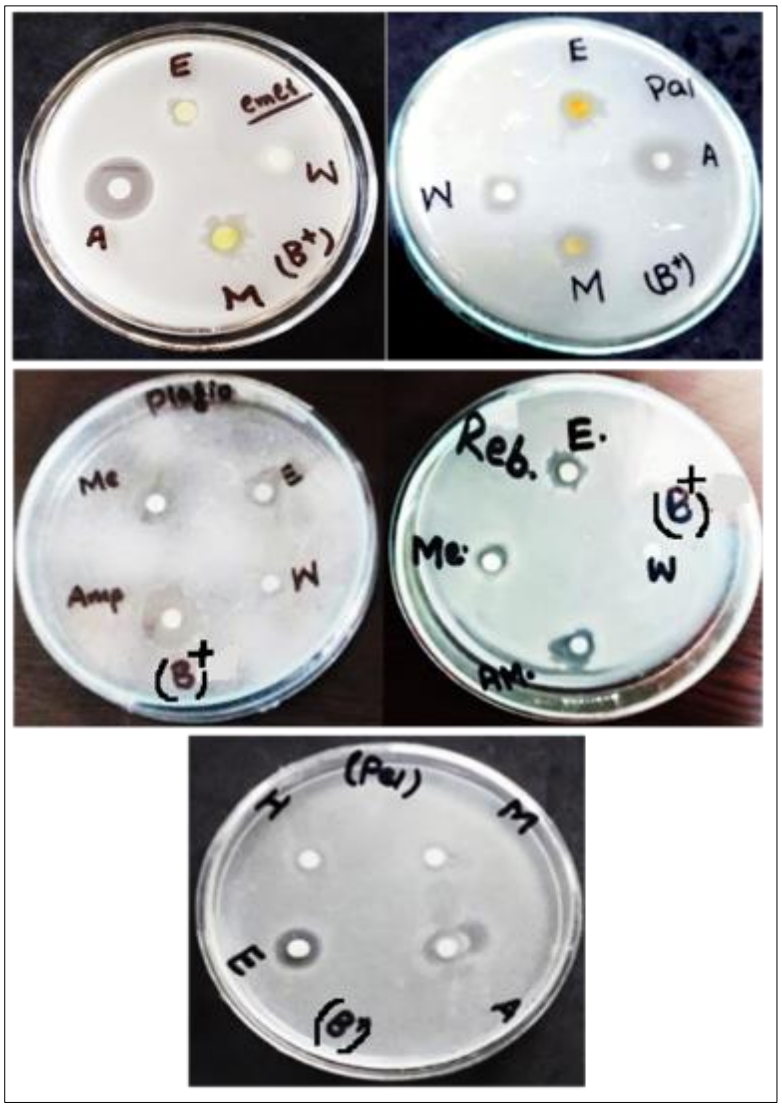


Key: E: Ethanol, M: Methanol, W/H: Water, A: Ampicillin, A⁻**:** *Achromobacter*, Emer: *M. emerginata,* Pale: *M. paleacea*, Plagio: *P. rupestre,* Reb: *R. hemisphaerica* and Pel: *P. epiphylla*

Figure 2 Antibacterial Activity of Liverwort Species against *A. xylosoxidans*

3.2. Antibacterial Activity of Liverworts against *B. licheniformis*

In case of gram- positive bacteria, ethanolic extract of *P. epiphylla* showed the highest activity and *P. rupestre* showed the lowest activity. In methanol extract, *M. emarginata* exhibited highest value and *P. epiphylla* exhibited lowest value. In aqueous extract, *M. paleacea* and *P. epiphylla* showed positive results (Figure 3).



Key: E: Ethanol, M: Methanol, W/H: Water, A: Ampicillin, B+**:** *Bacillus*, Emer: *M. emerginata,* Pale: *M. paleacea*, Plagio: *P. rupestre,* Reb: *R. hemisphaerica* and Pel: *P. epiphylla*

Figure 3 Antibacterial Activity of Liverwort Species against *B. licheniformis***.**

4. Discussion

In the present study, an effort was made to assess the antibacterial activity of liverworts against *B. licheniformis* and *A. xylosoxidans* by using disc diffusion protocol. This method is more reproducible than others as reported by Ibero-American Program of Science and Technology for Development [15]. *B. licheniformis* is a pathogenic bacteria that has been found associated with a range of human disorders such as peritonitis, enteric disease, septicemia, food poisoning and ophthalmitis [16]. *A. xylosoxidans* is an aerobic bacteria with diverse natural and acquired resistance against antibiotics [17]. This species has been reported as nosocomial pathogen that cause numerous human infections like bacteremia, endocarditis, inflammation, cystic fibrosis, pneumonia and urinary tract infection [18]. In current research work, the inhibitory activity of all tested liverwort species was noticeable against tested bacterial strains, although the activity was variable. Stronger and broader spectrum for the zone of inhibition was observed from the ethanol extract of the species. Maximum activity in ethanol is due to the high solubility of phytochemicals in this polar solvent as previously reported [19-21]. The extracts of *M. paleacea* showed noteworthy antibacterial activity against both bacterial strains. Diverse oil bodies have been reported in this species. These oil bodies are the source of lipophilic terpenoids and aromatic compounds which provide resistance against pathogens [22]. *M. paleacea* is also traditionally used in North America, China and Indonesia to cure inflammation caused by various pathogens [23]. Ethanolic extract of *M. emarginata* was most active against *A. xylosoxidans*. The highest antimicrobial potential might be due to the presence of Marchantin A, a cyclic bis (bibenzyl ether). It is a well-known compound which possesses diverse antimicrobial, antioxidant, anti-cancerous and anti-inflammatory activities [24]. Similarly, ethanolic extract of *P. epiphylla* showed

maximum antibacterial activity against *B. licheniformis*. To the best of our knowledge, antibacterial activity of *P. epiphylla* was carried out for the first time. However, ethno-medicinal usage of this species has been reported from literature. In India and Canada, it is used to cure mouth and sore infections which indicate the presence of antimicrobial compounds that provide resistance against pathogens [25]. The results showed that a significant antibacterial activity was noted for the ethanol and methanol extract of *R. hemisphaerica* against both strains. These findings are in conformity with the previous studies [26]. Compared to other species, zone of inhibition was less in case of *P. rupestre.* Lowest activity might be due to the potential differences in bacterial strains and experimental procedures. Present study showed that aqueous extract of most of the species was susceptible for both bacterial strains. This is due to the less solubility of phytochemicals in water as compared to organic solvents [27]. Overall, the antibacterial activity shown by gram- negative bacteria was less than that of gram- positive bacteria. The resistance shown by gram- negative bacteria was due to the presence of impenetrable cell wall [28-29]. This is also important from medicinal point of view because antibacterial compounds which are normally used in therapy are active mainly against Gram-positive bacteria [30].

5. Conclusion

From the above results of current research work, it has been concluded that tested liverworts possess strong defensive ability against pathogenic bacteria. Among all species, *M. emarginata* and *P. epiphylla* showed most promising results. This study project will be helpful to open a fresh avenue for the use of these liverworts species in novel drug development.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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