

## Research Paper

### **Taxonomic Characterization and Antibacterial Activity of some Mosses of District Bagh Azad Jammu and Kashmir (Western Himalaya), Pakistan**

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#### **Abstract**

District Bagh is a biodiversity rich area of Azad Jammu and Kashmir. However, the exploration of bryo-flora has been neglected due to many reasons. The current research project is the first attempt to explore moss flora of the area. Total eight species of mosses, namely *Rhodobryum roseum*, *Funaria hygrometrica*, *Tortula subulata*, *Grimmia montana*, *Hypopterygium flavolimbatum*, *Fissidens adianthoides*, *Phelonotis fontana* and *Erythrodonium julaceum* have been collected from the area. Antibacterial activity of three different solvent extracts (ethanol, methanol, and water) was evaluated against *Bacillus licheniformis* (Gram-positive) and *Achromobacter xylosoxidans* (Gram-negative) by following disc diffusion protocol. All extracts of *P. fontana*, *T. sabulata* and *G. montana* showed positive results, whereas maximum susceptibility was demonstrated by *E. julaceum*. The aqueous extract of most of the species was inactive against both bacterial strains. The current research study will be helpful to make contribution in bryophyte diversity of the area with special reference to Azad Jammu and Kashmir. The results of antibacterial activity showed the presence of strong defensive mechanism in these moss species that can be further investigated for novel drug development.

## **Introduction**

District Bagh is a hilly resort that lies in Western Himalaya with sub-tropical to moist temperate climate. It is situated at a distance of 80 Km from Muzaffarabad (Chughtai et al., 2018). It is located between 33°-36° North latitudes and 73°-75° East longitudes. Total area of Bagh is 1368 square kilometer, which is approximately 10% of total area of Azad Jammu and Kashmir. The general altitude is between 1500-2500m above sea level. Average annual precipitation is 1500mm and temperature is 21°C (Shaheen et al., 2013).

Bryophytes are the second largest group of kingdom plantae that are very small in size and encompasses 20,000-25,000 species worldwide. They are divided in to three major classes, including mosses, hornworts and liverworts (Olasoji et al., 2019). Mosses are the most diverse group of bryophytes which have approximately 17,000 species across the world. They have a dominant gametophytic generation with multicellular rhizoids. Morphological characters that differ them from other two groups of bryophytes include lack of elaters and capsule with stomata and columella (Huttunen et al., 2018).

Due to quick multiplication rate, bacteria acquired resistance against various antibiotics. According to World Health Organization, antimicrobial resistance is among ten major health concerns. Therefore, it possesses a serious challenge for the researchers and pharmaceutical industries to discover proficient antimicrobials and efficient drugs in order to cure numerous nosocomial infections (Peters et al., 2019). However, there are many problems concerned with synthetic drugs like high cost, low production, inefficient access and financial emergency. Most people around the world prefer herbal medicines over synthetic drugs due to their safe use and less side effects (Semerjyan et al., 2021). Medicinal plants possess a variety of secondary metabolites and peptides which are the source of potential antibacterial and antioxidant agents from centuries. It attracts the interest of researchers in their expedition for novel drug development (Chandra and Prasad, 2017; Erb and Kliebensteinb, 2020).

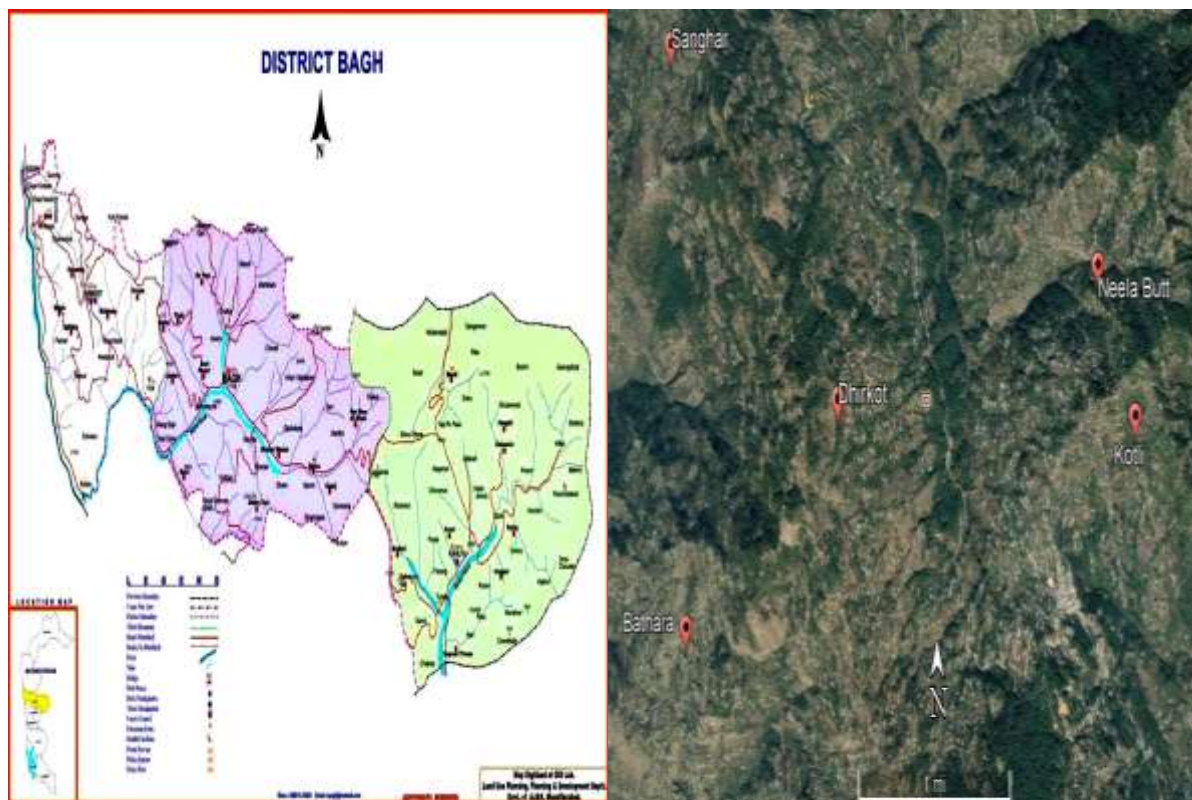
One of the prominent characteristics of bryophytes is that they are usually not harmed by insects and pathogens as they possess a vast array of phytochemicals (Aruna and Krishnappa, 2018; Ludwiczuk and Asakawa, 2020). A variety of biological active compounds have been extracted and characterized from bryophytes comprising; phenyl-propanoid, volatile terpenoids, phenylquinones, flavonoids, bibenzyl, fatty acid derivatives, sulfur-and nitrogen-containing compounds (Sabovljevic et al., 2016; Peters et al., 2019). A wide range of extract from the bryophytes exhibited antimicrobial and antioxidant activities (Cianciullo et al., 2021). However, due to their small size, lack of commercial worth and discreet place in the ecosystem, they are treated as hidden paragon to the world.

To the best of our knowledge, there is no previous record available on the exploration of moss flora of District Bagh. Therefore, the objective of the current research work is to explore moss species of the area and to assess the possible inhibitory potential of these identified mosses.

## Materials and Methods

### Selection of Sites

Five different sites of District Bagh were selected for the collection of mosses including Sanghar, Bathara, Neela Butt Dhirkot and Kotli during March to August, 2020 (Figure 2).



**Figure 1:** Color figure can be visited at (<http://www.google.com/imgres>).

### Sample collection and processing

The necessary field data (altitude, voucher number, Global Positioning System coordinates, locality and habitat) was recorded on the spot (Table 1). The collected specimens were dried in air and placed in plastic zipper bags. The specimens were then brought to the laboratory and gently washed with distilled water. After drying, samples were gently grounded into powder and then stored for further processing. Each specimen was properly processed for herbarium deposition. Species were identified by reviewing taxonomic structures of various parts and also by comparing recorded characters with different bryo-floras. Accession number was assigned (Table 1) and then specimens were deposited to the AKASH Herbarium, Department of Botany, University of Azad Jammu and Kashmir as future reference.

**Figure 2:** Map of study area showing localities of sampling sites.

**Table 1. Geographical information of collected species.**

Voucher Number	Species Name	Family Name	Altitude (m)	Locality	GPS Coordinates	Habitat	Collection Date	Accession Number
SN-001	<i>R. roseum</i>	Bryaceae	1827	Neela Butt	N 34°01.134' E 073°35.194'	Soil in forest floor	21 Mar, 2020	2745
SN-002	<i>F. hygrometrica</i>	Funariaceae	1510	Bathara	N 34°03.801' E 073°31.521'	Rocks, Soil, Building walls	10 Mar, 2020	2756
SN-003	<i>T. subulata</i>	Pottiaceae	1651	Dhirkot	N 34°03.034' E 073°34.611	Soil along road side, rocks	25 Mar, 2020	2750
SN-004	<i>G. montana</i>	Grimmiaceae	1938	Sanghar	N 34°05.211' E 073°33.782'	Rocks	15 May, 2020	2759
SN-005	<i>H. flavolambatum</i>	Hookeriaceae	1800	Sanghar	N 34°01.200' E 073°35.193'	Soil in forest floor	21 Mar, 2020	2762
SN-006	<i>F. adianthoides</i>	Fissidentaceae	1705	Neela Butt	N 34°03.021' E 073°34.836'	Moist Soil	21 Mar, 2020	2755
SN-007	<i>P. fontana</i>	Bartramiaceae	1980	Sanghar	N 34°05.231' E 073°33.732'	Soil of forest floor	16 May, 2020	2753
SN-008	<i>E. julaceum</i>	Entodontaceae	1730	Kotli	N 34°02.998' E 073°34.332'	Epiphyte	20 June, 2020	2743

### **Micro and macroscopic study**

Length and width of various parts i.e., leaf, stem and rhizoids were measured by using standard ruler. Whereas, length and width of spores, peristome teeth and various cells were measured with the help of compound microscope. Macro-morphological features of different parts were examined by using stereomicroscope (Model: IM-SZ-500). Photomicroscope (Model: MT4300H) was used for microscopic observation. For this, fresh slides of different parts were prepared. Light micrographs were also taken with the help of photomicroscope. All measurements were obtained three times and then average was taken.

### **Extract preparation**

Grounded sample was dissolved in separate solvent (ethanol, methanol and water) in 1:10 ratio. Soaking was done for three days, and crude extract was stored at 4°C (Magtoto et al., 2015).

### **Antibacterial activity**

For the assessment of antibacterial activity of mosses, disc diffusion protocol was followed (Ansar et al. 2013). Two bacterial strains *A. xylosoxidans* and *B. licheniformis* were used for this purpose. These bacterial strains were obtained from microbiology laboratory (Quaid-i-Azam University, Islamabad). Fresh culture for each strain was prepared. Crude extract of each sample was then re-dissolved in relevant solvent. Culture plates comprising agar and bacterial suspensions were solidified for 20 minutes. Sterile filter paper discs (6mm) were gently placed to the marked position in the petri plate and then incubated at 37°C for about 24 hours. Ampicillin was used as a positive control. The zone of inhibition was measured in millimeter with standard ruler. Every step was repeated three times and an average was taken.

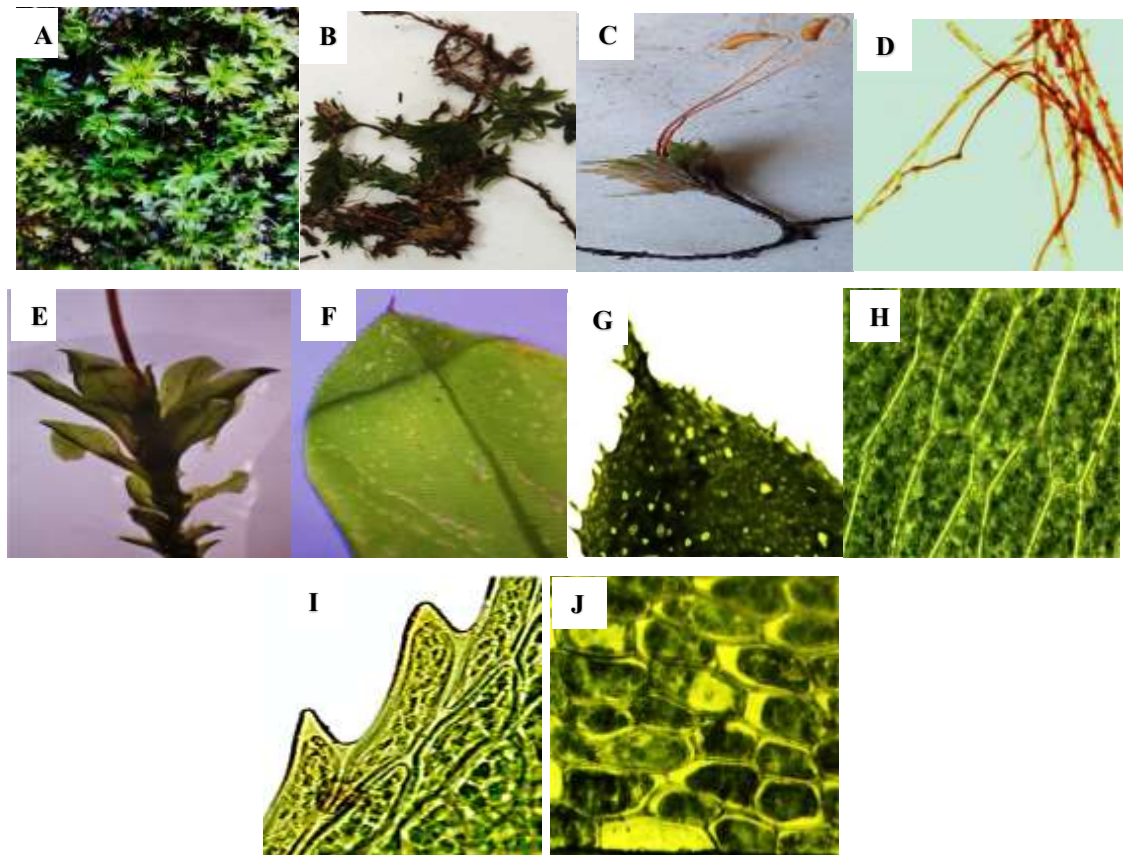
### **Statistical analysis**

The results of antibacterial assay were expressed in term of mean and standard error of mean of three replicates by using Microsoft Excel.

## **Results**

### **1. *Rhodobryum roseum* Hedw.**

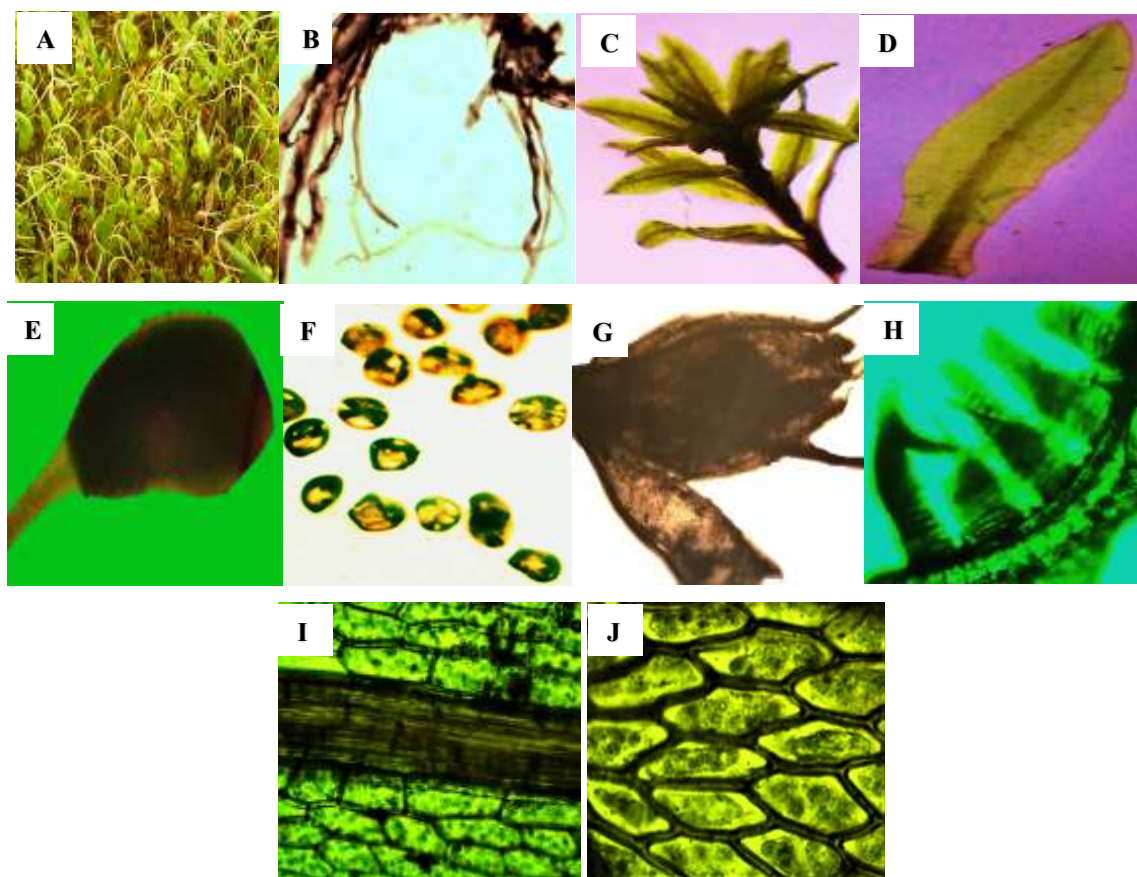
Plant has shining appearance and grows in the form of dense patches. Rhizoids are light brown and 3-5 mm long. Stem is 1-3 cm long, with stem leaves in rosettes, 20-22 in number and light green in color. Leaves are 3- 7.5 mm in length and 1-1.8 mm in width. Margins are revolute up to mid leaf. Apex of the leaf is acute. When the plant is dry, the leaves become curved and expended after rehydration. Perichaetial leaves with costa are sub-percurrent to percurrent, sometime short excurrent. Capsules are elongated and 3-5 mm long. Seta is elongated and reddish in color. Spores are rounded in shape and 16-20 µm long. Marginal cells are elongated, irregular; median cells irregular and allar cells are square to hexagonal (Figure 3).



**Figure 3.** *R. roseum* A) Patch of plant on natural habitat B) Plant when dry C) Single plant with capsule D) Rhizoids (4X). E) Arrangement of leaves F) Leaf (10X). G) Leaf apex (10X). H) Allar cells (40X). I) Marginal cells (40X). J) Median cells (40X).

2. *Funaria hygrometrica* Hedw.

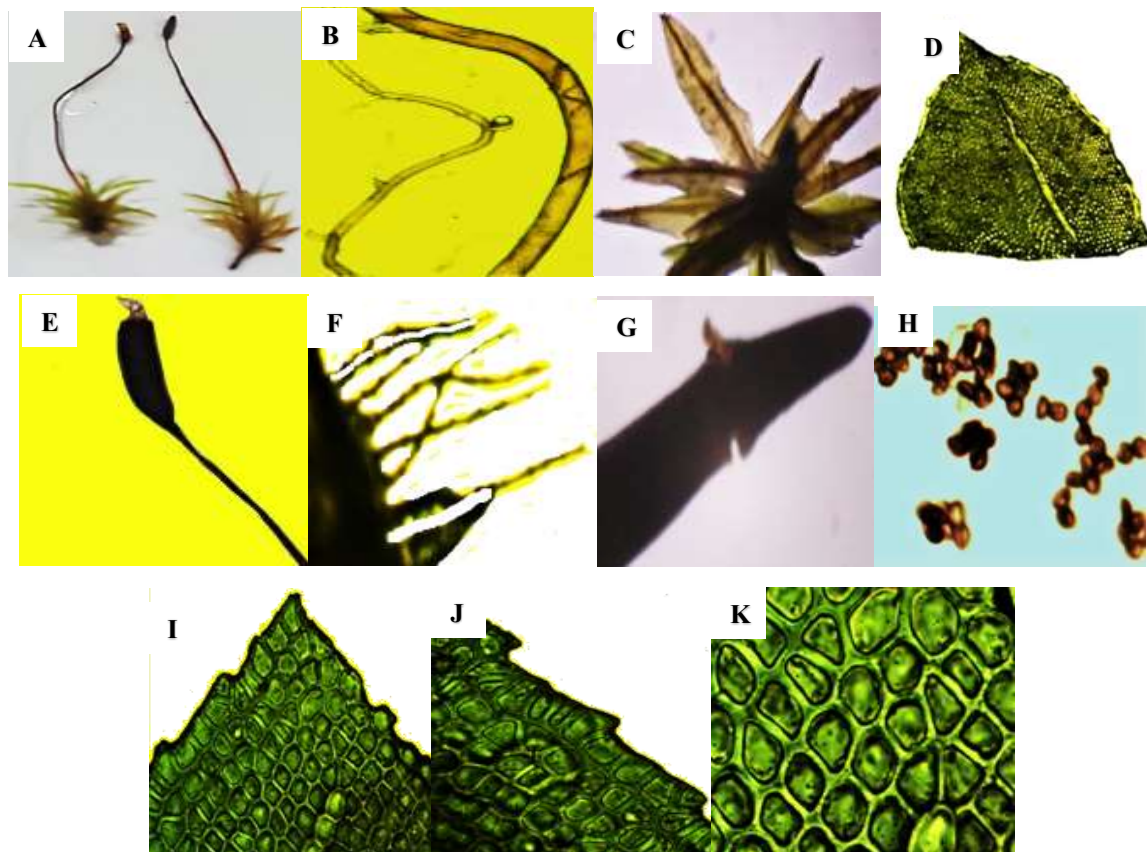
*Funaria* is an acrocorpous moss species that is 4-8 mm long and 3 mm broad, light- yellowish green in color and found in tufts. Rhizoids are smooth that reach up to the height of 4 mm. Stem is short, slender, erect with rosette of leaves. Leaves are bulbiform, crowded with upper leaves that are larger in size, while lower leaves are small. Leaves are usually concave, oblong- ovate to broadly obovate, erect to scatter, 3 mm in length and 1 mm in width. Margins are entire to somewhat serrulate; apex is acute; costa strong, sub-percurrent to short ex-current. Seta is slender, flexuous and 15-30 mm long. Capsule is asymmetrical, pyriform, curved to straight and 2- 3.5 mm long. Operculum is slightly convex; peristome teeth brown, papillose- striate and appendiculate distally. Calyptra smooth and cucullate. Spores are small, spherical in shape, smooth and 12-21  $\mu\text{m}$  long. Marginal cells are irregular whereas, median cells are elongated and hexagonal (Figure 4).



**Figure 4.** *F. hygrometrica* A) Plant habitat. B) Rhizoids (10X). C) Leaf arrangement D) Single leaf (10X). E) Seta and capsule (10X). F) Spores (40X). G) Perichaetial leaves (10X). H) Peristome teeth (10X). I) Costa and median cells (40X). J) Marginal cells (40X).

### 3. *Tortula subulata* Hedw.

*T. subulata* is a perennial and acrocarpous species that is light green to yellowish green in color, medium size (3-3.5 mm) and found in dense turfs. Rhizoids are dark brown, erect, aseptate and 2.5 mm long. Stem is short, cylindrical, and up to 3 mm long. Leaves are oblong-lanceolate to long-elliptical, yellowish in color, arranged in globose fashion, 2.7 mm long and 0.4-0.5 mm wide. Apex is acute; margins are entire, re-curved at the base; costa is short and ex-current. Sexually the species is autoecious. Seta is smooth, elongated, reddish brown in color and 1-3 cm long. Capsule is erect, cylindrical and 4-6 mm long. Peristome teeth are 1700-2000  $\mu\text{m}$  lengthwise. Spores are rounded, finely papillose and 12-18  $\mu\text{m}$ . Marginal cells are irregular in shape and 60-70  $\mu\text{m}$  in length. While, median cells are square to rectangular in shape and 60-65  $\mu\text{m}$  in length (Figure 5).

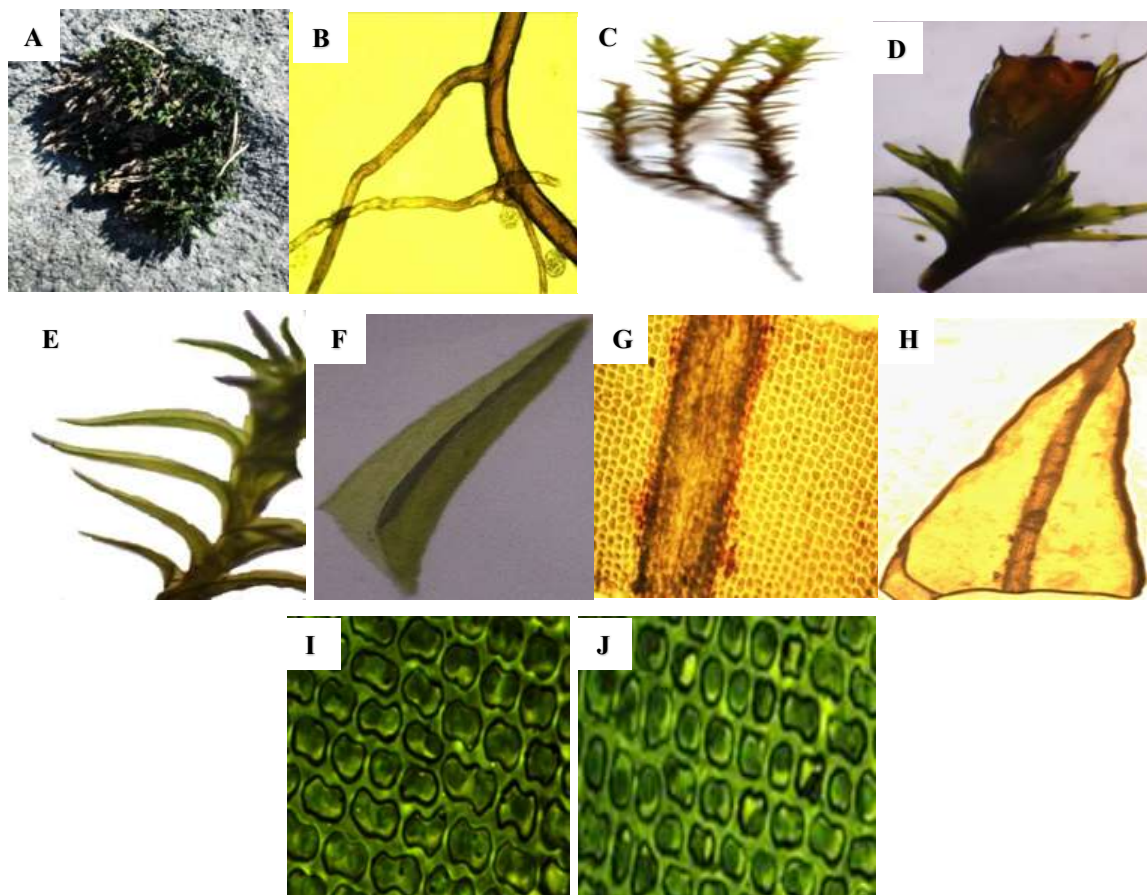


**Figure 5.** *T. subulata* A) Patch of plant on the natural habitat B) Rhizoids (40X). C) Arrangement of leaves D) Leaf (10X). E) Seta and capsule (10X). F) Peristome teeth (40X). G) Operculum (4X). H) Spores (4X). I) Leaf apex (100X). J) Marginal cells (100X). K) Median cells (100X).

#### 4. *Grimmia montana* Bruch and Schimper, Bryol.

Plant appears as yellow green to dark green or nearly blackish when dry and found in silvery cushions. Rhizoids are yellowish to dark brown and 2-2.5 mm long. Stem is 1-1.5 cm long, with weak central stands. Leaves are scarcely lanceolate, sometimes ovate- lanceolate, 1-2 mm long and bowl-shaped keeled. Margins of the leaf are plane, rarely incurved distally, with prominent costa. Sexually, *G. montana* is a dioicous species. Perichaetial leaves are small, seta is straight and 2-3 mm long. Capsule is rarely present, oblong, and yellow to brown. Operculum is rostelliate, while peristome teeth are fully developed. Laminal cells are rounded to irregular in shape and 20-30  $\mu\text{m}$  long. Basal cells are elongated to rectangular and 25-35  $\mu\text{m}$ . Marginal cells are rectangular to quadrate and 40-50  $\mu\text{m}$  in length (Figure 6).

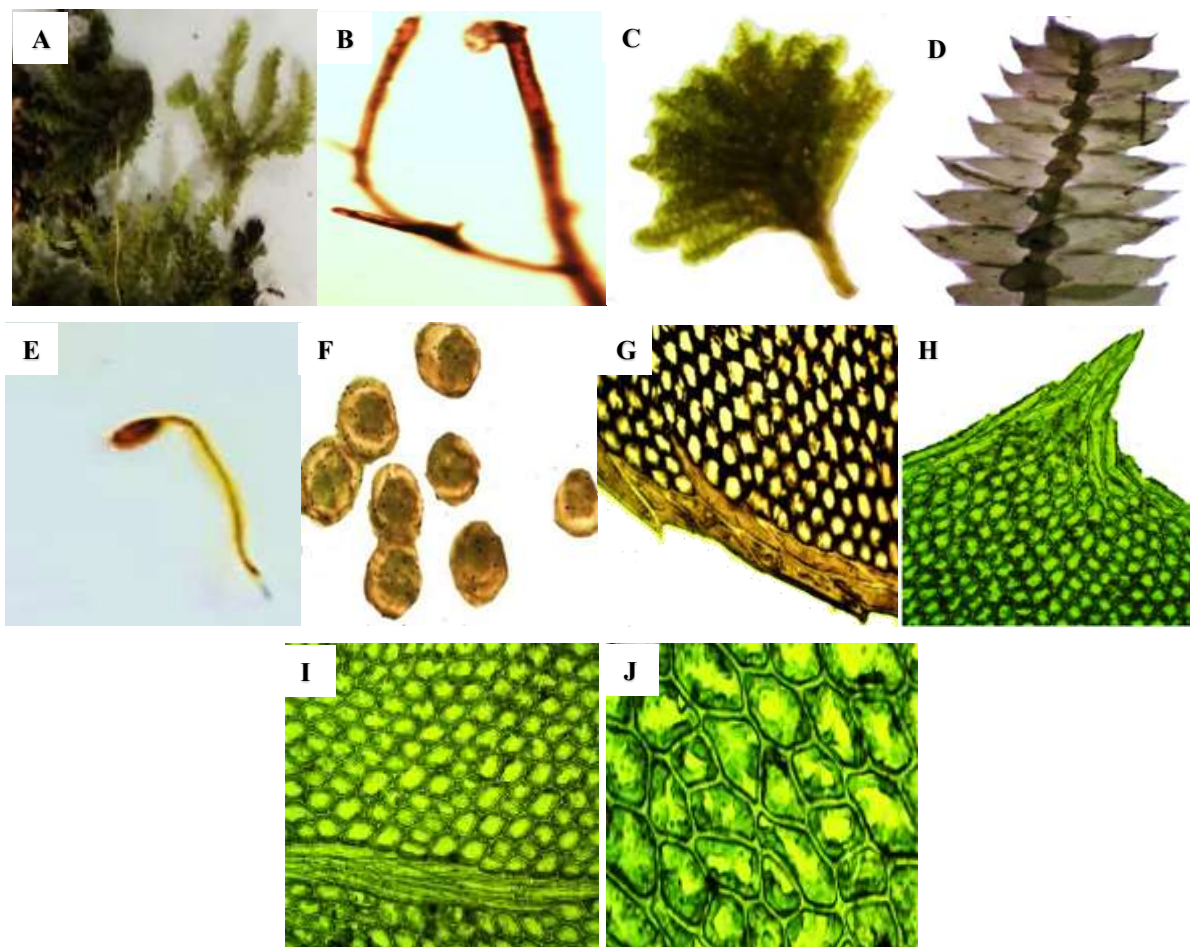




**Figure 6.** *G. montana* A) Plant patch on natural habitat B) Rhizoids (10X). C) Single plant when wet D) Keel E) Arrangement of leaves F) Single leaf G) Costa (4X). H) Leaf (10X). I) Median cells (100X). J) Basal cells (40X).

##### 5. *Hypopterygium flavolimbatum*, Muller & Hal.

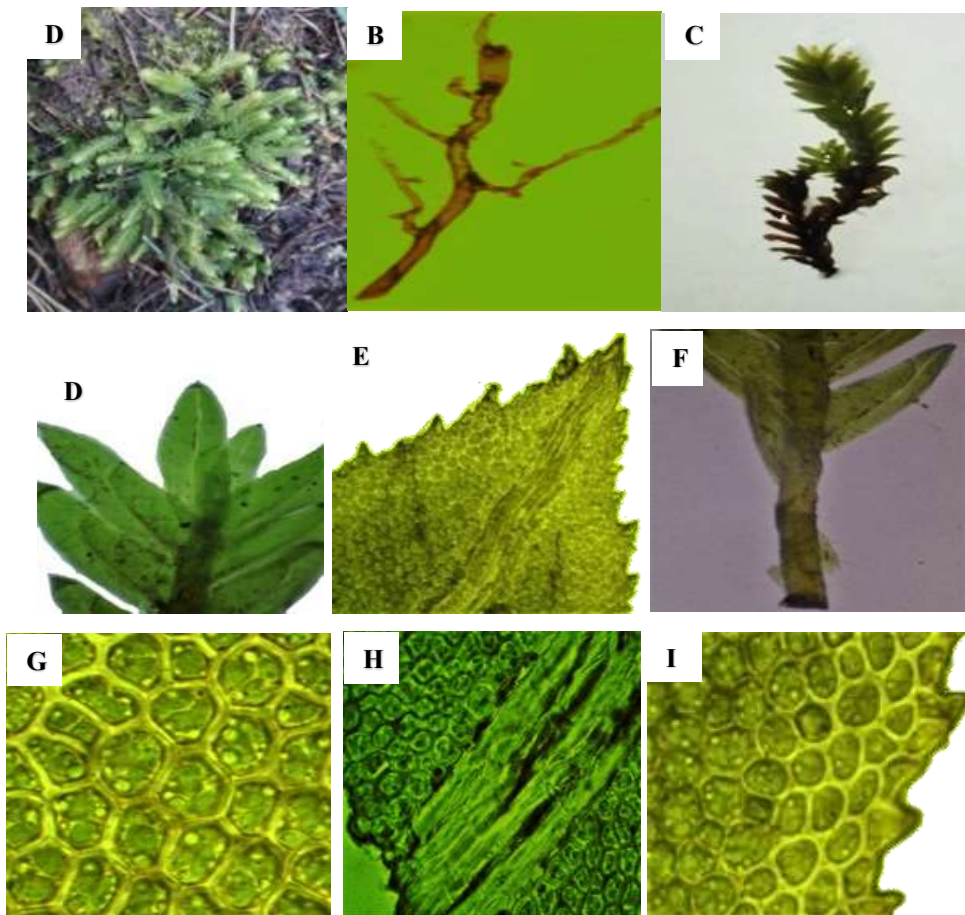
The leaves of *H. flavolimbatum* has shining appearance. Plant is medium size (50-65 mm) and pinnate- umbellate. Rhizoids appear smooth, dark brown, deeply attached with soil and 3 mm long. Stem is light green with hard texture. Leaf has basal fronds and stipe in 8 rows. Leaf is dainty green or yellowish green in color, ovate to acuminate, 1.4-1.8 mm in length and 0.9 mm in width. Margins of the leaf are smooth to serrate in distal, apex is acute- apiculate with costa distributed shortly. Parichaetial leaves are absent. Seta is smooth, papillose distally and 1.5-2.5 cm long. Capsule is oval shape, 1.5 mm and mature in early summer. Operculum is rostrate and 1- 1.5 mm in length. Marginal cells are rhombic in shape and 25-35  $\mu\text{m}$  long. Median cells are quadrate- isodiametric and 30-40  $\mu\text{m}$  (Figure 7).



**Figure 7. *H. flavolimbatum*** A) Plant patch on the natural habitat B) Rhizoids (10X). C) Single leaf D) Arrangement of leaves E) Seta and capsule (10X). F) Spores (10X). G) Marginal cells (10X). H) Leaf apex (10X). I) Costa (40X). J) Median cells (100X).

6. *Fissidens adianthoides* Hedwig.

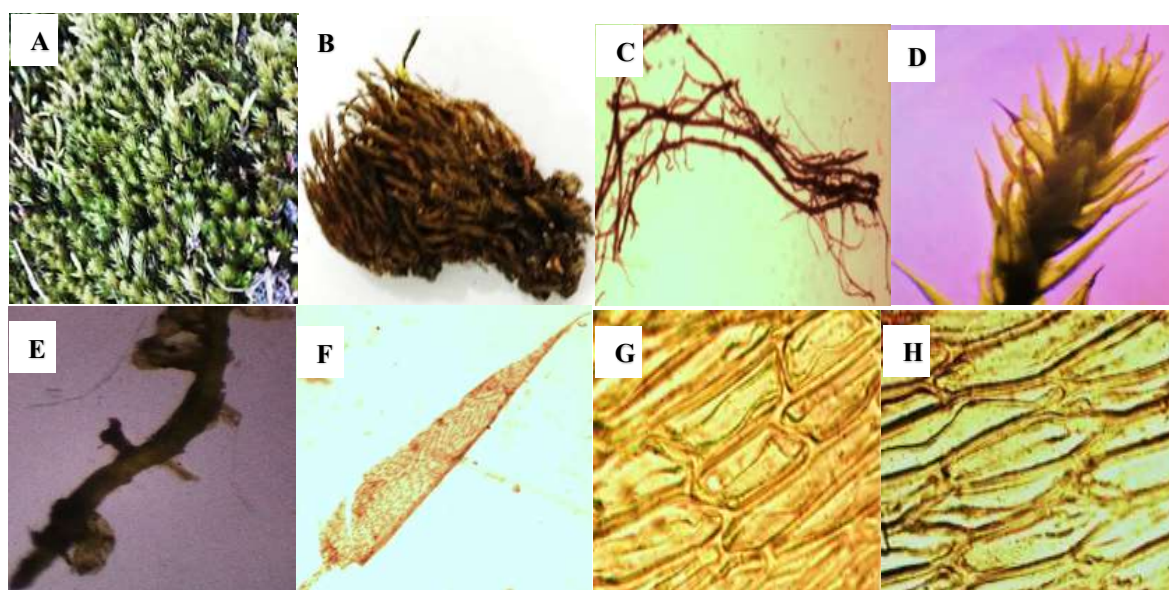
Leaves are light green- yellowish in color, present in turfs and 7-8 cm long. Stem is branched, hard, without axillary nodules and yellowish in color. Leaves occur in 30 pairs, mostly acute or sometime obtuse and elongated- lanceolate. Margins are crenulate-serrulate, sometime elimbate, marginal rows almost 1-5 in number and usually thin. Costa is percurrent and few cells apart from the apical cells of leaf. Sexual stage of plant was not observed during collection. Apical cells are elongated, rounded to rectangular in shape. Marginal cells are irregular to slightly rounded, up to 25  $\mu\text{m}$  long. Median cells are rounded in shape and 30-38  $\mu\text{m}$  long (Figure 8).



**Figure 8.** *F. adianthoides* A) Plant patch on the natural habitat B) Rhizoids (4X). C) Single plant when wet D) Leaf arrangement E) Leaf apex (10X). F) Stem (10X) G) Median cells (40X). H) Allar cells (100X). I) Marginal cells (40X).

7. *Phelonotis fontana* Hedw.

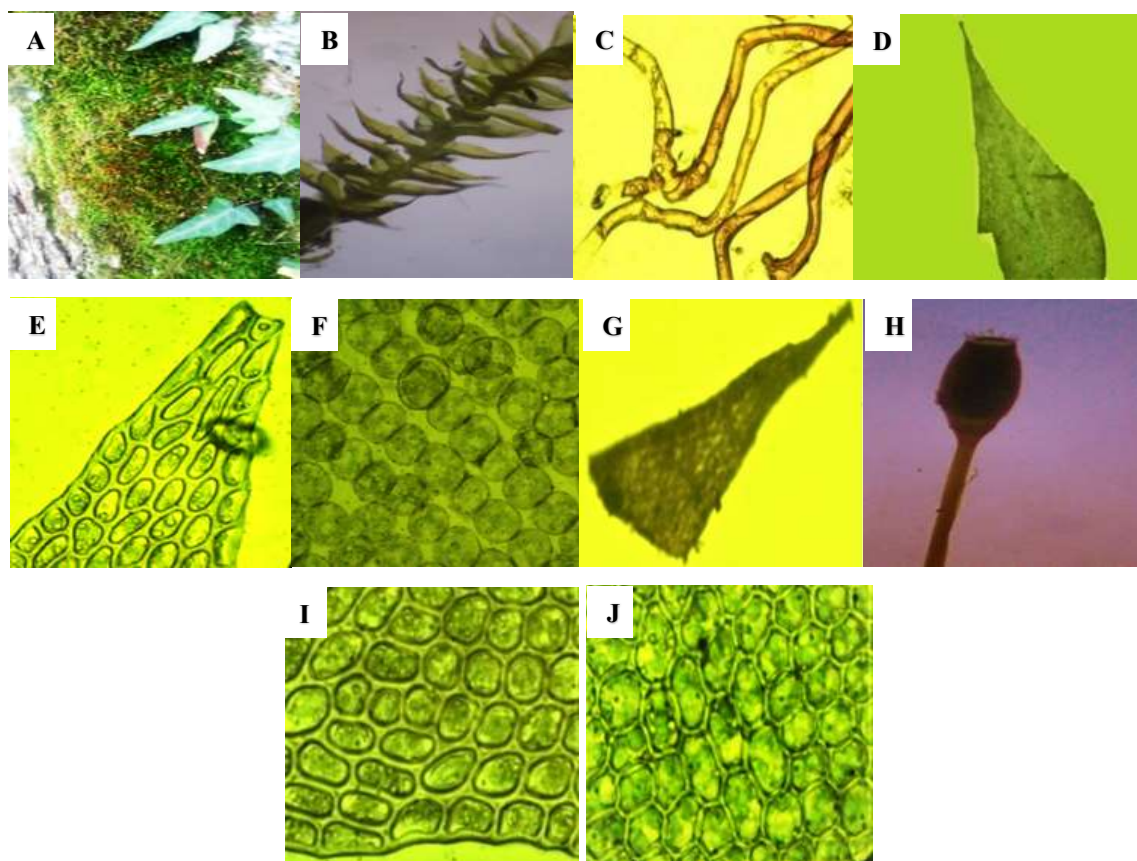
Plant occur in clump to mats, small to moderate in size and reddish- yellowish or greenish in color. The height of the plant is 12-20 cm. Stem is simple, erect or diverged and 12-18 cm in length. Leaves are ovate to lanceolate in shape, yellowish green- brown in color and 0.6-3  $\mu\text{m}$  long. Margins are reevaluate to serrulate. Apex is acute to acuminate. Costa is prominent and ex-current. The reproductive parts of plant were not collected during field survey. Transverse section of leaves indicated that the alar cells are regular hexagonal and 30-40  $\mu\text{m}$  long. Basal cells are rectangular to oblong, 15-30  $\mu\text{m}$  long and 10-12  $\mu\text{m}$  wide. Median cells are elongated, hexagonal and 50  $\mu\text{m}$  long (Figure 9).



**Figure 9.** *P. fontana* A) Plant patch on its natural habitat B) Dry patch C) Rhizoids (4X). D) Arrangement of leaves (10X). E) Stem (10X). F) Leaf (4X). G) Median cells (100X). H) Marginal cells (100X).

#### 8. *Erythrodontium julaceum* Hampe.

Plant is rigid, dark green, present in mats, carticolous- saxicolous and slender to discreetly robust. The length of the plant is 18-20 mm. Stem is light green in color, elongated, prostrate and 8-12 mm in length. Leaves are dense, terete, ovate- lanceolate, present parallel to one another concave, oblong to ovate, 1.05 mm long and 0.6 mm wide, with narrow and short tip. Costa is yellow and 40-50% of the leaf length. Margins of the leaf are entire to serrulate. Plant is sexually dioicous. Seta is erect, dark brown in color and 5 mm long. Capsule is elongated and appear orange to yellow during maturation. Perichaetial leaves small in size. Operculum is conical, usually with tapering end, 5 mm in width and 8mm in length. Calyptra is long and 5 mm in length. Spores are spherical in shape, brown in color and 8  $\mu\text{m}$  long. Marginal cells are elongated, kidney shape and 8-10  $\mu\text{m}$  in length. Median cells are hexagonal and 7-9  $\mu\text{m}$ . Apical cells are long, oval- dome shape, cylindrical and 12-15  $\mu\text{m}$  long (Figure 10).



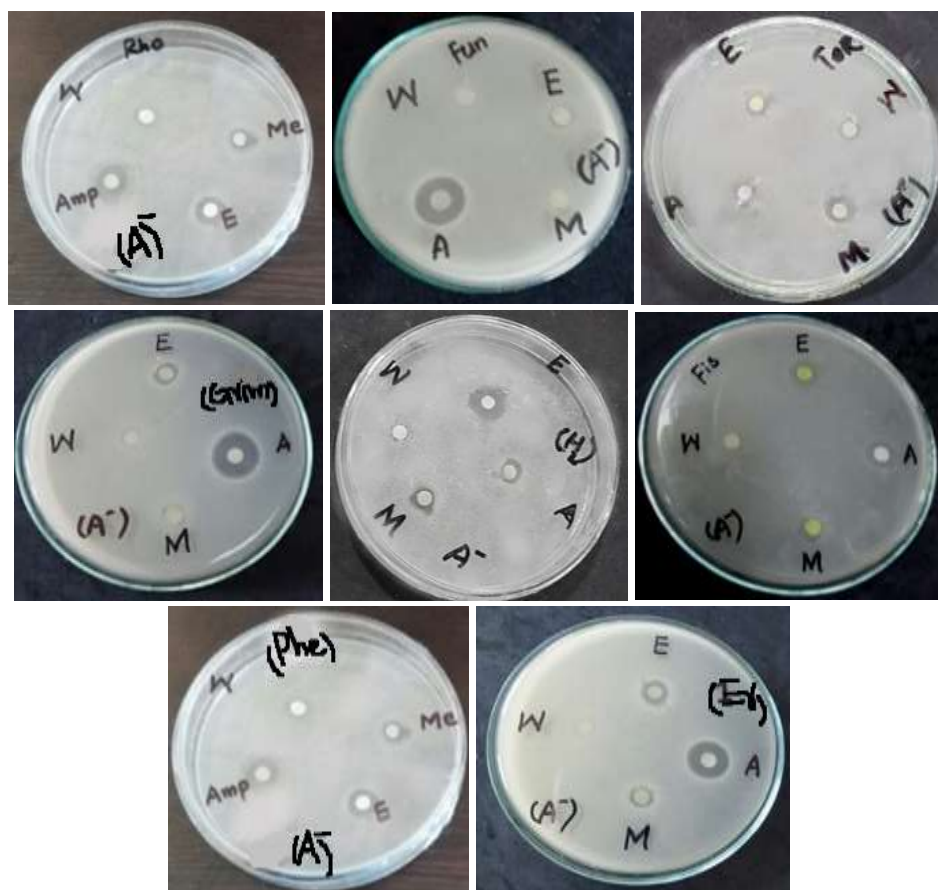
**Figure 10.** *E. julaceoum* A) Natural habitat of plant B) Leaf arrangement C) Rhizoids (4X). D) Leaf (10X). E) Leaf apex (10X). F) Spores (10X). G) Operculum (4X). H) Seta and capsule I) Marginal cells J) Median cells (40X).

### Antibacterial Characterization

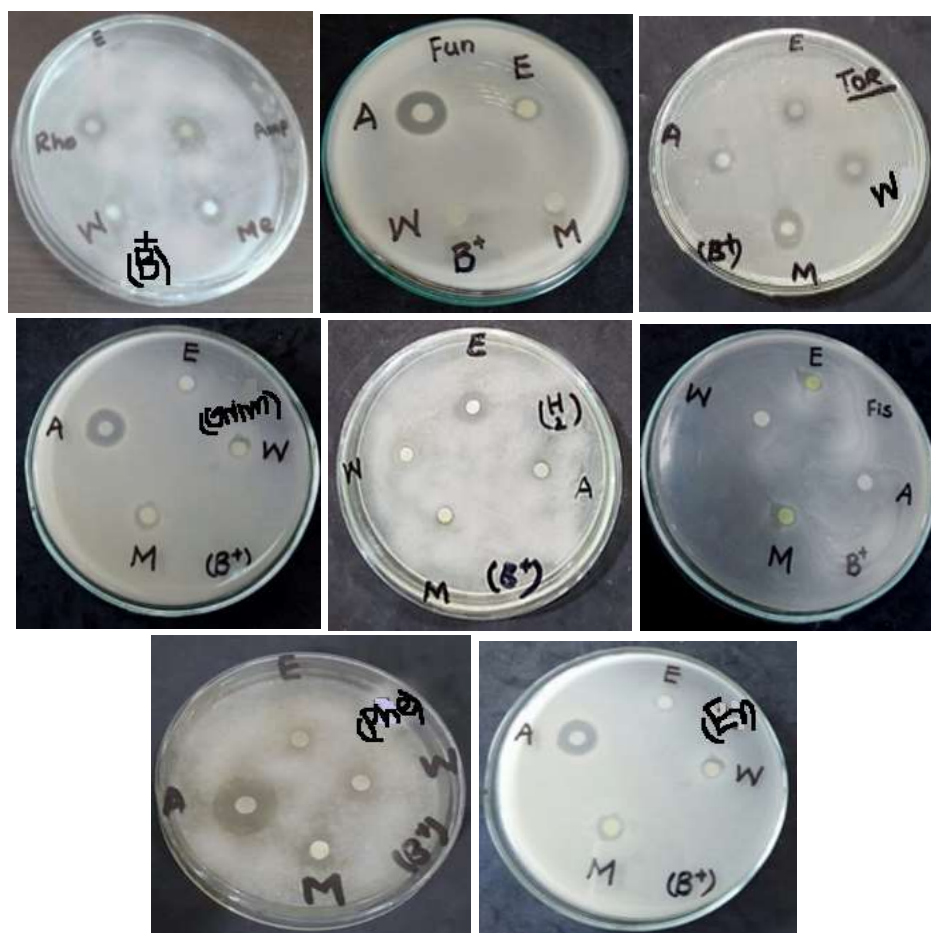
The results of antibacterial activity showed that crude extract of moss species has significant influence on the tested bacterial strains (Table 2). Stronger and diverse spectrum for the zone of inhibition was observed with ethanol extract. The maximum antibacterial activity for ethanol extract was observed in *H. flavolimbatum* ( $14 \pm 0.67$ ) against *A. xylooxidans*. *R. roseum* ( $12 \pm 0.33$ ) and *P. fontana* ( $10 \pm 0.67$ ) also exhibited significant results. For methanol extract, highest zone of inhibition was observed in *T. subulata* ( $14 \pm 0.88$ ) against *B. licheniformis*. Whereas, *R. roseum*, *H. flavolimbatum* and *P. fontana* also showed significant results against both bacterial strains. In aqueous extract, only *T. subulata* and *P. fontana* showed resistance.

**Table 2. Antibacterial activity of moss extracts in different solvents.**

Species Name	Bacterial Strains					
	<i>A. xylosoxidans</i>			<i>B. licheniformis</i>		
	Ethanol	Methanol	Water	Ethanol	Methanol	Water
<i>R. roseum</i>	12 ±0.33	09 ±0.33	-	11 ±1.15	12 ±0.33	-
<i>F. hygrometrica</i>	07 ±0.88	-	-	07 ±0.88	-	-
<i>T. subulate</i>	07 ±1.15	09 ±0.88	07 ± 0.58	08 ±0.58	14 ±0.88	09 ±0.33
<i>G. montana</i>	09 ±0.58	07 ±0.67	07 ±0.33	07 ±0.33	08 ±0.88	07 ±0.58
<i>H. flavolimbatum</i>	14 ±0.67	09 ±1.15	-	14 ±0.67	08 ±0.58	-
<i>F. adianthoides</i>	08 ±0.88	07 ±0.58	-	09 ±0.33	09 ±0.33	-
<i>P. fontana</i>	10 ±0.67	08 ±0.33	07 ±0.33	07 ±0.58	10 ± 0.67	08 ±0.58
<i>E. julaceum</i>	10 ±0.33	07 ±0.33	-	-	08 ± 0.67	07 ±1.15
Ampicillin (Control)		23 ±0.33			18 ±1.15	



**Figure 11.** Antibacterial Activity of Moss Species against *A. xylosoxidans* Key: E: Ethanol, M: Methanol, H/W: Water, A: Ampicillin, A: *Achromobacter*, Rho: *R. roseum*, Fun: *F. hygrometrica*, Tor: *T. subulate*, Grim: *G. montana*, H<sub>2</sub>: *H. flavolimbatum*. Fis: *F. adianthoides*, Ph: *P. fontana*, Er: *E. julaceum*.



**Figure 12.** Antibacterial Activity of Moss Species against *B. licheniformis* Key: E: Ethanol, M: Methanol, H/W: Water, A: Ampicillin, A<sup>-</sup> *Achromobacter*, Rho: *R. roseum*, Fun: *F. hygrometrica*, Tor: *T. subulata*, Grim: *G. montana*, H<sub>2</sub>: *H. flavolimbatus*. Fis: *F. andianthoides*, Ph: *P. fontana*, Er: *E. julaceum*.

## Discussion

District Bagh is a biodiversity rich area including lower non-vascular plants. However, bryophytes are mostly mistreated in biodiversity studies due to their minuscule nature and identification problem. The current research work is the first effort to collect moss species of the area and to study their taxonomic and antibacterial characteristics. Eight species were collected from the area between 1510-1980m altitudes. These species belonging to families; Bryaceae, Funariaceae, Pottiaceae, Grimmiaceae, Hookeriaceae, Fissidentaceae, Bartramiaceae and Entodontaceae. The maximum number of species was collected from Sanghar that is located at highest altitude. It has been find out that diversity of bryophytes increased along the altitudinal gradient (Bergamini et al., 2001). During morphological study, various parts of the leaf like tip, margins, base and costa were thoroughly examined with the help of light microscope. Similarly, different parts of sporophyte like, foot, seta and capsule were also investigated. The dissimilarities in the above mentioned parts are considered as the base line of discrimination between different genera.

In the current research work, an effort was also made to evaluate the antibacterial potential of moss species against *A. xylooxidans* and *B. licheniformis* by disc diffusion procedure. This protocol

was selected because it is more reproducible as compared to others (Sequeda et al., 2019). The results of antimicrobial assessment showed that different moss extracts retain different impact on the growth of bacteria. The potential difference of various extract for the zone of inhibition is due to the presence of wide range of antimicrobial compounds with different intensity and action spectrum as described by (Kandpal et al., 2016).

It has been find out that ethanol extract had highest potency as compared to other solvents against both bacterial strains. These results are in conformity with other research works that also reported stronger zone of inhibition in the ethanol extract (Negi and Chaturvedi 2016; Kandpal et al., 2016). All extracts of *P. fontana*, *T. sabulata* and *G. montana* showed positive results against both bacteria. Antibacterial activity of these species was carried out for the first time. Native people of India and North America used *P. fontana* for the healing of wounds and burns (Mohandas and Kumaraswamy, 2018) It is also used to cure adeno-phyryngitis and as antidotal and antipyretic (Greeshma et al., 2016). This indicates the presence of bioactive compounds in this species. Of all the assessed species, maximum susceptibility was demonstrated by *E. julaceum* and *F. hygrometrica*. This may be attributed to the lower number of potent phytochemicals present in these species. *R. roseum* showed stronger activity in methanol and ethanol extracts against both bacteria. These findings are in conformity with the previous study (Singh et al., 2020).

In general, gram- negative bacteria showed more resistance as compared to gram- positive bacteria. This is due to the existence of impenetrable cell wall in gram- negative bacteria (Altuner et al., 2014; Muhsinah et al., 2022). The aqueous extract of most of the species was inactive against both gram- positive and gram- negative bacteria. This may be because of the reason that the phytochemicals are slightly soluble in water as compared to organic solvents. Similar results have been discussed by (Nikolajeva et al., 2012). Aqueous extract of *T. sabulata* showed highest activity. These results agree with the findings of (Saxena and Yadav 2018). This study showed that *T. sabulata* might have some phytochemical compounds which could be dissolve in water.

## **Conclusion**

The current research project is the first attempt to explore moss flora of District Bagh, Azad Jammu and Kashmir. Shape of leaf, costa, margins, leaf apex and cell shape proved to be useful character to distinguish between different species. Results showed that all species of mosses has the ability to inhibit the growth of bacteria. Present study will open a fresh Avenue for the future researchers to use these identified moss species for biochemical characterization. Evaluation of antibacterial activity will be fruitful to provide a baseline for the use of mosses in novel drug development.



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