

Antimicrobial activity of *Taxithelium nepalense* (Schwägr) Broth in various solvents by agar well diffusion method

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Abstract: Bryophytes are the primitive non-vascular plants seen in wet areas. They possess unique phytochemical compounds having therapeutic potential. Therefore, the present study was carried out to analyze the phytochemicals in the bryophyte, *Taxithelium nepalense* (Schwägr) Broth. in various solvents like acetone, ethyl acetate and distilled water and also check the antimicrobial effect against ten bacteria. Studies reveal that *T. nepalense* possesses valuable secondary metabolites like alkaloids, phenols, xanthoproteins etc. The extract of acetone *Taxithelium nepalense* shows highest antimicrobial activity against *Salmonella enteritis* and least activity against *Shigella sonnei* in ethyl acetate extract.

Keywords: Antimicrobial activity, Agar well diffusion method, Soxhlet extraction, Zone of inhibition.

1. INTRODUCTION

Bryophytes are the simplest and primitive plants occupy the position between thallophyta and pteridophyta. Bryophytes have been investigated extensively for active constituents and pharmacological activity. They are antimicrobial activity (Mitre *et al.*, 2004), anti-cancerous activity (Xiao *et al.*, 2004), anti-oxidant capacity (Abhijith Dey and Jitendra Nath De, 2012) etc. Several research works are carried out on the pharmacological effect of the bryophytes (Neelam Mewari and Padma Kumar, 2008, Kanga *et al.*, 2007)

Infectious diseases are communicable or contagious resulting from the infection of growth of pathogens like bacteria in an individual organism. Now a days infectious diseases remain one among the leading cause of death worldwide. In recent years there is an important role for plants with antimicrobial activity, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms (O'Neill, 2014). Researchers have been conducted various experiments with the extracts of various plants for screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. The mosses and liverworts are medicinal plants and are said to possess certain biological activity and effects Garnier *et al.*, 1969; Suire, 1972; Ding, 1982; Wu, 1982; Ando and Matsuo, 1984). *Taxithelium nepalense* is a common corticolous bryophyte mostly seen on the wet areas and also widely distributed in India, China and Philippines etc. (Gangulee's flora of Western regions of mosses). In this study we studied on the antibacterial effect of the bryophyte *Taxithelium nepalense* (Schwägr) Broth. against ten bacterial culture.

2. MATERIALS AND METHODS

Collection of bryophyte

Collection was made as far as possible with the help of suitable instruments (chisel, knife etc). Collected bryophyte is temporarily stored in plastic bags to prevent desiccation and the protection of reproductive parts and the specimens deposited in the herbarium of Bishop Moore College Mavlikkara (BMC.B-05)

Identification of bryophyte

Collected bryophyte is carefully separated from unwanted substances and washed thoroughly with distilled water. Identification of bryophyte was made with the help of suitable literature like Gangulee's mosses of eastern India and adjacent regions. The identification was mainly based on morphological, anatomical and reproductive characters.

Preparation of the extract of *Taxithelium nepalense*

Taxithelium nepalense was collected from the various places of the Alappuzha district. The identified plant was washed and air dried (especially with sporophyte). Dried plant is powdered and it is dissolved in a suitable solvents (here acetone, ethyl acetate and distilled water are used) for the extraction of the phytochemical compounds using Soxhlet apparatus. After 24 hours of the continuous cycle in the apparatus, we got the extract in various solvents (William B. Jensen, 2007). Extracts are used for the phytochemical analysis and antimicrobial screening

Phytochemical analysis of the bryophyte

The extracts of bryophyte were subjected to phytochemical analysis using methodology of Sofowora (1982) and Kepm.

The major pharmaceutically valuable phytochemical compounds investigated in the present study were:

- ❖ Alkaloids
- ❖ Carboxylic acids
- ❖ Coumarins
- ❖ Flavonoids
- ❖ Phenols
- ❖ Proteins and free amino acids
- ❖ Quinones
- ❖ Resins
- ❖ Saponins
- ❖ Sterols , phytosterols and triterpenoidal sapogenins
- ❖ Tannins
- ❖ Xanthoproteins
- ❖ Sugars

Antimicrobial activity of bryophyte

Bacterial culture

Ten bacterial strains are used to test the antimicrobial effect of the bryophyte extract. They are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Xanthomonas campestris*, *Mycobacterium* spp. etc.

Screening of antimicrobial activity

Antimicrobial activity is accessed by agar well diffusion method (Perez *et al.*, 1990). Steps involved in the screening are

1. 10 sterile petriplates are maintained
2. Prepare agar medium (1.6g Mueller Hinton agar and 0.5 g agar bacteriological dissolved in 200 ml distilled water) and sterilize the medium in autoclave
3. Pour the agar medium to the petriplate with the thickness of 0.5 cm. wait for few minutes to solidify.
4. Prepare the wells using well cutter in .6 cm diameter.
5. Swab the bacterial cultures on the agar plate.

6. Pour the extract (dissolved in the DMSO solution) to the wells.
7. Place the antibiotic disc in the center of the wells.
8. Incubate the extract filled petriplates for 12 hours in the incubator.
9. Measure the zone of inhibition.
10. Compare the diameter of inhibited areas of different extract.

3. RESULT AND DISCUSSION

The collected sample was identified as *Taxithelium nepalense* (Schwägr) Broth. and it belongs to the family sematophyllaceae, order hypnobryales, class musci. These are seen in different habitats depending upon the environmental conditions. A very little studies were conducted on this bryophyte (Plate-1).

Phytochemical analysis of *Taxithelium nepalense*

The study shows that, bryophyte extracts contain alkaloids, flavonoids, quinones, sugars, proteins and amino acids etc. but do not contain carboxylic acids, resins, steroids and saponins etc. (**Table: 1**) Presence of the common compounds reveals that, bryophytes shows various biological activities as similar to that of higher plants.

Table 1: Phytochemical analysis of bryophyte

Phytochemical Constituents	<i>Taxithelium nepalense</i>		
	A	EA	W
Alkaloids	-	+	-
Carboxylic acids	-	-	-
Coumarins	+	+	-
Flavonoids	-	-	-
Phenol	+	+	-
Proteins & aminoacids	-	+	-
Quinones	-	-	-
Resins	-	-	-
Saponins	-	-	-
Steroids	-	-	-
Tannins	+	-	-
Xanthoproteins	-	+	-
Sugars	+	+	+

Antimicrobial activity of *Taxithelium nepalense*

Table: 2 shows the antimicrobial activity of the *Taxithelium nepalense* (Schwägr) Broth. Acetone extract of *Taxithelium nepalense* shows highest activity against *Salmonella enteritis* is 19 mm and the least activity against *Enterobacter aerogense* is 12 mm. The ethyl acetate extract of *T. nepalense* shows maximum activity against *Salmonella enteritis* is 17 mm and the lowest activity shows against two bacteria like *Xanthomonas campestris* and *Mycobacterium* spp. is 12 mm. Distilled water extract of *T. nepalense* shows highest activity against 3 bacteria like *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritis* is 14 mm and the least activity shows against *Shigella sonnei* and *Enterobacter aerogenes* is 11 mm.

From this study we can conclude that *Salmonella enteritis* shows the highest activity in acetone, the zone of inhibition is 19 mm. The second highest activity shown by the *Escherichia coli*. the zone of inhibition is 18 mm. Also *Shigella sonnei* shows the least activity in ethyl acetate. Its zone of inhibition is 10 mm. Here the chloramphenicol is used as the positive control.

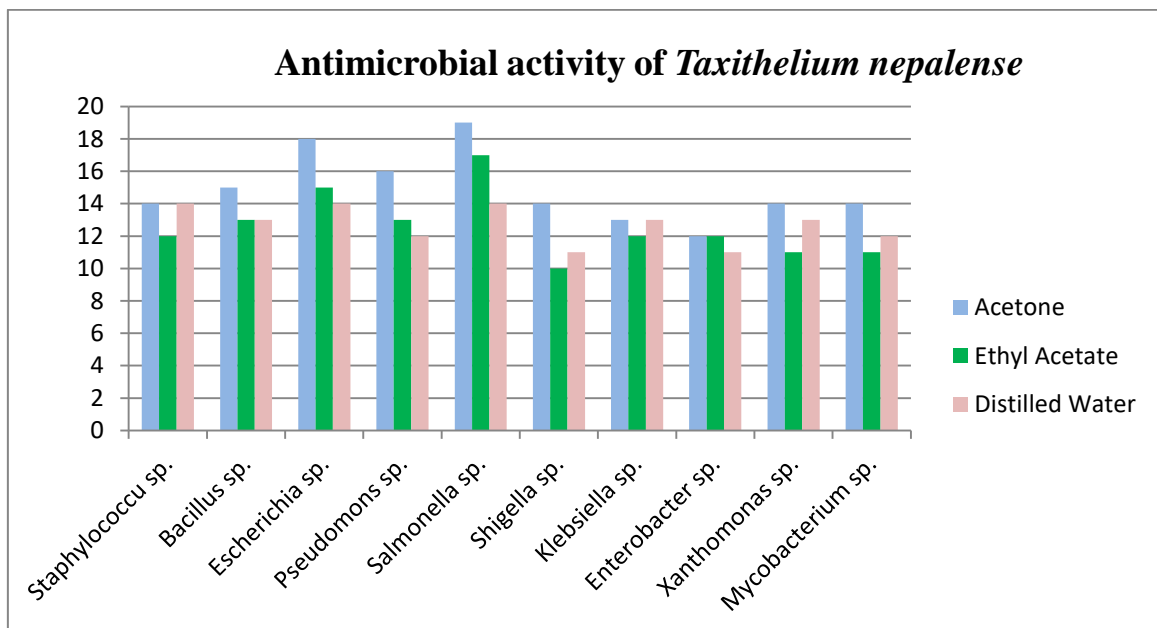
Table 2: Antimicrobial activity of *Taxithelium nepalense*

ORGANISMS	ACETONE	ETHYL ACETATE	DISTILLED WATER	DMSO
<i>Staphylococcus aureus</i>	14	12	14	-
<i>Bacillus subtilis</i>	15	13	13	-
<i>Escherichia coli</i>	18	15	14	-
<i>Pseudomonas aeruginosa</i>	16	13	12	-
<i>Salmonella enteritis</i>	19	17	14	-
<i>Shigella sonnei</i>	14	10	11	-
<i>Klebsiella pneumoniae</i>	13	12	13	-
<i>Enterobacter aerogenes</i>	12	12	11	-
<i>Xanthomonas campestris</i>	14	11	13	-
<i>Mycobacterium</i> spp.	14	11	12	-

In this study the identified bryophyte *Taxithelium nepalense* is selected for phytochemical screening and other biological studies. Bryophytes rank second after the flowering plants, among major group of land plants (Crum, 2001) with an estimated 20,000 species worldwide. They are found in all ecosystems except marine but their ecological roles are significant. Only limited works are carried out under this particular group hence few information is available in different journals. This is because the difficulties in the collection, preservation and further process.

Bryophytes normally grow in humid or damp habitats, they are relatively free from microbial attacks because, which constitute small molecules of secondary metabolites (Asakawa Y, 2001). Antimicrobial activities of crude extracts of bryophyte were screened by using solvents like acetone, ethyl acetate and distilled water against 10 different bacteria, from this results it is clear that all the crude extracts are found against various microorganisms. The extracts of *Taxithelium nepalense* in acetone shows highest antimicrobial against *Salmonella enteritis* (zone of inhibition is 19 mm). So this bryophyte extract can be used in medical field for future (Plate 2).

Graph: 1

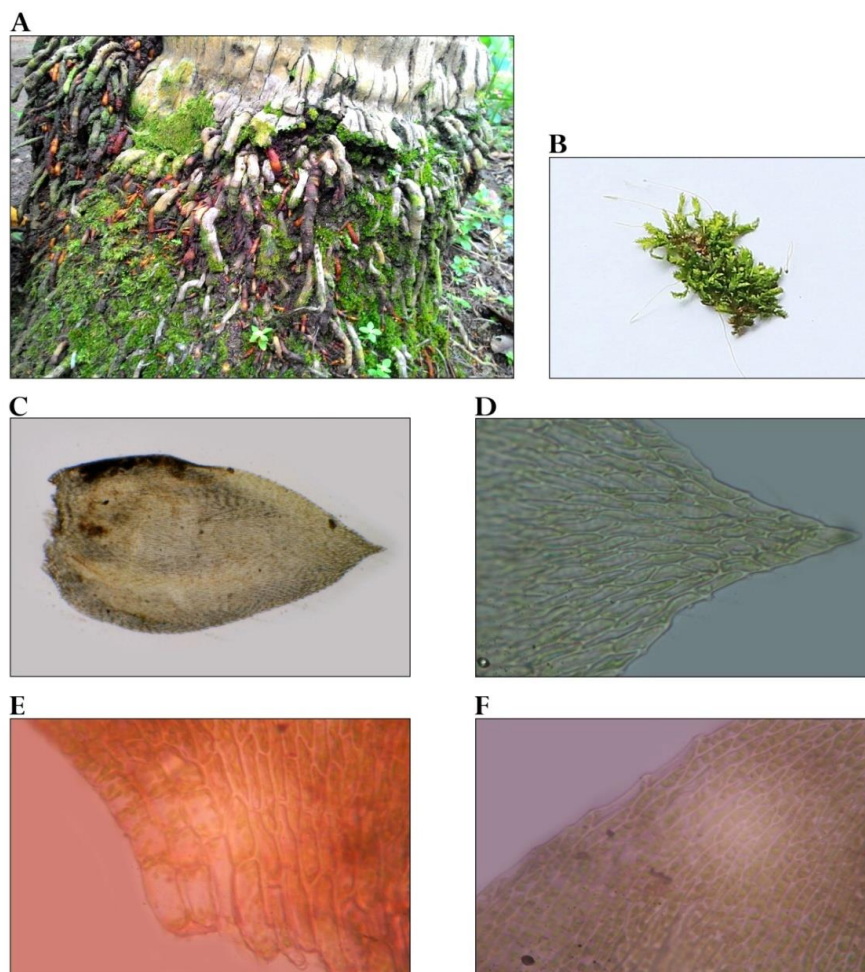


4. SUMMARY AND CONCLUSION

Bryophytes rank second after the flowering plants among the major group of green land plants. Bryophyte was identified based on their morphological, anatomical and reproductive characteristics. Phytochemical analysis reveals that bryophyte extract contains alkaloids, coumarins, phenol, proteins, aminoacids, xanthoproteins and sugars etc. Traditionally medicinal plants are used for the treatment of various diseases due to the phytochemical compounds present in it. Our study reveals that bryophytes contain phytochemicals just like to higher plants (Bent S, 2008). Screening of antimicrobial studies reveals that *Taxithelium nepalense* shows maximum activity against *Staphylococcus aureus*

Plate 1

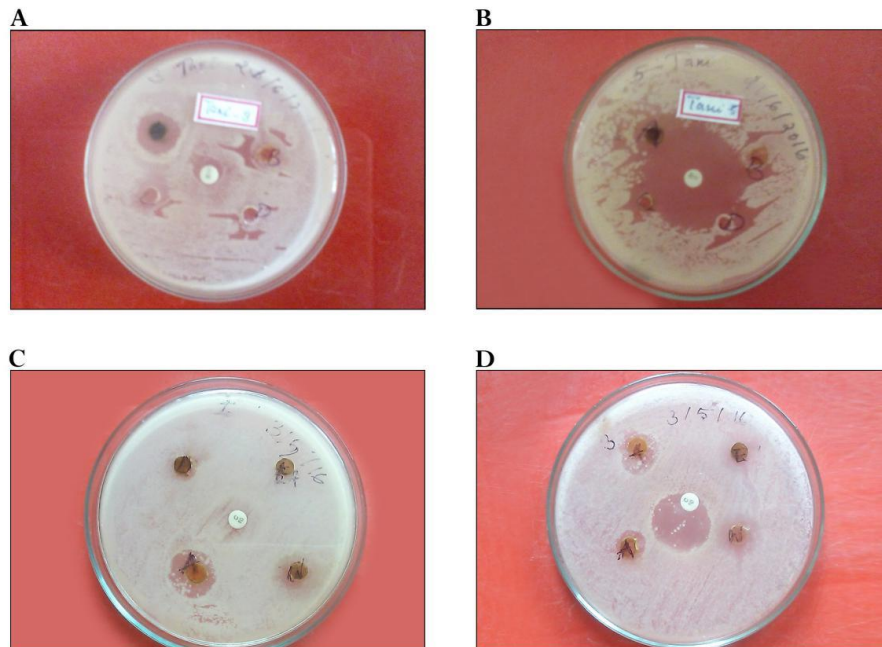
Taxithelium nepalense (Schwagr) Broth.



- A Habitat
- B Habit
- C Single leaf
- D Apical cells
- E Basal cells
- F Marginal Medium cells

Plate.2

Antimicrobial activity of *Taxithelium nepalense*



A Antimicrobial activity of *Taxithelium nepalense*

aganist *Escherichia coli*

B Antimicrobial activity of *Taxithelium nepalense*

aganist *Bacillus subtilis*

C Antimicrobial activity of *Taxithelium nepalense*

aganist *Salmonella enteritis*

D Antimicrobial activity of *Taxithelium nepalense*

aganist *Pseudomonas aeruginosa*

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