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Research Article

# SCREENING AND EVALUATION OF ANTIMICROBIAL AGENTS FROM *FUNARIA* SP. AGAINST VARIOUS PATHOGENS

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**ABSTRACT**

Objective: The present study was carried out to evaluate the biological active phytochemical compounds from moss of Berijam Lake, Kodaikanal, India.

Methods: Acetone and methanol extracts of moss were obtained using soxhlet extractor and the obtained extracts were evaluated for various biological activities. The combined effect of commercially available antibiotics and moss extracts was also investigated by disk-diffusion ~~assay~~ method against various Gram positive and Gram negative bacteria. The free radical scavenging activity of moss extracts were studied by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Results: The maximum zone of inhibition was found against Gram negative pathogens whereas weak inhibitory activity was investigated against *Staphylococcus aureus*. The combined effect of chloramphenicol with acetone and methanol extracts exhibited pronounced antimicrobial effect against *Bacillus subtilis*, *Proteus mirabilis* and *Escherichia coli.* The phytochemical tests revealed the presence of carbohydrates in *Funaria* sp. and the acetone extract showed effective antioxidant activity when compare to methanol extract using free radical scavenging assay.

Conclusion: The present study reveals that the bryophytes from Berijam Lake possess potential antimicrobial and antioxidant activity.

**Keywords:** synergistic effect, bryophytes, clinical pathogens, antibiotics

## INTRODUCTION

The continuing and ever-increasing antibiotic resistant among pathogenic microorganisms has become a worldwide concern. The overuse of antibiotics in humans and its use as growth promoters in food of animals have contributed to antibiotic resistance [1]. Plants have been exploited most from the ancient times as they are the important source of herbal medicines and therapeutic phytochemical agents [2]. To combat antimicrobial resistance, in recent years numerous studies have been conducted with various medicinal plants for the discovery of new antimicrobial agents. Bryophytes belong to the group of the oldest known terrestrial plants, which includes liverworts, hornworts and mosses. They are traditionally known medicines in China, Europe, North America and India to treat cardiovascular diseases, skin diseases and burns [3]. Due to their small morphological characters, difficulty in collecting pure samples of bryophytes was the reasons for its phytochemical negligence [4] but the biological active compounds is the only element that helped bryophytes to survive in today's flora [5]. The main reason for this negligence is that they do not possess nutritional value. However, a number of bryophytes have been extensively used as medicinal plants in China, to cure bruises, burns, snake bites, external wounds, pulmonary tuberculosis, neurasthenia, fractures, convulsions, scald, uropathy, pneumonia [6, 7, 8, 9, 10, 11].

The development of new and sophisticated techniques, have played an important role in finding additional resources of raw material for pharmaceutical industry [12]. According to previous reviews, there are evidences confirming that mosses contain various useful phytochemicals which has attracted the attention of botanist and pharmaceutical industry. Several types of alkaloids, tannins, steroids, terpenoids, flavanoids and phenolic compounds from moss species have been isolated and characterized. It has been reported that alkaloids, flavonoids, biflavonoids, and isoflavonoids from bryophyte extract possess effective antibacterial, antifungal activity against pathogenic microorganisms [13, 14, 15]. The volatile phenol compounds extracted from bryophytes act as antioxidants, which can quench reactive free radicals, thus prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases as well as aging [16].

According to literature survey, there is dearth of supporting evidence on antimicrobial agents from bryophytes of Berijam Lake, Kodaikanal. The present study was aimed to screen and evaluate biological active compounds from acetone and methanol extract of mosses against clinical pathogens.

## MATERIALS AND METHODS

### Sample Collection

The sample was collected from the trees at Berijam Lake, near Kodaikanal, India in the month of January 2012. Moss was found growing on dense patches in moist area around the tree trunk and on the forest floor. Phyllodes were green in color and colorless branched rhizoids with oblique septa were found. On the apex of the main plant antheridia was borne on the lateral branch archegonia was situated.

The collected moss was identified as *Funaria* sp. by Shembaganur Museum of Natural History, Kodaikanal, India. The sample was packed and transported to laboratory. *Funaria* sp. was dried in shade and finally ground into a fine powder using mortar and pestle.

### Preparation of moss extracts

Finely ground moss (30 g) was extracted using acetone and methanol in a Soxhlet extractor, care was taken not to exceed the boiling point of the solvent. The extracts were filtered through Whattman Filter Paper No.1 and then concentrated under reduced atmospheric pressure. The dry extracts were stored at -20°C. The extracts were further dissolved in 5% dimethyl sulphoxide (DMSO) for further experimental analysis.

### Phytochemical Tests

The qualitative phytochemical analysis of moss extract was carried out to detect the presence of alkaloids (Mayer's test, Wagner's test

and Hagner's test), carbohydrates and glycosides (Molish's test, Fehling's test, Benedict's test), proteins and amino acids (Millon's test, Biuret's test, Ninhydrin test), fixed oil and fat (Spot test and saponification test), phenolic compounds (Ferric chloride test, Gelatin test, Lead acetate test and Alkaline reagent test) using the method followed by Parekh and Chanda [17].

**Test Organisms and Antibiogram study**

The clinical pathogens used in this study were *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Salmonella* sp., *Shigella* sp., *Enterococci* sp., *Pseudomonas aeruginosa, Klebsiella pneumonia*. All the clinical isolates were acquired from Microbial Biotechnology Laboratory, SBST, VIT University, Vellore, India. Bacterial isolates were maintained on Nutrient agar and antibiotic resistance of clinical pathogens was investigated on Muller-Hinton agar by disc-diffusion method.

**Thin layer chromatography**

The silica coated plates (Merck) were used to identify retention factor ($R_f$) of the acetone and methanol extracts. The various combinations of solvents like hexane: diethyl ether: formic acid (130:100:20), hexane: ethyl acetate (80:20, 60:40, 90:10), chloroform: methanol (80:20, 60:40, 90:10), ethyl acetate: Methanol (80:20, 60:40, 90:10). The TLC plates were then observed in Ultra violet chamber using long UV (400nm). The $R_f$ values were calculated and based on these values, the components present in the extracts were determined.

**Antimicrobial activity**

The sensitivity of acetone and methanol extract of *Funaria* sp. was analyzed for antibacterial activity against clinical pathogens by Kirby-Bauer method. The clinical isolates were swabbed onto Muller-Hinton agar plates, wells were punctured onto the agar plate. 50 mg/ml of dried acetone and methanol extract of moss with different concentrations (25 µl, 50 µl, 75 µl and 100 µl) were loaded onto the wells. The petriplates were incubated for 24 h, and the zone of inhibition was measured around the wells.

**Synergistic study**

The combined effect of moss extracts and commercially available antibiotics was studied by disc-diffusion method. The test organisms were swabbed onto Muller-Hinton agar, 10 µl of 50 mg/ml acetone and methanol extract was impregnated on antibiotic disc. The antibiotic disc penicillin (10 µg/disc), streptomycin (10 µg/disc), vancomycin (30 µg/disc), tetracycline (10 µg/disc), ampicillin (10 µg/disc), oxacillin (1 µg/disc), methicillin (10 µg/disc), chloramphenicol (10 µg/disc) and clindamycin (30 µg/disc) with moss extract were incubated at 37°C for 24 h. The zone of inhibition was measured and fold-increase percentage was calculated using formula: Fold increase percentage = [(b-a)]*100, where a=antibiotic, b=acetone or methanol extract.

**Antioxidant assay**

The acetone and methanol extracts of moss was analyzed for free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Various concentrations of the extracts were prepared and mixed with 0.5 mM DPPH solution. The reaction was allowed to occur at 37°C for 30 min in dark. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}]/100$, where $A_S$ is the absorbance of the solution when the sample extract has been added at a particular level, and $A_{DPPH}$ is the absorbance of the DPPH solution [18].

**RESULTS**

The extracts of *Funaria* sp. exhibited effective antibacterial activity against various clinical pathogens compared to antibiotics. The Gram negative pathogens appear to be susceptible towards acetone extracts whereas methanol extracts effectively inhibited both Gram-positive and Gram-negative pathogens. The weak activity was manifested against *Staphylococcus aureus*. Table 1 summarizes the antimicrobial effect of *Funaria* sp. extracts against various pathogens. The moss extracts exhibited maximum inhibition effect against Gram-negative clinical pathogens such as *Klebsiella pneumonaie*, *Escherichia coli*, *Shigella* sp. and *Salmonella* sp. The preliminary phytochemical test has revealed the presence of carbohydrates in *Funaria sp.* extract (Table 2) which was further analyzed by calculating $R_f$ values as shown in Table 3. The $R_f$ value obtained with various solvent system which was similar to cholorophyll b, xanthophylls and carotene. The combined effect of commercially available antibiotics and moss extracts showed pronounced antimicrobial activity against *Proteus mirabilis, Bacillus subtilis, Escherichia coli*, *Alcaligens* sp., *Klebsiella pneumonia* and *Shigella* sp. as shown in Table 4. The antioxidant activity was analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and acetone extracts exhibited greatest free radical scavenging activity when compared to methanol extracts as shown in Table 5.

**Table 1: Zones of inhibition obtained with acetone extract of moss *Funaria* sp. against clinical pathogens**

| Clinical pathogens | Streptomycin (control) | Acetone Extract (µL) | | | | Methanol Extract | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 |
| *Escherichia coli* | 13 | 13 | 18 | 18 | 22 | 10 | 12 | 14 | 15 |
| *Salmonella* sp. | 19 | - | - | - | - | 12 | 11 | 13 | 12 |
| *Staphylococcus aureus* | 20 | - | - | - | - | 7 | 11 | 12 | 13 |
| *Enterococcus* sp. | 20 | - | - | - | - | - | - | - | - |
| *Klebsiella pneumonia* | 15 | 16 | 14 | 13 | 18 | 11 | 12 | 12 | 15 |
| *Proteus mirabilis* | 15 | - | - | - | - | - | - | - | - |
| *Pseudomonas aeruginosa* | 12 | - | - | - | - | - | - | - | - |
| *Shigella* sp. | 13 | 12 | 12 | 12 | 20 | - | - | - | - |

**Table 2: Phytochemical profile of Moss *Funaria* sp. extract**

| Phytochemical Screening | Test | Acetone Extract | Methanol Extract |
|---|---|---|---|
| Alkaloids | Mayer's Test | - | - |
| | Wagner's Test | - | ++ |
| | Hager's Test | - | - |
| Carbohydrates and Glycosides | Molish's Test | + | + |
| | Fehling Test | - | - |
| | Benedict's Test | - | + |
| Protein and Amino acids | Milon's Test | - | - |
| | Biuret Test | - | - |
| | Ninhydrin Test | - | - |
| Phenols | Ferric chloride Test | - | - |
| | Gelatin Test | - | - |
| | Lead acetate Test | - | - |
| | Alkaline reagent Test | - | - |

| | | | |
|---|---|---|---|
| Fixed oils and Fats | Spot Test | - | - |
| | Saponification Test | - | - |
| Saponins | Foam Test | - | - |

**Table 3: $R_f$ values of *Funaria* sp. Extracts.**

| Solvent System | Concentration | $R_f$ of Methanol Extract | | $R_f$ of Acetone Extract | |
|---|---|---|---|---|---|
| | | $R_f$ 1 | $R_f$ 2 | $R_f$ 1 | $R_f$ 2 |
| Hexane: Diethylether: Formic acid | 130:100:20 | 0.36 | - | - | - |
| | 60:40:00 | 0.81 | 0.86 | 0.81 | 0.88 |
| | 80:20:00 | 0.93 | - | 0.77 | 0.93 |
| Chloroform: Methanol | 90:10:00 | 0.76 | 0.8 | - | - |
| | 60:40:00 | - | - | - | - |
| | 80:20:00 | 0.84 | 0.88 | - | - |
| Hexane: Ethylacetate | 90:10:00 | 0.43 | 0.57 | - | - |

**Table4**: **Synergistic study of moss *Funaria* sp. against various pathogens.**

| Test Organisms | Antibiotic | Diameter zone of inhibition (mm) | | | Fold increase percentage [(b-a)]*100 | |
|---|---|---|---|---|---|---|
| | | Antibiotic (a) | Acetone extract (b) | Methanol extract (b) | Acetone extract (b) | Methanol extract (b) |
| *Pseudomonas aeruginosa* | Met | - | - | - | - | - |
| | Chl | 11 | 10 | 10 | -9 | -9 |
| | Ox | - | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | - | - | - | - | - |
| *Enterococcus* sp. | Met | - | - | - | - | - |
| | Chl | 30 | 25 | 32 | -16.6 | 6.6 |
| | Ox | 19 | 11 | 12 | -42.1 | -36.8 |
| | Cli | - | - | - | - | - |
| | Amp | 30 | 30 | 30 | 0 | 0 |
| *Escherichia coli* | Met | - | - | - | - | - |
| | Chl | - | 23 | 17 | 100 | 100 |
| | Ox | - | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | - | - | - | - | - |
| *Salmonella* sp. | Met | - | - | - | - | - |
| | Chl | 32 | 22 | 24 | -31.2 | -25 |
| | Ox | - | - | - | - | |
| | Cli | - | - | - | - | - |
| | Amp | 30 | 35 | 29 | 16.6 | -3.3 |
| *Klebsiella pneumonia* | Met | - | - | - | - | - |
| | Chl | 15 | 17 | 20 | 13.3 | 33.33 |
| | Ox | - | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | - | - | - | - | - |
| *Shigella* sp. | Met | - | | | | |
| | Chl | - | 20 | 18 | 100 | 100 |
| | Ox | - | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | - | - | - | - | - |
| *Proteus mirabilis* | Met | - | 10 | 10 | 100 | 100 |
| | Chl | 32 | 25 | 22 | -21.8 | -31.25 |
| | Ox | - | 11 | 13 | 100 | 100 |
| | Cli | 20 | 10 | 10 | -50 | -50 |
| | Amp | 34 | 22 | 24 | -35.2 | -29.4 |
| *Staphylococcus aureus* | Met | - | - | - | - | - |
| | Chl | 25 | 17 | 18 | -32 | -28 |
| | Ox | - | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | 25 | - | - | - | - |
| *Bacillus subtilis* | Met | - | - | - | - | - |
| | Chl | 8 | 20 | 28 | 150 | 250 |
| | Ox | 16 | - | - | - | - |
| | Cli | - | - | - | - | - |
| | Amp | 23 | - | - | - | - |
| *Alacigenes* sp. | Met | - | 12 | - | 100 | - |
| | Chl | 27 | 27 | 32 | 0 | 18.51 |
| | Ox | 15 | 18 | 14 | 20 | -6.67 |
| | Cli | 15 | 20 | 20 | 33.33 | 33.33 |
| | Amp | 33 | 24 | 19 | -27.27 | -42.42 |

**Note: met=methicillin, chl=choloramphenicol, ox=oxacilin, cli=clindamycin, amp=ampicillin**

**Table 5: Antioxidant activity of moss *Funaria* sp. extract**

| S. No. | Concentration (µg/ml) | Acetone extract | | Methanol extract | |
|---|---|---|---|---|---|
| | | Abs 517nm | Radial scavenging activity (%) | Abs 517nm | Radial scavenging activity (%) |
| 1 | 50 | 0.023 | 68.49 | 0.123 | -68.49 |
| 2 | 100 | 0.039 | 46.57 | 0.097 | -32.87 |
| 3 | 150 | 0.048 | 34.24 | 0.079 | -8.21 |
| 4 | 200 | 0.05 | 31.5 | 0.041 | 43.83 |
| 5 | 250 | 0.069 | 5.4 | 0.019 | 73.97 |

## DISCUSSION

In the present study, the biological activities of bryophytes of Berijam Lake, India were investigated. The acetone and methanol extracts were obtained and these extracts were further used all through the study for the determination of antimicrobial and antioxidant activities. The extracts obtained from *Funaria* sp. exhibited effective antimicrobial activity against all the pathogens. The Gram-negative bacteria were found to be more resistant than Gram-positive bacteria [19] and causes diseases such as pneumonia, urinary and respiratory tract infection, nosocomial pathogens and opportunistic infections [20]. In the present investigation, extracts from *Funaria* sp. from Berijam Lake manifested commendable antimicrobial effect against Gram-negative clinical pathogens. It has been previously reported that the differences in antimicrobial effect of plant species is due to its phytochemical constituents among various plants [21]. The methanol extract exhibited greater antibacterial activity when combined with choloramphenicol and clindamycin against Gram-negative bacteria. The synergistic antibacterial effect with combination of moss extracts with chloromphenicol showed pronounced activity than other antibiotics. The free radical scavenging activity was analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and acetone extracts exhibited greatest free radical scavenging activity as compare to methanol extracts. In the present method, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The number of reduced molecules DPPH was monitored measuring the absorbance decrease at 515–528 nm [22] and free radical scavenging activity was determined by the discoloration of the DPPH solution [18]. The present work reveals that the acetone and methanol extracts of bryophytes from Berijam Lake, India posses effective antimicrobial, antioxidant and synergistic activity against Gram-negative and Gram-positive clinical pathogens.

## CONCLUSION

The present investigation reveals the biological potential of *Funaria* sp. of Berijam Lake, India and it can be used as antimicrobial agent in pharmaceutical associations. The biological active constituents present in *Funaria* sp. manifested good antimicrobial, antioxidant and synergistic activity against various clinical pathogens. Furthermore studies are required for separation of phytochemical compounds and their biological properties.

## CONFLICT OF INTEREST STATEMENT

No conflict of interest to declare.

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