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# Antimicrobial Screening of the Solvent Extracts of *Marchantia polymorpha* against Some Pathogenic Strains

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**Abstract** **Original Research Article**

*Marchantia polymorpha* is a shade loving species of bryophytes prefers to grow in low temperature and moderate humidity, where presence of thick forest canopy. *Marchania polymorpha* used in tribal regions for several medicinal applications. Based on its importance, this species is evaluated for antimicrobial activity against four bacterial stains such as *Bacillus subtilis* and *Streptococcus mutans* (Gram positive), *Klebsiella pneumonia* and *Salmonella enterica* (Gram negative) and two fungal pathogens such as *Candida albicans, Rhizopus oryzae*) by Agar Well Diffusion method. Plant extracts were obtained successfully with hexane, chloroform, methanol and water using Soxhlet extraction apparatus. Results of this study reveal that methanol extracts of *M. polymorpha* thallus showed the significant antimicrobial activity against all bacterial and fungal pathogens. These studies on bryophyte species indicated that the presence of active constituents which can be exploited for the production of novel drugs for the benefit of the humanity.

**Keywords:** Bryophyte, *Marchantia polymorpha*, Antimicrobial Activity, Well Diffusion Method.



## INTRODUCTION

There is an increasing demand of biodiversity from natural resources for therapeutic drugs. So far, many chemically unique compounds, with different biological activities have been isolated and a number of medicines and extracts from various plant species have proven inhibitory activity against human, animal and plant pathogens. India has a rich diversity of plant species with applications in medicine and treatment of several ailments of human beings. Bryophytes are lower group of plant kingdom which grows in evergreen tropical and sub-tropical regions of India. Local communities are utilizing these bryophytes for treating different ailments of human beings (Murthy *et al*., 2011; Narasimha Rao and Srinivasa Rao, 2013; Narasimha Rao and Reshmi, 2014; Narasimha Rao and Dora, 2019).

Based on its medicinal properties some researchers across the globe conducted experimental and anti-microbial activities on different species of bryophytes. Bryophytes have been used in various formulations of herbal medicines for treatment of ailments related to human beings and livestock. Bryophytes are rich in many active constituents like polysaccharides, amino acids, oligosaccharides, sugar alcohols and phenolic compounds (Nagashima *et al.*, 2003). Fatty acids reported from *Marchantia* spp. responsible for antibacterial activity (Krishnan and Murugan, 2017). Acetone extract of *Lunularia cruciata* (Marchantiaceae) showed antibacterial activity against both Gram-positive and Gram-negative bacteria (Basile *et al*., 1998). In tribal regions people are utilizing many species which are not known to the outside of these regions. *Marchantia polymorpha* L. is thalloid liverwort belongs to Marchantiaceae family. This species is preferred to grow on shaded moist soil and banks of the running streams along the Eastern Ghats of India. Visakhapatnam district of Eastern Ghats of India offers good growth for bryophytes especially *Marchantia polymorpha* (Narasimha Rao and Dora, 2019). In the present investigation an attempt was made to evaluate the antimicrobial activity of *M. polymorpha* against four bacterial and two fungal strains.

## MATERIALS AND METHODS

### Collection of plant material

Plant materials of *M. polymorpha* were collected from Anathagiri hills of Eastern Ghats of India, Visakhapatnam district. Ananthagiri which is a hill station situated in the Eastern Ghats at a distance of 75 km from Vishakhapatnam with 18°11' N latitudes and

82°59' E longitudes, and approximately 19 km from Araku valley.

**Test organisms**

The selected bacterial strains were obtained from Microbial Type Culture and Collection (MTCC) from Institute of Microbial Technology, Chandigarh, India. The microorganisms such as two Gram positive strains (*Bacillus subtilis* and *Streptococcus mutans*), two Gram negative (*Klebsiella pneumonia* and *Salmonella enterica*) and two fungal strains (*Candida albicans* and *Rhizopus oryzae*) were selected for carryout the antimicrobial activities.

**Preparation of Plant Extracts**

The epiphytes and other deposits were removed from the bryophytic plant and then the specimens are shade dried. The shade dried plant materials were chopped into small pieces and coarsely powdered. The coarsely powdered material was weighed and extracted with hexane, chloroform, methanol and water in sequential order of polarity using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2 ml of solvent was used. The extracted solvents were filtered though Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuo at $40^0$C) using a rotary evaporator. The residue obtained was designated as crude extract and was stored in a freezer at $-20^0$ C until bioassayed. The plant extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentrations (100 mg/ml, 150 mg/ml and 200 mg/ml) and filtered through a 0.45 µm membrane filter and stored in sterile brown bottles at $20^0$ C until bio assayed.

***In vitro* Antibacterial Activity Assays:**

The antimicrobial activity of the hexane, chloroform, methanol and water extracts of each sample was evaluated by using Agar Well Diffusion Method of Murray *et al.*, (1995) modified by Olurinola, (1996). 20ml of sterilized agar medium (Nutrient Agar Media for bacteria and Potato Dextrose Agar or PDA for fungi) was dispensed into sterile universal bottles. These were then inoculated with 0.2 ml of bacterial cultures, media was mixed gently and poured into sterile petri dishes and it is allowed to solidify. Then the 4 uniform wells were made in each petri dish by using a sterilized number 3-cup borer (6mm diameter). The wells were filled with 50—µl of the extract concentration of 100mg/ml, 150mg/ml, 200mg/ml and control (DMSO) and allowed diffusion for 45 minutes. The plates were incubated at 37° C for 24 hours for bacteria and 25° C for 48 hours for fungi. The diameter of the inhibition zone was measured in millimeters. Antibacterial and antifungal activity was expressed in terms of activity index (AI), and the experiment was carried out in duplicates.

## RESULTS AND DISCUSSION

Anti-bacterial and anti-fungal activities of four solvent extracts of hexane, chloroform, methanol and water (100 mg/ml, 150 mg/ml and 200 mg/ml) of *M. polymorpha* were evaluated against tested bacterial and fungal strains. The antimicrobial activity was rated (Alves *et al*., 2000) based on the value of zones of inhibition given below.

<9mm - inactive
9–12mm-lessactive
13–18mm- active
>18mm- veryactive

In the present investigation there was a gradual increase in the zone of inhibition from 100 to 200 mg/ml, with highest at 200 mg/ml concentration of plant extract. Hence only 200 mg/ml dosage level results were analyzed. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive.

**Antimicrobial Activity of *M. polymorpha*:**

Fig 1 represents the comparison among four solvents of *M. polymorpha* plant parts of 100mg/ml of hexane, chloroform, methanol and water extracts. Fig 2 represents the comparison among four solvents of *M. polymorpha* plant parts of 150mg/ml of hexane, chloroform, methanol and water extracts. Fig 3 represents the comparison among four solvents of *M. polymorpha* plant parts of 200mg/ml of hexane, chloroform, methanol and water extracts.

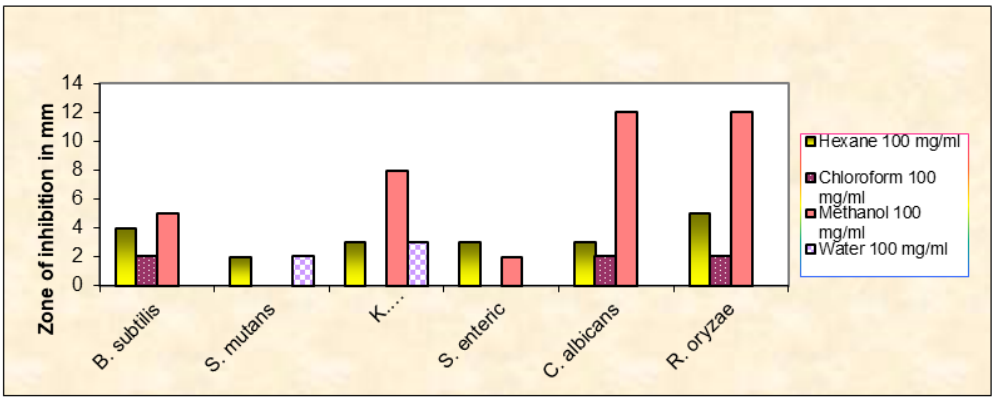


**Fig 1: Antimicrobial Activity of *M. polymorpha* (100 mg/ml)**

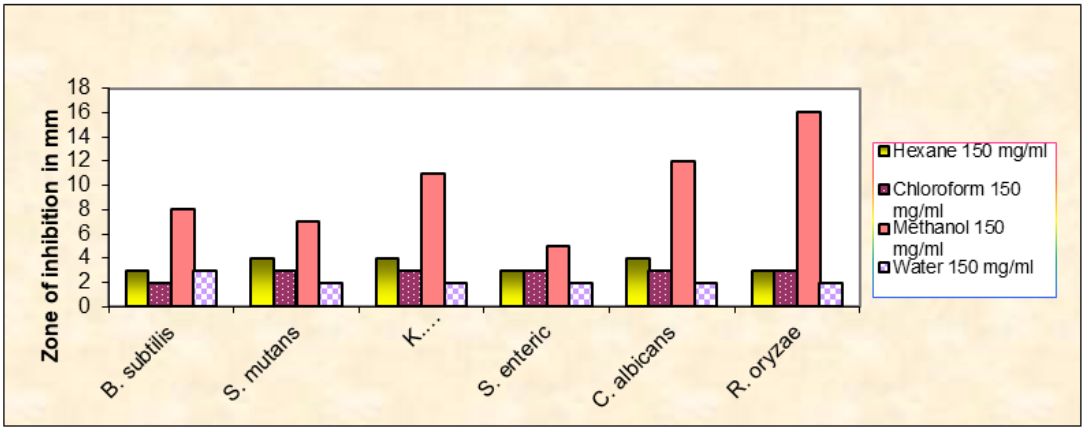


**Fig 2: Antimicrobial Activity of *M. polymorpha* (150 mg/ml)**

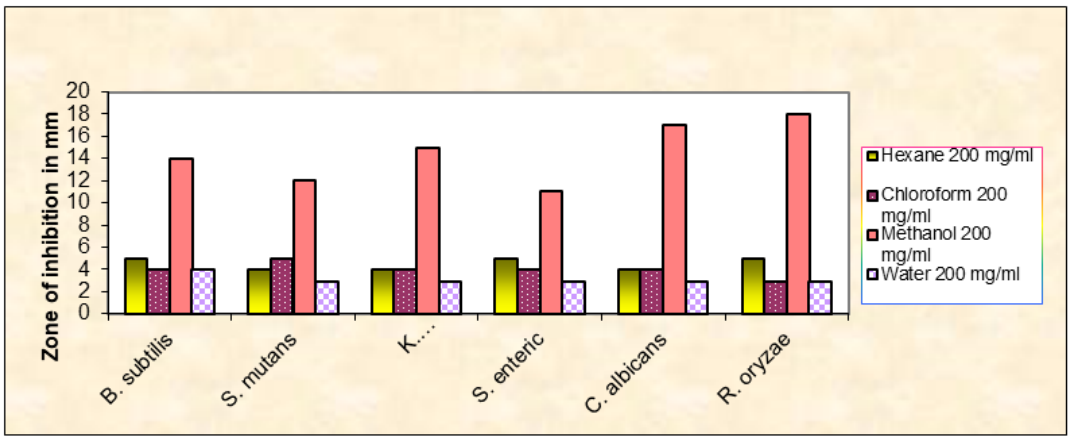


**Fig 3: Antimicrobial Activity of *M. polymorpha* (200 mg/ml)**

Highest level of antimicrobial activity was recorded for the methanol extracts of *M. polymorpha*, against fungal strains such as *Candida albicans* (18 mm) followed by *Rhizopus oryzae* (17 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *Klebsiella pneumonia (15 mm),* followed by *Bacillus subtilis (14* mm). Less antibacterial activity observed against *Streptococcus mutans* (12 mm) followed by *Salmonella enterica (11* mm) (Fig 1, 2 & 3). Whereas less antibacterial activity was recorded for the hexane, chloroform and water extracts against all bacterial and fungal strains. The results in the present study are in accordance with the antimicrobial activity studies made by Mewari and Kumar (2008), they reported that the inhibition zone produced by methanol extracts of *M. polymorpha* against *Staphylococcus aureus* pathogen was almost 20 mm. Methanol extract also exhibited high antibacterial activity against *Proteus mirabilis* (19.3 mm), with moderate activity against *E. coli* (9 mm). The results in the present study are in accordance with the antimicrobial activity studies made by Singh *et al*., (2006), evaluated the antimicrobial activity on ethanolic extracts of fifteen bryophytes, against eleven bacterial and eight fungal strains. *Sphagnum junghuhnianum, Barbula javanica, Barbula arcuata, Brachthecium populeum, Brachythecium rutabulum, Mnium marginatum* and *Entodon cf rubicundus* were found to be most active against all the pathogens tested. In another study by Singh *et al.,* (2011) reported that the chloroform fractions of liverworts were more active against Gram negative strains while butanol fractions of mosses had significant activity against Gram positive bacteria. *Staphylococcus aureus* was the most sensitive strain of those tested with the butanol fraction of *M. marginatum* (moss), with the strongest inhibition zone.

## CONCLUSION

The results revealed that methanol extracts of *M. polymorpha* thallus extracts have greater potential compounds against used microorganisms and they can be used as novel antimicrobial agents. *M. polymorpha* is an ethano-medicinally important bryophyte as a potent to cure bacterial infections paving the way for its use by the practitioners of modern healthcare systems.

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