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# Antibacterial Activity of *Anoectangium clarum* Mitt. (Bryophyta: Pottiaceae) against some Pathogenic Bacteria

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**Abstract**

In recent years bryophytes have emerged as potential plants for many bioactive phytochemicals with antimicrobial efficacies. In present study antibacterial activity of *Anoectangium clarum* Mitt. has been assessed using various extracts. For this assessment agar diffusion method is used against selected pathogenic fungus. Tetracycline is used as positive control. The ethanolic extract of moss exhibited a superior effect than the extract prepared in methanol. The utmost effect was observed against *Escherichia coli* followed by *Salmonella typhimurium* and *Bacillus subtilis*. This study projects *Anoectangium clarum* as an eco-friendly antibacterial agent.

**Keywords:** *Anoectangium clarum,* antibacterial activity, extract, moss, well diffusion method.

## Introduction

Plants are treasured source of natural products for upholding normal health of human beings. Conventional therapeutic systems of Ayurveda, Unani and Sidha are the prevalent verification of their use in therapy. In the last few decades, with more rigorous researches for therapies based on natural systems, plants are used extensively to cure different diseases especially dermal ailments [1]. The search for unexplored plants or plant group with substantial antimicrobial action has attained massive importance these days, due to a growing concern about the attainment of antibiotic- resistance by the pathogenic microorganism.

According to a report of World Health Organization (WHO) approximately 80% of folks from developing nations use conservative systems of natural medicine, which are dependent on the compounds present in medicinal plants. Thus, there is need to investigate more and more plants for their phytochemical and antioxidant possessions, protection and effectiveness with experimental rationale [2].

Bryophytes, the group of first land plants, include liverworts, hornworts, and mosses. These usually considered as one of pioneers, colonize habitats with damp or exceptionally inconsistent conditions. Liverworts and mosses have been used customarily in Indian culture curing many skin related diseases due to their remarkable antimicrobial activity [3]. In Chinese medicine system the use of these miniature plants dates back to 400 years ago. Species of *Fissidens* and *Polytrichum* were used as hair growth stimulating drugs and diuretic [4]. Furthermore, North American Indians used moss taxa like, *Polytrichum juniperinum*, *Bryum* spp., *Mnium* spp.and *Philonotis* spp. to cure bruises, burns and injuries [5].

Many species of bryophytes are known that are not affected by bacteria, fungi, larvae of insect, etc. [6] because they possess compounds like phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, and few aliphatic compounds that offer defense against these pathogenic organisms, therefore, along with

other large plants, bryophytes are also considered as potential medicinal plants [6, 7]. Asakawa [8] stated that the presence of secondary metabolites in bryophytes provide antibacterial properties. Afterward, various studies have also validated that bryophytes extracts appear to function as antimicrobial agents in nature.

Many other bryophytes elsewhere also exhibit antimicrobial potential against many strains of bacteria and fungi [9-15]. Even though profits are well recognized for their therapeutic significance yet they are not utilized effusive, predominantly in Indian sub-continent. Only a few workers from India have assessed the antimicrobial potential of these amphibians of the plant kingdom to some extent [16-20]. Various Indian bryophytes like *Plagiochasma rupestre* [21], *Targionia hypophylla* [22], *Entodon nepalensis* [23], and *Hyophila rosea* [24] have recently been evaluated for antimicrobial activitiy and exhibited substantial antifungal and antibacterial activity.

This study intends to examine a common moss- *Anoectangium clarum* (Order: Pottiales M. Fleisch.; Family: Pottiaceae Schimp.), for its antibacterial potential against some selected strains of pathogenic bacteria with a vision to find out their probable medicinal use. The selected moss species is well known in Rajasthan and Punjab Plains [25-26].

## Materials and methods

### Plant material

Plant materials of this study were collected from the Mount Abu, Rajasthan, at an altitude of 1400 m, 72.7083°E 24.5925°N, in July 2014. Specimens taken: BURI-7860301; Legit.: A. Alam and S. C. Sharma; Det.: A. Alam) are placed in the Banasthali University Rajasthan India (BURI), Banasthali Vidyapith, India.

### Test Microorganism

The pathogenic bacteria were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. *Escherichia coli* (MTCC 118) a

Gram-negative; *Bacillus subtilis* (MTCC 619) a Gram-positive bacterium; *Salmonella typhimurium* (MTCC 98) Gram-negative, non-spore-forming and motile enterobacteria.

**Solvent Extract preparation**

The plant materials (15 g) were dried, powdered and extracted with ethanol, methanol and distilled water separately in orbital shaker at 120 rpm for 24 h. The extracts were concentrated and dried in a vacuum. For the assessment of antibacterial activity, the dried extracts were dissolved in relevant solvents.

**Test for antibacterial assay**

Microbial suspension was set in sterile usual saline and attuned to 0.5 Macfarland standard ($10^8$ Cfu/ml). All selected strains were inoculated unvaryingly on labelled plates. Wells were prepared using sterile cork borer (diameter of 6mm) and poured with 100 µl of extract. At 37 °C the plates were incubated for 24 h. Antibacterial activity was analysed by gauging the diameter of inhibition zones (mm) formed after incubation. For positive control antibiotic Tetracycline (10µg/ml) was used [27]. Different solvents alone were used as negative control for each test organism.

**Results**

A variety of extracts of *Anoectangium clarum* demonstrated diverse antibacterial activity against three selected strains of bacteria (Table 1). The inhibition zones were recorded to be considerably different from each other ($p>0.05$). Utmost activity was found in the ethanol extract against *Escherichia coli* and *Salmonella typhimurium* (ZI:-12 and 10 mm, respectively) followed by extract prepared in methanol. The zone of inhibition was not significantly different against *Bacillus subtilis* utilizes methanolic and ethanolic extract. The aqueous extract showed slightest efficiency against all taken bacterial strains. In case of aqueous extract Utmost activity was recorded against *E. coli* and lowest against *B. subtilis.* While, in case of the standard antibiotic tetracycline (10µg/ml), the Zone of Inhibition (ZI) was found to be 8, 10 and 18 against *E. coli*, *B. subtilis*, and *S. typhiurium*, respectively.

Minimum Inhibitory Concentration (MIC) of ethanolic and methanolic extract was nearly beyond normal against all the selected bacteria. The aqueous extract exhibited slightest activity in terms of MIC against all the selected bacteria. In case of positive control, the MIC was observed to be 7 and 3 µg/ml against *Escherichia coli* and *Bacillus subtilis*, respectively.

**Discussion**

This analysis is the first report on the antibacterial activity of *Anoectangium clarum*. Aqueous, ethanolic and methanolic were made individually to evaluate their antibacterial effectiveness. Even though all the extracts showed different levels of antagonistic activity in opposition to all three bacterial strain, the ethanolic extract was assessed best in comparison to other two extracts. The possible reason behind this might be the varying solubility of various plant metabolites in different solvents, in this manner differential antibacterial activity was observed. The methanolic and ethanolic extract showed the substantial activity, suggesting easier isolation and subsequent solubilzation of antibacterial components from *Anoectangium clarum* in both the solvents.

**Conclusion**

The results confirmed that *Escherichia coli* was extremely responsive test bacterium followed by *S. typhimurium* and *B. subtilis*. This shows the occurrence of those phytochemicals, which are more effective against gram negative bacteria than gram positive bacteria. Unadventurously antibiotics are reported usually more active in opposition to gram positive than gram negative bacteria [28]. Conversely the antibacterial activity of this moss species was found to be more active against gram negative bacteria. This highlights the significance of various commonly growing mosses as an antibacterial agent [29]. Now more research is needed for uncomplicated isolation of bioactive phytochemicals from this plant and development of feasible scaling up method for possible threpeutical use against these pathogenic bacteria.

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**Table 1: Antibacterial activity of *Anoectangium clarum* extracts (Zone of Inhibition in mm)**

| Extracts | Bacterial strains | | | | | |
|---|---|---|---|---|---|---|
| | ***E. coli*** (MTCC 118) | | ***S. typhimurium*** (MTCC 98) | | ***B. subtilis*** (MTCC 619) | |
| | IZ (mm) | MIC (µg/ml) | IZ (mm) | MIC (µg/ml) | IZ (mm) | MIC (µg/ml) |
| **Aqueous** | $7^{c}$ | $952^{a}$ | $5^{c}$ | $1112^{a}$ | $2^{b}$ | $1195^{a}$ |
| **Methanol** | $10^{b}$ | $876^{b}$ | $8^{b}$ | $995^{b}$ | $5^{a}$ | $978^{b}$ |
| **Ethanol** | $12^{a}$ | $862^{b}$ | $10^{a}$ | $976^{b}$ | $6^{a}$ | $963^{b}$ |

$^{a–fc}$ Mean values represented by the same letters within the same column are not significantly different at $p>0.05$.

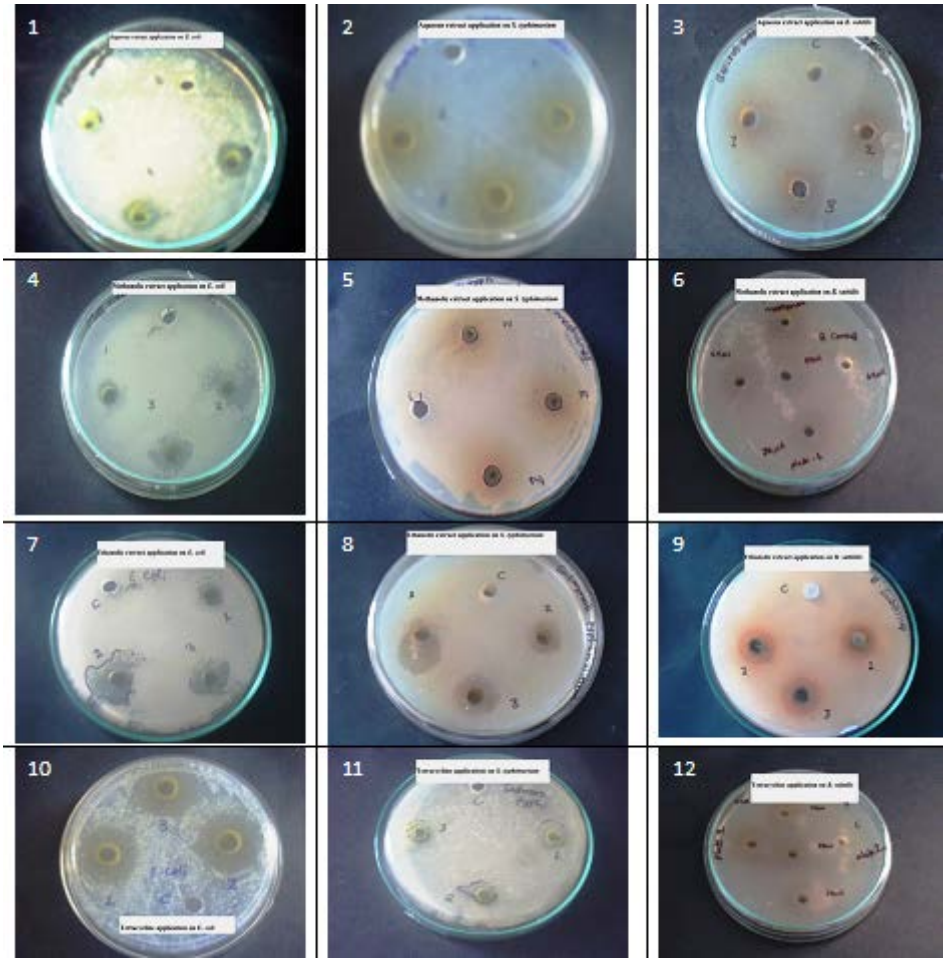


**Plate 1: Antibacterial activity of *Anoectangium clarum* extract with different combinations: 1-3. Aqueous, 4-6. Methanolic, 7-9. Ethanolic, 10-12.Tetracycline, against *Escherichia coli* (1, 4, 7, 10), *Salmonella typhimurium* (2, 5, 8, 11) & *Bacillus subtilis* (3, 6, 9, 12).**

## References

[1] Flowers, S (1957) Ethnobryology of the Gosiute Indians of Utah. Bryologist. 60, 11–14.

[2] Ellof, JN (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60, 1-6.

[3] Frahm, JP (2004) New frontiers in bryology and lichenology: Recent developments of commercial products from bryophytes. Bryologist. 107, 277-283.

[4] Asakawa, Y (2001) Recent advances in phytochemistry of bryophytesacetogenins, terpenoids and bis (bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. Phytochemistry. 56, 297-312.

[5] Ilhan, S., Savaroglu, F., Colak, F., Iscen, C and Erdemgil, F (2006) Antimicrobial activity of *Palustriella commutata* (Hedw.) Ochyra extract (Bryophyta). Tr. J. of Biology. 30, 149-152.

[6] Asakawa, Y (1981) Biologically active substances obtained from bryophytes. J. Hattori Bot. Lab. 50, 123-142.

[7] Asakawa, Y (1988) Biologically active substances found in hepaticae. S. 277 ff. In A.U. Rahman (Ed.), Studies in Natural Products Chemistry. Elsevier, Amsterdam.

[8] Basile, A., Giordano, S., Lopez-Saez, JA and Castaldo, CR (1999) Antibacterial Activity of pure flavonoids isolated from mosses. Phytochemistry. 52, 1479-1482.

[9] Basile, A., Vuotto, ML., Ielpo, MTL, Moscatiello, V., Ricciardi, L., Giordano, S and Castaldo, CR (1998b) Antibacterial Activity in *Rhynchostegium riparioides* (Hedw.) Card. extract (Bryophyta). Phytother. Res. 12, 146-148.

[10] Banerjee, RD (2001) Antimicrobial activities of bryophytes: A Review. In: Perspectives in Indian Bryology. Eds. Nath V. and Asthana A. K. Bishen Singh Mahendra Pal Singh Publisher, Dehradun, India. 55-74.

[11] Frahm, JP and Kirchhoff, K (2002) Antifeeding effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against slug Arion lusitanicus. Cryptogamie Bryol. 23, 271-275.

[12] Scher, JM, Speakman, JB, Zapp, J aand Becker, H (2004) Bioactivity guided isolation of antifungal compounds from the liverwort *Bazzania trilobata* (L.) Gray SF. Phytochemistry. 65, 2583-2588.

[13] Sabovljevic, A., Sokovic, M., Sabovljevic, M and Grubisic, D (2006) Antimicrobial activity of *Bryum argenteum*. Fitoterapia. 77, 144-145.

[14] Dülger, B., Hacıolu, N and Uyar, G (2009) Evaluation of Antimicrobial Activity of some Mosses from Turkey. Asian J. Chem. 21(5), 4093-4096.

[15] Gupta, KG and Singh, B (1971) Occurrence of antibacterial activity in moss extracts. Res. Bull. Punjab Univ. Sci. 22, 237-39.

[16] Banerjee, RD and Sen, SP (1979) Antibiotic activity of bryophytes. Bryologist. 82, 141-153.

[17] Kumar, K., Singh, K K, Asthana, AK and Nath, V (2000) Ethnotherapeutics of bryophyte *Plagiochasma appendiculatum* among the Gaddi tribes of Kangra valley, H.P., India. Pharma. Biol. 38, 353-356.

[18] Subhisha, S and Subamoniam, A (2005) Antifungal activities of a steroid from *Pallavicinia lyellii*, a liverwort. Ind. J. Pharmacol. 37, 304-308.

[19] Singh, M., Rawat, AKS and Govindarajan, R (2007) Antimicrobial activity of some Indian mosses. Fitoterapia. 78, 156-158.

[20] Bodade, RG, Borkar, PS, Saiful Arfeen MD and Khobragade, CN (2008) *In vitro* Screening of bryophytes for antimicrobial activity. J. Med. Plants. 7(4), 23-28.

[21] Alam, A (2012) Antifungal activity of *Plagiochasma rupestre* (Forst.) Steph. Extracts. Researcher. 4(3), 61-64.

[22] Alam, A., Sharma SC and Sharma, V (2012a) *In vitro* Antifungal Efficacies of Aqueous Extract of *Targionia hypophylla* L. against growth of some pathogenic fungi. Int. Jour. Ayur. Herb. Med. 2, 229-233.

[23] Alam, A., Sharma, V., Sharma SC and Kumari, P (2012b) Antibacterial activity of the alcoholic extracts of *Entodon nepalensis* Mizush. against some pathogenic bacteria. Rep Opinion. 4(10), 44-47.

[24] Alam, A (2013) Antifungal efficacies of *Hyophila rosea* Williams (Bryophyta: Pottiaceae). Mycopath. 11 (1), 15-17.

[25] Alam, A., Pandey, S., Singh, V., Sharma, SC and Sharma, V (2014) Moss flora of Mount Abu (Rajasthan), India: An updated checklist . Trop. Plant Res. 1 (1), 8-13.

[26] Rawat, KK, Alam, A and Verma, PK (2015) Moss flora of Rajasthan and Punjab plains. Plant Science Today. 2(4), 154-158.

[27] NCCLS (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard Fifth Edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA.

[28] Anna, K and Brown, DF (2001) Quality assurance of antimicrobial susceptibility testing by disc diffusion. J. Antimicrob. Chemother. 4, 71 – 76.

[29] Alam, A., Shrama, V., Rawat KK and Verma, PK (2015) Bryophytes - The Ignored Medicinal Plants. SMU Medical Journal. 2(1), 299-315.

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