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Altitudinal variations in the phytochemical profile of *Hyophila involuta* (Hook.) A. Jaeg. from Rajasthan, India

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ABSTRACT

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When it comes to the phytochemical and antioxidant capacity of plants, bryophytes are the ones that have received the least attention. The phytochemistry of the bryophytes is still largely unknown, despite the fact that their diversity and antibacterial activity have been the subject of numerous researches. This could be a valuable source of chemicals of therapeutic significance. Even with its arid landscape and severe weather, the Indian state of Rajasthan has several areas where these bryophytes can be found. In the current study, populations of *Hyophila involuta* from Mount Abu, Ranthambore, and Banasthali Vidyapith—three such locales with varying altitudes—have been examined to determine the effect of altitudinal variations on phytochemical profiles.

Keywords: Bryophytes, Phytochemical, altitude, *Hyophila involuta*, variation, phenolic content, flavonoid.

INTRODUCTION

The indigenous people have long relied on bryophytes as the earliest land plants and amphibians of the plant kingdom to combat a diverse range of ailments, as evidenced by research (Alam et al. 2015). Despite its known antimicrobial properties, there has been a dearth of convincing evidence. However, scientists now believe these plants offer a rich source of novel medications and are a promising natural treatment option. Consequently, their phytochemistry has been extensively studied. Bryophytes have been evaluated for their potential as antioxidants, antimicrobials, and secondary metabolites. Their physiologically active molecules play a critical role in their defence mechanism against microbial infections due to the presence of unique phytochemicals. Despite numerous efforts in this area, many bryophytes such as liverworts, hornworts, and mosses have yet to be fully explored. Mosses are a highly significant lineage of plants with a unique life cycle, notable antimicrobial properties, and exhibit Poikilohydric habit. These tiny plantlets serve as a vital interface between the air and substrate, where climatic conditions can differ greatly from those observed during routine meteorological observations. Compared to higher plants, mosses offer several advantages, including a relatively simple structure, a haploid gametophytic dominant vegetative phase, fewer chromosomes, and less morphological variability throughout the growing season (Joshi & Alam 2023). Additionally, mosses have a broad distribution,

and their annual growth segments can indicate the presence of high concentrations of pollutants. With a high surface-to-volume ratio and ease of sampling, mosses are a valuable tool for soil management and environmental pollution monitoring. Furthermore, their rapid life cycle completion improves their usefulness in various ways (Adebiyi et al. 2012).

Hyophila involuta is a lawn-forming moss with upright shoots. On the shoot tip, the leaves form a rosulate group, which makes the shoot tip look like a little star. This moss is most widely spread in tropical regions. It grows on limestone rocks, moist house foundations (so-called "cement moss"), walls on the shore and temporarily flooded rocks (Aruna & Krishnappa 2015).

MATERIALS AND METHODS

Study area: The plant specimens were collected from different areas of Rajasthan, such as Banasthali Vidyapith Campus, at an altitude of 320 m, 25°41' to 26°24' N, 75°19' to 76°16' E; Mount Abu, western Rajasthan, at an altitude of ca. 1600 m, 24°31' to 24°43' N, 72°38' to 72°53' E; and Ranthambore Forest, at an altitude of ca. 400–500 m, 26.0173° N, and 76.5026° E, during August and September 2023 (Figure 1). As mosses tend to grow in close association with each other, they were collected carefully by taking only homogenous patches free from dead and decayed material. The collected specimens invariably belong to terrestrial and corticolous habitats such as tree surfaces, rocks, soil-covered rocks, wet rocks, etc. The study area was thoroughly explored, and the plant specimens were collected from various localities and sub-localities rich in bryoflora.

Collection of plant sample: Plant materials for this study were collected from three different areas of Rajasthan with varying altitudes, i.e. Banasthali Vidyapith Campus in Tonk, Mount Abu and Ranthambore (Figure 2), and are deposited in the Banasthali University Rajasthan India (BURI) Herbarium Banasthali Vidyapith, India [Herbarium number: BURI-1737/2024 (Banasthali Vidyapith), BURI-1738/2024 (Mount Abu) and BURI-1736/2024 (Ranthambore)]. The plant specimens were collected carefully according to standard procedure with the help of a sharp-edged knife. The gathered materials were kept in brown paper

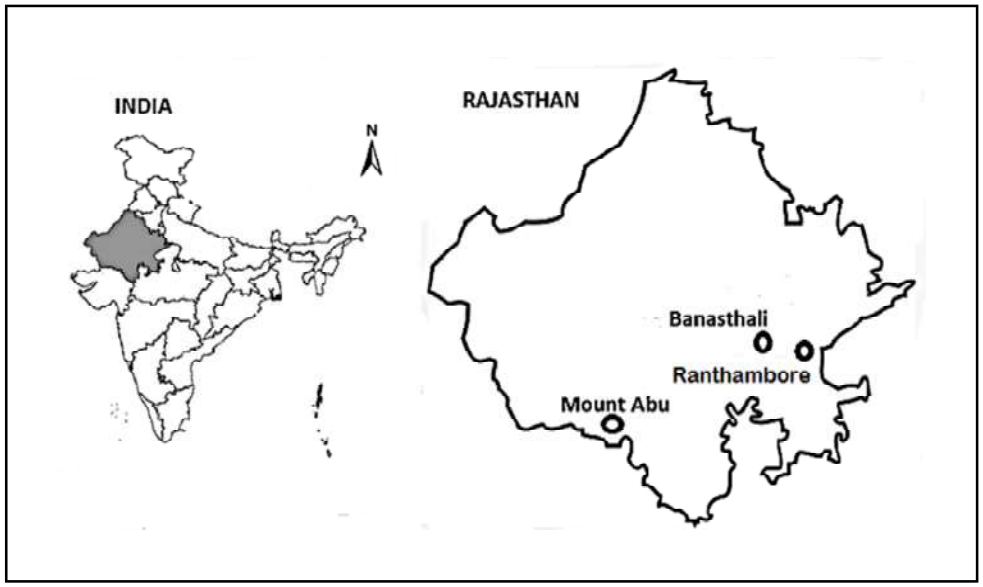
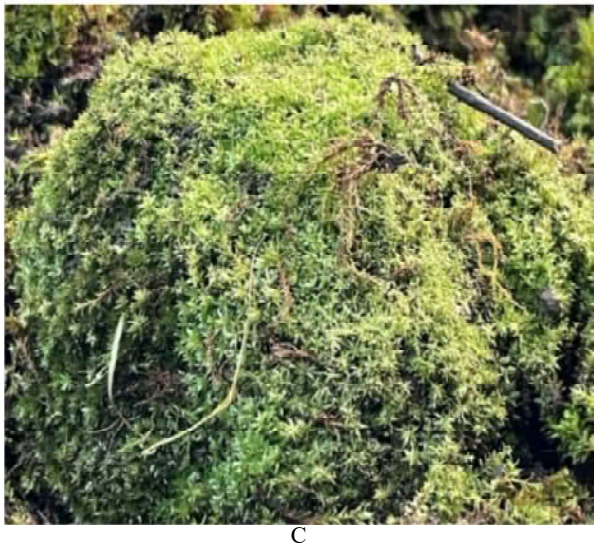


Figure 1. Location of three areas of Rajasthan from where plant specimens were collected. 1. Banasthali Vidyapith Campus at an altitude of 320 m (25°41' to 26°24' N, 75°19' to 76°16' E); 2. Mount Abu, western Rajasthan at an altitude of ca. 1600 m (24°31' to 24°43' N, 72°38' to 72°53' E); and 3. Ranthambore Forest, at an altitude of ca. 400–500 m, 26.0173° N, and 76.5026° E).

A

B



C

Figure 2. Populations of *Hyophila involuta* collected from 3 different altitudes. **A.** Banasthali Vidyapith (Altitude: ca. 320 m). **B.** Mount Abu (Altitude: ca. 1600 m). C. Ranthambore (Altitude: ca. 400–500 m).

packets directly in the case of dried specimens, and for wet specimens, blotting paper was used to soak up excess moisture and to dry the specimens. For the morphological study, the collected plant specimens were air dried at room temperature and kept in brown paper herbarium packets (size: 15 × 10 cm). Each herbarium packet was labelled with comprehensive information such as the herbarium specimen number and collection site, including the name of the major locality, altitude, plant habitat, date of collection, and collector's name. The herbarium packets were then kept in herbarium boxes (size: 37.5 × 15 cm). The labels now include the names of the identified plants and their determinators. Plant materials were kept at 4 degrees Celsius until the isolation procedures were completed. The remaining plant specimens were used for further observations.

Preparation of plant extract: The plant material was collected and cleaned with running tap water, followed by autoclaved water to eliminate dirt. After drying for 10 days at room temperature, fresh plant materials were pulverized using liquid nitrogen on a mortar pestle. The dirt-free plant powder was stored at 4°C in an airtight container for future usage (Mehra & De 2017).

Extraction: 10 grams of powdered plant material were loaded in a thimble made from Whatman filter paper (No-1) and placed in the Soxhlet assembly's extraction chamber for 12 hours, with 150 ml of methanol poured into the boiling flask. The flow of ice-cold water in the condenser portion of the built Soxhlet apparatus was kept constant. With a heating mantle's help, the boiling flask temperature was observed at 40–45° C. A cyclic movement of the solvent was noticed between the extraction chamber, the boiling flask, and the thimble. This cyclic flow was maintained for 20 to 25 cycles until the solvent in the extraction chamber was colourless.

Phytochemical Screening: To identify the various classes of active chemical constituents, the qualitative screening for the phytochemicals was carried out. To

do so, extracts were prepared from solvents, specifically methanol, for different altitudes of the plant. The solvent was then evaporated using a heating mantle at a temperature of 40ºC. To ensure a comprehensive screening, plants with variation in their altitude were used. The screening involved the use of standard prescribed methods. The results of the screening were recorded as present (++) for appreciable amount, (+) for trace amount, and (–) for completely absent. These results were tabulated in Table 1.

Quantitative Screening: Total phenolic content (TPC): The Folin-Ciocalteu technique was used to determine the plant extract's total phenolic and flavonoid content (Aiyegroro & Okoh 2010). Gallic acid and a methanolic solution of the samples were prepared at 1 mg/ml each. The reaction mixture was produced by mixing 0.5 mL of methanolic extract solution, 2.5 mL of water-dissolved 10% Folin-Ciocalteu reagent, and 2.5 mL of 7.5% $NaHCO_3$. Instead of the plant samples, 0.5 mL of methanol was used for the blank. The samples were incubated for 45 minutes at 45 degrees Celsius on a thermostat. The absorbance was measured at 765 nm. Results were presented as gallic acid equivalent (GAE) of mg/g for each gram of dry weight.

Total flavonoid content (TFC): The total flavonoid content was determined using the method described by Srinivasan et al. (2014). Using the aluminium chloride technique, quercetin was chosen as a benchmark for assessing the flavonoid concentration. Extracts and quercetin were produced in methanol (1 mg/mL). A spectrophotometer was used to measure the absorbance at 415 nm. Results were given in milligrams of quercetin equivalent (mg/g QE) per gram of dry-weight extracts.

Table 1. Phytochemical evaluation of *Hyophila involuta* in three different altitudes of Rajasthan.

Phytoconstituents	Banasthali Vidyapith	Mount Abu	Ranthambore
Phenols Ellagic acid test	++	+	+
Alkaloids Dragendroff's test	++	+	+
Flavonoids Flavonoid test	++	+	+
Cardiac Glycosides Kellar-Killani test	–	–	–
Sterols Liebermann-Burchard test	++	+	+
Saponins Froth formation test	++	+	+
Anthraquinone Borntrager's test	++	+	+

(++ (highly present), + (less present), - (Absent).

Table 2. Quantitative evaluation of *Hyophila involuta* in three different altitudes of Rajasthan. India.

Variables	Banasthali Vidyapith	Mount Abu	Ranthambore
Total Phenolic content (mg/g GAE)	0.57±0.009	0.53±0.006	0.56±0.005
Total flavonoid content (mg/g QE)	0.5±0.008	0.12±0.004	0.47±0.002

(The averages and standard deviations of n = 3 independent experiments are the data).

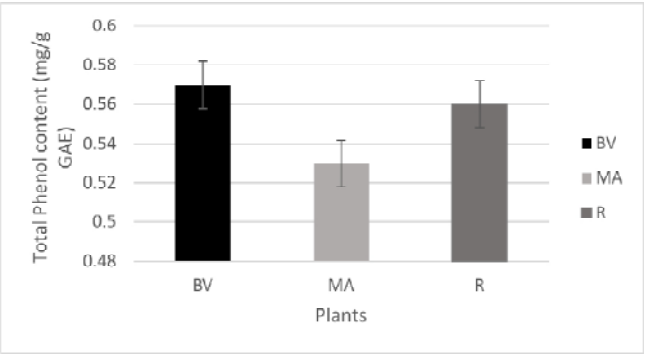


Figure 3. Total phenol content of *Hyophila involuta* in three different regions of Rajasthan, India.

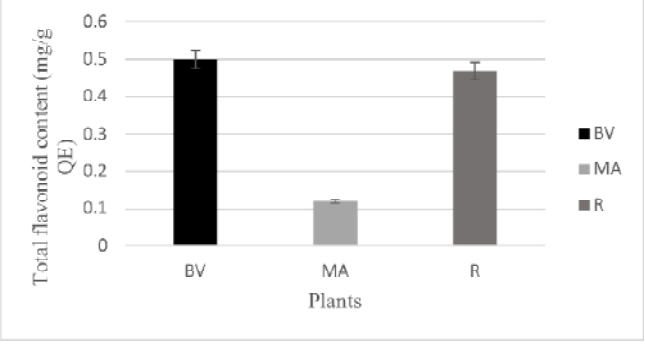


Figure 4. Total flavonoid content of *Hyophila involuta* in three different altitudes of Rajasthan, India.

RESULTS

Phytochemical Screening: The methanolic extract of *Hyophila involuta* plant was found to contain multiple phytoconstituents. Interestingly, the plant sample collected from Banasthali Vidyapith exhibited significantly better results when compared to samples from other altitudes such as Mount Abu and Ranthambore. Notably, the presence of cardiac glycosides was not detected in any of the plant samples from the aforementioned altitudes. After careful analysis, it was concluded that the ideal outcome was achieved with the plant collected from Banasthali Vidyapith (Table 1).

Quantitative Screening (TPC and TFC): Table 2 displays the phenolics and flavonoid contents present in the methanol fractions of plants gathered from different altitudes. The highest concentration of total phenolics and flavonoids was observed in the Banasthali Vidyapith region, followed by Ranthambore and Mount Abu regions. In terms of extraction yield, the samples were obtained in descending order of Banasthali Vidyapith > Ranthambore > Mount Abu. This information indicates that the Banasthali Vidyapith region is the most suitable location for collecting plants with high levels of phenolics and flavonoids (Table 2).

DISCUSSION

TPC and TFC Phenols are the usual phytoconstituents in plants, and their constituents have antioxidant properties. The antioxidant capacity of phenolic compounds has been studied extensively in the treatment of a variety of ailments, including diabetes, inflammation, neurogenerative disease, cardiac disease and cancer (Soobrattee et al. 2005). In this present study the maximum TPC was obtained in *Hyophila involuta* collected from Banasthali Vidyapith (0.57±0.009 mg/g GAE) and lower yield was obtained in Mount Abu (0.53±0.006 mg/g GAE) (Figure 3). Whereas, maximum TFC yield (0.5±0.008 mg/g QE) was also obtained with methanol and lower yield in Mount Abu (0.12±0.004 mg/g QE) was obtained (Figure 4, Table 2). The plant collected from Ranthambore has shown moderate results for both TPC (0.56±0.005 mg/g GAE) and TFC (0.47±0.002). Hence, the current study on *Hyophila involuta* showed more or less similar outcomes as reported earlier (Karim et al. 2014).

CONCLUSION

The study conducted on *H. involuta* has revealed important information about its phytochemistry through qualitative and quantitative analysis. This also depicted the variability in the plant's phytochemicals at different altitudes. The findings suggest that this moss has significant potential as a natural source of antioxidants, making it a promising candidate for inclusion in future herbal formulations aimed at boosting immunity and preventing infections. These results also highlight the importance of altitudinal variations on the phytochemical profiles, as at lower altitudes, maximum TPC was obtained, which shows that under abiotic stress, the production of secondary metabolites shows a noticeable increase. This outcome would be useful to identify new sources of natural compounds with therapeutic potential from stressed bryophyte species.

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REFERENCES

Adebiyi A.O., Oyedeji A.A., Chikwendu E.E. & Fatoke O.A. 2012. Phytochemical screening of two tropical moss plants, *Thuidium gratum* P. Beauv and *Barbula indica* Brid, grown in the Southwestern ecological zone of Nigeria. American Journal of Analytical Chemistry 3: 836–839.

Aiyegoro O.A. & Okoh A.I. 2010. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complementary and Alternative Medicine 10(1): 21. https://doi.org/10.1186/1472-6882-10-21

Alam A., Sharma V., Rawat K.K. & Verma P.K. 2015. Bryophytes–The ignored medicinal plants. SMU Medical Journal 2(1): 299–316.

Aruna K.B. & Krishnappa M. 2015. Phytochemical screening of lawn-forming moss *Hyophila involuta* in Kuvempu University Campus, Shivamogga District, Karnataka. International Journal of Research in Pharmacy and Life Sciences 3: 825–828.

Joshi S. & Alam A. 2023. Bryomonitoring of atmospheric elements in *Sphagnum* sp. commonly growing bryophyte in the Indian Himalayan region of Uttarakhand. Nova Geodesia 3(2): 127–127.

Karim F.A., Suleiman M., Rahmat A., M. A. & Bakar 2014. Phytochemicals, antioxidant and antiproliferative properties of five moss species from Sabah, Malaysia. International Journal of Pharmaceutical Sciences 6: 292–297.

Mehra Y.K. & De K. 2017. Determination of phytochemical, total flavonoids and antioxidant activity of methanolic extract of *Pisum sativum*. International Journal of Innovative Pharmaceutical Sciences and Research 5(8): 1–12.

Soobrattee M.A., Neergheen V.S., Luximon-Ramma A., Aruoma O.I. & Bahorun T. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis 579(1–2): 200-213.

Srinivasan B., Krishnan R. & Sundarapandian S. 2014. Preliminary Phytochemical screening and spectroscopic analysis of *Ormocarpum sennoides* DC. International Journal of Pharmaceutical Sciences 5: 216–220.