

**QUALITATIVE PHYTOCHEMICAL PROFILING OF IN VITRO
PROPAGATED *Marchantia polymorpha* AND *Marchantia polymorpha*
subsp. *montivagans* FROM MT. KITANGLAD, BUKIDNON,
PHILIPPINES**

Aires Jean E. Duave^{1*} and Lesley C. Lubos²

¹Lantapan National High School-Senior High School, Poblacion, Lantapan,
Bukidnon, 8722, Philippines

²Bukidnon State University, Poblacion, Malaybalay City, Bukidnon 8700,
Philippines

*Corresponding author: airesjean.eroisa001@deped.gov.ph

ABSTRACT – *Marchantia* species contain diverse secondary metabolites that have been investigated for their potential biological and biotechnological significance. However, limited information is available on the qualitative phytochemical composition of *Marchantia* taxa propagated under in vitro conditions. This study determined the qualitative phytochemical profile of in vitro-propagated *Marchantia polymorpha* and *Marchantia polymorpha* subsp. *montivagans* established from specimens collected in Mt. Kitanglad, Bukidnon, Philippines. Healthy gametophytes collected across nine elevational gradients were established under aseptic in vitro conditions using a sterilized garden soil and coco peat substrate (1:1). Following the culture period, harvested thalli were subjected to ethanolic extraction and qualitative phytochemical screening by an accredited laboratory using standard procedures. Eight classes of secondary metabolites were screened. Both taxa tested positive for flavonoids, tannins, saponins, and phenolic compounds, with differences in reaction intensity between taxa. *Marchantia polymorpha* showed a strongly positive (+++) reaction for flavonoids and weakly positive (+) reactions for tannins, saponins, and phenolic compounds, whereas *Marchantia polymorpha* subsp. *montivagans* showed a moderately positive (++) reaction for tannins and weakly positive (+) reactions for flavonoids, saponins, and phenolic compounds. Alkaloids, glycosides, terpenoids, and oxalates were not detected in either taxon. The findings establish the presence of several secondary metabolite classes in the in vitro-propagated *Marchantia* taxa examined, particularly flavonoids, tannins, saponins, and phenolic compounds. These results provide baseline phytochemical information that may support subsequent studies on the biological activities, antibacterial properties, conservation, and biotechnological applications of these taxa.

Keywords: *in vitro* propagation, *Marchantia polymorpha*, *Marchantia polymorpha* subsp. *montivagans*, phytochem

INTRODUCTION

The Philippines is recognized as one of the world's megadiverse countries, with diverse climatic conditions, topography, and ecological characteristics that support the growth of numerous plant species, including bryophytes, which are among the early-diverging lineages of land plants and perform important

ecological functions within terrestrial ecosystems (Glime, 2017; Renzaglia et al., 2020). Among bryophytes, the genus *Marchantia* has received considerable scientific attention because of its ecological significance and diverse secondary metabolites. Previous studies have reported the occurrence of flavonoids, terpenoids, phenolic compounds, alkaloids, glycosides, tannins, and saponins in *Marchantia* and related bryophytes, highlighting their importance in ecological interactions and natural-product research (Asakawa, 2022; Asakawa & Ludwiczuk, 2017). These metabolites have been associated in the literature with various biological activities, including antioxidant, antimicrobial, antifungal, anti-inflammatory, and cytotoxic effects, although such activities require direct experimental evaluation for specific taxa and extracts.

Although vascular plants remain widely investigated as sources of medicinal compounds, bryophytes offer an alternative group for phytochemical research because their distinctive chemical profiles are associated with evolutionary adaptation and environmental conditions (Sabovljević et al., 2016). Their secondary metabolites may contribute to defense against microbial pathogens, herbivores, ultraviolet radiation, and other environmental stresses (Asakawa, 2022). Consequently, bryophyte chemistry has increasingly been explored for its potential contribution to natural-product and biotechnological research. However, much of the available phytochemical research has focused on naturally occurring specimens, while comparatively limited attention has been given to the chemical composition of bryophytes propagated under controlled laboratory conditions (Coelho et al., 2021). This limitation is particularly relevant to *Marchantia*, whose phytochemical composition may be influenced by environmental conditions such as topography, physicochemical characteristics, and nutrient availability.

In vitro propagation provides a controlled approach for producing plant material while supporting ex situ conservation and potentially reducing repeated collection from natural populations. For bryophytes, this approach is particularly applicable because many liverworts possess strong regenerative capacities that allow their vegetative tissues to develop under suitable culture conditions (Sabovljević et al., 2016; Asakawa, 2022). Controlled propagation can also provide a consistent supply of biomass for subsequent laboratory investigations. However, the chemical composition of material produced under in vitro conditions cannot be assumed to be equivalent to that of naturally occurring material because changes in environmental conditions, nutrient availability, and physiological responses may influence secondary metabolite production. Thus, establishing the phytochemical profile of in vitro-propagated material is necessary to determine which classes of secondary metabolites are detectable under controlled culture conditions and to provide baseline information for subsequent investigations.

Despite the growing interest in bryophyte chemistry and in vitro propagation, information on the qualitative phytochemical composition of in vitro-propagated *Marchantia* remains limited. Existing studies have extensively addressed the morphology, taxonomy, distribution, ecological diversity, and chemistry of bryophytes, but comparatively fewer studies have characterized the secondary metabolites present in *Marchantia* material produced under controlled culture conditions (Coelho et al., 2021; Puglisi & Sciandrello, 2023). Moreover, limited information is available on the phytochemical profile of locally collected *Marchantia* taxa after establishment and propagation under in vitro conditions. Addressing this gap is important because qualitative phytochemical screening can identify the classes of secondary metabolites present in cultured material and provide a basis for determining which compounds warrant further quantitative, pharmacological, or antibacterial investigation. However, phytochemical screening alone does not establish biological activity or demonstrate equivalence between in vitro-propagated and naturally occurring specimens; such questions require separate comparative and bioactivity-based investigations.

In this context, the present study aimed to determine the qualitative phytochemical profile of two in vitro-propagated *Marchantia* taxa, namely *Marchantia polymorpha* and *Marchantia polymorpha* subsp. *montivagans*, established from specimens collected in Mt. Kitanglad, Bukidnon, Philippines. Specifically, the study aimed to assess the presence of eight classes of secondary metabolites—alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and oxalates—in the cultured specimens through qualitative phytochemical screening. The study was limited to determining the detectable classes and reaction intensities of these secondary metabolites and did not assess their concentrations, biological activities, antibacterial effects, or equivalence with naturally occurring specimens.

METHODOLOGY

Research Design, Sampling, and Taxonomic Identification

The study employed a descriptive research design to determine the qualitative phytochemical profile of in vitro-propagated *Marchantia polymorpha* and *Marchantia polymorpha* subsp. *montivagans* collected from Mt. Kitanglad, Bukidnon, Philippines. Field sampling was conducted across nine elevational bands at approximately 1,200, 1,400, 1,600, 1,800, 2,000, 2,200, 2,400, 2,600, and 2,800 masl. At each elevation, a 20-m × 2-m modified belt transect was established and divided into eight 5-m × 1-m subplots for systematic collection. Healthy, green, and actively growing gametophytes were purposively collected, while damaged, diseased, or decomposed thalli were excluded. The nine elevations represented collection sites rather than biological replicates of the phytochemical analysis; specimens from the different elevations were not separately propagated or analyzed according to elevation. Taxonomic identification was performed by a bryologist from Bukidnon State University based on morphological characteristics and relevant taxonomic references. No voucher specimen number or herbarium repository accession was available, and molecular authentication was not conducted.

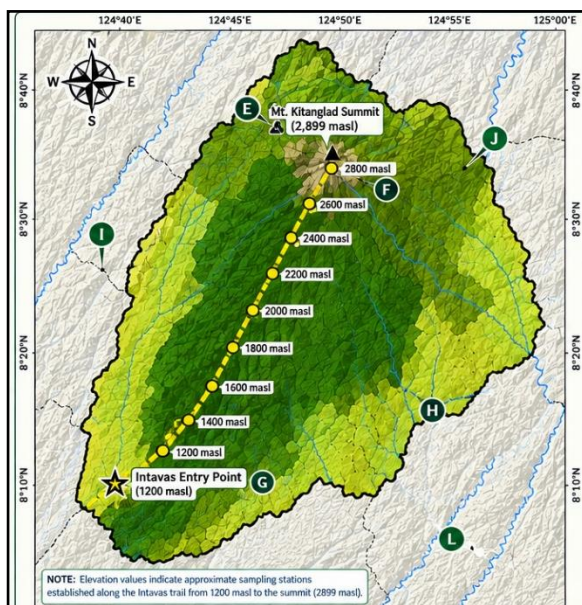


Figure 1. Map of the study site.

In Vitro Propagation and Sample Preparation

The collected explants were cleaned, surface-sterilized with 70% ethanol for 5–10 seconds, and rinsed with sterile distilled water before inoculation. In vitro propagation was conducted using a 1:1 mixture of sterilized garden soil and coco peat as the culture substrate. Five culture replicates were established for each taxon, with six explant clumps per replicate, and maintained at 20–25°C under controlled laboratory conditions. The cultures were monitored weekly for contamination, survival, regeneration, and growth throughout the culture period. At harvest, the regenerated thalli were rinsed, air-dried under shaded and well-ventilated conditions until constant weight, and prepared for phytochemical extraction. The cultured materials were handled as taxon-level samples because the specimens collected from the nine elevations were combined during culture establishment rather than maintained as separate elevation treatments.

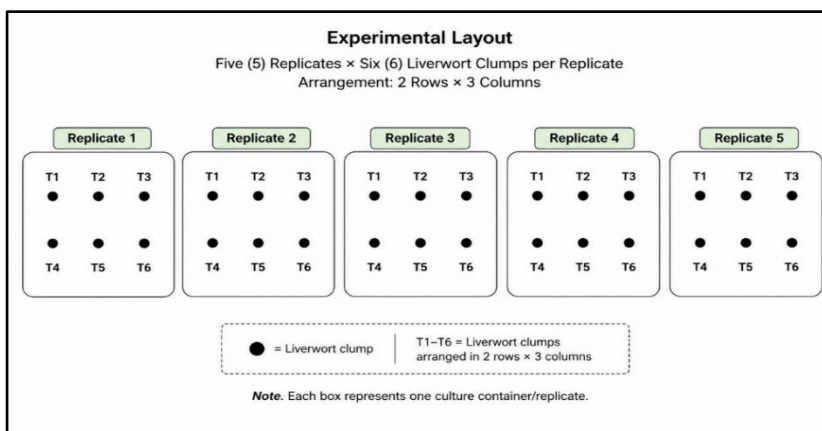


Figure 2. Experimental layout.

Ethanollic Extraction and Qualitative Phytochemical Screening

The dried in vitro-propagated thalli approximately 60–70 grams were submitted to WVN Research and Laboratory Supply, Davao City, Philippines, for extraction and qualitative phytochemical analysis. The laboratory pulverized the dried material, subjected it to ethanolic extraction through maceration, filtered the extract, concentrated the filtrate using a rotary evaporator, and prepared the resulting crude ethanolic extract for screening. Eight classes of secondary metabolites—alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, and oxalates—were qualitatively assessed using the laboratory's established procedures. Because the laboratory provided the analytical results but did not provide the researchers with the detailed SOP specifying the plant material-to-solvent ratio, extract amount per assay, individual reagents and test procedures, controls, and assay replication, these parameters could not be independently documented. The official laboratory report was therefore used as the basis for reporting the presence or non-detection and reaction intensity of the screened metabolites. Since only in vitro-propagated materials were analyzed and no corresponding wild specimens were subjected to the same screening, the phytochemical results were interpreted as exploratory, taxon-level qualitative observations rather than evidence of phytochemical retention, equivalence, concentration changes, or biological activity. Table 1 presents the qualitative phytochemical interpretation based on WVN Testing and Research Laboratory.

Table 1. Interpretation for Qualitative Phytochemical Profiling.

Symbol	Interpretation	Description
+++	Strongly Positive	Indicates a strong reaction, suggesting a high presence or abundance of the phytochemical constituent in the extract.
++	Moderately Positive	Indicates a moderate reaction, suggesting a considerable amount of the phytochemical constituent.
+	Weakly Positive	Indicates a slight reaction, suggesting the presence of the phytochemical constituent in low concentration.
–	Negative	Indicates no observable reaction and the absence of the phytochemical constituent.

Source: WVN Testing and Research Laboratory, Davao City, Bukidnon; Nortjie, E., Basitere, M., Moyo, W., & Ray, S. S. (2022). *Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review*. *Plants*, 11(15), 2011. <https://doi.org/10.3390/plants1115201>

RESULTS AND DISCUSSION

Qualitative Phytochemical profile

Table 2 presented the qualitative phytochemical profile of *Marchantia polymorpha* and *Marchantia polymorpha* subsp. *montivagans* based on the laboratory analysis conducted by WVN Research and Laboratory Supply. The screening examined eight selected chemical classes using qualitative procedures. As presented in Table, both taxa yielded positive reactions for flavonoids, tannins, saponins, and phenolic compounds, although differences were observed in reaction intensity.

Table 2. Phytochemical Testing Result.

Test Parameters	Results	
	<i>Marchantia polymorpha</i>	<i>Marchantia polymorpha</i> subsp. <i>Montivagans</i>
Alkaloids	-	-
Flavonoids	++	+
Tannins	+	++
Saponins	+	+
Glycosides	-	-
Phenols	+	+
Terpenoids	-	-
Oxalate	-	-

Marchantia polymorpha showed a strongly positive (+++) reaction for flavonoids and weakly positive (+) reactions for tannins, saponins, and phenolic compounds, whereas *M. polymorpha* subsp. *montivagans* showed a moderately positive (++) reaction for tannins and weakly positive (+) reactions for flavonoids, saponins, and phenolic compounds. Both taxa yielded negative (–) reactions for alkaloids, glycosides, terpenoids, and oxalates. As presented in Table 1, the two taxa therefore exhibited a generally similar qualitative phytochemical pattern, with differences in reaction intensity particularly for flavonoids

and tannins. The shared detection of these four chemical classes indicates that the ethanolic extracts of the examined thalli produced detectable reactions corresponding to flavonoids, tannins, saponins, and phenolic compounds under the laboratory conditions employed.

The differences in reaction intensity provide a basis for describing the qualitative chemical responses of the two taxa. The strongly positive (+++) flavonoid reaction in *M. polymorpha* represents a more pronounced assay response than the weakly positive (+) reaction observed for *M. polymorpha* subsp. *montivagans*, whereas the latter exhibited the stronger response for tannins. These symbols, however, should be understood as qualitative reaction intensities and not as measurements of metabolite concentration. A positive reaction indicates that compounds capable of producing the corresponding assay response were detectable in the extract, but it does not establish the amount of the compound present or identify the specific molecules responsible for the reaction. Qualitative phytochemical screening is therefore useful for preliminary characterization, whereas compound identification and concentration determination require more specific analytical approaches (Nortjie et al., 2022; Sen et al., 2023). This distinction prevents the reaction intensity from being interpreted as a direct measure of the abundance or potency of the detected constituents.

The detection of flavonoids, tannins, saponins, and phenolic compounds in both taxa is generally consistent with reports describing diverse specialized metabolites in liverworts and *Marchantia*. Previous studies have documented flavonoids, phenolic compounds, tannins, bibenzyls, bisbibenzyls, and other chemical constituents among bryophytes (Asakawa et al., 2022; Sen et al., 2023). Studies involving *M. polymorpha* have likewise reported a diverse chemical composition, although the compounds detected may vary according to taxonomic characteristics, developmental stage, environmental history, extraction conditions, and analytical procedures (Giardini et al., 2022; Ginin et al., 2025; Kozłowska et al., 2025). Thus, the present findings are comparable with the broader phytochemical profile reported for liverworts while providing preliminary information specifically on the chemical classes detectable in the two cultured *Marchantia* taxa examined.

The contrasting qualitative responses for flavonoids and tannins may reflect differences in the chemical constituents extracted from the two taxa, although the present methodology does not allow the specific basis of these differences to be determined. Taxon-specific biochemical characteristics may contribute to variation in chemical profiles, but further compound-specific and quantitative analyses would be necessary to verify such differences. The nine elevational bands used during field collection likewise should not be interpreted as phytochemical treatments. They represented collection sites from which source materials were obtained, while the collected specimens were subsequently established and propagated without maintaining separate phytochemical cultures according to elevation. Consequently, the present results cannot be used to determine an elevational effect on phytochemical composition. If elevation is to be examined as a factor affecting phytochemical variation, future investigations should maintain samples separately by elevation and include appropriate biological replication during both propagation and phytochemical analysis.

The negative reactions for alkaloids, glycosides, terpenoids, and oxalates provide additional information on the qualitative profile of the extracts. However, these findings indicate only that no detectable reaction was obtained under the analytical conditions used and should not be interpreted as definitive evidence of the complete absence of these compounds. Qualitative screening outcomes can be influenced by extraction efficiency, solvent characteristics, reagent specificity, and detection capability. Such factors are particularly relevant when comparing qualitative results generated through different procedures or laboratories. Oxalates were included because they formed part of the laboratory's screening

panel; however, they are chemically distinct from the major secondary-metabolite groups such as flavonoids, tannins, saponins, and alkaloids. Their inclusion is therefore treated as part of the broader chemical screening rather than as evidence that oxalates constitute another secondary-metabolite class in the same sense as the other groups examined.

The detected phytochemical classes may be relevant to the broader chemical characterization of *Marchantia*, but their biological significance cannot be established from the present qualitative results alone. Although previous studies have reported biological activities associated with particular compounds from *Marchantia* and other bryophytes (Giardini et al., 2022; Kozłowska et al., 2025; Ginin et al., 2025), the current screening did not identify individual compounds, determine their concentrations, or directly evaluate antibacterial, antioxidant, therapeutic, or plant-defense activities. Therefore, the detection of flavonoids, tannins, saponins, and phenolic compounds should be interpreted strictly as evidence of detectable phytochemical classes in the extracts examined, rather than as evidence of biological activity. Establishing such activities would require separate, appropriately designed bioassays and, preferably, characterization of the compounds responsible for the observed effects.

As a whole, the findings provide preliminary baseline information on the qualitative phytochemical characteristics of the in vitro-propagated *M. polymorpha* and *M. polymorpha* subsp. *montivagans* examined in this study. The detection of flavonoids, tannins, saponins, and phenolic compounds in their ethanolic extracts indicates that these phytochemical classes were detectable under the analytical conditions employed, while the non-detection of the other screened classes further characterizes the qualitative profile of the extracts. These findings should be regarded as a preliminary qualitative phytochemical characterization rather than evidence of pharmacological potential, antibacterial activity, pharmaceutical efficacy, or preservation of the original phytochemical profile of naturally occurring plants. Similarly, the possible use of in vitro propagation as an approach for sustainable production of *Marchantia* material remains a potential application that requires further assessment. More comprehensive studies involving replicated quantitative analyses, compound-specific characterization, bioactivity assays, and comparisons between naturally collected and in vitro-propagated materials are needed to establish the chemical composition, biological properties, and practical or sustainability-related applications of these taxa.

CONCLUSION

The study established a preliminary qualitative phytochemical profile of the in vitro-propagated *Marchantia polymorpha* and *M. polymorpha* subsp. *montivagans* collected from Mt. Kitanglad, Bukidnon, Philippines. The ethanolic extracts of both taxa showed detectable flavonoids, tannins, saponins, and phenolic compounds, with differences in qualitative reaction intensity for flavonoids and tannins, while alkaloids, glycosides, terpenoids, and oxalates were not detected under the laboratory conditions employed. These findings provide baseline information on the phytochemical classes detectable in the cultured thalli examined; however, the qualitative results do not establish metabolite concentrations, specific compound identities, or biological activities. Thus, the study represents an initial phytochemical characterization rather than evidence of antibacterial, pharmacological, or pharmaceutical efficacy. The potential application of in vitro propagation for sustainable production of *Marchantia* materials likewise remains subject to further investigation. Future studies involving quantitative and compound-specific analyses, replicated assays, bioactivity testing, and comparisons with naturally collected materials are recommended to provide a more comprehensive understanding of the phytochemical characteristics and potential applications of these taxa.

ACKNOWLEDGEMENT

The researchers sincerely express their gratitude to Bukidnon State University for the institutional support and assistance extended throughout the conduct of this study. Special appreciation is given to the Botanical Garden and Herbarium for providing valuable support in the collection, identification, and handling of the plant materials, and to the Plant Tissue Culture Laboratory for the assistance and facilities that supported the in vitro propagation of the *Marchantia* taxa. The researchers also gratefully acknowledge all individuals, laboratory personnel, technical staff, validators, and other contributors whose time, expertise, assistance, and encouragement contributed to the successful completion of this study.

Above all, the researchers offer their deepest gratitude to Almighty God for His guidance, wisdom, strength, protection, and blessings throughout the entire research process. His grace and providence made it possible to overcome the challenges encountered and bring this study to completion.

STATEMENT OF AUTHORSHIP

The authors declare that they jointly contributed to the conceptualization, design, implementation, analysis, interpretation, and writing of this study. All authors participated in reviewing and revising the manuscript and approved the final version for submission. The authors further declare that the work is original, that all sources used have been appropriately acknowledged and cited, and that no part of the manuscript has been published elsewhere or submitted for simultaneous consideration in another publication.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data include the qualitative phytochemical screening results and other relevant study records. Additional information may be provided subject to institutional policies and appropriate conditions for data sharing.

REFERENCES

- Asakawa, Y., & Ludwiczuk, A. (2017). Chemical constituents of bryophytes: structures and biological activity. *Journal of natural products*, 81(3), 641- 660.
- Asakawa, Y., Ludwiczuk, A., Novakovic, M., Bukvicki, D., & Anchang, K. Y. (2022). Bis-bibenzyls, bibenzyls, and terpenoids in 33 genera of the Marchantiophyta (Liverworts): Structures, synthesis, and bioactivity. *Journal of Natural Products*, 85(3), 729–762. <https://doi.org/10.1021/acs.jnatprod.1c00302>
- Bowman, J. L., Kohchi, T., Yamato, K. T., et al. (2017). Insights into land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell*, 171(2), 287–304.e15. <https://doi.org/10.1016/j.cell.2017.09.009>
- Coelho, M. C., Gabriel, R., Hespanhol, H., Borges, P. A. V., & Ah-Peng, C. (2021). Bryophyte diversity along an elevational gradient on Pico Island (Azo
- Giardini, S., Bellini, E., Bandoni, E., Saba, A., & Sanità di Toppi, L. (2022). Tools for in vitro propagation/synchronization of the liverwort *Marchantia polymorpha* and application of a

- validated HPLC-ESI-MS-MS method for glutathione and phytochelatin analysis. *Stresses*, 2(1), 136–145. <https://doi.org/10.3390/stresses2010011>
- Ginin, S., Dinev, T., Grozeva, N., Memdueva, N., & Tzanova, M. (2025). A review of bryophytes: Enzymatic and non-enzymatic antioxidants as a key for their pharmacological potential and green-biotechnological application. *Antioxidants*, 15(1), Article 16. <https://doi.org/10.3390/antiox15010016>
- Glime, J. M. (2017). *Bryophyte Ecology* (Vols. 1–5). Michigan Technological University. <https://digitalcommons.mtu.edu/bryophyte-ecology/>
- Kozłowska, M., et al. (2025). *Marchantia polymorpha* as a source of biologically active compounds. *Molecules*, 30(3), 558. <https://doi.org/10.3390/molecules30030558>
- Muñoz, M., Díaz, O., Reinún, W., Winkler, A., & Quevedo, R. (2019). Slow growth in vitro culture for conservation of Chilotanum potato germplasm. *Chilean journal of agricultural research*, 79(1), 26-35.
- Nortjie, E., Basitere, M., Moyo, W., & Ray, S. S. (2022). Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants*, 11(15), 2011. <https://doi.org/10.3390/plants11152011>
- Puglisi, M., & Sciandrello, S. (2023). Bryophyte Diversity and Distribution Patterns along Elevation Gradients of the Mount Etna (Sicily), the Highest Active Volcano in Europe. *Plants*, 12(14), 2655. <https://doi.org/10.3390/plants12142655>
- Puttick, M. N., Morris, J. L., Williams, T. A., Cox, C. J., Edwards, D., Kenrick, P., Pressel, S., Wellman, C. H., Schneider, H., Pisani, D., & Donoghue, P. C. J. (2018). The interrelationships of land plants and the nature of the ancestral embryophyte. *Current Biology*, 28(5), 733–745.e2. <https://doi.org/10.1016/j.cub.2018.01.063>
- Renzaglia, K. S., Villarreal, J. C., & Piatkowski, B. T. (2020). Bryophytes: Origin and early evolution of land plants. *American Journal of Botany*, 107(7), 1023–1035. <https://doi.org/10.1002/ajb2.1490>
- Sabovljević, M. S., Sabovljević, A. D., Ikram, N. K. K., Peramuna, A., Bae, H., & Simonsen, H. T. (2016). Bryophytes—an emerging source for herbal remedies and chemical production. *Plant genetic resources*, 14(4), 314-327.
- Sen, K., Khan, M. I., Paul, R., Ghoshal, U., & Asakawa, Y. (2023). Recent advances in the phytochemistry of bryophytes: Distribution, structures and biological activity of bibenzyl and bisbibenzyl compounds. *Plants*, 12(24), 4173. <https://doi.org/10.3390/plants12244173>

