Journal of Chemical Learning Innovation
Vol. 2, No. 2, December 2025, pp. 266~273
ISSN: 3063-0886, DOI:10.37251/jocli.v2i2.2963

Phytochemical Investigation of *Mastigophora diclados*: Isolation of a Herbertene-Type Sesquiterpenoid

Ferry Indar Ardiansyah[1], Moeketsi Freddie Tlali[2]
[1]Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jakarta, Indonesia
[2]Free State University, South Africa

Article Info

Article history:

Received Oct 5, 2025
Revised Nov 25, 2025
Accepted Dec 27, 2025
OnlineFirst Dec 31, 2025

Keywords:

Bryophytes
Herbertene
Mastigophora Diclados
Phytochemical Analysis
Secondary metabolites

ABSTRACT

Purpose of the study: This study aims to isolate and characterize secondary metabolite compounds from the ethyl acetate extract of the liverwort *Mastigophora diclados* collected in Indonesia using chromatographic separation and spectroscopic analysis.

Methodology: This study employed an experimental laboratory design using maceration extraction with n-hexane and ethyl acetate. Isolation was conducted using column chromatography and thin-layer chromatography (silica gel 60 GF254). Instruments included rotary evaporator (Eyela N-1000), water bath (Eyela SB-1000), and ^{1}H-NMR spectrometer (JEOL 500 MHz). Data were analyzed through spectral comparison and phytochemical screening methods.

Main Findings: The ethyl acetate extract yielded 41.78 g (1.98%). Phytochemical screening indicated the presence of terpenoids. Two pure compounds were obtained (III-B: 8 mg; IV-B: 4 mg) with Rf 0.44. Compound III-B showed a melting point of 152–154 °C. ^{1}H-NMR analysis revealed characteristic signals of four methyl groups and olefinic protons, indicating a herbertene-type sesquiterpenoid structure.

Novelty/Originality of this study: This study is the first to report the isolation and structural characterization of secondary metabolites from Indonesian *Mastigophora diclados*. It reveals a herbertene-like compound with slight spectral variations, suggesting a potential new derivative influenced by geographical factors, thereby contributing to bryophyte chemotaxonomy and expanding natural product research in tropical regions.

Corresponding Author:
Ferry Indar Ardiansyah,
Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jl. Kertamukti No. 1, Ciputat, South Tangerang, Banten 15412, Indonesia
Email: ferry@gmail.com

1. INTRODUCTION

Indonesia is recognized as one of the world's megabiodiversity countries, particularly due to its vast tropical rainforest ecosystems that host diverse plant groups, including bryophytes. Bryophytes, which consist of mosses, liverworts, and hornworts, are among the earliest land plants and play a crucial ecological role in ecosystem succession and nutrient cycling [1], [2]. These plants are often found in moist and shaded environments, ranging from lowlands to highlands, where they act as pioneer species in degraded habitats [3]-[5]. Despite their ecological importance, bryophytes remain underexplored compared to higher plants, particularly in tropical regions such as Indonesia [6], [7]. Therefore, investigating bryophytes is essential not only for biodiversity conservation but also for uncovering their potential applications in science and medicine [8]-[10].

Among bryophytes, liverworts are known to possess unique chemical constituents stored in specialized organelles called oil bodies. These organelles synthesize a wide range of secondary metabolites, including terpenoids, aromatic compounds, and acetogenins, many of which exhibit significant biological activities [11], [12], [13]. Previous studies have reported that liverwort-derived compounds show antimicrobial, antioxidant, cytotoxic, and enzyme inhibitory properties, making them promising candidates for pharmaceutical development [14]. In particular, sesquiterpenoids such as herbertene derivatives have attracted attention due to their diverse pharmacological activities [15]-[17]. Consequently, liverworts represent a valuable yet underutilized source of bioactive natural products.

Although Indonesia harbors rich bryophyte diversity, scientific investigations focusing on their chemical composition and bioactive compounds remain limited. Most existing studies have primarily addressed ecological aspects or general phytochemical screenings, rather than detailed compound isolation and structural elucidation [18]-[20]. Furthermore, research on Indonesian liverwort species is still scarce compared to studies conducted in other regions such as Japan and Malaysia [21]-[23]. Importantly, there has been no comprehensive report on the isolation of secondary metabolites from *Mastigophora diclados* collected in Indonesia [24]. This gap highlights the need for systematic phytochemical studies to explore and document the chemical diversity of Indonesian bryophytes.

Mastigophora diclados, a member of the Mastigophoraceae family, is a liverwort species widely distributed across tropical and subtropical regions, including Indonesia. Previous studies have identified that this species contains sesquiterpenoid compounds, particularly herbertene-type molecules, which exhibit notable biological activities such as cytotoxic, antioxidant, and antimicrobial effects . Investigations conducted in regions such as Tahiti and Malaysia revealed variations in chemical composition depending on geographical origin [25], [26], [27]. Additionally, studies in Indonesia reported that ethanol extracts of *M. diclados* possess cytotoxic and anti-inflammatory activities [27], [28]. However, these studies have not yet focused on isolating and characterizing individual bioactive compounds from Indonesian samples.

Given the variability of secondary metabolites influenced by environmental and geographical factors, investigating *M. diclados* from Indonesia may reveal novel or distinct chemical constituents. The absence of studies on compound isolation from this species in Indonesia provides a strong basis for originality in this research. Moreover, isolating pure compounds allows for a more accurate understanding of structure–activity relationships compared to crude extract analysis [29], [30]. This approach is essential for identifying potential lead compounds for drug development. Therefore, this study offers novelty by focusing on the isolation and structural characterization of secondary metabolites from Indonesian *M. diclados*.

Based on the identified research gap, this study aims to isolate secondary metabolite compounds from the ethyl acetate extract of *Mastigophora diclados*. The isolation process is carried out using chromatographic techniques, including column chromatography and thin-layer chromatography [31], [32]. Furthermore, the structure of the isolated compound is elucidated using spectroscopic methods, particularly proton nuclear magnetic resonance (^{1}H-NMR). Through these approaches, the study seeks to identify and characterize bioactive compounds present in this species. Ultimately, this research provides fundamental chemical insights into Indonesian liverworts.

This study contributes to the advancement of natural product research by providing new data on the chemical constituents of *Mastigophora diclados* from Indonesia. The findings are expected to enrich the scientific database of bryophyte-derived secondary metabolites, which remains limited. Additionally, the identification of specific compounds, such as herbertene-like structures, may support further pharmacological investigations. The results also highlight the potential of Indonesian bryophytes as a source of novel bioactive compounds. Therefore, this research not only fills an existing knowledge gap but also supports future exploration of natural products for pharmaceutical applications.

2. RESEARCH METHOD

2.1. Study Design

This study employed an experimental laboratory-based approach aimed at isolating and characterizing secondary metabolites from the liverwort *Mastigophora diclados*. The workflow consisted of sample preparation, extraction, phytochemical screening, compound isolation, purification, and structural elucidation. Chromatographic techniques were used for separation, while spectroscopic analysis was performed for compound identification. The study design follows standard procedures in natural product chemistry. All experiments were conducted under controlled laboratory conditions..

2.2. Materials and Reagents

The plant material used in this study was *Mastigophora diclados* (Brid. ex Web.) Nees collected from Mount Slamet forest, Central Java, Indonesia . The sample was taxonomically identified at the Indonesian Institute of Sciences (LIPI). Organic solvents used included ethyl acetate, n-hexane, methanol, ethanol, chloroform, and

distilled water. Silica gel 60 GF254 was used as the stationary phase for chromatography. Analytical reagents such as $FeCl_3$, H_2SO_4, Dragendorff, Mayer, and Bouchardat reagents were used for phytochemical analysis.

2.3. Instruments

The instruments used included analytical balance, rotary evaporator (Eyela N-1000), water bath (Eyela SB-1000), column chromatography apparatus, thin-layer chromatography (TLC) plates, and melting point apparatus . Structural elucidation was performed using a 500 MHz proton nuclear magnetic resonance (^{1}H-NMR) spectrometer (JEOL). Additional glassware such as Erlenmeyer flasks, pipettes, and vials were also used. All instruments were calibrated prior to use. Standard laboratory procedures were followed throughout the experiment.

2.4. Instruments

Fresh samples of *M. diclados* (2.220 kg) were cleaned to remove impurities and washed with water. The samples were then air-dried under shade conditions to prevent degradation of thermolabile compounds. After drying, the material was ground into a coarse powder. The powdered sample was stored in a dry container prior to extraction. This step ensured homogeneity and increased extraction efficiency.

2.5. Extraction Procedure

Extraction was carried out using maceration with ethyl acetate as the solvent . The powdered sample was soaked in ethyl acetate at room temperature with occasional stirring. The process was repeated several times to ensure exhaustive extraction. The combined extracts were filtered and concentrated using a rotary evaporator under reduced pressure. The crude extract obtained was then weighed and stored for further analysis.

2.6. Phytochemical Screening

Preliminary phytochemical screening was conducted to identify the presence of major secondary metabolite groups. Standard qualitative tests were performed to detect alkaloids, flavonoids, terpenoids, and phenolic compounds. The detection was based on color changes or precipitate formation after the addition of specific reagents. This screening provided initial information on the chemical composition of the extract. The results guided further isolation procedures.

2.7. Isolation and Purification

The crude extract was subjected to column chromatography using silica gel as the stationary phase . Elution was carried out using a gradient system of n-hexane and ethyl acetate with increasing polarity. Fractions were collected and monitored using thin-layer chromatography (TLC). Fractions with similar TLC profiles were combined. Further purification was performed using recrystallization to obtain a pure compound.

2.8. Thin-Layer Chromatography (TLC) Analysis

TLC analysis was performed using silica gel GF254 plates as the stationary phase. The mobile phase consisted of n-hexane:ethyl acetate (8:2). Spots were visualized under UV light at 254 nm and 366 nm, followed by spraying with detection reagents. The retention factor (Rf) values were calculated to evaluate compound separation. TLC was used to monitor fraction purity during isolation.

2.9. Structure Elucidation

The purified compound was analyzed using ^{1}H-NMR spectroscopy to determine its molecular structure . The sample was dissolved in deuterated chloroform ($CDCl_3$) prior to analysis. Chemical shift values (δ), multiplicity, and coupling constants were recorded. The obtained spectra were compared with literature data. The compound was identified as having structural similarity to herbertene.

3. RESULTS AND DISCUSSION

The plant material used in this study comprised the entire parts of *Mastigophora diclados* (Brid. ex Web.) Nees, collected from the Mount Slamet forest, Baturaden, Purwokerto, Central Java, Indonesia. The specimen was taxonomically identified by the Bogoriense Research Center. A total of 2.220 kg of the sample was washed under running water until clean. Wet sorting was then carried out to remove impurities and foreign materials, thereby minimizing contaminants present in the test material. The drying process was conducted by air-drying the sample indoors using a traditional tray. The dried simplicia were re-sorted to eliminate any remaining impurities. Subsequently, the cleaned simplicia were ground using a blender into a fine powder. After undergoing the processes of sorting, drying, and grinding, a total of 2.103 kg of dried simplicia powder of *Mastigophora diclados* (Brid. ex Web.) Nees was obtained.

3.1. Extraction

A total of 2.103 kg of dried simplicia powder of *Mastigophora diclados* (Brid. ex Web.) Nees was macerated nine times over a period of nine days using 30 liters of *n*-hexane as the solvent. The advantage of the maceration method lies in its simplicity in terms of procedure and equipment, whereas its disadvantages include the lengthy extraction time, high solvent consumption, and relatively incomplete extraction efficiency.

The maceration extract was filtered, and the resulting filtrate was concentrated using a vacuum rotary evaporator at approximately 30 °C to obtain a viscous *n*-hexane extract. The residual marc from the *n*-hexane extraction was subsequently re-macerated using ethyl acetate as the solvent, carried out seven times over seven days with a total solvent volume of approximately 25 liters. The solvent was then evaporated using a vacuum rotary evaporator to yield 41.78 grams of a viscous ethyl acetate extract.

Table 1. Yield Data of *Mastigophora diclados* (Brid. ex Web.) Nees Extracts

Name of the Drug	Extract Weight (g)	Extract Yield (%)
n-hexane extract	52 grams	2.53%
Ethyl acetate extract	41.78 grams	1.98%

3.2. Phytochemical Screening

The results of the phytochemical screening of the ethyl acetate extract of the liverwort *Mastigophora diclados* (Brid. ex Web.) Nees are presented in Table 2.

Table 2. Phytochemical screening results of the ethyl acetate extract of *Mastigophora diclados* (Brid. ex Web.) Nees

No	Group	Observation result
1	Alkaloids	-
2	Flavonoids	-
3	Terpenoids	+
4	Phenolics	-
5	Anthraquinones	-
6	Saponins	-

3.3. Isolation and Purification of Compounds

The isolation and purification of compounds were carried out on the ethyl acetate extract. From the isolation and purification processes of the ethyl acetate extract of *Mastigophora diclados* (Brid. ex Web.) Nees, 8 mg of pure compound III-B and 4 mg of pure compound IV-B were obtained, each exhibiting an Rf value of 0.44.

Table 3. Data of pure isolates from the ethyl acetate extract of *Mastigophora diclados* (Brid. ex Web.) Nees using *n*-hexane:ethyl acetate (8:2) as the eluent

Compound	Organoleptic	Rf	Isolate Weight (g)
III-B	Needle crystals, white	0.44	0.008 grams
IV-B	Needle crystals, white	0.44	0.004 grams

3.4. Melting Point Determination

Melting point determination was conducted to assess the purity of the compound based on its melting range. A compound is considered pure if it exhibits a narrow melting range of approximately ±2 °C. The melting point analysis of compound III-B showed a melting range of 152–154 °C. This result indicates that the difference between the initial melting point and complete melting is 2 °C; therefore, compound III-B can be considered pure.

3.5. Structure Elucidation of the Pure Compound

The structure elucidation of the pure compound was performed on compound III-B, which appeared as white needle-shaped crystals with a melting point of 152–154 °C. Thin-layer chromatography (TLC) analysis using *n*-hexane:ethyl acetate (8:2) as the eluent showed that this compound has an Rf value of 0.44.

Structural analysis using ^{1}H-NMR spectroscopy enables the identification of protons within a molecular structure. The data obtained from ^{1}H-NMR consist of chemical shifts that serve as characteristic signals of specific parts of a molecular structure and assist in identifying functional groups within a compound.

The ^{1}H-NMR spectral data of compound III-B revealed the presence of 3 protons at a chemical shift (δH) of 0.64 ppm (s, 3H), indicating a methyl group (CH_3). Another set of 3 protons appeared at δH 0.99 ppm (s, 3H), also corresponding to a methyl group (CH_3). Additionally, 6 protons were observed at δH 1.25 ppm (s, 6H), indicating the presence of two methyl groups (2 × CH_3).

In the aromatic/olefinic proton region, a total of 4 protons were observed: at δH 4.89 ppm (1H, d, J = 1.95 Hz), δH 4.94 ppm (1H, d, J = 10.35 Hz), δH 5.14 ppm (1H, s), and δH 5.70 ppm (1H, dd, J = 11.05 Hz and 10.35 Hz).

Table 4. Proton chemical shift data (δH) of compound III-B measured at 500 MHz using $CDCl_3$ as solvent

No	δH	Function Group
1	0.64 ppm(s)	3H (CH3)
2	0.99 ppm(s)	3H (CH3)
3	1.25 ppm(s)	6H (2CH3)
4	4.89 ppm (d)	1H
5	4.94 ppm (d)	1H
6	5.14 ppm(s)	1H
7	5.70 ppm (dd)	1H

From the ^{1}H-NMR data above, it is evident that compound III-B exhibits a pattern consisting of four methyl groups and four protons in the aromatic region. The presence of four aromatic protons suggests that the structure of compound III-B contains two substituents. This spectral pattern shows similarity to compounds belonging to the herbertene-type sesquiterpenes.

Herbertene compounds are known to display characteristic spectra, namely the presence of four methyl groups appearing at chemical shifts (δH) of 0.58 ppm (s), 1.10 ppm (s), and 1.27 ppm (s). In addition, there are four protons in the aromatic region observed at chemical shifts (δH) of 6.70–7.15 ppm (m) [33].

Table 5. Comparison of the proton chemical shift (δH) of compound III-B with Herbertene

δH		Function Group
Herbertene	Isolated Compounds	
0.58 ppm(s)	0.64 ppm (s)	3H (CH3)
1.10 ppm(s)	0.99 ppm (s)	3H (CH3)
1.27 ppm(s)	1.25 ppm (s)	6H (2CH3)
6.70-7.15 ppm (m)	4.89 – 5.73 ppm (m)	1H

Berdasarkan hasil data instrumen yang diperoleh, senyawa III-B memiliki karakteristik spektrum yang mirip dengan senyawa golongan sesquitriterpen yaitu herbertene. Dilihat dari data spektrum ^{1}H-NMR dimana senyawa III-B memiliki ciri struktur yang mirip dengan herbertene yaitu terdapat 4 gugus metil pada pergeseran kimia (δ_H) = 0,64 ppm (*s*, 3H), 0,99 ppm (*s*, 3H) dan 1,25 ppm (*s*, 6H). Kemudian terdapat gugus 4 proton pada area aromatis yaitu pada pergeresan kimia (δ_H) = 4,89 ppm (1H *d, J*=1,95 Hz), pada pergeseran kimia (δ_H) = 4,94 ppm (1H *d, J*=10,35 Hz), pada pergeseran kimia (δ_H) = 5,14 ppm (1H s) dan pada pergeseran kimia (δ_H) = 5,70 ppm (1H *dd, J*=11,05 Hz dan 10,35 Hz).

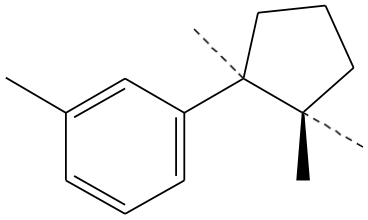

Figure 1. Herbertene structure (Source: Matsuo, et al, [34])

The results of this study indicate that the ethyl acetate extract of *Mastigophora diclados* contains terpenoid compounds that were successfully isolated into pure compounds with characteristics resembling herbertene. The use of a semi-polar solvent such as ethyl acetate proved effective in extracting sesquiterpenoid compounds, which generally exhibit intermediate polarity in bryophytes. This finding is consistent with the work of Asakawa [25], who reported that liverworts predominantly produce secondary metabolites in the form of terpenoids soluble in semi-polar solvents. In addition, a recent study by Łukasz Ludwiczuk confirmed that liverworts possess a rich chemical profile of bioactive sesquiterpenes with potential pharmaceutical applications [35]. Therefore, the findings of this study align with global trends in the exploration of natural products from bryophytes.

Furthermore, chromatographic and spectroscopic analyses revealed that the isolated compound exhibits structural characteristics typical of herbertene, marked by the presence of methyl groups and olefinic protons. This structural pattern has been widely reported in the genus *Mastigophora* as a major component of its secondary metabolites. Research by Yoshinori Asakawa demonstrated that herbertene exhibits biological activities such as

cytotoxic and antimicrobial effects [25]. Moreover, a study by Ii et al. reported the cytotoxic activity of liverwort extracts against cancer cells [36]. Thus, the presence of herbertene-like compounds in this study strengthens the pharmacological potential of *M. diclados*.

Interestingly, slight differences in chemical shift values (δH) compared to literature data were observed, indicating the possibility of structural variations or novel derivatives. These variations may be influenced by environmental factors such as habitat conditions, altitude, and climate [37], [38]. According to Janice Glime [39], ecological factors play a significant role in the biosynthesis of secondary metabolites in bryophytes. A similar observation was reported by Natália Martins et al. [40] who found that geographical variation can lead to significant chemical diversity. Therefore, this study opens up the possibility of discovering new compounds that warrant further investigation.

The main novelty of this research lies in the successful isolation and characterization of secondary metabolite compounds from *Mastigophora diclados* originating from Indonesia, which has not been previously reported. Unlike earlier studies that mainly focused on biological activity tests or crude extract profiling, this study successfully isolated pure compounds and identified their structural characteristics using a spectroscopic approach. In addition, this research provides evidence that geographical variation can influence the chemical profile of a species, thereby enriching the understanding of bryophyte chemotaxonomy. Another novel aspect is the indication of a potential new herbertene derivative based on differences in the ^{1}H-NMR spectrum compared to standard references. Thus, this study offers an original contribution to the field of natural product chemistry, particularly in the exploration of tropical bryophytes.

The implications of this study are significant for the development of natural product chemistry and pharmaceutical sciences. The identification of herbertene-like compounds opens opportunities for further studies on specific biological activities, such as anticancer, antimicrobial, and antioxidant properties that have been reported for similar compounds. In addition, these findings reinforce the potential of Indonesia's biodiversity as a source of new drug candidates based on natural products. Practically, the isolation method used in this study can serve as a reference for the exploration of bioactive compounds from other lower plants. In a broader context, this research also supports efforts in bioprospecting and biodiversity conservation in Indonesia.

Despite the successful isolation and characterization of the compound, several limitations should be noted. First, the structural identification was based solely on ^{1}H-NMR data, which does not provide a fully comprehensive structural elucidation without support from advanced techniques such as ^{13}C-NMR, 2D-NMR, or mass spectrometry. Second, the amount of compound obtained was relatively small (mg-scale), limiting further comprehensive biological activity testing. Third, this study did not explore the structure–activity relationship (SAR). Additionally, environmental factors influencing metabolite variation were not analyzed in depth. Therefore, further studies are required to strengthen the validity and expand upon these findings.

4. CONCLUSION

From 10 grams of the ethyl acetate extract of *Mastigophora diclados* (Brid. ex Web.) Nees, 8 mg of pure compound III-B was obtained. Based on the ^{1}H-NMR analysis, compound III-B exhibits a structural framework similar to herbertene. Further studies are required on the isolation of secondary metabolites from this plant, as several potentially active fractions still offer opportunities for the discovery of additional compounds. Moreover, more comprehensive instrumental analyses are recommended, including FTIR, LC-MS, ^{13}C-NMR, DEPT, HMBC, and HMQC, to achieve a more complete structural elucidation.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to all parties who contributed to the completion of this research. Special thanks are extended to the laboratory staff and colleagues for their technical assistance and valuable discussions throughout the study. The authors also acknowledge the Bogoriense Research Center for their support in plant identification.

This research was supported by institutional funding from the Faculty of Teacher Training and Education, Universitas Jambi. The authors are also grateful for any additional financial support and facilities provided by the respective institutions that made this research possible.

AUTHOR CONTRIBUTIONS

Conceptualization, F. I. Ardiansyah and M. F. Tlali; Methodology, F. I. Ardiansyah and M. F. Tlali; Software, F. I. Ardiansyah; Validation, F. I. Ardiansyah and M. F. Tlali; Formal Analysis, F. I. Ardiansyah; Investigation, F. I. Ardiansyah; Resources, M. F. Tlali; Data Curation, F. I. Ardiansyah; Writing – Original Draft Preparation, F. I. Ardiansyah; Writing – Review & Editing, F. I. Ardiansyah and M. F. Tlali; Visualization, F. I. Ardi.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

Not applicable.

REFERENCES

[1] J. Hao and L. M. Chu, "Vegetation types attributed to deforestation and secondary succession drive the elevational changes in diversity and distribution of terrestrial mosses in a tropical mountain forest in Southern China," 2021. doi: 10.3390/f12080961.

[2] J. M. Glime, "Roles of bryophytes in forest sustainability—positive or negative?," 2024. doi: 10.3390/su16062359.

[3] S. H. Watts, N. Barsoum, K. J. Park, and A. S. Jump, "Why the present is not the key to past or future: moving beyond restricted relict habitat conditions to improve outcomes in mountain woodland restoration," *Restor. Ecol.*, vol. 33, no. 7, Sep. 2025, doi: 10.1111/rec.70130.

[4] P. da S. Finamore *et al.*, "Nigerian politicians, discipline, integrity, character and the rule of law: Application versus financial spending in 2019 federal elections," *J. Chem. Inf. Model.*, vol. 53, no. February, p. 2021, 2021, doi: 10.13140/RG.2.2.19482.59846.

[5] F. Wittmann *et al.*, "A review of the ecological and biogeographic differences of Amazonian floodplain forests," 2022. doi: 10.3390/w14213360.

[6] J. Sun, B. Liu, H. Rustiami, H. Xiao, X. Shen, and K. Ma, "Mapping Asia plants: Plant diversity and a checklist of vascular plants in Indonesia," 2024. doi: 10.3390/plants13162281.

[7] J. Patiño *et al.*, "Unveiling the nature of a miniature world: a horizon scan of fundamental questions in bryology," *J. Bryol.*, vol. 44, no. 1, pp. 1–34, Jan. 2022, doi: 10.1080/03736687.2022.2054615.

[8] S. Yadav *et al.*, "Bryophytes as modern model plants: an overview of their development, contributions, and future prospects," *J. Plant Growth Regul.*, vol. 42, no. 11, pp. 6933–6950, 2023, doi: 10.1007/s00344-023-10986-1.

[9] R. Motti, A. D. Palma, and B. de Falco, "Bryophytes used in folk medicine: An ethnobotanical overview," 2023. doi: 10.3390/horticulturae9020137.

[10] M. Commisso, F. Guarino, L. Marchi, A. Muto, A. Piro, and F. Degola, "Bryo-activities: a review on how bryophytes are contributing to the arsenal of natural bioactive compounds against fungi," 2021. doi: 10.3390/plants10020203.

[11] S. C. Bhatla and M. A. Lal, "Secondary Metabolites BT - Plant Physiology, Development and Metabolism," S. C. Bhatla and M. A. Lal, Eds., Singapore: Springer Nature Singapore, 2023, pp. 765–808. doi: 10.1007/978-981-99-5736-1_33.

[12] H. S. Elshafie, I. Camele, and A. A. Mohamed, "A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin," 2023. doi: 10.3390/ijms24043266.

[13] Y. Delgado *et al.*, "Biomedical effects of the phytonutrients turmeric, garlic, cinnamon, graviola, and oregano: A comprehensive review," 2021. doi: 10.3390/app11188477.

[14] M. A. Elfaky, S. R. M. Ibrahim, N. A. Obaid, and R. Wasfi, "Biofilms of pathogenic bacteria," in *Perspectives of quorum quenching in new drug development*, CRC Press, 2024, pp. 1–33.

[15] Y. Asakawa, "Phytochemistry of Bryophytes: Biologically Active Compounds and Their Uses as Cosmetics, Foods, and in Drug Development BT - Phytochemistry of Bryophytes," A. D. Kinghorn, H. Falk, S. Gibbons, Y. Asakawa, J.-K. Liu, and V. M. Dirsch, Eds., Cham: Springer Nature Switzerland, 2025, pp. 1–399. doi: 10.1007/978-3-031-77795-0_1.

[16] V. P. Sülsen, "Sesquiterpene lactones and diterpenes: promising therapeutic candidates for infectious diseases, neoplasms and other chronic disorders," 2021. doi: 10.3390/molecules26051251.

[17] Y.-H. Ma, X.-X. Dou, and X.-H. Tian, "Natural disesquiterpenoids: an overview of their chemical structures, pharmacological activities, and biosynthetic pathways," *Phytochem. Rev.*, vol. 19, no. 4, pp. 983–1043, 2020, doi: 10.1007/s11101-020-09698-1.

[18] R. Barthwal and R. Mahar, "Exploring the significance, extraction, and characterization of plant-derived secondary metabolites in complex mixtures," 2024. doi: 10.3390/metabo14020119.

[19] S. P. Gaudêncio *et al.*, "Advanced methods for natural products discovery: bioactivity screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools, and structure elucidation," 2023. doi: 10.3390/md21050308.

[20] S. Mondal, M. Das, S. Debnath, B. K. Sarkar, and G. Babu, "An overview of extraction, isolation and characterization techniques of phytocompounds from medicinal plants," *Nat. Prod. Res.*, vol. 40, no. 7, pp. 1939–1961, Apr. 2026, doi: 10.1080/14786419.2024.2426059.

[21] N. C. S. Ruklani and S. C. K. Rubasinghe, "Species richness patterns, hotspots and phytogeographic affinities of thalloid liverworts (Marchantiophyta) and hornworts (Anthocerotophyta) of Sri Lanka," *Trop. Ecol.*, vol. 62, no. 2, pp. 218–233, 2021, doi: 10.1007/s42965-020-00132-3.

[22] G. E. LEE, H. Y. TANG, T. PÓCS, D. C. CARGILL, and N. SEVANAKUN, "Data to the Malaysian liverwort flora, V: Marchantiopsida of Malaysia with emphasis on the genus *Cyathodium* (Cyathodiaceae)," *Hattoria*, vol. 15, pp. 27–41, 2024, doi: 10.18968/hattoria.15.0_27.

[23] F. SUSILO, N. PASARIBU, S. SYAMSUARDI, and E. S. SIREGAR, "Diversity and distribution of liverworts in Mount Sibuatan, North Sumatra, Indonesia," *Biodiversitas J. Biol. Divers.*, vol. 23, no. 9, Sep. 2022, doi: 10.13057/biodiv/d230921.

[24] A. S. Nugraha *et al.*, "Indonesian marine and its medicinal contribution," *Nat. Products Bioprospect.*, vol. 13, no. 1, p. 38, 2023, doi: 10.1007/s13659-023-00403-1.

[25] Y. Asakawa, "Chemosystematics of the Hepaticae," *Phytochemistry*, vol. 65, no. 6, pp. 623–669, 2004, doi: https://doi.org/10.1016/j.phytochem.2004.01.003.

[26] M. Museli, O. Clus, P. Ortega, I. Milimouk, and D. Beysens, "Physical, chemical and biological characteristics of dew and rainwater during the dry season of tropical islands," 2021. doi: 10.3390/atmos12010069.
[27] M. Guerrero-Esperanza, R. Hernández-Peña, J. Molina-Torres, and J. J. Ordaz-Ortiz, "Non-targeted metabolomics for origin differentiation of cured vanilla pods (Vanilla planifolia) by LC-MS and GC–MS," *Microchem. J.*, vol. 216, p. 114795, 2025, doi: 10.1016/j.microc.2025.114795.
[28] K. W. Samarakoon, A. I. Kuruppu, J.-Y. Ko, J.-H. Lee, and Y.-J. Jeon, "Structural characterization and anti-inflammatory effects of 24-methylcholesta-5 (6), 22-diene-3β-ol from the cultured marine diatom Phaeodactylum tricornutum; Attenuate inflammatory signaling pathways," 2023. doi: 10.3390/md21040231.
[29] E. F. Queiroz, D. Guillarme, and J.-L. Wolfender, "Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: from metabolite profiling to pure chemical entities," *Phytochem. Rev.*, vol. 23, no. 5, pp. 1415–1442, 2024, doi: 10.1007/s11101-024-09928-w.
[30] B. Lin and G. Huang, "Extraction, isolation, purification, derivatization, bioactivity, structure–activity relationship, and application of polysaccharides from White jellyfungus," *Biotechnol. Bioeng.*, vol. 119, no. 6, pp. 1359–1379, Jun. 2022, doi: 10.1002/bit.28064.
[31] T. Kowalska and M. Sajewicz, "Thin-layer chromatography (TLC) in the screening of botanicals–its versatile potential and selected applications," 2022. doi: 10.3390/molecules27196607.
[32] W. Parys, M. Dołowy, and A. Pyka-Pająk, "Significance of chromatographic techniques in pharmaceutical analysis," 2022. doi: 10.3390/pr10010172.
[33] I. Z. Rakhmatullin, S. V Efimov, A. V Klochkov, O. I. Gnezdilov, M. A. Varfolomeev, and V. V Klochkov, "NMR chemical shifts of carbon atoms and characteristic shift ranges in the oil sample," *Pet. Res.*, vol. 7, no. 2, pp. 269–274, 2022, doi: https://doi.org/10.1016/j.ptlrs.2021.10.001.
[34] A. Matsuo, S. Yuki, M. Nakayama, and S. Hayashi, "(–)-Herbertene, an aromatic sesquiterpene with a novel carbon skeleton from the liverwort Herberta adunca," *J. Chem. Soc. Chem. Commun.*, no. 16, pp. 864–865, 1981, doi: 10.1039/C39810000864.
[35] F. Li, D. Bi, X. Liang, R. Luo, H. Zhuang, and L. Wang, "Alkaloids from the stem barks of Erythrina stricta," *Phytochemistry*, vol. 170, p. 112220, 2020, doi: https://doi.org/10.1016/j.phytochem.2019.112220.
[36] N. Li, C.-C. Zhu, H.-M. Xiao, and K.-J. Wang, "Norlignan derivatives from Curculigo breviscapa," *Fitoterapia*, vol. 81, no. 6, pp. 528–531, 2010, doi: https://doi.org/10.1016/j.fitote.2010.01.012.
[37] H. Gong, M. Yang, C. Wang, and C. Tian, "Leaf phenotypic variation and its response to environmental factors in natural populations of Eucommia ulmoides," *BMC Plant Biol.*, vol. 23, no. 1, p. 562, 2023, doi: 10.1186/s12870-023-04583-3.
[38] L. Zhao *et al.*, "Effects of environmental factors on the spatial distribution pattern and diversity of insect communities along altitude gradients in Guandi Mountain, China," 2023. doi: 10.3390/insects14030224.
[39] J. M. Glime, *Bryophyte Ecology*. Michigan Technological University, 2021. [Online]. Available: https://digitalcommons.mtu.edu/oabooks/4
[40] F. G. Martins, A. Melo, and S. F. Sousa, "Identification of New Potential Inhibitors of Quorum Sensing through a Specialized Multi-Level Computational Approach," 2021. doi: 10.3390/molecules26092600.