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Phytochemistry Profile and Antioxidant Activity of *Dumortiera hirsuta* (Sw.) Nees from Gumitir, East Java

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ABSTRACT

Dumortiera hirsuta, a thalloid liverwort, predominantly grows on the ground floor of coffee plantations in Gumitir, Jember District, East Java, and is known for its rich phytochemical content. This study aimed to comprehensively profile the volatile and non-volatile compounds present in the methanol extract of *D. hirsuta* and evaluate its antioxidant activity. The thallus was macerated using 96% methanol (1:10 ratio), followed by analysis using Gas Chromatography–Mass Spectrometry (GC–MS) and Liquid Chromatography–Mass Spectrometry Quadrupole Time-of-Flight (LC–MS QTOF). GC–MS identified 37 volatile compounds, with terpenes (29%), phenols (21%), and fatty acids (13%) as dominant classes. Several potent antioxidant sesquiterpenoids, including caryophyllene, guaiene, and aromadendrene derivatives, were notably abundant, along with unique compounds such as phytol, benzoic acid, pyrocatechol, and furanones. LC–MS analysis detected 15 non-volatile secondary metabolites, predominantly flavonoids (e.g., kaempferol-3-O-β-D-glucuronide, luteolin, leucocyanidin), phenolics (sesamol, euparin), and terpenoids (brefeldin A, E-p-coumaric acid), of which nine are well-documented for their antioxidant properties. These compounds were identified with high accuracy (mass error ±4 ppm) across positive and negative ion modes. Antioxidant potential was confirmed through the DPPH radical scavenging assay, which yielded a moderate IC_{50} value of 101.13 ppm and a strong dose-response correlation (R^2 = 0.9526). The favourable microclimatic conditions of Mount Gumitir likely contributed to the phytochemical richness observed. Collectively, these findings highlight *D. hirsuta* as a chemically diverse bryophyte with promising antioxidant constituents, supporting its potential application in pharmacological development and natural antioxidant sourcing.

1. Introduction

Dumortiera hirsuta (Sw.) is a thalloid liverwort that thrives in mountainous regions with humid conditions. It is widely distributed across the Asia-Pacific region. However, it is relatively rare in Europe, where it is only found in specific locations, including France, Ireland, Italy, Portugal (Azores, Madeira, and mainland), Spain (Canary Islands and mainland), and Great Britain (England, Northern Ireland, and Scotland). This limited distribution in Europe is primarily influenced by habitat suitability, as environmental factors play a crucial role in determining the presence of *D. hirsuta*. (Forrest *et al.* 2011; Durant-Archibold *et al.* 2018 Callaghan 2020).

In Indonesia, *D. hirsuta* has been recorded in several regions, including Mount Gumitir, Jember

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District, East Java. This area is known for its high plant diversity, attributed to its significant rainfall intensity, which maintains consistently moist soil conditions, an essential factor for the growth of *D. hirsuta* (Luthfiah *et al.* 2021). Despite its widespread presence, *D. hirsuta* remains underutilized in Indonesia. However, previous studies have demonstrated that this liverwort contains a diverse range of bioactive phytochemicals with potential medicinal applications (Durant-Archibold *et al.* 2018). The major phytochemical constituents of *D. hirsuta* include flavonoids, phenolics, and terpenoids, which have been reported to exhibit antioxidant properties. Chromatographic analysis using Fourier-transform infrared spectroscopy (FTIR) further identified 20 metabolites with peak area percentages exceeding 0.5%, suggesting their potential as significant bioactive compounds (Yadav *et al.* 2022).

Phytochemical profiling of *D. hirsuta* can be conducted using advanced chromatographic techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). GC-MS is primarily used for identifying volatile secondary metabolites, while LC-MS is suitable for detecting non-volatile secondary metabolites that cannot evaporate. Both techniques offer rapid and precise detection of complex phytochemical compounds, with GC-MS providing an untargeted screening approach and LC-MS enabling more targeted compound identification (Faizi *et al.* 2014; Shah *et al.* 2021; Oktavianawati *et al.* 2024). Given their efficiency and reliability, these techniques are recommended for the comprehensive phytochemical analysis of *D. hirsuta* extracts.

One of the key medicinal properties of *D. hirsuta* is its antioxidant activity. Antioxidants are crucial in neutralizing free radicals and preventing oxidative stress, thereby supporting immune function and reducing the risk of various diseases (Faizi *et al.* 2014; Shah *et al.* 2021). The antioxidant activity of plant extracts is commonly measured using UV-Vis spectrophotometry, which determines the absorbance of light at specific wavelengths (Hepokur *et al.* 2020). The presence of bioactive compounds in *D. hirsuta* suggests its potential applications as an anticancer and antibacterial agent. For instance, kaempferol, a flavonoid found in *D. hirsuta*, has been reported to exhibit antioxidant properties that may help reduce colon cancer progression in experimental models (Liu *et al.* 2018). Additionally, research by Luthfiah *et al.* (2021) demonstrated that *D. hirsuta* from Mount Gumitir possesses antibacterial activity, as indicated by the formation of clear inhibition zones against pathogenic bacterial strains such as *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*.

The growing demand for plant-based medicinal products highlights the importance of exploring alternative natural resources. Bryophytes, including *D. hirsuta*, have been recognized as valuable sources of bioactive metabolites with pharmaceutical potential. According to Cianciullo *et al.* (2022), bryophytes can serve as medicinal plants due to their diverse metabolite profiles, offering promising alternatives to traditional herbal medicine.

2. Materials and Methods

2.1. Plant Material

Samples of the liverwort *Dumortiera hirsuta* were collected from Mount Gumitir, East Java, Indonesia, at an elevation of 930 meters above sea level. The abiotic parameters at the time of collection were recorded as follows: ambient temperature 27.3°C, light intensity 505 lux, and relative humidity 60.8%. Species identification was confirmed by bryologist Florentina Indah Windadri (National Research and Innovation Agency, BRIN), and a voucher specimen was deposited in Herbarium Jemberiense (Biology Department, Jember University) for reference. Following collection, the samples were thoroughly rinsed with running tap water to remove adhering soil and substrate particles. The cleaned thalli were then air-dried at room temperature (~25°C) for 60 minutes to reduce excess surface moisture prior to extraction and further analysis.

2.2. Plant Extraction

The study of *D. hirsuta* was carried out using the maceration method. A total of 30 g of air-dried thallus was ground into fine powder using a blender and soaked in 300 mL of 96% methanol (1:10 w/v) at room temperature. The initial maceration was conducted for 72 hours with occasional stirring once daily to enhance solvent penetration and metabolite diffusion. After the first maceration, the mixture was filtered through Whatman No. 1 filter paper, and the plant residue was subjected to two consecutive remaceration steps using fresh methanol solvent under identical conditions, yielding a total of three sequential maceration cycles. All filtrates were pooled and concentrated under reduced

pressure using a rotary evaporator to remove the solvent. The resulting crude extract was stored in a sealed vial at 4°C until further phytochemical and bioactivity analyses.

2.3. Determination of Phytochemical Composition

2.3.1. GC-MS

The GC-MS analysis was conducted in three stages. The first stage involved sample preparation, where the pure extract of *D. hirsuta* liverwort was filtered using a membrane syringe filter to separate solids from liquids before chromatographic analysis, preventing column clogging. In the second stage, 1 µL of the filtered extract was injected into the GC-MS injector chamber using the split injection method. The split injection method, also known as partial injection, allows only a portion of the sample to enter the partition column. In contrast, the remaining sample is discarded and directed to the cleaning column (septum purge) (Chasteen *et al.* 2000).

The third step entailed determining the type of secondary metabolite components using Shimadzu's GC-MS-QP 2010 Plus instrument set. This instrument set is equipped with a split injector set at 290°C, an MS detector temperature of 280°C, and an Rtx-50 column (0.25 mm inner diameter, 30 m length, and 0.25 µm thickness). The oven temperature program was as follows: an initial temperature of 80°C held for 10 minutes, ramped at 5°C/min to 230°C, and held for an additional 10 minutes. The carrier gas was high-purity helium, used at a constant flow rate of 3.0 mL/ min. The injection volume was 1 µL, delivered in split mode with a split ratio of 1:10 (Ulum *et al.* 2023; Amalini *et al.* 2025). The resulting analytical results on mass spectrometry were interpreted using a database in the form of Wiley 9, which contains more than five million reference chemical compounds (Rajesh *et al.* 2016).

2.3.2. LC-MS

The identification of non-volatile secondary metabolites in *D. hirsuta* was performed using liquid chromatography coupled with tandem mass spectrometry (Xevo G2-XS QTof MS) at PT Saraswanti Indo Genetech Laboratory, Bogor, Indonesia. A total of 0.1 g of crude extract was dissolved in methanol and adjusted to a final volume of 10 mL. The solution was homogenized and filtered through a 0.22 µm GHP/PTFE membrane filter before injection. Chromatographic separation was conducted using an ultra-performance liquid chromatography (UPLC) system equipped with a reverse-phase C18 column maintained at 40°C, while the autosampler was kept at 15°C. The injection volume was 10 µL, and the mobile phase consisted of solvent A: 0.1% formic acid in water, and solvent B: 0.1% formic acid in acetonitrile, applied under a gradient elution at a flow rate of 0.6 mL/min. Mass spectrometric detection was performed using a quadrupole time-of-flight (QTOF) mass spectrometer operated in Tof-MSE acquisition mode, with electrospray ionization (ESI) in both positive and negative ion modes (ESI^+/ESI^-) to maximize coverage of different compound classes. The scan range was set from m/z 50 to 1200 Da (Yunita *et al.* 2022). This dual-ionization strategy enabled the detection of a broad spectrum of metabolites, including flavonoids, phenolics, and terpenoids, based on their ionization behavior and mass fragmentation patterns (Shah *et al.* 2021).

2.3.3. Antioxidant Activity

The antioxidant potential of *D. hirsuta* methanolic extract was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, a standard method for evaluating free radical neutralization (Martin-Puzon and Rivera 2015). A DPPH stock solution was prepared by dissolving 0.001 g of DPPH powder in 25 mL of PA-grade methanol; this solution was stored in a dark bottle and used as a stable source of free radicals for the assay. The plant extract was initially prepared at a concentration of 1000 ppm in methanol and further diluted to obtain working concentrations of 200, 160, 120, and 80 ppm. For each assay, 0.3 mL of extract solution was combined with 1.3 mL of the DPPH solution in test tubes, mixed thoroughly, and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with methanol serving as the blank. A mixture of 1 mL methanol and 1 mL DPPH solution was used as the control. Ascorbic acid was included as a positive control. The percentage of DPPH radical inhibition was calculated using the equation: Inhibition radical scavenging (%) = (Ab (control) - Ab (sample)) / Ab (control) × 100%

where Ab (control) is the absorbance of the control, and Ab (sample) is the absorbance of the test sample. The IC_{50} value, indicating the concentration of extract required to scavenge 50% of the DPPH radicals, was determined by plotting the inhibition percentage against concentration and applying linear regression analysis using Microsoft Excel (Amalini *et al.* 2025). Antioxidant strength was interpreted based on IC_{50} values as follows: <50 µg/mL = strong, 50–100 µg/mL = moderate, 100–

250 µg/mL = weak, and >250 µg/mL = very weak or inactive (Nurhaeni and Hardi 2019).

3. Results

3.1. Phytochemical Compound Detected with GC-MS

The GC-MS analysis of *D. hirsuta* extract identified 40 distinct peaks representing the volatile phytochemical/metabolite compounds present in the sample. These peaks correspond to the intensity of detected metabolites and indicate their relative abundance in the extract. In addition to intensity, each peak also provides information on the retention time, which represents the time required for each compound to be separated during the chromatographic process (Figure 1).

GC-MS analysis identified 37 different metabolite compounds in *D. hirsuta*. The successfully identified compounds comprised 29% terpenes, 21% phenols, 6% ketones, 2% hydrocarbons, 13% fatty acids, and 6% amino acids. The following table presents the list of metabolite compounds detected by GC-MS (Table 1).

3.2. Phytochemical Compound Detected with LC-MS

Analysis with Liquid Chromatography–Mass Spectrometry Quadrupole Time-of-Flight (LC-MS QTOF) was employed to identify the chemical constituents of the *D. hirsuta* methanol extract based on accurate mass measurement and fragmentation patterns. The fragmentation spectra provided diagnostic fragment ions corresponding to each compound's molecular structure,

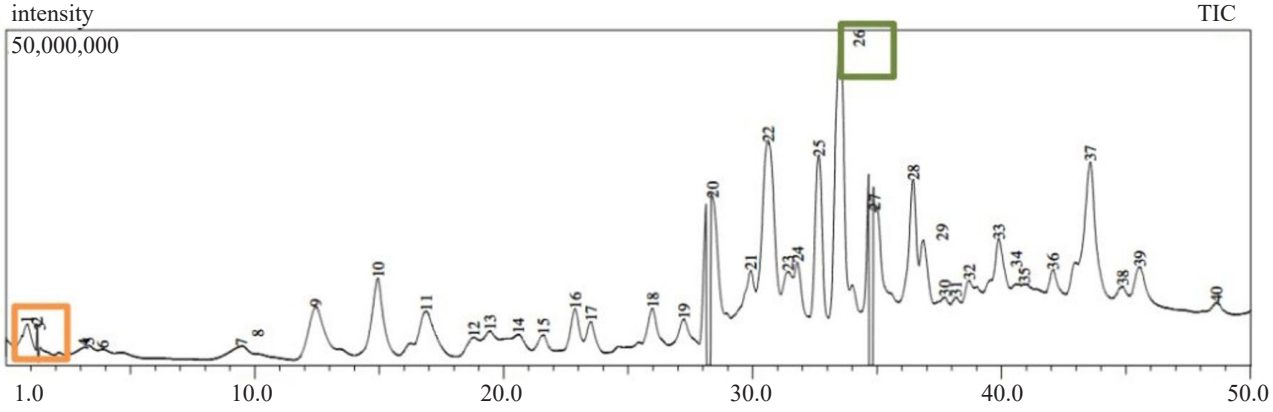

Figure 1. Chromatography GC-MS of *D. hirsuta* methanol extract from Gumitir

Table 1. Compound Identification by GC-MS Analysis

R. Time	Area %	Metabolite compounds	PM/SM	Groups
0.848	0.52	2-Acetyl-2-Methyl-Succinonitrile	-	-
1.253	0.08	L-Alanine, ethyl ester (CAS) Alanine ethyl ester*	PM	Amino acid
1.363	0.34	L-(-)-Asparagin	PM	Amino acid
3.148	0.16	acetic acid, 2-methylpropyl ester (CAS) Isobutyl acetate	PM	Fatty acid
3.327	0.15	Alpha.-Methylcyclohexane-Methyl Amine	-	-
3.888	0.11	Pyrazine, 2,6-dimethyl- (CAS) 2,6-Dimethylpyrazine	-	-
9.475	0.86	2(3H)-Furanone, dihydro- (CAS) Butyrolactone	SM	Phenol
10.140	0.27	2(5H)-Furanone	SM	Phenol
12.455	3.14	1,2-Cyclohexanedione (CAS) 1,2-Dioxocyclohexane	SM	Ketone
14.958	3.20	7-Oxabicyclo[2.2.1]hept-5-en-2-one	-	-
16.893	2.72	Benzenesulfonic acid, 4-hydroxy- (CAS)	SM	Phenol
18.808	0.71	3-Furanol, tetrahydro- (CAS) 3-Hydroxytetrahydrofuran	SM	Phenol
19.458	1.09	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	SM	Phenol
20.595	1.32	1,2,3-Propanetriol (CAS) Glycerol	-	-
21.597	0.67	Oxetane, 2-propyl- (CAS) 2-N-Propyl-Oxetan	-	-
22.887	1.37	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	SM	Ketone
23.510	0.87	Bicycloelemene	SM	Sesquiterpenes
25.991	1.68	Benzoic acid (CAS) Retardex	SM	Hydrocarbon
27.247	1.10	Benzaldehyde, 4-methyl- (CAS) p-Tolualdehyde	SM	Phenol

Table 1. Continued

R. Time	Area %	Metabolite compounds	PM/SM	Groups
28.405	5.12	1,2-Benzenediol (CAS) Pyrocatechol	SM	Phenol
29.948	3.33	Cycloheptan, 4-Methylen-1-Methyl-2-(2-Methyl-1-Propen-1-Yl)-1-Vinyl- (Humulen-"V)	-	-
30.637	8.63	1(10),4-aromedenedradiene***	SM	Sesquiterpenes
31.443	2.13	1H-Indene, octahydro-2,3a,4-trimethyl-2-(1-methylethyl)-, (2.alpha.,3a.beta.,4.beta.,7a.beta.)-(+)- (CAS) Fukinane	SM	Monoterpenes
31.822	2.22	alpha.-Guaiene	SM	Sesquiterpenes
32.679	4.80	1(10),4-aromedenedradiene***	SM	Sesquiterpenes
33.539	10.20	2(1H)-Naphthalenone, 7-ethynyl-4a,5,6,7,8,8a-hexahydro1,4a-dimethyl (1.alpha.,4a.beta.,7.beta.,8a.alpha.)- (CAS)**	SM	Sesquiterpenes
34.912	5.83	(-)-Isoledene	SM	Sesquiterpenes
36.468	4.82	(-)-Caryophyllene oxide***	SM	Monoterpenes
36.873	2.70	Aromadendrenepoxide-(I)	SM	Monoterpenes
37.722	1.39	(-)-Caryophyllene oxide***	SM	Monoterpenes
38.185	0.93	(t-2-Isopropenyl-c-5-methyl-r-1-cyclopentyl)methylketone	-	-
38.714	2.47	9-Octadecanoic acid (Z)- (CAS) Oleic acid***	PM	Fatty acid
39.913	4.45	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	PM	Fatty acid
40.598	1.42	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl- (CAS) 1,2,3,4,4A,5,6,8A-Octahydro-4A,8- Dimethylnaphthalene	SM	Sesquiterpenes
40.915	2.40	Isospathulenol	SM	Sesquiterpenes
42.087	2.29	Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl) Propionate	SM	Phenol
43.583	9.02	9-Octadecenoic acid (Z)- (CAS) Oleic acid	PM	Fatty acid
44.865	1.41	Octadeca-9,12-Dienoic Acid Methyl Ester***	PM	Fatty acid
45.566	3.47	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol	PM	Diterpenes
48.639	0.64	5-(P-Anisyl)-3-Methyl-2-Pentenonitrile	-	-

*(Lowest peak), **(Highest peak), ***(Indicates that the same or structurally similar compound was detected at multiple retention times), PM (Primary metabolite), SM (Secondary metabolite)

facilitating precise annotation of metabolites detected at distinct retention times. The LC-MS chromatogram of the methanol extract revealed multiple peaks, each representing individual chemical entities, as shown in Figure 2. These peaks were interpreted to identify compounds based on their observed m/z values, theoretical masses, and fragmentation consistency, with detailed compound information summarized in Table 2.

A total of fifteen secondary metabolites were identified, comprising flavonoids, phenolic compounds, and terpenoids. Flavonoids constituted the most abundant group with eight compounds, including kaempferol-3-O-β-D-glucuronide, consistently observed in both positive (m/z 463.0864) and negative (m/z 461.0712) ESI modes, confirming its robust ionization behavior. Other flavonoids detected in negative mode included chrysanthemin (m/z 447.0926), kaempferol 3-O-β-D-glucuronopyranosyl methyl ester (m/z 475.0882), kushenol B (m/z 491.2440), leucocyanidin (m/z 305.0659), luteolin (m/z 285.0393), and sophoranodichromane B (m/z 407.1876). These compounds exhibited low mass error values (–4.0 to +3.0 ppm), reflecting high identification reliability. Two phenolic compounds were identified in negative ion mode: euparin (m/z 463.0864) and sesamol (m/z 137.0235), both known for their antioxidative potential. The terpenoid class included five metabolites: dihydrocostunolide (m/z 235.1688, positive mode), borneol-2-O-β-D-glucoside (m/z 315.1808), brefeldin A (m/z 279.1596), carabrone (m/z 247.1331), and E-p-coumaric acid (m/z 163.0394), all detected in negative mode. Mass error values across these identifications ranged from 3.8 to +0.2 ppm, within acceptable limits for QTOF validation.

3.3. Antioxidant Activity of *D.hirsuta* Extract

The antioxidant activity of *D. hirsuta* extract was assessed using the DPPH radical scavenging assay, which revealed both qualitative and quantitative evidence of free radical neutralization. Upon addition of the extract to the DPPH solution, a visible colour change from deep violet to yellow was observed, particularly prominent at the highest tested concentration (400 ppm), indicating the presence of antioxidant constituents capable of reducing DPPH radicals. Quantitative analysis using UV-Vis spectrophotometry at 517 nm demonstrated a concentration-dependent increase in radical inhibition, with higher extract concentrations yielding greater scavenging

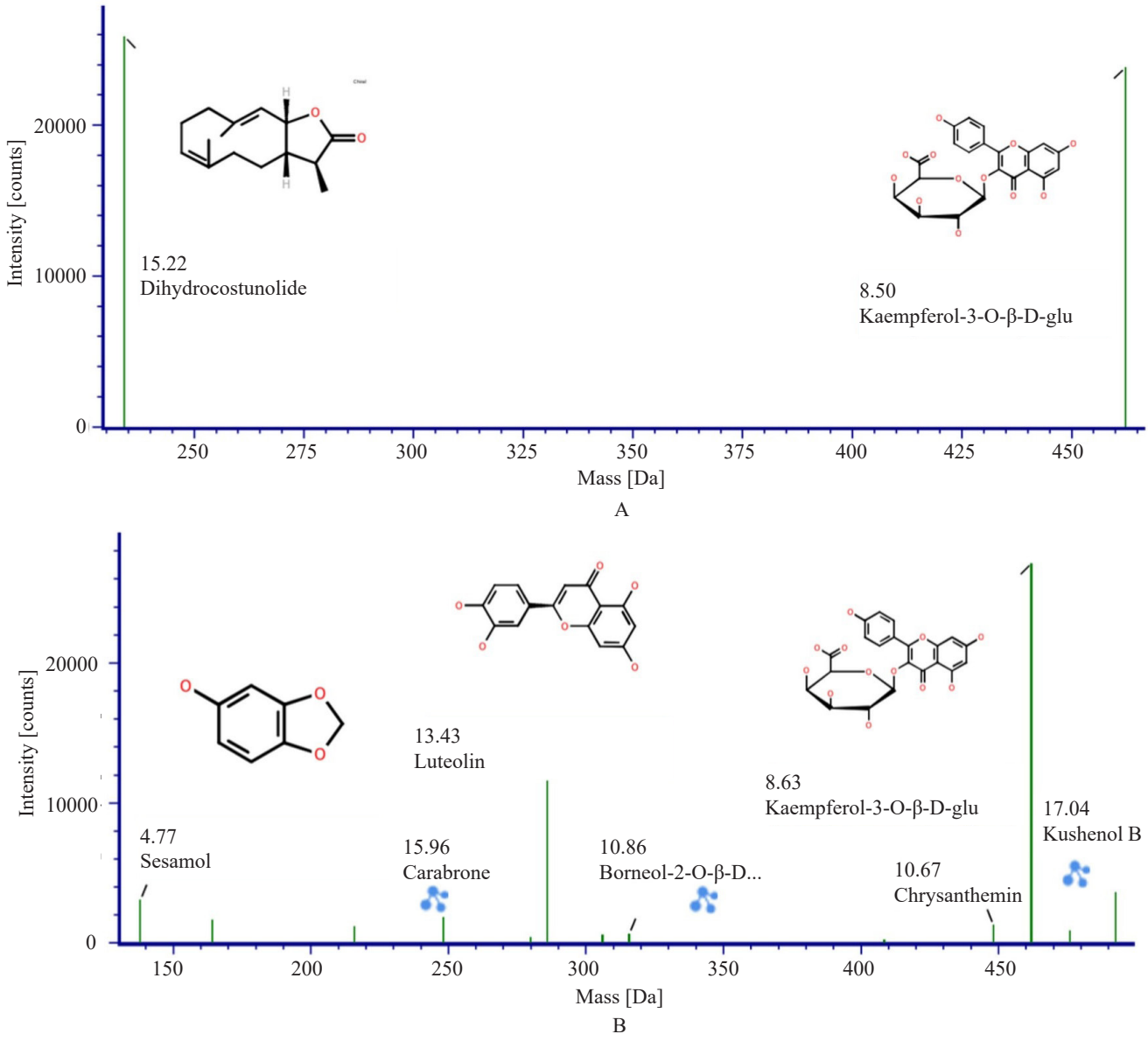

Figure 2. Fragmentation spectra obtained from LC-MS QTOF analysis of Dumortiera hirsuta methanol extract from Gumitir: (A) positive ionisation mode (ESI+) and (B) negative ionisation mode (ESI–)

Table 2. Compound identification by LC-MS QTOF analysis

RT (min)	Molecular formula	Adducts	Observed m/z	Theoretical mass (ppm)	Mass error(ppm)	Metabolite compounds	ESI mode	Group
8.50	C21H18O12	+H	463.0864	33.3	-0.5	Kaempferol-3-O-β-D-glucuronide	(+)	Flavonoid
10.67	C21H20O11	-H	447.0926	33.3	-1.5	Chrysanthemin	(-)	Flavonoid
10.52	C22H20O12	-H	475.0882	33.3	0.1	Kaempferol 3-O-β-D-glucuronopyranosyl methyl ester	(-)	Flavonoid
8.63	C21H18O12	-H	461.0712	33.3	-2.8	Kaempferol-3-O-β-D-glucuronide	(-)	Flavonoid
17.04	C30H36O6	-H	491.2440	33.3	0.2	Kushenol B	(-)	Flavonoid
6.60	C15H14O7	-H	305.0659	33.3	-2.6	Leucocyanidin	(-)	Flavonoid

Table 2. Continued

RT (min)	Molecular formula	Adducts	Observed m/z	Theoretical mass (ppm)	Mass error(ppm)	Metabolite compounds	ESI mode	Group
13.43	C15H10O6	-H	285.0393	33.3	-4.0	Luteolin	(-)	Flavonoid
18.56	C25H28O5	-H	407.1876	33.3	3.0	Sophoranodichromane B	(-)	Flavonoid
9.20	C13H12O3	-H	463.0864	33.3	-3.3	Euparin	(-)	Phenol
4.77	C7H6O3	-H	137.0235	33.3	-6.4	Sesamol	(-)	Phenol
15.22	C15H22O2	+H	235.1688	33.3	-2.1	Dihydrocostunolide	(+)	Terpenoid
10.86	C16H28O6	-H	315.1808	33.3	-1.6	Borneol-2-O-β-D-glucoside	(-)	Terpenoid
15.18	C16H24O4	-H	279.1596	33.3	-2.1	Brefeldin A	(-)	Terpenoid
15.96	C15H20O3	-H	247.1331	33.3	-3.3	Carabrone	(-)	Terpenoid
7.91	C9H8O3	-H	163.0394	33.3	-3.8	E-p-Coumatic acid	(-)	Terpenoid

effects. Each concentration was tested in triplicate (n = 3), and the mean inhibition percentages were plotted to obtain a regression equation ($y = 0.3464\times + 14.97$, $R^2 = 0.9526$), from which the IC_{50} value was calculated to be 101.13 ppm, indicating moderate antioxidant potency (Figure 3).

4. Discussion

4.1. Volatile Metabolites Identified in *D. hirsuta*

The GC-MS analysis of *D. hirsuta* extract from Mount Gumitir identified 37 phytochemical compounds, comprising both primary and secondary metabolites, with 22 classified as secondary metabolites. This phytochemical profile demonstrates strong congruence with previous studies on *D. hirsuta* subsp. hirsuta and *D. hirsuta* subsp. nepalensis from Panama, which also reported sesquiterpenoids as major constituents (Durant-Archibold *et al.* 2018). Shared compounds between both populations include sesquiterpenes such as caryophyllene, guaiene, and aromadendrene derivatives, along with oxygenated terpenes including caryophyllene oxide, isoledene, and aromadendrenepoxide, which were notably abundant in the Gumitir samples. However, the Gumitir population displayed greater phytochemical richness, evidenced by the presence of additional compound classes such as fatty acids (e.g., oleic and octadecadienoic acids), aromatic acids and alcohols (e.g., benzoic acid, 1,2-benzenediol), furanones, and nitrogen-containing pyrazines. Unique compounds such as phytol and naphthalenone derivatives were also exclusively detected in this population, suggesting potential chemotypic differentiation or metabolomic responses to local ecological pressures.

In particular, the Gumitir *D. hirsuta* exhibited several volatile secondary metabolites with well-documented antioxidant activities (Table 3), reinforcing its value as a promising natural source of free radical scavengers. Among them, furanone derivatives, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, are known for their effective radical-quenching activity through hydroxyl-mediated electron donation and conjugated ring systems (Chen *et al.* 2021). Benzoic acid contributes additional antioxidant potential due to its aromatic carboxylic structure, commonly used in food and pharmaceutical preservation (Komarayati *et al.* 2018). Pyrocatechol (1,2-benzenediol), a dihydroxybenzene, is particularly effective in neutralising reactive oxygen species (ROS) due to its ortho-positioned hydroxyl groups (Kosobutskii 2014). Furthermore, sesquiterpenoids such as fukinane and aromadendrenepoxide-(I) are associated with significant antioxidant capacity by stabilising reactive intermediates (Cascaes *et al.* 2022). Another compound, methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl) propionate, a synthetic phenolic derivative, also exhibited radical-inhibiting properties and may serve as a stable antioxidant scaffold in pharmaceutical applications (Li *et al.* 2014). Collectively, these findings provide strong evidence that *D. hirsuta* from Mount Gumitir is a chemically diverse bryophyte with promising antioxidant constituents.

4.2. Non-Volatile Metabolites Identified in *D. hirsuta*

Liquid Chromatography–Mass Spectrometry (LC–MS) analysis of the methanolic extract of *D. hirsuta* from Mount Gumitir successfully identified fifteen secondary metabolites, grouped into flavonoids, phenolic compounds, and terpenoids. Among these, nine metabolites have been well-documented for their antioxidant activity based on previous pharmacological studies (Table 4). These findings highlight the extract's potential as a natural antioxidant source, contributing to the scientific validation of *D. hirsuta* in ethnomedicine.

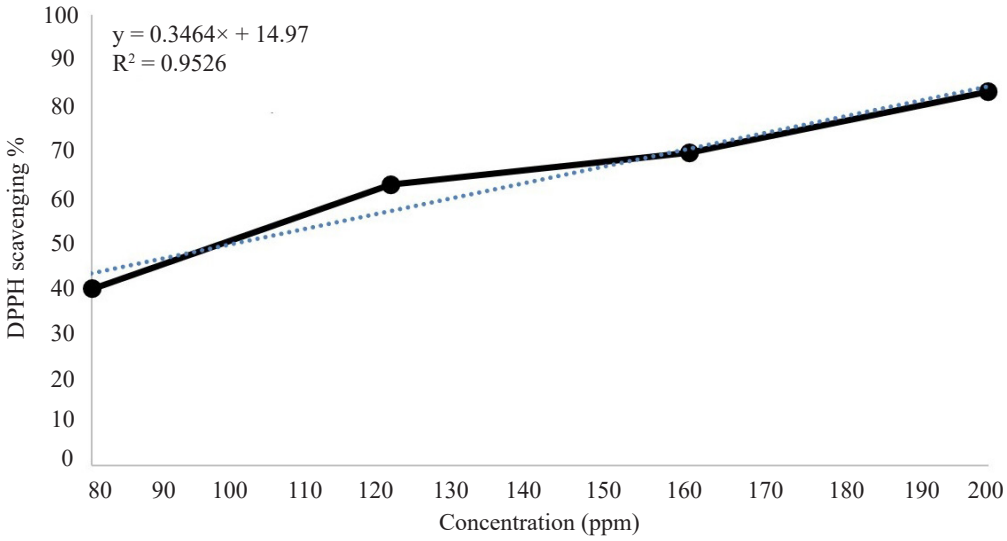

Figure 3. Antioxidant activity of *D. hirsuta* extract

Table 3. Antioxidant-associated volatile secondary metabolites detected in *Dumortiera hirsuta* by GC–MS

Metabolite compounds	References
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	Chen *et al*. 2021
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Chen *et al*. 2021
Benzoic acid (CAS) Retardex	Komarayati *et al.* 2018
1,2-Benzenediol (CAS) Pyrocatechol	Kosobutskii, V.S. 2014
1H-Indene, octahydro-2,3a,4-trimethyl-2-(1-methylethyl)-, (2.alpha.,3a.beta.,4.beta.,7a.beta.)-(+)- (CAS) Fukinane	Cascaes *et al.* 2022
Aromadendrenepoxide-(I)	Cascaes *et al*. 2022
Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl) Propionate	Li *et al*. 2014

Table 4. Antioxidant-associated non volatile secondary metabolites detected in *Dumortiera hirsuta* by LC–MS

Metabolite compounds	Group	Reference
Kaempferol-3-O-β-D-glucuronide	Flavonoid	Lim *et al*. 2023
Kaempferol 3-O-β-D- glucuronopyranosyl methyl ester	Flavonoid	Lim *et al*. 2023
Kushenol B	Flavonoid	Fan *et al.* 2018
Leucocyanidin	Flavonoid	Rafa Zubair *et al*. 2018
Luteolin	Flavonoid	Imran *et al.* 2019
Euparin	Phenol	Ezzatzadeh and Hossaini 2019
Sesamol	Phenol	Shah *et al*. 2019
Brefeldin A	Terpenoid	Wang *et al.* 2023
E-p-Coumatic acid	Terpenoid	Boo 2019

The flavonoid group dominated the antioxidant-active compounds, with kaempferol-3-O-β-D-glucuronide and kaempferol 3-O-β-D-glucuronopyranosyl methyl ester identified as major constituents. Both were previously reported to exert potent antioxidant and anti-inflammatory effects by inhibiting ROS formation and modulating immune responses through microglial suppression (Lim *et al.* 2023). Their simultaneous detection in both positive and negative ionisation modes further supports their abundance and stability in the extract.

Kushenol B, another flavonoid detected, has been reported to possess strong antioxidant capacity alongside other therapeutic properties (Fan *et al.* 2018). Similarly, leucocyanidin, a compound structurally similar to vitamin C, exerts antioxidant and antibacterial effects through free radical neutralisation and mucosal protection (Rafa

Zubair *et al.* 2018). Luteolin, a widely studied flavonoid, was also identified and is known to scavenge reactive oxygen species (ROS) and inhibit cancer cell proliferation by modulating oxidative stress and suppressing epidermal growth factor (EGF) signalling (Imran *et al.* 2019).

Two phenolic compounds, euparin and sesamol, were also detected and are known for their antioxidative activity. Sesamol, in particular, contains a benzodioxole ring that efficiently scavenges hydroxyl radicals, enhancing its biological efficacy as an antioxidant (Shah *et al.* 2019). Euparin contributes further antioxidant potential, although its detailed mechanism remains less well-characterised (Ezzatzadeh & Hossaini 2019).

Among the terpenoids, brefeldin A and E-p-coumaric acid (also referred to as E-p-coumatic acid) were identified with known antioxidant capacity. Brefeldin A exhibits dual antioxidant and anticancer roles, with its ability to regulate ROS levels and induce apoptosis through the production of isothiocyanates (Wang *et al.* 2023). E-p-coumaric acid is a hydroxycinnamic acid derivative known to suppress oxidative stress by neutralising free radicals and protecting cellular biomolecules (Boo 2019).

Together, these compounds demonstrate that *D. hirsuta* methanolic extract possesses a diverse phytochemical composition rich in antioxidant agents. The co-occurrence of flavonoids, phenolic acids, and terpenoids with low mass error (<±4 ppm) and high ionisation reliability provides a robust chemical basis for its potential application in oxidative stress-related therapeutic interventions. This study significantly advances current understanding by identifying specific antioxidant compounds through a high-resolution LC-MS approach, in contrast to previous works that only offered qualitative profiles without detailed metabolite annotation (Dogra *et al.* 2024).

4.3. Antioxidant Activity of *D. hirsuta* Extract

The results of the DPPH assay clearly demonstrate that the methanol extract of *D. hirsuta* exhibits notable antioxidant potential, as indicated by the visible colour change from deep violet to yellow and the quantitative increase in radical scavenging activity with higher extract concentrations. The linear relationship between concentration and percentage inhibition, with a high correlation coefficient (R^2 = 0.9526), reflects a reliable dose-dependent effect. The calculated IC_{50} value of 101.13 ppm categorizes the extract as having moderate antioxidant activity (Nurhaeni and Hardi 2019), suggesting that *D. hirsuta* is a promising source of natural antioxidants. The lower the IC_{50} value, the higher the antioxidant activity of the extract (Nuraeni and Sembiring 2018).

The abiotic conditions of the sample collection site might influence the results of antioxidant activity. The abiotic conditions of Gunung Gumitir at the sample collection location are highly conducive to the survival of *D. hirsuta*. The sample collection site at Gunung Gumitir has a temperature of 27.3°C, light intensity of 505 lux, and humidity of 60.8%. These abiotic conditions suggest that the site is not excessively hot and is shaded by several tree species' canopies. Based on previous research conducted by Khusni *et al.* (2018), environmental conditions that are not excessively hot due to the shading effect influence the antioxidant activity of a plant. This ecological context reinforces the finding that *D. hirsuta* from the shaded environment of Gunung Gumitir exhibits moderate antioxidant potential, highlighting the interplay between environmental factors and the bioactivity of bryophyte extracts.

D. hirsuta contains secondary metabolite compounds with a high potential for antioxidant activity in the future. The phytochemical compounds that exhibit strong antioxidant activity are primarily found in the phenolic and flavonoid groups. These compounds stabilize free radicals by donating hydrogen atoms. This stabilization process reduces the rate of auto-oxidation caused by free radicals, thereby preventing oxidative damage through the action of secondary metabolites as antioxidants (Amin *et al.* 2016).

The antioxidant activity found in *D. hirsuta* extract suggests that this liverwort has the potential for further development as an anticancer agent. Based on research by Liu *et al.* (2018), *D. hirsuta* has anticancer activity against Colon HT-29 for animal models, as evidenced by a reduction in HeLa cancer cells in mice following treatment with *D. hirsuta* extract. Similarly, research by Cianciullo *et al.* (2022) showed that *D. hirsuta* extract possesses antitumor properties due to its terpenoid content, which also exhibits antioxidant activity. In India, research on the antioxidant activity of *D. hirsuta* subsp. hirsuta has been developed using the DPPH assay method. The extract of *D. hirsuta* demonstrated the highest radical scavenging activity of 71.19% at a concentration of 400 µg/mL, which falls into the high antioxidants category. Therefore, this study's extraction or analytical methods can be further developed in Indonesia (Dogra *et al.* 2024). This research, with its

improvements in more optimal methods, serves as a step forward in developing antioxidants in East Java, starting with *D. hirsuta* from Gumitir Mount.

This study provides a comprehensive metabolomic profile of *D. hirsuta* from Mount Gumitir through integrated GC–MS and LC–MS analyses, revealing its potential as a rich natural source of antioxidant compounds. The GC–MS analysis identified 37 volatile metabolites, comprising primarily terpenes (29%), phenols (21%), and fatty acids (13%), with sesquiterpenoids such as caryophyllene, guaiene, and aromadendrene derivatives being particularly abundant. Notably, the phytochemical richness of the Gumitir population surpassed that of previously studied populations, with the presence of unique constituents such as phytol, pyrocatechol, furanones, and benzoic acid, many of which possess well-documented radical-scavenging capabilities.

Complementing these findings, LC–MS analysis of the methanol extract identified 15 secondary metabolites, including nine with proven antioxidant activity spanning flavonoids, phenolic compounds, and terpenoids. Key antioxidant constituents such as kaempferol-3-O-β-D-glucuronide, leucocyanidin, luteolin, sesamol, and E-p-coumaric acid were detected with high mass accuracy (±4 ppm) in both positive and negative ion modes, affirming their robust ionisation and analytical reliability.

The antioxidant potential of the extract was further validated by the DPPH radical scavenging assay, which exhibited a dose-dependent response with a moderate IC_{50} value of 101.13 ppm. The extract's activity was visibly indicated by colour change and strongly correlated with concentration ($R^2 = 0.9526$), confirming its efficacy in neutralising free radicals. These findings are further supported by the conducive microclimatic conditions of the Gumitir site, characterised by moderate temperature, canopy shading, and high humidity, which likely enhance secondary metabolite biosynthesis.

Altogether, the antioxidant-rich phytochemical profile of *D. hirsuta* highlights its pharmacological promise, particularly for applications in oxidative stress mitigation and potential anticancer development. This study also underscores the value of East Java's tropical liverworts as underexplored reservoirs of bioactive metabolites, laying the groundwork for future drug discovery and conservation-oriented bioprospecting efforts.

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