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LC-MS/MS Metabolite Profiling of Meistera aculeata (Roxb.) Škorničk. & M.F. Newman (Susube) and Preliminary Brine Shrimp Lethality Screening

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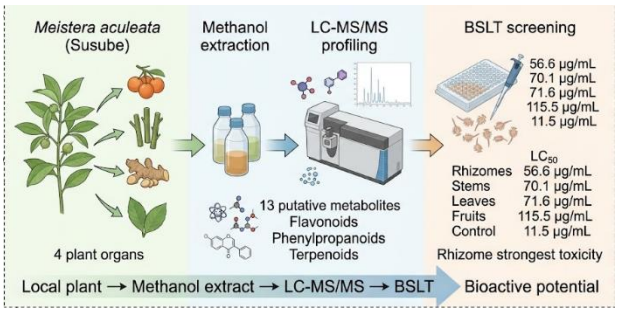
ABSTRACT



Meistera aculeata (Roxb.) Škorničk. & M.F. Newman is a species of the Zingiberaceae family distributed in Southeast Sulawesi, Indonesia, particularly in Konawe Regency. The fruits are traditionally consumed and used as a culinary spice by the Tolakinese community. However, information regarding its metabolite composition and biological activities remains limited. This study aimed to profile putative metabolites using LC–MS/MS and to evaluate preliminary lethality using the Brine Shrimp Lethality Test (BSLT). Dried powders of rhizomes, stems, leaves, and fruits were extracted by maceration with methanol. Metabolite annotation was performed using LC–MS/MS, while cytotoxic potential was assessed through BSLT. The analysis tentatively detected 13 metabolites dominated by flavonoids, phenylpropanoids, and terpenoids. Methanol extracts of rhizomes, stems, leaves, and fruits showed toxic activity with LC_{50} values of 56.6 ± 9.9, 70.1 ± 8.1, 71.6 ± 9.3, and 115.5 ± 13.5 µg/mL, respectively, compared with $K_2Cr_2O_7$ as a positive control (LC_{50} 11.5 ± 1.6 µg/mL). Literature comparison suggests that although these metabolites have been reported in other plant species, their occurrence in *M. aculeata* is reported here for the first time. The BSLT results indicate preliminary cytotoxic activity, suggesting the presence of bioactive compounds. These findings support further chemotaxonomic and pharmacological investigations to identify potential marker compounds and expand knowledge of metabolites within the Zingiberaceae family, particularly the genus *Meistera*.

Keywords: *Meistera aculeata; Zingiberaceae; LC-MS/MS; chemotaxonomy; brine shrimp lethality test*
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INTRODUCTION

Indonesia possesses exceptionally high biodiversity, including medicinal plants that hold great potential as sources of raw materials for pharmaceutical preparations. One of the major challenges faced by the national pharmaceutical sector is its heavy reliance on imported active pharmaceutical ingredients (APIs), which account for 90%. This condition not only affects national resilience in the health sector but also hampers the development of an independent and sustainable pharmaceutical industry [1][2]. Hence, the exploration and characterization of bioactive compounds from local plants as alternative sources of pharmaceutical raw materials have become highly urgent.

The Zingiberaceae family serves as a valuable source of various bioactive phytochemicals. It includes approximately 52 genera and 1,300 species of aromatic, perennial flowering herbs characterized by creeping horizontal or tuberous rhizomes [3]. Meistera is a genus within the Zingiberaceae family, comprising 47 identified species worldwide. One of the newly discovered species of this genus is Meistera aculeata (Roxb.) Škorničk. & M.F. Newman. This plant is distributed from Sri Lanka and India across the Indo-Chinese region to Sundaland [4][5]. Meistera aculeata (M. aculeata) was initially described under the name Amomum aculeatum. It was subsequently reassigned to the genus Meistera based on phylogenetic analysis [4]. In Indonesia, this plant is found exclusively in Southeast Sulawesi, where it is locally known as Susube by the Tolakinese. The fruit is traditionally consumed raw and occasionally used as a cooking spice.

Previous studies have reported that the ethanol extract of M. aculeata fruit contains secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and terpenoids. Those compounds exhibits potential toxicity with an LC_{50} value of 683 ppm [6]. However, these studies were limited to general phytochemical screening and preliminary cytotoxicity assay, without providing detailed liquid chromatography tandem mass spectrometry (LC-MS/MS) based metabolite profiling across different plant organs. In addition to M. aculeata, another Meistera species (M. chinensis) identified in Southeast Sulawesi is locally known as “walay” by the Tolakinese [7]. This species contains alkaloids, flavonoids, phenolics, tannins, and triterpenoids, which demonstrates strong toxic activity. Several bioactive compounds have been structurally identified from this species based on LC-MS/MS analysis, including phillgenin, 2-methoxyacofinic acid, feroxidin, (E)-hexadecyl ferulate, and spinasterol [8]. From a chemotaxonomic perspective, M. aculeata has great potential to share similar chemical constituents with M. chinensis. However, no comprehensive study has yet validated this assumption using comparative LC–MS/MS-based metabolite profiling, leaving the chemotaxonomic framework of the genus Meistera largely unresolved.

The identification of secondary metabolites in plants can be carried out using various analytical techniques. One of the methods frequently employed is liquid chromatography tandem mass spectrometry (LC-MS/MS) [9]. The advantages of LC-MS/MS lie in its high sensitivity and resolution, enabling the separation and detection of compounds in small quantities based on their mass-to-charge ratios and fragmentation patterns [10][11]. Therefore, this technique has been widely applied in plant metabolomics studies, biomarker discovery, and the exploration of bioactive compounds with potential as natural drug candidates [12][13]. Therefore, the objective of this study is to comprehensively identify the chemical constituents of M. aculeata (fruit, stem, rhizome, and leaves) using LC–MS/MS and its preliminary cytotoxic activity.

METHODS

1. Material

The samples of *M. aculeata* (Roxb.) Skornick. and M.F. Newman were collected

from Totombe Jaya Village, Konawe Regency, Southeast Sulawesi. The plant specimen was authenticated by the Biology Research Center, Life Sciences Research Organization, with a voucher specimen recorded as B085/V/DI.05.07/9/2021.The chemicals used were technical methanol distilled before use, potassium dichromate ($K_2Cr_2O_7$), distilled water, seawater, and *Artemia salina* Leach larvae. The instruments used were glassware, oven, analytical balance, micropipette, rotary evaporator, and a binocular microscope.

2. Procedures

a. Preparation and Extraction

The freshly rhizomes, stems, fruits (peeled), and leaves were cleaned, cut into small pieces, and dried in an oven at 40 °C. Once dried, all samples were ground into fine powder for extraction. A total of 100 g of each sample was extracted by maceration using technical methanol with a sample:solvent ratio of 1:2 for 3 x 24 hours. Every 1x2 hours the sample was stirred and the solvent was renewed by filtering the extract (the filtrate was separated), the residue was added with solvent again. The filtrate obtained was then collected and evaporated to obtain a concentrated extract.. These methanol extracts were subsequently used for LC-MS/MS qualitative analysis and preliminary cytotoxicity assay.

b. Compounds Identification by LC-MS/MS

The compound analysis by LC-MS/MS was performed in Advanced Characterization Laboratories, National Research and Innovation Institute through E-Layanan Sains, Badan Riset dan Inovasi Nasional in Serpong, Indonesia. The method was adopted from our previous study with modifications [14]. The phytochemical constituents of the methanol extract of *M. aculeata* were separated on a reverse-phase column (ACQUITY UPLC BEH C8, 1.7 µm × 100 mm) using a gradient elution system composed of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The separation was performed at a flow rate of 0.3 mL/min and a column temperature of 40°C for 16 minutes, with the gradient conditions set at 95% A (0–1 min), 60% A (8 min), 100% B (11–13 min), and 5% B (16 min). The resolved peaks were analyzed using a Xevo G2-XS QTof mass spectrometer operated in positive electrospray ionization (ESI) mode. Mass spectra were acquired over the range of m/z 50–1,200 with a source temperature of 120°C and a desolvation gas flow of 1,000 L/h at 500°C. The detected m/z values were further processed using UNIFI software and annotated by comparison with online mass databases (PubChem and ChemSpider).

c. Brine Shrimp Lethality Test (BSLT) assay

The brine shrimp lethality test (BSLT) was performed at the Pharmacy Laboratory, Faculty of Science and Technology, Universitas Sembilanbelas November Kolaka, following previously reported procedures with slight modifications [15], [16]. Methanol extracts of M. aculeata (fruits, stems, rhizomes, and leaves) were prepared and transferred into 96-well microplates (F-bottom). Serial dilutions were performed by adding 100 µL of the extract stock solution

(2000 µg/mL) into a well containing 100 µL of artificial seawater, followed by thorough mixing. This procedure was repeated to obtain a series of concentrations at 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000, and 2000 µg/mL for the extracts, positive controls (K2Cr2O7), and solvents (DMSO). Subsequently, 100 µL of Artemia salina larval suspension that contains 10 larvae (±1–2 individuals due to handling variability) obtained from seawater hatching media (48h incubation time and yeast as a food source) was added into each well. The plates were then incubated for 24 hours at room temperature (22–29 °C). Each treatment was carried out in triplicate. After incubation, the number of dead larvae (immobile) was counted in each well using a binocular microscope (12.5×), followed by counting the total number of larvae. The mortality percentage was calculated based on the actual number of larvae per well. The mortality percentage of A. salina larvae was calculated using formula (1).

$$\% \text{ mortality} = \frac{number\ of\ death\ larvae}{number\ of\ initial\ larvae} \times 100\% \quad (1).$$

d. Analysis of Toxicity

The cytotoxicity data obtained from the bioassay were analyzed using Minitab 17 software to determine the LC_{50} value. LC_{50} is defined as the concentration at which 50% lethality occurs, calculated using a linear regression model. The percentage of larval mortality was first transformed into probit values, while the concentrations of the extract were converted into their logarithmic form. A regression analysis was then performed by plotting the probit values (Y) against the logarithm of concentrations (X). From the regression equation generated by Minitab, the concentration corresponding to probit 5 was calculated, representing the LC_{50} value. The obtained LC_{50} value was subsequently interpreted to classify the cytotoxic activity of the extract according to standard toxicity guidelines [15].

RESULT AND DISCUSSION

1. Identification of compounds using LC-MS/MS

LC-MS/MS instrumentation is a highly appropriate method for detecting the components of a compound in plant extracts. The UPLC chromatograms revealed the separation of chemical compound peaks in each *M. aculeata* extract (Figure 1), observed within a retention time (RT) range 0-16 minutes. The separation of compounds occurs because each chemical constituent exhibits a different migration rate depending on its interactions with the mobile and stationary phases [17]. The separation is characterized by the formation of distinct compound peaks. Peaks appearing at specific retention times were further analyzed by MS/MS based on their molecular weights and fragmentation patterns, allowing the tentative determination of molecular identities (name and structure). The chromatograms also indicate similarities in chemical composition among samples, as reflected by comparable retention times.

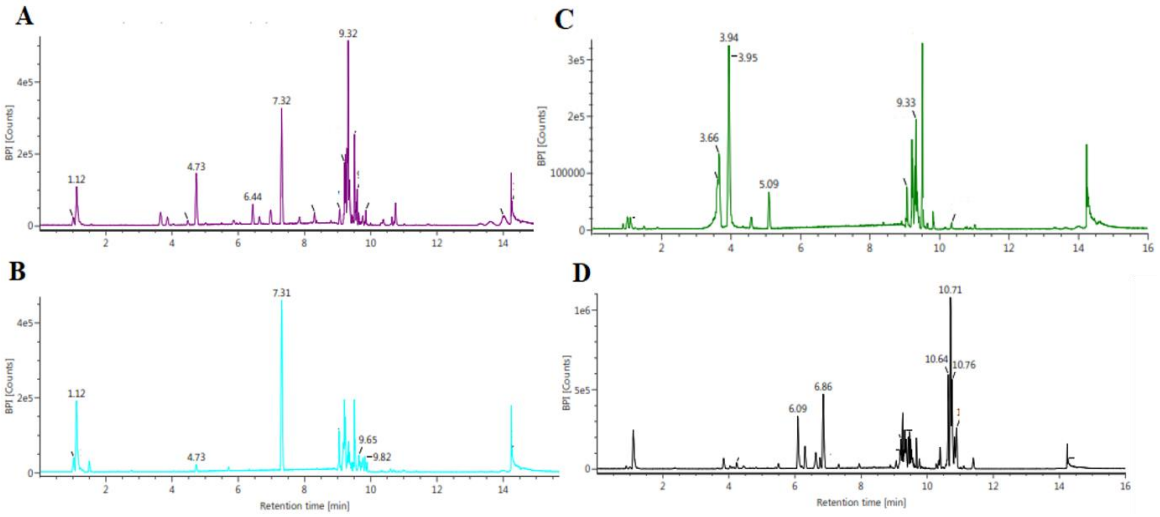

Figure 1. UPLC Chromatogram of compounds in the methanolic extract of M. aculeata (A)stems; B) rhizome; C) fruits; D) leaves).

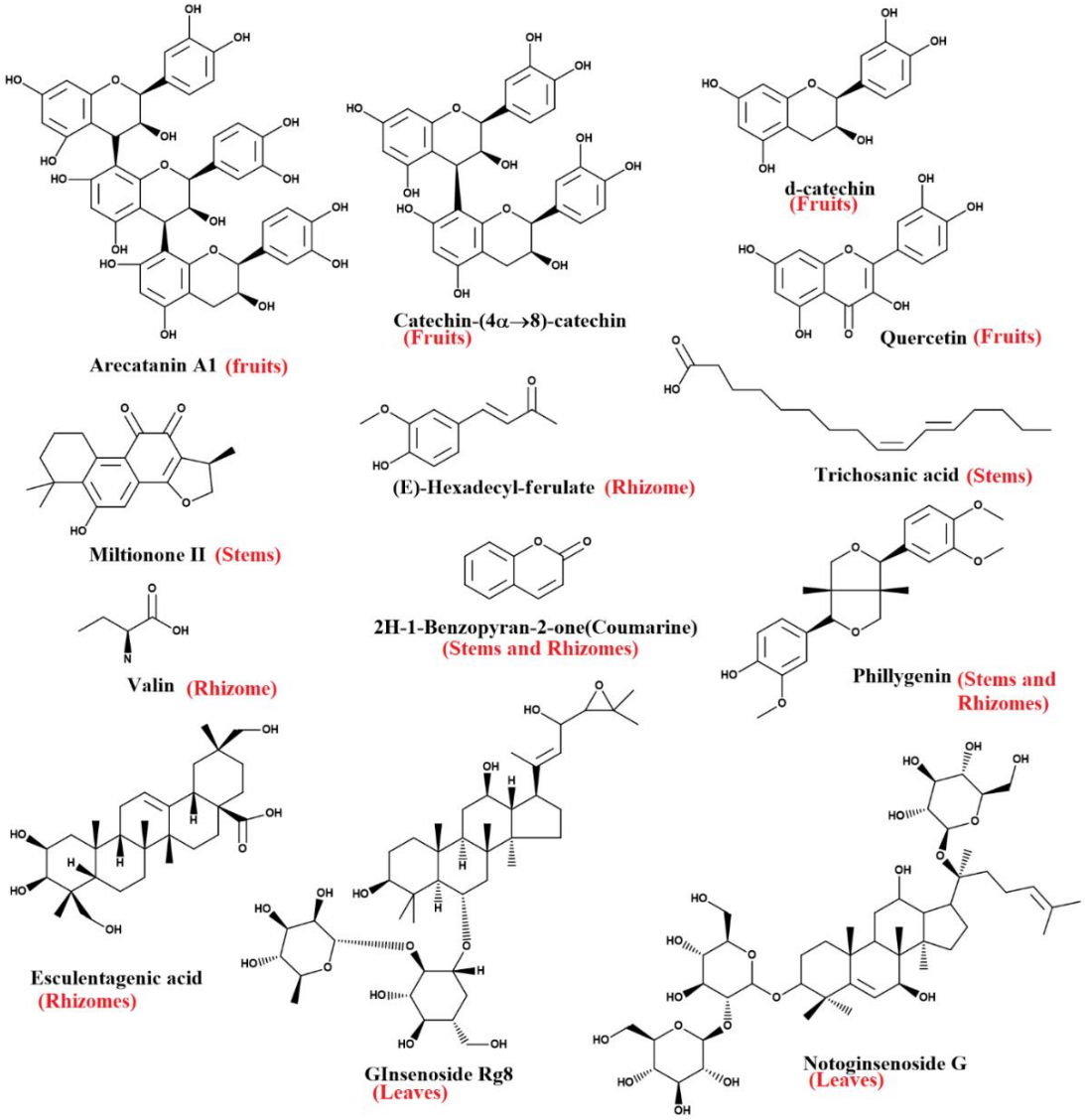

Figure 2. The compounds structure from M. aculeata (Roxb.) Skornick. & M.F.Newman

Table 1. The compounds identified from the methanol extract *M. aculeata* (Roxb.) Skornick. & M.F.Newman based on LC-MS/MS

RT (min)	(+)-ESI (m/z)	MS/MS Fragmentation (m/z)	Neutral mass (Da)	Formula	Compound name	Group
Fruits						
3,66	579.1499 $[M+H]^+$	427/409/289/139/127	578.14243	$C_{30}H_{26}O_{12}$	Catechin-(4α 8)-catechin	Flavonoid
3.94	291.0861 $[M+H]^+$	289/139/123	290.07904	$C_{15}H_{14}O_6$	d-Catechin	Flavonoid
4,00	867.2129 $[M+H]^+$	577/289/245/127	866.20581	$C_{45}H_{38}O_{18}$	Arecatannin A1	Flavonoid
5.10	303.0502 $[M+H]^+$	247/153	302.04265	$C_{15}H_{10}O_7$	Quercetin	Flavonoid
9.30	520.3396 $[M+Na]^+$	478/184/139/98	497.35051	$C_{31}H_{47}NO_4$	CC 1	Unknown
Stems						
1.13	381.0797 $[M+Na]^+$	275/118	358.09000	$C_{15}H_{18}O_{10}$	CC 2	Unknown
4,74	147.0439 $[M+H]^+$	-	146.03678	$C_9H_6O_2$	2H-1-Benzopyran-2-one (Coumarin)	Fenil propanoid
6.44	313.1435 $[M+H]^+$	295/181/147/133	312.13616	$C_{19}H_{20}O_4$	Miltionone Ⅱ	Terpenoid
7.32	373.1649 $[M+H]^+$	355/137/123/105	372.15729	$C_{21}H_{24}O_6$	Phillygenin	Fenil Propanoid
9.32	279.2321 $[M+H]^+$	261/184/95	278.22458	$C_{18}H_{30}O_2$	Trichosanic acid	Fatty acid
Rhizomes						
1.1	118.0860 $[M+H]^+$	104/87	117.07898	$C_5H_{11}NO_2$	Valine	Amino acid
4.73	147.0438 $[M+H]^+$	-	146.03678	$C_9H_6O_2$	2H-1-Benzopyran-2-One (coumarin)	Fenil propanoid
7.32	373.1650 $[M+H]^+$	355/137/123/105	372.15729	$C_{21}H_{24}O_6$	Phillygenin	Fenil propanoid
9,77	419.3162 $[M+H]^+$	401/163/147/123	418.30831	$C_{26}H_{42}O_4$	(E)-Hexadecyl-ferulate	Fenil propanoid
9.82	505.3507 $[M+H]^+$	475/413/149	504.34509	$C_{30}H_{48}O_6$	Esculentagenic acid	Terpenoid
leaves						
6.10	983.5183 $[M+Na]^+$	781/619/457/439	960.52938	$C_{48}H_{80}O_{19}$	Notoginsenoside G	Terpenoid
6.86	821.4673 $[M+Na]^+$	781/763/619/457	798.47656	$C_{42}H_{70}O_{14}$	Ginsenoside Rg8	Terpenoid
10.71	887.5685 $[M+H]^+$	284	886.55950	$C_{54}H_{78}O_{10}$	CC 3	Unknown
10.76	917.5791 $[M+H]^+$	885/639/551	916.57006	$C_{55}H_{80}O_{11}$	CC 4	Unknown
10.64	903.5626 $[M+H]^+$	885/625	902.55441	$C_{54}H_{78}O_{11}$	CC 5	Unknown

***CC=Candidate Compound**

Based on the results of the LC-MS/MS analysis, 13 compounds were successfully identified (name and structure). Table 1 shows the distribution of compounds and their groups in each organ of *M. aculeata*. The fruit contains predominantly flavonoids, the rhizome and stem contain phenylpropanoids and a small amount of

terpenoids, amino acid, fatty acid. The leaves contain terpenoids. In addition, there are 5 candidate compounds whose names and structures are not yet known. Figure 2 shows the structures of the 13 identified compounds. Differences in the composition of compounds in each plant organ are due to metabolic processes that depend on the organ's physiological function and environmental interactions [18][19][20]

In the fruit of *M. aculeata*, most of the compounds are flavonoids (Catechin-(4α 8)-catechin; d-Catechin; Arecatannin A1; and Quercetin) and CC 1 with the molecular formula $C_{31}H_{47}NO_4$ (m/z 520.3396; RT 9.30 min). Flavonoids are a group of compounds that can be found in plants and are distributed in various parts of plant tissue (fruit, leaves, stems, roots) [21].

Some of the most common flavonoid compounds show cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral activities such as apigenin, galangin, hesperetin, kaempferol, myricetin, naringenin, and quercetin [22]. They are even used in the cosmetics industry such as pigments and as a source of natural antioxidants in the pharmaceutical industry [23]. For plants of *M. aculeata* species, the compounds Catechin-(4α 8)-catechin; d-Catechin; Arecatannin A1; and Quercetin were first reported.

The stems of *M. aculeata* contain compounds from the phenyl propanoid group (2H-1-Benzopyran-2-one (Coumarin) and Phillygenin), terpenoids (Miltionone II), and the fatty acid Trichosanic acid. Coumarin has a benzopyrone skeleton commonly found in medicinal plants and a broad spectrum of biological activities such as anti-inflammatory, antimicrobial, antiviral, antihypertensive, antimalarial, anti-inflammatory, and antioxidant [24][25]. Phllygenin is a lignan compound from the phenyl propanoid group. This compound can act as an anti-inflammatory, anticancer, antibacterial, and antiviral [26][27][28]. Miltionone II is a terpenoid that was first reported in the *Salvia miltiorrhiza* (Labiatae) plant [29][30] and has activity to inhibit scarring [31], anti-inflammatory, antioxidant, anticancer, neurological disorders and some cardio vascular diseases [32]. Trichosanic acid fatty acid or known as Punicic acid is very abundant in pomegranate plants (*Punica granatum*). This compound has activity to prevent blood clotting [33] and neurological disorders [34].

A similar case is found in the rhizome *M. aculeata* rhizomes, which contains coumarin and phillygenin. However, several additional compounds are present, includin (E)-hexadecyl-ferulate, esculentagenic acid, and the amino acid valine. (E)-hexadecyl-ferulate has antioxidant activity [35][36]. Esculentagenic acid, a member of the terpenoid (triterpenoid) group, has anti-inflammatory [37] and antiplasmodial activity [38]. Valine has also been detected in *M. aculeata* rhizomes. Therefore, *M. aculeata* rhizomes can be developed as a source of essential amino acid nutrition.

In the leaves, terpenoid compounds (Notoginsenoside G and Ginsenoside Rg8) and 3 candidate compounds with molecular masses of $C_{54}H_{78}O_{10}$ (m/z 887.5685; RT 10.71 min), $C_{55}H_{80}O_{11}$ (m/z 917.5791, RT

10.76 min), and $C_{54}H_{78}O_{11}$ (m/z 903.5625 RT 10.64 min) were detected. Notoginsenoside G and Ginsenoside Rg8 compounds were first reported to be isolated in ginseng plants (*Panax ginseng* and *Panax quinquefolium*) which have tonic effects and natural immunomodulatory agents [39][40]. These compounds were also detected in *M. aculeata* for the first time.

Based on previous research on the same genus, several compounds were detected in the methanolic rhizome extract of *Meistera chinensis* from Southeast Sulawesi using the LC-MS/MS [8]. These two plants have two similar compounds, namely Phillygenin and (E)-Hexadecyl-ferulate. Comparison of compound components from *M. aculeata* and *M. cninensis* is shown in **Error! Reference source not found.** T herefore, chemotaxonomically,
the compound composition of *M. aculeata* and *M. chinensis* is similar. Therefore, a comprehensive study is urgently needed to strengthen the compound diversity in the genus Meistera, especially its marker compounds.

Table 2. Comparison of retention time from the same compound components in the rhizomes of *M. aculeata* and *M. chinensis*

Species	RT (min)	Mass observed (m/z)	Formula	Compound name
Meistera aculeata	7.32	373.1650	$C_{21}H_{24}O_6$	Phillygenin
	9,77	419.3162	$C_{26}H_{42}O_4$	(E)-Hexadecyl-ferulate
***Meistera chinensis* [8]**	7.04	373.1645	$C_{21}H_{24}O_6$	Phillygenin
	9.69	419.3151	$C_{26}H_{42}O_4$	(E)-Hexadecyl-ferulate

2. Toxicity*: Brine Shrimp Lethality Test (BSLT)* screening

Table 3. The LC_{50} Value of *Meistera Aculeate*

Sample	LC_{50} (µg/mL)	Cytotoxicity Classification [15]
Fruits	115.5 ± 13.5	Toxic
Stems	70.1 ± 8.1	Toxic
Rhizomes	56.6 ± 9.9	Toxic
Leaves	71.6 ± 9.3	Toxic
Control ($K_2Cr_2O_7$)	11.5 ± 1.6	Very toxic

BSLT is an initial test used to detect bioactive compounds that have the potential as anticancer drugs. One of the parameters used to determine the toxic properties of a plant is the LC_{50} value. In the BSLT assay, the Lethal Concentration 50% (LC_{50}) represents the concentration of a substance such as an extract, compound, or chemical that induces mortality in 50% of the *Artemia salina* larval population within a specified exposure period, typically 24 hours. The LC_{50} value provides a critical parameter in assessing the toxic potential of a sample by quantifying its lethality toward *A. salina* nauplii. A lower LC_{50} value denotes higher toxicity and greater bioactive potential, thereby providing a valuable preliminary indicator in the early-stage screening of natural compounds prior to more advanced

toxicity evaluations in cellular or animal models.

The results of the toxicity test (LC_{50} value) on *M. aculeata* plants are shown in Table 3. Based on the resulting LC_{50} data, it shows that the greatest toxic properties are produced in the methanol extract of the rhizome, followed by the stem, leaves, and fruit with LC_{50} values of 56.6;, 70.1;, 71.6;, and 115.5 µg/mL, respectively, when compared to the positive control $K_2Cr_2O_7$ of 11.5 µg/mL. The addition of the extract caused the death of *Artemia salina*, which experienced a disorientation of motion (irregular movements).

The death of Artemia salina larvae is caused by the drastic concentration gradient across the cell membrane. This change allow compound to become toxic spread throughout the larval body [41]. Early symptoms include irregular movements or loss of coordination (disorientation). This indicates a disturbance in the nervous or neuromuscular system, such as affecting cellular ion channels or neurotransmitter activity. Movement disorders accelerate functional failure in essential functions such as swimming and feeding, ultimately leading to mortality [42]. The detrimental metabolic effects occurred rapidly and were observed within 24 hours, resulting in up to 50% mortality [41]. Based on the results of cytotoxic testing on the M. aculeata methanolic extract, the LC50 was < 1000 ppm and categorized as toxic. According to [15], an extract shows toxic activity in BSLT if the extract can cause the death of 50% of test animals at a concentration of less than 1000 ppm.

Based on the comparison of the LC50 values of all parts of the plant in Table 3., it shows that the greatest cytotoxic properties are in the rhizome, followed by the stem and leaves. On the contary, the smallest cytotoxicity is in the fruit. The cytotoxic ability is influenced by the components of the compounds contained in each part of the plant. In the rhizome, phenyl propanoid and terpenoid compounds were detected, as was the case in the stem, followed by the leaves where only terpenoids were detected. In the fruit, the majority are composed of flavonoid compounds and have the smallest cytotoxic properties compared to the stem, rhizome, and leaves. Different plant parts contain distinct compositions of secondary metabolites, which may lead to variations in their biological activities, including cytotoxic effects [43]. Therefore, a comprehensive study of cytotoxic activity in *M. aculeata* plants is needed in the future.

CONCLUSION

In this study, *Meistera aculaeta* plants showed a diverse chemical compound profile with preliminary lethality activity in BSLT. Based on LC-MS/MS analysis, the compound profile of *M. aculeata* is dominated by phenylpropanoids (2H-1-Benzopyran-2-one/coumarin; Phillygenin), terpenoids ((E)-Hexadecyl-ferulate; Miltionone II; Esculentagenic acid Ginsenoside Rg8; and Notoginsenoside G), flavonoids (Catechin-(4α 8)-catechin; d-Catechin; Arecatannin A1; and Quercetin) and some fatty acid compounds Trichosanic acid and amino acid valine. In addition, there are five candidate compounds with unidentified structures and observed masses of 520.3396 (m/z)

$C_{31}H_{47}NO_4$; 381.0797 (m/z) $C_{15}H_{18}O_{10}$; 887.5685 (m/z) $C_{54}H_{78}O_{10}$; 917.5791 (m/z) $C_{55}H_{80}O_{11}$; 903.5626 (m/z) $C_{54}H_{78}O_{11}$. The results of the BSLT analysis showed that the *M. aculeata* plant has cytotoxic activity based on the LC_{50} values of methanol extracts of rhizomes, stems, leaves, and fruits of 56.6,; 70.1,; 71.6,; and 115.5 µg/mL, respectively, when compared to the positive control $K_2Cr_2O_7$ of 11.5 µg/mL. Chemotaxonomic studies are required to determine the marker compounds of the genus Meistera and comprehensive further plant bioactivity studies to determine more specific pharmacological potential.

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