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Exploring the Metabolites of *Boesenbergia plicata* from Pulau Tuba: A Dereplication Approach

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Boesenbergia plicata (Zingiberaceae) or "wild ginger" can be exclusively found in limestone rainforest, Pulau Tuba, Langkawi, Malaysia. To date, no chemical constituent has been reported from this plant. Thus, this research was conducted to explore the metabolites present in this unique plant for future pharmacological assessments. A dereplication strategy was implemented for metabolite annotation. The dried leaves and stems of *B. plicata* were macerated in hexane, dichloromethane, and methanol, and concentrated at 40°C. The dichloromethane extracts of the stems and leaves of *B. plicata* were introduced into a solid-phase extraction (SPE) column, before being profiled using ultra-high-performance liquid chromatography (UHPLC) and mass spectrometry. This was followed by database export, database building, and metabolites processing using Sirius software. A total of 27 and 19 metabolites were detected in the *B. plicata* leaves and stems, respectively. The selected metabolites in the leaves part were targeted for isolation work. The leaf extract was introduced into the Sephadex column, medium-pressure liquid chromatography (MPLC), preparative HPLC, and recycling HPLC. Two metabolites namely *epi*-loliolide (**1**) and loliolide (**2**) were isolated and characterized. The purification of the metabolites validated this dereplication technique. Future work on the bioactive metabolites from the plant is warranted.

Keywords: *Boesenbergia plicata*; Sirius; *epi*-loliolide; loliolide; Zingiberaceae; dereplication

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Plants contain diverse and complex metabolites. Isolation and purification of these metabolites in the crude extract is extremely challenging due to the complexity of the chemical structure, isomeric, and very closely eluted metabolites. Nowadays, the dereplication strategy has become a widely used tool as it provides rapid screening of the metabolites contained in the complex crude extract [1]. By implementing this strategy, the amount of time needed to isolate the metabolite can be greatly reduced, thus saving energy and money due to solvent usage.

Zingiberaceae family, commonly referred to as the ginger family, has over 1,300 species that are classified into approximately 52 genera, usually found throughout tropical Asia, Africa, and the Americas [2]. The plants belonging to the Zingiberaceae family are known for their creeping horizontal or tuberous rhizomes and are an important source of spices, particularly ginger. The Zingiberaceae family has been reported to produce various classes of metabolites such as phenylpropanoid, terpenoid, polyphenol, diarylheptanoid, phenylbutenoid, flavonoid, and essential oil [3–10].

Genus *Boesenbergia* consists of more than 80 species and is distributed in Southeast Asia, China, and India [11–14]. They are typically found in extremely humid, shaded places, often near streams or in swampy conditions [15]. Phenolic, flavonoid, terpenoid, and essential oil are among the metabolites reported thus far [16–22].

Meanwhile, *Boesenbergia plicata* (Ridl.) Holttum *var*. lurida (Ridl.) Holttum or "wild ginger" is considered a rare species of Zingiberaceae as it is limited to the limestone rainforest of Pulau Tuba, Langkawi, Malaysia. To date, no single metabolite has been reported from this plant. Thus, this research was carried out to explore the metabolites present in this unique plant for future pharmacological assessments.

EXPERIMENTAL

Chemicals and Reagents

The analytical grade solvents such as hexane, dichloromethane, and methanol (Elite Advanced Materials Sdn. Bhd., Selangor, Malaysia) were used for plant extraction and fractionation using the Sephadex column. The HPLC grade solvents such as acetonitrile and methanol (Fisher *ChemAlert*® Guide, Fisher Scientific Korea Ltd., Seoul, Korea) were used in the SPE procedure and instrumentation such as

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UHPLC, MPLC, preparative HPLC, and recycling HPLC. The MS grade solvent of acetonitrile and formic acid (Fisher Scientific, Czech Republic) was used in the MS analysis while the deuterated methanol (Merck, Darmstadt, Germany) was used for the NMR analysis. Ultra-pure water was purified through the arium® pro system (Sartorius Malaysia Sdn. Bhd., Malaysia).

Instrumentations

The analysis of the crude extract of *B. plicata* was carried out using a Thermo Scientific analytical ultra-high performance liquid chromatography (UHPLC) system. This UHPLC comprises a Dionex UltiMate 3000 pump, a column compartment, an autosampler, a diode array detector (DAD), and an automated fraction collector.

The fractionation was performed using a YFLC W-Prep 2XY dual channel medium pressure liquid chromatography (MPLC) system equipped with a Yamazen pump, a parallel fraction collector FR-260, a collection tube rack, a column stand, and an ultraviolet detector (UV). The samples were further fractionated using preparative HPLC comprising a Waters 2545 pump, a prep degasser, a column compartment, a Waters 2707 autosampler, a Waters 2998 photodiode array detector, and an automated fraction collector.

The purification of chemical constituents was performed on a Japan Analytical Industry Co., Ltd (JAI LC 9063) preparative recycling HPLC equipped with an injection port, L-7150 pump, column compartment, 3702 UV detector, and SS-250F2 pen recorder with paper chart.

The mass spectra were acquired using Thermo Scientific Vanquish UHPLC and Thermo Scientific™ Orbitrap Fusion™ Tribid™ Mass Spectrometer.

The NMR analysis was recorded on Bruker Ascend™ 600 at Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns), Malaysia. ^{1}H NMR measured at 600 MHz and ^{13}C NMR at 150 MHz. The chemical shift of the spectrum was reported in ppm while the coupling constant was given in Hz. Bruker TopSpin 3.0 software was used to set the parameters of the experiment as well as process the spectra. MestReNova software was used to view, process, and analyze the NMR spectra.

Extraction of Plant Material

The leaves and stems of *Boesenbergia plicata* were collected in trail, wet, and slightly sandy, Gua Wang Buluh at Pulau Tuba, Langkawi, Malaysia. The authentication of the plant species was conducted by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia. The voucher specimen (voucher code: PT02/19) was deposited at the herbarium of Universiti Kebangsaan Malaysia. The leaves and stems were air-dried at room temperature before further dried using an oven at 40 °C. The dried leaves (106.02 g) and stems (24.69 g) were macerated separately for a day in hexane, before being concentrated using a rotary evaporator at 40°C. Next, the sample was successively soaked in dichloromethane and methanol for another three days and concentrated at 40°C. The weight of each crude extract was as follows: leaves (hexane: 0.87 g; dichloromethane: 1.33 g; methanol: 5.39 g) while stem (hexane: 0.10 g; dichloromethane: 0.27 g; methanol: 0.99 g). Their percentage of extractive yields was as follows: leaves (hexane: 0.82%; dichloromethane: 1.25%; methanol: 5.08%) while stem (hexane: 0.41%; dichloromethane: 1.09%; methanol: 4.01%). Finally, these extracts were stored in the refrigerator before the next analysis.

Sample Preparation for LC-MS Analysis

The dichloromethane extract of *B. plicata* leaves and stems (20 mg each) was weighed and dissolved in acetonitrile and ultra-pure water (90:10, v/v%), before being filtered using a 0.45 µm PTFE filter. The column (TMstrata® C18-E, Phenomenex) was activated using ultra-pure water and acetonitrile (3 mL each) before being conditioned using 3 mL of acetonitrile and ultra-pure water (90:10, v/v%). The sample (20 mg/mL) was then introduced into the column and added with 2 mL of acetonitrile and ultra-pure water (90:10, v/v%). The eluate was collected and concentrated using a rotary evaporator at 40°C to yield leaves (7.59 mg) and stems (5.87 mg) samples.

Both extracts were optimized using an analytical UHPLC system. The mobile phases consisting of ultra-pure water (solvent A) and acetonitrile (solvent B) were applied in the system, using a constant flow rate of 1 mL/min and an injection volume of 10 µL. They are monitored at four different wavelengths 210 nm, 254 nm, 260 nm, and 280 nm using Chromeleon 7 software. The chromatographic separation was achieved using an analytical XBridge C18 (250 mm × 4.6 mm, 5 µm, Waters, Ireland). The method was set as follows: 0-27 min, from 10 to 100% B; 27-32 min, at 100% B; 32-33 min, from 100 to 10% B; 33-38 min, at 10% B.

The pre-treated samples of leaves and stems of *B. plicata* were further analyzed using an Orbitrap mass spectrometer. An analytical Accucore™ Vanquish™ C18 (100 x 2.1 mm, 1.5 µm, Thermo Scientific, Lithuania) column was used for chromatographic separation. Both solvents were added with 0.1% of formic acid. The analysis was conducted in positive ion mode with 1 µL of injection volume. The flow rate was kept at 0.2 mL/min using a similar gradient of UHPLC. The mass spectrometer was run at 60,000 and 15,000 orbitrap resolutions at full and MS/MS scan modes, respectively. Electro-spray ionization

(ESI) was used as the ion source. The spray voltage at 3,500 V, the ion transfer tube temperature at 300ºC, the sheath gas at 35 arb, the auxiliary gas at 7 arb, the vaporizer temperature at 275ºC, and the RF lens at 60% were used in this system. The scan range covered from *m/z* 100 to 1000. The orbitrap detector was employed in full and tandem scan modes. The instrument control and data processing were performed using FreeStyle™ software 1.6.

Database Export

A licensed Dictionary of Natural Products (DNP) database was chosen to import metabolites from the Zingiberaceae family, genus *Boesenbergia*, and *Boesenbergia plicata*. The code of Z.R.19400, specifically designed for the Zingiberaceae family was used to obtain the family database, using a type of organism property, while all text and biological source properties were applied for the genus and species, respectively. The hits were exported to an Excel file (.xls) formatted text containing the chemical name, synonym, molecular formula, accurate mass, and SMILES. The SMILES was cleaned up manually and eventually saved as a comma delimited (csv) file. This file was viewed through DataWarrior for further check-up on their structures.

Data Processing using MZmine

Thermo raw data of crudes (leaves and stems) and blank were converted into the mzML file using MSConvert from ProteoWizard. Then, these mzML raw data were imported into the MZmine 3 software. The project was saved first using the mzmine format before further data processing. The crude chromatogram was then visualized to determine the noise level. The next step involved MS detection for MS levels 1 and 2 using both blank and crude data. Centroid mass detector was selected and the polarity was set to positive. The noise level for MS levels 1 and 2 was adjusted to 1.0E6 and 0.0E0, respectively. A chromatogram building was then created, followed by a chromatogram resolver. The isotope filter, alignment, and gap-filling were then applied before being exported to Sirius as a mgf file.

Dereplication Procedure

Sirius's academic account was first created. The dereplication strategy begins by importing the mgf file into the Sirius software. The software was ensured to connect to the web server. The databases were built by adding the cleaned-up SMILES of metabolites from Zingiberaceae and *Boesenbergia*, respectively, into the software. All listed features were set to adduct $[M+H]^+$, before computed. Then, the Sirius button was activated. The elements in the molecular formula were only allowed for hydrogen (H), carbon (C), nitrogen (N), and oxygen (O). The instrument was set to orbitrap with an MS2 mass accuracy of 15 ppm. MS/MS isotope score was set to "SCORE". All databases available in the Sirius software such as Bio Database, Biocyc, CHEBI, COCONUT, EcoCyc Mine, GNPS, HMDB, HSDB, KEGG, KEGG Mine, KNApSAcK, Maconda, MeSH, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, YMDB, YMDB Mine, ZINC bio, SuperNatural, and the two developed databases, were used for the annotation. Next, the Zodiac button was activated, followed by fingerprint prediction, structure database search, and compound class identification (CANOPUS) buttons. Finally, the compute button was selected, and the job was allowed to finish processing.

Isolation of *Epi*-loliolide and Loliolide

The dichloromethane extract of the leaves part of *B. plicata* (50 mg) was introduced into the Sephadex® LH-20 (Sigma-Aldrich, USA) column for chlorophyll removal. An isocratic elution of dichloromethane and methanol (1:1) was applied to the column to yield six fractions (F1-F6). The combined fractions 2 and 3 (25.12 mg) were selected for the next separation step.

The other portion of dried leaves extract of *B. plicata* (1.26 g) was pre-mixed with silica obtained from a packed inject column by Yamazen Corporation (ODS 2L) and dried using a rotary evaporator at 40 °C. The pre-coated sample was introduced into a Hi-Flash™ 2L (26 x 150 mm) column connected to an MPLC system. Fully automated dual-channel preparative liquid chromatograph software was used in this system. The flow rate was maintained at 40 mL/min. The method was as follows: 0-3 min, at 10% B; 3-30 min, from 10 to 100% B; 30-59 min, at 100% B. The fractionation yielded 16 fractions (F1-F16). The combined fractions F1 and F2 (185.7 mg) were selected for the next separation step.

The combined fractions from Sephadex and MPLC (50 mg/mL) were further fractionated using a preparative high-performance liquid chromatography (HPLC) system. Empower software was used for instrument control and data processing. The separation was analyzed using a preparative Eclipse XDB-C18 (250 mm × 21.2 mm, 7 µm, Agilent, USA), using a flow rate of 16.0 mL/min and the injection volume of 500 µL. A similar gradient as the UHPLC system was used throughout the analysis. This step yielded 11 fractions.

Fractions 2 (2.14 mg) and 3 (2.47 mg) from preparative HPLC were further purified using preparative recycling HPLC. The separation was carried out by using a preparative JAIGEL-ODS-AP (250 mm × 20 mm, 15 µm, JAI, Japan) reversed-phase column. An isocratic elution of acetonitrile (ACN) and ultrapure water (29:71, v/v%) and (31:69, v/v%) were used for the purification of F2 and F3, respectively. The sixth and fifth cycles were required to get pure *epi*-loliolide (**1**) (0.45 mg) and loliolide (**2**) (0.26 mg), respectively.

RESULTS AND DISCUSSION

Dereplication Analysis

A total time of 38 minutes was required to obtain the comprehensive profiling of *B. plicata* leaves and stems. Figure 1 shows the base peak chromatogram of dichloromethane extract of leaves of *B. plicata*. A total of 27 metabolites were annotated in the leaves of *B. plicata*, which comprised 20 fatty acids, one phenolic, and two metabolites in each of the following: alkaloid, terpenoid, and flavonoid. Fatty acid was the most abundant metabolite found in the leaves extract, representing 74.1% of the total metabolites (Table 1). Terpenoid, namely loliolide, was detected twice in this extract, at the retention time of 5.32 and 5.96 minutes. Since LC-MS has its limitations in predicting the exact structure of isomeric metabolites, isolation is warranted. This research successfully identified those metabolites as *epi*-loliolide (**1**) and loliolide (**2**).

Figure 2 shows the base peak chromatogram of dichloromethane extract of stems of *B. plicata*. A total of 19 metabolites were detected in the stems of *B. plicata*, which comprised two alkaloids, 14 fatty acids, two terpenoids, and one phenolic. Similar to leaves part, fatty acid was also the most abundant metabolite detected in the stems extract, representing 73.7% of the total metabolites (Table 1). The listed structure proposal of the leaves and stems extracts of *B. plicata* are displayed in Appendix A and B, respectively.

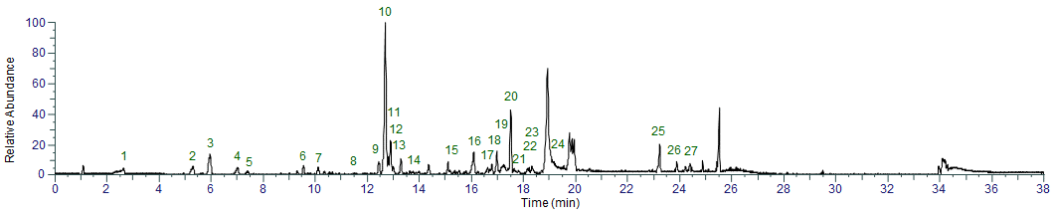


Figure 1. The base peak LC-MS chromatogram of dichloromethane extract of *B. plicata* leaves.

Table 1. The classes of metabolites in the dichloromethane extract of stems and leaves of *B. plicata*.

Metabolite	Leaves (%)	Stems (%)
Alkaloid	7.4	10.5
Terpenoid	7.4	10.5
Fatty acid	74.1	73.7
Flavonoid	7.4	-
Phenolic	3.7	5.3

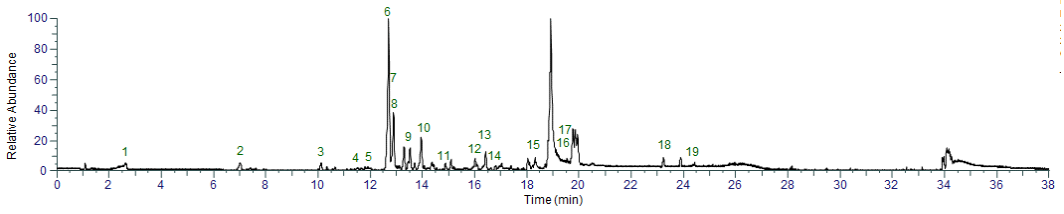


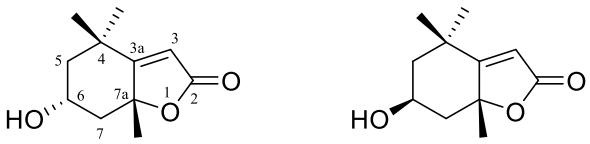
Figure 2. The base peak LC-MS chromatogram of dichloromethane extract of *B. plicata* stems

The mass error for the leaves and stems of *B. plicata* was below 5 ppm. The metabolites from both plant parts were detected within a retention time range of two to 25 minutes. While most of the metabolites annotated did not match the imported databases of DNP, they did align with *in-house* databases. Based on the dereplication results, metabolites **1** and **2** are only present on the leaves part of *B. plicata*. These monoterpene lactones are absent in the part of the stems.

Characterization of Metabolites

Purification of selected fractions from the dichloromethane extract of *B. plicata* leaves yielded two metabolites named *epi*-loliolide (**1**) and loliolide (**2**). The metabolites were characterized by NMR and MS analyses. The spectral data was compared with the literature and displayed good agreement [23].

Metabolite **1** was isolated as colourless gum, and its ESI-MS spectrum showed a molecular ion peak at *m/z* 197.1176 $[M+H]^+$, corresponding with the molecular formula $C_{11}H_{16}O_3$. The ^1H NMR (Table 3) spectrum exhibited 16 protons including three methyl protons [δ_H 1.31 (6H, overlap, H-4α and H-4β), 1.62 (3H, *s*, 7a)], one olefinic proton [δ_H 5.81 (1H, *s*, H-3)], and one hydroxyl proton [δ_H 4.12 (1H, *tt*, *J*=11.4, 4.2 Hz, α-H_{ax})]. From ^{13}C NMR, there were 11 carbon signals in metabolite **1** (Table 3) including three methyls (δ 23.9, δ 24.4, and δ 29.3), a hydroxyl group (δ 63.9), and a lactone carbonyl (δ 172.6). Comparison with literature confirming metabolite **1** as *epi*-loliolide [23].



1 **2**

Figure 3. Structures of *epi*-loliolide (**1**) and loliolide (**2**).

Table 2. The comparison of ^1H and ^{13}CNMR of metabolite **1** with the previous report [23].

Position	^1H NMR (*δ* ppm)		^{13}C NMR (*δ* ppm)	
	Observed (600 MHz, CD_3OD)	(Gangadhar et al., 2020) [23] (400.1 MHz, $CDCl_3$)	Observed (150 MHz, CD_3OD)	(Gangadhar et al., 2020) [23] (100.6 MHz, $CDCl_3$)
1	-	-	-	-
2	-	-	172.6	171.6
3	5.81, *s*, 1H	5.71, *s*, 1H	112.3	113.3
3a	-	-	182.5	180.8
4	-	-	34.8	35.0
5	1.32, *overlap*, 1H, β-H_{ax} 2.03, *dq*, 1H, *J*=12.6, 2.4 Hz, α-H_{eq}	1.33, *t*, 1H, *J*=12.8 Hz, β-H_{ax} 2.04, *brd*, 1H, *J*=12.8 Hz, α-H_{eq}	49.3	49.7
6	4.12, *tt*, 1H, *J*=11.4, 4.2 Hz, α-H_{ax}	4.13, *tt*, 1H, *J*=11.5, 4.1 Hz, α-H_{ax}	63.9	65.0
7	1.44, *t*, 1H, *J*=11.4 Hz, β-H_{ax} 2.50, *dq*, 1H, J=12.0, 2.4 Hz, α-H_{eq}	1.51, *t*, 1H, *J*=11.9 Hz, β-H_{ax} 2.54, *brd*, 1H, *J*=11.8 Hz, α-H_{eq}	47.4	47.3
7a	-	-	87.2	86.5
4α-CH_3	1.31, *overlap*, 6H	1.26, *s*, 3H	23.9	25.0
4β-CH_3		1.31, *s*, 3H	29.3	29.9
7a-CH_3	1.62, *s*, 3H	1.58, *s*, 3H	24.4	25.6

Table 3. The comparison of ^{1}H and ^{13}NMR of metabolite **2** with the previous report [23].

Position	^{1}H NMR (δ ppm)		^{13}C NMR (δ ppm)	
	Observed (600 MHz, CD_3OD)	(Gangadhar et al., 2020) [23] (400.1 MHz, $CDCl_3$)	Observed (150 MHz, CD_3OD)	(Gangadhar et al., 2020) [23] (100.6 MHz, $CDCl_3$)
1	-	-	-	-
2	-	-	173.1	171.5
3	5.77, *s*, 1H	5.69, *s*, 1H	111.9	112.9
3a	-	-	184.3	182.7
4	-	-	35.8	35.9
5	1.55, *dd*, 1H, *J*=14.4, 3.6 Hz, α-H_{ax} 2.02, *tt*, 1H, *J*=14.4, 2.4 Hz, β-H_{eq}	1.53, *dd*, 1H, *J*=14.5, 3.3 Hz, α-H_{ax} 1.97, *brd*, 1H, *J*=14.6 Hz, β-H_{eq}	46.6	47.3
6	4.24, *m*, 1H, α-H_{eq}	4.33, *m*, 1H, α-H_{eq}	65.8	66.8
7	1.76, *d*, 1H, *J*=3.6 Hz, α-H_{ax} 2.44, *tt*, 1H, *J*=13.8, 2.4 Hz, β-H_{eq}	1.79, *m*, 1H, α-H_{ax} 2.45, *brd*, 1H, *J*=14.1 Hz, β-H_{eq}	45.0	45.6
7a	-	-	87.6	86.7
4α-CH_3	1.30, *s*, 3H	1.27, *s*, 3H	29.6	30.6
4β-CH_3	1.49, *s*, 3H	1.46, *s*, 3H	25.6	26.5
7a-CH_3	1.78, *s*, 3H	1.78, *s*, 3H	26.0	27.0

Metabolite **2** was obtained as white amorphous powder, and produced a molecular ion peak at *m/z* 197.1172 $[M+H]^+$, consistent with the molecular formula $C_{11}H_{16}O_3$. The ^{1}H NMR (Table 4) spectrum exhibited 16 protons including three methyl protons [δ_H 1.30 (3H, *s*, H-4α), 1.49 (3H, *s*, H-4β)], 1.78 (3H, *s*, 7a)], one olefinic proton [δ_H 5.77 (1H, *s*, H-3)], and one hydroxyl proton [δ_H 4.24 (1H, *m*, α-H_{eq})]. From ^{13}C NMR, there were 11 carbon signals in metabolite **2** (Table 4) including three methyls (δ 25.6, 26.0, and 29.6), a hydroxyl group (δ 65.8), and a lactone carbonyl (δ 173.1). Metabolite **2** is identified as loliolide based on comparison with literature data [23].

Metabolite **1** has not been extensively found in the plant kingdom. It was reported from the ethyl acetate of aerial parts of *Eirmocephala megaphylla* of the Asteraceae family, ethanolic extract of aerial parts of *Excoecaria cochinchinensis* (Euphorbiaceae), and methanol extract of leaves of *Annona muricata* (Annonaceae) [24–26]. In addition, this metabolite has been found in algae such as *Tisochrysis lutea*, *Ulva lactuca*, and *Sargassum horneri* [23, 27, 28] as well as seaweeds of *Dictyota coriacea* and *Ascophyllum nodosum* [29,30]. It is interesting to note that this metabolite has also been discovered in sea sponges such as *Axinella sinoxea* [31].

Metabolite **1** showed antibabesial, anti-melanogenesis, anti-hepatocellular carcinoma (HepG2), and anti-inflammatory activities. This metabolite exhibited antibabesial activity against *Babesia gibsoni* with an IC_{50} value of 10.0 mg/mL [25]. It also displayed anti-melanogenesis with 22.6 % inhibition at 30 µg/mL [29]. Furthermore, this metabolite demonstrated anti-HepG2 by reducing cell viability and showed lower toxicity against a non-tumoral stromal cell line (S17) [23]. This metabolite showed an anti-inflammatory effect in human periodontal ligament cells stimulated with PG-LPS, attributed to the control of PKA/CREB signaling [28].

Metabolite **2** has been identified in the chloroform extract of leaves of *Arnica montana* as well as *Eirmocephala megaphylla* and *Artemisia princeps* (Asteraceae) [24,32,33], ethanol extract of leaves of *Mondia whitei* (Apocynaceae) [34], whole plant of acetone extract of *Phyllanthus urinaria* (Phyllanthaceae) [35], methanol extract of leaves of *Annona muricata* and *Duguetia pycnastera* (Annonaceae) [26, 36], as well as ethanol extract of aerial parts of *Vicia tenuifolia* (Fabaceae) [37]. In short, metabolite **2** has been found in several plant families such as Asteraceae, Apocynaceae, Phyllanthaceae, Annonaceae, and Fabaceae. Besides plants, this metabolite has been reported from algae such as *Cladostephus spongiosus* and *Sargassum horneri* [38, 39], soft coral such as *Briareum excavatum* [40], and seaweeds of *Codium tomentosum* and *Ascophyllum nodosum* [30, 41].

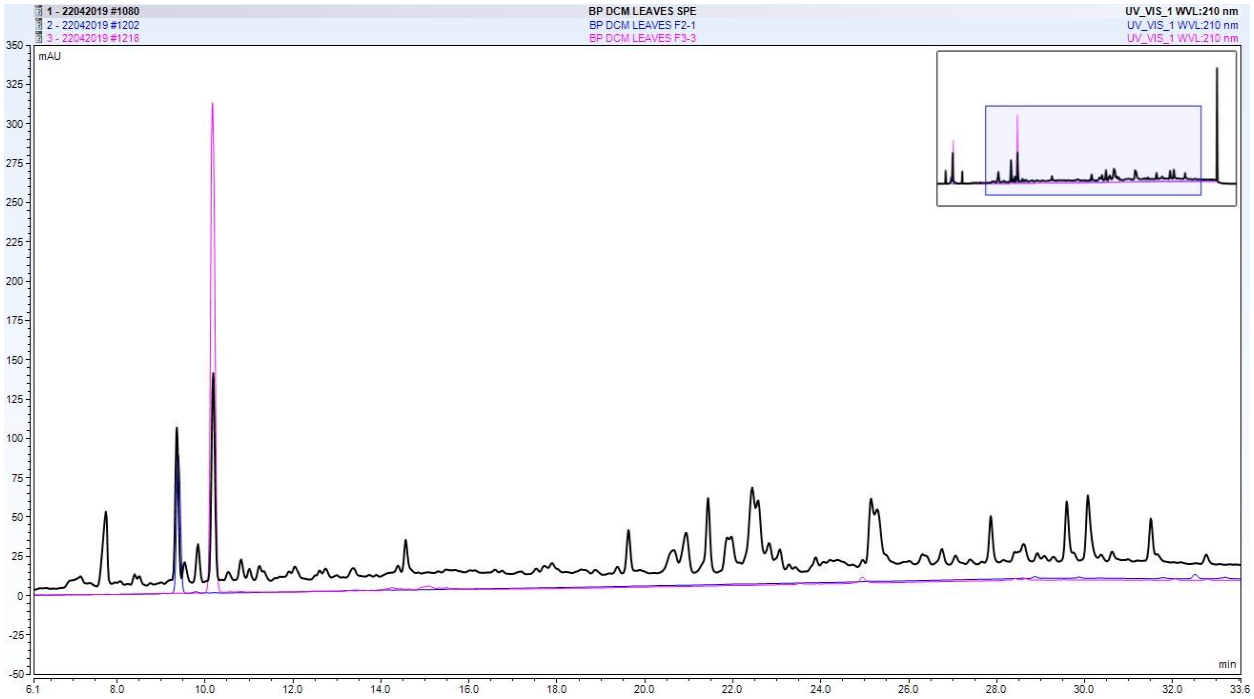


Figure 4. Comparison of UHPLC chromatogram of *epi*-loliolide and loliolide with the crude extract of *B. plicata* leaves

Several biological activities have been reported on this metabolite such as anti-viral, antiapoptosis, antiscratching, neuroprotective, cytotoxic, anti-adipogenic, and anti-inflammatory. Metabolite **2** inhibited HCVcc infection in a dose-dependent manner concentration with an EC_{50} of 2.48 ± 0.6 µM [35]. It also reduced the expression of caspases 3, 8, and 9, which are related to ROS-induced apoptosis, and showed an increase in cell migration and wound scratch closure in HaCaT cells at 100 µM [42]. Furthermore, the metabolite exhibited a neuroprotective effect in the presence of 6-OHDA neurotoxin, resulting in a 23.70 ± 7.77% increase in cell viability at 50 µM and 41.06 ± 6.31% at 100 µM [41]. It also displayed a cytotoxic effect against HCT-116 and MDA-MB231 cells using an MTT assay with viability of more than 95% when treated with 30 µM [37]. It was discovered that this metabolite inhibited the adipogenic differentiation of human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) via stimulating Wnt/β-catenin, which in turn led to AMPK-mediated inhibition of PPARγ activity [43]. Besides, it showed the ability to inhibit inflammation by nitric oxide suppression through the downregulation of iNOS and pro-inflammatory cytokines [39].

Figure 4 shows the comparison between the isolated metabolites **1** (blue colour) and **2** (pink colour) with the dichloromethane crude extract of *B. plicata* leaves (black colour). These two metabolites are the major metabolites and thus might be the marker metabolites contained in the leaves part.

CONCLUSION

This research successfully annotated 27 and 19 metabolites from the dichloromethane extract of *B. plicata* leaves and stems, respectively. The leaves comprised two alkaloids, two terpenoids, 20 fatty acids, two flavonoids, and one phenolic, while the stems comprised two alkaloids, 14 fatty acids, two terpenoids, and one phenolic. Two metabolites named *epi*-loliolide (**1**) and loliolide (**2**) were successfully isolated from *B. plicata* leaves, and thus identified and validated the dereplication strategy. To the best of our knowledge, this is the first report on the isolation of *epi*-loliolide in the Zingiberaceae family whereas loliolide in the genus *Boesenbergia*.

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REFERENCES

1. Gaudêncio, S. P., Bayram, E., Bilela, L. L., Cueto, M., Díaz-Marrero, A. R., Haznedaroglu, B. Z., Jimenez, C., Mandalakis, M., Pereira, F., Reyes, F. and Tasdemir, D. (2023) Advanced methods for natural products discovery: Bioactivity

screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools, and structure elucidation. *Marine Drugs*, **21(5)**, 308.

2. Taylor, T. N., Taylor, E. L. and Krings, M. (2009) Flowering plants. In *Paleobotany The Biology and Evolution of Fossil Plants, Academic Press*, 873–997.

3. Ly, T. N., Shimoyamada, M., Kato, K. and Yamauchi, R. (2003) Isolation and characterization of some antioxidative compounds from the rhizomes of smaller galanga (*Alpinia officinarum* Hance). *Journal of Agricultural and Food Chemistry*, **51(17)**, 4924–4929.

4. Zhou, B., Guo, M. -J., Zhao, X. -M., Li, X. -L., Liu, S. -H., Shen, X. -C. and Zhang, N. -L. (2024) Terpenoids from *Alpinia galanga* and their acetylcholinesterase inhibitory activity. *Natural Product Research*, 1–8.

5. Bian, M. -Q., Kang, J., Wang, H. -Q., Zhang, Q. -J., Liu, C. and Chen, R. -Y. (2014) Three new norsesquiterpenoids from the seeds of *Alpinia galanga*. *Journal of Asian Natural Products Research*, **16(5)**, 459–464.

6. Alolga, R. N., Wang, F., Zhang, X., Li, J., Tran, L. -S. P. and Yin, X. (2022) Bioactive compounds from the Zingiberaceae family with known antioxidant activities for possible therapeutic uses. *Antioxidants*, **11(7)**, 1281.

7. Ma, J., Jin, X., Yang, L. and Liu, Z. -L. (2004) Diarylheptanoids from the rhizomes of *Zingiber officinale*. *Phytochemistry*, **65(8)**, 1137–1143.

8. Jitoe, A., Masuda, T. and Nakatani, N. (1993) Phenylbutenoid dimers from the rhizomes of *Zingiber cassumunar*. *Phytochemistry*, **32(2)**, 357–363.

9. Gilli, C., Orlowska, E., Kaiser, D., Steyrer, J., Rathgeb, A., Lorbeer, E., Brecker, L. and Schinnerl, J. (2014) Diarylheptanoids, flavonoids and other constituents from two neotropical *Renealmia* species (Zingiberaceae). *Biochemical Systematics and Ecology*, **56**, 178–184.

10. Natta, L., Orapin, K., Krittika, N. and Pantip, B. (2008) Essential oil from five Zingiberaceae for anti food-borne bacteria. *International Food Research Journal*, **15(3)**, 337–346.

11. Flora of China (2000) *Boesenbergia* Kuntze. In *Flora of China*, 367–368.

12. Sirirugsa, P. (1992) A revision of the genus *Boesenbergia* Kuntze (Zingiberaceae) in Thailand. *Natural History Bulletin of the Siam Society*, **40**, 67–90.

13. Saensouk, S. and Larsen, K. (2001) *Boesenbergia baimaii*, a new species of Zingiberaceae from Thailand. *Nordic Journal of Botany*, **21(6)**, 595–598.

14. Aishwarya, K., Vinitha, M. R., Thomas, G. and Sabu, M. (2015) A new species of *Boesenbergia* and rediscovery of *B. rotunda* (Zingiberaceae) from India. *Phytotaxa*, **197(3)**, 186–196.

15. Jing, L. J., Mohamed, M., Rahmat, A. and Abu Bakar, M. F. (2010) Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda, Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*). *Journal of Medicinal Plants Research*, **4(1)**, 027–032.

16. Hiserodt, R. D., Franzblau, S. G. and Rosen, R. T. (1998) Isolation of 6-, 8-, and 10-gingerol from ginger rhizome by HPLC and preliminary evaluation of inhibition of *Mycobacterium avium* and *Mycobacterium tuberculosis*. *Journal of Agricultural and Food Chemistry*, **46(7)**, 2504–2508.

17. Jaipetch, T., Kanghae, S., Pancharoen, O., Patrick, V. A., Reutrakul, V., Tuntiwachwuttikul, P. and White, A. H. (1982) Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*): Isolation, crystal structure and synthesis of (±)-boesenbergin A. *Australian Journal of Chemistry*, **35(2)**, 351–361.

18. Ching, A. Y. L., Wah, T. S., Sukari, M. A., Lian, G. E. C., Rahmani, M. and Khalid, K. (2007) Characterization of flavonoid derivatives from *Boesenbergia rotunda* (L.). *The Malaysian Journal of Analytical Sciences*, **11(1)**, 154–159.

19. Taechowisan, T., Chanaphat, S., Ruensamran, W. and Phutdhawong, W. S. (2014) Antibacterial activity of new flavonoids from *Streptomyces* sp. BT01; an endophyte in *Boesenbergia rotunda* (L.) Mansf. *Journal of Applied Pharmaceutical Science*, **4(4)**, 8–13.

20. Bailly, C. (2022) Toward the use of *Boesenbergia rotunda* extracts and the chalcone panduratin A to treat periodontitis. *Journal of Oral Biosciences*, **64(2)**, 183–192.

21. Theanphong, O., Mingvanish, W. and Jenjittikul, T. (2022) Chemical composition and antioxidant, anti-tyrosinase and anti-inflammatory activities of essential oil from *Boesenbergia longiflora* (Wall.) Kuntze. *Agriculture and Natural Resources*, **56(2)**, 363–372.

22. Win, N. N., Kyaw, M. M., Prema, Ngwe, H., Ito, T., Asakawa, Y., Okamoto, Y., Tanaka, M., Abe,

I. and Morita, H. (2019) Dinorcassane diterpenoid from *Boesenbergia rotunda* rhizomes collected in lower Myanmar. *Chemistry and Biodiversity*, **16(4)**, e1800657.

23. Gangadhar, K. N., Rodrigues, M. J., Pereira, H., Gaspar, H., Malcata, F. X., Barreira, L. and Varela, J. (2020) Anti-hepatocellular carcinoma (HepG2) activities of monoterpene hydroxy lactones isolated from the marine microalga *Tisochrysis lutea*. *Marine Drugs*, **18(11)**, 567.

24. Borkosky, S., Valdés, D. A., Bardón, A., Díaz, J. G. and Herz, W. (1996) Sesquiterpene lactones and other constituents of *Eirmocephala megaphylla* and *Cyrtocymura cincta*. *Phytochemistry*, **42(6)**, 1637–1639.

25. Yamada, K., Subeki, Nabeta, K., Yamasaki, M., Katakura, K. and Matsuura, H. (2009) Isolation of antibabesial compounds from *Brucea javanica*, *Curcuma xanthorrhiza*, and *Excoecaria cochinchinensis*. *Bioscience, Biotechnology, and Biochemistry*, **73(3)**, 776–780.

26. Abdul Wahab, S. M., Jantan, I., Haque, M. A. and Arshad, L. (2018) Exploring the leaves of *Annona muricata* L. as a source of potential anti-inflammatory and anticancer agents. *Frontiers in Pharmacology*, **9**, 661.

27. Chung, Y., Jeong, S., Lee, I. -K., Yun, B. -S., Lee, J. S., Ro, S. and Park, J. K. (2021) Regulation of p53 activity by (+)-epiloliolide isolated from *Ulva lactuca*. *Marine Drugs*, **19(8)**, 450.

28. Kim, E. -N., Nabende, W. Y., Jeong, H., Hahn, D. and Jeong, G. -S. (2021) The marine-derived natural product epiloliolide isolated from *Sargassum horneri* regulates NLRP3 via PKA/CREB, promoting proliferation and anti-inflammatory effects of human periodontal ligament cells. *Marine Drugs*, **19(7)**, 388.

29. Ko, R. K., Kang, M. -C., Kim, S. S., Oh, T. H., Kim, G. -O., Hyun, C. -G., Hyun, J. W. and Lee, N. H. (2013) Anti-melanogenesis constituents from the seaweed *Dictyota coriacea*. *Natural Product Communications*, **8(4)**, 427–428.

30. Chi, H., Qi, X., Wang, X., Wang, Y., Han, X., Wang, J. and Wang, H. (2021) Preparative separation and purification of loliolide and epiloliolide from *Ascophyllum nodosum* using amine-based microporous organic polymer for solid phase extraction coupled with macroporous resin and prep-HPLC. *Analytical Methods*, **13(16)**, 1939–1944.

31. Kouchaksaraee, R. M., Farimani, M. M., Li, F., Nazemi, M. and Tasdemir, D. (2020) Integrating molecular networking and ^{1}H NMR spectroscopy for isolation of bioactive metabolites from the persian gulf sponge *Axinella sinoxea*. *Marine Drugs*, **18(7)**, 366.

32. Holub, M., Samek, Z. and Poplawski, J. (1975) Loliolide from *Arnica montana*. *Phytochemistry*, **14(7)**, 1659.

33. Kim, H. (2019) Isolation and structure determination of matrix metalloproteinase inhibitors and antioxidants from Ligustrum japonicum Fructus, Zostera asiatica and Artemisia princeps. *Ph.D. thesis, Korea Maritime and Ocean University, Busan, Korea*.

34. Neergaard, J. S., Rasmussen, H. B., Stafford, G. I., Van Staden, J. and Jäger, A. K. (2010) Serotonin transporter affinity of (-)-loliolide, a monoterpene lactone from *Mondia whitei*. *South African Journal of Botany*, **76(3)**, 593–596.

35. Chung, C. -Y., Liu, C. -H., Burnouf, T., Wang, G. -H., Chang, S. -P., Jassey, A., Tai, C. -J., Tai, C. -J., Huang, C. -J., Richardson, C. D., Yen, M. -H., Lin, C. -C. and Lin, L. -T. (2016) Activity-based and fraction-guided analysis of *Phyllanthus urinaria* identifies loliolide as a potent inhibitor of hepatitis C virus entry. *Antiviral Research*, **130**, 58–68.

36. Nardelli, V. B., Silva de Souza, C. A., da Silva Chaar, J., Ferreira Koolen, H. H., Araújo da Silva, F. M. and Costa, E. V. (2021) Isoquinoline-derived alkaloids and one terpene lactone from the leaves of *Duguetia pycnastera* (Annonaceae). *Biochemical Systematics and Ecology*, **94**, 104206.

37. Yang, M. H., Ha, I. J., Ahn, J., Kim, C. -K., Lee, M. and Ahn, K. S. (2023) Potential function of loliolide as a novel blocker of epithelial-mesenchymal transition in colorectal and breast cancer cells. *Cellular Signalling*, **105(1)**, 110610.

38. El Hattab, M., Culioli, G., Valls, R., Richou, M. and Piovetti, L. (2008) Apo-fucoxanthinoids and loliolide from the brown alga *Cladostephus spongiosus* f. *verticillatus* (Heterokonta, Sphacelariales). *Biochemical Systematics and Ecology*, **36(5–6)**, 447–451.

39. Jayawardena, T. U., Kim, H. -S., Asanka Sanjeewa, K. K., Han, E. J., Jee, Y., Ahn, G., Rho, J. -R. and Jeon, Y. -J. (2021) Loliolide, isolated from *Sargassum horneri*; abate LPS-induced inflammation via TLR mediated NF-κB, MAPK pathways in macrophages. *Algal Research*, **56**, 102297.

40. Sung, P. -J., Chen, B. -Y., Chen, Y. -H., Chiang, M. Y. and Lin, M. -R. (2010) Loliolide: Occurrence of a carotenoid metabolite in the

octocoral *Briareum excavatum* (Briareidae). *Biochemical Systematics and Ecology*, **38(1)**, 116–118.

41. Silva, J., Alves, C., Martins, A., Susano, P., Simões, M., Guedes, M., Rehfeldt, S., Pinteus, S., Gaspar, H., Rodrigues, A., Goettert, M. I., Alfonso, A. and Pedrosa, R. (2021) Loliolide, a new therapeutic option for neurological diseases? *In vitro* neuroprotective and anti-inflammatory activities of a monoterpenoid lactone isolated from *Codium tomentosum*. *International Journal of Molecular Sciences*, **22(4)**, 1888.

42. Park, S. H., Kim, D. S., Kim, S., Lorz, L. R., Choi, E., Lim, H. Y., Hossain, M. A., Jang, S., Choi, Y. I., Park, K. J., Yoon, K., Kim, J. -H. and Cho, J. Y. (2019) Loliolide presents antiapoptosis and antiscratching effects in human keratinocytes. *International Journal of Molecular Sciences*, **20(3)**, 651.

43. Oh, J. H., Karadeniz, F., Jang, M. -S., Kim, H., Seo, Y. and Kong, C. -S. (2021) Loliolide from *Artemisia princeps* suppresses adipogenesis in human bone marrow-derived mesenchymal stromal cells via activation of AMPK and Wnt/β-catenin pathways. *Applied Sciences*, **11(12)**, 5435.

APPENDIX A

The metabolites proposal in the dichloromethane extract of *B. plicata* leaves

No.	Retention Time (min)	Annotation Source	Name	Formula	Sirius Score	Zodiac Score	Similarity	MS^1	MS^2	Mass Error (ppm)
1	2.65	Biocyc, CHEBI, COCONUT, HMDB, HSDB, KEGG, KNApSAcK, MeSH, NORMAN, Natural Products, PubChem, PubMed, ZINC bio	Caprolactam	$C_6H_{11}NO$	100.000 %	100.000 %	96.21%	114.0913	97.0644, 96.0802, 95.0485, 86.0598, 81.0570, 79.0537, 77.0381, 72.0803, 70.0649, 69.0694, 68.0491, 67.0537, 56.0493, 55.0539, 54.0335, 53.0383, 46.0285, 44.0128, 43.0540, 42.0336, 41.0383	0.35
2	5.32	CHEBI, COCONUT, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural, ZINC bio	Isomers of loliolide	$C_{11}H_{16}O_3$	100.000 %	100.000 %	72.00%	197.1175	179.1063, 161.0957, 151.1115, 146.0721, 143.0851, 137.0957, 135.1164, 133.1007, 123.1163, 121.0643, 119.0850, 117.0694, 109.1007, 107.0850, 105.0694, 95.0486, 93.0694, 91.0538, 85.0643, 83.0489, 81.0693, 79.0538, 69.0695, 67.0539, 59.0489, 57.0696, 55.0540, 43.0177, 41.0384	1.43
3	5.96	CHEBI, COCONUT, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural, ZINC bio	Isomers of loliolide	$C_{11}H_{16}O_3$	100.000 %	100.000 %	72.00%	197.1175	179.1063, 161.0957, 151.1115, 146.0721, 143.0851, 137.0957, 135.1164, 133.1007, 123.1163, 121.0643, 119.0850, 117.0694, 109.1007, 107.0850, 105.0694, 95.0486, 93.0694, 91.0538, 85.0643, 83.0489, 81.0693, 79.0538, 69.0695, 67.0539, 59.0489, 57.0696, 55.0540, 43.0177, 41.0384	1.43
4	7.03	PubChem	2-[2-(Octylamino)ethoxy]ethanol	$C_{12}H_{27}NO_2$	100.000 %	100.000 %	80.00%	218.2116	200.2005, 106.0856, 102.0907, 88.0751, 71.0851, 70.0647, 62.0597, 58.0647, 57.0695, 45.0332, 44.0492, 43.0540, 42.0336	0.67
5	7.42	PubChem	PEG-3 caprylamine	$C_{14}H_{31}NO_3$	99.998%	100.000 %	82.98%	262.2377	244.2263, 200.2006, 102.0907, 80.0751, 71.0851, 70.0647, 57.0695, 45.0334	0.12

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6	9.56	CHEBI, COCONUT, HMDB, KEGG, PubChem, SuperNatural, ZINC bio	Traumatic acid	$C_{12}H_{20}O_4$	100.000 %	100.000 %	92.70%	229.1434	175.1107, 165.1275, 157.1007, 149.0954, 147.1162, 133.1008, 129.0698, 123.1161, 121.1005, 119.0848, 109.0641, 107.0853, 105.0693, 99.0801, 97.0641, 95.0849, 93.0694, 91.0537, 85.0648, 83.0851, 81.0693, 79.0538, 69.0696, 67.0538, 55.0539	0.15
7	10.13	PubChem	2-[Decyl(2-hydroxyethyl)amino]ethanol	$C_{14}H_{31}NO_2$	100.000 %	100.000 %	87.90%	246.2430	228.2327, 106.0856, 102.0908, 88.0751, 71.0852, 70.0647, 62.0598, 58.0649, 57.0695, 45.0332, 44.0492	1.00
8	11.50	PubChem	1-Cycopentyl-3-octylurea	$C_{14}H_{28}N_2O$	100.000 %	100.000 %	76.88%	241.2274	111.1167, 97.1006, 85.1007, 83.0849, 73.0391, 72.0803, 71.0853, 69.0694, 61.0392, 57.0695, 55.0538	0.16
9	12.45	COCONUT, KEGG Mine, Natural Products, PubChem, PubMed	8-[4-Oxo-5-(pent-2-en-1-yl)cyclopent-2-en-1-ylidene]octanoic acid	$C_{18}H_{26}O_3$	100.000 %	100.000 %	75.94%	291.1960	273.1842, 255.1741, 217.1584, 199.1476, 185.1324, 183.1162, 175.1111, 171.1159, 163.1115, 161.0955, 159.1163, 157.1005, 149.0954, 147.0798, 145.1006, 143.0849, 135.0798, 133.1004, 131.0849, 129.0693, 125.0952, 123.0800, 121.0641, 119.0848, 117.0692, 111.0793, 109.0641, 107.0848, 105.0693, 97.1005, 95.0849, 93.0693, 91.0536, 85.0641, 83.0849, 81.0693, 79.0537, 69.0694, 67.0538, 57.0331, 55.0538	1.82
10	12.71	NORMAN, PubChem, PubMed	Lauryldiethanolamine	$C_{16}H_{35}NO_2$	100.000 %	100.000 %	100.00%	274.2741	256.2631, 230.2475, 212.2372, 106.0856, 102.0906, 88.0750, 71.0850, 70.0646, 62.0596, 58.0647, 57.0694, 55.0538	0.16
11	12.81	PubChem	2-Aminotetradecan-1-ol	$C_{14}H_{31}NO$	100.000 %	100.000 %	75.00%	230.2479	212.2374, 85.1007, 71.0851, 62.0597, 58.0648, 57.0695, 55.0540, 45.0332, 44.0492, 43.0540	0.26

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12	12.90	PubChem	PEG-3 lauramine	$C_{18}H_{39}NO_3$	100.000 %	100.000 %	90.21%	318.3015	256.2631, 102.0907, 88.0751, 71.0850, 70.0646, 59.0486, 58.0650, 57.0695	3.88
13	13.00	PubChem	2-(Dodecylamino)-2-(hydroxymethyl)propane-1,3-diol	$C_{16}H_{35}NO_3$	100.000 %	100.000 %	87.50%	290.2695	272.2584, 242.2479, 122.0810, 118.0861, 104.0705, 88.0755, 86.0599, 85.1010, 83.0857, 81.0697, 74.0599, 71.0854, 60.0443, 58.0650, 57.0698, 56.0494	1.83
14	13.77	Zingiberaceae, Biocyc, CHEBI, COCONUT, HMDB, KEGG, KNApSAcK, MeSH, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, SuperNatural, YMDB Mine, ZINC bio	Campheride	$C_{16}H_{12}O_6$	99.957%	100.000 %	100.00%	301.0716	286.0473, 269.0438, 258.0515, 257.0435, 241.0513, 230.0569, 229.0493, 213.0543, 202.0622, 183.0288, 165.0175, 161.0591, 153.0177, 139.0381, 137.0224, 135.0433, 121.0643, 107.0482	3.12
15	15.20	NORMAN, PubChem	Tetradecyl diethanolamine	$C_{18}H_{39}NO_2$	100.000 %	100.000 %	99.17%	302.3062	284.2951, 106.0856, 102.0907, 88.0751, 85.1009, 71.0850, 70.0646, 57.0695	2.80
16	16.11	PubChem	Tetraethylene glycol, nonyl ether	$C_{17}H_{36}O_5$	99.941%	100.000 %	81.82%	321.2640	195.1222, 177.1116, 151.0958, 133.0853, 107.0700, 89.0591, 85.1006, 71.0850, 69.0696, 57.0695	1.40
17	16.81	Biocyc, CHEBI, COCONUT, KEGG Mine, MeSH, NORMAN, Natural Products, PubChem, PubMed, SuperNatural	Parinaric acid	$C_{18}H_{28}O_2$	100.000 %	100.000 %	86.71%	277.2166	235.1681, 219.1737, 199.1470, 175.1483, 171.1161, 163.1475, 161.1316, 159.1165, 157.1010, 151.1123, 149.1318, 147.1162, 145.1005, 143.0845, 135.1161, 133.1006, 131.0848, 129.0693, 123.1163, 121.1005, 119.0849, 117.0692, 109.1005, 107.0848, 105.0692, 97.1005, 95.0849, 93.0692, 91.0536, 85.0645, 83.0850, 81.0693, 79.0537, 77.0382, 71.0489, 69.0694, 67.0537, 57.0695, 55.0539	1.43
18	16.99	PubChem	(9Z,12Z)-Octadeca-9,12-dien-17-ynoic acid	$C_{18}H_{28}O_2$	100.000 %	100.000 %	89.29%	277.2168	219.1741, 203.1427, 175.1482, 171.1158, 163.1477, 161.1317,	2.15

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									159.1162, 157.1003, 149.1319, 147.1163, 145.1007, 143.0844, 135.1162, 133.1006, 131.0848, 129.0694, 123.1161, 121.1006, 119.0849, 117.0693, 109.1005, 107.0849, 105.0693, 97.1004, 95.0849, 93.0693, 91.0537, 85.0645, 83.0850, 81.0693, 79.0537, 77.0380, 71.0487, 69.0694, 67.0538, 57.0695, 55.0539	
19	17.24	MeSH, PubChem, PubMed	Tetraethyleneglycol monodecyl ether	$C_{18}H_{38}O_5$	99.963%	100.000 %	84.96%	335.2791	195.1230, 177.1123, 151.0964, 141.1638, 133.0856, 107.0698, 96.1166, 89.0593, 85.1007, 83.0851, 73.0646, 71.0851, 57.0695	0.30
20	17.52	CHEBI, COCONUT, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural, ZINC bio	Asperphenamate	$C_{32}H_{30}N_2O_4$	2.462%	3.723%	98.01%	507.2284	256.1327, 252.1013, 238.1221, 224.1065, 134.0958, 117.0692, 105.0332	1.12
21	17.65	Biocyc, COCONUT, KEGG Mine, KNApSAcK, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, SuperNatural, ZINC bio	Kaempferol 7,4’-dimethyl ether	$C_{17}H_{14}O_6$	99.929%	100.000 %	95.63%	315.0873	300.0636, 299.0545, 283.0611, 272.0679, 271.0605, 255.0646, 254.0591, 244.0730, 243.0653, 241.0486, 229.0493, 227.0703, 226.0627, 223.0391, 216.0787, 215.0705, 198.0658, 197.0436, 179.0341, 167.0340, 161.0595, 153.0546, 151.0392, 135.0439, 123.0436, 121.0647, 107.0492, 95.0490	3.14
22	18.23	Biocyc, CHEBI, COCONUT, HMDB, KEGG, KNApSAcK, MeSH, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, SuperNatural, YMDB Mine, ZINC bio	Linolenic acid	$C_{18}H_{30}O_2$	100.000 %	100.000 %	90.34%	279.2321	261.2209, 243.2100, 237.1847, 223.1685, 219.2105, 219.1738, 209.1533, 205.1585, 195.1376, 191.1429, 187.1477, 181.1221, 179.1423, 177.1633, 177.1269, 173.1321, 167.1062, 165.1268, 163.1474, 163.1109, 161.1321, 159.1164, 157.1475, 153.0905, 151.1475, 149.1319, 147.1162,	0.88

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									145.1004, 143.1161, 141.1318, 139.1161, 137.1318, 135.1161, 133.1004, 131.0845, 129.1005, 127.0848, 125.1004, 123.1161, 121.1004, 119.0855, 117.1005, 115.0848, 113.0692	
23	18.34	PubChem	1-*O*-ethyl 3-*O*-(2-ethylhexyl) propanedioate	$C_{13}H_{24}O_4$	99.999%	100.000 %	77.94%	245.1746	189.1121, 171.1012, 133.0486, 115.0382, 87.0434, 57.0694	0.55
24	19.57	CHEBI, COCONUT, HMDB, KEGG, MeSH, Natural Products, PubChem, PubMed, SuperNatural, ZINC bio	13-Oxo-9,11-octadecadienoic acid	$C_{18}H_{30}O_3$	100.000 %	100.000 %	95.50%	295.2274	277.2163, 259.2049, 249.2207, 241.1947, 231.2096, 221.1534, 217.1940, 207.1374, 199.1480, 193.1221, 189.1269, 179.1427, 177.1263, 175.1474, 171.1164, 165.1266, 163.1475, 161.1318, 157.1002, 153.0906, 151.1110, 149.1318, 147.1161, 145.1011, 137.1318, 135.1160, 133.1004, 123.1163, 121.1003, 119.0848, 117.0691, 111.0795, 109.1003, 107.0848, 105.0691, 97.1004, 95.0847, 95.0485, 93.0692, 91.0536, 83.0848, 81.0692, 79.0535, 77.0537	2.14
25	23.25	PubChem	Ethyl 2-[4-(dibutylamino)-2-hydroxybenzoyl]benzoate	$C_{24}H_{31}NO_4$	52.395%	70.588%	74.76%	398.2330	369.1937, 314.1393, 296.1283, 285.0991, 268.0959, 267.0884, 266.0798, 252.0656, 250.0857, 240.1013, 239.0942, 149.0226, 85.1006, 57.0695, 55.0539	1.05
26	23.90	Biocyc, CHEBI, COCONUT, EcoCyc Mine, HMDB, KEGG Mine, KNApSAcK, MeSH, NORMAN, Natural Products, PubChem, PubMed, SuperNatural, YMDB Mine	Hexadecanamide	$C_{16}H_{33}NO$	100.000 %	100.000 %	100.00%	256.2636	116.1063, 102.0906, 100.0751, 97.1008, 95.0850, 88.0751, 85.1007, 83.0850, 81.0697, 74.0595, 72.0440, 71.0850, 70.0646, 69.0694, 67.0538, 60.0804, 58.0647, 57.0695, 56.0492, 55.0538	0.43

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27	24.42	Biocyc, CHEBI, COCONUT, EcoCyc Mine, HMDB, HSDB, KEGG, Maconda, MeSH, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, SuperNatural, YMDB Mine, ZINC bio	Oleamide	$C_{18}H_{35}NO$	100.000 %	100.000 %	100.00%	282.2796	240.2678, 153.1268, 139.1113, 135.1162, 128.1064, 125.1316, 125.0953, 123.1166, 121.1006, 114.0908, 111.1163, 109.1005, 107.0849, 100.0750, 97.1006, 95.0849, 93.0693, 91.0908, 85.1007, 83.0850, 81.0694, 79.0538, 77.0867, 72.0802, 71.0851	1.63

APPENDIX B

The metabolites proposal in the dichloromethane extract of *B. plicata* stems

No.	Retention Time (min)	Annotation Source	Name	Formula	Sirius Score	Zodiac Score	Similarity	MS^1	MS^2	Mass Error (ppm)
1	2.64	Biocyc, CHEBI, COCONUT, HMDB, HSDB, KEGG, KNApSAcK, MeSH, NORMAN, Natural Products, PubChem, ZINC Bio	Caprolactam	$C_6H_{11}NO$	100.000 %	100.000 %	96.21%	114.0912	97.0641, 96.0801, 95.0458, 86.0595, 81.0568, 79.0537, 77.0383, 72.0803, 70.0648, 69.0694, 68.0490, 67.0537, 55.0539, 55.0175, 54.0335, 53.0383, 44.0492, 44.0129, 43.0540, 42.0336, 41.0383	1.24
2	7.04	Pubchem	2-(Nonylamino)propane-1,3-diol	$C_{12}H_{27}NO_2$	100.000 %	100.000 %	79.39%	218.2114	200.2013, 106.0854, 102.0908, 88.0751, 71.0850, 70.0646, 62.0598, 58.0649, 57.0695, 55.0538, 45.0332, 44.0492, 43.0540, 42.0335	0.25
3	10.12	Pubchem	2-[Decyl(2-hydroxyethyl)amino]ethanol	$C_{14}H_{31}NO_2$	100.000 %	100.000 %	95.90%	246.2427	228.2298, 106.0856, 102.0908, 88.0751, 85.1008, 71.0850, 70.0646, 68.0491, 58.0651, 57.0695, 45.0334, 44.0492	0.23
4	11.50	PubChem	1-Cyclopropyl-3-decylurea	$C_{14}H_{28}N_2O$	100.000 %	100.000 %	77.91%	241.2274	111.1160, 97.1006, 85.1007, 83.0849, 73.0391, 72.0803, 81.0851, 72.0803, 61.0392, 57.0695, 55.0538	0.16
5	11.92	PubChem	13-Aminotridec-8-enoic acid	$C_{13}H_{25}NO_2$	100.000 %	100.000 %	77.92%	228.1958	211.1698, 210.1848, 193.1589, 175.1482, 165.1627, 151.1480, 149.1332, 147.1163, 141.0907, 137.0959, 135.1166, 133.1010, 129.0902, 125.0955, 123.1163, 121.1007, 119.0852, 115.0751, 111.1164, 111.0800, 109.1008, 107.0851, 105.0698,	0.02

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								101.0592, 99.0796, 97.1008, 95.0851, 93.0695, 85.0646, 83.0851, 81.0694, 79.0539, 71.0489, 69.0695, 67.0539, 57.0330, 55.0539	
6	12.72	NORMAN, PubChem, PubMed	Lauryldiethanolamine	$C_{16}H_{35}NO_2$	100.000 %	100.000 %	99.17%	274.2740	256.2633, 230.2475, 212.2374, 106.0856, 102.0907, 88.0751, 86.0595, 74.0596, 72.0803, 71.0850, 70.0646, 69.0694, 68.0490, 62.0596, 60.0443, 58.0647, 57.0695, 56.0492, 55.0538	0.20
7	12.83	PubChem	14-Aminotetradecan-1-ol	$C_{14}H_{31}NO$	100.000 %	100.000 %	75.00%	230.2480	212.2375, 85.1007, 71.0851, 62.0596, 58.0648, 57.0695, 55.0540, 44.0492, 43.0540	0.70
8	12.90	PubChem	PEG-3 lauramine	$C_{18}H_{39}NO_3$	100.000 %	100.000 %	82.19%	318.3015	300.2914, 256.2633, 212.2371, 150.1126, 132.1013, 102.0907, 88.0751, 86.0960, 85.1006, 84.0804, 74.0596, 72.0801, 71.0850, 70.0646, 69.0697, 68.0492, 58.0647, 57.0695, 56.0491, 55.0540	3.88
9	13.47	PubChem	Brefeldin C	$C_{16}H_{24}O_3$	100.000 %	100.000 %	78.42%	265.1799	247.1699, 229.1598, 219.1749, 211.1484, 205.1593, 201.1642, 191.1072, 189.1641, 187.1484, 185.1323, 179.1069, 177.1640, 177.0912, 175.1486, 169.1017, 165.0912, 163.1486, 161.1325, 159.1168, 151.0753, 149.1323, 149.0960, 147.1167, 145.1010, 139.0751, 135.1169, 135.0803, 133.1011, 131.0853, 123.0801, 123.0438, 121.1008, 119.0852, 117.0696,	0.30

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									109.1008, 107.0851, 105.0695, 97.1008, 95.0851, 93.0695, 83.0852, 81.0695, 69.0695, 67.0452, 55.0540	
10	13.96	PubChem	2-[2-Hydroxyethyl(tridecyl)amino]ethanol	$C_{17}H_{37}NO_2$	100.000 %	100.000 %	99.17%	288.2902	270.2783, 244.2637, 106.0856, 102.0907, 88.0751, 72.0803, 71.0851, 70.0646, 69.0695, 68.0490, 62.0597, 60.0440, 58.0648, 57.0695, 55.0539	1.72
11	14.89	COCONUT, KNApSAcK, Natural Products, PubChem, SuperNatural, ZINC bio	Asperglaucide	$C_{27}H_{28}N_2O_4$	55.206%	56.839%	96.64%	445.2126	385.1891, 252.1014, 224.1066, 194.1172, 177.0908, 152.1071, 134.0959, 117.0692, 105.0329	0.94
12	16.04	PubChem	2-(Hexadecylamino)propane-1,3-diol	$C_{19}H_{41}NO_2$	100.000 %	100.000 %	74.63%	316.3220	157.1002, 145.1011, 131.0847, 119.0852, 107.0848, 106.0856, 105.0692, 95.0847, 93.0695, 91.0542, 88.0751, 81.0695, 79.0540, 71.0850, 70.0647, 69.0697, 57.0695, 55.0539	3.15
13	16.44	PubChem	2-[2-Hydroxyethyl(pentadecyl)amino]ethanol	$C_{19}H_{41}NO_2$	100.000 %	100.000 %	97.54%	316.3212	298.3107, 106.0856, 102.0908, 88.0751, 85.1008, 71.0851, 70.0646, 58.0646, 57.0695	0.62
14	16.94	NORMAN, PubChem	Heptadecylamine	$C_{17}H_{37}N$	100.000 %	100.000 %	81.11%	256.2997	85.1008, 71.0850, 60.0804, 58.0647, 57.0696, 45.0571	0.69
15	18.34	PubChem	1-*O*-ethyl 3-*O*-(2-ethylhexyl) propanedioate	$C_{13}H_{24}O_4$	99.999%	100.000 %	77.37%	245.1746	189.1124, 171.1006, 133.0489, 115.0382, 87.0434, 57.0695	0.55
16	19.57	PubChem	Octadeca-8,10-dien-12-ynoic acid	$C_{18}H_{28}O_2$	100.000 %	100.000 %	86.58%	277.2167	235.1682, 219.1733, 199.1479, 189.1275, 185.1318, 179.1064, 175.1473, 171.1159, 165.1272, 161.1321, 159.1169, 157.1012, 151.1111, 149.1320, 147.1162, 145.1005, 143.0851,	1.79

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								137.0593, 135.1161, 133.1006, 131.0851, 129.0691, 125.0957, 123.1161, 121.1005, 119.0848, 117.0689, 109.1005, 107.0848, 105.0693, 97.1007, 97.0640, 92.0849, 95.0485, 93.0693, 91.0537, 85.0643, 83.0851, 81.0693, 79.0537, 71.0853, 69.0694, 67.0538, 57.0694, 55.0539, 53.0383	
17	19.66	COCONUT, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural, ZINC Bio	Andrograpanin	$C_{20}30_8O_3$	100.000 %	100.000 %	90.63%	319.2282	219.1372, 217.1220, 215.1792, 205.1220, 203.1071, 193.1222, 189.1633, 187.1464, 179.1055, 175.1486, 171.1167, 161.1325, 159.1169, 153.0905, 149.1319, 147.1161, 145.1005, 137.1321, 135.1164, 133.1005, 131.0855, 125.0602, 123.1161, 121.1005, 119.0849, 113.0595, 109.1005, 107.0850, 105.0693, 99.0435, 95.0850, 93.0692, 91.0537, 83.0849, 81.0693, 79.0539, 69.0695, 67.0539, 57.0697, 55.0542	4.49
18	23.25	CHEBI, COCONUT, MeSH, NORMAN, PubChem, PubMed	Diethylamino hydroxybenzoyl hexyl benzoate	$C_{24}H_{31}NO_4$	93.774%	100.000 %	73.33%	398.2328	369.1938, 314.1390, 296.1282, 269.1044, 268.0961, 267.0882, 266.0799, 250.0861, 240.1012, 239.0940, 149.0226, 138.0904, 83.0849, 57.0695	0.54
19	24.41	Biocyc, CHEBI, COCONUT, EcoCyc Mine,	Oleamide	$C_{18}H_{35}NO$	100.000 %	100.000 %	100.00%	282.2798	177.1642, 149.1321, 139.1117, 135.1163, 128.1071, 125.0955,	2.34

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HMDB, HSDB, KEGG, Maconda, MeSH, NORMAN, Natural Products, Plantcyc, PubChem, PubMed, SuperNatural, YMDB Mine, ZINC bio	121.1007, 114.0907, 111.1161, 109.1007, 107.0849, 100.0751, 97.1007, 95.0850, 93.0694, 86.0594, 85.1007, 83.0850, 81.0694, 79.0537, 72.0807, 71.0851, 69.0695, 67.0539, 57.0695, 55.0539