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Phytochemical Identification of *Albertisia papuana* Becc. Leaf Methanolic Extract through Liquid Chromatography Tandem Mass Spectrometry Data Analysis

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The leaves of *Albertisia papuana* Becc. (Menispermaceae) is traditionally used by the people of Borneo as rice wine's flavor enhancer and utilized for the treatment of symptoms associated with hypertension, stroke, and cancer. Despite its potential, this plant's part remains largely unexplored from a scientific standpoint. Thus, this work aimed to profile and identify the phytochemicals of the methanolic leaf extract of *A. papuana* Becc. through liquid chromatography tandem mass spectrometry-based data analysis with MZmine and global natural products social molecular networking (GNPS) platforms. The present study managed to identify a total of 21 phytochemicals from different classes of compounds including alkaloids, flavonoids, terpenoid and several other phytochemicals. Of these, three compounds (nicotiflorin **11**, isorhoifolin **12**, and genistein **17**) are first time reported in the family meanwhile 18 compounds (5'-deoxy-5'-(methylsulfinyl) adenosine **1,** coclaurine **2**, magnoflorine **3** isoschaftoside **4**, reticuline **5,** isovitexin **6,** sinapic acid **7**, dicoumaroyl spermidine **8,** apigenin **9,** loliolide **10,** liriodenine **13**, moupinamide **14**, paprazine **15,** ferulic acid 1**6,** n-acetylanonaine **18**, 13*S*-Hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid **19**, 2-hydroxy-3-(2-hydroxyacetoxy) propyl palmitate **20**, and monoelaidin **21**) are new to the genus and the species. Some of the identified phytochemicals such as moupinamide **2**, apigenin **9** and magnoflorine **11** have been previously reported to exhibit biological activities related to hypertension, stroke, cancer treatment, and flavor enhancing properties of certain foods. The findings provide evidence to support the plant's traditional uses.

Keywords: Traditional use; LCMS; MZmine; GNPS; alkaloid; flavonoid

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Malaysia boasts a wealth of plant diversity that can be attributed to the nation's distinctive geographic location, equatorial climate, and diverse topography. Notably, the indigenous communities have relied extensively on plants for sustenance, medicinal applications, shelter, and cultural rituals [1, 2]. This has resulted in the transmission of knowledge and traditions related to plants across generations [3,4]. Malaysia is estimated to host around 15,000 species of flowering plants, among which is *Albertisia papuana* Becc., a plant from the family of Menispermaceae [5]. *A. papuana* is a liana type species that can grow to a height of 2-5 meters [6]. This species is native to various regions, including Borneo, Cambodia, Jawa, Malaya, Maluku, New Guinea, Sulawesi, Sumatera, and Thailand as it thrives in the wet tropical biome [7]. In Malaysian Borneo (Sabah), the plant goes by the local names "Pokok ajinomoto," "Tapa," "Tapa tahambia," and "Tapa bohuang". Meanwhile, in Indonesia, it is known as "Sengkubak" and "Bekai" [8].

A. papuana has been utilized for both culinary and medicinal purposes. Dusun ethnic in Sabah used the leaves of this plant as a bittering agent in the preparation of rice wine known as 'tapai'. Besides that, it is also widely used as a natural food flavour enhancer by the people of Kalimantan [6, 8-10]. Traditionally, the plant has been used for the treatment of symptoms related to hypertension, stroke, cancer and tumor [10-12]. The plant's leaves have been reported to contain umami components, contributing to its flavor-enhancing properties [12]. The leaf extract was reported to exhibit antiplasmodium and cytotoxic activities as well as a suppressor for withdrawal symptoms in morphine-addicted mice [13-14]. The root of the plant possesses cytotoxic activity against

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T47D cell lines [13]. The stems of the plant have been reported to contain bisbenzylisoquinoline alkaloid while the roots have been reported to contain volatile substances analyzed using GCMS [15, 16]. Although the leaves of the plant are traditionally more utilized, the phytochemical of this plant's part is less explored.

Over the last two decades, novel technologies and methods have emerged thus consequently enhancing the speed and precision of phytochemical analysis. The utilization of liquid chromatography coupled with mass spectrometry (LCMS) for structural identification has gained popularity due to its capacity for high-throughput analysis, and comprehensive coverage of phytochemicals [17]. LCMS stands out as an optimal method for examining plant chemicals due to its versatility, sensitivity, and capability to separate and detect a wide range of polarities of compounds, including significant secondary metabolite categories [18]. Tandem MS analysis assumes significance as it provides both precursor and fragment ion data, enabling the annotation, identification, and dereplication of phytochemicals by supplying detailed structural information [19]. The analysis can be facilitated with phytochemical annotation tools such as MZmine and Global Natural Products Social Molecular Networking (GNPS) [20].

In view of scarce chemical information on the leaves of *A. papuana*, the aim of this work was to identify the phytochemicals of the methanolic leaf extract of the title plant through tandem LCMS-based data analysis applying MZmine and GNPS platforms. This paper discussed in detail the analysis process.

EXPERIMENTAL

Chemicals and Materials

Analytical grade solvents (AR) of methanol (MeOH) and dichloromethane (DCM) were used for the extraction, cleanup process and solid-liquid extraction were supplied from Elite Advanced Materials Sdn Bhd (EAM), Malaysia. Sephadex® LH-20 was used for the sample treatment and purchased from Sigma-Aldrich, Sweden. Methanol (MeOH) and acetonitrile (MeCN) of LCMS grade were purchased from Merck, Germany. Ultra-pure water (UPW) was sourced from the arium® pro ultrapure water system by Sartorius, Malaysia.

Plant Materials

The leaves of *A. papuana* were collected in December 2021 from Kampung Donggiluang, Keningau, Sabah (Malaysia), situated at coordinates 5°31'02.8"N 116° 14'25.4"E. It was authenticated by a certified botanist named Mr. Johnny Gisil from Universiti Malaysia Sabah (UMS). The voucher specimen MF 0001 was lodged at the Herbarium of the Institute for Tropical Biology and Conservation (IBTP), UMS. Following collection, the plant materials were meticulously sorted to eliminate undesirable components, cleaned with laboratory wipes, sectioned into small fragments, subjected to oven-drying (40 ºC), and subsequently pulverized using an electrical grinder (IKA, Staufen, Germany).

Preparation of Extract

The plant powder (455 g) was macerated with ratio of 1:10 (powdered sample: MeOH) for 72 hours at room temperature. The extract was then filtered through Whatman No 1 filter paper, and concentrated under reduced pressure at 40°C using a rotary evaporator (R-125 BUCHI, Flawil, Switzerland) yielding 30 g of dark solid extract. The extract of was subjected to a cleanup process through size exclusion column chromatography packed with Sephadex LH-20 and isocratically eluted with MeOH:DCM (1:1).

The cleanup extract (6 g), was further subjected to trituration with two different solvents: DCM and MeOH. This process resulted in the formation of two trituration-derived extracts, namely methanol-triturated (A) and dichloromethane-triturated (B) extracts. Subsequently, these extracts were concentrated using a vacuum evaporator and yielded extracts of weights 4.6 g (A) and 1.1 g (B), respectively. Extract B was stored at 4 ℃, while extract A was subjected to LCMS/MS analysis.

LCMS/MS Analysis

The extract A (1.0 mg) was dissolved in a mixture of MeOH and UPW (50/50, v/v) to give a concentration of 1000 ppm. Then, it was filtered through 0.22 μm syringe filter and centrifuged at 13,000 rpm for 10 min to separate the supernatant from the residue. The supernatant was transferred into 1.5 mL vial and subjected to LCMS analysis.

The LCMS analysis was conducted on Thermo Scientific™ Vanquish™ Horizon UHPLC hyphenated with Orbitrap Fusion MS detector with electrospray ionization (ESI) in positive mode. Separation was accomplished on an Accucore TM Vanquish C18+ Dim. (2.1 × 100 mm, 80 Å, 1.5 μm particle size) column. Mobile phase A was UPW and mobile phase B was LCMS grade MeCN. A constant flow rate of 0.8 mL/min was used and the mobile phase gradient: min 0; 5% B, min 18; 95% B, min 23, 95% B, min 23.1; 5% B, min 30; 5% B was applied to achieve a good baseline separation. The column was then equilibrated with mobile phase A for 15 min before the next injection. The column oven was preheated to 35°C. Full scan mode was used to record all the masses in the range of 100–600 m/z with ≤ 2.0 ppm mass error [21]. In addition to the full scan, data-dependent MS/MS fragmentation was recorded for the 5 tallest peaks on each spectral scan with various collision energies. The spectrum was viewed on FreeStyle Software. The data obtained from the LCMS analysis was then processed using several available platforms which are MZmine and GNPS.

Data Acquisition, Processing, and Metabolite Identification

The spectral data were converted into mzML format using Proteo Wizard 3.0 and were then imported to MZmine software version 3.2.8 for data processing which includes mass detection, chromatogram building, chromatogram deconvolution, alignment and gap filling. Centroid detector threshold was used for the detection of mass ion peaks with the noise level set to 1.0×10^5 for MS level 1 while the noise level set to 0 for MS level 2. Then, ADAP Chromatogram Builder [22] was used for chromatogram building (min group size in # of scans = 5, group intensity threshold = 2.0×10^5, min highest intensity = 5.0×10^5 and scan to scan accuracy = 0.0020 m/z or 10 ppm). The chromatogram deconvolution used Local minimum feature resolver algorithm (chromatographic threshold: 85 %, search minimum in RT range: 0.05 min, minimum absolute height: 5 x 10^5, minimum ratio of peak top/edge: 2, peak duration range: 0-1 min and minimum # of data points: 5). The deconvolution peaks were subsequently subjected to deisotoping using 13C isotope filter (m/z tolerance: 0.001 m/z or 3.0 ppm, retention time tolerance: 0.02 absolute (min), monotonic shape, maximum charge: 2, and representative isotope: most intense and never remove feature with MS^2). Following that, the isotopic peaks finder was configured specifically for the element's hydrogen, carbon, nitrogen, oxygen, sulphur, sodium, chlorine, bromine and potassium. The parameter was set to m/z tolerance: 0.0001 m/z or 5.0 ppm, maximum charge of isotope m/z is 2 and single most intense for search in scans. The feature lists were aligned using the join aligner with m/z tolerance: 0.001 m/z or 5.0 ppm, weight for m/z: 3, retention time (RT) tolerance: 0.05 absolute (min), weight for RT: 1 and mobility weight: 1.

The processed data was exported to GNPS in two formats which are a table containing the intensities of LCMS ion features (CSV), and an MS/MS spectral summary file that contained a list of MS/MS spectra associated with the LCMS ion features (MGF). These files were then utilized as input for the Feature-based Molecular Networking (FBMN) tool, with the parameter set to its default value. Upon clicking "Submit," the FBMN job was initiated, and the outputs were examined on GNPS.

RESULTS AND DISCUSSION

Prior to the LCMS/MS analysis, the extract was subjected to a cleanup process through size exclusion column chromatography packed with Sephadex LH-20 and isocratically eluted with MeOH: DCM (1:1). The purpose of this practice is the removal of potential interference particularly the large-sized molecules, chlorophyll.

The current research utilized FBMN approach due to its successful record for identifying natural compounds from diverse origins [23 - 25]. As demonstrated in Figure 1, the analysis performed using this technique managed to identify 21 phytochemicals from the methanolic leaf extract of *A. papuana*. Furthermore, the confidence level of this identification aligns with Level 2, in accordance with the classification proposed by Schrimpe-Rutledge et al. [26]. The comprehensive characteristics of the identified phytochemicals are outlined in Table 1. This includes information such as their library class, cosine value, spectral and library m/z values, ionization method employed, instrumentation details, and ion source used. These phytochemicals are identified from the crude extract belonging to different classes of phytochemicals including, alkaloids, flavonoids, terpenoid, and several other phytochemicals.

Of the 21 identified phytochemicals, seven are belong to alkaloids class of compounds. These alkaloids include coclaurine **2**, magnoflorine **3**, reticuline **5**, dicoumaroyl spermidine **8**, liriodenine **13**, paprazine **15**, and N-acetylanonaine **18**. It's worth noting that all of these alkaloids represent novel discoveries not only within the species but also within the genus. Interestingly, except for dicoumaroyl spermidine **8**, and paprazine **15** all of the identified alkaloids in this study are related to isoquinoline-derived alkaloids. As mentioned earlier, the stem of the plant has been reported to contain bisbenzylisoquinoline alkaloid. The present findings underscore the abundance of isoquinoline-derived alkaloids in the leaves of the plant. Most of the identified alkaloids have been reported to exhibit various pharmacological activities. For instance, reticuline **5** which has been isolated from the leaves of *Pachygone ovata* (Menispermaceae) reported to possess hyperthermic, spinal convulsant actions and a central nervous system stimulant [27]. Magnoflorine **3** isolated from *Cissampelos pareira* (Menispermaceae) exhibits various biological activities including anti-diabetic, anti-inflammatory, neuropsycho-pharmacological, immunomodulatory, hypotensive, antioxidant, and antifungal effects [28,29]. Previous research has reported that the presence of bitter-taste compounds such as alkaloids could play a role in enhancing the flavor of certain foods [30]. The existence of the alkaloids in the leaf of the plant could play some role in the traditional uses of the plant's part mentioned earlier i.e., treatment of symptoms associated to hypertension, stroke, cancer, and flavor enhancer properties.

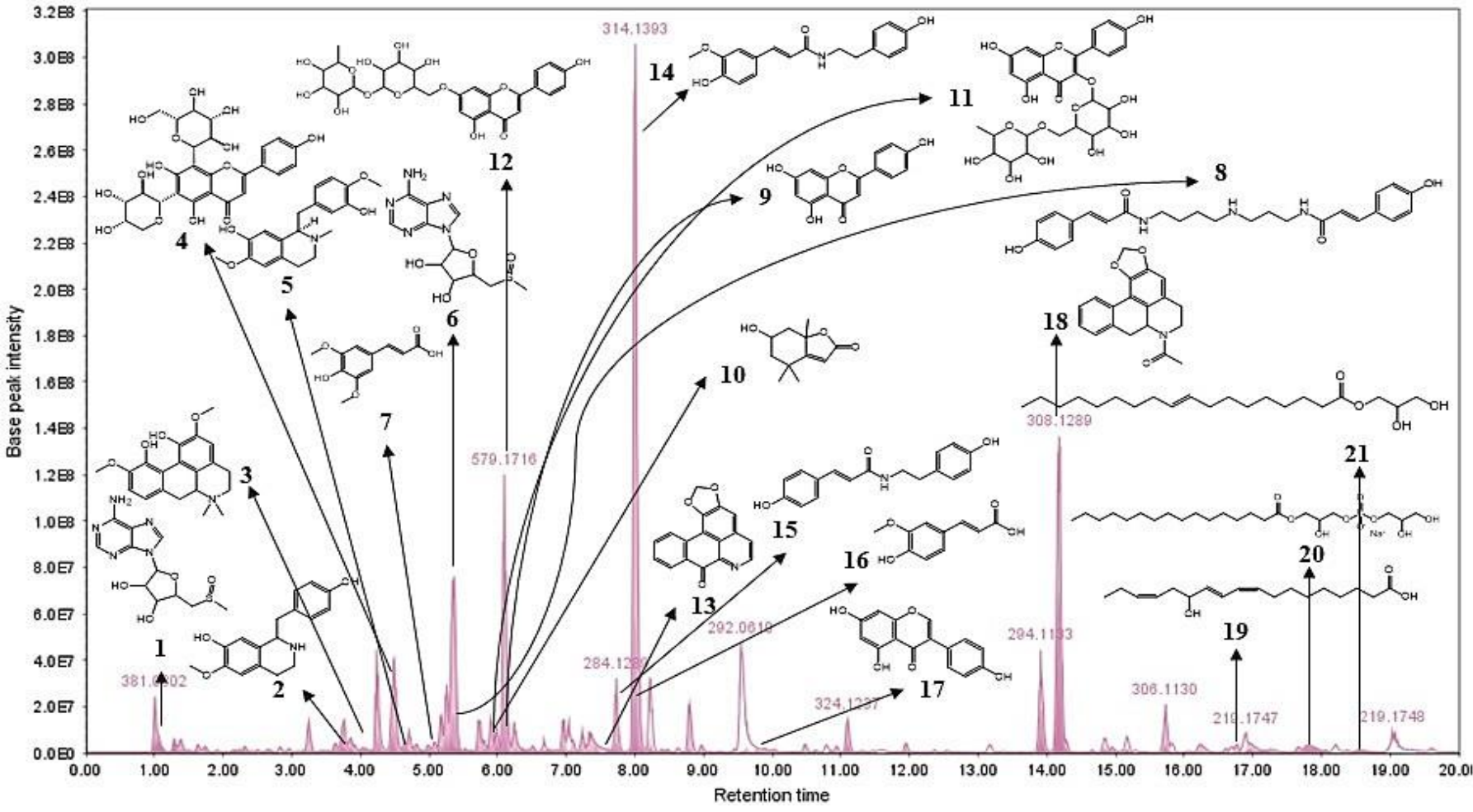

Figure 1. Identified phytochemicals from GNPS based on TIC.

Table 1. Library hits from Feature-Based Molecular Networking in GNPS.

*No.	Compound Name	Cluster Index	Library Class	Cosine	MZ Error or PPM	Spectral m/z / Library m/z	Instrument	Data Source	Ion Source
1	5'-Deoxy-5'-(methylsulfinyl) adenosine	50	Bronze	0.89	8	314.09/314.09	qTof	Claudia Maier	LC-ESI
2	Coclaurine	204	Gold	0.81	0	286.14/286.14	Orbitrap	Wolfender	ESI
3	Magnoflorine	251	Gold	0.90	8	343.17/343.17	Orbitrap	BMDMS-NP	ESI
4	Isoschaftoside	259	Bronze	0.71	0	565.15/565.15	qTof	Massbank	ESI
5	Reticuline	269	Bronze	0.87	2	330.17/330.17	qTof	KOOLEN/ANGOLINI	LC-ESI
6	Isovitexin	347	Bronze	0.90	6	433.11/433.11	qTof	Data from P Dorrestein	ESI
7	Sinapic Acid	358	Bronze	0.84	2	207.07/207.07	Orbitrap	Trent Northen	LC-ESI
8	Dicoumaroyl Spermidine	367	Bronze	0.75	2	438.24/438.24	qTof	Massbank	ESI
9	Apigenin	394	Bronze	0.91	2	271.06/271.06	LC-Q-TOF/MS	Putative ReSpect Match	ESI
10	Loliolide	407	Bronze	0.74	2	197.12/197.12	Orbitrap	Lihini Aluwihare	LC-ESI
11	Nicotiflorin	409	Bronze	0.95	0	595.17/595.17	ESI-QTOF	MoNA	N/A
12	Isorhoifolin	416	Bronze	0.97	1	579.17/579.17	Orbitrap	Trent Northen	LC-ESI
13	Liriodenine	556	Gold	0.99	14	276.07/276.07	Orbitrap	BMDMS-NP	ESI
14	Moupinamide	589	Bronze	0.98	2	314.14/314.14	Orbitrap	Pieter Dorrestein	LC-ESI
15	Paprazine	568	Gold	0.90	4	284.13/284.13	Orbitrap	BMDMS-NP	ESI
16	Ferulic acid	594	Bronze	0.94	1	177.06/177.06	Q-TOF	Data from Suryasarathi Dasgupta	ESI
17	Genistein	666	Bronze	0.95	1	271.06/271.06	Hybrid FT	Massbank	ESI
18	N-Acetylanonaine	786	Gold	0.91	6	308.13/308.13	qTof	Sang Hee SHIM Kyo Bin Kang	LC-ESI
19	13*S*-Hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid	865	Bronze	0.92	2	277.22/277.22	HCD	Data from Wolfender/Litaudon	ESI
20	2-Hydroxy-3-(2-hydroxyacetoxy) propyl palmitate	898	Bronze	0.87	0	485.09/485.29	qTof	O Laprevote	LC-ESI
21	Monoelaidin	914	Bronze	0.73	2	339.29/339.29	qTof	Data from Wolfender/Litaudon	ESI

*The compound numbering is based on the retention time in TIC.

Apart from the alkaloids, several flavonoids have also been identified from the leaf extract including isoschaftoside **4**, isovitexin **6**, apigenin **9**, nicotiflorin **11**, isorhoifolin **12**, and genistein **17**. The flavonoids nicotiflorin **11**, isorhoifolin **12**, and genistein **17** are newly identified within the family, adding to the significance of these findings. Apigenin **9** has been isolated from *Tinospora crispa* (Menispermaceae) [31]. This flavonoid has been reported to play a role in the improvement of cognitive performance in Alzheimer's disease, reduce demand for analgesic in knee osteoarthritis, and suppresses the in vivo growth of prostate cancer by targeting β-catenin and insulin-like growth factor-I-signaling pathways [32-34]. The traditional use of the plant's leaves in the treatment of symptoms related to hypertension could be attributed to the presence of these flavonoids.

Other phytochemicals that have been identified in the extract were sinapic acid **7**, moupinamide **14**, ferulic acid **16**, 13*S*-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid **19**, 2-hydroxy-3-(2-hydroxyacetoxy) propyl palmitate **20**, and monoelaidin **21**. These phytochemicals have been previously reported from the family Menispermaceae but new to the genus *Albertisia* and species *A. papuana*. For example, moupinamide **14**, which gave the most intense peak in the total ion chromatogram (TIC) of the extract has been isolated from the stem of *Tinospora cordifolia* (Menispermaceae) and exhibited antibacterial activity [35].

The structure confirmation of the identified phytochemicals was further supported through the library hits comparison where a mirror match between the experimental and library mass spectra was achieved. For instance, taking the isoquinoline-derived alkaloid **3** as an example its mirror spectral match displays a high similarity (gold level) to the experimental data (Figure 2) with a cosine value of 0.90. Furthermore, its diagnostic fragmentation (Figure 3) is consistent with the pattern proposed for **3** whereby its molecular ion peak initially produced a fragment ion at m/z of 298 ($C_{18}H_{17}O_4^+$) due to the neutral loss at m/z 45 $(CH_3)_2NH$ [36]. This occurred when the isoquinoline ring was opened and both the amino group and two methyl groups were eliminated. This characteristic fragmentation pattern is a key feature associated with aporphine alkaloids [37, 38]. The base peak for the fragment ions was generated at m/z 266 ($C_{17}H_{13}O_3^+$) and the loss of CO was obtained at m/z 238 ($C_{16}H_{15}O_2^+$). Taking into account the library hits, mirror spectral match, and diagnostic fragmentation, this particular phytochemical has been putatively annotated, and thus identified as magnoflorine.

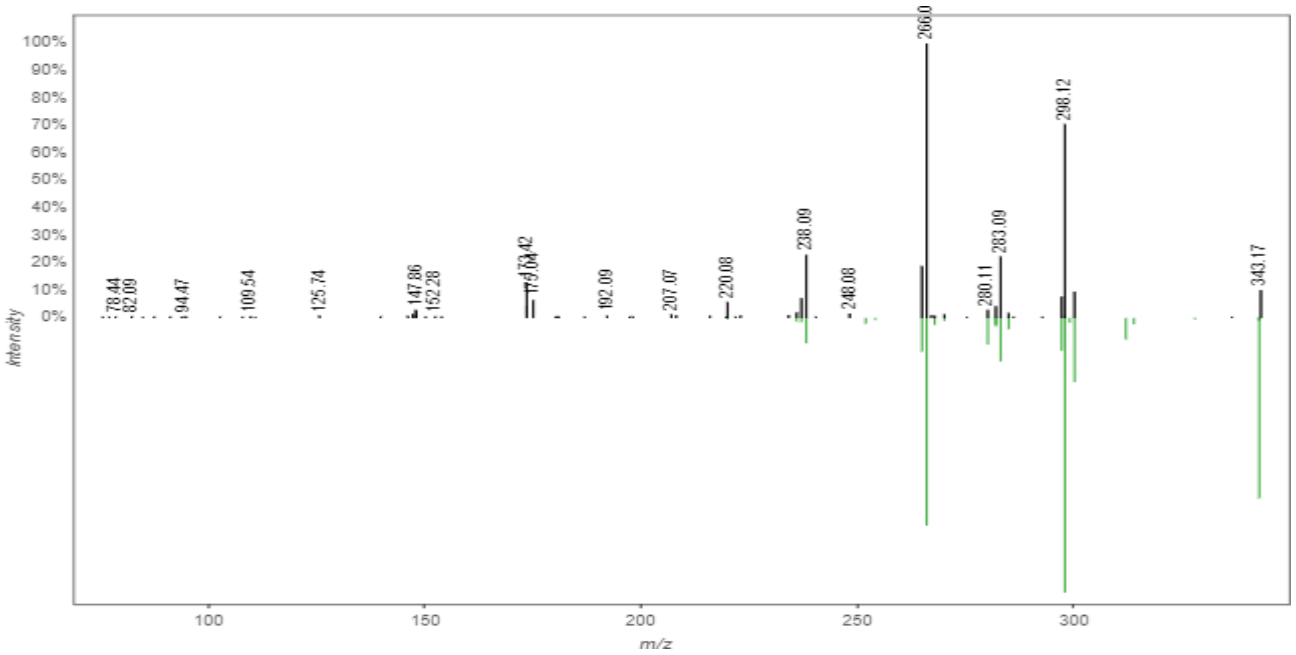

Figure 2. Mirror match of **3** with the reference spectra with gold library class and 0.9 cosine value.

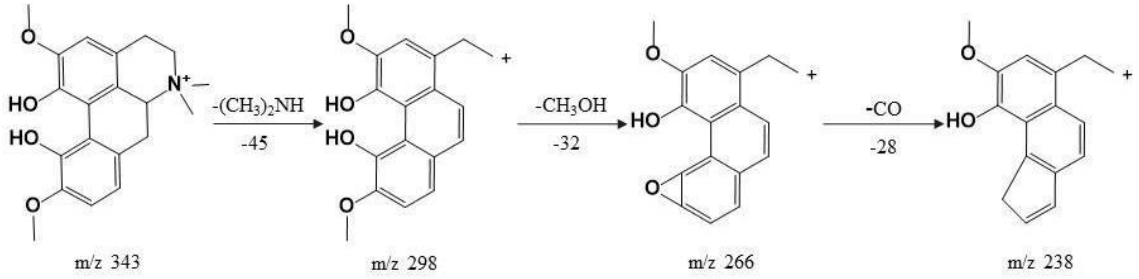

Figure 3. The diagnostic fragmentation analysis on phytochemical **3**.

CONCLUSION

A total of 21 phytochemicals were annotated in the leaves of *A. papuana* belonging to the classes of alkaloids, flavonoids, and terpenoids as well as several other phytochemicals. Some of the identified phytochemicals were found to correlate with the plant's traditional uses supported by their previously reported related biological activities. It is recommended that the isolation and characterization of the phytochemicals should be carried out to validate the findings of the present study as well as investigate their other biological importance.

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