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Metabolites Annotation of Dichloromethane Extract of *Kibatalia maingayi* Woods using Orbitrap High-Resolution Mass Spectrometry

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Kibatalia maingayi (Hook.f.) Woodson belongs to the Apocynaceae family and is known as "jelutong pipit". A preliminary study in this laboratory showed that the stem bark extract of *K. maingayi* inhibited the growth of *Plasmodium falciparum* with a high selectivity index, and the wood extract rich in alkaloids. To date, only one metabolite has been isolated from this plant. Further biological investigation on this plant is constrained by the lack of knowledge regarding its chemical components. Therefore, a dereplication approach was proposed to annotate and identify the known metabolites so that the isolation work can be directed toward new and/or potential known metabolites. The dichloromethane woods extract of *Kibatalia maingayi* was pre-treated with a C-18 solid-phase extraction and profiled using UHPLC, followed by LCMS analysis. Databases of metabolites from the Apocynaceae, genus *Kibatalia* and *Kibatalia maingayi* were constructed in Compound Discoverer 3.1 software. Nodes such as predicted compositions, mzCloud, ChemSpider, Mass Lists, mzLogic, and/or FISh scoring were applied to annotate metabolites. A total of 20 metabolites were detected in the woods part of *Kibatalia maingayi*, comprising 14 indole alkaloids and 6 fatty acids. Indole alkaloids such as penduflorines A to E from the Apocynaceae family were reported to possess antiplasmodial activity. As indole alkaloids are known to have good pharmacological potential, they will become the targeted metabolites for the next isolation and pharmacological studies.

Key words: *Plasmodium*; *Kibatalia maingayi*; dereplication; compound discoverer

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Malaria is one of the top three communicable diseases that affected billions of people, especially in the Sub-Saharan Region. According to World Health Organization, almost half of the world's population was at risk of malaria in 2021, making it one of the global health issues. There were about 247 million cases of malaria in 2021, with about 619,000 deaths reported, where most of the reported deaths were among young children [1]. *Plasmodium falciparum* has developed resistance towards the last defense drug used to treat malaria namely artemisinin, especially in Greater Mekong Subregion, urging the scientific community to search for new lead candidates [2]. With human mobility, it is feared that this strain will spread to neighboring regions. It is not long before a total resistance developed against artemisinin, thus it is important to search for new lead candidates for malaria treatment.

Natural products are sources of important drugs, in particular quinine and artemisinin. The plants have been used traditionally to treat malaria and fever for centuries. Many newer drugs today were synthetic derivatives of quinine and artemisinin. Natural product chemistry research has evolved significantly during the last few years, mainly due to the introduction of new technologies and the integration of established areas. The application of new technologies at each step in the investigation process for biologically-active natural products has resulted in more automated, high through-put, and comprehensive experimental conditions. In particular, the investigation involves the extraction of the plant material, the chemical and biological profiling of the plant extracts, the isolation and purification of natural products, and the structural elucidation of the pure isolated compounds.

The chemical profiling of plant extracts is an important step in the overall process and is significantly facilitated by the introduction of hyphenated techniques in the natural products field such as tandem mass spectrometry (MS/MS). This

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powerful technique has allowed the rapid profiling of complex plant extracts before any isolation step. Moreover, it is selective and highly sensitive compared to those other techniques [3]. Tandem MS/MS generates fragment ions that can be used further in the database searching to annotate and identify the metabolites in the plant extract. This rapid identification of known compounds in the extract refers to dereplication. The technique makes identification faster, and only targeted isolation is performed, saving time and greatly reducing the expensive consumption of solvent. The previous study showed that ultra-high-performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry provided fast screening of selected indole alkaloids such as harmine, harmaline, ajmalicine, and yohimbine [4].

Compound Discoverer is one of the licensed applications that use accurate mass data, isotope pattern matching, and mass library spectral searches for the structural annotation of small metabolites [5]. This qualitative data processing uses nodes such as predicted compositions, mzCloud, ChemSpider, Mass List, mzLogic, and/or FISh scoring to annotate metabolites. mzCloud uses automatic searches for online mass spectral databases using MS^n scan, ChemSpider uses ChemSpider database search for matching metabolites, mzLogic measures how similar a putative structure close to a matching structure in the database while Fragment Ion Search (FISh) scoring uses fragmentation scan to propose the structure. Recently, 414 metabolites have been annotated in nine mixed plant extracts using Compound Discoverer 3.1 software [6].

Apocynaceae, or dogbane family, consists of approximately 410 genera and over 5,000 species of tropical trees, shrubs woody climbers, and herbs [7]. Apocynaceae is a source of various classes of alkaloids, including indole alkaloids, aporphine alkaloids, mono-terpene alkaloids, pyrrolizidine alkaloids, steroidal alkaloids, tropane alkaloids, indolequinoline alkaloids, β-carboline alkaloids and many more [8]. Many important drugs were developed from various classes of alkaloids from the Apocynaceae family, such as vincristine, vinblastine, yohimbine, ajmaline, atropine, reserpine, ellipticine, ibogaine, and many more [9–15].

Kibatalia is a small genus in the Apocynaceae family, of about 15 species. *Kibatalia* can only be found in Southeast Asia, from Thailand to Indonesia and the Philippines to the east [16]. *Kibatalia* species are everlasting or occasionally enormous blooming plants that may grow up to 45 m or 65 m tall, have a straight trunk, no branches for approximately 30 m to 40 m lengths, have a circumference of up to 120 cm, and usually has short, massive, and broad roots with 1.5 m thickness [16]. Several classes of compounds were reported for *Kibatalia* sp. such as steroidal alkaloids, triterpenes, and coumarin [17,18].

Kibatalia maingayi (Hook.f.) Woodson (Apocynaceae) is commonly known as "jelutong pipit", "bintuas", "mentaos", and "pelai liling" [16,19]. This plant is a tree that can grow up to 40 m and can be found in lowland and lower montane forests [20]. It is widely distributed in Peninsular Malaysia, Sumatra, Borneo, the Philippines, Thailand, and Singapore [20,21]. The previous report on phytochemical screening on *K. maingayi* showed that the leaves have a low content of alkaloids and flavonoids, and moderate content of terpenes, while the bark showed a low content of flavonoids [22]. On contrary, preliminary phytochemical screening in this laboratory revealed that the wood extract of *K. maingayi* contained alkaloids. To the best of our knowledge, only one metabolite, known as maingayine, was isolated from this plant species. However, this paper was published under its synonym name, *Paravallaris maingayi* [23].

In continuation of our interest in new hit candidates of the alkaloidal moiety, *K. maingayi* (Apocynaceae) was evaluated for antiplasmodial activity. In a recent screening in this laboratory for antiplasmodial activity using *P. falciparum* K1 strain, the chloroform extract of the stem bark of *K. maingayi* showed antiplasmodial activity *in vitro* with an IC_{50} value of 1.1 µg/mL ± 1.0 µg with a selectivity index of 86.8. The wood extract showed weaker activity, however, the wood extract showed the presence of a higher abundance of alkaloids compared to stem bark. Thus, the wood extract of *K. maingayi* is selected for the annotation of metabolites using the dereplication approach in the present study. This approach will facilitate targeted isolation and characterization of new and/or potential known metabolites. This approach can save time, energy, and solvent consumption.

EXPERIMENTAL

Chemicals and Reagents

Analytical grade solvents (hexane, dichloromethane, and methanol) were used for the extraction of plant material while HPLC grade solvents (propan-2-ol, acetonitrile) were used for solid phase extraction method, UHPLC, and MS^n profiling. The solvents were purchased from RCI Labscan Limited, Bangkok, Thailand. Ultra-pure water was obtained from the arium® pro ultra-pure water system (Sartorius, Malaysia).

Collection of Plant Materials

The woods part of *K. maingayi* were collected from Bukit Nanas Forest Reserve, Kuala Lumpur, Malaysia. The plant was identified by Dr. Shamsul Khamis of Universiti Kebangsaan Malaysia.

Plant Extraction

The dried ground woods (468.27 g) of *K. maingayi* were macerated in hexane for one day, with 12 hours

of stirring and another 12 hours without stirring at room temperature. The filtrate was then filtered using Whatman filter paper number 1 into the Erlenmeyer flask. Next, the sample was successively macerated in dichloromethane and methanol, and the procedure was repeated three times, followed by filtration into their respective flasks. All filtrates were concentrated using a rotary evaporator at 40 °C to give hexane (1.0877 g), dichloromethane (0.7032 g), and methanol (23.1666 g) extracts. The crude extracts were stored in the refrigerator at -4 °C before analysis.

Sample Preparation

The crude extract (20 mg) was weighed and dissolved in acetonitrile (ACN) and propan-2-ol (50:50, v/v%), and filtered through a 0.45 µm PTFE filter. The sample (20 mg/mL) was pre-treated using a solid phase extraction method (TMstrata® C18-E from Phenomenex) before high-resolution mass spectrometry analysis. The filtrate was dried using a rotary evaporator at 40 °C. The sample stock solution was prepared by dissolving 5.3 mg in suitable solvents. Then, the stock solution was further diluted to a concentration of 250 ppm for MS^n profiling.

UHPLC Profiling

The analysis of a pre-treated wood extract of *K. maingayi* was carried out by using Thermo Scientific analytical ultra-high-performance liquid chromatography (UHPLC) system. This UHPLC is comprised of a Dionex UltiMate 3000 pump, a column compartment, an autosampler, a Diode Array Detector (DAD), and an automated fraction collector. Chromeleon 7 software was used for instrument control and data processing. The separation was carried out by using an analytical XBridge C18 (250 mm × 4.6 mm, 5 µm, Waters, Ireland). The injection volume was 10 µL, at the flow rate of 0.8 mL/min. The analysis was carried out using gradient elution, eluted with ultrapure water (solvent A) and ACN (solvent B). The gradient used was as follows: 0-30 min, from 10 to 100% B; 30-35 min, at 100% B; 35-37 min, from 100 to 10% B; 37-42 min, at 10% B. The profile was monitored at 200 nm.

UHPLC-Orbitrap Analysis

The dichloromethane woods extract of *K. maingayi* was analyzed using Thermo Scientific Vanquish UHPLC coupled to a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ Mass Spectrometer. FreeStyle™ software 1.6 was used for instrument control and data processing. The separation was carried out by using an analytical Accucore™ Vanquish™ C18 column (100 x 2.1 mm, 1.5 µm, Thermo Scientific, Lithuania). Positive ion mode was used throughout the analysis. The injection volume was 1 µL. The automated injection and a similar profiling gradient were used during the analysis. Both solvents A and B were added with 0.1% formic acid. The flow rate was maintained at 0.2 mL/min. The mass spectrometer was operated at 60,000 and 15,000 orbitrap resolutions in full and MS^2 scan modes, respectively. The ion source was electro-spray ionization (ESI). The spray voltage in positive mode was 3,500 V, an ion transfer tube temperature of 300 °C, sheath gas of 35 arb, auxiliary gas of 7 arb, vaporizer temperature of 275°C, and RF lens of 60%. The scan range was from *m/z* 150 to 1500. The orbitrap detector was used in full scan and MS^2 scan modes while the ion trap detector was used in MS^3 scan mode.

Database Export

A database called CHEMnetBASE: Dictionary of Natural Products (DNP), retrieved from an access Universiti Teknologi MARA library was used to import all metabolites obtained from the Apocynaceae family, genus *Kibatalia* and *Kibatalia maingayi*. The specific property was used to get the maximum total hit of metabolites. Type of organism property was used to search metabolites from the family of Apocynaceae. The specific code, Z.Q.04000, was selected, representing angiosperms (dicotyledons) from the Apocynaceae family. The chemical name, synonym, molecular formula, and accurate mass of those hits were exported to an excel file (.xls) formatted text and eventually saved as csv (comma delimited) file. The metabolite structures were saved in MDL molfile format. In contrast, all text and biological source properties were used for the genus *Kibatalia* and *Kibatalia maingayi*, respectively. Since *Kibatalia* is synonymous with *Paravallaris*, the later genus name was then included in the metabolite search.

Database Building

The excel files of Apocynaceae, *Kibatalia*, and *Kibatalia maingayi*, previously exported from DNP, were loaded into the Compound Discoverer software under the Mass List section. The structures of metabolites were manually added, one by one, using MDL molfile, to the excel file.

Dereplication Process

The dereplication strategy started by creating a new study and analysis in the Compound Discoverer 3.1 software. Next, the study name was created and the studies folder containing raw data and blank were selected. The workflow of "Natural Product\Natural Product Unknown ID w Online and Local Database Searches" was chosen. After that, the unprocessed MS^n raw data and its blank files in positive ion mode were added to the study and were assigned as sample and blank, respectively. The following settings were made: mass tolerance, 5 ppm; intensity tolerance, 30%; and ions selection, [2M+H]+1, [M+H]+1, [M+Na]+1, [M+K]+1. The minimum element count was set to carbon (C) and hydrogen (H), while the maximum element counts for C, H, oxygen (O), and nitrogen (N) were set to their default settings. Databases

of ChemBank, ChemSpiderman, DrugBank, MassBank, Molbank, Nature Chemistry, NIST, PubMed, Royal Society of Chemistry, and Web of Science were selected for the dereplication process as well as the three previously developed databases. The search formula was set to by formula or mass. Nodes such as predicted compositions, mzCloud, ChemSpider search, Mass Lists search, and/or mzLogic were used to annotate metabolites. The system was then run until fully completed. FISh scoring was then applied to all listed structure proposals.

RESULTS AND DISCUSSION

In this study, the chemical profiling of the crude extract was first developed using UHPLC before introduction into a high-resolution mass analyzer (HRMS), Orbitrap. HRMS was used to rapidly annotate the metabolite in the dichloromethane extract of *K. maingayi* woods. The annotation of metabolites is based on accurate mass and fragmentation ions that will be compared with the developed databases and standard *in-house* databases in the Compound Discoverer software. The analysis of the crude extract of *K. maingayi* revealed the presence of metabolites with molecular weights ranging from 278 to 444 g/mol.

The base peak chromatogram of the dichloromethane woods extract of *K. maingayi* was shown in Figure 1. There were 20 metabolites detected in this plant extract, comprising 14 alkaloids and 6 fatty acids. Alkaloid was the dominant compound found in the crude extract, showing 80% of the total metabolites (Figure 2). The alkaloids identified were of the indole alkaloid class. The other metabolites are from fatty acids which represent 20% of the total metabolites. They are shown by peak numbers 12, 16 to 20.

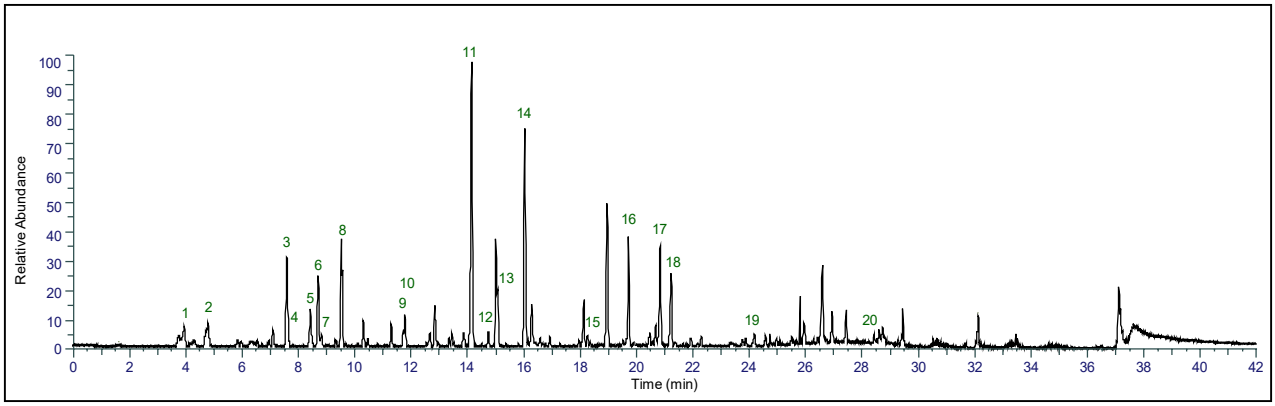

Figure 1. The base peak chromatogram of dichloromethane extract of woods part of *Kibatalia maingayi*

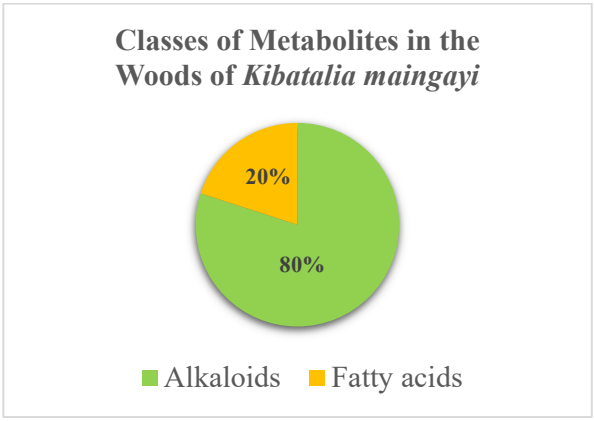

Figure 2. The pie chart of the classes of metabolites identified in the dichloromethane woods extract of *K. maingayi*

Table 1 showed the detailed structure proposal of each metabolite that was detected in the dichloromethane woods extract. The mass error was below 5 ppm. The metabolites were detected between the retention time of 3 to 29 minutes. Most of the metabolites hit the Mass List or Apocynaceae (family) database while the other metabolites did not match the family database but matched the mzCloud and/or ChemSpider search. The percentage of mzCloud best match was quite high which was more than 85%. This showed that the proposed metabolite matched the online mass spectral databases to that percentage. In contrast, FISh scoring matches metabolites to the libraries based on the fragmentation scan. The uses of fragmentation scan reduced from hundreds of structures proposal to a few numbers of metabolites. It should be noted that there is no acceptable score for FISh scoring. It depends solely on the fragmentation spectra. FISh scoring annotates the full spectrum tree, use general fragmentation rules, and uses the fragmentation libraries [5].

The priority of annotation source was given to the Mass List databases, followed by mzCloud and ChemSpider. Based on Table 1, coronaridine matched the Mass List and was supported by the mzCloud database, which gives a 95.8% best match score. Figure 3 displays the coronaridine mzCloud result, and the green color denotes a match between the sample (top) and the database (bottom). The MS/MS fragmentation pattern of coronaridine was shown in Figure 4. The loss of the methoxy group and its side chain was observed. All fatty acids except brassidic acid matched mzCloud and were supported by the ChemSpider database, which might confirm the annotation. Many MS^2 fragmentation scans matched the online mass spectral database (Table 1). Only one [2M+H]+1 ion appeared, corresponding to the major peak of peak number 11. This might be observed due to the higher concentration of the sample. Their MS^3 was labeled as an asterisk (Table 1).

There are five confidence levels of metabolite identification that were proposed by Schrimpe-Rutledge [24]. Level 1 is the highest confidence level where it identifies metabolite using a minimum of two properties such as tandem MS/MS, LC, or NMR of reference standard under the same experimental conditions, level 2 is the putative identification that uses fragmentation data match to MS/MS spectrum libraries, without standard, level 3 is the tentative structure that uses MS^1 database match, level 4 is the molecular formula candidates that use isotope abundance distribution, charge state, and adduct ion determination while level 5 is the unique feature that uses mass measurement accuracy, ±ppm. All metabolites are putatively identified (Level 2). Compound Discoverer 3.1 software gives preliminary ideas on what metabolites might be present in the crude extract.

Several subclasses of alkaloids were identified in the present study, including indole alkaloids, namely 18-hydroxyyohimbine, sitsirikine, 16-episitsirikine, 16,17-dihydrosecodin-17-ol, 17-*O*-acetyl-19,20-dihydrovoachalotine, coronaridine, 11-hydroxyhedranthine, eleganine A, suaveolenine, henrycinol A, and 17-acetylsarpagine, and monoterpenoid indole alkaloid, namely ervatamine, and 20-epiervatamine. The indole alkaloids annotated in the present study are widely studied for various biological activities, including antiparasitic activities. Indole alkaloids, such as yohimbine showed antiplasmodial activity [25] while monoterpene indole alkaloid, ervatamine, was reported to inhibit *Leishmania amazonensis* [26].

Indole alkaloids from the Apocynaceae family have been previously shown to inhibit *Plasmodium* spp. A mixture of penduflorines A and B exhibited higher antiplasmodial activity against the two strains of *P. falciparum* (3D7 and Dd2) compared to penduflorines C to E [27]. The other indole alkaloids such as alstonisine [28], 20-*epi*-dasycarpidone [29], 16-demethoxycarbonyl voacamine [30], and strychnobaillonine [31] also exhibited antiplasmodial activity.

18-Hydroxyyohimbine was identified in the methanol extract of the root bark of *Rauvolfia mannii* (synonym: *R. cumminsii*) [32], the root bark of *R. nitida* [33], and roots of *R. mombasiana* [34]. Ervatamine was reported in the ethanol extract of leaves and stem bark of *Tabernaemontana pandacaqui* (synonym: *Ervatamia orientalis*) [35], and stem and leaves of ethanolic extract of *T. divaricata* (synonym: *E. divaricata*) [36] while 20-epiervatamine was isolated in ethanol extract of stem bark of *T. pandacaqui* [35] and stem and leaves of ethanolic extract of *T. divaricata* [36].

Sitsirikine was found in *Vinca rosea* [37] and ethyl acetate extract of leaves, stem bark, and root bark of *Strychnos pungens* of Loganiaceae family [38] while 16-episitsirikine was reported in the seeds of *Aspidosperma album* [39]. 16,17-Dihydrosecodin-17-ol has been identified in the methanolic extract of roots of *Rhazya orientalis* [40]. 17-*O*-Acetyl-19,20-dihydrovoachalotine has been identified in the root bark of *Voacanga chalotiana* [41].

Coronaridine has been identified in the ethanolic extract of root bark [42] and the benzene extract of the stem of *T. divaricata* [43], methanol extract of the whole plant of *T. divaricata* (synonym: *E. coronaria*) [44], benzene extract of the root of *T. oppositifolia* [43], methanol extract of wood and stem bark of *T. alternifolia* (synonym: *E. heyneana*) [45], dichloromethane extract of the root of *T. ternifolia* [46], ethanolic extract of leaves and twigs of *T. pandacaqui* (synonym: *E. pandacaqui*) [47], petroleum ether of fruits of *Tabernanthe iboga* (synonym: *Tabernanthe pubescens*) [48], dichloromethane extract of root bark of *T. hystrix* [49], and ethyl acetate extract of stem bark of *T. buchtieni* (synonym: *Peschiera buchtieni*) [50]. Coronaridine showed cytotoxic activity against P-388 lymphocytic leukemia (ED_{50} = 0.43 µg/mL)

[45], and weak antituberculosis activity against *Mycobacterium tuberculosis* (MIC = 82.64 µg/mL) [46].

11-Hydroxyhedranthine has been identified in the methanolic extract of leaves of *Callichilia barteri* (synonym: *Hedranthera barteri*) [51]. Eleganine A has been identified in the methanol extract of leaves of *T. elegans* and showed apoptosis induction activity in human hepatoma HuH-7 cells [52]. Suaveolenine has been identified in the ethanol extract of the trunk of *Melodinus cochinchinensis* (synonym: *M. suaveolens*) [53]. There was a report on the isomers of tetraphylline pseudoindoxyl, ajmalicine pseudoindoxyl [54] while tetrahydroalstonine pseudoindoxyl has been identified in ethanol extract of leaves and stem bark of *Kopsia pauciflora* [55]. Demethylcorynantheine has been identified in the stem bark of *Rauvolfia mombasiana* [56].

Henrycinol A has been identified in the ethanolic extract of roots of *Melodinus cochinchinensis* [57]. 17-Acetylsarpagine has been identified in the roots of *Alstonia yunnanensis* [58]. α-Eleostearic acid has been identified in the seed oil of *Momordica charantia* of Cucurbitaceae [59] and seed oil of *Centranthus macrosiphon* of Caprifoliaceae [60]. 13(S)-HOTrE has been found in *Pseudo-nitzschia multistriata* [61].

9-Oxo-10(E),12(E)-octadecadienoic acid has been identified in the fruiting bodies of ethyl acetate extract of *Gomphus floccosus* of Gomphaceae [62]. The compound showed weak antifungal activity against *Phomopsis obscurans* and *Plasmopara viticola* (IC_{50} = 26 µM and 65 µM, respectively) compared to standard captan (IC_{50} = 2 µM and <1 µM, respectively) [62]. Palmitoyl ethanolamide and brassidic acid have been found in *Brassica napus* and *Monascus purpureus*, respectively [63,64]. Erucic acid has been identified in *Physalia physalis* [65].

It is interesting to note that many indole alkaloids annonated in the present study were also reported in various alkaloid-rich plants in the subfamily Rauvolfioideae, and tribes Vincae, Tabernaemontaneae, and Melodineae. Meanwhile, *Kibatalia* genus is categorized under the subfamily Apocynoideae and tribe Malouetieae [66]. Isolation and characterization of a known compound for validation of the present study shall be warranted to further elucidate the relationship between the two subfamilies in the Apocynaceae family.

CONCLUSION

A total of 20 metabolites were detected in the woods part of *Kibatalia maingayi*. They were comprised of 14 alkaloids and 6 fatty acids. These alkaloids were of the indole alkaloids class. Indole alkaloids like penduflorines A to E are known to have good pharmacological potential such as antiplasmodial activity. Therefore, it is recommended that the isolation and characterization of the indole alkaloids type of metabolite should be carried out to validate the dereplication of the *K. maingayi* extract as well as investigate their biological importance, especially malaria.

Table 1. The metabolite proposal in the dichloromethane extract of woods part of *Kibatalia maingayi* using Compound Discoverer 3.1

No.	Retention Time (min)	Annotation Source	Name	Formula	Molecular Weight	FISh Coverage	Mass error	MS^1	MS^2/MS^{3*}	mzCloud Best Match/mzLogic Score*
1	3.976	Mass List	18-Hydroxyyohim-bine	$C_{21}H_{26}N_2O_4$	370.18950	29.73	0.66	371.19705	293.16476, 275.15466, 171.09087, 169.07544, 117.06931	N/A
2	4.771	Mass List	Isomers of ervatamine	$C_{21}H_{26}N_2O_3$	354.19560	25.00	3.56	355.20325	295.17932, 225.10197, 199.08582, 160.07494, 92.04902, 67.05390	N/A
3	7.614	Mass List	Isomers of sitsirikine	$C_{21}H_{26}N_2O_3$	354.19560	20.53	3.56	355.20020	337.19052, 305.16434, 281.12808, 277.16983, 263.15338, 249.13832, 234.12727, 223.12270, 209.10635, 182.09622, 180.08003, 170.09566, 169.07536, 142.06470	N/A
4	7.689	Mass List	16,17-Dihydrosecodin-17-ol	$C_{21}H_{28}N_2O_3$	356.21044	64.29	1.24	357.21722	340.19193, 325.19031, 307.18088, 297.19675, 171.09099, 144.08012	N/A
5	8.452	Mass List	17-*O*-Acetyl-19,20-dihydrovoachal-otine	$C_{24}H_{30}N_2O_4$	410.22130	11.40	1.81	411.22855	393.21808, 353.18484, 351.20590, 339.16876, 335.17444, 321.15884, 293.16391, 277.17032	N/A
6	8.725	Mass List and mzCloud	Coronaridine	$C_{21}H_{26}N_2O_2$	338.20094	11.03	4.49	339.20810	309.15924, 307.18063, 279.18527, 223.12320, 171.09126, 159.09093	95.8

7	8.846	MassList	11-Hydroxyhedrant-hine	$C_{21}H_{24}N_2O_5$	384.16887	28.57	0.91	385.17572	367.16373, 353.14862, 339.16916, 335.13840, 325.15326, 206.08026	N/A
8	9.558	Mass List	Eleganine A	$C_{21}H_{24}N_2O_4$	368.17436	56.16	2.06	369.18085	351.16858, 337.15356, 309.15845, 146.05966, 138.09088	N/A
9	11.744	Mass List	Suaveolenine	$C_{21}H_{22}N_2O_4$	366.15865	34.21	1.89	367.16614	339.17169, 322.14502, 307.14517, 289.13434, 279.14984, 265.13373, 214.08705, 202.08536, 130.06482	N/A
10	11.801	Mass List	Isomers of tetraphylline pseudoindoxyl	$C_{21}H_{24}N_2O_4$	368.17436	47.37	2.06	369.18127	351.16953, 341.18536, 323.17496, 309.15918, 291.14969, 281.16406, 224.12704, 130.06458	N/A
11	14.169	Mass List	Demethylcoryn-antheine	$C_{21}H_{24}N_2O_3$	352.17864	81.25	0.14	705.36505 [2M+H]+1	353.18469 / 335.05, 324.12, 322.28, 321.09, 294.08, 293.07, 291.75, 277.02, 267.13, 250.00, 223.21, 196.43*	N/A
12	14.761	mzCloud and ChemSpider	8-{3-Oxo-2-[(2E)-2-penten-1-yl]-1-cyclopenten-1-yl}octanoic acid	$C_{18}H_{28}O_3$	292.20424	41.86	1.36	293.21161	275.19989, 257.18900, 247.20491, 239.17874, 229.19489, 223.13193, 205.12134, 191.14261, 177.12735, 173.13176, 165.12672, 159.11641, 85.06445	85.6
13	15.094	Mass List	19,20-Didehydro-6-hydroxyervatam-ine	$C_{21}H_{24}N_2O_4$	368.17436	30.56	2.06	369.18106	351.16861, 341.18555, 337.15405, 323.17511, 309.15955, 281.16452, 172.07500	N/A
14	16.056	Mass List	Henrycinol A	$C_{27}H_{28}N_2O_4$	444.20529	23.53	0.85	445.21313	385.19049	N/A, 26.7*
15	18.281	Mass List	17-Acetylsarpagine	$C_{21}H_{24}N_2O_3$	352.17919	35.00	1.40	353.18643	321.15878, 293.16385, 264.13708	N/A
16	19.733	mzCloud and ChemSpider	α-Eleostearic acid	$C_{18}H_{30}O_2$	278.22495	49.30	1.33	279.23245	261.22150, 243.21104, 237.18483, 223.16917, 219.21060, 209.15364, 205.15860, 201.16469, 195.13771, 191.14282, 187.14771, 181.12178, 179.14241, 177.12712, 173.13205, 167.10622, 165.16335, 165.12749, 163.14758, 163.11121, 161.13184, 151.14751, 149.09535, 147.11613, 141.09062, 135.08012, 127.07526, 105.06956, 95.04873	97.7
17	20.830	mzCloud and ChemSpider	13(S)-HOTrE	$C_{18}H_{30}O_3$	294.22017	43.55	2.28	295.22742	277.21561, 111.07989	93.4
18	21.209	mzCloud and ChemSpider	9-Oxo-10(E),12(E)-octadecadienoic acid	$C_{18}H_{30}O_3$	294.22017	41.94	2.28	295.22729	277.21567, 249.22101, 175.14771	97.9
19	24.163	mzCloud and ChemSpider	Palmitoyl ethanolamide	$C_{18}H_{37}NO_2$	299.28285	100.00	1.42	300.28983	283.26358, 282.27875, 239.23772, 109.10095	98.6
20	28.420	ChemSpider	Brassidic acid or erucic acid	$C_{22}H_{42}O_2$	338.31902	22.86	1.59	339.32645	321.31448, 303.30399, 219.21057, 195.17337, 163.14734, 143.10690, 121.10061	N/A

Note: N/A, not applicable

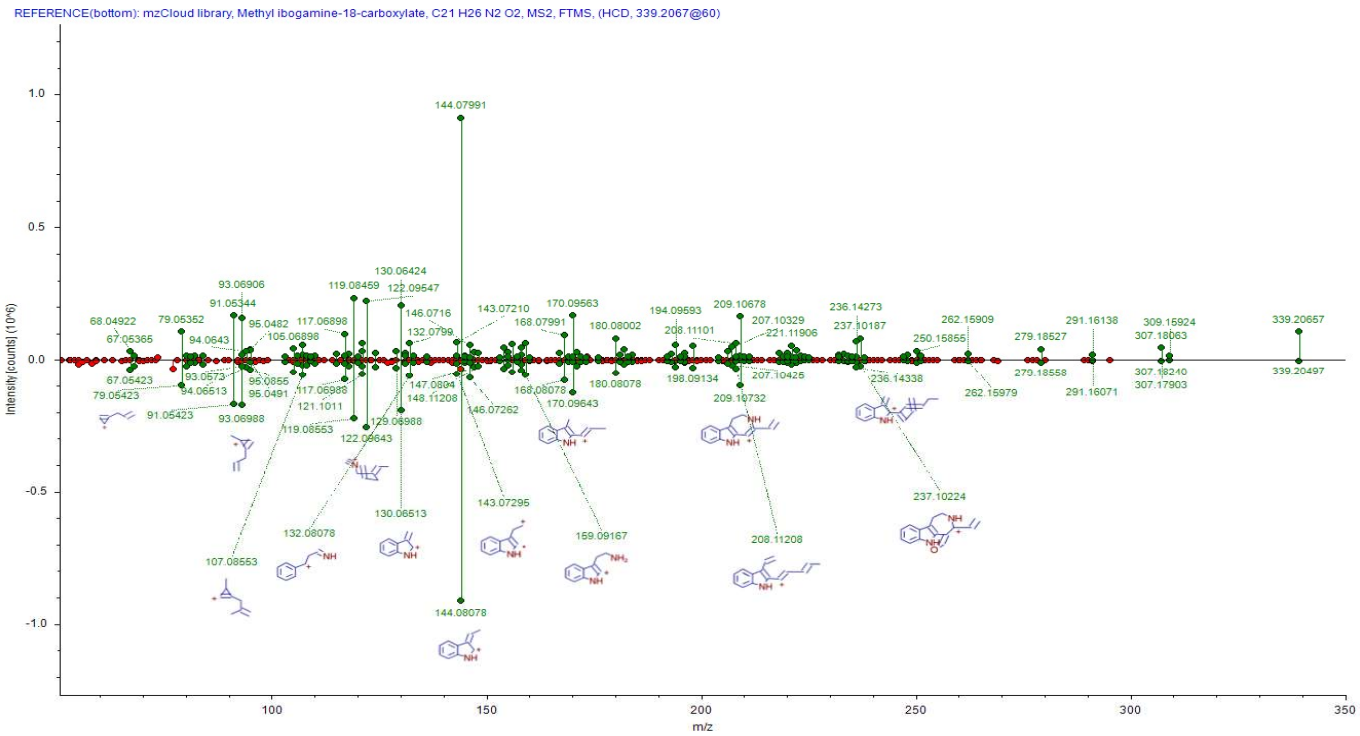

Figure 3. The mzCloud result of coronaridine

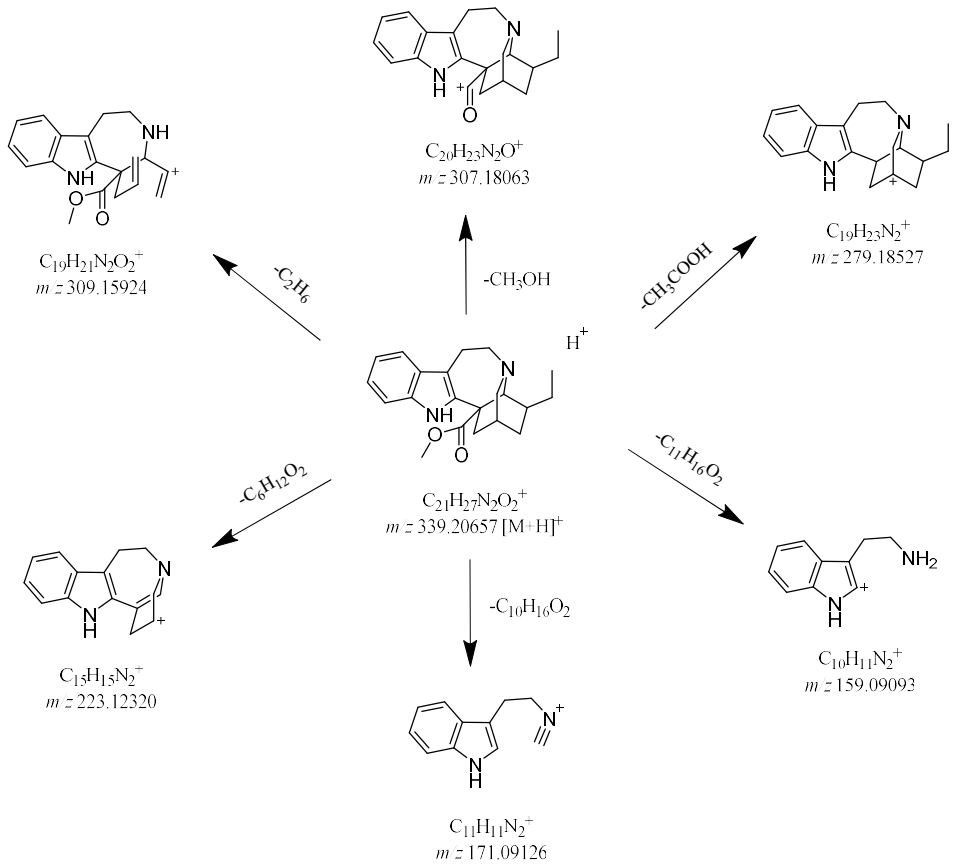

Figure 4. The MS/MS fragmentation result of coronaridine

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