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Volatile and Non-Volatile Metabolites Profiling of the Chloroform Extract of Marine Sponge *Clathria reinwardti* via Mass Spectrometry

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ABSTRACT

Metabolites are organic molecules produced by metabolic processes in living organisms and are responsible for various cellular functions. They are obtained from both terrestrial and marine organisms. Among marine organisms, sponges are an important source of metabolites. *Clathria reinwardti* Vosmaer is a demosponge belonging to the order Poecilosclerida and the family Microcionidae. The study aims to profile the metabolites of the chloroform extract of the marine sponge *C. reinwardti* using mass spectrometry analysis. A sponge sample was collected from the east coast of Sulug Island, Sabah, Malaysia. Total phenolic and flavonoid contents were determined. The composition of these extracts was further elucidated through qualitative biochemical screening, Fourier transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-quadrupole time-of-flight mass spectrometry (LT-qTOF-MS) analyses. The extracts displayed total phenolic and flavonoid content values. The qualitative biochemical screening tests indicated the presence of alkaloids and steroids. The GC-MS analysis indicated the presence of different metabolites of various natures, which include 2,5-bis(1,1-dimethylethyl) phenol,

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pentadecane, octacosane, eicosane, tetracosane, and cholestanol. The LC-qTOF-MS analysis indicated the presence of thymine, C16 sphinganine, hericene B, phylloquinone, 24-norcholesterol, palmitic amide, oleamide, solanidine, suillin, 9-thiastearic acid, and isoamijiol. The detected metabolites have been reported to have different pharmacological activities such as anti-inflammatory, antimicrobial, anti-diabetic, anti-cancer, antioxidant, anti-haemorrhagic, cytotoxic, neuroprotective, and chemopreventive. The finding highlights that the chloroform extract of *C. reinhardt* is an important source of metabolites with potential benefits to humans and animals, particularly in the aquaculture industry. Further isolation, purification, and characterization of the detected metabolites are needed for future studies.

Keywords: *Clathria reinhardt,* GC-MS, LC-QTOF-MS analysis, marine sponge, metabolites, seafood security

INTRODUCTION

Metabolites are small organic molecules resulting from various metabolic processes within living organisms (Lu et al., 2017). These molecules have various functions, including the formation of cellular structures, energy sources, signalling molecules, defence, and inter-organism interactions (Altaf-Ul-Amin et al., 2018). Metabolites have received much attention in biomedical research because of their potential therapeutic applications and diverse pharmacological properties (Qiu et al., 2023). They have exhibited various biological activities, including antimicrobial, antioxidant, anticancer, and anti-inflammatory (Hamzalioğlu & Gökmen, 2016).

Metabolites can be obtained from both terrestrial and marine sources. From the history of marine metabolites research, marine invertebrates, such as sponges, sea slugs, and soft corals, have been the major contributors to the novel source of natural bioactive compounds for the current trend in drug discovery (Varijakzhan et al., 2021). Among marine organisms, sponges are one of the richest sources of pharmacological metabolites such as alkaloids, terpenoids, steroids, phenolics, and flavonoids. Over 5,300 different bioactive products are known from marine sponges and their associated microorganisms, along with more than 200 new metabolites to be reported annually (Laport et al., 2009). Several drugs originated from sponges that had entered clinical trials and been approved. For instance, the development of cytarabine (Ara-C) for cancer treatment and vidarabine as an antiviral agent (Mayer et al., 2010).

Among the diverse types of marine sponges, *Clathria reinwardti* is the targeted sponge in this study. The *C. reinwardti* Vosmaer is classified under the phylum Porifera and the family Microcionidae. These sponges exhibit branching and sprawling morphologies that extend horizontally, resembling the intricate structure of branching tree roots (Ashok et al., 2019). Apart from their morphology, they also display orange colours, ranging from vibrant, bright, salmon-like orange colors to dark and dull brown-yellowish tones (Figure 1).

The *C. reinwardti* marine sponge has been documented in various regions, including Vietnam (Tai et al., 2021; Trang et al., 2022), Thailand (Dechsakulwatana et al., 2022), Indonesia (Sugrani et al., 2019), India (Venkateshwar et al., 2005), Singapore (S. C. Lim, 2008), and Malaysia (Ocean Biodiversity Information System [OBIS], n.d.).

Figure 1. Underwater photograph of *Clathria reinwardti* at the sampling site of Sulug Island, North Borneo, Sabah, Malaysia

To the author's knowledge, only a handful of authors have published on the composition of the bioactive compounds of *C. reinwardti*, with the reported metabolites limited to a maximum of seven compounds (Tai et al., 2021; Trang et al., 2022; Venkateshwar et al., 2005). In these studies, nuclear magnetic resonance spectroscopy (NMR) was mainly used to analyze the compounds. However, the inherent limitations of NMR spectroscopy, such as low sensitivity and the inability to quantitatively analyze trace compounds, may result in fewer compounds being detected (Wang et al., 2021), leading to a limited metabolite profile.

To date, no studies have been conducted on the metabolite profiling of *C. reinwardti* specifically in Sabah, in the Coral Triangle region. Filling this research gap is crucial because it is known that variations in location and environmental parameters affect the composition of bioactive compounds in marine sponges (Melawaty & Pasau, 2015). Therefore, this study is important to contribute to the fundamental understanding of *C. reinwardti* and serve as a reference for identifying the main compounds. Consequently, the study aims to evaluate the metabolite profile of chloroform extracts from *C. reinwardti* in Borneo waters using GC-MS and LC-qTOF-MS analyses.

METHODOLOGY

Chemicals and Reagents

The chemicals and reagents utilized to conduct this study include chloroform, n-hexane, catechin, folin-ciocalteau solution, hydrochloric acid (HCl), iodine, potassium iodide (KI), sulphuric acid (H_2SO_4), benzene, ammonia, sodium hydroxide (NaOH), acetone, high-performance liquid chromatography (HPLC)-grade methanol, distilled water, and Toyobo MagExtractor-Genome-Kit. The chemicals and reagents were purchased from Sigma Chemicals (Sigma-Aldrich Corporation, USA), Merck (USA), and J. T. Baker (Avantor, USA).

Sample Collection

Approximately 1 kg of *C. reinwardti* was collected in September 2023 from a site located on the east coast of Sulug Island, Sabah, Malaysia (5.9571°N, 115.9963°E) at a depth of 15 m. Concurrently, *in-situ* water parameters were measured using a YSI water quality meter (Professional Plus Multiparameter, Xylem Analytics, USA): water temperature (29.5°C), pH (7.69), salinity (31.31 ppt), dissolved oxygen (89%), total dissolved solids (31,330 mg/L), and turbidity (0.12 NTU). The necessary permits for sample collection in Sabah were obtained from the Sabah Biodiversity Centre (SaBC) (License No.: JKM/MBS.1000-2/13 JLD. 2(47)), the Sabah Parks Research Permit (Application: SPRP-220; Letter Ref. No.: TTS 100-6/2 Jld. 30), and the Department of Fisheries (DoF) (License No.: JPIN/BPP:100-24 Klt.7 (102).

Following collection, the samples were carefully placed in labeled zip-lock bags, cleaned, and rinsed with distilled water, and subsequently stored in a -80°C freezer for further analysis facilitation. The sample was deposited at the Borneo Marine Research Institute, Universiti Malaysia Sabah Herbarium, and assigned the voucher specimen number IPMB-Pf 01.00002. Additionally, DNA isolation from the collected sample was performed for identification purposes.

Extraction

The *C. reinwardti* sample was dried at room temperature and then ground into fine powder using a heavy-duty grinder. The powdered sample was extracted with HPLC-grade methanol, chloroform, and distilled water in a 1:10:10:10 (w/v/v) ratio using the orbital shaking method at 27°C for 72 hr. The extract was then filtered with a metal mesh, a cheesecloth strainer, and Whatman filter paper (125 mm) before separation with a separatory funnel. Three layers were formed, including the chloroform layer known as the non-polar layer (lowest layer), which was collected as chloroform crude extract. In contrast, the macromolecule layer and polar layer (mixture of methanol and distilled water layer) were discarded as a sea salt removal approach. The extract was dried with a rotary evaporator before lyophilization using a freeze dryer to obtain the crude extract. Subsequently, the crude extracts were then stored at a temperature of -80^{o}C for further analysis, and the extraction yield was calculated using Equation 1 (Yong et al., 2018).

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ the\ extract\ obtained\ \times 100\%}{Mass\ of\ the\ dry\ material\ taken\ for\ extraction} \quad [1]$$

Determination of Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) of the diluted extract was determined with the Folin-Ciocalteau method (Velioglu et al., 1998), while the total flavonoid content (TFC) was evaluated using the aluminum chloride colorimetric method (Zou et al., 2004).

Qualitative Metabolites Screening

The qualitative metabolite screening of the chloroform crude extract of *C. reinwardti* was conducted to assess the presence of different metabolites, including alkaloids, anthraquinones, saponins, and steroids (Harborne, 1998).

FTIR Analysis

The FTIR analysis was performed using a Bruker Alpha II Compact FTIR Spectrophotometer (Bruker, Germany). The crude chloroform extract was placed directly in contact with the test crystal window surface during data acquisition. Identification of the chemical class present was analyzed based on the FTIR spectrum range of previous studies (Nandiyanto et al., 2019).

GC-MS Analysis

About 1 µl of the hexane-reconstituted sample was injected into an HP-5MS capillary column (30 m × 0.25 mm with a film thickness) using a GC-MS system (Agilent Technologies, USA). Helium (99.999%) was used as a carrier gas at a constant flow rate of 1.0 ml/min. The injector temperature was set at 250°C, and the oven temperature gradient started at 40°C and held for 3 min, then ramped from 40 to 200°C at a rate of 3°C/min and held for 3 min at 200°C. The system operated in splitless mode with electron ionization (EI), and compound identification was done using the National Institute of Standards and Technology (NIST) database, matching mass spectra with the highest scores to identify the target compound (Shah et al., 2014). Each compound obtained was further reviewed with previous studies for reported bioactivities.

LC-qTOF-MS Analysis

The chloroform crude extract of *C. reinwardti* was reconstituted with methanol, and the concentration was adjusted before the LC-qTOF-MS analysis. A 1.0 µl reconstituted sample was chromatographically separated with an Agilent Zorbax Eclipse XDB-C18 reversed-phase column (Agilent Technologies, USA). The column was maintained at 25°C, and a flow rate of 500 µl/min during analysis. The mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The LC gradient followed the previously established program (Shah et al., 2020). Briefly, the gradient started at 5% solvent B for 5 min, progressed to 100% solvent B in 15 min, and was kept for 5 min. Later, the column was conditioned as an initial for 5 min before the subsequent injection. The MS signals were obtained, following the previous methodology with minor modifications (Ling et al., 2018). Data was acquired between an *m/z* of 100 and 1,500, with positive and negative heated electrospray ionization (ESI) deployed at 3,500 and

–3,500 V, respectively. Then, the acquired data was processed with Agilent MassHunter Qualitative Analysis software (version 10), and the compounds were identified with the built-in molecular feature algorithm and matched against the METLIN Metabolomics Database and Library databases. Each compounds obtained were further reviewed with previous studies for reported bioactivities.

RESULTS

Multiple analyses were undertaken in this investigation into the metabolic profiling of *C. reinwardti* sponge chloroform extracts. Initially, TPC and TFC were assessed. Subsequently, the composition of these extracts underwent further elucidation through qualitative biochemical screening, FTIR, GC-MS, and LC-qTOF-MS. The detailed results of these analyses are presented in subsequent sections.

The Percentage Yield, TPC, and TFC

The chloroform extract of *C. reinwardti* yielded 2.45%. The concentration of TPC of *C. reinwardti* chloroform extract was determined according to the equation ($y = 3.5407x + 0.0542$, $R^2 = 0.9962$) as gallic acid equivalent (mg GAE/g extract). The total phenolic content was recorded at 0.117 ± 0.008 mg GAE/g, respectively. The TFC was calculated by the equation ($y = 0.2315x + 0.0024$, $R^2 = 0.9976$), obtained by the calibration curve as catechin equivalents (mg CAE/g extract). The total flavonoid content resulted in 1.320 ± 0.087 mg CAE/g.

Qualitative Metabolites Screening of *C. reinwardti* Extract

The qualitative metabolites screening of *C. reinwardti* chloroform extract indicated the presence of alkaloids and steroids, while anthraquinone and saponins were absent.

FTIR Profile

FTIR analysis (Figure 2) showed a broad and weak absorption band of aliphatic amine stretch (N-H) recorded around 3339.74 cm^{-1}. The strong C-H stretch absorption band was observed around 2920.50 cm^{-1} and the medium band at 2852.89 cm^{-1}. Both these values fall within the reference range of methylene C-H stretch, indicating the presence of aliphatic methylene in the *C. reinwardti* chloroform extract. The adsorption band illustrated the presence of a carbonyl compound (C=O) at 1726.84 cm^{-1}. The co-occurrence of C-H (around 2852.89 cm^{-1}) and C=O stretch (1726.84 cm^{-1}) indicates the presence of an aldehyde group. A weak absorption band at 1636.85 cm^{-1} was attributed to the imine group. In contrast, the weak band around 1545.22 cm^{-1} suggested the presence of amide compound (C=O stretching or N-H bending) vibration in *C. reinwardti* chloroform extract (CONR2). The

fingerprint region (500–1,500 to 1,500 cm^{-1}) was not included as the sample tested was not a pure compound but a crude extract. The reference range was referred to (Nandiyanto et al., 2019; Sadat & Joye, 2020) as listed in Table 1.

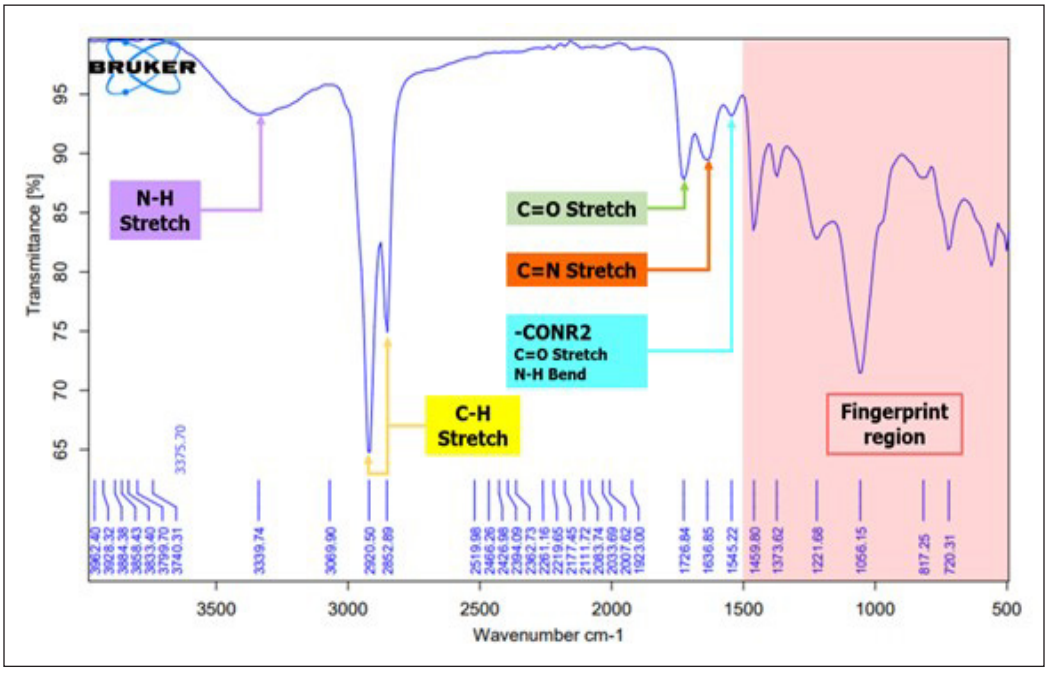

Figure 2. Fourier transform infrared spectroscopy spectrum showing a functional group of *Clathria reinwardti* chloroform extract

Table 1
Identified functional group of the Fourier transform infrared spectroscopy (FTIR) spectrum for Clathria reinwardti *chloroform extract based on the reference range*

Recorded absorption band	Reference range		Functional group
3339.74	3360-3310	N-H	Aliphatic secondary amine
2920.50	2935-2915	C-H	Aliphatic methylene (alkane)
2852.89	2800-2700	C-H	
1728.84	1740-1725	C=O	Carbonyl
1636.85	1690-1590	C=N	Imine
1545.22	1580-1510	-CONR2	Amide
1459.80	Fingerprint region		
1373.62			
1221.68			
1056.15			
817.25			
720.31			

GC-MS Analysis

GC-MS analyses of metabolites carried out in the chloroform extract of *C. reinwardti* are displayed in Table 2. Ten (10) metabolites of various functional groups, such as phenol, alkane, and sterol, are listed. Figure 3 displays the GC-MS chromatogram, while Figure 4 illustrates the structures of the identified metabolites from the chloroform extract.

Table 2
List of metabolites detected in the chloroform extract of Clathria reinwardti *via gas chromatography-mass spectrometry analysis*

No.	Retention time (min)	Metabolites name	Class	Molecular formula	Area (%)
1	26.325	2,5-bis(1,1-Dimethylethyl)phenol	Phenolic	$C_{14}H_{22}O$	1.63
2	34.199	Pentadecane	Alkane	$C_{15}H_{32}$	0.54
3	38.004	Eicosane	Alkane	$C_{20}H_{42}$	2.25
4	39.789	Heneicosane	Alkane	$C_{21}H_{44}$	3.00
5	41.557	Docosane	Alkane	$C_{22}H_{46}$	6.79
6	43.628	Tetracosane	Alkane	$C_{24}H_{50}$	9.16
7	46.306	Pentacosane	Alkane	$C_{25}H_{52}$	10.81
8	47.199	Cholestanol	Steroid	$C_{27}H_{48}0$	9.37
9	49.802	Hexacosane	Alkane	$C_{26}H_{54}$	9.99
10	54.443	Heptacosane	Alkane	$C_{27}H_{56}$	8.10

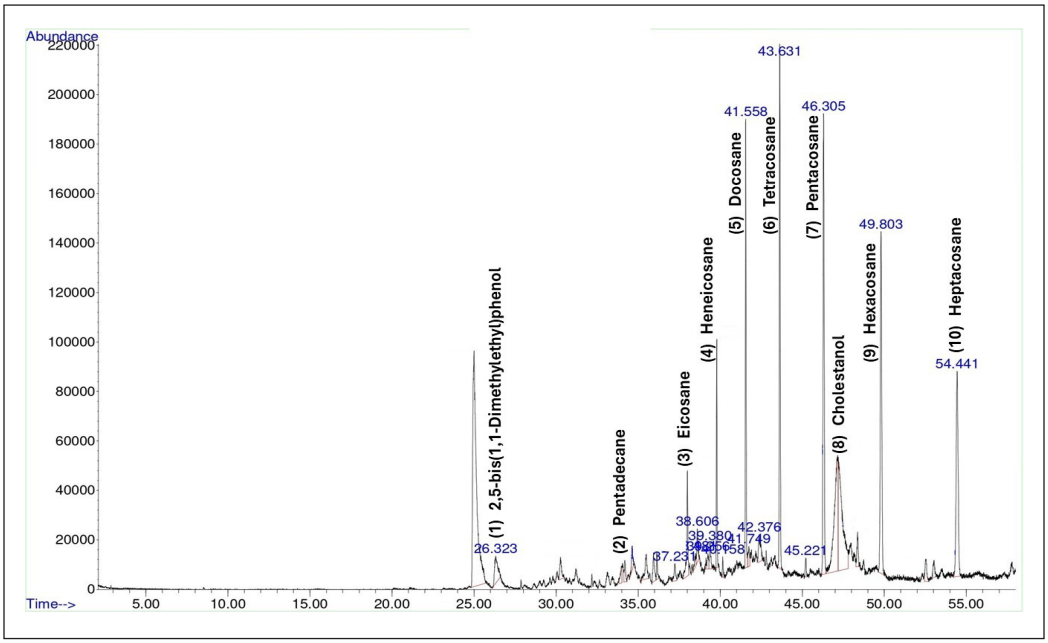

Figure 3. Chromatogram of *Clathria reinwardti* chloroform extract via gas chromatography-mass spectrometry analysis

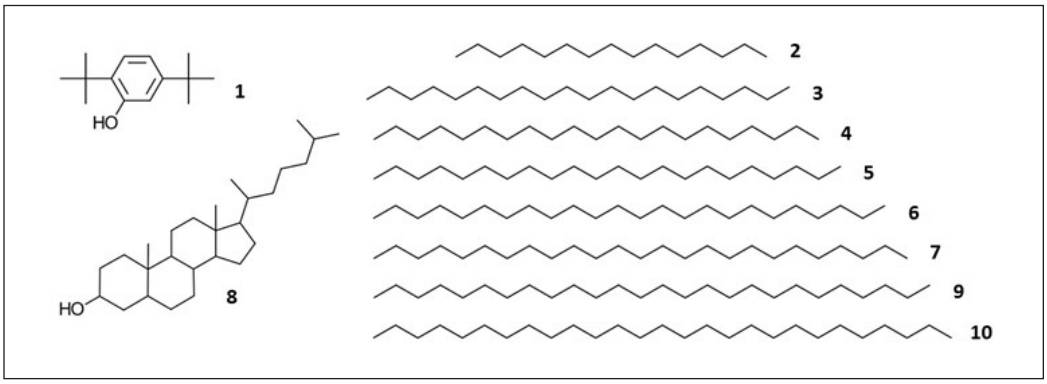

Figure 4. Structures of the detected metabolites in the chloroform extract of *Clathria reinwardti* via gas chromatography-mass spectrometry analysis

The total compounds detected in the chloroform extract of *C. reinwardti via* GC-MS analysis were classified into three chemical classes: phenol (10%), sterol (10%), and predominantly alkane (80%).

LC-qTOF-MS Analysis

(a) Positive Mode of LC-qTOF-MS

LC-qTOF-MS analysis via positive mode carried out in the chloroform extract of *C. reinwardti* is displayed below. Thirty (30) metabolites of different functional groups, such as fatty acids, terpenoids, amino acids, and steroids, are tabulated (Table 3)**.** Figure 5 presents the structure of metabolites identified in the chloroform extract of C. reinwardti using the positive mode of LC-qTOF-MS analysis.

(b) Negative Mode of LC -qTOF-MS

LC-qTOF-MS analysis via negative mode carried out in the chloroform extract of *C. reinwardti* is displayed below. Seven (7) metabolites of different functional groups, such as fatty acids, macrolides, and terpenoids, are tabulated (Table 4). Figure 6 depicts the structure of metabolites detected in the chloroform extract using the negative mode of LC-qTOF-MS analysis.

Overall, thirty (30) and seven (7) metabolites were identified through LC-qTOF-MS analysis via positive and negative modes, respectively. In the positive mode, the compounds can be classified into various chemical classes, dominated by vitamins (24%), followed by steroids (14%). Other reported chemical classes identified include terpenoid (10%), peptide (7%), ketone (7%), fatty amide (7%), aromatic (7%), fatty acid (4%), dicarboxyl acid (4%), amino acid (4%), lactone (3%), flavonoid (3%), sphingolipid (3%), and phenolic (3%).

Table 3
List of metabolites detected in the chloroform extract of Clathria reinwardti *via the positive mode of liquid chromatography-quadrupole time-of-flight mass spectrometry analysis*

No.	Retention time (min)	*m/z*	Metabolites	Class	Molecular formula	Mass error (ppm)
11	1.39	127.0497	Thymine	Purine	$C_5H_6N_2O_2$	1.16
12	7.48	254.1164	Ethyl 3-methyl-9H-carbazole-9-carboxylate	Aromatic	$C_{16}H_{15}NO_2$	4.98
13	12.42	274.273	Hexadecasphinganine	Sphingolipid	$C_{16}H_{35}NO_2$	4.56
14	12.66	181.1204	5-methyl-octanoic acid	Fatty acid	$C_9H_{18}O_2$	-4.61
15	13.16	214.0869	Benzyl nicotinate	Aromatic	$C_{13}H_{11}NO_2$	-1.85
16	13.30	277.1767	Kikkanol A	Terpenoid	$C_{15}H_{26}NO_3$	2.86
17	13.31	295.1874	3-methyl-tetradecanedioic acid	Dicarboxylic acid	$C_{15}H_{28}O_4$	2.87
18	14.97	189.1236	*N*-Alpha-acetyllysine	Amino acid	$C_8H_{16}N_2O_3$	-1.92
19	17.22	279.1537	4-Hydroxy-6-methylpyran-2-one	Lactone	$C_6H_6O_3$	4.12
20	17.23	205.0822	Gly Glu	Peptide	$C_7H_{12}N_2O_5$	-2.02
21	17.27	506.3183	Lys Trp Arg	Peptide	$C_{23}H_{36}N_8O_4$	1.09
22	18.05	600.4635	Hericene B	Phenolic	$C_{37}H_{58}O_5$	-2.34
23	18.28	468.3827	Phylloquinone	Vitamin	$C_{31}H_{46}O_2$	3.18
24	19.37	305.2464	Isoamijiol	Terpenoid	$C_{20}H_{32}O_2$	1.85
25	19.64	395.3284	24-Norcholesterol	Steroid	$C_{26}H_{44}O$	1.88
26	19.75	256.2628	Palmitic amide	Fatty Amide	$C_{16}H_{33}NO$	2.98
27	19.84	282.2779	Oleamide	Fatty Amide	$C_{18}H_{35}NO$	4.52
28	20.12	589.426	Caffeoylcycloartenol	Steroid	$C_{39}H_{56}O_4$	-1.38
29	20.27	398.3415	Solanidine	Steroid	$C_{27}H_{43}NO$	4.73
30	20.30	451.2813	25-Hydroxyvitamin D3-26,23-lactone	Vitamin	$C_{27}H_{40}O_4$	1.24
31	20.32	463.2806	Suillin	Terpenoid	$C_{28}H_{40}NO_4$	1.33
32	20.46	397.3099	25-Hydroxy-23,23,24,24-tetradehydrovitamin D3	Vitamin	$C_{27}H_{40}O_2$	-1.69
33	20.47	419.2959	1α,25-Dihydroxy-3-deoxy-3-thiavitamin D3	Vitamin	$C_{26}H_{42}O_2S$	-0.15
34	20.84	628.1864	Robinetin 3-rutinoside	Flavonoid	$C_{27}H_{30}O_{16}$	-1.12
35	20.96	386.3768	3-Deoxyvitamin D3	Vitamin	$C_{27}H_{44}$	3.80
36	21.04	479.3143	1α,22,25-Trihydroxy-23,24-tetradehydro-24a,24b-dihomo-20-epivitamin D3	Vitamin	$C_{29}H_{44}O_4$	-2.86
37	21.44	413.2655	Apocholic acid	Steroid	$C_{24}H_{38}O_4$	0.92
38	21.71	326.3407	Henicos-6-en-11-one	Ketone	$C_{21}H_{40}O$	4.01
39	21.87	451.3199	24,25-Dihydroxyvitamin D2	Vitamin	$C_{28}H_{44}O_3$	-4.17
40	24.62	338.3402	N-Cyclohexanecarbonylpentadecylamine	Ketone	$C_{22}H_{44}NO$	4.83

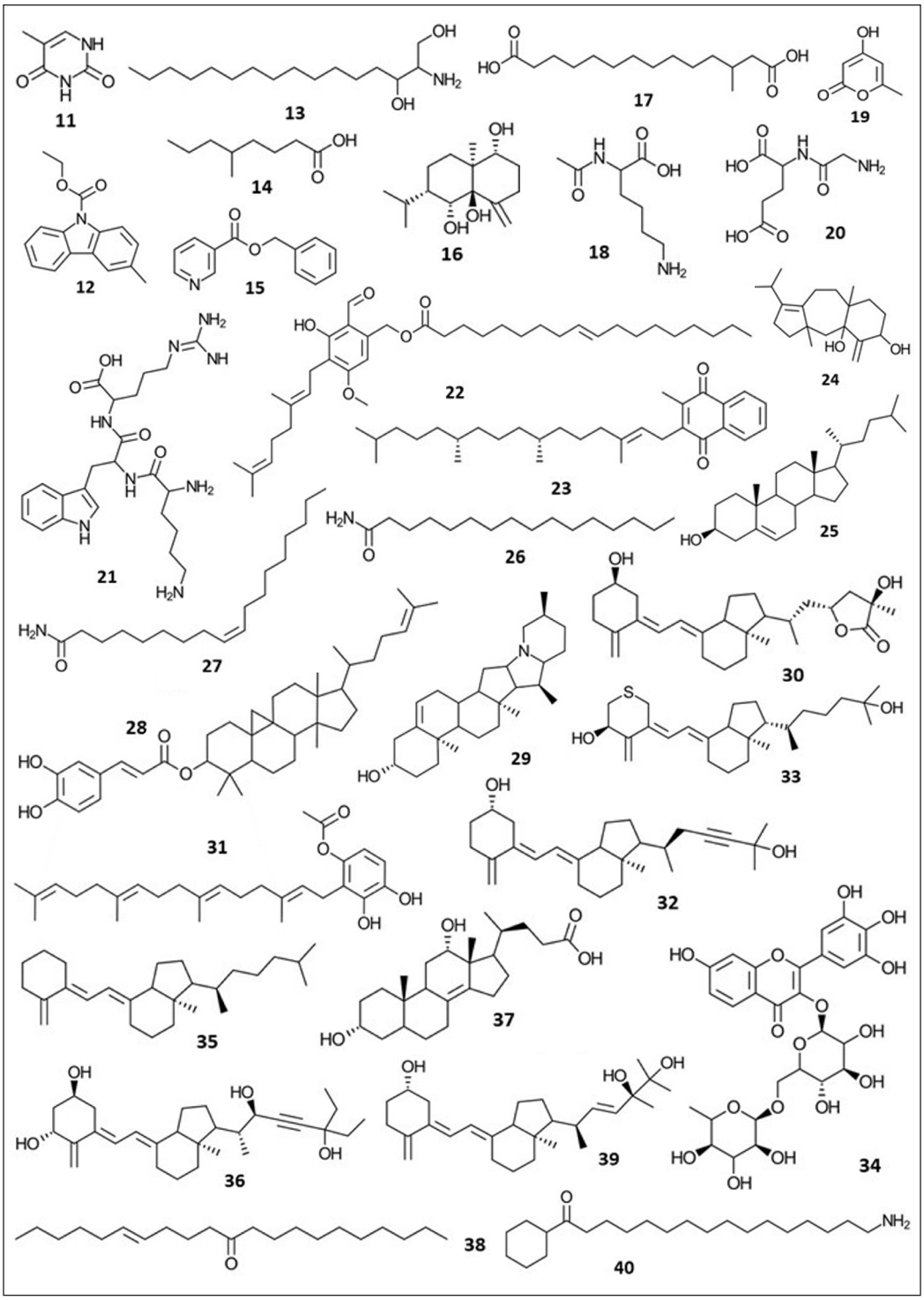

Figure 5. Structure of the metabolites detected in the chloroform extract of *Clathria reinwardti* via the positive mode of liquid chromatography-quadrupole time-of-flight mass spectrometry analysis

Table 4
List of metabolites detected in the chloroform extract of Clathria reinwardti *via the negative mode of liquid chromatography-quadrupole time-of-flight mass spectrometry analyses*

No.	Retenion time (min)	*m/z*	Metabolites name	Class	Molecular formula	Mass error (ppm)
41	14.33	571.3272	6,8a-Seco-6,8a-deoxy-5-oxoavermectin "2b" aglycone	Macrolide	$C_{33}H_{48}O_8$	1.01
42	15.92	317.2137	8-Hydroxyicosa-5,9,11,14,17-pentaenoic acid	Fatty acid	$C_{20}H_{30}O_3$	-4.66
43	16.16	343.2294	13-Hydroxydocosa-4,7,10,14,16,19-hexaenoic acid	Fatty acid	$C_{22}H_{32}O_3$	-4.44
44	16.34	319.2291	11-Hydroxyicosa-5,8,12,14-tetraenoic acid	Fatty acid	$C_{20}H_{32}O_3$	-3.87
45	17.13	599.3212	5-Oxoavermectin "2a" aglycone	Macrolide	$C_{34}H_{48}O_9$	2.04
46	18.54	301.2193	9-Thiastearic acid	Fatty acid	$C_{17}H_{34}O_2S$	4.41
24	19.33	303.2331	Isoamijiol	Terpenoid	$C_{20}H_{32}O_2$	0.03

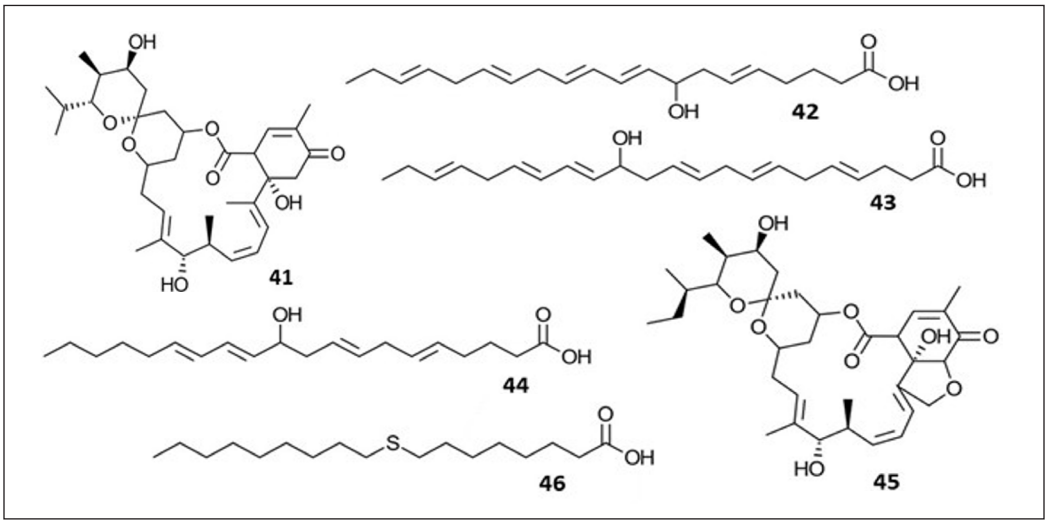

Figure 6. Structure of the metabolites detected in the chloroform extract of *Clathria reinwardti* via the negative mode of liquid chromatography-quadrupole time-of-flight mass spectrometry analysis

Compared to the positive mode, the negative mode of LC-qTOF-MS has detected fewer compounds (7 compounds). The chemical classes of the compounds are also fewer, comprised of terpenoid (14%), macrolide (29%), and dominated by fatty acid (57%).

DISCUSSION

The percentage yield of *C. reinwardti* obtained from chloroform extracts is 2.45%. The yield is lower compared to the methanol extract performed previously (Trang et al.,

2022). It is suggested that the compounds of *C. reinwardti* may have a higher affinity for a high-polarity solvent (methanol). For the chloroform extract, the TPC (0.117 ± 0.008 mg GAE/g) and TFC (1.320 ± 0.087 mg CAE/g) obtained complement the metabolite profiling. The GC-MS analysis (Table 2) has identified a phenol compound, namely, 2,5-bis(1,1-dimethylethyl) phenol, whereas flavonoid (robinetin 3-rutinoside) was identified via LC-qTOF-MS positive mode (Table 3). However, the TPC values of the chloroform extract of *C. reinwardti* are lower compared to the average TPC values (10.02 mg GAE/g extract) reported previously for 47 marine sponges (Oogarah et al., 2020). Aside from the use of different extraction solvents, the variations compared to the reported study may also be attributed to differences in sampling locations and environmental parameters, which can significantly influence the chemical composition of sponge-derived compounds (Melawaty & Pasau, 2015). This discrepancy is plausible, as the previous study collected a sample from Mauritius, located in the southwest Indian Ocean, whereas the present study's sample was obtained from the east side of Sulug Island, Malaysia. These geographic and ecological differences likely contributed to the presence of distinct compounds in sponges, therefore resulting in variation in TPC values. The qualitative screening of the chloroform extract of *C. reinwardti* revealed the presence of alkaloids and steroids, which were further confirmed through metabolite profiling using GC-MS and LC-qTOF-MS analysis. This finding aligns with previous studies, where alkaloids and steroids have been frequently identified in the genus *Clathria* (Capon et al., 2001; Chakraborty & Francis, 2021; Ohta et al., 1993; Woo et al., 2017). However, anthraquinones and saponins were not detected in the chloroform extract. This absence was consistent with subsequent GC-MS and LC-qTOF-MS analyses.

FTIR analysis of chloroform extract revealed absorption bands corresponding to different functional groups, including amine, alkane, carbonyl, imine, and amide. Subsequently, GC-MS analysis indicated that 80% of the compounds detected were alkanes (Figure 4). Additionally, the presence of the carbonyl group might be attributed to the fatty acid, which constitutes 57% of the metabolite class detected via LC-qTOF-MS negative mode.

Furthermore, the chloroform extract of *C. reinwardti* was analyzed through GC-MS for metabolite profiling. Among the 10 detected compounds, nine have demonstrated pharmacological effects, including 2,5-bis(1,1-dimethylethyl) phenol, which has exhibited antibacterial and anti-inflammatory properties (Phillips et al., 2015). Pentadecane has displayed anti-inflammatory, analgesic, and antipyretic activities (Okechuwuo, 2020). Heptacosane has shown antibacterial effects (Khatua et al., 2016). Heneicosane has been recognized for its antimicrobial properties (Vanitha et al., 2020). Eicosane has displayed anti-inflammatory, analgesic, and antipyretic effects (Okechuwuo, 2020). Hexacosane has exhibited antimicrobial properties (Rukaiyat et al., 2015). Tetracosane has displayed cytotoxic and apoptotic effects (Uddin et al., 2012). Pentacosane has shown antimicrobial properties (Carev et al., 2023). Cholestanol has induced apoptosis of corneal endothelial cells and lens epithelial cells and has exhibited cytotoxicity (Inoue et al., 1999; Miyashita et al., 2008).

The positive and negative modes of LC-qTOF-MS analysis indicated 36 metabolites, of which 23 have been reported to have bioactive properties. Some of the important pharmacologically active metabolites include thymine, which has been involved in pyrimidine metabolism (Qu et al., 2024). Hexadeca sphinganine has exhibited anti-cancer, nematocidal, and antibacterial activities (Gao et al., 2016; M. W. Lim et al., 2023; Reid et al., 2019). The 5-methyl-octanoic acid has inhibited α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), a subtype of AMPA-type glutamate receptors, and this inhibition has shown potential therapeutic interventions for Alzheimer's disease (Dunn et al., 2023). Benzyl nicotinate has acted as a vasodilator (Abramović et al., 2008). Kikkanol A has demonstrated inhibitory activity against rat lens aldose reductase (Yoshikawa et al., 1999). N-Alpha-acetyllysine has served as a biomarker for membrane nephropathy (MN) and IgA nephropathy (IgAN) identification (Qu et al., 2024). The 4-Hydroxy-6-methylpyran-2-one has served as a chemical platform (Kim et al., 2023). Hericene B has exhibited anti-diabetic properties and reduced the risk of gastric ulceration (Lv et al., 2021). Phylloquinone has been known for its antihemorrhagic and osteoporosis prevention properties (Bus & Szterk, 2021). Isoamijiol has acted as an antioxidant (M. W. Lim et al., 2023). Palmitic amide has shown antioxidant and anti-neuroinflammatory effects (M. W. Lim et al., 2023; Ngu et al., 2022). Oleamide exhibits anti-inflammatory, hypolipidemic, and chemopreventive effects against Alzheimer's disease, induces deep sleep, and upregulates appetite (Ameamsri et al., 2020; Cheng et al., 2010). Solanidine has acted as an anticancer agent against the Herpes simplex virus, an antitumor, and a protease inhibitor for SARS-CoV-2 (Minorics et al., 2011; Morillo et al., 2020; Zhao et al., 2021). Suillin has inhibited cell proliferation and induced apoptosis in human cancer cells and serves as a chemo-venting agent for the treatment of neurodegenerative diseases like Alzheimer's (Andrade et al., 2022; F.-Y. Liu et al., 2009). Robinetin 3-rutinoside has served as an inhibitor of tyrosinase (Pervaiz et al., 2022). The 3-Deoxyvitamin D3 has exhibited anti-inflammatory properties (Cheema et al., 2017). Apocholic acid demonstrates antimicrobial activity (Chua et al., 2023). The 9-thiastearic acid has inhibited stearoyl-CoA desaturase (G. Liu, 2009). Isoamijiol has displayed antioxidant and insecticidal properties (Kilic et al., 2021; M. W. Lim et al., 2023). Metabolites derived from *C. reinhardtii* may offer benefits to both humans and animals, particularly in aquaculture, which could contribute to enhancing seafood security.

CONCLUSION

Among marine organisms, sponges are an important source of metabolites. *Clathria reinhardt* Vosmaer is a marine sponge belonging to the family Microcionidae. The chloroform extracts displayed total phenolic and total flavonoid content values. The qualitative biochemical screening tests indicated the presence of alkaloids and steroids. The

GC-MS analysis indicated the presence of different metabolites of various natures, which include 2,5-bis(1,1-dimethylethyl) phenol, pentadecane, eicosane, tetracosane, cholestanol, etc. The LC-QTOF-MS analysis indicated the presence of different metabolites, which include thymine, hexadeca sphinganine, hericene B, phylloquinone, 24-norcholesterol, palmitic amide, oleamide, solanidine, suillin, 9-thiastearic acid, and isoamijiol. These compounds have been reported for various pharmacological activities, such as anti-inflammatory, antimicrobial, anti-diabetic, anticancer, antioxidant, anti-hemorrhagic, cytotoxic, neuroprotective, and chemopreventive. Thus, the chloroform extract of *C. reinhardt* is an important source of metabolites. Further isolation, purification, and characterization of the detected metabolites are needed for future studies.

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REFERENCES

Abramović, Z., Šuštaršič, U., Teskač, K., Šentjurc, M., & Kristl, J. (2008). Influence of nanosized delivery systems with benzyl nicotinate and penetration enhancers on skin oxygenation. *International Journal of Pharmaceutics*, *359*(1–2), 220–227. https://doi.org/10.1016/j.ijpharm.2008.03.014

Altaf-Ul-Amin, M., Kanaya, S., & Mohamed-Hussein, Z.-A. (2018). Investigating metabolic pathways and networks. In S. Ranganathan, M. Gribskov, K. Nakai, C. Schönbach (Eds.), *Encyclopedia of bioinformatics and computational biology* (Vol. 3, pp. 489–503). Academic Press. https://doi.org/10.1016/B978-0-12-809633-8.20140-4

Ameamsri, U., Chaveerach, A., Sudmoon, R., Tanee, T., Peigneur, S., & Tytgat, J. (2020). Oleamide in *Ipomoea* and *Dillenia* species and inflammatory activity investigated through ion channel inhibition. *Current Pharmaceutical Biotechnology*, *22*(2), 254–261. https://doi.org/10.2174/1389201021666200607185250

Andrade, J. M., Pachar, P., Trujillo, L., & Cartuche, L. (2022). Suillin: A mixed-type acetylcholinesterase inhibitor from *Suillus luteus* which is used by Saraguros indigenous, southern Ecuador. *PLOS One*, *17*(5), e0268292. https://doi.org/10.1371/journal.pone.0268292

Ashok, A., Arasumuthu, A., K, Diraviya Raj, K., & Patterson Edward, J. K. (2019). *Clathria reinwardtii* - An Active competitor on the coral communities of Gulf of Mannar Marine Biosphere Reserve - Southeast India. *Think India Journal*, *22*(14), 15163–15179.

Bus, K., & Szterk, A. (2021). Relationship between structure and biological activity of various vitamin K forms. *Foods*, *10*(12), 3136. https://doi.org/10.3390/foods10123136

Capon, R. J., Miller, M., & Rooney, F. (2001). Mirabilin G: A new alkaloid from a southern Australian marine sponge, *Clathria* species. *Journal of Natural Products*, *64*(5), 643–644. https://doi.org/10.1021/NP000564G

Carev, I., Gelemanović, A., Glumac, M., Tutek, K., Dželalija, M., Paiardini, A., & Prosseda, G. (2023). *Centaurea triumfetii* essential oil chemical composition, comparative analysis, and antimicrobial activity of selected compounds. *Scientific Reports*, *13*, 7475. https://doi.org/10.1038/s41598-023-34058-2

Chakraborty, K., & Francis, P. (2021). Callypyrones from marine Callyspongiidae sponge *Callyspongia diffusa*: Antihypertensive bis-γ-pyrone polypropionates attenuate angiotensin-converting enzyme. *Natural Product Research*, *35*(24), 5801–5812. https://doi.org/10.1080/14786419.2020.1837819

Cheema, A. K., Mehta, K. Y., Fatanmi, O. O., Wise, S. Y., Hinzman, C. P., Wolff, J., & Singh, V. K. (2017). A metabolomic and lipidomic serum signature from nonhuman primates administered with a promising radiation countermeasure, gamma-tocotrienol. *International Journal of Molecular Sciences*, *19*(1), 79. https://doi.org/10.3390/ijms19010079

Cheng, M.-C., Ker, Y.-B., Yu, T.-H., Lin, L.-Y., Peng, R. Y., & Peng, C.-H. (2010). Chemical synthesis of 9(*Z*)-octadecenamide and its hypolipidemic effect: A bioactive agent found in the essential oil of mountain celery seeds. *Journal of Agricultural and Food Chemistry*, *58*(3), 1502–1508. https://doi.org/10.1021/jf903573g

Chua, R. W., Song, K. P., & Ting, A. S. Y. (2023). Characterization and antimicrobial activities of bioactive compounds from endophytic *Trichoderma asperellum* isolated from *Dendrobium* orchids. *Biologia*, *79*, 569-584. https://doi.org/10.1007/s11756-023-01562-9

Dechsakulwatana, C., Rungsihiranrut, A., Muangchinda, C., Ningthoujam, R., Klankeo, P., & Pinyakong, O. (2022). Biodegradation of petroleum oil using a constructed nonpathogenic and heavy metal-tolerant bacterial consortium isolated from marine sponges. *Journal of Environmental Chemical Engineering*, *10*(6), 108752. https://doi.org/10.1016/J.JECE.2022.108752

Dunn, E., Zhang, B., Sahota, V. K., & Augustin, H. (2023). Potential benefits of medium chain fatty acids in aging and neurodegenerative disease. *Frontiers in Aging Neuroscience*, *15*, 1230467. https://doi.org/10.3389/FNAGI.2023.1230467

Gao, H., Qi, G., Yin, R., Zhang, H., Li, C., & Zhao, X. (2016). *Bacillus cereus* strain S2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. *Scientific Reports*, *6*, 28756. https://doi.org/10.1038/srep28756

Hamzalioğlu, A., & Gökmen, V. (2016). Interaction between bioactive carbonyl compounds and asparagine and impact on acrylamide. In V. Gökmen (Ed.), *Acrylamide in food: Analysis, content and potential health effects* (pp. 355–376). Academic Press. https://doi.org/10.1016/B978-0-12-802832-2.00018-8

Harborne, A. J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.

Inoue, K., Kubota, S., & Seyama, Y. (1999). Cholestanol induces apoptosis of cerebellar neuronal cells. *Biochemical and Biophysical Research Communications*, *256*(1), 198–203. https://doi.org/10.1006/BBRC.1998.9497

Khatua, S., Pandey, A., & Biswas, S. J. (2016). Phytochemical evaluation and antimicrobial properties of *Trichosanthes dioica* root extract. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 410–413.

Kilic, M., Orhan, I. E., Eren, G., Okudan, E. S., Estep, A. S., Bencel, J. J., & Tabanca, N. (2021). Insecticidal activity of forty-seven marine algae species from the Mediterranean, Aegean, and Sea of Marmara in connection with their cholinesterase and tyrosinase inhibitory activity. *South African Journal of Botany*, *143*, 435–442. https://doi.org/10.1016/j.sajb.2021.06.038

Kim, M. S., Choi, D., Ha, J., Choi, K., Yu, J.-H., Dumesic, J. A., & Huber, G. W. (2023). Catalytic strategy for conversion of triacetic acid lactone to potassium sorbate. *ACS Catalysis*, *13*(21), 14031–14041. https://doi.org/10.1021/ACSCATAL.3C02775

Laport, M. S., Santos, O. C. S., & Muricy, G. (2009). Marine sponges: Potential sources of new antimicrobial drugs. *Current Pharmaceutical Biotechnology*, *10*(1), 86–105. https://doi.org/10.2174/138920109787048625

Lim, M. W., Yow, Y.-Y., & Gew, L. T. (2023). LC-MS profiling-based non-targeted secondary metabolite screening for deciphering cosmeceutical potential of Malaysian algae. *Journal of Cosmetic Dermatology*, *22*(10), 2810–2815. https://doi.org/10.1111/jocd.15794

Lim, S. C. (2008). *A guide to sponges of Singapore*. Science Centre Singapore.

Ling, Y. S., Lim, L. R., Yong, Y. S., Tamin, O., & Puah, P. Y. (2018). MS-based metabolomics revealing Bornean *Sinularia* sp. extract dysregulated lipids triggering programmed cell death in hepatocellular carcinoma. *Natural Product Research*, *34*(12), 1796-1803. https://doi.org/10.1080/14786419.2018.1531288

Liu, F.-Y., Luo, K.-W., Yu, Z.-M., Co, N.-N., Wu, S.-H., Wu, P., Fung, K.-P., & Kwok, T.-T. (2009). Suillin from the mushroom *Suillus placidus* as potent apoptosis inducer in human hepatoma HepG2 cells. *Chemico-Biological Interactions*, *181*(2), 168–174. https://doi.org/10.1016/J.CBI.2009.07.008

Liu, G. (2009). Stearoyl-CoA desaturase inhibitors: Update on patented compounds. *Expert Opinion on Therapeutic Patents*, *19*(9), 1169–1191. https://doi.org/10.1517/13543770903061311

Lu, W., Su, X., Klein, M. S., Lewis, I. A., Fiehn, O., & Rabinowitz, J. D. (2017). Metabolite measurement: pitfalls to avoid and practices to follow. *Annual Review of Biochemistry*, *86*, 277–304. https://doi.org/10.1146/annurev-biochem-061516-044952

Lv, G., Song, X., & Zhang, Z. (2021). Protective effect of the ethanol extract from *Hericium erinaceus* against ethanol-induced gastric ulcers. *Polish Journal of Food and Nutrition Sciences*, *71*(3), 333–341. https://doi.org/10.31883/pjfns/141560

Mayer, A. M. S., Glaser, K. B., Cuevas, C., Jacobs, R. S., Kem, W., Little, R. D., McIntosh, J. M., Newman, D. J., Potts, B. C., & Shuster, D. E. (2010). The odyssey of marine pharmaceuticals: A current pipeline perspective. *Trends in Pharmacological Sciences*, *31*(6), 255–265. https://doi.org/10.1016/j.tips.2010.02.005

Melawaty, L., & Pasau, K. (2015). The profile of secondary metabolites of sponge *Clathria reinwardtii* extract as a result of Fe accumulation in Spermonde Archipelago. *Advances in Biological Chemistry*, *5*, 266–272.

Minorics, R., Szekeres, T., Krupitza, G., Saiko, P., Giessrigl, B., Wölfling, J., Frank, É., & Zupkó, I. (2011). Antiproliferative effects of some novel synthetic solanidine analogs on HL-60 human leukemia cells *in vitro*. *Steroids*, *76*(1–2), 156–162. https://doi.org/10.1016/j.steroids.2010.10.006

Miyashita, H., Kai, Y., Nohara, T., & Ikeda, T. (2008). Efficient synthesis of α- and β-chacotriosyl glycosides using appropriate donors, and their cytotoxic activity. *Carbohydrate Research*, *343*(8), 1309–1315. https://doi.org/10.1016/j.carres.2008.02.012

Morillo, M., Rojas, J., Lequart, V., Lamarti, A., & Martin, P. (2020). Natural and synthetic derivatives of the steroidal glycoalkaloids of *Solanum* genus and biological activity. *Natural Products Chemistry and Research*, *8*, 371.

Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret ftir spectroscope of organic material. *Indonesian Journal of Science and Technology*, *4*(1), 97–118. https://doi.org/10.17509/ijost.v4i1.15806

Ngu, E.-L., Tan, C.-Y., Lai, N. J.-Y., Wong, K.-H., Lim, S.-H., Ming, L. C., Tan, K.-O., Phang, S.-M., & Yow, Y.-Y. (2022). *Spirulina platensis* suppressed iNOS and proinflammatory cytokines in lipopolysaccharide-induced BV2 microglia. *Metabolites*, *12*(11), 1147. https://doi.org/10.3390/metabo12111147

Ocean Biodiversity Information System. (n.d.). Clathria *(Thalysias)* reinwardti *Vosmaer, 1880*. https://mapper.obis.org/?taxonid=167774

Ohta, S., Okada, H., Kobayashi, H., Oclarit, J. M., & Ikegami, S. (1993). Clathrynamides A, B, and C: Novel amides from a marine sponge *Clathria* sp. that inhibit cell division of fertilized starfish eggs. *Tetrahedron Letters*, *34*(37), 5935–5938. https://doi.org/10.1016/S0040-4039(00)73818-4

Okechuwuo, P. N. (2020). Evaluation of anti-inflammatory, analgesic, antipyretic effect of eicosane, pentadecane, octacosane, and heneicosane. *Asian Journal of Pharmaceutical and Clinical Research*, *13*(4), 29–35. https://doi.org/10.22159/ajpcr.2020.v13i4.36196

Oogarah, P. N., Ramanjooloo, A., Doorga, J. R. S., Meyepa, C., van Soest, R. W. M., & Marie, D. E. P. (2020). Assessing antioxidant activity and phenolic content of marine sponges from Mauritius waters. *International Journal of Pharmacognosy and Phytochemical Research*, *12*(3), 123–131.

Pervaiz, I., Hasnat, M., Ahmad, S., Khurshid, U., Saleem, H., Alshammari, F., Almansour, K., Mollica, A., Rengasamy, K. R. R., Zainal Abidin, S. A., & Anwar, S. (2022). Phytochemical composition, biological propensities, and *in-silico* studies of *Crateva adansonii* DC.: A natural source of bioactive compounds. *Food Bioscience*, *49*, 101890. https://doi.org/10.1016/j.fbio.2022.101890

Phillips, S., Rao, M. R. K., Prabhu, K., Priya, M., Kalaivani, S., Ravi, A., & Dinakar, S. (2015). Preliminary GC-MS analysis of an Ayurvedic medicine "Kulathadi Kashayam". *Journal of Chemical and Pharmaceutical Research*, *7*(9), 393-400.

Qiu, S., Cai, Y., Yao, H., Lin, C., Xie, Y., Tang, S., & Zhang, A. (2023). Small molecule metabolites: Discovery of biomarkers and therapeutic targets. *Signal Transduction and Targeted Therapy*, *8*, 132. https://doi.org/10.1038/s41392-023-01399-3

Qu, Y., Wang, Y., Hu, Z., Su, C., Qian, C., Pan, J., Zhu, Y., & Shi, A. (2024). Role of metabolomic profile as a potential marker to discriminate membranous nephropathy from IgA nephropathy. *International Urology and Nephrology*, *56*, 635-651. https://doi.org/10.1007/s11255-023-03691-1

Reid, T., Kashangura, C., Chidewe, C., Benhura, M. A., Stray-Pedersen, B., & Mduluza, T. (2019). Characterization of anti-*Salmonella typhi* compounds from medicinal mushroom extracts from

Zimbabwe. *International Journal of Medicinal Mushrooms*, *21*(7), 713–724. https://doi.org/10.1615/INTJMEDMUSHROOMS.V21.I7.80

Rukaiyat, M., Garba, S., & Labaran, S. (2015). Antimicrobial activities of hexacosane isolated from *Sanseveria liberica* (Gerome and Labroy) plant. *Advancement in Medicinal Plant Research*, *3*(3), 120–125.

Sadat, A., & Joye, I. J. (2020). Peak fitting applied to fourier transform infrared and raman spectroscopic analysis of proteins. *Applied Sciences*, *10*(17), 5918. https://doi.org/10.3390/APP10175918

Shah, M. D., Venmathi Maran, B. A., Haron, F. K., Ransangan, J., Ching, F. F., Shaleh, S. R. M., Shapawi, R., Yong, Y. S., & Ohtsuka, S. (2020). Antiparasitic potential of *Nephrolepis biserrata* methanol extract against the parasitic leech *Zeylanicobdella arugamensis* (Hirudinea) and LC-QTOF analysis. *Scientific Reports*, *10*, 22091. https://doi.org/10.1038/s41598-020-79094-4

Shah, M. D., Yong, Y. S., & Iqbal, M. (2014). Phytochemical investigation and free radical scavenging activities of essential oil, methanol extract and methanol fractions of *Nephrolepis biserrata*. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(9), 269–277.

Sugrani, A., Natsir, H., Djide, M. N., & Ahmad, A. (2019). Antibacterial and anticancer activity of protein sponges collected from the waters of Kapoposang Island of South Sulawesi, Indonesia. *International Research Journal of Pharmacy*, *10*(1), 82–87.

Tai, B. H., Hang, D. T., Trang, D. T., Yen, P. H., Huong, P. T. T., Nhiem, N. X., Thung, D. C., Thao, D. T., Hoai, N. T., & Kiem, P. V. (2021). Conjugated polyene ketones from the Marine sponge *Clathria* (Thalysias) *reinwardti* (Vosmaer, 1880) and their cytotoxic activity. *Natural Product Communications*, *16*(9). https://doi.org/10.1177/1934578X211043732

Trang, D. T., Hang, D. T. T., Dung, D. T., Bang, N. A., Huong, P. T. T., Yen, P. H., Tai, B. H., Cuc, N. T., An, D. H., Nhiem, N. X., Thung, D. C., & Kiem, P. V. (2022). Chemical constituents of *Clathria reinwardti*. *Vietnam Journal of Chemistry*, *60*(S1), 21–26. https://doi.org/10.1002/vjch.202200053

Uddin, S. J., Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum*. *Pharmaceutical Biology*, *50*(10), 1276–1280. https://doi.org/10.3109/13880209.2012.673628

Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., Punitha, V. N., Vidhya, E., & Praseetha, P. K. (2020). Heneicosane—A novel microbicidal bioactive alkane identified from *Plumbago zeylanica* L. *Industrial Crops and Products*, *154*, 112748. https://doi.org/10.1016/j.indcrop.2020.112748

Varijakzhan, D., Loh, J.-Y., Yap, W.-S., Yusoff, K., Seboussi, R., Lim, S.-H. E., Lai, K.-S., & Chong, C.-M. (2021). Bioactive compounds from marine sponges: Fundamentals and applications. *Marine Drugs*, *19*(5), 246. https://doi.org/10.3390/md19050246

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *46*(10), 4113–4117. https://doi.org/10.1021/jf9801973

Venkateshwar, G. T., Krishnaiah, P., Malla Reddy, S., Srinivasulu, M., Rama Rao, M., & Venkateswarlu, Y. (2005). Chemical investigation of the marine sponges *Clathria reinwardti* and *Haliclona cribricutis*. *Indian Journal of Chemistry*, *44B*, 607–610.

Wang, Z.-F., You, Y.-L., Li, F.-F., Kong, W.-R., & Wang, S.-Q. (2021). Research progress of NMR in natural product quantification. *Molecules*, *26*(20), 6308. https://doi.org/10.3390/molecules26206308

Woo, J.-K., Ha, T. K. Q., Oh, D.-C., Oh, W.-K., Oh, K.-B., & Shin, J. (2017). Polyoxygenated steroids from the sponge *Clathria gombawuiensis*. *Journal of Natural Products*, *80*(12), 3224–3233. https://doi.org/10.1021/acs.jnatprod.7b00651

Yong, Y. S., Lim, S.-C., Lee, P.-C., & Ling, Y.-S. (2018). Sponges from North Borneo and their bioactivity against human colorectal cancer cells. *Borneo Journal of Marine Science and Aquaculture*, *2*, 40–47. https://doi.org/10.51200/bjomsa.v2i0.1215

Yoshikawa, M., Morikawa, T., Murakami, T., Toguchida, I., Harima, S., & Matsuda, H. (1999). Medicinal medicinal flowers. I. Aldose reductase inhibitors and three new eudesmane-type sesquiterpenes, Kikkanols A, B, and C, from the flowers of *Chrysanthemum indicum* L. *Chemical and Pharmaceutical Bulletin*, *47*(3), 340–345. https://doi.org/10.1248/cpb.47.340

Zhao, D.-K., Zhao, Y., Chen, S.-Y., & Kennelly, E. J. (2021). *Solanum* steroidal glycoalkaloids: Structural diversity, biological activities, and biosynthesis. *Natural Product Reports*, *38*, 1423–1444. https://doi.org/10.1039/d1np00001b

Zou, Y., Lu, Y., & Wei, D. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L . *in vitro*. *Journal of Agricultural and Food Chemistry*, *52*(16), 5032–5039. https://doi.org/10.1021/jf049571r