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Potent Secondary Metabolites From Marine Sponge Extracts: Isolation, Characterization and Medicinal Applications

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KEYWORDS

Extract n-Hexane; Marine Sponges; Medicinal Potential; Natural Product; Secondary Metabolite

ABSTRACT:

Introduction: This study explores new and unique bioactive compounds from the marine sponges. This is important to do, considering the population of Indonesian sponges is abundant and diverse, By analyzing the colors of marine sponges, we graded those with bright or light colors, typically have a more complete cell structure and would contain more beneficial metabolic compounds. We successfully analyzed extracts for the presence of secondary metabolites in these marine sponges collected.

Objectives: This study aimed to identify the secondary metabolites in marine sponges collected from Indonesia with pharmaceuticals and medicinal potentials

Methods: This study employed maceration method for extract preparation. GC-MS data used for metabolic component identification, and FTIR spectra for confirmation of functional groups in organic compounds of interest.

Results: The secondary metabolite components obtained are in the form of these cholest-5-en-3-ol accounted for 25.48% of the total metabolites followed by benzenedicarboxylic acid (12.70%), pentadecanoic acid (7.89%) and Ergosta-5.22-dien-3-ol (7.78%). These substances hold potential as biomaterials for growth and stamina enhancement well as antibacterial and antifungal properties. It's noted that marine sponges, especially those with bright or light colors (*Clatria reinwardti*), typically have more complete cell structure and contain more beneficial metabolic compounds. Probably there would be more diverse and abundant microbial communities associated with them. This study generate sets of data, which would be useful for follow up research in isolation, analysis and valuation of bioactive compounds such as steroids, carboxylic acids, aldehydes or their derivatives for pharmaceutical and medicinal purposes.

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Conclusions: These substances are generally included in the group of carboxylic acid compounds which have the potential as medicinal biomaterials, especially to increase growth and endurance have antibacterial and antifungal properties.

1. Introduction

Being a tropical paradise, Indonesia is well known to possess rain forests as well as marine ecosystem as a remarkable biological resources (Bayona et al., 2020). Surrounded by the sea, the nation also finds opportunity to benefit from an extensive marine ecosystem that is home to a wide range of marine organisms including sponges (Hu et al., 2015). These marine sponges have been reported as a potential source of biological materials with a wide range of benefits (Lee & Tran, 2021; Kamaruddin, 2021). The search for secondary metabolites in marine sponges continues to inspire researchers for several reasons including: (1) the evolution of marine sponges has changed over time enduring alterations in their habitat caused by fluctuating natural conditions, resulting in a flourishing population of marine sponges in Indonesia (Elfahmi & Kayser, 2014). In general, these sponges are able to adapt to changes in their habitat by producing diverse bioactive compounds (De Voogd, 2007); (2) Marine sponges have a unique way of life in which they obtain nourishment by filtering the sediment of the seafloor (Hitora et al., 2011); (3) marine sponges are diverse in their distinctive forms and varied colours, demonstrating the significant metabolic potential of these organisms (Burridge et al., 2020; Marzuki et al., 2021a); and (4) sponge growth is very slow, reaching only approximately 2-5 cm per year (Maarisit et al., 2017); and (5) marine sponges have a unique ability to establish a symbiotic relationship with bacteria, where these microorganisms perform bioremediation functions, including biodegradation and biosorption of global trend pollutants (GTPs), such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, microplastics, pesticide residues, media wastes, and domestic wastes (Marzuki et al., 2022).

These factors indicate that marine sponges have a high potential as a source of secondary metabolites needed by the pharmaceutical industry (Abdel et al., 2014). Chronic diseases, such as cancer, Alzeimer's, and other neurodegenerative diseases, are a major concern in the medical field. Research has shown that these diseases are numerous and tend to be complex, with no truly effective cure currently available (Alanzi & Alsalhi, 2023). In addition to discovering substances for treatment, the pharmaceutical, cosmetic, food, and beverage industries are exploring marine organisms, including sponges for biomaterials used in the development of various pharmaceutical products, including treatments for chronic diseases (Page et al., 2005; Altmann, 2017).

2. Objectives

Researchers continue to investigate natural biomaterials that have the potential to boost the immune system and increase endurance (Martin et al., 2009; Widyarti et al., 2020). In addition, research has explored the potential applications of food additives and cosmetics to fulfill the needs of human life (Fristiohady et al., 2021). Marine sponges have a unique ability to produce a variety of bioactive compounds, largely due to their evolutionary adaptation for survival (Fiore et al., 2013), such as chemicals that defend against predators and competitors, including pollutants such as GTP (Marzuki et al., 2021b; Carroll et al., 2020). The unique habitats of sponges support a diverse microbial community, which also contributes significantly to the chemical diversity found in these organisms. This makes sponges a rich source of compounds with antibacterial, antiviral, antifungal, anticancer, and anti-Alzheimer properties, making them potential candidates for medicinal applications.

Numerous compounds derived from marine sponges have been reported, such as halicondrin, discormolide, hermiasterlin, and arasnastatin-A (Adam et al., 2020). The production of secondary metabolites by marine sponges is likely to be influenced by specific nutritional factors, and typically exhibits a mutualistic relationship with various microorganisms throughout the life cycle of marine sponges (Rohde et al., 2012). Secondary metabolites are biosynthetic compounds derived from primary metabolites and are generally produced by organisms for self-defence against harsh environmental conditions or predators (Karuppusamy, 2009).

This research was conducted with the objectives of (1) collecting and identifying marine sponge species from different localities in Indonesia, (2) preparing n-hexane extracts from the collected specimens, (3) identifying

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possible compounds present in the extracts, (4) initially screening for bioactive compounds with medicinal potential that could also be used in pharmaceutical, cosmetic, and other industries (Marzuki et al., 2021c). The novelty of this research lies in illustrating the steps for identifying potential compounds from sponge species, providing data and material for research into the production of targeted bioactive compounds from sponges.

3. Methods

Materials and methods

Three different types of sea sponges were collected from Barrang Caddi beach. In addition, various materials including methanol (CH_3OH), n-hexane (C_6H_{14}), sodium sulphate (Na_2SO_4), potassium permanganate ($KMnO_4$) solution, distilled water, sterile seawater, ethanol (CH_3CH_2OH) 70%, nickel chloride hexahydrate ($NiCl_2.6H_2O$), nickel (II) sulphate hexahydrate ($NiSO_4.6H_2O$), boric acid (H_3BO_3) and sodium dodecyl sulphate ($NaC1_2H_{25}SO_4$) were used in the experiments. The main equipment used was analytical instruments including GC-MS, FTIR, and a Neubauer haemocytometer. Additional equipment used included an analytical balance, dumbbells, centrifuge, vacuum rotary evaporator, portable water (quality AZ 8361, AZ Instrument Corp., Taichung Taiwan), rotary evaporator, diving equipment, including an Olympus Tg6 type underwater digital camera, buoyancy regulator, tank mask, snorkel, GPS, gloves, gauges, fins and bed commander life jacket, lyophilizer, a microscope was also used.

Sponge identification by morphological method

Sampling was performed in the year 2022 using a random method. Sampling time occurred between 8:00 PM and 11:00 PM utilizing underwater digital camera equipment. Samples were coded, and field data observations, including coordinates, temperature, salinity, pH, and several other parameters categorized as characteristics of the sampling points, were recorded. Samples of the collected marine sponges were morphologically identified using a guidebook (Hooper & Soest, 2002) combined with expert field analysis according to the instructions of the Poripera database (Abdelrahman et al., 2023). The sponge specimen was prepared by cutting the mesohyl components into approximately 2 cm, washed with 70% ethanol, and then examined under a microscope at 40x magnification. In addition, small pieces of the sponge were sterilised using sterile seawater that had been filtered through 0.2µm filter, then mixed, and the resulting cell suspension was examined under a microscope to determine the cell type and structure (Marzuki et al., 2021d).

Extract Preparation

The extraction method used in this study was the maceration technique. Each sponge sample was cleaned, sprayed with sterile seawater, cut into small pieces, and then weighed. The sponge sample was then placed in a maceration container and soaked in methanol as the a solvent. Soaking was carried out for 10 days with daily stirring to homogenize and maximize extraction of chemical constituents from the sample (Malaka et al., 2021). It was then drained and the methanol extract (free of sponge biomass) was obtained. The methanol extract was then concentrated using a rotary evaporator, resulting in a pasty extract (thick extract). Approximately 1g of the thick methanol extract was diluted with 10 mL of methanol, and re-extracted with n-hexane using a separating funnel. This n-hexane extract was then ready for the chemical constituent and functional group analysis using GC-MS and FTIR (Juwita et al., 2021).

GC-MS Analysis of extract composition

The HP 6890 GC-MS instrument was used to detect and quantify of the chemical constituents in the sponge extract. A volume of 1µL of previously diluted n-hexane extract sample was injected into the GC-MS instrument. The column used was a capillary type HP-5MS model 5% (Agilent 19091S-433) with a length of 30m, a diameter of 250m and a thickness of 0.25m. The carrier gas, helium, was operated at a pressure of 10.5psi and a total flow rate of 140mL/min with a split ratio of 1:50 (Marzuki et al., 2021e). The eluted compounds were detected using a mass detector, and the spectra of the known compound component were compared with the National Institute of Standards and Technology (NIST) library (Chong et al., 2019). The similarity of the analysed compounds was determined using the NIST, which provides information on the compound name, area and qualitative abundance of each eluted component (Swierts et al., 2018).

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Detection of Functional Groups of Compounds in the Extract by FTIR

The methanol/n-hexane extract of each sponge sample was analysed in pellet form with the addition of KBR (Watanabe 2007). A Shimadzu brand FTIR spectrophotometer of type IR Spirit-T with a resolution of 0.9cm-1, a DLTG model detector, a signal-to-noise ratio of 30,000 :1, and external dimensions of 250d mm x 390w x 210h and equipped with a temperature controller was used.

Data analysis

The data collected through observation and analysis included sponge sampling points, species of each sample, cell structure and morphology, chromatograms, abundance, quantity, similarity level, retention time data, and names of metabolites from GC-MS readings, as well as characterising functional group of each potential component selected based on FTIR readings (Marzuki et al., 2024). Determination of potential chemical components is based on GC-MS display chromatograms with two parameters, namely quality above 90% and the highest quantity. The selected components are further investigated using FTIR data to determine the functional group as a characteristic of the component (Yang & Liang, 2024; Zhang et al., 2017).

4. Results

Sampling was carried out in the shallow waters around Barrang Caddi Island, which is part of the Spermonde Islands group and is located within the administrative area of Makassar City. Table 1 and Figure 1, show the habitat characteristics of the marine sponge samples collected during this process.

Table 1. Characteristic of ocean sampling points

Code sample	Temperature (^{0}C)	Salinity (‰)	pH	Coordinate		Depth MSL (m)	Distance from the Beach (m)	TDS (mg/L)	EC (ds/m)
				E	S				
A	29.4	29.4	7.3	-5.044496°	119.321139°	5.40	± 340	8.21	16.12
B	30.8	29.2	7.3	-5.049456°	119.330629°	6.10	± 285	8.17	16.69
C	29.5	29.3	7.3	-5.044496°	119.321139°	6.25	± 365	8.23	15.87

TDS: total dissolved solid, EC: electrical conductivity, MSL: mean sea level.

As shown in Table 1, the overall habitat of the marine sponge samples was similar to the general condition of marine waters, indicating that the data for salinity, temperature, dissolved solids, and electrical conductivity at the sampling site were standard and stable. None of the observed parameters mentioned above indicated any potential influence on the growth and development of the marine sponge samples (Marzuki et al., 2020; Emelda et al., 2022).

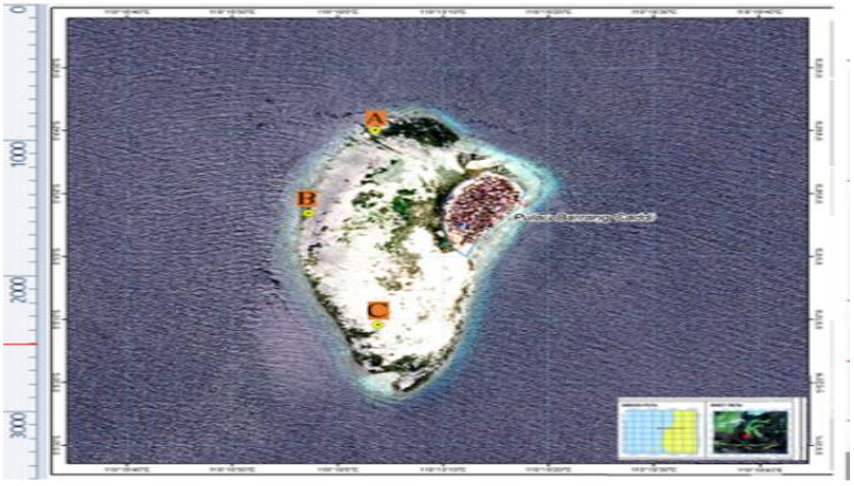

Figure 1. Map of sampling location around Barrang Caddi island. Site A, B, and C respectively sampling points of sponge samples of A, B, and C.

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The morphology of sponges, especially the color and body shape aspects of the three collected samples, are illustrated in live photos taken in their natural habitat on the seabed, presented below (Figure 2). All samples show bright and light colors, indicating that the presence and diversity and variation of chemical components or better known as secondary metabolites are very likely to be present in samples with specific types. This condition has been widely reported by previous researchers (Blunt et al., 2011; Nhiem et al., 2013).

Figure 2. Photo of deep-sea sponge samples (sampling points), the results of morphological analysis found that the species of each sample identified as: A) *Callyspongia* sp; B) *Clathria reinwardti*; and C) *Petrosia* (*Petrosia*) *nigricans*

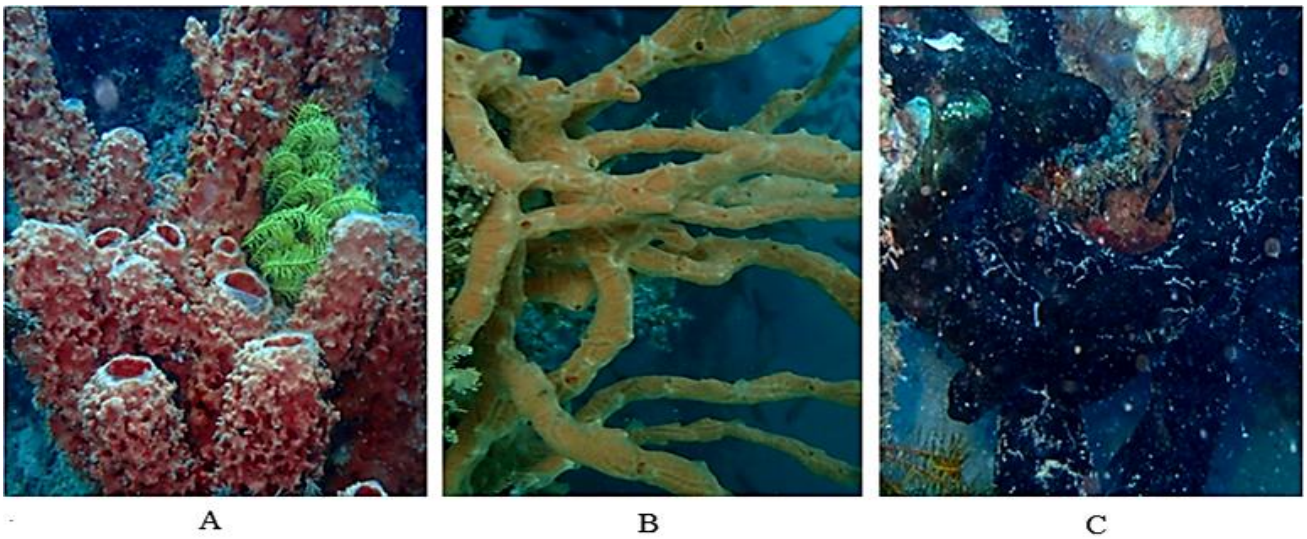
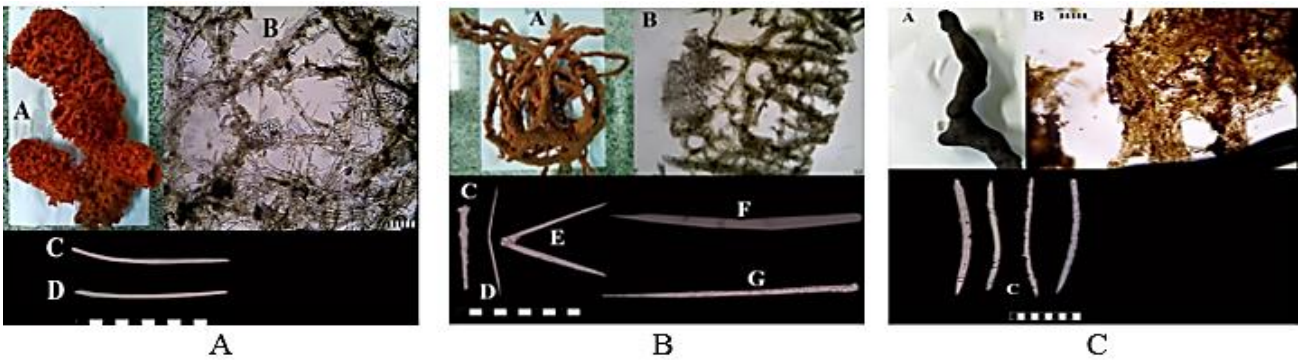


Figure 3. Structure and morphology of sea sponge samples: A) Callyspongia sp. B) Clathria reinwardti, and C) Petrosia (Petrosia) nigricans



A = A) Photograph of *Callyspongia* sp. on the surface; B) example skeleton of the genus *Callyspongia* for comparison; C) and D) *styles spicules* B, C, D, scales 1 = 100µm.

B = A) Photograph of sea sponge *Clathria reinwardti*, B) example skeleton of the genus *Clathria* for comparison; C) *acanthostyles*; D) *oxeote;* E) *toxa;* F) *styles* and G) *subtylostyles spicules* B, C, D, E, F, G, scales 1 = 100 µm.

C = A) Photograph of the sea sponge *Petrosia* (*Petrosia*) *nigricans* Lindgren; B) example skeleton of *Petrosia* genus for comparison; C) *oxea* and *strongylote spicules* B, C, scales 1 = 100µm.

The product obtained from each sponge sample was divided into two different types of extracts namely, the methanol extract and the n-hexane extract as shown in Figures 4-6.

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Figure 4. Extration of sponge samples by maceration using 96% methanol A) *Callyspongia* sp. B) *Clathria reinwardti*, and C) *Petrosia* (*Petrosia*) *nigricans*

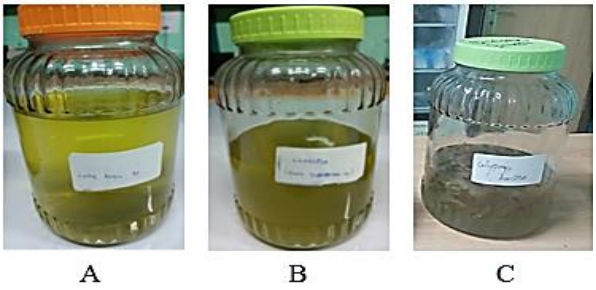


Figure 5. Aqueous extract (methanol macerate) of sponge, A) *Callyspongia* sp. B) *Clathria reinwardti*, and C) *Petrosia* (*Petrosia*) *nigricans*

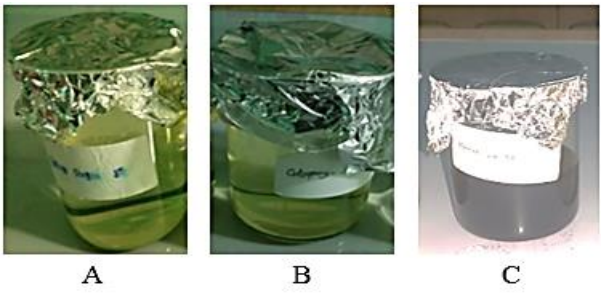
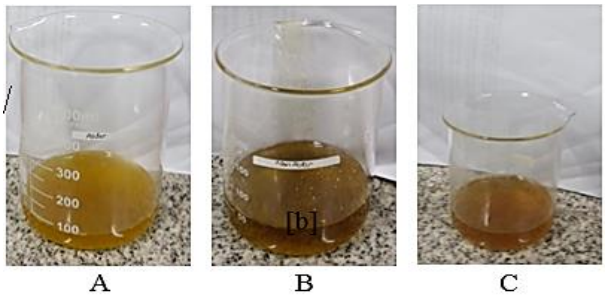


Figure 6. n-hexane extraction: A) *Callyspongia* sp. B) *Clathria reinwardti*, and C) *Petrosia* (*Petrosia*) *nigricans*



Extraction or separation of polar and non-polar chemical components using n-hexane solvent in a separatory funnel was performed to obtain the n-hexane extract of the sea sponge sample (Figure 6). This simple procedure aimed to investigate the chemical component and identify potential secondary metabolites as medical biomaterials or for other applications (Shafeian et al., 2022; Hasan et al., 2023).

5. Discussion

Sponge morphology profile

Morphological examination of the sea sponge samples (Figure 3) effectively identified the species and cellular composition of each sample. Analysis of the cell structures of the three sample types revealed that sample B (*Clathria reinwardti*) had the most complex structure, in contrast to samples A and C. The cell structures of sample B were identified as acanthostyles, toxa, oxeote, and subtylostyles, which were absent in the other samples (Figure 3). This suggests that sample B may have a greater diversity of secondary metabolites compared to samples A and C (Freeman et al., 2016; Marzuki et al., 2024). The colour of marine sponges can reflect the presence of bioactive compounds such as terpenoids, alkaloids and polyphenols (Freeman et al., 2016). For example, brightly coloured sponges (yellow, red, and orange) often contain more complex metabolites than neutrally coloured sponges (Brinkmann et al., 2017; Marzuki et al., 2021a). Sponges contain natural pigments, such as melanin, carotenoids, and flavonoids that impart specific colours (Erwin et al., 2012; Hardoim & Costa, 2014). These pigments sometimes act as secondary metabolites with antimicrobial and antioxidant properties (He et al. 2020). The colour of marine sponges is often associated with the presence of secondary metabolites produced either by the sponge itself or by its symbiotic microorganisms (Blunt et al., 2011; Freeman et al., 2016).

Analysis of medicinally potential substances

The chemical composition of the marine sponge samples was analyzed for secondary metabolites, including retention time, abundance, quantity, quality, and type of components. The aim of this analysis was to identify potential metabolic components that could be classified as secondary metabolites with possible applications in drugs, pharmaceuticals, cosmetics, and food, in particular those from the steroid, carboxylic acid and aldehyde groups that are naturally contained in the n-hexane extract of marine sponges (Shafeian et al., 2022; Balansa et al., 2024). The GC-MS readings and analytical results of the potential metabolites are presented in Tables 2-4, including those with potential uses as medicinal, pharmaceutical, cosmetic, food flavouring materials, and herbal stamina enhancers. The chemical composition of sample A which belongs to the sponge *Callyspongia* sp., is gien in Table 2.

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Table 2. Secondary metabolic components compotition of n-hexane extract in sample A (*Callysponge* sp).

Number	Peak number	Retention Time	Quantity (%)	Quality (%)	Component
1	1	13.299	0.272	96	Phenol, 2,4-bis(1,1-dimethylethyl)
2	6	15.382	0.424	98	Tetradecanoic acid
3	7	15.682	6.883	93	***2-(phenylmethylene)-innamaldehyde
4	10	16.218	0,368	95	2-Ethylhexyl salicylate
5	11	16.285	0.997	99	i-Propyl tetradecanoate
6	12	16.567	0.373	95	Pentadecanoic acid
7	13	16.698	5.313	99	***Hexamethyl-pyranoindane
8	16	17.150	0.493	99	Hexadecanoic acid
9	17	17.270	1.803	90	Trifluoroacetic acid
10	19	17.521	2.150	93	Dibutyl phthalate
11	27	18.572	3.716	99	9-Octadecenoic acid
12	34	19.118	0.201	98	3-(4-methoxyphen)
13	48	21.763	0.264	91	1,2-Benzenedicarboxylic acid
14	69	26.547	21.715	97	***Cholest-5-en-3-ol

Analysis of the polar chemical constituents identified 38 peaks, with 14 of which had an abundance or similarity of at least 90% (Table 2). The abundance of metabolites highly similar to those in the *Callyspongia* sp. sample was 44.33%. Of the 24 suspected secondary metabolites, 55.67% had a similarity level of less than 90%. The potential dominance or abundance of the 14 components and three components was analysed: 2-(phenyl-methylene)-innamaldehyde, hexamethyl-pyranoindane, and cholest-5-en-3-ol were identified as having potential medicinal activities (Zhou et al., 2010; Sahidin et al., 2018).

The chemical composition of metabolites in sample B (*Clatria reinwardti*) was comparable to that of sample A in terms of the pattern and composition of the secondary metabolites. The only difference was in the number of potential components identified and the overall abundance of metabolic components (He et al., 2020; Adam et al., 2020). A detailed summary of the analytical results for sample B is given in Table 3.

Table 3. Secondary metabolic component compotition of n-hexane extract in sample B (*Clatria reinwardti*).

Number	Peak number	Retention time	Quantity (%)	Quality (%)	Component
1	1	15.956	1.184	98	Methyl 13-methyltetradecanoate
2	3	16.763	1.794	94	Dioctadecyl ester
3	4	17.143	7.892	97	***Pentadecanoic acid
4	6	17.664	0.796	93	Hexadecanoic acid
5	9	18.746	1.436	98	Methyl stearate
6	10	20.240	1.743	92	N-ethyl-1,3-dithioisoindoline
7	11	21.753	2.158	93	1,2-Benzenedicarboxylic acid
8	13	21.755	2.701	91	3-Nitrophthalic acid
9	14	24.032	1.601	94	2-P-Nitrophenyl-1,3,4-Oxadiazol-5-one
10	15	24.044	2.664	97	2-Methyl-5H-dibenz[b,f]azepine
11	17	24.366	1.130	95	1,2-Benzisothiazol-3-amine
12	19	24.915	1.831	92	2-Naphthalene-sulfonic acid

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13	21	26.531	1.028	91	Tetrasiloxane
14	22	26.545	25.483	95	***Cholest-5-en-3-ol
15	23	26.622	0.569	91	Trimethylsilane
16	24	26.667	1.916	92	Hexamethyl-imethylsiloxane cyclic trimer
17	25	27.135	7.783	94	***Ergosta-5,22-dien-3-ol
18	26	27.897	6.436	90	***Pregna-5,16-dien-20-one
19	27	27.957	2.237	91	Acetamide, N-[4-(trimethylsilyl)phenyl
20	26	27.981	2.355	95	Decamethyltetrasiloxane
21	27	29.350	1.815	92	2-(Acetoxymethyl)-3-biphenylene
22	33	29.377	5.611	92	***gamma.-Sitosterol/ Stigmast-5-en-3-ol

The total number of peaks or components was 71, but only 22 metabolic components showed a similarity ≥ 90%. The combined abundance of these 22 chemical components was 80.79%. There were 49 secondary metabolic components (19.21%), with a quantification or similarity level was < 90% (Table 3). Of the 22 identified metabolites, only 5 were dominant based on their abundance: Pentadecanoic acid, Cholest-5-en-3-ol, Ergosta-5,22-dien-3-ol, Pregna-5,16-dien-20-one, and Gamma-Sitosterol/ Stigmast-5-en-3-ol (Otha et al., 2013; Santiago et al., 2019; Utami et al., 2024).

The analysis of sample B (Table 3) showed that the *Clatria reinwardti* sample had a greater diversity of metabolic components than the *Callyspongia* sp and *Petrosia (Petrosia) nigricans* samples (Gabriel et al., 2015). This finding supports the initial hypothesis derived from the morphological examination of sample B, which revealed a more complex cellular structure. The complexity of a sponge's cellular structure typically corresponds to the diversity and variation of its metabolic components, including pigmentation variations that serve as metabolic markers (Maarisit et al., 2017). The metabolic components observed in sample C, corresponding to the *Petrosia (Petrosia) nigricans*, were also broadly similar to the metabolic patterns and compositions observed in samples A and B (Kaliaperumal et al., 2023). A detailed breakdown of these comparisons is given in Table 4.

Table 4. Secondary metabolic component composition of n-hexane extract in sample C (*Petrosia* (*Petrosia*) *nigricans*)

Number	Peak number	Retention Time	Quantity (%)	Quality (%)	Component
1	4	16.281	1.543	98	i-Propyl tetradecanoate
2	6	17.144	6.721	99	Hexadecanoic acid
3	7	17.410	0.896	93	Palmitinic acid
4	8	17.700	2.647	94	Ethyl tridecanoate acid
5	10	17.944	3.405	91	i-Propyl hexadecanoate
6	12	18.566	5.097	99	9-Octadecenoic acid
7	13	18.748	1.324	99	Methyl stearate
8	14	19.071	0.969	96	Oleic acid
9	15	19.247	1.128	95	Ethyl octa decanoate
10	18	20.239	2.247	95	2-Ethylhexyl trans-4-methoxycinnamate
11	21	21.755	12.702	91	***1,2-Benzenedicarboxylic acid
12	25	24.043	4.667	92	2-Methyl-5H-dibenz[b,f]azepine
13	26	24.366	3.133	90	1,2-Benzisothiazol-3-amine
14	27	24.915	1.731	91	2-Naphthalene-sulfonic acid
15	28	25.952	3.567	90	Methyl 3,5-bis(ethylamino)benzoate

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16	30	26.555	6.968	98	***Cholest-5-en-3-ol
17	31	27.135	7.784	96	***Ergosta-5,22-dien-3-ol
18	33	27.896	2.411	90	12-Hydroxyabieta-8
19	34	27.967	5.766	93	Acetamide, N-[4-(trimethylsilyl)phenyl

The total number of components in the study was 39 of which 19 (72.84%) were identified as potential secondary metabolic compounds based on a similarity level of ≥ 90% (Setiawan et al. 2016). The remaining 20 components (27.14%) were classified as secondary metabolic, derivative, transition, or impurity components (Setiawan et al. 2016). Three of the 19 components were recognised as the dominant metabolic components in the GC-MS chromatograms and are believed to exhibit specific metabolic activities, medicinal properties, or potential applications as herbal ingredients to enhance stamina production (Nhiem et al., 2013; Reverter et al., 2018). These constituents include 1,2-benzenedicarboxylic acid, cholest-5-en-3-ol, and ergosta-5,22-dien-3-ol (Velu et al., 2018).

Functional group analysis of dominant metabolic components by FTIR

Infrared spectroscopic data were analyzed to confirm the dominant functional groups as characteristic components of the metabolic profile, in particular the chemical components found in the n-hexane extract of the three sponge samples analysed. The methanol extract of sample A showed 27 peaks, suggesting the presence of different chemical components in the metabolic profile of *Callyspongia* sp. However, the analysis of the methanol extract revealed only three dominant components, which had a similarity level of less than 90%. Therefore, it was considered insignificant and unsuitable for potential metabolic screening (Gauvin et al., 2004; Villegas et al., 2019).

The primary metabolic constituent in sample A (*Callyspongia* sp.), the n-hexane extract, was 2-(phenylmethylene)-innamaldehyde, an organic compound belonging to the aldehyde class. This substance has a carbonyl group (C=O), an aromatic carbon double bond (C=C), and a carbon-hydrogen bond (C-H), thus forming an aldehyde group, which is a defining characteristic. FTIR spectra were recorded at wave numbers 1700 cm^{-1}, 1750 cm^{-1}, and 860 cm^{-1}, indicating the presence of carbonyl groups (C=O), aromatic carbon bonds (C=C), and C-H aldehyde (Juwita et al., 2021). Functional groups typically found in hexamethyl-pyranoindane compounds include the carbonyl carbon ketone group (C=O), aromatic carbon skeleton bond (C=C), and aromatic hydroxyl functional group (O-H) (Marzuki et al., 2021c; Abraham et al., 2021).

The FTIR chromatogram confirmed the presence of spectra with wave numbers 1720 cm^{-1}, 1540 cm^{-1}, and 3450 cm^{-1} indicating the presence of C=O, C=C, and O-H groups respectively (Ruiz et al., 2013). The most abundant substance in sample A is cholest-5-en-3-ol, which is a cholesterol compound (Marzuki et al., 2021d). This compound has characteristic functional groups such as ketones, ethers, alkenes, and alkanes. Based on the FTIR spectrum analysis, the presence of functional groups was confirmed at 1760 cm^{-1}, 1250 cm^{-1}, 1610 cm^{-1}, and 1930 cm^{-1}. According to the data obtained from these functional groups, the n-hexane extract of sample A of Callyspongia sp. contained three dominant metabolic components (Pailee et al., 2017; Abraham et al., 2021; Kaliaperumal et al., 2023).

Spectral analysis of the n-hexane extract from the *Clatria reinwardti* sponge sample revealed five dominant components, including pentadecanoic acid. This compound has characteristic functional groups such as carbonyl carboxylate (C=O), hydroxyl (O-H), and alkane (C-H), and the FTIR chromatogram confirmed the presence of spectra with wave number values corresponding to these functional groups at 1700 cm^{-1}, 3600 cm^{-1}, and 1340 cm^{-1} respectively. The functional groups characteristic of the cholest-5-en-3-ol/cholesterol component correspond to those of the components in sample A, as previously described by Gauvin et al (2004) and Utami et al. (2024). Ergosta-5,22-dien-3-ol; Pregna-5,16-dien-20-one; and gamma-sitosterol/stigmast-5-en-3-ol, all share similar molecular structures and patterns with cholesterol (Marzuki et al., 2021e). These three metabolic components have relatively similar functional groups responsible for their chemical properties, including hydroxyl groups (O-H), carbonyl groups from ketones (-C=O), carbon double bonds (C=C-), and

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carbon-hydrogen bonds from alkanes (C-H). The functional groups have spectra with wave numbers in the range of 3650 cm^{-1},1640 cm^{-1}, and 2940 cm^{-1} (Ruzicka & Gleason, 2008; Galitz et al., 2021).

Sample C contained Petrosia (Petrosia) nigricans as the predominant component. The main functional group present in this sample is 1,2-benzenedicarboxylic acid, which is characterised by two carbonyl groups from ketones (C=O), two hydroxyl groups (O-H), and aromatic carbon-carbon double bonds (C=C) (Hitora et al., 2011; He et al., 2015). This functional group produces a spectrum at wave numbers of 1720 cm^{-1}, 3220 cm^{-1}, and 1550 cm^{-1}. Cholest-5-en-3-ol was also a dominant compound in samples A and B, and thus the characteristic functional groups and the resulting spectrum were identical. Similarly, ergosta-5,22-dien-3-ol is a prominent component in the n-hexane extract of Clatria reinwardti sponge, and its functional groups and spectra have been described previously (Hentschel et al., 2002; Adam et al., 2020).

Potential metabolites for drug development

Analysing the primary metabolic components present in each sample, we identified three components in samples A, B, and C. Table 5 lists these primary metabolic components and their possible biological activities.

Table 5. Potential medicinal secondary metabolite components and biological activities

Sample Code	Sponge sample name	Analysis parameters	Components of n-hexane extract
A	*Callyspongia* sp.	Number of chemical components	14/38 compound components
		Dominant chemical component	1) 2-(phenylmethylene)-innamaldehyde 2) Hexamethyl-pyranoindane 3) Cholest-5-en-3-ol
		Potential benefits and biological activity	1) Food flavoring, fragrance, mosquito repellent 2) Fragrances, detergents 3) Growth, increased stamina
B	*Clatria reinwardti*	Number of chemical components	22/71 compound components
		Dominant chemical component	1) Pentadecanoic acid 2) Cholest-5-en-3-ol/ 3) Ergosta-5,22-dien-3-ol 4) Pregna-5,16-dien-20-one 5) gamma.-Sitosterol/ Stigmast-5-en-3-ol
		Potential benefits and biological activity	1) Immunity, anti-fungal 2) Growth, increased stamina 3) Dietary supplements, stamina, vit precursors. D 4) Strengthens the bladder and prevents miscarriage 5) Facilitates urine output, increased stamina
C	*Petrosia (Petrosia) nigricans*	Number of chemical components	19/39 compound components
		Dominant chemical component	1) 1,2-Benzenedicarboxylic acid 2) Cholest-5-en-3-ol 3) Ergosta-5,22-dien-3-ol
		Potential benefits and biological activity	1) Anti-pathogenic bacteria (AHL)

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			2) Growth, stamina-boosting herbs 3) Dietary supplements, stamina, vit precursors. D

Based on the functional groups that appeared to determine the nature of the metabolic components in the sea sponge sample (Table 5), all organic compounds contained alcohol, aldehyde, carboxylic acid, and ester groups. The metabolic components consisted of four elements: ergosta-5,22-dien-3-ol, pregna-5,16-dien-20-one, gamma-sitosterol/stigmas-5-en-3-ol, and hexametyl-pyrnoindane (Tanokashira et al., 2016), which were identified in sample A and B. Sample A also included 2-(phenylmethylene)-innamaldehyde as the only aldehyde group compound. Sample B contained pentadecanoic acid as the only carboxylic acid group, while sample C contained 1,2-benzenedi carboxylic acid. Cholest-5-en-3-ol, the only ester group compound, was present in all samples (Zhou et al., 2015; Watanabe et al., 2007).

The potential applications of these metabolic components are extensive and varied, including their use in the food industry, dietary supplements, and herbs to enhance stamina, growth, and immunity. Some of these compounds (Table 5) have biological activities as antibacterial and antifungal agents (Du et al., 2021; Emelda et al., 2018; Gung & Omollo, 2008). The results of the morphological analysis and chemical identification suggested a correlation between the number and brightness of colours in sponges and the type and variety of metabolic components present (Armus et al., 2021; Snehlata et al., 2018). Therefore, it is recommended to select marine sponges for the identification of metabolic components for specific application by selecting sponges with a variety of bright and vivid colours (Marzuki et al., 2021e; Ruiz et al., 2013).

6. Conclusions

The major aims of this study was to identify the secondary metabolites in marine sponges collected from Makassar, Indonesia. By analyzing the metabolic components of marine sponges through morphological examination, component identification, and apparent functional groups, valuable conclusions can be drawn as follows: (1) Sponge sample B (*Clatria reinwardti*) has a more diverse range of metabolic components than sample A (*Callyspongia* sp.) and sample C (*Petrosia (Petrosia) nigricans*); (2) the organic compounds successfully identified in the sponge metabolites belong predominantly belong to the alcohol/phenol group, esters, carboxylic acids, and aldehydes; (3) cholest-5-en-3-ol (25.48%), benzenedicarboxylic acid (12.70%), pentadecanoic acid (7.89%), and ergosta-5,22-dien-3-ol (7.78%) were the most abundant metabolites found in all samples; (4) Several metabolites of marine sponges have been identified as potential endurance and growth-enhancing biomaterials, as well as exhibiting antibacterial and anti-fungal biological activities; and (5) Brightly colored sponges typically possess a more intricate and complete cell structure and may harbor a range of beneficial metabolites. This study provides valuable data for further research to isolate and evaluate bioactive compounds such as steroids, carboxylic acids, aldehydes or their derivatives for pharmaceutical and medicinal purposes.

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Conflict of Interest

There is no conflict of interest in this research.

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