Bioactive Compounds of Marine Sponge *Amphimedon viridis* and *Aaptos pernucleata* Using GC-MS Analysis

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Abstract. Marine sponges are rich sources of bioactive compounds with a wide range of structures and potential applications. This study aims to examine the bioactive compounds in marine sponges *Amphimedon viridis* and *Aaptos pernucleata* by using GC-MS analysis of ethanol extracts. The GC-MS analysis revealed that *A. viridis* contained diethyl phthalate (30.73%), cholesterol (12.21%), desmosterol (3.25%), hexadecenoic acid methyl ester (3.44%), 9-octadecenoic acid methyl ester (2.18%), and gamma sitosterol (1.33%). The major compounds found in *A. pernucleata* were diethyl phthalate (28.86%), bis (2-ethylhexy) phthalate (18.71%), 9-octadecenoic acid, 12-hydroxy-methyl ester (10.07%), cholecalciferol (8.24%), and cyclopenta [g]-2-benzopyran, 1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (2.90%). These bioactive compounds have promising potential in the development of new pharmaceuticals and therapeutic agents. This study provides an overview of the chemical composition of ethanol extract from marine sponges *A. viridis* and *A. pernucleata*, as well as their beneficial effect on biological activity.

Keywords: *Amphimedon viridis, Aaptos pernucleata,* chemical compound, ethanol extract, GC-MS

1. Introduction

In the medical world, sponges and their associated microorganisms present an enormous potential for exploration. An improper exploration, however, will destroy habitats and will be extremely costly. In addition to natural products, secondary metabolites are chemical compounds that are produced by living organisms like plants, invertebrates, and bacteria. Clinical applications of these technologies are widespread and contribute significantly to modern medicine [1]. Due to the fact that the ocean contains a variety of living organisms, and it covers more than 70 percent of the earth's surface, natural products can be researched in the ocean [2]. Researchers discovered 2659 marine natural products in sponges between 2010 and 2019. As a result, 47.2% of all marine natural products invertebrates are found in this species. Several studies have demonstrated that sponges contain high levels of bioactive chemicals, which has led to their description as chemical factories or gold mines [4]. The sponge phylum Porifera dates back over 580 million years, making it one of the oldest groups of animals [5,6]. Feeding on bacteria and particles the size of bacteria, they are attached to a hard

substrate in the ocean. The sponge also plays a role in water filtration. Sponge is an essential component of the benthic zone worldwide, found in freshwater, marine, intertidal, and deep sea environments [7].

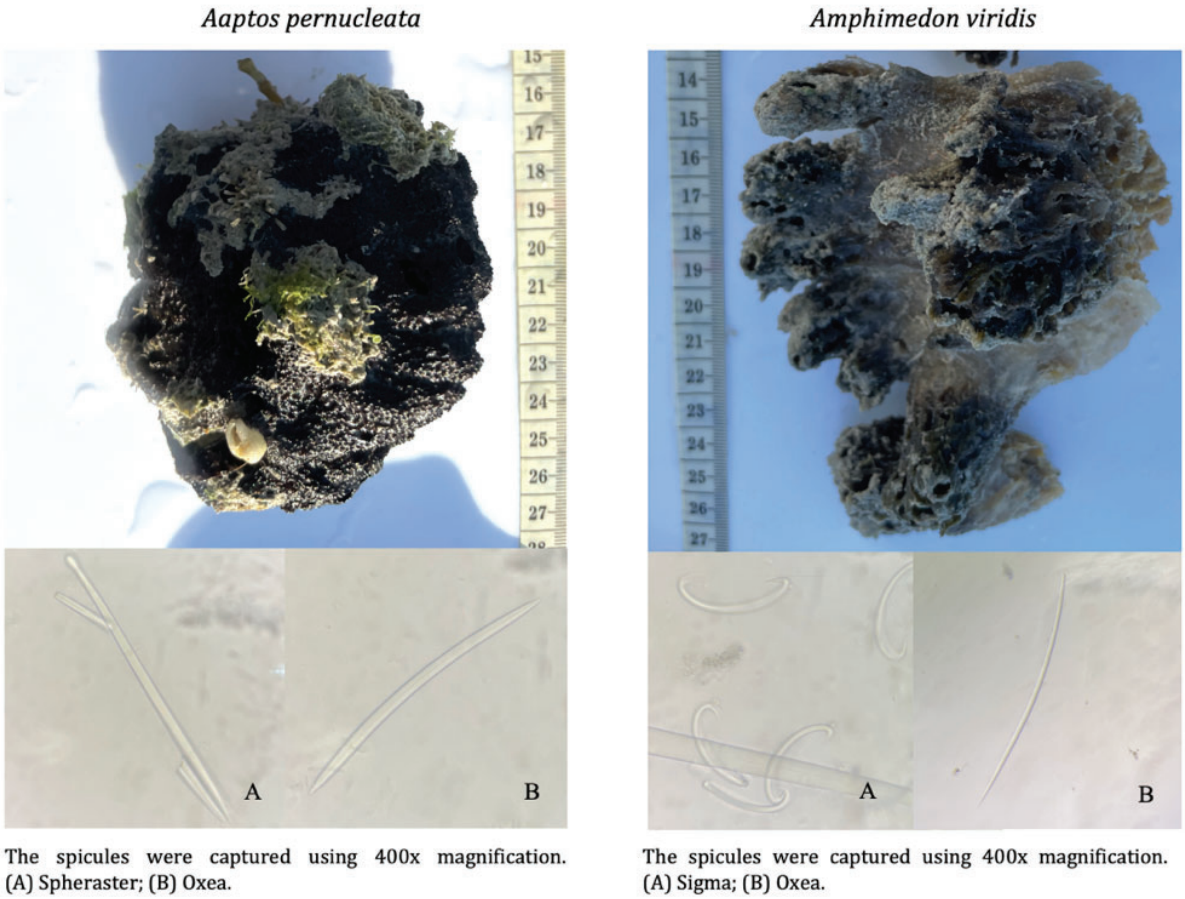
They are classified in the families Demospongiae, Hexactinellida, Homoscleromorpha, Hymedesmiidae, and Calcarea, with approximately 15,000 species identified in these families [9,10]. It is also due to the close relationship sponges have with bacteria, fungi, and viruses that they continue to be so interesting [11]. It has been reported that microorganisms create a variety of symbiotic relationships with hosts, accounting for 50–60% of host biomass [12]. These associations include extracellular exosymbioses, extracellular endosymbioses, intracellular symbioses, and intranuclear symbioses. The interaction between sponges and microorganisms is complicated due to the metabolites produced by sponges, which selectively increase microbial populations [13]. Several environmental stresses, including intense competition, overgrowth, toxicity, infection, and predation, have led to the evolution of secondary metabolites as sponge defense mechanisms [14]. Currently, more than 50 compounds have been identified as active substances, including sterols, alkaloids, terpenes, fatty acids, amino acid derivatives, and cyclic peptides. This study investigated the potential for developing new drugs from marine sponge-derived bioactive compounds. However, researchers have increasingly discovered that these weapons are not constructed by sponges but rather by microorganisms [15]. Sponge interiors and surfaces contain more nutrients than seawater, providing an ideal environment for microorganisms to grow. Chemical defense mechanisms of sponges are also developed by microorganisms. Furthermore, sponges produce a large number of secondary metabolites to repel predators, reduce competition with other sessile marine organisms, and to deter nest predators [17]. Marine sponges have positive effects on tumors in terms of chemoprevention and chemotherapy. *Tethya crypta* sponge was discovered by Bergmann & Feeney in the 1950s as a source of the anticancer agent vidarabine.In marine sponges, secondary metabolites have been found to have positive effects on tumors in terms of chemoprevention and chemotherapy. Bergmann & Feeney isolated the anticancer agent vidarabine from *Tethya crypta* sponge in the 1950s [18].

A widely distributed Indo-Pacific sponge, *Amphimedon viridis*, has been extensively investigated due to its unique secondary metabolite profile. Several studies have reported that diketopiperazines and other nitrogen-containing heterocyclic compounds have been isolated from this sponge. There is evidence that it contains several potent toxic compounds, including purified mixtures of halitoxin and amphitoxin, both of which are highly bioactive pyridinium alkaloids [19]. Fish are likely to be affected by these compounds in terms of their antifeedant properties. Two species of sympatric wrasse have been demonstrated to be deterred by the combined crude organic extracts of *A. viridis* [20]. However, the effects of spicules as potential deterrents have not been investigated. *A. viridis* and *A. pernucleata* are marine sponge species known for their diverse bioactive compounds [21,22]. These compounds have garnered significant attention due to their potential pharmaceutical and therapeutic applications [23,24]. The genus Aaptos contains sponges such as Aaptos suberitoides which have been studied for decades. Alkaloids extracted from these types of sponges, such as aaptamine, have been shown to exert strong anticancer activity in a variety of cancer cells by inhibiting proliferation, apoptosis, and proteasome activity [25]. This study aims to analyze the bioactive compounds within *Amphimedon viridis* and *Aaptos pernucleata* marine sponges extracted with ethanol using GC-MS.

2. Materials and Methods

2.1 Sample Collection and Identification

Sponge samples were collected from the mangrove habitat area on the West Coast, Pari Island, Thousand Islands, DKI Jakarta. *Amphimedon viridis* and *Aaptos pernucleata* marine sponges were identified macroscopically and microscopically. Macroscopic identification includes the morphological form and texture of the sponge, such as size, oscula, consistency (solid, soft, hard), body surface, and color [26], while microscopic identification of sponges was done by observing the spicules of the sponge as seen in Figure 1. The material was cleaned using distilled water to remove residual chlorine, and then the spicules were observed under a microscope [27,28]. Genus-level identification follows Van Soest & Hooper [29], and species-level identification based on spicule observations follows Ekins et al., [30]. A distribution and status assessment was conducted by using the World Porifera Database (WPD) by Van Soest et al., [31] and the World Register on Marine Species (WoRMS) (http://www.marinespecies.org).



The spicules were captured using 400x magnification. (A) Spheraster; (B) Oxea.

The spicules were captured using 400x magnification. (A) Sigma; (B) Oxea.

Figure 1. The spicules morphology of *Aaptos pernucleata* and *Amphimedon viridis*.

2.2 Sample Preparation

The sponge was first washed thoroughly with fresh water to remove dirt particles, salt content, and substrate in the extraction stage. The sponge was cut into small pieces and crushed using a blender. The sample was macerated using ethanol (96%) with a sample-to-solvent ratio of 1:3 (w:v). The sample was soaked for three nights; then, the filtrate was meticulously separated through a sterile cloth, ensuring sample purity. At 40°C, the filtrate was filtered on Whatman 40 filter paper and evaporated. Subsequently, the crude extract was collected into an airtight plastic micro tube and stored in a refrigerator for further chromatographic analysis. Chromatographic analysis is used to separate and identify the different compounds present in the crude extract.

2.3 GC-MS analysis of Amphimedon viridis and *Aaptos pernucleata*

The chemical composition of ethanol extracts from *A. viridis* and *A. pernucleata* samples was determined using GC-MS (Gas Chromatography interfaced with Mass Spectrometer). Injecting sample components was made possible by a split injector on the Agilent Technologies 5977B. The oven temperature was raised by 6°C/min from 70°C to 260°C. Helium was flown at a rate of 1.0 mL/minute. Peaks were obtained through the analysis of mass spectra. The oven temperature was raised from 70°C to 260°C at 6°C/min. It was carried with helium at a flow rate of 1.0 mL/min. Mass spectra were analyzed to obtain the peaks. Compounds were identified by comparing their molecular weights and molecular masses with the NIST and PubChem NCBI libraries.

3. Results and Discussion

Analyzing bioactive compounds from marine sponges from *Amphimedon viridis* and *Aaptos pernucleata* using Gas Chromatography-Mass Spectrometry (GC-MS) can provide detailed insights into their chemical profile. GC-MS is particularly effective for identifying volatile and semi-volatile organic compounds. Chromatograms and mass spectra can be generated based on gas chromatography for the identification of secondary metabolites in plants and animals. Quantification of the determination volatile chemical compounds in 90% ethanol extract is shown in Table 1 and Table 2. As shown in Figures 2 and 3, the chemical structure of *Aaptos pernucleata* and *Amphimedon viridis* can be summarized. The total bioactive compounds found in the ethanol extract of *Aaptos pernucleata* were 15 bioactive compounds. While the ethanol extract of *A. viridis* had 19 bioactive compounds. The major compounds found in *A. pernucleata* are diethyl phthalate (28.86%), bis (2-ethylhexy) phthalate (18.71%), 9-octadecenoic acid, 12-hydroxy-methyl ester (10.07%), cholecalciferol (8.24%), and cyclopenta [g]-2-benzopyran, 1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (2.90%). In *A. viridis* sponge, the major compounds found were diethyl phthalate (30.73%), cholesterol (12.21%), desmosterol (3.25%), hexadecenoic acid methyl ester (3.44%), 9-octadecenoic acid, methyl ester (2.18%), and gamma sitosterol (1.33%).

Table 1. GC-MS analysis of the bioactive compounds in *Aaptos pernucleata's* ethanol extract.

No.	Compound Name	Area (%)	Quality	Biological activities	References
1	Diethyl Phthalate	28.86	98	anticancer	[33]
2	Cyclopenta [g]-2-benzopyran, 1,2,4,6,7,8-	2.90	99	antitumor	[34]

	hexahydro-4,6,6,7,8,8-hexamethyl				
3	Hexadecanoic acid, methyl ester	2.37	99	antimicrobial, antioxidant	[35,36]
4	9-Octadecenoic acid, methyl ester	1.14	99	antiproliverative, antioxidant	[37]
5	4-Hydroxy-2-methyl-6-oxo-5-phenyl-1,6-dihydro-pyridine-3-carboxylic acid ethyl ester	1.39	47	anticancer	[38]
6	9-Octadecenoic acid, 12-hydroxy-methyl ester, [R-(Z)]	10.07	94	anticancer, antioxidant	[39]
7	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)	1.08	72	No activity reported	-
8	Icosa-9,11-diyne	1.50	90	No activity reported	-
9	Cyclobutane, (1-methylethylidene)	2.36	30	antimicrobial	[40]
10	Bis (2-ethylhexy) phthalate	18.71	91	Antibacterial, antioxidant, anticancer	[41,51]
11	4-Hydroxy-5-(3-methyl-5-phenyl-2H-pyrazo)-4yl) benzene-1,2-dicarbonitrile	7.18	51	No activity reported	-
12	Bicyclo [8.2.0] dodecane-11-one, 12,12-dichloro-, (1R*,10S*)	1.25	11	No activity reported	-
13	Cholestrol	1.95	95	anticancer	[42]
14	Cholecalciferol	8.24	98	anticancer	[43]
15	2H-1-Benzopyran-2-one, 7-(4-methyl-5phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl	2.92	35	No activity reported	-

Tabel 2. GC-MS analysis of the bioactive compounds in *Amphimedon viridis's* ethanol extract.

No.	Compound Name	Area (%)	Quality	Biological activities	References
1	Diethyl Phthalate	30.73	98	anticancer	[33]
2	Cyclopenta [g]-2-benzopyran, 1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl	4.13	99	antitumor	[34]

3	Pentadecanoic acid, 14-methyl, methyl ester	1.11	97	Antidiabetic, antiviral	[44]
4	9-Hexadecanoic acid, methyl ester, (Z)	1.02	99	Anticancer, antioxidant	[45]
5	Hexadecanoic acid, methyl ester	3.44	99	antimicrobial, antioxidant	[35,36]
6	9-Octadecenoic acid, methyl ester	2.18	99	antiproliverative, antioxidant	[37]
7	Methyl stearate	2.68	99	Antioxidant, anticancer	[46]
8	5,8,11,14-Eicosatetraenoic acid, methyl ester	1.06	99	No activity reported	-
9	1,2-Cyclohexanediol, cyclic sulfite	1.88	25	No activity reported	-
10	dl-Chimyl alcohol	5.83	92	No activity reported	-
11	Methyl 7,10,13,16-docosatetraenoate	1.01	97	No activity reported	-
12	15-Tetracosenoic acid, methyl ester	1.59	99	antitumor	[47]
13	1,22-Docosanediol	3.45	25	No activity reported	-
14	Methyl-5,22-dien-3-ol, (3.beta)	1.26	98	No activity reported	-
15	Cholesta-5,22-dien-3-ol, (3.beta)	1.35	94	No activity reported	-
16	Cholesterol	12.21	99	anticancer	[42]
17	Desmosterol	3.25	99	anticancer	[48]
18	Ergosta-5,234 (28)-dien-3-ol, (3.beta)	2.33	99	anticancer	[49]
19	Gamma sitosterol	1.33	99	anticancer	[50]

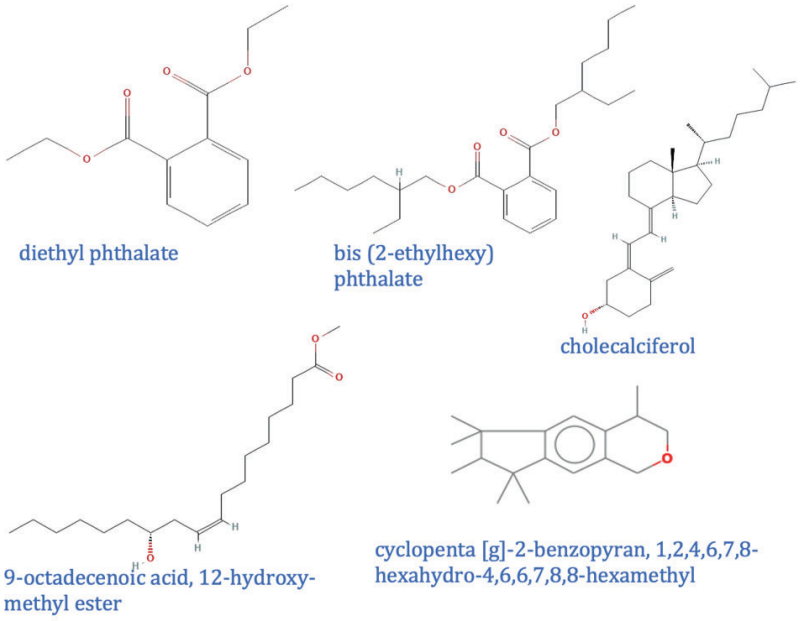


Figure 2. A summary of the chemical structure of *Aaptos pernucleata* with an extract of ethanol.

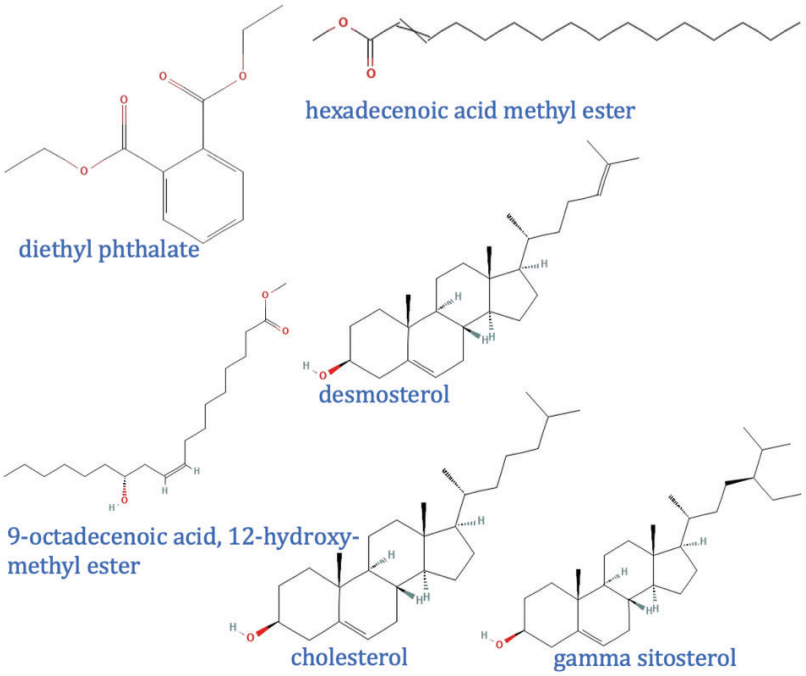


Figure 3. A summary of the chemical structure of *Amphimedon viridis* with an extract of ethanol.

The results of the chromatogram in the ethanol extract of *Aaptos pernucleata* and *Amphimedon viridis* sponges using GC-MS analysis are shown in Figure 4 and Figure 5. Many of the volatile compounds, such as fatty acid compounds, were found in the ethanol extract of *A. pernucleata* and *A. viridis* sponges. Fatty acids are an important source of bioactive compounds, including 9-octadecenoic acid, hexadecenoic acid, pentadecanoic acid, and gamma sitosterol. According to the GC-MS results, the ethanol extract of *A. pernucleata* and *A. viridis* sponges contains a wide range of volatile compounds, including terpenoids and fatty acids. One of the volatile bioactive compounds is included in the monoterpene and sesquiterpene compound groups. In addition to exerting antioxidant effects, bis(2-ethylhexyl) phthalate exhibits antitumor properties [50]. For example, a study published from Moushumi Priya & Jayachandran [51] demonstrated that bis(2-ethylhexyl) phthalate (BEHP) inhibited the proliferation of human erythroleukemic K562 cells. Aside from regulating mitochondrial enzymes, BEHP was capable of inducing caspase-dependent apoptosis. Bis-(2-ethylhexyl) phthalate was evaluated as an antibacterial and larvicidal compound against *Culex quinquefasciatus* Say larvae. It exhibited significant acetylcholinesterase inhibition activity and DNA damage [52].

Moreover, gamma-sitosterol (γ-Sitosterol) is an effective anticancer steroid [53]. IC_{50} values of 493.31 g/mL and 696.61 g/mL, respectively, have been demonstrated for isolation of γ-Sitosterol from *Acacia nilotica* extract in human breast cancer cell MCF-7 and human lung cancer cell A549 after 48 hours of treatment [53]. Gamma-sitosterol works by interfering with the growth and spread of cancer cells through multiple pathways. It has been shown to inhibit cell proliferation by blocking specific signaling pathways that cancer cells rely on for growth. Gamma-sitosterol can induce apoptosis in cancer cells, promoting their programmed cell death and reducing tumor size. Bioactive compounds from marine sponges present several challenges, primarily due to these organisms' complex and diverse chemical nature. The goal of extracting pure compounds is to separate them from a mixture of structurally similar compounds by utilizing sophisticated extraction techniques. Moreover, the difficult extraction and production of these valuable compounds are complicated by the limited availability of sponge materials and the intricate symbiotic relationships within their natural habitats.

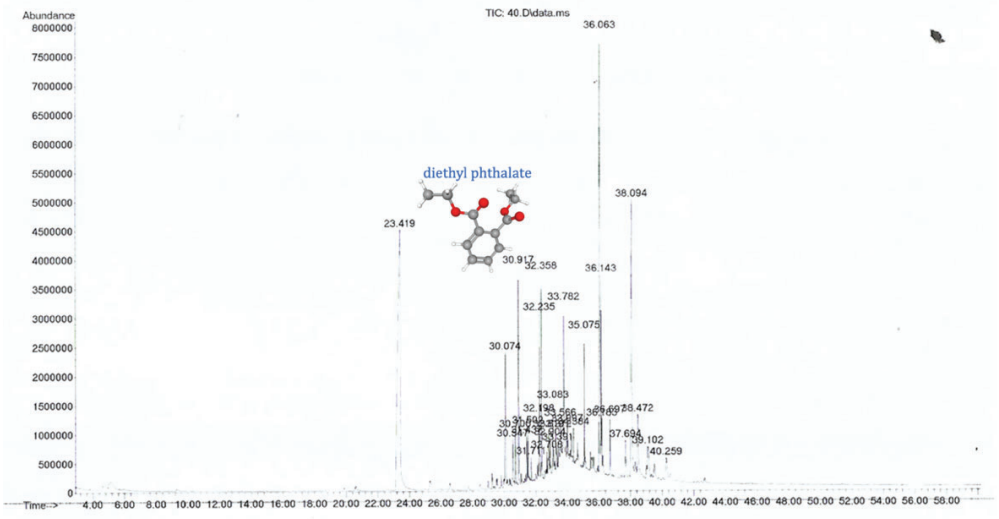


Figure 4. Determination of chromatogram of *ethanol extract* of *Amphimedon viridis* using GC-MS.

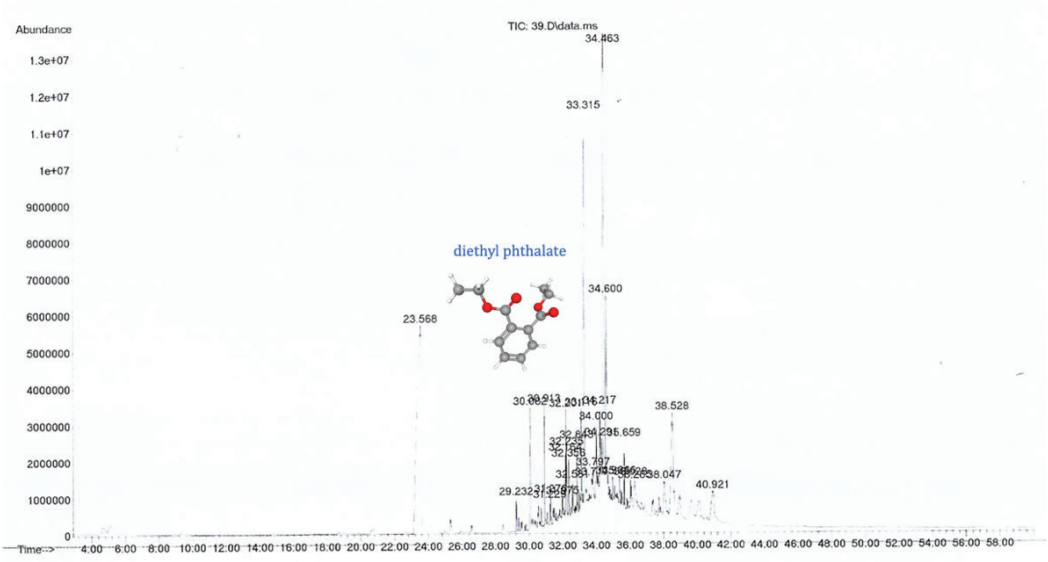


Figure 5. Determination of chromatogram of *ethanol extract* of *Aaptos pernucleata* using GC-MS.

4. Conclusion

As determined by GC-MS analyses of *A. viridis*, the most abundant compounds were diethyl phthalate (30.73%), cholesterol (12.21%), desmosterol (3.25%), hexadecenoenic acid methyl ester (3.44%), 9-octadecenoenic acid, methyl ester (2.18%), and gamma sitosterol (1.33%). One of the major components present in *A. pernucleata* was diethyl phthalate (28.86%), followed by bis (2-ethylhexy) phthalate (18.71%), 9-octadecenoic acid, 12-hydroxy-methyl ester (10.07%), cholecalciferol (8.24%), and cyclopenta [g]-2-benzopyran, 1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (2.90%). There are several biologically active compounds found in the ethanol extracts of marine sponges *A. viridis* and *A. pernucleata*, including antioxidants, antimicrobians, antiproliferatives, antivirals, and antidiabetics. Further studies are needed to explore the potential of the bioactive compounds from the extracted ethanol of marine sponges *Aaptos pernucleata* and *Amphimedon viridis* in biological activity and toxicity profiles. The impact of these compounds on various cell types should be assessed in future research with in vitro cytotoxicity tests.

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