Volume 5 Number 2 (2025)
January-June 2025
Page: 2302-2310

E-ISSN: 2745-4584
https://ejournal.insuriponorogo.ac.id
DOI: 10.37680/almikraj.v5i02.6215

AL MIKRAJ
Jurnal Studi Islam dan Humaniora

Cytotoxicity and Identification of Active Compounds in Sea Urchas (Diadema Setosum) in the Waters of Sinar Hading Village

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Submitted: Revised: 2025/05/20; Accepted: 2025/06/17; Published: 2025/09/05

Abstract

Indonesia boasts a rich marine biodiversity, including approximately 950 species of sea urchins, 84 species belonging to 48 genera and 21 species, found in Indonesian waters. This study aimed to determine the cytotoxicity level and identify active compounds in sea urchins (Diadema setosum) obtained from the waters of Sinar Hading Village, Lewolema District, East Flores Regency. The cytotoxicity test was conducted using the Brine Shrimp Lethality Test (BSLT) method with Artemia salina larvae. Identification of active compounds was performed using Gas Chromatography-Mass Spectrometry (GC-MS). The results showed that the sea urchin extract had an LC50 value of 94.51 ppm, which is considered moderately toxic. GC-MS analysis identified several active compounds, including trichloroethylene (3.9%), dimethyl disulfide (14.97%), toluene (100%), ethene 1,1,2,2-tetrachloro (25.54%), and several other toxic organochlorine compounds. These findings indicate that Diadema setosum has potential as a source of bioactive compounds for use in pharmaceuticals and biotechnology.

Keywords Bioactive Compounds, Cytotoxicity, Diadema Setosum, GC-MS,

INTRODUCTION

Indonesia boasts a rich marine biodiversity, including approximately 950 species of sea urchins, 84 species belonging to 48 genera and 21 species, found in Indonesian waters.[1] Among them, Diadema setosum is found abundantly in the waters off Sinar Hading, Lewolema District, East Flores Regency. Diadema setosum is a marine animal from the phylum Echinodermata, lacking a backbone or invertebrate, with a shelled body, a round body, and spines. Diadema setosum is a vital marine animal, considered key to the material cycle and energy flow in waters. Sea urchins, as marine life, are used to

[1] Ogello, E., Muthoka, M., & Outa, N. (2024). Exploring regenerative aquaculture initiatives for climate-resilient food production: Harnessing synergies between technology and Agroecology. *Aquaculture Journal*, 4(4), 324-344.

measure seawater quality (bioindicators). A moderate population of Diadema setosum sea urchins in an ecosystem plays a role in preventing excessive microalgal growth by consuming the algae, providing space for coral reef growth.[2]

The shells and spines of Diadema setosum sea urchins can be used as decoration, organic fertilizer, and dye. Diadema setosum sea urchins also have the potential to produce antimicrobial, antioxidant, and bioactive compounds, including peditoxin, which are used in the pharmaceutical industry.[3]

Sea urchins are known to produce toxic secondary metabolites that function as a defense mechanism. This venom contains bioactive compounds with potential uses in the medical, pharmaceutical, and agricultural fields. Research on the cytotoxicity of sea urchins has been conducted previously, but specific studies on the Diadema setosum species from the Sinar Hading waters are limited.[4] Therefore, this study was conducted to test the toxicity and identify the active compounds in the sea urchin extract. Bioactive compounds are secondary metabolites produced by microorganisms. Bacteria that are tolerant to high concentrations, these secondary metabolites can be produced to defend against pathogenic fungi and viruses.[5]

Chariou et al. (2020) defines active compounds as specific chemical compounds found in the animal body as medicinal substances that have physiological effects on other organisms, often referred to as bioactive compounds.[6] Active compounds are substances that have the power or ability to prevent various adverse conditions during metabolism,

[2] Pilnick, A. R., O'Neil, K. L., Moe, M., & Patterson, J. T. (2021). A novel system for intensive Diadema antillarum propagation as a step towards population enhancement. *Scientific Reports*, *11*(1), 11244.

[3] Bernt, J, schrroter, Mockl, Ha Gasteiger (2020). Analiysis of gas permeation phenomena in a PEM water electrolyzer operated at high current density. Meksiko.

[4] Daid, C, Carslaw, Stuart, K, Grange. (2013). Using merorological normalisation to detect interventions in air quality time series. University of york

[5] Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial pathogenesis*, *124*, 198-202.

[6] Chariou, P. L., Ortega-Rivera, O. A., & Steinmetz, N. F. (2020). Nanocarriers for the delivery of medical, veterinary, and agricultural active ingredients. *Acs Nano*, *14*(3), 2678-2701.

prevent health problems, and maintain human health (Suharto et al., 2012). Diadema setosum is a species of long-spined sea urchin belonging to the Deadematidae family. It is a distinctive sea urchin with very long, hollow spines that are mildly venomous.

Diadema setosum differs from other Diademas by the five distinctive white spots found on its body. This species can be found throughout the Indo-Pacific region, from Australia and Africa to Japan and the Red Sea.[7] Although capable of inflicting a painful sting when stepped on, sea urchins are only mildly venomous and do not pose a serious threat to humans.

Kingdom: Animalia

Phyllum: Echinoderms

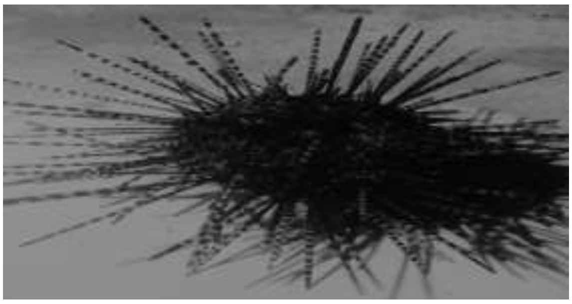
Class: Echinodea

Order: Cidaroidea

Family: Diadematidae

Genus: Diadema

Species: Diadema setosum.



Unlike starfish and brittle stars, Diadema setosum lacks arms. The body of the Diadema setosum is spherical, with a hard, calcareous shell covered with spines.

The spines are arranged in longitudinal rows and are movable. The mouth is located on the underside facing downward, and the anus is located upward at the top of the rounded shell. Diadema setosum is characterized by its black color with black spines

[7] Leliaert, F., Payo, D. A., Gurgel, C. F. D., Schils, T., Draisma, S. G., Saunders, G. W., ... & De Clerck, O. (2018). Patterns and drivers of species diversity in the Indo-Pacific red seaweed Portieria. *Journal of Biogeography*, *45*(10), 2299-2313.

extending upward for defense, while the lower part serves as a means of locomotion.[8] It has five white dots on the upper part, with one white dot between each segment.

According to Musfirah (2018),[9] Diadema setosum is characterized by long, sharp, and brittle spines all over its body. It has a round, jet-black body, five gonopores, and a very bright, shiny or glowing appearance. It lives in coral, algae, sand, and seagrass, where it can attach its ambulacral foot.

METHOD

The study was conducted from June to August 2025 in the waters of Sinar Hading Village. Preparation was conducted at the Fisheries Product Technology Laboratory of the Indonesian Fisheries Science Institute (IKTL), while GC-MS identification was performed at the Bioferakmaka Study Center Laboratory of the Bogor Agricultural University (IPB).

The sample consisted of 3 kg of sea urchins (Diadema setosum). Preparation was carried out by drying at 75°C for 24 hours, then grinding. Extraction was carried out using the maceration method using 75% ethanol at a ratio of 1:10. Cytotoxicity testing was performed using the BSLT method using Artemia salina larvae, while compound identification was performed using GC-MS.

RESULTS AND DISCUSSION

Cytotoxicity tests showed that Diadema setosum extract had an LC50 value of 94.51 ppm, making it moderately toxic to Artemia salina. This indicates the extract contains bioactive compounds with significant biological effects.

Table 1. Sample name and Identity

Sample name	**Sample identity and condition**	**Parameter**	**Results**	**Unit Analysis**	**technique**

[8] Voulgaris, K., Varkoulis, A., Zaoutsos, S., Stratakis, A., & Vafidis, D. (2021). Mechanical defensive adaptations of three Mediterranean sea urchin species. *Ecology and Evolution*, *11*(24), 17734-17743.

[9] Musfirah N. H. 2018. Struktur Komunitas Bulu Babi (*Echinoidea*). Sulawesi Selatan.

Solid sea urchin	extraction	Toxicity LC50	94.51	ppm	BLST

The results were then calculated as the Lethal Concentration (LC50) of the sea urchin extract. BLST data showed an LC50 value, where a concentration of 94.51 ppm could cause 50% mortality of Artemia salina. An extract is categorized as active and toxic if it can cause 50% mortality of A. salina. Very toxic (<1 ml), toxic (1-10 ml), moderately toxic (10-100 ml), and less toxic (>100 ml).

Table 2. GC-MS analysis identified several compounds, including:

No.	Sample type	**Rt**	Heavy molecule	Compound	Content %
1	Sea urchin *(Diadema setosum)*	3,463	91,71	Trichloroethylene	3,9
2		4,404	96,44	Disulfide, dimethyl	14,97
3		4,968	96,04	Toluene	100
4		38,31	53,97	Bis (chloromethyl) sulfide	0,47
5		11,438	74,52	Methene, borochloro	4,66
6		5,119	81,9	Ethene 1,1,2-trichloro	1,81
7		10,347	95,61	Ethene, 1,1,2,2-tetrachloro	25,54
8		11,438	74,52	Methene, dichloro-	4,66
9		4,09	96,11	Chloromethyl methyl sulfide	3,24
10		21,645	60,38	4-amino-2,6 dichloropyride	2,98

The existence of these compounds opens up opportunities for the use of sea urchins as a source of bioactives in the pharmaceutical field, although their toxic nature is also a challenge.

The cytotoxicity assay using the Brine Shrimp Lethality Test (BSLT) revealed that the ethanol extract of *Diadema setosum* demonstrated moderate toxicity, with an LC50 value of 94.51 ppm. This value indicates that the extract possesses bioactive potential capable of inducing significant biological responses, as concentrations below 100 ppm are considered to have a notable cytotoxic effect. The findings suggest that the extract contains compounds with potential pharmacological importance, supporting previous studies that highlighted the role of sea urchins as natural sources of secondary metabolites with defensive and medicinal properties.

Further analysis using Gas Chromatography-Mass Spectrometry (GC-MS) successfully identified several active compounds within the sea urchin extract. The dominant components included toluene (100%), ethene 1,1,2,2-tetrachloro (25.54%), and dimethyl disulfide (14.97%), alongside smaller concentrations of trichloroethylene, bis (chloromethyl) sulfide, and chloromethyl methyl sulfide. Many of these compounds belong to the group of organochlorine and sulfur-containing compounds, which are known for their cytotoxic, antimicrobial, and pesticidal properties. The diversity of the compounds detected indicates a complex chemical profile that may account for the moderate toxicity observed in the BSLT assay.

These findings highlight the potential of *Diadema setosum* as a natural source of bioactive molecules that could be developed for applications in pharmaceutical and biotechnological fields. However, the presence of toxic organochlorine compounds also emphasizes the need for careful evaluation regarding their safety and selectivity. The identification of both beneficial and hazardous compounds indicates that while *D. setosum* holds promise as a reservoir of cytotoxic agents, further studies on purification, isolation, and biological activity testing are necessary to determine its therapeutic relevance and reduce the risks associated with its toxicity.

The cytotoxicity results of *Diadema setosum* extract, with an LC50 value of 94.51 ppm, categorize it as moderately toxic. This finding aligns with the theory that marine organisms, particularly echinoderms, produce secondary metabolites as a defense mechanism against predators and pathogens. Previous studies, such as those by Khalil et al. (2022), emphasized that bioactive compounds in sea urchins often demonstrate significant biological activity, including cytotoxic and antimicrobial effects.[10] The moderate toxicity observed in this study confirms that *D. setosum* is consistent with other echinoderm species, which are widely acknowledged as reservoirs of pharmacologically

[10] Khalil, E. A., Swelim, H., El-Tantawi, H., Bakr, A. F., & Abdellatif, A. (2022). Characterization, cytotoxicity and antioxidant activity of sea urchins (Diadema savignyi) and jellyfish (Aurelia aurita) extracts. *Egyptian Journal of Aquatic Research*, *48*(4), 343-348.

active compounds.

The GC-MS analysis revealed the presence of compounds such as toluene, ethene 1,1,2,2-tetrachloro, and dimethyl disulfide, which are known to possess strong cytotoxic and antimicrobial properties. Similar findings were reported by Liu et al. (2022), who noted that secondary metabolites derived from marine organisms often contain organochlorine and sulfur-containing compounds with defensive and therapeutic potential.[11] From a theoretical standpoint, the identification of these compounds supports the concept that bioactive molecules from marine sources play dual roles: ensuring the survival of the organisms in their natural habitat while offering potential pharmacological applications. This duality underlines the ecological importance of sea urchins as both key species in marine ecosystems and sources of novel bioactive substances.

However, the discovery of organochlorine compounds such as trichloroethylene and bis (chloromethyl) sulfide also raises safety concerns, as these compounds are associated with toxicity and environmental persistence. This observation resonates with the argument of Monteiro et al. (2019), who highlighted that not all bioactive compounds are inherently safe for therapeutic use, and their pharmacological potential must be balanced against their toxicological risks.[12] Consequently, while the current findings affirm the potential of *D. setosum* as a valuable marine resource, they also stress the necessity of further purification and characterization studies. Isolating specific beneficial compounds while minimizing toxic components will be essential for translating these findings into safe pharmaceutical applications.

CONCLUSION

The sea urchin extract Diadema setosum from the waters of Sinar Hading Village exhibited cytotoxicity with an LC50 value of 94.51 ppm. GC-MS analysis revealed the presence of bioactive compounds such as trichloroethylene, disulfide, dimethyl, toluene, bis (chloromethyl) sulfide, methene, borochloro, ethene, 1,1,2-trichloro, ethene, 1,1,2,2-

[11] Liu, Z., Li, M., Wang, S., Huang, H., & Zhang, W. (2022). Sulfur-containing metabolites from marine and terrestrial fungal sources: Origin, structures, and bioactivities. *Marine Drugs*, *20*(12), 765.

[12] Monteiro, J., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2019). Pharmacological potential of methylxanthines: Retrospective analysis and future expectations. *Critical reviews in food science and nutrition*, *59*(16), 2597-2625.

tetrachloro, methene, dichloro-, methene, dichloro-, chloromethyl methyl sulfide, and 4-amino-2,6-dichloropyride. This study confirms the potential of Diadema setosum as a source of bioactive compounds that can be further utilized in the pharmaceutical and biotechnology fields.

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