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Potential Bioactive Compounds of Starfish *Archaster typicus* and *Nardoa tuberculata* Extract that Inhibits the Growth of Histamine-Producing Bacteria

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ABSTRACT

Histamine-producing bacteria in fishery products pose a significant risk to food safety and public health. Beyond their role as a food source, marine organisms represent a valuable reservoir of bioactive compounds with both economic and therapeutic potential. However, limited scientific data exist regarding the ability of starfish-derived bioactive extracts to inhibit histamine-producing bacteria. This study investigated the antibacterial potential of the starfish extracts from Maluku waters, Indonesia, against histamine-producing bacteria. Using an exploratory approach, the bioactive components and inhibitory activities of *Archaster typicus* and *Nardoa tuberculata* extracts were examined. Phytochemical analysis revealed that *A. typicus* extract contains flavonoids, tannins, and saponins, while *N. tuberculata* extract contains flavonoids and steroids. Both extracts demonstrated strong inhibitory activity against *Clostridium perfringens* at a concentration of 100µg/ mL. Notably, *A. typicus* extract exhibited significantly greater and more consistent inhibition of *Enterobacter aerogenes* across all tested concentrations compared to *N. tuberculata*. These findings underscore the potential of the starfish extracts as a promising source of natural antibacterial agents, supporting future research and development in food safety and natural product discovery.

INTRODUCTION

The decline in food quality due to spoilage caused by microorganisms is a global issue since it not only reduces edibility but also increases the risk of diseases and toxins resulting from biological agents **(Winnett *et al.*, 2014)**. One of the key microorganisms involved in food spoilage is histamine-producing bacteria. L-histidine decarboxylase can only produce histamine, a biogenic amine, from L-histidine with pyridoxal-5'-phosphate as a cofactor **(Patel *et al.*, 2023)**. The formation of histamine in fish is influenced by the presence of specific bacteria. **Sabry *et al.* (2019)** reported several histamine-producing bacteria commonly found in fish, including *Enterobacter aerogenes*, *Enterobacter cloacae*, *Raoultella planticola*, and *Citrobacter freundii*. The level of histamine formation

depends on the concentration of histidine in fish tissues and the abundance of bacteria capable of producing histidine decarboxylase. According to **Mahmoud *et al.* (2023)**, histamine is formed from the decarboxylation of free histidine by bacteria possessing the histidine decarboxylase (HDC) enzyme.

While histamine-producing bacteria are not always pathogenic, they can contribute to food spoilage and pose health risks. Pathogenic microorganisms, on the other hand, are a significant cause of diseases in humans and other living organisms. Efforts to mitigate the effects of these pathogens have focused on identifying chemical compounds that can inhibit their growth and activity **(Runtuwene, 2017)**. A current challenge is the growing resistance of bacteria to conventional antibiotics, highlighting the need for alternative antibacterial agents **(World Health Organization, 2023)**.

Starfish, as a potential source of bioactive compounds, have gained attention in this regard. **Madigan *et al.* (2012)** and **Runtuwene (2017)** define antibacterial agents as substances that inhibit bacterial growth or kill bacteria by disrupting their metabolism. Starfish, belonging to the phylum Echinodermata, have been extensively studied by organic chemists, biochemists, and pharmacologists as a promising source of marine natural products containing bioactive components. Among the macrozoobenthos found in coastal and marine environments, starfish play a number of crucial roles **(Suryanti *et al.*, 2018)** and are not only a food source but also a potential reservoir of economically valuable bioactive compounds.

Several studies have demonstrated the ecological and economic potential of starfish. For instance, they play a crucial ecological role as detritivores **(Setyowati *et al.*, 2017)**, serve as reef and seagrass associates in food chains, and contribute to beach cleaning by breaking down organic material **(Alfatmadina *et al.*, 2019)**. Economically, certain starfish species are used in food processing, handicrafts, and decorations due to their attractive patterns, colors, and shapes **(Mbana *et al.*, 2020)**. Starfish are also known to contain bioactive components such as alkaloids, steroids, flavonoids, saponins, and ninhydrin **(Ivanchina *et al.*, 2011)**. These compounds exhibit various biological activities, including antioxidant **(Agustina, 2012)**, antibacterial **(Juariah, 2014)**, anti-inflammatory, antifungal, and immunostimulatory properties **(Achmad *et al.*, 2014)**. For example, *Astropecten spinulosu*s collected from the Mediterranean Sea near Alexandria, Egypt, demonstrated antimicrobial potential **(Ibrahim *et al.*, 2020)**, while *Linckia laevigata* extracts containing saponins and flavonoids showed antibacterial activity against *Clostridium perfringens* and *Enterobacter aerogenes* **(Mailoa *et al.*, 2024)**. Although research on the antimicrobial activity of starfish is still limited, several studies have confirmed their potential. **Shimizu *et al.* (1990)** extracted bioactive compounds from *Asterias forbesi* and *Asterina pectinifera,* which inhibited influenza virus replication in chicken embryos. Similarly, **Prabhu and Bragadeeswaran (2013)** reported significant antimicrobial activity in *Ophiocoma marmorata* extracts. Marine echinoderms, including starfish, have attracted attention for their bioactive compounds, which exhibit

antimicrobial, antifungal, antiprotozoal, antiviral (including anti-HIV), anthelmintic, and anticancer properties **(Zapata & Amemiya, 2000; Datta *et al.*, 2015)**.

Given the above data, this study aimed to explore the potential of starfish from Maluku waters as a source of antibacterial agents, addressing the need for novel natural compounds to combat bacterial resistance and improve food safety.

MATERIALS AND METHODS

1. Starfish sampling and identification

Starfish *Archaster typicus* and *Nardoa tuberculata* samples were taken from the Waisarisa Village waters, West Seram Regency. Species were identified using the World Register of Marine Species (WoRMS). Sample preparation, extraction, and antibacterial activity testing were performed at the Fisheries Product Technology Laboratory of Fisheries and Marine Sciences Faculty, while qualitative bio-active component was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University.

2. Bioactive compounds extraction

The starfish samples were washed thoroughly with clean running water and cut into small pieces. Bioactive compounds were extracted using the maceration method with methanol as the solvent. Maceration is a simple extraction technique where powdered samples are soaked in a solvent, allowing the solvent to penetrate cell walls and dissolve active compounds **(Suryanto, 2012)**. Methanol was chosen for its effectiveness in extracting bioactive compounds and its suitability as a negative control in antibacterial activity tests **(Dash *et al.*, 2011)**.

3. Phytochemical testing

Phytochemical tests were conducted to identify secondary metabolites in the starfish extracts. The following qualitative tests were performed:

3.1 Flavannoid test

Two milliliters (ml) of the concentrated starfish extract were heated, followed by the addition of 1ml of alcohol, 1ml of concentrated HCl, and 0.05g of magnesium powder. The mixture was shaken vigorously, and a color change to red or orange indicated the presence of flavonoids **(Meila, 2017)**.

3.2 Steroid test

Three drops of strong sulfuric acid and ten drops of acetic anhydride were combined with two milliliters of the extract. The presence of steroids was verified by a change in hue to either blue or green **(Harborne, 1987)**.

3.3 Saponnin Test

Ten milliliters of boiling water were combined with two milliliters of the extract, allowed to cool, and then violently shaken for ten seconds. The formation of stable foam (1–10cm high) lasting more than 10 minutes, even after adding 1 drop of 2N HCl, indicated the presence of saponins **(Supomo *et al.*, 2016)**.

3.4 Tannin test

Two ml of the extract was treated with 1% $FeCl_3$ solution. A blackish-green color change confirmed the presence of tannins **(Mutiara & Wildan, 2014)**.

3.5 Phenolic test

Two ml of the extract was mixed with 3–4 drops of 1% iron (III) chloride solution. The formation of a strong green or blue color indicated the presence of phenolics **(Harborne, 1987)**.

3.6. Antibacterial activity testing

Antibacterial activity was evaluated using the disc diffusion method (Kirby-Bauer method). Sterile paper discs (6mm in diameter) were impregnated with 20µl of starfish extract at concentrations of 100, 50, and 25%. Tests were performed in duplicate against *Clostridium perfringens* (ATCC 19408, Gram-positive) and *Enterobacter aerogenes* (ATCC 13048, Gram-negative). Amoxicillin was used as a positive control, while dimethyl sulfoxide (DMSO) served as the negative control. After incubation at 35°C for 24–48 hours, the diameter of the inhibition zones was measured using a caliper **(Volk & Wheeler, 1988)**.

RESULTS AND DISCUSSION

1. Morphological description and habitat of starfish

The intertidal zone, or coastal area, is a region influenced by tidal fluctuations, characterized by physical and chemical factors that support diverse marine life. This zone exhibits high biodiversity due to its dynamic environment **(Tunny *et al.*, 2021; Nasruddin, 2022)**. Among the notable inhabitants of coastal and marine ecosystems are

echinoderms, which include five classes, one of which is Asteroidea (starfish) **(Hartati *et al.*, 2018)**.

Starfish samples collected from Waisarisa Village were identified as *Archaster typicus* and *Nardoa tuberculata* based on the World Register of Marine Species (WoRMS). Their morphological descriptions and habitats are summarized in Table (1).

Table 1. Morphological description and habitat of starfish found in Waisarisa Village

Species	**Morphological Description**		**Microhabitat**
Archaster typicus		This species has five flat arms with pointed tips. The oral side features a mouth, ambulacral grooves, and cylindrical tube feet. The aboral side includes a madreporite and anus. The body is covered with white, blunt spine.	*A. typicus* inhabits intertidal zones with fine sandy substrates. It is distributed across the southern Indian Ocean, Mascarene, eastern Africa (Madagascar), Maldives, Bay of Bengal, eastern India, northern Australia, the Philippines, China, Japan, the South Pacific, and Hawaii **(Clark & Rowe, 1971).**
Species	**Morphological Description**		**Micro habitat**
Nardoa tuberculata		This species has a star-shaped body with five arms, brown coloration, and white tubercles on the upper surface.	*N. tuberculata* is commonly found in seagrass beds and coral reef ecosystems.

2. Bioactive compound extraction and phytochemical analysis of *Archaster typicus* and *Nardoa tuberculata*

Extraction is a selective process that isolates desired compounds from a mixture using a solvent. The choice of solvent is critical, as it influences the efficiency and selectivity of bioactive compound extraction **(Sartika *et al.*, 2013)**. The maceration method was chosen for its simplicity and ability to prevent thermal degradation of heat-sensitive compounds, such as flavonoids **(Sa'adah & Nurhasnawati, 2015)**. During maceration, the cell walls break down due to pressure differences, allowing secondary metabolites to dissolve in the solvent **(Lenny, 2006)**. The maceration process lasted for 3–24 hours, followed by evaporation using a rotary evaporator at 40–43°C to obtain concentrated extracts **(Wendersteyt *et al.*, 2021)**.

Bioactive compounds are active substances in functional foods responsible for beneficial metabolic reactions. These compounds, such as alkaloids, flavonoids, steroids, triterpenoids, saponins, and tannins, exhibit antimicrobial, anticancer, antioxidant, and emulsifying properties. The phytochemical screening results for *Archaster typicus* and *Nardoa tuberculata* extracts are presented in Table (2).

Table 2. Phytochemical screening results

Bioactive Compound	*Archaster typicus* Extract	*Nardoa tuberculata* Extract	Result (Color)
Flavonoids	+	+	(+) Orange
Tannins	+	-	(+) Blackish green
Steroids	-	+	(+) Blue or green
Phenolics	-	-	(+) Dark green
Saponins	+	-	(+) Foam formation

Note:
(+) = Detected
(-) = Not detected

The results indicate the presence of secondary metabolites, including flavonoids, tannins, steroids, and saponins, in the extracts. Phenolic compounds were not detected. Ethanol is a solvent that is easily available, efficient, environmentally safe, and has a high extraction rate **(Jiménez-Moreno *et al.*, 2019; Chen *et al.*, 2020)**. Flavonoids, detected in both species, form complexes with magnesium ions, resulting in an orange color change. Flavonoids inhibit bacterial growth by disrupting cell membranes and energy

metabolism **(Cushnie & Lamb, 2005; Amalia *et al.*, 2017)**. Saponins, detected in *A. typicus*, act as surfactants, destabilizing bacterial cell membranes and causing cell lysis **(Sugianitri, 2011)**. Tannins, also found in *A. typicus*, inhibit bacterial enzymes and disrupt cell wall synthesis **(Ngajow *et al.*, 2013)**. Steroids, detected in *N. tuberculata*, interact with bacterial membrane lipids, causing leakage and cell death **(Madduluri *et al.*, 2011)**.

3. Antibacterial activity of *Archaster typicus* and *Nardoa tuberculata* extracts

The antibacterial activity of the extracts was evaluated using the disc diffusion method (Kirby-Bauer) at concentrations of 25, 50, and 100%. Amoxicillin was used as a positive control, while distilled water served as the negative control. The results are summarized in Table (3).

Table 3. Antibacterial activity results

Test Bacteria	Ekstract Type	Concentration	Inhibition Zone (mm)	Criteria **(Lomboan *et al.*, 2021)**
Clostridium perfringens	*Archaster typicus*	25	12,9	Strong
		50	16,6	Very Strong
		100	19,6	Very Strong
		Amoxicillin (+)	22,0	Very Strong
		Aquadest (-)	0	none
	Nardoa tuberculata	25	13,7	Strong
		50	15,9	Strong
		100	16,9	Very Strong
		Amoxicillin (+)	22,0	Very Strong
		Aquadest (-)	0	none
Enterobacter aerogenes	*Archaster typicus*	25	12,4	Strong
		50	15,5	Strong
		100	19,3	Very Strong
		Amoxicilin (+)	15,0	Strong
		Aquadest (-)	0	none
	Nardoa tuberculata	25	8,1	Moderate
		50	8,7	Moderate
		100	9,3	Moderate
		Amoxicilin (+)	15,0	Moderate
		Aquadest (-)	0	none

The results demonstrate that *Archaster typicus* extract was more effective in inhibiting both Gram-positive (*Clostridium perfringens*) and Gram-negative (*Enterobacter aerogenes*) bacteria compared to *Nardoa tuberculata* extract. The inhibition zones increased with higher extract concentrations, indicating dose-dependent activity. Gram-positive bacteria were more susceptible due to their simpler cell wall structure, which lacks the outer membrane present in Gram-negative bacteria **(Radji, 2011)**. The antibacterial activity of the extracts is attributed to their bioactive compounds, including flavonoids, tannins, saponins, and steroids, which disrupt bacterial cell membranes, inhibit enzyme activity, and cause cell lysis **(Madduluri *et al.*, 2011; Sugianitri, 2011; Bobbarala, 2012)**.

CONCLUSION

The study revealed that *Archaster typicus* extract contains flavonoids, tannins, and saponins, while *Nardoa tuberculata* extract contains flavonoids and steroids. Both extracts exhibited significant antibacterial activity against *Clostridium perfringens* and *Enterobacter aerogenes*, demonstrating their potential as natural antimicrobial agents. Further research is recommended to elucidate the chemical structures and mechanisms of action of these bioactive compounds.

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