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Screening Antibacterial Activity in Soft Coral Extract *Sinularia* sp. from Pahawang Island Waters, Lampung

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ABSTRACT

Sinularia sp. is one of the most widespread and abundant types of soft coral in Indonesian waters. The soft coral *Sinularia* sp. haves many bioactive compounds to use as a patogen antibacterial agent. The aim of this research was to compare the antibacterial activity from the soft coral *Sinularia* sp. against the pathogenic bacterial strain *Escherichia coli.* Samples of the soft coral *Sinularia* sp. were collected by SCUBA diving equipment at around Pahawang Island, Pesawaran Regency, Lampung, Indonesia, in July 2025. In this study, two species of soft corals, *Sinularia polydactyla* and *Sinularia flexibilis*, were found at the research site. The extract yields for soft coral species used methanol resulted in higher values compared to ethyl acetate and hexane. The antibacterial activity of the extract soft corals against the pathogenic bacteria *E. coli* used ethyl acetate solvent better than methanol and hexane. Soft coral extract for *Sinularia* sp. with ethyl acetate solvent can be used as antibacterial candidates against the pathogenic bacteria *E. coli*.

INTRODUCTION

Coral reefs are one of the characteristic ecosystems of tropical waters built by coral animals living in symbiosis with zooxanthellae algae (Yuan *et al*. 2024; Arabeyyat *et al*. 2024). Specifically, the coral reef ecosystem is comprised of two main groups of coral, hard coral and soft coral (Kennedy *et al*., 2020). Hard corals, the main group of corals, produce calcium carbonate, while soft corals belong to the order Alcyonacea and do not produce a calcium carbonate skeleton (Ng *et al*., 2021). Soft corals and hard corals together provide habitat and shelter for various marine organisms, play a role in the food chain and ecosystem balance, and support marine biodiversity (Moynihan *et al*., 2022). Currently, the existence of the coral reef ecosystem is increasingly threatened by various factors, including natural factors such as earthquakes and tsunamis, as well as anthropogenic factors such as destructive fishing using explosives and poisons, irresponsible marine tourism, environmental pollution, shipping activities, and coastal development (Praneeth *et al*., 2025). This condition degrades the quality and threatens the sustainability of the coral reef ecosystem. Therefore, we must undertake significant efforts to prevent and reduce damage, including sustainable conservation

initiatives to preserve the coral reef ecosystem.

One conservation effort to maintain the sustainability of the coral reef ecosystem provides an understanding that this ecosystem has vital functions, such as its potential to generate economic added value through natural bioactive compounds from soft coral secondary metabolites that benefit the pharmaceutical and health sectors (Fine *et al*., 2019). Secondary metabolites are compounds that do not directly play a role in an organism's growth or reproduction, but instead help the organism survive in its environment. In soft corals, secondary metabolites function as a defense against predators, aid in camouflage, and act as an adaptation mechanism against extreme environmental conditions. Besides being vital for the survival of soft corals, these secondary metabolite compounds also offer many benefits to humans (Veríssimo *et al*., 2021; Jusuf *et al*., 2022 and Goura *et al*., 2025). Thus, maintaining and preserving the coral reef ecosystem also indirectly protects a warehouse of bioactive compounds that benefit human health and welfare in the future.

One type of soft coral that is well known is *Sinularia* sp. *Sinularia* sp. is one of typed of soft coral the most widespread and abundant in Indonesian waters (Putra *et al*., 2012). *Sinularia* sp. is often found on shallow coral reef flats and in bright waters. Morphologically, this soft coral has a stalk like or fanlike body with a soft texture (Rozirwan *et al*., 2020). The soft coral *Sinularia* sp. haves many uses as an antibacterial, antifungal, antiviral, antitumor, and anticancer agent because it possesses bioactive compounds such as terpenoids, alkaloids, polyketides, and polypeptides (Qin *et al*., 2018). The aim of this research was to compare the antibacterial activity of bioactive compounds from the soft coral *Sinularia* sp. against the pathogenic bacterial strain *Escherichia coli.*

METHODS

Sample Collection

Samples of the soft coral *Sinularia* sp. were collected by SCUBA diving equipment at a depth of approximately 10 m around Pahawang Island, Pesawaran Regency, Lampung, Indonesia, in July 2025. The soft coral samples were cleaned from debris with distilled water, placed in a cooling box, and then transported to the laboratory for next proces.

Sample Preparation

After arrived at the laboratory, the soft coral samples were cleaned again by distilled water, cut into small pieces with a knife, weighed, and placed into a measuring cylinder for extraction.

Extraction

The soft coral tissue was extracted by the maceration method, where the soft coral tissue was soaked in methanol, ethyl acetate, and hexane solvents at a ratio of 500 g wet weight to 1,000 ml of solvent for 7 days. The solution mixture was filtered to use vacuum pump and millipore filter paper. The filtrate was dired, weighed, and calculated for yield (Kusuma *et al*., 2023).

Bacterial Culture

Origin pathogenic *E. coli* bacterial cultures (100 µl) were inoculated aseptically into test tubes contained 5 ml of Nutrient Broth (NB). The tubes were closed and shaken slowly to distribute the bacteria evenly within the incubation media for 24 hours at 37°C. After 24 hours of growth, a small amount of the culture was taken from the Nutrient Broth (NB) and inoculated into Nutrient Agar (NA) used by the streak plate or line planting method. Incubation was carried out for 24 hours at 37°C until colonies were formed from pathogenic *E. coli*

bacterial.

Suspension

Fresh bacterial colonies (100 µl) were collected from Nutrient Agar (NA), usually 18–24 hours after incubation. The colonies were placed in 3-5 ml of sterile saline solution in a tube. A sterile loop was used to scrape the colonies and mix them. The tube was shaken slowly with a vortex until the bacterial cells were evenly distributed. This resulted in a turbid bacterial suspension. Turbidity adjustment was performed with McFarland standards.

Antibacterial Test

Antibacterial test was used by the Kirby-Bauer or paper disc method. 1 ml of the *E. coli* pathogenic inoculum was taken aseptically from the Nutrient Broth (NB) and poured into a petri dish contained Nutrient Agar (NA). Paper discs 0,5 mm were used. The paper discs were dipped into extracts of specific concentrations, using various solvents according to the treatment groups. Using tweezers, the paper discs were placed onto the NA media that had been inoculated with *E. coli*. It was ensured that the discs did not move and adhered well to the agar surface. All Petri dishes were incubated at 37°C for 24 hours. The zone of inhibition (the clear area around the paper disc) was observed daily until the third day. The diameter of the inhibition zone was measured using a ruler or calipers. The inhibition zone indicated the effectiveness of the extract in inhibiting the growth of the pathogenic *E. coli* bacteria (Kusuma *et al*., 2026).

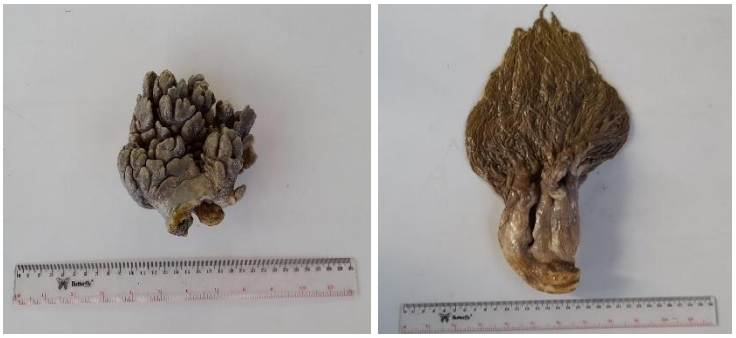
Data Analysis

Analysis of the yield data from extraction used maceration calculating the percentage ratio between the weight of the extract obtained and the initial weight used, whereas zone of inhibition analysis is performed by measuring the diameter of the clear zone formed around a paper disc on agar media cultivated with pathogenic bacteria. This clear zone was an indicator of the bacteria's sensitivity to the antibacterial agent being tested.

RESULTS

Soft Coral Identification

In this study, two species of soft corals, *Sinularia polydactyla* and *Sinularia flexibilis*, were found at the research site. The soft coral species *S. polydactyla* was formed large colonies that spread across the reef flat in shallow depths. This species inhabited sandy stone and stony sand substrates. In the study site, *S. polydactyla* created colonies that expanded across the seabed, reaching sizes of over two meters. These soft coral colonies attached themselves to dead corals. The tentacles occupied the upper part of the colony, forming finger-like shapes with branches. The colony exhibited a light brown color with a smooth, branchless base. A whitish color was turned when the coral was touched, and it was changed to black when it was cut. *S. flexibilis* was characterized as a type of soft coral that was formed by individual colonies and was found living in few quiet areas. At the research site, this soft coral was discovered only in a few locations with very limited numbers. The colonies were formed from existing individuals. A yellowish-brown color was exhibited by the colonies, which were shaped into branched tentacles. Long and soft tentacles were possessed, with branches grown from the base to the tip of the stem, so they were swayed strongly when hit by currents. A black color was turned when the coral was cut, and a pale white color was observed on the tentacles and stems. The soft coral species was presented in Figure 1.



(a) *Sinularia polydactyla* (b) *Sinularia flexibilis*

Figure 1. The Soft Coral Species (a) *Sinularia polydactyla* and (b) *Sinularia flexibilis*

Yield Extract

The yield extraction results showed varying values in methanol, ethyl acetate, and hexane solvents. The yield of soft coral extracts was presented in Table 1.

Table 1. The Yield of Soft Coral Extracts

Species	Yield (%)		
	Methanol	Ethyl Acetate	Hexane
S. polydactyla	2.1	0.6	0.3
S. flexibilis	3.2	2.1	0.6

The methanol extract of the soft coral *S. polydactyla* yielded 2.1%, while ethyl acetate produced 0.6% and hexane resulted in 0.3%. Meanwhile, *S. flexibilis* yielded 3.2% with methanol, 2.1% with ethyl acetate and 0.6% with hexane. The highest extract yield came from *S. flexibilis* used methanol at 3.2%, whereas the lowest occurred in *S. polydactyla* used hexane at 0.3%. The yield extract of *Sinularia* sp. ranged from 9-24% used methanol, 3-23% used ethyl acetate, and 0.6-22% used hexane (Setyaningsih *et al*., 2012; Nurhayati *et al*., 2012; Katiandagho *et al*., 2019; Lumbu *et al*., 2022; Kusuma et al., 2023). The yield extracts of *Sinularia polydactyla* ranged from 1.37-1.40% used methanol, 0.48-0.81% used ethyl acetate, and 1.55-2.18% used hexane, while *Sinularia flexibilis* ranged from 2.00-2.90% used methanol, 1.00-1.80% used ethyl acetate, and 2.00-2.89% used hexane (Rozirwan *et al*., 2014).

Antibacterial Activity

The antibacterial activity of soft coral extracts against the pathogenic bacteria *E. coli* was presented in Table 2.

Table 2. The Antibacterial Activity of Soft Coral Extracts Against the Pathogenic *E. coli*

Species	Inhibition Zone (mm)			
	Methanol	Ethyl Acetate	Hexane	Chloramphenicol (Control)
S. polydactyla	8.33	11.93	8.07	18.4
S. flexibilis	9.07	11.37	0	20.2

The antibacterial activity of *S. polydactyla* soft coral extract against the pathogen *E. coli* showed 8.33 mm with methanol, 11.93 mm with ethyl acetate, and 8.07 mm with hexane. Meanwhile, *S. flexibilis* produced 9.07 mm with methanol and 11.37 mm with ethyl acetate, but exhibited no antibacterial activity at all with hexane. The antibacterial activity against *E. coli* reached its highest point in *S. polydactyla* used ethyl acetate at 11.93 mm, while *S. flexibilis* used hexane showed the lowest result with no activity. Antibacterial activities of *Sinularia sp.*

against the pathogen *E. coli* were observed to range from 1-13 mm used methanol, 3-15 mm used ethyl acetate, and 5-17 mm used hexane (Setyaningsih *et al*., 2012; Nurhayati *et al*., 2012; Tanod *et al*., 2018; Katiandagho *et al*., 2019; Lumbu *et al*., 2022; Kusuma *et al*., 2023). Antibacterial activities of *Sinularia polydactyla* against *E. coli* were recorded to range from 10.20-11.45 mm used methanol and 13.3-14.7 mm used ethyl acetate, while no activity was observed using hexane; meanwhile, for *Sinularia flexibilis*, a range of 13-13 mm was observed by ethyl acetate, whereas no activity was detected using methanol or hexane (Rozirwan *et al*., 2014).

DISCUSSION

The solubility of bioactive substances was heavily influenced by the ability of the solvents. The yield value of the extracts was determined by the solvent polarity factor, as different active substances were dissolved by solvents with varying polarities. Varying results were shown by the yield of soft coral extracts. The differences in these yield values were suspected to be caused by the solvent properties. Methanol was categorized as an alcohol compound; its high solubility was facilitated by the formation of hydrogen bonds between methanol and water molecules. Hexane was described as a straight-chain alkane that was characterized by its inability to form hydrogen bonds, making it insoluble in water. Ethyl acetate was identified as an ester compound where a semi-polar state was created by its dipole moment. The extract yields for both soft coral species used methanol resulted in higher values compared to ethyl acetate and hexane because methanol exhibited a high polarity that enabled it to dissolve a wider range of bioactive compounds. A higher extract yield was produced by methanol compared to ethyl acetate and hexane due to its high polarity and universal nature, which allowed a greater variety of secondary metabolites to be dissolved. Important bioactive compounds were effectively extracted by methanol; because it was composed of both polar hydroxyl and non-polar methyl groups, bioactive components with a broader polarity range were able to be absorbed. Additionally, the diffusion of the solvent into sample cells was facilitated by its relatively low viscosity, allowing the total amount of dissolved substances to be optimized (Yahyaoui *et al*., 2017).

The antibacterial activity of the extracts from two types of soft corals against the pathogenic bacteria *E. coli* was showed to be greater when ethyl acetate solvent was used, compared to methanol and hexane. This was consistent with previous research, in which bioactive compound extracts from the soft coral *Sinularia* sp. were found to be highly effective as antibacterial agents against the pathogen *E. coli* (Radjasa *et al*., 2007; Wang *et al*., 2009; Putra *et al*., 2012; Wei *et al*., 2013; Putra *et al*., 2016; Mousavi *et al*., 2023). This proved that the semi-polar ethyl acetate solvent performed better than polar methanol and non-polar hexane in extracting bioactive antibacterial compounds. A polarity level between polar and non-polar was possessed by ethyl acetate, which was allowed for the maximal extraction of complex bioactive antibacterial compounds. Due to its semi-polar characteristics, ethyl acetate was made a universal solvent in the extraction process of natural products. Both polar compounds, such as flavonoids, and non-polar compounds, like steroids or terpenoids, were dissolved by the polar carbonyl group and non-polar hydrocarbon chain of ethyl acetate (Sukmawati *et al*., 2019; Akbar *et al*., 2021; Suarjana *et al*., 2025; Faradin *et al*., 2025; Subandrate *et al*., 2024; Royani *et al*., 2025; Azzahra *et al*., 2025).

CONCLUSION

Two types soft corals of *Sinularia* sp. are found in the Pahawang Island waters of are *S. polydactyla* and *S. flexibilis*. The extract yield from these soft corals with methanol can produce higher than with ethyl acetate and hexane. The ethyl acetate solvent can show higher antibacterial activity in soft coral extracts than with methanol and hexane. Soft coral extract for *Sinularia* sp. with ethyl acetate solvent can be used as antibacterial candidates against the pathogenic bacteria *E. coli*.

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