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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF SOFT CORAL *Nephthea* sp. EXTRACT ISOLATED AGAINST *Escherichia coli*

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ABSTRACT

This study aimed to evaluate the antibacterial potential of Nephthea sp. extract against Escherichia coli and to analyze the phytochemical constituents present in Nephthea sp. using various solvents. The extraction process was carried out using the maceration method with three different solvents: n-hexane, ethyl acetate, and methanol. The resulting extracts were analyzed through phytochemical screening and antibacterial activity assays. The results showed that Nephthea sp. extract contained several classes of bioactive metabolites, including alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins. Antibacterial testing demonstrated that the ethyl acetate extract produced the largest inhibition zone, measuring 3.16 mm. Based on the ANOVA test results, the 25% concentration showed a significant effect on the inhibition zone. Based on phytochemical analysis, the ethyl acetate extract contained triterpenoid compounds, whereas the other solvents did not

INTRODUCTION

The marine environment is recognized for its exceptionally high biodiversity and unique physicochemical conditions, making it a rich source of natural compounds with diverse chemical structures and biological activities (Carroll et al., 2025). In recent years, numerous studies have reported the successful isolation of bioactive compounds from marine organisms, highlighting their significant potential for pharmaceutical

applications, including antibacterial, anticancer, and immunomodulatory agents (Carroll et al., 2025). Soft corals, one of the major groups of marine invertebrates, are an important source of secondary metabolites with strong pharmacological activity. Various compounds, such as terpenoids, steroids, and alkaloids, produced by soft corals have been reported to possess significant antimicrobial, anti-inflammatory, antiviral, and antioxidant activities (Abdelhafez et al., 2021).

Bioactive compounds from soft corals have continued to attract research attention, particularly in health, due to their broad spectrum of biological activities, including potential as antibacterial agents against pathogens such as *Escherichia coli*. Infections caused by *E. coli*, especially those belonging to the diarrheagenic *E. coli* (DEC) group, remain a major cause of acute diarrhea in children in developing countries, contributing significantly to high morbidity and mortality rates among infants and young children (Gomes et al., 2016).

The production of secondary metabolites by soft corals is strongly influenced by the environmental conditions of their marine habitats. Marine organisms living in more polluted environments tend to produce higher amounts of secondary metabolites as an adaptive response to environmental stress (Yurchenko et al., 2021). Factors such as ocean depth, temperature, salinity, pH, light intensity, and nutrient availability also affect the variation and bioactivity of the compounds produced by soft corals(Karthikeyan et al., 2022).

Recent studies have shown that soft coral genera such as *Sinularia* sp., *Dampia* sp., and *Nephthea* sp. are rich in bioactive compounds, including saponins, phenolic compounds, triterpenoids, alkaloids, and sesquiterpenes and steroids, as identified through phytochemical screening (Fahrudin et al., 2025). In addition, recent investigations of *Nephthea* sp. revealed the presence of alkaloids, steroids/terpenoids, and flavonoids/phenolics. These compounds exhibited antibacterial activity against Staphylococcus aureus and Escherichia coli based on disc diffusion assays *(Rozirwan et al., 2015)*. The chemical complexity within this genus makes it a promising subject for further exploration as a potential antimicrobial agent and a marine-derived source of candidate metabolites in marine biotechnology.

Soft corals originating from West Nusa Tenggara, particularly from Kolo Beach in Bima City, have not yet been investigated for their bioactive compound content using

phytochemical tests or antibacterial activity assays. Based on this gap, the present study was conducted as a preliminary investigation to explore the bioactive potential of *Nephthea* sp., identify the chemical constituents through phytochemical screening, and evaluate its antibacterial activity against selected test bacteria.

MATERIALS AND METHODS

Sample Collection

Samples of the soft coral *Nephthea* sp. were collected from the waters of Kolo Beach, Bima, at a depth of 3–8 meters using scuba diving techniques. The sampling site was located at approximately 8.45° S and 118.74° E, based on map interpretation. The collected specimens were identified to the genus level based on their morphological characteristics, including a tree-like colony structure with a soft, flexible texture, a branching capitulum, and polyps distributed along the terminal branches, all typical features of the genus *Nephthea*. Identification was conducted using standard taxonomic keys and relevant literature on soft corals. The samples were cut using a stainless-steel knife, placed in plastic sample bags, and transported in a cool box to the laboratory for further analysis.

Sample Extraction

The soft coral samples were extracted using a sequential maceration technique. First, the samples were thoroughly washed, air-dried, cut into small pieces, and ground into a fine powder (simplicia). Sequential maceration was then carried out using n-hexane, ethyl acetate, and methanol as solvents, each for 24 hours. The extracts were filtered and concentrated using a rotary vacuum evaporator to obtain crude extracts, which were subsequently stored at room temperature until further analysis.

Metabolite Detection

Qualitative analysis of secondary metabolites was performed to identify the presence of alkaloids, flavonoids, triterpenoids/steroids, tannins, and saponins using specific test tube reagents. The procedure followed modified methods from (Luringunusa et al., 2023).

Antibacterial Activity Test

Antibacterial activity against *Escherichia coli* was evaluated using the disc diffusion method. The *E. coli* strain used in this study was obtained from the Faculty of Food Technology and Agroindustry, Universitas Mataram. The extracts were tested at concentrations of 15%, 20%, and 25%, with DMSO serving as the negative control and chloramphenicol as the positive control. The bacterial suspension was adjusted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar (MHA) plates. Sterile paper discs impregnated with the extracts were placed on the agar surface and incubated for 24 hours at 37 °C. The inhibition zones formed around the discs were measured to assess antibacterial efficacy. Antibacterial activity was indicated by the formation of inhibition zones surrounding the paper discs. The diameters of the inhibition zones were measured with a vernier caliper. The inhibition zone data were analyzed by calculating the mean inhibition zone values. The inhibition zone data were expressed as mean ± standard deviation from three independent replicates (n = 3). Statistical analysis was performed using analysis of variance (ANOVA).

$$\mathrm{Dzb} = \frac{(\mathrm{Dv} - \mathrm{Dc}) + (\mathrm{Dh} - \mathrm{Dc})}{2}$$

Notes:

Dzb = Diameter of the clear (inhibition) zone

Dv = Vertical diameter

Dh = Horizontal diameter

Dc = Disc diameter

Data Analysis

The inhibition zone data were analyzed quantitatively and descriptively. A one-way ANOVA was conducted to determine the significance of differences in antibacterial activity among extract concentrations. If a significant difference ($p < 0.05$) was found, a post hoc Tukey HSD test was performed to determine differences between groups.

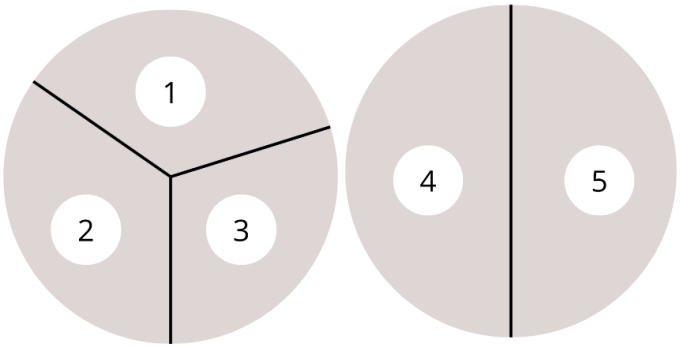

Figure 1. Plate design for antibacterial activity testing (Nurhayati et al., 2020). Antibacterial activity assay of soft coral extracts (*Nephthea* sp.) against *E. coli* : 1 = 15% concentration, 2 = 20% concentration, 3 = 25% concentration, 4 = positive control, 5 = negative control.

RESULTS AND DISCUSSION

The extraction of the soft coral *Nephthea* sp. in this study was performed using a sequential maceration method with three solvents in succession: n-hexane, ethyl acetate, and methanol. Variation in solvent polarity enables the isolation of a broader range of bioactive compounds, as each solvent dissolves specific compounds based on its polarity.

Based on the extraction yield results, the methanol extract produced the highest yield of 7.38%, followed by ethyl acetate at 3.5% and n-hexane at 3.24%. The yield value reflects the efficiency of each solvent in extracting compounds from the soft coral.

According to Roy et al. (2021), the extraction yield indicates the amount of secondary metabolites extracted by the solvent, although it does not specify the types of compounds obtained. Differences in yield values are influenced by the polarity of the solvents used. The higher yield observed in the methanol extract is attributed to methanol's polar nature, which enables it to dissolve a wide range of organic compounds. Furthermore, Gil-Martín et al. (2022) stated that polar solvents are more volatile, facilitating their removal from the extract.

The results of the antibacterial activity test of the *Nephthea sp.* soft coral extract against *E. coli* are presented in Figure 2. The inhibition zones formed indicate the antibacterial activity of each extract against the test bacteria. Based on the ANOVA results, concentration had a significant effect on the inhibition zone (p-value = 0.0000 < 0.05). Based on the measurements, the *Nephthea* sp. extract prepared with ethyl acetate as the solvent produced the largest inhibition zone of 3.16 mm (Figure 3).

Phytochemical analysis (Table 1) revealed that the ethyl acetate extract contained triterpenoid compounds, whereas the other solvents did not. Triterpenoids are known to disrupt the integrity of the bacterial outer membrane by interacting with porins (transmembrane proteins), forming strong polymeric bonds that damage the porins and ultimately inhibit bacterial growth (Chama et al., 2023). Based on Chung PYK's (2022) research, triterpenoids, including α-amyrin and betulinic acid, can dismantle MRSA biofilms and suppress cellular metabolic processes. This supports the possibility of a non-porin-based antibacterial pathway that plays an important role in targeting biofilm-associated resistance.

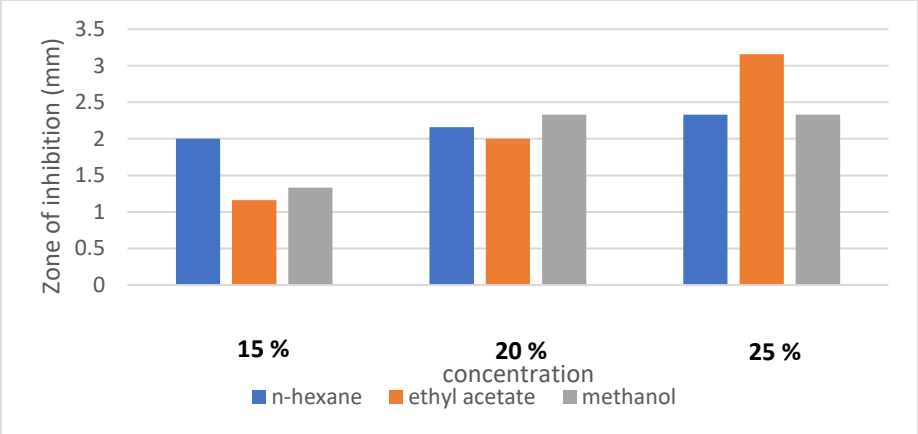

Figure 2. Inhibition zones of various Nephthea sp. soft coral extracts at concentrations of 15%, 20%, and 25% against Escherichia coli.

In addition to triterpenoids, alkaloids, flavonoids, and tannins were also detected. According to Rusli et al. (2019), flavonoids isolated from *Ficus variegata* exhibited antibacterial activity. *Escherichia coli* is classified as a Gram-negative bacterium. Studies conducted by do Nascimento & da Costa (2025) on the antibacterial properties of flavonoids against Gram-negative pathogens have demonstrated that several flavonoid compounds, such as liricidin-7-O-hexoside, licoflavone C, and dorsmanins, exhibit significant inhibitory activity. Their findings indicate that these flavonoids can interfere with key cellular processes, including membrane integrity, energy metabolism, and protein synthesis. A study by Zhang et al. (2025) found that flavonoids can inhibit bacterial growth in various mechanisms, such as destroying cell walls and membrane structures, affecting their normal morphology, inhibiting the synthesis or function of nucleic acids and proteins, inhibiting biofilm formation, reducing the expression of

virulence factors, interfering with bacterial signal transduction, and inhibiting bacterial efflux pumps.

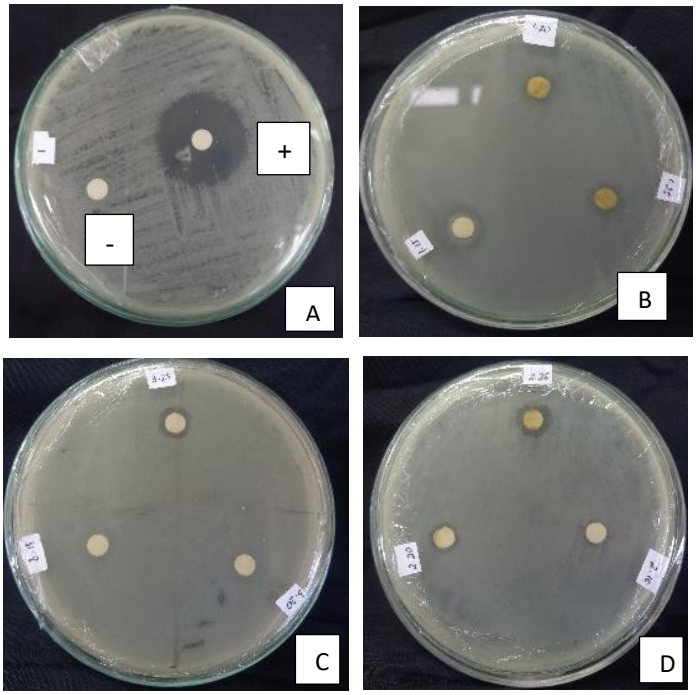

Figure 3. The inhibition zones formed on the *E. coli* growth medium by the n-hexane, methanol, and ethyl acetate extracts of *Nephtea* sp. Note: A(positive and negative control); B (n hexane); C (methanol); D (ethyl acetate)

Table 1. Results of Phytochemical Analysis of *Nephthea* sp.

Group of compounds	Reagent	*Nephthea* sp. extract		
		N-hexane	Ethyl acetat	Methanol
Alkaloids	Wagner's reagent	+	+	-
	Mayer's reagent	+	+	+
Flavonoids	Magnesium powder + HCl	+	+	+
Triterpenoids	Chloroform + Acetic anhydride +	-	+	-
Steroids	Sulfuric acid	+	-	-
Tannins	$FeCl_3$	+	+	+
Saponins	HCl	-	-	+

Note: (+) Positive, (–) Negative

The ethyl acetate extract of the soft coral also contained tannins. Tannin compounds, a group of polyphenols widely found in plant extracts, have been reported to exhibit antibacterial activity against various pathogenic bacteria, including E. *coli*, through several mechanisms such as disruption of the cell membrane and biofilm matrix, formation of complexes with bacterial enzymatic proteins that interfere with cellular metabolism, and inactivation of cell adhesins, thereby suppressing bacterial growth and viability. According to Hamzah et al. (2019), tannins can inhibit biofilm formation and

disrupt established biofilms of Gram-negative bacteria, such as *E. coli* and *Pseudomonas aeruginosa.* Tannins have the capacity to inhibit and disrupt both monospecies and polymicrobial biofilms, including those formed by *E. coli*, *P. aeruginosa*, and *S. aureus*, which is relevant to antibacterial mechanisms that disrupt the biofilm matrix and extracellular polymeric substance (EPS) structure. Tannins can inhibit bacteria by disrupting the structure of the cell membrane, inhibiting essential microbial enzymes, and interfering with nutrient uptake (such as sugars and amino acids), thereby suppressing bacterial growth (Kaczmarek, 2020)

Steroids were detected only in the n-hexane extract. Although it did not exhibit the highest activity, the n-hexane extract was able to inhibit *E.coli* with an inhibition zone of 2.33 mm. (Putra et al., 2021)have reported that steroid compounds isolated from the soft coral *Sinularia polydactila* exhibit antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *E. coli*. Steroids can interact with the cell's phospholipid membrane, which is permeable to lipophilic compounds, thereby decreasing membrane integrity and altering cell membrane morphology, leading to cell fragility and lysis (Sapara *et al.*, 2016).

Saponins were detected only in the methanol extract. Although it did not exhibit the highest activity, the 25% methanolic extract of the soft coral Nephthea sp. was able to inhibit *E.coli* with an inhibition zone of 3.33 mm. According to Stan et al. (2021), natural secondary metabolites, including saponins and steroids, have been widely reported to exhibit antimicrobial activity against various pathogenic bacterial strains through mechanisms such as disruption of cell membrane integrity and inhibition of cellular metabolic processes, indicating their potential for development as novel antibacterial agents with low toxicity. Saponin compounds exhibit antibacterial activity primarily through disruption of the bacterial cell membrane; their detergent-like properties reduce surface tension and membrane permeability, form complexes with membrane proteins, and compromise membrane integrity, resulting in leakage of proteins and ions from the cell, impairment of substance transport and cellular metabolism, and ultimately bacterial cell death (Putri et al., 2020)

This study is expected to serve as a foundation for further research exploring the bioactive compounds of Nephthea sp. and their potential applications in various health-related fields beyond antibacterial activity. The selection of samples from Kolo Beach,

Bima, is particularly relevant, as environmental factors such as water quality, temperature, salinity, and anthropogenic pressure may influence the production and diversity of secondary metabolites in marine organisms. These environmental variations can lead to differences in chemical profiles compared to those of the same genus collected from other geographic locations. Therefore, investigating Nephthea sp. from this specific area may provide novel insights into its bioactive potential. Furthermore, compound isolation is necessary to identify the specific antibacterial constituents present in the extract.

CONCLUSION

Antibacterial testing demonstrated that the ethyl acetate extract produced the largest inhibition zone, measuring 3.16 mm. Based on the ANOVA results, concentration had a significant effect on the inhibition zone. Phytochemical analysis revealed that the ethyl acetate extract contained triterpenoid compounds, whereas the other solvents did not.

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