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Phytochemical Compounds and Antibacterial Test of *Theonella cylindrica* against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT
Infectious diseases are a health problem faced by developing countries like Indonesia. This disease is caused by bacteria that attack humans. Improper use of antibiotics can cause bacterial resistance to antimicrobials. Antimicrobial resistance is the most significant public health threat, resulting in greater demand for finding new effective antimicrobials from natural products such as sea sponges. This research aimed to test the antibacterial activity of *Theonella extract* against *Staphylococcus aureus* and *Escherichia coli* and determine the class of chemical compounds contained therein. Research stages include sampling, sample preparation and extraction, qualitative phytochemical screening, making solutions, media creation, and antibacterial testing. The research showed that sponges metabolite compounds consisted of alkaloids, flavonoids, tannins, saponins, and phenolics. The diameter of the inhibition zone at concentrations of 80% and 100% showed potential antibacterial activity but more strongly inhibited the growth of *Staphylococcus aureus* bacteria than *Escherichia coli* bacteria.
Keywords: Phytochemical screening; antibacterial*; Theonella cylindrica; Staphylococcus aureus; Escherichia coli*

INTRODUCTION

Infectious diseases currently represent a formidable global health challenge (Baker et al., 2022). These maladies are primarily instigated by bacterial pathogens that assail human hosts. Antibiotics constitute the most frequently administered pharmacological agents in the management of infections. Consequently, the burgeoning incidence of infectious diseases has led to a commensurate escalation in antibiotic utilization (Dharmayani et al., 2023). Regrettably, a significant proportion of antibiotic misuse stems from self-medication practices. Such inappropriate antibiotic deployment can precipitate bacterial resistance, posing a substantial threat to public health (Gultom et al., 2021).

Staphylococcus aureus is a pivotal Gram-positive pathogen, a member of the Staphylococcaceae family, characterized by its spherical morphology and arrangement in grape-like clusters. Its approximate diameter spans from 0.5 to 1.5µm; it is non-motile, non-spore-forming, and exhibits facultative anaerobic growth, typically forming cohesive aggregations. Furthermore, *S. aureus* is recognized as a principal human pathogen, renowned for its remarkable adaptability to diverse host environments and capacity to induce a broad spectrum of distinct infections (Gherardi, 2023). Indeed, *Staphylococcus aureus* is among the predominant contributors to global morbidity and mortality attributable to infectious processes (Cheung et al., 2021).

Escherichia coli is a Gram-negative, rod-shaped bacterium belonging to the Enterobacteriaceae family (Pakbin et al., 2021). Certain pathogenic strains of *E. coli* are causative agents of various infectious diseases, including intestinal infections such as diarrhea and dysentery, as well as extraintestinal manifestations like urinary tract infections, respiratory tract infections, meningitis, and sepsis.

Pathogenic *E. coli* strains contribute significantly to global morbidity and mortality, thereby substantially burdening public health (Pokharel et al., 2023; Kaper et al., 2004).

Antimicrobial resistance arises when bacterial adaptations render therapeutic agents less effective in treating infections (Tuttle et al., 2021). AMR has emerged as one of the most formidable public health threats of the 21st century, frequently termed the "Silent Pandemic," necessitating urgent intervention. Without proactive measures, projections indicate that 2050 AMR could become a leading global cause of mortality (Tang et al., 2023). This escalating resistance underscores the critical imperative to discover novel and effective antimicrobial compounds from alternative sources. Approximately 50% of new drugs are antibiotics derived from natural products, with marine environments representing an abundant reservoir of biologically and pharmaceutically active natural compounds (Anteneh et al., 2021). The diverse secondary metabolites produced by marine organisms have garnered considerable research interest, owing to their unique chemical structures and intriguing pharmacological activities. Consequently, marine ecosystems are anticipated to be a pivotal resource for the pharmaceutical sector in developing the next generation of antimicrobial agents (Srinivasan et al., 2021).

Marine sponges are primitive sessile animals remarkably rich in biologically active compounds (Jamaludin et al., 20230. As sessile invertebrates, sponges lack an innate immune system or overt physical defense structures such as spines or shells. Consequently, their primary defense mechanism resides in the biosynthesis of diverse secondary metabolites, which facilitate their adaptation to challenging environmental conditions (Ortigosa-Palomo et al., 2024). Secondary metabolites from marine sponges have been successfully isolated from various geographical locations. Compounds identified from marine sponges, including nucleoside derivatives, terpenoids, polyethers, alkaloids, macrolides, and peptides, exhibit a wide array of biological activities, notably anti-inflammatory, anticancer, and antimicrobial properties (Anteneh et al., 2021). While previous research has explored the antibacterial properties of sponges, the specific chemical compounds responsible for antibacterial activity within *Theonella* sp. genus sponges remain largely un-investigated. Therefore, this study aims to identify *Theonella* sp. extract's chemical compounds and evaluate *Theonella cylindrica* extract's antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

METHODS

Plant collection and extraction

Theonella cylindrica were collected from the marine waters of Likupang, North Minahasa, at coordinates N 01º44'35.6"; E 124º58'38.2" from a depth of 5–10 m, utilizing diving aids (mask, snorkel, fins, and oxygen tank). The collected samples were washed with fresh water to remove surface salinity. Subsequently, the cleaned sponge samples were sectioned into small cubes using a knife, immersed in ethyl acetate solution, and transported to the Research Laboratory of the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, UNSRAT, Sam Ratulangi University, Manado.

The sponge samples were extracted using the maceration method with ethyl acetate as the solvent. At room temperature, sample immersion was performed at a

1:3 ratio for 24 hours. The extraction process was homogenized using an automatic shaker. Subsequently, the extract will be concentrated and evaporated using a rotary vacuum evaporator to yield a viscous extract. This extract was then subjected to liquid-liquid fractionation using hexane, ethyl acetate, and methanol solvents to obtain three fractions: an n-hexane fraction (non-polar), an ethyl acetate fraction (semi-polar), and an ethanol fraction (polar). The resulting fractions were subjected to phytochemical screening and antibacterial activity assays.

Phytochemical screening

Alkaloid

The alkaloid test was performed according to Douglas *et al.* (Sangi et al., 2008). A 50-100 mg sample of the sponge extract was treated with sufficient chloroform, followed by the addition of 10 mL ammonia and 10 mL chloroform. The resulting solution was then filtered into a test tube, and 10 drops of 2N H_2SO_4 were added to the filtrate. The mixture was regularly shaken and allowed to stand for several minutes until two layers formed. The upper layer was transferred in 1 mL aliquots into three separate test tubes. Several drops of Mayer's, Wagner's, and Dragendorff's reagents were added to each tube, respectively. The formation of a precipitate indicates the presence of alkaloids: Mayer's reagent yields a white precipitate, Wagner's reagent produces a brown precipitate, and Dragendorff's reagent results in an orange precipitate (Ningsih et al., 2016).

Flavonoid

The flavonoid test was conducted following the method described by Cai (Sangi et al., 2008). A 50 mg sample of the sponge extract was extracted with 5 mL of ethanol and heated in a test tube for five minutes. Subsequently, several drops of concentrated HCl were added, followed by 0.2 g of magnesium powder. A positive result is indicated by the appearance of a dark red color within 3 minutes.

Tannin

The tannin test was performed according to Miranda (Sangi et al., 2008). A 50 mg sample of the sponge extract was submerged in ethanol, and then 2-3 drops of 1% $FeCl_3$ solution were added. The formation of a bluish-black or green coloration evidences a positive result.

Saponin

The saponin test was carried out as per Simes *et al.* (Sangi et al., 2008). A 50 mg sponge sample was placed into a test tube, distilled water was added to immerse the sample fully, and the mixture was boiled for 2-3 minutes. After cooling, the mixture was vigorously shaken. A stable foam indicates a positive result.

Triterpenoid and Steroid

The triterpenoid and steroid test were conducted according to Briggs (Sangi et al., 2008). A 50-100 mg sample of the sponge extract was immersed in glacial acetic acid. After 15 minutes, six drops of the solution were transferred to a test tube, and

2-3 drops of concentrated sulfuric acid were added. The presence of triterpenoids is indicated by the development of red, orange, or purple coloration, while the formation of a blue color identifies steroids.

Phenolic

Before identifying phenolic compounds, a continuous extraction was performed using a Soxhlet apparatus with ether as the solvent to dissolve fats and chlorophyll present in the breadfruit leaves. The resulting ether extract displayed a greenishblack color; however, it did not react with 5% $FeCl_3$, indicating the absence of phenolic compounds in the ether extract. Following ether extraction, sequential extractions were carried out with 90% methanol and 50% methanol to bind polar components. A 1 mL aliquot of the methanol extract, after adding 5% $FeCl_3$, exhibited a color change from brownish-yellow to brownish-orange, confirming the presence of phenolic compounds.

Sample Preparation

The fermentation samples were heated on a thermoblock at 80ºC for 2 hours, then centrifuged at 6000 rpm for 5 minutes. The centrifuge results were diluted to 10^0, 10^{-2} dan 10^{-4}.

Sterilization of Tools and Nutrient Agar Media

The tools and materials used in this antibacterial activity study were sterilized beforehand using an autoclave.

Inoculation of Bacteria on Slanted Agar Media

The test bacteria were taken with a sterile needle and then planted on slanted agar media by scratching. They were then incubated in an incubator at a temperature of 37°C for 24 hours. The same treatment was performed on each type of test bacteria.

Preparation of Turbidity Standard Solution (McFarland Solution)

99.5 ml of 0.36 N H2SO4 solution was mixed with 0.5 ml of 1.175% $BaCl_2.2H_2O$ solution in an Erlenmeyer flask. The mixture was then shaken until a turbid solution was formed. This turbidity is used as the turbidity standard for the test bacteria suspension.

Preparation of Test Bacteria Suspension

The inoculated test bacteria are taken with a sterile wire loop and suspended in a tube containing 2 ml of 0.9% NaCl solution until the turbidity is the same as the McFarland solution turbidity standard. The same treatment is carried out on each type of test bacteria.

Preparation of Test Media

The base layer is made by pouring 10 ml of NA from the base media into nine petridishes, then left to solidify. After solidifying, seven steel spacers are placed on the surface of the base layer and arranged so that the observation areas do not overlap. Next, the bacterial suspension was mixed into the NA culture medium.

After that, 25 ml of the suspension and culture medium mixture was poured into each petri dish with the spacers as the second layer. Next, the spacers were removed aseptically from the petri dishes, ultimately forming wells for the antibacterial test.

In-vitro Antibacterial Activity Assay

Preparation of Positive and Negative Control Solutions

The positive control was prepared from a 500 mg Ciprofloxacin tablet. One Ciprofloxacin tablet was crushed, then weighed to correspond to 500 mg. Subsequently, 10 mg of Ciprofloxacin powder was dissolved in 10 mL of sterile distilled water to obtain a Ciprofloxacin solution at a concentration of 50 µg/50µL-sterile distilled water served as the negative control.

Media Preparation

a. Slant Agar Medium

In an Erlenmeyer flask, the Nutrient Agar (0.46 g) was dissolved in 20 mL of distilled water (corresponding to 23 g/1000 mL). The mixture was then homogenized using a stirrer over a water bath until boiling. Aliquots of 5 mL were dispensed into each of three sterile test tubes, which were then sealed with aluminium foil. The media were sterilized in an autoclave at 121°C for 15 minutes, then allowed to cool at room temperature for approximately 30 minutes until the media solidified at a 30°C inclination. The slant agar medium was utilized for bacterial inoculation.

b. Basal Medium and Broth Medium

The basal medium was prepared by weighing 2.8 g of Nutrient Agar and dissolving it in 100 mL of distilled water (23 g/1000 mL) in an Erlenmeyer flask. Concurrently, the broth medium was prepared by weighing 5.75 g of NA and dissolving it in 250 mL of distilled water (23 g/1000 mL) in an Erlenmeyer flask. Following dissolution, each medium was homogenized using a stirrer over a water bath until boiling. The homogenized media were then sterilized in an autoclave at 121°C for 15 minutes and cooled to approximately 45-50°C. The basal and broth medium were employed to prepare the test medium as the basal layer and the second layer, respectively.

Distilled water was the solvent for the extract samples, sterile distilled water served as the negative control, and a Ciprofloxacin solution of 50 µg/50 µL was used as the positive control. Each concentration series (20, 40, 60, and 80%) was dispensed into separate wells at 50 µL per well. The Petri dishes were then incubated at 37°C for 24 hours.

Observation and Measurement

Observations were conducted following a 24 hours incubation period. The presence of a clear zone signifies bacterial susceptibility to the tested antibiotic or other antibacterial agents, quantified by the diameter of the inhibition zone. The diameter of the inhibition zone was measured in millimetres (mm) using a scaled ruler, calculated by subtracting the 7 mm diameter of the well from the total diameter. Subsequently, the antibacterial potency of the inhibition zone was categorized based on the classification system proposed by Davis and Stout (Davis

and Stout, 1971). The formation of an inhibition zone is characterized by the appearance of a clear area surrounding the disk. The strength of the inhibitory potency, as indicated by the inhibition zone, can be evaluated using the classification presented in **Table 1**.

Table 1. Classification of Inhibitory Zones

Inhibitory zone diameter (mm)	Inhibitory Potency
<5 mm	Weak
5 – 10 mm	Moderate
11 – 20 mm	Strong
>21 mm	Very Strong

RESULTS AND DISCUSSION

Phytochemical Screening Results

Phytochemical analysis was conducted utilizing visualization techniques involving color changes and precipitation to ascertain the secondary metabolite content within the extracts. **Table 2** presents the qualitative analysis of secondary metabolite compounds identified in *Theonella* extracts, obtained by applying various solvents. The extract underwent liquid-liquid fractionation using hexane, ethyl acetate, and methanol solvents, yielding three distinct fractions: an n-hexane fraction (non-polar), an ethyl acetate fraction (semi-polar), and an ethanol fraction (polar). These fractions were subsequently subjected to qualitative phytochemical analysis. The phytochemical screening results indicated that the sponge extracts, across all three solvents, contained alkaloids, flavonoids, tannins, saponins, and phenolics. The phytochemical findings in this study align with previous research, confirming the diverse secondary metabolite profile of *Theonella swinhoei* and *Theonella cf. cupola*. This prior work also substantiated the presence of crucial secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolics (Jamaludin et al., 2023). The observed antibacterial activity is correlated with the phytochemical compounds inherent in *Theonella*, which serve as a protective shield against bacterial pathogens. Specifically, alkaloids, flavonoids, tannins, saponins, and phenolics can inhibit bacterial proliferation by disrupting bacterial cell walls (Mohammed Atiyah et al., 2022).

Table 2. Phytochemical Test of *Theonella cyclindrica* Extract

Metabolite	Ethanol	Ethyl Acetate	n-Hexane
Alkaloid (Dragendorf, Wagner, Meyer)	+++	+++	+++
Flavonoid	+	+	+
Tanin	+	+	+
Saponin	+	+	+
Steroid	-	-	-
Triterpenoid	-	-	-
Phenolic	+	+	+

Antibacterial Activity

The antibacterial activity assay was conducted using the agar well diffusion method. This method was chosen for its relative speed, simplicity, and ease of execution. The principle of the well diffusion method involves measuring the diameter of the inhibition zone formed around wells containing the extract. The antibacterial activity of *Theonella cylindrica* sponge extracts was tested against *Staphylococcus aureus* and *Escherichia coli*. This study employed Ciprofloxacin 500 mg, a broad-spectrum antibiotic, as a positive control. The inclusion of a positive control is crucial for evaluating the validity of the testing methodology. The antibacterial assay results for the sponge extracts against *S. aureus* and *E. coli*, utilizing three different solvents, are presented in **Table 3**.

Table 3. Antibacterial Activity of *Theonella cylindrica*

Isolates	Concentration (%)	Mean Inhibition Zone Diameter (mm) ± SD	Category
Staphylococcus aureus Ethanol	K – (Aquadest)	0 ± 0	Weak
	20	9.3 ± 0.28	Moderate
	40	10.3 ± 0.28	Moderate
	60	11.5 ± 0.28	Strong
	80	12.3 ± 0.28	Strong
	100	14.3 ± 0.28	Strong
	K + (Ciprofloxacin)	32.5 ± 0.00	Very Strong
Staphylococcus aureus Ethyl Acetate	K – (Aquadest)	0 ± 0	Weak
	20	8.5 ± 0.00	Moderate
	40	9.8 ± 0.35	Moderate
	60	11.2 ± 0.28	Strong
	80	12.5 ± 0.00	Strong
	100	13.5 ± 0.00	Strong
	K + (Ciprofloxacin)	32.3 ± 0.28	Very Strong
Staphylococcus aureus n-Hexane	K – (Aquadest)	0 ± 0	Weak
	20	8.5 ± 0.00	Moderate
	40	8.8 ± 0.28	Moderate
	60	9.5 ± 0.00	Moderate
	80	11.5 ± 0.50	Strong
	100	12.5 ± 0.00	Strong
	K + (Ciprofloxacin)	32.2 ± 0.28	Very Strong
Escherichia coli Ethanol	K – (Aquadest)	0 ± 0	Weak
	20	8.3 ± 0.28	Moderate
	40	8.5 ± 0.00	Moderate
	60	10.0 ± 0.00	Moderate
	80	10.5 ± 0.00	Moderate
	100	11.5 ± 0.00	Strong
	K + (Ciprofloxacin)	18.2 ± 0.28	Strong
Escherichia coli Ethyl Acetate	K – (Aquadest)	0 ± 0	Weak
	20	8.5 ± 0.00	Moderate
	40	8.3 ± 0.35	Moderate
	60	9.3 ± 0.35	Moderate
	80	10.2 ± 0.28	Moderate
	100	11.3 ± 0.28	Strong
	K + (Ciprofloxacin)	18.2 ± 0.28	Strong

Isolates	Concentration (%)	Mean Inhibition Zone Diameter (mm) ± SD	Category
Escherichia coli	K – (Aquadest)	0 ± 0	Weak
n-Hexane	20	7.8 ± 0.35	Moderate
	40	8.2 ± 0.28	Moderate
	60	9.0 ± 0.00	Moderate
	80	9.7± 0.28	Moderate
	100	10.5 ± 0.00	Moderate
	K + (Ciprofloxacin)	18.2± 0.28	Strong

Table 3 presents the mean inhibition zone diameters and standard deviations for *Staphylococcus aureus* and *Escherichia coli*, based on triplicate treatments at concentrations of 20%, 40%, 60%, 80%, and 100%. The obtained results demonstrate the potential of the sponge extract to inhibit the growth of both *S. aureus* and *E. coli*. The positive control, the broad-spectrum antibiotic Ciprofloxacin, exhibited the largest mean inhibition zone diameter against *S. aureus* at 32.5 mm. "Broad-spectrum" refers to a compound's activity against a wide range of bacterial strains, encompassing both Gram-positive and Gram-negative bacteria (World Health Organization, 2014).

For the ethanol fraction, the inhibition zone diameters against *S. aureus* at 80% and 100% concentrations were 12.3 ± 0.28 mm and 14.3 ± 0.28 mm, respectively. The ethyl acetate fraction showed inhibition zone diameters of 12.5 ± 0.00 mm and 13.5 ± 0.00 mm at 80% and 100% concentrations, while the n-hexane fraction yielded inhibition zones of 11.5 ± 0.50 mm and 12.5 ± 0.00 mm at the same concentrations. Based on the inhibition zone diameters observed for the ethanol, ethyl acetate, and n-hexane fractions at 80% and 100% concentrations, and in accordance with the classification by Davis and Stout (Davis & Stout, 1971), these fractions can be categorized as exhibiting strong inhibitory potency. Specifically, strong inhibition zones were observed at 100% concentration for the ethanol and ethyl acetate fractions, with diameters of 11.5 ± 0.00 mm and 11.3 ± 0.28 mm, respectively. At lower concentrations, a moderate inhibitory potency was observed. Conversely, the n-hexane fraction consistently exhibited only moderate inhibitory potency across all tested concentrations.

The results of this antibacterial activity assay are consistent with previous findings in the literature. Research by Mengko *et al.* (Mengko et al., 2022) demonstrated that the ethanol extract of *Theonella swinhoei* sponge possesses vigorous antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. Similarly, Lamatenggo *et al.* (Lamatenggo et al., 2018) investigated the antibacterial activity of *Theonella* extracts using various solvents, reporting strong inhibitory effects against Gram-positive and Gram-negative bacteria. Furthermore, Kuo *et al.* (Kuo et al., 2019) corroborated these findings by culturing 700 isolates (comprising 606 bacteria and 94 actinomycetes), which represent symbiotic microorganisms of marine invertebrates associated with *Theonella* as sources of bioactive compounds. Tests conducted on various bacterial species demonstrated potent broad-spectrum antibacterial activity.

The results indicate that an increase in extract concentration correlates with an enlargement of the mean inhibition zone diameter. This phenomenon is attributed to higher extract concentrations containing more active antibacterial compounds,

enhancing inhibitory effects (Achmad et al., 2024). The secondary metabolites present in the sponge extract act as antibacterial agents. Terpenoids and saponins are reported to damage bacterial cell membranes by increasing membrane permeability, thereby inducing bacterial cell disruption. Flavonoids exert their effects by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, energy metabolism, and porin transport within the cell membrane. Phenolic compounds exert their mechanism by denaturing and coagulating proteins, consequently inhibiting bacterial growth. Alkaloids are known to inhibit nucleic acid synthesis and enzyme activity. Furthermore, tannins interfere with bacterial cell walls and inhibit essential enzymes (Cane et al., 2023; Sasanti et al., 2025).

CONCLUSION

Phytochemical analysis revealed that the extract of *Theonella cylindrica* sponge contained a spectrum of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolics. Subsequent antibacterial assays revealed a concentration-dependent inhibitory effect, with the 80% and 100% extract concentrations demonstrating significant zones of inhibition. Furthermore, the extract exhibited differential efficacy across bacterial types, manifesting a markedly more potent growth suppressive effect against *Staphylococcus aureus* compared to *Escherichia coli*.

REFERENCES

Achmad, M.N., Masengi, A.S.R., Posangi, J., Fatimawali, Mambo, C.D. (2024). Skrining Fitokimia dan Uji Aktivitas Antibakteri Ekstrak Etanol Daun Binahong (*Anredera cordifolia* (Ten.) Steenis) Terhadap *Streptococcus mutans* dan *Escherichia coli*. *JBL* 14, 1–13. https://doi.org/10.35799/jbl.v14i3.54394.

Anteneh, Y.S., Yang, Q., Brown, M.H., Franco, C.M.M. (2021). Antimicrobial Activities of Marine Sponge-Associated Bacteria. *Microorganisms* 9, 171. https://doi.org/10.3390/microorganisms9010171.

Baker, R.E., Mahmud, A.S., Miller, I.F., Rajeev, M., Rasambainarivo, F., Rice, B.L., Takahashi, S., Tatem, A.J., Wagner, C.E., Wang, L.-F., Wesolowski, A., Metcalf, C.J.E. (2022). Infectious disease in an era of global change. *Nat Rev Microbiol* 20, 193–205. https://doi.org/10.1038/s41579-021-00639-z.

Cheung, G.Y.C., Bae, J.S., Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* 12, 547–569. https://doi.org/10.1080/21505594.2021.1878688.

Cane, H.P.C.A., Musman, M., Yahya, M., Saidi, N., Darusman, D., Nanda, M., Rizki, D.R., Puspita, K. (2023). Phytochemical screening and antibacterial activity of ethnomedicinal plants from Gayo Lues Highland, Indonesia. *J Pharm Pharmacogn Res* 11, 117–128. https://doi.org/10.56499/jppres22.1526_11.1.117

Davis, W.W., Stout, T.R. (1971). Disc Plate Method of Microbiological Antibiotic Assay. *APPL. MICROBIOL*. 22.

Dharmayani, N.K.T., Isnaini, Ulfa, M., Sudirman, Yuanita, E., Sariningsih, B.N. (2023). Antibacterial Activity of Marine Sponge (*Stylotella* sp.). *JPPIPA,*

pendidikan ipa, fisika, biologi, kimia 9, 4801–4805. https://doi.org/10.29303/jppipa.v9i6.3839.

Gherardi, G. (2023). Staphylococcus aureus Infection: Pathogenesis and Antimicrobial Resistance. *IJMS* 24, 8182. https://doi.org/10.3390/ijms24098182.

Gultom, E.S., Sitompul, A.F., Situmorang, A.D. (2021). Identifying Sponge Symbiont Bacterial with Antibacterial Activity against Multi-Drug Resistant Organism (MDRO) Bacteria from Sea Waters in Sibolga, North Sumatra Indonesia. *Biosfer: Jurnal Tadris Biologi* 12, 169-184. http://ejournal.radenintan.ac.id/index.php/biosfer/index.

Jamaludin, N.A., Bakar, K., Saidin, J. (2023). In Vitro Biological Activity of Three Marine Sponges From *Theonella* and *Haliclona* Genera Collected From Bidong Island, Terengganu, Malaysia. *MAB* 52, 51–59. https://doi.org/10.55230/mabjournal.v52i2.2559.

Kaper, J.B., Nataro, J.P., Mobley, H.L.T. (2004). Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2, 123–140. https://doi.org/10.1038/nrmicro818.

Kuo, J., Yang, Y.-T., Lu, M.-C., Wong, T.-Y., Sung, P.-J., Huang, Y.-S. (2019). Antimicrobial activity and diversity of bacteria associated with Taiwanese marine sponge *Theonella swinhoei*. *Ann Microbiol* 69, 253–265. https://doi.org/10.1007/s13213-018-1414-3

Lamatenggo, D.A., Wewengkang, D.S., Simbala, H.E.I. (2018). Aktivitas Antimikroba Ekstrak Spons *Theonella swinhoei*. *PADA* 7.

Mengko, K.R., Wewengkang, D.S., Rumondor, E.M. (2022). Uji Aktivitas Ekstrak Etanol Spons *Theonella swinhoei* Terhadap Pertumbuhan Bakteri *Escherichia coli* Dan *Staphylococus aureus. Pharmacon* 11, 1231.

Mohammed Atiyah, M., Shnawa Jasim, H., Mohammed Atiyah, H. (2022). Phytochemical Screening and Anti-Bacterial Activities Aqueous & Alcoholic Extracts of *Averrhoa bilimbi* Leaf against Bacteria Isolated From Oral Cavity. *Archives of Razi Institute*. https://doi.org/10.22092/ari.2022.357207.1996

Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399, 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

Ningsih, D.R., Zusfahair, Z., Kartika, D. (2016). Identification of Secondary Metabolites Compounds and Antibacterial Activities on The Extract of Soursop Leaf. *Molekul* 11, 101. https://doi.org/10.20884/1.jm.2016.11.1.199.

Ortigosa-Palomo, A., Quiñonero, F., Ortiz, R., Sarabia, F., Prados, J., Melguizo, C. (2024). Natural Products Derived from Marine Sponges with Antitumor Potential against Lung Cancer: A Systematic Review. *Marine Drugs* 22, 101. https://doi.org/10.3390/md22030101.

Pakbin, B., Brück, W.M., Rossen, J.W.A. (2021). Virulence Factors of Enteric Pathogenic *Escherichia coli*: A Review. *IJMS* 22, 9922. https://doi.org/10.3390/ijms22189922.

Pokharel, P., Dhakal, S., Dozois, C.M. (2023). The Diversity of *Escherichia coli* Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *Microorganisms* 11, 344. https://doi.org/10.3390/microorganisms11020344.

Sangi, M., Runtuwene, M.R.J., Simbala, H.E.I. (2008). Analisis Fitokimia Tumbuhan Obat Di Kabupaten Minahasa Utara.

Sasanti, A.D., Widanarni, W., Sukenda, S., Wahjuningrum, D., Yuhana, M., Setiawati, M. (2025). Phytochemical screening and antibacterial activity test of *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts against *Aeromonas hydrophila*. *Biodiversitas* 26. https://doi.org/10.13057/biodiv/d260409

Srinivasan, R., Kannappan, A., Shi, C., Lin, X. (2021). Marine Bacterial Secondary Metabolites: A Treasure House for Structurally Unique and Effective Antimicrobial Compounds. *Marine Drugs* 19, 530. https://doi.org/10.3390/md19100530

Tang, K.W.K., Millar, B.C., Moore, J.E. (2023). Antimicrobial Resistance(AMR). *Br J Biomed Sci* 80, 11387. https://doi.org/10.3389/bjbs.2023.11387.

World Health Organization. (2014). Antimicrobial resistance: global report on surveillance. *World Health Organization*, Geneva.