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Antibacterial Ability of Seaweed Endophytic Bacteria (*Turbinaria ornata*, *Sargassum crassifolium*, and *Sargassum polycystum*) Against Skin Disease Agents

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Abstract

Bioactive substances that can prevent the growth of pathogenic germs can be produced by endophytic bacteria This study aims to identify seaweed endophytic bacteria (*Turbinaria ornata*, *Sargassum crassifolium*, *Sargassum polycystum*) and their potential antibacterial activity against skin disease agents (*Staphylococcus aureus* and *Staphylococcus epidermidis*). In February 2023 (the rainy season), seaweed was harvested from the waters off Panjang Island in Jepara Regency, Central Java, Indonesia. Endophytic bacterial isolates that had been effectively isolated and purified were directly tested for antibacterial activity. Three isolates with the best antibacterial activity were extracted using the ethyl acetate solvent. The extract samples will be put through additional antibacterial testing using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and paper disc diffusion procedures. Using the thin layer chromatography (TLC), the class of chemical components in the extract was determined. A molecular technique based on the 16s rRNA gene (1,400 bp) was used to identify endophytic bacterial isolates. Three species of seaweed yielded 25 isolates of endophytic bacteria: 11 from *T. ornata*, 7 from *S. crassifolium*, and 7 from *S. polycystum*. To.09.pp, To.10.pp, and Sc.06.pp were the 3 isolates with the highest antibacterial activity in the direct challenge test. Zone of inhibition results from subsequent antibacterial testing using paper disc diffusion ranged from medium to very strong intensity. To.10.pp sample's MIC test yielded the best results, with *S. aureus* and *S. epidermidis* concentrations of 937.5 and 1,875 µg/mL, respectively. MBC test yielded the highest findings, with *S. aureus* and *S. epidermidis* concentrations of 1,875 and 1,875 µg/mL, respectively. The To.10.pp sample yielded terpenoids, alkaloids, and flavonoids, according to TLC analysis of chemical constituent groups. *Acinetobacter indicus* strain 80-1-2 and *Vibrio harveyi* strain B14-1 were identified as potential endophytic bacteria from *T. ornata*, and *Acinetobacter indicus* strain 80-1-2 from *S. crassifolium*.

Keywords: Antibacterial, Endophytic bacteria, Seaweed, Skin disease

Introduction

One of the initiatives to improve and develop the potential of bioactive compounds obtained from natural products is the exploration of biological resources in Indonesian marine waters. Numerous studies have been conducted on various microbe types, particularly those from particular or niche habitats, as producers of bioactive chemicals. The potential for the synthesis of bioactive chemicals is highlighted by microbes connected to marine creatures [1]. High diversity has been seen in the interactions of bacteria with seaweed, including endophytic bacteria [2].

An abundance of biological resources, such as seaweed, may be found in the sea around Jepara. According to related studies, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Micrococcus luteus* were all susceptible to the antibacterial effects of seaweed extract obtained from Jepara marine waters [3]. Seaweed extract also demonstrated antibacterial action against *S. aureus* and *P. aeruginosa* [4]. Marine bacteria are reported to produce secondary metabolites that help the host [5]. The presence of related microorganisms, such as endophytic bacteria, is suggested to support the ability of seaweed extract to have antibacterial characteristics [6-8] and the immune response of fish [9].

Endophytic bacteria have the capacity to produce a variety of enzymes and substances from their host either independently or together [10]. Endophytic bacteria promote host growth and increase their resistance to environmental stress [11]. The interaction is mutually beneficial because the host provides a place to live [12]. Endophytic bacteria are known to produce bioactive chemicals that are effective against pathogenic germs and have various positive health benefits, including antibacterial, anticancer, antiviral, antifungal, antidiabetic, immunosuppressive, and antimalarial properties [13]. Pathogenic bacterial growth is supposedly inhibited by endophytic bacteria found in *T. ornata*, *S. crassifolium*, and *S. polycystum*.

Pathogenic bacteria *S. aureus* and *S. epidermidis* are widely distributed on the scalp of people with dandruff [14]. It is uncertain whether endophytic bacteria exist in *T. ornata*, *S. crassifolium*, and *S. polycystum* or what part they play in the development of dandruff by *S. aureus* and *S. epidermidis*. It is necessary to conduct more research on the endophytic bacteria found on *T. ornata*, *S. crassifolium*, and *S. polycystum* seaweed that was obtained from Jepara maritime waters and their capacity to inhibit *S. aureus* and *S. epidermidis* bacterial activity. Seaweed extract to determine its antibacterial activity, but did not check the activity of endophytic bacteria. This study aims to identify seaweed endophytic bacteria (*Turbinaria ornata*, *Sargassum crassifolium*, *Sargassum polycystum*) and their potential antibacterial activity against skin disease agents (*Staphylococcus aureus* and *Staphylococcus epidermidis*)

Materials and methods

Materials

Three species of seaweed were collected and identified based on key morphological determinations and reported literature, namely *Turbinaria ornata*, *Sargassum crassifolium*, and *Sargassum polycystum*. On Panjang Island in Jepara during the rainy season in February 2023, brown seaweed predominates. Seaweeds of the species *T. ornata*, *S. crassifolium*, and *S. polycystum* were gathered from the maritime environments of Panjang Island in the Indonesian province of Jepara in Central Java. Three types of seaweed were used to isolate endophytic bacteria. The assays in this study utilized the test microorganisms *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* FNCC 0048.

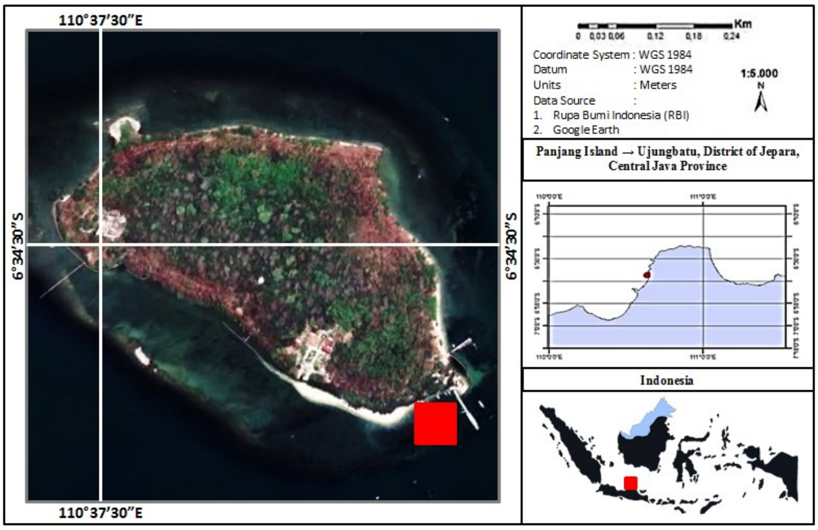


Figure 1 Seaweed sampling location (*Turbinaria ornata*, *Sargassum crassifolium*, dan *Sargassum polycystum*). Red point (6°34'41.29"S, 110°37'50.22"T).

Seaweed sampling

Seaweeds from Panjang Island, including *Sargassum polycystum*, *Sargassum crassifolium*, and *Turbinaria ornata*, were successfully gathered. The thallus and holdfast of the seaweed were sampled together [15]. Seaweed samples were kept in a coolbox that contained ice at a 1:1 (w/w) ratio [16].

Isolation and purification endophytic bacteria

Seaweed samples from *Sargassum polycystum*, *Sargassum crassifolium*, and *Turbinaria ornata* were cut into 5 cm lengths after being rinsed or washed under sterile running water for approximately 15 min. Epiphytic bacteria were sterilized for 1 min in a 70 % ethanol solution, 1 min in a 5.25 % NaClO solution, and 3 times in sterile water [17]. The sterilized samples were then crushed with a mortar and pestle, diluted with concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}, and planted in ZMA medium by pouring (pour plate method), which was then incubated at 29 ± 2 °C for 24 - 48 h [15]. If the endophytic bacterial isolates developed and displayed various colony morphologies, they were then purified to obtain a single colony. Each bacterial isolate has a unique code.

Screening of endophytic bacteria in the production of antibacterial compounds

By using a direct challenge test, endophytic bacteria's initial antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* was first identified. The swab method (sterile cotton bud) was used to inoculate the test microorganisms on MHA medium. Seawater was used as the solvent for growing endophytic bacterial isolates on NB medium. The *S. aureus* and *S. epidermidis* cultures on MHA medium were exposed to a direct challenge test by placing 6 mm-diameter paper discs that had been dripped with a 20 µl liquid culture of endophytic bacteria on them. The cultures were incubated for 24 consecutive hours at 29 ± 2 °C [18]. The next testing phase will continue with 3 endophytic bacterial isolates that either form the biggest inhibition zone or have a high level of inhibitory activity.

Antibacterial activity test on pathogenic bacteria

Selection of endophytic bacteria by diffusion method (paper discs)

In 1 L of NB medium, endophytic bacteria were cultivated before being incubated at 29 ± 2 °C for 96 h. By macerating liquid cultures with ethyl acetate as a solvent, endophytic bacteria's bioactive components were bound and extracted. A rotary evaporator will be used to concentrate the extract, which will then be used in the subsequent test. The endophytic bacteria's ethyl acetate extract was diluted with DMSO organic solvent. In a sterile Petri dish container, the extract with a concentration of 30 mg/mL was pipetted up to 20 µl and drip-coated on sterile paper discs with a diameter of 6 mm [15]. The extract was then left for 60 min in laminar air flow to absorb on the paper discs. After being raised on NB medium, the test bacteria *S. aureus* and *S. epidermidis* were subsequently implanted in a swab (sweep) with a sterile cotton bud on MHA media [18]. The culture was covered with paper discs dripping with extract, 10 µg of chloramphenicol served as a positive control, and DMSO served as a negative control [19]. Gram-positive bacteria can be examined with the antibiotic chloramphenicol [20]. Using a caliper, the inhibition zone was measured following the 24-h incubation period at 29 ± 2 °C.

Minimum inhibitory concentration (MIC) method

Using a 96-well plate and the microdilution method, the MIC test was conducted. Endophytic bacterial concentrated extracts were dissolved in DMSO at a concentration of 30 mg/mL. 100 µl of MHB medium were poured into each well. 50 µl of MHB media was placed in the first row. The negative control well contained 50 µl of DMSO, the positive control well contained 50 µl of chloramphenicol, and the remaining wells each contained 50 µl of bacterial extract. From the first dilution, which was 7,500 µl /mL, to the 12th dilution, serial dilution was done. To balance the quantity with other wells, the wells in the 12th dilution will be discarded. Each well received an inoculation of a 100 µl test bacterial solution with a cell density of 0.5 McFarland Standard. At 29 ± 2 °C, the plates were incubated for 24 h. turbidity in the wells is used to measure antibacterial activity. Wells that are clear and does not form bacterial colonies at the bottom of the well show that antibacterial agents are inhibiting the test bacterium [21]. A positive control was performed using the antibiotic chloramphenicol at an initial concentration of 0.10 mg/mL in sterile water, while a negative control was performed using simply DMSO.

Minimum bactericidal concentration (MBC) method

Staphylococcus aureus and *Staphylococcus epidermidis*, the test microorganisms, were inoculated on NA media in a petri dish to perform the MBC test. The test microorganisms were added to NA medium, which was then cultured for 24 h at 29 ± 2 °C. The MBC value is given as the lowest concentration at which the test microorganisms do not grow [21].

Identification of antibacterial compounds by thin layer chromatography (TLC) method

To separate compound mixtures, the Thin Layer Chromatography (TLC) method was used. With eluents of various polarity values, a mixture of chemicals is separated [22]. Eluents such as methanol, which is polar, ethyl acetate, which is semi-polar, and n-hexane, which is non-polar, are employed to extract endophytic bacteria. The eluent ratio employed was 1:6:2. The stains generated on the TLC plate were then observed using visible light and UV light with a wavelength of 365 nm. Spraying 10 % $FeCl_3$ reagent (for flavonoids), dragendorff (for alkaloids), and Liebermann Burchard (for steroid terpenoids) was used to determine the chemical group. Using 10 % $FeCl_3$ as the reagent, flavonoids produced favorable results that generated orange or red [23]. Dragendorff reagent results that were favorable for alkaloid compounds produced orange, orange, and brown [24]. The development of blue violet or violet red, terpenoid red, and steroid blue during terpenoid compound testing using the Liebermann Burchard reagent is a sign of success [25,26].

Molecular identification of potential isolates

A molecular technique based on the 16s rRNA gene's (1,400 bp) identification was used to identify probable isolates. The steps in molecular identification included extracting total DNA using the QIAamp DNA Stool Mini Kit, isolating DNA, and amplifying DNA using 16s rRNA primers (around 1,400 bp). The kit protocol is followed for the PCR conditions, which also include the initial denaturation, denaturation, annealing, and polymerization phases. The PCR product will then be purified using DNA Clean & Concentrator, followed by sequencing and data analysis [27]. Beginning with manual base sequence editing in the BioEdit program, the base sequence is then matched or blasted against the NCBI database, followed by alignment or alignment of species data. Using the Neighbor Joining (NJ) technique and Mega11 software, create phylogenetic trees.

Results and discussion

Seaweed sampling

In February 2023 (the rainy season), 3 varieties of seaweed were taken from the waters surrounding Panjang Island in the Jepara Regency of Central Java, Indonesia. On open beaches, seaweed develops and clings to the dead coral substrate. *Turbinaria ornata*, *Sargassum crassifolium*, and *Sargassum polycystum* were the sample findings, according to the literature and the key of determination (**Table 1**) [28-30].

Table 1 Morphological identification of seaweed samples.

No	Seaweed	Description
1.		*Turbinaria* sp. → This seaweed is classified as part of the Phaeophyceae family of brown seaweeds. Small disc-shaped holdfasts, spinning branching forms on the main stem, and cylindrical, leathery, erect stems are all characteristics of the genus Turbinaria. The leaves of this species have a funnel form. The serrated edge of the leaf. On the stem, the leaves are arranged in a circle. The seaweed's morphological features identify it as *Turbinaria ornata*. Jepara waters have been reported to contain the seaweed species *Turbinaria ornata* [31,32].
2.		*Sargassum* sp. 1 → This seaweed is classified as part of the Phaeophyceae family of brown seaweeds. The elliptic to oval leaf thallus, serrated, wavy edges, and curved or tapering edges are characteristics of the genus Sargassum. Vesicles are oval or spherical up until they start to float. This species has regular alternate branching and a kind of cylindrical cauloid form. Phylloids range in shape from oval to oblong. The serrated edge of the leaf. The leaves have rounded, duplicated tips. The seaweed is *Sargassum crassifolium*, according to its morphological features. In the waters around Jepara, the seaweed species *Sargassum crassifolium* has been discovered [33].

No	Seaweed	Description
3.		*Sargassum* sp. 2 → This seaweed is a member of the Phaeophyceae family of brown seaweeds. habitat on the underlying coral reef. The elliptic to oval leaf thallus, serrated, wavy edges, and curved or tapering edges are characteristics of the genus Sargassum. The thallus of the main branch is bigger and darker than that of the other branches. Visually, the thallus seems light brown. The leaf's apex is sharp, obtuse, and acuminate. The leaf margins range from bisserrate to serrate to integer. The circular to acuminate basal leaves. *Sargassum polycystum* is the name of the seaweed based on its morphological features. Jepara waters have been reported to contain the *Sargassum polycystum* type of seaweed [30,33].

Note: The black line indicator shows a 5 cm long scale, the red line shows a 1 cm long scale.

Three species of seaweed, *Turbinaria ornata*, *Sargassum crassifolium*, and *Sargassum polycystum*, were discovered as a result of seaweed sampling on Panjang Island in Jepara. On Panjang Island in Jepara during the rainy season in February 2023, brown seaweed predominates. This is a result of the marine environment's activity, which does not support other seaweed life. Other seaweeds' ability to grow is constrained by physical causes like powerful waves and currents. In order for brown seaweed to survive in the harsh marine environment, it needs a robust structure and a strong holdfast. The samples of seaweed that were obtained were hard and had a firm grip on the dead coral substrate.

Isolation and purification endophytic bacteria

Using the morphological determination key, the endophytic bacterial isolates that had grown were purified, yielding a total of 25 isolates, of which 11 were from *Turbinaria* sp., 7 were from *Sargassum* sp. 1, and 7 were from *Sargassum* sp. 1 (**Table 2**).

Table 2 Results of morphological determination.

Isolat	*Form*	*Margin*	*Elevation*	*Size*	*Pigmentation*
To.01.pp	Filamentous	Filamentous	Convex	Moderate	Non Pigmentation
To.02.pp	Circular	Entire	Raised	Moderate	Non Pigmentation
To.03.pp	Irregular	Entire	Convex	Large	Non Pigmentation
To.04.pp	Circular	Entire	Raised	Small	Non Pigmentation
To.05.pp	Circular	Entire	Convex	Small	Non Pigmentation
To.06.pp	Circular	Entire	Convex	Small	Non Pigmentation
To.07.pp	Circular	Entire	Convex	Punctiform	Non Pigmentation
To.08.pp	Circular	Undulate	Convex	Punctiform	Non Pigmentation
To.09.pp	Circular	Entire	Raised	Punctiform	Non Pigmentation
To.10.pp	Circular	Entire	Convex	Large	Non Pigmentation
To.11.pp	Irregular	Filamentous	Flat	Large	Non Pigmentation
Sc.01.pp	Irregular	Lobate	Flat	Large	Non Pigmentation
Sc.02.pp	Irregular	Entire	Flat	Large	Non Pigmentation
Sc.03.pp	Circular	Undulate	Convex	Punctiform	Non Pigmentation
Sc.04.pp	Circular	Entire	Convex	Small	Non Pigmentation
Sc.05.pp	Irregular	Undulate	Flat	Punctiform	Non Pigmentation
Sc.06.pp	Circular	Undulate	Raised	Moderate	Non Pigmentation

Isolat	*Form*	*Margin*	*Elevation*	*Size*	*Pigmentation*
Sc.07.pp	Irregular	Entire	Convex	Large	Non Pigmentation
Sp.01.pp	Irregular	Undulate	Raised	Large	Non Pigmentation
Sp.02.pp	Irregular	Undulate	Flat	Large	Non Pigmentation
Sp.03.pp	Circular	Entire	Flat	Punctiform	Non Pigmentation
Sp.04.pp	Irregular	Lobate	Flat	Large	Non Pigmentation
Sp.05.pp	Irregular	Entire	Raised	Moderate	Non Pigmentation
Sp.06.pp	Circular	Entire	Raised	Moderate	Non Pigmentation
Sp.07.pp	Rhizoid	Rhizoid	Umbonate	Moderate	Non Pigmentation

Note: The letter code "To" indicates the isolate comes from *Turbinaria ornata*, "Sc" for *Sargassum crassifolium*, "Sp" for *Sargassum polycystum*. The code number is for the distinguishing mark of isolates originating from the same seaweed. The letter code "pp" indicates the isolate was collected from the waters of Panjang Island, Jepara.

Screening of endophytic bacteria in the production of antibacterial compounds

To ascertain their potential antibacterial activity, endophytic bacterial isolates were put through direct challenge testing on the test microorganisms *Staphylococcus epidermidis* and *Staphylococcus aureus*. The test was carried out once, so to ensure the effectiveness of inhibition zone observations were carried out at 18, 24 and 48 h to observe the consistency of contact time. The antibacterial activity of 25 isolates varied in its outcomes (**Table 3**). Three isolates with the codes To.09.pp, To.10.pp, and Sc.06.pp were chosen for additional testing based on the antibacterial activity (inhibition zone) generated.

Table 3 The activity of the associated bacteria in the direct challenge test on the test bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Isolate code	Inhibition zone diameter(mm)					
	18 h		24 h		48 h	
	S. epidermidis	*S. aureus*	*S. epidermidis*	*S. aureus*	*S. epidermidis*	*S. aureus*
To.01.pp	10	9	9	10	9	10
To.02.pp	10	8	9	9	9	9
To.03.pp	-	-	-	-	-	-
To.04.pp	9	8	10	8	-	8
To.05.pp	11	13	11	15	11	15
To.06.pp	12	11	12	11	12	-
To.07.pp	11	10	9	8	9	8
To.08.pp	13	8	13	-	13	-
To.09.pp	15	9	15	9	15	10
To.10.pp	14	13	14	13	14	13
To.11.pp	13	14	10	14	10	-
Sc.01.pp	8	10	-	10	-	-
Sc.02.pp	-	10	-	10	-	10
Sc.03.pp	-	-	-	-	-	-
Sc.04.pp	10	12	9	12	9	12
Sc.05.pp	8	11	9	11	9	11

Isolate code	Inhibition zone diameter(mm)					
	18 h		24 h		48 h	
	S. epidermidis	*S. aureus*	*S. epidermidis*	*S. aureus*	*S. epidermidis*	*S. aureus*
Sc.06.pp	10	13	10	13	10	13
Sc.07.pp	8	-	8	-	8	-
Sp.01.pp	-	11	8	11	8	11
Sp.02.pp	12	13	8	12	8	-
Sp.03.pp	10	10	10	12	10	-
Sp.04.pp	13	9	10	10	10	-
Sp.05.pp	-	-	-	-	-	-
Sp.06.pp	8	10	10	10	10	-
Sp.07.pp	8	9	10	9	10	-

Note: Units in millimeters, diameter of paper discs (6 mm) are included in the inhibition zone observation, code "-" for no inhibition zone/missing inhibition zone.

Antibacterial activity test on pathogenic bacteria

Selection of endophytic bacteria by diffusion method (paper discs)

Different outcomes were seen when endophytic bacterial extracts were tested for antibacterial activity against the test germs *S. epidermidis* and *S. aureus*. The largest inhibitory zone measured 29 mm, while the smallest measured 6 mm. Based on the intensity in relation to the diameter of the inhibition zone (ZOI), the outcomes of antibacterial testing employing the diffusion method (paper discs) will be categorized [34].

Table 4 Diffusion method test results (paper discs).

Isolate code	Inhibition zone area (mm)		Inhibition zone intensity	
	S. epidermidis	*S. aureus*	*S. epidermidis*	*S. aureus*
To.09.pp	10	9	strong	medium
To.10.pp	9	29	medium	very strong
Sc.06.pp	12	6	strong	medium

Note: Units in millimeters, the diameter of paper discs (6 mm) are not included in the inhibition zone observations. The ZOI categories are very strong (> 20 mm), strong (10 - 20 mm), medium (5 - 10 mm), and no response (< 5 mm).

Minimum inhibitory concentration (MIC) method

There were discrepancies in the findings of the minimum inhibitory concentration (MIC) test on endophytic bacterial extracts related to antibacterial activity against the test germs *S. epidermidis* and *S. aureus*. **Table 5** displays the outcomes of the minimum inhibitory concentration (MIC) test.

Table 5 Minimum inhibitory concentration (MIC) test results.

Extract	Highest concentration (µg/mL)	Dilution to -		Lowest concentration (µg/mL)	
		S. epidermidis	*S. aureus*	*S. epidermidis*	*S. aureus*
To.09.pp	7,500	2	2	3,750	3,750
To.10.pp	7,500	3	4	1,875	937.5
Sc.06.pp	7,500	3	2	1,875	3,750

Minimum bactericidal concentration (MBC) method

There were discrepancies in the findings of the minimum bactericidal concentration (MBC) test on endophytic bacterial extracts related to antibacterial activity against the test germs *S. epidermidis* and *S. aureus*. **Table 6** displays the outcomes of the minimum bactericidal concentration (MBC) test.

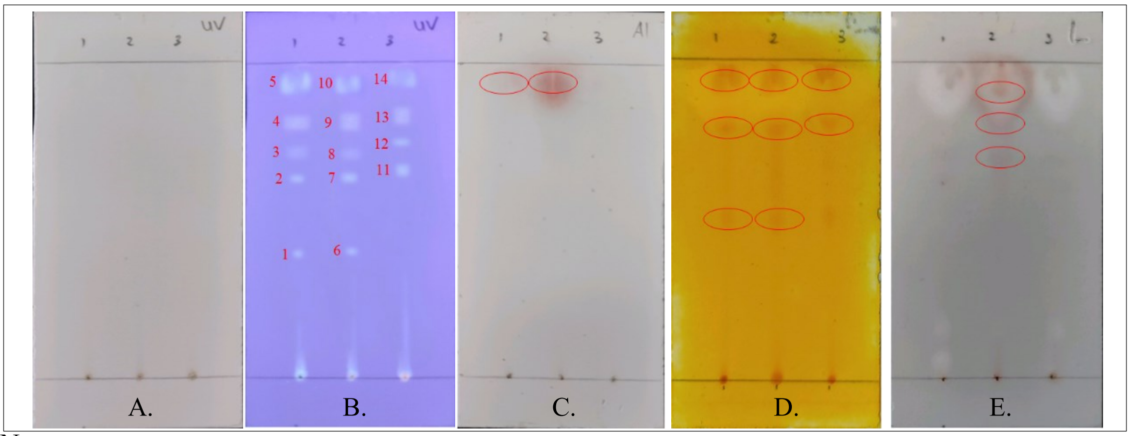
Table 6 Minimum bactericidal concentration (MBC) test results.

Extract	Highest concentration (µg/mL)	Dilution to -		Lowest concentration (µg/mL)	
		S. epidermidis	*S. aureus*	*S. epidermidis*	*S. aureus*
To.09.pp	7,500	2	1	3,750	7,500
To.10.pp	7,500	3	3	1,875	1,875
Sc.06.pp	7,500	2	1	3,750	7,500

Identification of antibacterial compounds by thin layer chromatography (TLC) method

The Thin Layer Chromatography (TLC) approach for identifying antibiotic chemicals in putative endophytic bacterial extracts revealed 14 stains on the TLC silica gel plate. Visualization with visible light does not reveal stains, however visualization with 365 nm-long UV light does reveal the spots that are produced. Endophytic bacterial extract To.09.pp produced 5 spots (0.35, 0.62, 0.7, 0.82 and 0.9), To.10.pp produced 5 spots (0.35, 0.6 , 0.75, 0.82 and 0.88), and Sc.06.pp produce 4 spots (0.62, 0.75, 0.82 and 0.88) (**Figure 1**).

The presence of flavonoid compounds was detected using $FeCl_3$ 10 % reagent, which resulted in a crimson stain on the TLC silica gel plate. The Rf_{10} = 0.88 stain revealed the presence of flavonoids in the endophytic bacterial extract To.10.pp. At Rf_5 = 0.9, the To.09.pp sample reacts with a faintly red tint. Using the Dragendorff reagent, it was possible to detect the presence of alkaloid compounds by seeing a color change reaction on the TLC silica gel plate, namely an orange stain. To.09.pp, an extract of an endophytic bacterium, was reactive at Rf_1 = 0.35, Rf_3 = 0.7, and Rf_5 = 0.9. Reactive values for the To.10.pp sample were Rf_6 = 0.35, Rf_8 = 0.75, and Rf_{10} = 0.88. At Rf_{12} = 0.75 and Rf_{14} = 0.88, the Sc.06.pp sample was reactive. Testing for the presence of terpenoid/steroid compounds using Liebermann Burchard reagent showed a color change reaction on the TLC silica gel plate, namely a red stain. The stain reaction turns red indicative of the presence of terpenoid compounds. Endophytic bacterial extract To.10.pp showed the presence of terpenoid compounds in Rf_8 = 0.75; Rf_9 = 0.82; and Rf_{10} = 0.88 (**Figure 1**).



Note:
Rf1 = 0,35
Rf_2 = 0,62
Rf_3 = 0,7
Rf_4 = 0,82
Rf5 = 0,9
Rf_6 = 0,35
Rf_7 = 0,6
Rf_8 = 0,75
Rf9 = 0,82
Rf_{10} = 0,88
Rf_{11} = 0,62
Rf_{12} = 0,75
Rf13 = 0,82
Rf_{14} = 0,88

Figure 1 Visualization results of thin layer chromatography (TLC) testing on endophytic bacteria extracts. A. TLC visualization in visible light, B. TLC visualization in UV light with a length of 365 nm, C. Flavonoid testing, D. Alkaloid testing, and E. Terpenoid-steroid testing. Code number 1 = To.09.pp; 2 = To.10.pp; and 3 = Sc.06.pp.

In comparison to To.09.pp and Sc.06.pp, samples of endophytic bacteria To.10.pp obtained the best antibacterial testing results using paper discs, MIC, and MBC diffusion techniques. The To.10.pp sample exhibits the biggest variance of compound groups, including flavonoids, alkaloids, and terpenoids, according to the results of the identification of chemical compound groups using TLC.

Turbinaria ornata, from which the endophytic bacterial extracts To.09.pp and To.10.pp are derived, is a type of seaweed. Because they can combine with extracellular proteins to form complex compounds, flavonoid compounds can act as antibacterial agents by compromising the integrity of bacterial cell membranes. The class of flavonoid chemicals has the ability to directly damage both gram-positive and gram-negative bacterial envelopes, as well as critical molecular targets in the target microorganism. Antibiotic resistance could become an increasingly important issue for the flavonoid group [35]. In addition to their antibacterial properties, flavonoids are also said to have anti-inflammatory, antioxidant, anti-tumor, and anti-fungal properties [36-40] and against Multi Drugs Resistants [41].

Extracts from To.09.pp, To.10.pp, and Sc.06.pp were discovered to contain alkaloid chemicals. More alkaloid groups are present in the endophytic bacterial extracts To.09.pp and To.10.pp, which are derived from the same seaweed, *Turbinaria ornata*. In addition to a sizable number of natural chemical compound groups and their structural diversity and complexity, the alkaloid compound group consists of heterocyclic nitrogen-containing compounds. The alkaloid family of substances has an antibacterial mechanism that involves preventing the development of bacterial cell walls, bacterial metabolism, altering the permeability of cell membranes, and inhibiting the creation of nucleic acids and proteins [42].

To.10.pp extract, which is an extract made from the endophytic seaweed *Turbinaria ornata* bacterium, contained the terpenoid chemical group. Isoprene is the fundamental structural component of a number of oxygenated hydrocarbons that belong to the terpenoid chemical group [43]. The terpenoid chemical compound family possesses antibacterial, antiviral, antimalarial, antitumor, anti-inflammatory, prevents treating cardiovascular disease, and hypoglycemic activity [44]. It also improves transdermal absorption and has anticancer and antiviral properties. The target bacteria's cell wall is destroyed by the terpenoid chemical group, which acts as an antibacterial agent. By interfering with the peptidoglycan component of the bacterial cell, the bacterial cell wall is destroyed, resulting in cell lysis damage and the death of the target bacteria [45].

Molecular Identification of Potential Isolates

Three possible endophytic bacterial isolates were successfully subjected to 16s rRNA gene amplification (1,400 bp). The findings demonstrate that all DNA bands have a 1,500 bp length. The outcomes revealed DNA bands that were clean and free of impurities (**Figure 2**).

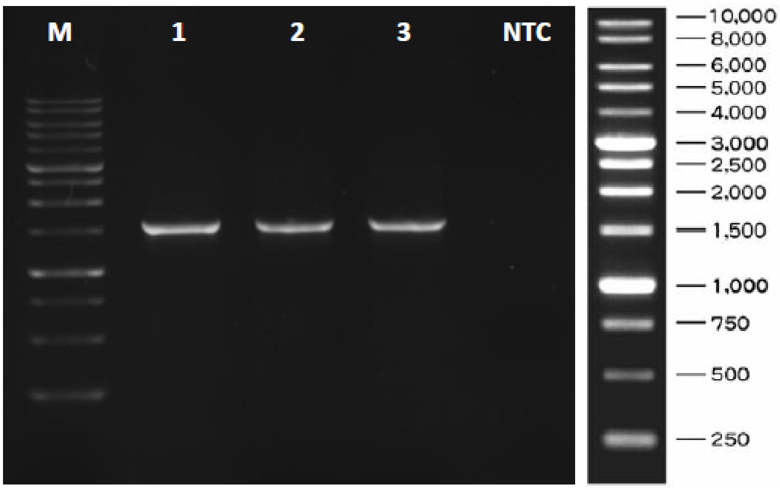


Figure 2 Photo Gel Results - PCR product; 1.5 µL of PCR product was assessed by electrophoresis with 1 % TBE agarose; M, 1 Kb DNA ladder (loaded 1.5 µL); code 1 = To.09.pp, 2 = To.10.pp, and 3 = Sc.06.pp.

Table 7 Gene-based sequence similarity of 16s rRNA (~ 1,400 bp) from potential endophytic bacteria using BLAST-N in NCBI.

Isolate code	*Nearest species*	*% similarity*	*Accession*
To.09.pp	*Acinetobacter indicus* strain 80-1-2	100.00 %	CP041295.1
To.10.pp	*Vibrio harveyi* strain B14-1	99.65 %	MK102612.1
Sc.06.pp	*Acinetobacter indicus* strain 80-1-2	99.00 %	CP041295.1

It was very successful to identify probable endophytic bacterial isolates using 16s rRNA gene-based molecular approaches (1,400 bp), which revealed the relationships between endophytic bacterial isolates. Using the Neighbor-Joining (NJ) approach and the 16s rRNA gene sequence, the phylogenetic relationships between endophytic bacteria and adjacent bacterial species were established. Molecular identification of 3 possible endophytic bacteria, To.09.pp (*Acinetobacter indicus* strain 80-1-2), To.10.pp (*Vibrio harveyi* strain B14-1), and Sc.06.pp (*Acinetobacter indicus* strain 80-1-2), based on the 16s rRNA gene (1,400 bp) (**Table 7**).

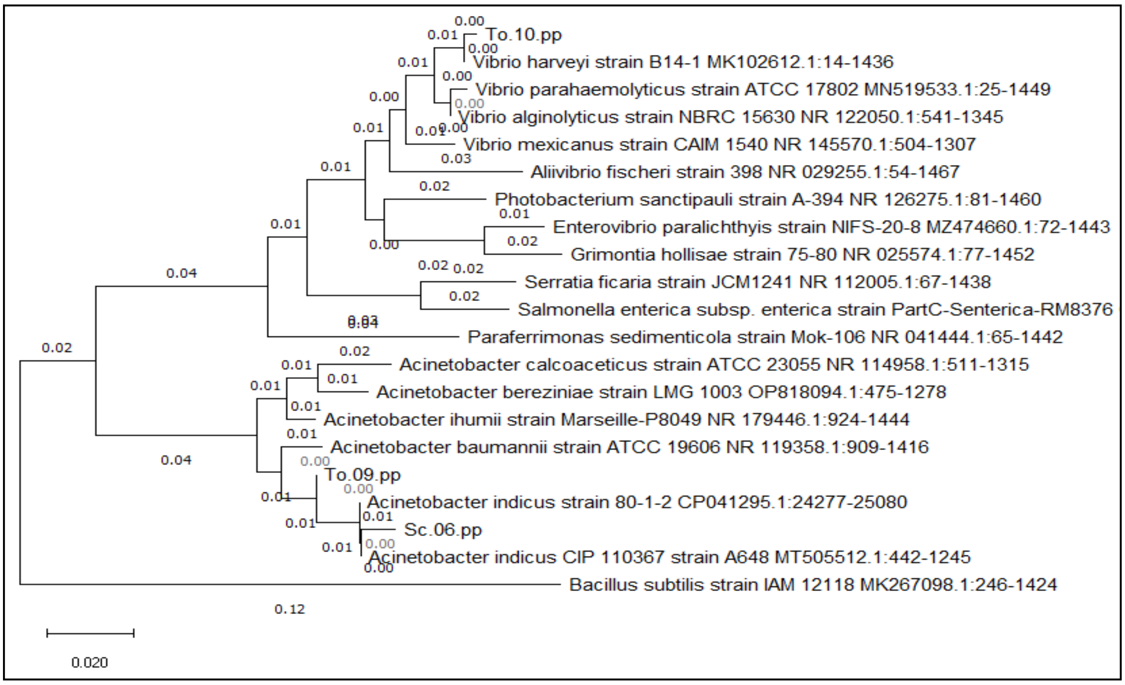


Figure 3 Potential endophytic bacterial phylogeny tree based on 16s rRNA gene sequence using Neighbor joining (NJ) method. Isolates coded To.09.pp, To.10.pp, and Sc.06.pp are potential endophytic bacterial isolates.

The endophytic bacteria To.09.pp, To.10.pp, and Sc.06.pp were 3 specifically chosen isolates. *Acinetobacter indicus* strain 80-1-2, *Acinetobacter indicus* CIP 110367 strain A648, and *Acinetobacter baumannii* strain ATCC 19606 are the closest species in isolates To.09.pp (*Turbinaria* sp.) and Sc.06.pp (*Sargassum* sp. 1), respectively. *Vibrio harveyi* strain B14-1, *Vibrio parahaemolyticus* strain ATCC 17802, and *Vibrio alginolyticus* strain NBRC 15630 make up the isolate To.10.pp (*Turbinaria* sp.). Two in-groups and one out-group may be seen in the cladogram produced by the Neighbor-Joining (NJ) method. There are 2 primary clades within the in-group; clade 1 comprises 12 species and clade 2 has 8 species. *Bacillus subtilis* strain IAM 12118 serves as the out-group. Vibrio, Aliivibrio, Photobacterium, Enterovibrio, Grimontia, Serratia, Salmonella, and Paraferrimonas are the 8 genera that make up Clade 1. Acinetobacter is the only genus found in Clade 2. The NJ technique of proving kinship is based on branch length. The branch length of the bacterial isolates To.09.pp and Sc.06.pp is 0.00. With a branch length of 0.040, isolate To.10.pp differs from isolates Sc.06.pp and To.09.pp (**Figure 3**).

Acinetobacter indicus strain 80-1-2 and *Vibrio harveyi* strain B14-1 have been shown through related research to have antibacterial activity against *S. aureus* and *S. epidermidis* bacteria. Marine-isolated *A. indicus* bacteria exhibit anti-microbial, anti-biofilm action against MRSA, and anti-proliferative properties [46]. MRSA, *E. coli*, and *C. albicans* are all susceptible to the antimicrobial effects of alkaloid compounds derived from the genus Acinetobacter [47].

Because the Vibrio genus is the most prevalent genus connected to the association between bacteria and seaweed, the discovery of *V. harveyi* strain B14-1 that is associated with seaweed validates earlier studies [2]. Because the genus Vibrio has both positive and negative effects on seaweed, the relationship between the 2 has not been definitively established adverse effects on macroalgae, such as white rot and green spot rot [48]. Seaweeds benefit from the activities of the genus Vibrio. The Vibrio genus creates a number of chemical substances to sustain the life of its host, seaweed.

Furthermore, the antibacterial test results showed the capability producing an inhibition zone against *S. aureus* in *Xylocarpus granatum* [49] and *Holuthuria atra* [50]. The surface of the seaweed is shielded against illnesses, herbivores, and polluting organisms by chemical substances produced by the Vibrio genus, such as antisettlement and Quorum Sensing (QS) inhibitors [51]. This explanation explains the continuing relationship between the host, seaweed, and the genus Vibrio [52].

Conclusions

Against *Staphylococcus aureus* and *Staphylococcus epidermidis*, endophytic bacteria isolated from the seaweeds *Turbinaria ornata*, *Sargassum crassifolium*, and *Sargassum polycystum* have antibacterial action. Endophytic bacteria are able to produce flavonoids, alkaloids, and terpenoids. The quest for natural chemical compounds that are used in relation to human skin disease pathogens could be a future endeavor.

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