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Journal of Multidisciplinary Applied Natural Science Vol. 5 No. 2 (2025) https://doi.org/10.47352/jmans.2774-3047.262
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Research Article

In Vitro Anti-Acne Activity and Bioactive Compound Analysis of *Sargassum cristaefolium* Ethanolic Extract from Teluk Awur Jepara

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Received : September 19, 2024	Revised : March 13, 2025	Accepted : April 10, 2025	Online : May 26, 2025

Abstract
Acne is a prevalent skin disorder, particularly among adolescents, causing significant physical and psychological impacts. Ongoing efforts are dedicated to acne treatment by developing therapeutic agents with enhanced effectiveness while minimizing side effects. In this study, we aimed to assess the potential of *Sargassum cristaefolium* sourced from Teluk Awur Jepara, extracted using ethanol, in combating acne through its *in vitro* antibacterial and antioxidant attributes. Utilizing the disk diffusion method, our findings demonstrated the extract's efficacy against *Cutibacterium acne, Staphylococcus epidermidis,* and *Staphylococcus aureus*. The minimum inhibitory concentration and minimum bactericidal concentration were determined to be 50 and >100 mg mL^{-1} against *C. acnes*, 25 and 100 mg mL^{-1} against *S. epidermidis*, and 25 and 100 mg mL^{-1} against *S. aureus*, respectively. Additionally, bacteriolytic assays confirmed the extract's ability to partially lyse bacterial cells, particularly *S. aureus* and *S. epidermidis* at 2× MIC, while *C. acne* exhibited partial lysis until the 6th hour, followed by an increase in absorbance, possibly due to cellular debris aggregation or bacterial persistence due to incomplete lysis. Furthermore, the extract exhibited notable free radical scavenging properties against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), with IC_{50} values of 645.35 ± 9.52 and 574 ± 33.52 µg mL^{-1}, respectively. The *S. cristaefolium* ethanol extract exhibited a total phenol content of 14.17 ± 0.39 mg GAE g^{-1} and a sulfate content of 10.99 ± 0.45%. Liquid chromatography-mass spectrometry (LC-MS) analysis identified bioactive compounds, including carotenoids, terpenoids, steroids, flavonoids, chromenols, and fatty acids, all of which demonstrate substantial potential as antioxidants and antibacterial agents against acne-causing bacteria. Therefore, ethanolic extract of *S. cristaefolium* demonstrates potential as an anti-acne treatment due to its antibacterial and antioxidant properties.

Keywords: *Sargassum cristaefolium,* anti-acne, antibacterial activity, antioxidant activity

1. INTRODUCTION

Acne is a skin disease often experienced by adolescents and it can persist for years and cause scars on the face [1]. This disease caused by increased sebum secretion, follicular hyperkeratinization, and bacterial colonization [2]. Numerous bacteria responsible for acne exhibit both Gram-positive and Gram-negative characteristics; however, predominant among them are Gram-positive strains, notably *Cutibacterium acne, Staphylococcus epidermidis*, and *Staphylococcus aureus* [3]. These microorganisms derive their sustenance from sebum by metabolizing triglycerides into free fatty acids. The aggregation of bacteria and the secretion of virulence factors contribute to the inflammatory response associated with acne [4]. Moreover, the metabolic processes of these bacteria result in the generation of reactive oxygen species (ROS), which inflict damage on inflamed tissues [5].

Acne has been treated using antibiotics, chemical peeling agents [6], and retinoids [7]. However, long-term use of these treatments can cause side effects such as antimicrobial resistance, collagen vascular disease, inflammatory bowel disease [8], and immunological hypersensitivity [9]. The utilization of chemical peeling agents may induce immediate side effects such as irritation and a burning sensation, or delayed effects like hyperpigmentation and acneiform eruptions [10]. Conversely, retinoids have the potential to lead to dry skin, irritation, peeling, and heightened sensitivity to sunlight as a result of skin thinning [7]. Consequently, there arises a necessity for an acne treatment remedy that exhibits superior therapeutic efficacy while minimizing adverse reactions. An efficacious anti-acne agent should possess antibacterial, antioxidant, and anti-inflammatory properties [11].

Sargassum sp. is a brown seaweed widely distributed in the ocean and contains bioactive

Table 1. The diameter of the inhibition zone of *S. cristaefolium* ethanolic extract against *C. acnes, S. epidermidis,* and *S. aureus.*

Sample	Concentration	Zone of inhibition (mm)		
		C. acnes	*S. epidermidis*	*S. aureus*
S.cristaefolium ethanolic extract	5,000 µg disc^{-1}	11.75±0.13^{aA}	14.79±0.38^{aB}	14.83±0.40^{aB}
Clindamycin	5 µg disc^{-1}	19.25±0.18^{b}	22.88±0.34^{b}	23.75±0.38^{b}
Nutrient broth medium	-	0	0	0
Solvent control (25% ethanol)	-	0	0	0

Note : Statistical analysis was performed using ANOVA followed by post-hoc tests (DMRT) to determine the significance of differences between the groups : *C. acnes* (F = 3627.08, p = 0.000), *S. epidermidis* (F = 750.172, p = 0.000), and *S. aureus* (F = 776.634, p = 0.000). Post-hoc tests revealed no significant differences between the ethanolic extract of *S. cristaefolium* and the positive control for *S. epidermidis* and *S. aureus* (p = 1.000), but significant differences were observed for *C. acnes* (p = 0.881). Different lowercase notations in same column indicate that the samples differ significantly. Different uppercase notations in same row suggest that the inhibition zones between bacteria are significantly different.

compounds that can be used to treat diseases [12]. *Sargassum* sp. has been reported to show potential anti-acne activity. Research by Kok et al. [1] showed the potential of *S. polycystum* as an antibacterial against *P. acne*, antioxidant, and anti-lipase activity. The research findings highlighted the antimicrobial properties of phlorotannin *Eisenia bicycles* against *P. acne, S. aureus, S. epidermidis,* and *Pseudomonas aeruginosa* [2]. Yim et al., delved into a study focusing on the inhibitory impact of *Sargassum miyabei* Yendo on skin inflammation induced by *C. acne* [12]. Harharah et al., documented the antibacterial efficacy of *S. cristaefolium* sourced from Batu Layar Beach, Lombok, against *S. epidermidis* [13]. Nevertheless, the assortment of seaweed from diverse locations can yield varying bioactive compounds. Research conducted by Prasedya et al. unveiled that samples of the identical *Ulva lactuca* exhibited distinct antioxidant activities, total phenolics, and flavonoid content depending on their geographical origins in Batu Layar, Tanjung Aan, and Seriwe [14].

The coastal region of Teluk Awur in Jepara City boasts an abundance of brown seaweed known as *Sargassum sp*. According to Rahmani et al., the full potential of *Sargassum sp*. remains untapped. Furthermore, the bioactive compounds derived from *S. cristaefolium* in Teluk Awur Jepara have yet to be documented [15]. Hence, this study endeavors to assess the capabilities and scrutinize the bioactive components of *S. cristaefolium* ethanolic extract from Teluk Awur Jepara, as a viable remedy for acne treatment.

2. MATERIALS AND METHODS

2.1. Materials

Sargassum cristaefolium J. Agardh was harvested from Teluk Awur Jepara, Central Java, Indonesia. The seaweed was authenticated with the seaweed identification certificate number No. 39.25.9/UN1/FFA.2/BF/PT/2023. Bacterial strains of *Cutibacterium acnes* (*P. acnes* ATCC 6919), *S. epidermidis* ATCC 12228, and *S. aureus* ATCC 29213 were procured from PT Agritama Sinergi Inovasi. Other materials utilized included clindamycin, ampicillin, nutrient broth (Merck), nutrient agar (Merck), ethanol, sodium chloride, ascorbic acid (Smartlab), 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Aldrich), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, Sigma Aldrich), potassium persulfate (Loba Chemie), trolox, methanol (Merck), Folin-Ciocalteu reagent (Merck), gallic acid (Merck), and sodium carbonate (Merck).

2.2. Methods

2.2.1. Extraction

The extraction methodology described in the study by Harharah et al. [13] involved modifications to include filtration and freeze-drying techniques. *S. cristaefolium* underwent a thorough washing with fresh water and was subsequently dried in ambient conditions for 4-5 d until the seaweed reached a maximum water content of 15% (according to SNI 2690: 2015 standards). The

seaweed sample was then finely ground into a powder (60 mesh) using a miller. Subsequently, 400 g of the seaweed powder were macerated in 96% ethanol (in a 1:4 ratio) for 24 h at room temperature, shielded from direct sunlight, and stirred intermittently. The resulting mixture was filtered using Whatman paper no. 42. The filtrate underwent evaporation through a rotary evaporator (operated at 40 °C, 60 rpm). The concentrated extract was then subjected to freeze-drying to yield a crude extract.

2.2.2. Antibacterial Activity

2.2.2.1. Determination of Inhibition Zones

Determination of inhibition zone through the diffusion method utilizing paper discs, as outlined by Prabowo et al. [16], with a modification involving double layer agar. A 100 µL suspension of *C. acnes, S. epidermidis,* and *S. aureus* (at a concentration of 10^8 CFU mL^{-1}) was dispensed into 5 mL of soft agar (nutrient broth (NB) + 0.7% agar) and subsequently vortexed. The soft agar containing the bacterial inoculum was gently poured onto the nutrient agar (NA) petri dish and left to solidify. Paper discs (6 mm in diameter) were impregnated with 50 µL of ethanol extract of *S. cristaefolium* (100 mg mL^{-1}), clindamycin as the positive control (100 µg mL^{-1}), NB as the negative control, and 25% ethanol as the solvent control, and then dried. The final concentration of ethanolic extract of *S. cristaefolium* was 5,000 µg $disc^{-1}$, while clindamycin was 5 µg $disc^{-1}$. Subsequently, the paper discs were aseptically positioned on the soft agar. The petri dish containing the bacteria was then placed in an incubator at 37 °C for 24 h. The antibacterial efficacy was quantified as the mean diameter of inhibition in mm. The determination of the inhibition zone was carried out in triplicate.

2.2.2.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of MIC by microdilution method as described Mustarichie et al. [17]. Columns 1–12 of a 96-well microplate were filled with 100 µL of NB. Column 1 served as the negative control, while column 12 acted as the positive control containing NB and bacteria. In column 2, 100 µL of *S. cristaefolium* ethanol extract at a concentration of 100 mg mL^{-1} was added. Subsequently, a dilution process was carried out by transferring 100 µL from column 2 to 3, repeating this step until column 11, and finally removing 100 µL from the last dilution. Within columns 2 to 12, 10 µL of test bacteria at a concentration of 10^8 CFU mL^{-1} were introduced. The microplate containing bacteria was then placed in an incubator at 37 °C for 24 h. The MIC was determined using a microplate reader (Glomax, Promega Type GM3000) set at a wavelength of 600 nm. The MIC represents the lowest sample concentration capable of inhibiting bacterial growth. To ascertain the MBC, three concentrations higher than the MIC were tested. A volume of 5 µL of bacterial suspension along with samples that displayed no bacterial growth were inoculated into NA media and incubated at 37 °C for 48 h (*C. acnes*), and 37 °C for 24 h (*S. epidermidis* and *S. aureus*). The absence of bacterial colonies in the media indicated the MBC.

2.2.2.3. Bacteriolytic Assay

Bacteriolytic assay refers to the research of Isnansetyo and Kamei [18]. Bacterial cultures (*C. acnes* ATCC 6919, *S. epidermidis* ATCC 12228, and *S. aureus* ATCC 29213) from NA medium were grown into NB medium then washed twice with sterile 0.9% NaCl using a centrifuge at 3,500 rpm for 20 min. The absorbance was adjusted to 0.1 using a UV-VIS spectrophotometer (GENESYS 10S UV-VIS, Germany) at 660 nm. Bacterial cell suspensions (5 mL each) were poured into sterile test tubes, and ethanolic extract of *S. cristaefolium* at various concentrations (MIC and 2×MIC) and ampicillin (1 µg mL^{-1}) as positive controls. Untreated bacterial suspensions were used as negative controls. The concentration of ethanol in each tube was less than 0.1% (v/v). The tubes were incubated using a water bath shaker (37 °C, 120 rpm). Absorbance was measured at a wavelength of 660 nm every 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h. Relative absorbance was calculated by dividing each sample absorbance by the negative control absorbance. Each treatment was conducted in triplicate.

2.2.3. Antioxidant Activity

Table 2. MIC and MBC values of *S. cristaefolium* ethanolic extract against *C. acnes*, *S. epidermidis*, and *S. aureus*.

Sample	MIC (mg mL^{-1})			MBC (mg mL^{-1})		
	C.acnes	*S.epidermidis*	*S.aureus*	*C.acnes*	*S.epidermidis*	*S.aureus*
S.cristaefolium ethanolic extract	50	25	25	>100	100	100
Clindamycin	25	50	50	50	100	100

2.2.3.1. DPPH Free Radical Scavenging Activity

The antioxidant activity of extracts were measured spectophotometrically by using DPPH refers to the method of Vora et al. [19]. A DPPH solution of 0.1 mM was prepared dissolving 3.9 mg of DPPH powder into 100 mL of methanol. A total of 1 mL of DPPH stock solution was mixed with 1 mL of *S. cristaefolium* ethanolic extract solution at different concentrations (200, 400, 600, 800, 1,000, and 1,200 µL mL^{-1}). A mixture of 1 mL methanol and 1 mL DPPH stock solution was used as a control. Ascorbic acid concentrations of 50, 100, 150, 200, 250, and 300 µg mL^{-1} were used as standard reference compounds. The solution was incubated in the dark for 30 min. Absorbance was measured using a UV-VIS spectrophotometer (GENESYS 10S UV-VIS, Germany) at a wavelength of 517 nm. Each sample was tested in triplicate. The inhibition percentage was calculated using the following formula 1.

$$\text{DPPH } scavenging\ effect\ (\%) = \left[\frac{(\text{A control} - \text{A sample})}{\text{A control}}\right] \text{x}100\% \quad \textbf{(1)}$$

The concentration of extract that can inhibit 50% of radical activity (IC_{50}) was measured using the Microsoft Excel application through a linear regression graph of each extract and standard solution.

2.2.3.2. ABTS Radical Cation Decolorization Assay

Antioxidant activity testing using ABTS followed the method as described by Blanc et al. [20] with modifications to the amount of sample testing. The stock solution was produced by mixing 7 mM ABTS with 2.4 mM potassium persulfate in equal amounts (1:1) for 12–16 h at room temperature in the dark to produce radical cations ($ABTS^{\cdot+}$). The test was conducted by mixing 1.85 mL of sample in 50 mL of ethanol to obtain an absorbance of 0.700 ± 0.02 A at 734 nm. Ethanol extract of *S. cristaefolium* with concentrations of 200, 400, 600, 800, and 1000 µg mL^{-1} were taken 75 µL each and reacted with 1,425 µL $ABTS^{+}$ at room temperature for 2 h in the dark and measured the absorbance using a UV-VIS spectrophotometer at a wavelength of 734 nm and assayed in triplicate. Control using 75 µL aquadest and reacted with 1,425 µL $ABTS^{+}$ at room temperature for 2 h in the dark. Trolox was used as a standard with a 100–600

µM concentration. $ABTS^+$ inhibition (%) was calculated using the formula 2.

$$\text{ABTS } inhibition\ (\%) = \left[\frac{(\text{A control} - \text{A sample})}{\text{A control}}\right] \text{x}100\% \quad (2)$$

The concentration of seaweed extract that shows 50% inhibition of ABTS radical activity is IC_{50}.

2.2.4. Determination of Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu assay method described by Alagan et al. [21]. The gallic acid standard curve was prepared by dissolving 1 mg of gallic acid in 10 mL of distilled water as a stock solution of 100 µg mL^{-1} and varying the concentrations of 10, 20, 30, 40, 50 µg mL^{-1}. A sample of *S. cristaefolium* extract of 1 mg was dissolved in 1 mL of distilled water. The reagent blank used distilled water. The standard solution, extract sample, and blank, each taken as much as 1 mL, were mixed with 0.2 mL of Folin-Ciocalteau reagent, vortexed, then incubated at room temperature for 5 min. Next, 1 mL of 15% sodium carbonate and 2 mL of distilled water were added to the above mixture, vortexed, and incubated at room temperature for 90 min. Absorbance was measured using a UV-VIS spectrophotometer at 750 nm. Samples were analyzed in triplicate. Total phenolic content was expressed as mg Gallic equivalents/g extract.

2.2.5. Determination of Sulfate Content

The sulfate content of the extract was determined using the $BaCl_2$-gelatin method based on Koh et al. [22]. Preparation of $BaCl_2$-gelatin reagent by dissolving 0.5 g of gelatin in 100 mL of distilled water, which was homogenized with a hot plate stirrer at a temperature of 60–70 °C. Then 0.5 g of $BaCl_2$ was added and homogenized using a hot plate stirrer. $BaCl_2$-gelatin reagent can be stored at a temperature of 4 °C. The standard solution used 0.05 g of K_2SO_4 dissolved in 50 mL of distilled water. This standard K_2SO_4 solution was diluted with 200, 400, 600, 800, and 1000 µg mL^{-1} concentrations. A sample of *S. cristaefolium* ethanolic extract, as much as 10 mg, was dissolved in 5 mL of 4 M HCl. The homogeneous solution was then hydrolyzed at 90 ° C for 2.5 h. A total of 0.1 mL of the hydrolysis results of the *S. cristaefolium* and the standard solution of K_2SO_4 were added with 1.9 mL of 3% TCA and 0.5 mL of $BaCl_2$-gelatin reagent. The mixture was homogenized using a vortex, and its absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 360 nm. The analysis was conducted in triplicates.

2.2.6. Identification of Compounds using Liquid Chromatography Mass Spectrophotometry (LC-MS)

LC-MS analysis was conducted utilizing a UPLC -MS (Ultra Performance Liquid Chromatography) system equipped with a QToF analyzer and positive electrospray ionization as the ionization source, coupled with a column in Acquity C_{18} form (1.8 µm; 2.1×100 mm). The eluent employed was a blend of A (water and 5 mM ammonium formic) and B (acetonitrile and 0.05% formic acid) utilizing a gradient elution system. The source temperature was maintained at 100 °C, while the desolvation temperature was set at 350 °C. The *S. cristaefolium* extract sample was isolated using the solid phase extraction (SPE) technique, followed by dissolution in 10 mL absolute methanol in a volumetric flask. Subsequently, 5 µL of the solution was introduced into the UPLC-MS system. The chromatogram results were processed using Masslynx software version 4.1 (Waters, Massachusetts, USA). Component identification was based on m/z ratios measured in Masslynx, PubChem (https://pubchem.ncbi.nlm.nih.gov/), HMDB, or chemspider.com.

2.2.7. Data Analysis

This research presents data including the mean and standard deviation (SD). Statistical analyses were conducted using SPSS statistics 27, with a significance level set at 5% probability ($p < 0.05$). The analysis involved three replicates of yield, inhibition zone diameter, IC_{50} of free radical scavenging activity, and IC_{50} radical cation decolorization activity, initially computed using Microsoft Excel and further analyzed through one-way analysis of variance (ANOVA). Levene's homogeneity test was utilized to assess variance homogeneity, while the Shapiro-Wilk test was employed to evaluate data normality. The Microsoft Excel software analyzed three replicates and determined results for MIC, MBC, total phenolic content, and sulfate content.

Table 3. Antioxidant activity (DPPH and ABTS) of *S. cristaefolium* ethanolic extract and standard.

Materials	IC_{50} antioxidant activity (µg mL^{-1})	
	DPPH	ABTS
S. cristaefolium ethanolic extract	645.35±9.52	574.79±33.52
Ascorbic acid	29.36±1.36	-
Trolox	-	296.79±0.69

3. RESULTS AND DISCUSSIONS

3.1. Ethanolic Extract of S. cristaefolium

The yield of *S. cristaefolium* extracted using ethanol is 2.13 ± 0.07% in paste form. According to Prasedya et al., the extract of *S. cristaefolium*, obtained through maceration with ethanol as the solvent and a sample particle size exceeding 250 µm, demonstrates a yield of 2.05% [23]. Based on the findings of Sipahutar et al., the *S. polycystum* macerated with ethanol had a yield of 2.73% [24]. In contrast to the study conducted by Juliana et al., the extraction of *S. cristaefolium* using methanol resulted in a yield of 6.91% [25]. The 3-stage extraction process of *S. cristaefolium*, utilizing hexane, ethyl acetate, and methanol as solvents, resulted in a yield of 1.99 ± 0.03%, as reported by Susilo et al. [26]. Discrepancies in yield outcomes can be attributed to variations in particle sizes, solvent polarity, extraction duration, temperature [23], water content, and the geographical locations of seaweed collection [27].

3.2. Antibacterial Activity

3.2.1. Inhibition Zone

The results of the inhibition zone of *S. cristaefolium* ethanolic extract against *C. acne, S. epidermidis*, and *S. aureus* are shown in Table 1. In this investigation, the inhibitory effect of clindamycin against *S. epidermidis* and *S. aureus* was deemed highly potent, whereas against *C. acnes,* it exhibited a robust efficacy. The determination of these classifications aligns with the criteria outlined by Davis and Stout, who categorize the inhibitory zones into 4 levels based on their diameter; specifically, if the inhibition zone measures 20 mm or more, 10–20 mm, 5–10 mm, and <5 mm, they are designated as very potent, potent, moderate, and weak, respectively [28]. Clindamycin manifests its inhibitory action by impeding protein synthesis through the inhibition of the peptidyl transferase center within the 50S subunit of bacterial ribosomes [29].

Table 1 illustrates that the ethanolic extract of *S. cristaefolium* exhibits an inhibition zone ranging from 11.75±0.13 to 14.83±0.40 mm, meeting the required standards, though at a concentration 1,000 times greater than clindamycin. In a study conducted by Harharah et al., employing the ethanolic extract of *S. cristaefolium* sourced from Batu Layar Lombok at a concentration of 100%, an inhibition zone of 9.46±1.60 mm was observed [13]. These findings suggest that the ethanolic extract of *S. cristaefolium* from Taluk Awur Jepara exhibits heightened potential as an antibacterial agent.

The inhibition zone of *S. aureus* was not significantly different from the inhibition zone of *S. epidermidis*. *Staphylococcus epidermidis* and *S. aureus* are members of the same genus, so they have the same main phenotypic properties [30]. The cell wall thickness of *S. aureus* is 24±70 nm, while the cell wall thickness of *S. epidermidis* is 33±15 nm [31]. This demonstrates that the cell wall of *S. aureus* is comparatively thinner than that of *S. epidermidis*, resulting in a larger diameter of the extract inhibition zone against *S. aureus*. Surprisingly, the inhibition zone of *C. acnes* is even smaller than both aforementioned bacteria, despite belonging to the same Gram-positive group. *Cutibacterium acnes* possesses a distinctive cell wall and envelope characterized by the presence of phosphatidylinositol, triacylglycerol, and numerous lipids. The composition of *C. acnes*' cell wall primarily consists of peptidoglycan, with a unique peptide chain composition containing L-acid L-diaminopelic acid and D-alanine, setting it apart from other Gram-positive bacteria. The

amalgamation of these amino acids fortifies the bacterial cell wall, rendering it more resilient and impervious to specific antibacterial agents. Lee, et al. [2], highlighted that a methanol extract derived from brown seaweed, *Eisenia bicylis*, at a concentration of 5 mg disc^{-1}, yielded an impressive inhibition zone of 14 mm against *S. aureus* (KCTC 1927) and *S. epidermidis* (KCTC 1370). In contrast, the inhibition zone against *P. acnes* (KCTC 3314) measured 8 mm.

3.2.2. MIC and MBC Determination

Based on Table 2, the antibacterial efficacy of the ethanolic extract of *S. cristaefolium* against *C. acne* is 2,000 times less potent, while against *S. epidermidis* and *S. aureus,* it is 500 times less potent compared to the antibiotic clindamycin. Blaskovich et al. [6] highlighted that 'non-antibiotic' acne medications have the potential to combat acne through various mechanisms, including bacterial inhibition, albeit with a reduced impact compared to recognized antibiotics like clindamycin, vancomycin, tetracycline, erythromycin, oxacillin, and dapsone. The potential efficacy ranges from 2–64 mg mL^{-1}, representing a 1,000-fold decrease compared to designated antibiotics. Discrepancies in antibacterial mechanisms between the ethanolic extract of *S. cristaefolium* and the antibiotic clindamycin may contribute to variations in antibacterial efficacy observed in this study. Xie et al. documented that the ethanolic extract of *Meconopsis quintuplinervia* Regel (EMQ) exhibited a MIC value of 12.5 mg mL^{-1} and MBC of 100 mg mL^{-1} against *P. acne*, while displaying a MIC value of 12.5 mg mL^{-1} and MBC of 50 mg mL^{-1} against *S. aureus* [32].

The MIC and MBC values of *S. cristaefolium* ethanolic extract surpassed those reported in previous studies on brown seaweed. Prasedya et al., in their research utilizing the ethanolic extract of *S. cristaefolium* from the western shores of Lombok, demonstrated a reduced MIC value against *S. aureus* at 1,302 µg mL^{-1} [33]. Furthermore, Pouladi et al. exhibited the antibacterial efficacy of the methanolic extract of *S. cristafolium* against *E. coli* at 100 µg mL^{-1}, *P. aeruginosa* at 50 µg mL^{-1}, and *L. monocytogenes* at 50 µg mL^{-1} [34]. Discrepancies in the MIC and MBC values of brown seaweed against these bacteria can potentially be attributed to variations in extraction techniques, solvents utilized [35], inoculum density, and differences in the duration of measurements [36]. Kok et al. [1], documented the MIC of the methanol fraction of *S. polycystum* extract against *P. acnes* at 250 µg mL^{-1} with an MBC of 500 µg mL^{-1}. Radhika et al., highlighted that fractionated seaweed exhibited superior antibacterial properties compared to crude extracts [37]. In this study, *S. cristaefolium* ethanolic extract exhibits a relatively lower antibacterial potency, indicating the necessity for optimization in its formulation. The practical implications of these findings include the need to enhance the extract's skin penetration and stability. Additionally, adjustments to the concentration levels will be required to ensure both the safety and therapeutic efficacy of the extract in topical applications. This study adds novelty by focusing on *S. cristaefolium* from Teluk Awur Jepara, a region not previously studied, and demonstrating its enhanced antibacterial efficacy against acne-related bacteria. The geographical focus of this research contributes new insights into the variability of seaweed bioactive compounds and their antibacterial potential, addressing a gap in the literature by exploring local seaweed sources for acne treatment.

3.2.3. Bacteriolytic Assay

Cell lysis, also known as cellular disruption, refers to the breakdown or destruction of the cell membrane to release intracellular components such as DNA, RNA, proteins, and organelles. In the bacteriolytic assay, the presence of living bacterial cells in a solution causes it to become turbid, leading to high light absorption. A higher optical density (OD) value indicates a greater number of viable bacteria in the solution, resulting in increased light scattering or absorption beyond the spectrophotometer's detection limit. Conversely, bacterial lysis reduces the number of viable bacterial cells in the solution, consequently lowering light absorption and turbidity as measured by absorbance on the spectrophotometer.

This study utilized ampicillin at a concentration of 1 µg mL^{-1} as a positive control, based on previous studies such as Peechakara et al., which commonly use this concentration to evaluate

ampicillin's antimicrobial efficacy against *S. aureus* and other bacteria [38]. This concentration aligns with standard MIC values (0.6–1.0 mg L^{-1}) for effective inhibition, targeting bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs) [38]. Ampicillin was chosen as the positive control instead of clindamycin because of the distinct mechanisms of action between these two antibiotics. Clindamycin inhibits bacterial protein synthesis by targeting peptidyl transferase, which disrupts protein synthesis, thereby inhibiting bacterial growth and preventing reproduction [29].

The ethanol extract of *S. cristaefolium* also demonstrated the capacity to lyse *S. epidermidis* and *S. aureus* cells, evident through a reduction in the absorbance of bacterial cell suspensions. The ethanol extract of *S. cristaefolium* effectively lysed *S. aureus* and *S. epidermidis* bacterial cells at the optimal concentration using 2× MIC extract. This phenomenon can be attributed to the increased concentration of active ingredient compounds present in the extract. The *C. acnes* cellular lysis assay exhibited a decline in absorbance until the 6th hour of measurement, followed by a subsequent rise. This phenomenon may be attributed to variations in bacterial susceptibility to antibacterial agents [39], stemming from distinctive structural characteristics of individual bacterial cell walls.

3.3. Antioxidant Activity

3.3.1. DPPH Free Radical Scavenging Activity

Ascorbic acid and *S. cristaefolium* ethanolic extract exhibited notable antioxidant properties in terms of DPPH radical scavenging activities, with IC_{50} values of 29.36±1.36 and 645.35±9.52 µg mL^{-1}, respectively (Table 3). The IC_{50} value of ascorbic acid signifies its classification as a potent antioxidant. Ascorbic acid, characterized as a secondary antioxidant, contains 2 hydrogen atoms capable of neutralizing free radicals and enhancing their stability. This investigation reveals that the ethanolic extract of *S. cristaefolium* possesses limited antioxidant efficacy. The limited efficacy is due to the use of crude extract, which contains a mixture of compounds, some of which may not contribute to antioxidant activity. Furthermore, the research also addresses the potential for improving the extract's efficacy by highlighting the need for purification techniques, such as fractionation, to enhance its antioxidant potency for practical topical applications.

The research results have a higher IC_{50} value of DPPH antioxidants compared to the research of Prasedya et al. [23] which was reported to have an IC_{50} value of 438.10±1.32 µg mL^{-1}. This is because the particle size of the powder used for extraction in the research of Prasedya et al. [23] is smaller than the research findings. The reduced particle size of the powder utilized for extraction contributes to an elevation in phytochemical content. This leads to a heightened potential to engage with free radicals, thereby preventing their transformation into a stable, non-reactive state [23]. In comparison to the IC_{50} value of the DPPH antioxidant methanol extract of *S. cristaefolium* as documented by Rohimat et al. [40], a higher IC_{50} value (1,603 µg

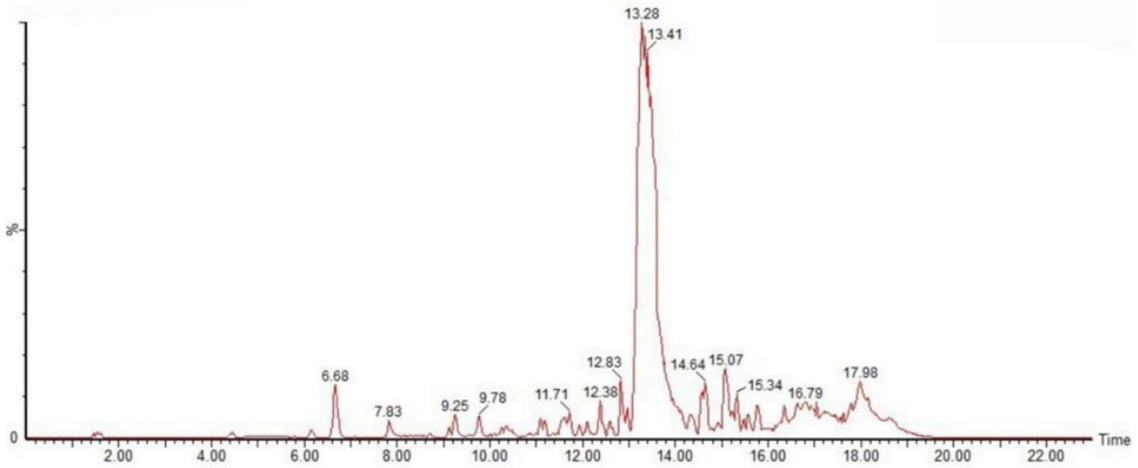

Figure 1. LC chromatogram of *S. cristaefolium* ethanolic extract.

mL^{-1}) was reported [40]. Discrepancies in harvest age, drying techniques, and environmental conditions in which the seaweed thrives may account for this disparity [41].

3.3.2. ABTS Radical Cation Decolorization Assay

Antioxidant activity assessment utilizing ABTS is predicated on the capacity of antioxidant compounds to stabilize free radical compounds through the donation of proton radicals. This contrasts with the DPPH technique, which relies on electron transfer reactions and hydrogen atom uptake reactions as marginal reaction pathways [42]. Trolox and the ethanolic extract of *S. cristaefolium* exhibited ABTS antioxidant activity with IC_{50} values of 296.79±0.69 and 574.79±33.52 µg mL^{-1} (Table 3). Trolox, a synthetic compound derived from vitamin E (α-tocopherol), demonstrates potent antioxidant properties by counteracting peroxyl and alkoxyl radicals [43]. Based on these criteria, it is evident that the ethanolic extract of *S. cristaefolium* displays minimal antioxidant activity. The findings of this investigation (IC_{50} 574.79±33.52 µg mL^{-1}) are lower when compared to the reported IC_{50} values of antioxidants from the *S. cristaefolium* extract by Sunarwidhi, et al. (2258±8.90 and 1873±7.19 µg mL^{-1}), but higher than those reported by Prasedya et al. (321.5±2.13 µg mL^{-1}) [23]. They suggested that the impact of powder particle size has been documented to significantly influence the antioxidant activity of the extract.

The decolorization activity of ABTS radical cations produces secondary antioxidants, while the DPPH free radical scavenging activity produces primary antioxidants. The IC_{50} value of the antioxidant activity of the extract using the ABTS method is lower compared to that of DPPH. This suggests that the antioxidant mechanism of the ethanolic extract of *S. cristaefolium* is primarily influenced by secondary antioxidants. These findings are further substantiated by the outcomes of LC-MS analysis, which reveal the presence of secondary antioxidant compounds such as carotenoids, flavonoids, terpenoids, chromenol, and xanthophyl.

3.4. Determination of TPC

The results of the standard curve measurement for gallic acid yielded a linear regression equation of $y = 0.0194x + 0.012$, with an impressive R^2 value of 0.9985. The total phenolic content found in the ethanolic extract of *S. cristaefolium* was determined to be 14.17±0.39 mg GAE g^{-1}. A study by Erniati et al. [42] indicated that the total phenolic content in *S. crassifolium* and *S. binderi* was comparatively lower at 8.71±0.10 and 9.02±0.12 mg GAE g^{-1}, respectively. Phenolics are antioxidant components that neutralize free radicals by virtue of their capacity to donate hydrogen atoms to these reactive species. Phenolics possess structural attributes that serve to counteract the damaging effects of free radicals [44]. The potency of antioxidants is directly correlated with the phenolic content, as evidenced by a lower IC_{50} value [45]. In this investigation, the total phenolic content in the ethanolic extract of *S. cristaefolium* was measured at 14.17±0.39 mg GAE g^{-1}, with an IC_{50} antioxidant value of 645.35±9.52 µg mL^{-1} using the DPPH method and 574.79±33.52 µg mL^{-1} using the ABTS method. A separate study by Puspita et al. revealed that *S. polycystum*, extracted with a 75% ethanol, exhibited a lower total phenolic content of 5.8±0.6% and a higher IC_{50} antioxidant value (indicating lower antioxidant activity) of 5.20±5.90 µg mL^{-1} [46]. The substantial antioxidant activity observed in this research was also attributed to the presence of other bioactive compounds such as carotenoids, flavonoids, and terpenoids, as identified in the LC-MS analysis of the ethanolic extract of *S. cristaefolium*, detailed in Table 4.

3.5. Sulfate Content

Sulfate content determines the potential of various bioactive properties such as antioxidant capacity [22], antibacterial [47], and anti-inflammatory [48]. The K_2SO_4 standard curve measurement results obtained a linear regression equation $y = 0.0005x + 0.0104$ with an R^2 value of 0.998. Based on the calculation results, the sulfate content in the ethanolic extract of *S. cristaefolium* was 10.99±0.45%. Research by Wang et al. showed that *S. cristaefolium* extracted using water and then ethanol to obtain pure fucoidan had a sulfate content of 13.58±0.62% [49]. This can be caused by differences in the extraction methods used, which can affect the selectivity and effectiveness of extraction of certain compounds in

Table 4. The results of metabolite identification of *S. cristaefolium* extract using LC-MS.

No	Rt (min)	Measured m/z	Calculated m/z	%Area	Formula	Name	Grups	Function	Ref.
1	4.445	1.870.609	1.870.606	0.19	$C_8H_{10}O_5$	Erinapyrone C	Dihydropyranone	Moderate activity against Gram positive bacteria [59]	Link
2	5.098	2.271.081	2.271.072	0.23	$C_{15}H_{14}O_2$	Flavanol	Flavonoid	Antioxidant, antibacterial, anti-inflammatory [58]	Link
3	6.68	1.971.183	1.971.178	2.24	$C_{11}H_{16}O_3$	Loliolide	Monoterpenoid hydroxylactone	Anti-inflammatory, antiaging, antioxidant[55], and antibacterial [60]	Link
4	7.848	3.412.155	3.412.117	1.03	$C_{22}H_{28}O_3$	Canrenone	Steroid	Antiandrogenic [61]	Link
5	11.124	2.551.754	2.551.749	0.8	$C_{18}H_{22}O$	3-(4-Methylbenzylidene) camphor	Bicyclic monoterpenoids	Chemical sunscreen agent: UV organic filter [62]	Link
6	11.652	599.41.00	599.41.00	1.27	$C_{40}H_{54}O_4$	Halocynthiaxanthin	Carotenoid	Antioxidant [63]	Link
7	11.714	3.992.533	3.992.535	1.27	$C_{25}H_{34}O_4$	Variabilin	Diterpenoid	Anti-inflammatory, antimicrobial [64]	Link
8	12.108	4.252.691	4.252.692	0.16	$C_{27}H_{36}O_4$	(R)-Sargachromenol	Chromenol	Antioxidant and anti-inflammatory [57]	Link
9	12.376	2.772.168	2.772.168	0.79	$C_{18}H_{28}O_2$	Stearidonic acid	Fatty acid Omega-3	Anti-inflammatory [65]	Link
10	13.338	6.414.205	6.414.206	62.35	$C_{42}H_{56}O_5$	Halocynthiaxanthin acetate	Carotenoid	Antioxidant [66]	Link
11	15.096	5.814.003	5.813.995	4.47	$C_{40}H_{52}O_3$	Phoenicoxanthin	Xanthophylls	Photoprotective dan antioxidant [54]	Link
12	16.347	6.414.212	6.414.206	1.08	$C_{42}H_{56}O_5$	Halocynthiaxanthin acetate	Carotenoid	Antioxidant [62]	Link

seaweed, such as fucoidan [22]. In the study, the extract was still a crude extract with extraction using ethanol solvent and maceration method. Different methods for isolating fucoidan from seaweed can produce variations in sulfate composition at the end of fucoidan extraction [22]. Additional research has indicated elevated levels of sulfate, with fucoidan derived from brown seaweed *Saccharina latissim*a exhibiting sulfate contents ranging from 14.4 to 31.6% [50], and 18.64±1.43% in *S. crassifolium* [51], contingent upon geographical location and harvest season [50]. This suggests that refined fucoidan extracts yield heightened sulfate concentrations. Furthermore, variances in sulfate levels can be attributed to species distinctions [51].

3.6. Identification of Compounds using LC-MS

LC-MS analysis generates large spectral data or chromatograms, which are essential for identifying and quantifying compounds present in a sample. Figure 1 illustrates a typical chromatogram obtained from LC-MS analysis. The peaks in the chromatogram represent different compounds present in the sample, each characterized by its retention time and mass-to-charge ratio. Ethanol extract of *S. cristaefolium* has various bioactive compounds. Table 4 shows the compound constituents present in the ethanolic extract of *S. cristaefolium* that potentially contribute to the treatment of acne. The ethanolic extract of *S. cristaefolium* encompasses bioactive compounds such as carotenoids, terpenoids, steroids, flavonoids, chromenol, and fatty acids.

Halocynthiaxanthin acetate is a carotenoid [52] identified as the major compound, occupying 62.35% of the total area. Halocynthiaxanthin exhibits antioxidant properties, demonstrating both hydroxyl radical scavenging and singlet oxygen quenching capabilities [53]. Through meticulous analysis, the ethanolic extract of *S. cristaefolium* revealed the presence of phoenicoxanthin, constituting 4.47% of the area. This particular compound, phoenicoxanthin, showcases potential as a photoprotective and antioxidative agent in diverse cosmetic formulations [54]. Moreover, the compound loliolide was identified at 2.24%. Loliolide, a monoterpenoid hydroxy lactone, is renowned for its anti-inflammatory, antioxidant, and anti-aging properties, promoting wound healing and mitigating the surge of reactive oxygen species triggered by UVB exposure. Various studies [55] underscore loliolide's efficacy in combating oxidative stress, melanin production, and aging, while Radman et al. corroborate its antioxidative and anti-inflammatory attributes [56]. Additionally, the presence of Sargachromenol, a monoterpenoid compound, manifests potent antioxidant and anti-inflammatory effects [57]. Table 4 further highlights the existence of flavonoid compounds, a class of secondary polyphenolic metabolites renowned for their antioxidant and anti-inflammatory activity [58].

Based on the bacteriolytic cell assay results, the antibacterial mechanism of the ethanolic extract of *S. cristaefolium* by lysing bacterial cells. This is supported by the results of compound identification using LC-MS in Table 4. All diterpenoid compounds, including variabilin, can be against Gram-positive bacteria. The antibacterial mechanism of diterpenoids is by damaging the bacterial cell membrane. Insertion and disruption of the bacterial cell membrane are lipophilic and cause lysis in bacteria [67]. In addition, flavonoids also have an antibacterial mechanism that works on the cell membrane of Gram-positive bacteria, resulting in disruption or damage to phospholipid bilayers. Lipophilicity is a crucial factor responsible for the antibacterial activity of flavonoids [68]. Furthermore, a compound with the potential to exhibit antibacterial properties in the ethanolic extract of *S. cristaefolium* is loliolide. As indicated by Grabarczyk et al., loliolide has demonstrated antibacterial efficacy against a spectrum of bacteria including *S. aureus, S. epidermidis, E. coli, Enterobacter cloacae, Klebsiella pneumoniae*, and *P. aeruginosa* [60]. Through the compound identification via LC-MS, it was elucidated that carotenoid compounds exert their antibacterial effect not through bacterial cell lysis but by enhancing cell membrane permeability, leading to cytoplasmic leakage [69]. Hence, it becomes imperative to undertake a bacterial cell membrane permeability assay to unveil other potential mechanisms of the *S. cristaefolium* ethanolic extract as an antibacterial agent. Additionally, stearidonic acid was identified in the ethanolic extract of *S. cristaefolium*. Functioning as an anti-inflammatory

agent, stearidonic acid inhibits the synthesis of leukotriene B4 (LTB4), a pivotal mediator in the inflammatory cascade. LTB4 and prostaglandin E2 play crucial roles in the initiation of acne lesions and are notably present in the sebaceous glands of acne-affected facial skin. Inhibiting LTB4 leads to a remarkable 70% reduction in acne lesion inflammation [65]. The findings of this investigation propose that compounds derived from *S. cristaefolium* hold promise as a potential pharmaceutical component against acne-associated bacteria.

3.7. Antibacterial and Antioxidant Mechanisms of S. cristaefolium Ethanolic Extract

The ethanolic extract of *S. cristaefolium* exhibits antibacterial activity, primarily due to terpenoids such as variabilin. These compounds disrupt bacterial cell membranes, causing cell lysis, especially against Gram-positive bacteria. The antibacterial mechanism is attributed to the disruption and insertion of lipophilic compounds into the bacterial membrane, leading to cell death [68]. In addition to terpenoids, flavonoids also contribute to antibacterial activity by interacting with bacterial cell membranes, which results in the disruption or degradation of phospholipid bilayers. Lipophilicity plays a key role in the antibacterial activity of flavonoids [69]. Another important compound, loliolide, was identified in the extract and shown to exhibit significant antibacterial activity against *S. aureus, S. epidermidis, E. coli, Enterobacter cloacae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. Loliolide increases bacterial membrane permeability, leading to cytoplasmic leakage [60] [68].

For antioxidant activity, the extract was evaluated using DPPH radical scavenging and ABTS radical cation decolorization assays. The IC_{50} value for ABTS was lower, indicating stronger antioxidant potential compared to DPPH. This is consistent with the role of secondary antioxidants, such as carotenoids, flavonoids, terpenoids, chromenol, and xanthophyll, identified through LC-MS analysis. These compounds contribute significantly to the antioxidant capacity of the extract. Furthermore, the ethanolic extract of *S. cristaefolium* contains stearidonic acid, which exhibits anti-inflammatory properties by inhibiting leukotriene B4 synthesis. This compound is involved in reducing skin inflammation and significantly decreases lesion inflammation by 70% [65].

4. CONCLUSIONS

S. cristaefolium ethanolic extract demonstrates potential as an anti-acne treatment due to its antibacterial and antioxidant properties. The extract showed antibacterial activity with MIC values of 50 mg mL^{-1} against *C. acnes*, and 25 mg mL^{-1} against *S. epidermidis* and *S. aureus*. Additionally, its antioxidant capacity, with IC_{50} values of 645.35±9.52 µg mL^{-1} for DPPH and 574.79±33.52 µg mL^{-1} for ABTS. The antibacterial action is attributed to the lysis of bacterial cells, while secondary antioxidants in the extract help scavenge free radicals, reducing skin cell damage. Key bioactive compounds such as loliolide, variabilin, flavonol, erinapyrone c, halocynthiaxanthin acetate, and phoenicoxanthin contribute to both antibacterial and antioxidant activities.

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Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Thanks are extended to the Directorate of Research, Technology, and Community Service, Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for funding this research through the 2024 Master's Thesis Research program [2869/ UN1/DITLIT/PT.01.03/2024]. This article is part of the first author's thesis. The first author also thanks for the scholarship from the KOREA ODA Project on the 'Korea-Indonesia Marine Technology Cooperation Research Center (20220512)' and 'Establishment of the Integrated Ocean Fisheries Technology Training Center and the Enhancement of Capacity Building in Indonesia (PG54670)' the Korea-Indonesia MTCRC Program, 2024.

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