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The Profiling of Bioactive Compounds from Sargassum Sp. Extract and the Antibacterial Potential on Tembe Nggoli Woven Fabric Against Staphylococcus aureus and Escherichia coli

Muh. Nasir¹*, Olahairullah¹, Faturrahman², Wanda Qoriasmadillah³, Saprini Hamdiani⁴, Ruslan⁵

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ABSTRACT

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Tembe Nggoli is a traditional woven fabric from Bima City, Indonesia, produced using natural cotton yarn with hygroscopic properties that enhance comfort but also support bacterial growth, potentially leading to skin irritation or infections. The aim of this study was to evaluate the potential of bioactive compounds from Sargassum sp. as antibacterial agents and their role in enhancing the functional properties of Tembe Nggoli fabric. Bioactive compounds were extracted through microwave-assisted extraction (MAE) using ethyl acetate, methanol, and n-hexane, followed by liquid-liquid fractionation. The chemical profiles were subjected to rigorous analysis using Fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). FT-IR analysis confirmed the presence of functional groups such as hydroxyl (O-H), alkyl (C-H), carbonyl (C=O), and ether (C-O), signifying the presence of various bioactive constituents. However, GC-MS analysis identified hexadecanoic acid as the predominant compound, which was well-documented for the established antibacterial properties. The ethyl acetate extract had the most significant inhibition against S. aureus, with zones measuring 1.20 mm at 80% concentration (Agar Well Diffusion) and 1.16 mm at 40% concentration (paper disk). The extract had a diminished activity against E. coli, suggesting enhanced resistance. The results showed that Sargassum sp. extract, particularly the type derived from ethyl acetate, could enhance the antibacterial properties of cotton-based textiles such as Tembe Nggoli. The use of natural antibacterial agents would enhance the hygienic quality of the fabric as well as contribute to the sustainable development and added value of traditional textile products.

Keywords: Antibacterial, Sargassum sp. seaweed, Tembe Nggoli Woven Fabric

Introduction

Indonesia is an archipelagic nation comprising vast marine areas dominated by oceans. ¹ These expansive marine ecosystems harbor rich biodiversity, such as macroalgae, that remains largely unexplored. ² The genus *Sargassum* is a prominent group of macroalgae, abundant and rich in bioactive compounds. Species of *Sargassum* are widely distributed across Indonesia, particularly in areas adjacent to the Pacific and Indian Oceans, such as the Lombok and Sumbawa islands in West Nusa Tenggara. Despite the substantial availability of *Sargassum* in Bima Regency, the potential has not been fully harnessed. The high biomass of this species, coupled with the nearly year-round availability, presents a promising opportunity for sustainable resource management. ³

*Corresponding author. E mail: nasir.unswa25@gmail.com Tel: +6285253506723

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Seaweed species from the genus Sargassum have gained substantial research interest because of their rich composition of bioactive molecules, including polysaccharides, polyphenols, and fatty acids, which are known to exhibit diverse biological activities, encompassing antioxidant, antiviral, and antibacterial properties.4 The exploration of these bioactive compounds presents an opportunity for the development of sustainable natural antibacterial agents, offering a viable alternative to synthetic chemicals. The use of Sargassum sp. for antibacterial production holds considerable promise, particularly in the textile industry, where there is a growing interest in enhancing the functional properties of fabrics. An important application is the incorporation of the functional properties into woven fabrics, which represent a traditional handicraft passed down through generations in the archipelago area. Additionally, woven fabric is recognized as an Intangible Cultural Heritage (ICH) by UNESCO. The diversity of these woven products can be distinguished by the motifs, raw materials for yarn, dyes, and the tools used in the production.

Tembe Nggoli woven fabric is a distinguished product from Bima City, characterized by unique patterns and attributes that differentiate it from products of other areas. Furthermore, the widespread appeal extends across various demographics, both locally and internationally. The primary raw material for Tembe Nggoli is cotton yarn derived from natural fibers. However, the inherent limitations of cotton fabric include the hygroscopic properties, which lead to being an ideal medium for bacterial proliferation. Bacterial colonization in cotton fibers can lead to unpleasant odors, structural degradation of the fabric, discoloration, and diminished mechanical properties, including reduced tensile

¹Department of Biology Education, Faculty of Teacher Training and Education, Nggusuwaru University, Bima, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, Indonesia

³Biodiversity Warriors, The Indonesian Biodiversity Foundation, Jakarta, Indonesia

⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, Indonesia

⁵Department of Chemistry Education, Faculty of Teacher Training and Education, Nggusuwaru University, Bima, Indonesia

strength.⁶ Cotton fabric may act as vectors for the transmission of bacteria responsible for skin infections, particularly through direct skin contact.⁷

The development of comfortable functional textile materials with high quality has gained significant attention in recent studies. These functional textiles can protect against and reduce the accumulation of harmful pollutants on textile surfaces, including dust, colored compounds, and pathogenic microbes, as well as offer protection from ultraviolet (UV) radiation. Investigations into functional textiles assessed antibacterial, self-cleaning, UV-protective, and superhydrophobic materials over the years. Considering that antibacterial functional textiles have been widely studied, the antibacterial agents predominantly used are metal oxides such as Titanium dioxide (TiO2), Silver oxide (Ag), Copper(II) oxide (CuO), and Zinc oxide (ZnO), associated with high production costs. Io

Advancements in textile materials have expanded beyond the application of synthetic chemicals that are commonly available. Recent studies have explored the use of natural agricultural waste, which offers a more environmentally sustainable alternative. Agricultural by products, such as the wood of *Berberis vulgaris* L., have been utilized in the dyeing and functional treatment of cotton textiles. To enhance the adhesion of natural dyes to the fabric, citric acid is used to introduce carboxyl functional groups onto the cotton fibers. Under optimized cross-linking conditions, the treated fabrics exhibit improved wash durability, resistance to light-induced fading, and significant antibacterial activity against both Gram-negative and Gram-positive bacterial strains.¹¹

The demand for textiles treated with environmentally friendly antibacterial agents has become more critical because of the need to address the increasing concern over microbial contamination. Numerous medicinal plants are known to contain potent antimicrobial compounds. The proliferation of harmful bacteria in fabrics has led to the advancement of antibacterial textiles, with a growing emphasis on fabric preservation and user protection.¹² To address the aforementioned issue, this research investigates the potential application of natural antibacterial agents derived from the bioactive constituents of Sargassum sp., a marine macroalga recognized for its promise as a renewable and sustainable resource within algal biotechnology. The study involves the profiling of bioactive compounds extracted using ethyl acetate, methanol, and n-hexane solvents, followed by an assessment of their antibacterial activity against Staphylococcus aureus and Escherichia coli when incorporated into Tembe Nggoli woven fabric.

This study used microwave-assisted extraction (MAE) followed by liquid-liquid fractionation to isolate bioactive compounds from *Sargassum* sp. The two methods were selected due to the efficiency in preserving the integrity and potency of thermolabile compounds. ¹³ Moreover, the extract samples were analyzed using FT-IR spectroscopy and GC-MS considered well-established methods for identifying functional groups and molecular structures in complex biological matrices. ¹⁴ Antibacterial activity was evaluated against *S. aureus* and *E. coli* using the agar well diffusion and paper disk diffusion methods, which provided a reliable and reproducible process of assessing inhibition zones. The choice of the two bacterial strains was based on the clinical relevance as common causes of skin and soft tissue infections. ¹⁵

This study presents a novel approach by utilizing *Sargassum sp.* extract as a natural antibacterial agent to improve the hygienic quality of Tembe Nggoli woven fabric. The novelty lies in the integration of marine-derived bioactive compounds with traditional woven textiles, which has not been previously explored. While *Sargassum sp.* has been studied for its antibacterial properties in biomedical and food-related applications, its application in enhancing the functional value of cultural fabrics, such as Tembe Nggoli, represents a new and innovative direction. This approach not only contributes to the development of eco-friendly textile finishing methods but also promotes the preservation and modernization of local heritage crafts through scientific innovation.

To date, limited research has addressed the incorporation of marinederived bioactive compounds into traditional Indonesian textiles, thereby offering a unique contribution to both biomedical applications and the innovation of cultural heritage fabrics. Additionally, the adoption of environmentally sustainable extraction methods is consistent with the principles of sustainable development in the production of valuable natural compounds. The outcomes of this study are anticipated to enhance the strategic use of *Sargassum sp.* as a bioresource, fostering the advancement of functional antibacterial textiles with significant potential for global market expansion.

Materials and Methods

Chemicals and Reagents

The reagents and solvents used were hydrochloric acid (HCl, 37%, Merck, Germany), ethanol (95%, Merck, Germany), methanol (99.9%, Merck, Germany), n-hexane (98%, Merck, Germany), ethyl acetate (99.8%, Sigma-Aldrich, USA), dimethyl sulfoxide (DMSO, 99.9%, Sigma-Aldrich, USA), and distilled water. Phytochemical reagents included boric acid, oxalic acid, anhydrous acetic acid, ether, chloroform, sulphuric acid, acetone, Dragendorff's reagent, Mayer's reagent, and ferric chloride (FeCl₃), which were all analytical grade (Merck, Germany). Media components comprised Sabouraud Dextrose Agar (SDA), Nutrient Agar (NA), Nutrient Broth (NB), dextrose, and peptone (Himedia, India). The bacterial strains used were both *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Collection and Identification of Plant Material

A specimen of *Sargassum* sp. seaweed was collected on 16 June 2024 from the Wane Beach area in Bima, Indonesia (GPS coordinates: -8.808600547709833, 118.65613662180456). Plant identification and verification were conducted by Dr. Mursal Gazali, M.Si., from the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Mataram. A voucher specimen was deposited under the number UNRAM-SG-0124. The seaweed was processed in combination with Tembe Nggoli woven fabric sourced from local artisans.

Preparation of Sargassum sp. Seaweed Sample

Fresh *Sargassum* sp. was thoroughly rinsed with tap water to remove debris, soaked in 1% HCl for 2 hours, and rinsed with distilled water until pH became neutral. The sample was dried under shade (aerated conditions), ground using a blender (Philips HR2116, China), and sieved to obtain a fine powder.

Extraction of Bioactive Compounds

Approximately 10 g of dried *Sargassum* powder was extracted using 100 mL of 95% ethanol through MAE (Panasonic NN-DF383B, Japan) at 2450 MHz for 10–30 minutes in intervals of 5 minutes. The extract was filtered and concentrated using a rotary evaporator (Heidolph Laborota 4000, Germany) at 40°C until a viscous mass was obtained.

Fractionation of Bioactive Compounds

The ethanol extract was suspended in 100 mL methanol and sequentially fractionated with 100 mL *n*-hexane and 100 mL ethyl acetate by vigorous shaking and phase separation using a separatory funnel. The organic phases were collected, and each solvent was readded until a clear extract was obtained. Each solvent fraction was evaporated using a rotary evaporator.

FT-IR Analysis

FT-IR analysis was performed using a PerkinElmer Spectrum Two FT-IR spectrophotometer (USA). Furthermore, dried extract was mixed with KBr and compressed into pellets. Spectra were recorded over the 4000–400 cm⁻¹ range and analyzed by comparison to standard reference to identify functional groups. ¹⁶

Identification of Bioactive Compounds with GC-MS

GC-MS analysis was carried out using a Shimadzu QP2010 Ultra system (Japan) equipped with a BP-5MS capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness). The temperature program for the oven was set to increase from 60°C to 230°C at a rate of 5°C per minute.

Helium was used as the carrier gas, maintained at a constant flow rate of 1.0 mL/min. A 2.0 μL sample was injected in split mode with a split ratio of 5:1. The injector and ion source temperatures were set at 250°C and 230°C, respectively. Mass spectra were recorded over a $\emph{m/z}$ range of 40–700, and compound identification was achieved by comparing the acquired spectra with entries in the NIST 2017 mass spectral library. 17

In-vitro Evaluation of the Antibacterial Potential of Bioactive Compounds

Agar Well Diffusion Assay

A bacterial suspension equivalent to a 0.5 McFarland standard was spread evenly onto Sabouraud Dextrose Agar (SDA) plates using sterile cotton swabs. Wells, each 7 mm in diameter, were then created in the agar using a sterile borer. Into each well, 100 μL of the extract, previously diluted in DMSO, was added. The plates were incubated at 30°C for 24 hours, and the resulting inhibition zones were measured. The experiment was conducted in triplicate, with 50% DMSO as the negative control.

Paper Disk Assay

Sterile filter paper discs (6 mm) were soaked in extract solutions for 15 minutes and positioned on inoculated SDA media. After 24 hours of incubation at 30°C, inhibition zones were measured using a digital caliper, and each concentration was tested in triplicate.

Coating on Woven Fabric

Tembe Nggoli fabric was cut into 10×10 cm pieces and immersed in extract solution from the most active fraction. These samples were agitated in a shaker (IKA KS 260 Basic, Germany) at 150 rpm for 24 hours and dried at 70° C in an oven (Memmert UN55, Germany).

Antibacterial Activity Test on Woven Fabric

The antibacterial test included positioning woven fabric coated with *Sargassum* sp. extract alongside a control woven fabric disc over cultures of *S. aureus* and *E. coli.* Inhibition zone measurements were obtained following a 24-hour incubation period, after which observations were conducted after 72 hours. ¹⁸

Statistical Analysis

All experiments were conducted in triplicate, with the results presented as means \pm standard deviations (mean \pm SD). A two-way analysis of variance (ANOVA) was applied to examine the statistical differences between the treatment groups, with a particular focus on the interaction between solvent type and extract concentration. A p-value of less than 0.05 was considered statistically significant. Statistical computations were carried out using Microsoft Excel. This method allowed for the identification of significant factors affecting antibacterial performance and provided a quantitative framework for the interpretation of the results.

Results and Discussion

FT-IR spectroscopy (KBr, thin film) of the *Sargassum sp.* extract using ethyl acetate, methanol, and *n*-hexane showed consistent absorption bands, suggesting the presence of similar bioactive compounds across solvents. A broad absorption band observed between 2400 and 3400 cm⁻¹ corresponded to O–H stretching, typical of carboxylic acids. The presence of alkanes was confirmed by C–H stretching vibrations in the area of 2800–3000 cm⁻¹. A strong, sharp peak at 1750 cm⁻¹ was assigned to the C=O stretching of ester groups, while the C–O stretching vibration appeared as a strong band in the 1000–1300 cm⁻¹ area. Additionally, polysaccharides and isomeric carbohydrates were signified by absorption bands in the range of 1200–700 cm⁻¹. These results suggested the presence of hydroxyl, alkyl, ester, and polysaccharide functional groups in the extract.

The chemical profile of *Sargassum* sp. was elucidated using GC-MS, as illustrated in Figure 2. Table 1 presents the principal compounds detected, including their retention times (RT), peak areas, molecular formulas, and molecular weights. Compound identification was conducted by correlating the obtained spectral data with the reference standards available in the National Institute of Standards and Technology (NIST) database. The examination of the ethyl acetate extract revealed the presence of ten distinct bioactive components. Specifically, the predominant compounds in this extract included hexadecanoic acid (17.97%), 1,10-decanediol (5.01%), neophytadiene (4.78%), and octadecanoic acid (3.09%).

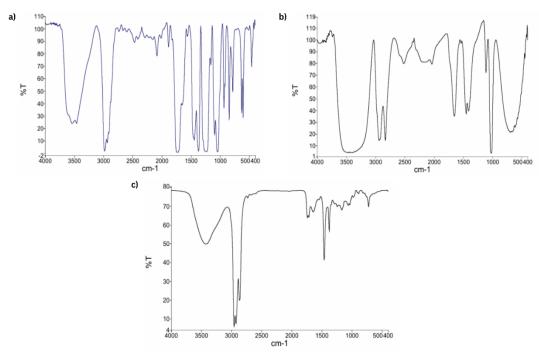


Figure 1: FTIR analysis of Sargassum sp. a) Ethyl acetate; b) Methanol; c) N-hexane

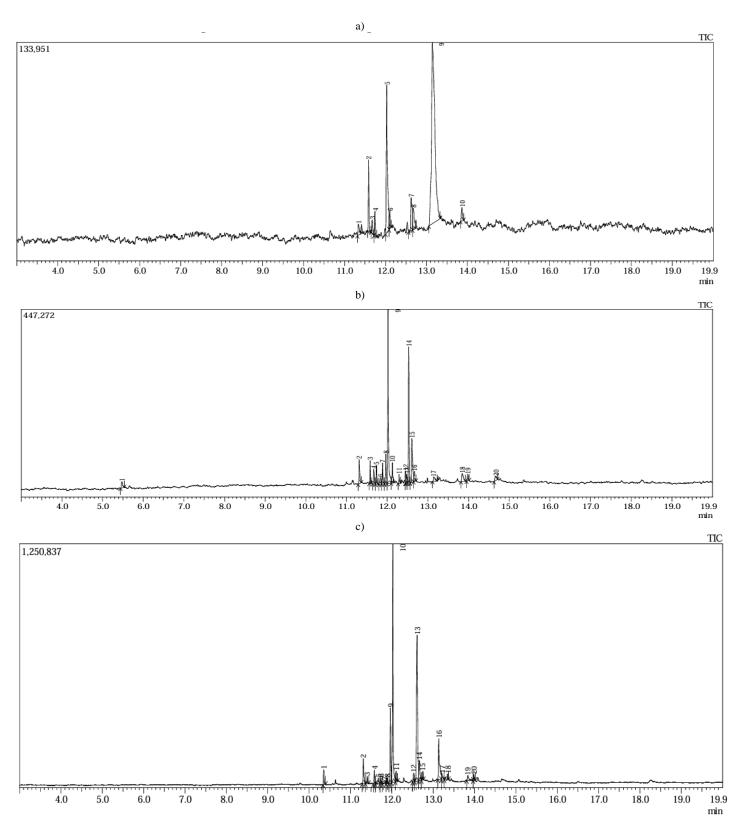


Figure 2: Bioactive Compound Chromatogram with GC-MS of Sargassum sp. a) Ethyl acetate; b) Methanol; c) N-hexane

Table 1: GC-MS analysis of *Sargassum* sp. ethyl acetate solvent

Compound Name	Retention Time	Molecular Formula	Molecular Weight (g/mol)	Area (%)	
Hexadecanoic acid (CAS) Palmitic acid	11.335	C ₁₆ H ₃₂ O ₂	256.42	1.57	
NEOPHYTADIENE	11.586	$C_{20}H_{38}$	278.51	4.78	
NEOPHYTADIENE	11.674	$C_{20}H_{38}$	278.51	1.25	
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-	11.740	$C_{20}H_{40}O$	296.53	1.63	
[R*,R*-(E)]]- (CAS)					
Hexadecanoic acid (CAS) Palmitic acid	12.028	$C_{16}H_{32}O_2$	256.42	17.97	
1-Nonadecene (CAS)	12.110	C19H38	266.50	1.56	
1,10-Decanediol (CAS) Decane-1,10-diol	12.625	$C_{10}H_{22}O_2$	174.28	5.01	
Octadecanoic acid (CAS) Stearic acid	12.680	$C_{18}H_{36}O_{2}$	284.48	3.09	
Unknown	13.144	-	-	61.56	
Hexadecanoic acid, 2-hydroxy-1-	13.863	$C_{19}H_{38}O_4$	330.50	1.57	
(hydroxymethyl)ethyl ester (CAS) 2					
	Hexadecanoic acid (CAS) Palmitic acid NEOPHYTADIENE NEOPHYTADIENE 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Hexadecanoic acid (CAS) Palmitic acid 1-Nonadecene (CAS) 1,10-Decanediol (CAS) Decane-1,10-diol Octadecanoic acid (CAS) Stearic acid Unknown Hexadecanoic acid, 2-hydroxy-1-	Hexadecanoic acid (CAS) Palmitic acid NEOPHYTADIENE 11.586 NEOPHYTADIENE 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R- 11.740 [R*,R*-(E)]]- (CAS) Hexadecanoic acid (CAS) Palmitic acid 12.028 1-Nonadecene (CAS) 12.110 1,10-Decanediol (CAS) Decane-1,10-diol 12.625 Octadecanoic acid (CAS) Stearic acid 12.680 Unknown 13.144 Hexadecanoic acid, 2-hydroxy-1- 13.863	Hexadecanoic acid (CAS) Palmitic acid 11.335 C16H32O2 NEOPHYTADIENE 11.586 C20H38 NEOPHYTADIENE 11.674 C20H38 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R- 11.740 C20H40O [R*,R*-(E)]]- (CAS) Hexadecanoic acid (CAS) Palmitic acid 12.028 C16H32O2 1-Nonadecene (CAS) 12.110 C19H38 1,10-Decanediol (CAS) Decane-1,10-diol 12.625 C10H22O2 Octadecanoic acid (CAS) Stearic acid 12.680 C18H36O2 Unknown 13.144 - Hexadecanoic acid, 2-hydroxy-1- 13.863 C19H38O4	Hexadecanoic acid (CAS) Palmitic acid 11.335 C ₁₆ H ₃₂ O ₂ 256.42 NEOPHYTADIENE 11.586 C ₂₀ H ₃₈ 278.51 NEOPHYTADIENE 11.674 C ₂₀ H ₃₈ 278.51 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R- 11.740 C ₂₀ H ₄₀ O 296.53 [R*,R*-(E)]]- (CAS) Hexadecanoic acid (CAS) Palmitic acid 12.028 C ₁₆ H ₃₂ O ₂ 256.42 1-Nonadecene (CAS) 12.110 C ₁₉ H ₃₈ 266.50 1,10-Decanediol (CAS) Decane-1,10-diol 12.625 C ₁₀ H ₂₂ O ₂ 174.28 Octadecanoic acid (CAS) Stearic acid 12.680 C ₁₈ H ₃₆ O ₂ 284.48 Unknown 13.144 -	

Bioactive compounds extracted from *Sargassum* sp. using methanol as a solvent comprised a total of 20 distinct compounds, as presented in Table 2. The predominant compounds identified in this solvent included hexadecanoic acid (29.72%), 1-dodecanol, 3,7,11-trimethyl- (16.95%), 9,12-octadecadienoic acid (10.58%), and 9-hexadecenoic acid (5.29%). The extraction of bioactive compounds from *Sargassum sp.* using *n*-hexane solvent generated a total of 20 distinct compounds, as presented in Table 3. The predominant compounds identified in this extract included hexadecanoic acid (30.96%), 9-hexadecenoic acid (27.89%), and sulfuric acid, 5,8,11-heptadecatrienyl methyl ester (8.53%).

Three extract samples of *Sargassum sp.* obtained using different solvents were evaluated for the antibacterial activity against *S. aureus* and *E. coli*, as presented in Figure 3. The results showed that all three were rich in bioactive compounds capable of inhibiting bacterial growth. The sensitivity of the bacterial strains to *Sargassum sp.* varied, as evidenced by the increasing diameter of the inhibition zones corresponding to higher sample concentrations. The highest values on the red chart in Figure 3 signified that the extract using ethyl acetate had the most pronounced antibacterial efficacy, except for the observation found in the paper disk assay against *E. coli*.

Table 2: GC-MS analysis of Sargassum sp. methanol solvent

Peak	Compound Name	Retention Time	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	Benzene, ethyl- (CAS) EB	5.476	C8H10	106.17	1.77
2	Tetradecanoic acid (CAS) Myristic	11.312	$C_{14}H_{28}O_2$	228.37	4.18
	acid				
3	NEOPHYTADIENE	11.581	$C_{20}H_{38}$	278.51	2.80
4	Unknown	11.670	-	-	2.27
5	2-Hexadecen-1-ol, 3,7,11,15-	11.737	C18H34O	270.47	2.73
	tetramethyl-, [R-[R*,R*-(E)]]- (CAS)				
	P				
6	Unknown	11.831	-	-	1.77
7	Hexadecanoic acid, 15-methyl-,	11.886	$C_{18}H_{36}O_{2}$	284.4772	3.13
	methyl ester (CAS) METHYL-15-M				
8	9-Hexadecenoic acid (CAS)	11.966	$C_{16}H_{30}O_{2}$	254.41	5.29
9	Hexadecanoic acid (CAS) Palmitic	12.018	$C_{16}H_{32}O_{2}$	256.4241	29.72
	acid				
10	2-Hexadecen-1-ol, 3,7,11,15-	12.127	C20H40O	296.53	3.56
	tetramethyl-, [R-[R*,R*-(E)]]- (CAS)				
11	Sulfuric acid, 5,8,11-heptadecatrienyl	12.289	$C_{18}H_{30}O_{2}$	278.43	1.82
	methyl ester (CAS) HEPTA				
12	1-Hexadecanol, 3,7,11,15-	12.449	C18H38O	298.54	1.95
	tetramethyl- (CAS) Dih				
13	Cyclopropaneoctanoic acid, 2-hexyl-,	12.475	$C_{22}H_{38}O_2$	334.54	1.72
	methyl ester (CAS) M				

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14	1-Dodecanol, 3,7,11-trimethyl- (CAS)	12.530	C ₁₅ H ₃₂ O	228.414	16.95
	Hexahydrofarnesol				
15	9,12-Octadecadienoic acid (Z,Z)-	12.606	$C_{18}H_{32}O_2$	280.452	10.58
	(CAS) Linoleic				
16	Octadecanoic acid (CAS) Stearic acid	12.667	$C_{18}H_{36}O_{2}$	284.48	2.09
17	Sulfuric acid, 5,8,11-heptadecatrienyl	13.140	$C_{18}H_{30}O_{2}$	278.43	1.79
	methyl ester (CAS) HEPTA				
18	PENTADECANOIC ACID, 2-	13.842	C ₁₅ H ₃₄ O ₃	250.43	3.04
	HYDROXY-1-(HYDROXYMETHY				
19	1,2-Benzenedicarboxylic acid, dioctyl	13.982	C ₂₄ H ₄₀ O ₄	392.58	1.17
	ester (CAS) Dioctyl phthalate				
20	9,12-Octadecadienoyl chloride, (Z,Z)-	14.668	C ₁₈ H ₃₃ ClO	316.84	1.66

Table 3: GC-MS analysis of Sargassum sp. n-hexane solvent

Peak	Compound Name	Retention Time	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	Phenol, 3,5-bis(1,1-dimethylethyl)-	10.356	$C_{14}H_{22}O$	206.32	1.66
	(CAS) 3,5-Di-tert-butylphen				
2	Tetradecanoic acid (CAS) Myristic	11.312	$C_{14}H_{28}O_2$	228.37	3.99
	acid				
3	9-Eicosene, (E)- (CAS)	11.409	C18H36	280.5316	1.05
4	2-Hexadecen-1-ol, 3,7,11,15-	11.583	$C_{20}H_{40}O$	296.53	1.56
	tetramethyl-, $[R-[R^*,R^*-(E)]]$				
5	Unknown	11.674	-	-	1.15
6	Unknown	11.739	-	-	0.68
7	Hexadecanoic acid, 15-methyl9,9-	11.840	$C_{16}H_{32}O_2$	256.43	0.50
	Epoxymethano-6,6-dimethyl-3,4-				
	undecadien-2,10-dione				
8	Hexadecanoic acid, 15-methyl-,	11.887	$C_{18}H_{36}O_{2}$	284.48	0.64
	methyl ester (CAS) METHYL-1				
9	9-Hexadecenoic acid (CAS)	11.968	$C_{16}H_{30}O_2$	254.41	9.94
	Palmitoleic acid				
10	Hexadecanoic acid (CAS) Palmitic	12.026	$C_{16}H_{32}O_2$	256.4241	30.96
	acid				
11	1-Nonadecene (CAS)	12.105	$C_{19}H_{38}$	266.505	1.42
12	2-Hexadecen-1-ol, 3,7,11,15-	12.525	C18H36O	270.49	1.12
	tetramethyl-, [R-[R*,R*-(E)]]- (CAS)				
13	9-Hexadecenoic acid (CAS)	12.609	$C_{16}H_{30}O_{2}$	254.41	27.89
14	Octadecanoic acid (CAS) Stearic acid	12.667	$C_{18}H_{36}O_{2}$	284.484	3.62
15	1-Nonadecene (CAS)	12.740	C19H38	266.51	1.00
16	Sulfuric acid, 5,8,11-heptadecatrienyl	13.135	$C_{18}H_{30}O_{2}$	278.43	8.53
	methyl ester (CAS) HEPTA				
17	HEPTADECENE-(8)-CARBONIC	13.215	$C_{18}H_{36}O_2$	284.49	1.27
	ACID-(1)				
18	1-Nonadecene (CAS)	13.351	C19H38	266.51	0.97
19	Hexadecanoic acid, 2-hydroxy-1-	13.835	C19H38O4	330.50	1.26
	(hydroxymethyl)ethyl ester (CAS)				
20	1,2-Benzenedicarboxylic acid, dioctyl	13.982	$C_{18}H_{38}O_4$	390.56	0.79
	ester (CAS) Dioctyl phthalate				

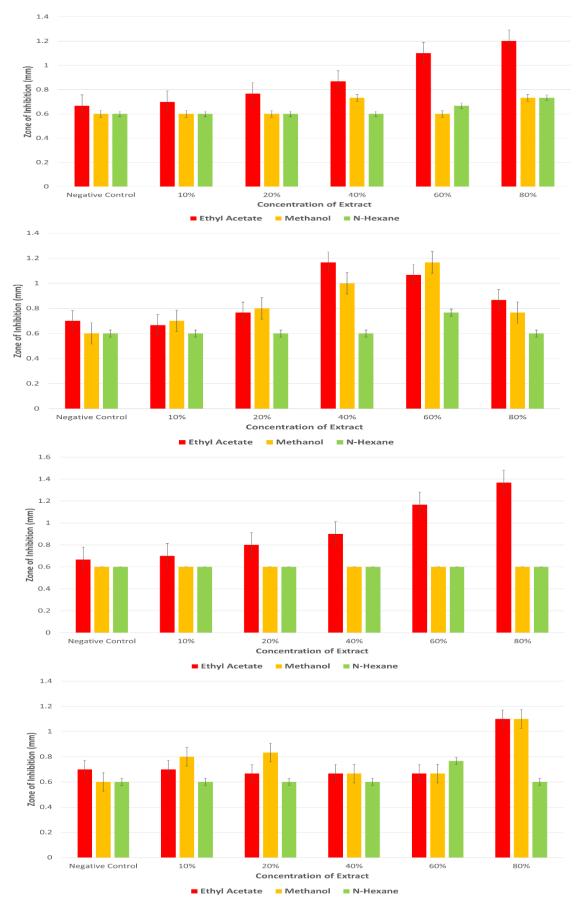


Figure 3: Antibacterial activity in *Sargassum* extract, sp. a) Agar Well Diffusion and b) Paper disk for *S. aureus*, as well as c) Agar Well Diffusion and d) Paper disk for *E. coli*

GC-MS analysis was conducted in this study using ethyl acetate, methanol, and n-hexane solvents. All three extract samples of Sargassum contained hexadecanoic acid, also known as palmitic acid, as the predominant compound. Previous investigations on Sargassum polycystum identified palmitic acid in conjunction with other fatty acids, including myristate, oleic acid, pentadecanoic acid, and behenic acid. These used GC-MS to assess the phytochemical constituents present in the extract of n-hexane, dichloromethane, and methanol solvents, determining that methanol had the highest extraction efficiency. The identification of palmitic acid and other fatty acids suggested considerable antibacterial potential in the extract of S. polycystum. 19 Additionally, investigations into Sargassum ilicifolium and Sargassum angustifolium showed that palmitic acid constituted a significant proportion of the total fatty acids, accounting for 37.44% in S. ilicifolium and 23.05% in S. angustifolium. 20 An analysis of Sargassum wightii extract showed the presence of phytochemicals, including phenolic compounds and flavonoids, which had substantial antibacterial activity against Pseudomonas aeruginosa.²¹

The extract obtained using ethyl acetate had the highest antibacterial effectiveness against both *S. aureus* and *E. coli*, with the largest inhibition zones observed at a concentration of 0.8 mg/mL. This result shows that semi-polar solvents such as ethyl acetate are more effective in extracting antibacterial bioactive compounds compared to polar (methanol) or non-polar (n-hexane) solvents, which is further supported by statistical analysis. For *S. aureus*, both the agar well and paper disk diffusion methods showed statistically significant differences among

the solvent types (p < 0.05). The paper disk method showed a significant effect of extract concentration (p = 0.0097), suggesting a dosedependent response. However, only the agar well method for E. coli showed a significant difference among the solvents (p = 0.0067). These discrepancies suggest that the testing method can influence the sensitivity of antibacterial activity detection. The paper disk appears to be more responsive to variations in concentration. In Gram-negative bacteria such as E. coli, the diffusion of active compounds may be limited by the complex structure of the outer cell wall, diminishing the observable effect of concentration. The results show that Sargassum sp. extract, particularly the type produced with ethyl acetate, possesses promising potential as a natural antibacterial agent against both Grampositive and Gram-negative bacteria. This supports the applicability in the development of functional textiles such as Tembe Nggoli, which may serve to prevent microbial contamination in humid tropical environments.

The antibacterial activity of *Sargassum* sp. was assessed in the application to Tembe Nggoli woven fabric composed of cotton. Treatments were administered using an extract that had the largest and smallest antibacterial zones as determined in prior antibacterial evaluations. According to Figure 4, antibacterial zones were derived from the ethyl acetate and *n*-hexane extract samples. The zone of inhibition produced by the ethyl acetate extract was significantly larger compared to the *n*-hexane extract, suggesting efficacy against both Gram-positive and Gram-negative bacteria. These results were consistent with previous studies reporting that ethyl acetate extract had the highest antibacterial potency.

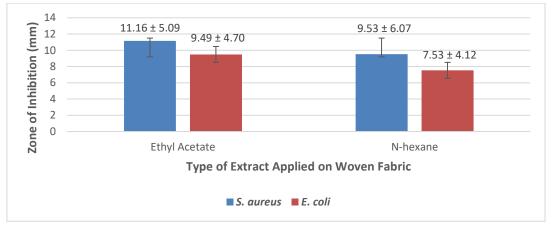


Figure 4: Antibacterial activity of Sargassum sp. extract on Tembe Nggoli woven fabric

The antibacterial activity was evaluated using both agar well diffusion and paper disk methods, showing notably greater effectiveness against Gram-positive bacteria than Gram-negative strains. This discrepancy in susceptibility is largely attributed to the resistance mechanisms inherent to Gram-negative bacteria, such as *Escherichia coli*. The cell wall structure of these bacteria, characterized by an outer lipoprotein layer, a middle peptidoglycan layer, and an inner lipopolysaccharide membrane, forms a robust barrier that limits the penetration of antimicrobial agents.²²

The significant antibacterial activity observed in the ethyl acetate extract is hypothesized to originate from the presence of specific compounds functioning as inhibitors, which are absent in the extract derived from other solvents, thereby clarifying the differential effects. The ethyl acetate extract shows superior solubility of the antibacterial compounds derived from *Sargassum* sp. compared to those extracted using alternative solvents. The results diverge from previous studies that identified methanol extract with the highest antibacterial efficacy. This variation in antimicrobial activity may be attributed to several factors, including the specific compounds present in the organism, the timing and method of sampling, the efficacy of the extraction processes in isolating active metabolites, and the tests conducted. A study found that the methanol extract of *Sargassum gracilis* effectively inhibited the growth of *Bacillus mesentericus*. Additionally, significant antibacterial

activity against *S. aureus* was identified for the methanol extract of *Sargassum sp.*, suggesting this algal species contains potent antibacterial compounds effective on certain pathogenic bacteria in humans. Supporting evidence from other investigations shows that the methanol extract of *Sargassum muticum* comprises various phenolic compounds, which are instrumental in combating invasive bacteria and mitigating environmental stressors, thereby enhancing plant defense mechanisms.²³

Groups identified in this study clarified the antibacterial mechanisms of algal compounds, particularly dieckol, a natural phlorotannin present in certain species of brown algae. Dieckol exerts antibacterial effects through several mechanisms, which include the disruption of bacterial cell wall integrity, interference with the peptidoglycan structure, induction of osmotic imbalance, promotion of leakage of intracellular constituents, and inhibition of key cellular processes such as DNA replication, transcription, protein synthesis, and enzymatic activity, ultimately leading to the death of the bacterial cells. ²⁴ The results of the antibacterial assay aligned with the findings from the FT-IR analysis, which detected various functional groups commonly associated with biologically active constituents, including hydroxyl, carboxyl, phenolic, ester, ether, and alkane groups. In addition, the presence of antimicrobial activity is often attributed to certain long-chain fatty acids, particularly unsaturated types such as oleic, linoleic, and

palmitoleic acids (C₁₆-C₂₀), as well as saturated fatty acids like stearic and palmitic acids.²⁵

The results of GC-MS analysis correlate with the antibacterial properties of *Sargassum* sp. extract. Furthermore, the organic compounds identified through the analysis are classified into several categories, including alkanes, alkenes, alkanols, and carboxylates, all of which have antibacterial activity. These compounds were hypothesized to function synergistically to inhibit bacterial proliferation. The structural configuration of alkenes, characterized by double bonds, facilitates the interaction with various molecular entities. Alkanols possessing hydroxyl groups are capable of bridging hydrophobic and hydrophilic interactions, while carboxylates compromising carboxyl groups can form bonds with amine groups. Both alkanols and carboxylic acids have distinctive inhibitory mechanisms, namely, alkanols may destabilize proteins, compromise cell membrane integrity, induce dehydration, and function as detergents or mediators between hydrophilic and hydrophobic interfaces. ²⁶

Hexadecanoic acid was identified in this study as the predominant compound among the three extract samples analyzed. A previous investigation reported that hexadecenoic acid had significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria.²⁷ The antibacterial activity of carboxylate compounds is largely due to their capacity to disrupt protein synthesis, an essential mechanism for bacterial growth and replication, by targeting the bacterial ribosomes. Additionally, the extract may inhibit the formation of the bacterial cell wall, which is particularly effective against Grampositive bacteria owing to their thicker peptidoglycan layer. The extract may also interfere with nucleic acid biosynthesis and replication, while simultaneously inducing oxidative stress through its antioxidant properties, resulting in the production of reactive oxygen species that are harmful to bacterial cells. These combined mechanisms likely contribute to the antibacterial properties of Sargassum sp., potentially arising from the synergistic effects of the diverse bioactive compounds present in the extract.28

Considering the significant potential of *Sargassum* sp. as a natural coating material for indigenous woven fabric, this study is consistent with previous investigations using *Mentha piperita* extract to formulate antibacterial agents for cotton applications. Although the full extent of the antibacterial mechanisms of *M. piperita* is not yet completely understood, it is believed that this plant disrupts the structural integrity of the phospholipid bilayer, interacts with enzymes and proteins associated with the membrane, and acts as a proton exchanger, thereby affecting the pH gradient across the membrane. To gain a deeper understanding of these mechanisms, further research employing scanning electron microscopy is recommended.²⁹

Cotton fabric coated with plant extract has a reduced potential for causing human skin irritation. The presence of *E. coli*, which proliferates on human skin or cotton substrates, is associated with the release of endotoxins originating from the metabolic processes. Additionally, the application of chemical disinfectants aimed at suppressing bacterial growth can lead to skin irritation. This investigation underscores the potential of plant-derived natural phytochemicals with antibacterial properties, while minimizing the risk of skin irritation. Future research should aim to evaluate the effectiveness of cotton fabrics coated with these extracts, using in-vivo animal models or human skin cell cultures, to provide further evidence supporting the non-irritating antibacterial effects of the phytochemicals.³⁰

The traditional protection of textiles from microbial contamination previously depended on synthetic antimicrobial agents, such as triclosan, metal salts, phenolic compounds, and quaternary ammonium compounds, which are readily available commercially. However, the safety profile of these substances is not assured because of the tendency to initiate undesirable side effects. Bio-based materials are increasingly considered a viable alternative for antimicrobial applications. Therefore, this study investigates the potential of *Sargassum* sp. exploiting locally abundant resources and simultaneously enhancing the cultural significance of Tembe Nggoli woven fabric. The bioactive compounds extracted from plant sources offer new possibilities in the context of health-oriented textile exploration. Recent developments show the effectiveness of bioactive antimicrobial coatings applied to

cotton fabrics, a technology that is increasingly recognized for the ability to produce textiles with improved aesthetic and functional properties. 31

Sargassum sp. application in Tembe Nggoli woven fabric represents a progressive and sustainable method to enhance the functional properties of textiles. The hygroscopic properties possessed by cotton provide an environment conducive to microbial proliferation, potentially leading to fabric deterioration, unpleasant odors, and skin infections. Cotton fabric treatment with natural bioactive compounds derived from Sargassum sp. holds promise for mitigating these issues by providing antibacterial protection while preserving the natural integrity and cultural significance of Tembe Nggoli woven fabric.

The application method detailed in this study, which includes immersing the fabric in a bioactive compound for a duration of 24 hours followed by heat drying, may benefit from further optimization. Extended heat exposure has the potential to compromise certain bioactive compounds, diminishing the efficacy. Therefore, investigating alternative application methods, such as microencapsulation where bioactive compounds are incorporated in a controlled release matrix, could enhance the antibacterial properties without needing prolonged thermal exposure.

A significant issue observed relates to the variability in *E. coli* resistance to *Sargassum* sp. extract. This shows the need for future investigations to concentrate on the identification of specific bioactive compounds in the extract possessing the capability to effectively target Gram-negative bacteria. More fractionation and purification of the bioactive constituents, followed by individual assays, will clarify the precise mechanisms constituting the antibacterial activity. The synergistic effects arising from the combination of the natural extract with established antibacterial agents or metal nanoparticles need exploration because of the potential to enhance efficacy against a broader range of bacterial strains.

This study emphasizes the antibacterial properties of *Sargassum* sp. extract without addressing the potential implications for the mechanical properties of the fabric substrate. Future investigations should determine whether the application of the bioactive compounds influences the tensile strength, durability, and texture of the fabric. The adverse effect of the antibacterial treatment on the flexibility or softness of the fabric may diminish the commercial viability.

Based on the environmental and sustainability considerations associated with the use of seaweed-derived extract, this study presents novel strategies for the development of eco-friendly textiles. Sargassum sp. which is prevalent in numerous coastal areas can act as a renewable source of natural antibacterial agents, corresponding with the global shift towards more sustainable manufacturing practices. Further investigations are still essential to enhance this technology and assess the commercial viability, which includes evaluating the costeffectiveness of large-scale seaweed extraction and examining the longterm stability of the bioactive compounds in daily textile applications. The significant antibacterial potential of Sargassum sp. when applied to cotton woven fabric is clarified in this study. However, several areas need more optimization and comprehensive investigation. The observed high efficacy against S. aureus is promising, yet the comparatively lower effectiveness against E. coli presents an issue. Future studies should prioritize the refinement of the extraction process, the exploration of alternative application methods, as well as the evaluation of the long-term effects on the functional and mechanical properties of the treated fabric. The incorporation of sustainable practices into this process corresponds with the growing focus on environmental stewardship, offering the dual advantages of enhanced functionality and environmental sustainability.

Conclusion

In summary, the present study demonstrated that *Sargassum* sp. extracts, as characterized by FT-IR and GC-MS analyses, impart antibacterial properties to Tembe Nggoli fabric, with hexadecanoic acid identified as a major bioactive compound. The ethyl acetate extract demonstrated the most pronounced antibacterial activity against *S. aureus*, while *E. coli* exhibited notable resistance. These findings underscore the potential of *Sargassum* sp. as a natural source for the

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functionalization of traditional textiles in sustainable applications. Future research should concentrate on isolating and characterizing specific active compounds responsible for the antibacterial effects, optimizing the extraction and application processes, and conducting long-term evaluations of the fabric's performance, durability, and safety under practical usage scenarios. Furthermore, an exploration of the environmental impact and biodegradability of the treated fabrics could support their integration into eco-friendly textile innovations.

Conflict of Interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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