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Research Article

Pigmented Gastropods-Assosiate Bacteria: an Innovation in Natural Antioxidants, Antibacterials, and Sun Protection

Marwa Irfan Hanif[1], Delianis Pringgenies[2*], Wilis Ari Setyati[2]

[1]Master's Programme in Marine Sciences, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang 50275, Indonesia
[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang 50275, Indonesia

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ABSTRACT

This research investigates the ocean's potential as a source of natural active ingredients with antioxidant, antibacterial, and sun protection properties. Marine gastropods are known to have symbiotic relationships with associated bacteria capable of producing pigments, which hold promise for the cosmetic and health sectors. The study aims to identify and analyze pigments produced by bacteria linked to marine gastropods and evaluate their effectiveness as antioxidants, antibacterials, and sun protectants. The method involved isolating and analyzing pigments from gastropod-associated bacteria, followed by tests to assess their properties. Results revealed two gastropod species: *Telescopium telescopium* (Linnaeus, 1758) and *Cassidula nucleus* (Gmelin, 1791), from which 21 bacterial isolates were obtained—10 from *T. telescopium* and 11 from *C. nucleus*. Among these, *Micrococcus yunnanensis*, a bacterium with high pigment production, was successfully isolated. At 1,000 µg/mL, its pigment's crude extract had low antioxidant activity and had a low SPF category as a photoprotective agent. Antibacterial tests showed efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. HPLC and FTIR analysis suggested the pigment contains carotenoid compounds. These findings highlight the potential of *M. yunnanensis* pigment for sustainable health and cosmetic applications.

1. Introduction

In today's scientific and technological advancement world, there is a fast-growing interest in exploring natural resources to find bioactive compounds with wide-ranging applications in the health and cosmetics industries. One of the natural resources being actively researched is gastropods, a group of invertebrate animals that includes snails. Gastropods, particularly those in relationships with associated pigmented microorganisms, present significant potential for use as natural ingredients in health and beauty products, including antioxidants, antibacterials, and sun protection agents (Setyati *et al.* 2023; Kusmita *et al.* 2023).

Gastropods are known for having various defense mechanisms, both physical and chemical. They inhabit diverse environments, from land to marine settings, exposing them to threats like UV radiation, pathogens, and environmental pollutants. Some gastropods produce toxic or unpleasant chemicals to ward off predators. These substances can also protect them from microbes and environmental stress (Kamyab *et al.* 2020; Mawardi *et al.* 2023). An important adaptation in some gastropod species is their ability to form associations with pigmented microorganisms. These microorganisms protect the gastropods from UV radiation through pigment production and create bioactive compounds with antioxidant and antibacterial properties (Wijaya *et al.* 2021).

The pigments produced by these symbionts are quite diverse, including carotenoids, melanin, and other pigments that protect against UV rays and have strong antioxidant properties. For instance, carotenoid pigments are well-known for their ability to neutralize free radicals and prevent cell damage (Brotosudarmo *et al.* 2021).

* Corresponding Author
E-mail Address: delianispringgenies@lecturer.undip.ac.id

Pigment, especially melanin, also has similar protective qualities, safeguarding cells from damage caused by UV radiation and oxidative stress. Eumelanin from the bacteria *Streptomyces lasalocidi* has the potential as a photoprotective agent (Asril *et al.* 2025).

Research has also significantly focused on the antibacterial properties of gastropods and their symbionts. Various studies indicate that extracts from pigmented gastropods and their symbionts can inhibit the growth of several pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Patel *et al.* 2020). This natural antibacterial has the potential to be used as an alternative to traditional antibiotics, which often result in bacterial resistance (Surya *et al.* 2025).

Several studies on a pigment's bioactivity from bacteria especially from marine bacteria, have been conducted, the pigmented bacteria that have been studied are derived from soft coral (Idris *et al.* 2014; Sibero *et al.* 2019; Cahlia *et al.* 2023; Kusmita *et al.* 2023), seagrass (Nugraheni *et al.* 2010), and seaweed (Arlita *et al.* 2013). However, no studies have yet explored the potency of pigmented bacteria from mangrove gastropods. With growing worries about the side effects of synthetic chemicals and rising antibiotic resistance, research into natural resources like gastropods and pigmented symbionts is gaining importance. Using natural ingredients not only offers sustainable solutions to health and environmental issues but also aligns with the trend of consumers becoming more aware of the need for safer and more eco-friendly products (Pringgenies *et al.* 2024).

Therefore, it is important to further explore the potential of gastropods with pigmented symbionts as sources of natural antioxidants, antibacterials, and sun protection. This research will not only expand our understanding of the chemical and biological diversity of gastropods but also create opportunities for innovation in developing natural health and beauty products that are both effective and safe. Additionally, by focusing on natural bioactive compounds, we hope to find new, sustainable solutions to global health and environmental challenges. Based on this, the research aims to identify the characteristics of pigments produced by bacteria associated with marine gastropods and assess their potential as antioxidants and antibacterials.

2. Materials and Methods

2.1. Sample Isolation

Bacteria were sampled and isolated from gastropod samples collected in Mangkang Waters, Semarang City, Indonesia. Astronomically at 110°19'1"E-110°19'4"E and 6°56'59"S-6°57'10"S. Gastropods have been identified according to Dharma (2005). The gastropod samples were washed with sterile seawater, and then 1 gram of each type was taken, crushed, and mixed to create a uniform solution before performing serial dilutions (Kurahman *et al.* 2020). From the diluted samples, 100 µl was spread onto Marine Nutrient Agar plates using the Spread Plate method. The plates were incubated for 48 hours at 34°C.

2.2. Characterization and Purification of Bacteria

The macroscopic morphological characteristics of bacterial colonies were assessed after incubating them for 24-48 hours by examining their size, shape, colony edge, surface elevation, and color (Hikmawati *et al.* 2019). Bacteria that grew successfully and displayed color were purified using the streak plate method. Bacterial colonies were collected using an inoculating loop and streaked onto Marine Nutrient Agar. The Petri dish containing the bacteria for purification was then incubated at 28°C for 48 hours (Pringgenies *et al.* 2021).

2.3. Extraction of Associated Bacterial Compounds and Total Carotenoid Content

Carotenoids from pigmented-associated bacteria were cultured on Marine Nutrient Agar media. The developed bacteria were collected and placed in a centrifuge tube containing methanol solvent, where they were allowed to macerate for 24 hours. Afterward, the sample was vortexed for 15 minutes to break down the bacterial cells. The samples were then centrifuged at 10,000 rpm for 10 minutes. This centrifugation step was repeated until the pellet turned pale in color. The supernatant was then filtered using filter paper and transferred into a vial, which was dried with N_2 gas. The total carotenoids present in the extract were determined using spectrophotometry at a wavelength of 470 nm and calculated using the Gross (1991) equation (as referenced by (Arlita *et al.* 2013; Kusmita *et al.* 2023).

2.4. Bacterial Identification

DNA was extracted from selected bacteria using 10% Chelex. Subsequently, PCR amplification of the 16S rRNA was performed with an initial denaturation at 95°C for 180 seconds. This was followed by denaturation at 95°C for 45 seconds, repeated over 34 cycles. Each cycle included annealing at 54°C for 1 minute, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes. The universal

primers used for this process were Eubacteria gen16S rRNA Gene Primers 27F and 1492R, with the sequences 27F (5'-AGAGTTTGATCMTGGCTCAG-3') as the forward primer and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') as the reverse primer. Electrophoresis was conducted using 1% agarose gel at 100V for 30 minutes, and the results were visualized under UV light. DNA sequencing was performed using PCR cycles with Big Dye Terminator v.3.1, followed by automatic sequence analysis. The data obtained were compared with the Basic Local Alignment Search Tool (BLAST) database for identification purposes, according to studies by Sari *et al.* (2021) and Idris *et al.* (2014).

2.5. Antioxidant Activity Test

The bacterial pigment's antioxidant activity was tested using a modified version of the Akbar *et al.* (2018) method. This involved preparing a 3 mL stock solution of DPPH at 0.2 mM, which was then mixed with 1 ml of the pigment sample solution at a concentration of 1,000 µg/mL, 500 µg/mL, 250 µg/mL, dan 125 µg/mL. Absorbance measurements were conducted following the procedure described by Rabima and Pangaman (2020) using an Elysa reader at the maximum DPPH absorption wavelength of 520 nm. Afterward, the percentage of inhibition and IC_{50} value was calculated.

2.6. Photoprotection Activity Test

The SPF (Sun Protection Factor) value was determined using a Shimadzu UV 1280 UV-Vis spectrophotometer by measuring the concentration of a 1,000 ppm PA methanol extract at UV B wavelengths, specifically from 290 to 320 nm, at intervals of every 5 nm, with PA methanol serving as a blank. The absorbance value (A) was calculated to obtain the SPF value using the Mansur equation according to (Putri *et al.* 2025).

The percentages for Erythema Transmission (%Te) and Pigmentation Transmission (%Tp) were calculated using a Shimadzu UV 1280 UV-Vis spectrophotometer. This involved measuring a 1,000 ppm solution of PA methanol extract. Measurements for %Te were taken at wavelengths between 292 nm and 317 nm, and for %Tp at wavelengths between 322 nm and 372 nm, with readings taken every 5 nm. Methanol PA was used as a blank for these measurements (Abdassah *et al.* 2015).

2.7. Antibacterial Activity Test of Pigment Extract of Associated Bacteria

Antibacterial testing was conducted using the disc diffusion method. Bacterial pigment extracts were applied to paper disks in various concentrations (250 µg/disk, 500 µg/disk, and 1,000 µg/disk), with each application consisting of 35 µL. The test was performed on the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which had been cultivated on Nutrient Agar media. The paper disks were placed on the media containing the test bacteria and then incubated at a temperature of 28°C. Observations were made over 48 hours to determine the presence of a clear zone, which was measured using a calliper (Kusmita *et al.* 2023).

2.8. Spectrophotometric Analysis

Spectrophotometric analysis of bacterial extracts was conducted by examining the 400 to 600 nm wavelength spectrum, which corresponds to the carotenoid wavelength, as referenced by Arlita *et al.* (2013).

2.9. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The bacterial extract solution from the gastropod was analyzed using an Agilent Cary 360 FTIR, which was equipped with an ATR platinum diamond sampling stage module with a ZnSe element. The spectrum was measured over a wavelength range of 4,000 to 650 cm^{-1}, with 24 scans and a resolution of 4 cm^{-1} (Irnawati *et al.* 2021).

2.10. High-Pressure Liquid Chromatography (HPLC) Analysis

High-Performance Liquid Chromatography (HPLC) was performed using a TM-C18 Dikma Diamosil Column measuring 5 µm; 250 mm × 4.6 mm, with a Waters 2695 system. The mobile phase consisted of Methanol, Sterile Water, Dichloromethane, and Acetonitrile in a ratio of 70:4:13:13 (v/v/v/v) and was pre-filtered. The HPLC analysis was conducted over a wavelength range of 100 to 800 nm for 70 minutes. The analysis proceeded at a 1.0 mL/min flow rate at room temperature, and the chromatogram was monitored at 480 nm. UV-visible wavelength peaks were directly obtained using a photodiode-array (PDA) detection system, according to published methods. This method is used to confirm

that there's any carotenoid compound in the extract and predict the compound (Lu *et al.* 2010).

3. Results

3.1. Bacteria Association Isolation

The sample identification results indicate that the gastropods are *Telescopium telescopium* (Linnaeus, 1758) and *Cassidula nucleus* (Gmelin, 1791). The analysis of these two specimens revealed 21 isolates: 10 from the gastropod host *Telescopium telescopium* (Linnaeus, 1758) and 11 from the gastropod host *Cassidula nucleus* (Gmelin, 1791), as shown in Table 1.

3.2. Characterization and Purification of Bacteria

A total of 21 bacterial isolates were obtained from two gastropod hosts. Among them, there were three pigmented bacterial isolates: one with the code MK.TT.2, which was obtained from the gastropod host *T. telescopium*, and two with the codes MK.CN.3 and MK.CN.6, which were obtained from the gastropod host *C. nucleus* (Table 2).

Table 1. Identification of gastropod samples

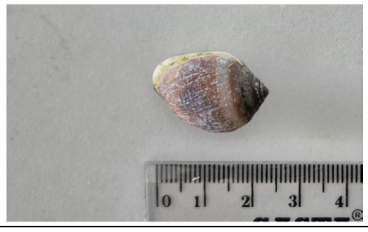
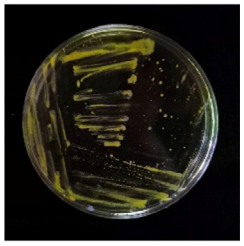
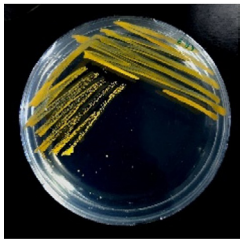
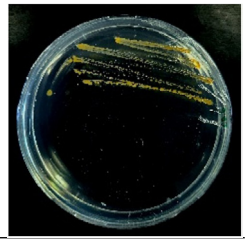
Sample code	Mollusk species	Sample image	Bacterial isolates isolated
MK.TT	*Telescopium telescopium* (Linnaeus, 1758) (Dharma 2005)		10
MK.CN	*Cassidula nucleus* (Gmelin, 1791) (Dharma 2005)		11

Table 2. Selected pigmented bacteria

Sample code	Host species	Colony color	Sample image
MK.TT.2	*Telescopium telescopium*	Yellow	
MK.CN.3	*Cassidula nucleus*	Orange	
MK.CN.6	*Cassidula nucleus*	Orange	

3.3. Extraction of Associated Bacterial Compounds and Total Carotenoid Content

The extraction of associated bacteria produced yellow and orange extracts, corresponding to the color of the bacterial colonies. The calculation of carotenoid content shows that the highest carotenoid concentrations were found in MK.TT.2, MK.CN.3, and MK.CN.6 (Figure 1).

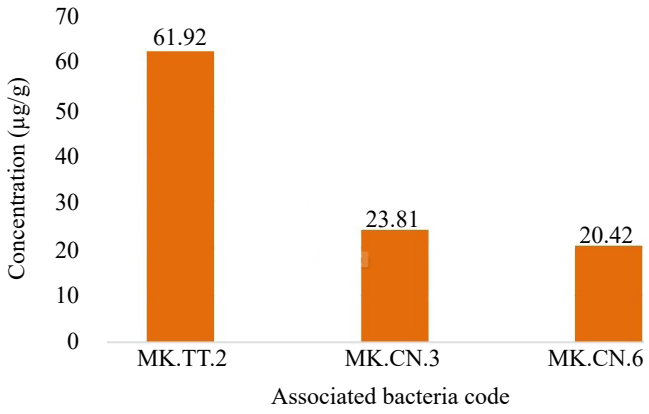


Figure 1. Total carotenoid content of gastropod-associated bacteria

3.4. Bacterial Identification

The bacteria associated with the MK.TT.2 strain were identified as having the highest carotenoid content. Results from the amplification of 16S rRNA indicate that the MK.TT.2 bacterial isolate can produce a DNA fragment that is 1,500 base pairs long compared to the DNA marker (Figure 2).

The analysis results using BLAST on isolate MK.TT.2 show that it is similar to the bacterial species *Micrococcus yunannensis* (Figure 3), with an identification percentage of 99.71% (Table 3).

3.5. Antioxidant Activity Test of Associated Bacterial Extracts

The antioxidant activity test results of the crude extract of *M. yunannensis* bacterial pigments at a concentration of 1,000 µg/mL indicated that the bacterial extract shows potential as an antioxidant, achieving a maximum DPPH scavenging percentage of 75.13% (Table 4).

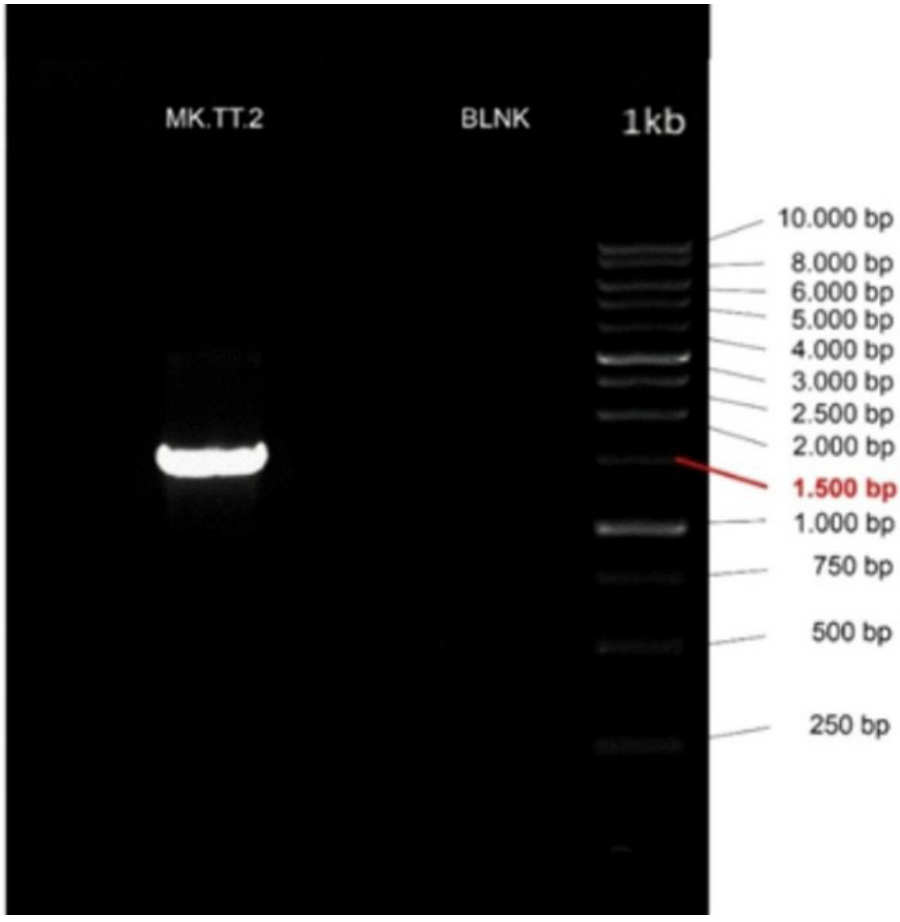


Figure 2. Visualization of DNA electrophoresis isolate MK.TT.2

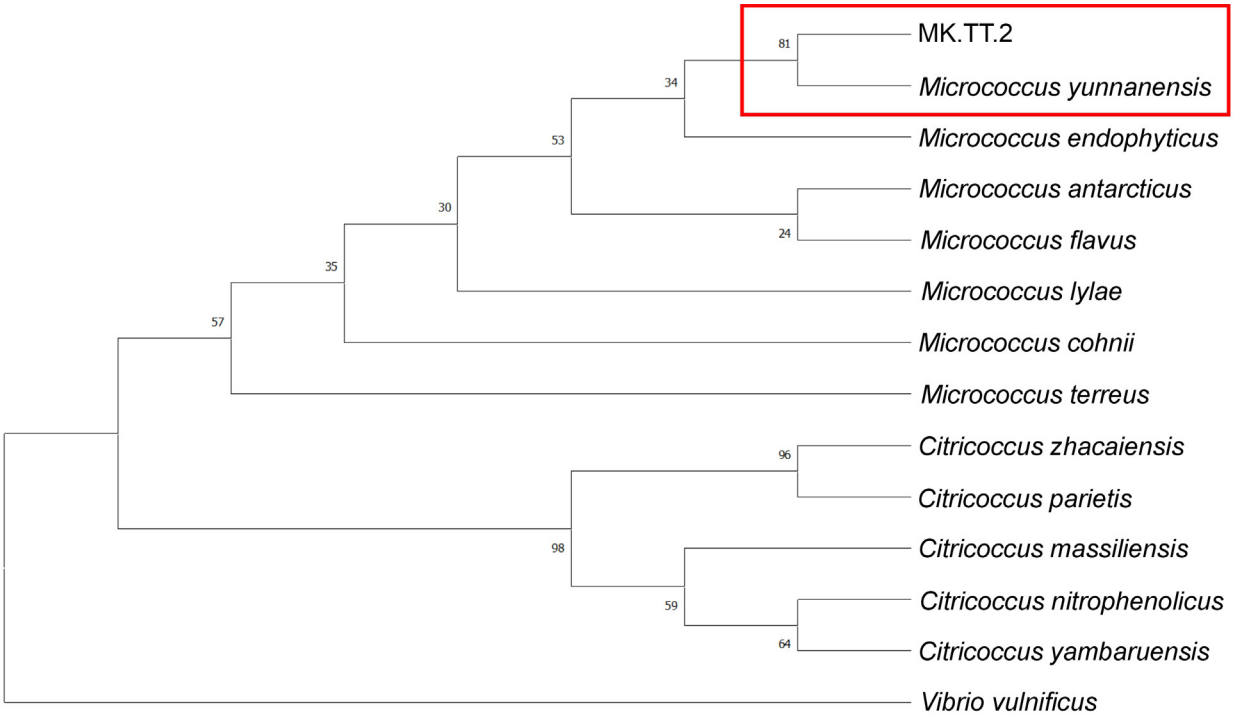


Figure 3. Phylogenetic tree from 16S rRNA sequencing results of isolate MK.TT.2

Table 3. Percent identification and identification results on BLAST

Isolate code	Identification results	%Ident	Query cover (%)	Accession number
MK.TT.2	*Micrococcus yunnanensis*	99.71%	99.71	NR_116578.1
-	*Micrococcus endohyticus*	99.35%	99.35	NR_044365.1
-	*Micrococcus antarcticus*	98.48%	98.48	NR_025285.1
-	*Micrococcus lylae*	98.26%	98.26	NR_026200.1
-	*Micrococcus flavus*	98.33%	98.33	NR_043881.1

Table 4. Results of antioxidant activity of associated bacterial extracts

Isolate code	Konsentrasi (ppm)	% Inhibition	IC_{50} (µg/mL)	Category
MK.TT.2	125	27.23	357	Weak
	250	34.83		
	500	61.77		
	1,000	75.13		

3.6. Photoprotection Activity Test of Associated Bacterial Extracts

The results of the photoprotection activity test of the crude extract of the bacterial pigment *M. yunannensis* showed that SPF was categorized in Low Protection based on the category, which was indicated by its ability as a UV A Sunblock in the %Pigmentation Transmission parameter (Table 5). This photoprotection activity test was carried out at an extract concentration of 1,000 µg/mL, which has the best %Inhibition value.

Table 5. Photoprotection activity test of associated bacterial extracts

Parameter	Result	Category
SPF	7.10	Low Protection
%Te	18.24	Tanning
%Tp	35.63	UV A Sunblock

3.7. Antibacterial Activity Test of Associated Bacterial Extracts

The antibacterial activity test of the crude extract from the bacterial pigment *M. Yunnanensis* indicated that the largest clear zone was observed at a concentration of 1,000 µL. However, an inhibitory zone was also formed at a concentration of 250 µL in both test bacteria, *P. aeruginosa* and *S. aureus*. The results showed that the pigment extract had a larger inhibition zone against *S. aureus*, demonstrating antibacterial properties on both test bacteria, which were bacteriostatic (Table 6).

3.8. Identification of Compound Content using a UV-Vis Spectrophotometer

Using a Shimadzu UV-Vis spectrophotometer, analysis of crude extracts from associated bacteria revealed spectra with three peaks in the wavelength

Table 6. Antibacterial activity test of *M. Yunnanensis* pigment extract

Isolate code	Concentration (µg/disk)	Zone of inhibition (ZOI) (mm)			
		P. aeruginosa		*S. aureus*	
		24 h	48 h	24 h	48 h
Methanol (-)	-	-	-	-	-
Amoxicillin (+)	20	15.0±0.20	14.2±0.14	21.2±0.52	20.3±0.42
MK.TT.2	250	5.2±0.02	3.6±0.02	5.7±0.04	4.3±0.04
	500	6.4±0.04	5.4±0.09	7.2±0.08	5.4±0.03
	1,000	8.0±0.12	6.9±0.07	8.5±0.05	7.7±0.08

range of 300 to 600 nm (Figure 4). These three peaks indicated that the extract from the bacteria associated with *M. yunnanensis* contained carotenoids.

3.9. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The results of the Fourier-Transform Infrared Spectroscopy (FTIR) test on the crude extract of bacteria were conducted to identify the functional groups present in the extract. The resulting graph displays data on wavelength and transmission. The FTIR analysis revealed that there are nine peaks in the extract. These analysis results are shown in Figure 5 and Table 7.

The results of identifying the functional groups in the MK.TT.2 isolate extract, determined from the wave number values, were obtained using the Vibrational Mode Stretching group according to the references in Table 7.

3.10. High-Pressure Liquid Chromatography (HPLC) Analysis

The results from the HPLC chromatogram indicated that the compounds were successfully separated at 480 nm, showing six peaks in the chromatogram (Figure 6). The chromatogram results for the crude extract of the associated bacterial pigment *M. yunannesis* were identified by retention and peak that showed in the PDA module. The carotenoid content was predicted by comparing the spectrum of each chromatogram peak with existing references.

The compound content prediction analysis results were determined by examining the maximum absorbance peak data at each dominant peak identified on the HPLC chromatogram. The predicted results for the compound extract of the MK.TT.2 bacterial isolate are shown in Table 8.

4. Discussion

The gastropods *T. telescopium* and *C. nucleus* from the mangrove ecosystem are seen as having significant potential. The gastropod *T. telescopium*, commonly known as the mangrove snail, is often used as a food ingredient and has antioxidant properties (Purwaningsih *et al.* 2019). On the other hand, the gastropod *C. nucleus* contains high levels of protein and calcium, making it a promising food source, as per research by Oktavia *et al.* (2023). Research by Wijaya *et al.* (2021) found that bacteria associated with mangrove gastropods in the Mangkang waters of Semarang, namely *Micrococcus* sp. exhibit antibacterial activity against *Bacillus cereus* and *Escherichia coli*. Marine bacteria, or those associated with marine organisms, are believed to possess similar abilities to their hosts. The identification and purification process yielded three bacterial isolates from two gastropod samples, which exhibited yellow and orange colors, indicating the presence of carotenoids. Velmurugan *et al.* (2020) note that carotenoids in bacteria can be identified by the yellow, orange, and red colors visible in their colonies. The carotenoid content analysis revealed that the crude pigment extract from isolate MK.TT.2 had the highest carotenoid content.

The identification results of the bacterium *T. telescopium* MK.TT.2 were conducted using the 16s rRNA gene, which serves as a universal molecular marker for species-level classification. DNA amplification indicated a single band with a length of 1500 base pairs. According to Kusharyati *et al.* (2020), a bacterium's taxonomy typically has a sequence and base pair length between 1,500 and 1,550. The BLAST analysis revealed that the bacteria MK.TT.2 is a species of *Micrococcus yunnanensis*, with an identity similarity of 99.71%. Hidayaturohman *et al.* (2024) report that the bacterium *M. yunnanensis* is found in marine ecosystems and is linked to corals. Tizabi and Hill (2023) state that the genus *Micrococcus*, part of the phylum Actinobacteria, contains potential bioactive compounds valuable for drug discovery. Jabeen *et al.* (2022) explain that *M. yunnanensis* can endure harsh environmental conditions and survive high salinity levels in marine waters. The marine bacterium *M. yunnanensis* produces yellow pigments

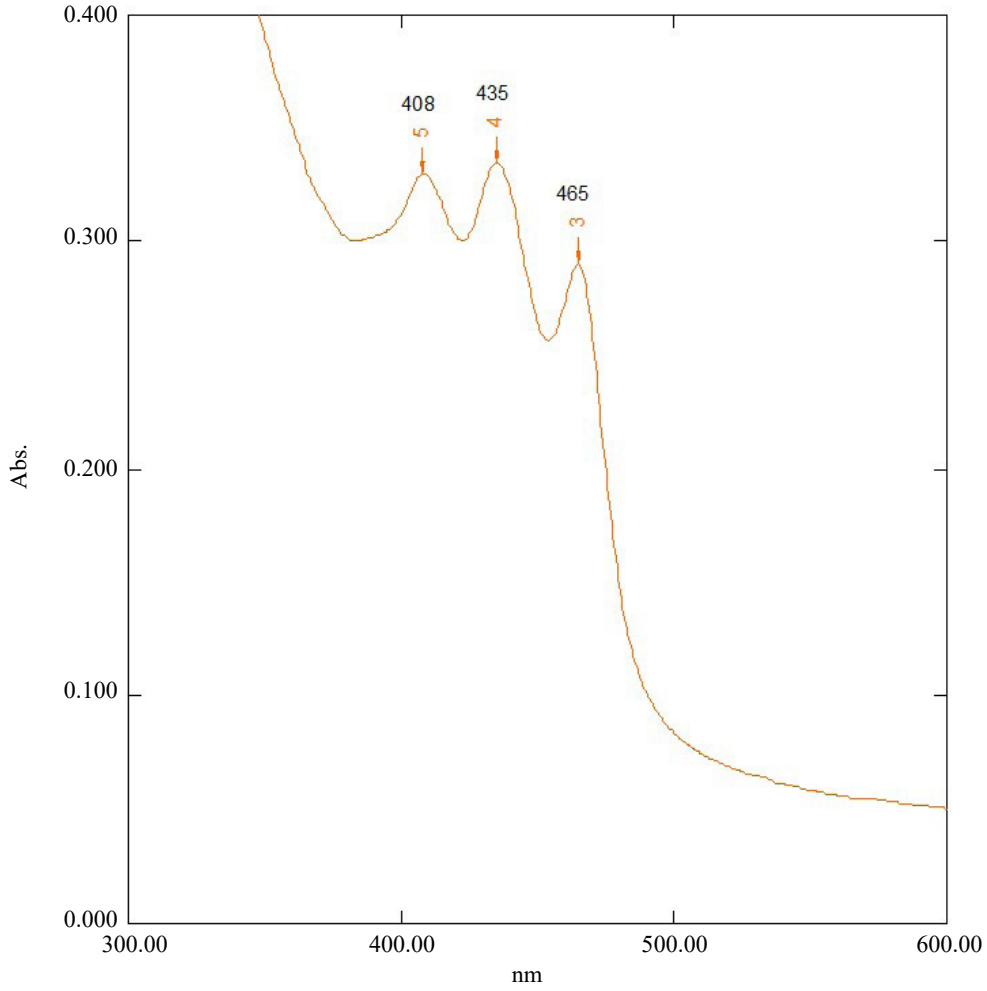


Figure 4. A pattern in the UV-Vis spectrum was obtained from the *M. yunnanensis* bacterial extract with Peak Points at 408, 435, and 465, indicating the presence of the carotenoid pigment group

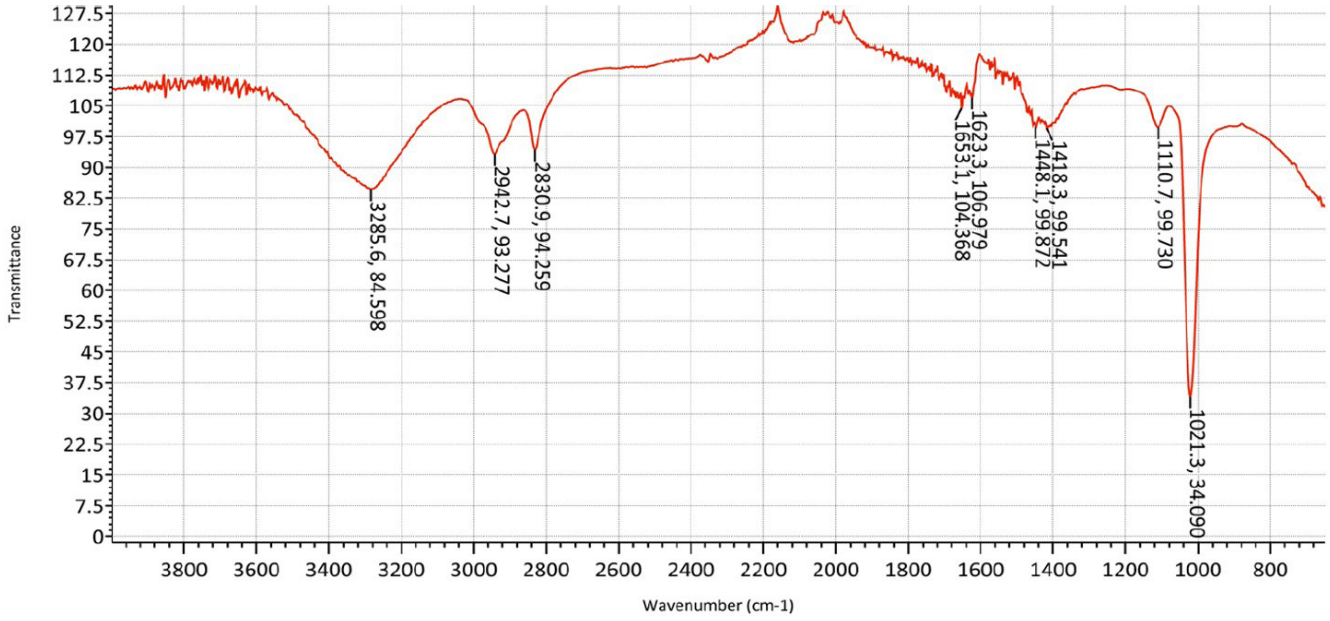


Figure 5. Graph of FTIR test results for associated bacterial extracts

Table 7. FTIR analysis of associated bacterial extracts

Peak	Wave number (cm^{-1})	Function group	Vibrational mode	Reference
1	1021.3	C – O	Stretching	Bose *et al.* 2023
2	1110.7	C – O – C	Stretching	Sitzia *et al.* 2024
3	1418.3	C – H	Bending	Barathiraja *et al.* 2016
4	1448.1	CH2 / CH3	Bending	Kustrin *et al.* 2020
5	1623.3	C = C	Streching	Bose *et al.* 2023
6	1653.1	C = C C = O	Stretching	Doley and Barthakur, 2022; Revathy *et al.* 2015
7	2830.9	C – H	Stretching	Revathy *et al.* 2015
8	2942.7	C – H	Stretching	Revathy *et al.* 2015
9	3285.6	O – H	Stretching	Revathy *et al.* 2015

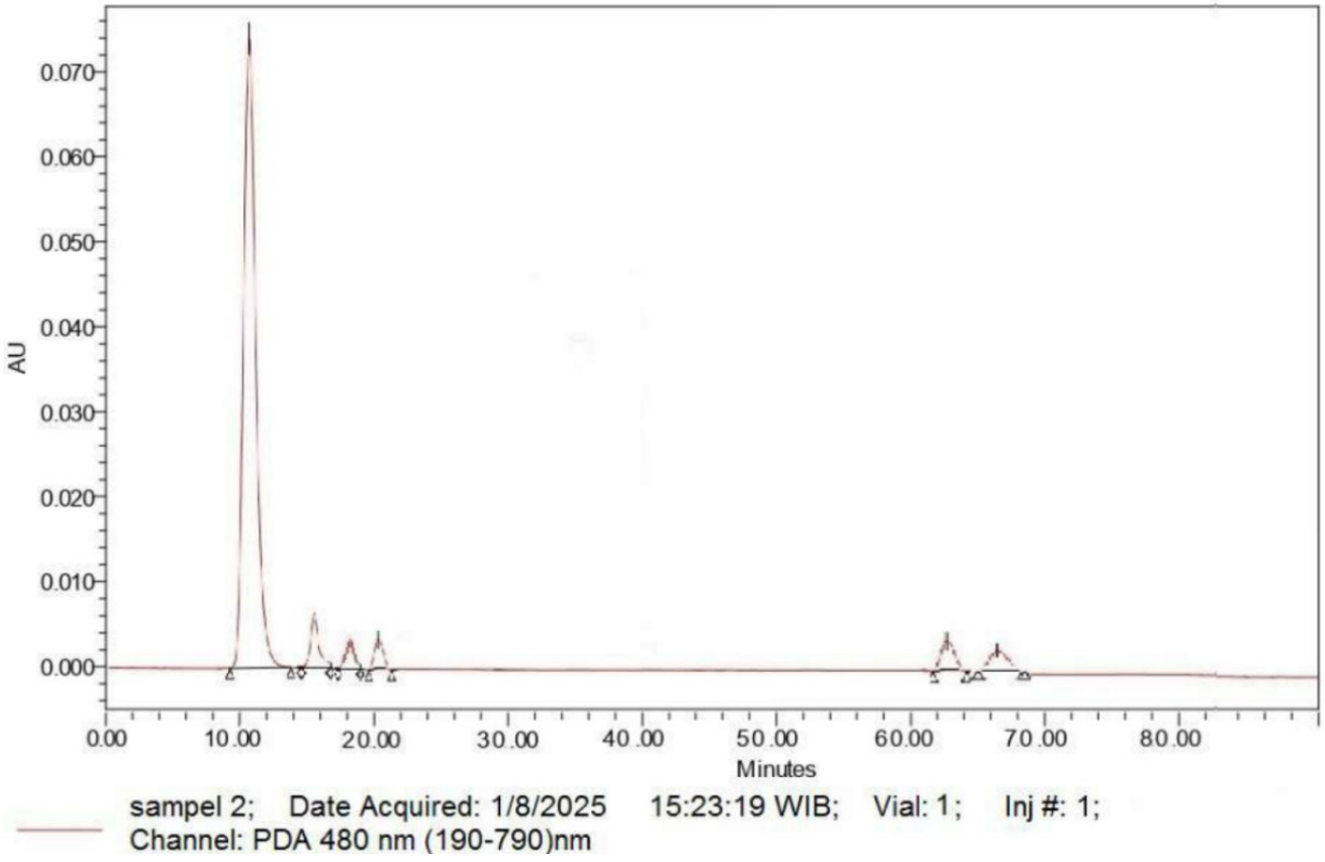


Figure 6. Chromatogram profile of *M. yunnanensis* associated bacterial

Table 8. Photoprotection test results of MK.TT.2 association bacterial extracts

HPLC peak	RT*	Area	Peak	Peak reported (reference)	Compound name
1	10,945	218.943	477	477 (Rahmalia *et al.* 2022)	Trans-Astaxanthin
2	14,964	30.941	462	469 (Lu *et al.* 2010)	Dehydro-Astaxanthin
3	18,013	27.365	458	458 (Marian *et al.* 2008)	3,3℃-dihydroxyisorenieratene
4	21,226	29.873	454	454 (Idris *et al.* 2014)	Prasinoxanthin
5	63,114	42.311	286	NA	Not Identified
6	67,198	47.891	430,451,480	430,452,478 (Weber *et al.* 2007)	β - carotene

*RT:Retention Time

categorized as carotenoid pigments, specifically sarcinaxanthin (Osawa *et al.* 2010; Smitha and Nath 2017). This carotenoid pigment has antioxidant abilities that are rated higher than β-carotene. Apart from having carotenoid pigments, the crude extract of *M. yunnanensis* bacterial pigments is reported to have antibacterial properties against Multidrug-Resistant *Staphylococcus aureus* bacteria (Ranjan and Jadeja 2017), *Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Bacillus subtilis* (Hidayaturohman *et al.* 2024).

The *M. yunnanensis* bacteria were roughly extracted, resulting in a yellow solution. The spectrum pattern of this solution was then analyzed using a UV-Vis spectrophotometer. The results showed peaks at 408, 435, and 465 wavelengths, suggesting the presence of carotenoids in the extracted solution. According to Arlita *et al.* (2013), Extracts that show maximum absorbance at wavelengths of 411, 437, or 464 suggest the presence of a carotenoid group in the extract. Carotenoid, in general, has a spectrum peak between 400–600 nm. Based on the FTIR spectrum test, there is an O-H group at a wavelength of 3285.6, which likely originates from the solvent. At wavelengths between 2,800 cm^{-1} and 3,000 cm^{-1}, the presence of a C-H group was identified, along with various functional groups such as C=C, C=O, and CH2/CH3. These functional groups are the major functional groups of carotenoids (Trivedi *et al.* 2017; Lismeri *et al.* 2023). Different from Abubakar *et al.* (2025), some peaks are missing in 1725 cm-1, which indicates the presence of C=O. These detected clusters are identical to the carotenoid clusters and spectra produced by *Bacillus clausii* bacteria in the research conducted by Korumilli and Mishra (2014). The High-Performance Liquid Chromatography (HPLC) results, based on research conducted by Lu *et al.* in 2010, showed nearly identical chromatographic outcomes, with the highest chromatogram peak likely predicted as the astaxanthin compound. The prediction of the presence of prasinoxanthin compounds in crude extracts of bacterial pigments aligns with the Fourier-transform infrared spectroscopy (FTIR) results, which indicate the presence of xanthophyll-type carotenoid compounds through C=O stretching. Prasinoxanthin is a carotenoid compound belonging to the xanthophyll group, as noted by Revathy *et al.* in 2015. Astaxanthin and β-carotene, which are suggested to be present in the extract, are types of carotenoids known for their antioxidant capabilities, according to research by Dang *et al.* (2024) and Kusmita *et al.* (2023). This HPLC result confirms that there is a carotenoid compound in the extract.

The antioxidant test showed that the crude pigment extract at a concentration of 1,000 ppm achieved a 75.13% DPPH inhibition rate. The test results indicated that the pigment could change the purple color of DPPH to yellow. According to Bufka *et al.* (2024), carotenoids contain double bonds in their molecular structure, allowing them to neutralize free radicals by donating an electron to Reactive Oxygen Species. Research by Kusmita *et al.* (2021) found that crude methanol extracts of bacterial pigments from soft coral at 1,000 ppm had an inhibition rate of 77.23% and an SPF value of 6.63. An SPF test and an Erythema Transmission test conducted on the crude extract from the associated bacteria *M. yunnanensis* showed that at 1,000 ppm, the highest SPF was 7.10, which provides low skin protection according to Asril *et al.* (2025). Susanti and Lestari (2019) state that an SPF value of 7.10 means that when applied for sun protection, it increases the skin's natural resistance to the sun by 7.10 times. The study found that the *M. yunnanensis* bacterial extract has a %Transmission for UV-B (%Te) of 18% and a %Transmission for UV-A (%Tp) of 35.64%. These findings suggest that the extract could offer UV-B and UV-A protection. A %Te value of 18% falls into the Tanning category. According to Whenny *et al.* (2015), fast tanning, or the Tanning category, occurs when a compound can block UV-B without causing redness, although it may still lead to rapid skin pigmentation. The %Tp Transmission results are classified in the Sunblock category, indicating that the samples can effectively block UV-A rays. According to Abdassah *et al.* (2015), sunblock can demonstrate that a compound sensitive to UV A. The known %Tp value indicates that the crude extract from *M. yunnanensis* can protect skin from UV A. This extract categorizes low protection against UV B because it has Tanning category in %Te. Natural conditions and competition for food sources force marine bacteria and microorganisms to compete against harmful and harmless microorganisms. According to Saubenova *et al.* (2024), carotenoid pigments not only protect microorganisms from UV radiation but also help them defend against other organisms. Tests on antibacterial activity have shown that the crude extract of the bacterial pigment *M. yunnanensis* in concentration of 1,000 µg/disk can inhibit the growth of bacteria such as *P. aeruginosa* with ZOI value is 8.0±0.12 mm and *S. aureus* with ZOI value is 8.5±0.05 mm. Compared with Kusmita *et al.*

(2023), carotenoid extract from bacteria *Virgibacillus* sp. derived from soft coral with concentrations in 1,600 µg/disk with ZOI value of 10.90±0.011 mm against *Escherichia coli* and 15.09±0.011 mm against MRSA (Multidrug Resistance *Staphylococcus aureus*). This result shows that the pigment extract from gastropod-associated bacteria has the potential as an antibacterial agent. According to Karpinski *et al.* (2021), carotenoids increase the permeability of the outer membrane of bacterial cells, altering the structure of efflux proteins in gram-negative bacteria. Additionally, carotenoid compounds can accumulate Reactive Oxygen Species (ROS), leading to oxidative damage to bacterial cell membranes. Another mechanism of carotenoids is to prevent the formation of biofilms in bacteria. Kusmita *et al.* (2023) reported that carotenoids can penetrate porins (transmembrane proteins) in bacterial cell walls, forming polymer bonds that damage these porins and reduce cell permeability.

In conclusion, the study concluded that the pigmented bacteria found with gastropods, identified as *Micrococcus yunnanensis*, have a crude extract that can inhibit antioxidants by 75.13% at a concentration of 1,000 ppm, resulting in an SPF of 7, %Te of 18%, and %Tp of 35%. Additionally, the crude extract from *M. yunnanensis* shows potential as an antibacterial agent against the pathogenic bacteria *P. aeruginosa* and *S. aureus*. Tests using HPLC and FTIR suggest that the bacterial pigment's crude extract may contain carotenoid compounds.

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