Saudi Pharmaceutical Journal 31 (2023) 101680

HOSTED BY

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Short communication

Sun protection and antibacterial activities of carotenoids from the soft coral *Sinularia* sp. symbiotic bacteria from Panjang Island, North Java Sea

Lia Kusmita [a], Annisa Nur Prasetyo Edi [a], Yuvianti Dwi Franyoto [a], Mutmainah [a], Sri Haryanti [a], Agustina Dwi Retno Nurcahyanti [b]

[a] *STIFAR Yayasan Pharmasi Semarang Letjend Sarwo Edhie Wibowo Km 1, Semarang, Indonesia*
[b] *Department of Pharmacy, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia*

ARTICLE INFO

Article history:
Received 26 February 2023
Accepted 14 June 2023
Available online 20 June 2023

Keywords:
Carotenoids
Bacterial symbiont
Sinularia sp.
Antioxidant
Antibacterial
Sun protection
Virgibacillus

ABSTRACT

Carotenoids have shown beneficial applications in cosmetology, pharmacology, and medicine. However, environmental stress in the marine environment can trigger the production of unique secondary metabolites, such as carotenoids. These compounds can also be sustainably produced by symbiotic bacteria. We hypothesized that the soft corals in tropical regions may produce diverse biological secondary metabolites, including carotenoids, both by the host organism and their bacterial symbiont. The unique carotenoids may provide promising biological activity such as antioxidant, UV photoprotector, and antibacterial activities. To this end, we isolated and characterized the carotenoids isolated from the bacterial symbiont of *Sinularia* sp., a soft coral from Panjang Island, North Java Sea, strain 19. PP.Sc.13. Bacterial identification was performed using DNA barcoding of the 16S rRNA region. Identification of carotenoids was carried out using a spectrophotometer, High-Performance Liquid Chromatography (HPLC), and attenuated total reflection fourier-transformed infrared (ATR-FTIR) spectroscopy. The antioxidant activity was estimated using the diphenylpicrylhydrazyl (DPPH) method, while the Sun Protection Factor (SPF) and % transmission of erythema and pigmentation were determined based on colorimetric methods. The antibacterial activity assay was carried out using the agar diffusion method against two multidrug-resistant bacteria. The bacterial symbiont was identified as *Virgibacillus* sp. and the carotenoids isolated from this symbiont exhibited significant antioxidant activity and extra sun protection effect, thus categorized as UVA sunblock. Furthermore, the isolated carotenoids exhibited antibacterial activities against Methicillin Resistant-*Staphylococcus aureus* (MRSA) and Multidrug-resistant (MDR) *Escherichia coli*. This study provides evidence of the carotenoids produced by the soft coral bacterial symbiont *Virgibacillus* sp., which may be used as an antioxidant, sun protection, and antibacterial agent. Further investigation of the *de novo* biological production of carotenoids by *Virgibacillus* sp. is warranted.

1. Introduction

Carotenoids are common naturally occurring secondary metabolites with the ability to protect organs, tissue, and cells from free radicals and lipid peroxide (Young and Lowe, 2018), including the preclinical and clinically demonstrated decrease in blood triglyceride levels (Zhuang et al., 2022). In cosmetology, carotenoids have been applied to protect and nurture skin health (Meléndez-Martínez et al., 2019). Carotenoids provide photoprotection, reduce scavenging singlet oxygen and peroxyl radicals, and thus decrease sensitivity against UV-induced erythema (Sies and Stahl, 2004; Zaytseva et al., 2021a).

Carotenoids derived from marine organisms, including seaweed, microalgae, marine animals, and hard and soft corals, possess a unique chemical structure and physiochemical characteristics (Galasso et al., 2017; Setiyono et al., 2019a). Soft corals are enriched with secondary metabolites, including a unique class of carotenoids. However, the harvesting of corals can damage the environment; thus, an alternative strategy is to explore symbiotic bacteria associated with soft corals since these symbionts also produce secondary metabolites like their host (Burgess et al., 2003;

E-mail address: adr.nurcahyanti@atmajaya.ac.id (A. Dwi Retno Nurcahyanti)
Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2023.06.013
1319-0164/

Mendes-Silva et al., 2020; Murti et al., 2016a; Setiyono et al., 2019b). While this approach may prevent negative environmental effects, the development of natural products for the pharmaceutical and cosmetology industries may be challenging. Culturing methods and natural product synthesis induction must ensure the expression of biosynthetic genes to produce natural products resembling those in their natural habitat (Atanasov et al., 2021).

Many carotenoids produced from soft coral symbiotic bacteria have shown pronounced potential as a commercial commodity due to their biological activities (Galasso et al., 2017; Vílchez et al., 2011), including antioxidant (Kusmita et al., 2021b, 2017; Mutiara et al., 2017), anti-aging (Kusmita et al., 2021a), and antibacterial activities (Kusmita et al., 2021b; Murti et al., 2016b). (Fayez et al., 2022) recently reported the carotenoids produced by *Virgibacillus halodenitrificans* bacteria with biological activity. Another study reported the anticancer and antiviral activities of the carotenoids of *Natrialba* sp. M6 (Hegazy et al., 2020), which showed that carotenoids derived from bacteria can be utilized in pharmaceutical and cosmetology applications.

The biological potency of the soft-coral *Sarcophyton* sp. bacterial symbiont from the North Java Sea was identified, including the carotenoids derived from this bacterium (Kusmita et al., 2017). This study furthers our knowledge of the distribution of *Sinularia* sp. and its carotenoids-derived bacterial symbiont from the same area (Ray Steven et al., 2022; Van Ofwegen, 2000a) and discusses the potency of the carotenoids extracted as antioxidant, sun protection, and antibacterial agents.

2. Materials and methods

2.1. Sampling

Samples of soft coral *Sinularia* sp. were collected from Panjang Island by scuba divers at a depth of approximately 2 m (Fig. 1) between January - February 2019. Samples were placed in a plastic container with sterile seawater and stored in a coolbox (Kusmita et al., 2017; Nugraheni et al., 2010a). Images of the *Sinularia* sp. samples were taken *in situ* at the surface using a Canon Power Shot S50 underwater camera (see Fig. 2).

Fig. 2. Sinularia sp.

2.2. Isolation of bacterial symbionts from soft corals

Bacterial symbionts were isolated using the distribution method (Radjasa et al., 2009). Briefly, soft corals were rinsed using sterile seawater and cut into pieces, then serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) from which 1 mL of the sample was plated onto Zobell 2216E agar. The Petri dishes were incubated at 30 °C for 1 – 2 days and the colored bacterial colonies on the agar surface were separated with the streak method to obtain pure bacterial strain (Burgess et al., 2003; Murti et al., 2016b; Radjasa et al., 2009).

2.3. Amplification of DNA barcode loci, 16S rRNA, and phylogenetic tree construction

The genomic DNA of the selected bacterial strain was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The forward (5′- CTC CTA CGG GAG GCA GCA G-3′) and reverse (5′-GTG CCA GCM GCC GCG GTA A-3′) primers were used to amplify the 16S rRNA region of the bacterial DNA. The PCR mixture contained 1 µl of genomic DNA, 1 µl of 20 pmol from each primer,

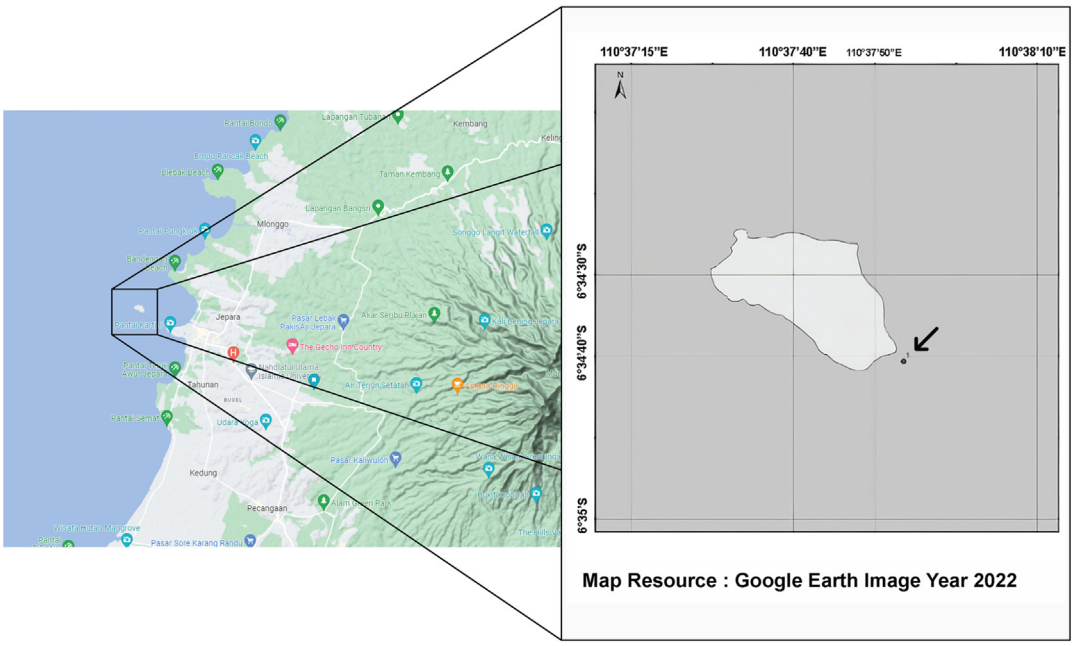

Fig. 1. Sampling location.

12.5 µl of MyTaq Red Mix 2x (Bioline), and 9.5 µl of ddH_2O. The PCR cycles were initial denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 15 s, 52 °C for 15 s, and 68 °C for 45 s. The quality of the PCR product was checked using 1% agarose gel before sequencing (Radjasa et al., 2013) and aligned with 35 sequences according to the MUSCLE algorithm. A maximum likelihood phylogenetic tree based on the 16S rRNA gene was constructed for the phylogenetic analyses using the GTR + G + I model with 1000 bootstrap replications using MEGA 7.0 (Kumar et al., 2016).

2.4. Pigment extraction and total carotenoids content

The carotenoids from the symbiont bacteria were extracted in methanol. The pigment-containing fraction was filtered, evaporated, and dried under a N_2 stream (Kusmita et al., 2017; Nugraheni et al., 2010b) before the total carotenoids content was determined using a UV–Vis spectrophotometer at a wavelength of 470 nm. Total carotenoids were calculated using the Gross equation (1990) as follows:

$$\mu g\,\text{carotenoid}/g = \frac{A \times V(mL) \times 10^6}{A_{1cm}^{1\%} \times 100 \times G(gr)}$$

where A = Absorbance, V = Total extract volume, $A_{1cm}^{1\%}$= 2500 (β-carotene Extinction Coefficient in Hexane), and G = Sample Weight.

2.5. Identification of carotenoids

2.5.1. Spectrophotometry analysis

Initial identification of carotenoids was carried out using a variant CARY 50 multiple beam spectrophotometer at a wavelength of 300 – 600 nm (Rodriguez-Amaya and Kimura, 2004a).

2.5.2. High-Performance Liquid Chromatography (HPLC) analysis

Carotenoids were then identified using analytical – HPLC (Shimadzu analytical-UFLC, Kyoto, Japan) using Symmetry C8 column (150 × 4.6 mm, 3.5 µm particle size, 100Å pore size) (Water, Milford, MA, USA) with two eluents as mobile phase. Eluent A was composed of 50% methanol, 25% acetonitrile, and 25% pyridine solution (0.25 M, pH 5) (v/v), while eluent B was composed of 20% methanol, 60% acetonitrile, and 20% acetone with a flow rate of 1 mL per min^{-1}. The separation was performed using the gradient system: 100% of eluent A from 0 to 22 min, followed by 40% eluent B at min 22 – 28, 5% eluent A (min 28 – 38), and 100% eluent A at min 40 – 50 at a flow rate of 1 mL/min with the column oven temperature set at 30 °C. The carotenoids were detected with a diode array detector at 450 nm (Shimadzu SPD M20A, 190–800 nm) according to published methods (Setiyono et al., 2019a).

2.5.3. Attenuated total reflection - fourier transform infrared spectroscopy (ATR-FTIR) analysis

Carotenoids were also identified using Agilent Cary 630 FTIR spectroscopy model no. G8043AA, equipped with the ATR platinum diamond sampling stage module ZnSe. The spectra were recorded in the 600–4,000 cm^{-1} range and spectral analysis was done using the Agilent MicroLab Expert software.

2.6. Determination of antioxidant activity with the DPPH (1,1-diphenyl-2-picrylhydrazyl) method

The extract was dissolved in methanol and serially diluted. Methanol was used as the blank solution, while the sample solution consisted of 4 mL DPPH plus 1 mL extract. The blank and samples were incubated for 30 min in the dark and the absorbance was measured at 517 nm using a single UV–visible spectrophotometer Shimadzu 1240 to calculate the antioxidant activity (Mukherjee et al., 2017) as follows:

$$\%Inhibitory = \frac{[DPPH]_0 - [DPPH]_s}{[DPPH]_0} \text{X}100\%$$

where$[DPPH]_0$ = Initial DPPH concentration and$[DPPH]_s$ = Final DPPH concentration remaining.

2.7. Determination of the sun protection factor (SPF)

Sun protection was assessed *in vitro* with a UV–Vis spectrophotometer at 290–320 nm. The average absorption was set at 5 nm intervals and the carotenoids extract was used at the IC_{50} for antioxidant activity. The SPF value was calculated using the Mansyur equation (Gonzalez et al., 2007; Liandhajani et al., 2013; Pelizzo et al., 2012) and categorized according to Table 1:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \text{abs}(\lambda)$$

where CF = correction factor, EE = Spectrum of erythema effect, I = spectrum of the sun's intensity, and Abs = Absorbance of the sample. The value of EE (λ) × I (λ) is constant, as shown in Table 2.

2.8. Determination of % transmission of erythema and pigmentation

The % transmission of erythema was determined by the absorption of the carotenoids extract at 292–372 nm every 5 nm (Abdassah et al., 2015a). The transmission (T) was calculated with the absorption value (A) using the formula:

$$A = -\log T$$

While the transmission of erythema (Te) was calculated using the formula:

$$Te = T \times Fe$$

where Fe is the erythema flux value at 290–320 nm (Table 3).

The value of flux erythema was used to calculate the sunscreen effect (Ee) using the formula:

$$Ee = (T \times Fe)$$

While the erythema transmission % is calculated using the formula:

$$\%\,\text{transmission of erythema (Te)} = \frac{Ee}{\sum Fe} = \frac{\sum(T \times Fe)}{\sum Fe}$$

To calculate the % transmission of pigmentation (Tp), the flux of pigmentation (Fp) was determined based on the absorbance measured in the 320–375 nm range (Table 3) (Barel et al., 2001). The transmission of pigmentation (Tp) was calculated using the following formula:

$$Tp = T \times Fp$$

While the pigmentation transmission (% Tp) was calculated using the formula:

Table 1
Prediction of SPF.

SPF	Protection category
2–4	Minimum
4–6	Moderate
6–8	Extra
8–15	Maximum
≥15	Ultra

Table 2
The normalized product function used in the calculation of sun protection factor (SPF).

Wavelength (λ nm)	EE × I (Normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	**1**

Table 3
The flux erythema (Fe) and pigmentation on sunscreen.

Wavelength (λ nm)	Flux of erythema (Fe)	Flux of pigmentation (Fp)
290 – 295	0.1105	–
295 – 300	0.6720	–
300 – 305	1.0000	–
305 – 310	0.2008	–
310 – 315	0.1364	–
315 – 320	0.1125	–
320 – 325	–	0.1079
325 – 330	–	0.1020
330 – 335	–	0.0936
335 – 340	–	0.0798
340 – 345	–	0.0669
345 – 350	–	0.0570
350 – 355	–	0.0488
355 – 360	–	0.0456
360 – 365	–	0.0356
365 – 370	–	0.0310
370 – 375	–	0.0260

$$\%\,\text{transmision of pigmentation (Tp)} = \frac{\text{Ep}}{\sum \text{Fp}} = \frac{\sum(\text{T} \times \text{Fp})}{\sum \text{Fp}}$$

The flux erythema and pigmentation at various wavelengths are presented in Table 3 and the sunscreen category based on %Te and Tp is determined according to Table 4.

2.9. Determination of antibacterial activity

2.9.1. Bacterial strains and culture conditions

Escherichia coli and *Staphylococcus aureus* were obtained as clinical isolates from the Department of Microbiology, Faculty of Medicine, Diponegoro University, and subjected to the antimicrobial susceptibility test (AST) according to the recommended method from Clinical and Laboratory Standard Institute (CLSI). *E. coli* was confirmed resistant to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and intermediate resistant to cefotaxime. Whereas *S. aureus* was resistant to cefoxitin, thus categorized as methicillin resistant-*Staphylococcus aureus* (MRSA). Each strain was then cultured to a density of 0.5 McFarland, which is equal to 1×10^8 colony-forming units per milliliter (CFU/mL) and diluted (1: 100) to provide a final concentration of 1×10^6 CFU/mL for further analysis.

Table 4
The category of sunscreen activity.

Category	Transmitted UV light range (%)	
	% erythema	% pigmentation
Sunblock	<1	3–40
Extra protection	1–6	42–86
Suntan	6–12	45–86
Tanning	10–18	45–86

2.9.2. Antibacterial test

The antibacterial activity was assessed based on the previous agar diffusion method (Murti and Radjasa, 2012; Radjasa et al., 2007). A dilution series of the carotenoids extract (4%, 6%, and 8% m/v) was prepared and plated onto Manitol Salt Agar (MSA; Himedia, India) or Eosyn Methylene Blue (EMB; Himedia, India) containing 1×10^6 CFU/mL bacteria suspension on the surface of the plate. The MSA ingredients consisted of meat extract, peptone, NaCl, phenol red, mannitol, and agar. While the ingredients of EMB consisted of gelatin pancreas, eosin, methylene blue, lactose, and dipotassium phosphate. MSA and EMB media were prepared according to the manufacturer's procedures. Ciprofloxacin 0.05% (b/v) served as the positive control. The microbial plate was then incubated at 37 °C for 24 h and the growth inhibition zones were measured.

2.10. Statistical analysis

The statistical analyses for the carotenoid bacterial content and carotenoids characterization were performed according to a previous study (Setiyono et al., 2019a). Three replicates for each assay were performed. The IC_{50} value was determined using a dose–response curve. The variance and significance analyses were calculated using the Tukey test with a significant difference of $p < 0.05$.

3. Results

3.1. Bacterial symbiont isolation and characterization

Sinularia sp. was collected from Panjang Island, Jepara Regency, Central Java, Indonesia (Fig. 1-2). Six bacterial isolates were obtained (Table 5) and four isolates contained carotenoids as indicated by the yellow/orange color (Fig. 3). These bacteria were then used for further analysis.

Among the four carotenoids-producing bacteria (Fig. 3), we selected 19. PP.Sc.13 based on the high intensity of their yellow/orange pigment, indicating a high content of carotenoids. The characterization of bacteria was then performed at the molecular level using the amplification of DNA barcode loci, 16S rRNA. Sequence analysis of this loci revealed 100% homologous to *Virgibacillus* sp. strain CARE V34. The sequence was deposited into GenBank under accession number MN880491.1. The phylogenetic tree was constructed using MEGA version 7.0 (Molecular Evolutionary Genetics Analysis) software to confirm the BLAST results and characterize the relationship between related species based on the intermolecular characteristics (Felix et al., 2011). Fig. 4 shows sample 1–3, corresponding to bacteria 19. PP.Sc.13, with a close relationship with other bacteria in the genus *Virgibacillus*.

3.2. Carotenoids bacterial content and characterization

The carotenoid content of the four isolated bacteria (19. PP. Sc.13 – 19. PP.Sc.16) were determined individually, where 19. PP. Sc.13 exhibited the highest carotenoid content (Fig. 5). The spectrum of the carotenoids extract from the 19.PP.Sc.13 bacteria is presented in Fig. 6, showing two maximum absorptions at 487.90 nm and 458.50 nm, indicative of a carotenoid mixture or compound (Rodriguez-Amaya and Kimura, 2004). Also, the HPLC analysis of carotenoids from the bacteria symbiont 19. PP.Sc.13 revealed three peaks at the retention's times (Rt) of 2.59, 2.75, and 4.62 with maximum absorbance at λ range of 450 – 490 nm, strongly suggesting the absorbance of carotenoids (Fayez et al., 2022; Finkel'shtein, 2016; Scott, 2001) (Table 6, Fig. 7). Following the spectrophotometer and HPLC analyses, the FTIR spectra was characteristic of carotenoids with benzene absorption at 700 – 750 cm^{-1}, while that at 2800 – 3000 cm^{-1} corresponds to C–H

Table 5
Bacterial isolates from *Sinularia* sp. 19.PP.Sc.1.

Number	Color	Colony size (cm)	Form	Edge	Elevation
1	Light brown	1.81	Round	Wavy	Big
2	Cloudy white	2.56	Round	Stringy	Big
3	Yellow	0.15	Round	Fine	Convex
4	Yellow	0.25	Round	Fine	Convex
5	Yellow	0.05	Round	Fine	Convex
6	Yellow	0.10	Round	Fine	Big

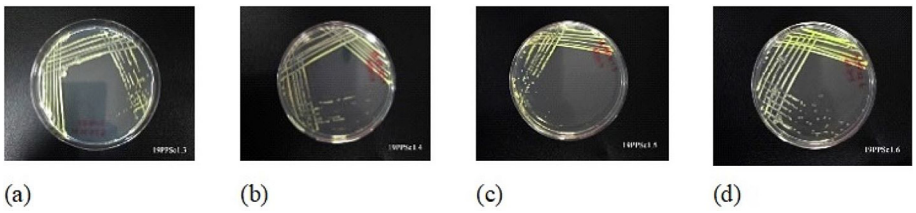

Fig. 3. Carotenoids producing bacteria (a) 19. PP.Sc.13, (b) 19.PP.Sc.14, (c) 19.PP.Sc.15, and (d) 19 PP.Sc.16.

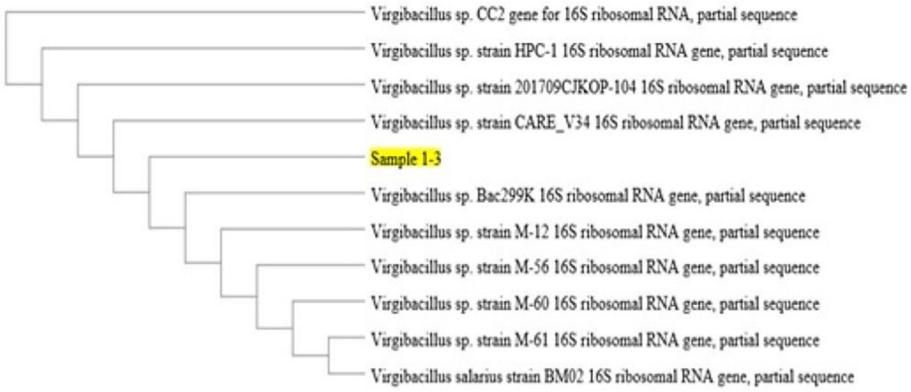

Fig. 4. Bacterial phylogenetic tree of 19. PP.Sc.13.

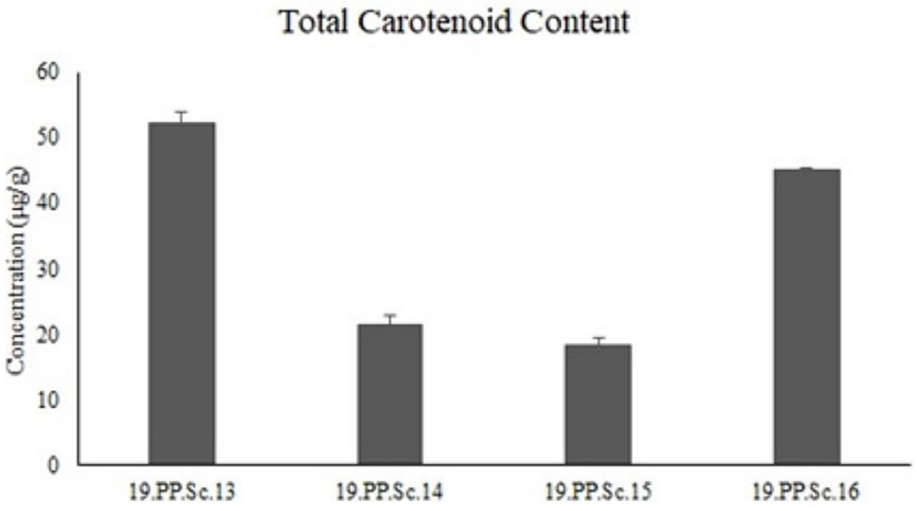

Fig. 5. Total carotenoids content of 19.PP.Sc.13–19.PP.Sc.16.

stretching vibration (Lóránd et al., 2002). The stretching vibration of some functional groups such as CH_3, CH_2, C = C, C = O, and OH was detected. Strong intensity spectrums at 1267 cm^{-1} were detected as stretching vibration of C – H and at 1457 cm^{-1}, while the stretching vibration of C = O was found at 1733 cm^{-1} (Fig. 8, Table 7). The spectra profile was different from that of the carotenoids isolated from *Virgibacillus halodenitrificans* from Wadi El-Natrun Salt Lakes (Fayez et al., 2022).

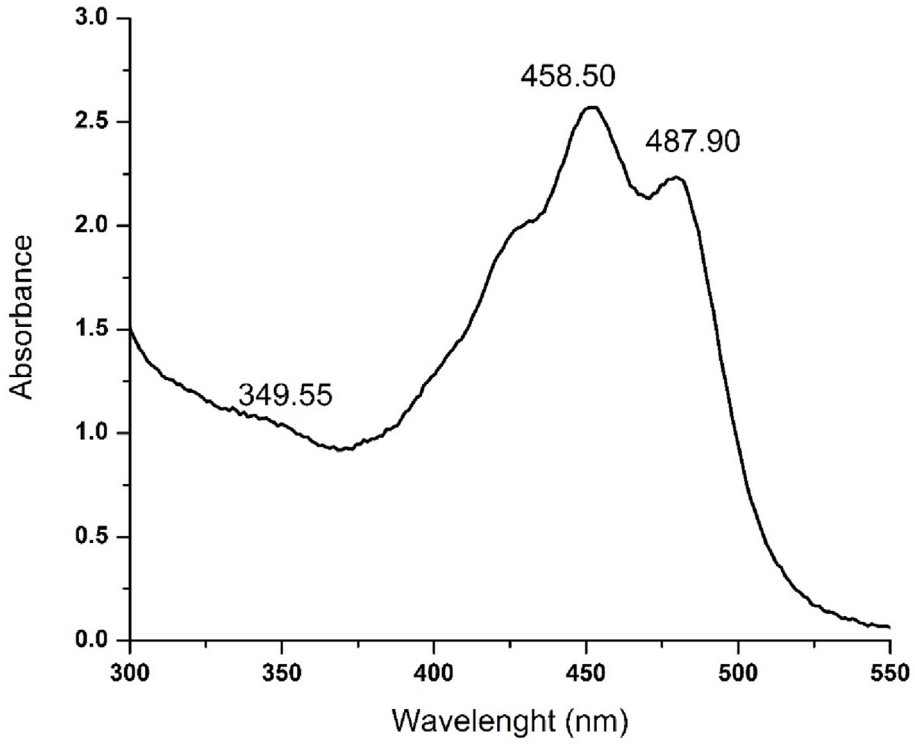

Fig. 6. The spectrum of carotenoids from the bacteria symbiont 19.PP.Sc.13.

Table 6
Carotenoids identification of bacterial symbionts of 19.PP.Sc.13.

Sample	tR	Component	λmaks			Reference
19.PP.Sc.13	2.59	Unidentified	(4 2 6)	458	487	Setiyono (2019)
	2.75	Unidentified	(4 2 4)	448	473	
	4.62	Unidentified		465		

3.3. Antioxidant activity

Carotenoids are well-known natural products with antioxidant activity. Total carotenoids extracted from 19.PP.Sc.13 had an IC_{50} of 506 ppm to inhibit free radicals DPPH, which was not significantly different from the β-carotene standard (IC_{50} 510 ppm) (Fig. 9).

3.4. Sun protection activity of the carotenoids extract

The assessment of the sun protection activity of the carotenoids extract based on the IC_{50} concentration (506 ppm) and three indicator parameters included the Sun Protection Factor (SPF), % Transmission of erythema (Te), and % Transmission of pigmentation (Tp). Based on standard parameter criteria, carotenoids extracted from the bacterial symbiont were categorized as SPF with extra protection, indicating the UVA sunblock category (Table 8) (Abdassah et al., 2015b).

3.5. Antibacterial activity of the carotenoids extract

The antibacterial activity of the carotenoids extracted was tested against MRSA and Multidrug-resistant (MDR) *E. coli*. Based on the inhibition zone diameter, the carotenoids extract showed a greater inhibitory effect against MRSA than MDR *E. coli* (Table 9, Fig. 10). A greater concentration of the carotenoids extract exerted greater inhibition zone against the pathogenic bacteria in a dose-dependent manner.

4. Discussion

Sinularia sp. is distributed across the central Indo-Pacific, Western Australia, and the Red Sea (Van Ofwegen, 2000b; van Ofwegen et al., 2013). Among well-studied secondary metabolites of the genus *Sinularia* is the class of terpenoids and its derivatives (Hsu et al., 2018; Kamel and Slattery, 2008a; Rodrigues et al., 2019), norterpenoids, and steroids/steroidal glycosides among others (Yan et al., 2021a). Some terpenoids and their derivatives exhibit biological activities and potency for drug development (Kamel and Slattery, 2008b; R Steven et al., 2022; Yan et al., 2021b), including antimicrobial activities (Aceret et al., 1998).

Besides the biological activity of the sponge itself, bacterial symbionts associated with the sponge are of interest as they share similar secondary metabolites, and consequently the biological activity ruled by those active compounds. In the current study, we identified the bacterial symbiont associated with *Sinularia* sp. as *Virgibacillus* sp., a Gram-positive bacterium that belongs to the Family Bacillaceae, known as anaerobic with chains and small cream/yellowish-white colonies on Tryptic Soy Agar media (Heyndrickx et al., 1998). This genus has been reported in hard corals (Ayuningrum et al., 2020), marine sediments (Xu et al., 2018), surface seawater (Peng et al., 2009), as well as in the deep-sea (Chang et al., 2021). *Virgibacillus* sp. was also associated with the

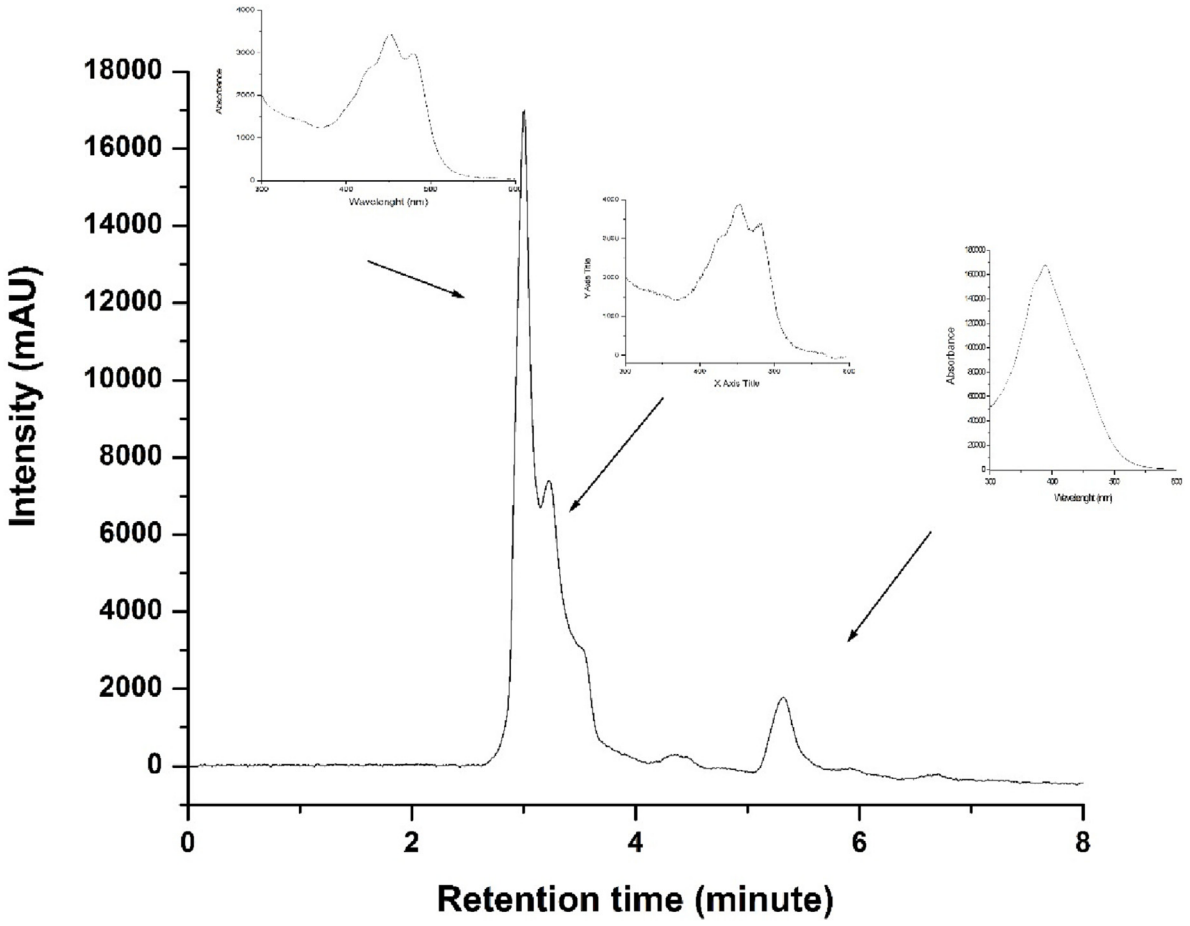

Fig. 7. HPLC profile of carotenoids from the bacteria symbiont 19.PP.Sc.13.

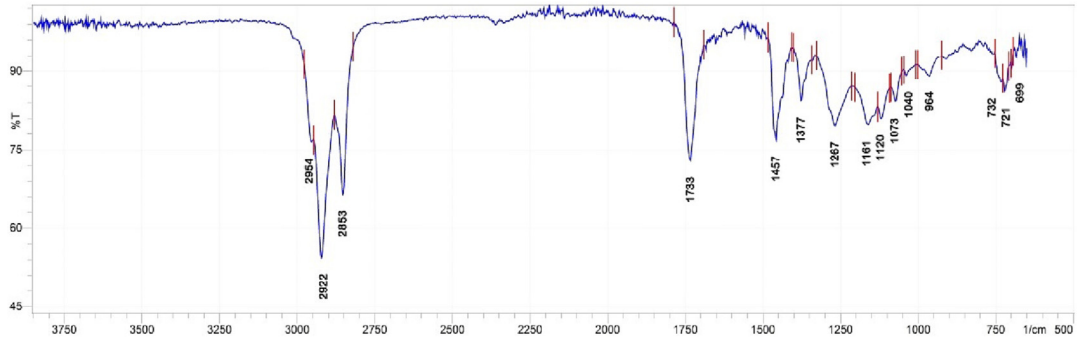

Fig. 8. ATR-FTIR spectrum of carotenoids extracts from bacterial symbiont 19.PP.Sc.1.3.

sponge *Callyspongia diffusa* and exhibited free radicals scavenging activity *in vitro* attributed to the presence of alkaloids, terpenoids, reducing sugars, and anthraquinones (Arunachalam and Amirtham Jacob Appadorai, 2013).

Furthermore, the carotenoid content of *Virgibacillus* sp. has been recently reported for its biological activities, including antibacterial and antifungal activities (Fayez et al., 2022). The color of this bacteria represents the content of carotenoids in which several studies have reported great antioxidant activity, making them lead compounds for pharmaceutical and nutraceutical sources (Galasso et al., 2017; Kusmita et al., 2021b; Shindo and Misawa, 2014). The carotenoids content of *Sinularia* sp., including their bacterial symbiont *Virgibacillus* sp., has not been comprehensively studied. (Kusmita et al., 2017) reported that the carotenoids extract of the bacterial symbiont, namely *Pseudoalteromonas shioyasakiensis*, *Pseudoalteromonas rubra*, *Virgibacillus salaries*, and *Pseudoalteromonas spongiae*, from the soft coral *Sarcophyton* sp. had the highest antioxidant activity compared to the carotenoids from other types of bacterial symbionts. In the current study, the ATR-FTIR analysis showed the presence of unsaturated moiety of identified carotenoids extracts, indicating the potential antioxidant activity (Al-Mamary et al., 2021; Chen et al., 2020; Mezzomo and Ferreira, 2016; Xavier and Pérez-Gálvez, 2016). The antioxidant activity of the carotenoids from our extract exhibited comparable antioxidant activity to standard β-carotene (Fig. 9), in accordance with the extra UV-light protector activity (Table 8). The ability to

Table 7
ATR-FTIR spectra of carotenoids extracts from bacterial symbiont 19.PP.Sc.1.3.

Peak area (%)	Begin [1/cm]	End [1/cm]	Width [1/cm]	Position (Max) [1/cm]	Functional Group
0.9	708	727	19	721	C – H
0.9	727	753	26	732	C – H
2.2	924	1001	76	964	C = C
0.4	1008	1046	37	1040	CO – O – CO
1.5	1053	1088	35	1073	C – O
1.3	1092	1129	37	1120	C – O
4.1	1129	1204	75	1161	C – O
13.2	1213	1327	114	1267	C – O
4.7	1342	1401	60	1377	O – H
14.8	1407	1483	76	1457	C – H
20.5	1690	1787	97	1733	C = O
12.2	2820	2879	60	2853	C – H
21.3	2879	2946	67	2922	C – H
1.9	2946	2978	32	2954	C – H

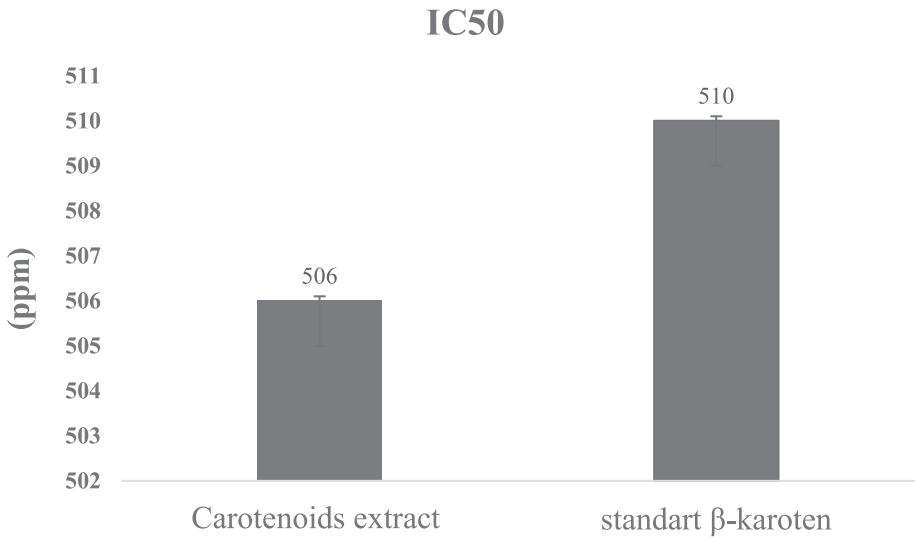

Fig. 9. Antioxidant activity of the carotenoids extract of the symbiont bacteria 19.PP.Sc.13 and β-carotene as the positive control.

Table 8
The assessment of the sun protection of the 19.PP.Sc.13 carotenoid extract.

	Value	Category
SPF	6.04	Extra Protection
% Te	33.19	–
% Tp	32.25	UVA Sunblock

Table 9
Inhibition zone diameter representing antibacterial activity of the carotenoids extracted from the bacterial symbiont 19.PP.Sc.13.

MDR bacteria	Concentration of carotenoid extract (%)	Inhibition zone (mm)
E.coli	4	8,86 ± 0.008
	6	9,44 ± 0.01
	8	10,90 ± 0.011
	Ciprofloxacin 0.05	20.63 ± 0.009
MRSA	4	12.48 ± 0.007
	6	13.76 ± 0.009
	8	15.69 ± 0.034
	Ciprofloxacin 0.05	21.92 ± 0.017

quench singlet oxygen and free radicals enable carotenoids to act as photo and UV-light protector from cell and tissue damage (Zaytseva et al., 2021b).

Besides antioxidants, marine bacteria must compete and survive in the presence of diverse pathogenic and non-pathogen organisms as unique antimicrobial substances can be secreted and utilized as antimicrobial lead compounds. Carotenoids fits in porins (transmembrane proteins) in the outer membrane of the bacterial cell wall, forming strong polymer bonds and destroying the porin, thereby reducing bacterial cell wall permeability and inhibiting bacterial growth (Evans and Cowan, 2016). Moreover, the anti-biofilm activity of marine bacterial carotenoids from *Virgibacillus halodenitrificans* has been reported (Fayez et al., 2022). The antibacterial activity observed in the current study suggested that the carotenoids extracted from *Virgibacillus* sp. may block bacterial transmembrane proteins and inhibit the formation of bacterial biofilms, leading to the inhibition of bacterial growth. Although this study showed potential biological activities of carotenoids extracted from *Virgibacillus* associated with *Sinularia* sp., further investigation using *in vivo* models followed by clinical studies is a prerequisite for translational Pharma Valley of Death and cosmetology products, which are usually limited by the conversion from *in vitro* models to clinical results (Brunt and Burgess, 2018).

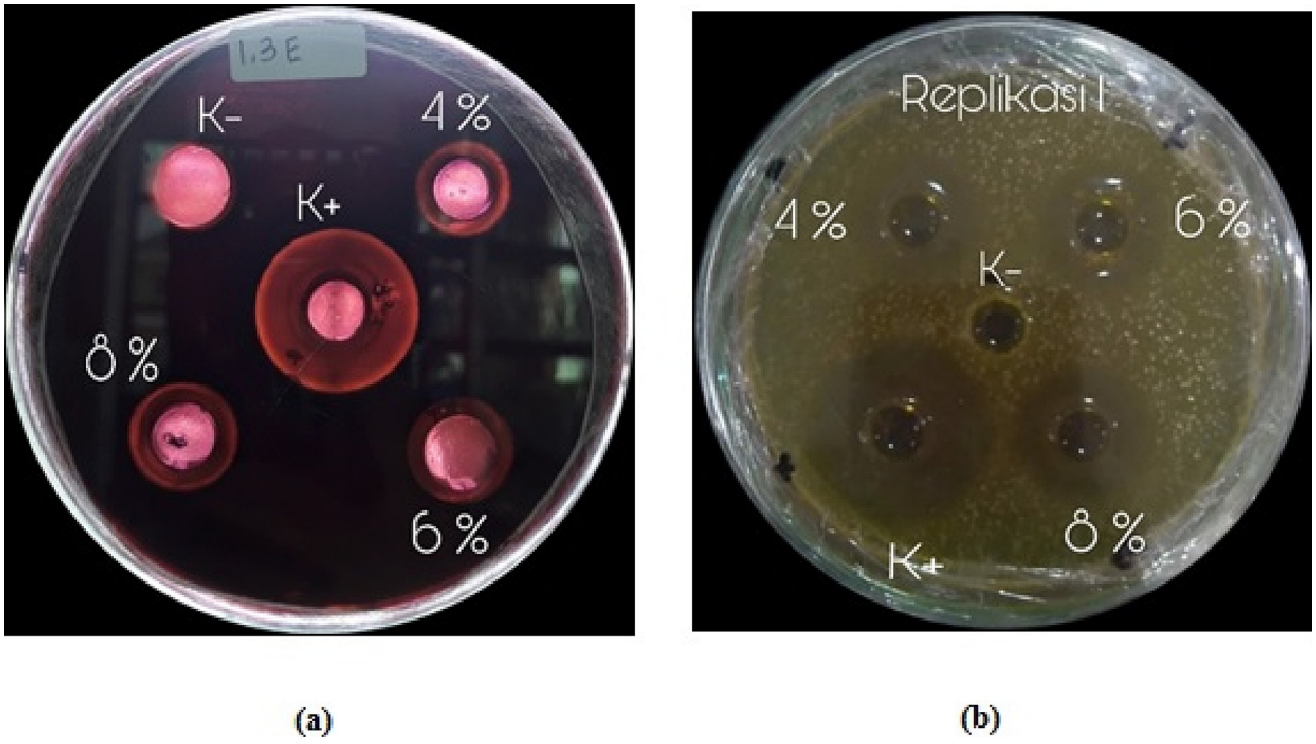

Fig. 10. Antibacterial activity of the carotenoids extract of bacterial symbiont 19.PP.Sc.13 against (a) MDR *E. coli* and (b) MRSA.

5. Conclusion

The carotenoids extracted from the soft coral *Sinularia* sp. from Panjang Island, North Java Sea, were identified as the bacterial symbiont *Virgibacillus* sp. The carotenoids extracted exhibited significant antioxidants, sun protection capability, and growth inhibitory activities against two strains of MDR bacteria. This study provides empirical evidence of the carotenoids produced by marine bacteria from tropical regions. Further investigation into methods of culturing the host organism and associated bacteria to mimic their natural habitat as well as developing *de novo* biological fabrication of bacterial carotenoids is warranted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Yayasan Pharmasi Semarang (No. 05/LPPM/EDW-SW/PDL/VI/2022) and the Ministry of Research, Technology, and Higher Education, Indonesia (No.0647/III/LPPM.PM.10.02/6/2022).

References

Abdassah, M., Aryani, R., Surachman, E., Muchtaridi, M., 2015a. In-vitro assessment of effectiveness and photostability avobenzone in cream formulations by combination ethyl ascorbic acid and alpha tocopherol acetate. J Appl Pharm Sci 5, 070–074. https://doi.org/10.7324/JAPS.2015.50611.

Aceret, T.L., Coll, J.C., Uchio, Y., Sammarco, P.W., 1998. Antimicrobial activity of the diterpenes flexibilide and sinulariolide derived from Sinularia flexibilis Quoy and Gaimard 1833 (Coelenterata: Alcyonacea, Octocorallia). Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 120, 121–126. https://doi.org/10.1016/S0742-8413(98)00032-2.

Al-Mamary, M.A., Moussa, Z., Al-Mamary, M.A., Moussa, Z., 2021. Antioxidant activity: The presence and impact of hydroxyl groups in small molecules of natural and synthetic origin. Antioxidants. https://doi.org/10.5772/INTECHOPEN.95616.

Arunachalam, K., Amirtham Jacob Appadorai, R., 2013. Antioxidant potential and biochemical evaluation of metabolites from the marine bacteria Virgibacillus sp. associated with the sponge Callyspongia diffusa. Free Radicals Antioxid. 3, 47–51. https://doi.org/10.1016/J.FRA.2013.04.004.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M., Rollinger, J.M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E.A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G.K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., Diederich, M., Dinkova-Kostova, A.T., Efferth, T., El Bairi, K., Arkells, N., Fan, T. P., Fiebich, B.L., Freissmuth, M., Georgiev, M.I., Gibbons, S., Godfrey, K.M., Gruber, C.W., Heer, J., Huber, L.A., Ibanez, E., Kijjoa, A., Kiss, A.K., Lu, A., Macias, F. A., Miller, M.J.S., Mocan, A., Müller, R., Nicoletti, F., Perry, G., Pittalà, V., Rastrelli, L., Ristow, M., Russo, G.L., Silva, A.S., Schuster, D., Sheridan, H., Skalicka-Woźniak, K., Skaltsounis, L., Sobarzo-Sánchez, E., Bredt, D.S., Stuppner, H., Sureda, A., Tzvetkov, N.T., Vacca, R.A., Aggarwal, B.B., Battino, M., Giampieri, F., Wink, M., Wolfender, J.L., Xiao, J., Yeung, A.W.K., Lizard, G., Popp, M.A., Heinrich, M., Berindan-Neagoe, I., Stadler, M., Daglia, M., Verpoorte, R., Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery 2021 20:3 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Ayuningrum, D., Muchlissin, S.I., Trianto, A., Radjasa, O.K., Sabdono, A., 2020. Crude extract from a hardcoral-associated bacterium Virgibacillus salarius PHC-44-04 inhibiting growth of Multidrug-Resistant Enterobacter aerogenes human pathogen. Asian Journal of Natural Product Biochemistry 18, 82–87. https://doi.org/10.13057/BIOFAR/F180205

Barel, A., Paye, M., Maibach, H., 2001. Handbook of Cosmetic Science and Technology. Marcel Dekker Inc, New York.

Brunt, E.G., Burgess, J.G., 2018. The promise of marine molecules as cosmetic active ingredients. Int. J. Cosmet. Sci. 40, 1–15. https://doi.org/10.1111/ICS.12435.

Burgess, J., Boyd, K., Armstrong, E., Jiang, Z., Yan, L., Berggren, M., May, U., Pisacane, T., Granmo, A., Adams, D., 2003. The development of a marine natural product-based antifouling paint. Biofouling 19, 197–205. https://doi.org/10.1080/0892701031000061778.

Chang, R.H., Yang, L.T., Luo, M., Fang, Y., Peng, L.H., Wei, Y., Fang, J., Yang, J.L., Liang, X., 2021. Deep-sea bacteria trigger settlement and metamorphosis of the mussel Mytilus coruscus larvae. Scientific Reports 2021 11:1 11, 1–10. https://doi.org/10.1038/s41598-020-79832-8.

Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., Kim, C.K., 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Scientific Reports 2020 10:1 10, 1–9. https://doi.org/10.1038/s41598-020-59451-z.

Mendes-Silva, T. de C.D., Andrade, R.F. da S., Ootani, M.A., Mendes, P.V.D., Sá, R.A. de Q.C. de, Silva, M.R.F. da, Souza, K.S., Correia, M.T. dos S., Silva, M.V. da, Oliveira, M.B.M. de, Mendes-Silva, T. de C.D., Andrade, R.F. da S., Ootani, M.A., Mendes, P.

V.D., Sá, R.A. de Q.C. de, Silva, M.R.F. da, Souza, K.S., Correia, M.T. dos S., Silva, M. V. da, Oliveira, M.B.M. de, 2020. Biotechnological potential of carotenoids produced by extremophilic microorganisms and application prospects for the cosmetics industry. Adv Microbiol 10, 397–410. https://doi.org/10.4236/AIM.2020.108029.

Evans, S.M., Cowan, M.M., 2016. Plant products as antimicrobial agents. Cosmetic and Drug Microbiology 12, 205–231. https://doi.org/10.3109/9781420019919-17.

Fayez, D., Youssif, A., Sabry, S., Ghozlan, H., Eltarahony, M., 2022. Carotegenic Virgibacillus halodenitrificans from Wadi El-Natrun Salt Lakes: Isolation, optimization, characterization and biological activities of carotenoids. Biology (Basel) 11, 1407. https://doi.org/10.3390/BIOLOGY11101407/S1.

Felix, F., Nugroho, T.T., Silalahi, S., Octavia, and Y., 2011. Screening of Indonesian original bacteria Vibrio sp as a cause of shrimp diseases based on 16S ribosomal DNA-technique 3, 85–99.

Finkel'shtein, E.I., 2016. Modern Methods of Analysis of Carotenoids (Review). Pharm Chem J 50, 96–107. https://doi.org/10.1007/S11094-016-1405-2/METRICS.

Galasso, C., Corinaldesi, C., Sansone, C., 2017. Carotenoids from marine organisms: Biological functions and industrial applications. Antioxidants 6. https://doi.org/10.3390/ANTIOX6040096.

Gonzalez, H., Tarras-Wahlberg, N., Strömdahl, B., Juzeniene, A., Moan, J., Larkö, O., Rosén, A., Wennberg, A.M., 2007. Photostability of commercial sunscreens upon sun exposure and irradiation by ultraviolet lamps. BMC Dermatol. 7, 1–9. https://doi.org/10.1186/1471-5945-7-1.

Hegazy, G.E., Abu-serie, M.M., Abo-elela, G.M., Ghozlan, H., Sabry, S.A., Soliman, N.A., Abdel-fattah, Y.R., 2020. In vitro dual (anticancer and antiviral) activity of the carotenoids produced by haloalkaliphilic archaeon Natrialba sp . M6. Sci Rep 1–14. https://doi.org/10.1038/s41598-020-62663-y.

Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G., Logan, N.A., 1998. Virgibacillus: A new genus to accommodate Bacillus pantothenticus (Proom and Knight 1950). Emended description of Virgibacillus pantothenticus. Int. J. Syst. Bacteriol. 48, 99–106. https://doi.org/10.1099/00207713-48-1-99.

Hsu, F.Y., Wang, S.K., Duh, C.Y., 2018. Xeniaphyllane-derived terpenoids from soft coral Sinularia nanolobata. Mar. Drugs 16, 40. https://doi.org/10.3390/MD16020040.

Kamel, H.N., Slattery, M., 2008a. Terpenoids of Sinularia.: Chemistry and biomedical applications. Pharm. Biol. 43, 253–269. https://doi.org/10.1080/13880200590928852.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/MOLBEV/MSW054.

Kusmita, L., Mutmainah, N.F.N., Sabdono, A., Trianto, A., Radjasa, O.K., Pangestuti, R., 2021a. Characteristic evaluation of various formulations of anti-aging cream from carotenoid extract of bacterial symbiont Virgibacillus salarius strain 19.PP.Sc1.6. Cosmetics 8. https://doi.org/10.3390/cosmetics8040120.

Kusmita, L., Mutiara, E.V., Nuryadi, H., Pratama, P.A., Wiguna, A.S., Radjasa, O.K., 2017. Characterization of carotenoid pigments from bacterial symbionts of soft-coral Sarcophyton sp. from North Java Sea. Int. Aquat. Res, 9, 61–69. https://doi.org/10.1007/s40071-017-0157-2.

Kusmita, L., Nuryadi, H., Widyananto, P.A., Muchlissin, S., Sabdono, A., Trianto, A., Radjasa, O.K., 2021b. Bioactivity of carotenoid produced by soft coral symbiotic microorganisms from panjang and karimunjawa island, Centra Java, Indonesia. Biodiversitas 22, 732–740. https://doi.org/10.13057/biodiv/d220226.

Liandhajani, Immaculata Iwo, M., Sukrasno, Soemardji, A.A., Hanafi, M., 2013. Sunscreen activity of α -mangostin from the pericarps of Garcinia Mangostana Linn Liandhajani, Maria Immaculata Iwo, Sukrasno, Andreanus A. Soemardji, Muhammad Hanafi. J Appl Pharm Sci 3, 70–73. https://doi.org/10.7324/JAPS.2013.3610.

Lóránd, T., Molnár, P., Deli, J., Tóth, G., 2002. FT-IR study of some seco- and apocarotenoids. J. Biochem. Bioph. Methods 53, 251–258. https://doi.org/10.1016/S0165-022X(02)00113-6.

Meléndez-Martínez, A.J., Stinco, C.M., Mapelli-Brahm, P., 2019. Skin carotenoids in public health and nutricosmetics: The emerging roles and applications of the UV radiation-absorbing colourless carotenoids phytoene and phytofluene. Nutrients 11. https://doi.org/10.3390/NU11051093.

Mezzomo, N., Ferreira, S.R.S., 2016. Carotenoids functionality, sources, and processing by supercritical technology: A review. J. Chem. 2016. https://doi.org/10.1155/2016/3164312.

Mukherjee, T., Bose, S., Mukhopadhyay, S.K., 2017. Antioxidant properties of the carotenoid extracts of three Deinococcus-Thermus phylum bacteria, Meiothermus sp. strains RP and TP and Thermus sp. strain YY from Paniphala hot spring. India. Nutrire 42, 1–11. https://doi.org/10.1186/s41110-017-0032-3.

Murti, P.D.B., Radjasa, O.K., 2012. Antibacterial activity of bacterial symbiont of soft coral Lobophytum sp. againts MDR bacteria Escherichia coli and Staphyllococcus aureus. Journal of Coastal Development 15, 297–302.

Murti, D.B., Susanto, A., Radjasa, O.K., Rondonuwu, F.S., 2016a. Pigments characterization and molecular identification of bacterial symbionts of brown algae Padina sp. collected from Karimunjawa Island 21, 59–64. https://doi.org/10.14710/ik.ijms.21.2.59-64.

Mutiara, E.V., Kusmita, L., Pratiwi, N.A., Nirmala, M.I.R., Radjasa, O.K., 2017. Sun screen protector activity from bacterial symbiont carotenoid of soft coral sarcophyton sp. Adv. Sci. Lett. 23, 6424–6427. https://doi.org/10.1166/asl.2017.9642.

Nugraheni, S.A., Khoeri, M.M., Sabdono, A., Radjasa, O.K., 2010a. Antibacterial activity of bacterial symbionts of softcoral Sinularia sp. against pathogenic resistant Bacteria. Journal of Coastal Development 13, 113–118.

Pelizzo, M., Zattra, E., Nicolosi, P., Peserico, A., Garoli, D., Alaibac, M., 2012. In Vitro Evaluation of Sunscreens: An Update for the Clinicians. ISRN Dermatol 2012, 1–4. https://doi.org/10.5402/2012/352135.

Peng, Q.Z., Chen, J., Zhang, Y.Q., Chen, Q.H., Peng, D.J., Cui, X.L., Li, W.J., Chen, Y.G., 2009. Virgibacillus zhanjiangensis sp. nov., a marine bacterium isolated from sea water. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 96, 645–652. https://doi.org/10.1007/S10482-009-9381-0/METRICS.

Radjasa, O.K., Khoeri, M.M., Darusallam, C.C., Trimasanto, H., Sudoyo, H., 2013. Bacterial symbionts of reef invertebrates: Screening for anti-pathogenic bacteria activity. Biodiversity 14, 80–86. https://doi.org/10.1080/14888386.2013.774937.

Radjasa, O., Salasia, S., Sabdono, A., Weise, J., Imhoff, J., Lammler, C., Risk, M., 2007. Antibacterial activity of marine bacterium Pseudomonas sp. associated with soft coral Sinularia polydacta against Streptococcus equi Subsp. zooepidemicus. Int. J. Pharmacol. 3, 170–174. https://doi.org/10.3923/ijp.2007.170.174.

Radjasa, O., Limantara, L., Sabdono, A., 2009. Antibacterial activity of a pigment producing-bacterium associated with Halimeda sp. from eland-locked marine lake Kakaban. Indonesia. Journal of Coastal Development 12, 100–104.

Rodrigues, I., Miguel, M., Mnif, W., 2019. A brief review on new naturally occurring cembranoid diterpene derivatives from the soft corals of the genera Sarcophyton, Sinularia, and Lobophytum since 2016. Molecules 24. https://doi.org/10.3390/MOLECULES24040781.

Rodriguez-Amaya, D.B., Kimura, M., 2004a. HarvestPlus Handbook for Carotenoid Analysis. HarvestPlus Technical Monographs 59.

Scott, K.J., 2001. Detection and Measurement of Carotenoids by UV/VIS Spectrophotometry. Current Protocols in Food Analytical Chemistry 00, F2.2.1-F2.2.10. https://doi.org/10.1002/0471142913.FAF0202S00.

Setiyono, E., Heriyanto, Pringgenies, D., Shioi, Y., Kanesaki, Y., Awai, K., Brotosudarmo, T.H.P., 2019a. Sulfur-containing carotenoids from a marine coral symbiont Erythrobacter flavus strain KJ5. Mar Drugs 17. https://doi.org/10.3390/MD17060349.

Shindo, K., Misawa, N., 2014. New and rare carotenoids isolated from marine bacteria and their antioxidant activities. Mar. Drugs 12, 1690–1698. https://doi.org/10.3390/MD12031690.

Sies, H., Stahl, W., 2004. Carotenoids and UV protection. Photochem. Photobiol. Sci. 3, 749–752. https://doi.org/10.1039/B316082C.

Steven, R., Humaira, Z., Natanael, Y., Dwivany, F.M., Trinugroho, J.P., Dwijayanti, A., Kristianti, T., Tallei, T.E., Emran, T.B., Jeon, H., Alhumaydhi, F.A., Radjasa, O.K., Kim, B., 2022. Marine microbial-derived resource exploration: Uncovering the hidden potential of marine carotenoids. Mar. Drugs 20. https://doi.org/10.3390/MD20060352.

Steven, R., Humaira, Z., Natanael, Y., Dwivany, F., Trinugroho, J., Dwijayanti, A., Kristianti, T., Tallei, T., Emran, T., Jeon, H., Alhumaydhi, F., Radjasa, O., Kim, B., 2022. Marine microbial-derived resource exploration: uncovering the hidden potential of marine carotenoids. Mar. Drugs 20, 352. https://doi.org/10.3390/MD20060352.

van Ofwegen, L.P., Benayahu, Y., Mcfadden, C.S., 2013. Sinularia leptoclados (Ehrenberg, 1834) (Cnidaria, Octocorallia) re-examined. Zookeys 272, 29. https://doi.org/10.3897/ZOOKEYS.272.4406.

Van Ofwegen, L.P., 2000a. Status of knowledge of the Indo-Pacific soft coral genus Sinularia May, 1898 (Anthozoa: Octocorallia), in: Proceeding 9th International Coral Reef Symposium. pp. 1–5.

Van Ofwegen, L.P., 2000b. Status of knowledge of the Indo-Pacific soft coral genus Sinularia May, 1898 (Anthozoa: Octocorallia), in: Proceeding 9th International Coral Reef Symposium. pp. 1–5.

Vílchez, C., Forján, E., Cuaresma, M., Bédmar, F., Garbayo, I., Vega, J.M., 2011. Marine Carotenoids: Biological functions and commercial applications. Mar. Drugs 9, 319. https://doi.org/10.3390/MD9030319.

Xavier, A.A.O., Pérez-Gálvez, A., 2016. Carotenoids as a Source of Antioxidants in the Diet. Subcell. Biochem. 79, 359–375. https://doi.org/10.1007/978-3-319-39126-7_14.

Xu, B., Hu, B., Wang, J., Lan, Y., Zhu, Y., Dai, X., Huang, L., Huang, Y., Du, W., 2018. Virgibacillus indicus sp. nov. and Virgibacillus profundi sp. nov, two moderately halophilic bacteria isolated from marine sediment by using microfluidic streak plates. Int. J. Syst. Evol. Microbiol. 68, 2015–2023. https://doi.org/10.1099/IJSEM.0.002782/CITE/REFWORKS.

Yan, X., Liu, J., Leng, X., Ouyang, H., 2021a. Chemical diversity and biological activity of secondary metabolites from soft coral genus Sinularia since 2013. Mar. Drugs 19. https://doi.org/10.3390/MD19060335.

Young, A.J., Lowe, G.L., 2018. Carotenoids—Antioxidant Properties. Antioxidants 7. https://doi.org/10.3390/ANTIOX7020028.

Zaytseva, A., Chekanov, K., Zaytsev, P., Bakhareva, D., Gorelova, O., Kochkin, D., Lobakova, E., 2021a. Sunscreen effect exerted by secondary carotenoids and mycosporine-like amino acids in the aeroterrestrial chlorophyte coelastrella rubescens under high light and uv-a irradiation. Plants 10, 2601. https://doi.org/10.3390/PLANTS10122601/S1.

Zhuang, C., Yuan, J., Du, Y., Zeng, J., Sun, Y., Wu, Y., Gao, X.H., Chen, H.D., 2022. Effects of oral carotenoids on oxidative stress: A systematic review and meta-analysis of studies in the recent 20 years. Front. Nutr. 9, 481. https://doi.org/10.3389/FNUT.2022.754707/BIBTEX.