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An orange pigment from the marine bacterium *Paracoccus haeundaensis* SAB E11 as a prospective source of natural antioxidants

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Abstract. *Abubakar H, Astuti RI, Listyowati S, Batubara I, Wahyudi AT. 2022. An orange pigment from the marine bacterium* Paracoccus haeundaensis *SAB E11 as a prospective source of natural antioxidants. Biodiversitas 23: 4730-4737.* Bacterial pigment extracts are the source of many natural antioxidant substances. The present study aimed to assess the antioxidant activity of an orange pigment derived from a marine bacterium, identified as *Paracoccus haeundaensis* SAB E11, analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(ethylbenzothiazoline-6-sulfonic acid) (ABTS). Methanol-pigmented crude extract of *P. haeundaensis* SAB E11 exhibited the best DPPH scavenging activity with an IC_{50} value of 54.7 μg/mL, while the *n*-hexane crude pigment extract of the bacterium reduced ABTS optimally with an IC_{50} value of 24.46 μg/mL. The analysis of antioxidant compounds in *P. haeundaensis* SAB E11 was identified by combining liquid chromatography with tandem mass spectrometry (LCMS/MS). The five antioxidant compounds from methanol and *n*-hexane crude pigment extract of *P. haeundaensis* SAB E11 have been detected. Polyphenols were found to be the major compound, such as 3,5,3',4'-tetrahydroxystilbene, scopoletin, and liquiritigenin. The other compounds were dihydroactinidiolide (terpenes) and ricinoleic acid (a fatty acid compound). The orange-colored extract of marine bacterium *P. haeundaensis* SAB E11 could be promising pharmacological capabilities as an antioxidant.

Keywords: ABTS, antioxidant, DPPH, marine bacterium, pigment extract

INTRODUCTION

Cell metabolism, poor lifestyle, and environmental condition are prominent aspects of presenting oxidants in the cells. Oxidants are free radicals raised from oxygen, nitrogen, and sulfur that generate molecules with unpaired electrons. Consequently, the molecules are unstable and chemically reactive with other molecules to form unwanted chain reactions. Reactive Oxygen Species (ROS) are typically produced as metabolic cells, including hydroxyl radicals, hydrogen peroxide, nitric acid, or superoxide anions. However, ROS molecules are reactive to other macromolecules such as protein, lipid, and nucleic acid, causing cell damage (Ali et al. 2020; Andrés et al. 2021). Antioxidants are produced in cells to prevent oxidation by neutralizing free radicals with donating electrons. Nevertheless, antioxidants are overpowered by the increased number of free radicals resulting in oxidative stress (di Meo and Venditti 2020). Several cases have shown that oxidative stress causes degenerative diseases such as diabetes mellitus, Alzheimer's, cancer, and hypertension (Nandi et al. 2019).

Nowadays, plants are widely consumed as a source of natural antioxidants. However, many studies show that bacteria i.e. tiny organisms, produce antioxidant compounds. In addition, bacteria have a short life cycle, ease of culture, and easy subject to genetically engineering indicating that bacteria are potential sources of antioxidants. Bacteria produce antioxidant compounds such as phenolic, pigments, polysaccharides, and organic acids (Milke et al. 2018; Hamidi et al. 2019; Pérez-gálvez et al. 2020). Derivatives of polyphenolic and pigments are reported to have the highest antioxidant activity. Several polyphenolic compounds carry antioxidant activities. The compounds are simple phenols, phenolic acids, hydroxycinnamic acid, coumarins, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, and lignins. The intensity of antioxidant activity of the polyphenol complex is related to the degree of polymerization of the polyphenol. In general, lower levels of polymerization react with more excellent antioxidant activity and present other bioactive activities (Rudra et al. 2021). Another interesting antioxidant compound is pigment, a dye molecule that absorbs specific wavelengths of light and reflects the visible light spectrum in the wavelength range between 380-750 nm (Ramesh et al. 2019; Kusmita et al. 2021). The pigment colors of the bacterial cells play an essential role in physiological and molecular functions. In heterotrophic bacteria, the pigment plays a role in bacterial growth sustainability in the habitat. A pigment such as carotenoids can protect bacterial cell

damage caused by ultraviolet (UV) radiation (Reis-Mansur et al. 2019).

The habitat of the bacteria affects the production of bioactive compounds. Dynamic conditions of the marine ecosystem prospect the bacteria to synthesize bioactive compounds, particularly pigments containing antioxidant molecules, to defend themselves from outside influences like UV radiation (Genç et al. 2020). Many studies confirm the potential of pigmented marine bacteria as the source of antioxidants, the latest of which is the yellow-pigmented bacterium *Erythrobacter* sp. strain SDW2 was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(ethylbenzothiazoline-6-sulfonic acid) (ABTS), showed the inhibitory values of 73.4±1.4% and 84.9±0.7%, respectively (Picot et al. 2022). In addition, the pigment 2,2'-dihydroxy-astaxanthin produced by *Brevundimonas scallop* has better activity than the standard astaxanthin in reducing DPPH (Liu et al. 2020). In the present study, the potential antioxidant activity from an orange pigment derived the marine bacterial strain SAB E11 has been explored by identification and characterization, *in vitro* antioxidant activities analysis, and determination of the antioxidation compounds. Accordingly, the outcomes of the investigation imply that the orange-colored extract from the marine bacterium *Paracoccus haeundaensis* SAB E11 has promising pharmaceutical capabilities as an antioxidant.

MATERIALS AND METHODS

Bacterial strain and media culture

The marine orange pigmented bacterial strain SAB E11 has been successfully isolated in previous study from sponge *Jaspis* sp. (Raja Ampat Island, Papua-Indonesia) (Abubakar et al. 2011). The bacterial strain was routinely cultured in seawater complete (SWC) medium (3 mL glycerol, 5g peptone, 1g yeast extract, 750 mL seawater, and 250 mL distilled water).

Characterization of the bacteria

Bacterial strain SAB E11 was grown in solid SWC (2% w/v agar) medium for five days to obtain the pigment colonies. Morphology of the colony was characterized by shape, margin, elevation, size, texture, and pigmentation. Gram staining was performed using the standard Gram reaction to determine the bacterium's Gram type and cell body shape. Additional physiological tests performed were hemolysis and catalase. Hemolysis assay for pathogenic indication was conducted by culturing on blood sheep agar medium, while catalase enzyme performance was tested by H_2O_2.

Molecular identification

The genomic DNA of the bacterial strain SAB E11 was extracted using Presto Mini gDNA Bacteria Kit (Geneaid, Taiwan) based on the manufacturer's guidelines. The 16S rRNA gene were amplified using primers 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi et al. 1998). The PCR reaction was performed using MyTaq HS Red Mastermix (Bioline, United Kingdom) in 30 cycles with PCR condition pre-denatured at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec, extension at 72°C for 1 min 30 sec, and post-extension at 72°C for 7 min. The PCR products were visualized in 1% agarose (v/w) for 45 min at 70 V and sequenced at First BASE (Selangor, Malaysia). The gene sequence was aligned to the NCBI GenBank's database using BLAST program (Basic Local Alignment Search Tools) (www.ncbi.nlm.nih.gov/BLAST/). The phylogenetic tree was constructed based on the MOLE-BLAST with sequences of 10 bacterial strains derived from NCBI using MEGAX.

Pigment crude extraction

Bacterial strain SAB E11 was cultured in the primary medium (1000 mL) then harvested after incubation for 5 days. Bacterial cultures were then centrifuged at 6000 g for 15 min. The pigmented cells were extracted until colorless with a sonicator (Bransonic 1510E-MT) at 42 kHz using methanol, ethyl acetate, chloroform, and *n*-hexane as the solvents in a ratio of 1:5 (cell mass: solvent) (w/v). Afterward, they were centrifuged at 6000 g for 15 min. The extract solutions were evaporated using a rotary evaporator at 45°C. The dry pigment crude extracts were stored at 4°C.

Antioxidant activity

The antioxidant activities screening was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method referring to Prastya et al. (2018) and Batubara et al. (2020). Preparation was made by making a stock of pigment crude extracts with 10000 µg/mL concentration in dimethyl sulfoxide (DMSO).

DPPH

A total 100 µg/mL of the pigment crude extracts in various concentrations (10, 25, 50, 100, 200, 300, 400, 500, and 1000 µg/mL) were put 100 µL into a microwell plate (Costar 96) and added with 100 µL of DPPH solution (125 µM in methanol), then incubated for 30 min at room temperature in the dark. Ascorbic acid was used as a positive control. The absorbance value of the samples was measured using an ELISA microtiter plate reader spectrophotometry (EPOC, USA) at 517 nm. The percentage of inhibition of each extract was calculated using the following formula:

$$\%\ inhibition = \left(1 - \left(\frac{A_{sample} - A_{control}}{A_{blank} - A_{control}}\right)\right) \times 100\%$$

Where: A_{sample} is the absorbance value of DPPH with pigmented crude extract, $A_{control}$ is the absorbance of DPPH with ascorbic acid, and A_{blank} is the absorbance of methanol. The percentage value of inhibition was analyzed with the linear regression model to determine inhibitory capacity (IC_{50}).

ABTS

The ABTS solution (7mM) was oxidized with 2.46 potassium peroxide sulfate and incubated in the dark for 12-16 hrs. Subsequently, the solution was set to an absorbance of 0.7 (±0.02) at 734 nm. A total of 100 µL of ABTS was reacted with 100 µL of pigment crude extracts in various concentrations (10, 25, 50, 100, 200, 300, 400, 500, and 1000 µg/mL) of yield extract samples in a microwell plate (Costar 96), then incubated in a dark room for 30 min. The absorbance value of the sample was measured using an ELISA microtiter plate reader spectrophotometry (EPOC, USA) at 734 nm. The percentage of inhibition of each pigment sample was calculated using the following formula:

$$\%\ inhibition = \left(1 - \left(\frac{A_{sample} - A_{control}}{A_{blank} - A_{control}}\right)\right) \times 100\%$$

Where: A_{sample} is the absorbance value of DPPH with pigmented crude extract, $A_{control}$ is ABTS absorbance with trolox, and A_{blank} is the absorbance of methanol. The percentage value of inhibition was analyzed with a linear regression model to determine the Inhibitory Capacity (IC_{50}).

UV-Vis analysis

The pigment extract with antioxidant activity was weighed as much as 20 mg and dissolved in 5 mL of the solvent used at the extraction time. The measured visible light absorption spectrum was analyzed at 200-800 nm wavelengths, using a visible spectrophotometer (Hitachi U-2800) to detect the maximum wavelength absorption.

LCMS/MS analysis

Identification of antioxidant compounds of pigmented crude extracts of bacterial strain SAB E11 was conducted with Waters, USA's LCMS/MS devices. Chromatographic separation conditions using an LC system consisting of Ultra Performance Chromatography (UPLC) type ACQUITY UPLC®H-Class System (Waters, USA) measuring by C18 (1.8 µm, 2.1×100 mm) high strength silica (HSS) column with a separation temperature at 50°C. The moving elements were distilled water + 5 mM formic acid (A) and Acetonitrile + 0.05 % formic acid (B). The chromatographic flow rate was 0.2 mL/min for 23 min, with a total volume of the injected sample of 5 µL. The mass spectrometry used was the electrospray ionization (ES) type Xevo G2-S Qtof (Waters, USA) in the positive mode ion. The analyzed mass values ranged from 50-1200 m/z, and the desolvation gas flow used was 793 L/h with collison energy of 4 Volts, while the Rampt collison energy used was 25-60 Volts. Chromatogram data and mass spectrum values were obtained by MassLinx V4.1 software. Confirmation of mass spectrum values was used by spectral database https://massbank.eu and https://hmdb.ca sites.

Statistic analytical

Antioxidant activity data were obtained with three replications. Statistical analysis of experimental data used ANOVA followed by Duncan's test to determine the significant difference between the mean numbers, using SPSS 26 software. P>0.05 was statistically considered a significant difference.

RESULTS AND DISCUSSION

Characterization of the bacterium

SAB E11 showed a dense pigmentation with orange colonies on a solid SWC medium after five days of incubation at 27°C. Colony characteristics of SAB E11 isolate were round shape; lobate edge; convex elevation; moderate size; mucoid texture (Figure 1). SAB E11 was Gram-negative with spherical rod-shaped (coccobacillus) cells. The hemolysis test was negative, while the catalase test was positive (picture not shown).

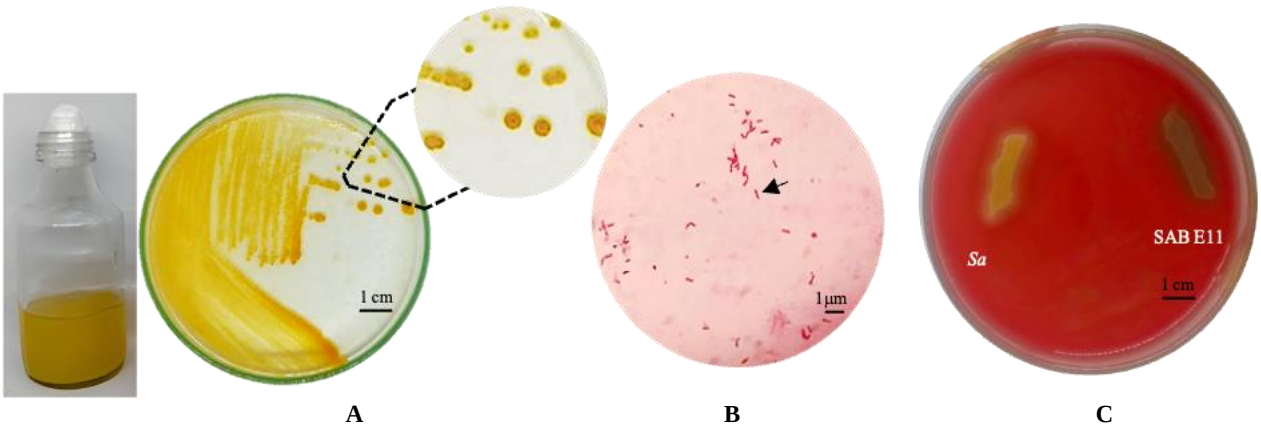


Figure 1. Characteristics of bacterial strain SAB E11 (a) Growth on SWC broth and the pigmented colonies in agar plate after the 5 days of incubation; (b) Gram-negative staining; (c) negative haemolysis, *Staphylococcus aureus* (Sa) as positive control.

Molecular identification

The 16S rRNA gene sequence of bacterial strain SAB E11 was found similar to *P. haeundaensis* BC74171 (homology: 99.41%; E-Value: 0.0; Accession Number: NR_025714.1). The sequence of bacterial strain SAB E11 has been deposited in NCBI with accession number ON810609. A phylogenetic tree was constructed based on 16S rRNA gene sequences using MOLE-BLAST with a neighbor-joining model and 1000x bootstrap. The phylogenetic tree placed *P. haeundaensis* SAB E11 in the same cluster as *Paracoccus hibiscisoli* THG-T2.31 (NR_157668.1), *P. marcusii* MH1 (NR_44922.1), *P. carotinifaciens* E-396 (NR_024658.1) and *P. haeundaensis* BC74171 (NR_025714.1) (Figure 2).

Pigment crude extraction

The physical characteristics and yield percentage extract of *P. haeundaensis* SAB E11 using methanol, ethyl acetate, chloroform, and *n*-hexane were varying (Table 1). The yield extracts appeared in the color range of yellow, orange, and red, while the consistencies were sticky, dry, and oily (Figure 3). The yield extracted by chloroform was the highest yield at 8.06%, followed by methanol (5.39%), *n*-hexane (2.48%), and ethyl acetate (2.38%).

Antioxidant activity

The antioxidant activity was expressed in the IC_{50} value. The IC_{50} value is a standard value for the lowest concentration that could inhibit 50% of DPPH and ABTS radicals. *Paracoccus haeundaensis* SAB E11 extracts, from all of the solvents used for extraction, showed antioxidant activities (Figure 4). The methanol extract had the highest antioxidant activity because it can reduce DPPH and ABTS radicals with an IC_{50} values of 54.70±1.64 and 46.12±2.21μg/mL, respectively. However, the *n*-hexane extract had the highest activity (IC_{50} value of 24.46±0,17 μg/mL) against ABTS radicals but not against DPPH (IC_{50} value of 257.09±10.83 μg/mL). Meanwhile, ethyl acetate and chloroform extracts had weak antioxidant activity against DPPH and ABTS with an IC_{50} value more than 300 μg/mL.

Table 1. Physical characteristics of pigment extracts of *Paracoccus haeundaensis* SAB E11 extracted by several organic solvents

Solvents Extracts	Colors	Consistency	Yield (%)
methanol	reddish-orange	sticky	5,39
ethyl acetate	orange	sticky	2,38
chloroform	orange	dry	8,06
n-hexane	reddish-orange	Semisolid, oily	2,48

Figure 3. Appearance of pigment extract from SAB E11 which was extracted using several solvents, namely methanol (A), ethyl acetate (B), chloroform (C), and *n*-hexane (D)

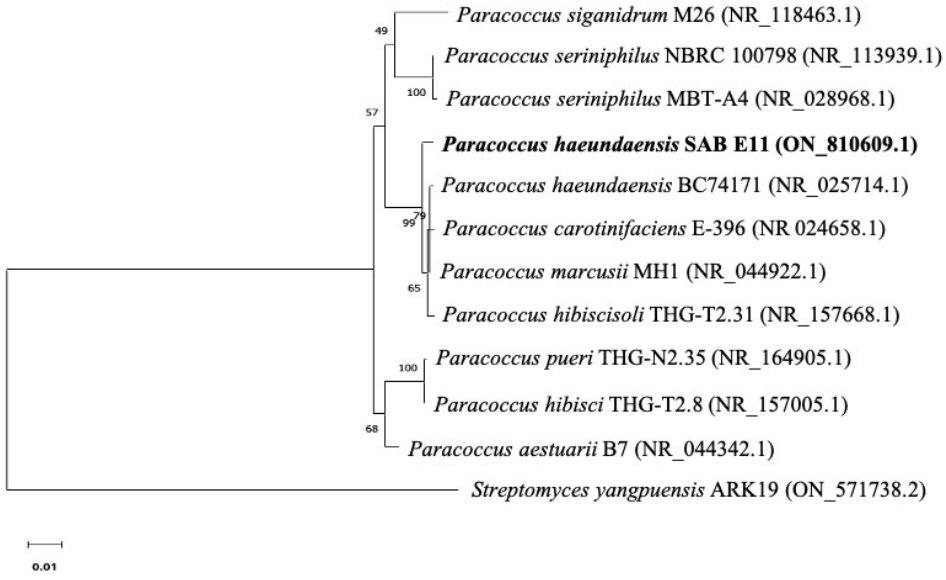


Figure 2. The neighborhood-joining phylogenetic tree based on the 16S rRNA gene sequence shows the position of bacterial strain SAB E11 isolate among the reference species (reference species was obtained from the NCBI database). The phylogenetic tree was constructed using MOLE-BLAST NCBI with a bootstrap of 1000 replications and the bar scale indicating a genetic distance of 0.01

UV-Vis analysis

The extracts with the best antioxidant activities based on DPPH and ABTS tests were identified using a visible spectrophotometer (Hitachi U-2800). The maximum wavelength (λ_{max}) of methanol-pigmented crude extracts of *P. haeundaensis* SAB E11 was 449 nm and shoulders at 473 nm. In the same time, the *n*-hexane extract had a single peak at 450 nm (Figure 5).

Identification antioxidant compounds by LCMS/MS analysis

The chromatographic analysis of *P. haeundaensis* SAB E11 was carried out to identify potential contributors to the antioxidant properties of the methanol and *n*-hexane pigmented crude extract (Figure 6). The four compounds in methanol pigmented crude extract, which were 3,5,3',4'-tetrahydroxystilbene (9.97%), scopoletin (1.79%), liquiritigenin (1.82%), and dihydroactinidiolide (1.04%) have been extracted (Table 2). Meanwhile, the antioxidant substances of *n*-hexane-pigmented crude extract consist of dihydroactinidiolide (1.30%), which was also detected in methanol extract with the same retention time (RT) at 9.10, and ricinoleic acid (7.41%). The electrospray ionization (ES) in positive ion mode to further analyze the MS/MS spectra to corroborate the identification of antioxidant chemicals in *P. haeundaensis* SAB E11 has been applied. The spectral pattern 3,5,3',4'-tetrahydroxystilbene was confirmed at m/z 245.0820, followed by scopoletin (195.0506), liquiritigenin (257.0818), dihydroactinidiolide (181.1228), and ricinoleic acid (299.2591).

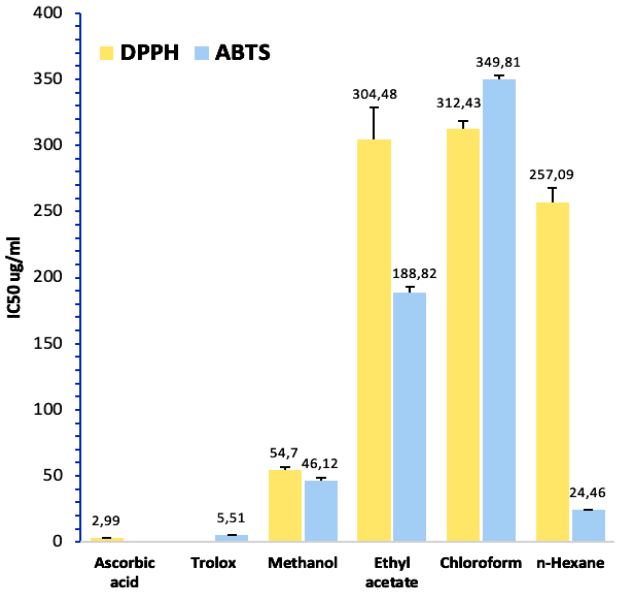
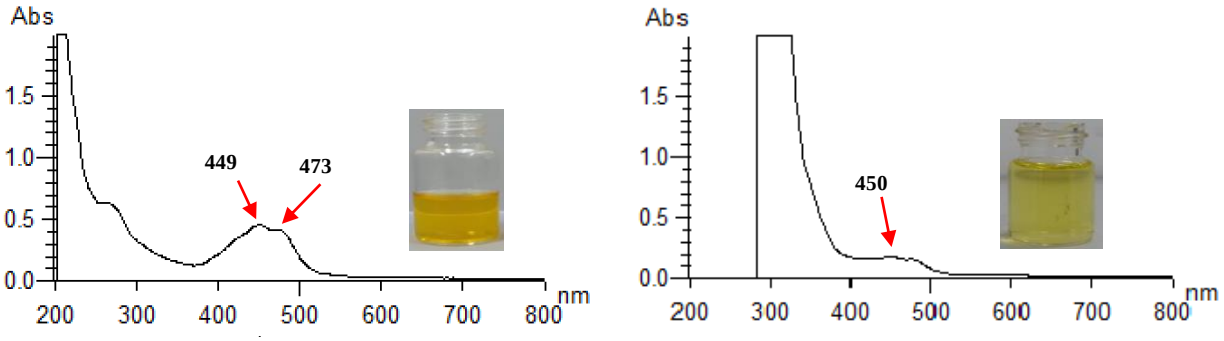


Figure 4. Antioxidant activities of methanol, ethyl acetate, chloroform, and *n*-hexane pigmented crude extracts against DPPH and ABTS radicals. Ascorbic acid and trolox were used as the positive control. Data are presented as the mean ± standard deviation (SD), n = 3



A **B**

Figure 5. Wavelength scans of (A) methanol and (B) *n*-hexane pigmented crude extract *Paracoccus haeundaensis* of SAB E11

Table 2. Antioxidant compounds in *Paracoccus haeundaensis* SAB E11 by LCMS/MS

Extract	RT (min)	Area (%)	Molecular formula	Ion (m/z)	Proposed identification	Fit conf (%)
methanol	9.96	9.97	$C_{14}H_{12}O_4$	245.0820	3,5,3',4'-Tetrahydroxystilbene	99.99
	5.23	1.79	$C_{10}H_8O_4$	195.0506	Scopoletin	98.95
	7.10	1.82	$C_{15}H_{12}O_4$	257.0818	Liquiritigenin	98.24
	9.10	1.04	$C_{11}H_{16}O_2$	181.1228	Dihydroactinidiolide	99.69
n-hexane	9.10	1.30	$C_{11}H_{16}O_2$	181.1228	Dihydroactinidiolide	99.69
	13.94	7.41	$C_{18}H_{34}O_3$	299.2591	Ricinoleic Acid	99.99

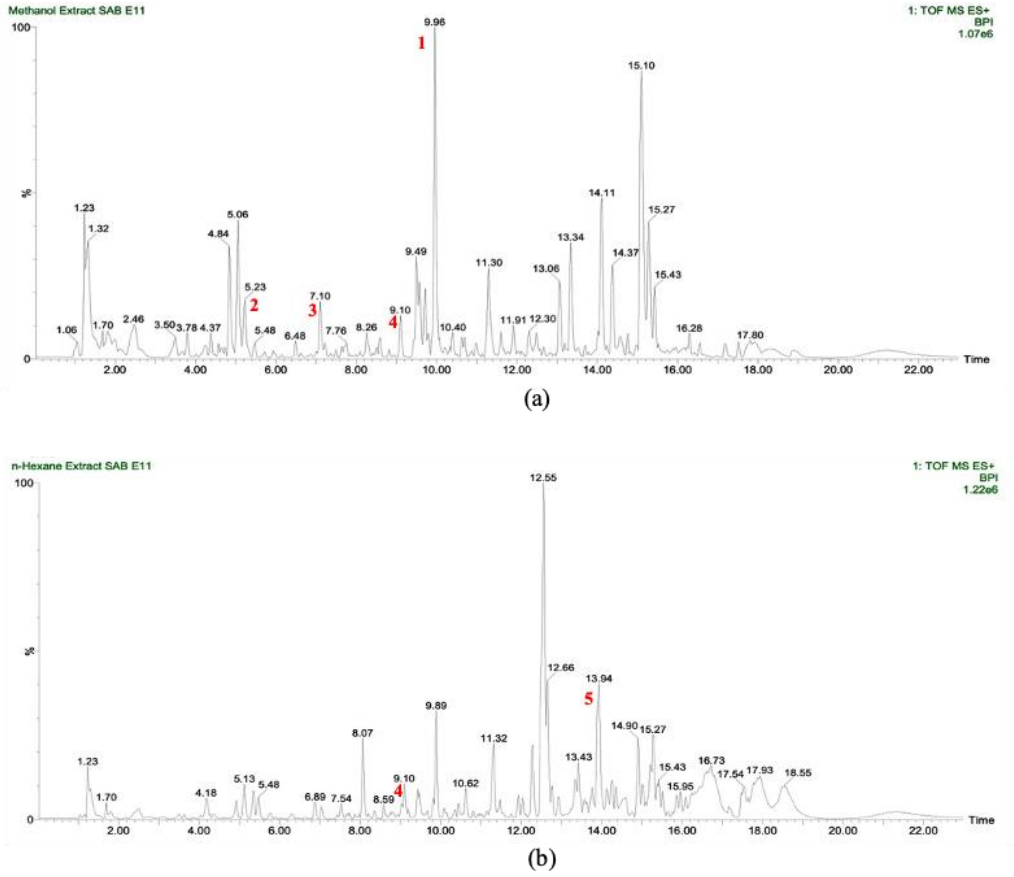
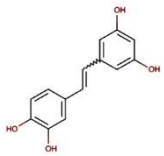
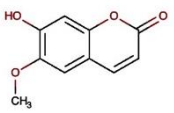
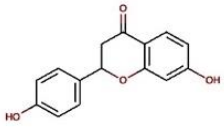
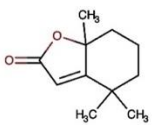
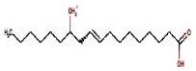


Figure 6. Base peak intensity (BPI) chromatogram of (a) methanol and (b) *n*-hexane pigmented crude extract of SAB E11. Peaks are labeled as follow, 1: 3,5,3',4'-tetrahydroxystilbene; 2: scopoletin; 3: liquiritigenin; 4: dihydroactinidiolide; 5: ricinoleic acid

Table 3. Compounds found in *Paracoccus haeundaensis* SAB E11 by LCMS/MS with antioxidant activity reported in previous study

Compound name	Compound structure	Compound group	Antioxidant activity	References
3,5,3',4'-Tetrahydroxystilbene		Stilbenes	DPPH radical scavenging activity	Osamudiamen et al. (2020)
Scopoletin		Coumarin	DPPH radical scavenging activity	Kübra et al. (2022)
Liquiritigenin		Flavonoid	Activation of SOD and GSH with inhibition of NOX2 and NOX4 expression	Shi et al. (2020)
Dihydroactinidiolide		Terpene	Increase of mRNA levels of Nrf2 and HO1	Das and Devi (2021)
Ricinoleic Acid		Fatty acid	DPPH radical scavenging activity	Park et al. (2020)

Discussion

In the present study, the marine *P. haeundaensis* SAB E11 produced a bright orange pigment observed from the color of the colonies. The molecular identification was performed using 16S rRNA gene sequence analysis. *Paracoccus haeundaensis* SAB E11 strain showed the closest homology to *P. haeundaensis* BC74171 with 99.41% similarity. Phylogenetically, *Paracoccus* is a genus belonging to α-Proteobacteria. *Paracoccus haeundaensis* BC74171 is a marine bacterium with characteristics of orange-pigmented, rod-shaped, aerobic, non-motile, positive catalase, and carotenoids-producing, mainly astaxanthin (Lee et al. 2004).

Based on the results of the *in-vitro* antioxidant capacity, it was found that the methanol-pigmented crude extract of *P. haeundaensis* SAB E11 demonstrated the best antioxidant activities against DPPH and ABTS radicals with IC_{50} values of 54.70±1.64 and 46.12±2.21 μg/mL, respectively (Figure 4). This results is exceeded the ethyl acetate extract derived from non-pigmented *Bacillus* sp. SAB-E 41 collected from the same sample as *P. haeundaensis* SAB E11 with IC_{50} value of 402.40±16.55 and 358.13±13.22 μg/mL for DPPH and ABTS assay (Prastya et al. 2018). Following a review of the literature, the antioxidant activity of *P. haeundaensis* BC74171 has been analyzed by DPPH assay using extracellular metabolic which capped with materials of gold nanoparticle (AuNPs). The data showed that scavenging activity was 73±3.0% at 320 μg/mL (Patil et al. 2019). Interestingly, the intracellular pigmented crude extract of *P. haeundaensis* SAB E11 presented superior antioxidant activities compare to the extracellular metabolic of *P. haeundaensis* BC74171. In addition, a study by Mesrian et al. (2021) stated that brownish-orange extract derived from marine actinomycetes *Micromonospora tulbaghiae* SCA54.P2 had lower scavenging activity against DPPH radicals compared to the orange pigment of *P. haeundaensis* SAB E11.

The colors of the pigmented crude extract of *P. haeundaensis* SAB E11 were determined by maximum wavelength. Measurement of it was carried out for the extracts with potential antioxidant activities. We detected the spectral pattern of the pigmented crude extracts at wavelengths between 200-800 nm, which showed the presence of peaks of maximum absorbance. Methanol and *n*-hexane pigmented crude extract of *P. haeundaensis* SAB E11 showed peaks at 449 and 450 nm. Previous research explained that the 400-500 nm region was considered the carotenoid fingerprint region (Sharma and Ghoshal 2020). The prominent peak in the methanol and *n*-hexane pigmented crude extract of *P. haeundaensis* SAB E11 was indicated as β-carotene based on the studies by Kaur et al. (2019) and Allahkarami et al. (2021).

LCMS/MS analysis was conducted for methanol and *n*-hexane pigmented crude extract of *P. haeundaensis* SAB E11. The present investigation was focused to determine the substances which have antioxidant activities. Based on the analysis, the crude pigment extract was rich in polyphenols, such as 3,5,3’,4’-tetrahydroxystilbene, scopoletin, and liquiritigenin. The other compounds were dihydroactinidiolide and ricinoleic acid, which in this case included terpenes and fatty acids, respectively. Some compounds found in *P. haeundaensis* SAB E11 have been reported to have antioxidant activity (Table 3).

The 3,5,3’,4’-Tetrahydroxystilbene, a synonym name of Piceatannol, had an enormous abundance among the other antioxidant compounds and showed the highest peak in the chromatogram of methanol pigmented crude extract of *P. haeundaensis* SAB E11. The molecule was also found in *Ensifer* sp. KSH1 (Gram-negative bacterium) and *Arthrobachter* sp. KSH3 (Gram-positive bacterium) isolated in a medium supplemented by resveratrol, phenol, or 4-hydroxyphenylacetic acid as a carbon source (Furuya et al. 2019). However, the antioxidant activity of the piceatannol production of both bacteria was not analyzed.

Scopoletin has been known as a *phytoalexin* found in several kinds of plants. The synthesis of scopoletin is correlated to stress conditions such as mechanical injury, drying, and resistance to microbial invasion (Gnonlonfin et al. 2012). Meanwhile, liquiritigenin is a chiral flavonoid with one or more stereogenic centers. Dihydroactinidiole is one of C11-terpene lactones which occur from the biological or degradation of carotenoids (Hamid et al. 2017). This molecule was found to be a minor compound in *P. haeundaensis* SAB E11. However, we found the compound in the methanol and *n*-hexane pigmented crude extract of *P. haeundaensis* SAB E11 at the same RT (9.10). It indicates that dihydroactinidiole can be extracted using polar and non-polar solvents. The last compound, ricinoleic acid, is the main component of castor oil extracted from *Ricinus communis* L. seeds, ranging from 87%-92% of all fatty acids in the oil (Pabiś and Kula 2016).

In conclusion, the LCMS/MS analysis revealed that the compounds contained in the pigment extract were dominated by phenolic compounds such as 3,5,3',4'-tetrahydroxystilbene, scopoletin, and liquiritigenin. Other compounds identified were dihydroactinidiolide and ricinoleic acid which included in terpenes and fatty acids, respectively. Thus, the results of the present study suggested that an orange-colored extract from the marine bacterium *P. haeundaensis* SAB E11 has a promising antioxidant activity.

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