HAYATI
Journal of Biosciences

Vol. 31 No. 1, January 2024 200-210
DOI:10.4308/hjb.31.1.200-210
ISSN: 1978-3019
EISSN: 2086-4094

Identification, Characterization and Antioxidant Activity of Yellowish-Orange Pigments Actinobacteria

Farhana Halimah Rusyda[1], Irmanida Batubara[2,3], Yulin Lestari[1,3*]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[3]*Tropical Biopharmaca Research Center, IPB University, Bogor 16128, Indonesia*

ARTICLE INFO

Article history:
Received June 2, 2023
Received in revised form August 5, 2023
Accepted August 10, 2023

KEYWORDS:
DPPH,
inhibitory concentration,
metabolite,
luteolin

ABSTRACT

Actinobacteria are Gram-positive bacteria that can produce various secondary metabolites, including pigments that have potential as antioxidants. This study assessed the characteristics of yellowish-orange pigmented actinobacteria. The antioxidant activity of their extract pigment was tested by DPPH and analyzed by LC-MS. The identification results based on the 16S rRNA gene showed that the HRA isolate had 99.91% similarity value to *Streptomyces gramineus* JR-43, the HVA isolate had 99.22% similarities value to *S. tendae* ATCC 19812 and the HVB isolate had 81.18% similarities value to *Rhodococcus ruber* DSM 43338. Meanwhile, AGM 2.2 and 2.3 isolates had similarities to *S. xanthophaeus* NRRL B-5414 with similarity values of 98.95% and 99.82%, respectively. The Inhibitory Concentration (IC_{50}) of actinobacterial crude extract pigments ranges from 53.38 µg/ml to 184.38 µg/ml. The HVB isolates with the major compound luteolin, have the highest antioxidant activity. The crude extract pigment of HVB isolates may consider rich in luteolin and has potency as an antioxidant.

1. Introduction

The tendency towards health problems is increasing, so non-toxic resources are widely considered in various industrial fields. Natural pigments are renewable, biodegradable, environmentally friendly, and known for their use in cosmetics, food ingredients, textile dyeing, and pharmaceutics (Shahid *et al.* 2013). Actinobacteria are Gram-positive bacteria with 51-70% G+C DNA content which can produce a wide variety of secondary metabolites such as pigments (Ventura *et al.* 2007; Fernandes *et al.* 2021). Actinobacteria can produce various types of pigments, including yellow to orange. Some familiar examples of yellowish-orange pigments are carotenoids, flexirubin, ankaflavin, and anthraquinones. Microorganisms' production pigments can be extracellularly or intracellularly (Aruldass *et al.* 2018). Pigments produced by bacteria have antioxidant capacity and protect bacteria from oxidative damage (Correa-Llantén *et al.* 2012).

* Corresponding Author
E-mail Address: yulinl@apps.ipb.ac.id

Antioxidants are compounds that can prevent free radical oxidation processes. The antioxidant activity can be determined by DPPH assay. DPPH (α, α-diphenyl-β-picrylhydrazyl) free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. In the DPPH assay, the compound or extract is mixed with the DPPH solution, and the absorbance is recorded after a certain period (Baliyan *et al.* 2022).

In recent, there have been several studies of antioxidant activity in actinobacteria in Indonesia, including the antioxidant activity of endophytic actinobacteria in mangosteen peel (Larasati *et al.* 2020), *Xylocarpus granatum* (Ariansyah *et al.* 2019; Ratte *et al.* 2022), *Eleutherine palmifolia* (Shabira *et al.* 2022). In addition, the study of pigmented bacteria has also been reported, including bacteria found in seaweed *Kappahycus alvarezii* (Arlita *et al.* 2013), mangrove sediment bacteria (Riyanti *et al.* 2019), and marine bacteria (Abubakar *et al.* 2022). However, to the best our knowledge studies related to the antioxidant activity of pigmented actinobacteria in Indonesia have rarely been reported. This study aimed to characterize pigmented actinobacteria, measure

the antioxidant activity of extracts pigments, and analyze the crude extracts pigment by LC-MS.

2. Materials and Methods

2.1. Sample Collection

Actinobacterial isolates were obtained from the mangrove rhizosphere at Cengkrong Beach of Trenggalek East Java, Indonesia, from red soil in the campus (IPB University) forest, and endophytic actinobacterial isolates from mangosteen peel identified by Larasati *et al.* (2020).

2.2. Isolation and Purification of Actinobacteria

Soil samples were dried by air drying or using the oven at 60°C for 2 hours. About 1 g of soil sample was suspended in 10 mL of 0.85% NaCl and incubated in shaking incubators at 120 rpm for 2 hours. The soil suspension was then diluted 10^{-1} to 10^{-6} with duplicates. 100 µL of soil suspension from each dilution was inoculated on humic acid vitamin agar (HV), added with 20 ppm nalidixic acid and 50 ppm nystatin, and then incubated at room temperature for 3 weeks. Isolates showing different colony morphological characters were purified on ISP 2 medium added with 20 ppm nystatin (Janatiningrum and Lestari 2022).

2.3. Morphological Characterization of Actinobacteria

Morphological characterization of pigment-producing actinobacteria was carried out by growing purified bacteria on four different media: ISP (International Streptomyces Project) 2, ISP 3, ISP 4, and YSA (Yeast Starch Agar). Characterization was carried out by identifying based on morphology colony and the aerial mycelium's pigmentation using a stereomicroscope. Actinobacterial spore chains were observed using a light microscope at 400× magnification (Larasati *et al.* 2020).

2.4. Molecular Identification of Actinobacteria

There were five isolates of actinobacteria with yellowish-orange pigment which were identified using the 16S rRNA gene. The total bacterial genome was isolated using a bacterial isolation kit (PrestoTM Mini Kit, Geneaid). Genomic DNA was amplified using Polymerase Chain Reaction (PCR) with 16S specific primers for actinobacteria 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Bruce *et al.* 1992) and 16Sact1114R(5'-GAGTTGACCCCGGCRGT-3') (Martina *et al.* 2008). PCR reactions were carried out in a total volume of 50 µL, containing 2.5 µL of GoTaq Green (Promega) DNA polymerase enzyme, 2.5 µL of each primer (10 pmol), 15 µL of nuclease-free water, and 5 µL of template DNA. The PCR conditions used were pre-denaturation (95°C, 2 minutes), denaturation (95°C, 30 seconds), annealing (55°C, 30 minutes), extension (72°C, 1 minute), and final extension (72°C, 7 min) for 30 cycles (Janatiningrum *et al.* 2018). The 16S rRNA gene sequence was compared to the sequence database in GenBank using the NCBI web. Phylogenetic trees were constructed using MEGA X software, bootstrap analysis of 1000 repetitions using the neighbour-joining method (Felsenstein 1985; Saitou and Nei 1987). Lactobacillus plantarum C88 has been incorporated as an outgroup in the phylogenetic reconstruction.

2.5. Pigment Extraction of Actinobacteria

Each agar culture from the five actinobacterial isolates was taken using a sterile plastic straw as many as 16 spheres (diameter = 0.7 cm), then put into 1,000 ml ISP2 liquid medium and incubated at room temperature using a rotary shaker at 100 rpm for 14 days. Then the liquid cultures of actinobacteria were centrifuged at 4,000 rpm for 30 minutes at 4°C. The pellets were added with methanol and then heated in water at 50°C. Finally, the methanol layer was evaporated with a temperature of 50°C.

2.6. UV-Vis Analysis of Crude Extract Pigment Actinobacteria

About 0.03 g crude pigment extract of actinobacterial pigment was dissolved in 3 ml methanol, and its spectrum was measured at a wavelength of 190-800 nm (Passos dan Saraiva 2019). The standard used for comparison is the UV Vis spectrum of 4 mg/ml β-carotene (Riyanti *et al.* 2019).

2.7. Antioxidant Activity Test of Crude Extract Pigment Actinobacteria

An antioxidant assay was conducted using the DPPH (2.2-diphenyl-1-picrylhydrazyl) method. The antioxidant activity test method was conducted based on Batubara *et al.* (2009). First, the DPPH solution was prepared by mixing 5 mg of DPPH and 100 ml of ethanol. 1 ml of each sample of the extract and ascorbic acid as the positive control were then reacted with 1 ml of 125 µM DPPH solution. The

absorbance of the mixed solution was measured using a spectrophotometer at a wavelength of 517 nm. Inhibition values are regressed to obtain Inhibitory Concentration (IC_{50}) values.

2.8. LCMS Analysis

The extract of pigment of actinobacteria was analysed by Ultra-Performance Liquid Chromatography (UPLC) coupled to a Xevo G2-S QToF-MS (Waters, USA) using electrospray ionization. Water + 5 mM Ammonium Formic (A) and Acetonitrile + 0.05 % Formic acid (B) was used for the mobile phase. 5 µL of the extract was injected into the LC column High Strength Silica (HSS) with type ACQUITY UPLC® HSS C18 (1.8 µm 2.1 × 100 mm). First, the mass spectrophotometry was determined with two-generation quadrupole time-of-flight mass spectrophotometry in positive ion mode. Then, the results were analysed using Masslynx 4.1 software.

2.9. Statistical Analysis

Data were statistically evaluated using one-way analysis of variance (ANOVA) by SPSS, while the significant difference was verified using the Tukey test at $P<0.05$.

3. Results

3.1. Diversity of Pigmented Actinobacteria

The five actinobacterial isolates used in this research work came from various habitats. HVA and HVB isolates were isolated from mangrove soil, HRA was from forest red soil in IPB university area, AGM 2.2 and AGM 2.3 were endophytes actinobacterial isolated from mangosteen peel.

Selected actinobacteria grow well in all media HRA, HVA, AGM 2.2 and AGM 2.3 isolates had dry colonies, aerial mycelium with a chalky texture, powdery and white to grey (Figure 1). Pigmentation of the mycelial substrate of actinobacterial isolates showed that the color of HRA isolates was dark yellow, HVA was bright yellow, HVB was orange, AGM 2.2 and AGM 2.3 were pale creams (Figure 2).

Based on microscopic observations, the HVB isolate rarely produce spores, while the other 4 actinobacterial isolates produced more spores. HVA, AGM 2.2 and AGM 2.3 isolates had spiral spore chains, while HRA isolates had straight to flexuous (rectiflexibiles) spore chain (Figure 3).

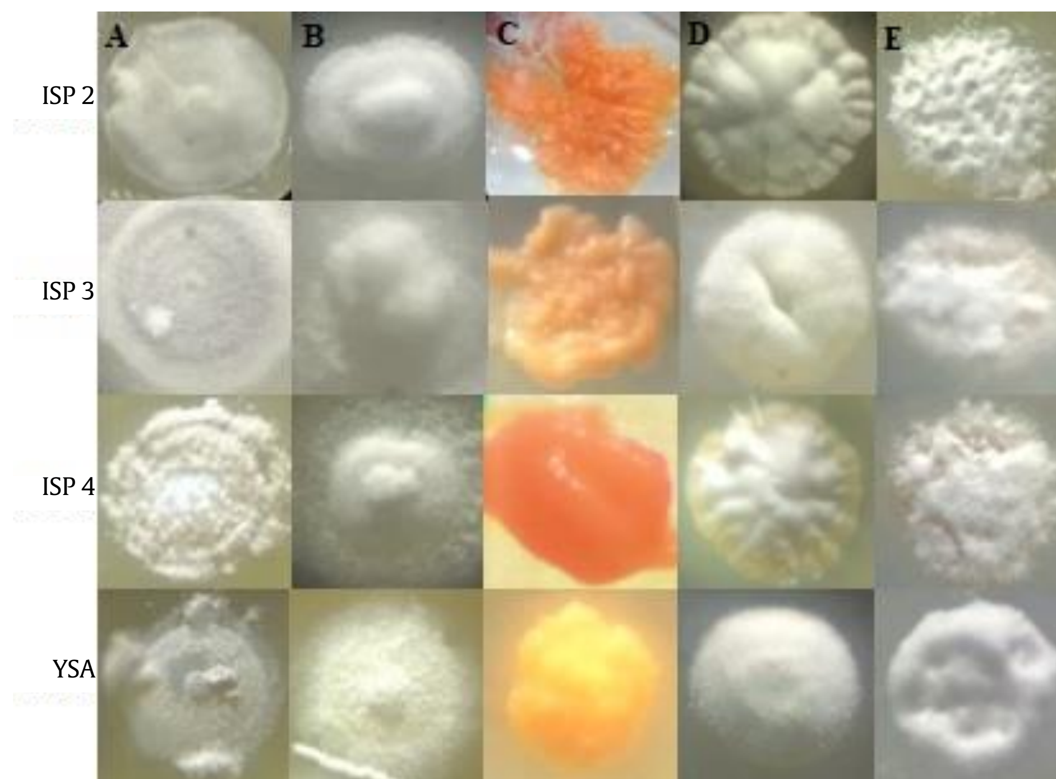

Figure 1. Pigmented actinobacteria morphology colonies in various media (top to bottom): ISP 2, ISP3, ISP4 and YSA. (A) HRA, (B) HVA, (C) HVB, (D) AGM2.2, (E) AGM2.3

Figure 2. Pigmentation of actinobacterial substrate mycelium aged 14 days on ISP 2 media: (A) HRA, (B) HVA, (C) HVB, (D) AGM 2.2, (E) AGM 2.3

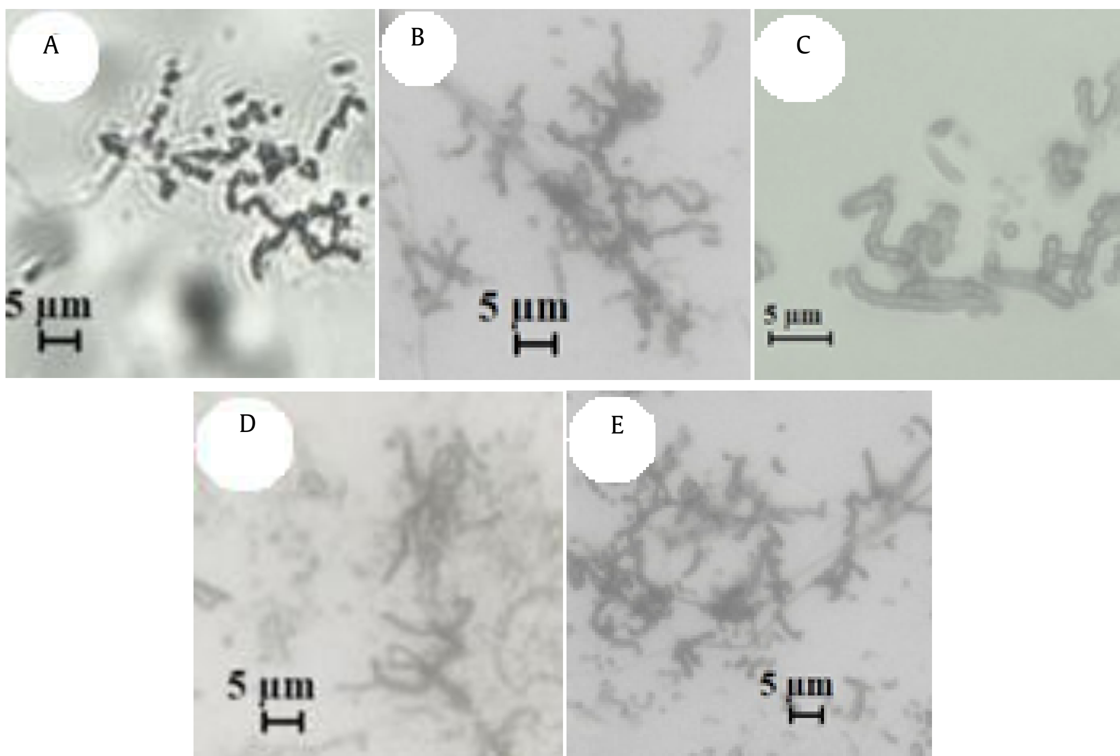

Figure 3. Spore chains and actinobacterial cell shape aged 14 days on ISP 2 media: (A) HRA, (B) HVA, (C) HVB, (D) AGM 2.2, (E) AGM 2.3

3.2. Molecular Identity Based on 16S rRNA Gene

The PCR products of the three isolates were detected as DNA fragments of 1,080 bp using 1% agarose gel electrophoresis (Figure 4).

BLAST analysis on the 16S rRNA gene of pigmented actinobacteria, as shown in Table 1, shows that the HRA isolate had a 99.91% similarity value to Streptomyces gramineus JR-43. The HVA isolate had 99.22% similarities value to *S. tendae* ATCC 19812. The HVB isolate had 81.18% similarities value to *Rhodococcus ruber* DSM 43338. While, 2 other isolates, i.e. AGM2.2 and AGM2.3 have been previously identified and show similarities to *S. xanthophaeus* NRRL B-5414 with similarity values of 98.95% and 99.82%, respectively. Bacteria *Lactobacillus plantarum* C88 have been incorporated as outgroups in phylogenetic reconstruction (Figure 5). The bar at the bottom of the Figure 5 provides a scale for the phylogenetic tree. In this case the line segment with the number 0.07 shows the length of branch that represents an amount genetic change of 0.07. On a phylogenetic tree, nodes represent the most recent common ancestor between two taxa. The numbers next to each node represent a measure of support for the node. These are given as percentages, where 100 represents maximal support.

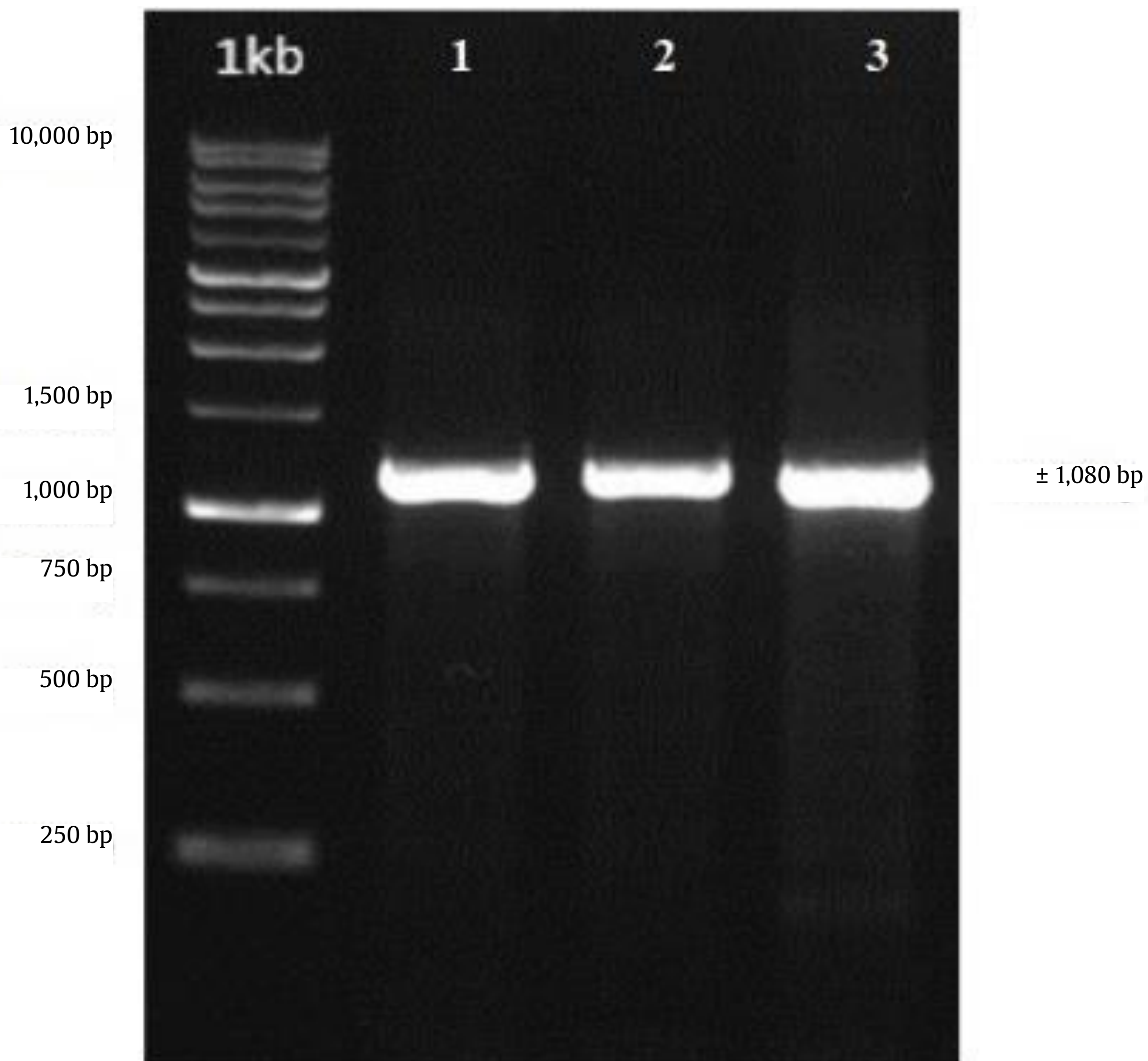

Figure 4. Results of PCR amplification (±1,080 bp) using actinobacteria specific primers 27F and 16Sact1114R, 1 kb marker, wells 1-3, namely HRA, HVA, HVB

Table 1. BLAST analysis on 16S rRNA gene of pigmented actinobacteria

Code	Accession number	Species	Strain	Similarity (%)
HRA	NR_109017.1	*Streptomyces gramineus*	JR-43	99.91
HVA	NR_025871.2	*Streptomyces tendae*	ATCC 19812	99.22
HVB	NR_026185.1	*Rhodococcus ruber*	DSM 43338	81.18

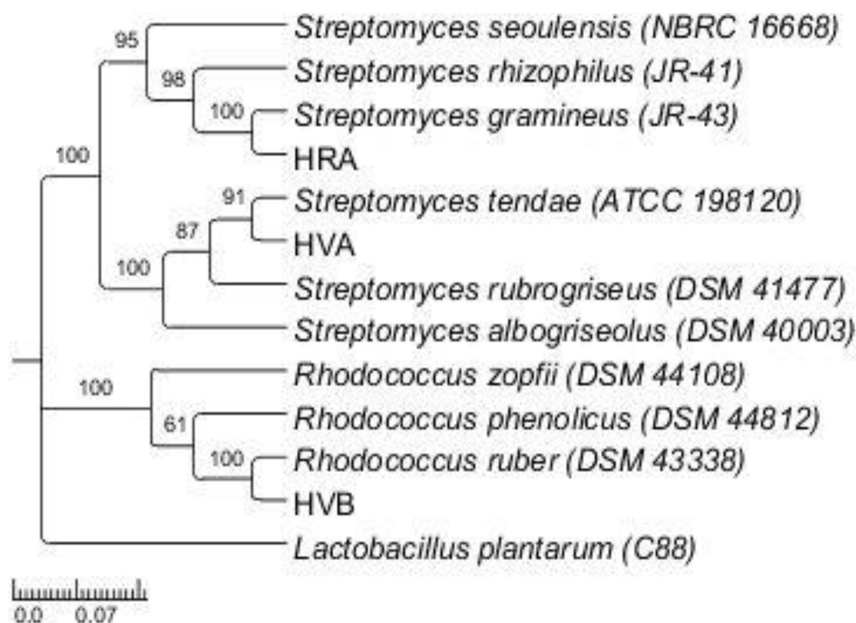

Figure 5. Phylogenetic tree of pigmented actinobacteria on 16S rRNA gene

3.3. Characterization of Crude Extract Pigment Actinobacteria

The UV-Vis spectrum of actinobacterial pigment crude extract was compared to the UV-Vis spectrum of the β-carotene standard. The maximum wavelength of β-carotene is 450 nm, while at crude pigment extract of HRA isolates was 411 nm; HVA isolates was 663 nm; HVB isolates 459 nm; AGM 2.2 isolate was 663 nm; and AGM 2.3 isolate was 664 nm (Figure 6).

3.4. Antioxidant Activity of Crude Extract Pigment of Actinobacteria

The activity of antioxidants was expressed in Inhibitory Concentration (IC_{50}) value. HVB isolate pigment extract had the highest antioxidant activity compared to the other isolate, but not as good as the ascorbic acid as a positive control (Table 2).

3.5. Crude Extract Pigment Characteristics

The HVB isolate pigment extract which had the highest antioxidant activity was further identified to determine the content of bioactive compounds contained in the pigment extract. LCMS analysis of the HVB isolate pigment extract is shown in Figure 7.

Based on LC-MS chromatogram of crude pigment extract of HVB isolate, there were 3-hydroxyhexadecanoic acid (peak 10.15), luteolin (peak 11.12), and n-desmethylclozapine (peak 12.02) detected. Based on the literature, all three compounds have antioxidant activity. Luteolin was the most dominant compound found in the pigment extract of HVB isolates. The characteristics of the major compound obtained from LC-MS analysis is shown in Table 3.

Table 2. IC_{50} value of actinobacterial pigment crude extract

Code	IC_{50} (μg/ml)
HRA	141.31±4.23[c]
HVA	54.72±1.76[b]
HVB	53.38±2.15[b]
AGM 2.2	184.38±5.13[e]
AGM 2.3	168.52±7.29[d]
Ascorbic acid	5.62±0.16[a]

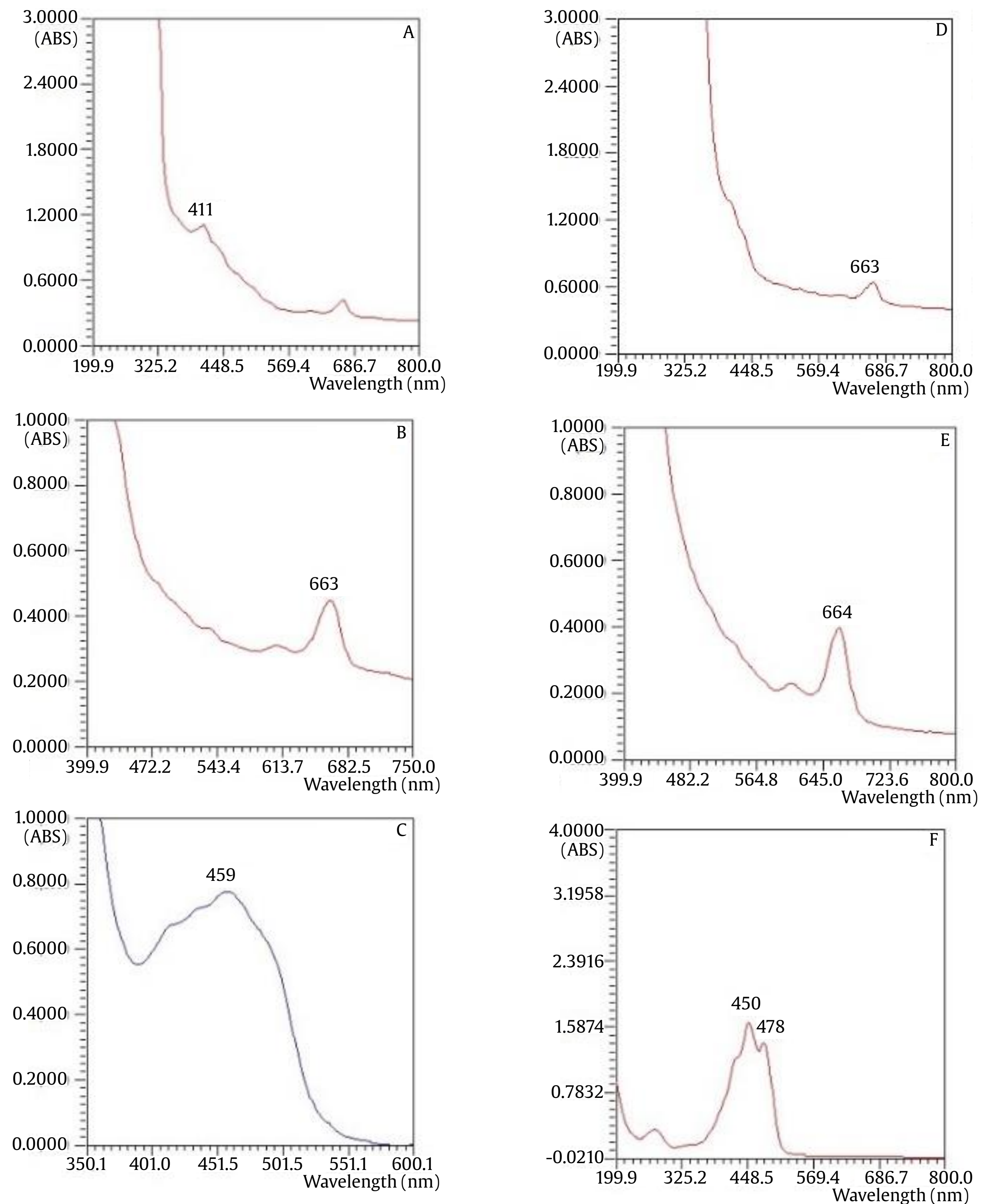

Figure 6. UV-Vis spectrum of actinobacterial pigment extract at a wavelength of 200-800 nm: (A) HRA, (B) HVA, (C) HVB, (D) AGM 2.2, (E) AGM 2.3, (F) β-carotene

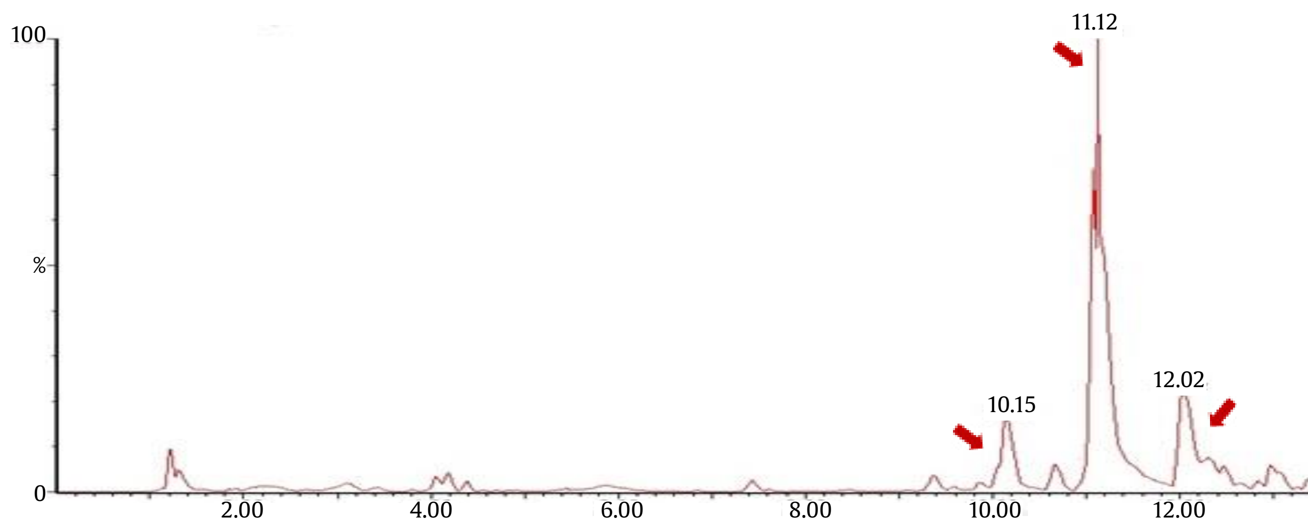

Figure 7. LC-MS chromatogram of crude pigment extract of HVB isolates

Table 3 Major compounds found in HVB isolate crude pigment extract by LC-MS analysis

Compound	Formula	Molecular weight (g/mol)	Retention time (min)	Biological activity (reference)
Luteolin	$C_{15}H_{10}O_6$	286.24	11.12	Antioxidant (Lin *et al.* 2008)
Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.42	10.15	Antioxidant (Lim *et al.* 2023)
N-Desmethylclozapine	$C_{17}H_{17}CIN_4$	312.80	12.02	Antioxidant (Caruso *et al.* 2020) and Antipsychotics (Natesan *et al.* 2006)

4. Discussion

Most of the actinobacteria colonies belonged to the genus of *Streptomyces*. *Streptomyces* characteristics rough surface colony, powdery textured, lumpy and granular, white or gray in color, and able to produce various kinds of pigments (Gebreyohannes *et al.* 2013). The difference in aerial mycelium and substrate mycelium colour is affected by the characteristics of species, source of carbon and nitrogen in the growth medium (Li *et al.* 2016). *Streptomyces* has a tiny spore and is composed of three types of spore chains, straight to flexuous (rectiflexibiles), open loop (Relinaculam-Apertum), and open or closed spiral (Spira) (Barka *et al.* 2016). HVA, AGM 2.2, and AGM 2.3 isolates had spiral spore chain types, while HRA isolates had rectiflexibiles spore chain types. HVB isolates have the characteristics of rod-shaped cells and reddish-orange color. Bacteria of the genus *Rhodococcus* have various colony surface characteristics, such as rough, smooth, and mucoid, and have various colony pigmentations, yellow, pink, salmon, orange, or red, and some variants are colourless (Bell *et al.* 1998; Yamshchikov *et al.* 2010).

Further identification using a molecular approach was conducted by 16S rRNA gene. Based on BLAST analysis, HRA, HVA, AGM 2.2, and AGM 2.3 isolates showed a closed relationship to *Streptomyces*, while the HVB isolate was closed to *Rhodococcus*. The phylogenetic tree results showed that HRA isolates had similarities with *S. gramineus* and HVA had similarities with *S. tendae*. While HVB isolates had low similarities with *R. ruber*, which may be further studied for its novelty. According to Larasati *et al.* (2020) AGM2.3 and AGM 2.3 isolates were in the same group and were closely related to *S. xanthophaeus*. As outgroup, *L. plantarum* C88 belong to gram-positive bacteria which has antioxidant activities (Zhang *et al.* 2013). *Streptomyces* is the largest genus in actinobacteria phylum and widely used in the health sector because it can produce various secondary metabolites. *Streptomyces gramineus* JR-43 has antibacterial activity against the pathogenic bacteria (Lee *et al.* 2012). *Streptomyces tendae* can produce ferulic acid (Ferreira *et al.* 2007). The antioxidant action mechanism of ferulic acid is complex, mainly based on inhibiting the formation of reactive oxygen species (ROS) and neutralize free radicals (Zduńska *et al.* 2018). *Streptomyces xantophaeus* has antioxidant

activity (Larasati *et al.* 2020). Apart from the genus *Streptomyces*, the genus *Rhodococcus* also belongs to the *Actinobacteria* phylum. Genus *Rhodococcus* is widely used in microbial biotechnology because of their metabolic versatility, ability to degrade of organic compounds, and resistance to various stress conditions (Cappelletti *et al.* 2020). *Rhodococcus ruber* is one of *Rhodococcus* that can produce carotenoids and have antioxidant activity (Zheng *et al.* 2013).

UV vis spectrum analysis was used to characterize the actinobacterial pigment extracts. β-carotene UV-Vis spectrum was 450 nm (Lichtenthaler and Buschmann 2001). UV Vis spectrum of crude extract pigment HRA isolate 411 nm and HVB isolate 459 nm categorized in carotenoids group. Carotenoids are diverse natural pigments and range their light absorption between 400 and 500 nm (Britton 1995). Three types of carotenoids that actinobacteria can produce are carotenoids of C50 chain length, carotenoids with aromatic end groups, and keto carotenoids like canthaxanthin or monocyclic keto-γ-carotene derivatives. Species from the genus *Rhodococcus* are the only known *Actinobacteria* with a simultaneous pathway to aromatic and to keto carotenoids (Sandmann 2021). The UV Vis spectrum of HVA 663 nm, AGM 2.2 663 nm and AGM 2.3 664 nm indicate the presence of flavonoids. According to Rani *et al.* (2016) and Omara *et al.* (2022) absorptions at 651, 660, 663, 664 and 665 nm indicate the presence of flavonoids. Flavonoids are a group of secondary metabolite compounds often found in plant and bacterial extracts. Endophytic bacteria can produce secondary metabolite such as those contained in their host plants by the horizontal gene transfer (HGT) (Tiwari and Bae 2020). HGT is known to have shaped the evolutionary history and diversity of actinobacteria. Even in species, recent genome-wide studies indicate that actinobacteria are not immune to HGT (Park *et al.* 2019). HGT can allow the transmission of genetic material between phylogenetically distant species or between cytoplasmic organelles (Nielsen *et al.* 1998; Gao *et al.* 2014).

Antioxidant activity was divided into 5 groups based on the IC_{50} value. The differences in activity are very active (<50 µg/ml), active (50-100 µg/ml), moderate (101-250 µg/ml), weak (250-500) µg/ml) and inactive (<500 µg/ml) (Jun *et al.* 2003). HVB isolated pigment extract had the highest DPPH antioxidant activity with an average IC_{50} value of 53.38 µg/ml. The HVB isolate was closed relationship to *Rhodococcus*. Carotenoids in *Rhodococcus* cells are thought to have an antioxidant role (Zheng *et al.* 2013). Other studies from Madhukar (2021) reported that the carotenoids found in the pigment extract of *R. kroppenstedtii* isolates have antioxidant activity 67.0±0.2 mg AA/g. Based on the results of statistical analysis, the average IC_{50} value of the five actinobacterial pigment extracts showed a significant difference ($P<0.05$) to ascorbic acid.

The chemical profile of crude extract pigment of HVB identified by LC-MS. Based on the result, the crude extract pigment of HVB was rich in luteolin ($C_{15}H_{10}O_6$). Luteolin is a secondary metabolite that belongs to the group of flavonoids with diverse biological activities, such as antioxidant, anticancer (Lin *et al.* 2008; Kang *et al.* 2017). According to Lin *et al.* (2008) multiple mechanisms may underlie luteolin's antioxidant effect including by ROS scavenger through its oxidation, inhibits ROS-generating oxidase, exerting its antioxidant effect by protecting or enhancing endogenous antioxidants, inhibiting the enzymes that catalyse oxidation of the cellular components and therefore inhibit lipo-oxygenase reaction.

In conclusion, the crude pigment extract from the five isolates has antioxidant activity, and HVB isolates produce the highest activity. The antioxidant activity of HVB isolates may be due to the presence of luteolin as its major compound of crude extract pigment.

References

Abubakar, H., Astuti, R.I., Listyowati, S., Batubara, I., Wahyudi, A.T., 2022. An orange pigment from the marine bacterium *Paracoccus haeundaensis* SAB E11 as a prospective source of natural antioxidants. *Biodiversitas*. 23, 4730–4737. https://doi.org/10.13057/biodiv/d230940

Ariansyah, A., Batubara, I., Lestari, Y., Egra, S., 2019. Antioxidant and antiglycation activity of rhizosphere and endophytic actinobacteria of *Xylocarpus granatum*. *Biosaintifika*. 11, 202–210. https://doi.org/10.15294/biosaintifika.v11i2.20018

Arlita, N.R., Radjasa, O.K., Santoso, A., 2013. Identifikasi pigmen karotenoid pada bakteri simbion rumput laut *Caulerpa cupressoides* (Vahl) C. Agardh. *Diponegoro Journal of Marine Research*. 2, 68–77. https://doi.org/10.14710/jmr.v2i3.3134

Aruldass, C.A., Dufossé, L., Ahmad, W.A. 2018. Current perspective of yellowish-Orange pigments from microorganisms-a review. *Frontiers in Sustainable Food Systems*. 180, 168–182. https://doi.org/10.1016/j.jclepro.2018.01.093

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P., Chang, C.M., 2022. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa. Molecules*. 27, 1-19. https://doi.org/10.3390/molecules27041326

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J.P., Klenk, H.P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of *Actinobacteria. Microbiology and Molecular Biology Reviews*. 80, 1–43. https://doi.org/10.1128/mmbr.00044-16

Batubara, I., Mitsunaga, T., Ohashi, H. 2009. Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. *Journal of Wood Science*. 55, 230–235. https://doi.org/10.1007/s10086-008-1021-1

Bell, K.S., Philp, J.C., Aw, D.W.J., Christofi, N., 1998. A review: the genus *Rhodococcus. Journal of Applied Microbiology*. 85, 195–210. https://doi.org/10.1046/j.1365-2672.1998.00525.x

Britton, G., 1995. Structure and properties of carotenoids in relation to function. *FASEB Journal*. 9, 1551–1558. https://doi.org/10.1096/fasebj.9.15.8529834

Bruce, K.D., Bruce, K.D., Hiorns, W.D., Hobman, J.L., Hobman, J.L., Osborn, A.M., Osborn, A.M., Strike, P., Strike, P., Ritchie, D.A., 1992. Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. *Applied and Environmental Microbiology*. 58, 3413–3416.

Cappelletti, M., Presentato, A., Piacenza, E., Firrincieli, A., Turner, R.J., Zannoni, D., 2020. Biotechnology of *Rhodococcus* for the production of valuable compounds. *Applied Microbiology and Biotechnology*. 104, 8567–8594. https://doi.org/10.1007/s00253-020-10861-z

Caruso, G., Grasso, M., Fidilio, A., Tascedda, F., Drago, F., Caraci, F., 2020. Antioxidant properties of second-generation antipsychotics: focus on microglia. *Pharmaceuticals*. 13, 1–20. https://doi.org/10.3390/ph13120457

Correa-Llantén, D.N., Amenábar, M.J., Blamey, J.M., 2012. Antioxidant capacity of novel pigments from an antarctic bacterium. *Journal of Microbiology*. 50, 374–379. https://doi.org/10.1007/s12275-012-2029-1

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39, 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fernandes, C.J., Doddavarapu, B., Harry, A., Dilip, S.P.S., Ravi, L., 2021. Isolation and identification of pigment producing actinomycete *Saccharomonospora azurea* SJCJABS01. *Biomedical and Pharmacology Journal*. 14, 2261–2269. https://doi.org/10.13005/bpj/2326

Ferreira, P., Diez, N., Faulds, C.B., Soliveri, J., Copa-Patiño, J.L., 2007. Release of ferulic acid and feruloylated oligosaccharides from sugar beet pulp by *Streptomyces tendae. Bioresource Technology*. 98, 1522–1528. https://doi.org/10.1016/j.biortech.2006.06.004

Gao, C., Ren, X., Mason, A.S., Liu, H., Xiao, M., Li, J., Fu, D., 2014. Horizontal gene transfer in plants. *Functional and Integrative Genomics*. 14, 23–29. https://doi.org/10.1007/s10142-013-0345-0

Gebreyohannes, G., Moges, F., Sahile, S., Raja, N., 2013. Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*. 3, 426–435. https://doi.org/10.1016/S2221-1691(13)60092-1

Janatiningrum, I., Solihin, D.D., Meryandini, A., Lestari, Y., 2018. Comparative study on the diversity of endophytic actinobacteria communities from *Ficus deltoidea* using metagenomic and culturedependent approaches. *Biodiversitas*. 19, 1514–1520. https://doi.org/10.13057/biodiv/d190443

Janatiningrum, I., Lestari, Y., 2022. Enzyme production, antibacterial and antifungal activities of actinobacteria isolated from *Ficus deltoidea* Rhizosphere. *Biodiversitas*. 23, 1950–1957.https://doi.org/10.13057/biodiv/d230429

Jun, M., Fu, H.Y., Hong, J., Wan, X., Yang, C.S., Ho, C.T., 2003. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). *Journal Food Science*. 68, 2117–2122. https://doi.org/10.1111/j.1365-2621.2003.tb07029.x

Kang, K.A., Piao, M.J., Ryu, Y.S., Hyun, Y.J., Park, J.E., Shilnikova, K., Zhen, A.X., Kang, H.K., Koh, Y.S., Jeong, Y.J., Hyun, J.W., 2017. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). *Journal Food Science*. 51, 1169–1178. https://doi.org/10.3892/ijo.2017.4091

Larasati, F., Batubara, I., Lestari, Y., 2020. The presence of endophytic actinobacteria in mangosteen peel (*Garcinia mangostana*) and its antioxidant activity. *Biodiversitas*. 21, 1488–1497. https://doi.org/10.13057/biodiv/d210429

Lee, H.J., Han, S.I., Whang, K.S., 2012. *Streptomyces gramineus* sp. nov., an antibiotic-producing actinobacterium isolated from bamboo (*Sasa borealis*) rhizosphere soil. *International Journal of Systematic and Evolutionary Microbiology*. 62, 856–859. https://doi.org/10.1099/ijs.0.030163-0

Li, Q., Chen, X., Jiang, Y., Jiang, C., 2016. *Morphological Identification of Actinobacteria,* third ed. IntechOpen, Rijeka. https://doi.org/10.5772/61461

Lichtenthaler, H.K., Buschmann, C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*. 1, 31-38. https://doi.org/10.1002/0471142913.faf0403s01

Lim, M.W., Yow, Y.Y., Gew, L.T., 2023. LC-MS profiling-based non-targeted secondary metabolite screening for deciphering cosmeceutical potential of Malaysian algae. *J Cosmet Dermatol*. 22, 2810-2815. https://doi.org/10.1111/jocd.15794

Lin, Y., Shi, R., Wang, X., Shen, HM., 2008. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Current Cancer Drug Targets*. 8, 634–646. https://doi.org/10.2174/156800908786241050

Madhukar, C.V., 2021. Antimicrobial and antioxidant potentials of carotenoid pigment produced by indigenous novel soil isolate *Rhodococcus kroppenstedtii. World Journal of Environmental Biosciences*. 10, 29–34. https://doi.org/10.51847/9qrsrjytn2

Martina, K., Jan, K., Tamás, F., Ladislav, Č., Marek, O., Geneviève, L.G., Yvan, M.L., Markéta, S.M., 2008. Development of a 16S rRNA gene-based prototype microarray for the detection of selected actinomycetes genera. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*. 94, 439–453.https://doi.org/10.1007/s10482-008-9261-z

Natesan, S., Reckless, G.E., Barlow, K.B.L., Nobrega, J.N., Kapur, S., 2006. Evaluation of *N*-desmethylclozapine as a potential antipsychotic—preclinical studies. *Neuropsychopharmacol*. 32, 1540–1549. https://doi.org/10.1038/sj.npp.1301279

Nielsen, K.M., Bones, A.M., Smalla, .K, van Elsas, J.D., 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria–a rare event?. *FEMS Microbiology Reviews.* 22, 79–103. https://doi.org/10.1111/j.1574-6976.1998.tb00362.x

Omara, T., Kiprop, A.K., Kosgei, V.J., 2022. Isolation andcharacterization of compounds in ethanolic extract of *Albizia coriaria* (Welw ex. Oliver) leaves: a further evidence of its ethnomedicinal diversity. *Bulletin of the National Research Centre.* 46, 30. https://doi.org/10.1186/s42269-022-00716-0

Park, C.J., Smith, J.T., Andam, C.P., 2019. Horizontal gene transfer and genome evolution in the phylum *Actinobacteria*, in: Villa, T.G., Vinas, M. (Eds.), *Horizontal Gene Transfer.* Springer Nature, Switzerland, pp. 155-174. https://doi.org/10.1007/978-3-030-21862-1_5

Passos, M.L., Saraiva, M.L.M.F.S., 2019. Detection in UV-visible spectrophotometry: detectors, detection systems, and detection strategies. *Measurement: Journal of the International Measurement Confederation*. 135, 896–904. https://doi.org/10.1016/j.measurement.2018.12.045

Rani, N., Sharma, S., Sharma, M., 2016. Phytochemical analysis of *Meizotropis pellita* by FTIR and UV-VIS spectrophotometer. *Indian Journal of Science and Technology*. 9, 1–4. https://doi.org/10.17485/ijst/2016/v9i31/94875

Ratte, M., Batubara, I., Lestari, Y., 2022. Morphological characterization and antioxidant activity of actinobacteria from *Xylocarpus granatum* growing in mangrove habitat. *Biotropika.* 10, 1–10. https://doi.org/10.21776/ub.biotropika.2022.010.01.01

Riyanti., Rahmawati, M., Hidayati, N.V., Syakti, A.D., Radjasa, O.K., 2019. Analysis of carotenoids and the identification of mangrove sediment bacteria of segara anakan, Cilacap. *Ilmu Kelautan.* 23, 163–170. https://doi.org/10.14710/ik.ijms.23.4.163-170

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* 4, 406–425.

Sandmann, G., 2021. Carotenoid biosynthesis in the phylum *Actinobacteria. Advances in Experimental Medicine and Biology*. 1261, 175-181. https://doi.org/10.1007/978-981-15-7360-6_14

Shabira, A.P., Tjahjoleksono, A., Lestari, Y., 2022. Endophytic actinobacteria of *Eleutherine palmifolia* as antioxidant producer. *Biodiversitas.* 23, 4209–4215. https://doi.org/10.13057/biodiv/d230844

Shahid, M., Shahid-Ul-Islam., Mohammad, F., 2013. Recent advancements in natural dye applications: a review. *Journal of Cleaner Production*. 53, 310–331.https://doi.org/10.1016/j.jclepro.2013.03.031

Tiwari, P., Bae, H., 2020. Horizontal gene transfer and endophytes: an implication for the acquisition of novel traits. *Plants.* 9, 305. https://doi.org/10.3390/plants9030305

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F., van Sinderen, D., 2007. Genomics of actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol. Mol. Biol. Rev*. 71, 495–548.https://doi.org/10.3390/plants903030510.1128/mmbr.00005-07

Yamshchikov, A.V., Schuetz, A., Lyon, G.M., 2010. *Rhodococcus equi* infection. *The Lancet Infectious Diseases*. 10, 350–359. https://doi.org/10.3390/plants903030510.1016/S1473-3099(10)70068-2

Zduńska, K., Dana, A., Kolodziejczak, A., Rotsztejn, H., 2018. Antioxidant properties of ferulic acid and its possible application. *Skin Pharmacology and Physiology*. 31, 332–336. https://doi.org/10.3390/plants903030510.1159/000491755

Zhang, L., Liu, C., Li, D., Zhao, Y., Zhang, X., Zeng, X., Yang, Z., Li, S., 2013. Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. *International Journal of Biological Macromolecules.* 54, 270–275. https://doi.org/10.3390/plants903030510.1016/j.ijbiomac.2012.12.037

Zheng, Y.T., Toyofuku, M., Nomura, N., Shigeto, S., 2013. Correlation of carotenoid accumulation with aggregation and biofilm development in *Rhodococcus* sp. SD-74. *Analytical Chemistry.* 85, 7295–7301. https://doi.org/10.3390/plants903030510.1021/ac401188f