Methanol pigment extracts derived from two marine actinomycetes exhibit antibacterial and antioxidant activities

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Abstract. Mesrian DK, Purwaningtyas WE, Astuti RI, Hasan AEZ, Wahyudi AT. 2021. Methanol pigment extracts derived from two marine actinomycetes exhibit antibacterial and antioxidant activities. Biodiversitas 22: 4440-4447. In recent years, natural pigments produced by marine microorganisms, especially actinomycetes, have become an important source of medically valuable drugs and other active compounds. Based on that crucial fact, this study aimed to determine the toxicity, antimicrobial, and antioxidant activities of methanolic pigment extract derived from marine actinomycetes HV11.P3 and SCA54.P2. Both extracts exhibited high toxicity activity based on Brine Shrimp Lethality Test (BSLT). The results showed the lethal concentration 50% (LC₅₀) values of actinomycetes HV11.P3 and SCA54.P2 pigment crude extracts were 94.60 µg/mL and 131.22 mg/mL, respectively. The antimicrobial activity of those extracts was also tested against several microbes and showed the best results for inhibiting against Bacillus subtilis and Escherichia coli. Furthermore, we assessed the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the results showed the inhibitory concentration 50% (IC₅₀) values of actinomycetes HV11.P3 and SCA54.P2 pigment crude extracts were 231.08 µg/mL and 369.3 µg/mL respectively. By using GC-MS analysis, we identified chemical compounds of actinomycete pigment crude extracts, revealing that the most dominant compounds were 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- (CAS) (44.65%) for HV11.P3 and 5-Methoxyxypyrrolidin-2-one (30.23%) for SCA54.P2. The 16S rRNA gene sequence analysis showed that actinomycete HV11.P3 and actinomycete SCA54.P2 had the closest similarity to Micromonospora chalcea strain 1464-217L and Micromonospora tubaquisina strain TVU1, respectively. This study revealed that two pigment crude extracts derived from actinomycetes HV11.P3 and SVA54.P2 had antibacterial, antioxidant, and toxicity activities. The actinomycete SCA54.P2 pigment crude extract had the best activity as an antibacterial against E. coli ATCC 8739, while actinomycete HV11.P3 pigment crude extract had the best activity as an antioxidant.

Keywords: Actinomycetes, antimicrobial, antioxidant, pigment, toxicity

INTRODUCTION

In recent years, various problems in the health sector have emerged and attracted the attention of the world. These include resistance to several types of pathogens microbial and increased deaths due to infection with degenerative diseases (Prestinaci et al. 2015; Kharirie and Andriani 2020). Resistance occurs when bacteria, viruses, fungi, and parasites change continuously and reduce the effectiveness of the commonly used antimicrobial agent (Founou et al. 2017). Furthermore, infections that are difficult to treat will increase the risk of disease spread, disease severity, and mortality which have a long-term impact (Roope et al. 2019). Chronic bacterial, viral, and fungal infections might be causative factors for inflammatory and worsen degenerative diseases. Therefore, the discovery of new antimicrobial compounds should be considered in the present and the future.

Degenerative diseases as a result of cell damage due to the aging process or various other factors that affect the number of free radicals in the body (Sahardi and Makpol 2019). The accumulation of free radicals will initiate multiple diseases such as Alzheimer's, Parkinson's, atherosclerosis, cardiovascular disorders, hypertension, and diabetes mellitus type 2 (Stambler 2017). Naturally, the human body can reduce free radicals in a certain amount by antioxidant compounds that have been formed in the body. However, if oxidative stress occurs, which is an imbalance between the number of free radicals and the number of antioxidants in the body, the body needs an external source of antioxidants to prevent the oxidation of the essential constituents of cells (Al-Snafi 2017). The other bioactive compound that can act as a tool against degenerative disease is the toxic compound. It has been proven that the toxic compound tested with the brine shrimp lethality test (BSLT) has a good correlation with cytotoxic activity in some solid human tumors (Mclaughlin et al. 1998).

Actinomycetes are Gram-positive, filamentous aerobic bacteria with high G+C composition (Gong et al. 2018; Bhakayashree and Kannabiran 2018). Actinomycetes isolated from terrestrial were extensively researched, leading to the rediscovery of natural products that have been reported. On the other hand, marine actinomycetes are primarily a new resource of secondary metabolites including terpenes, peptides, sterols, fatty acids, alkaloids, amino acids and their derivatives (Liao et al. 2016; Pudi et al. 2016). The marine environment is capable of producing all of these compounds due to the enormous biodiversity.
and diverse environmental conditions (Bibi et al. 2020). Actinomycetes associated with marine sponges such as *Micromonospora* sp, have recently been used to produce many bioactive compounds with novel molecular scaffolds and significant pharmacological activities (Hifnawy et al. 2020). Thus, the genus *Micromonospora* is one of the prolific producer of secondary metabolites, especially as bioactive compounds.

The genus *Micromonospora* is a member of the *Micromonosporaceae* which includes 32 genera. This genus is found in various geographic environments such as soils, mangrove sediments, plants, marine habitats, and extreme environments (Trujillo et al. 2015; Hifnawy et al. 2020). More than 740 strains of *Micromonospora* have been identified to produce various bioactive compounds, one of the bioactive compound comes from the pigment (Berdy 2005). *Micromonospora* colonies have carotenoid pigments including yellow, orange, red, purple, brown, or black (Sineva et al. 2021). Some *Micromonospora* species with their soluble pigments are *M. chalcea* (yellow), *M. halophytica* (red-brown), *M. olivasterospora* (olive-green), *M. purpureochromoge* (dark-brown), and *M. rosaria* (wine-red) (Genilloud 2015). In the previous study, we have isolated two pigmented actinomycetes from marine sponges, namely actinomycetes HV11.P3 and SCA54.P2 (unpublished data). To determine the potency of pigment crude extract, this study aimed to test the antimicrobial, antioxidant, and toxicity abilities of the pigment methanol extracts produced by the marine actinomycetes HV11.P3 and SCA54.P2.

**MATERIALS AND METHODS**

**Material**

Two pigmented marine actinomycetes, namely actinomycete HV11.P3 (greenish-black) and actinomycete SCA54.P2 (orange) used in this study had been isolated from sponges collected from Pramuka Island, Kepulauan Seribu, Indonesia (5°44'46.3 S 106° 36'35.7 E). Five microbes were used for the antimicrobial test, namely *Bacillus subtilis* ATCC 19659, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231, which were obtained from IPB Culture Collection, IPB University (Bogor, Indonesia).

**Procedures**

**Pigment extraction**

The extraction of pigments refers to method described by Dawoud et al. (2020) with slight modifications. Each of Actinomycete was cultured in 1 L of ISP-4 broth medium (10 g Soluble Starch, 1 g MgSO₄·7H₂O, 1 g NaCl, 1 g K₂HPO₄, 2 g (NH₄)₂SO₄, 2 g CaCO₃, 0.001 g FeSO₄·7H₂O, 0.001 g MnCl₂·4H₂O, and 0.001 g ZnSO₄·7H₂O) and agitated continuously in orbital shaker at 100 rpm for 14 days at room temperature. The actinomycetes cultures were then centrifuged at 6000 rpm for 15 minutes. The cell biomass were then added with 1 L of methanol as a solvent and then heated at 50°C for 15 minutes in water bath until the cell became colorless. After that, the methanol phase was separated with the cell biomass by centrifugation at 6000 rpm for 15 minutes. The methanol solvent was evaporated using a rotary evaporator at 50°C. The crude extracts were collected and dissolved in 10% dimethyl sulfoxide (DMSO), and then stored at 4°C for further assay.

**Toxicity test**

The toxicity test was performed using Brine Shrimp Lethality Test (BSLT) according to Meyer et al. (1982). Twenty *Artemia salina* larvae were placed in each vial containing 4 mL of seawater and pigment crude extracts in 0, 10, 100, 250, 500, 750, and 1000 µg/mL. The vials containing larvae and pigment extracts were incubated under a light condition for 24 hours at room temperature. The dead larvae were counted, and the percentage of mortality was calculated using the following formula:

\[ \text{% Mortality} = \left( \frac{\text{Σ Sample larvae mortality} - \text{Σ Control larvae mortality}}{\text{Σ Total larvae}} \right) \times 100 \]

A linear regression model and probit analysis were used to determine the LC₅₀ value of each extract.

**Antimicrobial test**

The antimicrobial activity was performed using the disc diffusion method. The pigment extracts were tested against several clinical isolates. *B. subtilis* ATCC 19659, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, and *S. aureus* ATCC 6538 were inoculated into a Mueller-Hinton Agar medium, meanwhile *C. albicans* ATCC 10231 was inoculated into a Potato Dextrose Agar medium. A paper disc (6 mm) containing various concentrations of the extracts (250, 500, 750, and 1000 µg/mL) was placed on the surface of agar plate medium. Tetracycline (100 µg/mL) was used as a positive control and 10% dimethyl sulfoxide (DMSO) was used as a negative control. The plates were incubated for 24 hours at room temperature, and the diameter of inhibition zones formed was measured.

**Antioxidant test**

The antioxidant activity of the pigment extracts was determined using DPPH radical according to Batubara et al. (2009) with modification. Five hundred microliters of each extract at various concentrations (0, 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL) were mixed with 500 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (0.125µM in methanol). The mixture was incubated at room temperature for 30 minutes in the dark condition, then the absorbance of the sample was measured using a spectrophotometer at 514 nm (Thermo Electronics-Genesis 20, Thermo Fisher Scientific, USA). Ascorbic acid was used as a positive control. The percentage of inhibition was calculated using the following formula:

\[ \text{% Inhibition} = \left[ \frac{1}{(\text{Sample absorbance} - \text{Control absorbance})} \right] \times 100 \]

A linear regression model was used to determine the IC₅₀ value of each extract.
Chemical compound analysis

The pigment extract of the isolate HV11.P3 and SCA54.P2 was analyzed using Gas Chromatography in Agilent 5977B GC/MSD (Agilent Technologies, USA). 0.6 μL of extract solution diluted in methanol was injected into a column with HP-5MS column type (30 m x 250 μm x 0.25 μm). The initial oven temperature was 80°C and gradually increased at 15°C/min until 300°C and held for 20 minutes. Helium gas was used as a carrier at a 1 mL/min flow rate. The injection temperature was 300°C and the aux temperature was maintained at 300°C. The result was analyzed by the GC-MS Pyrolysis program (WILLEY9THN 08. L).

Molecular identification

The genomic DNA of Actinomycete HV11.P3 and SCA54.P2 were extracted using Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) following the protocol’s guidelines. The 16S rRNA gene (1480 bp) were amplified using primers 20F (5’- CAGGCCCTAACACATGCAAGTC-3’) dan 1500R (5’- GGCGGWTGTACAAAGGC-3’) (Weisberg et al. 1991). The PCR was performed using MyTaq™ HS Red Mix (Bioline, United Kingdom). The PCR conditions were pre-denaturation at 95°C for 2 min, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and post extension at 95°C for 7 min. PCR products were visualized using 1% (w/v) gel agarose electrophoresis and the DNAs were sequenced in First BASE (Selangor, Malaysia). The sequences were aligned to National Centre or Biotechnology Information (NCBI) database using Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/), and the phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis X (MEGA X) neighbor joining p-distance model with 1000 bootstrap replication.

RESULTS AND DISCUSSION

The pigment extraction

The Actinomycete HV11.P3 and SCA54.P2 showed greenish-black and orange colonies on ISP-4 agar medium at seven days of incubation at room temperature. The HV11.P3 and SCA54.P2 were cultured in ISP-4 broth medium and incubated for 14 days on a rotary shaker prior to the pigment extraction. The pigment crude extracts showed greenish-black for HV11.P3 and brownish-orange for SCA54.P2 (Figure 1). The pigment extract yield of both actinomycete HV11.P3 and SCA54.P2 were 0.23% and 0.18%, respectively (Table 1).

Toxicity of pigment extracts

Based on the BSLT test, both of the pigment extracts of actinomycete HV11.P3 and SCA54.P2 showed toxicity activity against Artemia salina. The toxicity was expressed as LC₅₀ value. LC₅₀ value is a standard for the lowest concentration that can kill 50% of test organisms (A. salina). The lower of LC₅₀ value indicated the better of toxicity. The LC₅₀ of actinomycete HV11.P3 and SCA54.P2 pigment extracts were 94.53 μg/mL and 131.22 μg/mL, respectively (Table 2).

Figure 1. The colonies appearance of actinomycetes on ISP-4 agar medium at 7 days of incubation at room temperature and the pigment crude extract yielded after 14 days of culturing in ISP-4 broth medium: A. Actinomycete HV11.P3; B. Actinomycete SCA54.P2
Table 1. The pigment extract yield derived from Actinomycete HV11.P3 and SCA54.P2.

| Pigment Extract  | Culture Volume (L) | Weight (g) | Yield (%) |
|------------------|--------------------|------------|-----------|
| Actinomycete HV11.P3 | 1                  | 2.33       | 0.23      |
| Actinomycete SCA54.P2 | 1                  | 1.82       | 0.18      |

Table 2. The toxicity of actinomycyte HV11.P3 and SCA54.P2 pigment extracts based on Brine Shrimp Lethality Test

| Pigment extract | /* */ | Replication 1 | Replication 2 | Replication 3 | Average |
|-----------------|-------|---------------|---------------|---------------|---------|
| Actinomycete HV11.P3 |       | 88.13         | 80.04         | 115.42        | 94.53 ± 15.14 |
| Actinomycete SCA54.P2 |       | 156.96        | 102.72        | 133.98        | 131.22 ± 22.23 |

Table 3. The antimicrobial activities of actinomycyte HV11.P3 and SCA54.P2 pigment extracts (750 μg/mL) against several tested microbes

| Pigment extract | * | Bacillus subtilis ATCC 19659 | Escherichia coli ATCC 8739 | Pseudomonas aeruginosa ATCC 15422 | Staphylococcus aureus ATCC 6538 | Candida albicans ATCC 10231 |
|-----------------|---|-----------------------------|-----------------------------|----------------------------------|-------------------------------|----------------------------|
| Actinomycete HV11.P3 |   | +                           | +                           | +                                | -                             | -                          |
| Actinomycete SCA54.P2 |   | +                           | ++                          | +                                | -                             | -                          |
Note: 0.1-5 mm (+), 6-10 mm (++), and 11-15 (+++)

Table 4. The antioxidant activities of actinomycyte HV11.P3 and SCA54.P2 pigment extracts against DPPH as free radical

| Pigment extract | * | Replication 1 | Replication 2 | Replication 3 | Average |
|-----------------|---|---------------|---------------|---------------|---------|
| Actinomycete HV11.P3 |   | 243.40        | 224.07        | 225.78        | 231.08 ± 8.74 |
| Actinomycete SCA54.P2 |   | 436.77        | 373.95        | 297.18        | 369.3 ± 57.08 |

Antimicrobial activities of pigment extracts

The pigment extracts of actinomycetes were tested against both Gram-positive and Gram-negative bacteria, as well as yeast. Based on the antimicrobial test, the pigment extract of actinomycyte HV11.P3 and SCA54.P2, exhibited antimicrobial activity against several tested microbes. The HV11.P3 pigment extract possessed positive activities against *B. subtilis*, *E. coli*, and *S. aureus*. On the other hand, SCA54.P2 pigment extract could inhibit *B. subtilis*, *E. coli*, and *P. aeruginosa* activity (Table 3). No inhibition zone was observed for both of those extracts against *C. albicans*.

Antioxidant activities of pigment extracts

Both of the pigment extracts derived from actinomycete HV11.P3 and SCA54.P2 showed scavenging activity against DPPH as free radical. Actinomycete HV11.P3 and SCA54.P2 pigment extracts exhibited an IC₅₀ value of 231.08 μg/mL and 369.30 μg/mL, respectively (Table 4). IC₅₀ is a standard value for the lowest concentration that can inhibit 50% activity of free radicals.

Chemical compound identification

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used for investigating the dominant extract compound. The dominant compound in actinomycete HV11.P3 pigment extract was 1H-Purine-2,6-dione,3,7-dihydro-1,3,7-trimethyl- (Figure 2; Table 5), while for actinomycete SCA54.P2 pigment extract the dominant compound was 5-Methoxyxypyrroloidin-2-one (Figure 3; Table 6).

Molecular identification

Amplification of 16S rRNA genes from actinomycete HV11.P3 and SCA54.P2 genome showed 1480 bp band after visualization by gel electrophoresis (Figure 4). Based on analysis of 16S rRNA gene sequences of the two actinomycetes exhibited the same closest similarity with *Micromonospora* genera (Table 7). The constructed phylogenetic tree of the two 16S rRNA sequences of the two actinomycetes showed their position among other actinomycetes. Actinomycete HV11.P3 showed the closest similarity with *Micromonospora chalcea* strain 1464-217L, while actinomycete SCA54.P2 showed the closest similarity with *Micromonospora tullbaghiae* strain TVU1 (Figure 5).
**Figure 2.** Chromatogram of chemical compounds of pigment extract derived from actinomycete HV11.P3 based on GC-MS analysis showing five dominant compounds as indicated by red arrows.

**Figure 3.** Chromatogram of chemical compounds of pigment extract derived from actinomycete SCA54.P2 based on GC-MS analysis showing five dominant compounds as indicated by red arrows.

**Table 5.** Five major chemical compounds of actinomycete HV11.P3 pigment extract and their biological activities.

| Chemical compound                                      | Peak area (%) | Activity                          | Reference                      |
|--------------------------------------------------------|---------------|-----------------------------------|--------------------------------|
| 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- (CAS)| 44.65         | Anti-inflammation                 | Rezaei et al. (2021)           |
| Cyclotrisiloxane, hexamethyl- (CAS)                    | 13.20         | Antimicrobial                     | Keskin et al. (2012)           |
| n-Hexadecanoic acid (CAS)                              | 11.10         | Antimicrobial, antioxidant,       | Krishnaveni et al. (2014)      |
| Octadecanoic acid (CAS)                                | 4.46          | Antibacterial                     | Pradheesh et al. (2017)        |
| 9-Octadecenoic acid                                    | 3.61          | Anti-hyperglycaemic               | Kapoor et al. (2019)           |
Our results are in line with the previous study that revealed these compounds have potent antibacterial and antibiotics effectivity. However, some antioxidant agents were reported to have potent antibacterial activity. The mechanisms involved in this process have been described previously (Naqvi et al. 2019; Dwyer et al. 2014). Interestingly, both our crude extracts also showed antioxidant and antibacterial activity. Our findings were consistent with research done using Streptomyces sp. VITSTK7 that possessed both antibacterial and antioxidant activities (Thenmozhi and Kannabiran 2012).

Furthermore, to understand the possible chemical compounds involved in their biological activities, we identified the chemical composition of the pigment extracts by GC-MS analysis. Some of those compounds include benzenes, alcohols, esters, fatty acids, and amino acids groups. The presences of n-Hexadecanoic acid (CAS), Octadecanoic acid (CAS), and 9,12,15-Octadecadienoic acid (Z.Z.Z) in our present study may be the main compounds that play a role in inhibiting microbial growth. Our results are in line with the previous study that revealed these compounds can inhibit the growth of bacteria such as E. coli (Gram-negative bacteria), S. aureus (Gram-positive bacteria), and the fungus Aspergillus flavus (Krishnaveni et al. 2014). Meanwhile, n-Hexadecanoic acid (CAS) may also play a significant role along with 5-Methoxypyrrolidin-2-one which may act as an antioxidant agent (Dascalu et al. 2020).

### Table 6. Five major chemical compounds of actinomycete SCA54.P2 pigment extract and their biological activities.

| Chemical compound                        | Peak area (%) | Activity               | Reference                      |
|------------------------------------------|---------------|------------------------|--------------------------------|
| 5-Methoxypyrrolidin-2-one                | 30.23         | Antioxidant            | Dascalu et al. (2020)          |
| 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-(CAS) | 24.27         | Anti-inflammation      | Rezaei et al. (2021)           |
| n-Hexadecanoic acid                      | 12.93         | Antibacterial          | Krishnaveni et al. (2014)      |
| 9,12,15-Octadecadienoic acid (Z.Z.Z)     | 12.26         | Antibacterial          | Ojinnaka et al. (2015)         |
| 4H-Pyran-4-one, 2-hydroxy-3-methyl-(CAS) | 5.05          | Anti-proliferative, antioxidant | Amatori et al. 2010            |

### Table 7. Molecular identification of the actinomycetes isolates based on the 16S rRNA gene

| Isolate                  | Closest relative          | Query cover (%) | E-Value | Percent identification (%) | Accession Number |
|--------------------------|---------------------------|-----------------|---------|---------------------------|------------------|
| Actinomycete HV11.P3     | Micromonospora chalcea    | 99              | 0.0     | 97.9                      | NR_036795.1      |
| Actinomycete SCA54.P2    | Micromonospora tulbaghiae | 100             | 0.0     | 100                       | NR_116241.1      |

### Figure 4. Gel electrophoresis of 16S rRNA genes amplified from actinomycete genomes. 1: actinomycete HV11.P3; 2: actinomycete SCA54.P2; M: Marker 1 Kb ladder

**Discussion**

In our study, HV11.P3 and SCA54.P2 were found to be capable of producing intracellular pigments that were greenish-black and orange, respectively. Based on the BSLT test, pigment extract of HV11.P3 and SCA54.P2 showed a high in vivo toxicity effect with an LC50 value of less than 150 µg/mL. BSLT test was used as preliminary test to search for an anticancer agent (Elsyana et al. 2016). Therefore, further study to evaluate the cytotoxic activity against human cells need to be done. Promising antimicrobial abilities will lead to great activity of toxicity (Pandit et al. 2018). Some compound such as antimicrobial peptide (AMP) has been found to have dual action as antimicrobial as well as cytotoxic activity (Felicio et al. 2017). Actinomycete SCA54.P2 and HV11.P3 pigment crude extracts showed a low to moderate activity against the tested bacteria. Both of the extracts could inhibit the Gram-positive and Gram-negative of the tested bacteria. However, none of these extracts had activity against C. albicans as a representative of the eukaryotic cell. Pigments derived from actinomycetes have been gaining much attention due to their therapeutic potencies, one of them is antimicrobial activities (Mumtaz et al. 2018).

In order to investigate their antioxidant activity, both of our pigment extracts were tested for their in vitro antioxidant activities. Our results revealed that actinomycete HV11.P3 and SCA54.P2 pigment extracts had IC50 value of 23.08 µg/mL and 369.30 µg/mL against DPPH radicals, respectively. The antioxidant may decrease the oxidative stress level on the cell. The low level of free radical is undoubtedly reduced the possibility of cell damage and antibiotics effectivity. However, some antioxidant agents were reported to have potent antibacterial activity. The mechanisms involved in this process have been described previously (Naqvi et al. 2019; Dwyer et al. 2014). Interestingly, both our crude extracts also showed antioxidant and antibacterial activity. Our findings were consistent with research done using Streptomyces sp. VITSTK7 that possessed both antibacterial and antioxidant activities (Thenmozhi and Kannabiran 2012).
Identification of the two actinomycete isolates was performed based on the 16S rRNA gene sequence. As a result, both actinomycetes belong to the genus *Micromonospora*. The actinomycete HV11.P3 showed a similarity percentage of 97.9% with *Micromonospora chalcea* strain 1464-217L, and actinomycete SCA54.P2 showed a 100% similarity percentage with *Micromonospora talbaghiae* strain TVU1. The genus *Micromonospora* is known to be able to produce various types of bioactive compounds such as oligosaccharides, terpenes, and lanthipeptides that have therapeutic activity as antibacterial (Back et al. 2021), antitumor and antioxidant (Nafie et al. 2021), antifungal (Zhao et al. 2010), and antivirus (Debbab et al. 2010).

Thus, the present results revealed that methanol extract of pigments derived from two actinomycetes identified as *Micromonospora chalcea* HV11.P3 and *Micromonospora talbaghiae* SCA54.P2 showed antibacterial, antioxidant, and toxicity activities. The best antibacterial activity of the pigment extract was shown by *M. talbaghiae* SCA54.P2 against *E. coli* ATCC 8739, while the *M. chalcea* HV11.P3 pigment extract showed the best activity as an antioxidant.

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