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# Endophytic actinobacteria of *Eleutherine palmifolia* as antioxidant producer

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**Abstract.** *Shabira AP, Tjahjoleksono A, Lestari Y. 2022. Endophytic actinobacteria of* Eleutherine palmifolia *as antioxidant producer. Biodiversitas 23: 4209-4215.* Endophytic actinobacteria can generate bioactive compounds similar to those produced by the host plant. The *Eleutherine palmifolia* (L.) Merr is an example of a widely utilized host plant in the Dayak community. Flavonoids, which have antioxidant effects, are among the bioactive compounds found in *E. palmifolia*. The antioxidant activity of bioactive compounds produced by endophytic actinobacteria of *E. palmifolia* remains unidentified. The aim of this study was to examine the antioxidant activity of bioactive compounds produced by endophytic actinobacteria of *E. palmifolia*. Antioxidant activity from isolates actinobacteria was determined quantitatively using the DPPH and ABTS methods. Identification of the types of bioactive compound in the samples was identified using TLC assay and bioautography test. Analysis of flavonoid levels was performed using colorimetric aluminum chloride ($AlCl_3$). The result showed that the extraction of six isolates of endophytic actinobacteria with ethyl acetate solvent had different yields. The extract of endophytic actinobacteria EPB 6 isolate produced the highest (0.013%) yield. The extract of endophytic actinobacteria EPB 3 isolate had the best antioxidant activity, with 53.85 mg/mL (DPPH) and 42.43 mg/mL (ABTS). The extract of endophytic actinobacteria EPB 3 isolate and *E. palmifolia* had the same blue spots in TLC analysis, indicating the presence of bioactive compounds flavonol and flavones groups. The bioautography test of EPB 3 isolate endophytic actinobacteria extract produced yellow color, indicating qualitatively antioxidant activity. The total flavonoid content of the EPB 3 isolate extract was also higher (25.30 QE/mg) than the other five isolates, while it was 46.03 QE/mg in *E. palmifolia* extract. It is clear from the present study that the endophytic actinobacteria extract can produce antioxidants.

**Keywords:** Antioxidant, endophytic actinobacteria, *Eleutherine palmifolia*, flavonoid

**Abbreviations**: ABTS: 2,2-azinobis-3-ethyl benzothiazoline-6-sulphonic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, $IC_{50}$: Half-maximal inhibitory concentration, TLC: Thin-layer chromatography, QE: Quercetin equivalen

## INTRODUCTION

Actinobacteria are gram-positive bacteria that vary in shape and size, have 70% guanine-cytosine in their genome, and are wide spread due to their ability to survive in diverse environments, including the soil, rhizosphere, and internal parts of plant tissues (Singh and Dubey 2015). Endophytic actinobacteria live as colonies in plant tissues and protect the host plant from diseases. Endophytic actinobacteria are also harmless to the host plant. Endophytic actinobacteria can produce bioactive chemicals similar to those produced by their hosts. These bioactive chemicals are known to have various properties (including antioxidant activity, antibiotics, and immunosuppressants), making them suitable for human consumption (Tan and Zou 2001; Millker et al. 2012; Singh and Dubey 2018). The antioxidant can counteract free radicals from the environment to prevent disorders in the human body, like reduced respiratory function, premature ageing, blood vessel obstruction, tumors, and breast cancer. The number of free radicals that enter the human body is not proportional to the number of available antioxidants, allowing it to cause excess free radicals (Ganie et al. 2013; Ahmadinejad et al. 2017). DPPH and ABTS are two commonly used techniques to measure antioxidant activity. The DPPH method can describe the body's defensive mechanism against free radicals, whereas ABTS approach is typically used to test the antioxidant activity of food ingredients. In DPPH method, antioxidant uses an electron transfer mechanism to give hydrogen to stabilize DPPH radicals, while in ABTS approach, it uses protons to stabilize ABTS radicals (Re et al. 1999; Prakash et al. 2001). Antioxidant activity indicates a value of 50% inhibitory capacity ($IC_{50}$). The lower the IC50 value of bioactive compound, the better its antioxidant activity. Artificial and natural antioxidants are the two types of antioxidants that help counteract free radicals. Natural antioxidants have fewer side effects than artificial antioxidants. Plants that produce flavonoids are examples of natural antioxidants including anthocyanins, flavonols, and isoflavones (Panche et al. 2016). Flavonoids are renowned for their antioxidant properties and biochemical effects on human health issues. The aluminum chloride colorimetric method is commonly used to determine the quantity of flavonoids in a bioactive compound. This approach works by forming bonds between flavonoids in the tested substances with $AlCl_3$ to produce color (Zuraida et al. 2017).

Flavonoid compounds can be extracted from various plant parts and are most commonly found in fruit and leaves. The bulbs of *E. palmifolia* contain flavonoids, quercetin, eleutherine, and gallic acid (Kumar and Pandey 2013; Kamarudin et al. 2020). *Eleutherine palmifolia* is found in Central Kalimantan at less than 700 masl. *Eleutherine palmifolia* is characterized by its lack of woodiness, 30 to 40 cm height, white blooms, and dark green linear leaves (Galingging 2007). The people in Kalimantan believe that *E. palmifolia* helps treat dysentery, skin abscess, diabetes Mellitus, hypertension, and breast cancer (Febrinda et al. 2013). The usage of *E. palmifolia* as traditional medicine has prompted research into bioactive chemicals with antioxidant activity in *E. palmifolia*, such as research conducted Lestari et al. (2019), that reported antioxidant activity and anticancer potential of *E. palmifolia*. Several previous studies reported the presence of endophytic microbes such as *Bacillus* and *Nitrosococcus* in *E. palmifolia*. In addition, endophytic molds, such as *Aspergillus* and *Geotrichum* were isolated from leaves of *E. palmifolia* (Reckow 2016; Arfah 2019). However, isolation of endophytic actinobacteria from *E. palmifolia* has not been previously reported. Information about the antioxidant activity of bioactive compounds from endophytic actinobacteria of *E. palmifolia* also is not widely known. Therefore, it is necessary to conduct research concerning the potential of endophytic actinobacteria of *E. palmifolia* as a producer of antioxidants. The aim of this study was to provide information on the use of endophytic microorganisms as well as the development of potential endophytic microorganisms with natural antioxidants as medicinal substances for the future pharmaceutical industry in Indonesia.

## MATERIALS AND METHODS

### Research materials

Endophytic actinobacterial isolates were isolated from *E. palmifolia* bulbs. *E. palmifolia* were obtained from Palangkaraya, Central Kalimantan. Endophytic actinobacteria were grown in humic acid-vitamin agar (HVA) media for 30 days and purified in inorganic salt starch agar (ISP 4) (Kalalinggi 2022).

### Extraction method

*Extraction of endophytic actinobacteria bioactive compounds*

The starter cultures were produced from six endophytic actinobacteria isolates rejuvenated on the ISP 2 agar medium. In addition, starter culture was generated by transferring one sphere of endophytic actinobacteria isolate with a diameter of 9 mm into 10 mL of ISP 2 liquid medium and shaked at 100 rpm for five days. The production culture was then created by mixing 10% of the starter culture inoculum with 1000 mL ISP 2 liquid media. In a shaker, the cells were incubated for ten days.

Cell biomass and supernatant in culture media were separated at 4000 rpm for 30 minutes at 4°C (Lertcanawanichakul et al. 2015). Liquid-liquid extraction was performed by adding ethyl acetate solvent in a 1:1 (v/v) ratio to the supernatant. The mixture was shaken for 30 minutes and transferred to a separating funnel until two layers were formed. The liquid-liquid extraction was repeated twice on the bottom layer generated. The top layer was then evaporated until a concentrated paste-like extract was formed.

*Extraction of* Eleutherine palmifolia *bioactive compounds*

In an oven, thin slice of *E. palmifolia* bulbs were dried at 50°C for 24 hours. Next, the dry samples were mashed with a blender until they became simplicia. 100 grams of *E. palmifolia* simplicia was taken and macerated in 500 mL of ethyl acetate for 2×24 hours (Laksmitawati 2019). Maceration was performed until a clear solution was obtained. The solution then evaporated until a concentrated paste-like extract was not formed.

### Antioxidant activity analysis

*Antioxidant activity determined by DPPH method*

Antioxidant activity of *E. palmifolia* extract and extracts of six endophytic actinobacteria isolates was measured using DPPH techniques with three replications. In DPPH method, each extract was first dissolved with methanol to make 1000 ppm solution, then diluted into five different concentration. Each sample solution was pippeted 100 µL and mixed with 100 µL of 125 µM DPPH solution (Batubara et al. 2009). Samples were then incubated in darkroom for 30 minutes. The absorbance of the samples was measured at a wavelength of 517 nm. The ascorbic acid was used as a standard solution and quantified using similar procedur mentioned in DPPH method. The antioxidant activity was identified using the following calculation:

$$\text{Inhibition (\%)} = 1 - \frac{AA - AC}{AB - AC} \times 100\ \%$$

Where: AA = absorbance of extract samples or ascorbic acid + DPPH; AB = absorbance of ethanol + DPPH; AC = absorbance of ethanol.

*Antioxidant activity determined by ABTS method*

The ABTS technique followed the previous method of Re et al. (1999) with little modification to analyze the antioxidant activity of *E. palmifolia* extract and extracts of six endophytic actinobacteria isolates. In ABTS method, 20 µL of extract sample was mixed with 180 µL ABTS solution. ABTS solution (7 mM) was reacted with 2.45 mM potassium peroxydisulfate ($K_2S_2O_8$), incubated for 16 hours and the absorbance was measured. The ABTS solution mixture is ready to use when the absorbance value reach 0.68. The samples were incubated in the darkroom for 30 minutes, and the absorbance of the samples was recorded at a wavelength of 734 nm. Ascorbic acid was prepared at a concentration of 100 ppm as a standard solution and quantified using similar procedur mentioned before. The antioxidant activity was measured using the following formula:

$$\text{Inhibition (\%)} = 1 - \frac{AA - AC}{AB - AC} \times 100\ \%$$

Where: AA = absorbance of extract samples or ascorbic acid + DPPH; AB = absorbance of ethanol + DPPH; AC = absorbance of ethanol.

**Thin-layer chromatography (TLC) assay**

0.004 g of six actinobacteria extracts and *E. palmifolia* extract were dissolved in 1 mL of methanol solvent (Prastya 2019). Extracts from the endophytic actinobacteria isolate with the highest $IC_{50}$ value was used. Endophytic actinobacteria isolate extract, and *E. palmifolia* extract were applied on $G_{60}$ $F_{254}$ silica gel plates measured at 10 × 2 cm and allowed to dry at room temperature. Sample extract was applied using an applicator such as a syringe (CAMAG® Linomat 5) then Rf value was analyzed using CAMAG® Reprostar 3 and WinCATS software. The mobile phase was the combination of methanol and chloroform eluents. *Eleutherine palmifolia* extract, and endophytic actinobacteria isolate extract has an methanol:chloroform ratio of 1:9 (v/v). The eluent was saturated for 30 minutes before use. The TLC plate was placed in the container containing the eluent. TLC plate was removed and dried once the eluent has reached the boundary line. The number of eluted plates was counted at the wavelengths of 254 nm and 366 nm.

**Bioautography antioxidant test**

The TLC plate used for thin-layer chromatography examination was sprayed with 5 mM DPPH solution and incubated in a dark environment for 30 minutes. For bioautography test, 2.5 mg DPPH dissolved with 50 mL ethanol. A yellow spot on the purple TLC plate showed that the tested extract had antioxidant activity. The Rf value was analyzed by detecting spot at UV light with wavelengths of 254 nm and 366 nm. The spot was detected at UV 254 nm and 366 nm, then compared its Rf value with the spot in the previous chromatogram.

**Total flavonoid content**

Each actinobacteria isolate extract was dissolved in DMSO (dimethyl sulfoxide), while *E. palmifolia* extract was dissolved in methanol solvent. The total flavonoid content was measured using the aluminum chloride ($AlCl_3$) colorimetric test as described by Mayur et al. (2010). A total of 60 µL ethanol was added to 10 µL of sample extracts, and 110 µL distilled water was added next. 10 µL of 10% $AlCl_3$ was added after five minutes of incubation, and 10 µL potassium acetate ($CH_3COONa$) 1M was added in the sixth minute. Then 120 µL of distilled water was added too. The mixture was homogenized and incubated at room temperature for 30 minutes. The sample absorbance was measured at a wavelength of 415 nm. The standard solution of quercetin was made at a concentration of 100 ppm stock solution, then diluted into several different concentration series using a similar procedure previously mentioned.

**Data analysis**

Test result data was calculated with Microsoft Excel to help determine the value of antioxidant activity. The test data were statistically analyzed using a one-way analysis of variance (ANOVA). The Games-Howell test with a 5% significance level was employed in further research to find the actual difference between treatments.

## RESULTS AND DISCUSSION

**Free radical scavenging activity**

In this study, six actinobacterial isolates were isolated from *E. palmifolia* bulbs. Each isolate was named EPB 1, EPB 2, EPB 3, EPB 4, EPB 5, and EPB 6. The percentage of extract yield influences the extract quality. The yield percentage of *E. palmifolia* was 0.070%, and the yield percentage of six *E. palmifolia* endophytic actinobacteria isolates ranged from 0.006 to 0.013% (Table 1). *E. palmifolia* and six endophytic actinobacterial isolates extract produced by the maceration extraction method. Extraction was carried out using ethyl acetate solvent. Various factors such as solvent type, temperature, and the solvent ratio can affect the extraction results. Some previous studies have shown that ethyl acetate is commonly used in the extraction process (Prastya 2019; Larasati et al. 2020). The cultures were extracted using ethyl acetate as a solvent because it can bind a wide range of bioactive compounds, polar and non-polar. Kalalinggi (2022) reported that the supernatant from endophytic actinobacteria isolates of *E. palmifolia* bulb had antioxidant activity of 38.8% to102.6%. The isolates used were the same as those used in present study. Identification of the isolates carried out in the form of morphological characterization. Molecular identification based on 16S rRNA gene has not yet been carried out. Before use, the isolates were cultured first until they reached the stationary phase. Since the endophytic actinobacteria had entered the stationary phase and had decreased nutrition during this period, the endophytic actinobacteria produced extra secondary metabolites to survive (Swarnalatha et al. 2015). The yield of endophytic actinobacteria extract obtained in this investigation ranged from 0.006% to 0.013%. The higher the yield obtained from an extract, the more bioactive compounds it has. But, the amount of product does not represent the antioxidant activity, therefore, antioxidant activity test was performed further.

The inhibition value obtained was calculated to determine the inhibitory capacity $IC_{50}$. The activity of sample extract in reducing 50% of free radical toxicity was expressed by $IC_{50}$ (Banjarnahor and Artanti 2014). The average $IC_{50}$ values of endophytic actinobacteria extract ranged from 53.85 to 100.28 mg/mL, while the average $IC_{50}$ value of *E. palmifolia* extract was 49.11 ppm in DPPH test (Table 2). The average $IC_{50}$ value of endophytic actinobacteria extract was significantly different ($p<0.05$) compared to ascorbic acid. EPB 3 isolate had the best IC50 value of 53.85 ppm compared to other endophytic actinobacteria extract. Endophytic actinobacteria isolate EPB 3 can produce bioactive chemicals similar to the *E. palmifolia* plant. In DPPH test, significant differences

($p<0.05$) were recorded in EPB 2 isolate extract. The average $IC_{50}$ values of endophytic actinobacteria extract ranged from 42.43 to 75.53 mg/mL, while the average $IC_{50}$ value of *E. palmifolia* extract was 36.11 ppm in the ABTS test (Table 3). The average $IC_{50}$ value of *E. palmifolia* extract and six endophytic actinobacteria extract was significantly different ($p<0.05$) than that of ascorbic acid. Among the six endophytic actinobacteria extracts, EPB 3 isolate had the best $IC_{50}$ value of 42.43 ppm. Endophytic actinobacteria isolate EPB 3 can produce bioactive chemicals similar to *E. palmifolia* plant. Significant differences ($p<0.05$) were observed in EPB 5 isolate extract in ABTS test.

The antioxidant activity of *E. palmifolia* extract and six endophytic actinobacteria extract to neutralize 50% of free radicals can be shown by the $IC_{50}$ value. According to Molyneux (2004), the value of $IC_{50}$ = 50-100 mg/mL is classified as having strong antioxidant activity. The antioxidant activity of the extracts of six isolates of endophytic actinobacteria in this study that tested using DPPH had a value of $IC_{50}$ = 50-100 mg/mL. Only the antioxidant activity value of EPB 3 isolate was classified as very strong with the value of $IC_{50}$ < 50 mg/mL in ABTS method test. When compared to five other endophytic actinobacteria isolates, endophytic actinobacteria EPB 3 extract showed the highest $IC_{50}$ value. *Eleutherine palmifolia* extract had very strong antioxidant activity in both DPPH and ABTS tests. Each isolate has a different mechanism and ability to neutralize free radicals, thus causing differences in the antioxidant activity produced.

The antioxidants of bioactive compounds transfer hydrogen atoms to free radicals in DPPH mechanism because DPPH has an unpaired electron. This mechanism converts DPPH to DPPH-H due to which purple color turns yellow (Pisoschi and Negulescu 2012). Antioxidants in the human body work by transferring a hydrogen atom (H) to neutralize free radicals as in DPPH mechanism (Apak et al. 2016). In contrast, in ABTS machanism, the bioactive antioxidant compounds supply protons to free radicals, and protons react with the cations in ABTS radicals. The color change in the ABTS solution from blue to colorless indicates that this mechanism has occured (Fogarasi et al. 2015; Munteanu and Apetrei 2021). Both treatments were designed to increase the stability of free radicals. There are benefits and drawbacks to each method of free radical testing. DPPH is simpler yet slower when reacting with samples, while ABTS is faster in reacting to samples, but it costs more (Prakash et al. 2001; Shalaby and Shanab 2013).

**Screening of bioactive compounds**

The amount and type of components in a bioactive molecule in a sample extract can be determined using TLC. This method separated compounds based on their solubility between two phases, the stationary phase, and the mobile phase. TLC silica plate served as fixed phase, while eluent served as mobile phase (Chakravarti and Chakravarti 2015). The endophytic actinobacteria extract had a different spot color from the *E. palmifolia* extract, as observed at 366 nm UV. A more colored spots were seen at UV 366 and easier to observe than UV 254. The *E. palmifolia* extract showed 6 spots of fluorescent blue colors with Rf values (0.07, 0.21, 0.38, 0.48, 0.62, 0.88). The extract of actinobacteria EPB 3 showed 5 blue and one red spots with Rf values (0.09, 0.33, 0.48, 0.62, 0.88) (Figure 1).

In a mixture of eluent methanol:chloroform (1:9 v/v), the endophytic actinobacteria EPB 3 isolate extract produced red, blue spot chromatograms. The *E. Palmifolia* extract produced blue and fluorescent blue spot chromatogram in a mixture of eluent methanol:chloroform (1:9 v/v). The red color shows the presence of anthocyanin compounds. Blue indicates flavonol, whereas fluorescent blue indicates isoflavone compounds (Markham 1988).

**Table 1.** The extract yield of *Eleutherine palmifolia* extract and six endophytic actinobacteria after maceration using ethyl acetate

| Isolates code | Extract | |
|---|---|---|
| | Weight (gram) | Yield (%) |
| EPB 1 | 0.123 | 0.006 |
| EPB 2 | 0.197 | 0.009 |
| EPB 3 | 0.216 | 0.011 |
| EPB 4 | 0.206 | 0.010 |
| EPB 5 | 0.248 | 0.012 |
| EPB 6 | 0.265 | 0.013 |
| *Eleutherine palmifolia* | 1.409 | 0.070 |

**Table 2.** Antioxidant activity of sample extracts measured by DPPH method

| Isolates code | Antioxidant activity of $IC_{50}$ (ppm) |
|---|---|
| EPB 1 | $62.30^{b}$ |
| EPB 2 | $88.29^{c}$ |
| EPB 3 | $53.85^{b}$ |
| EPB 4 | $69.11^{bc}$ |
| EPB 5 | $100.28^{d}$ |
| EPB 6 | $72.61^{bc}$ |
| *Eleutherine palmifolia* | $49.11^{b}$ |
| Ascorbic acid* | $4.74^{a}$ |

Note: * Standard solution; Results are the mean of three $IC_{50}$ ($n$ = 3); The numbers with different letters (a-d) in the same method showed significant differences statistically ($p<0.05$).

**Table 3.** Antioxidant activity of sample extracts measured by ABTS method

| Isolates code | Antioxidant activity of $IC_{50}$ (ppm) |
|---|---|
| EPB 1 | $53.42^{cd}$ |
| EPB 2 | $72.69^{d}$ |
| EPB 3 | $42.43^{b}$ |
| EPB 4 | $54.52^{c}$ |
| EPB 5 | $75.53^{c}$ |
| EPB 6 | $65.34^{bc}$ |
| *Eleutherine palmifolia* | $36.11^{b}$ |
| Ascorbic acid* | $4.39^{a}$ |

Note: * Standard solution; Results are the mean of three $IC_{50}$ ($n$ = 3); The numbers with different letters (a-d) in the same method showed significant differences statistically ($p<0.05$).

Anthocyanin, flavonol, and isoflavone belong to the flavonoid subclasses. The TLC method shows a specific pattern of compound fractions for various compounds. First, two single eluents that can separate the compounds to generate a spot chromatogram are chosen, then the two eluents are mixed in a specific ratio, and UV 254 nm and UV 366 nm are used to identify them. If irradiated with different wavelengths, then TLC silica plate can provide information on the pattern of different compounds. At UV 254 nm, only dark spots appear on the fluorescent silica plate because the light was well absorbed by the TLC silica plate, which had a fluorescent indicator. At the same time, the bioactive compounds at this wavelength are diminished. Silica plates cannot fluoresce at 366 nm UV light, and therefore the spot chromatograms are more visible in color (Artanti 2019). The Rf value (retardation factor) is the ratio of the distance traveled by the compound to the distance traveled by the eluent, used to help identify compounds. The Rf values of the two sample extracts evaluated in the TLC analysis were similar (0.48, 0.62, 0.88) at 254 and 366 nm, indicating that the bioactive compounds of the two samples were similar. If two different samples have the same Rf value, they are likely the same compound. The presence of flavonol compounds in the extracts of endophytic actinobacteria and *E. palmifolia* indicates the presence of bioactive compounds with antioxidant activity, implying that endophytic actinobacteria isolated from *E. palmifolia* have the potential to be used for medicinal purposes.

**Antioxidant bioautography**

Qualitative analysis of compounds active in reducing free radicals was carried out through bioautography test. The extract of endophytic actinobacteria EPB 3 isolate showed three yellow spots on the purple plate of DPPH radical. The Rf value of actinobacteria EPB 3 isolate was (0.09, 0.33, 0.62). *Eleutherine palmifolia* extract showed 4 yellow spots with Rf values (0.07, 0.21, 0.38, 0.88). The yellow color of the *E. palmifolia* extract spot was lighter than the yellow color of the endophytic actinobacteria extract spot. Ascorbic acid as positive control showed only one yellow spot with Rf values (0.03) (Figure 2).

A yellow color change after DPPH was applied in the bioautography test indicates spot chromatograms with antioxidant activity. If the plate remains purple after being sprayed with DPPH, in that case the spot chromatogram may not contain a bioactive compound with antioxidant activity. The hydrogen atom in the bioactive compound with antioxidant activity binds to DPPH radical, causing the purple color of DPPH radical to fade and become yellow. The more the antioxidant activity, the brighter the yellow color produced (Molyneux 2004).

**Total flavonoid content**

Colorimetric test with $AlCl_3$ was used to evaluate the total flavonoid content. The free radical scavenging activity might be attributed to the presence of flavonoid content. The flavonoid content in endophytic actinobacteria extracts ranged from 13.53 QE/mg to 25.30 QE/mg, while *E. palmifolia* extract had 46.03 QE/mg (Figure 3). EPB 3 isolates produced the highest flavonoid content than other endophytic actinobacteria extracts. Based on the result of analysis of variance, the six extracts of endophytic actinobacteria isolates were significantly different ($p<0.05$) from quercetin. *Eleutherine palmifolia* contains several bioactive compounds, including quercetin, so quercetin was chosen as a positive control in this test (Kamarudin et al. 2020). The value of flavonoid content also showed a significant difference ($p<0.05$) between *E. palmifolia* extract and endophytic actinobacteria isolate extracts.

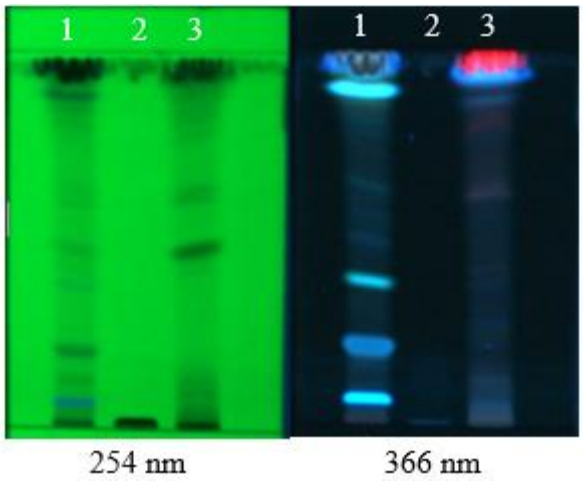


**Figure 1.** Thin-layer chromatography of *Eleutherine palmifolia* extracts and endophytic actinobacteria EPB 3 isolate extract. Samples 1 (*Eleutherine palmifolia*), 2 (ascorbic acid), and 3 (endophytic actinobacteria EPB 3 isolate)

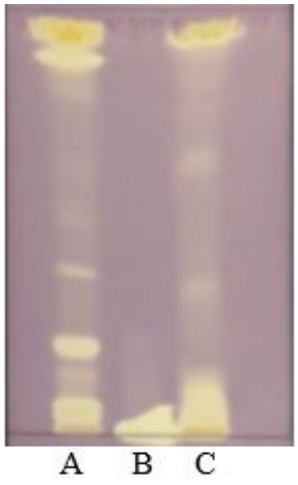


**Figure 2.** Bioautography of *Eleutherine palmifolia* extract and endophytic actinobacteria EPB 3 isolate extract. Samples: A *Eleutherine palmifolia*), B (ascorbic acid), and C (endophytic actinobacteria EPB 3 isolate)

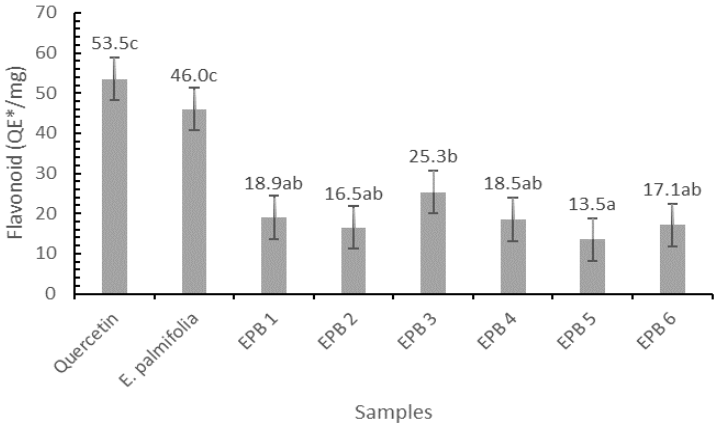


**Figure 3.** Total flavonoid content of six endophytic actinobacteria extracts, and *Eleutherine palmifolia* extract. Values represent the average of 3 repetitions. *QE = quercetin equivalent with $r^2$ = 0.9924; The numbers with different letters (a-c) showed significant different statistically ($p<0.05$)

The total flavonoid content of endophytic actinobacteria EPB 3 isolate extract was higher among the other five isolates. However, the total flavonoid content was still 2× lower than the total flavonoid content of *E. palmifolia* extract. This shows that actinobacteria can produce bioactive compounds similar to their host plant. Endophytic actinobacteria are known to be able to produce secondary metabolites with antioxidant activity, such as the endophytic actinobacteria of the plant *Garcinia mangostana* L. as a producer of flavonoids reported by Larasati et al. (2020). Flavonoid compound is a secondary metabolite that can scavenge free radicals. Intakes of flavonoids per day for men are 14.5 mg, and for women is 20.4 mg (Panunggal and Isdamayani 2015). The total flavonoid content of endophytic actinobacteria extract can meet the daily needs of flavonoids. The variety of each sample character can lead to differences in total flavonoid content. Flavonoid compounds have wave friction due to the hydroxy and keto group of the sample extract which react with $AlCl_3$ and produce a yellow color. The maximum absorbance at 415 nm can be an indicator of the presence of a flavonoid-AlCl3 complex to ensure that no other compounds absorb at that wavelength (Aminah et al. 2017). Quercetin has natural antioxidant activity and belongs to the flavonoid subclasses. Quercetin is used as a positive control because it belongs to the flavonoid group capable of binding to $AlCl_3$. This study is the first to publish data on antioxidant activity and flavonoid content of endophytic actinobacteria from *E. palmifolia*.

This study concludes that the extracts of endophytic actinobacteria generally displayed strong antioxidant activity in DPPH and ABTS tests. Endophytic actinobacteria EPB 3 isolate extract had the highest flavonoid content compared to the other five endophytic actinobacteria isolates. According to the thin-layer chromatography assay, endophytic actinobacteria extracts can produce a similar bioactive compound to *E. palmifolia* extract. This means that endophytic actinobacteria can generate bioactive compounds similar to those produced by the host plant.

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