Thin layer chromatography fingerprint, antioxidant, and antibacterial activities of rhizomes, stems, and leaves of *Curcuma aeruginosa* Roxb.

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**Abstract.** Fingerprints of 5 *temu hitam* (*Curcuma aeruginosa* Roxb.) accessions (Malang, Cirebon, Kuningan 1, Bogor, and Liwa) were determined by thin-layer chromatography (TLC) and compared to fingerprints of turmeric (*Curcuma longa* L), *temu putih* (*Curcuma zedoaria* (Christm.) Roscoe), and *temu lawak* (*Curcuma zanthorriza* Roxb.). Maceration method with ethanol as the solvent was used for extraction. The eluent used for fingerprint by TLC was chloroform:dichloromethane (9:1v/v). Five accessions of *temu hitam* show similar fingerprint patterns, but different in band thickness. *Temu hitam* rhizomes have bands of curcuminoid (Rf 0.22, 0.10, 0.03), and characteristic bands of Rf 0.42, 0.27, and 0.77, which can be distinguished from turmeric and *temu lawak* and Rf 0.13, which is different from *temu putih*. Leaves and stems of *temu hitam* can be distinguished from *temu putih*, turmeric, and *temu lawak* at Rf 0.60. Rhizomes of all plants reveal strong antibacterial activity against *Staphylococcus aureus* and antioxidant activity on DPPH radicals than its corresponding stems and leaves. Antibacterial and antioxidant activities were determined by microdilution and TLC-bioautography. Antibacterial activity of rhizomes of Cirebon and Kuningan 1 accessions are higher than that of other accessions (MIC= 250 μg/mL; MBC= 500 μg/mL), but lower as compared to that of *temu lawak* (MIC= 62.5 μg/mL; MBC= 250 μg/mL) and tetracycline (MIC=MBC= 15.63 μg/mL). Rhizome of Liwa accession exhibits the highest antioxidant activity (IC₅₀= 124.88 μg/mL) amongst all accessions, but lower than that of *temu lawak* (IC₅₀= 18.45 μg/mL), turmeric (IC₅₀= 18.82 μg/mL), and *temu putih* (IC₅₀= 94.35 μg/mL).

1. Introduction

*Temu hitam* (*Curcuma aeruginosa* Roxb.) is one type of curcuma species of Zingiberaceae family. This plant can be categorized as a medicinal plant because extracts and essential oils of *temu hitam* reported active as antibacterial, antiinflammation, antipyretic, and antinociceptive agents. The ethyl acetate extract of *temu hitam* can inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.

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and Bacillus subtilis [1] and its chloroform extract has bioactive compounds that act as antinociceptive, antinflammation, and antipyretic [2,3]. Essential oils of temu hitam's rhizomes also have antibacterial activity against Escherichia coli, Bacillus aureus, Staphylococcus aureus, and Pseudomonas aeruginosa [4]. In addition, essential oils of rhizomes, stems, and leaves of temu hitam can inhibit the growth of Streptococcus mutans and as biofilm degradator on teeth [5]. Besides those activities, ethanol and methanol extracts and essential oils of temu hitam's rhizomes also have antioxidant activity [6-8].

Various activities of temu hitam is related to its secondary metabolites. These metabolites are formed when the plants are under stress conditions, for self-protection, and defense [9-11]. Temu hitam was reported to contain secondary metabolites such as terpenoids, flavonoids, tannins, alkaloids, and saponins [7,12]. The content of secondary metabolites in various plants can be different depend on the diversity of plant physiological (organ development, the type of plant materials, seasonal variation, and wound mechanical or chemical), environmental conditions (climate, pollution, disease, and edaphic factor), geographic variation, genetics and evolution, storage, and the amount of plant material [13]. The number of compounds in any kind of plant drugs affected these factors need to be evaluated, compared, and quality controlled through fingerprint analysis. Fingerprint patterns indicate the overall profile of components because it can represent the diversity of components in medicinal plants [14].

Fingerprint analysis of temu hitam from three regions (Nagrek, Cikabayan, and Tawangmangu) was performed using liquid chromatography-mass spectrometer [12]. The ethanol extract of rhizomes of temu hitam from three regions have chemical components with different concentrations. Temu hitam from Nagrek has the greatest antioxidant potency and temu hitam from Cikabayan has the greatest toxicity. In this study, the profile of rhizomes, stems, and leaves of temu hitam with different accessions determined and compared with its species and other curcuma species through fingerprint analysis by thin layer chromatography. In addition, antibacterial and antioxidant activity of rhizomes, stems, and leaves of temu hitam and other curcuma species determined and associated with their components profile.

2. Methodology

2.1. Material

Five accessions of fresh temu hitam (Malang, Kuningan 1, Liwa, Bogor, and Cirebon accession) as main samples collection of Division of Plant Biotechnology Collection, Department of Agronomy and Horticulture, IPB in Experimental Garden Sukamantri, Sukamantri Village, Tamansari Subdistrict, Bogor, West Java, and temu lawak, turmeric, and temu putih as comparator samples (Table 1) were used as plant materials. All solvents that were used are analytical grade and obtained from Merck or Sigma-Aldrich (St. Louis, USA). Beside that, TLC plate silica gel 60 F_{254} (Merck, Darmstadt, Germany), standard of curcuminoid (ChromaDex, California, USA), vanillin-sulfuric acid were used for fingerprint analysis. Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), inoculant of Staphylococcus aureus (IPB Culture Collection, Faculty of Mathematics and Natural Sciences, IPB), tetracycline, and DMSO 20% were used for antibacterial assay. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and standard of ascorbic acid were used for antioxidant assay.

2.2. Procedure

2.2.1. Instrumentation. Fingerprint of thin-layer chromatography was carried out using a system of semi-automatic TLC sampler Linomat V (connected to nitrogen tank), twin-through chamber, and documentation device Reprostar 3 with winCATS version 1.2.3. planar chromatography software manager (Camag, Muttenz, Switzerland). Epoch microplate Spectrophotometer with Gen5 Data Analysis software (Biotek Instruments, Inc., Winooski, VT, USA).
2.2.2. Sample and standard preparation. Rhizomes, stems, and leaves of each samples were dried and powdered/pulverized before extracted with ethanol. Then powder and dried plant materials were extracted with a solvent (ratio of 1 g sample: 10 ml solvent) for 24 hours three times. The extracts were filtered and concentrated in a vacuum at 30-40 °C using rotary evaporator. Sample extracts were diluted with ethanol with a final concentration of 2% for fingerprint analysis. Curcuminoid standard solution was prepared by dissolving curcumin with ethanol with a final concentration of 0.2%.

Table 1. Five accessions of *temu hitam* of Division of Plant Biotechnology Collection, Department of Agronomy and Horticulture, IPB.

| Accessions code of *Curcuma aeruginosa* Roxb. | Sources | Accessions code of *Curcuma aeruginosa* Roxb. |
|-----------------------------------------------|---------|-----------------------------------------------|
| KUN1-JABAR | Cileuleuy Village, Cigugur Subdistrict, Kuningan, Jawa Barat | KUN1-JABAR |
| BOG-JABAR | Anyar Market, Bogor, Jawa Barat | BOG-JABAR |
| MAL-JATIM | Turirejo Village, Lawang Subdistrict, Malang, Jawa Timur | MAL-JATIM |
| LIWA-LAMP | Sebarus Village, Bukit Tinggi Subdistrict, Liwa, Lampung | LIWA-LAMP |
| CIR-JABAR | Kanoman Market, Cirebon, Jawa Barat | CIR-JABAR |
| *Curcuma longa* | Unit of conservation and cultivation of biopharmaca, Tropical Biopharmaca Research Center of IPB, Dramaga Bogor | *Curcuma longa* |

2.2.3. Thin Layer Chromatography (TLC)-fingerprint analysis. The ethanol extract of samples were applied to a TLC plate G60F254 with a volume of 10 μL using semiautomatic sampler CAMAG Linomat V. Then, the plates were drained and developed into a chromatography chamber with chloroform: dichloromethane (9:1v/v) as mobile phase. Temperature and humidity were not controlled, but in a controlled laboratory temperature condition. After the mobile phase reached the finish line (80 mm), plates were removed and air-dried. Furthermore, the plates were detected with UV lamp 254 nm, 366 nm, visible light, and vanillin-sulfuric acid reagent (visible light).

2.2.4. Antibacterial assay. The antibacterial assay was performed by CLSI (2012) methods with some modification [15]. The methods was broth microdilution method - Bacteria test used was Staphylococcus aureus with growing medium TSB. A 100 mL of each samples (concentration 15.63-2000 μg/mL), 100 μL TSB and 20 μL suspension of Staphylococcus aureus (incubated 24 hours) were put in well of steril 96-well plates. After that, the mixture in well was incubated at 37 °C for 24 hours. The minimum concentration of extract at which no visually detectable bacterial growth was described as the minimum inhibitory concentration (MIC). Next, 100 μl of each medium with no visually detectable bacterial growth was inoculated in 100 μl of fresh medium and incubated again for 24 hours at 37 °C. The minimum concentration at which there was no bacterial growth after the second inoculation was described as the minimum bactericidal concentration (MBC). DMSO solution of 20% used as a negative control and a positive control used tetracycline.

2.2.5. Antioxidant assay. The antioxidant activity was determined by two methods, which are microdilution and Thin Layer Chormatography (TLC)-bioautography. Microdilution - 100 μL DPPH solution of 125 μM added to 100 μL of ethanol extract samples with various concentrations of up to a total volume of 200 μL. The mixtures were incubated at 37 °C for 30 minutes. Then, samples absorbance were measured at 517 nm using *Epoch Microplate Spectrophotometer*. Ascorbic acid was used as a positive control. DPPH free radical trapping capacity was calculated [16].
**TLC-bioautography** - Plates that had been developed previously by TLC fingerprint method were sprayed with a solution of 5 mM DPPH and then incubated at 37 °C for several hours. Yellowish bands appeared indicating that the bands were active as an antioxidant and the plate background was purple. The chromatograms bands were compared with chromatogram that had been detected previously to determine the value of Rf bands that have antioxidant activity [17].

### 3. Results and discussion

Five accessions of temu hitam and three other plants (temu putih, temu lawak, and turmeric) determined in Bogoriense Herbarium, Biology Research Center, LIPI Cibinong. The results showed that five temu hitam accessions and three other plants are Zingiberaceae with different species, *Curcuma aeruginosa* Roxb. (Five accession temu hitam), *Curcuma zedoaria* (Christm.) Roscoe (temu putih), *Curcuma Zanthorriza* Roxb. (temu lawak), and *Curcuma longa* L. (turmeric).

The chemical components in medicinal plants relies heavily on the diversity of physiological, harvest condition, plant sources, drying processes, and other factors to ensure the integrity and authenticity of medicinal plants [13,18]. Fingerprint analysis can be used for classification and validation of plant species and multicomponent quality control of medicinal plants. Thin layer chromatography (TLC) was choosen because it provide patterns of fractions of compounds that are specific to each species, beside that the analysis is fast, simple, and relatively cheap [19]. This technique can be applied qualitatively and quantitatively with the presence or absence of a standard compound. Additionally, TLC can provide relatively different patterns of the compound when administered beams with different wavelengths, such as visible light, UV 366 nm and UV 254 nm, as well as the specific dye reagents so as to enrich the detection results (multidetection).

**Temu hitam** samples used in this study came from five different areas, but cultivated in the same specific area so-called as accession. In addition to temu hitam, three other plants, turmeric, temu putih, and temu lawak are used to distinguish component profile between temu hitam and the three other plants. By phenotype, temu hitam and the three plants have a similar visual appearance, especially among temu hitam, temu putih, and temu lawak, specially on the leaf shape and tinge of purple on the leaf stalk. However, the rhizome can be distinguished when still in the form intact.

Figure 1 shows the profile of compounds in rhizomes, leaves, and stems of five accessions of temu hitam and three other plants. All three parts of this plant has a different pattern because it has different type of plant material. The pattern of the compounds in the rhizome is dominated by yellow-green bands and blue in UV 366 nm. Meanwhile, the leaves and stems contain more red bands at UV 366 nm. Different band colors can indicate different compounds. According to Markham (1988), the blue color indicates the presence of flavonoids, flavonon, or flavonols, red as anthocyanidins compound, and greenish as Auran and flavones compound [20]. In UV 254 nm there are only dark bands due to the absorption of the compound to light will diminish and outages, this is because the stationary phase in the form of silica gel containing indicators of fluorescence that can absorb light at a wavelength of 254 nm so that only the stationary phase fluorescent [21]. However, the pattern of the component of three parts plant (rhizomes, stems, and leaves) looks different. In addition to using UV detection 254 and 366 nm, detection is also done with a specific dye reagents. Reagent vanilina-sulfuric acid has the ability to react with chromophore groups on the compound and can enlarge the wavelength (UV-Vis) so that the bands previously not seen in visible light (Figure 1) to be seen in visible light (Figure 2).

The ethanol extract on the same part of the five accessions have similar patterns, but has a different type of plant material. The pattern of the compounds in the rhizome is dominated by yellow-green bands and blue in UV 366 nm. Meanwhile, the leaves and stems contain more red bands at UV 366 nm. Different band colors can indicate different compounds. According to Markham (1988), the blue color indicates the presence of flavonoids, flavonon, or flavonols, red as anthocyanidins compound, and greenish as Auran and flavones compound [20]. In UV 254 nm there are only dark bands due to the absorption of the compound to light will diminish and outages, this is because the stationary phase in the form of silica gel containing indicators of fluorescence that can absorb light at a wavelength of 254 nm so that only the stationary phase fluorescent [21]. However, the pattern of the component of three parts plant (rhizomes, stems, and leaves) looks different. In addition to using UV detection 254 and 366 nm, detection is also done with a specific dye reagents. Reagent vanilina-sulfuric acid has the ability to react with chromophore groups on the compound and can enlarge the wavelength (UV-Vis) so that the bands previously not seen in visible light (Figure 1) to be seen in visible light (Figure 2).

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0.24 in comparison with four other accessions. Meanwhile, the abundance of the highest compound (Rf 0.70 and 0.37) on the stem owned by Cirebon accession (Figure 1c). Based on that, although the five temu hitam accessions came from different areas have been cultivated in the same specific area, the abundance or concentration of compounds still have differences.

![Figure 1](image1.png)

**Figure 1**: The pattern of the fingerprint chromatograms of ethanol extract of rhizome (a), leaves (b), and stem (c) with a mobile phase of chloroform-dichloromethane 9:1 (v/v). Detection: (i) visible light; (ii) UV 254 nm; and (iii) UV 366 nm. MAL: Malang Accession, LIW: Liwa Accession, BGR: Bogor Accession, CIR: Cirebon Accession, KUN: Kuningan 1 Accession, TMP: Temu Putih, TML: Temu lawak, KNY: Turmeric, and STD: Curcuminoid Standard

![Figure 2](image2.png)

**Figure 2**: The pattern of the fingerprint chromatogram of the ethanol extract of the rhizome (a), leaves (b), and stems (c) with a mobile phase of chloroform-dichloromethane 9:1 (v/v) Detection: vanillin-sulfuric acid visible light. MAL: Malang Accession, LIW: Liwa Accession, BGR: Bogor Accession, CIR: Cirebon Accession, KUN: Kuningan 1 Accession, TMP: Temu Putih, TML: Temu lawak, KNY: Turmeric, and STD: Curcuminoid Standard

Ethanol extract profiles of five accessions temu hitam difference with the three other plants (Figures 1-2). Rhizomes all plants contain curcuminoid so curcuminoid solution is used as a standard [22-26]. Curcuminoid standard used to view the distribution of the three type of curcuminoid. Three type curcuminoid in Figure 1 and 2a indicated the existence of three bands with Rf 0.22, 0.10, and 0.03. Rhizome five accession temu hitam and turmeric contains three bands on a standard but have different thickness intensity, while temu lawak contains only band with Rf 0.22 and 0.10 and temu putih contain bands with Rf 0.03 and 0.10.
Rhizome of *temu hitam* had characteristic band with Rf 0.42, 0.27, and 0.77 that distinguish it from the rhizome of *temu lawak* and turmeric, but the rhizome of *temu hitam* did not have band with Rf 0.73, 0.20, and 0.64 owned rhizome of *temu lawak* and 0.71 owned turmeric. Band with Rf 0.13 on the rhizome of *temu hitam* is a distinguishing band from rhizome temu putih. In addition, at 254 nm visualization rhizome *temu hitam* did not have band with Rf 0.35 owned turmeric and *temu putih* (Figure 3a). The ethanol extract of leaves of *temu hitam* can be distinguished from leaves three other plants in the band with Rf 0.06 owned *temu hitam* and Rf 0.60 that exist in other plants. Turmeric leaf can be distinguished by the leaves of *temu hitam*, *temu lawak*, and *temu putih* at 0.23 and 0.96 (contained in turmeric leaves). On the other hand, turmeric leaf did not have band with Rf 0.80 and 0.63 owned *temu hitam*, *temu lawak*, and *temu putih* (Figure 3b). The pattern of chemical components on the stems of *temu hitam* and other plants are not much different from the pattern of the leaves, like Rf 0.94, 0.70, and 0.45 are in both, as well as the characteristic band is same at Rf 0.60 (*temu lawak*, turmeric, and temu putih). However, the number of bands on the leaves more than stems (Figure 3b). Given these distinction bands, *temu hitam* can be discriminated from the three other plants and can avoid counterfeit products are made from *temu hitam* in a commercial application.

**Figure 3:** Pattern of the fingerprint chromatogram of the ethanol extract of the rhizome (a), leaves (b), and stem (c) with Rf information. Visualization (i) reagent vanillin-sulfuric acid in visible light, and (ii) UV 254 nm with a mobile phase of chloroform-dichloromethane 9:1 (v/v), (iii) UV 366 nm with a mobile phase of chloroform-dichloromethane 9:1 (v/v). MAL: Malang Accession, LIW: Liwa Accession, BGR: Bogor Accession, CIR: Cirebon Accession, KUN: Kuningan 1 Accession, TMP: Temu Putih, TML: Temu lawak, KNY: Turmeric, and STD: Curcuminoid Standard

The diversity of components of the profile indicates the potential bioactivity on the plant *temu hitam* the profile shows that *temu hitam* rhizome contains curcuminoid which have a wide range of bioactivity, such as antibacterial and antioxidant [27-29]. *Temu hitam* also contain terpenoids class that reported has antibacterial activity [4,30].

In this study, antibacterial and antioxidant activity of five accessions of *temu hitam* and three other plants were determined bioautography. Based on the results of the ethanol extract of *temu hitam* and other plants have inhibitory activity against *Staphylococcus aureus* with different MIC and MBC. The minimum inhibitory concentration (MIC) called also bacteriostatic, which is the lowest concentration that can inhibit the growth of bacteria. Meanwhile, the concentration that kills 99.9% of bacteria inoculant called minimum bactericidal concentration (MBC). MIC and MBC sample is determined by comparing the turbidity (visual observation) between the sample wells with positive control wells (tetracycline) and negative control (DMSO 20%). Turbid wells indicates there are bacteria grows and
components in the sample are able to inhibit the growth of bacteria, while the clear wells indicates components in the sample were able to inhibit even kill bacteria [31].

Table 2: The antibacterial activity of ethanol extract of rhizomes, stems, and leaves five *temu hitam* accessions and comparative plants

| Ethanol extracts of *temu hitam* comparator samples | MIC (µg/mL) | MBC (µg/mL) |
|----------------------------------------------------|-------------|--------------|
|                                                    | R | S | L | R | S | L |
| Kuningan 1                                         | 250 | 500 | 500 | 500 | * | 2000 |
| Cirebon                                            | 250 | 500 | 1000 | 500 | * | 2000 |
| Malang                                             | 500 | 1000 | 500 | 1000 | * | 2000 |
| Bogor                                              | 500 | 500 | 500 | 500 | * | 2000 |
| Liwa                                               | 250 | 500 | 500 | 1000 | * | 2000 |
| *Temu putih*                                       | 500 | 1000 | 500 | 2000 | * | 2000 |
| Turmeric                                           | 250 | 1000 | 500 | 1000 | * | 1000 |
| *Temu lawak*                                       | 62.5 | 500 | 500 | 250 | * | 2000 |
| Tetracycline                                       | 15.63 |         |     | 15.63 |     |     |
| DMSO 20%                                            | - |     |     | - |     |     |

(*)= Activity more than 2000 µg/mL; (-)= No activity; R= Rhizomes; S= Stems; L= Leaves

The highest antibacterial activity found in the rhizome followed by leaves and stems (Table 2). Rhizome *temu hitam* Cirebon and Kuningan 1 accession have the highest bacteriostatic and bactericidal activity with MIC and MBC respectively of 250 and 500 µg/mL. However, the antibacterial activity of ethanol extract of rhizome of *temu hitam* Cirebon and Kuningan 1 accession are not higher than the ethanol extract of *temu lawak* rhizome (MIC= 62.5 µg/mL, MBC = 250 µg/mL) and tetracycline as a positive control (MIC=MBC= 15.63 µg/mL).

Table 3. IC$_{50}$ value of *temu hitam* ethanol extract, comparative plants, and ascorbic acid

| Accessions of *temu hitam* comparator samples | Rhizomes | IC$_{50}$ (µg/mL) | Stems | Leaves |
|----------------------------------------------|----------|-------------------|-------|--------|
| Cirebon                                      | 131.40   | 315.81            | 193.84|        |
| Malang                                       | 131.56   | 561.25            | 163.05|        |
| Bogor                                        | 130.01   | 702.00            | 194.63|        |
| Liwa                                         | 124.88   | 573.38            | 160.58|        |
| Kuningan 1                                   | 146.71   | 481.46            | 211.49|        |
| *Temu putih*                                 | 94.35    | 1335.20           | 162.60|        |
| Turmeric                                     | 18.82    | 417.93            | 171.36|        |
| *Temu lawak*                                 | 18.45    | 305.06            | 157.17|        |
| Ascorbic acid                                |          | 3.22              |       |        |

Various compounds contained in the five accessions *temu hitam* profile makes this plant has a variety of activities, not only antibacterial activity, but other activities, such as antioxidants. The ethanol extract five accession *temu hitam* on different parts have different relative IC$_{50}$ values, the concentration that inhibit 50% radicals (Table 3). Based on the IC$_{50}$ value, the rhizome five accessions *temu hitam* had the highest activity than stems and leaves, but its activity is lower than turmeric, *temu lawak* and temu putih. This is also supported by the profile component of the rhizome that has a number of compounds (band) most. IC$_{50}$ value rhizomes and leaves of Liwa accession has the highest antioxidant activity than the other accessions, but on the stem, Cirebon accession which has the highest activity.
Besides determined by \( IC_{50} \) value, the antioxidant activity was also determined qualitatively using TLC-bioautography. In this method showed yellowish specific band with a purple background plate, yellow band emerged due to the reduction of DPPH and indicates that the band is active of antioxidants. Yellow band thickening showed stronger antioxidant activity [17]. Results showed \textit{temu hitam} and comparator plants rhizomes have thickest yellow bands and most visible than the leaves and stems. Rhizomes of Liwa and Bogor accessions have higher activity, this is due to the accessions of these have yellow bands with Rf 0.77 and 0.42 thicker than the other accessions (Figure 4a). Likewise, the leaves and stems, leaf of Liwa accession and stem of Cirebon accession showed yellow bands higher intensity than the other accessions (Figure 3a). These results are also supported by the results of fingerprint chromatogram pattern in Figure 1-2.

Figure 4. TLC-bioautogram of ethanol extract of rhizomes (a), leaves (b), and stems (c) with chloroform:dichloromethane 9:1 (v/v) as mobile phase. Detection by DPPH 5mM in visible light

At the five \textit{temu hitam} accessions, bands with active antioxidant are Rf of 0.93, 0.83, 0.77, 0.52, 0.42, 0.10, 0.13, and 0.03, while Rf of 0.77, 0.83, and 0.93 are most active. Meanwhile, the most active rhizome bands of \textit{temu lawak} are 0.64, 0.22, 0.10, and 0.93. Turmeric has active band similar to \textit{temu lawak}, just do not have active band at Rf 0.64 and 0.93, but there are active bands at Rf 0.88, 0.10. The thickest band at 0.77 found in \textit{temu putih} and have more active band at 0.35 and 0.42 (Figure 4b). On leaves and stems have less active bands of antioxidants than the rhizome. This is also evidenced by the IC\(_{50}\) value of the leaves and stems lower than the rhizome.

The leaves of \textit{temu hitam} has active band at Rf of 0.80, 0.94, 0.77, 0.70, 0.15 and 0.06. However, active bands are more clearly visible only in Liwa accession. Furthermore, characteristic band at 0.96 of turmeric has activity, whereas in \textit{temu lawak} and \textit{temu putih} the most active bands are 0.70 and 0.45 (Figure 4b). Stems have very weak antioxidant activity seen from the active band only seen in \textit{temu hitam} Cirebon accession, \textit{temu lawak}, and turmeric at 0.94 and 0.16, 0.65 (\textit{temu hitam} Cirebon), and 0.68 (\textit{temu lawak}) (Figure 4c).

The antioxidant activity of ethanol extract of \textit{temu hitam} rhizome lower than \textit{temu lawak}, \textit{temu putih}, and turmeric rhizome. It can be caused by the content of existing curcuminoid. Curcuminoid can be used as one factor for the level of antioxidant because three type of curcuminoid is active as an antioxidant. Based on Figure 4a curcuminoid content of the ethanol extract of \textit{temu hitam} rhizome lower than the other three rhizomes, especially in turmeric and \textit{temu lawak} rhizome. These results are also consistent with research Jitoe \textit{et al.} (1992) which states curcuminoid content of \textit{temu lawak} and turmeric rhizome higher than \textit{temu hitam} [32]. In the bioautogram shown a yellow bands curcuminoid at Rf of 0.10 and 0.03 on \textit{temu hitam} thinner even at 0.22 almost no activity (low intensity). Additionally, \textit{temu lawak} has an additional activity from Rf of 0.20, 0.64 and 0.73 bands and turmeric from Rf of 0.88. \textit{Temu putih} has bands at Rf of 0.77 and 0.10 thicker than \textit{temu hitam}. Bioautogram shown antioxidant activity is directly proportional to the IC\(_{50}\) value generated by the accession Liwa rhizomes that have the highest activity, but lower than \textit{temu lawak}, turmeric, and \textit{temu putih}.

4. Conclusion

The ethanol extracts of five accessions \textit{temu hitam} have different chromatographic fingerprints profiles between rhizomes, stems and leaves, but has a similar profile to the same part of plant.
Rhizome of five accession temu hitam and turmeric contains three bands of standard of curcuminoid with the different thickness intensity, while temu lawak contains only band with Rf 0.22 and 0.10 and temu putih with Rf 0.03 and 0.10. Rhizomes of temu hitam has characteristic bands at Rf 0.42, 0.27, and 0.77 that distinguish it from the rhizome of temu lawak and turmeric, and Rf 0.13 with temu putih rhizomes. Leaves and stems of five accessions temu hitam and comparator plants has similar fingerprint profile and band at 0.60 distinguishing between the stem and leaves of temu hitam with the leaves and stems of comparator plants.

Rhizomes of temu hitam and comparator plants have the highest antibacterial and antioxidant activity than their leaves and stems. The antibacterial activity of Cirebon and Kuningan 1 accessions rhizomes are stronger than the other accessions but lower than the rhizome of temu lawak and tetracycline (positive control). Based on the IC50 and TLC-bioautography, ethanol extract of rhizomes Liwa accession has strongest antioxidant activity than the other accessions, but lower than temu lawak, turmeric, and temu putih.

References
[1] Philip K, Malek S N A, Sani W, Shin S K, Kumar S, Lai H K, Serm L G and Rahman S N S A 2009 Am. J. Appl. Sci. 6 1613-7
[2] Reanmongkol W, Subhadhirasakul S, Khaisombat N, Feungnawakit P, Jantasila S and Khamjun A 2006 Songklanakarin J. Sci. Technol. 28 999-1008
[3] Hossain C F, Al-Amin M, Sayem A S Md, Siragee I H, Tunan A M, Hassan F, Kabir Md M, Sultana G N H 2015 BMC Complement. Altern. Med. 15 1-7
[4] Kamazeri Tg S A Tg, Samah O A, Taher M, Susanti D and Qaralleh H 2012 Asian Pac. J. Trop. Dis. 202-9
[5] Tambunan D Y S 2014 Minyak atsiri rim pang, batang, dan daun temu hitam (Curcuma aeruginosa Roxb.) sebagai antibakteri Streptococcus mutans dan penedegradasi biofilm pada gigi (Bogor: Bogor Agricultural University)
[6] Angel G R, Vimala B and Nambisan B 2012 IJPPR 4 69-73
[7] Nurcholis W, Khumaida N, Muhamad S, Maria B and Ardyani 2015 Int. J. Res. Ayurveda Pharm. 6 634-7
[8] Theanphong O, Mingvanish W and Kirdmanee C 2015 Bull. Health Sci. Tech. 13 6-16
[9] Mazid M, Khan T A and Mohammad F 2011 Biol. Med. 3 232-49
[10] Seafrr H and Wink M 2009 Biotechnol. J. 4 1684-703
[11] Wink M 1988 Theor Appl Genet. 75 225-33
[12] Septaningisih D A 2015 Identifikasi Komponen Temu Ireng (Curcuma aeruginosa) dengan Pola Sidik Jari Kromatografi Cair-Spektroskopi Massa dan Kemometrik (Bogor: Bogor Agricultural University)
[13] Figueiredo A C, Barroso J G, Pedro L G and Scheffer J J C 2008 Flavour Frag. J. 23 213-26
[14] Borges C N, Bruns R E, Almeida A A and Scarminio I S 2007 Anal. Chim. Acta 595 28-37
[15] Cockerill F R, Wikler M A, Alder J, Dudley M N, Eliopoulos G M, Ferraro M J, Hardy D J, Hecht D W, Hindler J A, Patel J B, Powell M, Swenson J M, Thomson R B, Traczewski M M, Turnidge J D, Weinstein M P and Zimmer B L 2012 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Ninth Edition vol 32 (Wayne, Pennsylvania: Clinical and Laboratory Standards Institute)
[16] Batubara I, Mitsunaga T, and Ohashi H 2009 J. Wood Sci. 55 230-5
[17] Salazar-Aranda R, Perez-Lopez L A, Lopez Arroyo J, Alanis-Garza B A, and Torres N W 2009 Evid. Based Complement. Alternat. Med. 2011 1-6
[18] Liang Y Z, Xie P, and Chan K 2004 J. Chromatogr. B 812 53-70
[19] Liu W J H 2011 Traditional Herbal Medicine Research Methods: Identification, Analysis, Bioassay, and Pharmaceutical and Clinical Studies (New Jersey: John Wiley & Sons, Inc.)
[20] Markham K R. 1988 Techniques of Flavonoid Identification, (New York : Academic Press)
[21] Sherma J and Fried B 2003 *Handbook of Thin-Layer Chromatography* (New York: Marcel Dekker Inc.)
[22] Lobo R, Prabhu K S, Shirwaikar A, Shirwaikar A 2009 *J. Pharm. Pharmacol.* 61 13-21
[23] Mau J, Lai E Y C, Wang N, Chen C, Chang C, Chyau C 2003 *Food Chem.* 82 583-91
[24] Jayaprabhakara G K, Rao L J M, Sakariah K K 2002 *J. Agric. Food Chem.* 50 3668-72
[25] Lechtenberg M, Quandt B, Nahrstedt A 2004 *Phytochem. Anal.* 15 152-8
[26] Bos R, Windono T, Woerdenbag H J, Boersma Y L, Koulman A and Kayser O 2007 *Phytochem. Anal.* 18 118-22
[27] Kowsalya R and Krishnaveni M 2011 *Ex J. Pure Appl. Microbiol.* 5 317-21
[28] Sivasothy Y, Sulaiman S F, Ooi K L, Ibarhim H and Awang K 2013 *Food Control* 30 714-21
[29] Jayaprabhakara G K, Rao L J and Sakariah K K 2006 *Food Chem.* 98 720-4
[30] Simoh S and Zainal A 2015 *Asian Pac. J. Trop. Biomed.* 5 412-7
[31] Basri D F and Fan S H 2005 *Indian J. Pharmacol.* 37 26-9
[32] Jitoe A, Masuda T, Tengah I G P, Suprapta D N, Gara I W and Nakatani N 1992 *J. Agric. Food Chem.* 40 1337-40