International Journal on Advanced Science Engineering Information Technology

Vol.12 (2022) No. 4
ISSN: 2088-5334

Antituberculosis Activity and Iron Chelation Ability of Brazilin Isolated from *Caesalpinia Sappan L.*

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***Abstract*—The present study was conducted to evaluate the anti-tubercular activity of *Brazilin* and to know the role of iron chelation on the anti-tuberculosis activity of *Brazilin*. *Anti-tuberculosis* activity in vitro was tested through MIC values and a reduction in the number of Mtb bacterial cells. The MIC and MBC tests utilized extent technique comprising four treatment groups; the positive control (Lowenstein-Jensen medium inoculated with Mtb), the negative control (LJ medium), the anti-tuberculosis drugs (rifampicin, isoniazid, ethambutol, and streptomycin), and *Brazilin* at concentration 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 ppm that were watched for about eight weeks. The iron chelation capability was assessed using atomic absorption spectrophotometer. The results indicated that the MIC from Brazilin is 128 ppm and MBC of 256 ppm. Brazilin at 128 ppm showed iron chelation of 32.96% capacity and can reduce up to 72% Mtb cells in 10^{-3} inoculum dilution. Iron levels at Brazilin 128 ppm (MIC) are higher than iron levels at concentrations of 256 ppm (MBC), indicating that *Brazilin* binds to iron. The binding of iron by *Brazilin* results in the unavailability of iron for Mtb, and causes suppression of Mtb growth, further resulting in Mtb cell death. These results exhibit that *Brazilin* can be used as an iron-chelating agent that might be advantageous in treating and controlling mycobacteria infection.**

Keywords*— Brazilin; iron chelation; *Anti-tuberculosis*; *Mycobacterium tuberculosis

Manuscript received 3 Sep. 2021; revised 7 Apr. 2022; accepted 14 May 2022. Date of publication 31 Aug. 2022.
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I. INTRODUCTION

As reported by World Health Organization (WHO) in 2015, Indonesia is a country with the highest infections of tuberculosis (TB) in Southeast Asia and ranked fifth in the world [1]. The increasing cases of TB were due to the existence of *Mycobacterium tuberculosis* (Mtb) strains resistant to multiple drugs (Multi-Drug Resistance; MDR). Recent research on anti-TB drug resistance found that multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB) was detected in 3.5% of new and 18% of previously treated TB cases. In 2017, according to WHO, an estimated 230,000 deaths from 558,000 emerging cases of MDR / RR-TB were found. Most cases and deaths occur in India and China. About 8.5% are cases of MDR-TB that are extensively resistant to TB (XDR-TB) [2].

Mtb strains or MDR-TB are resistant to at least two kinds of first-line anti-tuberculosis drugs, namely rifampicin and isoniazid. The resistance of Mtb strains to fluoroquinolone and at least one of the second-line ATBD (anti-tuberculosis drugs), i.e., kanamycin, capreomycin, and amikacin were extensive drug resistance (XDR-TB) [3], and it is defined as Totally Drug Resistance (TDR-TB), if Mtb strains are resistant to all ATBD.

Indonesia is well-known for its medicinal plants, and herbal medicine has closely related to great natural resources and traditions. *Sappan* wood (*Caesalpinia sappan L.*; Indonesia language: *secang*) is one of the medicinal plants used to treat wounds, bloody cough, *syphilis*, stop bleeding, treat *dysentery diarrhea*, and pain due to blood circulation disorders [4].

The chemical constituents of *Sappan* wood are of various structural types of phenolic components, including xanthone (1 type), *coumarin* (1 type), *chalcones* (3 types), *flavones* (2 types), *homoisoflavonoids* (3 types), and *Brazilin* [5]. *Brazilin* [(6a S-cis)-7,11b *dihydrobenzo* [b] *indeno* [1,2-d]pyran-3,6a,9,10 (6H)- t*etrol*], is a major and active compound in *sappan* wood [6]. Brazilin is known to have antioxidant

properties, and its catechol group can chelate iron [4], [7]. The activity against antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE), multi-drug resistant *Burkholderia cepacian* are shown by Brazilin isolated from *Caesalpinia sappan* [8]. Our past investigation demonstrated that the extract of *sappan* wood had recorded an anti-*mycobacterium tuberculosis* strain H37Rv capability and iron chelation activity. *Sappan* wood extract contains various compounds, and *Brazilin* is a major compound. However, the same capacity primarily originates from *Brazilin* is unknown.

Iron deprivation faced by MTB is the most critical environmental stress condition during the infection process caused by the absence of free iron availability in the human host. Iron deprivation faced by MTB is the most critical environmental stress condition during the infection process caused by the absence of free iron availability in the human host. According to Pal et al. [9], membrane homeostasis is disrupted due to iron deprivation which is shown by an increase in membrane permeability and membrane hypersensitivity, which is disturbed by agents that cause an increase in passive drug diffusion. In addition, TEM images show differences in the thickness of the cell envelope. The results showed a relationship between cellular iron and mycobacteria's drug susceptibility, suggesting iron is a determinant for combating MD. Mtb requires iron for its growth by producing a siderophore compound for chelating the iron. Therefore, phenolic compounds such as *Brazilin* are very likely to have potential as anti-tuberculosis due to their high affinity to iron, in addition to phenolic compounds' antibacterial properties. This study described Brazilin's characteristics extracted from *Caesalpinia sappan* and examined the role of iron chelation related to the anti-tuberculosis activity of *Brazilin*.

II. Materials and Methods

A. Extraction and Fractionation of Sappan Wood

Extraction was conducted by the maceration technique, performed three times for 24 hours. Fractionation and separation of brazilin compounds were performed by column chromatography. The brazilin compound is characterized by Ultraviolet-Visible (UV) and Infra-Red spectrophotometer. Then, to determine the brazilin purity in the isolates' High-Performance Liquid Chromatography (HPLC) is used. The data compared with the brazilin spectrum was examined by previous studies [7].

B. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Experiments using the proportion method are the gold standard. It has a sensitivity value, specificity, negative predictive value, and a positive predictive value of 100% [10]. [13] This experiment uses the proportion method of four treatment groups: The negative control group (LJ medium); The positive control group (LJ medium + MtbMtb); The LJ mediums added by Brazilin in various concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 ppm), and The LJ mediums added by anti-tuberculosis drugs (rifampicin, isoniazid, ethambutol, and streptomycin). Each treatment was conducted once (S = simple) and duplicated (D= Duplo). The MIC was assessed as the minimum concentration of Brazilin that produces as much as 20-100 Mtb colonies on the LJ medium, while the MBC was evaluated by the absence of Mtb on the LJ medium. LJ medium is used because it uses fresh egg and glycerol, egg in LJ medium as an iron source for Mtb'sMtb's growth.

C. Measurement of Iron Levels

The iron levels measurement was performed using atomic absorption spectrophotometer (AAS) [11]. Iron content was assessed in four treatment groups: 1. LJ medium; 2. LJ medium inoculated with Mtb; 3. LJ medium added by 128 ppm of Brazilin; and 4. Medium LJ was added by 128 ppm of Brazilin and inoculated with Mtb.

III. Results and Discussions

A. An Isolation, Characterization, and Determination of Brazilin Compounds

The isolation of brazilin compounds aimed to obtain pure compounds from the fractionation. The first phase of isolation was performed by column chromatography using ethyl acetate solvent: hexane at the ratio of 1:9. The result was followed by the separation of thin-layer chromatography (TLC) to obtain isolates with the same RF value. TLC results were obtained from the three groups of fractions with the same RF value, i.e., f1 = 2.2689 g, f2 = 0.6104 g and f3 = 2,338 g. The first suspected fraction containing brazilin compounds was purified. The second stage of the separation was done by column chromatography using hexane: ethyl acetate with a ratio of 2:8. The results of the TLC separation were then performed, and four fractions were obtained, namely f1, f2, f3, and f4, with a weight of 0.4889, 0.5770, 0.3032, and 0.0417 g respectively. In the second fraction, the major compounds which produced blue color fluorescent under UV light were identified, and then the pure isolates were characterized using IR and UV spectrophotometer, while the purity of the *Brazilin* isolate was determined with HPLC.

The spectrophotometer of IR showed several absorption areas in which the functional group of brazilin compound can identify, including the absorption in C-H group at 1000 -1300 wavelength that indicated the presence of C-O-C group and C-OH, at 1550-1650 wavelength that showed the presence of C = O group (Fig.1).

According to the result, the group observed in the absorption area is a functional group of brazilin compounds [12]. The UV-Vis spectrophotometer analysis on fraction 2 exhibited the maximum absorption peak at 204.5 nm and shoulder at 250 nm-300 nm (Fig.2). The maximum absorption peaks and shoulders exist as fraction 2 contains flavonoid derivates, which are brazilin compounds.

B. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Brazilin against Mycobacterium tuberculosis

MIC and MBC were assessed by the proportion method [13]. The proportion method is commonly used to examine anti-tuberculosis substances standardized by WHO. MIC and MBC tests were led to identify the antimicrobial activity by inhibiting bacterial growth.

The study used the proportion method by conducting culture on Lowenstein Jensen (LJ) medium add Brazilin in various concentrations. In the process, the proportion method takes 4 - 6 weeks for the results. The proportion method was used to obtain the MIC value, reducing the mycobacterium population in line with the increase in brazilin concentration.

Also, to analyze iron content in the control medium (without bacteria and *Brazilin*) and on medium containing brazilin concentration at MIC values. The method for testing the sensitivity of anti-tuberculosis drugs can also use the Rema (Resazurin Microtiter Assay) method. This method is a colorimetric method using the liquid medium Middlebrook 7H9. After incubation for seven days, adding resazurin to the medium will produce a color change indicating the growth of Mycobacterium. However, this method cannot analyze the mycobacterium population and iron chelation ability. Purnanamasari *et al.* [14] compared with the culture method, the Rema method had 80% sensitivity, 100% PPV (positive predictive value), and 97% NPV (negative predictive value). The feasibility study of the medium as a primary isolation media for mycobacteria, using MB BACT and Middlebrook 7h10 (MB7H10) and LJ medium, showed that the medium LJ was better and specific than the medium Middlebrook 7 H10 [15]. Antimicrobial susceptibility testing (AST) of Mtb has customarily been performed by the agar technique of proportion using the Lowenstein-Jensen (LJ) medium as a bacterial culture medium by including the concentration of the drug that has been suspended into the LJ medium.

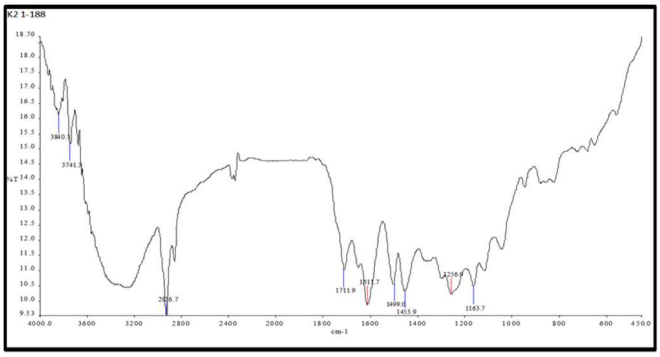

Fig. 1 Characterization of Brazilin using infra-red spectrum

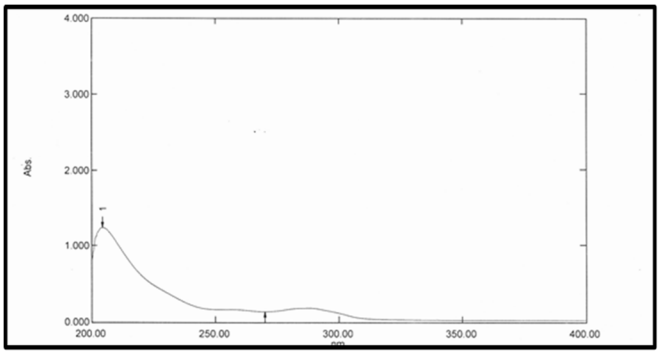

Fig. 2 Characterization of Brazilin using UV-Vis

TABLE I
MIC AND MBC OF MTB OF INOCULUM

Assay Material	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8	
	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}	10^{-3}	10^{-5}
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Positive Control	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
B 1 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 2 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 4 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 8 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 16 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 32 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 64 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 128 ppm	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
B 256 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B 512 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B 1024 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R 40 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I 0.2 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E 2 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S 4 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

B=brazilin; R=rifampicin; I=isoniazid; E=ethambutol; S=streptomycin

The lowest concentration of an antimicrobial agent to inhibit the growth of microbial is shown by MIC. The brazilin concentration identifies MIC to produce the growth of 20-100 colonies of Mtb. MIC is influenced by several factors, such as the growth medium component, age, and acidity (pH), the number of inoculums, the age of inoculum, the temperature, and incubation time for bacterial growth [16].

The MBC is the lowest concentration of an antimicrobial agent to kill the bacterium and is indicated by a total inhibition of bacterial growth in the medium. [17] The result of MIC and MBC of brazilin compounds against Mtb presented in Table 1. showed that the MIC of Brazilin for Mtb is 128 ppm and MBC of *Brazilin* for Mtb at 256 ppm on LJ medium. Compared to the control of LJ medium, Mtb grows in the fourth week, while adding brazilin compound at a concentration of 128 ppm in the LJ medium can delay the growth of Mtb until the fifth week. The presence of Mtb growth inhibition by the brazilin compound reduces the number of Mtb. There are no differences in the value of MIC and MBC against Mtb in populations with the dilution of inoculum 10^{-3} and 10^{-5}, and this result shows that Brazilin was effective in inhibiting the growth of Mtb. Brazilin belongs to

the class of flavonoids. The antibacterial mechanism of flavonoids consists of three different ways: direct kill, synergic antibiotic activation, and weakening pathogenicity of bacteria [18].

Flavonoids have demonstrated inhibitory activity towards various kinds of lactamases delivered by bacteria, which are the key enzyme that debilitate the common antibiotics. In any case, the antibacterial activity of flavonoids reasoned that hydroxyls at exceptional sites on the aromatic rings of flavonoids improve the action or hydroxyl groups on extraordinary sites are ideal for the action; for example, the 5,7-dihydroxyl replacement for flavone and flavanone, and 2' or 4' hydroxylation for chalcones [19].

The hydroxyl group also increased the activity of flavone at three (3) positions on the C ring. Flavonoids have the potential to increase the effectiveness of antibiotic treatment [20]. The ability of Brazilin to reduce the Mtb population is presented in Figures 3 and 4.

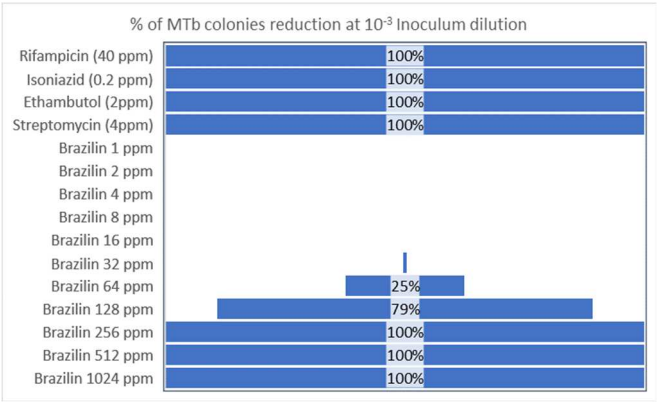

Fig. 3 Mtb colonies reduction at 10^{-3} inoculum dilution in various brazilin concentrations for 8 weeks

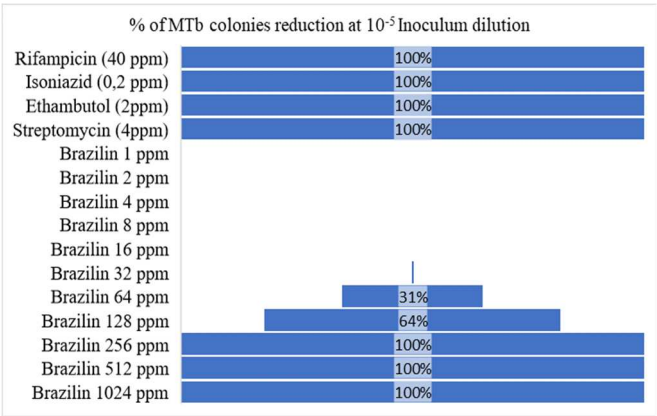

Fig. 4 Mtb colonies reduction at 10^{-5} inoculum dilution in various brazilin concentrations for 8 weeks

Figure 3 shows that the Mtb population at 10^{-3} dilution inoculum at 128 ppm and MIC, the number of the cell was reduced by 79 %, while at a concentration of 256 ppm, no Mtb grew or the Mtb population was reduced by 100%. MBC concentration at 256 ppm is the same as that of first-line anti-tuberculosis drugs (ATBD). Mtb in dilution of inoculum 10^{-5}, brazilin concentration of 128 ppm can reduce the population of Mtn to 64%. The MBC concentration is the same as the four types of the first line of anti-tuberculosis drugs (rifampicin, isoniazid, streptomycin, and ethambutol). The first line of antibiotics for anti-tuberculosis drugs has a high sensitivity to anti-tuberculosis than *Brazilin*.

C. The ability of brazilin compounds as iron chelates

Iron is a substance that TB bacteria need for their metabolism; the acquisition of Fe ions is necessary for the intracellular growth of Mtb [21]. The absence of iron is a critical environmental pressure condition experienced by MTB during the infection process due to the lack of free iron in the host.

TABLE II
IRON CONTENT IN LOWENSTEIN-JENSEN (LJ) MEDIUM WITH AND WITHOUT THE ADDITION OF BRAZILIN

Material Assay	**The Average of Iron Concentration (ppm)**
Medium LJ	4.55
Medium LJ + Brazilin 128 ppm	3.05 (32.96%)

Table 2. Showed the concentration of iron in LJ medium and LJ medium that was added with 128 ppm of Brazilin. The measurement of iron levels in LJ medium containing brazilin compound shows the ability of brazilin compound to chelate iron at 32.96%. The ability of chelation of brazilin structure is due to the bidentate ligand, which is a powerful scavenger for metal cation; *Brazilin* has a catechol group as well as a bidentate ligand capable of tight binding iron (III) at pH 7. Iron chelators such as Deferiprone (Ferriprox®) are bidentate ligands that are effective chelators for patients with thalassemia who can remove excess iron from the heart. But, it has major toxic side effects, including agranulocytosis, musculoskeletal and joint pains, gastric intolerance, and zinc deficiency [22], flavonoids having a catecholate group are more likely to interact with metals, particularly Fe [23].

One virulence factor of mycobacteria is siderophore production, which are low-molecular-weight compounds with a high affinity for iron. Siderophore is a complexing compound of Fe (3^{+}) ions or chelating iron produced by certain types of microbes [24]. The siderophore acts after being released into the extraciliary space within the macrophage. Siderophore then binds to the available iron molecule and reinternalizes it through specific receptors. Two types of siderophore are found in *mycobacteria*, *exochelins*, and *mycobactins*. *Pathogenic mycobacteria*, especially Mtb, only produce *mycobactin*, which contains salicylate molecules [25]. Brazilin's ability to chelate iron and Mtb compounds requires iron to be the basis of competition between Brazilin and Mtb for iron in the antimicrobial mechanism of *Brazilin* [26]. The iron needed by Mtb in the LJ medium for its growth is obtained from duck eggs, which are the main component of the LJ medium.

TABLE III
IRON CONTENT IN LJ MEDIUM WITH OR WITHOUT MYCOBACTERIUM TUBERCULOSIS

Assay	**The Average of Iron Concentration (ppm)**	
	Dilution 10^{-3}	**Dilution10^{-5}**
LJ + Mtb	0.005	0.103
LJ + Mtb + Brazilin 128 ppm	1.450	2.410
LJ + Mtb+ Brazilin 256 ppm	0.410	0.490

Iron levels at brazilin concentration 128 ppm (MIC) are higher than iron levels at concentrations of 256 ppm (MBC), which are (1.45; 2.41 ppm) and (0.41; 0.49 ppm) (table 3). The higher the *Brazilin* concentration in MBC, the lower the free iron content than the MIC free value. The concentration of free iron in LJ medium inoculated with Mtb and added 128 ppm of Brazilin was less than iron levels in the LJ medium without Brazilin. The same thing is also seen in table 3: the concentration of iron in 10^{-5} inoculum dilution was higher than the iron level in 10^{-3} inoculum dilution. It is indicated that Mtb growth requires iron as a nutrient. Anti-tubercular properties of Brazilin are thought to be due to the iron chelation properties of Brazilin, which may be caused by the presence of multiple OH groups and the catechol structure as an iron-binding site [27].

The Mtb growth on LJ medium containing brazilin compound and without brazilin compound began in the fifth and third weeks, respectively. The Mtb growth on LJ medium containing brazilin was slower due to iron binding competition between the *Brazilin* compound and Mtb. A reduced level of iron in LJ medium causes inhibition on Mtb growth. Furthermore, the ability to chelate iron was due to the siderophore of Mtb. Siderophore is a complexing Fe (3^{+}) ion compound or iron chelator produced by certain microbes [24]. The mechanism of the *Brazilin* compound to inhibit Mtb growth is due to iron chelation. The ability of this compound to chelate iron may reduce the availability of iron for Mtb growth. The low iron concentration of the LJ medium added by the *Brazilin* compound indicates that the iron binding capacity of Brazilin resulted in a lower absorption value than the LJ medium without the *Brazilin* compound. Mtb requires iron to synthesize 40 different enzymes in the Mtb genome [28]. Besides, iron is involved in electron transport, oxygen metabolism, and co-factor proteins involved in amino acid biogenesis. Iron is also essential for the DNA synthesis of ribonucleotide reductase [29]. According to Phelan et al. [21] and Kololli, et al., [30], the iron will enhance Mtb growth. The bacteria attack antibody-producing cells, specifically, the T cells and the phagocytic cells. Phagocytic cells can attack free radicals and lysozyme, while the T cell is a natural component that responds to Mtb infection [31]. Additionally, T cells consist of two types, the CD4 + T cells and CD8 + T cells. CD4 + T cells responded to the production of antibodies and macrophages, which is helper cells, whereas CD8+ T cells are killer cells specific to the Mtb infection. Indeed, Mtb in the penetration process will take over the role of CD8 + T cells as the body's defense against infectious Mtb [32].

The differences in the sensitivity between the *Brazilin* compound and ATBD (Anti-Tuberculosis Drug) were hypothetically due to the different mechanisms of inhibiting Mtb growth. Most ATBD has a direct antimicrobial mechanism on Mtb cells. For instance, rifampicin can inhibit the growth of Mtb by inhibiting the synthesis of enzyme transcription and gene expression. The inhibition of the synthesis enzyme transcription is due to the binding of rifampicin to the β-subunit of RNA polymerase [33]. On the other hand, the mechanism of isoniazid against Mtb is by inhibiting mycolic acid synthesis. Thus, the formation of Mtb cell walls is also inhibited. Indeed, isoniazid can inhibit the synthesis or metabolism of DNA, lipids, carbohydrates, and Nicotinamide adenine dinucleotide (NAD) of Mtb cells [34]. Nevertheless, ATBD inhibition against Mtb causes drug resistance to Mtb due to the mutation process of Mtb. Irregular ATBD consumption can lead to a pause of Mtb in its defense process against ATBD and contribute to the high drug resistance. Thus, *Brazilin* is an *anti-tuberculosis* through an indirect antimicrobial mechanism because the brazilin compound is not working on the physiological structure of Mtb [35].

Brazilin is a group of phenolic compounds that can inhibit bacterial growth by inhibiting DNA and protein synthesis [36]. Besides, flavonoids and Brazilin are phenolic compounds that have been widely used in medicine because of their considerable benefits for health, such as growth inhibition for several pathogens.

The differences of iron concentration identified in LJ medium with and without the addition of *Brazilin* and with and without the inoculation of Mtb. Based on the observations, the level of iron inoculation in LJ medium with Mtb was lower than LJ medium without the inoculation of Mtb. It occurred due to the competition between Mtb and Brazilin to chelate iron; hence, the iron binding process produces lower atomic absorption values. In contrast, the LJ medium without Mtb had lower iron chelation, resulting in a higher absorption value. The differences in iron levels are influenced by the percentage of iron binding in the sample, in which the iron levels in LJ medium negatively correlated with the percentage of iron binding.

Brazilin's ability to inhibit Mtb growth is also due to the nature of the phenolic compounds. The phenolic compounds may initiate irreversible harm to the cytoplasmic membrane and cell content coagulation, possibly leading to the inhibition of intracellular enzymes. Phenolic acids are accounted to have the ability to disrupt membrane integrity, as they cause resulting essential intracellular constituent leakage. Protein and RNA syntheses are also affected by flavonoids that may act by repressing both energy metabolism and DNA synthesis. As Brazilin is a group of phenolic compounds, the mechanism of phenolic as an antimicrobial is by inhibiting the synthesis of nucleic acids, cytoplasmic membrane function, energy metabolism, bacterial pili attachment, and biofilm formation, and inhibiting the synthesis of cellular membranes [37].

Polyphenols exhibit antibacterial properties; based on chemical structure; polyphenols are divided into two major: flavonoids and non-flavonoids. The structure of flavonoids is a carbon skeleton of diphenyl propane and two benzene rings (ring A and B) joined by a linear three-carbon chain. The pyran ring (ring C) is formed by an A benzene ring and a central three-carbon chain. Flavonoids are divided into subclasses depending on the degree of oxidation of the central pyran ring. Based on molecular structure, flavonoids can be divided into the following groups: flavanols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoids. Non-flavonoids are divided into benzoic acid derivatives such as gallic or protocatechuic acid, cinnamic acid derivatives such as *caffeic*, *ferulic*, or *coumaric acid*, and *stilbenes* such as resveratrol. Much research shows that polyphenolic compounds such as flavonoids or phenolic acids exhibit antimicrobial properties against microorganisms, including multi-drug resistance strains that are bactericidal or bacteriostatic. The action of phenolic compounds on bacterial cells is partly by damaging cell membranes, inhibiting

virulence factors such as enzymes and toxins, and suppressing biofilm formation. In addition, several natural polyphenols have synergistic properties when combined with general chemotherapy [38]. *Brazilin* is a flavonoid; it has antimicrobial potency because the catechol group inhibits energy metabolism by binding the iron for Mtb energy metabolism [27]. Other phenolic compounds, such as kaempferol, hesperetin, and biochanin, can inhibit the growth of other bacteria such as *Escherichia coli* by inhibiting the metabolism of liposomes [39]. Phenolic compounds in guava (*Psidium guajava*) can inhibit the growth of several pathogenic bacteria such as *Bacillus strearothermophillus* and *Pseudomonas fluorescence* by inhibiting the nucleic acid synthesis [40].

According to Safitri [7] *Sappan* wood extract (*C. sappan* L.) at the concentration of 200 mg /kg/ bw was able to reduce ferritin levels by 32% whereas, in satellites groups or silenced for one week, ferritin still decreased. It showed that the *Sappan* wood extract had a long-term effect, which showed iron chelating. MTB battles for the accessible restricted iron resource with the host and one of the techniques utilized is by extracting iron from iron storage molecules, for example, transferrin (TF)1, lactoferrin, and ferritin [9]. Administration of *Sappan* wood at a dose 200 mg/kg/ body weight (bw) also increased the transferrin to 102.27%, which had a similar ability as deferiprone. In rats (*Rattus norvegicus* L.), the administration of iron increased liver iron concentration (LIC) to 265.67 mg/kg/bw compared to the control group, 51.33 mg/kg/kg/bw or increased HIC by 417%. The condition is caused by the underlying accumulation of iron liver as the primary organ to regulate iron. Administration of *Sappan* wood extract at a dose of 200 mg/kg/bw also reduced iron levels in the liver by 41.7% within 28 days [4]. This result showed *Sappan* wood has iron chelating activity. The ability of brazilin compounds inhibits Mtb growth also confirmed by previous research, according to Safitri [27]. *Sappan* wood extract at a concentration of 100 ppm showed an inhibitory ability of MTB strain H37Rv growth at week six. In contrast, MTB growth containing *Sappan* wood extract was slower than MTB growth without the addition of *Sappan* wood. The growth of MTB in the LJ medium without *Sappanwood* extract was seen in the third week. The limited presence of iron in the LJ medium containing *Sappan* wood extract could cause slow growth.

Based on the results, Brazilin could be used as anti-tuberculosis due to its mechanism to inhibit Mtb extracellularly by chelating the iron. Moreover, phenolic properties in this compound can inhibit Mtb growth. These results can be used as the basis to solve the Mtb resistance problems to anti-tuberculosis drugs (ATBD) where ATBD are only able to inhibit the growth of bacteria intracellularly, allowing the occurrence of mutations that reduce ATBD capability to eradicate tuberculosis infections recently.

IV. Conclusions

Brazilin has a MIC at 128 ppm and MBC at 256 ppm to MTb. Brazilin also has properties as an iron-chelating agent. *Brazilin's anti-tuberculosis* mechanism is based on competition between iron-binding of Brazilin and iron-binding by Mtb. *Brazilin* belongs to the flavonoids class known to have antibacterial properties, such as the direct killing of the bacteria, synergic antibiotic activation, and weakening pathogenicity of bacteria. With these advantages, Brazilin could be used as an anti-tuberculosis drug due to its mechanism to inhibit Mtb extracellularly by iron chelation and can be developed into tuberculosis drugs to anticipate the increased MTb resistance to antibiotics.

Acknowledgment

The authors thank the Ministry of Research, Technology, and the Higher Education Republic of Indonesia (Kemenristekdikti) for supporting and funding this research through the Scheme of Applied Research of excellence of higher education (*Penelitian Terapan Unggulan Perguruan Tinggi*). We would like to thank Aya Sofia for technical supporting and the *Balai Laboratorium Kesehatan Provinsi Jawa Barat*, Indonesia, was given the MTB strain H37Rv.

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