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Antioxidant and Antibacterial Activity of Acetone Extract of Javanese Pepper Fruit (*Piper retrofractum* Vahl.)

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ABSTRAK

Lada Jawa (*Piper retrofractum* Vahl.) adalah rempah-rempah yang memiliki nilai ekonomi dan telah banyak digunakan sebagai ramuan jamu tradisional untuk melawan berbagai masalah kesehatan, seperti bronkitis, demam, tuberkulosis, diare, sebagai obat kumur dan afrodisiak. Sampai sekarang, tidak ada laporan tentang aktivitas antioksidan dan antibakteri dari ekstrak aseton buah lada Jawa. Oleh sebab itu, penelitian ini bertujuan untuk mengidentifikasi aktivitas antioksidan dan antibakteri ekstrak aseton buah lada Jawa yang berasal dari Jawa Barat. Aktivitas antioksidan ditentukan oleh metode DPPH (1,1-diphenyl-2-picrylhydrazil), sedangkan aktivitas antibakteri ditentukan dengan metode difusi agar menggunakan *Eschericia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* sebagai bakteri uji. Ekstrak aseton buah lada Jawa memiliki aktivitas antioksidan dengan nilai IC₅₀ 825 ppm. Sementara itu, penentuan aktivitas antibakteri pada ekstrak aseton buah lada Jawa menunjukkan zona inhibisi pada *Staphylococcus aureus*, yang tidak ditemukan pada bakteri uji lain.

Kata Kunci: Javanese Pepper; Antioksidan; Antibakterial

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ABSTRACT

Javanese Pepper (*Piper retrofractum* Vahl.) has been known as economically valuable herb and has been traditionally used as folk medicine (jamu) against various kinds of health disorders, such as bronchitis, fever, tuberculosis, diarrhea, as mouthwash, and aphrodisiac. Until now, there is no report about antioxidant and antibacterial activity of acetone extract of Javanese pepper fruit originated from West Java. Therefore, this study analyzes antioxidant and antibacterial activities of acetone extract of Javanese pepper fruit originated from West Java. Antioxidant activity determined by DPPH (1,1-diphenyl-2-picrylhydrazil) radical scavenging method, while antibacterial activity determined by agar diffusion method with *Eschericia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* as test bacteria. Acetone extract of Javanese pepper fruit is proven to possess antioxidant activity with IC₅₀ value of 825 ppm. Meanwhile, antibacterial activity determination of acetone extract of Javanese pepper fruit shows inhibition zone on *Staphylococcus aureus*, but no inhibition found for another tested bacteria.

Kata Kunci: Javanese Pepper; Antioxidant; Antibacterial

1. INTRODUCTION

Indonesia is well-known for its herb and spices biodiversity. There are more than 20000 herb species spread all over Indonesia territory [1]. Almost 50% of Indonesian citizen using herb to prevent and treat various kind of disease [2]. Plants from Piperaceae family have been known as herb that can be used to cure many diseases. One of Piperaceae family plant which grow in Indonesia is Javanese pepper (*Piper retrofractum* Vahl.).

Javanese pepper is a scrub which spread in tropical and sub-tropical region. The fruit is green when still young and become red after mature. Plant and fruit of Javanese pepper showed at Figure 1 and 2. Not only in Indonesia, Javanese pepper can be found in Malaysia, Filipina, Thailand, Vietnam, China, dan India [3]. Javanese pepper used traditionally to treat bronchitis, fever [4], tuberculosis, diarrhoea, as mouthwash [5], and aphrodisiac [6]. Many researches were done to prove efficacy of Javanese pepper scientifically. Essential oil of Javanese pepper leaf has antibacterial property [5]. Amide and lignan from it stem

extract show significant antileishmanial activity [7]. Meanwhile, Javanese pepper fruit extract have anti-obesity [8], larvicide [9], and good neurotropic activity [10].

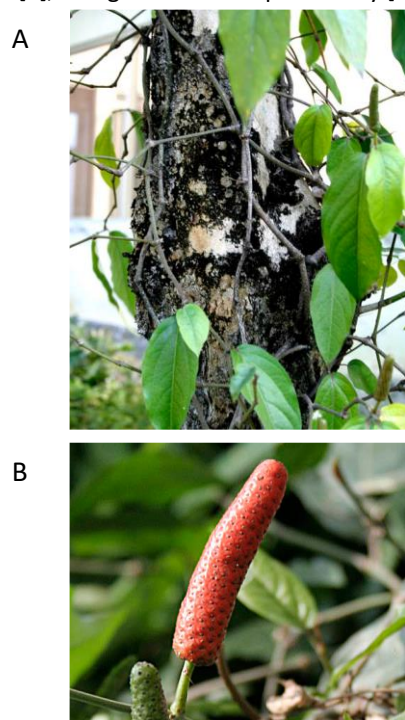


Figure 1. (A) Javanese pepper plant; (B) Javanese pepper fruit [11]

Many activities showed by Javanese pepper are related with its secondary metabolites contents [11]. Plants produces different secondary metabolites when grow at different locations. Javanese pepper contains many secondary metabolites group such as amides [12], alkaloids, steroids, flavonoids, saponins [13], phenolic acids, terpenoids, dan lignins [14]. Flavonoid compounds, phenolic acids, dan terpenoids have strong antioxidant activity [15]. Furthermore, flavonoids, phenolic acids, alkaloids and saponins also active as antibacterial agent [16-18]. Until now, there is no report about antioxidant and antibacterial activities of acetone extract of Javanese pepper fruit originated from West Java. Therefore, this research aims at determining antioxidant and antibacterial activities of acetone extract of Javanese pepper fruit originated from West Java.

2. METHODS

2.1 Apparatus

In this research we use glass apparatus such as simple distillation apparatus, Büchner funnel, beaker glass, volumetric flask, short funnel, stirring rod, volumetric pipette, vial bottle, petri dish, test tube, and graduate glass. Furthermore, we use instrumental apparatus such as analytical balance, heating mantle, vacuum rotary evaporator, and Shimadzu UV 1200 spectrophotometer.

2.2 Material

Dried Javanese pepper fruit obtained from Manoko Experimental Garden, in Kabupaten Lembang, West Java. Distilled acetone used for sample maceration. Pure methanol used as solvent in determination of antioxidant activity, while 1,1-diphenyl-2-picrilhydrazil (DPPH) used as free radical. Vitamin C used as comparison antioxidant compound. For determination of antibacterial activity, we use pure methanol as solvent and negative control. Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) used as bacterial growing medium. We use *Staphylococcus aureus*, *Streptococcus mutants*, *Escherichia coli*, and *Pseudomonas aeruginosa* as test bacterials.

2.3 Procedure

Sample Extraction

As much as 500 g of dried Javanese pepper fruit crushed and extracted by maceration. Acetone was used as solvent. Maceration was done for three times, with 24 h for each maceration. Extract was then filtered, and the solvent was evaporated by using vacuum rotary evaporator.

Determination of Antioxidant Activity

The acetone extract of Javanese pepper fruit was weighed as much as of 0.5 g, then dissolved into 50 mL volumetric flask to obtain 10000 ppm main solution. The main solution was pipetted as much as 2.5 mL, 2 mL, 1.5 mL, 1 ml and 0.5 mL into a 25 mL volumetric flask to obtain working solution of 1000 ppm, 800 ppm, 600 ppm, 400 ppm, and 200 ppm. Each of working solutions was reacted with 50 ppm DPPH solution at 4:1 ratio. DPPH solution 50 ppm was used as control. The test solution then incubated for 30 minutes in the dark. Absorbance measurement of test solution was done by three replication using Shimadzu UV 1200 spectrophotometer at 517 nm. Vitamin C used as comparison antioxidant compound. The inhibition percentage value calculated using Equation (1).

$$\text{Inhibition percentage} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (1)$$

Determination of Antibacterial Activity

Determination of antibacterial activity begins by preparing the test medium. As much as 21 g of MHB medium and 38 g of MHA medium dissolved in 1 L of aquadest, then sterilized by autoclave for 15 minutes at 121°C. MHB medium is used for bacterial regeneration, while MHA medium is used for antibacterial activity testing. A 250 µL bacterial suspension was mixed with 5 mL of MHB medium. 100 µL of the mixture pipetted then mixed with 20 mL MHA medium at temperature 40-50°C. The mixture was homogenized and allowed to solidify. Furthermore, MHA medium that have been solidified was perforated to make wells with a diameter of 5 mm. The extract was dissolved in methanol and made several concentrations of 0.625-80%. A total of 50 µL extract solution was pipetted into the well provided, then incubated for 24 h at 37°C. After incubation, culture was observed and was measured for its inhibitory zone. All testings were conducted in duplicate.

3. RESULTS AND DISCUSSIONS

3.1. Antioxidant Activity

The test results showed that acetone extract of Javanese pepper fruit has antioxidant activity. This can be seen from the difference between the absorption value of the control solution and the absorption of the test solution. The difference in absorption value is equivalent to the concentration of antioxidant compounds present in the extract. In Table 1, the absorption value of the test solution decreased as the concentration of extract increased. However, in contrast to the percentage of inhibition that increases. This is due to the increasing number of antioxidant compounds in the extract, the more DPPH radicals can be neutralized. If more DPPH radicals can be neutralized, the stronger the antioxidant activity.

Table 1. Antioxidant activity of acetone extract of Javanese pepper fruit

Extract Concentration (ppm)	Test Solution Absorban	Control Absorban (50 ppm)	Inhibition (%)	Regression Equation	IC ₅₀ (ppm)
200	0,239		19,53	y = 0,047x + 11,21	825
400	0,204		31,31		
600	0,179	0,297	39,73		
800	0,153		48,48		
1000	0,125		57,91	R ² = 0,996	

Table 2. Antioxidant activity of vitamin C

Vitamin C Concentration (ppm)	Test Solution Absorban	Control Absorban (40 ppm)	Inhibition (%)	Regression Equation	IC ₅₀ (ppm)
1	0,818		10,50	y =13,45x - 4,819	4,07
3	0,585	0,914	35,99		
5	0,403		55,91		
7	0,059		93,54		
				R ² = 0,982	

The influence of solvent type on antioxidant activity of Piper leaf extract or better known as Sirih has been studied by Nouri et al. (2014) [20]. As a result, the antioxidant activity of ethyl acetate, acetone, and methanol extract has inhibition value 55.51%, 81.77%, and 87.18%, respectively. The study showed that methanol as a more polar solvent than acetone and ethyl acetate, yielded extracts with the strongest antioxidant activity.

Acetone is an organic solvent that is relatively more nonpolar than methanol. It can be seen from the dielectric constant value, which is 20.7 for acetone and 32.7 for methanol [21] Compounds that are serve as antioxidants generally come from phenolic compounds class. Acetone will dissolve fewer phenolic compounds than methanol because phenolic compounds tend to be polar. Therefore, acetone extract of Javanese pepper fruit has a very large IC₅₀ value, which is 825 ppm. When compared with the

The antioxidant activity of acetone extract of Javanese pepper fruit differs significantly with antioxidant activity of vitamin C. The antioxidant activity of vitamin C is found in Table 2. Acetone extract of Javanese pepper fruit takes 825 ppm to produce 50% of inhibition, while vitamin C only requires 4.07 ppm to produce the same inhibitory value. This difference is caused because vitamin C is a pure compound that has proved very strong as an antioxidant. In contrast to the acetone extract of Javanese pepper fruit which are still a mixture of various compounds, so the activity of antioxidant compounds contained in the extract is covered by other compounds that are not serve as antioxidants. In addition, the type of solvent used in extraction process also affect the antioxidant activity of the extract obtained [19].

activity of antioxidant extract of other Piper genus plants, for example ethanol extract of *Piper crocatum* leaf which have IC₅₀ value 85 ppm [22], then acetone extract of Javanese pepper fruit seems to have antioxidant activity nearly tenfold weaker. According to Kamto et al. (2014), IC₅₀ < 50 ppm is very strong; 50 ppm < IC₅₀ < 100 ppm is strong; 100 ppm < IC₅₀ < 200 ppm is said medium; IC₅₀ > 200 ppm is weak [23]. So that the acetone extract of Javanese pepper fruit is included in the category of weak antioxidants.

3.2. Antibacterial Activity

The results showed that acetone extract of Javanese pepper fruit only gives zone of inhibition on *Staphylococcus aureus* bacteria. This inhibitory zone is characterized by a clear area around the well filled with the extract solution as shown in Figure 3. Negative control has no inhibitory zone,

so the inhibitory zone showed on the *Staphylococcus aureus* bacteria purely generated from acetone extract of Javanese pepper fruit. The inhibitory zone formed was measured in mm. The measurement of inhibitory zone diameter was done three times, in horizontal, vertical, and diagonal positions. The measurement results were then averaged.

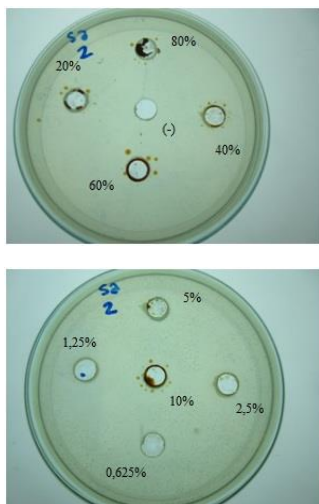


Figure 3. Inhibitory zone of acetone extract of Javanese pepper on *S. aureus* at 5-80% concentrations

Antibacterial activity of acetone extract of Javanese pepper fruit is closely related to the content of chemical compounds present in it. Javanese pepper fruit is known to contain a variety of secondary metabolite products from the class of alkaloids, flavonoids, saponins, steroids, and tannins [24]. Several classes of compounds, such as phenolic acid, flavonoids, tannins [16], alkaloids [17], and saponins [18] [25] have been known to have antibacterial activity. The existence of these compounds is believed to be responsible for the antibacterial activity produced by acetone extract of Javanese pepper fruit against *Staphylococcus aureus*.

Research on other *Piper* genus plant species showed that ethanol extract of *Piper crocatum* leaf or better known as Sirih Merah has antibacterial activity against *Staphylococcus aureus* [26]. However, the acetone extract of Javanese pepper fruit has higher activity because it started to provide inhibition at 5% concentration (equivalent to 5,000 ppm) with 5.4 mm inhibitory zone diameter, while ethanol extract of Sirih Merah leaf started to provide inhibition at 1,000,000 ppm with inhibitory zone diameter of 20.6 mm. Other studies showed that ethanol extract of *Piper betle* leaf [27] and ethyl acetate extract of *Piper hispidum* leaf [28] also have antibacterial activity against *Staphylococcus aureus*, but no activity against *Escherichia coli* and *Pseudomonas aeruginosa*.

Figure 4 shows the inhibitory zone produced by acetone extract of Javanese pepper fruit is observed at a concentration of 5%. The diameter of the inhibitory zone continues to increase as the concentration of extract increases. Antibacterial activity is said to be very strong if

the inhibitory zone diameter > 20 mm, it is said to be strong if the inhibitory zone diameter is between 10-20 mm, it is said to be medium if the inhibitory zone diameter is between 5-10 mm, and it is said to be weak if the inhibitory zone diameter < 5 mm [29]. Based on these references, the antibacterial activity of acetone extract of Javanese pepper fruit is classified as medium antibacterial agent because having inhibitory zone ranging from 5-7 mm after being reduced by the diameter of the well.

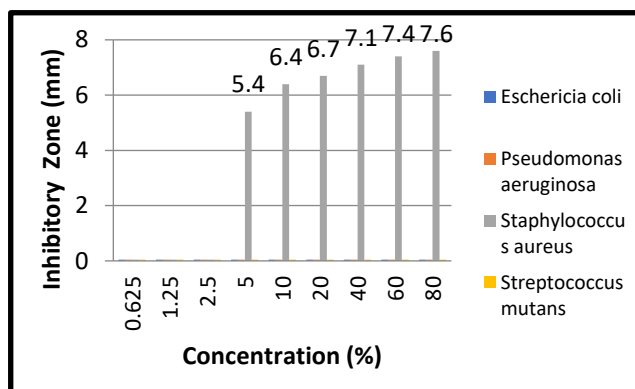


Figure 4. Antibacterial activity of acetone extract of Javanese pepper fruit against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. mutans*

4. CONCLUSION

The acetone extract of Javanese pepper fruit originated from West Java has antioxidant activity with IC₅₀ value of 825 ppm and antibacterial activity against *Staphylococcus aureus* with inhibitory zone diameter 5,4-7,6 mm at extract concentration 5-80%. For upcoming research, it is suggested to isolate secondary metabolites and to test the antioxidant and antibacterial activity of pure secondary metabolites that have been isolated.

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