Studii şi Cercetări Ştiinţifice 2014, **15** (2), pp. 107-112
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

ANTIBACTERIAL ACTIVITIES OF ETHYL ACETATE EXTRACT FROM *Plumeria Alba* STEM BARK

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Received: June, 12, 2014
Accepted: July, 08, 2014

Abstract: In current study, antibacterial compounds of ethyl acetate extract from white frangipani (*Plumeria alba*) stem bark were isolated and their antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* were investigated. The plant extract was prepared by maceration of 500 g of dried stem bark *P. alba* in ethyl acetate. A total of 26 g (5.2 %) of crude plant extract was obtained. The fractionation was carried out by using vacuum liquid chromatography column (VLCC), resulting of five fractions with amount of 0.42 g, 0.36 g, 0.61 g, 0.99 g and 4.15 g for fraction A, B, C, D, and E, respectively. These fractions and crude extract were applied to the Kirby-Bauer method using discs with various concentrations of 0.5, 1.0, 2.0, and 3.0 %, to examine their antibacterial activities against *E. coli* and *S. aureus*. The fraction B was found to be the most active, showing average zone of inhibition, at 3.0 % concentration, of 12.4 mm and 15.6 mm to *E. coli* and *S. aureus*, respectively. The phytochemical analysis result of fraction B indicated terpenoids compounds. In this experiment, chloramphenicol was used as a positive control.

Keywords: *antibacterial activity, Escherichia coli, Staphylococcus aureus, Plumeria alba*

INTRODUCTION

White frangipani (*Plumeria alba*) is a well-known plant that has been widely used as a traditional medicine in Indonesia to treat various diseases, such as constipation and dysentery. Dysentery is a stomach ailment that is caused by bacteria, commonly *E. coli* and *S. aureus*. Ethnobotanical study of white frangipani showed that the plant contains antibacterial compounds, especially against bacteria that cause dysentery.
Various studies of white frangipani extract's activity against diarrhea-causing microorganisms has been reported, but most of the extracts or essential oils of white frangipani were obtained from its flower or leaves [1 – 5]. Ekowati investigated the stem bark of the plant frangipani and reported that at the concentration of 1600-4000 ppm, the obtained extract has an antibacterial activity [6]. Results of other studies showed that at a concentration of 10%, *n*-hexane extract of the white frangipani stem bark have antibacterial activity with diameter of inhibition zone of 8.3 and 10 mm, against *E. coli* and *S. aureus*, respectively [7 – 8]. In addition, the methanol extract was also active as an antibacterial against *E. coli* and *S. aureus*.
In this study, the activity of the stem bark of plant *P. Alba* extracted with a semi-polar solvent was examined. The selection of bio-indicator was based on the difference bio-indicator species i.e., *E. coli* to represent Gram-negative bacteria and *S. aureus* as Gram-positive bacteria.

MATERIALS AND METHODS

Extraction and fractionations

The stem bark of *P. alba* was dried and crushed. A total of 500 g obtained dry powder was macerated with *n*-hexane and sequentially with ethyl acetate solvent. The crude extract was further fractionated by vacuum liquid chromatography column using silica gel stationary phase G-60 GF254 in gradient elution.

Identification of isolated compounds

Alkaloids test of *P. alba* stem bark and ethyl acetate extracts was performed using Meyer, Dragendorff, and Wagner reagent. For steroid test, the Liebermann-Burchard reagent was used. The occurrence of blue or green color indicates the presence of steroids, while the formation of red color indicates the triterpenoids compound. The existence of stable foam for 30 minutes after agitation is a sign of a saponin. Characterization of flavonoid was done by the addition of magnesium metal and HCl, and indicated by the appearance of red or purple color [9].

Antibacterial activity

Bioactivity assay was performed by Kirby-Bauer method using discs. Mueller-Hinton Agar (MHA) was used as media. One set of experiment consisted of six discs *i.e.* the positive control (chloramphenicol 30 $\mu g \cdot mL^{-1}$), negative control (ethyl acetate) and test sample with four different concentrations. Incubation was performed at 37 °C for 24 hours. Antibacterial activity was assessed by observing the diameter of inhibition zone of the applied bacteria. All tests were carried out in triplicate.

RESULTS AND DISCUSSION

The samples obtained from *n*-hexane maceration and ethyl acetate maceration of *P. alba* stem bark were 7 grams (1.4 %) and 26 grams (5.2 %), correspondingly. The ethyl acetate extract sample was then treated further to obtain isolated active compound by using VLCC. Preliminary qualitative analysis by phytochemical test was carried out to determine the group of secondary metabolites in plant. The results, as shown in Table 1, indicated that fresh sample of *P. alba stem bark* contained of alkaloid compounds, terpenoids, saponins and saponin terpenoids. The result of ethyl acetate extract showed no differences. These findings were similar to results obtained from other studies which reported that extracts oil and *P. alba* flower contained several types of secondary metabolites such as flavonoids, alkaloids, terpenoids, steroids, saponins and polyphenols [3, 5].

Table 1. *Phytochemical test results of fresh stem and ethyl acetate extracts of plants* P. alba

Chemical content	Reagent	Fresh Stem	Ethyl Acetate Extract
Alkaloid	Meyer	+	−
	Wagner	+	−
	Dragendorff	+	−
Steroid	Liebermann-Burchard	−	−
Terpenoid	Liebermann-Burchard	+	+
Saponin	Agitation	+	+
Saponin Steroid	Liebermann-Burchard	−	−
Saponin Terpenoid	Liebermann-Burchard	+	+
Flavonoid	0,5 Mg and HCl	−	−

Description: + = Containing secondary metabolites; − = Not containing secondary metabolites.

Antibacterial activity test of ethyl acetate extract of *P. alba* was conducted to determine how strong the extracts inhibit the bacteria growth, especially those that cause dysentery (*E. coli* and *S. aureus).* The corresponding alteration of disk inhibition zone correlates linearly with the antibacterial activity of samples. Antibacterial activity test of ethyl acetate extract and fractions was performed as many as three repetitions.
Average inhibition zone of antibacterial activities (in mm) for ethyl acetate extract against *E. coli* and *S. aureus* are presented in Table 2 and Table 3, respectively. Complementary picture showing bacterial inhibition zone images is depicted in Figure 1.

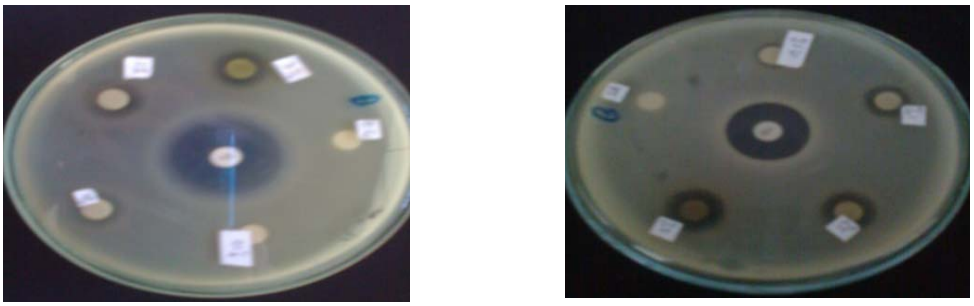

Figure 1. *Inhibition zone of ethyl acetate crude extract of* P. Alba *against* E. coli *(left)* S. aureus *(right)*

Table 2. *Ethyl acetate extract's inhibition zone of* P. alba *bark against* E. coli

Concentration (% w/v)	Inhibitory Zone (mm)					
	Crude	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
0.5	–	–	7.67	–	–	–
1.0	–	–	9.16	7.33	–	–
2.0	–	6.80	11.16	9.67	–	–
3.0	7.00	7.30	12.40	12.40	–	–
P	28.33	26.16	25.00	23.58	–	–
N	–	–	–	–	–	–

Note: P = positive control (chloramphenicol); N = negative control (ethyl acetate)

Results in Table 2 suggested that the crude extract of ethyl acetate was relatively less active against the bacteria *E. coli*, since the measured inhibition zone was only 7 mm at a concentration of 3 %. Furthermore, results at concentration of 0.5, 1, and 2 % did not show clear inhibition zone. Unlike the fractions which had antibacterial activity against bacteria *E. coli* (except fraction E), fraction B generally had the highest activity with inhibition zone of 7.67 mm at a concentration of 0.5 % and 12.40 mm at a concentration of 3%.

Table 3. *Ethyl acetate extract's inhibition zone of* P. alba *bark against* S. Aureus

Concentration (% w/v)	Inhibitory Zone (mm)					
	Crude	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
0.5	17.83	7.00	10.00	7.75	7.92	–
1.0	19.16	7.67	11.67	9.42	9.67	–
2.0	20.50	8.00	13.33	11.00	11.00	–
3.0	21.00	10.00	15.16	14.58	15.16	–
P	21.16	21.25	20.83	19.42	25.67	21.16
N	–	–	–	–	–	–

Note: P = positive control (chloramphenicol); N = negative control (ethyl acetate)

Results in Table 3 indicated that the ethyl acetate extract had high activity against *S. aureus* bacteria with diameter inhibition zone of 17.83 mm and 21 mm at a concentration of 0.5 % and 3 %, correspondingly. The ethyl acetate fractions, excluding fraction E, also showed the potential to inhibit the growth of *S. aureus* bacteria. The highest activity result was obtained from fraction B among five fractions, with disc inhibitory zone of 10 mm and 15.16 mm at a concentration of 0.5 % and 3 %, respectively. However, the clear zone was not significant compared with to crude extract, which was equal to 17.83 mm at a concentration of 0.5 % and 21.00 mm at a concentration of 3 %.

Experimental results suggested that the ethyl acetate extract fractions showed significant inhibition activity of bacteria growth. On the contrary, it had relatively less significant activity when compared to the positive control of chloramphenicol (inhibition activity ranged from 19.42 to 25.67 mm). Moreover, the results revealed that the ethyl acetate extract and fractions were more active in inhibiting the growth of bacteria *S. aureus* than *E. coli*. This was because of the differences in the composition and structure of the cell wall between the respective bacteria. The structure of the cell wall of gram-positive bacteria is simpler; consist of single-layered with a low lipid content (1−4 %) to

facilitate antibacterial material into cells. In contrast, structure of the cell wall of gram-negative bacteria is more complex, having three layers, namely the outer layer of lipoprotein, middle layer of lipopolysaccharide which acts as a barrier to the antibacterial material, and the inner layer of peptidoglycan with high lipid content in the amount of 11-12 %. The results also implied that fraction B had the greatest inhibition zone against both bacteria. Based on the test results of phytochemical analysis, fraction B contained of terpenoids compound. This result confirmed previous studies, which reported that the active compound in inhibiting the growth of bacteria *E. coli* and *S. aureus* was a compound similar to the methyl group kommet terpenoids [7 − 8, 10]. However, the inhibition of ethyl acetate extract of the stem bark against bacteria *E. coli* and *S. aureus* was much larger than the methanol extract and *n*-hexane. It was presumably due to the active compounds contained in *P. alba* was semi-polar compounds. Other studies reported that terpenoid compounds found in the ethyl acetate extract of Jengkol leaves were active in inhibiting the growth of *E. coli* and *S. aureus* [11]. In spite of that, the class of triterpenoid compounds contained in plant roots chloroform extract thick white intersection actively inhibited bacterial growth at a concentration of 500 ppm and 1000 ppm with inhibition zone of 1 mm and 4 mm of bacteria *S. aureus* and 0.5 mm and 2 mm of *E. coli*.

CONCLUSIONS

Antibacterial test results showed that the ethyl acetate extract of the *P. alba* stem bark and its fractions were active in inhibiting the growth of *S. aureus* and *E. coli.* Only fraction E samples did not show the antibacterial activity. The most active fraction of the antibacterial activity was fraction B, which at a concentration of 3 % gave disc inhibition zone of 14.20 mm and 15.16 mm of *E. coli* and *S. aureus*, respectively. According to phytochemical analysis results, fraction B of the ethyl acetate extract contained compounds of terpenoids class, which are active against bacteria *E. coli* and *S. aureus*. The inhibition of ethyl acetate extract of *P. alba* against the bacteria *E. coli* and *S. aureus* was higher than the inhibition of *n*-hexane extract and methanol.

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