Antibacterial Activity of Methanol Extracts and Compounds of Wualae (*Etlingera elatior*) Fruits from Southeast Sulawesi-Indonesia

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Abstract : Wualae (*Tolakinese*) or *Etlingera elatior* grows bulky in Southeast Sulawesi. The fruit of this plant is widely used as cooking spices and traditional medicine. Scientific studies of the fruit and its properties against certain diseases are still very limited. The aim of the article shares the chemical content of *E. elatior* fruits and its activity against various selected pathogenic bacteria. Isolation was performed by chromatographic methods, including Thin Layer Chromatography (TLC), Vacuum Liquid Chromatography (VLC), and Radial Chromatography (RC). Structure of the isolated compounds was elucidated by using spectroscopic techniques, i.e. IR and NMR-1D spectroscopy (^{1}H and ^{13}C-NMR) and comparing with similar data from the literature. The activity of the methanol extracts and the isolated compounds were evaluated against bacteria using the diffusion agar method. The tested bacteria included *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa*, *Staphylococcus aureus* ATCC 25923, *Bacilus subtilis*, *Streptococcus mutans* ATCC 25175 and *Salmonella enteric*. The result showed that two compounds have been isolated from *E. elatior* fruit, namely vanilic acid (1) and *p*-hydroxybenzoic acid (2). The compounds and crude extracts were most active against *S. mutans*. The data is a reference where the methanol extracts of *E.elatior* fruits can be developed into a mouthwash or toothpaste.

1 INTRODUCTION

Zingiberaceae is one of the common plants in Indonesia used as traditional medicines (Hartati et al, 2014). The genus *Etlingera* belongs to the Zingiberaceae family and contains approximately 150–200 species of worldwide distribution. Of these, much species of this genus have been recorded in Indonesia, including 48 species from Sulawesi and 6 species from Java (Poulsen, 2012). Species of this genus have been used in medicinal folklore to treat various oilments, and the presence of the volatile and non-volatile entities in these species has gained research interests among scientists.

Previous studies revealed the presence of phenylpropanoids, flavonoids, and phytosterols in the species of *Etlingera*. The leaves of *E. elatior* produced quinic acid-containing cinnamic acid derivatives, including 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid (chlorogenic acid), and 5-*O*-caffeoylquinic acid methyl ester (Chan et al, 2009a). In addition, its leaves also contained kaempferol-3-glucuronide, quercetin-3-glucuronide, quercetin-3-glucoside, and quercetin-3-rhamnoside (Williams et al, 1997). Moreover, leaves and rhizomes of *E. brevilabrum* and *E. sphaerochepala* var. *grandiflora* produced *β*-sitosterol and stigmasterol (Yahya et al, 2011; Mahdavi, 2014). The latter species also yielded a simple phenolic paeonol (Mahdavi, 2014). The stems of *E. calophrys* produced yakuchinone A, *p*-hydroxybenzoic acid and stigmasterol (Sahidin et al., 2018).

Different parts of *Etlingera* species also have proven to have promising biological activities. Leaves and stems of *E. brevilabrum* exhibited anticholesterol activity (Mahdavi, 2014), while the leaves and rhizomes of *E. elatior* performed antioxidant, antibacterial, and tyrosinase inhibitory activities (Williams et al, 1997; Ficker et al, 2003; Chan et al, 2008; Lachumy et al, 2010; Wijekoon et al, 2011; Chan et al, 2009b, Chan et al, 2007). Antibacterial and antioxidant activities was also exhibited by the leaves extract of *E. fulgens* (Ficker et al, 2003). Furthermore, antioxidant was also

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showed by methanol extract of *E. calophrys* stems (Sahidin et al., 2018). Other studies revealed the potency of *E. littoralis* rhizomes and *E. maingayi* leaves as antibacterial agents (Chiang et al, 2010). Moreover, essential oil of *Etlingera fenzlii* (Kurz) K. Schaum was safe for repellent source (Sudhakaran et al, 2016).

According to the above information, the chemistry and pharmacology aspects of *E. elatior* fruits have not been reported. Hence, the present work will facilitate and report the isolation and identification of chemical compounds from the methanol extract of *E. elatior* fruits, as well as their antibacterial activity.

2 MATERIALS AND METHODS

2.1 General Procedures

Instruments were used Cary Varian 100 Conc UV spectrophotometer, PerkinElmer Spectrum One FT-IR spectrophotometer, and JEOL ECP 500 NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). Chromatography techniques were performed using Kieselgel 60 F_{254} 0,25 mm, silica gel 60 GF_{254}, and silica 60 G (Merck, Darmstadt, Germany). TLC plates were derivatised using a cerium sulphate reagent (Merck, Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Merck (Darmstadt, Germany).

2.2 Sample

Fruits of *Etlingera elatior* were collected from the Wolasi Forest, South Konawe, South East Sulawesi, in November 2016 with No of Specimen EST02. The plant specimen was identified and stored in the Herbarium Bogoriense, Indonesia.

2.3 Extraction and Isolation

The dried powdered fruits of *E. elatior* (2.1 kg) was macerated with methanol (MeOH, 3 x 5.0 L, 24 h each time) at room temperature and yielded a dried methanol extract as dark green gum (80 g). This extract was further fractionated using a silica gel VLC (10 x 5 cm, 150 g), eluted with *n*-hexane–ethyl acetate (from 9:1 to 0:10) followed by pure MeOH, and gave 5 main fractions (F1-F5) with weight of 1.3, 4.1, 7.3, 6.2, and 29.6 g, respectively. Main fraction F3 was re-fractionated using a silica gel VLC (10 x 5 cm, 150 g) and gradiently eluted with *n*-hexane–ethyl acetate (from 7:3 to 0:10) and MeOH as mobile phases, to yield subfractions F31 (0.1 g), F32 (0.7 g), F33 (0.7 g), and F34 (4.4 g). Subfraction F32 was chromatographed using a silica gel RC with chloroform–MeOH (95:5) and pure MeOH as mobile phases, to produce pure compound **1** (0.03 g). Furthermore, subfraction F33 was further purified using the same method as compound **1** purification to get compound **2** (0.08 g).

2.4 Antibacterial Activity

The antibacterial assay was determined against *Bacillus subtilis* FNCC 0060, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and *Streptococcus mutans* ATCC 25175. The antibacterial test was conducted by the agar dilution method using the general procedure outlined by Thakurta (Sahidin *et al*, 2017). The cultural concentration of bacteria was (*B. subtilis* = 2.0 x 10^{8} cfu/mL, *E. coli*= 4.2 x 10^{8} cfu/mL, *P. aeruginosa* = 1.2 x 10^{8} cfu/mL, *S. enterica* = 2.0 x 10^{8} cfu/mL, *S. aureus*= 3.2 x 10^{7} cfu/mL and *S. mutans*= 1.2 x 10^{7} cfu/mL).

3 RESULTS

3.1 Physicochemical Property and Spectroscopic Data of The Isolated Compounds From E. Elatior Fruits

Two compounds (**1**–**2**) were successfully isolated and identified from the methanol extract of *E. elatior* fruits. Structures of these compounds were determined based on their physicochemical property and spectroscopic spectra of IR and NMR. These data were also compared with the same data reported in the previous studies.

Vanilic Acid (**1**); a white powder. Spectra of ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 10.87 (br, *s*), 8.40 (1H, *s*), 7.61 (1H, *dd*, 8.4, 1.9), 7.58 (1H, *d*, 1.,7), 6.93 (1H, *d*, 8.2), and 3.92 (3H, *s*). Spectra of ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 166.6 (C-7), 151.2 (C-4), 147.2 (C-3), 124.0 (C-6), 122.0 (C-1), 112.6 (C-5) and 55.4 (C-8).

p-Hydroxybenzoic acid (**2**); white amorphous powder. Spectra of ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.43 (1H, *s*), 7.91 (2H, d, J = 8.6 Hz, H-2/H-6), 6.92 (2H, d, J = 8.4 Hz, H-3/H-5). Spectra of ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 166.7 (C-7), 161.8 (C-4), 131.8 (C-2/C-6), 121.8 (C-1), 115.1 (C-3/C-5).

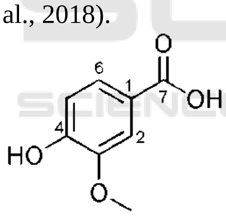
3.2 Antibacterial Activities Data

Table 1: Antibacterial activities of methanol extracts and the isolated compounds.

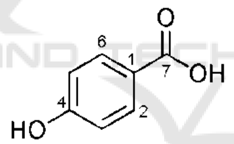
	Inhibition Zone (mm ± SD), [sample]= 100 µg/mL			
	Methanol extracts	**Vanilic Acid**	***p*-Hydroxybenzoic acid**	**Chloramphenicol**
B. subtilis	0.30±0.11	0.00±0.00	0.17±0.10	15.30±0.80
E. coli	0.25±0.18	0.38±021	0.83±0.15	10.60±0.65
P. aeruginosa	0.30±0.05	0.80±0.14	0.17±0.12	9.71±0.90
S. enterica	0.30±0.18	3.80 ±0.30	0.63±0.20	12.70±0.75
S. aureus	0.25±0.20	0.00±0.00	0.50±0.10	6.25±0.55
S. mutans	0.60±0.15	3.46±0.25	0.67±0.16	15.60±0.70

4 DISCUSSION

Vanilic acid (**1**) is firstly reported from *Etlingera* plants. Meanwhile, *p*-hydroxybenzoic acid (**2**) has been isolated from stems of *E. callophrys* (Sahidin et al., 2018).



vanilic acid (**1**)



p-hydroxybenzoic acid (**2**)

Those compounds were isolated from *E. elatior* fruits are known compounds, so the structures are determined by comparing the spectroscopic data of isolated compounds with similar data from references. For example, isolate **1**, the spectrum data of ^{1}H NMR and ^{13}C NMR has a high similarity parameter with vanilic acid (**1***) (Sheng et al, 2014). It can be concluded that compound **1** is vanilic acid, as displayed in **Table 2**.

Tabel 2: Spectra of ^{1}H and 13C NMR of isolate 1 and vanilic acid

No of C	***Isolate 1***		***Vanilic Acid*** **(Sheng *et al.*, 2014)***	
	δ_C	δ_H (∑H, *m*, *J* in Hz)	δ_C	δ_H (∑H, *m*, *J* in Hz)
C1	122.00.00	-	122.09.00	-
C2	115.06.00	7.58 (1H, *d*, 1,7)	115.05.00	7.56 (1H, *d*, 1.7)
C3	151.02.00	-	152.00.00	-
C4	147.02.00	8.4 (1H, *s*)	148.00.00	-
C5	112.06.00	6.93 (1H, *d*, 8,2)	113.04.00	6.91 (1H, *d*, 8.2)
C6	124.00.00	7.61 (1H, *dd*, 8,4, 1,9)	124.00.00	7.59 (1H, *dd*, 8.2, 1.77)
C7	166.06.00	10.87 (br, *s*)	167.05.00	-
C8	55.04.00	3.92 (3H, *s*)	56.03.00	3.82 (3H, *s*)

*Measured in acetone-d$_6$ (^{1}H, 400 MHz; ^{13}C NMR 100 MHz)

In the same way as structure determination of vanilic acid, the compound **2** is *p*-hydroxybenzoic acid (Sahidin et al., 2018).

Based on biological activity data in the Table 1, The activities of all samples both crude extracts and isolated compounds are lower than chloramphenicol (positive control) against some tested bacteria. Methanol extract of *E. elatior* fruit at concentration of 100 µg / mL (100 ppm) showed an interesting antibacterial activity especially in inhibiting the growth of *S. mutans* and *S. enterica*. This is supported by the activity of compound successfully isolated from the fruit of *E. elatior* which is vanilic acid that has inhibition zone (mm) against *S. mutans* and *S. enterica* are 3.46±0.25 and 3.80±0.30, respectively. The data are references where the methanol extracts of *E. elatior* fruits can be developed into a mouthwash or toothpaste and anti-*salmonellosis* diseases herbals.

5 CONCLUSION

Vanilic acid and *p*-hydroxybenzoic acid have been isolated and identified from the methanol extract of *E. elatior* fruits.. Of these, vanilic acid is firstly isolated from the genus *Etlingera*. On biological activities, potency of the crude methanol extracts as an antibacterial agent was especially toward *S. mutans* and *S. enterica* supported by the activity of vanilic acid.

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