



## Antibacterial and antioxidant potentials of methanol extract and secondary metabolites from Wualae rhizome (*Etlingera elatior*)

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### ABSTRACT

The rhizome of *Etlingera elatior* or Wualae (Tolakinese) has many advantages on traditional remedies and cooking in Sulawesi Tenggara. To support those advantages, two secondary metabolites derived from steroid and phenylpropanoic acid classes, stigmast-4-en-6 $\beta$ -ol-3-one (1) and p-coumaric acid (2), respectively, have been firstly isolated and identified from the *E. elatior* rhizome. Isolation of these two compounds was performed using several chromatography techniques, including thin layer chromatography (TLC), vacuum liquid chromatography (VLC) and radial chromatography (RC). Identification of isolates was carried out using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and comparing the spectroscopy data with the library. The potency of antibacterial of the methanol extract of Wualae rhizome and the isolates were evaluated against *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* FNCC 0060, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Salmonella enterica* ATCC 14028, and *Streptococcus mutans* ATCC 25175 using agar diffusion method. Antioxidant activity was evaluated against DPPH radicals (2,2-diphenyl-1-picrylhydrazyl). The results show that the antibacterial potential of Wualae methanol extract is better than compound 1 and 2. Furthermore, the antioxidant properties of Compound 2 (IC<sub>50</sub> 159.47  $\mu$ g/ml) was stronger than the antioxidant properties of Compound 1 (IC<sub>50</sub> 219.95  $\mu$ g/ml) and the methanol extract (IC<sub>50</sub> 586.38  $\mu$ g/ml).

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## 1. Introduction

Study on the phytochemical and pharmacological aspects of the traditional medicinal plants in Southeast Sulawesi are continuously undergoing at the Halu Oleo University, Faculty of Pharmacy. Some of the plants that have been studied including Dipterocarpaceae (Juliawaty et al., 2009; Sahidin et al., 2017), Euphorbiaceae (Sahidin et al., 2011; Sahidin et al., 2013; Sabandar et al., 2013), Fabaceae (Al Muqarrabun et al., 2013), Poaceae (Ruslin et al., 2013), Polygonaceae (Sahidin et al., 2014; Sahidin et al., 2015; Megantara et al., 2018; Ahmad et al., 2018), Anacardiaceae (Wahyuni et al., 2018) and Zingiberaceae (Sahidin et al., 2018).

Wualae (Tolakinese) or *Etlingera elatior* (Zingiberaceae) contains non-volatile and volatile compounds which are interesting to study. *E. elatior*, also known as “kecombrang” (Indonesia), “pacikala” (Bugisnese) or “honje” (Sundanese), has been used as a traditional medicine and a spice for cooking. The local communities in Sulawesi use its fruit as an additional ingredient for fish-based food such as “kapurung” (Bugis) and “sinonggi” (Tolaki). The flower extract of this plant has antioxidant properties (Sukandar et al., 2011) and its fruit extract is cytotoxic against murine leukemia P-388 cells (Rusanti et al., 2017). Leaves and rhizomes of the plant display antioxidant, antibacterial and inhibitory activities against tyrosinase (Ficker et al., 2003; Chan et al., 2008; Lachumy et al., 2010; Wijekoon et al., 2011; Chan et al., 2009a; Chan et al., 2007). Such activities shown by *E. elatior* are highly associated to its

chemical contents. The leaves produce derivatives of cinnamic acid such as 3-O-caffeoylquinic acid (chlorogenic acid), 5-O-caffeoylquinic acid (5-O-caffeoylquinic-methyl ester acid) (Chan et al., 2009b), 3-glucuronide, quercetin-3-glucuronide, quercetin-3-glucoside, and quercetin-3-rhamnoside (Williams and Harborne, 1997). In addition, the seeds contain 3,4-dihydroxy benzoic acid which has antioxidant property (Sukandar et al., 2018).

The above description informs that the phytochemical study of wualae rhizome (*E. elatior*) and its potential activities as antibacterial and antioxidant have not been reported yet. Therefore, this article presents the isolation, structure determination of the isolates, and the activity assays of the methanol extract and isolates against bacteria and DPPH (2,2-diphenyl 1-picrylhydrazyl).

## 2. Materials and Methods

### 2.1. General procedures

The isolation apparatus consisted of glass vacuum liquid chromatography (VLC), vacuum rotary evaporator, radial chromatography (Chromatotron). The <sup>1</sup>H and <sup>13</sup>C NMR spectra were measured on ECP 500 operating at 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C) in Chemistry Research Centre – LIPI Serpong.

### 2.2. Plant materials and chemicals

Rhizomes of Wualae (*E. elatior*) were taken from Sambeani village, South Konawe, Southeast Sulawesi on January 2018 and

determined at Biology Department, Faculty of Education, Halu Oleo University. Silica gel 60 GF<sub>254</sub> (Merck) was used for VLC, silica gel 60 PF<sub>254</sub> pre-coated aluminium plates was used for TLC, silica gel 60 PF<sub>254</sub> containing gypsum (Merck) was used for radial chromatography. All organic solvents used were technical grade and distilled before used.

### 2.3. Extraction and isolation

The dried rhizomes of *E. elatior* (2.1 kg) were powdered and macerated three times using methanol (MeOH, 3 x 5 l, 24 h) at room temperature to yield dark green-methanol extract (100 g). The extracts were fractionated using VLC (10 cm column diameter, 150 g silica gel), eluted with solvent mixtures of n-hexane:ethyl acetate (gradient from 9:1 to 0:10, v/v), terminated with MeOH to yield five fractions (Fa 2.3 g; Fb 6.1 g; Fc 7.8 g; Fd 8.2 g; and Fe 35.3 g). Afterwards, F2 fraction was fractionated using VLC (5 cm column diameter, 75 g silica gel) eluted with solvent mixtures of n-hexane:ethyl acetate (gradient from 7:3 to 0:10, v/v), terminated with MeOH to yield four sub-fractions (Fb1 0.1 g; Fb2 0.9 g; Fb3 0.9 g; and Fb4 3.4 g). Then, the sub-fraction of F23 was purified using radial chromatography, eluted with solvent mixtures of chloroform:MeOH (95:5, v/v), and washed with MeOH to yield compound 1 (0.03 g).

Compound 2 was obtained from F3 fraction using the same approach as compound 1. Briefly, F3 fraction was fractionated using VLC (5 cm column diameter, 75 g silica gel) eluted with solvent mixtures of n-hexane:ethyl acetate (gradient from 6:4 to 0:10, v/v), terminated with MeOH to yield five sub-fractions (Fc1 0.2 g; Fc2 0.6 g; Fc3 0.9 g; Fc4 1.0 g; Fc5 1.6 g). Then, the sub-fraction of Fc3 was purified using radial chromatography, eluted with solvent mixtures of n-hexane:ethyl acetate (75:25, v/v), and washed with MeOH to yield compound 2 (0.04 g).

### 2.4. Structure identification

The chemical structures of the isolates were determined using <sup>1</sup>H and <sup>13</sup>C NMR spectra and compared with the spectra of similar compounds from published reports.

### 2.5. Antibacterial assay

Antibacterial properties of the methanol extract and isolates were assayed according to the method described in Sahidin et al. (2013; 2019). The assay was against six bacteria including *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* FNCC 0060, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Salmonella enterica* ATCC 14028, and *Streptococcus mutans* ATCC 25175.

### 2.6. Antioxidant assay

The radical scavenger potency of samples including the methanol extracts, compound 1, 2 and standard (ascorbic acid) were assayed against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. The inhibition of the radicals was determined using the Bios method (Sahidin et al., 2018; 2019).

## 3. Results and Discussion

### 3.1. NMR spectra of isolated compounds

The NMR spectra of Stigmast-4-en-6 $\beta$ -ol-3-one (1) were described in Sahidin et al. (2019).

*p*-coumaric acid (2); white powder. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ <sub>H</sub> (ppm): 9,23 (1H, s), 7,60 (1H, d, 15,9, H-7), 7,54 (2H, d, J = 8,6 Hz, H-2/H-6), 6,90 (2H, d, J = 8,4 Hz, H-3/H-5), 6,33 (1H, d, 15,9, H-8). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ <sub>C</sub> (ppm): 167,4 (C-9), 160,1 (C-4), 144,6 (C-7), 130,0 (C-2/C-6), 121,8 (C-1), 115,8 (C-3/C-5), and 114,9 (C-8).

NMR spectra of <sup>1</sup>H and <sup>13</sup>C indicates two compounds from different classes of secondary metabolites, namely steroid and phenylpropanoic acid. Based on the comparison of spectroscopy data between the isolates and references, the compound 1 has high similarity with stigmast-4-en-6 $\beta$ -ol-3-one isolated from *E. elatior* (Habsah et al., 2005). Therefore, it can be agreed that the compound 1 is stigmast-4-en-6 $\beta$ -ol-3-one. Similarly, the compound 2 is *p*-coumaric acid since the spectra data have high similarity with *p*-coumaric acid which previously isolated from fruits of *E. elatior* (Sahidin et al., unpublished). Structures of the compounds are given in Fig. 1

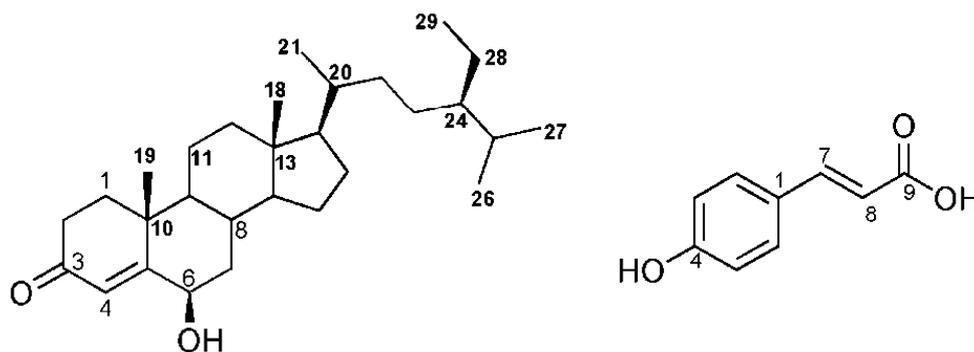


Fig. 1. Isolated compounds from methanol extract of *E. elatior* rhizome

### 3.2. Biological activities

The pharmacological studies previously showed that leaves and rhizomes of *E. elatior* possessed several activities as antioxidant, antibacterial and tyrosinase inhibitors (Ficker et al., 2003; Chan et al., 2008; Lachumy et al., 2010; Wijekoon et al., 2011; Chan et al., 2009a; Chan et al., 2007). These reports validated the local tradition of communities in North Kolaka, Southeast Sulawesi who use squished water from stem and rhizome of *E. elatior* to alleviate the typhoid fever (Research

Team, 2011). Furthermore, it is emphasised by the successful isolation of two compounds from the rhizomes. One of these compounds, stigmast-4-en-6 $\beta$ -ol-3-one (1), was isolated from the rhizome for the first time. Meanwhile, *p*-coumaric acid (2) has been previously isolated from fruits of *E. elatior* (Sahidin et al., unpublished).

The antibacterial properties of methanol extract and their isolated compounds from Wualae rhizome are displayed in Table 1.

**Table 1.** Antibacterial activity of methanol extract and isolated compounds from rhizome of *E. elatior*.

Bacteria	Inhibition zone (mm)			
	Methanol extract	Stigmast-4-en-6 $\beta$ -ol-3-one	p-coumaric acid	Chloramphenicol
<i>B. subtilis</i>	2.20 $\pm$ 0.10	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	15.30 $\pm$ 0.58
<i>E. coli</i>	1.60 $\pm$ 0.14	0.00 $\pm$ 0.00	1.75 $\pm$ 0.14	10.60 $\pm$ 0.34
<i>P. aeruginosa</i>	1.20 $\pm$ 0.20	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	9.71 $\pm$ 0.38
<i>S. enterica</i>	3.80 $\pm$ 0.24	0.00 $\pm$ 0.00	1.50 $\pm$ 0.20	12.70 $\pm$ 0.22
<i>S. aureus</i>	1.00 $\pm$ 0.10	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	6.25 $\pm$ 0.78
<i>S. mutans</i>	4.20 $\pm$ 0.12	0.00 $\pm$ 0.00	2.00 $\pm$ 0.26	15.60 $\pm$ 0.66

Sample concentration was 100  $\mu$ g/ml. Mean $\pm$ SD data were from triplicate experiment.

In general, Table 1. indicated that the positive control of chloramphenicol is more active toward tested bacteria than the methanol extract and the isolated compounds of *E. elatior*. Interestingly, the methanol extract displayed higher activity against the tested bacteria rather than the isolated compounds. This result shows the presence of antibacterial active compounds in methanol extract which, at that moment, have not been successfully isolated yet. The compound 2, p-coumaric acid, only displayed antibacterial activity against *E. coli*, *S. enterica*, and *S. mutans*, whereas compound 1, stigmast-4-en-6 $\beta$ -ol-3-one, displayed no activity. The compound structure is one of the various factors determining the ability of a compound to inhibit the bacterial growth. Accordingly, stigmast-4-en-6 $\beta$ -ol-3-one is not active as antibacterial probably due to its structure has no similarity to the structure of chloramphenicol. In contrast, p-coumaric acid showed antibacterial activity since its structure is more similar to chloramphenicol (Alves et al., 2013; Sahidin et al., 2019). The structure similarity between p-coumaric acid and chloramphenicol probably lies in the presence of the aromatic ring which is known to have an ability to interact with the peptidyl transferase, a pivotal enzyme in the bacterial chromosome. Inhibition of work of this enzyme will eventually prevent the growth of bacteria (Sahidin et al., 2019). The antioxidant properties of methanol extract and their isolated compounds from Wualae rhizome are determined by their ability to scavenge the DPPH radicals as shown in Table 2.

**Table 2.** Scavenging activity of methanol extract and compounds from rhizome of *E. elatior* against DPPH radicals.

Radical source	IC <sub>50</sub> ( $\mu$ g/ml)			
	Methanol extract	Stigmast-4-en-6 $\beta$ -ol-3-one	p-coumaric acid	Vitamin C
DPPH	586.38	219.95	159.47	37.98

Meanwhile, the methanol extract still contains a mixture of aliphatic and aromatic compounds in low concentration and therefore its scavenging activity is a little bit lower.

#### 4. Conclusion

Two compounds from methanol extract of Wualae (*Etingera elatior*) rhizome have been successfully isolated and identified, namely stigmast-4-en-6 $\beta$ -ol-3-one and p-coumaric acid. The antibacterial potential of Wualae methanol extract is better than stigmast-4-en-6 $\beta$ -ol-3-one and p-coumaric acid. Furthermore, the antioxidant property of p-coumaric acid (IC<sub>50</sub> 159.47  $\mu$ g/ml) was stronger than the antioxidant properties of stigmast-4-en-6 $\beta$ -ol-3-one (IC<sub>50</sub> 219.95  $\mu$ g/ml) and the methanol extract (IC<sub>50</sub> 586.38  $\mu$ g/ml).

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#### Conflict of interest

The authors declare no conflict of interest.

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