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by Prodi S1 FKG Unimus

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Gallic Acid From The Leaves of *Altingia excelsa* noronha as an antibacterial agent for *Enterococcus faecalis*

ABSTRACT

Abstract. *Enterococcus faecalis* is one of the bacteria which inhabit within dental pulp and periapical causing dental and oral diseases commonly in children. The aim of this study was to determine the antibacterial compound of *Altingia excelsa* leaves to inhibit the growth of *Entrococcus faecalis*. The study was true experimental laboratory design. Separating the ethyl acetate extract via their compounds using various chromatographic techniques. One compound was revealed, that was gallic acid. Antibacterial test against *Enterococcus faecalis* was done to determine inhibitory effect by its compound and MIC values showed of 15,63 µg/mL. The data were analyzed by ANACOVA assay. Gallic acid as the compound of *Altingia excelsa* leaves had a strong inhibitory effect on *Enterococcus faecalis*

Keywords: *Altingia excelsa*, *Enterococcus faecalis, antibacterial, apigenin*.

INTRODUCTION

Dental caries is a common oral dan dental disease which mostly found in Indonesian children. Based on the global oral health from WHO, global dental caries index in 12 years old children was 1.6 tooth which means that the average of person that experienced tooth decay was more than 1 tooth (Erik, 2013). Health survey in 2013 reported that prevalence of oral dan dental problem was 23.4%, followed with national prevalence of active caries was 43.4%. It was 50-70% dental caries prevalence in Indonesia which mostly toddlers (Soendoro, 2013).

Untreated primary dental caries can be quickly expanded and cause exposure of the pulp (Love and Jenkinson, 2002). The pulp that has been exposed become the entry pathway of microorganism that can cause an inflammation and if it is continues, it will cause a non vital pulp. A primary teeth with caries infection that reach pulp tissues will need an endodontic treatment. Clinical manifestation of teeth with periapical lesion or root canal infection usually gives symptoms of pain at night, with or without stimulation. Clinically, a periapical abscess or fistula and abnormal tooth mobility would give sensitive response to percussion and pressure examination (Jeffrey, 2012). There are many bacteria inhabit within root canal of primary tooth such as aerob, anaerob and facultative. But, mostly found bacteria is *Enterococcus faecalis*.

There are some antibacterial agent as a root canal sterilization that has been used to eliminate bacterial growth that still prescence after the biomechanical preparation and this matter related with periradicular healing (Sjogren et al. 1991). Rockle's, Tricresol Formalin, Cresophane, and CHKM are some of root canal antibacterial. Degradation amounts of bacteria inside the root canal can be examined with bacterial culture (Sathorn et al. 2007). However, the bacteria would become resistant to antibiotic causing primary failure of endodontic treatment in children (Refdanita et al. 2004). Therefore, discovery research is necessary to find new antibacterial agent which explore natural ingredients originated from medicinal plants.

Some medicinal plants contain antibacterial compounds such as phenols and phenolic compounds, quinones, flavones, flavonoids, tannins, coumarin, terpenoids, alkaloids, lectins, and polypeptides and their mixtures (Cragg and Newman, 2005). At present, trade in medicines derived from plants throughout the world reaches US $ 50 billion every year (Cragg and Newman, 2005). As the largest biodiversity in the world with more than 30 thousand species of plants, Indonesia has medicinal properties and potential for treating various diseases traditionally by utilizing these medicinal herbs. One of the herbs is *Altingia excelsa* nornha leaf which comes from Altingia genus and including Hammamelidaceae family (Anwar, 2018). Traditionally, Altingia is useful as a anti-pyretic, vitality enhancer, anti-inflammatory, cough and stomach medicine (Anwar, 2018). Scientifically, previous study reported to aim the benefits of *A. excelsa*, as potentially anti cancer[16] and antibacterial (Pangestika, 2017).

The prospecting drugs from natural ingredients contain chemical compounds and pharmacologically biological which need to be determined associated with their efficacy and usefulness. Isolation guided by biological tests is a chromatographic method to isolate chemical compounds which monitor its purity. The identification of antibacterial agents is directed not only based on ethnobotany and phytochemical studies but also in vitro antibacterial test. Therefore, further research to explore the potential of *A. excelsa* as antibacterial drugs is very important. Here, we show that antibacterial of *A. excelsa* leaves inhibit the growth of *Enterococcus faecalis* by its chemical compounds.

MATERIALS AND METHOD

1. Plant Materials

Altingia excelsa leaves which obtained from the Wayang Windu mountains, Pangalengan, Bandung was used and analyzed at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

2. Bacterial Strains

Enterococcus faecalis ATCC™ 19433 (Thermoscientific) were used in this study.

3. *Altingia excelsa* leaves extraction

Extraction and isolation of active compounds from *A. excelsa* leaves was carried out at the Organic Chemistry Laboratory of Natural Materials, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Garut University. Maceration was done for dry leaves of *A. excelsa* using methanol for 24 hours and carried out by mass storage three times. The maserat concentrated with a rotary evaporator until 225 g of concentrated methanol was achieved. Dissolving the concentrated methanol maserat into water and splitting using n-hexane were done to produce n-hexane extract and water. The n-hexane extract was divided and concentrated using rotary evaporator to achieve 167 g concentrated n-hexane extract. The obtained water layer was then divided using ethyl acetate to produce ethyl acetate extract and water. Ethyl acetate extract was divided and concentrated using rotary evaporator to obtain 145 g of concentrated ethyl acetate extract. These extracts were tested for antibacterial against *Enterococcus faecalis*.

4. Isolation of the most active compound

Thin Layer Chromatography (TLC) analysis was done to obtain five main fractions (A-E) and used to determine the antibacterial activity. MIC values of various fractions of the antibacterial test showed Fraction C was potential as an antibacterial. Vacuum Liquid Chromatography (VLC) was used to analyze Fraction C on the G60 silica gel stationary phase with the mobile phase n-hexane-ethyl acetate methanol with a gradient of 10% (v/v). Thin Layer Chromatography (TLC) analysis was done further to obtain five fractions (C1-C5), and they were tested for antibacterial activity showed that the C3 fraction gave potential antibacterial activity.

Column chromatography (KK) was done to separate further C3 fraction in stationary silica gel (70-230 mesh) with n-hexane mobile phase ethyl acetate with 10% (v/v) gradient to obtain four fractions of C31 to C34 which was tested for antibacterial activity. From the results of the antibacterial test, which has the potential as an antibacterial is the C32 Fraction. The C32 fraction was further separated by gradient column chromatography (KK) using an n-hexane acetone mobile phase to obtain four fractions namely C321 to C324. One of the fractions is C321 which is an amorphous solid which is then further recrystallized with a mixture of benzene: methanol (8: 2), a white crystal (9 mg) compound 1 is obtained.

5. Determination of compound structure

Isolated compounds determined physical properties include color and melting point. The chemical structure of compounds is determined based on spectroscopic data including (UV), infrared (IR), core magnetic resonance (NMR), and comparison with spectra data obtained from the literature. NMR spectrum measurements were carried out at the LIPI Serpong Chemical Research Center, and antibacterial tests were carried out at the Microbiology Laboratory, University of Muhammadiyah Semarang.

6. *Enterococcus faecalis* antibacterial test

The extract and isolated compounds were diluted in DMSO 1% and carried out by the dilution method to antibacterial test against *Enterococcus faecalis* using 96 wells (Iwaki-Japan) (Rahman et al. 2008). The parameters used were turbidity that occurred due to the growth of test bacteria at certain concentrations caused by the antibacterial activity of the extracts and isolates, therefore read with ELISA microplate reader (BioRed-Japan) at a wavelength of 630 nm.

RESULT AND DISCUSSION

To determine the antibacterial activity of various extracts produced against *E. faecalis* bacteria was carried out by the liquid dilution method (Rahman et al. 2008). Antibacterial activity of various extracts against *E. faecalis* bacteria is expressed by MIC value, referring to the criteria for the level of antibacterial activity of extracts of natural ingredients in testing for antibacterial properties (Ramya et al. 2009) as listed in table 1.

The result of antibacterial test, showed that ethyl acetate extract was the highest inhibitory effect, so that the ethyl acetate was continued to be isolated.

With the guide of antibacterial test for ethyl acetate was done by separation and purification until it obtained one compound.

Compound 1

White solids, t.l. 258-260 °C, UV (MeOH) λmaks nm 222, 271 and 404; IR (KBr) vmaks 3422, 1649, 1408, 1020 cm-1; 1H-NMR (CD3OD, 500 MHz) δH (ppm) 7,15 (1H, s, H-2, H-6); 13C-NMR (CD3OD, 500 MHz) δC (ppm) 122,2 (C-1), 110,7 (C-2, C-6), 145,9 (C-3, C-5), 138,6 (C-4), dan 167,7 (C-7). TOF MS ES+ m/z [M+H]+ 168,6684 calculation for C7H6O5, m/z 170,1195.

Chemical Compound Structure

Compound 1 was obtained as a white solid with a melting point of 258-260 ° C. The UV spectrum of compound 1 shows the presence of two absorption bands at λmax 271 and 222 nm. The absorption at λmax 271 nm indicates a π→ π * transition which was thought to be derived from the B band of the benzene group. Meanwhile, the absorption at λmax 222 nm was thought to originate from the carbonyl group in the presence of the n→ π * transition (R band).

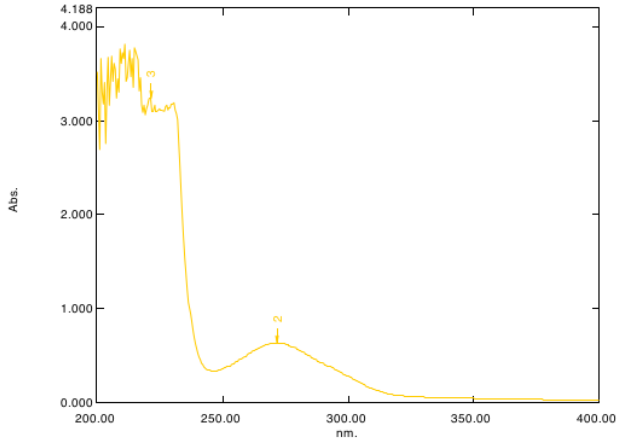

Figure 1. UV spectra

The IR spectrum of compound 1 showed the presence of an -OH group as evidenced by the appearance of the O-H strain observed at vmax 3422 cm^{-1} and the C-O strain at vmax 1021 cm^{-1}.

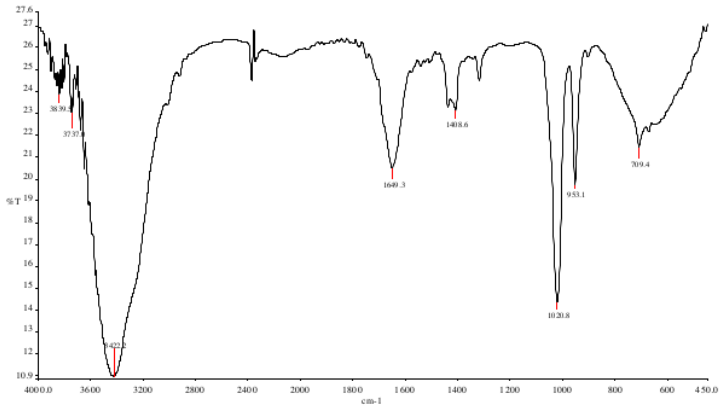

Figure 2. IR spectra

The 13C NMR spectrum showed the presence of seven carbon signals consisting of one carbonyl carbon resonating at δC167.6 ppm and a CH sp^2 signal resonating at δC145.9-110.1 ppm. In compound 1, there are three oxygenated quaternary carbons, namely δC145.9 ppm (C-3 and C-5) and δC138.6 ppm (C-4). Therefore, compound 1 showed a benzene framework with four substituents. Two carbon signals each contain two carbons (^{13}CNMR data), thus compound 1 has a symmetrical structure.

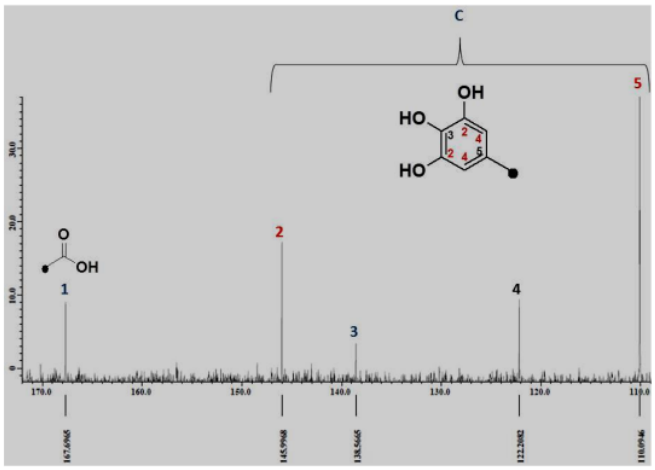

Figure 3. C NMR Spectra

The ^{1}H NMR spectrum of compound 1 showed the presence of a singlet proton signal resonating at δH 7.14 ppm. Therefore, the singlet proton signal represented two equivalent protons thereby amplifying the benzene framework with four substituents.

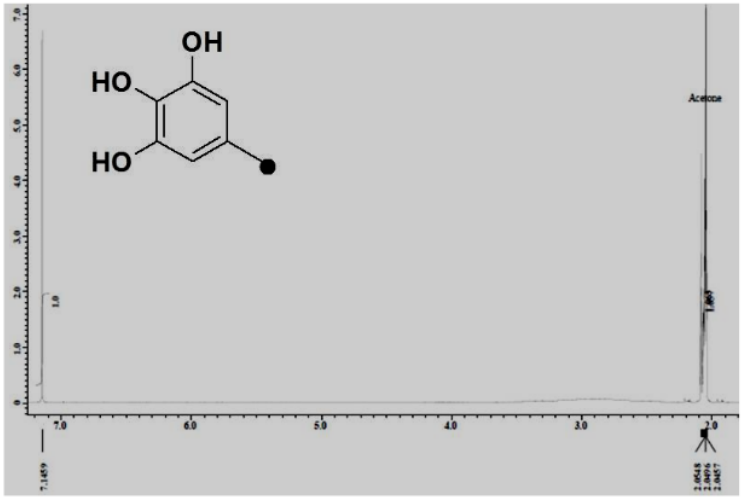

Figure 4. H NMR Spectra

The molecular formula for compound 1 was designated as C7H6O5 based on the TOF-MS ES^{-} spectra (m/z 168.8390 [M + H]$^{-}$, calculations for C7H6O5 m/z 170.1195) and NMR data so that five degrees of unsaturation were obtained. Comparison of the NMR data of compound 1 with 3,4,5-trihydroxy benzoic acid compounds (Gangadhar et al., 2011), showed that the two compounds were very high compatibility, so that definitively, compound 1 was identified as an acidic compound 3,4,5 -trihydroxy benzoate.

Table 1. Comparison compound 1 with gallic acid

Posisi C	Compound 1		3,4,5-trihidroksi benzoat acid	
	δ_C (ppm)	δ_H (ppm), $\sum$ H, mult, *J* (Hz)	δ_C (ppm)	δ_H (ppm), $\sum$ H, mult, *J* (Hz)
1	122,2	-	120,6	-
2	110,7	7,15 (1H; *s*)	108,9	7,07 (1H; *s*)
3	145,9	-	144,9	-
4	138,6	-	138,2	-
5	145,9	-	144,9	-
6	110,7	-	108,9	-
CO_2H	167,7	-	169,0	-

Table 2. MIC value various extract of Rasamala leave

Extract	MIC (*µ*g/mL)
Methanol	69.23
n-heksane	97.12
Ethyl acetate	12.25
Water	98.03

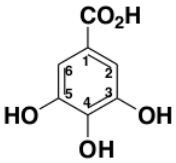

Figure 5. Chemical structure of 3,4,5 trihydroxy benzoate (gallic acid)

To determine the antibacterial activity of compound 1 against E. faecalis bacteria and also carried out by the liquid dilution method (Rahman et al. 2008). The antibacterial activity of compound 1 against *E. faecalis* is expressed by the MIC value, referring to the criteria for the level of antibacterial activity of natural compounds in testing for antibacterial properties (Pinho et al. 2014), which resulted in an MIC of 12.68 *µ*g/mL.

Gallic acid is a member of flavones (fenolic acid) and belongs to the subclass of flavonoids (Borges et al. 2013). Gallic acid as derivative of cinnamic acid is formed through the pathway of shikimic acid with 3-dehydrosikimic acid as a base ingredient. Gallic acid has been tested as antibacterial, including against *Staphylococcus aureus*, against *helicobacter pylori* (Pinho et al. 2014). It showed antimicrobial activities associated with various pathways within cytoplasmatic

membrane via destabilization, permeabilization, inhibitory enzyme by oxidized products which perhaps through reaction with sulfhydryl groups or more nonspecific interactions with proteins and inhibition the synthesis of nucleic acids for both Gram-negative and Gram-positive bacteria (Borges et al. 2013; Pinho et al. 2014; Junaidi and Anwar, 2018).

Gallic acid showed alteration mechanism to bacterial hydrophobicity facilitated by its physicochemical surface properties. Alteration of bacterial cells was induced by gallic acid resulting adjustment the polar, nonpolar, and electron acceptor (c +) of their components. It gave rise to differential ability for both increased electron acceptor as in Gram-positive and decreased electron acceptor as in Gram negative bacteria. It was also electrophilic and significantly depend on the bacterial surface components (Junaidi and Anwar, 2018; Rajamanickam et al. 2019). Due to characteristics of hydroxycinnamic acid via their propenoid side chain, it has antibacterial and less polar than the corresponding hydroxybenzoic acids. Thus, cell membrane now easily penetrated by its properties enabled through its transport (Rajamanickam et al. 2019).

CONCLUSION

In conclusion, the gallic acid which was isolated from *A. excelsa* leaves had inhibitory effect on *Enterococcus faecalis*.

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