Phytochemistry and Antibacterial Activity Evaluation of Genitri (Elaeocarpus ganitrus)

Retno Indriatie¹,², Siti Mudaliana¹, Febriyana Rizky Hapsari¹, Masruri MASRURI²*
¹UPT Materia Medica, Jl. Lahore Batu, Indonesia
²Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University. Jl. Veteran 65145 Malang, Indonesia

*Corresponding author: masruri@ub.ac.id

Abstract. The Elaeocarpus ganitrus has local name as genitri. It has been used traditionally as traditional medicine. This paper reported the phytochemistry and their antibacterial activity on Staphylococcus aureus and Escherichia coli. Several extracts have been afforded using high speed extraction technique using methanol, ethyl acetate and n-hexane as solvents. The extract is composed of alkaloid, tannin, and flavonoid. The negative result is afforded for sapoin test. The MIC of all extracts in both bacteria are above 10 mg/mL. Moreover, the quantitative analysis using spiking method using liquid chromatography found a quercetin and rutin in minor quantity of some sample extract.

1. Introduction
The plant of genitri (Javanesse) has a taxonomy name as Elaeocarpus ganitrus Roxb. Ex G. Don and a synonym as Elaeocarpus serratus L. It is categorized as a high plant which commonly grow in forest. The distribution of this plant is getting wider, not only in West and Central Java but also the other places in Indonesia [1]. Traditionally, part of genitri tree, such as seed, bark, leave, and flowers was applied and used as source of herbs and a medicine for some diseases [2, 3]; such as antidepressant [2], antihypertensive [4], antifungal [5], anti-diabetic [6], and some other mental disorder diseases [3].

Recent report by Singh et al. [7] disclosed antianxiety activity of the flower extract prepared from genitri flower. Several solvents were used to extract, such as using petroleum ether, chloroform, and ethanol. The ethanol extract showed antianxiety with a safe dose until 5000 mg/kg of animal body weight. Interestingly, using 25 mg/kg of doses still indicate the antianxiety activity. Further separation and purification from the extract gave a 3,3′,4′,5,7-penta-hydroxyflavone or known as quercetin composed the extract. This molecule belongs to the secondary metabolite class of flavonoid. Furthermore, this class of flavonoid was also able to be used for antibacterial agent. The mechanism relates to inactivation process of type III protein secretion substrate [8]. For example antibacterial activity of quercetin isolated from lotus leave [9], and also quercetin glycoside from narrow leaf vetch [10]. Quercetin also enhance the antibacterial activity by loading it in poly-lactic-co-glycolic acid nanoparticle both for in vitro and in vivo evaluation [11], and improve the antibacterial for silver nanoparticle [12]. The similar structure to quercetin glycoside reported previously [10] was known as
rutin (Fig 1). Rutin also has been reported active as antibacterial agents [13, 14]. In this paper, will be reported the phytochemicals of genitri seed extract grown in Malang, East Java and evaluation of the presence of flavonoid quercetin and rutin (structure in Fig 1) in the extract. And also, evaluation their antibacterial activity in *Staphylococcus aureus* and *Escherichia coli*.

![Figure 1. Genitri (*Elaeocarpus ganitrus*) seed and the molecular structure of quercetin and rutin.](image)

2. Experimental section

2.1. Material and chemical
Sample of genitri seed is collected from Balai Materia Medica Plants. The taxonomy name is assessed by the same institution. The fresh seed is washed, grinded, and dried in oven before extraction process. Isolate sample of bacteria *Staphylococcus aureus* ATCC25923 and *Escherichia coli* was prepared from the collection by the same institution. Other chemical used for research include antibiotic gentamycine, Mueller Hilton agar (MHA), BHI broth media, methanol, methanol 70%, ethyl acetate, and n-hexane.

2.2. Instrument analysis
The ultra-high-performance liquid chromatography was operated for flavonoid analysis using UFLC (Shimadzu). Solvent used as mobile phase is methanol/water (63:37) isocratic with flow rate of 1 mL/min and column C18 (5 um) with dimension of 4.6x150 mm, and UV detector set at 254 and 260 nm. The standard of quercetin and rutin applied for analysis was 200 ppm.

2.3. Extraction procedure
The procedure for extraction was undergone using speed extractor apparatus (Buchi E-916, Switzerland). A 5.0 g of seed dried powder of genitri in sample holder was placed in speed extractor apparatus. Then, 50 mL of methanol was flowed under nitrogen at the constant pressure of 100 bar. The process was circulated for three times or 3-cycles. The solvent extract was further evaporated under rotary high vacuum evaporator to afford methanol extract of genitri seed. Separated procedure was conducted using different solvents, i.e. methanol 70%, ethyl acetate, and n-hexane. These processes provided methanol 70% extract, ethyl acetate extract, and n-hexane extract, respectively. For further analysis, similar concentration of extract (10 mg/mL) were prepared using 10 mg of extract dissolved in 1.0 mL of water.
2.4. **Phytochemical evaluation**

2.4.1. **Flavonoid test**. 1.0 mL of extract sample in a 10 mL of reaction tube was added with water. This mixture was heated for 5 min, and 1.0 mL of aliquot was taken in reaction tube. This mixture was added with 5.0 mg of magnesium powder, and a few drops of 37% hydrochloric acid solution. Positive result of flavonoid was indicated by the formation of orange, pink, or red solution.

2.4.2. **Alkaloid test**. 1.0 mL of extract sample in a 10 mL of reaction tube was added with water. This mixture was heated for 5 min and divided into three portions in different reaction tube. Each tube was added with 1.0 mL of different reagents, i.e. Meyer reagent, Dragendorf reagent, and Libermann-Burchart reagent. The positive alkaloid test was indicated by the presence of a white precipitate.

2.4.3. **Saponin test**. 1.0 mL of extract sample in a 10 mL of reaction tube was added with water. This mixture was heated for 5 min. 1.0 mL of filtrate was added with hot water and shaken rigorously and added with 1.0 mL of 37% hydrochloric acid solution. The positive saponin was indicated by permanent foam.

2.4.4. **Tannin test**. 1.0 mL of extract sample in a 10 mL of reaction tube was added with water. This mixture was heated for 5 min, and 1.0 mL of aliquot was taken in reaction tube and added with 1.0 mL of iron(III) chloride solution. The positive result for tannin was indicated with the presence of blue or black precipitate.

2.5. **Antibacterial activity evaluation**

The procedure to evaluate the antibacterial activity of genitri seed extract used a well diffusion technique. Two types of bacteria were used, i.e. *Staphylococcus aureus* and *Escherichia coli*. The bacteria isolate was grown in BHI broth media and incubated at 37 °C for 24 hours. Then, the bacteria culture in MHA media was spread with cotton swap aseptically. The well in media was made by using cock borer which was a 6.0 mm in diameter. Each well was dropped with 100 uL of the tested extract sample, 100 uL of distilled water as a negative control sample (NC), and gentamycin 10 mg/mL as a positive control. Then, each well was incubated for 24 hours at 37 °C, and a clear area around the well was measured using digital micrometer as an antibacterial activity.

The minimum inhibitory concentration (MIC) was undertaken using similar procedure above. But several different concentrations of extracts were evaluated, i.e. 10, 5.0, 2.5, 1.25, 0.62, and 0.31 mg/mL. Each sample was tested using a 100 uL of volume. The MIC value was determined based on the lowest extract concentration that was unable to inhibit the bacteria growth.

| Extract sample | Flavonoid test | Tannin test | Saponin test | Alkaloid test |
|----------------|---------------|------------|-------------|--------------|
| genitri seed   | +             | +          | +           | +            |
| Methanol 70% extract | +     | +          | +           | +            |
| Methanol extract | +         | +          | +           | +            |
| Ethyl acetate extract | -        | -          | -           | -            |
| Hexane extract | -            | -          | -           | -            |

3. **Result and discussion**

Secondary metabolite class compounds are divided into phenolic, flavonoid, alkaloid, and terpenoid. Tannin and flavonoid are classified into phenolic. Meanwhile saponin is classified to terpenoid. Phenolic class is generally soluble in polar solvent such as metanol and water. But terpenoid, especially triterpenoid can be extracted using non polar solvent such n-hexane. The result for genitri
(Elaeocarpus ganitrus) seed phytochemical analysis is tabulated in Table 1. This analysis detects the secondary metabolite composed in the extract sample based on the color visualisation or precipitate resulted. The positive results indicate the presence of the secondary metabolites. The extracts of methanol and methanol 70%, indicate a positive result for flavonoid, tannin, saponin, and alkaloid test. However, the ethylacetate and n-hexane extract did not give a positive result. Previously, phenolic groups of flavonoid was also reported [3,7] from the ethanol extract of root and leaves part of genitri.

![Figure 2. Chromatogram of quercetin standard.](image)

![Figure 3. Chromatogram of methanol extract and spiking methanol extract with rutin.](image)

Further flavonoid analysis was undertaken by using liquid chromatography. The representative chromatogram was depicted in Fig 2 and Fig 3. The quercetin and rutin was used as standard. Both are detected at retention time 3.45 and 2.02 minute, respectively (Fig. 3). Meanwhile, the composition of
both flavonoid contained in each extract sample of genitri seed are summarized in Table 2. It was found that quercetin composed the methanol extract in the highest quantity (9.51%). Following by ethyl acetate (3.56%) and methanol 70% extract (2.24%). However, no quercetin was detected in the n-hexane extract. On the other hand, the highest rutin quantity was detected composed the methanol 70% extract (6.04%). Following by methanol extract (5.71%), and ethyl acetate extract (5.23%). And it was not found rutin in the n-hexane extract.

### Table 2. The quantity of phenolic quercetin contained in the genitri extract.

| Extract sample of genitri seed | Quercetin detected | Rutin detected |
|---------------------------------|--------------------|----------------|
|                                 | Retention time (min.) | Percentage (%) | Retention time (min.) | Percentage (%) |
| Methanol 70% extract            | 3.546              | 2.24%          | 2.026                  | 6.04%          |
| Methanol extract                | 3.477              | 9.51%          | 2.020                  | 5.71%          |
| Ethyl acetate extract           | 3.472              | 3.56%          | 2.011                  | 5.23%          |
| Hexane extract                  | -                  | 0              | -                      | 0              |
| Quercetin standard              | 3.453              | 95%            | -                      | -              |
| Rutin standard                  | -                  | -              | 2.021                  | 97%            |

In some reports, the presence of quercetin, rutin, and other flavonoid compounds correlated to their bioactivity [15–17], such as growth inhibitor of bacteria [9, 10, 14]. The antibacterial activity of the genitri extract is tabulated in Table 3, meanwhile the minimum inhibitory concentration (MIC) is summarized in Table 4. Each sample tested has a concentration of 10 mg/mL, including the standard antibiotic of gentamycine.

### Table 3. Antibacterial activity evaluation of the extract from genitri seed.

| Sample extract of genitri seed | Growth inhibition (mm) |
|--------------------------------|-------------------------|
|                                | *Escherichia coli*   | *Staphylococcus aureus* |
|                                | Genitri | PC | NG | Genitri | PC | NC |
| Methanol extract 70%           | 0       | 32.50±4.95 | 0 | 0       | 26.50±4.95 | 0 |
| Crude methanol extract         | 0       | 29.00±5.66 | 0 | 0       | 28.50±0.71 | 0 |
| Ethyl acetate extract          | 0       | 27.50±0.71 | 0 | 0       | 31.50±2.12 | 0 |
| Hexane extract                 | 0       | 21.00±4.24 | 0 | 0       | 26.50±3.54 | 0 |

Note: PC is positive control contains gentamycine 10 mg/mL. NC is negative control contains solvent without sample.

### Table 4. Minimum inhibitory concentration (MIC) of genitri extracts.

| Sample extract of genitri seed | Minimum inhibitory concentration (mg/mL) |
|--------------------------------|------------------------------------------|
|                                | *Escherichia coli* | *Staphylococcus aureus* |
| Methanol extract 70%           | >10                                      | >10                       |
| Crude methanol extract         | >10                                      | >10                       |
| Ethyl acetate extract          | >10                                      | >10                       |
| Hexane extract                 | >10                                      | >10                       |

The genitri extract with 10 mg/mL concentration did not inhibit the bacterial growth (Table 3). Meanwhile the gentamycine give a strong activity in both bacteria tested. Moreover, the minimum inhibitory concentration (MIC) has value above 10 mg/mL for all extract (Table 4).
4. Conclusion
The extract of genitri seed is composed of flavonoid, tannin, saponin, and alkaloid. All these secondary metabolites can be isolated using methanol 70%, methanol, and ethyl acetate as solvent. The flavonoid contain in genitri seed is quercetin, and detected in low concentration. However, the antibacterial evaluation to Staphylococcus aureus and Escherichia coli give insignificant activity.

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