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Antibacterial activities of *Elaeocarpus ganitrus* Roxb. leaves from Kebumen against *Escherichia coli* and *Shigella flexneri*

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ABSTRACT

Ganitri (*Elaeocarpus ganitrus* Roxb) is a plant known for its antibacterial properties, particularly in its leaves. The leaves of Ganitri contain antibacterial compounds such as flavonoids, alkaloids, and tannins. The objective of this study is to investigate the antibacterial activity and determine the minimum concentration of ethanol, methanol, and distilled water extracts from Ganitri leaves in inhibiting the growth of *Escherichia coli* and *Shigella flexneri* bacteria. A preliminary study of phytochemical with Thin Layer Chromatogram (TLC) and antibacterial study was conducted. The study was conducted in vitro by disc diffusion method with positive control of ciprofloxacin 50 µg/mL and negative control of sterile aquadest. The extract concentration series used were 10, 20, 30, 40, 50, and 100%. The extracts of ganitri leaves have antibacterial activities against *Escherichia coli* and *Shigella flexneri* bacteria. The TLC results were observed under visible light at 254 and 365 nm. Ethanol, methanol, and aquadest extracts of ganitri leaves produce spots that are almost comparable to the compounds of quercetin and tannic acid. The inhibitory zone range of the extracts in *Escherichia coli* bacteria is 10.33-19.66 mm with a strong category. In comparison, the inhibitory zone range of *Shigella flexneri* is 8.88-22.62 mm with a moderate-very strong category. ANOVA test results stated a significance value of $p = 0.001$ ($p < 0.05$). Therefore, it can be concluded that the ethanol, methanol, and aquadest extracts of ganitri leaves had significant differences. The minimum concentration of each extract in inhibiting the bacteria *Escherichia coli* and *Shigella flexneri* is 10%.

Keywords: *Elaeocarpus ganitrus* Roxb, *Escherichia coli, Shigella flexneri,* disc diffusion

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INTRODUCTION

Medicinal plants have been used for treatment since ancient times in human history. In recent decades, the pharmacological potential of medicinal plants has been evaluated and investigated for their potential health benefits (Kumar, 2014). Ganitri The plant that has potential that has pharmacological potential is the ganitri plant. Ganitri plantations *(Elaeocarpus ganitrus* Roxb.*)* in Indonesia can be found in Central Java, Indonesia, such as Kebumen District (Rohandi, 2014). Kebumen is an area that has a large potential for ganitri stands. Almost all districts in Kebumen have developed ganitri plants as staple crops on people's lands. The high public interest in ganitri plants is due to the very high selling price of dried ganitri seeds. Because of the high economic value of ganitri seeds, many farmers replace their coconut plants with ganitri plants. Generally, ganitri farmers can generate an income of up to 500,000 for a ganitri tree (Sinuraya, 2017).

The ganitri plant (*Elaeocarpus ganitrus* Roxb.), widely grown in Indonesia, has not been fully utilized by the local community. The part of the ganitri plant that is often used is only limited to the seed. Dried ganitri seeds are an export commodity with high selling prices in the international market, where almost 70% of the supply of ganitri seeds in the world market is supplied from Indonesia. This has been felt by almost the majority of people in the Kebumen and Cilacap districts (Asep, 2014).

The leave and seed parts of ganitri *(Elaeocarpus ganitrus* Roxb.*)* have traditionally been used for curing stress, anxiety, migraine, epilepsy, depression, asthma, hypertension, palpitations, arthritis, and various liver diseases (Kumar, 2014). In addition, ganitri (*Elaeocarpus ganitrus* Roxb.) also has immunostimulatory, anti-inflammatory, antimicrobial, antifungal, antidepressant, and antioxidant (Kumar, 2014). Ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* contain antibacterial compounds such as phenol, tannins, and flavonoids (Jayashree, 2016).

Flavonoid is the largest phenolic compound that has the ability as an antibacterial compound. Flavonoids in ganitri leaves will denature proteins in bacteria to become inactive through the process of protein clumping. Inactive proteins will destroy bacterial cell membranes. The tannin content in the ganitri leaf extract is able to inhibit bacterial growth by destroying the bacterial cell membrane permeability so that bacterial growth will be inhibited and the bacteria die (As'ari, 2016). Phenol is a compound that is toxic to microorganisms, including pathogenic bacteria. A high amount of phenolic component can be found in higher amounts in extracts with highly polar solvents such as dilute methanol, dilute ethanol, and distilled water than in concentrated methanol and ethanol (Jayashree et al., 2016).

Previous research has demonstrated that the aqueous and methanol extracts of ganitri leaves possess antibacterial properties against *Shigella* sp. (Jayashree, 2016). Additionally, other studies have reported the antibacterial activity of ethanol and methanol extract of ganitri leaves against *Escherichia coli*, utilizing the disc diffusion method (Pandey, 2016; Sharma, 2015). Notably, investigations conducted in Indonesia regarding the antibacterial activity of ganitri plants have primarily focused on ganitri seeds. The findings indicate that ganitri seeds exhibit antibacterial activity against *Escherichia coli* and *Shigella sp.* with a MIC of 10% (As'ari, 2016).

Studies on the antibacterial activity of ganitri leaves against pathogenic bacteria have never been done in Indonesia. It is important to conduct the research because plants of the same type can contain different compounds due to climate, temperature, soil fertility, and geographical factors. The antibacterial activity of ganitri leaves from other parts of Indonesia is not necessarily the same as the antibacterial activity of ganitri leaves from Indonesia. The objective of this study is to evaluate the antibacterial efficacy of ethanol, methanol, and aquadest extracts obtained from ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* against *Escherichia coli* and *Shigella flexneri* bacteria. The disc diffusion method will be employed for this purpose.

MATERIALS AND METHOD

Materials

The materials used in this study include ganitri leaves (*Elaeocarpus ganitrus* Roxb.) from Kebumen, 70% ethanol, 70% methanol, aquadest, filter paper, silica plate GF254, quaretin, tannic

acid, FeCl3, Mueller Hinton Agar, Whatmann paper No. 1, NaCl 0.9%, ciprofloxacin, aluminum foil and wrap plastic.

Methods

Ganitri Leaves extraction *(Elaeocarpus ganitrus* Roxb.*)*

Ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* used were obtained from Kebumen District. The plant was identified by Systematics Laboratory Plants, Faculty of Biology, Gadjah Mada University. Simplicia of ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* was extracted by maceration using 70% ethanol and methanol solvent for 72 hours. The maceration process using aquadest solvent was carried out for 24 hours. The results of maceration were evaporated using a rotary evaporator until a thick extract was formed (Kholifah, 2011; Pandey et al., 2016).

Thin layer chromatography Ganitri leaves extract *(Elaeocarpus ganitrus* Roxb.*)*

Thin Layer Chromatography was performed on flavonoid and tannins compounds contained in ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)*. The stationary phase used was the silica plate GF_{254} with a mobile phase in the form of n-buthanol: glacial acetic acid: aquadest (6: 2: 2). Comparative compounds used were quercetin (flavonoids) and tannic acids (tannins). $FeCl_3$ solution was used to spray. Chromatogram results were observed under visible light and UV light at 254 and 365 nm (Anderson, 2006; Sopiah, 2019).

Antibacterial activity of Ganitri leaf extract *(Elaeocarpus ganitrus* Roxb.*)*

The antibacterial activity of ganitri leaves extract was observed using the paper disc diffusion method. The ethanol, methanol, and aquadest extract concentrations of ganitri leaves used for testing were 10, 20, 30, 40, 50, and 100% dissolved using sterile aquadest. Whatmann No 1 filter paper used as a disc was first soaked in each extract concentration series, positive control (amoxicillin), and negative control solution (sterile aquadest). The prepared disc paper was then placed in a circle on the test media containing the bacterial suspension carefully. The prepared media was then incubated at 37 °C for 18-24 hours. Antibacterial testing of ganitri leaves extract was repeated for 3 replications. The inhibition zones arising were then measured and recorded using calipers (Ferdi, 2019; Pandey, 2016).

Data Analysis

The analysis of TLC results marked the presence of spots and calculated the Rf value. Positive results containing flavonoids or tannins are indicated by the presence of spots and the same color, similar to the comparison**.** In addition, the analysis of antibacterial activity*)* was repeated three times. The antibacterial activity value was expressed as an average of three replications ± Standard Deviation (SD). The test results were statistically analyzed using the SPSS version 16 program (Gama, 2017).

RESULT AND DISCUSSION

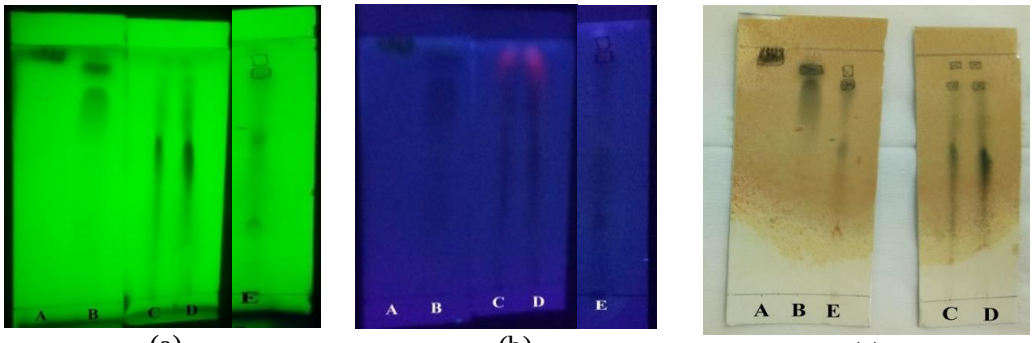
Ganitri leaves extraction *(Elaeocarpus ganitrus* Roxb.*)*

Ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* used were fresh and green leaves. Determination of plants was carried out with the aim to determine the species of plants used as samples. The ganitri plant parts used for plant determination were stems and leaves. The results of plant determination showed that the ganitri plants used belong to the species *Elaeocarpus ganitrus* Roxb. Ganitri leaves extraction was carried out using maceration method. The maceration method was preferred because it is simple and capable of extracting materials that are not resistant to heating, such as leaves. These three solvents were chosen because they are polar so they are expected to attract the active compounds in the ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)*. Maceration process using 70% ethanol and 70% methanol as solvent was carried out for 72 hours. Maceration process using aquadest solvent was carried out for 24 hours. It was done to avoid the growth of fungus on the aquadest solvent. The extracts obtained were then evaporated using a rotary evaporator at a temperature of less than 70 °C

because flavonoid, tannin, and fenol compounds tends not to be resistant to heating. The yield of ethanol, methanol, and aquadest extracts of ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* was 25.79; 31.79 and 23.38% respectively.

Thin layer chromatography

Thin layer chromatography was carried out on flavonoids, tannins, and phenols compounds in ganitri leaf extracts *(Elaeocarpus ganitrus* Roxb.*)*. The stationary phase used a silica GF_{254} plate with a comparative compound such as quercetin for flavonoids and tannic acid for tannins. The mobile phase consisted of n-buthanol: glacial acetic acid: aquadest (6: 2: 2) was chosen because it is polar and is able to separate the glycoside flavonoid compounds such as the flavonoids found in the ganitri leaves extracts *(Elaeocarpus ganitrus* Roxb.*)*(Dubey, 2018).



(a) (b) (c)

Figure 1. **TCL profile extracts of *Elaeocarpus ganitrus* Roxb. A: quercetin, B: tanic acid, C: ethanol extract, D: methanol extract, C: aqueous extract and their chromatogram spot visualization (a) UV 254 nm, (b) UV 365 nm,(c) $FeCl_3$ spray reagent**

The chromatogram results were observed using visible light as well as UV light at 254 nm and 365 nm. The ethanol, methanol, and distilled water extracts derived from ganitri leaves (Elaeocarpus ganitrus Roxb.) exhibited spots that were nearly comparable to the reference compounds, quercetin, and tannic acid (as shown in Table 2) (Mutammima, 2017). The data presented in Table 1 demonstrates that the ethanol, methanol, and distilled water extracts of ganitri leaves (Elaeocarpus ganitrus Roxb.) contain antibacterial compounds, including flavonoids and tannins.

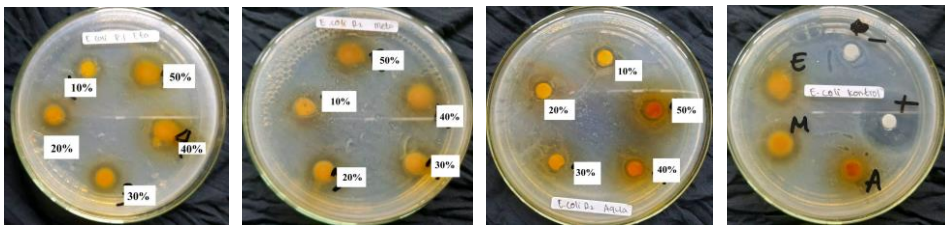
The results of the chromatogram in Figure 1 were sprayed using $FeCl_3$ solution as a marker of the presence of phenol compounds in the spots. The black color arised on the results of the chromatogram after spraying using $FeCl_3$ solution. This indicates the presence of phenol compounds.

Antibacterial activity of Ganitri leaves extract *(Elaeocarpus ganitrus* Roxb.*)*

Antibacterial activity tests of *Elaeocarpus ganitrus* Roxb extracts were conducted by using the disc diffusion method. The concentration of ethanol, methanol, and aqueous extracts were 10%, 20%, 30%, 40%, 50%, and 100%. In addition, the positive control used was amoxicillin 1%, which generally can be used to treat *Escherechia coli* and *Shigella flexneri* infection, and the negative control was sterile water. The results of the antibacterial activity test of *Elaeocarpus ganitrus* Roxb leaf extracts showed inhibition of the *Escherechia coli* and *Shigella flexneri* bacteria, as shown in Figure 2 and Figure 3. In the antibacterial assay, it was found that all extracts formed inhibition zones.

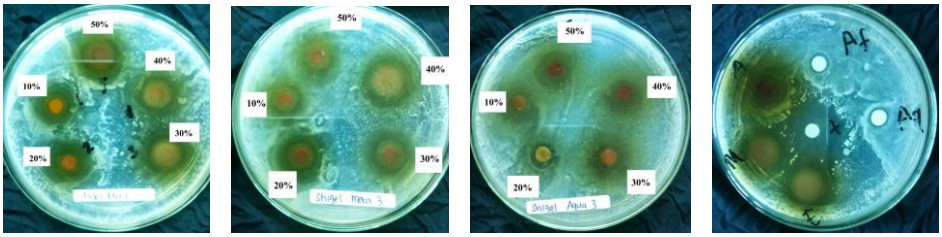
Table 1. TLC chromatograms of *Elaeocarpus ganitrus* Roxb extract

Sampel	Standart	Rf	Result		
			Visible Light	254 nm UV Light	366 nm UV Light
Quercetin (A)	0.91 – 0.98	0.95	Yellow	Black	Orange
Asam Tanat (B)	0.91 – 0.95	0.94	Yellow	Black	Black
Ethanol (C)	-	0.98	Yellow	Black	Orange
Metanol (D)	-	0.98	Yellow	Black	Orange
Aquadest (E)	-	0.91	Yellow	Black	Orange



(a) (b) (c) (d)

Figure 2. Inhibition zones of extract of *E.Ganitrus* against *Escherichia coli* (a) ethanol, (b) methanol, (c) aqueous, (d) positive and negative control



(a) (b) (c) (d)

Figure 3. Inhibition zones of extract of *E.Ganitrus* against *Shigella flexneri* (a) ethanol, (b) methanol, (c) aqueous, (d) positive and negative control

The findings of the antibacterial assay conducted on two test bacterial species are provided in Table 2. The results indicate that the various organic solvent extracts possess a broad-spectrum antibacterial activity, exhibiting different levels of sensitivity among the tested bacterial species. The negative controls, however, did not display any antimicrobial activity. Positive control (amoxicillin) showed inhibition zones of 20 mm and 18 mm, respectively. The data presented in Table 2 indicates that ethanol and methanol extract are more potent against all tested bacterial strains compared to the aqueous extract. Ganitri leaf extract *(Elaeocarpus ganitrus* Roxb.*)* has a relatively strong antibacterial activity with an inhibition zone range between 10 mm and 19 mm in *Escherichia coli* bacteria. The antibacterial activity of ganitri leaves extract *(Elaeocarpus ganitrus* Roxb.*)* is classified as moderate to very strong against *Shigella flexneri* bacteria with an inhibition zone ranges between 8 mm and 22 mm. The minimum concentrations of ethanol, methanol, and aquadest extracts of ganitri leaf *(Elaeocarpus ganitrus* Roxb.*)* in inhibiting the bacteria *Escherichia coli* and *Shigella flexneri* were 10%, respectively.

Table 2. The inhibition diameter measurement of *Escherichia coli* bacteria treated by ganitri *(Elaeocarpus ganitrus* Roxb.*)* extract

Concentration	Inhibition diameter of extract					
	***Escherichia coli* Bacteria**			***Shigella flexneri* Bacteria**		
	Ethanol	**Methanol**	**Aqueous**	**Ethanol**	**Methanol**	**Aqueous**
Ganitri extract 10%	12.48±0.33	11.87±0.00	10.33±0.33	14.26±0.00	13.60±0.00	8.88±0.00
Ganitri extract 20%	13.15±0.33	13.53±0.00	11.08±0.33	15.14±0.00	14.73±0.00	10.06±0.00
Ganitri extract 30%	14.77±0.33	14.44±0.00	11.81±0.33	16.98±0.00	15.64±0.33	10.41±0.33
Ganitri extract 40%	16.17±0.00	16.43±0.33	13.53±0.33	18.23±0.00	18.15±0.33	12.91±0.58
Ganitri extract 50%	17.95±0.58	19.66±0.33	15.59±0.33	20.02±0.58	18.88±0.33	17.06±0.58
Ganitri extract 100%	18.88±0.00	18.03±0.88	13.59±0.00	22.62±0.58	21.45±0.33	18.29±0.58
Positive control (amoxicillin)		20±0.88			18±0.68	
Negative control (sterile water)		0±0			0±0	

Antibacterial testing of ethanolic, methanol, and aquadest extracts of ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* against Escherichia coli and *Shigella flexneri* bacteria is comparable to Jayashree's (2016) and Sharma's (2015) research. Research by Jayashree (2016) demonstrated that methanolic and aquadest extracts of ganitri leaves have an inhibitory effect on *Shigella sp.* with a minimum concentration of extract of 50 µg/mL. Meanwhile, research conducted by Sharma (2015) showed that ganitri leaf extract has antibacterial activity against *Escherichia coli* bacteria with a minimum concentration of extract of 200 µg/mL extract. Research in Indonesia by As'ari (2016) regarding the antibacterial activity of ganitri seeds against *Escherichia coli* and *Shigella sp.* bacteria showed that ganitri seeds have a minimum concentration of extract to inhibit the growth of *Escherichia coli* and *Shigella sp.* with 10% have inhibition zones 0.34 mm and 0.26 mm. This shows that the antibacterial activity of ganitri leaves *(Elaeocarpus ganitrus* Roxb.*)* is more effective than the antibacterial activity of ganitri seeds.

The extracts exhibit antibacterial activity due to the presence of phytochemical compounds. Flavonoids function by forming complex compounds with extracellular proteins, which disrupt the integrity of the bacterial cell membrane (Torar et al., 2017). Alkaloids act as antibacterials by interfering with the peptidoglycan component within bacterial cells, preventing the formation of an intact cell wall layer and leading to cell death (Torar et al., 2017). Saponins possess strong, active compounds with soap-like properties, enabling them to reduce surface tension. When saponins bind to the cell surface, they cause damage to the cell membranes by increasing permeability, resulting in cell leakage and eventual death due to the loss of essential cell components (Torar et al., 2017). Tannins exert their antibacterial activity by shrinking cell walls or membranes, thereby disrupting bacterial cell permeability. This disruption hampers the ability of cells to carry out vital functions, causing growth inhibition or even cell death. Additionally, tannins can precipitate proteins, similar to phenolic compounds, contributing to their antibacterial effect (Torar et al., 2017).

Analysis of the research data was done using the SPSS 16 program. Through the One-Way Anova test, it was found that each extract had a significant difference in antibacterial activity against *Escherichia coli* and *Shigella flexneri* bacteria ($p<0.05$). This experiment indicated that antibacterial activity was concentration-dependent. On the other hand, there was no significant difference between the effect of aqueous extract on *Shigella flexneri* and positive control, indicated that the extract has the same effect as the positive control.

CONCLUSION

Ethanol, methanol, and aquadest extracts of ganitri leaves *(Elaeocarpus ganitrus* Roxb*.)* have moderate to very strong antibacterial activity, and the extracts are significantly different between each extract. The minimum concentration of the three extracts to inhibit the growth of *Escherichia coli* and *Shigella flexneri* is 10%.

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