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Research Article

Determination of Total Flavonoid and Antibacterial Activity of *Parameria laevigata* (Juss.) Moldenke Bark Extract Against *Staphylococcus saprophycitus* and *Escherichia coli*

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Abstract. *Parameria laevigata* (Juss.) Moldenke Bark is a natural ingredient containing metabolic secondary and empirically has been used to support health. This study aims to determine total flavonoid content of extract rapet bark using 70% and 96% ethanol by UAE (*Ultrasonic-Assisted Extraction*) for one hour at 40℃, determine antibacterial activity of extraxt rapet bark containing the highest total flavonoid content, and to determine the differences in antibacterial activity of extract rapet bark containing flavonoid content at concentrations 1, 3, 5% against *Staphylococcus saprophycitus* and *Escherichia coli*. The extraction process was carried out by UAE with a ratio 1:10. Extract results were subjected to ethanol free, color reaction test, TLC, determination of total flavonoid content, and the results of the highest total flavonoid content will be tested for antibacterial activity. The obtained ethanol content of 70% was 19.8737 ± 0.5828 mgRE/gram, 12.8389 ± 0.1687 mgRE/gram in ethanol 96%, and sequentially obtained antibacterial inhibition zones of 8.750 ± 0.025 mm; 9.855 ± 0.021; 12.460 ± 0.038 mm in *Staphylococcus saprophycitus* and 13.635 ± 0.029 mm; 14.545 ± 0.033 mm; 15.455 ± 0.021 in *Escherichia coli*.

Keywords: *Escherichia coli*; Flavonoid Total; Rapet Bark; *Staphylococcus saprophycitus*; UAE.

1. Introduction

pH ranges of vaginal is 4-5 during puberty and menopause, 3,5 – 4,5 during pregnancy. *Lactobacillus* is the vaginal flora that helps to maintain the vaginal ecosystem by inhibiting anaerobic microorganism, thereby preventing reproductive system disorders, including Urinary Tract Infections (UTI) which are commonly caused by *Staphylococcus saprophycitus* and *Escherichia coli* [1-4].

Rapet bark (*Parameria laevigata*) is a natural material that has been traditionally used to support health, such as for wound healing, relieving postpartum, treating diarrhea, and aiding to lost weight. These benefits are attributed to the presence of secondary metabolits in rapet bark, which is exhibit various pharmacological effects [5-9]. In a previous study, extraction with Ultrasonic-Assisted Extraction for 10 minutes using ethanol 70% and 96% as a solvents produced % yields of 9.86% and 6.69%. Antifungal test against *Candida albicans* at concentrations 0.375%, 0.625%, and 1.25% showed inhibition of fungal growth [5]. Folin-Ciocaleu test assay in another study reported 16.96 mg of total phenolic compounds[7]. Antibacterial test using chloroform and ethanol extracts at concentrations 0.25%, 0.5%, and 0.75% agains *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* using paper disk method showed that antibacterial activity was observed only in the 0.75% chloroform extract against *Staphylococcus aureus* [10].

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Based on the background, a research gap can be identified the antibacterial activity of ethanol extract against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtulis*requires higher concentrations to effectively inhibit bacterial growth, and no studies have yet determined total flavonoid content. Therefore, this study aims to determine total flavonoid content and evaluate the antibacterial activity of rapet bark (*Parameria laevigata*) against *Staphylococcus saprophycitus* and *Eschericia coli* using agar well diffusion method with increased sample concentrations

2. Methods

Equipment and Materials

The equipments used a digital balance, stopwatch, glassware laboratory, volumetric flasks, bunchner pump, bath sonicator, UV-Vis Spectrophotometer (Shimazdu 1789 and 1240), chamber TLC, capillary tubes, hot plate, yellow tips, blue tips, micropippets, calipers, autoclave, test tube, petri dishes, cylinder cups, inoculating loops, TLC plates (Silica Gel 60 GF_{254}.

The materials used a rapet bark (*Parameria laevigata*) from Kalikuto, Grabag, Magelang Regency, Central Java, Indonesia. Also we used ethanol 70% and 96% as a solvents. Reagents for preliminary phytochemical screening and TLC included $FeCl_3$, Mg, Rutin Hydrate, Amyl Alcohol, Gelatin, Chloroform, Acetic Anhydride, Ethyl Acetate, N-butanol, Formic Acid, Glacial Acetic Acid, H_2SO_4, 2N HCl, Methanol, Ethanol, Distilled Water, Mayer Reagent, Bauchardat Reagent, and Dragendorff Reagent. For antibacterial activity, material used included ½ Mc. Farland solution, Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA), Nutrient Broth (NB), Nutrient Agar (NA), Ciprofloxacin (K+), DMSO, and the bacteria used were *Staphylococcus saprophycitus* and *Escherichia coli*.

Preparation and Extraction

The preparation of simplicial included raw material processing, wet sorting, washing, slicing, drying, and dry sorting [11-12]. For the extraction, powdered rapet bark was weighed ± 100 g and placed into a beaker glass, then 1000 mL of 70% and 96% ethanol were added separately to each beakerglass (1:10). After preparation sample was completed, the extraction process was carried out using ultrasonic waves for 1 hour at 40℃. The extract was then filtered using a buchner pump to obtain the filrate. The obtained filtrate was subsequently evaporated using a rotary evaporator and waterbath at 40°C until a concentrated extract was obtained.

Extract Evaluation

Ethanol Free Test

This test was conducted to ensure the samples used for antibacterial activity testing were free of ethanol [13]. The procedure involved adding 1 mL of glacial acetic acid and H_2SO_4 to test tube containing the sample, then capping it with cotton, homogenizing and heating. If no ester odor detected, it indicates sample doesn't contain ethanol [14].

Preliminary Test and TLC Test

a) Phenolic

Sample + $FeCl_3$ 1% was added, and if color change to green, red, purple, blue, or dark black its indicates the presence of phenolic compounds in the sample [15]. TLC test used mobile phase Acetic Acid – Chloroform (9:1) and $FeCl_3$ for detection result a dark black spot [16].

b) Flavonoids

Sample + 10 mL of distilled water was added, heated, and then filtered. To obtained filtrate 0,1 g of Mg powder, 1 mL HCl and 2 mL amyl alcohol were added. The appearance of red,yellow, or orange colr in the amyl alcohol layer it indicates the presence of flavonoid compounds in the sample [15]. TLC test used mobilr phase Ethyl Acetate – Formic Acid – Glacial Acetic Acid – Water (100:11:11:26) and ammonia vapor as a detection produced yellow to brown spots [17].

c) Alkaloids

Sample + wagner's, dragendorff's, and mayer's reagents were added into the tube test. The addition of Wagner's reagents produces a light brown to yellow precipitate, Dragendorff's reagents produces a red to orange precipitate, and Mayer's reagaents produces a whitish-yellow precipitate. Sample considered to contain alkaloids if a complex precipitatae forms in at least two of reagents test used [16,18].

TLC test used mobile phase N-butanol – Glacial Acetic Acid – Water (40:40:10) and dragendorff's reagents as a detection producing brown to orange [17].

d) Tannins

Sample + 2-3 drops $FeCl_3$ 1%, NaCl 1%, and Gelatin solution 1% were added. A color change to blue, black, or green with $FeCl_3$ and white precipitate with NaCl and gelatin it indicates the presence of tannins compounds in sample [16]. TLC test used mobile phase Ethyl Acetate - Methanol - Water (77:13:10) and $FeCl_3$ as a detection showing dark black color [16].

e) Saponins

Sample + 10 mL of distilled water, then heated and cooled. After that, the sample was vigorously shake until foam formed (1-10 cm) and left to stand for 10 minutes, then 2N HCl was added. If the foam remains after adding 2N HCl, it indicates the presence of saponin compounds sample [18]. TLC test used mobile phase Chloroform – Glacial Acetic Acid – Methanol – Water (60:32:12:8) and Anisaldehyd asam sulfate as a detection produced purple [17].

f) Steroid Triterpenoids

Sample + Lieberman-Buchard (Acetic Anhydride and H_2SO4 reagent) was added to the tube test. A color change to green-blue indicates steroids, and red-purple color indicates the presence of triterpenoids [19-20]. TLC test used mobile phase Chloroform – Methanol - Water (65:25:4) and Anisaldehyd asam sulfate as a detection produced red-purple [17].

Total Flavonoid Content

The determination of total flavonoid content rapet bark refers to Indonesian Herba-Pharmacopeia second edition [21] with the following procedures:

Preparation of Test Solution

100 mg rapet bark into a 10,0 mL volumetric flask. Dissolve with ethanol and extraction for one hour using sonicator at 40℃, then filter into a 10.0 mL volumetric flask and dilute to the mark with absolute ethanol.

Preparation of Standart Solution

25 mg Rutin Hydrate into a 50.0 mL volumetric flask, dissolve with absolute ethanol up to the mark to obtain a 500 ppm stock solution. From this stock solution, prepare a standard solutions at concentration of 50, 100, 150, 200, and 250 ppm.

Preparation of Control Solution

1,5 mL absolute ethanol, 2.8 mL distilled water, and 0.1 mL Sodium Acetate.

a) Procedure

Pippete 0.3 mL of the test solution and standard solution into a test tube, then add 1.5 mL absolute ethanol, 2.8 mL distilled water, 0.1 mL AlCl 10%, and 0.1 mL Sodium Acetate into the test tube

Allow the mixture to atand for 30 minutes at room temperature, then measure the abs at 415 nm

Determine the Operating Time and Maximum Wavelength, then perform measurements on the standard and sample solutions.

Construct a calibration curve (Concentraction vs Absorbance)

$$\text{Content } (\mu g/g) = \frac{C \; X \; V \; X \; Fp}{W} \quad [22]$$

C = concentration compound in sample (μg/mL)
Fp = Dilution Factor
V = Volume sample (mL)
W = Weight sample (g)

Antibacterial

a) Preparation Medium

Nutrient Broth (NB): 1.95 g medium dissolve in 50 mL of destilled water in beakerglass, heat and stir until completely dissolved. Transfer medium into a erlenmeyer flask, stopper it, and sterilize using an autovlave 121°C 15 minutes.

Nutrient Agar (NA): 1.40 g medium dissolve in 50 mL of destilled water in beakerglass, heat and stir until completely dissolved. Transfer medium into a erlenmeyer flask, stopper it, and sterilize using an autoclave 121°C 15 minutes.

Mannitol Salt Agar (MSA): 21.6 g medium dissolve in 200 mL of destilled water in beakerglass, heat and stir until completely dissolved. Transfer medium into a erlenmeyer flask, stopper it, and sterilize using an autoclave 121°C 15 minutes.

Eosin Methylene Blue Agar (EMBA): 7.5 g medium dissolve in 50 mL of destilled water in beakerglass, heat and stir until completely dissolved. Transfer medium into a erlenmeyer flask, stopper it, and sterilize using an autoclave 121°C 15 minutes.

b) Preparation ½ Mc. Farland Solution

Composition of the ½ Mc. Farland :

0.5 mL 0.048M $BaCl_2H_2O$

99.5 mL 0.18M H_2SO_4

Pippete 0.5mL $BaCl_2H_2O$ and 99.5 mL H_2SO_4 into 100.0 mL volumetric flask. Then pippete 5.0 mL into 10.0 mL volumetric flask, fillto the mark with NB medium, homogenize. Mesure absorbance reaches 0.08 – 0.1 using UV-Vis Spectrophotometer at 625 nm [23]

c) Bacteria Revival

Prepare NA slant agar and tranfer one loop of pure bacteria (*Staphlococcus saprophycitus* and *Escherichia coli*) using streak inoculation and incubate at 37°C for 24 hours.

d) Preparation Bacterial Suspensions

One loop of bacteria and transfer into NB medium in a test tube. Incubate at 37°C for 24 hours. After incubation, measure abs at 625 nm and adjust to the ½ Mc.Farland.

e) Preparation of Positive Control

50.0 mg ciprofloxacin, dissolvein 100.0 mL volumetric flask using DMSO. Pippete 1.0 mL of this solution into 10.0 mL volumetric flask, dilute to the mark with DMSO.

f) Antibacterial Activity Test

Antibacterial activity test was conducted using extract with highest flavonoid content obtained from extraction (UAE). The extract was prepared at concentrations of 1%, 3%, and 5% tested against to *Staphylococcus saprophycitus* and *Escherichia coli*. For the test, 10 mL of growth medium for each bacteria was aseptically poures into petri dishes and allowed to solidify (first layer). Cylinder cups were then placed on the first layer. Pippete 10µL suspension *S.Saprophycitus* and 15µL suspension *E.coli* into of a medium, spread evenly like 8 pattern and allow it to solid. After solidification, remove cylinder cups and add 40µL extract ethanol 70% at concentrations 1%, 3%, 5%, DMSO (K-), Ciprofloxacin (K+), then incubate at 37°C for 24 hours. This procedure was repeated five times.

Data Analysis

Data for the determination of total flavonoid content in units of mgRE/gram of sample, meanwhile for antibacterial actibity were expressed in millimeters (mm) obtained from measurements of the inhibitions zone diameter using a caliper. Data processing was carried out using IBM SPSS Statistics 23 which is included normality test (Shapiro-Wilk Test) and homogenity test (Levene's Test of Homogenity of Variance). If data were normally distributed and homogeneous, the analysis wascontinued using One-Way ANOVA, followed by a Post Hoc Test.

3. Results and Discussion

This study was designed to determine of total flavonoid content in rapet bark extract obtained through by Ultrasonic-Assisted Extraction (UAE) method for 60 minutes at 40°C using 70% and 96% ethanol as a solvents. This study aims to evaluate antibacterial activity of the extract with the highest flavonoid content agains *Staphylococcus saprophycitus* and *Escherichia coli*, also this study in addition also sought to compare antibacterial activity of the extract at concentrations of 1%, 3%, and 5%.

The sample used in this study were collected from Kalikuto, Grabag, Magelang Regency using random sampling technique. Sample preparation involded process wet sorting, washing, cutting, drying, dry sorting, and grinding into powder. Powder was then sieved using a 60-mesh sieve to obtain uniform particle size and incrase the surface area. This enhacement promotes greater interaction between sample and solvent, thereby optimizing the extraction process in isolating secondary metabolite compounds [24-25]. Ultrasonic-Assisted Extraction (UAE) was employed as the extraction method because it operates through ultrasonic vibrations >2000 kHz that generate cavitation bubbless. These bubbles induce disruption of the cell walls, leading to enchanted plant diffusion and increased cell wall permeability thereby facilitating the extraction of bioactive compounds. This method offers several advantages, including producing a higher % yield in short time, it is also non destructive and non invasive sample [25-28].

The extraction results showed % yield of 11.78% in ethanol 70% and 6.20 in ethanol 96%. Based on these values, it canbe observed that factor influencing % yield is the polarity solvent which follows the principle of like dissolves like[24,25,29]. Then extract was subsequently tested to ensure it was free from residual ethanol to prevent false positive in antibacteriak activitiy. This step was necessary because ethanol is known have to ability denature proteib and disrupt bacterial cell [30]. Subsequentlty, color reaction test and TLC analysis were conducted to identify the compounds present in the sample using spesific reagents, with the results compared to literature. TLC aims to determine the separation of compounds based of differences in polarity following the principle of sample elution along the stationary phase to a certain limid inside a chamber saturated with eluent. As shown in table 1 sample was confirmed free ethanol and was found to contain secondary metabolites like phenolics, flavonoids, saponins, alkaloids, tannins, and triterpenoids. In addition different Rf values were obtained for each compounds because the differences by polarity and the amount of extract spotted on the plate. A compounds with a higher Rf values indicates lower polarity, where as a compounds with a low Rf indicates higher polarity [31-32]

Table 1. Ethanol Free Test, Color Reaction Test, TLC Test of *Parameria laevigata* Extract.

Description	Color Reaction		TLC		
	70%	96%	Spot Visualittaion	-/+	Rf
Ethanol Free Test	Ethanol Free	Ethanol Free			
Phenolics	+	+	Black	+	0.25
Flavonoids	+	+	Yellow	+	0.48
Alkaloids	+	+	Brown	+	0.28
Tannins	+	+	Black	+	0.16;0.33;0.86
Saponins	+	+	Purple	+	0.48;0.75
Steroid/Triterpenoids	/+	-/+	Purple	-/+	0.16;0.33

After preliminary test, the next step was the determination of total flavonoid content using a UV-Vis Spectrophotometer through a colorimetric method with $AlCl_3$ 10% and Sodium Acetat 1M as a reagents. $AlCl_3$ was added to induce a bathochromic effect causing the compounds wavelength into the visible regin, indicated by the solution changing color to yellow. This color change occurs due to the formation of a stable complex between the ketohyroxyl and a stable acid complex with ortho-hydroxyl, meanwhile sodium acetate was added to maintain the wavelength within the visible region[33]. The analysis of total flavonoid content was carried out using the calibration cure method beginning with the preparation of rutin standard solutions at concentrations of 50, 100, 150, 200, and 250 ppm which aims to determine Concentration Vs Absorbance as indicated by the linearity values.

Rutin was selected as a standard because it belongs to the flavonoid glycoside group and according to the Indonesian Herbal Pharmacopeia 2017. This is further supported by TLC analysis was showed in table 1, which confirmed the presence of rutin in the sample indicated by an Rf value of 0.48 falling within the literature range of 0.4 to 0.65 for rutin [34]. Based on these data and literature support, it canbe concluded that flavonoid compounds in the extract correspond to thereference in the Indonesian Herbal Pharmacopeia 2017 namely Rutin [21]. A 150 ppm standard solution was used to determine the wavelength in order to identify the maximum absorption wavelength and operating time sample. This helps establish period during which measurements remain stable indicating the formation of a stable complex, as evidenced by repeated absorbance values [35]. The maximum wavelength was found to be 416 nm with an operating time of 15 minutes, and the linear regression of Concentration Vs Absorbance obtained was y = 0.0026x + 0.1189 with a linearity value of 0.9991, as shown in table 2 and figure 1.

Table 2. Concentration Vs Absorbance (Rutin Hydarate Vs *Parameria laevigata* Extract).

Concentration (ppm)	Absorbance
50.16	0.251
100.32	0.387
150.48	0.498
200.64	0.634
250.80	0.779

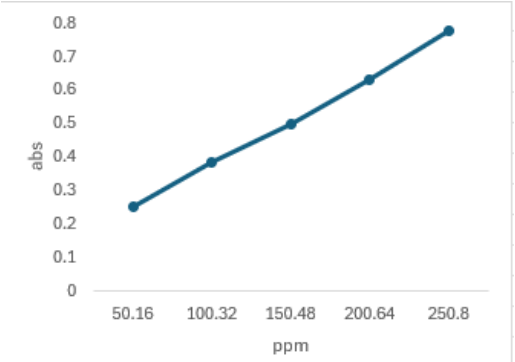

Figure 1. Curve Concentration Vs Absorbance.

The obtained linear regression equation was used to determine flavonoid rutin content in 1 gram sample, expressed in mgRE/g. The experiment was repeated five time to ensure accurate results as showed at table 3.

Table 3. Total Flavonoid Content from *Parameria laevigata* Extract.

Extract		Weight (mg)	Absorbance	Content (mgRE/g)	Average Content (mgRE/g)
Ethanol 70%	1	0.102	0.657	20.1914	
	2	0.1014	0.631	19.4242	
	3	0.0997	0.6	18.5595	**19.8737 ± 0.5828**
	4	0.100	0.625	19.3493	
	5	0.1017	0.636	19.556	
Ethanol 96%	1	0.1016	0.459	12.8748	
	2	0.1014	0.457	12.8243	
	3	0.1011	0.455	12.7863	**12.8389 ± 0.1687**
	4	0.102	0.466	13.0882	
	5	0.1009	0.45	12.621	

Based on table 3, it can be concluded that the extract with highest total flavonoid content is the ethanol extract 70%. This is influenced by the polarity of the solvents following the solbility principle like dissolve like, and the low Rf values observed in the TLC results of the sample also support the fact that most of the compounds in the sample are polar, which explains why they are more readily extracted in 70% ethanol [29].

Extract ethanol 70% was subjected to antibacterial activity against *Staphylococcus saprophycitus* and *Escherichia coli*. These bacteria was selected base on literature from clinical and experimental studies which indicate that they are infection like Urinary Tract Infection (ISK) in woman. The pathogenesis of these infections is associated with abnormsl pH vaginal, poor personal hygiene that allows uroopathogenic bacteria to enter the urinary tract, and the anatomical proximity of the uretra to the vulva [1], [36].

The well diffusion methoed was used in this stdy because the sample diffuse down to the lower layers, facilitating the measurementof clear zone. The principle of this method involves creating a hole in the medium using cylinder cup, into which the sample is introduced with the pour plate method for time efficiency. MSA and EMBA were used as the test media becase they are selective and differential media for the specific bacteria. MSA contains 7.5% salt which inhibits the growth of other bacteria, and when inoculated by *Staphylococcus saprophycitus* no color changed occurs because it cannot ferment mannitol. EMBA contains methylen blue and lactose, which inhibit gram positive bacteria. The appearance of a metallic green color after inoculation with *Escherichia coli* is due to acid production causing methylene blue precipitation and color uptake as a result of lactose fermentation by *Escherichia coli* [37-40]. Ciprofloxacin was used as the K+ because it is a broad spectrum antibacterial drug from the fluoroquinolone class and works by inhibiting enzyme in bacterial DNA replication, recombination, and repair. DMSO was used a K- because it can dissolve polar and non polar compounds in the sample and doesn't bacterisidal effects [41]. The purpose of using K- is to determine whether the solvents has any effect on the sample.

The results of the clearzone measurements are presents by table 4. According to the bacterial growth inhibition critaeria based on the classification by [42], a diameter of <5 mm is considered a weak response, 5-10 mm moderate, 10-20 mm strong, > 20 mm very strong. As shown in the table,the response increased proportionally with increasing sample concentrations. Testing against *Staphylococcus saprophycitus* showedthat 1% and 3% concentrations produced a moderate response, while 5% produced a strong response. For *Escherichia coli* even the 1% concentration already produced a strong response. This difference in inhibitory activity is related to the bacterial cellwall structure. *Staphylococcus saprophycitus* has a cell wall composed solely of peptidoglycan allowing active a compounds to penerate easily, meanwhile *Escherichia coli* has a more complex peptidoglycan structure which makes it more difficult for external substance to penerate [43]. Based on the test results, it can be concluded that the findings are consistent with theoretical, namely bacteria gram positive exhibit smaller inhibition zone than to gram negative bacteria.

Table 4. Antibacterial Activity of *Parameria laevigata Extract*.

Bacteria	Average Zone (mm)				
	1%	3%	5%	K+	K-
Staphylococcus saprophycitus	8.750 ± 0.025	9.855 ± 0.021	12.460 ± 0.038	13.650 ± 0.025	0.000
Escherichia coli	13.635 ± 0.029	14.545 ± 0.033	15.455 ± 0.021	17.575 ± 0.025	0.000

Based on the study, it can be observed that rapet bark contains various secondary metabolites, like phenolics, flavonoids, tannins, saponins, alkaloids, and triterpneoids. Antibacterial mechanism of these componds include damaging cell membrane and cytoplasm through passive diffusion, altering cell morphology, affecting cellular metabolism, inhibiting efflux pump, suppressing nucleic acid and enzyme synthesis, preventing bacterial adhesion, and binding to transmembrane proteins on the outer cell wall so this interaction leads to the disruption of transmembrane proteins ultimately reducing cell membrane permeability [44-49].

Quantative data from inhibition zones were than analyzed using statistical, including normality test (Shapiro-Wilk), homogenity test (Levene's Test of Homogenity of Variance), and parametric one way ANOVA if data were normally distributed and homogeneous.

This data is presented in table 5, and based on the results of the normality and homogeneity test showed a sig of 0.000 > 0.05 indicating that the data were normally distributed and homogeneous. Data that met there criteria were furthet analyzed using One-Way ANOVA to determine whether there were significant differences in effects between the sample and K+. ANOVA results showed a sig. 0.000 < 0.05, indicating a significant differences in antibact erial effects on both bacteria. Subsequently a Post Hoc Test was conducted to confirm whether there were significant differences between concentrations and K+ showed a sig. 0.000 < 0.05 that indicating were significant differences in effects among the concentrations compared to K+. Therefore, it can be conluded that nosample concentration exhubuted an inhibitory effect equivalent to the K+ in bacterial growth.

Table 5. Statictic Method Results Antibacterial Activity of *Parameria laevigata* Extract.

Bacteria		Shapiro Wilk		Levene's Test		Post Hoc Test	
Staphylococcus saprophycitus	1%	0.119	p > 0.05	0.616 > 0.05	1%	Vs 3%	0.000 < 0.05
	3%	0.314				Vs 5%	
	5%	0.492				Vs K+	
	K+	0.119					
Escherichia coli	1%	0.814	p > 0.05	0.675 > 0.05	3%	Vs 1%	0.000 < 0.05
	3%	0.421				Vs 5%	
	5%	0.314				Vs K+	
	K+	0.119					

4. Conclusions

The flavonoid content rapet bark which extracted using UAE obtained was 19.8737 ±0.5828 mgRE/g in ethanol 70% and 12.8389 ± 0.1687 mg/RE g in ethanol 96%, and the highest flavonoid content in ethanol 70% which also exhibited antibacterial activity against *Staphylococcus saprophycitus* and *Escherichia coli*. There were significant differences in antibacterial activity among the 1%, 3%, and 5% concentrations get the clear zone diameters were 8.750 ± 0.025 mm; 9.855 ± 0.021 mm and 12.460 ± 0,038 mm for *Staphylococcus saprophycitus* and 13.635 ± 0.029 mm; 14.545 ± 0.033 mm; 15.455 ± 0.021 mm for *Escherichia coli*. For future studes, it is necessary to conduct futher research on fractination of rapet bark using solvents with different polarity, evaluate pharmacological activity, and to carry out more detailed on specific secondary metabolites with antibacterial properties through isolation.

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request. No publicly archived datasets were generated or analyzed during the current study.

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