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Research Article

Total Flavonoid Levels in *n*-hexane and Ethyl Acetate Fractions of *Rosmarinus officinalis* L. Leaves and Their Antibacterial and Antioxidant Activities

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Abstract

The rosemary (*Rosmarinus officinalis* L.) is a plant of the Lamiaceae tribe that has not been widely studied regarding its pharmacological activity, known from previous studies to contain secondary metabolites of flavonoids. Flavonoids are phenol compounds with many pharmacological activities, including antibacterials and antioxidants. This study aims to determine the total flavonoid levels in *R. officinalis* leaves and their effect on antibacterial and antioxidant activities. This research began with the preparation of ethanol extract from *R. officinalis* leaves, then the fractionation of the extract produced *n*-hexane and ethyl acetate fractions. Total flavonoid levels were determined against both fractions by UV-Vis spectrophotometry. A test of the fraction's antibacterial activity against *Staphylococcus aureus* was performed using the disc diffusion method. The antioxidant test is carried out by the DPPH method. The total flavonoid content of the ethyl acetate fraction is 47.437 ± 1.947%, higher than the *n*-hexane fraction. Test antibacterial and antioxidant activity showed more significant results in the ethyl acetate fraction than in the *n*-hexane fraction. In conclusion, the total flavonoid levels of ethyl acetate fraction are directly proportional to the antibacterial and antioxidant activities of *R. officinalis* leaves.

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INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is one of the herbal plants belonging to the Lamiaceae family. In Indonesia, *R. officinalis* leaves have yet to be widely studied and are only known as a seasoning and food preservative. However, in several studies that have been carried out on *R. officinalis* leaves, it is known that the content of secondary metabolites is owned, one of which is flavonoids[1-3]. Flavonoid is one of the secondary metabolite compounds that belong to the phenol group and are found in all parts of the plant and have a C6-C3-C6 core structure[4].

Generally, flavonoids will bind to sugars, forming glycosides, which cause these compounds to dissolve more easily in polar solvents, such as methanol, ethanol, butanol, and ethyl acetate. In the form of glycans of a less polar nature, flavonoids will be more easily soluble in non-polar solvents, such as chloroforms and ethers[5,6]. The pharmacological activities of flavonoid compounds are very diverse, among which the most common is their activity as antibacterial and antioxidant. Flavonoids inhibit bacterial growth by damaging the cytoplasmic membrane, inhibiting protein synthesis from bacterial cell walls, nucleic acid synthesis, and energy metabolism[7].

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Staphylococcus aureus bacteria are one type of Gram-positive bacteria with a round and colonized shape, so they resemble the shape of grapes. This bacterium is also a normal skin and upper respiratory tract flora that can cause infectious diseases[8]. To treat bacterial infections, medical personnel will provide antibiotic therapy. However, the risk of antibiotic resistance events also increases with the increase in the number of infections that occur every year and the improper use of antibiotics. This is associated with the presence of factors such as improper use of the drug and improper length of use[9]. So, in this study, the antibacterial activity of *R. officinalis* leaves was tested against *S. aureus* growth.

A free radical is a compound or molecule with one or more unpaired electrons on its outer orbital. The presence of unpaired electrons causes the compound to be very reactive in looking for a partner, by attacking and binding to the electrons of the molecules around it. If the electrons bound by the free radical compound are ionic, the impact that arises is not so dangerous, but if free radicals from covalent bond compounds bind the electrons, it will be very dangerous[10]. The number of free radicals exceeding antioxidants will cause an imbalance between free radicals and endogenous antioxidants, known as oxidative stress. Oxidative stress conditions cause the body to need an intake containing a compound, antioxidants, that can capture and neutralize these free radicals so that further reactions that cause oxidative stress can stop and cell damage can be avoided or the induction of a disease can be stopped. The content of plants, such as flavonoids, can be used as a substitute for antioxidants that are disturbed in the body[11]. This research did qualitative and quantitative measurements of flavonoid presence in *R. officinalis* leaves and tested its antioxidant activity.

However, from the studies that have been carried out on *R. officinalis* leaves, no one has tested the pharmacological activity of *R. officinalis* leaves fractions. Therefore, in this study, fractionation of *R. officinalis* leaves extract was carried out using semi-polar solvents (ethyl acetate) and non-polar solvents (*n*-hexane). Research on herbal plant fractions showed more significant pharmacological activity than plant extracts. Guleria *et al*.[12] found that total phenolic and total flavonoid content was higher in ethyl acetate fraction than in ethanol extract of *Terminalia chebula*, and antibacterial activity was also higher in ethyl acetate fraction with a more expansive zone of inhibition against the bacteria. Aisyah *et al*.[13] found that ethyl acetate fraction and hexane faction had a higher antioxidant activity than the ethanol extract of *Angiopteris ferox* Copel. This study aimed to determine the total flavonoid levels of the n-hexane fraction and the ethyl acetate fraction of *R. officinalis* leaves extract from Bali, Indonesia. It also aimed to determine the influence of flavonoid levels on the antibacterial and antioxidant activity of each fraction.

MATERIALS AND METHODS

Materials

Plant materials were obtained from Bali (Bedugul-Tabanan Regency). Identification of the plant was carried out by the Indonesian Institute of Science, Bali Botanic Garden, Bali, Indonesia, with the voucher specimen number B-305/IPH.7/AP/XI/2020. *Staphylococcus aureus* FNCC 0047 isolates from Universitas Gadjah Mada, Indonesia. Another materials including Nutrient Agar (NA; Merck), Nutrient Broth (Merck), distilled water, 96% ethanol, *n*-hexane, ethyl acetate, NH3, CH3COOH, *n*-butanol, Mayer's reagent, KI, HCl, NaOH, chloroform, Na_2CO_3, K2Cr2O7, H_2SO_4, $FeCl_3$, $AlCl_3$, potassium acetate, violet crystalline solution, iodine solution, safranin solution, immersion oil, quercetin, 5% tween 80, and 3% amoxicillin. Instrument used was glassware (Iwaki), Petri dish, test tube (Iwaki), digital balance (Ohaus), split funnel, thin-layer chromatography (TLC) plate, TLC chamber (Camag), oven (Binder), autoclave, incubator (Binder), UV lamp, LAF Cabinet, and UV-Vis spectrophotometry.

Methods

Preparation of ethanol extract

A total of 600 g of simplicia was macerated with 96% ethanol, then allowed to stand 3 x 24 hours, which was occasionally stirred, filtered using flannel, and then the first filtrate was evaporated. The maceration residue was added 96% ethanol solvent back, allowed to stand for 3 x 24 hours, and stirred occasionally. The results of the re-maceration were filtered with a flannel cloth, and then the second filtrate was evaporated, and later, a thick extract of *R. officinalis* leaves ethanol was obtained[14].

Ethanol-free by color test

A total of 2 g of extract was added to 2 drops of concentrated H_2SO_4 and 1 mL of potassium dichromate solution. The ethanol content in the extract was indicated by a color change from orange to bluish-green[15].

Fractionation of ethanol extract from R. officinalis leaves

A total of 10 g of *R. officinalis* leaves ethanol extract was dissolved in 50 mL of distilled water, then added to 20 mL of 96% ethanol solvent, and filtered using filter paper. The filtration results were put into a split funnel, 100 mL *n*-hexane was added, shaken slowly for 5 minutes, and allowed to stand until the *n*-hexane and water layers were separated. The *n*-hexane layer in the upper layer was taken, and then the water layer at the bottom was repartitioned until the layer was color-cleared using *n*-hexane solvent. The process was then repeated with ethyl acetate solvent[16].

Phytochemical screening of flavonoid test by color reaction test

The extract was weighed at 0.2 g, then 10 mL of distilled water was added. The solution was heated for 5 minutes, cooled, then filtered. Five mL of filtrate was taken and put into a test tube, then added 0.1 g of magnesium powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol, then shaken and separated[17].

Flavonoids screening by TLC

The *n*-hexane and the ethyl acetate fractions were dissolved with solvents according to each fraction. The fraction was spotted on the TLC plate with a size of 4 x 10 cm at a distance of 1.5 cm from the bottom edge of the TLC plate using capillary pipes. The TLC plate was dried and diluted using an eluent of *n*-butanol : glacial acetic acid : distilled water with a ratio of 4 : 1 : 5 and a total volume of 20 mL. The elusion TLC plate was detected under a UV lamp of 254 and 366 nm, and then the appearance of spots using ammonia vapor was carried out[18,19].

Total flavonoid levels of n-hexane and ethyl acetate fractions

The mother liquor was prepared by weighing 10 mg of quercetin and then dissolving it with 10 mL of ethanol. Furthermore, a concentration series solution was made using a pipetted mother liquor of 0.2, 0.4, 0.6, 0.8, 1, and 1.2 mL, respectively, into a 10 mL measuring flask. Ethanol was added to the limit mark to make a solution with a concentration of 20, 40, 60, 80, 100, and 120 mg/L. Each standard solution of 20, 40, 60, 80, 100, and 120 mg/L was pipetted by 0.5 mL and fed into a 5 mL measuring flask, then added with 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 potassium acetate 1 M, and distilled water to the limit mark to obtain concentrations of 2, 4, 6, 8, 10, and 12 mg/L. The solution was allowed to stand at room temperature for 30 minutes then absorbance absorption was measured with a UV-Vis spectrophotometer at maximum wavelength. The *n*-hexane and ethyl acetate fractions of *R. officinalis* leaves extract were weighed as much as 25 mg and dissolved in ethanol on a 25 mL measuring flask until the limit mark. It was repeated three times with 0.5 mL of pipetted each, then added with 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of potassium acetate 1 M, and distilled water up to a volume of 5 mL. This solution was then allowed to stand at room temperature 25-30°C for 30 minutes and measured its absorbance on UV-Vis spectrophotometry at maximum wavelength[20].

Gram coloring of bacteria

The glass of the object was taken out with tongs and sterilized on the fire of the bunsen. The ose needle was heated with bunsen fire until the ose needle looked red; a drop of aqua dest was taken with an ose needle and placed on the glass of the object. The mouth of the Petri dish containing bacterial cultures was sterilized by aseptic means using bunsen fire, the ose needle was re-sterilized and the bacterial culture was taken. Bacterial cultures on the object glass were laid out in evenly circular motions; the object glass was dried with bunsen fire by passing it until it was dried and placed on a rack or dye container. Bacterial staining was carried out with four reagents: violet crystal, iodine, 95% alcohol, and safranin solutions. Each coloring was rinsed using distilled water. Areas that were not growing bacteria were cleaned using tissue to facilitate the drying process; The object glass was dried by rolling it until dry[21].

Antibacterial activity test against S. aureus by disc diffusion method

The bacterial suspension was applied with a sterile cotton swab on NA media in a Petri dish using the scratching method performed near the bunsen fire. Disc paper was soaked in each test solution for 30 minutes to 1 hour and placed on NA media containing bacterial cultures in a Petri dish using pre-sterilized tweezers. The antibacterial activity test of the *n*-hexane fraction and the ethyl acetate fraction of *R. officinalis* leaves ethanol extract used four concentration series: 3%, 6%, 9%, and

12%. Also, a media control containing only NA media in a Petri dish was created to observe whether mold or mold growth on NA media could damage the test results. All growth media were incubated at 37°C for 24 hours[22].

Antioxidant activity test by DPPH method

A total of 1000 mg/L for *n*-hexane fraction of *R. officinalis* leaves ethanol extract, ethyl acetate fraction of 100 mg/L, and quercetin solution of 100 mg/L were prepared. The amount of each *n*-hexane fraction 1000 mg/L was pipetted as much as 0.2, 0.6, 0.8, 1, and 1.2 mL were then put into a 10 mL volumetric flask to obtain concentrations of 20, 40, 60, 80, 100, and 120 mg/L, respectively. For ethyl acetate fraction and quercetin solution, 100 mg/L were pipetted as much as 0.1, 0.2, 0.3, 0.4, 0.5, and 0,6 mL were then put into a 10 mL volumetric flask to obtain concentrations of 1, 2, 3, 4, 5, and 6 mg/L, respectively. Ethanol 96% was used as the solvent. An amount of 5 mL of DPPH solution 40 mg/L was added to each volumetric flask, and then the volume was filled with ethanol to the marked line, allowed to stand in the dark for 30 minutes, then the absorbance was measured on a spectrophotometer with a wavelength of 517 nm[23].

Data analysis

Observation of bacteria was carried out using a microscope, which had previously been dripped with immersion oil. The inhibitory diameter of bacteria was measured on a clear zone formed around the disc paper using a ruler. The data from the DPPH assay were obtained from three repeated experiments (n = 3) and presented as the mean ± standard deviation (SD)[24].

RESULTS AND DISCUSSION

Extraction yield, ethanol-free, and R. officinalis leaves fractionation

As a result of the extraction of *R. officinalis* leaves by the maceration method, a dry extract with a percentage of amendments of 18.21% was obtained. The extract was tested ethanol-free to ensure that the *R. officinalis* leaves extract no longer contains ethanol, indicated by the absence of discoloration from orange to bluish-green (**Figure 1**)[15]. This indicates that the extract has been freed from ethanol content. Fractionation of *R. officinalis* leaves ethanol extract resulted in *n*-hexane and ethyl acetate fractions with a percentage yield of fractions of 3.7% and 15.3%. Results showed that the ethyl acetate fraction attracted more compounds from the ethanol extract of *R. officinalis* leaves.

Figure 1. The ethanol-free test result of *R. officinalis* extract.

Phytochemical screening and identification of flavonoids using TLC

Based on the test results, *n*-hexane and ethyl acetate fractions of *R. officinalis* leaves positively contain flavonoid compounds indicated by a change in color to orange (**Table I**). Mg and HCl will reduce the benzopyran nucleus in the flavonoid structure so that a red, yellow, or orange flavylium salt is formed[25].

Table I. The flavonoids screening.

Compound	*n*-hexane fraction	Ethyl acetate fraction
Flavonoids	+	+

+ : presence of compound

As a result of the fractionation of *R. officinalis* leaf extract, the *n*-hexane fraction and the ethyl acetate fraction were obtained. The TLC method then identified the two fractions using a quercetin comparison. The solvent is used because it includes a phase of motion commonly used in TLC to identify flavonoid compounds glycans and aglycone. Evaporation of TLC plates with ammonia can emphasize the intensity of color, according to the properties of flavonoid compounds that can glow when viewed with a UV light of 365 nm[26]. The results of flavonoid identification showed that the *n*-hexane fraction and the ethyl acetate fraction contained flavonoid compounds seen from the spots on the TLC plate (**Figure 2**). In addition, the Rf value of the *n*-hexane fraction and the ethyl acetate fraction are close to the Rf value of the quercetin standard of 0.98 (**Table II**).

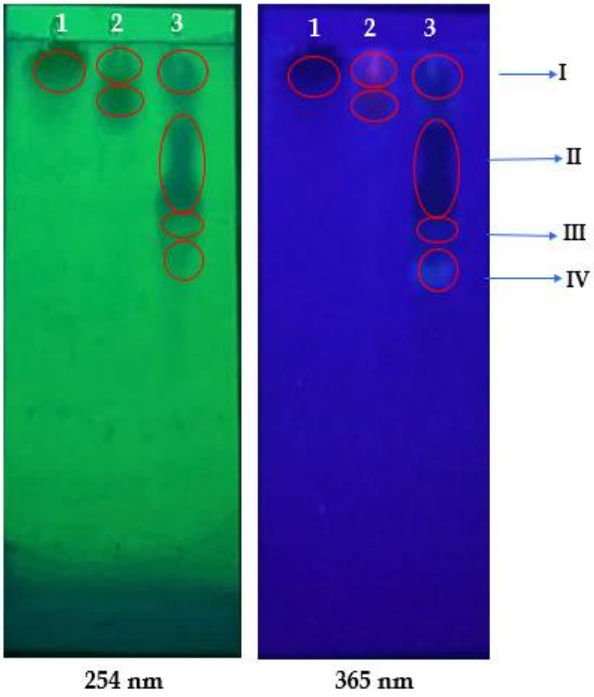

Figure 2. The chromatogram of (**1**) quercetin, (**2**) *n*-hexane, and (**3**) ethyl acetate fractions of *R. officinalis* leaves extract after evaporation with ammonia.

Table II. TLC-bioautography of *n*-hexane and ethyl acetate fractions of *R. officinalis* leaves.

Samples	Spot	Rf	The color at 254 nm	The color at 365 nm	Suspected types of flavonoids
Quercetin	I	0.98	Dim yellow-green fluorescence	Dark mauve	Flavonols
n-hexane fraction	I	0.97	Brownish-yellow	Red	Flavonols, chalcones
	II	0.87	Brownish-yellow	Red-black	Flavonols, chalcones
Ethyl acetate fraction	I	0.93	Blue-green fluorescence	Purple-blue fluorescence	Flavonols, flavones
	II	0.85	Blue-green fluorescence	Dark mauve	Flavonols
	III	0.62	Invisible	Purple blue	Isoflavones
	IV	0.56	Invisible	Blue-green fluorescence	Isoflavones

Total flavonoid levels of a fraction of R. officinalis leaves extract

Quercetin is used as a comparison standard in determining the total flavonoid levels against the *n*-hexane and the ethyl acetate fractions. Quercetin is included in the flavonoid group of flavonols, which has a keto group on the C-4 atom and a hydroxyl group in the C-3 or C-5 atom, which can bind to form a complex with $AlCl_3$[27]. The maximum wavelength measurement result obtained is 430 nm, similar to other study by Krisyanella *et al.*[28] that determined flavonoid levels using quercetin. Standard determination of the standard curve aims to determine the relationship between the concentration of the solution and its absorbance value so that a linear regression equation is obtained. The linear regression equation obtained from the absorbance value of the quercetin series solution (**Figure 3**) is $y = 0.0067x + 0.0225$ with a correlation coefficient value (R^2) of 0.9993. A value of R^2 close to 1 indicates that the standard curve is linear and there is a correlation between the solution concentration and the absorbance value; the higher the concentration, the higher the absorbance obtained[29].

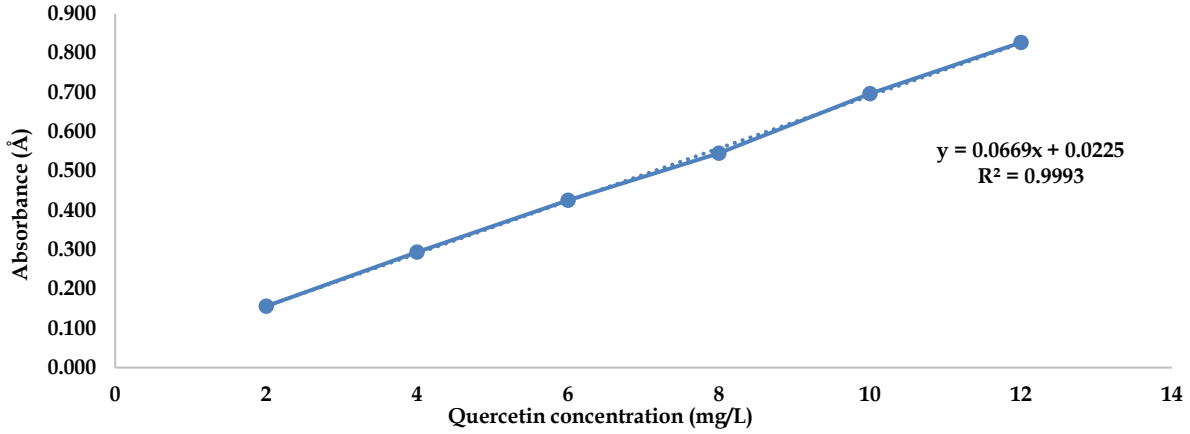

Figure 3. Quercetin standard curve.

In determining total flavonoid levels, adding potassium acetate aims to detect the presence of a 7-hydroxyl group[30,31]. Based on the results, total flavonoid levels of the *n*-hexane fraction were obtained by 19.129 ± 3.243%, and the ethyl acetate fraction by 47.437 ± 1.947% (**Table III**). These results show that ethyl acetate solvents can attract more flavonoid compounds than n-hexane as a non-polar solvent. The results are similar to another study by Suoth *et al*.[32], stating that the ethyl acetate fraction has higher flavonoid levels than the n-hexane fraction.

Table III. Flavonoid levels of total fractions of *n*-hexane and ethyl acetate fractions of *R. officinalis* leaves.

Fractions	Absorbance (Å)	Total flavonoid levels (mgQE/g)	Total flavonoid levels (%)
n-hexane	0.151 ± 0.022	191.290 ± 32.433	19.129 ± 3.243
Ethyl acetate	0.340 ± 0.013	474.373 ± 19.477	47.437 ± 1.947

Antibacterial activity of the fractions of R. officinalis extract against S. aureus

The Gram staining of bacteria showed that the bacteria were indeed *S. aureus*. From **Figure 4**, it can be seen that the bacteria are purple and have a colonized round shape. Gram-positive bacteria can bind to the primary paint because they have a thicker peptidoglycan layer than Gram-negative, and their cell walls are composed of heteropolymer proteins and sugars known as mureins. Mureins will become a barrier so the violet-iodine crystal complex cannot escape during the staining process[33,34]. In testing antibacterial activity, the disc diffusion method is used because the method is easy, simple, does not require a long time, does not require special equipment, and costs less[22]. The positive control was 3% amoxicillin, and the negative control was 5% tween 80. The selection of positive controls is based on its broad-spectrum nature with a mechanism of action preventing crosslinking of peptidoglycans in the late stages of cell wall synthesis, which is one of the antibacterial mechanisms by flavonoid compounds[35]. Tween 80 was used as a negative control following another study by Turahman and Sari[36] that stated that tween 80 did not produce an inhibitory zone in *S. aureus*. The selection of tween 80 is also because when manufacturing a series solution, the concentration is not all fractions can dissolve in polar solvents; the addition of tween 80 as a surfactant in the solution can lower the interface voltage of the solution so that it will improve the solubility process when making concentration series.

The results of antibacterial testing on the *n*-hexane and the ethyl acetate fractions with concentrations of 3%, 6%, 9%, and 12% showed moderate-strong inhibition of bacterial growth at concentrations of 9% and 12% (**Table IV**). This is associated with the total flavonoid levels possessed by each fraction, which is directly proportional to the antibacterial activity shown. In the negative control, there is no visible inhibition zone, and the positive control shows the strongest inhibitory power. The results of antibacterial testing were analyzed statistically using the Kruskal-Wallis test, which showed a value of 0.001 ($p < 0.05$). The results signaled a significant difference between positive control, negative control, *n*-hexane, and ethyl acetate fractions. The magnitude of antibacterial activity in the ethyl acetate fraction is due to the ability of ethyl acetate as a semipolar solvent containing more complex chemical compounds when compared to polar and non-polar fractions so that it can attract more flavonoid compounds. Flavonoid compounds have a strong ability to inhibit the growth of bacteria[37].

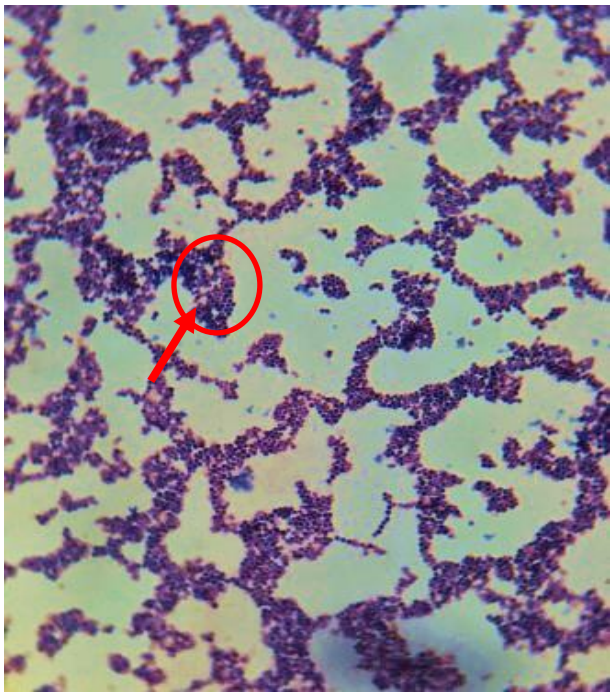

Figure 4. *Staphylococcus aureus* morphology. These bacteria included Gram-positive bacteria that were small and round in shape (coccus) and occurred as clusters appearing like a bunch of grapes on microscopy (red arrow).

Table IV. Inhibition zones diameter of *n*-hexane and ethyl acetate fractions against *S. aureus* FNCC 0047.

No.	Samples	Concentration (%)	$\bar{x}$ diameter of inhibition zones ± SD	Classification[38]	Sig.*
1	*n*-hexane	3	1.667 ± 0.577	Weak	0.001
		6	1.667 ± 0.577	Weak	
		9	6 ± 0.866	Medium	
		12	9.5 ± 1.322	Medium	
2	Ethyl acetate	3	2.166 ± 0.288	Weak	0.001
		6	3.5 ± 0.866	Weak	
		9	10 ± 1.322	Medium-strong	
		12	11.883 ± 0.577	Strong	
3	Positive control		14.166 ± 3.055	Strong	
4	Negative control		0 ± 0	No inhibition	

* Kruskal-Wallis statistical test, there are significant differences ($p < 0.05$)

Antioxidant activity of the fractions of R. officinalis extract by DPPH methods

Table V shows that the higher concentration of fraction (as an antioxidant) made an increase in inhibition of the DPPH oxidant. The increasing inhibition percentage for each fraction of *R. officinalis* extract as well as quercetin can be seen in **Figures 5** to **7**. This graph uses a linear regression equation to calculate the IC_{50} value, the concentration required to reduce the initial DPPH concentration by 50%. The ethyl acetate and *n*-hexane fractions of *R. officinalis* leaves extracts needed 5.486 and 66.293 mg/L, respectively, to decrease 50% of the DPPH oxidant. The antioxidant activity category of *R. officinalis* leaves extract based on IC_{50} value was very strong for ethyl acetate and strong for n-hexane fractions. The classification of antioxidant activities is divided into five: <50 (very strong), 50-100 (strong), 100-150 (moderate), 150-200 (weak), and >200 mg/L (very weak)[39].

In this research, quercetin was used as a positive control and is simply a known antioxidant. This will clearly show that this amount of sample has antioxidant activity compared to a certain amount of the control. A negative control is a mixture of methanol and DPPH 40 mg/L. Quercetin had high antioxidant activity, with a lower IC_{50} value of 3.751 mg/L. **Table V** showed ethyl acetate fraction of *R. officinalis* leaves extract had a higher IC_{50} value than the *n*-hexane, and its antioxidant activity was as strong as quercetin. This proved that fractions of *R. officinalis* leaves extract could scavenge free radicals of DPPH. The mechanism for scavenging DPPH radicals by antioxidant compounds is through donating hydrogen atoms where the incubation period of the sample mixed with the DPPH reagent is 30 minutes, causing the DPPH color to change from purple to yellow[23]. The color change was caused by the reduction of the conjugated double in DPPH due to the

presence of one electron by the antioxidant compound, which caused the unavailability of the electronic place to resonate where the change could be measured. The reaction process between antioxidant compounds and DPPH radicals occurs through hydrogen atom donation[40].

Table V. Absorbance value and inhibition percentage of *R. officinalis* leaves extract fractions.

Samples	Concentration (mg/L)	Absorbance (Å)	Percentage of inhibition (%)	IC_{50} (mg/L)	Category[39]
Quercetin	1	0.535 ± 0.025	16.822 ± 3.912	3.751	Very strong
	2	0.497 ± 0.02	22.682 ± 3.123		
	3	0.368 ± 0.005	42.802 ± 0.857		
	4	0.294 ± 0.004	54.314 ± 0.589		
	5	0.21 ± 0.015	67.382 ± 2.309		
	6	0.149 ± 0.004	76.82 ± 0.678		
Ethyl acetate fraction	1	0.4 ± 0	14.894 ± 0	5.486	Very strong
	2	0.392 ± 0.002	16.667 ± 0.325		
	3	0.343 ± 0	27.021 ± 0		
	4	0.324 ± 0	31.064 ± 0		
	5	0.272 ± 0.002	42.057 ± 0.325		
	6	0.181 ± 0.008	61.56 ± 1.784		
n-hexane fraction	20	0.368 ± 0.02	19.474 ± 4.442	66.293	Strong
	40	0.343 ± 0.008	24.872 ± 1.755		
	60	0.264 ± 0.015	42.231 ± 3.179		
	80	0.166 ± 0.005	63.53 ± 1.205		
	100	0.09 ± 0.026	80.16 ± 5.627		
	120	0.062 ± 0.023	86.360 ± 5.11		

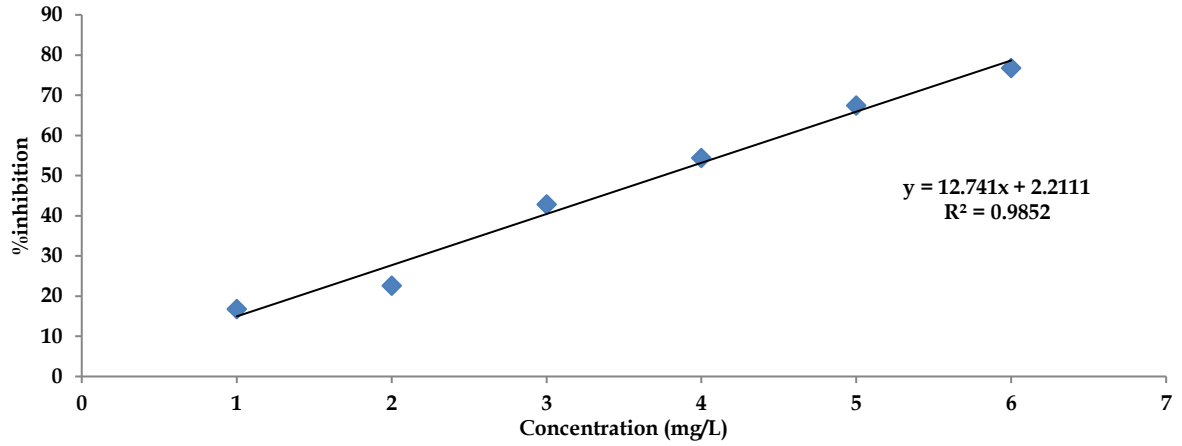

Figure 5. Inhibition percentage and concentration of quercetin.

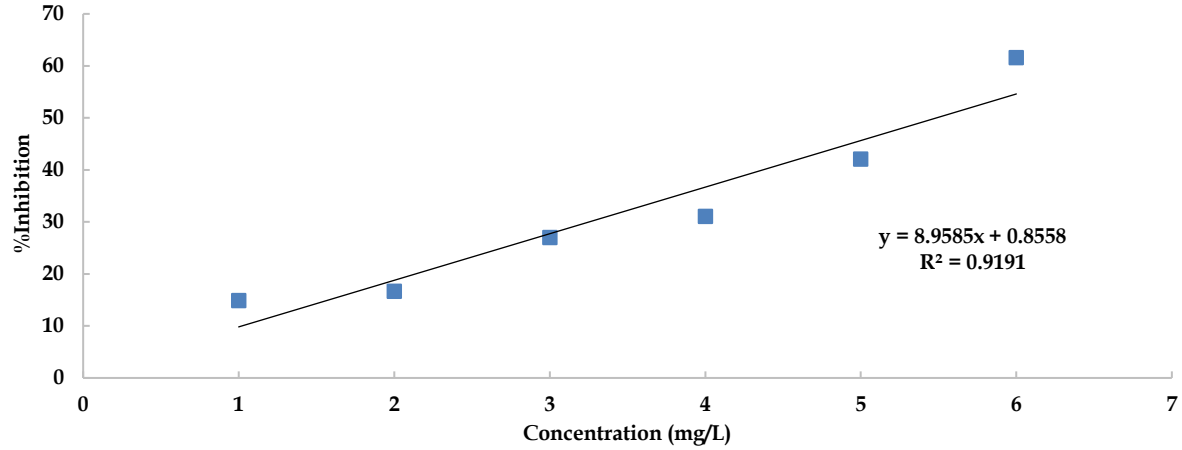

Figure 6. Inhibition percentage and concentration of ethyl acetate fraction.

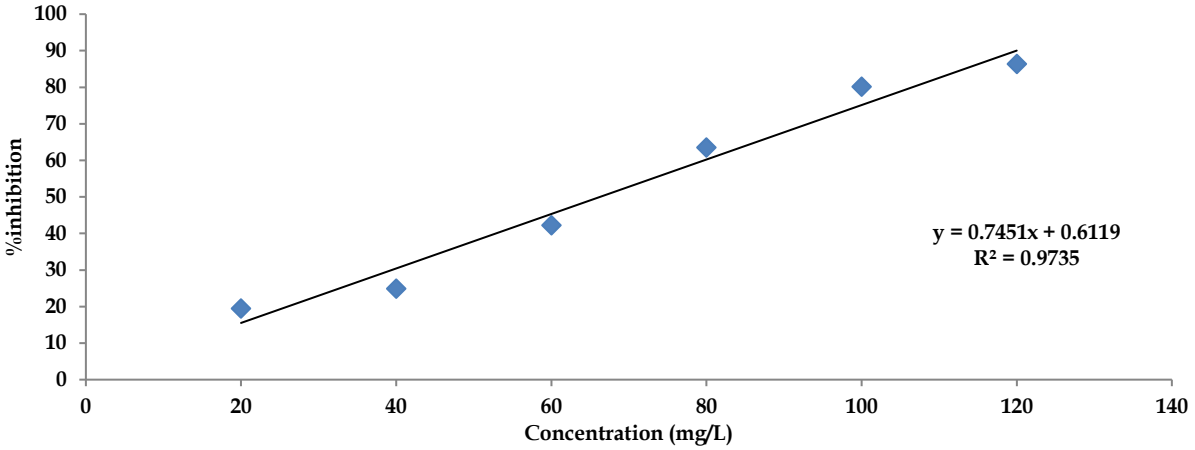

Figure 7. Inhibition percentage and concentration of *n*-hexane fraction.

CONCLUSION

The ethyl acetate fraction of *R. officinalis* has higher total flavonoid levels of 47.437 ± 1.947% than the *n*-hexane fraction. These are directly proportional to the antibacterial activity of the ethyl acetate fraction at a concentration of 12%, showing a more significant inhibition zone of 11.883 ± 0.577 mm than the *n*-hexane fraction. Antioxidant activity of ethyl acetate fraction with IC_{50} value of 5.486 mg/L, which is classified as very strong.

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AUTHORS' CONTRIBUTION

Conceptualization: Ni Ketut Esati
Data curation: Ni Putu Sudiasih, Ni Nyoman Dina Saniasih
Formal analysis: Ni Putu Sudiasih, Ni Nyoman Dina Saniasih
Funding acquisition: -
Investigation: Ni Putu Sudiasih, Ni Nyoman Dina Saniasih
Methodology: -
Project administration: -
Resources: -
Software: -
Supervision: Ni Ketut Esati, Elisabeth Oriana Jawa La
Validation: -
Visualization: -
Writing - original draft: Ni Putu Sudiasih
Writing - review & editing: Ni Ketut Esati, Elisabeth Oriana Jawa La

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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