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2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Analysis and Antibacterial Activities of Renggak Seed (*Amomum Dealbatum Roxb*)

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ABSTRACT

Natural sources have complex interaction mechanisms in providing inhibitory effects against various radical reactions and multi-drug resistance (MDR). *Amomum dealbatum* (Renggak in Lombok) seed phytochemistry and bioactivities have the potential to be investigated. This study aims to explore the bioactivity of extracts and fractions of renggak seeds in reducing reactive oxygen species and inhibiting bacterial growth to overcome MDR. The methods used to determine the level of free radical scavenging and antibacterial activity in this study were the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and agar diffusion methods. The results of the phytochemical tests of the ethanolic extract of Renggak seeds contain triterpenoids, alkaloids, saponins, and phenolics. Meanwhile, in the hexane fraction were found alkaloids, saponins, and phenolics. The results of antibacterial tests on *Staphylococcus aureus* using the agar diffusion method for ethanol and hexane extracts at concentrations of 20, 40 and 60% were 12.50±1.00, 19.00±0.00, and 21.8±0.76 for ethanol extracts, respectively. The hexane fraction was 5.33±0.57, 10.83±0.57, and 14.33±0.28 mm. For *Salmonella sp* bacteria, it was 9.00±1.00, 14.16±0.29, and 18.16±1.04 mm for ethanol extracts than was 7.50±0.50, 12.67±0.76, and 15.67±0.76 mm for hexane fractions. Moreover, the ethyl acetate fraction contains triterpenoids, alkaloids, saponins and phenolics which are responsible for giving antioxidant effects with an IC_{50} value of 66.515±2.37 ppm and is classified as a strong antioxidant to scavenging free radicals of DPPH. The higher the concentration of the fraction, the higher the inhibition of the growth of S*almonella sp* and *Staphylococcus aureus* bacteria. The ability of the extract and fraction to inhibit bacterial growth and reduce free radicals makes renggak seeds very potential for further investigation.

Keywords: *Amomum dealbatum*, antibacterial, antioxidant, extract, fraction

INTRODUCTION

A common health issue brought on by bacteria, fungus, viruses, or parasites is infection. Human infections are frequently caused by *Salmonella sp*. and *Staphylococcus aureus* germs. At the moment, the prevalence of multidrug resistance (MDR) and the presence of reactive oxygen species are closely linked to microbial infections. It has been determined by recent literature sources that multidrug resistance is on the rise. Furthermore, according to Alfei et al. (2024), reactive oxygen species (ROS) also activate this MDR. Free radicals are one type of ROS that can lead to cell damage and death in excess. At the lower levels, nevertheless, they play a crucial part in physiological processes and the regulation of several homeostatic cellular processes, such as growth, apoptosis, immunological response, and microbial colonization (Spooner). Hence, finding antibacterial and antioxidant substances, especially from natural resources that can inhibit microreactions in the physiological function of microorganisms that induce MDR is one strategy suggested in this study.

Renggak (*Amomum dealbatum Roxb*) is one of the herb plant species of the genus Amomum and belongs to the *Zingiberaceae* family. Renggak is commonly found in Lombok.

On the other hand, the fruit of Renggak is commonly used as a traditional medicine to treat diarrhea in Pangandaran, West Java (Kusuma et al., 2021a). A previous study reported that Renggak shows antimicrobial properties on *Escherichia coli, Bacillus cereus*, *Staphylococcus aureus*, and *Candida albicans*. In addition, Renggak has the potential as an antioxidant agent since they have a great capacity to scavenge free radicals form secondary metabolite compounds in Renggak. Bioactivities of Renggak can vary due to the presence of secondary metabolites. Previous studies reported that Renggak contains various secondary metabolites for instance alkaloids, steroids, flavonoids, terpenoids, and saponins. Nevertheless, the Renggak fruit consists primarily of water (55.19%), carbohydrates (34.51%), fiber (6.46%), ash (3.72%), protein (3.13%), and fat (2.87%) (Muliasari et al., 2019; Nufus, 2020).

Many pieces of work reported the bioactivities of almost all plant parts of Renggak. The ethanol extract of Renggak leaves has a medium antioxidant activity with an IC_{50} value of 149.59 ppm (Ayu et al., 2021). Renggak peel extract has also reported can inhibit *Propionibacterium acnes* and it has been formulated become an antiacne gel (Hariadi et al., 2022). In addition, Renggak peel extract can inhibit *Staphylococcus epidermidis* growth with an inhibition zone of 17.65 mm, along with a low antioxidant IC_{50} value of 244.904 g/mL was discovered in a previous study (Azim et al, 2023). Moreover, the ethanolic extract of Renggak fruits including peel was found to have cytotoxic activity with an LC_{50} value of 133.498 ppm (Putri, 2021). Of the many parts of the Renggak plant that are similar to the same genus namely Large cardamom (*Amomum subulatum Roxb*) has investigated particularly antioxidant and antibacterial activity (Auxilia et al., 2022). Nevertheless, The bioactivity of the Renggak seeds has not been widely studied. The bioactivities of Renggak seeds require further exploration to be used as alternative substances to overcome microbial infections that lead to MDR incidence, as a result of some of the aforementioned studies. Determining the antibacterial properties of Renggak seeds was the aim of this study. There has never been a report on the bioactivities of Renggak seed extracts or fractions. Therefore, this study will assess the antioxidant activity of Renggak seed as well as its effects on *Salmonella sp.* and *Staphylococcus aureus*.

METHODS

Materials and Instruments

The following are the laboratory equipment and materials used in this study, including analytical balance (durascale DAB-E223), Laminar Air Flow (Otto/5A-96), Oven (Memmert, AH-6), autoclave (Shenan), UV-Vis spectrophotometer (Shimadzu 1800i), Incubator (Memmert), water bath (HH-6), glassware (pyrex), petri dishes, ose, calipers, Renggak Seed, hexane p.a (Merck), ethyl acetate p.a (Merck), ethanol 96% (technical grade), distilled water, Hydrochloric Acid (HCl), Iron(III) chloride (FeCl3), Dragendorff's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) Zinc Powder p.a (TCL Kanto), Dimethyl sulfoxide (DMSO) p.a (Merck). MHA media (Himedia M173-500G), *Staphylococcus aureus ATCC 25922* and *Salmonella sp ATCC 14028.*

Procedures

Prepared simplicial 50 g of Renggak seeds were macerated using 250 mL ethanol 96% solvent, thus the ratio of simplicial and ethanol solvent 1:5 for three days. After three days, the maceration products were remacerated three times to maximize the extract than filtered and evaporated using a rotary evaporator to obtain an ethanol-condensed extract. Further, the condensed extract was dissolved with distilled water, then fractionated with a separating funnel with a solvent ratio of hexane:water (1:1) and ethyl acetate:water (1:1) subsequently obtaining hexane and ethyl acetate fractions, respectively. Each fraction was refracted for three times to maximize the results. Then, the ethanolic extract, hexane fraction, and ethyl acetate fraction were tested for phytochemical screening.

Phytochemical screening

Alkaloid test

Condensed extract and fractions were placed in a different tube and added with 1 mL of HCl 2N and aquadest 9 mL. The reaction tube boiled for two minutes. After reaching room temperature, the solution was filtered. The filtrate was divided into two tubes, the first tube was added with two drops of Mayer's solution and the added drops of Dragendorff's alkaloids reagent. The positive alkaloids show a white precipitate produced after Meyer's reaction and an orange color generated after Dragendorff's reaction, respectively (Malik et al., 2016).

Saponins test

Condensed extract and fractions were added with 10 mL of boiling water in different tubes. Then, the tubes were shaken violently for 10 seconds and then allowed to cool. If foam produced up to 10 cm high with good stability even though the addition of a drop of HCl 2N was indicated positive saponins (Febriani et al., 2021; Malik et al., 2016).

Flavonoid test

Condensed extract and fractions were diluted in 2 mL of 95% ethanol in different reaction tubes and added with 0.5 g of zinc powder and 2 mL of HCl 2 N, and leave for one minute. The creation of a red color indicates the presence of flavonoids (Noer et al., 2018).

Phenolics test

General tests for phenolic compounds were conducted by the following steps. Condensed extract and fractions were heated for about 15 minutes with 50 mL of distilled water, chilled, and filtered. The filtrate was put in a reaction tube and added with iron (III) chloride reagent. The production of a greenish-black color indicates a positive reaction of phenolic compounds (Noer et al., 2018).

Antimicrobial evaluation

Antimicrobial activity was evaluated using the agar diffusion method against *Staphylococcus aureus ATCC 25922* and *Salmonella sp. ATCC 14028*. Tested compounds were prepared with various concentrations from 20, 40, and 60% (w/v) in DMSO as a solvent. Meanwhile, ciprofloxacin and DMSO were used as positive and negative controls, respectively. The test agar plate (Mueller-Hinton Agar) was swabbed with a standardized McFarland concentration was equivalent to a bacterial cell suspension concentration of 1.5×10^8 CFU/mL of *Staphylococcus aureus ATCC 25922* and *Salmonella sp. ATCC 14028*. Then, paper discs containing a tested compound with a selected concentration were placed an amount of 40 µL of the fractions on the lawn of bacteria. After overnight incubation, the diameter of the zone of inhibited growth around the disk is measured (Wardania et al., 2020).

Antioxidant evaluation

Antioxidant evaluations were carried out qualitatively from the discoloration of the DPPH solution and quantitatively from the IC_{50} value. Determination of the IC_{50} value of antioxidants was carried out using the Molyneux method for the quantitative analysis (Taylor & Todd, 1995). The sample solutions were analyzed for the IC_{50} antioxidant activity using concentration series starting from 20, 30, 40, 50, and 60 ppm. 50 mM of DPPH and the tested compound were homogenized in a ratio (3:1) mL and were incubated ± 30 minutes in the dark room. Qualitatively, a positive result as an antioxidant was indicated by the formation of a color change from violet to pale yellow (Rahmawati et al., 2016). The absorbance of tested solutions was measured under wavelength 517.6 nm by UV-Vis Spectrophotometer. The percentage of DPPH inhibition is used to calculate antioxidant activity using the following formula:

$$\%Inhibition = \frac{Absorbance\ of\ the\ blank - Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ blank}\ x\ 100\%$$

The IC_{50} value is determined using the linear regression equation between % inhibition and concentration. The result from the line equation y = bx + a and the IC_{50} calculation is performed following the formula:

$$IC_{50} = (50\text{-}b) / a$$

Where: y = % Inhibition (50); a = Intercept (line intersection on the y-axis); b = Slopes; x = Concentration (IC_{50})

Data Analysis

Analysis data from the result of seed Renggak extract and fractions against *Staphylococcus aureus* and *Salmonella sp* bacteria by measuring the inhibition zone. According to Morales (Morales et al., 2003), The weak, medium, and strong categories have inhibition zone diameters < 5 mm, 6-10 mm, and 11-20 mm, respectively. Meanwhile, antioxidant evaluation data was considered the IC_{50} value of tested compounds. A chemical is considered to have strong antioxidant activity if its IC_{50} value is <50 ppm, strong if its IC_{50} value is 50-100 ppm, medium if its IC_{50} value is 100-150 ppm and weak if its IC_{50}150-200 (Moleyneux, 2024).

RESULTS AND DISCUSSION

Ethanol is an alcohol group solvent that was used as a solvent during the extraction of simplicial from Renggak seeds. The short chain of alcohol group solvent was selected as the sample solvent because it can dissolve both polar and non-polar substances. This solvent allows for the highest total extraction of compounds with antioxidant potential, such as flavonoids and natural phenolic compounds. Ethanol has an ethyl group ($-CH_2CH_3$) that can perform Van der Waals interactions with non-polar compounds and a hydroxyl group (OH) that produces hydrogen bonding interactions with polar compounds. Therefore, these solvents can provide fairly broad extraction gradients (Dias et al., 2019; Truong et al., 2019).

A phytochemical analysis of an ethanolic extract of Renggak seeds revealed the presence of triterpenoids, alkaloids, saponins, and phenolics or polyphenols as shown in Table 1. The content of secondary metabolites in extracts and fractions such as alkaloids, saponins, and tannins provide antibacterial effects (Kusuma et al., 2021)(Saptowo et al., 2022). Meanwhile, the presence of phenolics secondary metabolites in the ethyl acetate fraction gives this fraction great potential for investigation of its antioxidant activity (de Mello Andrade & Fasolo, 2014; Pandey & Rizvi, 2009; Stagos, 2019).

Table 1. Phytochemical test results of Renggak seed extracts on hexane and ethyl acetate

Secondary metabolites	Seed extracts	Hexane fraction	Ethyl acetate fraction
Triterpenoids	+	-	+
Alkaloids	+	+	+
Saponins	+	+	+
Phenolics	+	+	+

In the phytochemical tests, the results confirmed that the ethyl acetate fraction contained more secondary metabolites compared to the hexane fraction from Renggak seeds. The results were obtained since triterpenoids, alkaloids, saponins, and Phenolics have semi-polar properties that are easily dissolved in ethyl acetate solvent with similar polarity. Nevertheless, hexane solvent is a non-polar solvent, thus some secondary metabolites in Renggak seeds with semi-polar and polar properties were not dissolved in hexane. After confirming the secondary metabolites in ethanolic extract, ethyl acetate fraction, and hexane fraction of Renggak seeds, the antibacterial and antioxidant examination was conducted. The ethanolic extract and hexane fraction were used to examine the antibacterial properties of Renggak seeds. Meanwhile, the ethyl acetate fraction was used for determine antioxidant activity of Renggak seeds. The ethyl acetate fraction was further analyzed by our other colleagues, because it is a potential fraction to be explored, so in this article only DPPH radical scavenging analysis is presented.

Antibacterial examinations were conducted against *Staphylococcus aureus* and *Salmonella sp*. with several parameters including a tested compound with various

concentrations of 20, 40, and 60%; ciprofloxacin as a positive control; and DMSO as a solvent extract and negative control. The results of the antibacterial activity test of the ethanolic extract and the hexane fraction of the Renggak seeds were displayed in Table 2.

Table 2. Antibacterial activity of ethanolic extract and hexane fraction of Renggak seeds

Bacteria	Concentration (%)	Inhibition zone (mm) of ethanolic extract ±SD	Inhibition zone (mm) of hexane fraction ±SD	Positive Control (mm)±SD
Staphylococus aureus	20	12.5	6.0	29±0.00
		11.5	5.0	
		13.5	5.0	
		12.5±1.00	**5.33±0.57**	
	40	19.0	11.5	
		19.0	10.5	
		19.0	10.5	
		19±0.00	**10.83±0.57**	
	60	21.0	14.0	
		22.0	14.5	
		22.5	14.5	
		21.8±0.76	**14.33±0.28**	
Salmonella sp.	20	10.0	8.0	25±0.00
		9.0	7.0	
		8.0	7.5	
		9.0±1.00	**7.5±0.50**	
	40	14.0	13.5	
		14,5	12.0	
		14.0	12.5	
		14.17±0.29	**12.67±0.76**	
	60	18.5	15.0	
		19.0	16.5	
		17.0	15.5	
		18.16±1.04	**15.67±0.76**	

According to Table 2, both gram-positive (*Staphylococcus aureus*) and gram-negative (*Salmonella sp*.) bacteria are susceptible to the compounds contained in both ethanolic extract and hexane fraction of Renggak seeds. The antibacterial activity of the tested compound has concentration-dependent properties. The higher the concentration used, the greater the inhibition zone obtained. However, when compared with the positive control of ciprofloxacin, the inhibition zone of the positive control is still better than the ethanol extract and hexane fraction. As a preliminary study of the exploration of natural products, this research on renggak seeds needs to be reviewed again to obtain better data.

Based on the calculaton of the diameter of the inhibiton zone, the condensed extract and fraction were classifed as strong because they were > 10 mm and some were classifed as very strong > 20 mm (Azim et all, 2022). Ethanolic extract of Renggak seeds is considered to have strong antimicrobial activity in *S. aureus* in all concentrations tested. Meanwhile, the ethanolic extract of Renggak seeds is considered to have strong antimicrobial activity in Salmonella sp. at concentrations of 40 and 60%; and medium antibacterial activity at concentrations of 20%. Furthermore, the hexane fraction of Renggak seeds has lower antibacterial activity on *S. aureus* compared to the ethanolic extract. The hexane fraction gave a strong antimicrobial effect at a concentration of 60%; meanwhile, medium activity at concentrations of 20 and 40%. Nevertheless, the hexane fraction revealed medium antibacterial effects on Salmonella sp. at concentrations of 20%, but strong antibacterial

effects at concentrations of 40 and 60%. Overall, the antibacterial effects of ethanolic extract of Renggak seeds are greater in gram-positive (*S. aureus*). Generally, gram-positive bacteria have a thick peptidoglycan and it is suitable for compounds with polar and semipolar properties which are extracted to ethanol solvent to penetrate the cell wall of gram-positive bacteria (Azim et all, 2022). Meanwhile, hexane fractions have better antibacterial activity in gram-negative (*Salmonella sp.*) A gram-negative bacteria (Salmonella sp.) has thin peptidoglycan but contains rich lipopolysaccharides which are possible to penetrate by non-polar compounds that are fractioned to hexane parties (Azim et all, 2022).

The ethyl acetate fraction was selected to evaluate antioxidant activity since phenolic compounds is found based on phytochemical tests. Several sources state that the correlation between phenolic compound content and antioxidant activity is that the higher the phenolic content, the stronger the antioxidant activity (Gultom et al., 2021; Pujimulyani et al., 2010; Wardani et al., 2020). The ethyl acetate fraction here is focused on the antioxidant test because this fraction is a semi-polar component containing polyphenol compounds with hydroxyl groups that play a greater role in counteracting free radicals than antibacterial tests. This is reflected in the antibacterial analysis of the extract. The polyphenols are referred to flavonoids with abundant hydroxyl groups whose structure is easy to modify and to isolate for further investigated in organic synthesis.

The antioxidant activity of the ethyl acetate fraction of Renggak seeds showed fascinating results by showing discoloration of the DPPH solution from purple to pale yellow after the ethyl acetate fraction was added. The discoloration of DPPH occurred due to the interaction of the -OH groups in the aromatic chains of secondary metabolites for instance polyphenols and flavonoids with DPPH solution.

In radical scavenging studies, the mechanism of free radical suppression generally occurs through two important mechanisms, including hydrogen atom transfer (HAT) or single-electron transfer followed by proton transfer (SET-P). The HAT and SET-P are performed by the aromatic compound or phenolic compounds (Ar-OH). Those mechanisms are depicted by the analogy below (Gulcin & Alwasel, 2023).

$$\mathrm{ArOH} \rightarrow \mathrm{ArO^-} + \mathrm{H^+}\,(\mathrm{HAT})$$
$$\mathrm{ArOH} \rightarrow \mathrm{ArO^\bullet} + \mathrm{H^\bullet}\,(\mathrm{SET-PT})$$
$$\mathrm{ArOH} \rightarrow \mathrm{ArO^{\bullet +}} + \mathrm{e^-}$$
$$\mathrm{ArO^{\bullet +}} \rightarrow \mathrm{ArO^\bullet} + \mathrm{H^+}$$
$$\mathrm{ArO^-} + \mathrm{ROO^\bullet} \rightarrow \mathrm{ArO^\bullet} + \mathrm{e^-}$$
$$\mathrm{ArOH} + \mathrm{ROO^\bullet} \rightarrow \mathrm{ArO^\bullet} + \mathrm{ROO^-}\,(\mathrm{SPLET})$$

Scheme 1. Mechanism of HAT and SET-P Radical Assay (Gulcin & Alwasel, 2023)

That important interaction of -OH groups from secondary metabolites can scavenge free radicals from DPPH. New radicals are generated in the compound of secondary metabolites stabilized by intramolecular resonance (Azim et all, 2022). Antioxidant evaluation was carried out by analyzing the IC_{50} of the tested compound with various concentrations from 20, 30, 40, 50, and 60 ppm. The antioxidant evaluation results are displayed in Table 3.

Ethyl acetate fraction shows antioxidant activity with concentration-dependent properties as displayed in Figure 1 even though % inhibition fluctuates. In the linearity of graph below, the R^2 value for each repetition is 0.9394; 0.854 and 0.7843 which are then converted into r values to obtain 0.96; 0.92 and 0.88. The r value approaching 1 indicates that the relationship between % inhibition and concentration is a linear relationship. However, in this case, the linearity obtained is not good. One of the reasons this can happen since the concentration range or dilution factors used were too close. In addition, the possibility of data bias in this test also occurs. The dilution factors can affect the number of assay concentrations that are in the linear portion of the curve. The dilution factor is the factor that defines the spacing between adjacent test concentrations. Probability of this

process may affect the linierity of the graph below (Sebaugh, 2011). Measurements of the IC_{50} value of the tested compound were made at a wavelength of 517.6 nm with a DPPH concentration of 50 µM. Linear regression analysis of three repetitions yielded an average IC_{50} value of 66.515 mg/L that can be categorized as strong antioxidant activity according to Molyneux (Taylor & Todd, 1995).

Table 3. Antioxidant activity of the ethyl acetate fraction of Renggak seeds

M (ppm)	A_O	A_X			% IC			IC_{50} (mg/L)	IC_{50} (mg/L)	IC_{50} (mg/L)	IC_{50} (mg/L)
		1	2	3	1	2	3	1	2	3	x̄±SD
20	0.622	0.444	0.403	0.503	28.62	35.21	19.13	65.741	69.184	64.621	66.515±2.37 %RSD 3.57
30	0.622	0.389	0.38	0.411	37.46	38.91	33.92				
40	0.622	0.386	0.374	0.412	37.94	39.87	33.76				
50	0.622	0.353	0.327	0.329	43.25	47.43	47.11				
60	0.622	0.328	0.338	0.358	47.27	45.66	42.44				

*A_O = Blank DPPH Absorbance ; A_x= Absorbance of sampel ; %IC = % inhibition of sampel

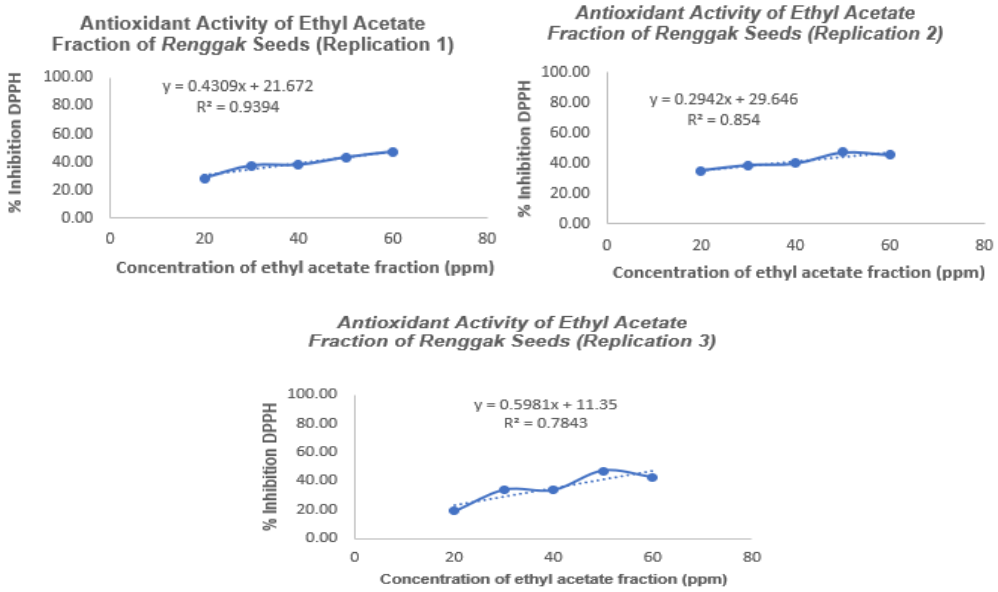

Figure 1. The curve of the relationship between % antioxidant inhibition and the concentration of the ethyl acetate fraction of Renggak seeds (*Amomum dealbatum* Roxb)

Table 4. Antioxidant Activity Category based on IC_{50} value (Molyneux, 2004)

IC_{50} Value	Antioxidant Activity Category
< 50 ppm	Very Strong
50-100 ppm	Strong
100-150 ppm	Medium
150-200 ppm	Weak

The antioxidant activity demonstrated by the ethyl acetate fraction of Renggak seeds extracts showed better results when compared to the Renggak peel extract with an IC_{50} of 244,904 ppm (weak antioxidant activity) (Azim, 2023). Meanwhile, the antioxidant activity of ethyl acetate fractions is still lower compared to other antioxidants based on literature such as quercetin, ascorbic acid, and tocopherol, which have IC_{50} values <50 ppm. In this research work. Positive control was not carried out, because it was a preliminary study to investigate the research potential of renggak seeds. Therefore, further research will be more focused on the ethyl acetate fraction with the appropriate comparison standard.

Polyphenolic compounds are important and responsible for antioxidant activity in the ethyl acetate fraction of Renggak seeds. Polyphenol compounds contain the polyhydroxy functional groups which is attached to an aromatic ring that can neutralize free radical species such as DPPH and prevent damage to cellular molecules. Structurally, polyphenolic compounds contain hydroxyl, methoxyl, and/or glycosyl groups, and it is very similar to flavonoid compounds. Previous studies also reported that both polyphenolic and flavonoid compounds have antioxidant activity (de Mello Andrade & Fasolo, 2014). This antioxidant activity was related to the content of secondary metabolites in the ethyl acetate fraction of Renggak seeds. Flavonoids and phenolics or polyphenols provide antioxidant effects due to the ability of free radical scavenging. However, polyphenolic compounds are better for antioxidant activity because of various structures and mechanisms of action.

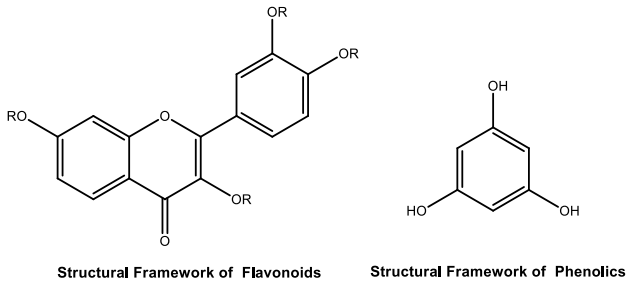

Figure 2. Structural framework of flavonoids and phenolics

Structurally, flavonoid compounds have hydroxyl groups (OH) that can act as free radical scavengings. However, some of the flavonoid compounds have their hydroxyl groups substituted by carbon atoms which causes the free radical scavenging power to decrease. While in polyphenol or phenolic compounds, of course, their hydroxyl groups are not substituted by other atoms, so the free radical quenching ability of polyphenol and phenolic compounds is better when compared to flavonoid compounds. The antioxidant properties of a compound correspond to some mechanisms of action. The antioxidant agent can act as hydrogen donors to directly react with radicals; reduce the production of radicals by inhibiting the enzyme activities; chelate metal ions that induce free radical production; inhibit oxidation reactions by increasing the activity of antioxidant enzymes; and generate synergistic antioxidant effects with other substances (Lv et al., 2021).

CONCLUSION

The secondary metabolites in Renggak (*Amomum dealbatum* Roxb) seeds such as triterpenoids, alkaloids, saponins and phenolics were successfully isolated to the ethanolic extract, hexane fraction, and ethyl acetate fraction. Ethanolic extract of Renggak seeds shows greater antibacterial effects on *Staphylococcus aureus* and hexane fraction of Renggak seeds gave better antibacterial activity on Salmonella sp. Meanwhile, the ethyl acetate fraction of Renggak seeds shows strong antioxidant activity in scavenging DPPH free radicals. The ethyl acetate fraction will be reviewed in more intens in further research.

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