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Potent Antioxidant Activity of Renggak (*Amomum dealbatum* Roxb.) Fruit and Its Antibacterial Effect Against *Klebsiella pneumoniae*

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Abstract
This study investigates the antioxidant and antibacterial activities of ethanolic extracts of renggak fruit (Amomum dealbatum Roxb.), an edible plant traditionally consumed in Lombok, Indonesia. The novelty of this research lies in the evaluation of fruit extracts of *A. dealbatum*, which have been rarely studied compared to other plant parts, particularly in relation to antioxidant potency and antibacterial activity against *Klebsiella pneumoniae*, a clinically relevant pathogen associated with severe pneumonia and antibiotic resistance. Extraction was carried out using a maceration method with 96% ethanol under controlled temperature and duration to preserve thermolabile compounds. Phytochemical screening revealed the presence of flavonoids, tannins, and terpenoids. Antioxidant activity was evaluated using the DPPH radical scavenging assay, yielding an IC_{50} value of 10.33 ppm, indicating very strong antioxidant activity compared to other reported *Amomum* species. Antibacterial activity was assessed using the agar well diffusion method at extract concentrations of 20–100%, showing moderate inhibitory effects against *K. pneumoniae* with inhibition zones ranging from 7.34 to 9.48 mm. These findings highlight renggak fruit as a promising natural source of antioxidant and antibacterial agents, supporting their potential application as functional food ingredients or natural therapeutics.

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INTRODUCTION

Renggak (*Amomum dealbatum* Roxb.) is a plant belonging to the Zingiberaceae family. This family comprises approximately 170 species that are widely distributed across China, India, and Southeast Asia, including Indonesia (Thu et al., 2021; Droop & Newman, 2014; Ding et al., 2020). In Asian countries, plants from the Zingiberaceae family are commonly utilized as spices and culinary ingredients due to their distinctive aroma and flavor (Thu et al., 2021; Alam & Singh, 2021). Numerous studies have reported that almost all parts of these plants such as rhizomes, stems, leaves, flowers, and fruits exhibit significant pharmacological properties (Alam & Singh, 2021; Pintatum & Laphookhieo, 2022; Praditha et al., 2020; Cai et al., 2021).

Previous investigations have demonstrated that species belonging to the genus *Amomum* contain a wide range of bioactive compounds with potential therapeutic applications (Alruhaili et al., 2023; Nufus, 2020). In Indonesia, *Amomum dealbatum* is known by different local names depending on the region; it is referred to as *wresah* in West Java and *renggak* in Lombok. Empirically, this plant has been traditionally used to treat various ailments, including eye inflammation, diarrhea, postpartum dizziness, and skin disorders (Minarno et al., 2023; Wulandari et al., 2021; Rahmawati et al., 2022; Nurcahyati & Ardiyansyah, 2018). In Lombok, West Nusa Tenggara, the fruit seeds of renggak are widely consumed as food due to their

distinctive aroma. Unlike other species within the same genus that are primarily used as spices, renggak fruit seeds can be eaten directly, highlighting their potential not only as a culinary ingredient but also as a functional food with therapeutic value. This potential is supported by their sufficient nutritional content and the presence of various secondary metabolites (Nufus, 2020; Muliasari et al., 2019; Azim et al., 2023).

Antioxidants play a crucial role in pharmacology and human health. They function as defense systems that protect the body from damage caused by reactive oxygen species (ROS), which are naturally produced during physiological processes. Antioxidants can be derived from endogenous sources within the body as well as from exogenous dietary sources (Ahmadi et al., 2022). Natural antioxidants, particularly phenolic compounds and flavonoids, are known for their biological activity and safety for human consumption. Consequently, extensive research has focused on plant-derived antioxidants (Subba et al., 2017).

To date, most scientific publications on the genus *Amomum* have primarily focused on volatile compounds, especially essential oils. However, emerging evidence suggests that non-volatile compounds, such as secondary metabolites, possess promising potential in phytochemical and pharmacological applications (Cai et al., 2021). Antioxidants, whether natural or synthetic, are known to prevent or delay cellular damage and are commonly found in fruits and vegetables. Traditionally, herbal medicines and dietary plants have served as the main sources of antioxidants, protecting communities from oxidative stress-related damage long before the advent of modern medicine (Yashin et al., 2017).

In addition to oxidative stress-related diseases, bacterial infections remain a major public health concern, particularly pneumonia. Pneumonia is an inflammatory condition of the lung parenchyma characterized by fluid and inflammatory cell accumulation within the alveoli and interstitial spaces. It predominantly affects infants and young children, although adults are also susceptible. Common symptoms include fever, shortness of breath, increased respiratory and heart rates, and greenish or thick sputum. One of the primary causative agents of pneumonia is *Klebsiella pneumoniae* (Herawati et al., 2019). Pneumonia continues to be a leading cause of mortality among children under two years of age. According to data from the Indonesian Ministry of Health in 2021, the national coverage of pneumonia cases among children under five reached 31.4%, while in West Nusa Tenggara Province, the prevalence remained high at 6.38%. The increasing occurrence of antibiotic resistance, resulting from bacterial adaptation and reduced drug efficacy, further exacerbates this problem.

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen and one of the major causes of community-acquired and hospital-acquired pneumonia (Paczosa & Mecsas, 2016; Martin & Bachman, 2018). This bacterium is associated with high morbidity and mortality rates, particularly in immunocompromised patients, infants, and the elderly (Navon-Venezia et al., 2017; Bengoechea & Sa Pessoa, 2019). The increasing prevalence of multidrug-resistant *K. pneumoniae* strains has become a global public health concern, necessitating the exploration of alternative antibacterial agents derived from natural products (Wyres & Holt, 2018; World Health Organization, 2017). Plant-based antibacterial compounds are considered promising due to their structural diversity, multiple mechanisms of action, and lower risk of resistance development (Cushnie et al., 2014; Górniak et al., 2019).

Secondary metabolites are known to play a significant role in scavenging free radicals and inhibiting oxidative damage. Previous studies by Mustariani et al. (2021) and Azim et al. (2023) reported that ethanol extracts of renggak leaves and fruit peels exhibited moderate to weak antioxidant activity, indicating the potential of this plant as a natural antioxidant source. Recent studies have reported antioxidant and antibacterial activities of several Amomum species, including *A. subulatum*, *A. compactum*, and *A. xanthioides*. However, most studies focus on essential oils, leaves, or fruit peels, while investigations on edible seed extracts of *Amomum*

dealbatum remain limited. Moreover, studies evaluating the antioxidant potency and antibacterial activity of *A. dealbatum* fruit against clinically relevant pathogens such as *K. pneumoniae* are still scarce. Based on these gaps, the research problem addressed in this study is how strong the antioxidant activity of ethanolic extracts of *A. dealbatum* fruit is and whether different extract concentrations exhibit antibacterial activity against *K. pneumoniae*. Therefore, this study aims to evaluate the antioxidant potency and antibacterial effects of renggak fruit extracts as a scientific basis for their potential development as functional food ingredients or natural antibacterial agents.

METHOD

Research Design

This study consisted of sample preparation, extraction, phytochemical screening, antioxidant activity evaluation, and antibacterial activity testing.

Sample Preparation

Renggak fruits (*Amomum dealbatum* Roxb.) were collected from several plantation areas in North Lombok Regency, West Nusa Tenggara, Indonesia. The fruits were washed, and the seeds were separated and oven-dried at 55 °C for 72 hours until constant weight was achieved. The dried seeds were used as simplicia for further analysis.

Extraction Procedure

The maceration method was selected to preserve thermolabile bioactive compounds. Extraction was conducted at room temperature (25–27 °C) to minimize degradation of phenolic compounds. The solvent-to-material ratio (1:5, w/v) and extraction time (24 hours, repeated three times) were optimized based on previous studies on Amomum species to ensure maximum extraction efficiency and representativeness of natural phytochemical composition (Cai et al., 2021).

Determination of Extraction Yield

The extraction yield (%) was calculated as the ratio between the weight of the obtained extract and the initial weight of the simplicia, multiplied by 100%. The yield was determined after solvent evaporation using a rotary evaporator at 40–60 °C (Syamsul et al., 2020).

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify major secondary metabolites, including flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids. The screening was carried out using standard colorimetric and precipitation methods with specific reagents, following previously reported procedures with minor modifications (Mustariani et al., 2021).

Antioxidant Activity Assay

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A 100 ppm DPPH solution was prepared in ethanol, and the maximum absorbance wavelength was determined using a UV–Vis spectrophotometer. The renggak fruit seed extract was tested at concentrations of 20, 40, 60, 80, and 100 ppm, while quercetin was used as a reference standard. Each sample solution was mixed with 1 mL of DPPH solution and diluted to 5 mL with ethanol. After incubation in the dark for 30 minutes at room temperature, absorbance was measured at the maximum wavelength. The percentage of inhibition was calculated, and IC_{50} values were determined using linear regression analysis based on the relationship between inhibition percentage and logarithmic concentration (Kamoda et al., 2021).

Antibacterial Activity Assay

Klebsiella pneumoniae used in this study was obtained from a laboratory stock culture. The bacterial suspension was prepared by adjusting turbidity to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using sterile physiological saline. The standardized suspension was evenly spread on Nutrient Agar plates prior to well formation to ensure uniform bacterial growth. Antibacterial activity against *Klebsiella pneumoniae* was assessed using the agar well diffusion method. Nutrient Agar medium was prepared and sterilized at 121 °C for 15 minutes. The ethanolic extract was tested at concentrations of 20%, 40%, 60%, 80%, and 100%. Amoxicillin and distilled water were used as positive and negative controls, respectively. Wells (5 mm diameter) were prepared in the agar plates, and 50 µL of each test solution was added. The plates were incubated at 37 °C for 24 hours, and antibacterial activity was evaluated by measuring the diameter of inhibition zones (Utami, 2017).

RESULTS AND DISCUSSION

Moisture Content of Renggak Fruit Seeds

The moisture content of renggak fruit simplicia was determined prior to extraction to ensure sample quality and stability. The results showed an average moisture content of 6.09%, which is below the maximum acceptable limit of 10% for simplicia materials. This indicates that the dried samples were suitable for further analysis and less susceptible to microbial growth during storage. High moisture content in simplicia may accelerate degradation and microbial contamination, potentially affecting extraction efficiency and phytochemical stability (Dhama et al., 2020).

Table 1. Moisture content of renggak fruit seed simplicia

Sample	Initial mass (g)	Final mass (g)	Moisture content (%)
1	2.0708	1.9447	6.0494
2	2.0055	1.8834	6.0882
3	2.0040	1.8801	6.1826
Average	–	–	**6.0978**

Phytochemical Screening

Qualitative phytochemical screening revealed that the ethanolic extract of renggak fruit contained flavonoids, tannins, and terpenoids, while alkaloids, saponins, and steroids were not detected. The presence of these secondary metabolites is consistent with previous studies on plants from the genus *Amomum*, which are known to be rich in phenolic and terpenoid compounds with biological activity (Cai et al., 2021; Alruhaili et al., 2023)

Table 2. Phytochemical Screening Results of Ethanolic Extract of Renggak Fruits (*Amomum dealbatum*)

No.	Phytochemical Test	Observation	Result
1	Flavonoids	Red–orange coloration	+
2	Alkaloids	No precipitate formed	−
3	Saponins	No stable foam	−
4	Tannins	Greenish-brown coloration	+
5	Steroids	No blue/green ring	−
6	Terpenoids	Reddish-purple coloration	+

Note: (+) detected; (−) not detected.

Flavonoids and tannins are widely recognized for their antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals. Terpenoids also contribute to antioxidant and antimicrobial activities through membrane disruption and oxidative stress modulation. The detected phytochemical profile suggests that the biological activities observed in this study are closely associated with these compounds, either individually or through synergistic interactions (Erwin et al., 2013). Similar phytochemical profiles rich in flavonoids and terpenoids have been reported in other Amomum species, supporting the role of these compounds in antioxidant and antibacterial activities (Cai et al., 2021; Alruhaili et al., 2023; Subba et al., 2017).

Antioxidant Activity

The antioxidant activity of renggak fruit extract was evaluated using the DPPH radical scavenging method at a maximum wavelength of 515 nm. The results demonstrated that the percentage of radical scavenging activity increased with increasing extract concentration, indicating a concentration-dependent antioxidant effect. This trend suggests that higher concentrations provide a greater amount of active compounds capable of neutralizing DPPH radicals.

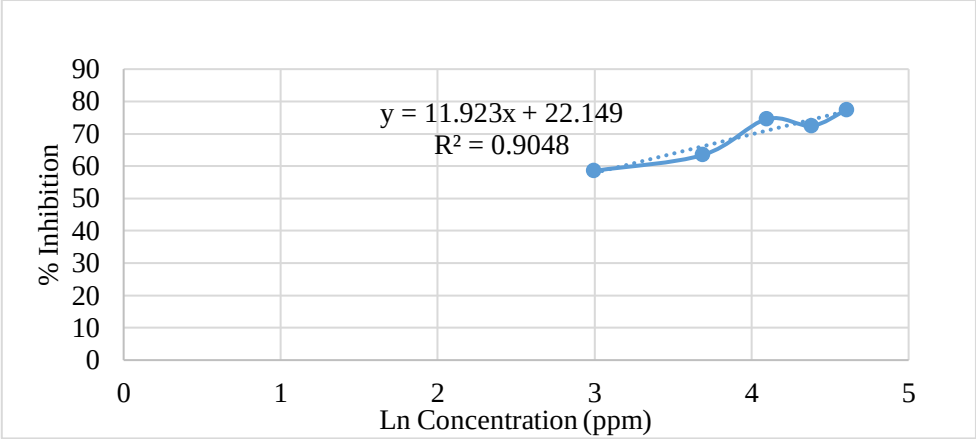

Figure 1. Linear Regression Curve for IC_{50} Determination of Renggak Fruit Extract

The ethanolic extract exhibited an IC_{50} value of 10.33 ppm, which categorizes it as a very strong antioxidant according to the classification proposed by Sharma et al. (2019) and Yashin et al. (2017), where compounds with IC_{50} values below 50 ppm are considered very strong antioxidants. For comparison, quercetin, used as a positive control, showed an IC_{50} value of 0.28 ppm, confirming its well-established antioxidant potency.

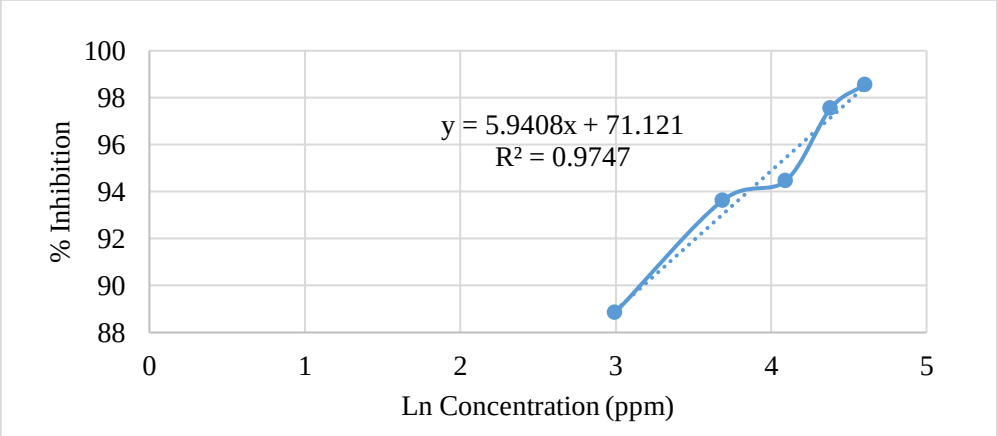

Figure 2. Linear regression curve of quercetin used as a reference standard for IC_{50} determination, illustrating the reliability of the DPPH method applied in this study.

The strong antioxidant activity observed in this study exceeds that reported for other parts of the same plant. Previous research by Mustariani and Hidayati (2021) reported an IC_{50} value of 149.59 ppm for ethanolic extracts of renggak leaves, categorized as moderate antioxidant activity. Similarly, Azim et al. (2023) reported weak antioxidant activity for renggak fruit peel extract with an IC_{50} value of 244.90 ppm. These comparisons indicate that renggak fruit possess superior antioxidant potential compared to other plant parts.

The mechanism of DPPH radical scavenging involves the reduction of DPPH radicals through hydrogen donation from hydroxyl groups present in phenolic compounds. As the number of available hydroxyl groups increases, the degree of DPPH reduction also increases, leading to higher antioxidant activity. This mechanism explains the observed decrease in DPPH absorbance and the color change from purple to yellow during the assay (Dewi, 2023).

Antibacterial Activity Against *Klebsiella pneumoniae*

The antibacterial activity of the ethanol extract of renggak fruits was evaluated using the agar well diffusion method. This method allows direct measurement of inhibition zone diameters and is widely used in antibacterial studies (Utami, 2017). All extract concentrations (20%, 40%, 60%, 80%, and 100%) exhibited inhibitory effects against *Klebsiella pneumoniae*. The mean inhibition zone diameters increased with extract concentration, ranging from 7.34 mm at 20% concentration to 9.48 mm at 100% concentration. Based on standard interpretation, these values fall into the moderate antibacterial activity category.

Table 5. Inhibition zone diameters (mm) of renggak fruit seed extract against Klebsiella pneumoniae using amoxicillin as a positive control.

Treatment	Replication I	II	III	IV	V	Mean ± SD (mm)	Category
Extract 20%	7.9	7.8	6.9	7.1	7.0	7.34 ± 0.47	Moderate
Extract 40%	7.8	7.7	7.5	7.5	7.8	7.66 ± 0.15	Moderate
Extract 60%	8.4	8.2	8.1	7.9	7.5	8.02 ± 0.36	Moderate
Extract 80%	8.1	8.3	8.2	8.0	8.4	8.20 ± 0.16	Moderate
Extract 100%	9.8	9.6	9.5	9.3	9.2	9.48 ± 0.24	Moderate
Positive control (Amoxicillin)	26	26	15	15	15	19.40 ± 6.02	Strong
Negative control (Distilled water)	0	0	0	0	0	0.00 ± 0.00	–

The positive control (amoxicillin) exhibited a strong inhibitory effect with a mean inhibition zone of 19.40 mm, while the negative control (distilled water) showed no inhibition. This confirms that the antibacterial activity observed was due to the bioactive compounds present in the extract. The antibacterial effect is attributed to the combined action of flavonoids, tannins, and terpenoids. Flavonoids can damage bacterial cell membranes and inhibit nucleic acid synthesis, tannins can inactivate enzymes and proteins, and terpenoids can disrupt membrane integrity (Cushnie et al. (2014); Górniak et al. (2019); Gustiana et al., 2022).

CONCLUSION

This study demonstrates, for the first time, that ethanolic extracts of edible renggak fruit seeds (*Amomum dealbatum Roxb.*) exhibit very strong antioxidant activity and moderate antibacterial effects against *Klebsiella pneumoniae*. The novelty of this research lies in the utilization of fruit seeds, which are traditionally consumed but rarely investigated scientifically, particularly against a clinically significant pathogen. The findings contribute to the growing evidence supporting *Amomum* species as valuable sources of natural bioactive compounds. The impact

of this study is the provision of scientific validation for renggak fruit as potential functional food ingredients and alternative natural antibacterial agents, especially in the context of increasing antibiotic resistance.

RECOMMENDATIONS

Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for the antioxidant and antibacterial activities of renggak fruits (*Amomum dealbatum* Roxb.). Quantitative analysis of phenolic and flavonoid contents, as well as in vivo antioxidant and antibacterial evaluations, should be conducted to confirm the biological efficacy and safety of the extract.

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