

Flavonoid content and antibacterial activity on Sabrang Onion Leaf infusion after Ultraviolet-B Irradiation

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Abstract. The Sabrang Onion or Dayak Onion (*Eleutherine palmifolia* Merr.) is traditionally used by the Dayak tribe of Kalimantan as herbal medicine, with most studies focusing on its tuber. However, the leaves also contain bioactive compounds, including flavonoids, which may possess antibacterial potential. This study aimed to determine the flavonoid content and antibacterial activity of Sabrang Onion leaf infusion against *Escherichia coli* following UV-B irradiation. Infusion was prepared by heating leaves at 90°C for 15 minutes, followed by thickening at 70°C for three days. Qualitative flavonoid tests were conducted using Mg-HCl reagent, and quantitative analysis using Thin Layer Chromatography (TLC). Antibacterial activity was assessed using the disc diffusion method at concentrations of 5% and 10%, with chloramphenicol as a positive control. UV-B irradiation for 1–2 hours increased flavonoid content and antibacterial activity, while 3-hour exposure resulted in reduced activity. TLC analysis showed increased flavonoids at 1–2 hours ($R_f = 0.885$), and decreased flavonoids at 3 hours ($R_f = 0.70$). The antibacterial activity correlated with flavonoid levels, indicating that UV-B exposure modulates bioactive compound production in Sabrang Onion leaves.

1 Introduction

The use of traditional medicine has grown rapidly in recent years. This development occurs because humans are practicing natural treatments. Many people are interested in treating various ailments with traditional medicine. One plant that has been cultivated, especially in Central Kalimantan, is the Sabrang Onion (*Eleutherine palmifolia* Merr.) [1]. In Indonesia, this plant is known as the Sabrang Onion, the Redenggy Onion, the Ghost Onion, the Dayak Onion, or the Arab Onion. The Sabrang Onion is a seasonal herb that grows as a vine, growing 30-40 cm tall. It has a pseudostem and a red, layered bulb, and is oval and elongated. Its green leaves are single, ribbon-shaped leaves with pointed tips and bases and smooth edges. Its flowers are compound, cylindrical, and have stalks approximately 40 cm long. Its roots are fibrous and light brown [2].

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Flavonoids are important secondary metabolites synthesized through the shikimic acid and malonic acid pathways and are widely distributed across plant tissues, including roots, stems, leaves, flowers, and bulbs [3]. Flavonoids sometimes exhibit antibacterial activity, and higher flavonoid concentrations correlate with increased inhibitory effects against pathogenic bacteria. This activity is associated with hydroxyl functional groups, which disrupt cellular structures and metabolic pathways in microbial cells [4]. However, flavonoid content in plants may vary considerably depending on environmental factors, extraction methods, and plant tissue type.

Exposure to abiotic stress—particularly ultraviolet-B (UV-B) radiation—is known to stimulate the accumulation of defensive secondary metabolites, including flavonoids [5]. UV-B activates photoreceptor-mediated signaling pathways and regulates genes associated with antioxidant metabolism [6]. Several studies report an increase in flavonoid content following short-term UV-B exposure in species such as buckwheat, *Betula pendula*, and *Arabidopsis thaliana* [6-7]. However, prolonged UV-B exposure may induce cellular damage, reduce photosynthetic efficiency, generate reactive oxygen species (ROS), and impair plant growth, suggesting that optimum exposure duration is critical [8].

Existing research on Sabrang Onion has focused primarily on ethanol-based maceration extraction techniques and antibacterial assays against *Staphylococcus aureus* and *Escherichia coli* [9]. Limited studies have examined aqueous extraction using the infusion method, which is suitable for heat-stable and water-soluble flavonoid compounds [10]. To date, no research has investigated the effect of UV-B exposure on flavonoid yield in Sabrang Onion leaf infusion preparations or subsequently evaluated their antibacterial potential.

2 Methods

2.1 Experimental design

This study was conducted using an experimental approach with a Completely Randomized Design (CRD). The treatment consisted of UV-B irradiation at four levels: 0 (control), 1, 2, and 3 hours, each with three replications.

2.2 Sterilization procedures

Heat-resistant tools were sterilized using moist heat at 121°C, 1 atm for 15 min in an autoclave. Non-heat-resistant materials were disinfected with 70% ethanol followed by flaming.

2.3 Plant cultivation and UV-B irradiation

Healthy and uniform bulbs were planted in polybags containing soil:rice husk charcoal:compost (1:1:1). Plants were acclimated for 10 days and irrigated once daily. UV-B treatment was applied at 311 nm for 1, 2, or 3 hours per day for three consecutive days. Leaves were harvested 10 days after treatment.

2.4 Sample preparation and infusion extraction

Harvested leaves were washed, chopped, and dried at 50°C for 36 hours. Dried material was powdered and extracted using an infusion method at a sample-to-solvent ratio of 1:10 in

distilled water at 90°C for 15 min (Ibrahim et al., 2019). The filtrate was concentrated in a water bath at 70°C to obtain a thick extract.

2.5 Qualitative flavonoid identification

Preliminary flavonoid screening was performed by adding Mg powder (0.1 g) and 3 drops of concentrated HCl to 1 mL extract. A color shift from red to orange indicated a positive flavonoid reaction.

2.6 Thin layer chromatography (TLC)

Quantitative analysis was conducted using TLC. Silica gel GF254 plates were activated at 100°C for 30 min. The mobile phase was ethyl acetate:methanol:water (12:2:2). Samples were spotted and developed to the solvent front. After drying, plates were sprayed with AlCl₃ and visualized under 254 nm UV. R_f values were calculated as:

$$R_f \text{ value} = (\text{sample distance}) / (\text{eluent distance})$$

2.7 Antibacterial activity assay

The antibacterial activity was assessed using the disk diffusion method. *E. coli* suspension was standardized to McFarland 0.5 (absorbance 0.08–0.13). Chloramphenicol served as the positive control, sterile distilled water as the negative control, and 5% extract solution as the test treatment. After 24-hour incubation at 37°C, inhibition zones were measured in millimeters.

2.8 Data analysis

Data were analyzed using two-way ANOVA. Significant results were followed by Kruskal–Wallis post-hoc testing to determine treatment differences.

3 Results and discussion

3.1 Qualitative Analysis of Flavonoids

The 1-hour UV-B sample showed no significant color change, while the control became reddish-black. The 2-hour and 3-hour UV-B samples presented a yellowish-orange color, confirming the presence of flavonoids (Figure 1). The underlying reaction involves the reduction of flavonoid structures by Mg/HCl, forming colored flavone or flavanone complexes [2].

Flavones and flavanones are commonly found in leaves [12]. The difference in color development is attributed to structural variations: flavones contain a C2=C3 double bond enabling π -conjugation, creating a stable chromophore that absorbs visible light and produces an orange color. Flavanones lack this conjugation, resulting in weaker color development [13].

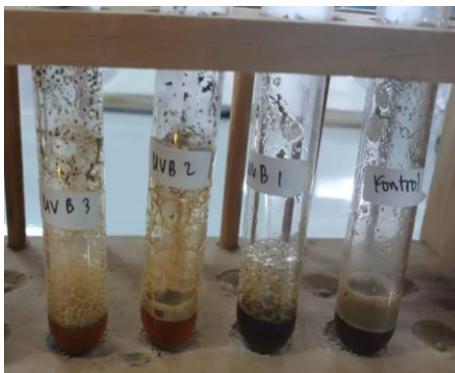


Fig.1. Color reagent test results

3.2 Analysis using Thin-Layer Chromatography (TLC)

Quercetin was used as a reference standard on TLC Analysis because it is one of the most abundant plant flavonoids and forms stable complexes with $AlCl_3$, improving visibility. Based on TLC result on Figure 2, the calculated R_f values are shown in Table 1. Quercetin exhibited an R_f of 0.91 as standard. UV-B treatments (1 and 2 hours) produced high R_f values (0.89), while the 3-hour treatment and control group had lower values (0.70 and 0.64, respectively). Long-term UV-B exposure may degrade phenolic compounds, as reported in hazelnut pollen where 3-hour UV-B reduced total phenolics by 22% [14].

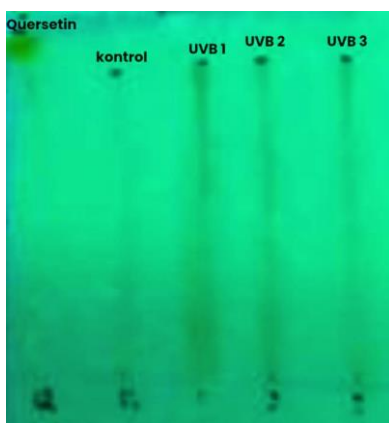


Fig.2. TLC test result

Table 1. Measurement results of visible spots on TLC

Treatment	Visible sample stain (cm)	R_f
Quersetin	4.15	0.91
Kontrol	2.7	0.64
UVB 1h	4	0.89
UVB 2h	4.05	0.89
UVB 3h	3.05	07

3.3 antibacterial activity against *Escherichia coli*

The highest inhibition zone was recorded in the 1-hour UV-B treatment (13 mm) (Figure 3), the calculated inhibition zone are shown in Table 2. The enhanced antibacterial effect in UV-B-treated samples may be attributed to increased stress-induced flavonoid synthesis. Interestingly, the 10% extract showed reduced antibacterial activity across treatments, possibly due to oxidation of flavonoids at higher concentrations [15].

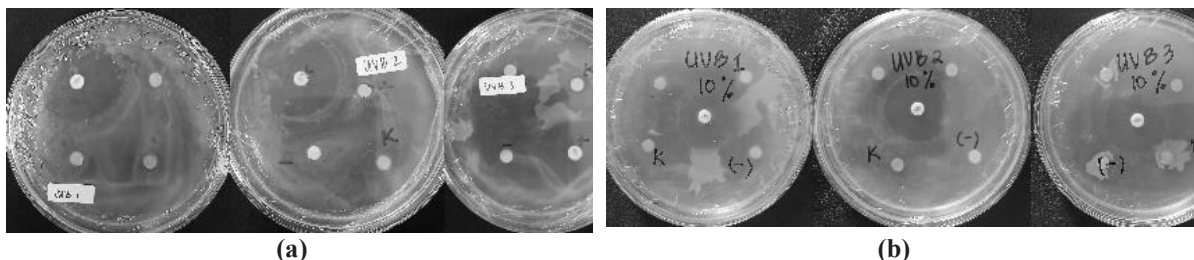
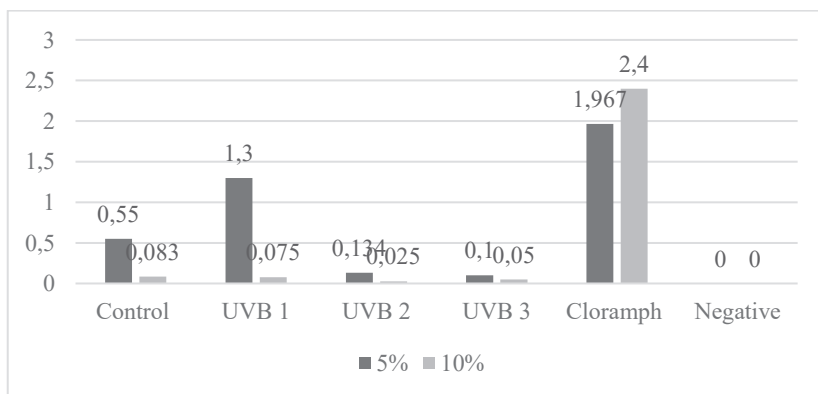


Fig.3. Results of antibacterial observations. **(a)** 5% antibacterial; **(b)** 10% antibacterial

The positive control (chloramphenicol) consistently produced clear inhibition zones ≥ 16 mm, confirming bacterial susceptibility, while the negative control exhibited no inhibition. Overall, the findings indicate that both UV-B exposure duration and extract concentration play critical roles in determining antibacterial activity. Short-term UV-B irradiation appears beneficial for enhancing bioactive compound production; however, excessive exposure may lead to oxidative damage and metabolite degradation. Future research should optimize UV-B duration, improve extract stability during storage, and evaluate additional extraction methods to maximize antibacterial potential.

Table 2. Inhibition zone measurement results

Sampel	Concentration 5% (cm)	Concentration 10% (cm)
UVB 1h	1.30	0.08
UVB 2h	0.13	0.05
UVB 3h	0.10	0.05



Graph 1. Bacterial inhibition zone

4 Conclusion

UV-B irradiation for 1–2 hours increased flavonoid concentration and antibacterial activity in *Eleutherine palmifolia* leaf infusion, while excessive irradiation (3 hours) resulted in decreased metabolite levels and bioactivity. Short-term UV-B stress may serve as a pre-harvest treatment to enhance medicinal properties. Further research should optimize irradiation parameters, explore active compound isolation, and compare extraction techniques.

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