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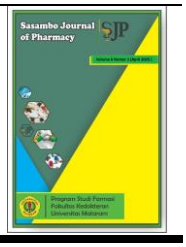

Post-harvest process influences antibacterial activity of *Clitoria ternatea* flower extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Butterfly pea is a plant with high potential for development as an antimicrobial agent. However, its antimicrobial potential is closely related to its phytochemical profile, which can be influenced by post-harvest processing. This study aimed to evaluate the effect of post-harvest processing on the antibacterial activity of butterfly pea flowers. Butterfly pea flower was prepared using three different methods: fresh, aerobic fermentation, and sun-dried. The samples were extracted by maceration using 96% methanol for 1 day with 3 re-extraction process. Phytochemical screening was conducted using colorimetric method with specific reagents for certain groups. Antimicrobial potential was tested using the well diffusion method on NA media in five different concentrations of extract. Extraction results showed that the three samples produced different extract yields, with sun-dried butterfly pea flower extract giving the highest yield (18%), followed by fresh extract (8%), and aerobic fermentation (0.8%). Phytochemical screening revealed that all three butterfly pea flower samples did not contain quinones and saponins. Additionally, alkaloids were not detected in the fermented extract. Antibacterial activity of the three butterfly pea flower extracts showed significantly different zone of inhibition, with the sun-dried extract consistently providing the largest zone of inhibition compared to fresh and fermented extracts against *Escherichia coli* and *Staphylococcus aureus*, with average inhibition zones of 8.67 - 11.17 ± 0.88 mm and 4.25 - 10.5 ± 1.64 mm, respectively. It can be concluded that the post-harvest processing affects the antibacterial activity of butterfly pea flowers.

Keywords: Butterfly pea flower; Post-harvest processing; Antibacterial activity.

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Introduction

Indonesia stands as a global hub of biodiversity, hosting 40,000 tropical plant species, with a remarkable 39% being native to the region. Many indigenous plants, including turmeric, ginger, and cloves, play a vital role in traditional Indonesian medicine, commonly referred to as "jamu" (Wirasisya & Hohmann, 2023). Among these native species is the butterfly pea flower (*Clitoria ternatea* Linn), which thrives in Indonesia's tropical climate and other sun-rich regions (Jamil et al., 2018; Oguis et al., 2019). Research has revealed that this flower contains a rich profile of compounds, including anthocyanins and various secondary metabolites such as phenolics, tannins, steroids, triterpenoids, saponins, and anthraquinones (Manjula et al., 2013). Its applications extend beyond its use as a natural colorant to include various medicinal purposes (Oguis et al., 2019).

Communities have long employed different parts of the butterfly pea plant in herbal remedies, particularly

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for fighting infections. Scientific studies have validated these traditional practices by demonstrating the plant's effectiveness against various disease-causing organisms (Khumairoh et al., 2020; Marpaung, 2020; Riyanto et al., 2019). Studies indicate that the flower exhibits strong antimicrobial properties against both gram-positive bacteria (including *B. cereus, B. subtilis, S. aureus*, and *S. faecalis*) and gram-negative bacteria (such as *E. coli, Klebsiella spp, P. aeruginosa, S. typhi, E. aerogenes*, and *P. mirabilis*), with impressive inhibition zones of 8-26 mm (Febrianti et al., 2022).

The effectiveness of the flower's antibacterial properties, however, varies depending on several factors, particularly the pos-harvest processing. The preparation technique significantly impacts the biological activity by altering the chemical composition of the final product (Horablaga et al., 2023). For instance, Wirasisya's 2018 study found that oven-dried samples of ashitaba (*Angelica keiskei*) showed enhanced antibacterial effects against *S. mutans* compared to sun-dried alternatives. Similarly, Mphahlele et al. (2016), comparing the antibacterial activity of freeze-dried with oven-dried *Punica granatum* L. samples, discovered that oven-dried plant bark demonstrated stronger antibacterial properties than freeze-dried samples. These findings suggest a clear correlation between preparation methods and antibacterial efficacy.

Despite these insights, there remains a gap in the research specifically examining how different post-harvest process affect the butterfly pea flower's antibacterial properties. This knowledge gap has prompted new research to investigate the relationship between various post-harvest process and their impact on the flower's antibacterial effectiveness.

Materials and Methods

Chemicals and reagents

Chemicals and reagents used in this study included technical grade of 96% methanol (Brataco, Indonesia), technical grade of 70% ethanol (Brataco, Indonesia), amyl alcohol (Sigma-Aldrich, Singapore), magnesium (Merck, Germany), $FeCl_3$ (Merck, Jerman), HCl (Merck, Germany), NaOH (Sigma-Aldrich, Singapore), Dragendorff (Sigma-Aldrich, Singapore), Liebermann-Burchard (Nitra Kimia, Indonesia), Stiasny (Nitra Kimia, Indonesia), gelatine (Nitra Kimia, Indonesia), nutrient agar (NA) media (Sigma-Aldrich, Singapore), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Singapore), dan amoxicillin (DexaMedica, Indonesia).

Sample collection and identification

Three kilograms of fresh butterfly pea flowers were collected from the Alang-alang Lebar District, Palembang City, South Sumatra, Indonesia. The collected samples were then botanically identified at the Herbarium Bandungense Laboratory of the School of Life Sciences and Technology (SITH), Bandung Institute of Technology, Bandung, West Java, Indonesia (No. 3148/IT1.C11.2/TA.00/2024).

Sample preparation and extraction

The harvested butterfly pea flowers were sorted, washed, and drained. The samples were then divided into 3 parts: 1 kg was sun-dried for 7 days, 1 kg underwent aerobic fermentation in an isolated container for 1 day, and the remaining portion was extracted in its fresh state. The prepared butterfly pea flower samples were then pulverized using a laboratory blender. Each 100 g of powder was macerated for 24 hours using technical grade 96% methanol at room temperature with occasional stirring. The extract was then filtered and concentrated using a vacuum rotary evaporator (Buchi, Germany) at 45°C and 70 rpm rotation. The extraction process was performed with 3 re-maceration repetitions. The obtained crude extract was then weighed, and the yield was calculated using the equation described by Utami et al. (2023):

$$\text{Extract yield (\%)} = \frac{\text{Weigh of crude extract}}{\text{Samples dry weigh}} \text{ x } 100$$

Preliminary phytochemical screening

Phytochemical screening of each butterfly pea flower extract was conducted to identify the presence of flavonoids, alkaloids, phenols, tannins, saponins, terpenoids, and quinones following the method described by Ayu et al. (2024).

Antibacterial activity assay

The antibacterial activity of each butterfly pea flower extract was tested against *Staphylococcus aureus* and *Escherichia coli* using well diffusion method. The bacterial suspension, standardized with 0.5 McFarland (equivalent to 1.5 x 10^8 CFU/mL), was inoculated onto NA media using a sterile cotton swab. Then, extract solutions at concentrations of 100%, 75%, 50%, 25%, and 12.5% (w/v) were placed into 6 mm diameter wells. The petri dishes containing the test materials were wrapped in aluminum foil and incubated at 37°C for 24 hours. Inhibitory activity was identified by measuring the clear zones around the wells. The experiment was conducted in 3 replications with 1000 ppm amoxicillin as a positive control and 10% DMSO as a negative control.

Data analysis

The inhibition zone of each butterfly pea flower extract was presented as mean values ± SD (n=3) and statistically analyzed using Two Way ANOVA and

Tukey's test (95% confidence level) on GraphPad Prism version 10.0.1 software.

Results and Discussion

Extraction

The extraction methodology plays a pivotal role in determining both the yield and quality of plant-derived extracts. When isolating bioactive constituents from botanical sources, the selection of extraction techniques and their corresponding parameters becomes a critical determinant of success. In this investigation, we employed maceration to extract compounds from butterfly pea flowers, revealing distinct variations in yield across different post-havest preparation methods. The quantitative analysis, visualized in **Figure 1**, demonstrates a clear correlation between post-havest process and extract yields. Our findings indicate that sun drying emerged as the most efficient method, generating the highest extract yield. This was followed by extracts obtained from fresh plant material, while aerobic fermentation produced comparatively lower quantities. These results underscore the significant impact that initial processing methods can have on the ultimate extraction efficiency of bioactive compounds from butterfly pea flowers.

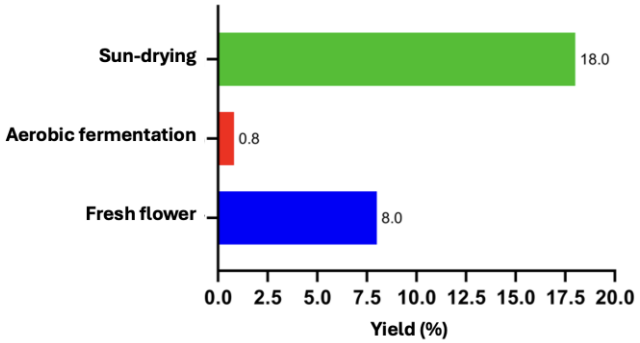


Figure 1. Extract yield of butterfly pea flower extract from various post-harvest process

The experimental findings demonstrate strong concordance with Herlina et al. (2024), revealing that solar dehydration techniques generated significantly higher yields (17.3%) than aerobic fermentation (14.3%) protocols of *Citrus limon* leaves. This disparity stems from fundamental differences in biochemical mechanisms: fermentation processes trigger enhanced enzymatic activity, leading to the modification and subsequent degradation of secondary metabolite compounds within plant tissues. Conversely, solar dehydration methodologies function by substantially reducing moisture content in the simplicia, effectively suppressing enzymatic activities responsible for both hydrolytic and oxidative degradation of secondary metabolites (Thirumdas & Annapure, 2020). This mechanistic difference explains the superior preservation of bioactive compounds in sun-dried sample.

Phytochemical screening

Colorimetric-based phytochemical screening was implemented to analyze the chemical composition of butterfly pea flower extracts through specific reagent interactions. The analytical results revealed distinct phytochemical profiles across sample preparations. Notably, alkaloid presence showed a gradient distribution: undetectable in fermented preparation, weakly present in fresh laves, and strongly manifested in dried sample, as documented in **Table 1**. While flavonoids, phenolics, and terpenoids maintained consistent presence across all samples, the sun-dried preparations demonstrated reduced chromogenic intensity. Notably, both saponin and quinone compounds were consistently absent across all sample preparations, as indicated by negative test outcomes.

Table 1. Preliminary phytochemical screening of butterfly pea flower extract from various post-harvest process

Secondary metabolite class	Post-harvest process		
	Fresh flower	Aerobic fermented flower	Sun-dried flower
Alkaloid	+	-	+++
Flavonoid	+++	++	+
Phenols	+++	++	+
Tannins	++	++	++
Saponins	-	-	-
Terpenoid	++	++	+
Quinones	-	-	-

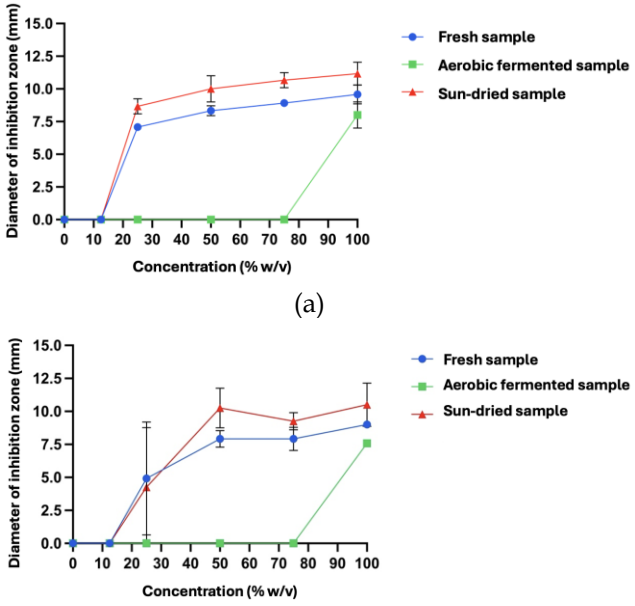
Remaks: (+++): Strong (++): Moderate; (+): Weak, (-): Undetected. *Note: Strong/weak category was assessed based on the color intensity and/or density of precipation observed.*

The study reveals that post-harvest process of butterfly pea flower exerts differential effects on secondary metabolite profiles. Alkaloid compounds demonstrate enhanced stability under thermal exposure, evidenced by intensified chromogenic responses in dried simplicia (de Nijs et al., 2017). High-temperature conditions catalyze the formation of novel alkaloid derivatives (i.e. tropane alkaloids) through compound degradation pathways, resulting in elevated alkaloid concentrations (Marin-Saez et al., 2019). This aligns with a study reported by Fitriyah & Ramadhani (2024) that showed butterfly pea contains wide range of alkaloids, one of which is tropanes alkaloids. Contrastingly, flavonoid, phenolic, and terpenoid compounds exhibit inverse thermal stability relationships. As reported by Herlina et al. (2024), direct solar exposure during drying

processes induces significant quantitative reductions in total phenolic (gallic acid equivalent) and flavonoid content (quercetin equivalent) of *Citrus limon* leaves. This phenomenon is attributed to the inherent reductive properties of these compounds, rendering them susceptible to thermally induced chemical transformations (El-Gamal et al., 2023). Terpenoid compounds display particular thermal sensitivity, readily undergoing oxidative degradation and volatilization under elevated temperature conditions (El-Gamal et al., 2023).

Antibacterial activity

The antibacterial assessment demonstrated differential inhibitory capacities among butterfly pea flower extracts prepared through various post-harvest process. The bacteriostatic efficacy exhibited concentration-dependent characteristics across all samples. Figure 2 illustrates that extracts derived from both sun-dried and fresh flowers demonstrated initial inhibitory activity at 25% (w/v) concentration against both bacterial strains, with inhibition zones displaying direct proportionality to concentration gradients. The observed zone of inhibition increased correspondingly with higher extract concentrations. Notably, fermented butterfly pea flower extracts exhibited antibacterial activity exclusively at the highest tested concentration (100% w/v).



Gambar 2. Antibacterial activity of buttefly pea flower extract against (a) *Staphylococcus aureus* dan (b) *Escherichia coli*. Data was presented as mean ± SD (n=3), and significantly different in a statistical analysis using *Two Way ANOVA*, Tukey's test (p<0,05). *Note: Amoxicillin 1000 ppm and DMSO 10% did not provide zone of inhibition.*

The antimicrobial analysis of butterfly pea flower extracts across varying post-harvest process demonstrated that sun-dried extracts consistently exhibited superior antibacterial efficacy against both bacterial strains, with fresh and fermented buttefly pea flower extracts showing progressively lower activity. Statistical analysis revealed significant differences in antibacterial potency among the samples, yielding a *p value* of 0.0036 (p<0.05). These findings conclusively demonstrate that post-harvest process significantly impacts the antibacterial properties of butterfly pea flower extracts.

The strong antibacterial activity of sun-dried butterfly pea flower extract is suspected to be caused by its relatively high alkaloid content compared to fermented and fresh butterfly pea flower extracts. As reported by Fitriyah & Ramadhani (2024), butterfly pea contains numerous alkaloids, including lupinine, alpha-erythroidine, beta-erythroidine, quinine, cephalotaxine, cephalotaxinone, and estradiol. Several studies have reported the antibacterial activity of alkaloid groups in inhibiting pathogenic bacteria (Othman et al., 2019; Sulaiman et al., 2022). According to Yan et al. (2021), alkaloid compounds have diverse mechanisms in providing antibacterial activity, ranging from inhibiting protein and nucleic acid synthesis, disrupting membrane and cell wall permeability, to inhibiting bacterial metabolic processes.

Conclusion

Antibacterial evaluation of butterfly pea flower extracts demonstrated that post-harvest process significantly influences bacterial growth inhibition for *Staphylococcus aureus* and *Escherichia coli*. Sun-dried plant material extract exhibited the most potent antibacterial activity, with substantially larger inhibition zones compared to fresh and aerobically fermented extracts. The mean inhibition diameters revealed remarkable differences: sun-dried extract zones ranged from 8.67 - 11.17 ± 0.88 mm, while fermented and fresh extracts showed zones of 4.25 - 10.5 ± 1.64 mm. These findings underscore the critical role of post-harvest process of butterfly pea folwer in maximizing its antibacterial efficacy.

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