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Fermentation Influences the Total Flavonoid Content and Antioxidant Activity of *Syzygium polyanthum*

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ABSTRACT

Introduction*: Syzygium polyanthum are widely recognized for their rich phytochemical profile, including flavonoids and phenolics. However, the bioavailability of these compounds can be limited, prompting exploration of methods to enhance their accessibility and potency.* ***Aims****: This study investigates the effects of fermentation on the bioactive compounds and antioxidant properties of Syzygium polyanthum.* ***Methods****: Three sample groups were examined: fresh leaves, leaves fermented for 3 days, and leaves fermented for 5 days. Qualitative and quantitative analyses were conducted to assess flavonoid content, phenolic compounds, and antioxidant activity in each sample.* ***Result****: The results demonstrated that fermentation significantly impacted these properties, with the 5-day fermented sample exhibiting the highest values: total flavonoid content of 279.850 mg QE/g, total phenol content of 135 mg GAE/g, and antioxidant activity with an IC_{50} of 51.89 ppm.* ***Conclusion****: These findings suggest that fermentation can be an effective method to enhance the nutritional and medicinal properties of Syzygium polyanthum leaves. This research provides valuable insights into the potential applications of fermented Syzygium polyanthum in the food and pharmaceutical industries, paving the way for further exploration of its health-promoting properties.*

KEYWORDS*: Syzygium polyanthum*, fermentation, TFC, TPC, antioxidant

INTRODUCTION

The Indonesian archipelago, stretching from Sabang to Merauke, is renowned for its rich biodiversity and abundance of medicinal plants. Among these, *Syzygium polyanthum* stands out as a particularly valuable species, traditionally used to address a range of health issues including high cholesterol, diabetes, hypertension, gastritis, and diarrhea (Dalimartha, 2007; Rahman et al., 2014). This plant's therapeutic potential is largely attributed to its diverse phytochemical profile, which includes flavonoids, terpenoids, phenolics, alkaloid, saponins, and antioxidant (Ismail & Ahmad, 2019).

The significance of *Syzygium polyanthum* lies in its phenolic and flavonoid compounds, which serve as potent antioxidants, which play a crucial role in protecting the body against free radical damage, potentially preventing a spectrum of diseases ranging from immune disorders to cancer and premature aging (Muscolo et al., 2024). However, the efficacy of these compounds is closely tied to their concentration and bioavailability within the plant. Recent studies have shed light on how fermentation can enhance the bioactive properties of various plants. For instance, Sintyadewi & Widnyani (2021) observed that fermentation duration significantly impacted the total flavonoid content in kombucha, with peak levels reached after 8 days. Similarly, Zhao et al. (2021) reported the alteration of polyphenol content in fermented plant-based food of both aerobic and anaerobic fermentation. Furthermore, the fermentation not only influenced the phenolic and flavonoid content of plants, but also affect their antioxidant activity index (Herlina et al., 2024).

These findings suggest that fermentation could be a promising method to augment the medicinal properties of *Syzygium polyanthum*. The fermentation process, however, is influenced by factors such as media composition, temperature, pH, and duration, has the potential to alter the plant's phytochemical profile, potentially enhancing its therapeutic value. Additionally, the fermentation results can also vary depending on its types, conditions, as well as the plant species. Given this context, our study aims to investigate how fermentation influences the total phenolic content, flavonoid levels, and antioxidant activity of *Syzygium polyanthum*. By exploring these effects, we hope to unlock new possibilities for maximizing the health benefits of this traditional medicinal plant, potentially leading to more effective natural remedies and nutraceuticals.

METHODS

Chemicals and Reagents

This study used several reagents both technical and analytical grade: Ethanol 96% (Technical grade, Smart Lab), Magnesium powder (Merck), HCl (Merck), $FeCl_3$ (Merck), $AlCl_3$ (Merck), Methanol (Merck), Quercetin (Sigma-Aldrich), Acetic acid (Merck), Gallic acid (Sigma-Aldrich), Folin-Ciocalteu (Merck), Natrium Carbonat (Merck), and DPPH (Sigma-Aldrich).

Sample Collection and Identification

Approximately 3.5 kg of fresh leaves of *Syzygium polyanthum* were harvested at Sukamaju district, Palembang, South Sumatera on May 2024. The sample was then botanically authenticated by the staff of Angga Dwiartama, PhD., laboratory of Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology, with identification number of 4054/IT1.C11.2/TA.00/2024.

Sample Preparation and Extraction

The prepared *Syzygium polyanthum* leaves were divided into three groups: fresh leaves, leaves fermented for 3 days, and leaves fermented for 5 days. The aerobic fermentation process involved stacking the leaves in a container, covering them with cloth, and storing them. One batch was fermented for 3 days, while another underwent the same process for 5 days. The fresh leaf group was prepared by simply cutting the cleaned leaves into small pieces. All leaf samples were then finely ground using a blender. The extraction of *Syzygium polyanthum* leaf simplicia followed a modified version of the method described by Utami et al. (2023). The extraction process employed maceration, where 200 g of each sample (fresh simplicia and aerobically fermented simplicia for 3 and 5 days) was soaked in 1 liter of 96% ethanol for 24 hours. After the initial soaking, the mixture was filtered to separate the residue from the filtrate. The residue underwent a second round of maceration using the same amount of 96% ethanol. This maceration process was repeated twice to ensure thorough extraction. The collected liquid extract was then concentrated using a rotary evaporator (Buchi, Germany) followed by water bath (Memmert, Germany), resulting in a thick crude extract. This concentrated extract was weighed, and its yield was calculated to determine the efficiency of the extraction process using the equation described by Setiawansyah et al. (2018), as follwos:

$$\%\text{Extract yield} = \frac{\text{Crude extract weight (g)}}{\text{Sample dry weight (g)}} \times 100$$

Total Flavonoid Content

The quantification of total content (TFC) was performed using the method outlined by Setiawansyah et al. (2024). In this procedure, 1 ml of a 100 ppm extract solution was combined with 1 ml of 10% $AlCl_3$ and 8 ml of 5% acetic acid. This mixture was incubated for 30 minutes before its absorbance was measured at a wavelength of 413 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). To ensure accuracy, each measurement was conducted in triplicate. The TFC was calculated and stated quercetin equivalent (mg QE/g extract) using the following formula:

$$\text{TFC} = \frac{\text{c x V x f}}{\text{m}}$$

Where

TFC	:	Total flavonoid content (mg QE/g)
c	:	Quercetin equivalence (µg/mL)
V	:	Total volume of extract (mL)
f	:	Dilution factor
m	:	Extract mass (g)

Total Phenolic Content

The quantification of total flavonoid content in *Syzygium polyanthum* leaf extract was conducted using an optimized protocol adapted from Herlina et al. (2024). This refined method involved precisely dissolving 5 mg of extract in 50 ml of methanol, followed by a carefully calibrated reaction where 1 ml

of this solution was combined with 1 ml of Folin-Ciocalteu reagent and 8 ml of distilled water. The resulting mixture was homogenized and transferred to a 10 ml volumetric flask, where 20% sodium carbonate solution was added to volume. After a critical 30-minute incubation period, the solution's absorbance was measured at 641 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). To ensure robust and reproducible results, all measurements were performed in triplicate. The TPC was calculated and stated as gallic acid equivalent (mg GAE/g extract) using the following formula:

$$TPC = \frac{c \times V \times f}{m}$$

Where

TPC : Total phenolic content (mg GAE/g)

c : Gallic acid equivalence (µg/mL)

V : Total volume of extract (mL)

f : Dilution factor

m : Extract mass (g)

Antioxidant Activity Assay

The determination of *Syzygium polyanthum* antioxidant activity was conducted following a method described by Setiawansyah et al. (2024). In this procedure, 25 mg of *Syzygium polyanthum* leaf extract was dissolved in 50 mL of analytical grade methanol in a volumetric flask to achieve a 500 ppm concentration. From this stock solution, a series of dilutions were prepared to obtain five distinct concentrations. For each concentration, 2 mL of the prepared solution was mixed with 2 mL of DPPH solution (100 ppm). These mixtures were then dark-room incubated for 20 minutes before their absorbance was measured at a wavelength of 513 nm using a UV-VIS spectrophotometer (Shimadzu, Japan). Each measurement was repeated three times to ensure accuracy with quercetin was used as control. The antioxidant activity was observed by calculating the radical scavenging activity according to the equation:

$$\text{Inhibition}(\%) = \frac{\text{Abs control} - \text{Abs samples}}{\text{Abs control}} \times 100\%$$

The IC_{50} value was determined by inputting the inhibition results obtained at various test solution concentrations into the regression equation Y = a + bx, where x represents the concentration of the test solution and y represents the percentage value. The antioxidant activity was presented as antioxidant activity indeks (AAI), calculated with the following formula:

$$AAI = \frac{\text{DPPH concentration}}{IC50}$$

Data Analysis

The data of TFC, TPC, and antioxidant were presented as mean ± SD and statistically analzed using One-Way ANOVA, Tukey's test in a GraphPad Prism 10.1 version.

RESULTS AND DISCUSSION

Effect of Fermentation on Extract Yield

The study on the fermentation of *Syzygium polyanthum* leaves reveals an interesting trend in extract yield (Figure 1), with fresh leaves showing the highest yield (7.85%), followed

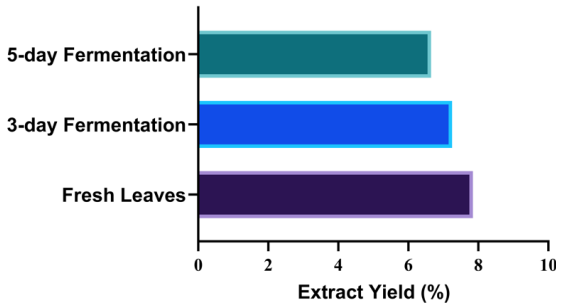

Figure 1. Extract yield of *Syzygium polyanthum* from various post-harvest process

by three-day fermentation (7.25%), and five-day fermentation (6.65%). This pattern suggests that while fermentation enhances certain bioactive compounds, it may lead to a reduction in overall extract yield. The higher yield observed in fresh leaves (7.85%) could be attributed to the intact cellular structure and the presence of a wide range of extractable compounds. This finding aligns with research by Azwanida (2015), who noted that fresh plant materials often contain higher levels of easily extractable compounds compared to processed ones. The high water content in fresh leaves may also contribute to easier extraction of water-soluble compounds. The slight decrease in yield after three days of fermentation (7.25%) suggests that the fermentation process begins to alter the leaf composition. This change could be due to the breakdown of some compounds by microbial activity, as observed by Adetuyi & Ibrahim (2014) in their study on fermented *Abelmoschus esculentus*. The fermentation process may initiate the conversion of complex molecules into simpler forms, potentially making some compounds more bioavailable but reducing the overall extractable mass. The further reduction in yield after five days of fermentation (6.65%) indicates a more pronounced effect of the fermentation process on the leaf composition. This decrease could be attributed to several factors. Firstly, the continued microbial activity may lead to the consumption of some extractable compounds as nutrients, as suggested by Hur et al. (2014) in their review of fermentation effects on plant-based foods. Secondly, the breakdown of cell walls and cellular components might result in the loss of some material during the fermentation process.

Interestingly, while the extract yield decreases with fermentation time, previous results showed an increase in specific bioactive compounds like flavonoids and phenols, as well as enhanced antioxidant activity. This inverse relationship between yield and bioactive compound concentration has been observed in other studies. For instance, Dulf et al. (2016) reported a similar trend in fermented plum by-products, where despite a decrease in extract yield, there was an increase in total phenolic content and antioxidant activity. This phenomenon could be explained by the concept of bioconversion during fermentation. As suggested by Martins et al. (2011), microbial enzymes can transform complex plant compounds into simpler, more bioactive forms. While this process may reduce the overall extractable mass, it can concentrate certain beneficial compounds, potentially explaining the enhanced bioactivity observed in the fermented samples despite

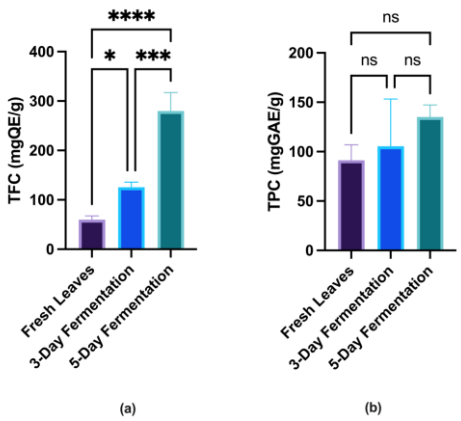

Figure 2. TFC (a) and TPC (b) of *Syzygium polyanthum* in various post-harvest process. Data are presented as mean ± SD (n=3). *significantly different ($p<0.05$); nsnot significantly different ($p>0.05$).

lower yields. The observed trend in extract yield highlights the complexity of the fermentation process and its effects on plant materials. It suggests that while fermentation can enhance certain desirable properties, it may come at the cost of reduced overall yield. This trade-off is an important consideration in the development of fermented plant-based products, where the balance between yield and bioactivity needs to be optimized.

Effect of Fermentation on TFC, TPC, and Antioxidant Activity

The results of this study demonstrate that fermentation significantly impacts the phytochemical profile and antioxidant activity of *Syzygium polyanthum* leaves. The observed increase in flavonoid and phenol content aligns with findings from similar studies on other plant materials. For example, Hur et al. (2014) reported that fermentation generally enhances the bioactive compound content and bioavailability in various food products. This dramatic increase in flavonoid content as depicted in Figure 2a (from 58.96 to 279.85 mg QE/g) and the more modest rise in phenol content in Figure 2b (from 90.30 to 135 mg GAE/g) over five days of fermentation suggest that the process particularly favors flavonoid synthesis. This findings is consistent with research by Martins et al. (2011) who found that microbial fermentation can lead to the biotransformation of phenolic compounds, often resulting in increased flavonoid content. Study conducted by Lasinskas et al. (2022) demonstrated that fermented leaves exhibited markedly elevated levels of quercetin-3-O-rutinoside (8.53%), myricetin (30.83%), luteolin (7.65%), and quercetin (18.52%). Additionally, fermentation also increased several phenolic compounds including benzoic acid (41.91%), and gallic acid (128.45%). However, aerobic fermentation also droped some phenolic compound levels including ellagic acid, *p*-coumaric acid, and cholorogenic acid (Lasinskas et al., 2023).

The non-linear trend observed in antioxidant activity (see Figure 3), with a slight decrease at 3 days followed by a significant improvement at five days, indicates a complex relationship between fermentation time and antioxidant properties. This pattern has been observed in other studies, such as that by Chu & Chen (2006), who reported similar fluctuations in antioxidant activity during the fermentation of peanut extracts. The initial decrease might be due to the breakdown of some antioxidant compounds, while the subse-

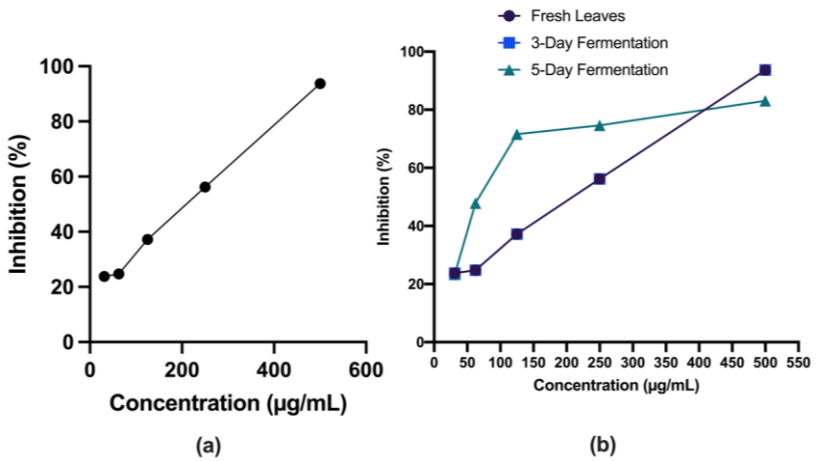

Figure 3. Relationship between sample concentration vs radical inhibition (%) of (a) Quercetin and (b) *Syzygium polyanthum* leaf extracts from various post-harvest process.

quent increase could be attributed to the formation of new, more potent antioxidant compounds. While the fermented *Syzygium polyanthum* samples showed improved antioxidant activity, they did not match the potency of quercetin. However, the significant improvement after five days of fermentation indicates that this process effectively enhances the leaves' antioxidant properties (Table 1). This aligns with findings by Herlina et al., (2024), who reported that fermentation can significantly increase the antioxidant activity of plant materials.

The antioxidant capacity of crude extracts generally demonstrates lower activity compared to pure compounds due to several key factors rooted in their chemical composition and molecular interactions. The primary reason lies in the complex matrix of crude extracts, which contains numerous compounds that can interact with and potentially inhibit each other's activities. According to research by Olszowy-Tomczyk (2020), these interactions often lead to antagonistic effects, where the presence of multiple compounds reduces the overall antioxidant efficacy compared to isolated pure compounds. The presence of interfering substances in crude extracts also plays a crucial role. Kumar et al. (2016) demonstrated that non-antioxidant compounds in crude extracts can compete for binding sites with antioxidant molecules, effectively reducing their capacity to neutralize free radicals. Furthermore, the concentration factor significantly impacts antioxidant performance. As highlighted by Nowak et al. (2022), pure compounds exist in higher concentrations per unit volume compared to their presence in crude extracts, where they are diluted by other

Table 1. Antioxidant activity index of *Syzygium polyanthum* leaf extract from various post-harvest process

Sampel	IC_{50} (ppm)	AAI
Quercetin	4.60[a]	21.74[b]
Fresh Leaves	146.54[a]	0.68[b]
3-day Fermentation	185.47[a]	0.54[b]
5-day Fermentation	51.89[a]	1.93[b]

Data are presented as mean ± SD (n=3) and statistically analyzed using One-Way ANOVA, Tukey's Test. [a,b]significantly different ($p<0.005$).

constituents. This dilution effect directly influences the ability to reach effective concentrations necessary for optimal antioxidant activity.

The positive correlation between flavonoid and phenolic content and antioxidant activity supports the widely accepted understanding that these compounds contribute significantly to antioxidant properties (Awwaliah et al., 2023). The enhancement through fermentation could be attributed to several factors, including the breakdown of cell walls, microbial metabolism producing new bioactive compounds, and enzymatic reactions releasing bound phenolics (Adebo & Medina-Meza, 2020; Melini & Melini, 2021).

Overall, this study demonstrates that fermentation is an effective method to enhance the flavonoid and phenol content, as well as the antioxidant activity of *Syzygium polyanthum* leaves. The optimal fermentation time appears to be around five days, but further research could explore longer fermentation periods or different fermentation conditions to potentially achieve even better results. These findings have important implications for improving the nutritional and medicinal value of S. polyanthum in food and pharmaceutical applications, as suggested by similar studies on other plant materials (Septembre-Malaterre et al., 2018).

CONCLUSION

Our findings demonstrated fermentations significantly influence the TFC and antioxidant activity of *Syzygium polyanthum*, in which longer fermentation durations resulted in higher TFC (279.850 mg QE/g) and a stronger AAI (1.93). However, the fermentation did not significantly influence TPC of *Syzygium polyanthum*, potentially due to the reduction of some phenolic compound which leads to the inbalance of the increase of overall TPC. Further research on other factors including temperature and the use of microbial fermentation are necessary to be undertaken for providing a more comprehensive understanding on how fermentation influence TFC, TPC, as well as the antioxidant activity of *Syzygium polyanthum*.

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