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A Biotechnological Approach to Flavonoid Enrichment of Red Andong (*Cordyline fruticosa* (L.) A. Chev.) Leaves via *Bacillus subtilis* Solid-State Fermentation

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Abstract. *Red andong (Cordyline fruticosa (L.) A. Chev.) is a medicinal plant widely used in Indonesian traditional medicine and is known to contain secondary metabolites such as flavonoids, saponins, and polyphenols with demonstrated pharmacological value. Flavonoids are of particular interest due to their antioxidant, antibacterial, anti-inflammatory, and antidiabetic properties. Solid-state fermentation using Bacillus subtilis has emerged as a biotechnological strategy to enhance phytochemical content and bioavailability. This study aimed to: (i) quantify the total flavonoid content (TFC) of red andong leaf extracts subjected to B. subtilis ITBCCB112-mediated solid-state fermentation at 24 and 48 hours, compared to non-fermented controls; (ii) evaluate extraction yield as a function of fermentation duration; and (iii) assess the organoleptic characteristics of fermented versus non-fermented extracts. Fresh leaves were fermented at 37°C, followed by maceration in 70% ethanol. TFC was determined spectrophotometrically using the $AlCl_3$ colorimetric method with quercetin as the standard. The results showed a progressive increase in TFC from 0.34% (non-fermented) to 0.96% (24 hours) and 1.36% (48 hours), expressed as quercetin equivalents. These findings indicate that B. subtilis-mediated fermentation is an effective biotechnological approach for enhancing flavonoid content in C. fruticosa, with 48 hours producing the highest TFC among the durations tested. This study contributes to the valorization of Indonesian medicinal plants through microbial bioconversion strategies, with potential applications in pharmaceutical and nutraceutical product development.*

Keywords: *Bacillus subtilis; bioconversion; Cordyline fruticosa; flavonoids; solid-state fermentation; medicinal plants.*

Type of the Paper: Regular Article.

1. Introduction

Medicinal plants form the backbone of primary healthcare in many developing nations, and Indonesia is recognized as one of the world's most significant repositories of botanical diversity [1,2]. The country harbors an estimated 30,000 vascular plant species, yet a large proportion remains incompletely characterized with respect to their phytochemical and pharmacological properties [3]. This remarkable biodiversity has underpinned a rich tradition of ethnomedicine, and over recent decades, scientific interest in Indonesian medicinal plants has intensified as researchers strive to validate traditional uses and optimize their therapeutic potential [4].

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Among Indonesia's diverse ethnomedicinal flora, red andong (*Cordyline fruticosa* (L.) A. Chev., family Asparagaceae) occupies a distinguished position. Known regionally as hanjuang (Sundanese), linjuang (Makassar), anderuwang (Lampung), and weluga (Ambonese), the plant has long been used in traditional medicine across the archipelago to treat dysentery, hemorrhage, febrile conditions, and inflammatory conditions [5,6]. Phytochemical studies have shown that red Andong leaves contain a broad range of bioactive secondary metabolites, including flavonoids, alkaloids, saponins, tannins, polyphenols, steroids, and triterpenoids, with total phenolic content reaching 102.6 mg GAE/g dry weight [5].

Flavonoids are among the most structurally diverse and pharmacologically important classes of plant secondary metabolites. These polyphenolic compounds, defined by a C6–C3–C6 carbon skeleton, comprise over 9,000 identified structures distributed across subclasses, including flavones, flavonols, flavanones, isoflavones, and anthocyanins [7,8]. Their biological effects are remarkably broad, they scavenge free radicals, disrupt bacterial membranes, modulate inflammatory cytokines, inhibit alpha-glucosidase, and suppress proliferation in cancer cell lines [9,10]. Quercetin, a flavonol found in *C. fruticosa*, contributes to the antioxidant activity of red andong extracts and is routinely used as the reference standard for spectrophotometric flavonoid assays [5,11].

Despite red andong's promising bioactive profile, raw material often contains flavonoids and related phytochemicals at concentrations too low to elicit robust therapeutic effects. These levels also fluctuate considerably depending on geographic origin, harvest time, and post-harvest processing [12]. To address this limitation, researchers have turned to biotechnological strategies that enhance both the yield and bioavailability of target phytochemicals from plant matrices. Among these approaches, microbial fermentation—particularly solid-state fermentation (SSF), stands out as a powerful, cost-effective, and environmentally sustainable option [13,14].

Fermentation boosts phytochemical content through multiple complementary mechanisms. The enzymes produced by fermenting microorganisms break down plant cell wall polysaccharides—cellulose, hemicellulose, and pectin, thereby releasing phenolic compounds that are trapped within the structural matrix [15,16]. At the same time, microbial glycosidases convert flavonoid glycosides into their more bioactive aglycone forms, while esterases cleave phenolic acids that are ester-linked to cell wall components, expanding the pool of extractable phenolics [17,18]. Together, these enzymatic activities increase the concentrations of bioavailable phytochemicals compared to what is obtained from non-fermented material.

Bacillus subtilis is a gram-positive, spore-forming soil bacterium with a long history of application in food biotechnology and industrial enzyme production. The organism produces a

wide range of extracellular hydrolytic enzymes, including subtilisin-type proteases, cellulases, hemicellulases, pectinases, amylases, and lipases, that work together to efficiently break down complex plant biomass [19,20]. Moreover, *B. subtilis* carries the Generally Recognized as Safe (GRAS) designation from the US Food and Drug Administration and is approved for food and fermentation applications in many countries, which makes it well-suited for pharmaceutical and nutraceutical use [21].

A growing body of evidence supports the effectiveness of *B. subtilis*-mediated fermentation for enhancing phytochemical content. Ali et al. [22] found that solid-state fermentation of *Moringa oleifera* leaves with *B. subtilis* progressively raised total phenolic content from 186.72 to 310.25 mg GAE/g over 48 hours, accompanied by improved antioxidant activity. Similar findings have been reported for soybean [23], buckwheat [24], and black rice [25], indicating that SSF with gram-positive bacteria is a broadly applicable strategy for phytochemical enhancement. Consistent with these observations, *B. subtilis* fermentation has been shown to significantly boost both antioxidant activity and flavonoid content in diverse plant leaf extracts compared to non-fermented controls [26], further supporting the broader applicability of this biotechnological approach.

The $AlCl_3$ colorimetric method is a standardized and reproducible technique for quantifying flavones and flavonols based on their ability to form stable aluminum chelate complexes, with quercetin serving as the reference standard [27]. This method is recognized in the Indonesian Herbal Pharmacopoeia *(Farmakope Herbal Indonesia, FHI)* and yields reliable comparative data across different sample preparations and fermentation conditions [28,29]. Accurately measuring TFC is essential, not only for assessing how the fermentation alters the phytochemical profile but also for establishing quality control standards in the future herbal product development.

The study had three main objectives: (i) to measure TFC in red andong leaf extracts after 24 and 48 hours of *B. subtilis* ITBCCB112-mediated SSF, compared to non-fermented controls; (ii) to determine how fermentation duration affects extraction yield; and (iii) to evaluate the organoleptic properties of fermented versus non-fermented extracts. The findings will provide a robust scientific basis for the biotechnological valorization of *C. fruticosa* and support the development of fermentation-enhanced standardized herbal preparations with improved therapeutic efficacy.

2. Materials and Methods

2.1. Plant material and botanical authentication

Fresh leaves of *Cordyline fruticosa* (L.) A. Chev. were collected from Karawang Regency, West Java, Indonesia in October 2025. Botanical identification was performed at Herbarium Jatinangorense, Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics

and Natural Sciences, Universitas Padjadjaran (UNPAD), Bandung, Indonesia, where a voucher specimen (Identification Sheet No. 51/HB/10/2025) was deposited for future reference. The leaves were then subjected to wet sorting, thoroughly washed under running water, surface-dried, and cut into uniform pieces to ensure homogeneous fermentation.

2.2. Preparation of bacterial inoculum

Bacillus subtilis ITBCCB112 was obtained from the Laboratory of Microbiology and Bioprocess Technology, Department of Chemical Engineering, Institut Teknologi Bandung (ITB), Indonesia (Certificate of Analysis No. 18/LAB.MTB/10/2025). The strain was revived on nutrient agar (Oxoid) and incubated at 37°C for 24 hours. Working inocula were prepared by suspending a loopful of biomass in sterile distilled water, and turbidity was adjusted to match McFarland 0.5 standard, which corresponds to a cell density of (1–2) × 10^8 CFU/mL [30]. Inoculum density was then confirmed spectrophotometrically at 625 nm, with absorbance falling within the validated range of 0.08–0.13 [31].

2.3. Solid-state fermentation

The solid-state fermentation (SSF) procedure was adapted from Ali et al. [22] with modifications suitable for *C. fruticosa* leaf substrate. Freshly sliced leaves (90 g per replicate batch) were sterilized by autoclaving at 121°C for 30 minutes, then equilibrated to 40 ± 3°C to prevent thermal inactivation of the inoculum. The sterile leaves were inoculated with 5 mL of *B. subtilis* starter suspension at (1–2) × 10^8 CFU/mL and mixed thoroughly to ensure uniform distribution of the inoculum throughout the substrate. Inoculated samples were incubated in hermetically sealed sterile glass jars at 37°C for either 24 or 48 hours. Non-fermented controls underwent identical autoclaving without inoculation. All treatments were conducted in triplicate (n = 3) to enable statistical analysis.

2.4. Extraction

After fermentation, plant material from each treatment was macerated in 70% ethanol (1:10 w/v) for 24 hours at room temperature with intermittent agitation every 6 hours. The macerate was filtered through Whatman No. 1 filter paper, and the clarified filtrate was centrifuged at 3,000 rpm for 10 minutes to remove residual particulates. The supernatant was concentrated under reduced pressure using a rotary evaporator (Heidolph Laborota 4001, Germany) at 50°C, followed by further evaporation on a water bath at 60°C to produce a viscous extract, which was designated as red andong leaf extract (RALE). Extraction yield (%) was calculated as the ratio of dried extract mass to starting fresh leaf mass, multiplied by 100.

The selection of 70% ethanol as the extraction solvent was because of its established effectiveness in recovering polyphenols and flavonoids from plant matrices. The hydroalcoholic

solvent system facilitates the solvation of both polar glycosylated flavonoids and less polar aglycone forms, thereby optimizing total flavonoid recovery [32]. Maesaroh et al. [33] previously demonstrated that 70% ethanolic extraction yielded superior flavonoid recovery from red andong leaves compared to 96% ethanol, further supporting this solvent selection.

2.5. Organoleptic evaluation

Each RALE was evaluated for color, aroma, and texture by two independently trained evaluators under standardized environmental conditions. The results were recorded descriptively and compared across fermentation treatments.

2.6. Quercetin calibration standard

A stock solution of quercetin (100 mg/100 mL in 96% ethanol P) was prepared and serially diluted to working concentrations of 10, 25, 50, 75, and 100 µg/mL. To construct the calibration curve, 2.0 mL of each standard was combined with 0.1 mL $AlCl_3$ (10% w/v), 0.1 mL sodium acetate (1 M), and 2.8 mL distilled water. After 30 minutes of equilibration at room temperature, absorbance was measured at 425 nm using a UV–Vis spectrophotometer (Genesys 10S, Thermo Scientific). Each concentration was measured in triplicate. A linear regression model was then fitted to the absorbance–concentration data [28,29].

2.7. Total flavonoid content determination

For TFC determination, 0.2 g of each RALE sample was dissolved in 25 mL 96% ethanol P with magnetic stirring for 30 minutes. The solution was then filtered into a 25 mL volumetric flask, and diluted to the mark with 96% ethanol P. Aliquots of 0.5 mL were transferred to individual vials and combined with 1.5 mL ethanol P, 0.1 mL $AlCl_3$ (10%), 0.1 mL sodium acetate (1 M), and 2.8 mL distilled water. After 30 minutes of equilibration, absorbance was recorded at 425 nm. All measurements were performed in triplicate. TFC was interpolated from the quercetin calibration curve and expressed as percentage quercetin equivalents (% QE) using Eq. (1) [29]:

$$F\,(\%) = (C \times V \times f \times 10^{-6} / m) \times 100 \quad (1)$$

where F = TFC (% QE); C = quercetin-equivalent concentration from calibration (µg/mL); V = total extract volume (mL); f = dilution factor; m = sample mass (g).

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD) of triplicate measurements (n = 3). Differences in TFC among the non-fermented, 24-hour, and 48-hour fermentation groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test, with $p < 0.05$ considered statistically significant. All statistical analyses were performed in Python using the SciPy and statsmodels libraries. The progressive

trend in TFC across fermentation durations is interpreted in the context of *B. subtilis* growth kinetics and enzymatic activity profiles reported in the literature.

3. Results and Discussion

3.1. Organoleptic characteristics

Organoleptic evaluation, conducted by two independently trained evaluators, revealed consistent color (brownish red), aroma (characteristic), and texture (viscous) across all RALE samples, regardless of fermentation duration. The consistent brownish-red coloration is primarily attributed to the high anthocyanin content of *C. fruticosa* leaves, which contain substantial quantities of cyanidin and delphinidin glycosides responsible for the distinctive red color of the leaves and their extracts [34]. The thermal processing steps used during extraction—rotary evaporation and water bath concentration—may have induced partial anthocyanin degradation or structural transformation, resulting in the brownish hue observed across all samples [35]. Fermentation did not noticeably alter the macroscopic sensory profile of the extracts, suggesting that although metabolic changes occurred at the molecular level, the overall organoleptic character remained intact—an important factor for end-user acceptability in herbal product formulations [36].

3.2. Extraction yield

Extraction yields across fermentation treatments are presented in Table 1. The non-fermented RALE (NFE) gave the highest yield (5.84%), followed by the 24-hour extract (EF24, 5.19%) and the 48-hour extract (EF48, 4.90%). The reduction in yield with increasing fermentation duration likely reflects microbial catabolism of non-phenolic plant constituents—primarily structural carbohydrates and simple sugars—during fermentation, which reduces the total extractable dry matter available for recovery during ethanolic maceration [37]. Bacterial metabolism consumes plant-derived carbon sources as energy substrates, thereby lowering total biomass dry weight without proportionally affecting the phenolic fraction, which is released rather than catabolized during early fermentation stages [15].

Table 1. Extraction yield (%) of RALE under different fermentation conditions.

Sample	Empty Container (g)	Container + Extract (g)	Extract Weight (g)	Yield (%)
NFE (RALE, Non-Fermented)	78.3197	81.2434	2.9237	5.84
EF24 (RALE, 24-h Fermented)	112.4266	115.0271	2.5985	5.19
EF48 (RALE, 48-h Fermented)	108.6645	111.1183	2.4538	4.90

Note: RALE = Red andong leaf extract; NFE = Non-Fermented Extract, EF24/EF48 = Extract Fermented for 24/48 h.

The inter-group yield differences observed in this study (< 1.0%) were relatively minor and are unlikely to have practical significance in large-scale production settings. Similar observations were reported by Ali et al. [22] for *B. subtilis*-fermented *Moringa oleifera*, where fermentation-induced yield changes were likewise modest despite substantial phytochemical enrichment. The 70% ethanol solvent system employed in this study is widely regarded as optimal for extracting both polar glycosylated flavonoids and less polar aglycone forms from plant matrices, consistent with the known solvent polarity preferences of different flavonoid subclasses [32]. This solvent choice was further supported by Maesaroh et al. [33], who demonstrated its superiority over 96% ethanol for total flavonoid recovery from the same plant species.

The inverse relationship between fermentation duration and extraction yield aligns with published findings on bacterial SSF of plant materials. Wink [12] noted that microbial degradation of plant matrices during fermentation involves progressive breakdown of structural biopolymers, releasing monomeric and oligomeric components that may then serve as substrates for further microbial catabolism. The net effect is a reduction in total extractable dry matter, even as phenolic compounds are liberated and concentrated. This dynamic highlights the importance of optimizing fermentation duration to balance maximal phenolic liberation against excessive substrate catabolism—a balance that appears to have been achieved at 48 hours in this study.

The quercetin calibration curve demonstrated excellent linearity over the concentration range 10–100 µg/mL, yielding the regression equation $y = 0.0071x + 0.0215$ ($R^2 = 0.9989$). The high coefficient of determination confirms adequate linearity and validates the method for TFC quantification across the tested concentration range, in accordance with International Council for Harmonisation (ICH) Q2(R1) guidelines for analytical method validation [38]. Quercetin was selected as the calibration standard because of its well-established role as a representative flavonol with well-characterized aluminum chelation properties, its widespread presence in *C. fruticosa*, and its designation as the reference compound for flavonoid quantification in FHI methodology [29,39].

The $AlCl_3$ colorimetric assay used in this study is specifically designed to quantify flavones and flavonols through the formation of stable, acid-resistant chelate complexes between Al^{3+} ions and the adjacent 3-OH/4-keto or 5-OH/4-keto groups found in these flavonoid subclasses [28]. Sodium acetate is incorporated to detect free 7-OH groups, thereby extending the specificity of the assay. However, the method does not detect flavanones or isoflavones because they lack the requisite hydroxyl-carbonyl chelation sites—a recognized limitation that should be considered when interpreting TFC values from plant matrices structurally diverse flavonoid profiles [40]. Future studies using HPLC-DAD or LC-MS/MS profiling would enable comprehensive identification and quantification of individual flavonoid species [41].

3.3. Total flavonoid content

Total flavonoid content (TFC) values across fermentation treatments are presented in Table 2. Fermentation duration produced a clear, statistically consistent, and progressive increase in TFC: 0.34 ± 0.012% (non-fermented), 0.96 ± 0.048% (24 h), and 1.36 ± 0.009% (48 h), all expressed as quercetin equivalents. These values represent 2.82-fold and 4.00-fold increases over the non-fermented controls at 24 and 48 hours, respectively. The low standard deviation—particularly at 48 h (± 0.009%)—indicates excellent inter-replicate reproducibility and confirm the robustness of the fermentation protocol. One-way ANOVA confirmed a highly significant effect of fermentation duration on TFC ($F(2,6) = 594.6$, $p < 0.0001$). Tukey's HSD post hoc test indicated that all pairwise comparisons among the non-fermented, 24-hour, and 48-hour groups were statistically significant ($p < 0.05$ for all pairs; Table 2), confirming that the observed increases in TFC were not due to random variation.

Table 2. Total flavonoid content (TFC) of RALE expressed as quercetin equivalents (%).

Sample	Mean ± SD (%)
NFE (RALE, Non-Fermented)	0.34 ± 0.012 [a]
EF24 (RALE, 24-h Fermented)	0.96 ± 0.048 [b]
EF48 (RALE, 48-h Fermented)	1.36 ± 0.009 [c]

Note: Values represent mean ± SD (n = 3); RALE = Red andong leaf extract; NFE = Non-Fermented Extract, EF24/EF48 = Extract Fermented for 24/48 h. Different superscript letters (a, b, c) within the Mean ± SD column indicate statistically significant differences among groups (one-way ANOVA with Tukey's HSD post hoc test, p < 0.05).

The fermentation-induced increase in TFC likely stems from several complementary mechanisms. *B. subtilis* produces a range of cell-wall-degrading enzymes, including endo- and exo- glucanases, hemicellulases, and polygalacturonases, which collectively disrupt the structural integrity of the plant cell wall during SSF [19,42]. This enzymatic deconstruction liberates flavonoids and other phenolics that are either physically entrapped within cellulose microfibrils or covalently bound to hemicellulose and lignin components via hydroxycinnamic acid bridges [15,16]. At the same time, *B. subtilis* β-glucosidase activity hydrolyzes flavonoid O-glycosides into their aglycone forms, which are more lipophilic and therefore more readily extracted by the 70% ethanol solvent system [17,18]. The overall effect is a marked increase in the concentration of $AlCl_3$-reactive flavonoid species in the final extract.

The difference in TFC between 24-hour and 48-hour fermented extracts (0.96 vs. 1.36%) can be explained by the growth kinetics of *B. subtilis*. According to Efendi et al. [43], *B. subtilis* displays a lag phase approximately from 0 to approximately 24 hours, an exponential growth phase from 24 to 36 hours, the onset of stationary phase at around 46 hours, and entry into decline phase after 50 hours. At 24 hours, the bacterium is still completing its lag phase, during which enzyme secretion has begun but has not reached maximal levels; this results in only partial cell wall

degradation and moderate flavonoid release. By 48 hours, the culture has moved through the exponential phase and entered early stationary phase, when the cumulative enzyme pool is at its highest and has acted on the substrate for a longer period. This temporal match between peak enzymatic activity and maximum flavonoid accumulation at 48 hours provides a plausible explanation for the observed TFC optimum.

The findings align closely with reports from the broader fermentation literature. Ali et al. [22] reported peak total phenolic content (TPC) in fermented *Moringa oleifera* at 48 hours (310.25 mg GAE/g) compared with 186.72 mg GAE/g in non-fermented controls, along with a corresponding peak in antioxidant activity—a pattern that closely mirrors the TFC trajectory observed in this study. Similarly, Bo et al. [25] documented significant increases in both total phenolic content and antioxidant capacity in *B. subtilis*-fermented black rice. Chen et al. [44] showed that *Lactobacillus brevis*-mediated fermentation of *Citrus aurantium* flowers produced markedly improved anti-tyrosinase, antioxidant, and antimicrobial activities, further supporting the broad applicability of bacterial SSF for phytochemical enhancement across diverse plant materials.

The correlation between TFC and improved bioactivity in fermented red andong extracts is well supported by mechanistic evidence. Flavonoids contribute to antioxidant activity primarily through electron donation, hydrogen atom transfer, and metal chelation, and their concentration largely determines the radical-scavenging capacity measured by DPPH and ABTS assays [45]. These findings are consistent with published evidence demonstrating that *B. subtilis* fermentation significantly elevates both TFC and antioxidant activity in plant leaf extracts [26], supporting the hypothesis that fermentation-induced flavonoid enrichment drives the observed improvement in bioactivity.

It is important to note that extending fermentation beyond the optimal window can lead to a decline in TFC, a phenomenon also reported in *B. subtilis*-fermented plant extracts when incubation extends beyond the enzymatic activity [26]. This decline likely stems from the progressive depletion of fermentable nutrients, a reduction in viable bacterial population and enzymatic output, and potential oxidative catabolism of liberated phenolics by bacterial laccases and peroxidases once alternative carbon sources are exhausted [37]. The 48-hour time point identified in this study represents a balanced condition that maximizes flavonoid liberation while minimizing the risk of phenolic degradation. Sulasiyah et al. [46] similarly found that optimal antioxidant enhancement in *Curcuma longa* fermented with *Aspergillus oryzae* occurred at an intermediate fermentation time, beyond which bioactivity declined.

From a translational standpoint, the 4.00-fold increase in TFC achieved through *B. subtilis* SSF represents a substantial improvement in the pharmacological value of red andong extracts with direct implications for herbal product development. *B. subtilis* carries the GRAS (Generally Recognized as Safe) designation and is approved for food and pharmaceutical applications in many countries [21], supporting the feasibility of integrating SSF into the upstream processing of *C. fruticosa*-based preparations. Yang et al. [47] demonstrated that bacterial fermentation can also enhance the sensory and flavor profiles of plant-based products, an additional factor to consider for consumer acceptance of fermented herbal formulations. Future research should focus on scaling up the SSF process in bioreactor systems, optimizing inoculum density and substrate-to-liquid ratio, conducting comprehensive HPLC-based characterization of individual flavonoid species across fermentation time points, and validating bioactivity in preclinical models.

Direct experimental support for this hypothesis comes from a companion study from the same plant species [48], in which red andong leaves were fermented with *B. subtilis* for 24, 48, and 72 hours and then evaluated for total polyphenol content (Folin–Ciocalteu assay), antioxidant capacity (DPPH and FRAP assays), and in silico target prediction. Total polyphenol content increased from 29.11 ± 0.90 mg GAE/g in non-fermented extracts to 63.03 ± 0.90 mg GAE/g (24 h) and 73.54 ± 0.98 mg GAE/g (48 h), before declining to 38.56 ± 0.82 mg GAE/g at 72 hours. The trend closely parallels the TFC pattern observed in this study, with both parameters peaking at 48 hours and decreasing thereafter—consistent with the fermentation kinetics discussed earlier. At the same time, DPPH radical-scavenging activity improved substantially with fermentation, as IC_{50} values decreasing from 39.297 µg/mL (non-fermented) to 13.159 µg/mL (24 h) and 10.488 µg/mL (48 h), before rising again to 37.872 µg/mL at 72 h—a lower IC_{50} value indicating stronger antioxidant activity. FRAP assay results corroborated this trend, confirming a progressive increase in reducing power that was most pronounced at 48 hours. In silico docking analysis from that study further suggested that fermentation-derived compounds, including phytol and α-tocopherol, may contribute to the observed antioxidant activity through modulation of CDK6, AR, and PKM targets. Collectively, these complementary findings provide direct chemical and bioactivity evidence—supporting the TFC data reported here—that 48 hours of *B. subtilis* fermentation produces the most favorable phytochemical and antioxidant profile among the durations tested, while fermentation beyond 48 hours is associated with a decline in both polyphenol content and antioxidant capacity.

4. Conclusions

Solid-state fermentation mediated by *Bacillus subtilis* ITBCCB112 significantly and progressively enhanced the total flavonoid content (TFC) of red andong (*Cordyline fruticosa*)

leaf extracts in a time-dependent manner. TFC increased from 0.34% (non-fermented) to 0.96% (24 h) and 1.36% (48 h), representing an approximately 4.0-fold enhancement, with differences among all three groups confirmed as statistically significant by one-way ANOVA and Tukey's HSD post hoc test ($p < 0.05$). Among the three durations evaluated, 48 hours produced the highest TFC; however, longer or intermediate durations were not tested, and this finding should not be interpreted as establishing a true process optimum. Extraction yield decreased only marginally with fermentation and remained within practical limits, while organoleptic characteristics were preserved across all treatments. These findings, together with corroborating antioxidant and polyphenol data from a companion study on the same plant material, indicate that *B. subtilis*-mediated SSF represents a promising and scalable biotechnological approach for the phytochemical valorization of *C. fruticosa*. Based on the conditions evaluated, 48 hours of fermentation is recommended as the duration producing the highest flavonoid content; however further studies incorporating additional time points, extraction-weight data, and direct antioxidant and chromatographic profiling of the present extracts are needed to establish a formally optimized processing parameter.

Abbreviations

$AlCl_3$	: Aluminum chloride
ABTS	: 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
CFU	: Colony-forming units
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
FHI	: Farmakope Herbal Indonesia
GAE	: Gallic acid equivalents
GRAS	: Generally Recognized As Safe
HPLC	: High-performance liquid chromatography
ICH	: International Council for Harmonisation
LC-MS/MS	: Liquid chromatography–tandem mass spectrometry
QE	: Quercetin equivalents
RALE	: Red andong leaf extract
SD	: Standard deviation
SSF	: Solid-state fermentation
TFC	: Total flavonoid content
TPC	: Total phenolic content
UV–Vis	: Ultraviolet–visible spectrophotometry.

Data Availability Statement

Data supporting the results reported in this paper are available from the corresponding author upon reasonable request.

CRediT Authorship Contribution Statement

Rian Maulana Muttaqin: Investigation, Data curation, Formal analysis, Writing – original draft. **Fahrauk Faramayuda**: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review and editing. **Soraya Riyanti**: Methodology, Validation, Resources, Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Use of AI in the Writing Process

The authors declare that AI writing assistance tools were not used in the preparation of this manuscript.

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