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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF RIPE *Muntingia calabura* FRUIT FERMENTED WITH *Saccharomyces cerevisiae*

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Abstract

Muntingia calabura fruit contains numerous phytochemicals with medicinal value; however, its utilization remains limited due to rapid ripening and poor storability. Fermentation using Saccharomyces cerevisiae is considered a promising approach to extend shelf life and preserve bioactive compounds. This study aimed to evaluate the phytochemical composition and antioxidant activity of fermented extracts from ripe M. calabura fruits. Phytochemical screening, the Folin–Ciocalteu method (for total phenolic content), the aluminum chloride colorimetric method (for total flavonoid content), and the DPPH radical scavenging assay were employed. The fermented product exhibited 8% ethanol, 8.5° Brix, and pH 4.0. The phytochemical constituents detected included saponins, polyphenols, cardiac glycosides, triterpenoids, and alkaloids. The total phenolic content was 95.87 ± 2.00 mg GAE/g, the total flavonoid content was 6.96 ± 0.21 mg QE/g, and antioxidant activity was expressed by an IC_{50} value of 203.9 ± 1.0 µg/mL. Fermentation of M. calabura fruit with S. cerevisiae effectively preserved, demonstrating strong potential for the development of natural fermented beverages and functional food products derived from this tropical fruit.

Keywords: *Antioxidant activity, flavonoids, phenolics, Muntingia calabura, Saccharomyces cerevisiae.*

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THÀNH PHẦN HÓA THỰC VẬT VÀ HOẠT TÍNH CHỐNG OXY HÓA CỦA QUẢ TRỨNG CÁ (*Muntingia calabura*) CHÍN LÊN MEN VỚI *Saccharomyces cerevisiae*

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Tóm tắt

Quả Muntingia calabura chín chứa nhiều hợp chất có giá trị dược học, tuy nhiên việc ứng dụng còn hạn chế do quả chín nhanh và khó bảo quản. Quá trình lên men bằng nấm men Saccharomyces cerevisiae là hướng khai thác tiềm năng giúp kéo dài thời gian sử dụng và bảo tồn các hợp chất có hoạt tính sinh học. Nghiên cứu này được tiến hành nhằm đánh giá thành phần hóa (học) thực vật và hoạt tính chống oxy hóa của dịch chiết lên men từ quả M. calabura chín. Các phương pháp được sử dụng bao gồm sàng lọc hóa thực vật, phương pháp Folin–Ciocalteu (xác định tổng hàm lượng phenolic), phương pháp so màu Aluminum chloride (xác định tổng hàm lượng flavonoid) và phép thử DPPH (đánh giá khả năng bắt gốc tự do). Kết quả cho thấy dịch lên men có nồng độ ethanol 8%, Brix 8,5^o, pH 4,0. Thành phần hóa thực vật được phát hiện gồm: saponin, polyphenol, glycoside tim, triterpenoid và alkaloid. Hàm lượng phenolic tổng đạt 95,87 ± 2,00 mg GAE/g, flavonoid tổng đạt 6,96 ± 0,21 mg QE/g, và hoạt tính chống oxy hóa thể hiện qua IC_{50} = 203,9 ± 1,0 µg/mL. Quá trình lên men quả M. calabura bằng/với S. cerevisiae đã mở ra tiềm năng trong phát triển các loại đồ uống lên men tự nhiên và thực phẩm chức năng từ loại quả nhiệt đới này.

Từ khóa: *Chống oxy hóa, flavonoid, phenolic, Muntingia calabura, Saccharomyces cerevisiae*

1. Introduction

Muntingia calabura L. (family Muntingiaceae), commonly known as Jamaican cherry is a tropical fruit-bearing plant native to Central and South America and widely distributed throughout Southeast Asia. Its fruits are rich in bioactive phytochemicals such as flavonoids, phenolics, saponins, triterpenoids, and alkaloids (Kathirvel, 2017; Vijayanand, 2018), which exhibit antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and gastroprotective properties (Saud et al., 2023; Zakaria et al., 2014). *M. calabura* fruit extract can regulate immunity through the stimulation of IgG production (Sujono et al., 2021). This fruit could protect and increase the viability of fibroblast cells (Nur et al., 2024). Despite these pharmacological potentials, its practical utilization of *M. calabura* remains limited due to the short shelf life and rapid spoilage of its ripe fruits.

Due to its high perishability at the ripe stage and during transportation, *M. calabura* fruit is primarily consumed fresh at the site of harvest or processed into jam and juice. Harshini et al. (2020) reported that ripe fruits intended for juice or jam production require blending with mausambi or apple to achieve acceptable sensory characteristics, with the shelf life extended to approximately 15 days for juice and up to 3 months for jam (Harshini et al., 2020). Although these processing approaches can enhance storability, they may result in a reduction or loss of the inherent bioactive compounds of *M. calabura* fruit due to the incorporation of additional ingredients or exposure to high processing temperatures. Alternatively, ethanol fermentation using *Saccharomyces cerevisiae* has been proposed as another viable method for beverage production (Nasution et al., 2022).

Fermentation using *Saccharomyces cerevisiae* has been recognized as an effective bioprocess to enhance the stability, bioavailability, and antioxidant properties of plant-derived products (Hur et al., 2014; Zhao et al., 2021). This approach can convert highly perishable fruits into functional beverages with improved nutritional and therapeutic value, while also stimulating sensory appeal due to the presence of a mild ethanol content. Therefore, the present study investigates the phytochemical composition and antioxidant activity of fermented extracts from ripe *M. calabura* fruits, aiming to support the development of natural fermented functional foods from this underutilized species.

2. Materials and methods

2.1. Chemicals and Materials

The chemicals used in the research such as: Gallic acid and Folin-ciocalteu regent were brought from Merck, potassium hydrogen phthalate from Panreac, sabouraud dextrose agar from Himedia, sodium carbonate and others from Merck.

Figure 1. The ripe fruit of *M. calabura*

Saccharomyces cerevisiae was brought from Saigon Yeast Company – Vietnam. *S. cerevisiae* was selected in this study because it is the most widely used yeast in the fermented food and beverage industry, with a high level of safety (GRAS status), stable ethanol-producing capability, and a rich enzymatic system that can contribute to the release of phenolic compounds. The use of a single yeast strain also allows for better control of the fermentation process and minimizes biological variability.

Ripe *M. calabura* fruit were collected from Binh Thuy Ward, Can Tho City. Figure 1 shows the image of ripe *M. calabura* fruit.

2.2. Fermented *Muntingia calabura fruit with Saccharomyces cerevisiae*

The fermentation of the fruits using *S. cerevisiae* was was performed following the method described by Nguyen et al. (2019) with minor modifications. Ripe fruits at maturity stage 3 were selected (Nasution et al. 2022), while damaged or spoiled fruits were discarded. The selected fruits were washed, drained, and blended with 30% (w/v) distilled water using a blender. The resulting slurry was supplemented with 0.15% (v/v) pectinase and left to stand for 30 minutes to facilitate tissue breakdown (Nguyen et al., 2019).

Figure 2. Ripening stages of *M. calabura* fruit

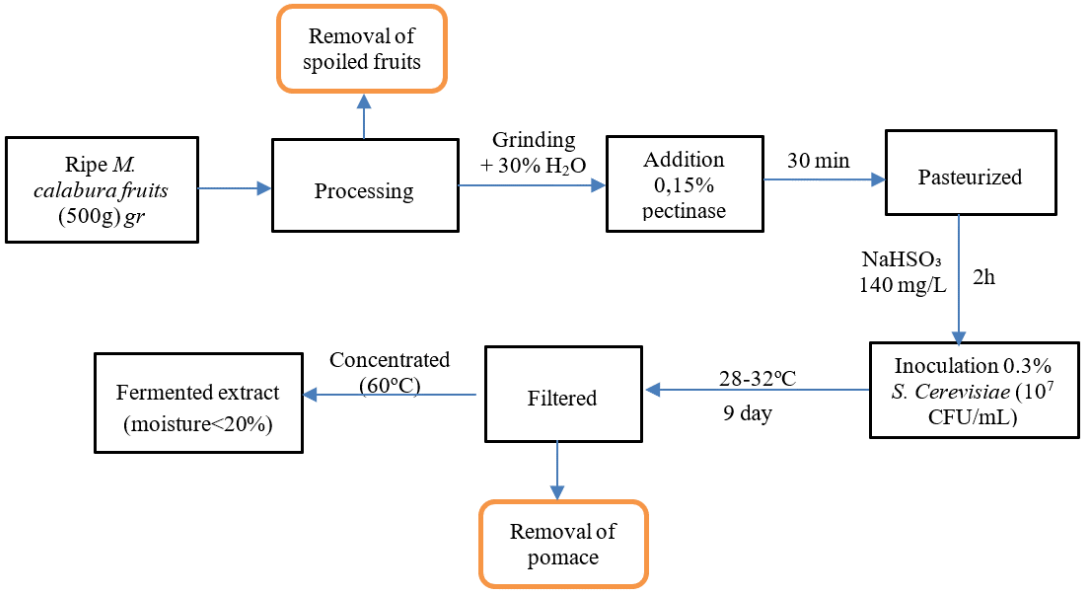

Figure 3. The fermentation process of ripe *M. calabura* fruit

The initial °Brix value and pH of the fruit mash were measured using a digital refractometer (Atago N1, Japan) and a pH meter (Adwa AD 1030, Romania), respectively, followed by pasteurization with a $NaHSO_3$ solution (140 mg/L) for 2 h. The pasteurized mash was then inoculated with *Saccharomyces cerevisiae* at an initial viable cell density of 3×10^7 CFU/mL, and fermentation was conducted under anaerobic conditions at room temperature (28–32 °C) for 9 days. The fermented mixture was then filtered to obtain the fermented extract, which was analyzed for Brix, pH, and ethanol content. Finally, the fermented extract was concentrated in a water bath at 60 °C until the moisture content was below 20% (Fig 3).

2.3. Tests for phytochemical screening

Phytochemical tests were carried out for: Saponins, polyphenols, cardiac glycosides, flavonoids, triterpenoids, and alkaloids. That was determined using standard qualitative tests. Saponins were detected by vigorous shaking of 1 mg extract in 5 mL distilled water, where persistent frothing indicated positivity (Rahim et al., 2024). Polyphenols were confirmed by a dark green to bluish-black color upon adding 5% $FeCl_3$. Cardiac glycosides produced a yellow to orange coloration with Baljet's reagent. Flavonoids were identified by heating 1 mg extract in 50% methanol, followed by the addition of magnesium and concentrated HCl, yielding a red to orange color. Triterpenoids were identified by the appearance of a golden-yellow layer upon adding concentrated H_2SO_4 (Salkowski's test). Alkaloids were confirmed by a light-yellow precipitate with Meyer's reagent (Shaikh & Patil, 2020).

2.4. Determination of total phenolic content and total flavonoid content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method (Feduraev et al., 2019). Briefly, 1 mL of each extract was mixed with 1 mL of 0.2 M Folin–Ciocalteu reagent and incubated for 3 min. Then, 1 mL of 10% (w/v) Na_2CO_3 solution was added, and the mixture was incubated in the dark at 40 °C for 30 min. Absorbance was measured at 765 nm using a Shimadzu UV-1800 UV–Vis spectrophotometer. A standard calibration curve was prepared with gallic acid, and results were expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicate, and data were presented as mean ± SD.

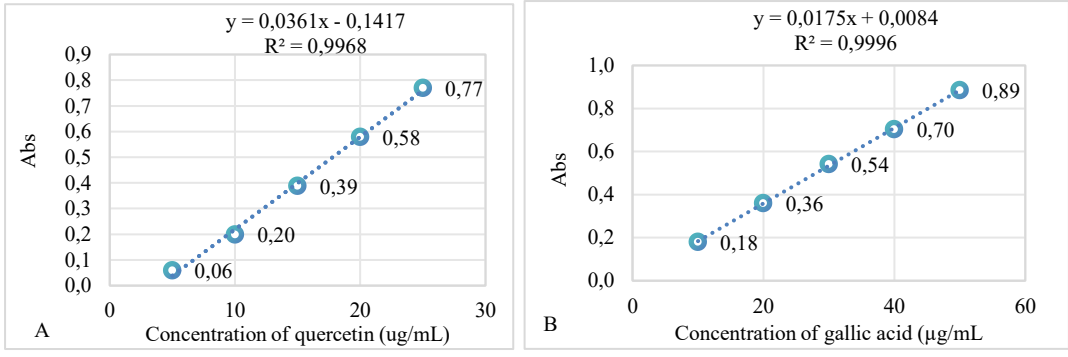

Figure 4. Linear equations of quercetin and gallic acid standards

A. Quercetin; B. Gallic acid

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Dewanto et al., 2002). In brief, 0.5 mL of ethanol extract was mixed with 0.3 mL of 10% (w/v) $AlCl_3$ and 0.3 mL of 10% (w/v) $NaNO_2$, followed by incubation at room temperature for 5 min. Absorbance was measured at 510 nm using the same UV–Vis

spectrophotometer. TFC was calculated from the calibration curve of quercetin and expressed as mg quercetin equivalents (QE)/g dry weight. Each measurement was performed in triplicate, and results were expressed as mean ± SD.

The total flavonoid content (TFC) and total phenolic content (TPC) were quantified based on linear regression equations obtained from the quercetin and gallic acid standards, respectively. Quercetin standard solutions (5-25 µg/mL) and gallic acid standard solutions (10-50 µg/mL) were used to establish linear calibration equations by plotting absorbance versus concentration, with a coefficient of determination (R^2) greater than 0.99. These equations were then applied to calculate the TFC (mg QE/g) and TPC (mg GAE/g) of the samples (Fig. 4)

2.5. Antioxidant Activity: DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay with slight modifications of the method by Khattak and Simpson (2008). For each assay, 1 mL of *M. calabura* fruit extract (fermented ripe fruit: 50–300 µg/mL; non-fermented ripe fruit: 50–600 µg/mL) was mixed with 0.25 mL of 0.6 mM DPPH in methanol. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was recorded at 515 nm using the same UV–Vis spectrophotometer. Ascorbic acid (5–40 µg/mL) served as the positive control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} x100$$

3. Results and discussion

3.1. Physicochemical characteristics after fermentation

After fermentation, the fruit extract exhibited an ethanol concentration of 8%**,** Brix value of 8.5°, and pH of 4.0. These values indicate that *S. cerevisiae* efficiently converted fermentable sugars into ethanol under anaerobic conditions while maintaining moderate residual sugar levels. Similar ethanol yields have been reported in fruit-based fermentations, such as guava and berry wines, with ethanol contents ranging from 6–10% (Nguyen et al., 2019; Hur et al., 2014).

The pH of the fermented wine produced was 4.0, which falls within the recommended range for fruit wines (2.8–4.0) as reported by Ribéreau-Gayon et al. (2021). This range is considered biologically safe because it inhibits the proliferation of undesirable bacteria that can cause spoilage. The final Brix value (8.5°) reflects partial sugar consumption, indicating incomplete fermentation — a desirable characteristic for low-alcohol functional beverages that retain mild sweetness and bioactivity. Thus, the fermentation conditions applied in this study successfully produced a beverage-like extract with balanced ethanol and acidity levels suitable for functional product development.

3.2. Phytochemical composition

Phytochemical screening revealed that the fermented extract of *M. calabura* retained major bioactive groups such as saponins, polyphenols, cardiac glycosides, triterpenoids, and alkaloids (Table 1). These results align with Ariffin et al., (2022) and Sowjanya, (2023), who identified similar phytochemical constituents in fresh *M. calabura* fruits. Moreover, Zhao et al., (2021) reported that yeast fermentation hydrolyzes glycosidic linkages, releasing more free phenolic forms and improving biological efficacy.

Table 1. The phytochemical composition of fermented ripe *M. calabura* fruit

Phytochemicals	Fermented fruit
Triterpenoids	+
Alkaloids	+
Flavonoids	+
Glycosids	+
Polyphenols	+
Saponins	+

3.3. The total phenolic content and total flavonoid content

The TFC of fermented ripe *M. calabura* fruit extract was determined based on the linear equation of quercetin standard $y = 0.0361x - 0.1417$, $R^2 = 0.9968$, and TPC based on the linear standard curve of gallic acid $y = 0.0175x + 0.0084$, $R^2 = 0.9996$. The results are shown in Table 2.

Table 2. TFC and TPC of fermented ripe *Muntingia calabura* fruits

Sample	TFC (mg QE/g)	TPC (mg GAE/g)
Fermented ripe *M. calabura*	6.96 ± 0.21	95.87 ± 2.0

The results presented in Table 2 indicate that the fermented ripe Muntingia calabura fruit exhibited substantially higher total polyphenol content (TPC, 95.87 ± 2.00 mg GAE/g) and total flavonoid content (TFC, 6.96 ± 0.21 mg QE/g) compared to values previously reported for fresh fruit. Zakaria et al. (2014) reported a TPC of approximately 72 mg GAE/g in the fresh fruit. In contrast, Pereira et al. (2018) reported a markedly lower TFC of 0.28 µg quercetin equivalents per gram of dry weight in dried fresh fruit extracts. These findings indicate that fermentation significantly enhances the phenolic and flavonoid compound content of this fruit. The increase in phenolic and flavonoid content can be explained by the enzymatic activity of Saccharomyces cerevisiae secreted, which can break down the structure of plant cell walls and release phenolic compounds bound to cellulose, hemicellulose, or pectin (Hur et al., 2014; Sarıtaş et al., 2024). In addition, fermentation promotes the conversion of complex phenolic compounds to lower molecular weight and higher biological activity forms (Hur et al., 2014). Therefore, fermentation not only acts as a biological pretreatment technology to increase extraction capacity but also contributes to enhancing the overall biological value of fermented products. Flavonoids are a group of compounds that are easily oxidized; however, fermentation environments can help stabilize them by lowering the pH and creating relatively anaerobic conditions, thereby limiting the degradation of flavonoids during processing (Ghosal et al., 2020).

The use of fermented *S. cerevisiae* has been shown to be effective in enhancing the content and bioavailability of phenolic and flavonoid compounds in various plant materials. Studies by Haile and Kang (2019) and Ghosal et al. (2020) showed that yeast fermentation not only increased TPC and TFC but also significantly improved the overall antioxidant activity of the product. Synthesizing these results confirms that the fermentation of M. calabura fruit with S. cerevisiae is a potential biostrategy to increase the functional value and applicability of the material compared to its original fresh form.

3.4. Antioxidant capacity

The antioxidant activity of the fermented fruit was evaluated using the DPPH free radical scavenging assay at concentrations ranging from 25 to 300 µg/mL, shown in Table 3 and Fig. 5.

Table 3. Results of the DPPH antioxidant survey of *M. calabura* fermented extract

Concentration (ug/mL)	Antioxidant activity (%)	
	Fermented ripe *M. calabura*	**Ascorbic acid**
5	x	1.39±3.28
10	x	9.26±2.90
20	x	32.06±2.58
25	4.48±4.24	x
30	x	58.12±1.35
40	x	85.10±0.60
50	12.14±4.76	x
100	26.57±1.08	x
200	49.71±1.18	x
300	72.59±0.13	x

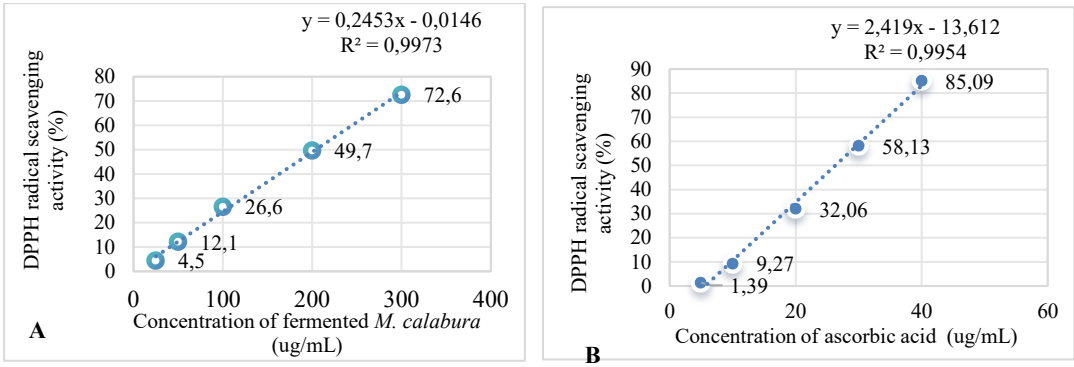

Figure 5. Antioxidant activity of *M. calabura* fruit extract and ascorbic acid at different concentrations

A. Fermented fruit extract; B. Ascorbic acid

The antioxidant activity of the fermented extract, with IC_{50} = 203.9 ± 1.0 µg/mL, indicates a moderate to strong free radical scavenging ability. Compared with the results from Suryani et al. (2021) ($IC_{50} \approx 315$ µg/mL for fresh fruit extract), fermentation clearly enhanced antioxidant potential. This improvement may be attributed to the formation of new phenolic derivatives and increased levels of free phenolics during yeast metabolism (Khattak & Simpson, 2008; Hur et al., 2014). These compounds act as effective electron donors, improving the neutralization of free radicals. The high TPC and TFC values observed in the fermented fruit are consistent with the strong antioxidant activity observed, indicating that the increased levels of phenolic and flavonoid compounds contribute to the enhanced bioactivity of the fermented product.

4. Conclusion

This study demonstrated that fermentation of *M. calabura* fruit using *S. cerevisiae* effectively produced a low-alcohol beverage (8% ethanol, 8.5° Brix, pH 4.5) with high phytochemical content and strong antioxidant capacity. The fermented extract retained key bioactive compounds including saponins, polyphenols, cardiac glycosides, triterpenoids, and alkaloids and exhibited enhanced levels of phenolic and flavonoid compounds. These results confirm that yeast fermentation not only preserves but also improves the bioactive potential of this fruit, making it a promising process for developing natural fermented functional beverages and nutraceutical products derived from tropical fruits. Although research has yielded promising results regarding the bioactivity of fermented products from M. calabura, some experimental factors and conditions have not yet been fully expanded or tested. Therefore, further research is needed, including experiments with different fermentation conditions compared with fresh fruit extracts, comparisons with other preservation methods, and, more importantly, in vivo testing to refine the data and enhance the product's practical feasibility.

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