



Research Article

PHYTOCHEMICAL CHARACTERIZATION, PHENOLIC STABILITY, AND INHIBITORY ACTIVITIES AGAINST α -AMYLASE AND α -GLUCOSIDASE OF A MALE *Carica papaya* L. FLOWER DECOCTION

Ho Linh Kieu Nhi¹, Vong Binh Long², Nguyen Tien Dung¹,

Nguyen Ngoc Phuong³, Nguyen Thi Thuong Huyen^{3*}

¹University of Science, Vietnam National University Ho Chi Minh City, Vietnam

²International University, Vietnam National University Ho Chi Minh City, Vietnam

³Ho Chi Minh City University of Education, Vietnam

*Corresponding author: Nguyen Thi Thuong Huyen – Email: huyennthh@hcmue.edu.vn

Received: June 12, 2026; Revised: June 22, 2026; Accepted: July 01, 2026

ABSTRACT

This study was conducted to evaluate the phytochemical composition, stability of phenolic compounds during frozen storage, and carbohydrate-digesting enzyme inhibitory activities of an aqueous decoction of male *Carica papaya* L. flowers harvested in Xuan Son Commune, Ho Chi Minh City, Vietnam. Qualitative screening showed that the decoction contained many biologically active secondary metabolites, with polyphenols, flavonoids, and tannins being the dominant groups of compounds. During storage at -20°C , phenolic compound levels gradually decreased over time but remained relatively stable for the first seven days. After 42 days of storage, flavonoids were the most stable of the three groups. The decoction inhibited both α -amylase ($\text{IC}_{50}=22.22\text{ mg/mL}$) and α -glucosidase ($\text{IC}_{50}=12.40\text{ mg/mL}$) in a concentration-dependent manner. The stronger inhibition of α -glucosidase suggests that the decoction may help regulate carbohydrate digestion and postprandial glycemic responses. Although its inhibitory potency did not reach that of acarbose, these findings provide preliminary evidence supporting the use of male papaya flowers as a natural source of phenolic compounds that merits further investigation. Notably, the phenolic composition of the decoction remained largely unchanged during storage at -20°C for up to 7 days.

Keywords: antihyperglycemic activity; male papaya flower; phenolic compounds; α -amylase inhibition; α -glucosidase inhibition

1. Introduction

Medicinal plants represent an important reservoir of bioactive compounds and have been extensively investigated for their potential contributions to the prevention and management of metabolic disorders. Among the species investigated, *Carica papaya* L.

Cite this article as: Ho, L. K. N., Vong, B. L., Nguyen, T. D., Nguyen, N. P., & Nguyen, T. T. H. (2026). Phytochemical characterization, phenolic stability, and inhibitory activities against α -amylase and α -glucosidase of a male *Carica papaya* L. flower decoction. *Ho Chi Minh City University of Education Journal of Science*, 23(7), 1576-1587. [https://doi.org/10.54607/hcmue.js.23.7.5867\(2026\)](https://doi.org/10.54607/hcmue.js.23.7.5867(2026))

stands out as a promising medicinal plant, with biological activities reported from several plant parts, including the leaves, seeds, and flowers (Agada et al., 2020; Marpaung et al., 2021; Roy et al., 2022; Ugbogu et al., 2023). In many regions of Vietnam and other countries, male papaya flowers are consumed as a traditional herbal remedy and are often associated with improved health and glycemic control (Dwivedi et al., 2020; Nga et al., 2020). These reported biological effects are likely related to the diverse phytochemicals present in papaya flowers, including polyphenols, flavonoids, alkaloids, and saponins (Srivastava et al., 2025).

Postprandial hyperglycemia is regarded as one of the major risk factors contributing to type 2 diabetes and metabolic disorders (Magliano & Boyko, 2021). Dietary starch is hydrolyzed to glucose by the enzymes α -amylase and α -glucosidase (Li et al., 2020). Accordingly, enzyme inhibition has become a promising approach for regulating postprandial hyperglycemia (Abubakar et al., 2019). Although synthetic inhibitors such as acarbose are used for this purpose, their long-term use often results in gastrointestinal disorders (Naveen & Baskaran, 2018), highlighting the need for alternative approaches based on natural products.

Besides biological activity, the stability of phytochemicals during storage is another important determinant of quality, efficacy, and reliability of herbal preparations. Specifically, phenolic substances, including polyphenols and flavonoids, are highly susceptible to degradation induced by heat, light, or oxygen. Consequently, prolonged storage can promote phenolic degradation, reducing antioxidant and other biological activities (Choulitoudi et al., 2021; Mrazkova et al., 2023). Storage at -20°C is often used to prolong the shelf life of medicinal plant extracts, but the degree to which phytochemicals are preserved depends on their chemical properties and stability. To date, little information is available in the literature on the stability and degradation kinetics of phytochemicals in aqueous decoctions obtained from male *Carica papaya* flowers under frozen storage conditions. It is also unclear whether changes in phytochemical constituents during storage influence the ability of male papaya flower preparations to inhibit α -amylase and α -glucosidase. Evaluating how storage affects phytochemical stability and enzyme inhibitory activity may help clarify the potential value of male papaya flower decoctions as a natural source of compounds for blood glucose management.

Consequently, this study was undertaken to identify the main bioactive phytochemical constituents of male papaya flower decoction, determine the stability of phenolics during frozen storage at -20°C , and evaluate its inhibitory potential against α -amylase and α -glucosidase *in vitro*. The findings advance current understanding of male papaya flower decoctions and support further research on their use as natural plant-based products for postprandial glycemic control.

2. Materials and methods

2.1. Plant materials

For this study, unopened, pale-green male papaya flowers rich in white latex were collected in July 2024 in Xuan Son Commune, Ho Chi Minh City (10°38'58.0"N, 107°18'57.5"E). Botanical identification of the plant material was conducted by MSc. Nguyen Thi Thanh Tam at the Faculty of Biology, Ho Chi Minh City University of Education.

2.2. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Reagents for phytochemical analyses included gelatin, NaCl, Pb(Ac)₂, Wagner's reagent, FeCl₃, Molisch's reagent, Biuret reagent, ninhydrin, vanillin, HClO₄, Folin–Ciocalteu reagent, Na₂CO₃, AlCl₃, and CH₃COOK. Reference standards included tannic acid, gallic acid, quercetin, atropine, glucose, albumin, cysteine/tyrosine, and oleanolic acid.

For enzyme inhibitory assays, α -amylase from porcine pancreas (Fluka, 10080), α -glucosidase from *Saccharomyces cerevisiae* (Sigma, G5003-100U), acarbose (Sigma, A8980-1G), p-nitrophenyl- α -D-glucopyranoside, starch, Lugol's solution, hydrochloric acid, disodium hydrogen phosphate dodecahydrate, and potassium dihydrogen phosphate were used. Chemicals were purchased from Sigma-Aldrich, Merck, Fluka, and Xilong.

2.3. Methods

2.3.1. Preparation of male papaya flower samples

Fresh male papaya flowers were transported to the laboratory, washed twice with distilled water, and screened to ensure uniformity. Dehydration was performed sequentially by shade-drying (48 hours, well-ventilated) and convection oven-drying (60°C) to constant weight. Finally, the dried medicinal materials were stored in sealed paper bags with desiccant packets and kept under cool, dry, and dark conditions.

2.3.2. Preparation of the decoction

The male papaya flower decoction was prepared using a traditional aqueous extraction method with slight modifications (Ho, 2005). Dried male papaya flowers were first weighed and rinsed twice by soaking in distilled water for 15 min each to remove impurities and facilitate hydration of the plant material. The soaking water was discarded prior to extraction.

The extraction process was conducted in two consecutive decoction cycles. In the first extraction, the plant material was mixed with 300 mL of distilled water and heated at 70–80°C for 90 min. The resulting extract was collected as the first decoction. The residual plant material was subsequently subjected to a second extraction using 200 mL of distilled water under the same temperature and extraction time conditions (70–80°C for 90 min). The second extract was then collected and combined with the first decoction.

After pooling, the extracts were centrifuged at 3,000 rpm for 10 min to remove insoluble residues. The supernatant was concentrated in a 50–60°C water bath to a 1:1 ratio

(g raw material/mL extract), and the resulting decoction was used for phytochemical and biological activity analyses.

The decoction was evaluated for its physical quality, storage stability, and biological activity. Visual inspection was performed to assess color, odor, homogeneity, and solution clarity, while storage stability was determined based on the absence of visible sediments, turbidity, and noticeable changes in appearance or odor. Only decoctions that fulfilled the quality requirements and demonstrated detectable biological activity in preliminary assays were retained for further investigation. The selected preparation was stored at -20°C , and samples were analyzed on days 0, 7, 14, 28 and 42.

2.3.3. Qualitative phytochemical screening of antioxidant compounds in the aqueous decoction

The aqueous decoction was qualitatively screened for the presence of major antioxidant-related phytochemicals using standard phytochemical assays based on characteristic color changes, precipitate formation, and foaming reactions. The investigated phytochemical groups included saponins (foam test), tannins (gelatin-salt test), flavonoids [lead acetate, $\text{Pb}(\text{Ac})_2$ test], alkaloids (Wagner's reagent test), polyphenols (FeCl_3 test), carbohydrates (Molisch's test), proteins (Biuret test), amino acids (ninhydrin test), and triterpenoids (vanillin-perchloric acid assay) (Maheshwaran et al., 2024).

The presence of each phytochemical group was determined based on the appearance of characteristic visual reactions compared with the corresponding controls. The results were semiquantitatively recorded as absent (–), detected (+), or strongly detected (++), reflecting the relative intensity of the observed reactions and the presumed abundance of the corresponding phytochemical constituents in the decoction (Maheshwaran et al., 2024). Positive and negative controls were included to validate the qualitative reactions.

2.3.4. Quantification of selected antioxidant phytochemicals in the aqueous decoction

To evaluate antioxidant-related phytochemical constituents in the decoction, three major phenolic groups, namely polyphenols, flavonoids, and tannins, were quantitatively determined. These compounds are widely recognized for their roles in free radical scavenging and cellular protection against oxidative stress (Panche et al., 2016a; Shahidi & Ambigaipalan, 2015a). Standard assays were used to determine the phytochemical composition of the decoction (Singleton et al., 1999).

Total phenolic content (TPC): The TPC was measured using the Folin-Ciocalteu assay. The decoction sample was mixed with Folin-Ciocalteu reagent and Na_2CO_3 , and the mixture was incubated in the dark for 30 minutes. Absorbance was then measured at 765 nm, and TPC was determined using a gallic acid standard curve and expressed as mg GAE/g (Makkar, 2003).

Total flavonoid content (TFC): The TFC was measured using the AlCl_3 colorimetric assay. The decoction sample was mixed with AlCl_3 and CH_3COOK , incubated in the dark

for 15 min, and its absorbance measured at 415 nm. TFC was calculated using a quercetin standard curve and expressed as mg QE/g DW (Mahboubi et al., 2013).

Total tannin content (TTC): The TTC was measured using a modified Folin-Ciocalteu assay. The decoction sample was mixed with the reagent mixture, incubated in the dark, and measured at 765 nm. TTC was calculated using a tannic acid standard curve (0-50 µg/mL) and expressed as mg TAE/g DW (Makkar, 2003).

2.3.5. *In vitro* α-amylase inhibitory activity assay

The α-amylase inhibitory activity was determined according to the method of Chakrabarti et al. (2014) with minor modifications. Briefly, 125 µL of the decoction at different concentrations was mixed with 175 µL phosphate buffer (1/15 M, pH 6.9) and 100 µL of α-amylase solution (0.5 mg/mL). The mixture was incubated at 37°C for 10 minutes in the dark. Subsequently, 100 µL starch solution was added, followed by further incubation under the same conditions for 10 minutes. The reaction was terminated by adding 500 µL HCl and 2000 µL Lugol's solution, and the absorbance was measured at 620 nm using a spectrophotometer.

Acarbose (0-35 µg/mL) was used as the positive control, while distilled water served as the negative control. All experiments were performed in triplicate. The percentage inhibition of α-amylase activity was calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Abs}_2 - \text{Abs}_1}{\text{Abs}_4 - \text{Abs}_3}\right) \times 100$$

- Abs₁: absorbance of the reaction mixture containing decoction sample, starch, and α-amylase;

- Abs₂: absorbance of the mixture containing decoction sample and starch without α-amylase;

- Abs₃: absorbance of the control mixture containing starch and α-amylase;

- Abs₄: absorbance of the blank mixture containing distilled water and starch.

2.3.6. *In vitro* α-glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity was determined according to the method of Agada et al. (2020) with minor modifications. Briefly, 50 µL of the decoction at different concentrations was mixed with 70 µL phosphate buffer (0.1 M, pH 6.9) and 40 µL α-glucosidase solution prepared in the same buffer. The mixture was incubated at 37°C for 20 minutes in the dark. Subsequently, 40 µL p-NPG solution was added, followed by further incubation under the same conditions for 17 minutes. The reaction was terminated by adding 70 µL Na₂CO₃ (0.2 M), and the absorbance was measured at 405 nm using a spectrophotometer.

Acarbose (0-100 µg/mL) was used as the positive control, while distilled water served as the negative control. All experiments were performed in triplicate. The percentage inhibition of α-glucosidase activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Absorbance of control (without decoction) and Absorbance of test (with decoction) denote the respective absorbance values. The IC_{50} value was determined based on the dose–response curve showing the correlation between concentration and percentage inhibition.

2.3.7. Statistical analysis

All experimental data were statistically analyzed using GraphPad Prism software version 8.4.3 (GraphPad Software, San Diego, CA, USA). Statistical comparisons among multiple groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple-comparisons test. Differences were considered statistically significant at $p < 0.05$. All quantitative results are presented as mean $\pm$ standard deviation (SD).

3. Results and discussion

3.1. Qualitative screening of antioxidant phytochemicals in the male papaya flower decoction

Table 1. Qualitative phytochemical screening of the male papaya flower decoction

Phytochemical compounds	Relative abundance	Color comparison	Positive control	Negative control
Polyphenol	++	-	+	-
Flavonoid	++	-	+	-
Alkaloid	+	-	+	-
Tannin	++	-	+	-
Saponin	+	-	+	-
Triterpenoid	-	-	+	-
Carbohydrate	+	-	+	-
Protein	+	-	+	-
Acid amin	+	-	+	-

Note: (+): detected; (++): strongly detected; (-): not detected

The phytochemical analysis results are presented in Table 1. Positive reactions were obtained for polyphenols, flavonoids, tannins, alkaloids, saponins, carbohydrates, proteins, and amino acids. Among these compounds, polyphenols, flavonoids, and tannins showed the strongest reactions (++), whereas the remaining groups produced weaker positive reactions (+). No reaction was observed for triterpenoids, which may be explained by their lipophilic nature and limited extraction into the polar aqueous medium.

Both controls showed the expected reactions, confirming that the qualitative assay performed as expected. Moreover, the color of the decoction had no influence on the outcome of the test because no interfering color appeared in the “color blank” section.

3.2. Quantitative analysis of antioxidant phytochemicals in the male papaya flower decoction

Figure 1 highlights the phytochemical stability of the decoction during a 42-day storage period at -20°C . The initial 7-day period showed high stability; however, the levels of polyphenols, flavonoids, and tannins then declined sharply, decreasing by nearly 30% by

day 28. Thereafter, this degradation plateaued, with the retention levels stabilizing at 74%, 76%, and 72%, respectively, at the end of the trial. Although flavonoids were more stable, the long-term freezing of the decoction reduced the phenolic content, which would likely affect its antioxidative activity.

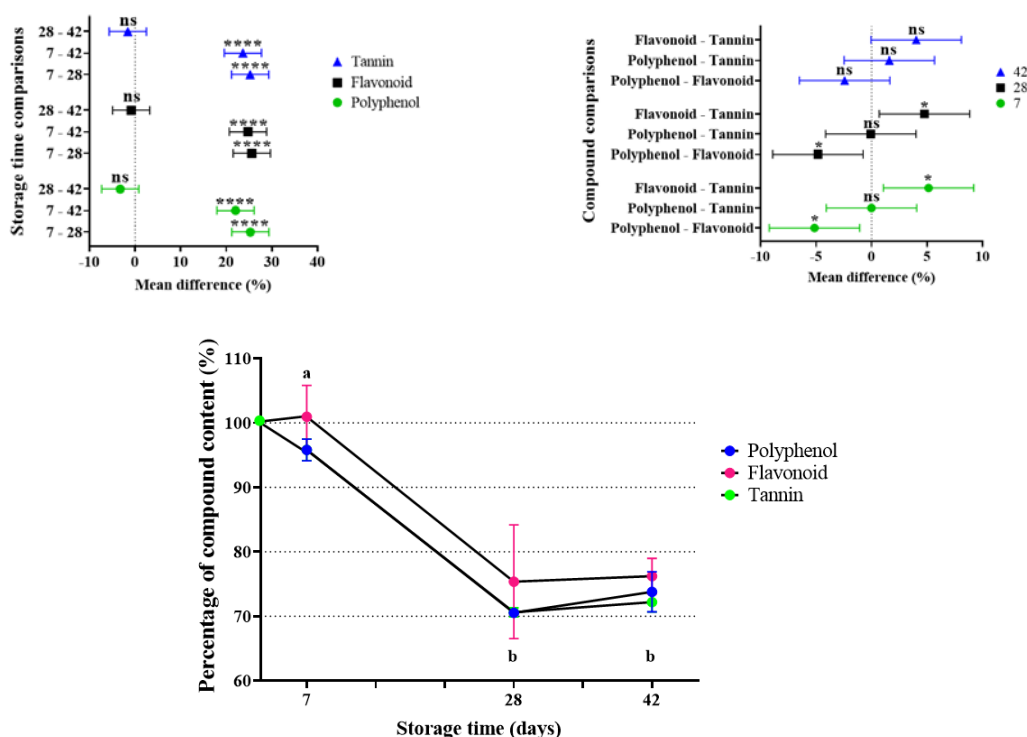


Figure 1. Changes in polyphenol, flavonoid, and tannin contents in the male papaya flower decoction during storage at -20°C for 42 days
ns, not significant; *, $p < 0.01$; ****, $p < 0.0001$. Error bars represent standard deviation ($n = 3$). Different lowercase letters indicate significant differences among storage times ($p < 0.05$).

3.3. α -amylase inhibition of male papaya flower decoction

Concentration-dependent inhibitory activities against α -amylase and α -glucosidase were observed for the aqueous decoction of male *Carica papaya* L. flowers (Figures 2 and 3). For α -amylase, the inhibition percentage increased from 18.25% to 64.88% as the decoction concentration increased from 10.89 to 27.23 mg/mL. The inhibition data were fitted to the regression equation $Y = 2.895x - 14.32$ ($R^2 = 0.9982$), and the IC_{50} value was determined to be 22.22 mg/mL (Figure 2B). Under the same experimental conditions, the positive control acarbose exhibited an IC_{50} value of 15.07 $\mu\text{g/mL}$ (Figure 2A).

Similarly, the α -glucosidase inhibition percentage increased from 20.67% to 72.73% when the decoction concentration was increased from 10.89 to 13.62 mg/mL. The inhibition data were described by the regression equation $Y = 19.48x - 191.6$ ($R^2 = 0.9975$), yielding an IC_{50} value of 12.40 mg/mL (Figure 3B). The positive control, acarbose, exhibited an IC_{50} value of 58.13 $\mu\text{g/mL}$ (Figure 3A).

The IC₅₀ value obtained for α -glucosidase was lower than that obtained for α -amylase, indicating a stronger inhibitory activity of the decoction toward α -glucosidase.

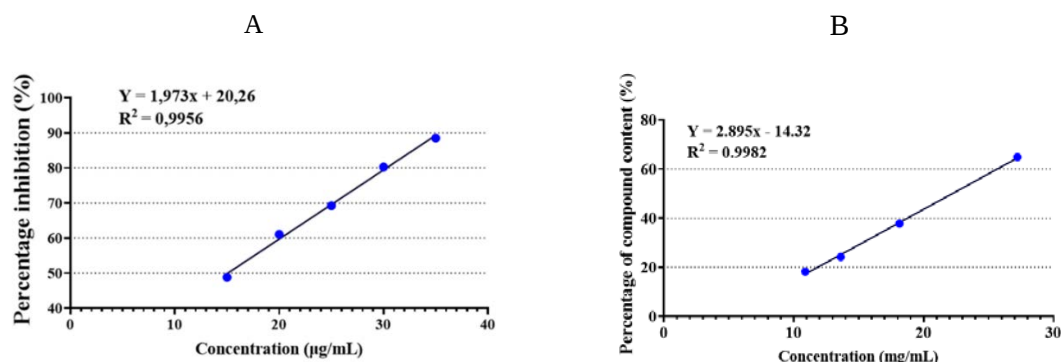


Figure 2. Percentage inhibition of α -amylase by acarbose (A) and the aqueous decoction of young male *Carica papaya* flowers (B)

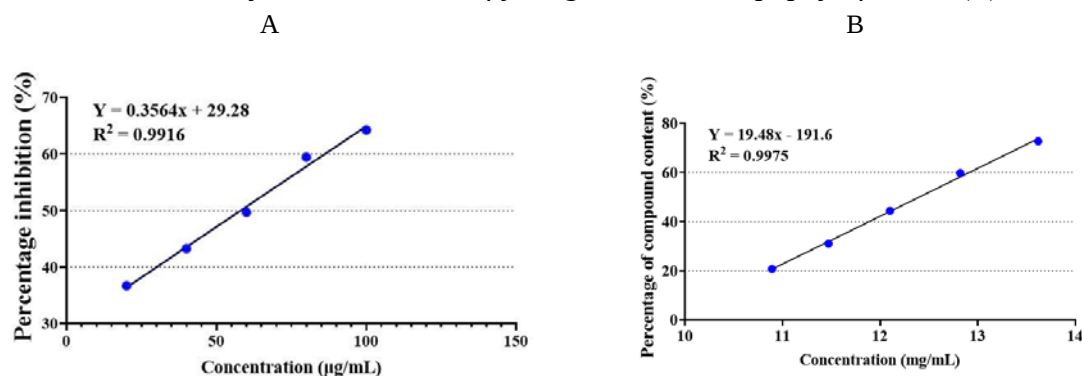


Figure 3. Percentage inhibition of α -glucosidase by acarbose (A) and the aqueous decoction of male *Carica papaya* flowers (B)

3.4. Discussion

The male papaya flower decoction demonstrated concentration-dependent inhibition of both α -amylase and α -glucosidase. Inhibition was stronger for α -glucosidase. The observed dual inhibition may reflect the synergistic action of the extract's phenolic pool, particularly flavonoids and tannins. These constituents may inhibit carbohydrate-hydrolyzing enzymes through complex hydrogen-bonding and hydrophobic interactions (Kalita et al., 2018; Martinez-Gonzalez et al., 2017; Sadeghi et al., 2024; Sohretoglu et al., 2018; Tadera et al., 2006).

Although the inhibitory potency of the decoction was lower than that of acarbose (Luthra et al., 2017), its activity appeared to be higher than that reported for extracts prepared from other parts of papaya plants (Abubakar et al., 2019; Agada et al., 2020; Marpaung et al., 2021). These findings suggest that the male flower is a promising source of antidiabetic phytochemicals, although direct comparisons among studies should be interpreted cautiously because of differences in plant material and experimental conditions.

Preferential inhibition of α -glucosidase ($IC_{50} = 12.40$ mg/mL) over α -amylase ($IC_{50} = 22.22$ mg/mL) may offer an advantage for controlling postprandial hyperglycemia. Acarbose effectively delays carbohydrate digestion by inhibiting both enzymes; however, excessive inhibition of pancreatic α -amylase allows a greater proportion of undigested carbohydrates to reach the colon, where bacterial fermentation can cause gastrointestinal adverse effects such as flatulence, abdominal distension, and diarrhea (DiNicolantonio et al., 2015). Therefore, the milder inhibition of α -amylase observed for the decoction may reduce the likelihood of these gastrointestinal effects while maintaining postprandial glucose control.

The biological activity of the decoction is likely influenced by the collective contribution of multiple phytochemicals rather than a single dominant constituent. Phenolic and flavonoid accumulation is known to vary with environmental conditions during plant growth, and differences in geographical origin or cultivation conditions may partly explain the variability reported among *C. papaya* extracts (Qaderi et al., 2023).

Because these phytochemicals underpin the decoction's antidiabetic potential (Panche et al., 2016b; Shahidi & Ambigaipalan, 2015b), maintaining their integrity is critical. Our stability data show that -20°C storage is effective for short-term preservation. However, as reported previously (Cao et al., 2021; Mrazkova et al., 2023), prolonged freezing slows but cannot completely prevent phenolic breakdown. Despite this gradual degradation, the relative stability of flavonoids over 42 days is encouraging.

Therefore, these results suggest that male papaya flower decoction is a promising natural source of enzyme inhibitors for postprandial glycemic control. Future studies should identify the active compounds and evaluate their effects *in vivo*.

4. Conclusion

In conclusion, the aqueous decoction from the flowers of male *Carica papaya* L. is a promising source of phenolic phytochemicals, especially polyphenols, flavonoids, and tannins. The decoction modulated carbohydrate digestion and showed stronger inhibition of α -glucosidase ($IC_{50} = 12.40$ mg/mL) compared to α -amylase ($IC_{50} = 22.22$ mg/mL). Although its overall inhibitory activity was lower than that of acarbose, its concentration-dependent activity highlights its potential for postprandial glycemic management. The decoction maintained its phenolic composition well when stored at -20°C for up to seven days, with flavonoids showing the greatest stability over time. Future *in vivo* studies should focus on identifying the key active compounds and verifying their safety and biological efficacy.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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THÀNH PHẦN HOÁ THỰC VẬT, ĐỘ ỔN ĐỊNH PHENOLIC VÀ HOẠT TÍNH ỨC CHẾ α -AMYLASE, α -GLUCOSIDASE CỦA NƯỚC SẮC HOA ĐU ĐỦ (*Carica papaya* L.)

Hồ Linh Kiều Nhi¹, Vòng Bính Long², Nguyễn Tiến Dũng¹,
Nguyễn Ngọc Phương³, Nguyễn Thị Thương Huyền^{3*}

¹Trường Đại học Khoa học Tự nhiên, Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

²Trường Đại học Quốc tế, Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

³Trường Đại học Sư phạm Thành phố Hồ Chí Minh, Việt Nam

*Tác giả liên hệ: Nguyễn Thị Thương Huyền – Email: huyenntth@hcmue.edu.vn

Ngày nhận bài: 12-6-2026; Ngày nhận bài sửa: 22-6-2026; Ngày duyệt đăng: 01-7-2026

TÓM TẮT

Nghiên cứu này được thực hiện nhằm đánh giá thành phần hóa thực vật, độ ổn định của các hợp chất phenolic trong quá trình bảo quản đông lạnh và hoạt tính ức chế enzyme tiêu hóa carbohydrate của nước sắc hoa đu đủ đực (*Carica papaya* L.) thu hái tại xã Xuân Sơn, Thành phố Hồ Chí Minh, Việt Nam. Kết quả sàng lọc định tính cho thấy nước sắc chứa nhiều hợp chất chuyển hoá thứ cấp có hoạt tính sinh học, trong đó polyphenol, flavonoid và tannin là các nhóm hợp chất chiếm ưu thế. Trong quá trình bảo quản ở -20°C , hàm lượng phenolic giảm dần theo thời gian nhưng vẫn tương đối ổn định trong 7 ngày đầu. Sau 42 ngày bảo quản, flavonoid là nhóm hợp chất có độ ổn định cao nhất trong ba hợp chất. Nước sắc thể hiện hoạt tính ức chế phụ thuộc nồng độ đối với cả α -amylase và α -glucosidase, với giá trị IC_{50} lần lượt là 22,22 mg/mL và 12,40 mg/mL. Hoạt tính ức chế α -glucosidase mạnh hơn cho thấy tiềm năng điều chỉnh quá trình tiêu hóa carbohydrate và kiểm soát đường huyết sau ăn. Mặc dù hiệu lực ức chế thấp hơn acarbose, nhưng kết quả nghiên cứu này vẫn cung cấp bằng chứng khoa học ban đầu về tiềm năng ứng dụng của nước sắc hoa đu đủ đực như một nguồn nguyên liệu tự nhiên giàu hợp chất phenolic. Hơn nữa, thành phần phenolic của nước sắc hầu như không thay đổi trong quá trình bảo quản ở -20°C trong tối đa 7 ngày.

Từ khoá: hoa đu đủ đực; hợp chất phenolic; hoạt tính hạ đường huyết; ức chế α -glucosidase; ức chế α -amylase