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EVALUATING ANTIOXIDANT ACTIVITY, ALPHA-GLUCOSIDASE INHIBITION, AND BIOACTIVE COMPOUNDS IN NOVEL HERBAL TEA BLENDS FEATURING *CAMELLIA HAKODAE* NINH FLOWERS

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Abstract – *Camellia hakodae Ninh (C. hakodae Ninh) flowers, an endemic medicinal species in Vietnam, have attracted interest for their potential use in functional food and phytotherapy. This study evaluated the antioxidant and α-glucosidase inhibitory activities, as well as quantified total polyphenol and flavonoid contents, of herbal tea-bag formulations prepared from C. hakodae Ninh flowers combined with traditional medicinal herbs. Antioxidant capacity and α-glucosidase inhibition were determined using DPPH radical scavenging and in vitro enzymatic assays, respectively, while total polyphenol and flavonoid contents were measured spectrophotometrically using gallic acid and quercetin standards. Among the four formulations, CT1 (containing 50% C. hakodae Ninh flower) exhibited the strongest bioactivities, with IC_{50} values of 288.41 ± 5.84 μg/mL for DPPH and 86.15 ± 2.45 μg/mL for α-glucosidase inhibition ($p < 0.05$). Its total polyphenol and flavonoid contents were 45.39 ± 1.82 mg GAE/g and 10.35 ± 0.48 mg QE/g, respectively. These results demonstrated that C. hakodae Ninh flowers, in combination with other herbal ingredients, contribute significantly to antioxidant and antidiabetic potential, supporting their development as functional herbal tea products for health promotion and glycemic control.*

***Keywords:* α-glucosidase inhibition, antioxidant activity, Camellia hakodae Ninh, herbal tea, phytotherapy, polyphenols.**

I. INTRODUCTION

The consumption of functional herbal teas has grown rapidly in recent years, especially in Asia, reflecting increasing demand for natural, health-promoting beverages [1]. With its exceptional biodiversity and long tradition of phytotherapy, Vietnam is well-positioned to contribute to this trend through the utilization of endemic medicinal plants.

Camellia hakodae Ninh (*C. hakodae* Ninh), known as the yellow camellia, is particularly noteworthy for its richness in polyphenols and flavonoid compounds associated with potent antioxidant, antidiabetic, and anticancer properties [2, 3]. While preliminary studies on *C. hakodae* Ninh have demonstrated significant biological potential [2, 3], systematic evaluations of this species in functional formulations remain scarce.

Yellow camellia species are recognized as prolific sources of secondary metabolites, including flavonoids, saponins, and polysaccharides, which underpin diverse pharmacological effects [4, 5]. Moreover, tea polyphenols are well documented to inhibit α-glucosidase, highlighting their role as natural agents in glycemic regulation [6].

This study, therefore, focused on novel tea-bag formulations in which *C. hakodae* Ninh flowers serve as the principal component, combined with red jujube (*Ziziphus jujuba*) and monk fruit (*Siraitia grosvenorii*), both traditionally valued for antioxidant and glycemic benefits [7]. The objective was to assess their antioxidant and α-glucosidase inhibitory activities, alongside

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quantifying polyphenol and flavonoid contents, thereby providing evidence for the development of functional herbal teas from this valuable endemic species.

II. LITERATURE REVIEW

Building on genus-level evidence, the flower of *C. hakodae* Ninh merits distinct attention, including golden camellia syntheses consistently place floral organs among the richest reservoirs of polyphenols and flavonoids (beyond leaf chemotypes), with complementary saponins, polysaccharides, and volatiles that together rationalize robust antioxidant and metabolism-modulating effects in infusion matrices [4, 5]. Species-specific data for *C. hakodae* Ninh strengthen this translational case: acute and sub-chronic toxicity profiles are acceptable – an essential prerequisite for food-format development – and preclinical work indicates attenuation of diet-induced adiposity and metabolic disturbance, suggesting benefits that extend past redox chemistry into metabolic regulation [2, 3].

Mechanistically, the flower's phenolic repertoire, including catechin- and gallic-acid-related scaffolds inferred from *Camellia* comparators, underwrites dual functionality, consisting of direct radical scavenging and α-glucosidase inhibition, both repeatedly mapped to phenolic abundance and substitution patterns in tea systems; structure-activity studies show that galloylation/hydroxylation tune binding at α-glucosidase active/allosteric sites, explaining formulation-level IC_{50} differences and guiding flower-forward blend design [6, 8, 9]. Interpreting these couplings demands assay discipline. Folin-Ciocalteu must be treated as a reducing-capacity metric with matrix-appropriate blanking and transparent gallic-acid calibration (mg GAE/g), while $AlCl_3$ colorimetry requires wavelength control and explicit quercetin calibration (mg QE/g). Being paired with non-linear (4PL) modeling and fit diagnostics for DPPH and enzyme inhibition, these practices yield defensible, cross-study-comparable inferences for *C. hakodae* Ninh flower infusions [10, 11, 14].

On this analytical basis, formulation choices are crucial. Floral tissues exhibit distinct extraction kinetics and sensory precursors versus leaves, so bioactivity gains are not strictly linear with loading-empirically justifying graded flower proportions, from formulation 1 to 4 (CT1–CT4) and the inclusion of co-ingredients that preserve palatability while broadening functional coverage. Monk fruit contributes high-intensity sweetness with emerging antioxidant/glycometabolic signals, and jujube adds polysaccharides/phenolics with tonic and metabolic relevance – together supporting a practice-oriented blueprint for flower-centered *C. hakodae* Ninh teas aimed at oxidative-stress mitigation and glycemic moderation [1, 7, 12, 13].

III. MATERIALS AND METHODS

A. Materials and equipment

Fresh flowers of *C. hakodae* Ninh were collected at full bloom in December 2024 from the Tam Dao area, Vinh Phuc Province, which is now part of Phu Tho Province. The raw materials were carefully cleaned to remove dust and damaged parts, then dried at 40^oC under controlled conditions to preserve bioactive compounds and natural aroma. The dried samples were ground into fine powder, with final moisture content determined as $8.02 \pm 0.11\%$. Four teabag formulations (CT1–CT4) were prepared by blending *C. hakodae* Ninh flower powder with selected medicinal ingredients, namely red jujube (*Ziziphus jujuba*) and monk fruit (*Siraitia grosvenorii*). Each tea bag contained 2 g of material and was sealed in food-grade filter paper.

Key reagents included DPPH radical (0.3 mM, Sigma-Aldrich, USA), α-glucosidase enzyme (0.2 U/mL, Sigma-Aldrich, USA), phosphate buffer (0.01 M, pH 7.0, Merck, Germany), p-nitrophenyl-α-D-glucopyranoside (pNPG, 3.0 mM, Sigma-Aldrich, United States), ascorbic acid (Drug Quality Control Institute, Ho Chi Minh City, Vietnam) and acarbose (Sigma-Aldrich, USA) as positive controls, aluminum chloride ($AlCl_3$, 10%, Merck, Germany), sodium acetate (CH_3COONa, 1 M, Merck, Germany),

Folin–Ciocalteu reagent (10%, Merck, Germany), and sodium carbonate (Na_2CO_3, 7.5%, Merck, Germany). Absorbance measurements were performed using a Thermo Fisher Scientific ELISA microplate reader (United States).

B. Experimental procedure

Extraction and infusion

In each formulation, 2 g of powdered material was infused in 100 mL of distilled water at 90^{o}C for 10 minutes, simulating typical consumer preparation. Four tea-bag formulations (CT1–CT4) were designed with increasing proportions of *C. hakodae* Ninh flowers at 50%, 60%, 70%, and 80% by mass, respectively, as detailed in Table 1. The remaining content in each formulation was equally divided between red jujube (*Ziziphus jujuba*) and monk fruit (*Siraitia grosvenorii*), with minor excipients included to improve stability and sensory properties. All formulations were previously optimized with respect to extraction conditions to ensure maximal recovery of bioactive compounds and preservation of natural aroma. Extracts were filtered and used immediately for subsequent analysis.

Table 1: Composition of herbal tea-bag formulations (CT1–CT4)

Formulation	*Camellia hakodae* Ninh (%)	Red jujube (*Ziziphus jujuba*) (%)	Monk fruit (*Siraitia grosvenorii*) (%)
CT1	50	25	25
CT2	60	20	20
CT3	70	15	15
CT4	80	10	10

Antioxidant activity (DPPH assay)

Radical-scavenging capacity was quantified by a microplate DPPH protocol (96-well), tracking the loss of the 517 nm chromophore after one-electron/H-atom transfer from antioxidants to DPPH, with ascorbic acid serving as reference [14, 15]. Test aliquots (225 μL) were mixed with DPPH working solution (75 μL, 0.3 mM in MeOH), incubated 30 minutes at 37^{o}C in the dark, and read at 517 nm.

For each concentration, percent radical scavenging was calculated as in Equation (1).

$$\%\text{DPPH scavenging} = 100 \times \frac{A_{blank} - A_{sample}}{A_{blank}} \quad (1)$$

A_{blank}: The absorbance of the reagent blank (DPPH + solvent, no sample);

A_{sample}: The absorbance of the reaction containing DPPH and the test solution.

Dose–response curves (% scavenging vs. concentration, μg/mL) were fitted to a four-parameter logistic (4PL) model, presented in Equation (2).

$$y = \text{bottom} + \frac{\text{top} - \text{bottom}}{1 + (\frac{x}{IC_{50}})^{\text{Hillslope}}} \quad (2)$$

The IC_{50} (μg/mL) was interpolated at 50% scavenging from the fitted curve using Equation (2). Each formulation was measured in sextuplicate independent preparations, each with technical replicates. Results are reported as mean $\pm$ SD; fits were accepted when $R^2 \geq 0.99$ and residuals showed no systematic trend. Quality controls included reagent-only blanks, sample-only blanks, and a positive-control curve (ascorbic acid) run in parallel.

α-Glucosidase inhibitory assay

Enzyme inhibition was assessed with yeast α-glucosidase using p-nitrophenyl-α-D-glucopyranoside (pNPG), an accepted in-vitro model to gauge postprandial glycemic modulation by polyphenol-rich beverages [8, 9]. Samples were prepared in 0.01 M phosphate buffer (pH 7.0). Reaction mixtures contained 100 μL sample and 100 μL enzyme (0.2 U mL^{-1}), pre-incubated for 5 minutes at 37^{o}C; adding 100 μL pNPG (3.0 mM) initiated the reaction (15 minutes, 37^{o}C), after which 1.5 mL Na_2CO_3 stopped the reaction. Absorbance of p-nitrophenolate was recorded at 405 nm, and acarbose served as the positive control.

Inhibition and IC_{50} values were computed analogously to the DPPH assay, using Equation (3).

$$\%\ \text{Inhibition} = 100 \times \frac{A_{blank} - A_{sample}}{A_{blank}} \quad (3)$$

A_{blank}: Absorbance of enzyme + substrate (no inhibitor/sample);

A_{sample}: Absorbance of enzyme + substrate + test solution at 405 nm.

Fit % inhibition (y) versus concentration (x, μg/mL) using a four-parameter logistic (4PL) model; extract IC_{50} at 50% inhibition. Sextuplicate independent runs were performed per formulation. Total flavonoid content

Total flavonoid content (TFC) was determined by the $AlCl_3$ complexation assay adapted to microplates (415 nm) with sodium acetate to stabilize the Al(III)–flavonoid chelate, as illustrated in Figure 1; quercetin served as the calibrant (3.2–50 mg L^{-1}), and results were expressed as mg QE g^{-1} dry extract [11, 16]. To reinforce specificity and reproducibility, the incubation (40 minutes at room temperature, in the dark) was controlled and verified the absorption maximum in the 410–430 nm region.

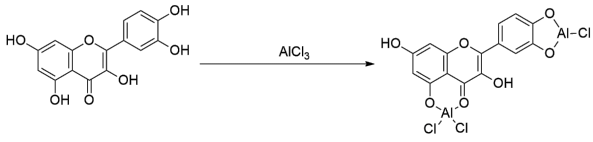

Fig. 1: Reaction mechanism of the sample with AlCl3 [11]

The mass- and moisture-corrected TFC was computed as Equation (4).

$$w_T = \frac{(A_T - A_0) \times d}{S_{std} \times m \times 1000 \times (1-h)} \qquad (4)$$

w_T: Total flavonoid content in medicinal herbs (mg QE/g medicinal herbs);

A_T: Absorbance of the test sample;

A_0: Absorbance at the point where the standard curve intersects the y-axis;

d: Dilution;

S_{std}: Slope of the standard curve;

m: Mass of the test sample (g);

h: Moisture of the medicinal herbs (%).

Total polyphenol content

Total polyphenol content (TPC) was measured by the Folin–Ciocalteu microplate assay (765 nm) with gallic acid as calibrant, reported as mg GAE g^{-1} dry extract [10, 17]. Samples (30 μL) were mixed with F–C reagent, pre-reacted for 5 minutes, alkalinized with Na_2CO_3, and incubated for 60 minutes in the dark before measurement.

Mass- and moisture-corrected TPC was computed analogously using Equation (5).

$$w_T = \frac{(A_T - A_0) \times d}{S_{std} \times m \times 1000 \times (1-h)} \qquad (5)$$

w_T: Total polyphenol content in medicinal herbs (mg GAE/g medicinal herbs);

A_T: Absorbance of the test sample;

A_0: Absorbance at the point where the standard curve intersects the y-axis;

d: Dilution;

S_{std}: Slope of the standard curve;

m: Mass of the test sample (g);

h: Moisture of the medicinal herbs (%).

Statistical analysis

All experiments were conducted with six independent replicates. Results were expressed as mean ± SD. IC_{50} values were obtained using 4PL nonlinear regression. Differences among formulations were evaluated using one-way ANOVA with Tukey's post-hoc test, and Pearson correlation was applied to examine associations between *C. hakodae* Ninh proportion and IC_{50}, TPC, and TFC. Statistical significance was set at $p < 0.05$.

IV. RESULTS AND DISCUSSION

A. Research results

Antioxidant activity

The antioxidant activity of four tea formulations was determined based on the linear equation of the standard substance ascorbic shown in Figure 2, with $y = 8.3039x - 0.2968$, $R^2 = 0.9941$, and expressed as IC_{50} values derived from DPPH radical scavenging assays (Figure 3).

All formulations exhibited dose-dependent DPPH radical scavenging activity. Error bars represent the standard deviation (SD) from three independent measurements (n = 3). CT1 demonstrated the strongest antioxidant activity among the tested formulations (IC_{50} = 288.41 ± 5.84 μg/mL), although it remained less potent than

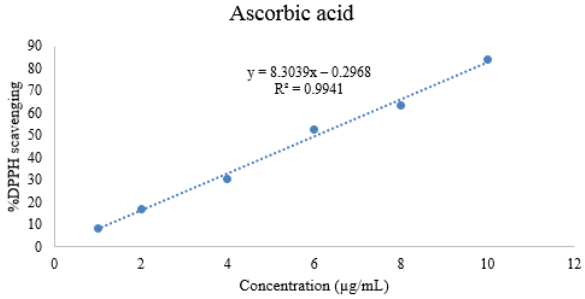

Fig. 2: Ascorbic acid calibration curve

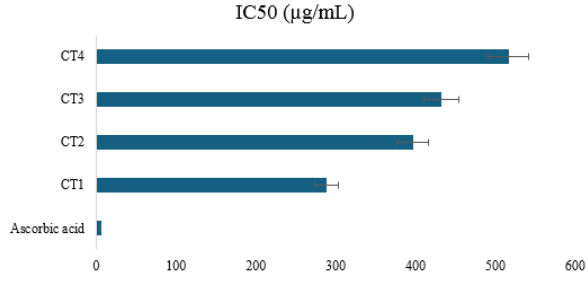

Fig. 3: DPPH antioxidant potency of formulations CT1–CT4 (IC_{50})

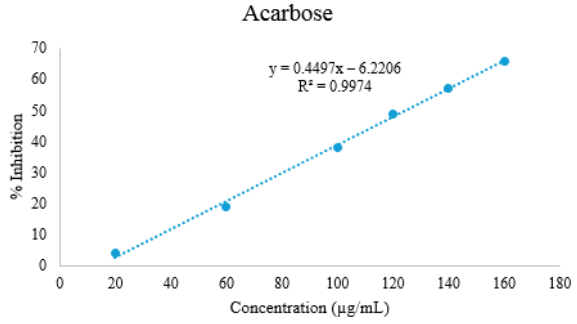

Fig. 4: Acarbose calibration curve

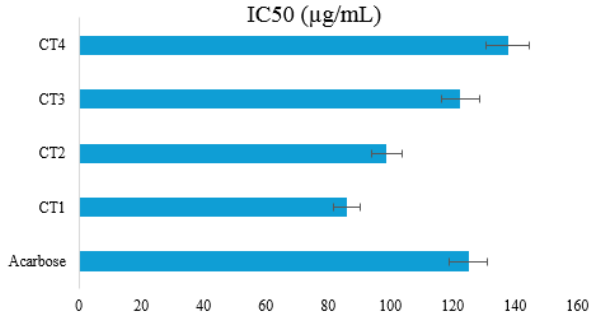

Fig. 5: α-Glucosidase inhibition of formulations CT1–CT4 (IC_{50})

the positive control ascorbic acid (6.06 ± 0.21 µg/mL). One-way ANOVA followed by Tukey's post-hoc test confirmed that CT1 exhibited significantly higher activity compared with the other formulations ($p < 0.05$). Other formulations showed moderate effects (IC_{50} range: 380–520 µg/mL). A significant negative correlation was observed between the concentration of *C. hakodae* Ninh and IC_{50} values (Pearson's correlation coefficient R = - 0.97, $p < 0.05$), confirming that higher proportions of *C. hakodae* Ninh enhance antioxidant activity.

α-Glucosidase inhibition

Figure 4 presents the linear calibration curve of the reference inhibitor was used to determine the α-glucosidase inhibitory activity of yellow camellia (*C. hakodae* Ninh) flower extracts, particularly, Acarbose y = 0.4497x – 6.2206, R^2 = 0.9974, and the corresponding IC_{50} values are presented in Figure 5.

CT1 showed the strongest α-glucosidase inhibitory activity with an IC_{50} of 86.15 ± 2.45 µg/mL, which was significantly lower than that of the positive control acarbose (125.02 ± 3.21 µg/mL, p = 0.018), while CT2–CT4 exhibited only moderate inhibition (IC_{50} = 98.71 – 137.66 µg/mL). Statistical analysis by one-way ANOVA and Tukey's post-hoc test confirmed significant differences among formulations ($p < 0.05$). The negative correlation between *C. hakodae* Ninh proportion and IC_{50} (R = – 0.94, $p < 0.05$) indicates that higher *C. hakodae* Ninh content enhances α-glucosidase inhibition.

Total flavonoid contents

Total flavonoid content in yellow camellia (*C. hakodae* Ninh) flowers was quantified using the linear calibration curve of the quercetin standard: y = 0.0171x – 0.0132; R2 = 0.9956 (Figure 6), and the flavonoid contents of the four formulations are summarized in Figure 7.

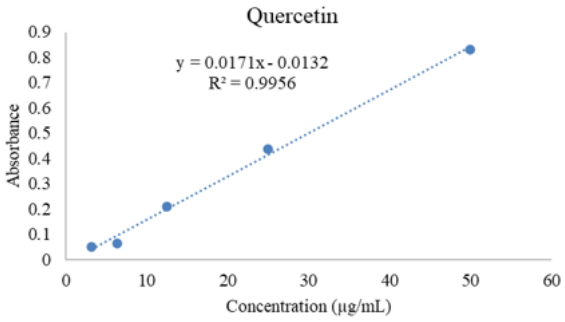

Fig. 6: Quercetin calibration curve

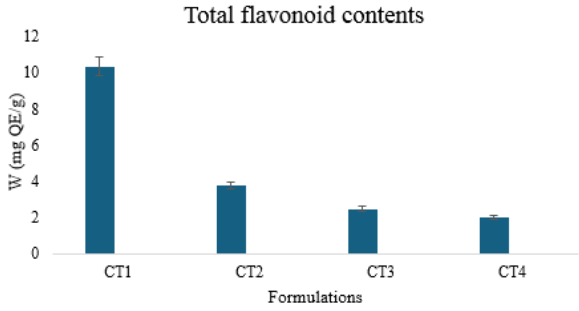

Fig. 7: Total flavonoid contents of formulations (CT1–CT4)

The total flavonoid content varied significantly among formulations (Figure 7). CT1 (containing 50% *C. hakodae* Ninh flower) exhibited the highest total flavonoid content (10.35 ± 0.48 mg QE/g), which was significantly greater than those of CT2 (3.75 ± 0.22 mg QE/g), CT3 (2.47 ± 0.19 mg QE/g), and CT4 (2.01 ± 0.17 mg QE/g) ($p < 0.05$, one-way ANOVA, Tukey's test). This descending trend (CT1 > CT2 > CT3 > CT4) suggests a diminishing return in flavonoid yield as the proportion of *Camellia* increases, possibly due to non-linear extraction efficiency and matrix effects from co-ingredients.

Notably, the inclusion of *Ziziphus jujuba* and *Siraitia grosvenorii* in CT1 may synergistically enhance flavonoid extraction. Statistical analysis confirmed that CT1 exhibited significantly higher TFC than CT2–CT4 ($p < 0.05$), supporting this synergistic effect. These co-ingredients likely contribute their own phenolics and improve extraction efficiency through matrix interactions. In contrast, CT4 (80% *Camellia* flower) did not show a proportional increase in TFC ($p > 0.05$), indicating a possible plateau in extractable flavonoids at higher *Camellia* ratios.

Total polyphenol contents

Total polyphenol content in yellow camellia (*C. hakodae* Ninh) flowers was quantified using the linear calibration curve of the acid gallic: y = 0.0121x + 0.0703; R2 = 0.9952, illustrated in Figure 8, and the polyphenol levels of the formulations are shown in Figure 9.

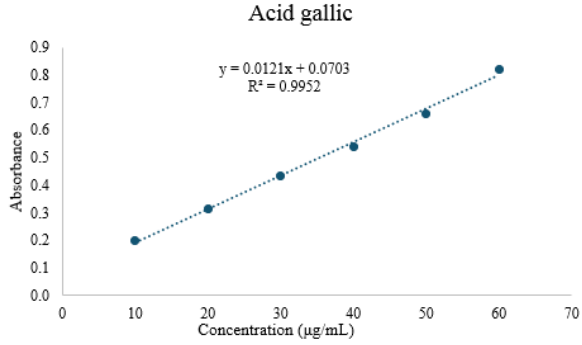

Fig. 8: Acid gallic calibration curve

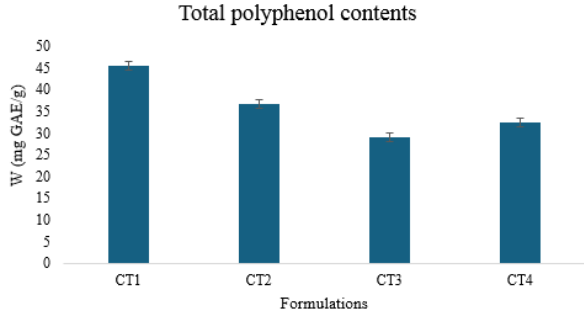

Fig. 9: Total polyphenol contents of formulations (CT1–CT4)

Total polyphenol content showed a clear peak in CT1 (45.39 ± 1.82 mg GAE/g), which was significantly higher than CT2–CT4 ($p = 0.012$, one-way ANOVA, Tukey's test) despite the increasing proportion of *C. hakodae* Ninh flower. This non-linear pattern suggests an extraction

optimum at a balanced composition, where co-ingredients such as *Ziziphus jujuba* and *Siraitia grosvenorii* may enhance polyphenol solubilization and matrix-assisted diffusion. Excessive flower loading (CT3–CT4) did not further increase extractable phenolics, possibly due to solvent saturation and reduced diffusivity.

Biologically, the elevated TPC in CT1 aligns with its strongest antioxidant capacity (lowest DPPH IC_{50}) and α-glucosidase inhibition, reinforcing the central role of phenolic compounds in both radical scavenging and enzyme modulation.

B. Discussion

This study demonstrates that tea blends incorporating *C. hakodae* Ninh flowers exhibit significant antioxidant and α-glucosidase inhibitory activities. The strong activity of CT1 aligns with its enriched polyphenol and flavonoid content, consistent with known mechanisms of radical scavenging and enzyme inhibition [1, 8, 9, 14, 15]. These findings are consistent with previous reports highlighting the correlation between total phenolics and antioxidant or antidiabetic effects in camellia and other herbal teas [6, 8, 14].

The IC_{50} values of CT1 against α-glucosidase were lower than or comparable to those of herbal teas containing *Camellia sinensis* or *Chrysanthemum* extracts [5, 6, 8], suggesting competitive potential. Importantly, the activity of CT1 was superior to that of acarbose, a clinically used α-glucosidase inhibitor, highlighting its potential as a natural alternative in glycemic control [7, 9, 12].

The potential clinical relevance of these herbal tea formulations is noteworthy. The findings indicate possible combined effects rather than proven synergistic interactions. The potent antioxidant activity suggests a role in combating oxidative stress-related conditions (e.g., reducing cellular damage associated with aging, cardiovascular disease, or chronic inflammation), while the marked α-glucosidase inhibition indicates antidiabetic potential (slowing carbohydrate digestion to moderate postprandial blood sugar spikes). Significantly, the best-performing formula outperformed acarbose – a pharmaceutical α-glucosidase inhibitor – *in vitro*, highlighting its promise as a natural adjuvant or alternative in managing hyperglycemia. However, translation to clinical use will require further validation. Future investigations should involve phytochemical profiling (HPLC, LC-MS), mechanism-based assays, and *in vivo* models.

V. CONCLUSION

In summary, this study's findings underscore the significant promise of *C. hakodae* Ninh flower-based herbal tea formulations as functional health-promoting beverages. Among the four formulations, CT1 (with a balanced 50% *Camellia* and 50% supportive herbs) consistently demonstrated the highest levels of bioactive compounds and the most potent biological activities. The enriched flavonoid and polyphenol content in CT1 translated into superior antioxidant capacity and α-glucosidase inhibitory effect, highlighting the importance of optimizing ingredient ratios. These results have practical implications for the development of functional herbal tea products. Particularly, incorporating *C. hakodae* Ninh flowers in synergy with complementary ingredients (such as jujube and monk fruit) can yield a tea blend with enhanced health benefits. The lead formulation (CT1) can be viewed as a prototype for a functional tea that might help mitigate oxidative stress and assist in glycemic control in a natural, dietary form.

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