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Pigments, phenolics, and bioactivities of various extracts from flowers of *Camellia quephongensis*

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Abstract

Camellia quephongensis Hakoda et Ninh, a plant species in the Theaceae family, is native to Vietnam. In this study, the effects of various solvents used for extracting *Camellia quephongensis* flowers on phenolic content, antioxidative, and in vitro anti-inflammatory activities were assessed. The highest total phenolic and flavonoid contents and antioxidant properties were observed for the ethyl acetate extract. Catechin and epicatechin in the ethyl acetate extract showed the highest concentrations (17.23 ± 0.57 mg/g and 3.86 ± 0.42 mg/g, respectively) whereas epigallocatechin gallate had the highest concentration in methanol extract (10.66 ± 0.33 mg/g). Most of the other phenolics were also found at greater concentrations in the ethyl acetate extract in comparison to the other extracts. The results also demonstrated that ethyl acetate displayed the strongest antioxidant potential assessed by ABTS and DPPH radical scavenging assays. The albumin denaturation inhibition assay showed that the ethyl acetate extract had the greatest efficacy, followed by the ethanol and methanol extracts. Chlorophyll and carotenoids were found in smaller amounts compared to those in other *Camellia* species' leaves. These findings provide deeper insights into obtaining extracts with high levels of bioactive compounds, antioxidative potential, and anti-inflammatory effects from *C. quephongensis*.

Keywords

albumin denaturation; antioxidant activity; *Camellia quephongensis;* chlorophyll; phenolics

Introduction

The yellow-flowered tea belongs to the *Camellia* genus, and approximately 52 species have been identified, with over 40 species found in Vietnam, primarily concentrated in the Southern region (1). Yellow-flowered tea, similar to green tea, is commonly used for tea production and can be consumed in fresh or dried forms, with a higher preference for and usage of its flowers over the leaves. The popularity of yellow-flowered tea stems from its health benefits, as suggested by previous studies: it possesses antioxidative properties that aid in treating throat ulcers, diarrhea, high blood pressure, and irregular menstruation, and serves as a preventive measure against cancer (2). Other studies have indicated its capacity to lower blood sugar and blood lipids (3, 4) reduce cholesterol levels in blood, and prevent arterial atherosclerosis (5). These activities are attributed to the presence of multiple bioactive components in yellow-flowered tea, such as polysaccharides, saponins, and phenolics (6, 7). Phenolics are a group of

molecules containing one or more aromatic rings attached to hydroxyl groups. These compounds can be categorized into different subclasses, including phenolic acids, flavonoids, coumarins, stilbenes and tannins. Studies have demonstrated that phenolics possess antioxidant properties, enabling them to counteract the harmful effects of reactive oxygen species (ROS) (8). ROS are involved in the release of nuclear transcription factor NF-κB, which subsequently triggers the formation of inflammatory cytokines and cyclooxygenase-2 (COX-2) (9). Consequently, the use of antioxidants, such as phenolics, can mitigate the impact of free radicals, inhibit the formation of NF-κB, and ultimately prevent the production of inflammatory mediators (10).

Camellia quephongensis is also a yellow-flowered tea recently discovered in 2002. This species resides in tropical forests along riverbanks, typically found at elevations ranging from 200 m to 300 m, characterized as a short-sized shrub, growing up to 4 m to 5 m tall, blooming with flowers of 5.5 cm to 6.5 cm in diameter every year in springtime. It was found in Que Phong district, Nghe An province, Vietnam's central region (11, 12). According to our previous research on this species, the extracts from its leaves contain high levels of phenolics, and catechins, exhibiting good antioxidative properties, and displaying inhibitory effects on a-amylase and albumin denaturation (13).

There has been limited information about the phytochemicals and bioactivities of *C. quephongensis* flowers. There is only one study that has reported the presence of several phenolics in extracts obtained from the flowers of this species (14). To our knowledge, other than this, no quantification of these components and determination of bioactivities of the flowers have been published. The current study aims to quantitatively identify phenolics, antioxidant potential, and albumin denaturation inhibitory capacity of various extracts of *C. quephongensis* flowers. The findings of this study will hopefully give insights into the potential health-endorsing properties of this plant and open up opportunities for its applications in the nutraceutical industry.

Materials and Methods

Plant collection and identification

Camellia quephongensis were collected from Que Phong, a district located in Nghe An, in January 2023. Authentication of the plant was conducted by Dr. Luong Van Dung at Dalat University, and a voucher specimen (CQ-0123) was deposited at the Institute of Applied Technology, Thu Dau Mot University, Binh Duong, Vietnam. Following collection, the flowers were meticulously washed to eliminate soil and debris, then subjected to drying at 45 °C for a day, ensuring moisture content was reduced to ≤ 8 percent, using a convective dryer. The dried samples were subsequently stored in a cool, dry environment for further analysis.

Determination of total phenolic content

The total phenolic content (TPC) in the extracts was determined following a previously established protocol (15). The procedure involved mixing 0.5 mL of diluted extract with 2.5 mL of 10% Folin-Ciocalteu reagent and the mixture was allowed to settle for 5 min in darkness. Afterward, 7.5% Na_2CO_3 (2 mL) was added, and the mixture was shaken and left to incubate in darkness for 60 min. Absorbance was then measured at 765 nm by using a spectrophotometer (Thermo Genesys 20 UV-VIS), with gallic acid serving as the standard compound. TPC was shown as mg of gallic acid equivalents per g of extract (mg GAE/g). Each sample was analyzed in triplicate.

Determination of total flavonoid content

Total flavonoid content (TFC) in extracts was evaluated by utilizing an aluminum chloride solution in a colorimetric method adapted from Nurcholis et al. with a minor modification (16). An aliquot (0.5 mL) from each extract was dispensed with 0.1 mL of aluminum chloride (10%), 1.3 mL of 96% ethanol, and 0.1 mL of 1 M sodium acetate. The mixture subsequently underwent a 40-minute incubation at room temperature in darkness. Absorbance was recorded at 415 nm by using a spectrophotometer (Thermo Genesys 20 UV-VIS). The quantification of TFC was presented as mg quercetin equivalents per g of extract (mg QE/g), with quercetin used as the standard compound. Each sample was analyzed in triplicate.

Analysis of phenolic compounds

The flower extracts were diluted with 80% methanol, and filtered through a 0.45 mm membrane into a vial before injection into a high-performance liquid chromatography system connected with a diode-array detector (HPLC-DAD). The separation was carried out on an Agilent Zorbax Eclipse XDB C18 column (4.6 ´ 150 mm, 5 mm). The mobile phase consisted of solution A (0.1% formic acid) and solution B (100% acetonitrile). The gradient elution was performed using a method previously developed by Nguyen et al. (13). The flow rate was 0.6 mL/min. Detection wavelengths were 295, 340, and 360 nm.

Determination of chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents of the flowers were evaluated using the method described by Lichtenthaler and Buschmann (17). One gram of flower powder was placed into a falcon tube, followed by adding 20 mL of acetone. The mixture was shaken in darkness for 24 hours. After filtering, the resulting solution was centrifuged at 5500 rpm for 15 min. The obtained solution was spectrophotometrically measured at wavelengths of 470, 662, and 645 nm. Chlorophyll a, b, and carotenoid concentrations were determined using the formulae:

Chlorophyll a (C_a) = (11.24 ´ A_{662} – 2.04 ´ A_{645})

Chlorophyll b (C_b) = (20.13 ´ A_{645} – 4.19 ´ A_{662})

Total carotenoid content = [1000 ´ A_{470} – (1.9 ´ CA + 63.14 ´ CB)]/214 V/M

where V and M denote solvent volume (ml) and sample weight (g).

Antioxidant activity

The ABTS ions were generated by combining an ABTS solution (7 mM) with potassium persulfate at a 1:1 ratio and the mixture underwent an incubation in darkness for 16 h. Fifty microliters of the *C. quephongensis* diluted extract were added to 3 mL of the newly prepared ABTS solution, and the mixture was then incubated in darkness for 30 min before being measured at a wavelength of 734 nm (18). For the DPPH assay, 0.5 mL of the diluted extract of *C. quephongensis* was added to 2.5 mL of 0.1 mM DPPH solution, followed by incubation in darkness for 30 min, then measured at a wavelength of 517 nm (19). Ascorbic acid served as the reference standard in the assays. IC_{50} (mg/mL) was employed to assess the ABTS and DPPH scavenging activities of the extracts.

Inhibition of albumin denaturation

A bovine albumin solution (0.16%, 1 mL) was mixed with 1 mL of a diluted extract and 2 mL of sodium acetate buffer (pH 5.5). The mixture underwent a 45-minute incubation at 37 °C. Subsequently, the temperature of the mixture was raised to 67 °C for 3 min. After cooling to room temperature, absorbance was measured at 660 nm. Diclofenac sodium served as a positive control (20).

Statistical analysis

All experiments were performed three times. This study employed ANOVA (Tukey's test) to determine whether pigments, phenolic levels, and bioactivities of the extracts significantly differed. Minitab 19 and Microsoft Excel 365 were used for data analysis.

Results and Discussion

Chlorophyll and carotenoid contents

Chlorophyll is the primary component responsible for the green color in the stems and leaves of the *Camellia* genus. The flowers of *C. quephongensis* contained a low chlorophyll content, with chlorophyll a and b levels of 17.45 – 21.88 µg/g and a total carotenoid content of 52.21 µg/g dry weight. Compared to the chlorophyll content in the stems and leaves of other species within the *Camellia* genus, such as in the leaves of *C. sinensis* (21), the chlorophyll content in the flowers of this species is notably lower.

Table 1: Chlorophyll and carotenoid contents of *C. quephongensis* flowers

Concentrations (µg/g dry weight)	Chlorophyll a	Chlorophyll b	Total carotenoid content
	21.88 ± 0.33	17.45 ± 1.67	52.21 ± 1.59

Phenolics

Phenolics and flavonoids represent the primary compound classes found in the *Camellia* genus, pivotal in exhibiting various bioactivities such as antioxidative, antibacterial, and anticancer properties of this genus (22). Table 2 presents the TPC and TFC results in methanol (ME), ethanol (ET), and ethyl acetate (EA) extracts. The findings showed that the TPC content in EA was the greatest (329.30 mg GAE/g), followed by that in methanol extract at 253.46 mg GAE/g, while ET had the lowest content in three extracts (164.87 mg GAE/g). The TPC in EA and ET are equivalent to TPC content in ethyl acetate and ethanol extracts of *C. nitidissima* Chi flowers (345.14 and 170.74 mg GAE/g, respectively) and higher than that in methanol extract from the leaves of *C. sinensis* (209.17 mg GAE/g) (21, 23). TFC in EA is 44.51 mg QE/g, whereas the lowest content is observed in ET at 8.54 mg QE/g. TFC in EA surpasses that in the methanol extract of *C. sinensis* leaves but falls short of the level in the ethanol extract of *C. sinensis* flowers (22, 24).

According to the data presented in Table 2, seven of the examined phenolics presented significantly higher concentrations in EA compared to those in the other extracts. The concentration of gallic acid in EA (21.79 ± 0.11 mg/g) was 5 – 6 times as high as those in ME and ET. EA also was much richer in ferulic acid compared to ME and ET. No significant differences in chlorogenic acid levels among the extracts were observed. EA contained 2 – 6 times as many catechins as ME and ET. Another catechin compound, namely epicatechin, was found to be more abundant in EA compared to the other extracts. In contrast, the concentrations of EGCG in the extracts followed the order: ME > ET > EA. The other flavonoids (i.e., rutin, quercetin, and kaempferol) were detected at the highest levels in EA than in the other extracts. Overall, the HPLC-DAD analytical results could in part explain the highest TPC of EA as discussed earlier. One study on *C. nitidissima* demonstrated ethyl acetate extract from its flowers had the highest TPC compared to the other extract (23). However, research has reported that ethyl acetate is not always an effective solvent to extract phenolics. Previously, we revealed that ethyl acetate extract obtained from *C. quephongensis* leaves was poor in phenolics (13). Regarding these points, the effectiveness of ethyl acetate generally depends on the specific plant material and the phenolic compounds present. Several factors that could explain why ethyl acetate was successful in extracting phenolics from *Camellia* flowers include its higher affinity for the specific types of phenolic compounds present in the flowers.

Antioxidant activity

The antioxidant potential of the extracts of *C. quephongensis* flowers was assessed using DPPH and ABTS methods as presented in Table 2. Regarding the antioxidative activities using DPPH and ABTS, EA exhibited superior antioxidative capabilities compared to the other extracts, with IC_{50} of 90.24 and 116.40 µg/mL, respectively. The weakest antioxidant activity was observed for ET with IC_{50} of 146.83 and 221.72 µg/mL. These results align with TPC and TFC found in the respective extracts. In comparison with the DPPH and ABTS antioxidative abilities of stems and leaves, which our group previously investigated, the flower's capabilities are inferior (13). In terms of ABTS antioxidative activity, the flower of *C. quephongensis* outperforms the methanol extract from the leaves of *C. fascicularis* (343.45 µg/mL) but is surpassed by methanol extracts from the leaves of *C. nitidissima* (64.24 µg/mL) and *C. sinensis* (26-37 µg/mL). However, the

Table 2. Phenolic contents of the *C. quephongensis* flower extracts

Phenolics		Concentrations		
		ME	ET	EA
Gallic acid	mg/g	3.53 ± 0.07 c	4.16 ± 0.06 b	21.79 ± 0.11 a
Chlorogenic acid		0.16 ± 0.00	0.16 ± 0.00	0.19 ± 0.02
Catechin		6.93 ± 0.73 b	3.37 ± 0.39 c	17.23 ± 0.57 a
Epicatechin		0.45 ± 0.07 c	1.44 ± 0.30 b	3.86 ± 0.42 a
EGCG		10.66 ± 0.33 a	8.50 ± 0.23 b	1.97 ± 0.16 c
Rutin		0.41 ± 0.02 b	0.44 ± 0.01 b	0.64 ± 0.02 a
Ferulic acid		0.82 ± 0.01 c	1.00 ± 0.05 b	2.09 ± 0.02 a
Quercetin		0.27 ± 0.01 b	0.26 ± 0.00 b	0.94 ± 0.02 a
Kaempferol		0.45 ± 0.00 b	0.51 ± 0.00 b	1.66 ± 0.04 a
TPC	mg GAE/g	253.46 ± 0.69 b	164.87 ± 0.08 c	329.30 ± 0.26 a
TFC	mg QE/g	14.96 ± 0.06 b	8.54 ± 0.79 c	44.51 ± 1.43 a

Data are presented as mean ± standard deviation of triplicate measurements. Different letters (a, b, c) show significant differences in phenolic contents among the extracts ($p < 0.05$).

DPPH method demonstrates weaker antioxidative abilities compared to those found in the leaves of *Camellia fascicularis* (14.07 µg/mL) and *C. nitidissima* (78.80 µg/mL) (23, 25).

Inhibition of bovine albumin denaturation

This assay can be employed to predict the potential anti-inflammatory activity of plant extracts (26). As shown in Table 3, IC_{50} values of all the extracts were higher than 500 µg/mL, significantly greater than that of diclofenac (84.40 ± 4.90 µg/mL). This indicates a weaker inhibitory effect of the extracts on albumin denaturation. A previous study revealed aqueous methanolic extract of C. *quephongensis* leaves had a strong capacity to protect albumin from denaturation (13). In one study, extracts derived from *C. sinensis* flowers exhibited a strong anti-inflammatory effect against acute inflammation and immunological liver inflammation. The observed anti-inflammatory properties were attributed to the suppression of nitric oxide (NO) production and the downregulation of mRNA expression for TNF-a and IL-1β. Flower extracts from another yellow camellia (*C. nitidissima*) were shown to have an anti-inflammatory activity because of 3-cinnamoyltribuloside detected in its phytochemical composition (27, 28).

In the present study, levels of pigments, phenolics, antioxidant activity, and albumin denaturation inhibitory effect of the *C. quephongensis* flower extracts were revealed. While this study is the first work to report quantitative data on phytochemicals in flowers of this *Camellia* species, it has several limitations. The determination of chlorophyll and carotenoid contents by spectrophotometry could be susceptible to interference from other compounds present in the sample, such as proteins, sugars, and phenolic compounds. These interferences can influence the accuracy of the measurements and lead to overestimation or underestimation of chlorophyll and carotenoid concentrations. Few phenolics in the sample were monitored in the study, leading to an incomplete picture of the overall phenolic composition of *C. quephongensis* flowers. This also limits the understanding of the sample's potential human health benefits.

Table 3. Antioxidant activities of *C. quephongensis* flower extracts

Sample	IC_{50}, µg/mL	
	ABTS	DPPH
ME	130.82 ± 0.49 b	104.59 ± 0.36 b
ET	221.72 ± 0.18 a	146.83 ± 0.50 a
EA	116.40 ± 0.37 c	90.24 ± 0.01 c
Ascorbic acid	63.39 ± 0.14 d	28.69 ± 0.12 d

Data are presented as mean ± standard deviation of triplicate measurements. Different letters (a, b, c) show significant differences in phenolic contents among the extracts ($p < 0.05$).

Table 4. Bovine albumin denaturation inhibitory activity of *C. quephongensis* flower extracts

Samples	Inhibition percentage*, %				IC_{50} (mg/mL)
	62.5 mg/mL	125 mg/mL	250 mg/mL	500 mg/mL	
ME	1.56 ± 0.39	1.52 ± 0.53	1.64 ± 0.38	24.32 ± 2.92	> 500
ET	8.31 ± 3.01	13.21 ± 2.37	19.41 ± 2.97	25.94 ± 2.80	> 500
EA	15.37 ± 5.15	15.66 ± 1.99	22.77 ± 1.32	31.46 ± 5.13	> 500
Diclofenac	n.a.	n.a.	n.a.	n.a.	84.40 ± 4.90

Data are presented as mean ± standard deviation of triplicate measurements. n.a.: not available.

Conclusion

This research is the first work to explore pigments, phenolic contents, antioxidants, and albumin denaturation inhibitory activities of various extracts from *C. quephongensis* flowers. Catechins were the most abundant phenolics in the flowers. Ethyl acetate extract had the highest total phenolic content (329.30 mg GAE/g). It also exerted the most potent DPPH and ABTS antioxidant activity among the extracts, with IC_{50} of 90.24 and 116.40 µg/mL. All the extracts presented a weaker protective effect against albumin denaturation. The results indicated potential applications of the flowers in the nutraceutical industry. Further studies are needed to explore other chemical classes and to assess the potential health benefits of the flowers.

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Authors' contributions

NTN carried out bioassays and drafted the manuscript. THDN participated in the design of the study performed the statistical analyses and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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