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Quantitative analysis of some chemical components in leaves and flowers of *Camellia luongii* Tran & Le in Vietnam

Phân tích định lượng thành phần hóa học trong lá và hoa của loài *Camellia luongii* Tran & Le ở Việt Nam

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Abstract

This study presents a comparative quantitative analysis of major bioactive compounds caffeine, polyphenols, saponins, and flavonoids in the leaves and flowers of *Camellia luongii*, a relatively unexplored *Camellia* species. The results indicate that the leaves of *C. luongii* contain significantly higher concentrations of caffeine (129.0 mg/100 g), total polyphenols (5260 mg/100 g), and total flavonoids (453.0 mg/100 g) compared to its flowers. When benchmarked against other *Camellia* species, such as *C. sinensis*, *C. japonica*, and *C. oleifera*, *C. luongii* demonstrates moderate caffeine content but remarkably high polyphenol levels relative to several species. These findings suggest the potential of *C. luongii* as a new source of natural antioxidants and bioactive compounds for functional food or nutraceutical applications.

Keywords: Caffeine; flavonoids; natural antioxidants; polyphenols; saponins.

Tóm tắt

Nghiên cứu này trình bày phân tích định lượng các hợp chất hoạt tính sinh học chính caffeine, polyphenol, saponin và flavonoid rong lá và hoa của *Camellia luongii*, một loài *Camellia* chưa được nghiên cứu trước đây. Kết quả chỉ ra rằng lá của *C. luongii* chứa nồng độ caffeine (129,0 mg/100 g), tổng polyphenol (5260 mg/100 g) và tổng flavonoid (453,0 mg/100 g) cao hơn đáng kể so với hoa của nó. Khi so sánh với các loài *Camellia* khác, chẳng hạn như *C. sinensis, C. japonica* và *C. oleifera*, *C. luongii* cho thấy hàm lượng caffeine vừa phải nhưng mức polyphenol cao đáng kể so với một số loài. Những phát hiện này cho thấy tiềm năng của *C. luongii* như một nguồn chất chống oxy hóa tự nhiên và hợp chất hoạt tính sinh học mới cho các ứng dụng thực phẩm chức năng hoặc dược phẩm chức năng.

Từ khóa: Caffeine; Polyphenol; Saponin; Flavonoid; Chất chống oxy hóa tự nhiên.

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1. Introduction

The *Camellia* genus, first described by Linnaeus in 1753, includes approximately 120 to 280 species, predominantly distributed throughout East and Southeast Asia. Vietnam and China are recognized as biodiversity hotspots for this genus, accounting for the highest number of species reported. Phytochemical and pharmacological investigations have largely centered on three key species with substantial commercial relevance *Camellia sinensis* (L.) Kuntze, *Camellia japonica* L., and *Camellia oleifera* Abel due to their extensive applications in tea production and edible oil extraction [19]. Among these, *Camellia sinensis* has been particularly well-characterized and contains a broad spectrum of secondary metabolites, such as alkaloids, terpenoids, steroids, saponins, and polyphenolic compounds. Numerous studies have confirmed that members of the genus *Camellia* possess a wide range of bioactivities, notably antioxidant, antimicrobial, antifungal, antiviral, and anticancer properties, suggesting their potential utility in pharmaceutical and nutraceutical contexts [16].

Camellia luongii Tran & Le is a recently identified yellow-flowered species endemic to Thai Nguyen Province, Vietnam, with its initial collection recorded in 2015. Historical accounts from residents suggest that the species once thrived in dense populations across mountainous regions, although it was largely overlooked at the time. Since 2011, however, there has been increasing exploitation of this plant, including the harvesting of flower buds (both immature and mature), dried flowers, fruits, and seeds for commercial purposes. Additionally, branches have been extensively collected for propagation in horticultural settings. As a result, the remaining wild populations predominantly consist of individuals regenerated from original rootstocks. The current condition of these wild trees is concerning, as they are often found beneath large canopy trees that were subsequently cut down during land clearing for industrial agricultural development.

To assess the potential applications of *Camellia luongii* in comparison with other tea species, this study conducted a quantitative analysis of key tea constituents, including caffeine, total polyphenols, total saponins, and total flavonoids, in both the leaves and flowers of the species. Notably, this represents the first reported quantification of these bioactive compounds in *Camellia luongii*, providing a foundational dataset for future research and potential utilization.

2. Materials and methods

2.1. Plant materials

In 2022, samples of *Camellia luongii*, including mature leaves (CLL), young leaf buds (CLB), and fruit pericarps (CLH), were collected from Na Chi Phang village, Lung Than commune, Si Ma Cai district, Lao Cai province, northern Vietnam. A voucher specimen was deposited at the Forest Industry Research Institute under the Vietnam Academy of Forest Sciences for documentation. The plant materials were rinsed thoroughly with tap water to remove debris and subsequently oven-dried at 60°C for 48 hours.

2.2. Extraction

The dried plant materials were subjected to triple extraction using 95% ethanol under ultrasonic-assisted conditions. The resulting ethanol solutions were concentrated under reduced pressure at 50°C using an IKA RV10 rotary evaporator (Germany) until complete solvent removal was achieved. The dried crude extracts were then preserved at 4°C for subsequent phytochemical and biological analyses.

2.3. Caffeine quantification

The sample was weighed into a 50 mL centrifuge tube, and approximately 40 mL of hot water was added. It was vortexed and sonicated in an ultrasonic bath at 80°C for 60 minutes. Subsequently, it was centrifuged at 6000 rpm for 5 minutes, and the supernatant was transferred to a 100 mL volumetric flask. The process was repeated with 40 mL of hot water, sonicated for 30 minutes. The combined supernatants were collected in a 100 mL volumetric flask and made up to the mark with water. The solution was filtered through filter paper and injected into the HPLC system [9].

HPLC Conditions

- C18 column (Waters) (250 mm × 4.6 mm × 5 µm) with a corresponding guard column
- PDA detector at 272 nm
- Mobile phase: H_2O: MeOH = 70 : 30
- Flow rate: 1 mL/min
- Injection volume: 20 µL

2.4. Quantification of total polyphenols

The sample was weighed into a 50 mL centrifuge tube. Approximately 30 mL of 70% methanol was added, vortexed, and hydrolyzed in a water bath at 70°C for 1 hour. It was then centrifuged at 6000 rpm for 5 minutes. The supernatant was decanted into a 50 mL volumetric flask. The process was repeated with 10–20 mL of 70% methanol. The combined supernatants were collected in a 50 mL volumetric flask and made up to the mark with 70% methanol. The solution was filtered through filter paper, reacted with the Folin-Ciocalteu reagent, and measured using a UV-Vis spectrophotometer at 737 nm [10].

2.5. Quantification of total saponins

Saponins in the sample were extracted using methanol, purified, and impurities were removed by shaking with n-butanol and water. The extract was defatted with ether, then dried, and the obtained mass was weighed to determine the saponin content [11].

2.6. Quantification of total flavonoids

Total flavonoids in the sample were extracted using ethanol. The extract was then reacted with $AlCl_3$ to form a colored complex in an alkaline medium and measured spectrophotometrically at 415 nm [11].

3. Results and discussion

The chemical composition analysis revealed significant differences between the leaves and flowers in terms of bioactive compound content (Table 1). Specifically, the caffeine content in the leaves (129.0 mg/100 g) was more than six times higher than in the flowers (21.2 mg/100 g), indicating a greater potential for physiological stimulation in the leaves. The total polyphenol content was similar between the two parts, with 5.26 g/100 g in leaves and 5.09 g/100 g in flowers. Regarding total saponins, the leaves contained 14.2 g/100 g compared to 13.1 g/100 g in the flowers, which may contribute to higher anti-inflammatory and antimicrobial activity in the leaves. Notably, the total flavonoid content in the leaves (453.0 mg/100 g) was more than four times that of the flowers (109.0 mg/100 g), suggesting a markedly superior antioxidant potential.

Table 1. Quantitative analysis of compounds characteristic of *Camellia luongii* species.

Analysis indicators	Unit	Leaves	Flowers
Caffeine	mg/100 g	129.0	21.2
Total polyphenols	mg/100 g	5260	5090
Total Saponins	mg/100 g	14.2	13.1
Total Flavonoids	mg/100 g	453.0	109.0

The quantitative analysis of *Camellia luongii* revealed significant differences in the distribution of bioactive compounds between leaves and flowers, as well as in comparison to other *Camellia* species. Notably, the caffeine content in the leaves of *C. luongii* (129.0 mg/100 g DW) (Table 1) was markedly lower than that found in commonly consumed *C. sinensis* teas, such as green tea (1830–2530 mg/100 g) and black tea (1430–3480 mg/100 g) (Table 2), suggesting its potential as a low-caffeine alternative. Conversely, *C. luongii* flowers contained even less caffeine (21.2 mg/100 g), further underscoring its suitability for health-conscious formulations.

Table 2. Summary of the total caffeine content of some *Camellia* species

Material	Content (mg/100g DW)	Solvent	Part
Mate tea *(Camellia sinensis)*	610 to 1320	Water	Leave [17]
White tea *(Camellia sinensis)*	2150 to 31000	Water	Leave [17]
Green tea *(Camellia sinensis)*	1830 to 2530	Water	Leave [17]
Black tea *(Camellia sinensis)*	1430 to 3480	Water	Leave [17]
China green tea *(Camellia sinensis)*	2722.4	Water	Leave [1]
China black tea *(Camellia sinensis)*	2709.5	Water	Leave [1]
Japan green tea *(Camellia sinensis)*	1883.1	Water	Leave [1]
Japan black tea *(Camellia sinensis)*	2165.0	Water	Leave [1]
Nepal green tea *(Camellia sinensis)*	1978.8	Water	Leave [1]
Nepal black tea *(Camellia sinensis)*	3671.5	Water	Leave [1]
Korea green tea *(Camellia sinensis)*	1977.5	Water	Leave [1]
Korea black tea *(Camellia sinensis)*	2485.2	Water	Leave [1]

In terms of total polyphenol content, *C. luongii* leaves (5260 mg/100 g) and flowers (5090 mg/100 g) exhibited levels considerably higher than those found in *C. japonica* leaves (74.62 mg/100 g) (Table 3) and comparable to mid-range values reported for *C. sinensis*-derived green and black teas. However, these values remained lower than the exceptionally high polyphenol concentrations found in the flowers of species such as *C. achrysantha* (17188 mg/100 g) and *C. pubipetala* (17000 mg/100 g), which may have specialized applications in antioxidant-rich formulations.

Table 3. Summary of total polyphenol content of some *Camellia* species

Material	Content (mg/100 g DW)	Solvent	Part
Camellia japonica	74.62	Methanol	Young leaves [6]
Camellia japonica	65.02	Methanol	flower buds [6]
Camellia japonica	62.42	Methanol	flowers [6]
Camellia oleifera	1.0333-2.3800	Petroleum ether	Fruit [8]
Black tea *(Camellia sinensis)*	1132	Water	Leave [13]
Green tea *(Camellia sinensis)*	1235.5	Water	Leave [13]
Black tea *(Camellia sinensis)*	1164	Ethanol	Leave [13]
Green tea *(Camellia sinensis)*	1346	Ethanol	Leave [13]
Camellia oleifera seed oil	0.34-5.88	*n*-Hexane	Seed [7]
Camellia tunghinensis	5988	Ethanol (75%)	Flower [15]
Camellia longzhouensis	14406	Ethanol (75%)	Flower [15]
Camellia pubipetala	17000	Ethanol (75%)	Flower [15]

Camellia quinqueloculosa	14796	Ethanol (75%)	Flower [15]
Camellia achrysantha	17188	Ethanol (75%)	Flower [15]
Camellia impressinervis	14210	Ethanol (75%)	Flower [15]
Camellia insularis	16506	Ethanol (75%)	Flower [15]
Camellia perpetua	7608	Ethanol (75%)	Flower [15]
Camellia micrantha	6326	Ethanol (75%)	Flower [15]
Camellia nitidissima	7422	Ethanol (75%)	Flower [15]
Camellia oleifera	1.26	SC–CO_2	Seed [12]

In terms of total polyphenol content, *C. luongii* leaves (5260 mg/100 g) and flowers (5090 mg/100 g) exhibited levels considerably higher than those found in *C. japonica* leaves (74.62 mg/100 g) (Table 4) and comparable to mid-range values reported for *C. sinensis*-derived green and black teas. However, these values remained lower than the exceptionally high polyphenol concentrations found in the flowers of species such as *C. achrysantha* (17188 mg/100 g) and *C. pubipetala* (17000 mg/100 g), which may have specialized applications in antioxidant-rich formulations.

Table 4. Summary of total flavonoid content of some *Camellia* species

Material	**Content (mg/100 g DW)**	**Solvent**	**Part**
Camellia oleifera	180 to 630	Petroleum ether	Fruit [8]
Black tea	633	Water	Leave [13]
Green tea	1698	Water	Leave [13]
Black tea	1950	Ethanol	Leave [13]
Green tea	3155	Ethanol	Leave [13]
Green tea	188	Water	Leave [4]
Camellia tunghinensis	320	Ethanol (75%)	Flower [15]
Camellia longzhouensis	506	Ethanol (75%)	Flower [15]
Camellia pubipetala	428	Ethanol (75%)	Flower [15]
Camellia quinqueloculosa	310	Ethanol (75%)	Flower [15]
Camellia achrysantha	536	Ethanol (75%)	Flower [15]
Camellia impressinervis	662	Ethanol (75%)	Flower [15]
Camellia insularis	826	Ethanol (75%)	Flower [15]
Camellia perpatua	842	Ethanol (75%)	Flower [15]
Camellia micrantha	346	Ethanol (75%)	Flower [15]
Camellia nitidissima	848	Ethanol (75%)	Flower [15]
White tea	50.26	Ethanol	Leave [5]
Green tea	21.69	Ethanol	Leave [5]
Camellia sinensis var *assamica*	191.40	Ethanol (75%)	Leave [14]
Camellia sinensis	3320	Water	Leave [3]

Total saponins were relatively low in both leaves (14.2 mg/100 g) and flowers (13.1 mg/100 g) of *C. luongii*, particularly when contrasted with *C. sinensis* seeds (187.91 mg/100 g) and *C. oleifera* seeds (74.12 mg/100 g) (Table 5). This suggests that *C. luongii* may not be a major source of saponins compared to other Camellia species, although its levels are higher than some reported floral values, such as those in *C. nitidissima* flowers (56.53 mg/100 g).

Table 5. Summary of total saponin content of some *Camellia* species

Material	Content (mg/g WD)	Solvent	Part
Camellia oleifera	74.12	60% ethanol	Seed [20]
Camellia sinensis	71.39	70% methanol	Green bud [2]
Camellia sinensis	38.05	70% methanol	Flowers bloomed [2]
Camellia sinensis	187.91	70% methanol	Freshly mature seed [2]
Camellia nitidissima	38.83	95% ethanol	Leave [18]
Camellia nitidissima	56.53	95% ethanol	Flower Leave [18]

In summary, *Camellia luongii* demonstrates a distinct phytochemical profile, characterized by low caffeine content, moderate levels of polyphenols and flavonoids, and low saponin concentrations. These characteristics suggest that *C. luongii* may be particularly suitable for functional food or beverage development targeting antioxidant benefits with minimal stimulant effects.

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Conflicts of Interest

The authors declare no conflict of interest.

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