

Research Article

Volatile compounds and antioxidant properties of ethanol extract of *Camellia yokdonensis*

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Abstract

Camellia yokdonensis is an endemic species in Vietnam. The present study reveals the composition of volatile compounds and antioxidant property of the ethanol extract obtained from the leaves of this plant. A total of eleven chemical components were identified in the leaf extract in which β -monolinolein (46.14%), glycerol β -palmitate (14.69%), phytol (11.99%), squalene (7.82%), γ -sitosterol (5.24%) are the major compounds. In addition, the studied extract also shows the potent DPPH free radical scavenging with the IC_{50} value of 6.04 μ g/mL, comparable to the positive control vitamin C, which showed the IC_{50} value of 1.56 μ g/mL.

Keywords: *Camellia yokdonensis*, Ethanol extract, Volatile components, DPPH, GC/MS

Introduction

The genus *Camellia*, belonging to the family Theaceae, comprises approximately 280 species (Hoi *et al.*, 2021). These members of this genus are predominantly found in southern China and northern Vietnam (Ming & Bartholomew, 2007; Hoi *et al.*, 2021) while some species were found in several Asian countries (Páscoa *et al.*, 2019). Several species within the *Camellia* genus have been reported as the significant economic importance because of their valuable derivatives, including oil seeds, tea, ornamental shrubs, and decorative plants (Yang *et al.*, 2016). *Camellia sinensis* (widely known as the tea plant), *Camellia japonica* (valued for its ornamental flowers), and *Camellia oleifera* (cultivated for its edible oil) have been known as the key species belonging to this genus. Furthermore, studies also demonstrated that some *Camellia* species possessed so many biological activities such as antioxidant, antitumor, and antimicrobial properties (Chitsazan, 2015). Notably, *Camellia* plants, majority of *C. sinensis*, *C. oleifera*, and *C. japonica*, are widely recognized as a natural source of antioxidant agents, with the leaves being the most extensively studied organ, either through aqueous extracts or various solvent extracts, including ethanol, methanol, butanol, acetone, n-hexane, ethyl acetate, acetonitrile, chloroform, and isopropanol (Teixeira & Sousa, 2021).

Camellia yokdonensis Dung bis & Hakoda, also known as “pink tea”, is the first published as a new species to science in 2007 that its distribution has been only found in Dak Lak province, Vietnam so far (Hakoda *et al.*, 2007). Studies also provided the biological activities and primary phytochemistry of this species. For instance, Hoang *et al.* (2024) reported the cytotoxicity and antioxidant capabilities of the methanol extract obtained from the *C. yokdonensis* leaves. Recently, Do *et al.* (2024) provided the primary phytochemistry and antioxidant property of the flower tea wine of *C. yokdonensis*. This indicates that the key properties of this species remain largely unexplored. Therefore, in this study, we present the volatile compositions

and antioxidant activity of the ethanol extract isolated from *C. yokdonensis* leaves for the first time.

Materials and methods

Specimen collection and processing

The specimen of *Camellia yokdonensis* Dung bis & Hakoda was collected in village 6, Ea Drang commune, Dak Lak province, Vietnam (Figure 1). Following collection, the *C. yokdonensis* leaf was washed, and dried in an oven at 50 °C until a constant weight was achieved. The dried material was then ground into a fine powder. Extraction was performed by macerating the powdered samples in 99% ethanol (Fisher, USA) at a 1:10 (w/v) ratio for 72 hours at room temperature. The mixture was subsequently filtered through filter paper to obtain the crude extract. To maximize extraction efficiency, the maceration and filtration steps were repeated twice. The combined filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at 45 °C to remove the solvent. The resulting concentrated extract was further dried at 45 °C until a constant mass was reached. The final dried extract was stored at 4 °C for subsequent analyses.

Gas chromatography-mass spectrometry (GC/MS)

The volatile compounds of the ethanol extract of the *C. yokdonensis* leaf were analyzed on a GC 2010 Gas Chromatograph (Shimadzu, Japan) combined with a GCMS-QP2010 single quadrupole mass spectrometer. The SLB-5MS column (30 m x 0.25 mm x 0.25 μ m) was used as the stationary phase and Helium with a flow rate of 0.7 mL/min was used as the carrier gas to determine the chemical composition of the ethanol extract. The sample was injected into the GC system using a 10:1 split-sampling method with a split time of 1 min. The injection temperature was set at 200 °C. The oven temperature was programmed to proceed from 50 °C for 2 min, and increased by 5 °C/min until reaching 80 °C, 5 °C/min until



Figure 1: *Camellia yokdonensis*. a) part of plant, b) leaves, c and d) flowers

reaching 150 °C, 10 °C/min to 280 °C. The ion source temperature was 240 °C, the interface temperature was 300 °C. The mass scan range of the MS was 35-500 m/z with a scanning frequency of 2 scans/sec. The spectral method was performed using Shimadzu GC/MS Postrun software which was used to generate data. To determine the chemical composition of the extract sample based on retention time, mass spectrum and peak area of the compound and compared between the mass spectra of the substances with the spectrum library (NIST library 2014). The percentage of substances identified was based on the peak area of that substance divided by the total peak area of all substances and multiplied by 100.

Antioxidant activity

The antioxidant activity of the ethanol extract obtained from the *C. yokdonensis* leaf was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Extracts were fully dissolved in 99.9% methanol (Fisher, USA) and diluted to appropriate concentrations. A volume of 0.5 mL of each sample solution was mixed with 2.0 mL of 0.076 mM DPPH (Alfa Aesar, USA) solution in methanol. The mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with methanol serving as the blank. All measurements were performed in triplicate. Ascorbic acid (Bio Basic, Canada) was used as the positive control. The antioxidant activity of the samples was expressed as the IC₅₀ value, defined as the concentration of the extract required to scavenge 50% of the DPPH free radicals. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\%DPPH = \left(\frac{A_0 - A_i}{A_0} \right) \times 100\%$$

Where

A₀ is the absorbance of the DPPH solution without sample
A_i is the absorbance of the DPPH solution with sample

Results and discussion

Chemical compositions of ethanol extract from *C. yokdonensis* leaf

The volatile compounds of the ethanol extract isolated from the *C. yokdonensis* leaf were shown in the Figure 2 and Table 1. Accordingly, a total of eleven components were isolated from the studied sample, including β-monolinolein (46.14%), glycerol β-palmitate (14.69%), phytol (11.99%), squalene (7.82%), γ-sitosterol (5.24%), cyclopropanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester (5.30%), pentadecanoic acid (4.13%), stigmasterol (1.70%), fumaric acid, decyl 2-dimethylaminoethyl octadecyl ester (1.50%), glycidyl palmitate (0.98%), and arachidonic acid (0.51%).

Studies demonstrated that some compounds isolated from the ethanol extract of the *C. yokdonensis* leaf possessed the biological activities. For instance, β-monolinolein, the most abundant compound in the extract, had an inhibitory effect on some bacterial and fungi strains, such as *Bacillus subtilis* (Kusumah *et al.*, 2020) *Phytophthora infestans*, and *Rhizoctonia repens* (Stoessel *et al.*, 1980). Furthermore, β-monolinolein was also demonstrated significant chemopreventive and anti-breast cancer properties. *In vitro* studies demonstrated its strong cytotoxic action against MDA-MB-231 breast cancer cells, with an IC₅₀ value of 13.2 μg/mL and clear evidence of apoptosis-related morphological changes. In an *in vivo* model of DMBA-induced mammary carcinoma, administration of β-monolinolein, particularly in combination with stigmasterol, markedly reduced tumor mass and volume, improved body weight, and mitigated overall tumor burden. Histological and immunohistochemical evaluations confirmed enhanced apoptosis through TUNEL assay and reduced proliferation based on lower Ki-67 expression. Furthermore, treatment with β-monolinolein restored abnormal elevations of glycoproteins, lysosomal enzymes, and tumor marker enzymes (γ-GT, LDH, ALP) to near-normal levels, reflecting suppression of tumor progression. Notably, these therapeutic effects were achieved without detectable toxicity in experimental animals, highlighting β-monolinolein as a promising natural compound for breast cancer prevention and treatment (Sofi *et al.*, 2018).

Glycerol β-palmitate, one of the major components in the *C. yokdonensis* leaf extract, has been demonstrated to have significant antifungal activity by interfering with fungal growth and survival. Studies suggest that it compromises membrane stability and alters essential metabolic pathways in pathogenic fungi, thereby inhibiting spore germination and restricting hyphal expansion. Such inhibitory effects have been documented across multiple phytopathogenic species, suggesting a function as a natural plant defense metabolite. Beyond its structural role as a lipid, β-palmitate therefore emerges as a biologically active compound with potential applications in sustainable crop protection and the design of eco-friendly antifungal strategies (Vyssotski *et al.*, 2015).

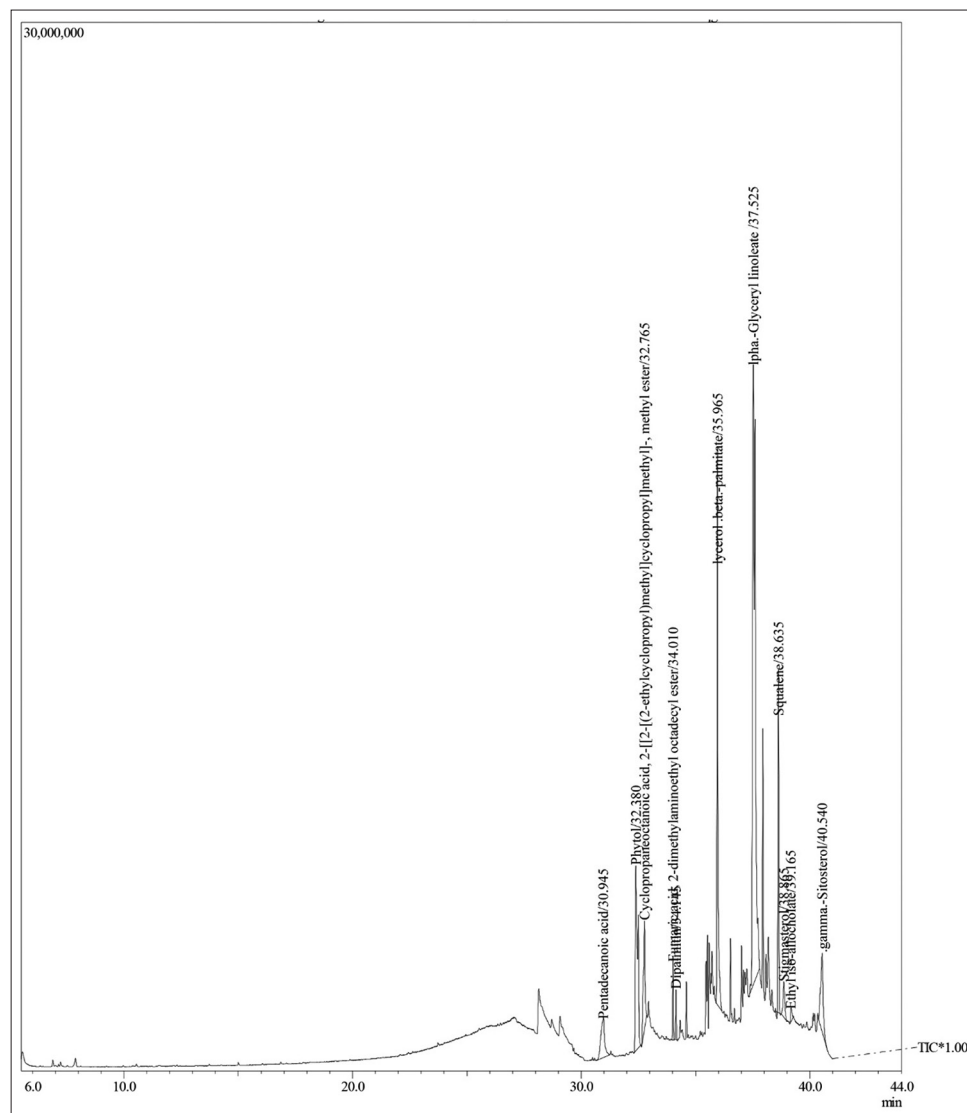


Figure 2: The GC chromatogram of ethanol extract from *C. yokdonensis* leaf

Table 1: The chemical components of ethanol extract from *C. yokdonensis* leaf

Peak	RT (time)	Compounds	Area	Area%
1	30.945	Pentadecanoic acid	13060862	4.13
2	32.380	Phytol	37898598	11.99
3	32.765	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl]-, methyl ester cyclopropyl] methyl]-, methyl ester	16768642	5.30
4	34.010	Fumaric acid, decyl 2-dimethylaminoethyl octadecyl ester	4729000	1.50
5	34.090	Glycidyl palmitate	3106717	0.98
6	35.965	Glycerol β -palmitate	46438053	14.69
7	37.525	β -Monolinolein	145867479	46.14
8	38.635	Squalene	24717097	7.82
9	38.865	Stigmasterol	5387949	1.70
10	39.165	Arachidonic acid	1605273	0.51
11	40.540	γ -Sitosterol	16562521	5.24
Total				100.00

Studies also provided the volatile compounds of the different extracts isolated from the other species belonging to the genus *Camellia* using GC/MS technique. For instance, the methanol extract of *C. japonica* leaf collected from Darjeeling Himalaya was found to be rich in squalene; lupeol; and 1,2-Benzenedicarboxylic acid, diethyl ester (Majumder *et al.*, 2020). The volatile compounds of the methanol, ethanol and ethyl acetate extracts obtained from the fruit shell of *C. oleifera* grown in China have also

reported (Xie *et al.*, 2018). Accordingly, the methanol extract showed γ -sitosterol; 5-hydroxymethylfurfural; and cis-vaccenic acid as the major compounds. The ethanol was identified as a mixture of 5-hydroxymethylfurfural; furfural; and pyrazole-4-carboxaldehyde, 1-methyl- whereas cis-vaccenic acid; n-hexadecanoic acid; and coniferyl aldehyde were the main components in the ethyl acetate extract (Xie *et al.*, 2018). The benzene/ethanol extract of the *C. japonica* leaf was also reported in which butyraldehyde,

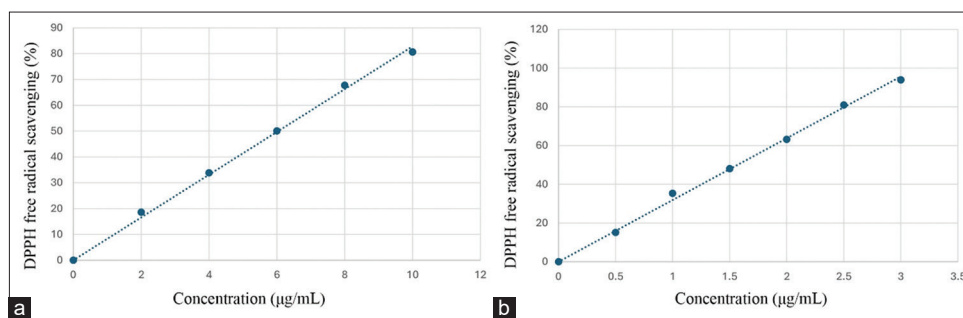


Figure 3: DPPH free radical scavenging of the ethanol leaf extract of *C. yokdonensis* a) leaf and b) vitamin C

semicarbazone; hexatriacontane; 1,6-anhydro-β-D-glucopyranose were the main compounds (Liu *et al.*, 2009).

The extract of the *C. sinensis* collected from India mainly contained anethole, and 1-monolinoleoylglycerol trimethylsilyl ether (Gupta & Kumar, 2017). Pradhan and Dubey (2021) showed the volatile compounds of the methanol extract from the *C. sinensis* leaf and the acetone extract from the *C. assamica* leaf (Pradhan & Dubey, 2021). Accordingly, the *C. sinensis* leaf extract was found to be rich in *n*-heptadecanol-1; 2-pentanone, 4-hydroxy-4-methyl-; and 7-Hexadecanoic acid, methyl ester, (*Z*) while 2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether; *N*(Trifluoroacetyl)*O,O',O''*tris(trimethylsilyl)epinephrine; and Tetracosamethyl cyclododecasiloxane were the abundant components in the *C. assamica* leaf extract (Pradhan & Dubey, 2021). The volatile compounds of the methanol extract of the mature leaves of *C. sinensis* were also identified using GC-MS analysis. Accordingly, this extract was found to be rich in caffeine; hexadecanoic acid, methyl ester; and 9, 12, 15-Octadecatrienoic acid methyl ester (*Z, Z, Z*) (Hasan *et al.*, 2024). Similarly, Hope *et al.* (2022) provided volatile compounds of the ethanol extract of the *C. sinensis* leaf was characterized by the predominance of caffeine, Naphthacene-5,12-dione, 6,11-dihydroxy-2,3,8,9-tetramethyl-; and Estr-1,3,5(10)-trien-17-ol, 2,3,4-trimethoxy-, (17β)- (Hope *et al.*, 2022).

Antioxidant activity of ethanol extract from *C. yokdonensis* leaf

The DPPH free radical scavenging of the ethanol leaf extract of *C. yokdonensis* leaf was presented in the Figure 3 with IC₅₀ value of 6.04 µg/mL (Figure 3a), comparable to the positive control vitamin C, which showed a IC₅₀ value of 1.56 µg/mL (Figure 3b).

Studies also provided the biological activities and primary phytochemistry of this species *C. yokdonensis*. For example, Hoang *et al.* (2024) showed that the methanol extract isolated from the *C. yokdonensis* leaf showed potent DPPH radical-scavenging properties with an EC₅₀ value of 19.37 µg/mL, comparable to the positive control vitamin C, which showed an EC₅₀ value of 12.22 µg/mL (Hoang *et al.*, 2024). Furthermore, this report also showed that the leaf methanol extract of this plant also possessed the cytotoxicity against 4 cell lines such as K562, MCF-7, A549, and HCC-J5 with IC₅₀ values of 48.82, 175.52,

172.90, and 138.00 µg/mL, respectively (Hoang *et al.*, 2024). Recently, Do *et al.* (2024) provided the primary phytochemistry and antioxidant property of the flower tea wine of *C. yokdonensis*. Accordingly, the flower tea wine of this species contained some bioactive compounds such as coumarin, flavonoid, proanthocyanin, tannin, and saponin. In addition, this flower extract also possessed the very strong DPPH radical-scavenging activity with an IC₅₀ value of 5.12 µg/mL while 2.64 µg/mL was the IC₅₀ value of the acid ascorbic (Do *et al.*, 2024).

Research has demonstrated that species within the *Camellia* genus have potent antioxidant properties, especially in scavenging DPPH radicals. For example, Chan *et al.* (2007) showed the strong DPPH radical-scavenging properties of the methanol extracts obtained from the shoots, young and mature leaves of *C. sinensis* with IC₅₀ values of 0.026, 0.030, and 0.037 mg/mL, respectively (Chan *et al.*, 2007). Similarly, Nor Qhairul Izzreen and Mohd Fadzelly (2013) provided the antioxidant activities of the various water extracts of *C. sinensis*. Accordingly, the shoots, young and mature leaves of the green tea possessed the DPPH radical-scavenging activities with IC₅₀ values of 0.03, 0.03, and 0.04 mg/mL while 0.03, 0.03, and 0.04 mg/mL were shown by the IC₅₀ values of the black tea towards the same organs (Nor Qhairul Izzreen & Mohd Fadzelly, 2013).

Chiou *et al.* (2015) showed that the ethanol extracts and other solvent fractions isolated from the different organs of *C. tenuiflora* possessed the strong antioxidant properties. Accordingly, the crude ethanol extract and its fractions, including methanol, *n*-butanol, and aqueous of the fruit shell showed DPPH radical scavenging activities with IC₅₀ values of 19.74, 7.34, 13.18, and 27.25 µg/mL, respectively. Furthermore, the corresponding seed extracts showed a DPPH radical scavenging capacities with IC₅₀ values of 14.30, 5.47, 15.55, and 5.82 µg/mL, respectively while 84.96, 14.38, 170.99, and 218.03 µg/mL, respectively were the IC₅₀ values seed pomace towards the same species (Chiou *et al.*, 2015). The methanol and ethanol extracts of the *C. japonica* leaf were also possessed the strong DPPH radical-scavenging activities with IC₅₀ values of 0.23 and 0.22 mg/mL, respectively (Moon & Kim, 2018).

Conclusion

The volatile composition and antioxidant capacity of the ethanol extract from the leaves of *C. yokdonensis*

is reported for the first time. The analysis revealed the presence of numerous bioactive compounds within the extract. Moreover, consistent with other members of the genus *Camellia*, the extract of *C. yokdonensis* exhibited remarkably strong antioxidant activity, highlighting its significant potential for future applications in the food and pharmaceutical industries.

Author contributions

Hoai Huong Dinh-Thi: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing - original draft. Duy H. Truong: Supervision, Formal analysis, Validation, Writing - review & editing. All authors approved the final manuscript.

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