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Camellia cattienensis: phytochemical and biological properties from the leaf extract

Camellia cattienensis: propiedades fitoquímicas y biológicas del extracto de hojas

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ABSTRACT

Keywords:
Antibacterial
Antioxidant
Cat Tien camellia
GC/MS

Camellia consists of many plants of high economic importance that are used in different fields, especially in the food industry. *Camellia cattienensis* is a rare species and is native to Vietnam. Studies on the phytochemical and biological properties of this species have been unknown so far. In this study, the chemical components of the acetone extract from *C. cattienensis* leaves and its fractions, including n-hexane, chloroform, and ethyl acetate, were investigated using gas chromatography-mass spectrometry assay. Accordingly, 2-pentanone, 4-hydroxy-4-methyl- was the most abundant compound in the acetone extract and the ethyl acetate fraction, while phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) and hexanedioic acid, bis(2-ethylhexyl) ester are the richest components in the chloroform and n-hexane fractions, respectively. Furthermore, the acetone extract was active against four tested bacteria, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* BAA750. The acetone extract of the *C. cattienensis* leaves also possessed DPPH, and ABTS free radical scavenging with the IC_{50} values of 91.63±1.88 and 13.32±0.49 µL, respectively. The outcomes of this study hold promise for potential applications of *C. cattienensis* leaves in food product development, especially in the future beverage industry.

RESUMEN

Palabras clave:
Antibacterianas
Antioxidantes
Cat tien camellia
GC/MS

La *Camelia* se compone de muchas plantas de alto valor económico y se utilizan en diferentes campos, especialmente en la industria alimentaria. *Camellia cattienensis* es una especie rara y originaria de Vietnam. Hasta el momento se desconocen los estudios sobre las propiedades fitoquímicas y biológicas de esta especie. En este estudio, se investigaron los componentes químicos del extracto de acetona de las hojas de *C. cattienensis* y sus fracciones, como n-hexano, acetato de etilo y cloroformo, mediante un ensayo de cromatografía de gases/espectrometría de masas. En consecuencia, 2-pentanona, 4-hidroxi-4-metil-fue el compuesto más abundante en el extracto de acetona y la fracción de acetato de etilo, mientras que fenol, 2,4-bis(1,1-dimetiletil)-, fosfito (3:1) y el ácido hexanodioico, el éster bis(2-etilhexílico) son los componentes más ricos en las fracciones de cloroformo y n-hexano, respectivamente. Además, se descubrió que el extracto de acetona era eficaz contra cuatro bacterias analizadas, incluidas *Klebsiella pneumoniae, Staphylococcus aureus,* y *Staphylococcus saprophyticus*. El extracto de acetona de las hojas de *C. cattienensis* también poseía eliminación de radicales libres DPPH y ABTS con valores de IC_{50} de 91,63±1,88 y 13,32±0,49 µL, respectivamente. Los resultados de este estudio son prometedores para aplicaciones potenciales de las hojas de *C. cattienensis* en el desarrollo de productos alimenticios, especialmente en la industria de bebidas en el futuro.

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Camellia is a large genus in the family of Theaceae, comprising over 280 species distributed in many countries around the world, and about 95 species have been found in Vietnam (Quach et al. 2021). Many members of the Camellia genus are plants of high economic value due to their byproducts, including oil seeds, tea, ornamental plants, and iconic flowering shrubs (Yang et al. 2016). Additionally, various solvent extracts obtained from Camellia plants have been reported to possess numerous pharmaceutical properties, including antibacterial, antifungal, antitumor, and antioxidant activities (Yang et al. 2016). Notably, *Camellia sinensis*, commonly known as the tea plant, is cultivated in tropical and subtropical regions. Additionally, leaf extracts from this species are recognized as the second most consumed beverage in the world (Chitsazan 2015). Moreover, they contain numerous beneficial compounds, such as catechins (Gaur and Bao 2021), steroids, alkaloids, polyphenols, and terpenoids (Anand et al. 2015). Furthermore, the oil extracted from certain *Camellia* species, particularly *C. oleifera* and *C. japonica*, is known as 'Asian olive oil' due to its high content of major chemical compounds, such as oleic acid and neutral lipids (Kim et al. 2014).

Camellia cattienensis Orel was first reported as a new species for the flora of Vietnam by Orel and Wilson (2011). This species, whose type specimen was collected from Cat Tien National Park, Dong Nai Province, Vietnam. To date, it is a rare plant and is found only in the type collection (Orel and Wilson 2011). The phytochemical and biological effects of this species have been unknown so far. This study, thus, provided the chemical constituents, antioxidants, and antibacterial activities of *C. cattienensis* for the first time.

MATERIALS AND METHODS

Materials

The leaf specimens of *Camellia cattienensis* were collected from Bau Sau station, Cat Tien National Park, (Dong Nai Province, Vietnam) by Van Hop Nguyen, where its type specimen was collected. The voucher specimen was CT22092022, and it was deposited in the herbarium of the Faculty of Natural Resources and Environment, Vietnam National University of Forestry-Dong Nai Campus, Vietnam.

Bacterial strains

Ten bacterial strains were used to identify the antibacterial activity of the studied species, including six Gram-negative strains (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311, *Klebsiella pneumoniae* ATCC 13883, *Klebsiella pneumoniae* ATCC 700603, *Shigella flexneri* ATCC 9199, *Enterobacter hormaechei* ATCC 700323) and four Gram-positive strains (*Staphylococcus saprophyticus* BAA750, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 13883).

Extraction procedures

The leaves of *C. cattienensis* were freshly harvested, well-washed, air-dried at 50 °C, and evenly ground into fine powder. 500 mL of acetone solution (99%, Thermo Fisher Scientific, USA) was used to immerse 100 g of powder for 72 h; after that, the supernatant was collected and filtered. The remaining solid matter underwent two more rounds of acetone extraction, and the end product was recovered by combining all the filtered fractions. The solvent was then eliminated in vacuum condition at 45 °C.

Three grams of acetone extract were dissolved in 30 mL of distilled water in an extraction flask. Then, 30 mL n-hexane was added, shaken, and left standing for layer formation. The hexane layer on the top was collected, and this procedure was performed again two more times to pick up 90 mL of the n-hexane extract. This extract was also eliminated under vacuum conditions at 45 °C to obtain the hexane fraction. The same process done above was used to obtain ethyl acetate and chloroform (Le et al. 2021).

Gas chromatography-mass spectrometry assays

The chemical constituents of the acetone extract and its fractions (n-hexane, ethyl acetate, and chloroform) from *C. cattienensis* leaves were determined using the TRACE 1310 Gas Chromatograph in conjunction with the ISQ 7000 mass spectrometer (Thermo Fisher Scientific, USA). The DB-5MS column (Agilent, USA) was used as a stationary phase with GC/MS, and run parameters were configured as previously described by Nguyen et al. (2023). Acquired mass spectral data were used to compare with the NIST 2017 library to determine the exact chemical compositions.

Determination of antibacterial activity

The disk diffusion method was employed to assess the antibacterial efficacy of the acetone extract from the studied species, following the Clinical and Laboratory Standards Institute guidelines (CLSI 2016). Acetone extracts at concentrations of 100, 150, and 200 mg mL^{-1} in 15% dimethyl sulfoxide were used for the test. As a positive control, a 10-µg gentamicin disk (Nam Khoa BioTek, Vietnam) was used, while 15% DMSO served as the negative control. The experiment was conducted in triplicate, and Fisher's least significant difference (LSD) and one-way analysis of variance (ANOVA) were employed for statistical analysis.

DPPH radical scavenging assay

DPPH radical scavenging activity of the acetone extract from the studied species was determined by DPPH assay (Nguyen et al. 2023) with slight modifications. Methanol 99.8% was used to dissolve the extract into different concentrations. The mixture, composed of 0.3 mL of extract and 3.7 mL of DPPH 0.1 mM was incubated at room temperature for 30 min in the dark. Absorbance measurement was done using a UV-Vis spectrophotometer (Genesys 20, USA) at 517 nm. DPPH radical scavenging activity was calculated as follows the Equation 1:

$$DPPH(\%) = \frac{A_o - A_i}{A_o} \text{ x } 100 \qquad (1)$$

Where A_o and A_i are the absorbance of the DPPH solution and the sample-DPPH mixture, respectively. The DPPH radical scavenging effect was determined by IC_{50} value in comparison with the control ascorbic acid.

ABTS radical scavenging assay

Antioxidant activity was determined by ABTS radical scavenging assay described by Re et al. (1999) with slight modifications. Initially, solution A comprised 7 mM ABTS and 2.45 mM $K_2S_2O_8$, was prepared and incubated at 37 °C for 18 h in the dark. Subsequently, a mixture of 3 mL of solution A, 0.1 mL of the studied extract, and 1.9 mL of acetone was made, followed by 15 min incubation in the dark. To assess the ABTS radical scavenging activity, the absorbance of the solution was measured at 734 nm using a UV-VIS spectrophotometer (UVS 2800, Labome, USA) equipped with UVWin6 Software. Ascorbic acid served as the reference standard, and the standard curve for ascorbic acid (0 to 15 ppm) was constructed. The sample concentration was determined from the standard curve equation and expressed as µg mL^{-1} ascorbic acid.

RESULTS AND DISCUSSION

Chemical components of the acetone extract and chloroform, ethyl acetate, and hexane fractions from *Camellia cattienensis*

The chemical components of the acetone extract and chloroform, ethyl acetate, and hexane fractions of *C. cattienensis* are shown in Table 1 and Figure 1. A total of 54 compounds were found in the four extracts studied. The acetone extract was found to be rich in 2-pentanone, 4-hydroxy-4-methyl (22.85%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (17.14%); neophytadiene (16.97%); stigmasterol (12.78%); α-amyrin (5.29%); and n-hexadecanoic acid (4.75%). The chloroform fraction possessed phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) (74.91%); n-hexadecanoic acid (8.30%); and 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (5.08%) as the major compounds. The ethyl acetate fraction was mainly composed of 2-pentanone, 4-hydroxy-4-methyl (54.68%); phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) (9.17%); n-hexadecanoic acid (8.25%); and 5-hydroxymethylfurfural (8.08%) whereas hexanedioic acid, bis(2-ethylhexyl) ester (30.89%); neophytadiene (25.21%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (11.58%); n-hexadecanoic acid (10.26%); and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (8.44%) were the major compounds in the n-hexane fraction.

Previous studies have also demonstrated the chemical compositions of various *Camellia* species collected from Vietnam. For example, the essential oil of the *C. longii* flower was found to be rich in α-eudesmol (16.1%), (E)-nerolidol (13.0%), and β-eudesmol (8.9%) (Tran et al. 2023). Hoang et al. (2014) determined 35 volatile compounds that could be the main factor responsible for the black tea's aroma quality (*Camellia sinensis*), of which α-ionone, ethyl caprylate, 3-hydroxy-β-damascone, β-ionone, 2(4H)-benzofuranone were the main factors contributing for this (Hoang et al. 2014). The seed oil of the *Camellia ninhii* was characterized by the predominance of oleic acid (45.43%), palmitic acid (27.83%), and *trans*-cinnamic acid (4.83%) (Tran et al. 2022).

Table 1. Chemical components of the acetone extract and its fractions.

STT	RT	Compounds	Relative percentage (%)			
			CAT	CATC	CATE	CATH
1	2.20	Acetic acid, butyl ester	-	-	1.55	-
2	2.41	3-Furaldehyde	-	-	0.74	-
3	2.43	3-Penten-2-one, 4-methyl-	1.77	-	-	-
4	2.48	2-Pentanone, 4-hydroxy-4-methyl-	22.85	-	54.68	0.24
5	2.82	Benzene, 1,3-dimethyl	-	-	0.15	-
6	3.04	2-Propenoic acid, butyl ester	-	-	1.65	-
7	3.09	1,3,5,7-Cyclooctatetraene	-	-	0.23	-
8	3.81	2-Heptanol, acetate	0.47	-	-	-
9	5.81	Ethanol, 2,2'-oxybis-, diacetates	0.10	-	-	-
10	6.33	Dodecane	-	-	1.35	-
11	6.43	2,6-Dimethyldecane	-	-	0.22	-
12	7.44	Linalool	0.38	-	-	-
13	7.96	Octanoic acid	-	-	0.42	-
14	8.03	2,6-Dimethyl-6-nitro-2-hepten-4-one	0.35	-	-	-
15	8.14	Benzoic acid	0.18	-	-	-
16	8.27	Azulene	-	-	0.69	-
17	8.51	Undecane, 2,6-dimethyl	-	-	0.22	-
18	8.61	Dodecane, 4-methyl	-	-	0.17	-
19	8.74	5-Hydroxymethylfurfural	0.20	-	8.08	-
20	9.02	Tetradecane, 5-methyl-	-	-	0.50	-
21	9.14	Dodecane, 2,6,11-trimethyl-	-	-	1.38	-
22	10.08	Undecane, 4,7-dimethyl-	-	0.56	-	-
23	10.84	1-Iodo-2-methylundecane	-	-	1.45	-
24	10.97	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	-	0.30	-	-
25	11.53	Phenol, 2,4,6-tri-tert-butyl-	-	0.62	-	-
26	12.19	Tetradecane, 2,6,10-trimethyl-	-	0.93	1.40	-
27	12.66	Benzyl Benzoate	-	0.79	-	-
28	12.96	Neophytadiene	16.97	2.13	-	25.21
29	13.36	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	-	5.08	1.43	-
30	13.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.14	-	-	11.58
31	13.57	n-hexadecenoic acid	4.75	8.30	8.25	10.26
32	14.45	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	-	-	2.01	-
33	14.46	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-	-	-	8.44
34	14.55	Octadecanoic acid	0.56	2.53	2.25	1.97
35	14.63	Phytol	1.34	-	-	1.01
36	14.72	9,12-Octadecadienoic acid (Z,Z)-	0.28	-	-	-
37	15.36	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	-	0.85	-	-
38	15.50	17-Pentatriacontene	0.35	-	-	-

Table 1

STT	RT	Compounds	Relative percentage (%)			
			CAT	CATC	CATE	CATH
40	15.66	Oleic Acid	0.29	-	-	-
41	16.18	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, 2-ethylhexyl ester	-	2.08	-	-
42	16.41	Bis(2-ethylhexyl) phthalate	-	-	-	1.61
43	16.59	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.51	-	-	-
44	16.70	Hexadecenoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	0.72	-	-	-
45	16.80	Oleic acid, 3-(octadecyloxy)propyl ester	0.73	-	0.18	-
46	16.83	Octadecanal, 2-bromo	0.65	-	-	-
47	17.26	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.58	-	-	-
48	18.15	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	-	-	-	2.35
49	18.54	Squalene	2.42	-	-	2.84
50	24.05	dl-α-Tocopherol	2.26	-	-	-
51	27.26	Stigmasterol	12.78	-	-	3.42
52	27.63	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	-	74.91	9.17	-
53	28.03	α-Amyrin	5.29	-	-	-
54	28.30	β-Sitosterol	4.30	-	-	-
		Total	98.22	99.08	98.17	98.82

Note: CAT: acetone extract, CATC: chloroform, CATE: ethyl acetate, and CATH: hexane.

Furthermore, a recent study reported that the leaf and flower of *C. tonkinensis* consisted of Zn (10.20 and 13.40 mg kg^{-1}) and saponin (58.30 and 87.10 mg g^{-1}) whereas various amino acids were also present in two organs of this plant, including aspartate, glutamate, serine, histamine, glycine, arginine, threonine, alanine, cysteine, tyrosine, valine, phenylalanine, methionine, leucine, isoleucine, proline, and lysine with the contents ranging from 3.512 to 42.087 g kg^{-1} (Dang et al. 2022).

Studies have identified the chemical compounds in various *Camellia* species extracts using different solvents and analyzed them with gas chromatography-mass spectrometry. For instance, the methanol extract isolated from *Camellia sinensis* leaves collected from Bangladesh mainly contained caffeine (27.44%), hexadecanoic acid, methyl ester (14.02%), 9,12,15-octadecatrienoic acid methyl ester (Z, Z, Z) (3.95%) (Hasan et al. 2024). Similarly, the chloroform: methanol extract of the *C. sinensis* leaves grown in Ambala Cantt., India was found to be rich in caffeine (48.21%), *trans*-13-octadecenoic acid (18.30%), *cis*-11-eicosenoic acid (15.15%) (Gupta and Kumar 2017). In addition, the methanol extract of *C. sinensis* leaves from Dehradun, India, was mainly composed of *n*-heptadecanol-1 (68.63%); 2-pentanone, 4-hydroxy-4-methyl- (3.82%); and 7-hexadecanoic acid, methyl ester, (Z) (2.32%) (Pradhan and Dubey 2021). The phytochemistry of the methanol extract of *C. sinensis* leaves collected from six regions of India was also investigated. Accordingly, the sample extracts from Moonar (Kerala) and Kodaikanal (Tamil Nadu) were characterized by the predominance of caffeine (46.25-57.17%); 1,2,3-benzenetriol (18.40-16.28%); and 1,3,4,5-tetrahydroxycyclohexanecarbonyl (13.96-9.64%). Meanwhile, the extracts grown in Ootacamund (Tamil Nadu) and Bengaluru (Karnataka) contained caffeine 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (56.48-57.52%);1,2,3-benzenetriol (28.36-21.55%) as the major compounds. Finally, 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (42.75-59.79%) as the most abundant compound in the extracts from Assam (Assam) and Kolkata (West Bengal) (Senthilkumar et al. 2015). Apart from that, the major constituents of the ethanol extract of the *C. sinensis* leaves from Uganda were caffeine (82.69%); naphthacene-5,12-dione, 6,11

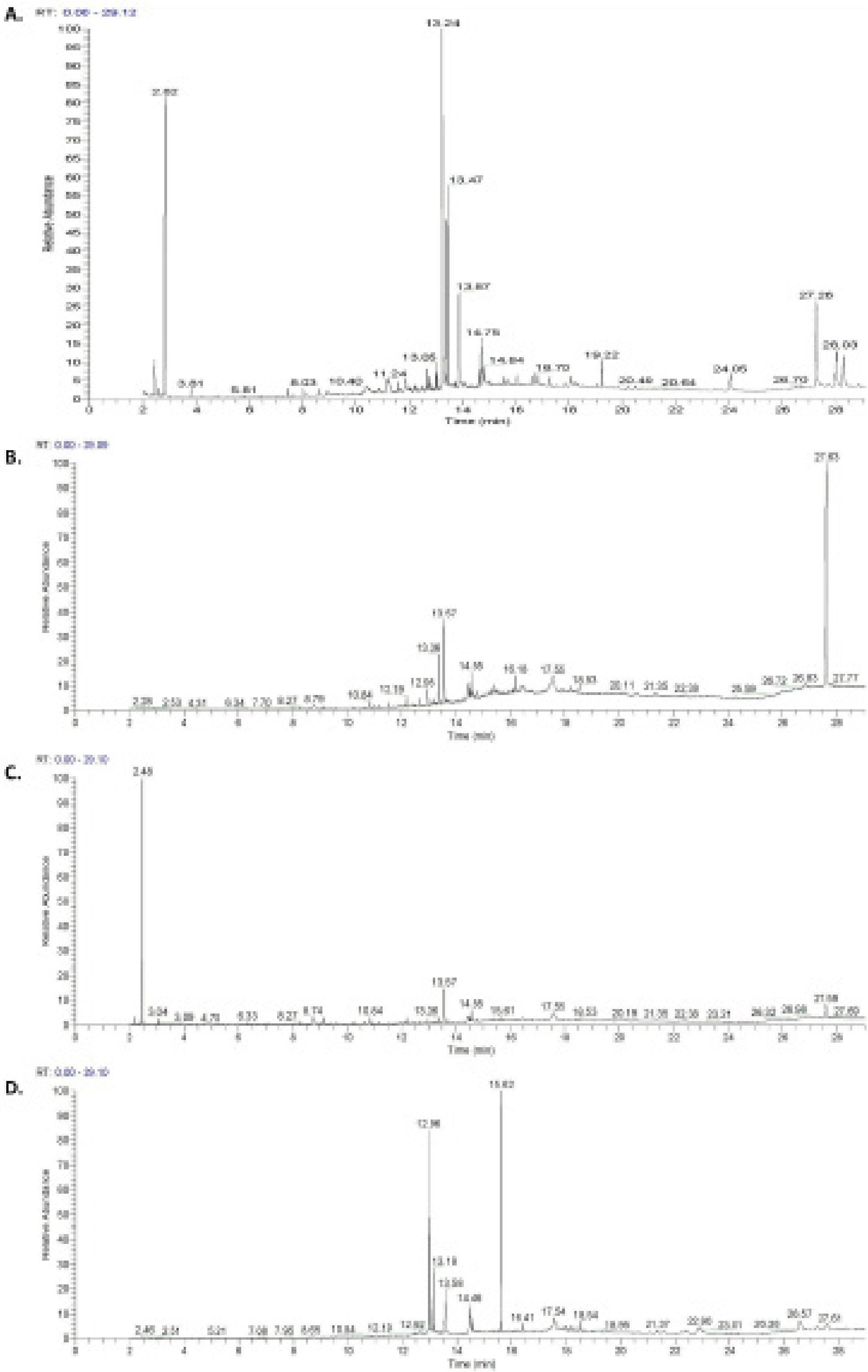

Figure 1. The GC chromatogram of **A.** acetone extract from *Camellia cattienensis* and **B**. chloroform, **C.** ethyl acetate, and **D**. hexane fractions from *Camellia cattienensis.*

-dihydroxy-2,3,8,9-tetramethyl- (3.73%); and estra-1,3,5(10)-trien-17-ol, 2,3,4-trimethoxy-, (17.β.)- (3.73%) (Hope et al. 2022).

The benzene-ethanol extracts of *Camellia oleifera* leaves collected from Hunan, China, were found rich in butyraldehyde, semicarbazone (11.58%); hexatriacontane (8.04%); and 1,6-anhydro-β-D-glucopyranose (7.54%) (Liu et al. 2009). The chemical constituents of different extracts of *C. oleifera* fruit grown in Hunan, China, were also reported. For instance, the methanol extract contained a mixture of γ-sitosterol (43.32%); 5-hydroxymethylfurfural (22.07%); and *cis*-vaccenic acid (12.71%). The ethanol extract was mainly composed of 5-hydroxymethylfurfural (58.78%) and furfural (4.74%), while *cis*-vaccenic acid (45.20%); *n*-hexadecanoic acid (10.98%); and 9-octadecenamide, (Z)- (5.82%) were the main compounds in the ethyl acetate extract (Xie et al. 2018). Moreover, the acetone extract of *Camellia assamica* leaves collected from Dehradun, India was characterized by the predominance of 2′,6′dihydroxyacetophenone, bis(trimethylsilyl) ether (17.58%); *N*(trifluoracetyl) *O*,*O*',*O*''tris(trimethylsilyl) epinephrine (15.83%); and tetracosamethyl cyclododecasiloxane (10.62%) (Pradhan and Dubey 2021).

Antibacterial activity of acetone extract from *Camellia cattienensis*

The antibacterial property of *C. cattienensis* acetone extract was presented in Table 2. Accordingly, the studied extract was found to be effective against four out of eight bacterial strains, including *K. pneumoniae* ATCC 700603, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, and *S. saprophyticus* BAA750. Overall, at a concentration of 200 mg mL^{-1}, the studied extract exhibits a stronger antibacterial effect against four bacterial strains in comparison with the remaining concentrations (Table 2).

Table 2. Antibacterial property of acetone extracts from *Camellia cattienensis*.

Bacterial strains	**Zone of inhibition (mm)**				
	100 (mg mL^{-1})	**150 (mg mL^{-1})**	**200 (mg mL^{-1})**	**Gentamycin**	**Negative control**
B. cereus ATCC 13883	-	-	-	14.17±0.29	-
E. coli ATCC 25922	-	-	-	13.33±0.58	-
E. hormaechei ATCC 700323	-	-	-	8.83±0.76	-
K. pneumoniae ATCC 700603	4.00±0.87^{a}	4.83±0.29^{a}	5.33±1.15^{a}	12.83±0.76^{b}	-
K. pneumoniae ATCC 13883	-	-	-	13.50±0.87	-
S. aureus ATCC 29213	2.50±0.50^{a}	4.00±0.50^{b}	4.33±0.58^{b}	13.67±1.53^{c}	-
S. aureus ATCC 25923	2.67±1.15^{a}	4.33±0.58^{a}	6.67±0.76^{b}	13.00±1.00^{c}	-
S. flexneri ATCC 9199	-	-	-	12.00±0.87	-
S. saprophyticus BAA750	2.33±0.58^{a}	2.50±0.50^{a}	3.67±0.29^{b}	13.33±0.58^{c}	-
S. typhimurium ATCC 13311	-	-	-	14.50±0.87	-

Different superscript lower-case letters in the same row denote significant difference (P<0.05). (-) Not active.

Studies provided the antimicrobial activities of different solvent extracts from *Camellia* species. For example, the chloroform: methanol extract of the *Camellia sinensis* leaves grown in Ambala Cantt., India had an inhibitory effect on *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* (Gupta and Kumar 2017). The methanol extract of the *C. sinensis* and *C. assamica* leaves from Dehradun, India and its various fractions such as water, ethanol, methanol, chloroform, and petroleum ether were reported to possess antimicrobial effects against *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans* (Pradhan and Dubey 2021). In another report, the antibacterial effects of the hot water extract isolated from the green, herbal, and black teas (*C. sinensis*) were also investigated. Accordingly, the first extract was found to be effective against three bacterial strains, including *Micrococcus luteus*, *Bacillus cereus*, and *Staphylococcus aureus*, while the latter extracts exhibited an inhibitory effect on *Micrococcus luteus* and *Bacillus cereus* (Chan et al. 2011).

The ethanol extract of *C. sinensis* leaves from Uganda was demonstrated to be effective against different pathogenic bacteria, especially *Salmonella* and *Escherichia coli* (Hope et al. 2022). Also, the leaf ethanol extract of this plant collected from Chennai, India, displayed activity against *Streptococcus mutans* and *Lactobacillus acidophilus* (Anita et al. 2015). The ethanol extract of *C. sinensis* leaves grown in Iran was effective against 30 *Escherichia coli* strains isolated from the urine cultures of patients in three hospitals in Zabol, southeastern Iran (Sepehri et al. 2014). Additionally, the organic acids and phenolic components from the seeds of *Camellia oleifera* cake, collected in Jiangxi, China, exhibited antimicrobial activity against six fungal and bacterial strains, including *Aspergillus oryzae*, *Rhizopus stolonifer*, *Mucor racemosus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (Zhang et al. 2020). Furthermore, another study demonstrated the antibacterial effects of the methanol extract fractioned into basic, neutral, and acid fractions obtained from *Camellia japonica* grown in Yeosoo, Korea. Accordingly, the methanol extract, neutral, and acid fractions displayed activity against some bacterial strains, including *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Kim et al. 2001).

Antioxidant activity of acetone extract from *Camellia cattienensis*

The antioxidant effects of the tested extract from *C. cattienensis* were shown in Figure 2. Accordingly, the extract had the DPPH, ABTS free radical scavenging with the IC_{50} values of 91.63±1.88 and 13.32±0.49 µg mL^{-1}, respectively.

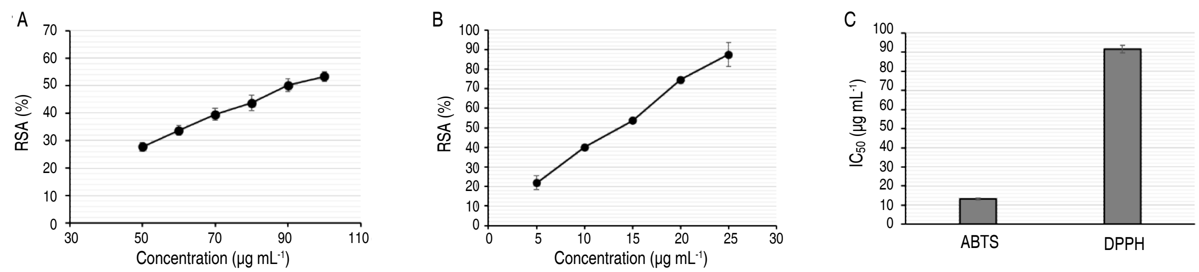

Figure 2. The free radical scavenging activity of the acetone extract from *Camellia catienensis* was measured using **A.** the DPPH assay, **B**. the ABTS assay, and **C**. the corresponding IC_{50} values.

Compared to other *Camellia* species, the results show moderate antioxidant potential. For example, the methanol extract of *C. sinensis* leaves from Bangladesh showed a stronger DPPH scavenging effect with an IC_{50} value of 69.51 µg mL^{-1}, indicating better antioxidant activity compared to *C. cattienensis* (Hasan et al. 2024). Conversely, the acetone extract of *C. oleifera* seed oil from Taiwan exhibited a much lower antioxidant effect, with only 4.46% inhibition at 200 µg mL^{-1}, significantly weaker than *C. cattienensis* (Lee and Yen 2006). Similarly, the ethyl acetate extract of *C. oleifera* seed oil showed only 5.92% inhibition, while the methanol extract performed better, with 66.50% inhibition at the same concentration (Lee and Yen 2006). In another study, the organic acids and phenolic components of *C. oleifera* seed cake from Jiangxi, China, demonstrated weaker antioxidant activity than *C. cattienensis*, with IC_{50} values of 184 mg L^{-1} and 103 mg L^{-1}, respectively (Zhang et al. 2020). Furthermore, the ethanol extract of *C. japonica* flowers from Korea demonstrated a DPPH inhibition of up to 60% at 50 µg mL^{-1} (Piao et al. 2011), while the ethanol extract of *C. japonica* leaves from Jeonnam, Korea, showed a stronger antioxidant effect with an IC_{50} value of 38.53 µg mL^{-1} (Yoon et al. 2017).

Overall, the acetone extract of *C. cattienensis* leaves displayed a moderate antioxidant effect, stronger than that of *C. oleifera* extracts but weaker compared to the more potent antioxidant effects of *C. sinensis* and *C. japonica* ethanol and methanol extracts. These differences likely reflect the variations in solvent choice, plant parts used, and regional factors influencing the phytochemical composition.

CONCLUSION

Camellia cattienensis is a rare species that, to date, has only been documented from the type collection at Nam Cat Tien National Park, Dong Nai Province, Vietnam. In the present study, a limited number of samples of this species were utilized for experimental purposes to prioritize its conservation. Furthermore, phytochemical, antioxidant, and antibacterial properties are recognized as prominent features in *Camellia* species. Therefore, the present study provides these characteristics for the first time from the acetone extract of *C. cattienensis*. As a result, the various acetone sub-extracts were found to contain several bioactive compounds, with 2-pentanone, 4-hydroxy-4-methyl-phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1); and hexanedioic acid, bis(2-ethylhexyl) identified as the most abundant compounds. The acetone extracts of *C. cattienensis* exhibited moderate effects against bacterial strains, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus*. Notably, this extract demonstrated potent antioxidant activity, as evaluated using the DPPH and ABTS assays, with IC_{50} values of 91.63±1.88 and 13.32±0.49 μg mL^{-1}, respectively. The results of analyses, such as the antioxidant and antibacterial activities of the n-hexane, chloroform, and ethyl acetate fractions, will be reported in a subsequent study. Given the versatile applications of various *Camellia* species in the food industry, the findings of this study contribute to a deeper understanding of the potential food uses of *C. cattienensis*, particularly in the beverage industry.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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