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Characterization and Evaluation of the In Vitro Antioxidant, α-Glucosidase Inhibitory Activities of *Camellia longii* Orel and Luu. (Theaceae) Flower Essential Oil and Extracts From Vietnam

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Abstract

Objective/Background: *Camellia longii* Orel and Luu was discovered for the first time in Cat Tien National Park of Lam Dong Province, Southern Vietnam, was determined as a new species. To the best of our knowledge, this is the first report on the phytochemical and bioactivity of this species. **Methods:** The chemical profile of essential oil (EO) prepared from *C. longii* flowers was determined by GC/MS analysis and comparison of the recorded retention indices and the corresponding literature values. The total phenolic and flavonoid contents in different flower extracts were estimated by Folin-Ciocalteu and aluminum chloride methods with a spectrophotometer, respectively. The antioxidant activities of the extracts were determined by various bioassays, including DPPH free radical scavenging, ferric reducing power, and cupric reducing antioxidant capacity. The α-glucosidase inhibitory activity of the extracts was evaluated using *p*-nitrophenyl α-D-glucopyranoside solution as a substrate and acarbose as the positive control. **Results:** Fifty-seven compounds were identified from the EO of *C. longii* flowers. Among these, the main components were α-eudesmol (16.1%), followed by (*E*)-nerolidol (13.0%), β-eudesmol (8.9%), τ-cadinol (6.5%), and γ-eudesmol (5.8%). The total phenolic and flavonoid contents were found high in ethyl acetate, *n*-butanol, and ethanol extracts (206.8-378.6 mg gallic acid equivalents/g crude extract and 298.5-390.3 mg rutin equivalents/g crude extract, respectively). These extracts were shown to possess potent antioxidant activities in the examined bioassays. As for the α-glucosidase inhibitory assay, the ethyl acetate, butanol, and ethanol extracts were shown to be more potent than acarbose with IC_{50} values of 2.16 ± 0.04, 1.95 ± 0.05, and 1.53 ± 0.04 μg/mL, respectively. **Conclusion:** The findings first provide the phytochemical profiles of *Camellia longii* Orel and Luu and its bioactivities, indicating moderate potentials in antioxidant activity and strong α-glucosidase inhibition.

Keywords

Camellia longii, antioxidant, α-glucosidase inhibitory, essential oil, extract

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Introduction

Camellia L. is the largest genus in the Theaceae family that comprises about 280 species worldwide.[1,2] This genus has a pantropical and subtropical distribution from the Himalayas to Japan and Indonesia.[3,4] The medicinal effects of *Camellia* have a long history dating back almost 5000 years and are reported to have antioxidant, antiinflammation, antimicrobial properties, and protective effects against tooth decay, diuretic, and asthma.[5] *Camellia longii* Orel and Luu. (Figure S1, supporting information) was described as new for the flora of Vietnam based on the specimen CT4 (VNM, NSW) which was collected from Cat Tien National Park of Lam Dong Province, Southern Vietnam. This species is distinct from all the other *Camellia*

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species by having campanulate or almost campanulate, intensely dark orange to red flowers with uneven whitish margins, 5-6 petals and 3-2 petaloids, filaments that are united with the petals and one another, a diamond-shaped ovary and compound 5-6 styles that are connate at the base.[4] As a new and rare species, phytochemical and bioactivity studies have not been reported in the literature. Therefore, this work was performed to identify phytochemical constituents, antioxidant, and α-glucosidase inhibitory activities of the flowers of *C. longii* collected in Vietnam.

Results and Discussion

Chemical Composition of Essential Oil

The average yield of essential oil (EO) from *C. longii* flowers was 0.06% (w/w), calculated on a fresh weight basis. This EO was a light-yellow liquid with a lower density than water. The total ion chromatogram recorded by GC/MS is displayed in Figure S2 (supporting information). Fifty-seven compounds, accounting for 88.7% were identified from the EO of *C. longii* flowers (Table 1). All the major components are sesquiterpene. The most abundant constituents present in this EO were *α*-eudesmol (16.1%), followed by (*E*)-nerolidol (13.0%), *β*-eudesmol (8.9%), *τ*-cadinol (6.5%), and *γ*-eudesmole (5.8%). The eudesmane-type sesquiterpenoids account for 31.6% of the EO content. A total of 34 constituents, accounting for 96.8% of *Camellia nitidissima* flower oil, were identified, and α-eudesmol (34.3%), γ-eudesmol (31.5%), and linalool (11.1%) were the primary components.[6]

Phytochemical Screening of Plant Extracts

The results of the phytochemical screening of different extracts of *C. longii* flowers are presented in Table 2. The results revealed the absence of alkaloids as well as amino acids in all the extracts. In contrast, all extracts contain tannins. Flavonoids, coumarins, terpenoids, reducing sugars, and polysaccharides are all found in ethyl acetate, *n*-butanol, and ethanol extracts. Besides, the presence of saponins in butanol and ethanol extracts and the absence of them in hexane and ethyl acetate extracts are confirmed by the Foam test.

Total Phenolic and Flavonoid Contents

The results from the determination of the total phenolic and flavonoid contents of all four extracts are shown in Tables 3 and Table 4. Total phenolic content (TPC) was expressed as milligram gallic acid equivalent per gram of crude extract. TPC is highest in ethyl acetate extract (378.6 ± 2.3) and lowest in hexane extract (8.0 ± 0.1). The concentration of flavonoids was described as mg of equivalent rutin per gram of crude extract. Total flavonoid content (TFC) is highest in butanol extract (390.3 ± 0.47) and lowest in hexane extract (88.6 ± 2.11). In general, high contents of total phenolic and flavonoid compounds are found in ethyl acetate, butanol, and ethanol extracts.

Phenolic and Flavonoid Compounds Analysis Using HPLC

In this study, eight phenolic acids and two flavonoids were analyzed using an HPLC-DAD system. Table 5 shows the results of the retention time, limit of detection (LOD), and limit of quantification (LOQ) of the examined phenolic compounds. The LOD and LOQ ranged from 0.01 to 2.46 μg/mL and 0.05 to 8.20 μg/mL, respectively. A linear regression method was employed to fit all the calibration data, resulting in a calibration coefficient R^2 greater than 0.997. Six out of eight phenolic acids were quantified in the ethanolic extract of *C. longii* flowers, with the average levels ranging from 6.69 to 29.66 mg/g extract. The two other acids, *p*-coumaric and ferulic acids, were not found in the sample. Quercetin was present in the extract, with a concentration averaging 0.32 mg/g, while its rutinoside form (ie, rutin) was not detected. The presence of the monitor compounds in the sample and a standard solution is shown in Figure S3 (supporting information). In a study described by Morikawa et al, the flowers of *Camellia sinensis* were found to be a rich source of quercetin glycosides.[7]

Antioxidant Activity

Antioxidant compounds can neutralize free radicals, thus protecting cells from reactive oxygen species. In this study, the scavenging activity and reducing powers of different extracts of *C. longii* flowers were determined. As for scavenging effects, the results showed that the ethyl acetate, butanol, and ethanol extracts showed higher activities, with IC_{50} ranging from 57.42 to 61.69 μg/mL, whereas the hexane extract displayed a weak activity, with an undetermined IC_{50} in the studied ranges (Table 6). The results were in line with the antioxidant activity of the tea extract from *C. sinensis,* ranging from 22.9 to 56.4%.[8] The ethanol extract of *C. nitidissima* possessed strong effects similar to those of ascorbic acid.[6]

However, the antioxidant activities of these three extracts were lower than those of ascorbic acid, the reference compound. These activities were correlated to phenolic and flavonoid content, with correlation coefficients of 0.85 and 0.96, respectively (Figure S4, supporting information), indicating that phenolic and flavonoid compounds largely contribute to the scavenging effects of *C. longii* flower extracts. These results were consistent with previous studies, which showed phenolic and flavonoid compounds were mainly responsible for the scavenging effects against free radicals.[9,10]

For metal ion reduction, ferric and cupric ions were tested, which compared the ability of extracts to donate electrons at different pHs. The results showed that ethyl acetate, butanol, and ethanol extracts effectively reduced cupric and ferric solutions, and the reducing powers of these extracts are in line with their concentration. The hexane extract, however, showed weak reducing activities even at a concentration of 1000 μg/mL

Table 1. Chemical Composition of the EO of *Camellia longii* Flowers.

Peak	RT (min.)	Compounds	RI (cal.)	RI (lit.)	Content (%)	Identification
1	3.77	3-hexanone	-	784	0.1	MS
2	3.85	2-hexanone	-	790	0.1	MS
3	3.94	3-hexanol	-	797	0.1	MS
4	7.14	*α*-pinene	938	937	0.2	MS, RI
5	7.56	camphene	954	952	0.1	MS, RI
6	8.34	*β*-pinene	980	979	0.3	MS, RI
7	8.75	*β*-myrcene	993	991	0.1	MS, RI
8	9.12	*α*-phellandrene	1005	1005	0.1	MS, RI
9	9.50	*α*-terpinene	1020	1017	0.1	MS, RI
10	9.72	*p*-cymene	1028	1025	0.2	MS, RI
11	9.85	limonene	1030	1027	1.2	MS, RI
12	9.94	eucalyptol	1036	1032	0.4	MS, RI
13	10.42	*trans*-*β*-ocimene	1053	1049	0.1	MS, RI
14	10.73	*γ*-terpinene	1063	1060	0.1	MS, RI
15	11.61	terpinolene	1091	1088	0.1	MS, RI
16	11.94	linalool	1100	1099	0.1	MS, RI
17	13.88	linderol	1170	1166	0.1	MS, RI
18	14.61	*α*-terpineol	1193	1189	0.2	MS, RI
19	15.44	fenchyl acetate	1224	1223	0.2	MS, RI
20	17.28	bornyl acetate	1289	1285	0.7	MS, RI
21	17.45	2-undecanone	1295	1294	0.1	MS, RI
22	18.67	*δ*-elemene	1343	1338	0.2	MS, RI
23	19.70	α-copaene	1381	1376	1.3	MS, RI
24	20.11	*β*-elemene	1396	1391	0.1	MS, RI
25	20.35	cyperene	1405	1399	0.1	MS, RI
26	20.59	*α*-gurjunene	1415	1409	0.2	MS, RI
27	20.84	β-caryophyllene	1425	1419	1.8	MS, RI
28	21.34	aromandendrene	1445	1440	0.1	MS, RI
29	21.44	selina-5,11-diene	1449	1447	0.1	MS, RI
30	21.69	α-humulene	1459	1454	0.5	MS, RI
31	21.88	alloaromadendrene	1466	1461	0.3	MS, RI
32	22.18	*γ*-gurjunene	1478	1473	0.4	MS, RI
33	22.25	*γ*-muurolene	1481	1477	1.0	MS, RI
34	22.38	germacrene D	1485	1481	0.3	MS, RI
35	22.48	aristolochene	1489	1487	0.7	MS, RI
36	22.53	*β*-selinene	1486	1486	0.6	MS, RI
37	22.77	viridiflorene	1499	1493	1.5	MS, RI
38	22.83	epizonarene	1502	1501	1.8	MS, RI
39	23.07	*β*-bisabolene	1510	1509	0.5	MS, RI
40	23.28	*γ*-cadinene	1517	1513	0.6	MS, RI
41	23.53	*β*-cadinene	1525	1518	4.3	MS, RI
42	23.61	*δ*-cadinene	1528	1524	2.3	MS, RI
43	23.81	cubenene	1534	1532	2.4	MS, RI
44	24.14	*α*-calacorene	1544	1542	0.4	MS, RI
45	24.74	(*E*)-nerolidol	1563	1564	**13.0**	MS, RI
46	25.55	globulol	1587	1583	1.5	MS, RI
47	26.04	guaiol	1601	1596	1.2	MS, RI
48	27.29	*epi*-cubenol	1628	1627	3.0	MS, RI
49	27.42	*γ*-eudesmol	1631	1631	**5.8**	MS, RI
50	27.89	*τ*-cadinol	1641	1640	**6.5**	MS, RI
51	28.06	*δ*-cadinol	1645	1645	3.3	MS, RI
52	28.25	*β*-eudesmol	1649	1649	**8.9**	MS, RI
53	28.40	*α*-eudesmol	1652	1653	**16.1**	MS, RI
54	28.65	*trans*-guai-11-en-10-ol	1657	1655	1.0	MS, RI
55	30.57	eudesm-7(11)-en-4-ol	1695	1692	0.2	MS, RI
56	32.15	*trans*-farnesol	1732	1722	0.8	MS, RI
57	33.55	xanthorrhizol	1764	1753	1.2	MS, RI
		Total			**88.7**	

Abbreviations: RT (min.), retention time; RI (cal.), retention indices calculated on HP-5MS Ultra-Inert column (relative to *n*-alkane, C_8-C_{26}); RI (lit.), retention indices from literature; MS, constituent identified by mass-spectra comparison; RI, constituent identified by retention index matching; EO, essential oil.

Table 2. Phytochemical Screening of Extracts of *Camellia longii* Flowers in Different Solvents.

Phytochemical compounds	Test with	HE	EAE	BE	EE
Flavonoids	Shinoda's test	-	+	+	+
	Ferric chloride	-	+	+	+
Coumarins	Sodium hydroxide	-	+	+	+
Tannins	Lead acetate	+	+	+	+
Saponins	Foam test	-	-	+	+
Terpenoids	Salkowski's test	-	+	+	+
Reducing sugars	Fehling's test	-	+	+	+
Polysaccharides	Lugol reagent	-	+	+	+
Amino acids	Ninhydrin reagent	-	-	-	-
Alkaloids	Mayer reagent	-	-	-	-
	Dragendorff reagent	-	-	-	-

Abbreviations: HE, *n*-hexane extract of flower of *Camellia longii*; EAE, ethyl acetate extract of flower of *C. longii*; BE, *n*-butanol extract of flower of *C. longii*; EE, ethanol extract of flower of *C. longii*; "+" presence; "−" absence.

Table 3. Total Phenolic Content.

Extracts	Equivalent mg gallic acid equivalents/g crude extract
HE	8.0 ± 0.1^{a}
EAE	378.6 ± 2.3^{b}
BE	206.8 ± 0.02^{c}
EE	208.6 ± 1.39^{c}

Abbreviations: HE, *n*-hexane extract of flower of *Camellia longii*; EAE, ethyl acetate extract of flower of *C. longii*; BE, *n*-butanol extract of flower of *C. longii*; EE, ethanol extract of flower of *C. longii*; n=3; results expressed as M±SD. Letters a, b, and c indicate the statistically significant difference among values ($p < .05$).

(Tables 7 and 8). These results are consistent with the above-mentioned scavenging effects.

α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of four different extracts of *C. longii* flowers and the reference drug acarbose at different concentrations were examined, and results are shown in Table 9. At 256 μg/mL concentration, α-glucosidase inhibitory activities of extracts are comparable to those of acarbose, except for hexane extract. The hexane extract is always the least active at each concentration. Noticeably, the ethyl acetate, butanol, and ethanol extracts retain strong inhibitory activity at 64 μg/mL, 16 μg/mL, and 4 μg/mL, while the inhibitory activity of acarbose drops sharply. The ethyl acetate, butanol, and ethanol extracts have IC_{50} values of 2.16 ± 0.04, 1.95 ± 0.05, and 1.53 ± 0.04 μg/mL, indicating that they are much more active than acarbose (IC_{50} of 134.56 ± 3.02 μg/mL). The IC_{50} values of *C. longii* extracts except for hexane extract were in the range of 1.53–2.16 μg/mL, which were clearly stronger than most of the reported natural extracts or compounds.[11] The α-glucosidase inhibitory activities of these three extracts were much stronger than extracts from *Roylea cinerea* (D. Don) Baill. (Lamiaceae), *Clematis grata* Wall. (Ranunculaceae), and *Cornus capitata* Wall. (Cornaceae), which have traditionally been used in the management of diabetes and various other diseases.[12] Zhang et al demonstrated that *C. nitidissima* Chi flowers extracts showed good α-glucosidase inhibitory activities.[13]

Table 4. Total Flavonoid Content.

Extracts	Equivalent mg rutin equivalents/g crude extract
HE	88.6 ± 2.11^{a}
EAE	298.5 ± 1.69^{b}
BE	390.3 ± 0.47^{c}
EE	327.9 ± 2.19^{d}

Abbreviations: HE, *n*-hexane extract of flower of *Camellia longii*; EAE, ethyl acetate extract of flower of *C. longii*; BE, *n*-butanol extract of flower of *C. longii*; EE, ethanol extract of flower of *C. longii*; n=3; results expressed as M±SD. Letters a, b, c, and d indicate the statistically significant difference among values ($p < .05$).

Experimental

Chemicals, Solvents, and Standards

All chemicals, solvents, and standards used in the present study were purchased from Merck and Sigma–Aldrich in the highest purity and analytical grades.

Plant Material

The fresh flowers of *C. longii* were collected in October 2022 from Cat Tien National Park, Lam Dong Province, Vietnam. The scientific name of the plant was authenticated by Dr Dang Van-Son, Institute of Tropical Biology, Vietnam Academy of Science and Technology. A voucher specimen (No. Dang 472) was kept in the herbarium of the Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan, District 3, Ho Chi Minh City, Vietnam.

Extraction of the EO of C. longii Flowers

Fresh flowers of *C. longii* (350 g) were cut into small pieces and hydrodistilled with a Clevenger-type apparatus for 4 h. The experiment was performed three times. The extracted EO was dried over anhydrous sodium sulfate and stored in a sealed vial at 4 °C before GC/MS analyses. The EO yield based on the fresh weight of the sample was calculated.[14–16]

Preparation of the Different Extracts of C. longii Flowers

The air-dried flowers (1000 g) were immersed in 90% ethanol (3000 mL) at room temperature three times (each for 72 h). The extract was filtered and evaporated under reduced pressure at 45 °C to give a residue of ethanol extract (96 g). This extract was then suspended in hot water, then allowed to cool, and partitioned with *n*-hexane, ethyl acetate, and *n*-butanol, successively. The resulting fraction was concentrated under reduced

Table 5. Phenolic Composition of the Ethanolic Extract of *Camellia longii* Flowers.

Phenolics	Retention time	LOD*, μg/mL	LOQ**, μg/mL	Concentrations, mg/g extract
Gallic acid	5.84	2.46	8.20	18.08 ± 0.19
Chlorogenic acid	11.92	0.90	3.02	6.69 ± 0.15
Caffeic acid	14.18	0.42	1.40	7.66 ± 0.06
p-Coumaric acid	18.77	0.23	0.76	< LOD
Ferulic acid	19.22	0.24	0.79	< LOD
2,4-Dihydroxybenzoic acid	20.60	0.99	3.32	29.66 ± 0.96
Salicylic acid	24.94	0.12	0.39	24.41 ± 2.74
Cinnamic acid	25.90	0.18	0.59	14.65 ± 0.05
Rutin	20.78	0.02	0.06	< LOD
Quercetin	25.89	0.01	0.05	0.32 ± 0.00

*, **: limit of detection and limit of quantification.

Table 6. Scavenging Activity of Different Extracts of *Camellia longii* Flowers.

Extracts	IC_{50} (μg/mL)
HE	$> 1000^{a}$
EAE	61.69 ± 1.22^{b}
BE	57.42 ± 1.11^{c}
EE	60.50 ± 1.19^{b}
Ascorbic acid	26.68 ± 0.42^{d}

Abbreviations: HE, *n*-hexane extract of flower of *Camellia longii*; EAE, ethyl acetate extract of flower of *C. longii*; BE, *n*-butanol extract of flower of *C. longii*; EE, ethanol extract of flower of *C. longii*; *n*=3; results expressed as M ±SD. Letters a, b, and c indicate the statistically significant difference among values ($p < .05$).

pressure to give *n*-hexane (10 g), ethyl acetate (34 g), and *n*-butanol (29 g) fractions.

Analysis of the EO of C. longii *Flowers*

The GC/MS analysis was performed using an Agilent Technologies 7890B GC System coupled with a 5977B MSD model and equipped with an HP-5MS Ultra-Inert column (30 m × 0.25 mm i.d. and 0.25 μm film thickness).[14] The injection volume was 1 μL and the split ratio was 1:25. The oven temperature program was programmed from 50 °C (2 min isothermal) to 150 °C (10 min isothermal) at a rate of 5 °C/min, continuing to increase 10 °C/min to 280 °C (finally 10 min isothermal). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injector, MS Quad, and transfer line temperatures were maintained at 300 °C, 150 °C, and 300 °C, respectively. In addition, the MS source was set at 230 °C. Mass spectra were recorded at 70 eV, the mass range was from m/z 50 to 550 at 2.0 scan/s. The identification of oil components was accomplished by comparing their retention indices and their recorded mass spectra with those from the literature (NIST 17 and Adams book).[17]

Phytochemical Screening of Flower Extracts

The phytochemical screening tests of the different extracts of *C. longii* flowers were conducted according to previous studies:[18–21] flavonoids test with Shinoda's test and ferric chloride, coumarins test with sodium hydroxide, tannins test with lead acetate, saponins test with foam test, terpenoids test with Salkowski's test, reducing sugars test with Fehling's test, polysaccharides test with Lugol reagent, amino acids test with Ninhydrin reagent, and alkaloids test with Mayer and Dragendorff reagents.

Determination of Total Phenolic and Flavonoid Content

The TPC of the flower extracts was determined using the Folin-Ciocalteu method as reported by Singleton et al,[22] while the TFC of the flower extracts was estimated using the aluminum chloride method as reported by Pękal and Pyrzynska.[23]

The mixture of sample and reagents was spectrometrically measured at 744 nm and 510 nm using a spectrophotometer (Shimadzu V-630), respectively. Total phenolic content and TFC were determined from the calibration curves of gallic acid ($Y = 0.0099\ X - 1.6523 \times 10^{-5}$, $R^2 = 0.9996$), and rutin ($Y = 8.9656 \times 10^{-4}\ X - 3.6977 \times 10^{-4}$, $R^2 = 0.9999$), respectively.

Phenolic and Flavonoid Compounds Analysis Using High-Performance Liquid Chromatography

The analysis of phenolics in the extracts was performed on a Shimadzu LC-2030C high-performance liquid chromatography system equipped with a diode-array detector (HPLC-DAD). A VertiSep™ GES C18 reverse-phase column (250 × 4.6 mm, 5.0 μm particle size) was used to chromatographically separate the phenolics. The mobile phase comprised methanol (A) and 1% formic acid in water (B), and the gradient profile of the mobile phase was based on a method previously described by Vu et al,[24] The detection was conducted at a wavelength of 295 nm for phenolic acids and 340 nm for flavonoids. The quantification of phenolics was based on their calibration curves, with concentrations ranging between 1 and 30 μg/mL. The correlation coefficients (R^2) of the calibration were above 0.99.

Table 7. Ferric Reducing Antioxidant Power (FRAP) of Different Extracts of *Camellia longii* Flowers.

	FRAP (OD 593 nm)						
Extracts	0 μg/mL	15.63 μg/mL	62.50 μg/mL	250 μg/mL	500 μg/mL	750 μg/mL	1000 μg/mL
HE	0	0	0	0.03 ± 0.01	0.05 ± 0.01	0.07 ± 0.00	0.14 ± 0.02
EAE	0	0.15 ± 0.01	0.37 ± 0.02	1.03 ± 0.06	1.14 ± 0.02	1.28 ± 0.01	1.34 ± 0.01
BE	0	0.12 ± 0.01	0.26 ± 0.04	0.86 ± 0.09	1.00 ± 0.01	1.17 ± 0.01	1.34 ± 0.00
EE	0	0.08 ± 0.01	0.26 ± 0.04	0.57 ± 0.03	0.82 ± 0.00	1.08 ± 0.03	1.32 ± 0.01

Abbreviations: HE, *n*-hexane extract of flower of *C. longii*; EAE, ethyl acetate extract of flower of *C. longii*; BE, *n*-butanol extract of flower of *C. longii*; EE, ethanol extract of flower of *C. longii*; *n*=3; results expressed as M±SD.

Table 8. Cupric Reducing Antioxidant Capacity (CUPRAC) of Different Extracts of *Camellia longii* Flowers.

	CUPRAC (OD 450 nm)						
Extracts	0 μg/mL	15.63 μg/mL	62.50 μg/mL	250 μg/mL	500 μg/mL	750 μg/mL	1000 μg/mL
HE	0	0	0.02 ± 0.00	0.05 ± 0.00	0.11 ± 0.01	0.17 ± 0.00	0.23 ± 0.01
EAE	0	0.12 ± 0.00	0.39 ± 0.00	0.91 ± 0.02	0.97 ± 0.00	1.02 ± 0.02	1.07 ± 0.01
BE	0	0.08 ± 0.00	0.26 ± 0.00	0.72 ± 0.02	0.98 ± 0.06	1.04 ± 0.03	1.07 ± 0.01
EE	0	0.07 ± 0.01	0.23 ± 0.02	0.71 ± 0.03	1.00 ± 0.01	1.04 ± 0.02	1.10 ± 0.01

Abbreviations: HE: *n*-hexane extract of flower of *C. longii*, EAE: ethyl acetate extract of flower of *C. longii*, BE: *n*-butanol extract of flower of *C. longii*, EE: ethanol extract of flower of *C. longii*, $n = 3$, results expressed as M ± SD.

Table 9. α-Glucosidase Inhibitory Activity of Various Extracts of *Camellia longii* Flowers.

	α-glucosidase inhibitory activity (%)					
Extracts	256 μg/mL	64 μg/mL	16 μg/mL	4 μg/mL	1 μg/mL	IC_{50} (μg/mL)
HE	81.5	69.5	19.5	13	0	45.28 ± 0.68
EAE	92	91	88	85.5	27.5	2.16 ± 0.04
BE	99	96	90	88	32.5	1.95 ± 0.05
EE	94	94	91	90	41.5	1.53 ± 0.04
Acarbose	93	26	3	0	0	134.56 ± 3.02

Abbreviations: HE: *n*-hexane extract of flower of *C. longii*, EAE: ethyl acetate extract of flower of *C. longii*, BE: *n*-butanol extract of flower of *C. longii*, EE: ethanol extract of flower of *C. longii*, IC_{50}: half maximal inhibitory concentration (%), $n = 3$, results of IC_{50} expressed as M ± SD.

Determination of Antioxidant Activity

The scavenging activity of different extracts of *C. longii* flowers (0-1000 μg/mL) was determined using different methods, including DPPH method,[25] ferric reducing power[26] and cupric reducing antioxidant capacity.[27] Ascorbic acid (0-100 μg/mL) was used as an analytical standard.

Determination of α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory assay was conducted following the method described in previous studies.[28–31] The inhibitory activities of test samples were determined on a 96-well plate. The test sample was diluted with DMSO and deionized water to a series of concentrations, from 4, 16, 64, to 256 μg/mL. Samples can be further diluted to determine activities at lower concentrations. Acarbose (4-256 μg/mL) was used as a positive control. Chemicals used in the assay include phosphate buffer 100 mM at pH 6.8; α-glucosidase 0.2 U/mL, test sample, and 2.5 mM *p*-nitrophenyl α-D-glucopyranoside solution. In the control sample, the test sample was replaced by a reaction buffer. The experiment was incubated at 37 °C. After 30 min, the reaction was quenched by adding the solution of Na_2CO_3. The absorbance of the reaction was determined on a BIOTEK instrument with a wavelength of 410 nm (A).

The inhibitory activity of the test samples on α-glucosidase was determined by the formula:

$$\text{Inhibition}(\%) = [A_{(\text{control})} - A_{(\text{test sample})}] / A_{(\text{control})} \times 100\%$$

IC_{50} (half maximal inhibitory concentration) is the concentration of the test sample that inhibits 50% of the α-glucosidase activity, which is calculated using Tablecurve software.

Statistical Analysis

Each experiment was run three times. The outcomes were displayed as M ± SD. Microsoft Excel was used to perform the ANOVA one way and the linear regression model (Microsoft, 2018). *P*-value under .5 was regarded as significant.

Conclusion

In conclusion, phytochemical investigation, antioxidant, and α-glucosidase inhibitory activities of the flowers of *C. longii* collected in Vietnam were demonstrated. The findings indicated the flowers contained various phytochemical classes and were particularly rich sources of phenolics and flavonoids, resulting in high antioxidant activities. The study provides an insight into the potential bioactivities of the plant, particularly the inhibitory effect on α-glucosidase, which is crucial for the development of natural antidiabetic agents. It also opens new possibilities for the plant in the food and nutraceutical industries.

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Statement of Human and Animal Rights

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Statement of Informed Consent

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Supplemental Material

Supplemental material for this article is available online.

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