

Essential oils and ethanol extract from *Camellia nitidissima* and evaluation of their biological activity

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Abstract *Camellia nitidissima*, a well-known species of yellow *Camellia*, has undergone commercial cultivation as a new tea resource recently. Herein, the composition, antioxidant and antimicrobial activities of the essential oil and ethanol extract of *C. nitidissima* were investigated. The essential oils from the leaves and flowers of *C. nitidissima* were obtained by hydro-distillation. A total of 56 and 34 constituents accounting for 77.5 and 96.8% of the oils were identified by GC–MS. Linalool (35.8%), phytol (7.9%), *cis*-geranyl acetone (7.3%) and methyl salicylate (6.8%) were found to be the primary components in the leaf oil, while the flower oil was rich in α -eudesmol (34.3%), γ -eudesmol (31.5%) and linalool (11.1%). The ethanol extract of *C. nitidissima* leaves contained 281.04 mg gallic acid equivalent/g of total phenols. The antioxidant activities of the two oils and extract were evaluated by DPPH and ABTS radical-scavenging assays. The IC₅₀ values varied from 17.4 (extract) to 720.3 μ g/mL (flower oil) for

DPPH and from 28.8(extract) to 889.6 μ g/mL (flower oil) for ABTS. Both essential oils exhibited moderate antioxidant activities, and the extract possessed strong effects close to ascorbic acid. Additionally, the antimicrobial activities of the oils and extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were evaluated by agar dilution assay. No considerable bactericidal activities were observed for either essential oil or extract compared with ampicillin and tobramycin standards. The results indicated the extract was more efficient than the two essential oils against *S. aureus* (MIC = 0.625 mg/mL) and *B. subtilis* (MIC = 1.25 mg/mL).

Keywords *Camellia nitidissima* · Extract · Essential oil · Antioxidant activity · Antibacterial activity

Introduction

While traditional tea is made from *Camellia sinensis*, that species is with white flowers and just one member of a subgroup of *Camellia* genus, yellow *Camellia* is another species with golden yellow flowers, comprising approximately 42 species and 5 variants, distributed in south China and Northern Vietnam (Liang 2007; Zhang and Ren 1998). When the flowers and leaves of yellow *Camellia* are made into tea, the drink has a unique aroma and taste compared to the traditional tea. Besides making tea, yellow *Camellia* plants are widely used as a Chinese medicine to treat various complaints, such as dysentery, sore throat, and hypertension, and for cancer prevention (Xiao and Xia 1991; Chen et al. 1993; Huang 1994). In 2010, yellow *Camellia* plants were authenticated as a new food resource by the SFDA of China. Therefore, investigation of the

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phytochemicals of yellow *Camellia* and their potential biological activity has great significance.

Camellia nitidissima (*C. nitidissima*), a well-known species of yellow *Camellia*, has undergone commercial cultivation as a new tea resource in recent years. Previous studies indicated that the chemical constituents and extracts from *C. nitidissima* possess multiple biological activities including antioxidant activity (Song et al. 2011), anticancer activity (Lin et al. 2013), and cytotoxicity (Qi et al. 2016) as well as inhibiting the formation of advanced glycation end-products (Wang et al. 2016). So far, there is no previous report of the volatile components from the flowers of *C. nitidissima*, and only two earlier researches investigated the oil composition from the leaves of *C. nitidissima* briefly (Huang et al. 2009; Cui et al. 2013). Studies on the essential oil, the phenolic components and their biological activities of *C. nitidissima* are still scarce. Therefore, the chemical composition and application potential of *C. nitidissima* awaits deeper research. In the present work, we investigate the chemical composition of the essential oils from the leaves and flowers of *C. nitidissima*, as well as the total phenolic content of this plant's extracts, and evaluate the antioxidant and antimicrobial activities of the essential oils and ethanol extract.

Materials and methods

Plant materials

Leaves and flowers of *C. nitidissima* were collected from Fangcheng Yellow *Camellia* National Natural Reserve (Guangxi province, China) during May–August 2016. An authenticated herbarium specimen of the plant was deposited at Guangxi Institute of Botany, Chinese Academy of Sciences. The leaves were air-dried in the shade for about 2 weeks, and then ground into pieces, and the flowers were freeze-dried and ground.

Preparation of the essential oil and ethanol extract

The two pulverized materials were subjected to water distillation using a Clevenger-type apparatus for 5 h each. Ten extraction runs were performed for each sample, and the essential oils were combined. The obtained oil was dissolved in *n*-hexane, dried over anhydrous sodium sulfate, and stored in a sealed vial at 4 °C prior to further analysis and investigation.

The powdered leaves of *C. nitidissima* (3 kg) were refluxing-extracted 3 times using 95% ethanol (3 × 5 L, 2 h each time). The combined extracts were evaporated under reduced pressure to remove ethanol, then the extract was suspended in 1 L of deionized water and extracted

with petroleum ether (60–90 °C, 3 × 1 L) to remove lipophilic components. The extract was concentrated, dried and stored in the dark place at 4 °C before use.

Gas chromatography (GC) analysis

The chemical composition of the essential oils was quantitatively analyzed on a Bruker 456 GC apparatus equipped with a flame ionization detector (FID) and a Bruker BR-5 ms fused silica capillary column (15 m × 0.25 mm ID with 0.25 μm film thickness). The injector and FID temperature were 280 and 230 °C, respectively. Helium was used as the carrier gas at a constant volume velocity of 1.0 mL/min. The oven temperature was maintained at 70 °C for 3 min initially and then increased to 250 °C at the rate of 4 °C/min, subsequently raised up to 270 °C at a rate of 20 °C/min and held at 270 °C for 3 min. The content of each constituent was expressed as a relative percentage of the total peak area.

Gas chromatography-mass spectrometry (GC–MS) analysis

The GC–MS analysis was carried out on a Bruker SCION™ TQ mass spectrometer equipped with a Bruker 456 GC and a fused silica capillary column Bruker BR-5 ms (15 m × 0.25 mm ID and 0.25 μm film thickness) under the following conditions. The oven temperature was set at 70 °C initially and kept for 3 min, then was programmed from 70 to 250 °C at 4 °C/min, then subsequently up to 270 °C at 20 °C/min and held isothermally at 270 °C for 3 min. The injector and interface temperature were maintained at 280 and 250 °C, respectively. The sample was injected in the split mode at a ratio of 1:20 using helium as carrier gas at 1.0 mL/min. Mass spectra were acquired over the range of 33–450 *m/z* using electron ionization (70 eV). The retention indices (RI) of the oil components were determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Van den Dool and Kratz 1963). The essential constituents were identified by comparing their retention indices and mass spectra reported in the literature (Adams 2007), NIST/EPA/NIH mass spectral library (2014) and Wiley registry of mass spectral data (9th edition).

Determination of total phenols and typical phenolic components

The total phenolic content of the extract was determined by the Folin-Ciocalteu method using gallic acid as a standard. The extract solution was transferred to a 25 mL volumetric flask. 2 mL of Folin–Ciocalteu reagent and 4 mL of 15% sodium carbonate solution were added and mixed

completely. The total volume was adjusted to 25 mL with distilled water. The mixture was reacted for 90 min at room temperature away from light, and absorbance was recorded at 760 nm. The total phenolic content of the extract was expressed as gallic acid equivalents in mg/g material.

Typical phenolic components of the sample from *C. nitidissima* leaves were analyzed on a LabAlliance HPLC system equipped with an Agilent TC-18 column (250 mm × 4.6 mm, 5.0 μm) at room temperature. The mobile phase was composed of acetonitrile (A) and 0.1% phosphoric acid aqueous solution (B). The linear gradient elution was: 0–2 min, 2% A; 2–10 min, 2–12% A; 10–25 min, 12–18% A; 25–30 min, 18–25% A; 30–50 min, 25–50% A; 50–60 min, 50–70% A; 60–85 min, 70–2% A. The flow rate was 1.0 mL/min. UV absorption was set at 270 nm.

Free radical-scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) were used to test the antioxidant activity of the essential oils and ethanol extract. DPPH assay was performed using the methods described by Blois and Braca with some modifications (Blois 1958; Braca et al. 2001). 100 μL of 0.1 mM DPPH solution was mixed with 100 μL of various concentrations of the sample in ethanol, and reacted for 30 min, away from light, at room temperature. The absorbance of the mixture at 517 nm was recorded on a microplate reader. The DPPH radical-scavenging effect was evaluated by the IC₅₀ value (the amount of sample needed to scavenge 50% of DPPH radicals), which was determined from the graph of percentage of inhibition plotted versus the sample concentration. Ascorbic acid was used as a reference compound and treated similarly.

The radical-scavenging activity of the essential oils and ethanol extract against ABTS was evaluated according to the procedure reported by Re and Chen with some modifications (Re et al. 1999; Chen et al. 2015). A 7.0 mM ABTS solution and a 2.45 mM K₂S₂O₈ solution were prepared in 10 mM sodium phosphate buffer (pH 7.4), separately. The reaction of these two solutions in 1:1 volume ratio was conducted to generate ABTS⁺ radical cations. This mixture was kept away from light for 12–16 h at room temperature until used. The ABTS⁺ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 195 μL of ABTS⁺ solution and 5 μL of the sample with various concentrations in ethanol were mixed evenly. After reaction for 10 min at room temperature, the absorbance at 734 nm was measured. The IC₅₀ value was obtained similarly to the DPPH assay. Again, ascorbic acid was employed as a reference standard.

Evaluation of antibacterial activity

Strains of *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 21216), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used to evaluate the bactericidal activity of the essential oils and ethanol extract by the agar dilution assay. The colony-forming units of each strain were counted and adjusted to 0.5 McFarland standard turbidity, an approximate inoculum concentration of 10⁸ CFU/mL.

The essential oils or ethanol extract were dissolved in ethanol to obtain an initial concentration of 10 mg/mL, and then two-fold diluted to prepare the nutrient agar plates with a series of concentrations of sample. The plates were spotted and incubated at 37 °C for 24 h. Ethanol was used as solvent control and ampicillin and tobramycin as positive controls. The antimicrobial activity was evaluated by the minimal inhibitory concentration (MIC), the lowest concentration of the sample inhibiting the visible growth of the tested bacteria after incubation. All tests were performed in duplicate.

Results and discussion

Chemical compositions of the essential oils

The treated leaves and flowers of *C. nitidissima* were hydro-distilled and yielded 0.21 and 0.79% essential oils, respectively. These two essential oils were qualitatively and quantitatively analyzed by GC and GC–MS. The identified compounds and their relative percentages of peak areas (more than 0.1%) are listed in Table 1 along with their retention indices. 56 constituents from the leaves were identified, representing approximately 77.5% of the total oil, revealing linalool (35.8%), phytol (7.9%), *cis*-geranyl acetone (7.3%), methyl salicylate (6.8%), *n*-hexanal (4.4%) and *trans*-linalool oxide (3.2%) as the major compounds. For the essential oil of *C. nitidissima* flowers, 34 components were characterized, amounting to 96.8% of the total oil composition, indicating that the flower oil is very abundant in α -eudesmol (34.3%), γ -eudesmol (31.5%) and linalool (11.1%). As seen from Table 1, the chemical composition of the essential oils from the leaves and flowers of this plant exhibited a remarkable diversity, although linalool and *trans*-linalool oxide were found in both oils at relatively high amounts. However, the previously reported oil compositions were markedly distinct from our results, probably because of differences in the plant material resources, oil extraction methods, the time of specimen collection. Huang et al. (2009) reported methyl salicylate (26.9%) and benzyl alcohol (5.9%) as the principal compounds in *C. nitidissima*, while the other main components reported in the present study, including

Table 1 Composition of the essential oils from the leaves and flowers of *C. nitidissima*

Constituents ^a	Mass data	RI ^b	Leaf Area (%) ^c	Flower
2,4-Dimethylhexane	114.2	735		1.23
<i>n</i> -Hexanal	100.2	772	4.41	
2-Formylfuran	96.1	804		0.13
2,5,5-Trimethyl-1-hexen-3-yne	122.2	816	0.25	
2-Hexenal	98.1	827	2.38	
3-Hexenol	100.2	838	0.80	
1-Hexanol	102.2	852	1.13	0.10
<i>cis</i> -4-Heptenal	112.2	879	0.14	
Heptanal	114.2	880	0.45	0.45
Benzaldehyde	106.1	942	0.26	0.25
3-Octenol	128.2	959	0.37	
Sulcatone	126.2	964	1.40	
2-Pentylfuran	138.2	979	1.24	
<i>trans,trans</i> -2,4-Heptadienal	110.2	982	0.37	
<i>trans</i> -2-(2-Pentenyl)furan	136.2	983	0.27	
Octanal	128.2	984	0.19	
<i>cis</i> -5-Isopropenyl-2-methyl-2-vinyltetrahydrofuran	152.2	985	0.18	
Benzeneacetaldehyde	120.2	1008		0.16
<i>trans,trans</i> -2,4-Octadienal	124.2	1012	1.34	
<i>trans</i> -2-Octenal	126.2	1034	0.26	
<i>trans</i> -Linalool oxide	170.3	1064	3.23	1.93
<i>cis</i> -Linalool oxide	170.3	1066	0.94	1.07
<i>trans,trans</i> -3,5-Octadien-2-one	124.2	1070	0.39	
Linalool	154.3	1082	35.81	11.13
Nonanal	142.2	1084		1.54
<i>trans</i> -5-Methyl-2-isopropyl-2-hexen-1-al	154.3	1107	0.15	
Lilac aldehyde A	168.2	1122		0.37
(<i>R,S</i>)-5-Ethyl-6-methyl-3E-hepten-2-one	154.3	1124	0.19	
<i>trans,cis</i> -2,6-Nonadienal	138.2	1125	0.21	
<i>cis</i> -2-Nonenal	140.2	1125	0.40	
2-Isopropenyl-5-methylhex-4-enal	152.2	1165		0.10
<i>cis</i> -3-Hexenyl butyrate	170.3	1167	0.16	
Methyl salicylate	152.2	1168	6.78	0.61
α -Terpinol	154.3	1172	1.21	1.06
Safranal	150.2	1173	0.18	
Decanal	156.3	1183	0.11	
β -Cyclocitral	152.2	1196	0.32	
Nerol	154.3	1209	0.23	0.13
<i>trans</i> -Geraniol	154.3	1232	0.48	0.47
<i>trans</i> -2-Decenal	154.3	1240	0.22	
α -Cyclocitral	152.2	1249	0.16	
<i>cis</i> -5,7-Dodecenyne	164.3	1259	0.29	
<i>p</i> -Isopropylbenzyl alcohol	150.2	1266		0.11
2,4-Decadienal	152.2	1270	0.15	
2-Methoxy-4-vinylphenol	150.2	1288		0.10
<i>trans,trans</i> -2,4-Decadienal	152.2	1291	0.67	
<i>trans</i> -2-Undecenal	168.3	1337	0.71	
<i>cis</i> - β -Damascenone	190.3	1361	0.13	
α -Ionone	192.3	1413	1.62	

Table 1 continued

Constituents ^a	Mass data	RI ^b	Leaf Area (%) ^c	Flower
Dehydro- β -ionone	190.3	1440	0.54	
Geranyl acetone	194.3	1452	7.29	
α -Guaiene	204.4	1453		0.27
<i>trans</i> - β -Ionone	192.3	1453	1.23	
Epoxy- β -ionone	208.3	1456	0.24	
<i>trans</i> -2-Octenyl tiglate	210.3	1474	0.16	
<i>cis,cis</i> -5,9-Tetradecadiene	194.4	1479	0.38	
3- <i>trans</i> -5- <i>cis</i> -Pseudoionone	192.3	1535	0.19	
<i>cis</i> - β -Guaiene	204.4	1535		0.14
Elemol	222.4	1544		0.44
<i>trans</i> -Nerolidol	222.4	1548	0.23	0.23
<i>cis</i> -3-Hexenyl benzoate	204.3	1550	0.34	
<i>trans,trans</i> -Pseudoionone	192.3	1562	0.34	
Guaiol	222.4	1589		1.42
5- <i>epi</i> -7- <i>epi</i> - α -Eudesmol	222.4	1608		0.81
<i>neo</i> -Intermedeol	222.4	1613		0.14
10- <i>epi</i> - γ -Eudesmol	222.4	1620		0.84
γ -Eudesmol	222.4	1627		31.52
Agarupiol	222.4	1630		1.94
α -Eudesmol	222.4	1644		34.28
Bulnesol	222.4	1655		2.31
<i>epi</i> - γ -Eudesmol	222.4	1660		0.15
Juniper camphor	222.4	1681		0.76
<i>trans</i> -Farnesol	222.4	1794	0.18	
Phytone	268.5	1842	0.72	
Farnesyl acetone	262.4	1895	0.69	
Isophytol	296.5	1940	0.35	
Phytol	296.5	2102	7.95	
Ethyl linoleate	308.5	2143		0.20
Ethyl linolenate	306.5	2145		0.36

^aConstituents listed in order of elution from a Bruker BR-5 ms column

^bRetention indices on a Bruker BR-5 ms column

^cRelative percentage, individual constituent in relation to total oil

linalool, phytol, *cis*-geranyl acetone and *n*-hexanal, were found in much lower amounts or did not exist at all in this plant. In another earlier report, 45 components, accounting for 51.6% of the total oil composition, were identified, and the major constituents observed in the present study were also not found (Cui et al. 2013).

Total phenolic content and typical phenolic compounds

The total content of phenols in the ethanol extract of *C. nitidissima* was determined as 281.04 ± 6.87 mg gallic

acid equivalent/g, indicating this plant is rich in phenolic compounds. Plenty of evidence has demonstrated the important physiological role of phenolic compounds, especially polyphenols from various plants, which are considered to be responsible for many biological activities. To better characterize the phenolic composition of *C. nitidissima* extract, several phenols were employed as standards for HPLC analysis, and six tea polyphenols (catechin, epicatechin, epigallocatechin, epicatechingallate, gallo catechingallate and epigallocatechin gallate), and three flavones (quercetin, quercetin-7-*O*- β -D-glucoside and kaempferol) were identified from the extract by comparing

the retention times of unknown peaks with those of authenticated reference standards. The content of each constituent mentioned above in *C. nitidissima* leaves was also determined by Soxhlet extraction. As shown in Table 2, this plant is very rich in quercetin-7-*O*- β -glucoside and quercetin. Also, it is interesting to note that, similarly to traditional green tea, *C. nitidissima* contains abundant polyphenols, with especially high amounts of epicatechingallate, epigallocatechingallate and galocatechingallate.

Antioxidant activity

The antioxidant activities of the essential oils and extract against DPPH and ABTS radicals were investigated. These were quantified by the IC₅₀ values, which are inversely proportional to the extent of activity, i.e. low values indicate a significant anti-radical activity via the scavenger effect. The IC₅₀ values of the extract, leaf oil and flower oil against DPPH radicals were 17.4, 164.8 and 720.3 μ g/mL, respectively, while the IC₅₀ values of the three tested samples against ABTS radicals ranged from 28.8 μ g/mL (extract) to 889.6 μ g/mL (flower oil). This variation in DPPH and ABTS-scavenging abilities can be attributed to the difference in chemical composition of the extract and essential oils. Compare to the IC₅₀ of ascorbic acid (the DPPH IC₅₀ and ABTS IC₅₀ were 13.2 and 18.3 μ g/mL, respectively), the oils obtained from *C. nitidissima* leaves and flowers exhibited low ability to scavenge free radicals in both the DPPH and ABTS tests. However, the extract showed strong antioxidant activity quite close to that of ascorbic acid. As illustrated in Table 2, the ethanol extract of *C. nitidissima* contains abundant polyphenolic constituents such as catechins and quercetins. The antioxidant capacity of phenolic compounds has been well

characterized, and arises from their ability as electron or hydrogen donors. Previous reports have demonstrated the good antioxidant performance of catechins (Mukhtar and Ahmad 2000; Higdon and Frei 2003; Shahidi et al. 1992) and polyphenolic flavonoids such as quercetin (Sowndhararajan and Kang 2013; Zhang and Lu 2006). Our present results confirmed that the ethanol extract of *C. nitidissima* possessed considerably better DPPH and ABTS-scavenging capacity than the essential oils from the plant. Therefore, we conclude that polyphenols in the ethanol extract are primarily responsible for the strong free radical-scavenging ability.

Antibacterial activity

Two Gram-positive bacteria (*S. aureus* and *B. subtilis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were selected to evaluate the in vitro bactericidal activities of the essential oils and extract of *C. nitidissima* by the agar dilution test. The essential oil from *C. nitidissima* leaves showed comparatively high activity against *S. aureus*, *B. subtilis* and *E. coli* (the MIC values were 0.625, 1.25 and 1.25 mg/mL, respectively), but not against *P. aeruginosa* (MIC > 10.0 mg/mL). The MIC against the *S. aureus* and *B. subtilis* of ethanol extract of *C. nitidissima* were 0.625 and 1.25 mg/mL, which exhibited better activity than the two Gram-negative bacteria strains (10 and 5 mg/mL for *E. coli* and *P. aeruginosa*). Among the three samples tested, the MIC values against the strains of the essential oil from *C. nitidissima* flowers were range from 2.5 to 10 mg/mL, showed a mild activity against the Gram-positive bacteria strains. However, when compared with standards (ampicillin and tobramycin, which MIC values against the four bacteria were 4.0–516 μ g/mL and 0.5–1.0 μ g/mL, respectively), neither the essential oils nor extract of *C. nitidissima* demonstrated considerable antimicrobial effect. As summarized in Tables 1 and 2, linalool, eudesmol and polyphenols are the main compounds in the leaf essential oil, flower essential oil and ethanol extract of *C. nitidissima*, respectively. Some previous investigations have revealed the good antimicrobial effects of linalool (Ebrahimabadi et al. 2010; Liu et al. 2012; Peana et al. 2002; Griffin et al. 1999), eudesmol (Costa et al. 2008) and certain polyphenols such as catechins (Maria John et al. 2011; Fernández et al. 2017; Xiong et al. 2017) and quercetins (Bonvicini et al. 2017; Basile et al. 2000). Our results suggest a possible antagonistic relationship between these predominant compounds and the other components of the oils, which seems to be responsible for the weak antimicrobial activity of the whole essential oils and the extract from *C. nitidissima* despite the activity of their major components in isolation.

Table 2 Content of several typical polyphenols in the leaves of *C. nitidissima*

Phenols ^a	Content ^b (μ g/g)
Epigallocatechin	41.3 \pm 0.8
Catechin	13.3 \pm 1.9
Epigallocatechin gallate	63.5 \pm 2.6
Epicatechin	39.7 \pm 1.1
Galocatechingallate	87.4 \pm 0.5
Epicatechingallate	240.6 \pm 3.6
Quercetin-7- <i>O</i> - β -glucoside	673.9 \pm 2.1
Quercetin	139.4 \pm 0.4
Kaempferol	30.6 \pm 0.8

^aPhenols listed in order of elution from an Agilent TC-18 column

^bValues are expressed as the mean \pm standard deviation (n = 3)

Conclusion

This work investigated the composition, antioxidant and antimicrobial activities of the essential oils from the leaves and flowers, and the ethanol extract, of *C. nitidissima*. The essential oils showed mild antioxidant activities and the extract exhibited strong antioxidant effects, while none of those three samples demonstrated considerable bactericidal activities against four bacteria tested. Their activities varied greatly as a result of their different chemical compositions. These findings may help researchers to better understand the chemical composition and activities of *C. nitidissima*, and have practical implications for the application potential of yellow *Camellia*.

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