



Spatial variation of volatile organic compounds and antioxidant activity of turmeric (*Curcuma longa* L.) essential oils harvested from four provinces of China

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ABSTRACT

The objective of this study was to investigate the spatial variation of volatile organic compounds and antioxidant activity of turmeric essential oils (TEOs) harvested from four provinces of China. The major chemical components of these TEOs were analyzed using headspace solid-phase micro-extraction gas chromatography-mass spectrometry. More than forty volatile organic compounds in TEOs were identified, which accounted for 82.09–93.64% of the oil components. The relative abundances of the main volatile organic compounds in TEOs at the genus level were visualized by a heat map. The antioxidant activity of the TEOs of five different origins was characterized by the DPPH free radical scavenging activity, in which the antioxidant activity of the TEOs from Guangxi was superior to those of other sources. Furthermore, the IC₅₀ values of the antioxidants TEOs collected from Guangxi, Sichuan, Yunnan, Changting, and Liancheng were 33.30, 42.5, 35.22, 63.27, and 39.96 mg/mL, respectively, which indicated the excellent free radical scavenging activity of those TEOs. Therefore, the TEOs might be considered as a natural antioxidant with potential applications in food and pharmaceutical industries.

1. Introduction

Curcuma longa Linn. (syn. *C. domestica* Valetton and *C. brog* Valetton), family *Zingiberaceae*, is known as "turmeric" worldwide, "Jianghuang" in Chinese, "kurkum" in Arabic, and "haldi" in Hindi and Urdu (Dosoky and Setzer, 2018; G. Singh et al., 2010). It is assumed that turmeric originated in China, and Buddhist monks or Chinese migration brought it to the Indian subcontinent (Stanojevic et al., 2015). Currently, turmeric is cultivated in Asian countries (i.e., China, Bangladesh, Thailand, Cambodia, Malaysia, Indonesia, and Philippines) and some parts of South America (Peru and Bolivia) (Sharma et al., 2021; Stanojevic et al., 2015). There are approximately 93–100 accepted *Curcuma* species, however, the exact number of species is still controversial, particularly considering the turmeric rhizomes harvested from different regions.

In this regard, turmeric rhizome contains two major classes of

secondary metabolites: phenolic curcuminoids and essential oils (EOs) (Stanojevic et al., 2015; Tosati et al., 2018). In addition to the curcuminoids, EOs from turmeric mainly consists of aromatic compounds and aliphatic terpenes, which are considered to have significant biological activities including antioxidant (Avaço et al., 2017), antibacterial (Avaço et al., 2017; Hu et al., 2017; Li et al., 2019), anti-inflammatory (Akinoyemi et al., 2018; Todén et al., 2017), anticancer (Cheng et al., 2012; Joshi et al., 2016; Kim et al., 2013), anti-hyperlipidemic (Ling et al., 2012; V. Singh et al., 2013) and antidiabetic role (Lekshmi et al., 2012; Shinichi et al., 2006; Tozo et al., 2005). In this case, hundreds of compounds have been identified from the turmeric essential oils (TEOs); however, the major components are *ar*-Turmerone, α -Turmerone, β -Turmerone, *ar*-Curcumene, and Curlone, followed by notable amounts of α -Zingiberene, α -Bisabolene, *ar*-Turmerol, β -Phellandrene, α -Phellandrene, α -Terpinene, *r*-Terpinene, Terpinolene, α -Sesquiphellandrene,

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β -Sesquiphellandrene, 1,8-Cineole, Caryophyllene oxide, and β -Bisabolene (Akinyemi et al., 2018; Avanzo et al., 2017; B, Kottarapat, & Ramadasan, 2011; Hwang et al., 2016; Kutti Gounder and Lingamallu, 2012; Naveen Kumar et al., 2016; Oyemitan et al., 2017; Stanojevic et al., 2015). Zhang et al. (2017) (Zhang et al., 2017) analyzed 81 components of TEO using gas chromatography-mass (GC-MS) from 20 different habitats in China, and a total of 81 chromatographic peaks were obtained, among which the main components were *ar*-Turmerone, β -Turmerone, α -Zingiberene, *ar*-Curcumene, and β -Sesquiphellandrene. Dosoky, Satyal, and Setzer (2019) (Dosoky et al., 2019) reported that TEO was obtained and analyzed by GC-MS. TEO volatiles were dominated by α -Turmerone, Curhone, *ar*-Turmerone, β -Sesquiphellandrene, α -Zingiberene, Germacrone, Terpinolene, *ar*-Curcumene, and α -Phellandrene. Xu et al. (2020) (Xu et al., 2020) analyzed the volatile components in turmeric samples from five major production areas of China using GC-MS. A total of the chemical components including *ar*-Turmerone, α -Turmerone, β -Turmerone, (E)-Atlantone, Caryophyllene, *ar*-Curcumene, (-)-Zingiberene, β -Bisabolene, β -Sesquiphellandrene and (6R,7R)-Bisabolene were identified. However, the comparative data on the chemical compositions of volatile organic compounds of TEOs stemmed from different regions are scarce, particularly lack of assessing tools with a high efficacy, such as headspace solid-phase micro-extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS). Moreover, considering the extraction of the different terpenoids and other compounds from EOs, HS-SPME-GC-MS would be a powerful tool that requires a minimal amount of sample. HS-SPME-GC-MS was chosen in this study because it can determine accurately, conveniently, and rapidly the chemical compositions of those TEOs. (Durant et al., 2014). Furthermore, HS sampling is a fundamental technique to characterize the volatile fraction of aromatic plants (Da Porto and Decortis, 2008).

In recent years, the evaluation of antioxidant potential of foods has received much attention. The considerable research efforts have been attached to the antioxidant and related antimicrobial, insecticidal, antifungal, and antioxidation properties. For example, the essential oil of turmeric rhizome showed major radical scavenging activity against DPPH free radical (Tsai et al., 2011). Furthermore, an obvious variation was demonstrated by the antioxidant activities of TEOs extracted from Chongqing and Guangdong of *C. longa*: high DPPH radical-scavenging activity was exhibited by the TEOs from Guangdong than Chongqing (Zhang et al., 2017). Since TEOs have been discovered to be toxic to fungi engaged in the deterioration of agricultural products, TEOs could serve as an alternative to synthetic pesticides for the control of food fungi and pests. The interest in their use has been increasing because they demonstrate lower risks for human health and the environment. TEOs would not leave residues in food, another growing concern of the population. Furthermore, very recently, the price of turmeric has been increased continuously with the increase in demand, and the quality variations of turmeric of geographic locations have become dramatically important. In this case, since the turmeric essential oil (TEO) is one of the main active components of turmeric, the studies on the spatial variation of the chemical composition and antioxidant activity of such a component would provide insights into the evaluation of turmeric quality.

The objective of this study was to evaluate the spatial variation of the chemical compositions and antioxidant activity of the TEOs harvested from four different provinces of China. The major chemical compositions of these TEOs were analyzed using headspace solid-phase micro-extraction gas chromatography-mass spectrometry HS-SPME-GC-MS. The multivariate analysis of the chemical compositions in these TEOs were performed using the hierarchical cluster analysis and principal component analysis (PCA). The antioxidant activity of the TEOs of four provinces was characterized by the DPPH-radical-scavenging activity to provide a certain scientific basis for the quality evaluation of this plant.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Shanghai Macklin Biochemical Co., Ltd. The ultrapure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was used in this study, provided by a Simplicity-UV water purification system (Millipore, Bedford, USA).

2.2. Plant materials and TEOs extraction

The *C. longa* rhizomes used in this study were harvested from five different origins in Guangxi, Sichuan, Yunnan, and Fujian provinces of China. The geographic information of these origins was shown in Table 1. TEOs of *Curcuma* species could be obtained by hydro- or steam-distillation of the fresh or dry rhizome. Alternatively, *Curcuma* volatiles have also been attained by solvent extraction or supercritical fluid extraction of the powdered rhizome. In this study, the fresh rhizomes of turmeric (*C. longa*) harvested from Liancheng and Changting, Fujian Province were washed in running water, dried in the air, and thinly grated for further utilization; while the dry rhizomes of *C. longa* obtained from Sichuan Province, Guangxi Province, and Yunnan Province were pulverized into fine powders. Then, all these treated dry and fresh rhizomes were passed through an 80-mesh sieve to obtain a uniform powder, respectively. TEOs were extracted by the hydrodistillation of 200 g of rhizomes for 5 h using a Clevenger apparatus (Commission, 2015). The TEOs were collected, and the water remaining after extraction was removed by adding anhydrous sodium sulphate (Na_2SO_4), followed by filtration. The TEOs were stored at 4°C in sealed glass vials and protected from light prior to chemical analysis and further use. The TEOs were denoted as LC, CT, SC, GX, and YN for the rhizomes of *C. longa* harvested from Liancheng and Changting, Fujian Province, Sichuan Province, Guangxi Province, and Yunnan Province accordingly.

2.3. Chemical analysis and identification of the major components of TEOs

HS-SPME-GC-MS was used for identifying thermally labile volatile compounds from TEOs. The PDMS/DVB fibers (57329-U, Supelco, USA) were used for the extraction of the volatile organic compounds of TEOs of five different origins. The fibers were conditioned for 12 min at 250°C in the GC/MS injector (GCMS-TQ8040, SHIMADZU, Japan) before SPME-GC/MS analysis. For TEOs of each origin, a small amount of TEOs was put in a 15 mL vial. The fiber coatings were embedded into the headspace to determine the values of the temperature and time set in the experiments. The temperature was set at 50°C while the incubation and extraction time was set at 10 and 30 min, respectively. The fibers containing the extracted volatile organic compounds of TEOs were injected into the GC/MS injector. Separation of TEOs was used in a Rxi-5Sil MS column ($30 \text{ m} \times 0.25 \text{ mm}, 0.25 \mu\text{m}$). The measurement of each sample using GC/MS equipped with an auto-sampler was set for about 40 min. The temperature of the injector and detector was 250 and 280°C , respectively. The initial temperature was kept at 50°C for 2 min, and then was gradually increased to 180°C at a temperature ramp rate of $5^\circ\text{C}/\text{min}$ and was dwelled at 180°C for 0 min; then the temperature was increased to 280°C at a rate of $10^\circ\text{C}/\text{min}$ and was held at 280°C for 2 min.

The gas chromatography and mass spectrometry (GC-MS) analyses were performed under the following conditions: the mass spectrometer was operated at an ionization voltage of 70 eV; the temperature of an ion source was 200°C ; the full mode range was conducted with a scan mass range of 35–550 amu; the carrier gas was helium, at a flow rate of 1.0 mL/min, and the split ratio was 100:1.

Table 1
Geographic and climatic information on the collected turmeric for the study.

Province	District	Locality	Longitude	Latitude	Accessions collected	Climate	Elevation
Guangxi	Baise	Napo	105° 84'	23° 41'	GX	subtropical monsoon climate	1681 m
Yunnan	Wenshan	Maguan	103° 52'	22° 42'	YN	subtropical monsoon climate	1447 m
Sichuan	Yibin	Yibin	104° 53'	28° 69'	SC	subtropical monsoon humid climate	422 m
Fujian	Liancheng	Chixi	116° 32'	25° 13'	LC	subtropical monsoon climate	375 m
Fujian	Changting	Tongfang	116° 57'	25° 88'	CT	subtropical monsoon climate	658 m

2.4. Identification of components

The components were identified according to the search and match of gas chromatographic retention indices, mass spectra with FFNSC 1.2., NIST14. and NIST14s. libraries, and the literature (Qin et al., 2007; Zhang et al., 2017). The peak area normalization method was used to calculate the relative amount of an individual component of the essential oil. The retention indices were calculated using a homologous series of *n*-alkanes C₁₀–C₂₅.

2.5. DPPH-radical-scavenging activity

The free-radical-scavenging activity of the TEOs was determined based on the scavenging activity of the stabilized DPPH radical according to the previous method of Cuendet (1997) with some modifications (Cuendet et al., 1997). Initially, 0.5 mL of TEOs dilutions in absolute ethanol at concentrations of 8, 12, 16, 20, and 24 mg/mL was added 2.5 mL of 24 µg/mL DPPH. 24 µg/mL DPPH was obtained by dissolving 12 mg of DPPH in absolute ethanol and diluting to a final volume of a 500 mL volumetric flask. After vigorously shaken, the mixing solution of TEOs and DPPH was allowed standing for 30 min in the dark at room temperature, following which the absorbance was recorded at 517 nm using an Ultraviolet–visible (UV–Vis) spectrophotometer (T6 New Century, Beijing Puxi General Instrument Co., Ltd.) (Zhou et al., 2017). The same amount of the absolute ethanol was added instead of the TEOs solution as a negative control. Each sample was tested three times for comparison. The DPPH radical scavenging capacity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_1 is the sample absorbance, and A_0 is the negative control absorbance at 517 nm, respectively. The IC₅₀ is the concentration of an antioxidant EOs at which 50% inhibition of DPPH free radical activity is observed. The lower IC₅₀ value indicates the greater overall effectiveness of the antioxidant.

2.6. Statistical analysis

Principal component analysis (PCA) was carried out with the substances presented in the TEOs using SIMCA 13.0. Heat map representing an unsupervised, hierarchical cluster analysis of the TEOs of five different origins by Origin 2019b 32Bit. The IC₅₀ values of the DPPH assay were calculated from GraphPad Prism 8.0 software.

3. Results and discussion

3.1. The total ion chromatograms of the turmeric essential oils of five different origins

The TEOs of five different origins were analyzed by the HS-SPME-GC-MS method. The total ion chromatograms (TICs) of TEOs collected from five different origins were shown in Fig. 1. The TICs represent the summed intensity across the entire range of masses being detected at every point in the analysis. From these chromatograms in Fig. 1, the main constituents demonstrated by the position of the peaks of the TEOs of the five different origins were quite similar although there were

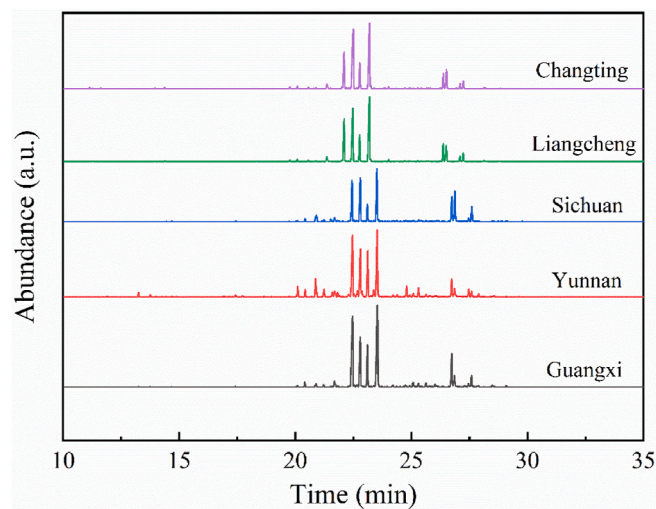


Fig. 1. The total ion chromatograms (TICs) of TEOs collected from different origins.

considerable differences in the chemical compositions of these TEOs, as evidenced by the responses of detected peaks, i.e., the intensity of the peaks.

3.2. Chemical compositions of the turmeric essential oils of five different origins

The major chemical compositions of the TEOs of five different origins were analyzed using HS-SPME-GC-MS, which were compiled in Table 2, respectively. A total of 40 peaks were identified, accounting for 82.08–93.66% composition of the volatile organic constituents. Sesquiterpenes (i.e., hydrocarbons and oxygenated) consisted of the highest composition (79.50–93.06%) of the TEOs of all five origins whereas monoterpenes (i.e., hydrocarbons and oxygenated) consisted of a comparatively lower composition (0.16–3.31%). Moreover, sesquiterpene hydrocarbons accounted for the highest composition in the TEOs of all five origins. The natural bicyclic sesquiterpenes demonstrated a high abundance in these TEOs and the potential anticancer activity of the natural bicyclic sesquiterpenes has been extensively studied in the literature (Abu-Izneid et al., 2020; Afoulous et al., 2013; Dahham et al., 2015; Pant et al., 2019).

The major components of the volatile organic compounds in the TEOs of five different origins were quite similar, as demonstrated in Fig. 1. β -Cedrene, *ar*-Curcumene, and α -Zingiberene represented the main components. However, the corresponding components in the TEOs of five different origins showed significantly different. β -Cedrene, the largest component identified in all five origins of the TEOs except Changting, is a member of the class of the compounds known as sesquiterpenoids. α -Zingiberene, the largest component identified in the TEOs from Changting, is a monocyclic sesquiterpene that is a generally predominant compound of the TEOs from many cultivars of ginger. Moreover, α -Zingiberene has a warm, woody-spicy, and very tenacious odor (Yeh et al., 2014). α -Zingiberene has been reported as a bioactive compound that is efficacious for anticancer (Bou et al., 2013).

Table 2
The chemical compositions of TEOs of five different origins in China.

Number	Compounds	Formula	RT ^b	RI ^c	Relative Content% (mean ± SD) ^a				
					Guangxi	Yunnan	Sichuan	Liancheng	Changting
1	β-Pinene	C ₁₀ H ₁₆	8.22	976	– ^d	0.45 ± 0.25 ^a	8.22	0.03 ± 0.01 ^b	0.06 ± 0.02 ^b
2	Eucalyptol	C ₁₀ H ₁₈ O	9.808	1032	0.05 ± 0.02 ^b	0.47 ± 0.23 ^b	0.24 ± 0.05 ^b	0.42 ± 0.07 ^b	3.25 ± 0.85 ^a
3	Camphor	C ₁₀ H ₁₆ O	13.245	1139	0.06 ± 0.01 ^b	0.64 ± 0.17 ^a	0.02 ± 0.01 ^b	–	–
4	DL-Isoborneol	C ₁₀ H ₁₈ O	13.753	1155	0.05 ± 0.01 ^b	0.37 ± 0.03 ^a	0.02 ± 0.01 ^b	–	–
5	2-Undecanone	C ₁₁ H ₂₂ O	17.422	1279	0.09 ± 0.02 ^b	0.36 ± 0.12 ^a	0.08 ± 0.03 ^b	0.03 ± 0.01 ^b	0.03 ± 0.01 ^b
6	2-Tridecanol	C ₁₃ H ₂₈ O	17.715	1289	0.01 ± 0.01 ^b	0.23 ± 0.13 ^a	–	0.02 ± 0.01 ^b	0.02 ± 0.01 ^b
7	β-Elemene	C ₁₅ H ₂₄	20.096	1375	0.34 ± 0.05 ^b	1.38 ± 0.62 ^a	0.25 ± 0.05 ^b	0.54 ± 0.08 ^b	0.47 ± 0.09 ^b
8	7-epi-Sesquithujene	C ₁₅ H ₂₄	20.413	1387	0.97 ± 0.08 ^{ab}	1.04 ± 0.28 ^a	0.72 ± 0.18 ^b	0.69 ± 0.05 ^b	0.69 ± 0.09 ^b
9	cis-α-Bergamotene	C ₁₅ H ₂₄	21.218	1414	0.45 ± 0.02 ^b	1.01 ± 0.50 ^a	0.61 ± 0.07 ^{ab}	0.25 ± 0.02 ^b	0.24 ± 0.02 ^b
10	β-Sesquiphellandrene	C ₁₅ H ₂₄	21.397	1419	0.50 ± 0.03 ^a	0.46 ± 0.15 ^a	0.55 ± 0.06 ^a	0.31 ± 0.02 ^b	0.27 ± 0.02 ^b
11	(E)-β-Farnesene	C ₁₅ H ₂₄	21.683	1428	1.70 ± 0.15 ^a	1.42 ± 0.31 ^a	1.37 ± 0.18 ^a	1.68 ± 0.02 ^a	1.50 ± 0.19 ^a
12	α-Humulene	C ₁₅ H ₂₄	21.813	1432	0.34 ± 0.03 ^a	0.57 ± 0.36 ^a	0.59 ± 0.04 ^a	–	–
13	ar-Curcumene	C ₁₅ H ₂₂	22.465	1452	22.80 ± 0.98 ^a	14.50 ± 1.83 ^{bc}	11.40 ± 1.98 ^d	15.45 ± 1.91 ^b	12.01 ± 0.79 ^{cd}
14	β-Eudesmene	C ₁₅ H ₂₄	22.678	1458	–	1.12 ± 0.44	–	–	–
15	α-Zingiberene	C ₁₅ H ₂₄	22.794	1462	12.33 ± 0.59 ^c	14.07 ± 5.58 ^c	15.57 ± 0.60 ^{bc}	20.94 ± 1.96 ^{ab}	24.41 ± 3.96 ^a
16	β-Bisabolene	C ₁₅ H ₂₄	23.109	1472	8.77 ± 0.24 ^a	8.50 ± 1.39 ^a	5.18 ± 0.55 ^c	8.35 ± 0.59 ^{ab}	7.07 ± 0.46 ^b
17	δ-Cadinene	C ₁₅ H ₂₄	23.372	1480	0.32 ± 0.07 ^a	–	–	0.08 ± 0.03 ^b	0.06 ± 0.03 ^b
18	β-Cedrene	C ₁₅ H ₂₄	23.537	1484	25.37 ± 0.43 ^a	17.04 ± 0.56 ^b	15.75 ± 1.23 ^b	25.89 ± 1.02 ^a	24.37 ± 1.22 ^a
19	cis-Sesquisabinene hydrate	C ₁₅ H ₂₆ O	24.202	1504	0.42 ± 0.01 ^b	0.47 ± 0.05 ^a	–	0.18 ± 0.01 ^c	0.14 ± 0.02 ^c
20	Humulene epoxide II	C ₁₅ H ₂₄ O	24.214	1504	–	–	0.37 ± 0.02	–	–
21	Germaacrene B	C ₁₅ H ₂₄	24.372	1508	0.14 ± 0.02 ^c	0.49 ± 0.03 ^b	0.63 ± 0.05 ^a	0.61 ± 0.02 ^a	0.50 ± 0.05 ^b
22	3,3,5,5-Tetramethylcyclopentene	C ₉ H ₁₆	24.515	1511	0.08 ± 0.01 ^{bc}	0.07 ± 0.01 ^c	0.13 ± 0.01 ^a	0.09 ± 0.01 ^b	0.07 ± 0.01 ^c
23	ar-Turmerol	C ₁₅ H ₂₂ O	24.742	1517	0.48 ± 0.07 ^b	–	0.75 ± 0.09 ^a	0.14 ± 0.04 ^c	0.09 ± 0.03 ^c
24	Caryophyllene oxide	C ₁₅ H ₂₄ O	24.917	1521	0.25 ± 0.01 ^b	0.34 ± 0.08 ^a	0.22 ± 0.01 ^b	0.04 ± 0.01 ^c	0.02 ± 0.01 ^c
25	Cryptomeridiol	C ₁₅ H ₂₈ O ₂	25.2	1527	–	0.17 ± 0.06	–	–	–
26	Epicurzerenone	C ₁₅ H ₁₈ O ₂	25.295	1530	0.88 ± 0.08	2.08 ± 0.30	–	–	–
27	Zingiberenol	C ₁₅ H ₂₆ O	25.622	1537	0.92 ± 0.05 ^a	0.66 ± 0.27 ^b	0.45 ± 0.05 ^{bc}	0.39 ± 0.04 ^c	0.31 ± 0.12 ^c
28	trans-Nuciferol	C ₁₅ H ₂₂ O	25.772	1541	0.33 ± 0.04 ^a	–	–	0.23 ± 0.02 ^b	0.18 ± 0.07 ^b
29	α-Acorenol	C ₁₅ H ₂₆ O	26.09	1548	–	0.31 ± 0.06 ^a	–	0.15 ± 0.02 ^b	0.12 ± 0.03 ^b
30	β-Ylangene	C ₁₅ H ₂₄	26.086	1548	0.30 ± 0.02	–	–	–	–
31	(E)-γ-Atlantone	C ₁₅ H ₂₂ O	26.35	1554	0.14 ± 0.02 ^b	–	0.58 ± 0.04 ^a	0.10 ± 0.03 ^b	0.09 ± 0.03 ^b
32	ar-Turmerone	C ₁₅ H ₂₀ O	26.737	1563	7.94 ± 0.57 ^{ab}	5.47 ± 1.43 ^c	9.90 ± 1.22 ^a	6.15 ± 0.56 ^{bc}	5.52 ± 1.72 ^c
33	Turmerone	C ₁₅ H ₂₂ O	26.853	1566	3.03 ± 0.37 ^c	3.12 ± 0.62 ^c	13.31 ± 1.70 ^a	5.87 ± 0.82 ^b	5.87 ± 0.57 ^b
34	(E)-α-Santalal	C ₁₅ H ₂₂ O	27.113	1572	–	0.22 ± 0.14	–	–	–
35	(E, E)-Germaacrene-3,7(11),9-trien-6-one	C ₁₅ H ₂₂ O	27.46	1580	0.75 ± 0.10 ^b	1.84 ± 0.32 ^a	1.39 ± 0.24 ^a	1.79 ± 0.10 ^a	1.62 ± 0.39 ^a
36	Curlone	C ₁₅ H ₂₂ O	27.588	1583	2.60 ± 0.26 ^b	1.69 ± 0.42 ^b	6.02 ± 0.80 ^a	2.64 ± 0.25 ^b	2.56 ± 0.81 ^b
37	Curdione	C ₁₅ H ₂₄ O ₂	27.889	1591	0.22 ± 0.06	0.90 ± 0.25	–	–	–
38	Curcumenol	C ₁₅ H ₂₂ O ₂	28.116	1596	0.05 ± 0.02 ^a	0.26 ± 0.12 ^a	–	0.05 ± 0.02 ^a	0.17 ± 0.27 ^a
39	Bisabolone	C ₁₅ H ₂₄ O	28.48	1604	0.39 ± 0.04 ^a	0.21 ± 0.08 ^a	0.24 ± 0.06 ^a	0.39 ± 0.02 ^a	0.46 ± 0.33 ^a
40	(E)-Atlantone	C ₁₅ H ₂₂ O	29.078	1616	0.25 ± 0.04 ^b	0.15 ± 0.05 ^c	0.41 ± 0.05 ^a	0.16 ± 0.02 ^c	0.14 ± 0.05 ^c
	Total identified (%)	93.34 ± 0.45	82.09 ± 1.78	86.74 ± 0.58	93.64 ± 0.62	92.28 ± 1.55			
	Sesquiterpene hydrocarbons	74.33 ± 2.08	61.61 ± 2.45	52.62 ± 4.74	74.78 ± 0.87	71.57 ± 5.55			
	Oxygenated sesquiterpenes	18.67 ± 1.64	17.89 ± 3.36	33.62 ± 4.19	18.28 ± 1.00	17.28 ± 4.27			
	Monoterpene hydrocarbons	–	0.45 ± 0.25	–	0.03 ± 0.01	0.06 ± 0.02			
	Oxygenated Monoterpenes	0.16 ± 0.04	1.48 ± 0.09	0.28 ± 0.09	0.42 ± 0.07	3.25 ± 0.85			

^a Relative content (%) is given as means ± SDs ($n = 3$); The different labelled letters (a, b, c, d) in a row indicated the values are significantly different among the TEOs of different origins ($P < 0.05$) by Duncan's Multiple Range Test.

^b RT is the abbreviation for retention time.

^c Retention indices (RI) represents the retention index obtained using the C10–C25 n-alkane series as the reference in the Rxi-5Sil MS column.

^d - represents that the component was not retrieved in the corresponding sample.

Furthermore, *ar*-Curcumene, a type of sesquiterpene, was the second-largest component identified in the TEOs from Guangxi and Yunnan. These components were concentrated in the period after the GC-MS peak, which was tentatively related to the relative amount of components volatility and extraction equilibrium of the extraction head. In addition, other chemical compounds identified in the lower

abundance in the TEOs of five different origins were β-Bisabolene, *ar*-Turmerone, Turmerone, Curlone, and (E)-β-Farnesene.

The results obtained in the present study were different from those of Hwang et al. (2016) (Hwang et al., 2016), in which the major constituents of TEO by GC-MS from Korea were α-Zingiberene (27.70–36.75%), *ar*-Turmerone (19.54–32.24%), β-Sesquiphellandrene (13.14–18.23%),

α -Turmerone (3.72–6.50%), β -Turmerone (2.86–5.60%), and β -Bisabolone (2.50–3.46%). In contrast, Mustapha et al. (2019) (Mustapha et al., 2019) reported that the major compounds in the TEO by GC-MS from Klang, Malaysia were Turmerone (35.46%), Cumene (20.61%), *ar*-Turmerone (13.82%), Cymene (0.90%) and Curcumenol (0.43%). Furthermore, Naveen Kumar, Venkataramana, Allen, Chandranayaka, Murali, and Batra (2019) (Naveen Kumar et al., 2016) reported that *ar*-Turmerone (53.1%), β -Turmerone (6.42%), α -Turmerone (6.15%), *ar*-Curcumenol (4.81%), β -Phellandrene (4.39%), α -Terpinene (3.28%), and Limonene (3.15%) were as major compounds in the TEO by GC-MS from the Ooty, Tamil Nadu, India. The distinct results could be caused by the TEO harvested from different origins and different measurement methods as well.

Heat map representing an unsupervised, hierarchical cluster analysis of the TEOs of five different origins. The heat map of volatile organic compounds in the TEOs at the genus level based on relative abundance was shown in Fig. 2. In the comparison of different origins of TEOs, the relative abundance of volatile organic compounds, especially Turmerone, Curlone, 3,3,5,5-Tetramethylcyclopentene, (E)- γ -Atlantone, Humulene epoxide II, *ar*-Turmerone, (E)-Atlantone and *ar*-Turmerol in SC were much higher than those in GX, YN, LC, and CT. In addition, β -Eudesmene, Cryptomeridiol, and (E)- α -Santalal were only detected in YN. In GX, the relative amount of volatile organic compounds, *trans*-Nuciferol, Zingiberenol, *ar*-Curcumenol, δ -Cadinene, and β -Ylangene were higher, whereas, in CT, Bisabolone, α -Zingiberene, and Eucalyptol were higher. The differences in these results could be attributed to the

TEOs harvested from different regions since the longitude, latitude, climate, and elevation of these regions are different. The cluster heat map clearly demonstrated the spatial variation of the relative abundance of volatile organic compounds collected from five different origins in the TEOs at the genus level.

To evaluate the quality variation and differentiate the volatile organic compounds in the TEOs of five different origins, the principal component analysis (PCA) was performed based on the normalized relative peak areas of 40 components (Fig. 3). PC1 could separate CT and LC from SC, YN, and GX. The volatile organic compounds Humulene epoxide II, Eucalyptol, α -Zingiberene, and Bisabolone in the TEOs were contributed to the separation of the CT and LC samples. The SC samples were clustered in the positive PC2 cluster, which was contributed by the volatile organic compounds in the following sequence: Curlone, (E)-Atlantone, *ar*-Turmerone, and Curcumenol (Fig. 3A). PC1 could separate CT and LC from SC, YN, and GX whereas PC3 could allow YN, CT, and LC to be differentiated from SC and GX. Moreover, the YN samples were clustered in the positive PC3 cluster, which was contributed by the volatile organic compounds with the following order: α -Acorenol, *cis*- α -Bergamotene, 2-Undecanone, and β -Elemene (Fig. 3B). These findings indicated that the volatile organic compounds of TEOs were closely correlated with the five geographic distributions among populations, and the harvest time as well as modes of processing. Additionally, the results in the previous studies have indicated that the volatile organic compounds in the TEOs are related to the employed peak areas and multivariate statistics methods.

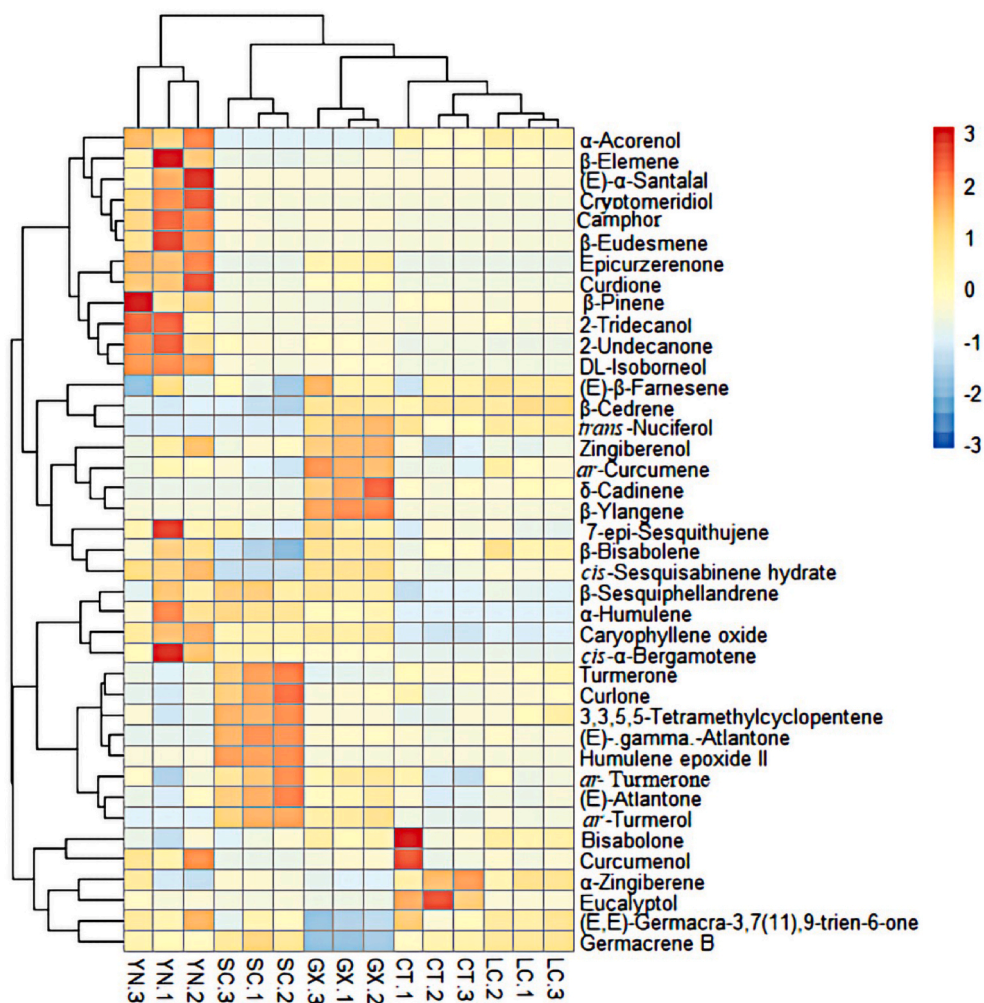


Fig. 2. HCA dendrogram associated with the heat map of the components of TEOs from Yunnan(YN), Sichuan(SC), Guangxi(GX), Changting(CT) and Liangcheng(LC).

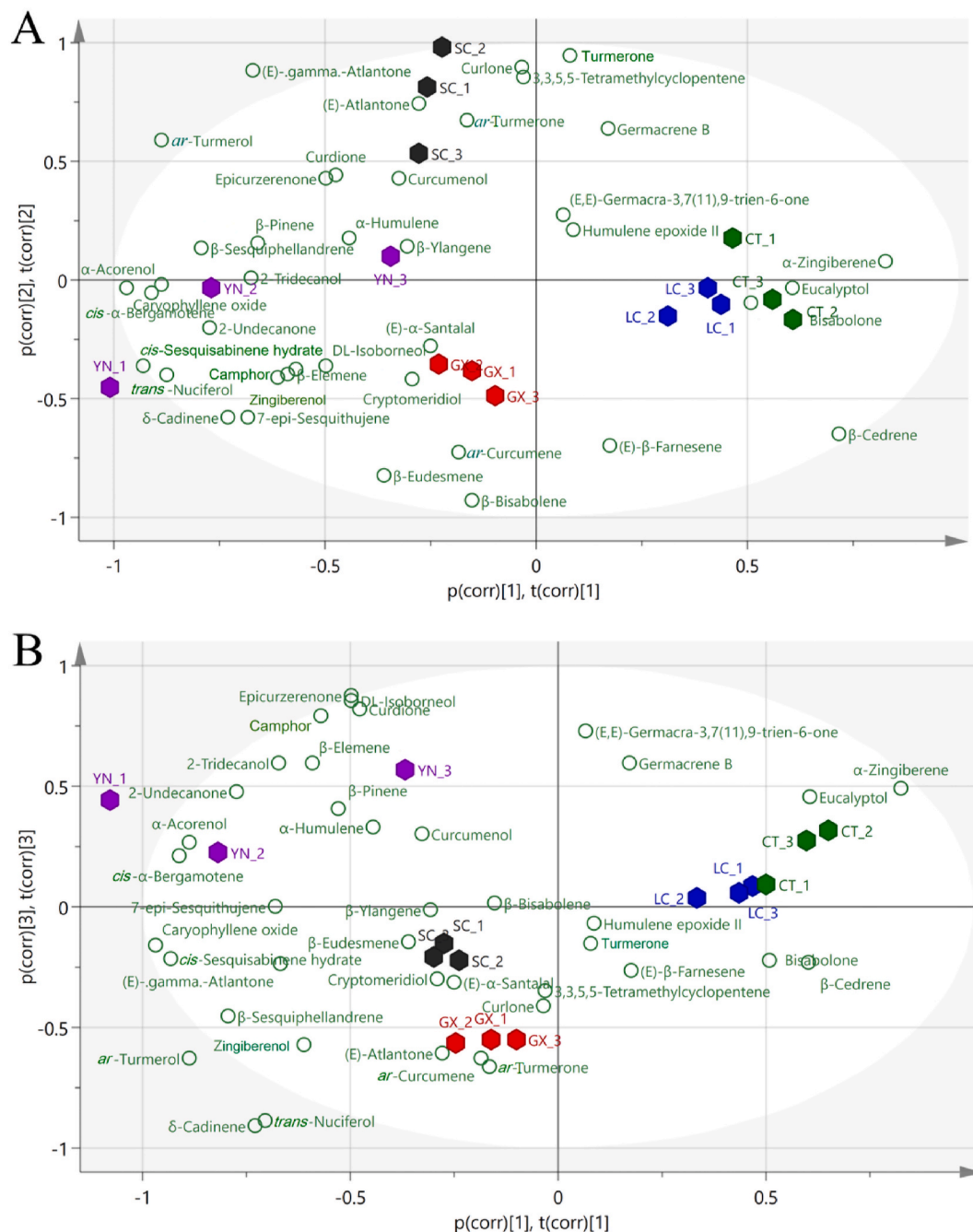


Fig. 3. Principal component analysis (PCA) score plot of the components in the TEOs of five different origins GX, YN, SC, LC, and CT. (A) The PCA score plot of the first two principal components (PC1 and PC2); (B) The PCA score plot of the first and third principal components (PC1 and PC3). GX, YN, SC, LC, and CT represented the TEOs from Guangxi, Yunnan, Sichuan, Liancheng, and Changting.

Furthermore, the taxon-based analysis could reveal the specific key phylotypes of the volatile organic compounds in the TEOs responding to the CT and LC. (Fig. 4). Compared with the CT group, the relative abundances of *ar*-Curcumene and β -Bisabolene were markedly increased in the GX group, whereas the relative abundances of Turmerone, Eucalyptol, and α -Zingiberene were significantly decreased (Fig. 4A). Compared with the CT group, the relative abundances of Curlone, Turmerone, and *ar*-Turmerone were notably increased in the SC group, whereas the relative abundances of β -Cedrene, β -Bisabolene, and Eucalyptol were significantly decreased (Fig. 4B). Compared with the CT group, the relative abundance of Epicurzerenone was dramatically increased in the YN group, whereas the relative abundances of β -Cedrene, Turmerone, and Eucalyptol were significantly decreased

(Fig. 4C). Compared with the CT group, the relative abundances of *cis*-Sesquisabinene hydrate and Germacrene B were remarkably increased in the LC group, whereas the relative abundances of Eucalyptol were significantly reduced (Fig. 4D). Compared with the LC group, the relative abundances of *ar*-Curcumene and *ar*-Turmerone were strikingly increased in the GX group, whereas the relative abundances of α -Zingiberene and Turmerone were significantly diminished (Fig. 4E). Compared with the LC group, the relative abundance of Turmerone, Curlone, and *ar*-Turmerone were markedly increased in the SC group, whereas the relative abundances of β -Cedrene, β -Bisabolene, and α -Zingiberene were significantly decreased (Fig. 4F). Compared with the LC group, the relative abundance of Epicurzerenone was particularly increased in the YN group, whereas the relative abundances of

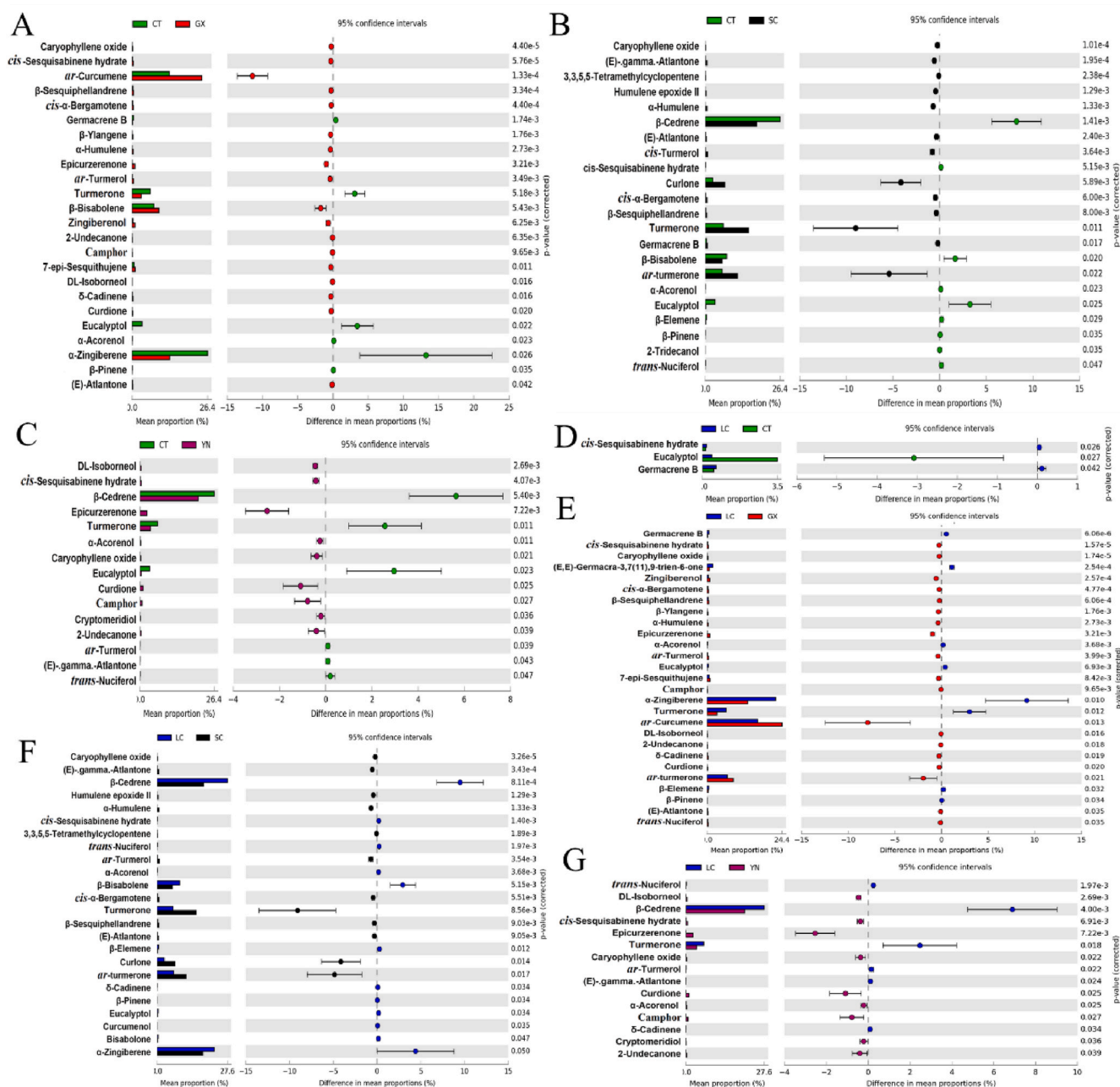


Fig. 4. The extended error bar plot identified the differences of volatile organic compounds in the TEOs among the mean proportions of bacterial taxa. The differences between groups were determined using a Welch’s *t*-test, while the Benjamini–Hochberg procedure was used to control the false discovery rate due to multiple tests. The corresponding p values were shown on the right sides. (A) CT (green) versus GX (red); (B) CT (green) versus SC (black); (C) CT (green) versus YN (purple pink); (D) LC (blue) versus CT (green); (E) LC (blue) versus GX (red); (F) LC (blue) versus SC (black); (G) LC (blue) versus YN (purple pink). The confidence intervals were provided to allow for the critical assessment of the biological relevance of the test results. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

β -Cedrene and Turmerone were significantly decreased (Fig. 4G).

3.3. Antioxidant activity of the TEOs of five different origins

The antioxidant activity of EOs from aromatic plants is mainly attributed to the active compounds present in them. This may be due to the high proportion of the main ingredients, or the presence of small amounts of other ingredients, or the synergy between them (Politeo et al., 2006). In this regard, DPPH has been widely used to evaluate the antioxidant capacity, which would change color from purple to yellow upon the acceptance of electrons/hydrogens, thus indicating the scavenging activity (Feng et al., 2014; H. Singh, Mittal, Kaur, Batish and

Kohli, 2009). Avança et al. (2017) (Avança et al., 2017) have reported that TEOs demonstrated dose-dependent DPPH-radical-scavenging activity, indicating that the oils could serve as a hydrogen donor antioxidant. As shown in Fig. 5, the scavenging activity was 41.41%, 32.56%, 37.39%, 36.58%, and 24.31% at the concentration of 24 mg/mL for the TEOs of Guangxi, Sichuan, Yunnan, Changting, and Liancheng, respectively. Moreover, the IC₅₀ value was 33.30, 42.5, 35.22, 39.96, and 63.27 mg/mL of TEOs from Guangxi, Sichuan, Yunnan, Liancheng, and Changting, respectively to quench DPPH free radicals (50% inhibition of DPPH free radical activity), which was different from the IC₅₀ value of 10.03 mg/mL reported by Avança et al. (2017) (Avança et al., 2017). The scavenging activity of DPPH free radical was the highest in the TEO

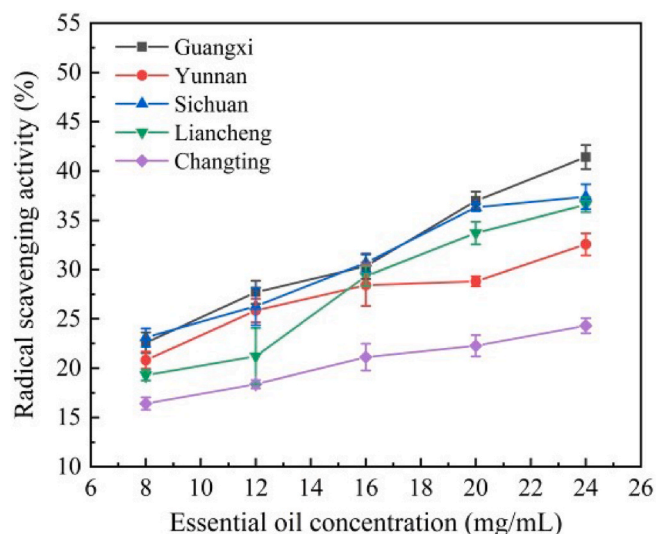


Fig. 5. The DPPH-radical-scavenging activity (%) of the TEOs of Guangxi, Yunnan, Sichuan, Liancheng, and Changting.

of Guangxi. The results indicated the significant scavenging activities of DPPH free radical at different concentrations for the TEOs of Guangxi, Sichuan, Yunnan, Changting, and Liancheng. In this case, the TEOs could reduce the concentration of DPPH free radical. The antioxidant activity indicated the curcumin-free TEOs could act as a proton donor and an antioxidant. The results reported here could demonstrate that the TEOs might be considered as the potential natural antioxidants, which could be applied as a part of daily supplements or additives to prevent oxidative stress that causes many degenerative diseases.

4. Conclusions

In summary, the spatial variations of the chemical compositions and antioxidant activity of turmeric (*Curcuma longa* L.) essential oils (TEOs) harvested from four provinces (i.e., five different origins) of China were investigated. The results indicated that the turmeric growing in China exhibited considerable differences in the chemical compositions of TEOs among different populations, thus indicating spatial variation. Moreover, the TEOs of five different origins demonstrated notably different antioxidant activities, in which the antioxidant activity of the TEOs from Guangxi was superior to that of other sources. Furthermore, TEOs might be considered as a natural antioxidant with potential applications in food and pharmaceutical industries.

CRedit authorship contribution statement

Yueyue Qiang: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Ruiru Si:** Conceptualization, Validation, Formal analysis, Visualization, Writing – review & editing. **Suo Tan:** Investigation, Formal analysis. **Hang Wei:** Investigation, Resources. **Biao Huang:** Writing – review & editing. **Miaohong Wu:** Resources. **Mengzhu Shi:** Resources. **Ling Fang:** Resources. **Jianwei Fu:** Conceptualization, Supervision, Project administration, Funding acquisition. **Shaoxiao Zeng:** Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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