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A comparative evaluation of chemical composition and antimicrobial activities of essential oils extracted from different chemotypes of *Cinnamomum camphora* (L.) Presl

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ABSTRACT: The purpose of this study is to determine the chemical composition of the essential oils of *Cinnamomum camphora* (L.) Presl leaves (CCPL) from 5 different habitats in China by GC-MS, and to evaluate their antimicrobial activities against 3 foodborne pathogens, using a paper disc diffusion method. A total of 30 compounds were identified with a predominance of oxygenated monoterpenes, including linalool (42.65%-96.47%), eucalyptol (39.07%-55.35%) and camphor (26.08%) as well as monoterpene hydrocarbons such as sabinene (6.18%-12.93%) and α-terpineol (8.19%-13.81%). Through cluster analysis, CCPL from 5 different habitats can be well divided into 2 categories. Combining with principal component analysis, the habitats can be better correlated with the chemical constituents of the essential oils. The antimicrobial activities of 5 extracted essential oils against 2 gram-negative bacteria and one gram-positive bacteria were assessed. It showed that the essential oil extracted from the CCPL harvested in Jinxi had the strongest antibacterial property. The results of this study provided basis for resource identification of CCPL and quality difference identification of essential oils. Research on the antibacterial properties of several pathogenic strains has proved its application value as a natural food preservative.

KEYWORDS: *Antimicrobial activities;* Cinnamomum camphora *(L.) Presl; Essential oils; Foodborne bacteria; Linalool*

RESUMEN: ***Evaluación comparativa de la composición química y las actividades antimicrobianas de los aceites esenciales extraídos de diferentes quimiotipos de* Cinnamomum camphora *(L.) Presl.*** El objetivo de este estudio es determinar la composición química de los aceites esenciales de hojas de *Cinnamomum camphora* (L.) Presl (CCPL) de 5 hábitats diferentes de China mediante GC-MS, y evaluar sus actividades antimicrobianas contra 3 patógenos transmitidos por los alimentos, utilizando un método de difusión de disco de papel. Se identificaron un total de 30 compuestos, con predominio de monoterpenos oxigenados, entre ellos linalol (42,65%-96,47%), eucaliptol (39,07%-55,35%) y alcanfor (26,08%) así como hidrocarburos monoterpenos como el sabineno (6,18%-12,93%) y α-terpineol (8,19%-13,81%). A través del análisis de conglomerados, los CCPL de 5 hábitats diferentes se pueden dividir bien en 2 categorías. En combinación con el análisis de componentes principales, los hábitats se pueden correlacionar mejor con los componentes químicos de los aceites esenciales. Se evaluaron las actividades antimicrobianas de 5 aceites esenciales extraídos contra 2 bacterias gramnegativas y una bacteria grampositiva. Se demuestra que el aceite esencial extraído del CCPL cosechado en Jinxi tenía la propiedad antibacteriana más fuerte. Los resultados de este estudio proporcionaron la base para la identificación de recursos de CCPL y la identificación de diferencias de calidad de los aceites esenciales. La investigación sobre las propiedades antibacterianas de varias cepas patógenas ha demostrado su valor de aplicación como conservante natural de alimentos.

PALABRAS CLAVE: *Aceites esenciales; Actividades antimicrobianas; Bacterias transmitidas por los alimentos;* Cinnamomum camphora *(L.) Presl; Linalol*

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1. INTRODUCTION

Food safety is a global concern, with at least 1 in 10 people falling ill yearly from the consumption of contaminated food and 2 million deaths occurring as a result, mostly children, according to the World Health Organization (WHO) (Alegbeleye *et al.*, 2018). Foods containing harmful bacteria, viruses, parasites or chemicals can cause more than 200 diseases, from diarrhea to cancer. *Salmonella sp*., *Staphylococcus sp*. and *Escherichia coli* account for 28, 7 and 5% of foodborne bacterial infections, respectively (Dussault *et al.*, 2014; Mutlu-Ingok *et al.*, 2020; Scallan *et al.*, 2011). Foodborne pathogens are widely distributed in the environment and are transmitted through various food vehicles, including vegetables, meats, poultry products, ready-to-eat foods and dairy products (He *et al.*, 2016; Lee *et al.*, 2018). Antimicrobials, such as antibiotics, are essential for treating infections caused by bacteria. However, in the last decade we have witnessed a dramatic increase in the proportion and an absolute number of bacterial pathogens resistant to multiple antibacterial agents (Roca *et al.*, 2015). The overuse and misuse of antibiotics in veterinary and human medicine have had their efficacy and acceptance compromised (Dannenberg *et al.*, 2019; Van Boeckel *et al.*, 2015). In this context, there is a need to develop alternative strategies for the effective control of microbial pathogens in food, especially natural ones rather than traditionally synthetic preservatives.

As an alternative to synthetic preservatives, essential oils have attracted particular attention due to their antibacterial properties, biodegradable nature and their potential for commercial application (Limam *et al.*, 2020; Liu *et al.*, 2006). *Cinnamomum camphora* (L.) Presl (CCP), a member of the *Lauraceae* family, is a valuable timber and economic forest species as a special product in southeastern China (Imai *et al.*, 2009). The essential oil from the roots, barks, branches, leaves and fruits of *Cinnamomum camphora* has long been prescribed as economic importance as a source of food preservative and additive and as raw materials for the cosmetic and pharmaceutical industries (Chen *et al.*, 2018; Jiang *et al.*, 2016). According to the different chemical components and the main constituents of essential oils from leaves, *Cinnamomum camphora* was classified into 5 different chemotypes, including linalool-type (58–92%), borneol-type (67–82%), camphor-type (54–97%), cineole-type (32–52%), and nerolidol-type (16–57%) (Guo *et al.*, 2017).

Biological activity depends on the type, chemical composition, and concentration of the spice or essential oils. In previous studies, CCPL essential oil was used in the preparation of a strong fungistatic agent against *C. cucurbitarum* infection (Pragadheesh *et al.*, 2013). It was also considered an environmentally friendly plant-based preservative to resist the decay of bamboo (Xu *et al.*, 2013). Marasini *et al.* (2015) summarized the values of the use of the plant and the use of the extracts from the leaves, seeds, and bark of *Cinnamomum camphora* for the remedies of bronchitis, bronchopneumonia, epilepsy and wound infections. The inhibitory effects of the inflammatory phenomena were investigated using *Cinnamomum camphora* (Lee *et al.*, 2006; Xiao *et al.*, 2020). The essential oil from CCPL has been found to possess contact and fumigant activities towards *Lasioderma. Serricorne* (a cigarette beetle) (Chen *et al.*, 2014), while linalool was found to be a significant contributor to the insecticidal and repellent activities of cotton aphids (Jiang *et al.*, 2016).

All the above-mentioned biological activities of the essential oils from CCPL are justified by the chemical composition of all parts of the plant. The essential oil which contains large amounts of active constituents such as linalool, camphor, borneol, eucalyptol and α-terpineol, is crucial to biological activities. To the best of our knowledge, despite all mentioned studies concerning the composition of the single type CCPL essential oil in antibacterial activity, the comparison of different chemotypes, especially different habitats has not yet been investigated. In view of the need to make better use of *Cinnamomum camphora* resources to develop future technology application and discover new antimicrobials, the objective of this study is to report the chemical composition of CCPL essential oils from 5 different habitats in China and to investigate their antimicrobial activities against 3 foodborne bacteria. Through principal component analysis and systematic cluster analysis, the relationship between the habitats of CCPL and the components of essential oils was clarified, which further provided research basis for resource identification of CCPL and quality difference identification of its essential oils.

2. MATERIALS AND METHODS

2.1. Plant materials and chemicals

5-year-old plants of *Cinnamomum camphora* (L.) Presl were selected, and the fresh CCPL was obtained from upper branches collected during October to November in 2018 from Jinxi (Jiangxi Province, China), Nanchang (Jiangxi Province, China), Chengdu (Sichuan Province, China), Mianyang (Sichuan Province, China), and Kunming (Yunnan Province, China). The plants were identified by Prof. Ming Yang from the Jiangxi University of Traditional Chinese Medicine.

The nutrient broth and nutrient agar were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents and other chemicals of analytical grade were purchased from Sigma Chemicals Co., Ltd.

2.2. Extraction of essential oils

Each essential oil was obtained from the fresh CCPL (150 g) by using a Clevenger-type apparatus as described by Ait Babahmad *et al.* (2018). In the extractions, the relationship between leaf mass and water volume was 3/50, at a temperature of 100 °C, for a period of 5 h. The obtained oils were dried over anhydrous sodium sulfate, weighed, and stored at 4 °C until use.

2.3. Gas chromatography-mass spectrometry

The determination of the chemical composition of the essential oils was made by means of an Agilent 7890A/5975C Gas Chromatography-Mass Spectrometer, equipped with a FID detector and a HP-5 ™ fused silica capillary column (30 m × 0.25 μm× 0.25 μm film thickness). The injector and detector temperature were set at 250 and 230 °C, respectively; while the detector operated in the electron-impact ionization (EI) mode with a mass scan range from m/z 40 to 400 at 70 eV. The ion source temperature was 230 °C. Helium was used as the carrier gas at a flow of 1 mL/min, and split ratio of 1:50. The oven temperature was programmed to start at 50 °C for 1 min, followed by an increase of 5 °C/min to 200 °C, then linearly increased by 10 °C/min to a final temperature of 250 °C, where it remained for 5 min.

The identification of components was mainly based on the comparison of their GC Kovat retention indices (RI), determined by reference to a homologous series of C7–C32 n-alkanes. GC retention times were also analyzed. Computer matching with NIST 11 library and comparison of the fragmentation patterns with those reported in the literature were also performed to ensure accuracy.

2.4. Antibacterial Activity

2.4.1. Microbial strains

A total of 3 bacterial species, selected as representative of foodborne bacteria, were tested: (1) 2 gram-negative bacteria, namely: *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC9027), and (2) one gram-positive bacteria, namely: *Staphylococcus aureus* (ATCC 25923). The bacteria were obtained from the Nanchang Institute of Microbiology.

2.4.2. Antimicrobial activity assay

The antimicrobial activity of essential oils was determined according to the NCCLS 2015 guideline. The strains were prepared with 0.9% sterile sodium chloride solution yielding the concentration of 1.5×10^8 CFU/mL (0.5 McFarland). The 0.2 mL aliquot inoculum was spread onto the surface of MacConkey-Sorbitol agar plates for *S. aureus*, *P. saeruginosa* and *E. coli* with sterile swabs. Sterile circular filter papers with a diameter of 6 mm were arranged on the plate and 6 μl of each EO was added to each disc. After 24 h of incubation at 37 °C, the inhibition zones were measured by a vernier caliper and expressed in mm. All experiments were performed in triplicate.

2.5. Data analysis

All analyses were conducted in triplicate. Data were analyzed using one-way analysis of variance ANOVA and Duncan's mean comparison test. The software (IBM SPSS Statistics 21) was used to calculate the eigenvalues and cumulative contribution rate of samples from different habitats by principal component analysis. Using Euclidean square distance as the measurement, a cluster analysis of samples from different habitats was carried out. Tukey test was used to detect significant differences ($p \leq 0.05$) among the mean values obtained from the antimicrobial activity assay.

3. RESULTS AND DISCUSSION

3.1. Characterization of essential oils

The essential oils of CCPL from 5 different habitats in China gave nearly colorless oil with a yield of 2.07% (Nanchang), light yellow oil with a yield of 1.52% (Jinxi), nearly colorless oil with a yield of 1.39% (Chengdu), light yellow oil with a yield of 1.11% (Kunming) and golden oil with a yield of 0.80% (Mianyang). The essential oil from Nanchang had the highest oil yield, while Mianyang had the lowest oil yield. Similar yields (1.83% and 1.3%) of essential oils extracted from CCPL were reported by Chen HP *et al.* (2014) and Satyal *et al.* (2013), respectively. The different yield obtained may be explained by different developmental stages and also by the environmental conditions as well as ontogenetic developments which influence the biosynthetic pathway of oil compounds (Jamali *et al.*, 2013; Kizil *et al.*, 2008).

The composition of the essential oils of CCPL were identified using GC–MS. The chemical compositions of the essential oils are reported in Table 1 with their retention index (RIs), the molecular formula and the relative areas of compounds. A total of 30 compounds were identified in the essential oils of CCPL from 5 different habitats. Among these compounds, only 5 compounds were identified in the essential oil of CCPL from Jinxi; while 17 compounds were identified in the essential oil of CCPL from Mianyang, Kunming, Chengdu and Nanchang.

According to the GC–MS quantitative analysis, the collected results highlighted the domination of the essential oil of CCPL from Jinxi by oxygenated monoterpenes, where linalool (96.47%) was distinguished as the chief component. Its proportion was more than twice that of Mianyang's. Among the 17 compounds identified in the leaves' essential oil from Mianyang, the main constituents in descending order of content were linalool (42.65%), camphor (26.08%), borneol (5.62%), nerolidol (3.59%), caryophyllene oxide (3.34%), bornyl acetate (3.12%) and eucalyptol (2.33%). The above main components accounted for 86.73% of the total amount of essential oil. A total of 17 components were detected in the essential oil of CCPL from Kunming, which accounted for 95.82%. They were mainly composed of the oxygenated monoterpenes eucalyptol (39.07%) followed by nerolidol (16.9%), 3,3,6-trimethyl-1,5-heptadine-4-one (13.42%) and α-terpineol (8.19%). Moreover, no significant difference was found between the quantitative and qualitative composition of the oils obtained from the CCPL of Chengdu or Nanchang. Comparing the composition of the essential oils of Chengdu and Nanchang, oxygenated monoterpenes (71.55 and 72.67%, respectively) and monoterpene hydrocarbons (26.41 and 24.89%, respectively) were found to be the important constituents in the study. For instance, linalool (42.65-96.47%) was identified as the major compound of the leaves' essential oil from Jinxi and Mianyang; while 3 others were dominated by eucalyptol (39.07-55.35%). Therefore, combined with the previous studies reported by Chen *et al.* (2018), the CCPL from Jinxi and Mianyang belonged to the linalool type and 3 others belonged to the cineole type because of rich linalool and eucalyptol, respectively. As natural linalool is an important source for domestic products, cosmetics and fragrance applications, this significant type of *Cinnamomum camphora* from Jinxi is economically and practically ideal (Amiri *et al.*, 2016; Herman *et al.*, 2016)

In fact, these differences in chemical compounds of essential oils in quantitative and qualitative terms could be caused by several factors such as environment (Mutlu-Ingok *et al.*, 2020), harvest time, local climate, extraction technique and variety (Harkat-Madouri *et al*., 2015). As essential oil of the *Cinnamomum* genus (*Lauraceae*) is an important source for chemical and pharmaceutical use (Dai *et al.*, 2020), various profiles of famous medicinal herbs such as *cinnamon* (*C. cassia* Presl), *Ceylon cinnamon* (*C. zeylanicum* Bl.), *Chai Gui* (*C. tamala)*, *sassafras* (*C. porrectum.*) and *Chuan Gui* (*C. wilsonii* Gamble) were previously described based on their major compounds of essential oils. Also, recent research works were focused on the *Cinnamomum* genus essential oils containing different major compounds. As shown in Table 2, aromatic compounds and oxygenated monoterpenes were reported as the main constituents in the essential oils of *Cinnamomum* genus. The essential oil also contained elements which characterize certain genera belonging to the *Cinnamomum* genus, such as linalool and camphor, which were the basis of the *Sect. Camphora* (Trew) Meissn. Compounds; whereas eugenol and borneol were the main compounds in *Sect. Cinnamomum*.

TABLE 1. Chemical compositions and relative area of CCPL essential oils from five different locations in China

No.	Compounds	Molecular formula	RI[a]	Relative area (%)				
				A	B	C	D	E
1	α-Thujene	$C_{10}H_{16}$	934	-[b]	-	0.43	0.74	0.72
2	α-Pinene	$C_{10}H_{16}$	942	-	1.78	2.33	4.38	4.11
3	Camphene	$C_{10}H_{16}$	959	-	1.08	-	-	-
4	Sabinene	$C_{10}H_{16}$	989	-	-	6.18	12.93	12.69
5	β-Pinene	$C_{10}H_{16}$	993	-	0.59	1.84	3.27	3.01
6	Myrcene	$C_{10}H_{16}$	1007	-	-	0.65	1.36	1.25
7	α-Terpinene	$C_{10}H_{16}$	1029	-	-	0.58	0.96	0.87
8	p-Cymene	$C_{10}H_{14}$	1037	-	0.57	-	-	-
9	D-Limonene	$C_{10}H_{16}$	1040	-	1.39	-	-	-
10	Eucalyptol	$C_{10}H_{18}O$	1042	-	2.33	39.07	52.2	55.35
11	γ-Terpinene	$C_{10}H_{16}$	1067	-	-	1.02	1.72	1.48
12	trans-4-Thujanol	$C_{10}H_{18}O$	1075	-	-	-	0.59	0.63
13	Terpinolene	$C_{10}H_{16}$	1094	-	0.87	-	0.39	0.34
14	Tricyclene	$C_{10}H_{16}$	1104	-	-	-	0.66	0.42
15	Linalool	$C_{10}H_{18}O$	1105	96.47	42.65	0.89	-	-
16	Camphor	$C_{10}H_{16}O$	1153	-	26.08	0.48	-	-
17	3-Methyldecane	$C_{11}H_{24}$	1159	0.55	-	-	-	-
18	Borneol	$C_{10}H_{18}O$	1180	-	5.62	-	-	-
19	Myrcenol	$C_{10}H_{18}O$	1181	-	-	0.72	1.04	0.82
20	Terpinen-4-ol	$C_{10}H_{18}O$	1195	-	-	2.58	3.91	3.25
21	α-Terpineol	$C_{10}H_{18}O$	1209	-	1.10	8.19	13.81	12.62
22	Bornyl acetate	$C_{12}H_{20}O_2$	1313	-	3.12	-	-	-
23	β-Caryophyllene	$C_{15}H_{24}$	1444	0.58	-	1.00	0.65	0.78
24	α-Humulene	$C_{15}H_{24}$	1482	-	-	0.54	0.84	0.87
25	β-Selinene	$C_{15}H_{24}$	1516	-	0.89	-	-	-
26	3,7,11,11- Tetramethylbicyclo[8.1.0]2,6-undecadiene	$C_{15}H_{24}$	1526	1.69	-	-	0.55	0.78
27	Nerolidol	$C_{15}H_{26}O$	1590	-	3.59	16.90	-	-
28	Spathulenol	$C_{15}H_{24}O$	1604	0.70	1.51	-	-	-
29	Caryophyllene oxide	$C_{15}H_{24}O$	1610	-	3.34	-	-	-
30	1,5-Heptadien-4-one,3,3,6-trimethyl-	$C_{10}H_{16}O$	1763	-	1.25	13.42	-	-
	Total identified		99.99	97.76	96.82	100	99.99	
	Oxygenated monoterpenes		96.47	82.15	65.35	71.55	72.67	
	Monoterpene hydrocarbons		-	6.28	13.03	26.41	24.89	
	Oxygenated sesquiterpenes		0.70	8.44	16.90	-	-	
	Sesquiterpenes hydrocarbons		2.27	0.89	1.54	2.04	2.43	
	Aliphatic compounds		0.55	-	-	-	-	

Notes: Means of relative area (%) are the average of three determinations (n = 3).
CCPL, *Cinnamomum camphora* (L.) Presl leaves.
A, essential oil of camphor leaves from Jinxi, (Jiangxi Province, China).
B, essential oil of camphor leaves from Mianyang, (Sichuan Province, China).
C, essential oil of camphor leaves from Kunming, (Yunnan Province, China).
D, essential oil of camphor leaves from Chengdu, (Sichuan Province, China).
E, essential oil of camphor leaves from Nanchang, (Jiangxi Province, China).
RI[a], Retention index
-[b], not detected

Table 2. The major compounds of essential oils, classification, geographical distribution and extraction techniques.

Plant	Major compounds	Classification	Geographical distribution	Extraction techniques	Reference
Cinnamomum porrectum	Safrole (93.92%)	aromatic compounds	China, Pakistan, India, Malaysia and Indonesia	HD	(Sukcharoen *et al.*, 2017)
Cinnamomum kanehirae	Linalool (64.4%)	oxygenated monoterpenes	Taiwan (China)	HD	(Cheng *et al.*, 2015)
Cinnamomum septentrionale	Camphor (30.53%)	oxygenated monoterpenes	China	HD	(Yang *et al.*, 2017)
Cinnamomum agasthyamalayanum	Camphor (70.8%)	oxygenated monoterpenes	India	HD	(Sriramavarathara-jan *et al.*, 2016)
Cinnamomum cassia Presl	(E)-cinnamaldehyde (79.39%)	aromatic compounds	China, India, Laos, Vietnam and Indo-nesia	HD	(Sun *et al.*, 2016)
Cinnamomum zeylanicum	Benzyl benzoate (64.36%)	aromatic compounds	Sri Lanka, China and tropical Asian countries.	HD	(Lobo *et al.*, 2018)
Cinnamomum tamala	Eugenol (52.54%)	aromatic compounds	China, Nepal, Bhutan, and India	HD	(Heer *et al.*, 2016)
Cinnamomum japonicum Sieb.	Borneol (41.91 and 36.15% for SFMD–HE and HD, respectively)	oxygenated monoterpenes	China, Korea and Japan	SFMD–HE and HD	(Zhao *et al.*, 2018)

Notes: HD, hydrodistillation.
SFMD–HE, solvent-free microwave-assisted distillation followed by homogenate extraction.

3.2. Principal component analysis of essential oils

3.2.1. Correlation analysis of chemical constituents of CCPL essential oils from five different habitats in China

According to the analysis of the correlation matrix of CCPL essential oils from 5 different habitats in China (as shown in Table 3), the following conclusions can be drawn: (1) the similarity of CCPL essential oils between Jinxi and Mianyang was very large, which showed that there was little difference between the 2 samples; (2) the similarity of CCPL essential oils between Kunming, Chengdu and Nanchang was large, which indicated that the 3 sample components were less different. Generally, the correlation coefficient was more than 0.6, which can be considered a significant correlation.

3.2.2. Principal component analysis of CCPL essential oils from five different habitats in China

The percentage of the peak area values corresponding to 30 retention indexes was taken as the

Table 3. The correlation matrix of essential oils from five different habitats in China

Column heading	Correlation Matrix					
		Jinxi	**Mianyang**	**Kunming**	**Chengdu**	**Nanchang**
Correlation	**Jinxi**	1.000	0.840	-0.059	-0.066	-0.063
	Mianyang	0.840	1.000	-0.041	-0.071	-0.066
	Kunming	-0.059	-0.041	1.000	0.863	0.867
	Chengdu	-0.066	-0.071	0.863	1.000	0.999
	Nanchang	-0.063	-0.066	0.867	0.999	1.000

Notes: The analysis of the correlation matrix of *Cinnamomum camphora* (L.) Presl leaves' essential oils from 5 different habitats were based on the average of three determinations.

Table 4. Initial eigenvalues and contribution rate of principal components in CCPL essential oils

The principal components	Initial eigenvalues		
	eigenvalues	variance contribution rate (%)	cumulative contribution rate (%)
1	19.039	63.464	63.464
2	7.391	24.637	88.101
3	3.444	11.479	99.580

Notes: CCPL, *Cinnamomum camphora* (L.) pressed leaves.
The analysis of initial eigenvalues and contribution rate of principal components in CCPL essential oils were based on the average of three determinations.

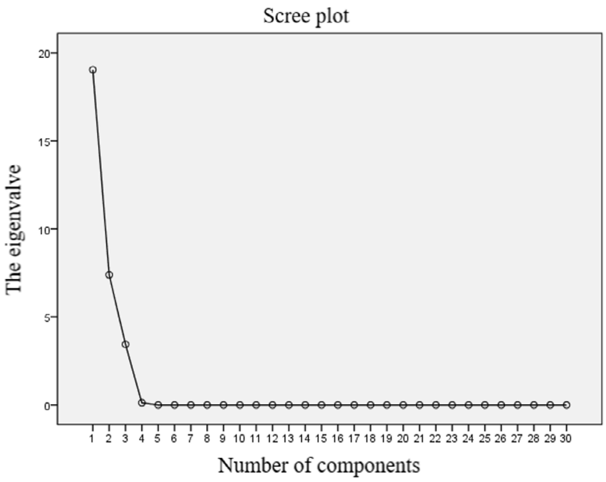

Figure 1. The scree plot of eigenvalues of 30 components.

Notes: The results of scree plot of eigenvalues of 30 components were based on the average of three determinations.

analysis object, marking the percentage of 30 peak area values in turn as A1-A30. After the data were standardized, PCA analysis was conducted. As can be seen from Table 4, the characteristic values of the first 3 principal components were all greater than 1 and the cumulative contribution rate to the total variance was 99.58%, indicating that the first 3 factors played a leading role in influencing the evaluation index of the volatile quality and can objectively reflect the quality represented by the volatile components of CCPL. Generally, the eigenvalue of each scree in the scree plot in Fig.1 was the basis of the characteristic quantity value, which was used to determine the number of principal components to be retained. It can be seen from Fig.1 that the difference among the eigenvalues of factors 1, 2 and 3 was large; while the difference among factors 4-30 was small. A preliminary conclusion can be drawn that most of the information can be generalized by retaining 3 factors. Therefore, the first 3 principal components should be selected for comprehensive comparison.

The information provided by the 3 principal components accounted for 99.580% (> 85%) of the total information, which were 63.464, 24.637 and 11.479%, respectively. The eigenvectors corresponding to the first principal component (PC1) were larger in compounds 2, 5, 21, 6, 4, 1, 7, 11, 24, 10, 20, 19 and 15, indicating that the first principal component was greatly affected by these components. Interestingly, linalool (15) showed the highest relative content in Jinxi and Mianyang. The second principal components corresponding to the larger load were 22, 9, 3, 29, 8, 18 and 25. The above 7 components were not detected in CCPL essential oils from Kunming, Chengdu or Nanchang. The third principal components corresponding to a larger load were compounds 27 and 30, which had no significant influence on the differentiation of the essential oils of *Cinnamomum camphora* from Kunming.

3.2.3. Cluster analysis of CCPL essential oil from five different habitats in China

According to Figure 2, the cluster can be divided into 2 categories: first was No.1 and No.2, i.e. CCPL essential oils from Jinxi and Mianyang; the second was No.3, No.4 and No.5, i.e. CCPL essential oils from Kunming, Chengdu and Nanchang. Among the 5 samples, linalool was 96.47 and 42.65% from Jinxi and Mianyang, respectively, and it had components that were not detected in some other 3 areas. Therefore, it was classified into one group by systematic clustering. Further combined with the principal component analysis results, it can be seen that there were up to 13 components of CCPL essential oils from Kunming, Chengdu and

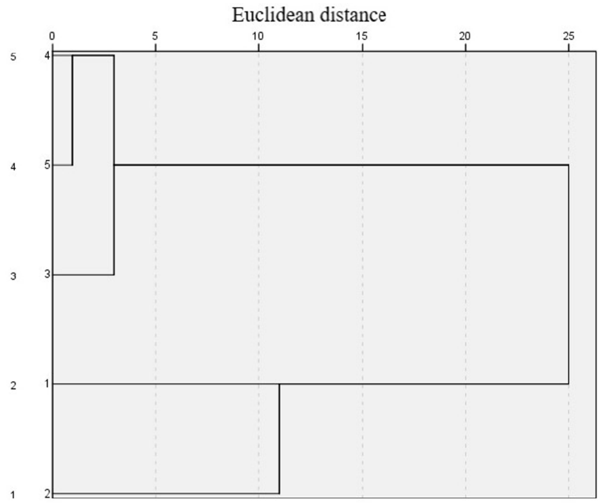

Figure 2. Cluster analysis of CCPL essential oil from five different habitats in China.

Notes: The results of cluster analysis of CCPL essential oil from five different habitats were based on the average of three determinations. CCPL, Cinnamomum camphora (L.) Presl leaves.
1, essential oil of camphor leaves from Jinxi, (Jiangxi Province, China).
2, essential oil of camphor leaves from Mianyang, (Sichuan Province, China).
3, essential oil of camphor leaves from Kunming, (Yunnan Province, China).
4, essential oil of camphor leaves from Chengdu, (Sichuan Province, China).
5, essential oil of camphor leaves from Nanchang, (Jiangxi Province, China).

Nanchang, and their relative contents were similar, and therefore they were classified into one category by systematic clustering.

3.3. Antibacterial activity of essential oils

The antimicrobial properties of CCPL essential oils from 5 different habitats in China were assessed against one gram-positive bacteria (*S. aureus*) and 2 Gram-negative bacteria (*E. coli* and *P. aeruginosa*) by the paper disc diffusion method which was used to measure the diameter of the inhibition zone. As shown in Table 5, the obtained data suggested that all essential oils possessed different degrees of inhibition against all tested strains, apart from CCPL EO from Kunming, which had no inhibitory effects on *P. aeruginosa*. Among the 5 essential oils, Jinxi's showed the strongest antibacterial properties (with 14.29±0.02mm as IZ) especially when tested against *S. aureus;* while the other 4 essential oils showed low bacteriostasis (6.30±0.02-8.79±0.09). Furthermore, the EO of Jinxi's on *E. coli* and *P. aeruginosa* had larger inhibition zones (11.33±0.18mm and 7.89±0.09mm, respectively), compared to the other 4 essential oils (from 6.16±0.03 mm to 9.14±0.02 mm and from 6.47±0.15 mm to 7.37±0.02 mm, against *E. coli* and *P. aeruginosa,* respectively). Moreover, considering the antibacterial properties, the essential oils from Mianyang, Chengdu and Nanchang showed no statistical differences ($p > 0.05$) against *S. aureus* and *E. coli,* respectively.

Table 5. The antibacterial activity of CCPL essential oils from 5 different habitats in China.

Essential oils	Average value of inhibition zone (mm)		
	S. aureus	**E. coli**	**P. aeruginosa**
A	14.29±0.02a	11.33±0.18a	7.89±0.09a
B	8.73±0.05b	8.93±0.05b	6.47±0.15d
C	6.30±0.02c	6.16±0.03c	NT
D	8.79±0.09b	9.14±0.02b	7.37±0.02b
E	8.68±0.09b	9.05±0.04b	6.82±0.09c

Notes: CCPL, *Cinnamomum camphora* (L.) Presl leaves.
A, essential oil of camphor leaves from Jinxi, (Jiangxi Province China).
B, essential oil of camphor leaves from Mianyang, (Sichuan Province China).
C, essential oil of camphor leaves from Kunming, (Yunnan Province China).
D, essential oil of camphor leaves from Chengdu, (Sichuan Province China).
E, essential oil of camphor leaves from Nanchang, (Jiangxi Province, China).
NT, Not tested
* Tukey test was used to detect significant differences ($p \leq 0.05$) among the mean values obtained from three replicates performed. Different letters in each column indicate significance (* $p < 0.05$).

Previous studies have shown that the most diverse group of isoprenoids, sesquiterpenes and monoterpenes accounted for the largest proportion of essential oils in plants (Sarikurkcu *et al.*, 2018). In addition, Chen *et al.* (2018) reported that different major monoterpenoids primarily led to differences in different chemical types of volatile oils. Therefore, the similar antibacterial effects of CCPL essential oils from Chengdu and Nanchang can be attributed to the similar proportion of the major compound eucalyptol (52.2 and 55.35%, respectively). As for the EO from Mianyang, the high concentration of other constituents, such as linalool (42.65%) and camphor (26.08%) also contributed to its synergistic effects on antimicrobial activity. This antibacterial potency of the EO from Jinxi can probably be explained by the known antimicrobial effects of linalool as it was identified as the major compound (96.47%) in the essential oil of CCPL from Jinxi. Indeed, Herman *et al.* (2016) confirmed that the combination of tested essential oil with linalool showed a greater efficacy than the essential oil and linalool separately against Gram-negative bacteria including *P. aeruginosa* and *E. coli*, also against *C. albicans* (fungus). This conclusion is strongly supported by a study by Schmidt *et al.* (2012), who proved the antibacterial activity of linalool chemotype essential oil, which can be related to the interaction of the main constituent linalool. The study by Chen *et al.* (2020) also showed that the essential oil from *C. camphora,* which is rich in linalool had good activity against MRSA, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Salmonella gallinarum* and *Escherichia coli*.

Generally, comparing the gram-negative bacteria, gram-positive bacteria proved to be more sensitive to the essential oils. As a matter of fact, the essential oils from different habitats had the strongest inhibitory effects on *S. aureus* and the weakest inhibition on *P. aeruginosa*. Moreover, Kunming's oil had the minimal inhibition zones (6.30±0.02mm and 6.16±0.03mm against *S. aureus* and *E. coli,* respectively) on both tested strains, and even no effect on *P. aeruginosa*. This variation in antibacterial behavior could be explained by the fact that the cell wall structure of bacteria was different. Gram-negative has a special component of the outer membrane layer embedded with lipopolysaccharide (Nisar *et al.*, 2018; Otoni *et al.*, 2014). The addition of an outer lipophilic membrane increased the penetration of hydrophobic compounds (linalool, eucalyptol and camphor) through the membrane (Fisher *et al.*, 2006).

4. CONCLUSIONS

In this study, CCPL essential oil of from Jinxi is a promising source of linalool, which was proved to have strong antimicrobial activities, which make it a natural food preservative due to its high concentration of linalool (96.47%). Based on principal component analysis and systematic cluster analysis, a comprehensive comparison of 30 compounds contained in CCPL essential oils from 5 different locations showed that the chemical components of Jinxi and Mianyang were significantly different from those from Kunming, Chengdu and Nanchang, providing a basis for resource substitution. The antibacterial activity obtained from CCPL essential oils from 5 different habitats in China were mostly attributable to rich bioactive oxygenated monoterpene hydrocarbons, such as linalool, eucalyptol and camphor. The essential oils were natural products, and can inhibit the growth and reproduction of foodborne pathogens (*S. aureus*, *E. coli* and *P. aeruginosa*) in this test, hereby serving as a basis to justify and guide further work aiming toward suitable concentrations of these essential oils or their bioactive components for the application of CCPL extract as a natural food preservative.

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6. ETHICAL STATEMENTS

This study does not involve any human or animal testing.

7. CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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