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Characterizing the essential oil composition and assessing the antioxidant and antimicrobial properties of two compositae taxa: *Gerbera delavayi* Franch. and *Gerbera piloselloides* (L.) Cass

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Introduction: *Gerbera piloselloides* (L.) Cass. and *Gerbera delavayi* Franch. are increasingly recognized for their medicinal properties, particularly among ethnic minority communities in southern China, where they are used for heat-clearing, detoxification, cough relief, lung expulsion, and asthma alleviation. Despite their traditional use, these species have been subjected to limited research regarding their biological activities, leaving a gap in scientific understanding.

Methods: This study was designed to investigate the essential oil (EO) compositions, as well as the antioxidant and antimicrobial properties of *G. piloselloides* and *G. delavayi*. The EOs were extracted via hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant potential was assessed through ABTS and DPPH free radical scavenging assays, along with the ferric reducing antioxidant power (FRAP) method. The antimicrobial activity was evaluated against five bacterial strains, including two Gram-positive (*Staphylococcus aureus*, *Listeria*) and three Gram-negative (*Salmonella*, *Escherichia coli*, *Pasteurella multocida*) species, using the broth microdilution technique.

Results: The essential oil from *G. piloselloides* (EOgp) yielded 0.14% and was found to contain 24 compounds. It demonstrated high antioxidant activity in the ABTS assay and exhibited the strongest antibacterial effect against *Listeria in vitro*. In contrast, the essential oil from *G. delavayi* (EOgd) had a higher yield of 0.26% and contained a more complex composition with 100 compounds. It showed superior antioxidant activity in both the DPPH and FRAP assays and also demonstrated the highest antibacterial activity against *Listeria*.

Discussion: The findings of this study confirm that both *G. piloselloides* and *G. delavayi* possess significant potential as natural sources of antioxidants and antibacterial agents, warranting further exploration for their development into therapeutic products.

KEYWORDS

essential oil, *Gerbera delavayi* Franch., *Gerbera piloselloides*, chemical composition, antioxidant activity, antibacterial activity

1 Introduction

The relentless progression of antibiotic resistance, particularly among multidrug-resistant bacterial strains, poses a significant threat to global health, underscoring the urgent need for the continuous development and discovery of new antimicrobial materials (Baran et al., 2023). While the "Antibiotic Era" may be waning, the potential of medicinal plants as a source for novel antimicrobial agents remains a beacon of hope. Through the intricate process of photosynthesis, plants synthesize a wealth of organic matter and secondary metabolites, which exhibit a broad spectrum of biological activities. These compounds lay the pharmacological groundwork for the prevention and treatment of diseases (Petric et al., 2020; Rehman et al., 2020). With many medicinal plants recognized for their safety, efficacy, and minimal side effects, the exploration of their bioactive compounds for antimicrobial properties is not only imperative but also a promising avenue in the fight against multidrug-resistant bacteria (Bouarab Chibane et al., 2019).

Essential oils (EOs), a type of secondary metabolite produced by aromatic plants, exhibit a spectrum of biological activities, including antibacterial, antioxidant, anti-inflammatory, enzyme inhibitory, sedative, anxiolytic, and antidepressant properties (Zengin et al., 2019; Liu Y. et al., 2024). EOs are utilized as natural remedies for the treatment of infectious diseases and as flavoring agents in food, offering a green and healthy alternative (Coelho et al., 2023; Wu et al., 2024). Due to their remarkable biological activities, EOs from medicinal plants are of great interest to scientists seeking to identify new phytochemical bioactive molecules that align with biodiversity and medicinal needs (Oliveira de Veras et al., 2020).

Gerbera Cass., a member of the Compositae family (Mutisieae Cass.), comprises approximately 80 species ranging from Africa to East Asia, with 20 species found in China, predominantly in the southwestern region (Zhao et al., 2024). *Gerbera piloselloides* (L.) Cass. and *Gerbera delavayi* Franch. are perennial herbs within the Gerbera genus. *G. piloselloides* is known for its heat-clearing, detoxifying, cough-relieving, phlegm-resolving, and circulation-regulating properties (Zhao et al., 2022; Liu C. et al., 2024). Traditionally, it is used in southwestern China to treat cough and sore throat when mixed with honey and also serves as a flavoring agent in winemaking and meat cooking due to its pleasant aroma (Zhou et al., 2022). The plant's bioactive compounds, including caffeic acid derivatives, parasorboside derivatives, coumarins, and flavonoids, have been isolated through activity-guided isolation (Wang et al., 2014). The EO of *G. piloselloides*, EOgp, has been analyzed by GC-MS and found to contain fatty acids, terpenes, and aromatic compounds (Tang et al., 2003).

G. delavayi, found in open areas and forest margins at altitudes of 1800 to 3200 meters, was historically known as "ignited flowers" or "fireweed" due to its leaf's combustion-supporting properties (Xu et al., 2017). The soft fiber on the back of its leaves is used in hand-weaving (Zheng et al., 2017). Beyond its use in spinning, *G. delavayi* holds significance in medicine and ornamental purposes. Gerbera species in China are noted for their antitussive, antipyretic, hemostatic, circulatory, and anti-inflammatory effects (Wu et al., 2005). The ethanol extract of *G. delavayi* has led to the isolation of two new coumarin compounds, gerdelavins A and B, along with 13 known compounds (Liu et al., 2010). Coumarins, characterized by their benzopyrone core, interact with various enzymes and receptors in organisms through weak bonds, conferring a broad range of medicinal potential, including antibacterial, antitumor, and anticoagulant activities (Balewski et al., 2021; Citarella et al., 2024).

In this context, our study endeavors to delve deeper into the properties of two lesser-studied Gerbera species, *G. piloselloides* and *G. delavayi*. The objective was to assess the EO compositions and to explore their antioxidant and antimicrobial potential. Notably, there is a paucity of literature documenting the biological activities of the EOs from these two plant species. Consequently, this investigation stands as the first comprehensive examination of the biological activities of the extracted EOs from *G. piloselloides* and *G. delavayi*, marking a significant contribution to the existing knowledge base.

2 Materials and methods

2.1 Plant material

To obtain a comprehensive representation of the chemical profile, the entire plants of *G. piloselloides* and *G. delavayi*, encompassing leaves, stems, roots, and rhizomes, were collected

Abbreviations: EO, essential oil; FRAP, ferric reducing antioxidant power; ABTS, 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EOgp, essential oil from Gerbera piloselloides; EOgd, oil from Gerbera delavay; TPC, total polyphenolic content.

from the Stone Forest region of China. Plant materials from two Gerbera species, were meticulously collected in the Stone Forest region of China. The sampling locations were at elevations of 2316 m (24°81′10.55″ N, 103°30′12.83″ E) for *G. piloselloides* and 1689 m (24°46′27.55″ N, 103°17′18.83″ E) for *G. delavayi*, within Shilin County, Kunming, Yunnan Province, in July 2019. The taxonomic identification of these species was conducted by the Professor Huifeng Sun from Heilongjiang University of Chinese Medicine in Harbin, China.

For posterity and to facilitate future studies, voucher specimens were meticulously archived in the Herbarium of the College of Veterinary Medicine. The voucher numbers assigned to *G. piloselloides* and *G. delavayi* are 2031 and 2032, respectively. Following collection, the herbal materials were subjected to natural drying at room temperature. Subsequently, they were finely pulverized using a grinder and preserved at a refrigerated temperature of 4 °C, awaiting subsequent utilization in experimental procedures.

2.2 Extraction of essential oils

The essential oils (EOs) from *G. piloselloides* and *G. delavayi* were extracted using the hydrodistillation method, as described by Semerdjieva et al. (2019). For the extraction process, a precise amount of 100 grams of dried plant material was combined with 1000 mL of distilled water in a flask. The extraction was conducted for a duration of 8 h, commencing once the water reached boiling point. Following extraction, the EOs were separated from the aqueous phase with ethyl ether, dried over anhydrous sodium sulfate, filtered, and then subjected to evaporation of the ethyl ether in an oven at 40 °C for one hour. The resulting EO was transferred to amber vials and stored at -20 °C. The yield percentage (w/w) of the oil was determined based on the initial weight of the plant material used.

2.3 GC/MS analysis

The compositional analysis of the EOs was performed using an Agilent Technologies Gas Chromatograph model 7697A, equipped with a triple quadrupole detection system and a split-splitless injection port. The chromatographic separation was achieved on a HP-5MS fused silica capillary column (30 m × 250 μm × 0.25 μm) coupled with an Agilent MS Detector. The column temperature program began at 40 °C for 5 min, followed by an increase to 280 °C at a rate of 10 °C/min. An injection volume of 0.8 μL was used with a split ratio of 1: 20, and helium was employed as the carrier gas at a constant flow rate of 20 mL/min. Mass spectra were acquired at an electron energy of 70 eV, with the ion source temperature set at 250 °C. The mass spectra data were recorded within the mass-to-charge ratio (*m/z*) range of 44-550.

The identification of the EO compounds was accomplished by comparing their retention times and mass spectra with reference data in the NIST mass spectra library. The relative percentage contents of the individual compounds were quantified based on the peak areas in the GC-MS chromatograms, following the methodology described by Thabet et al. (2022).

The identification of the EO compounds was accomplished by comparing their retention times and mass spectra with reference data in the NIST mass spectra library. The retention indices were calculated using the linear retention index method, with a mixture of n-alkanes (C8-C20) at a concentration of 1 μg/mL as the reference compounds. The relative percentage contents of the individual compounds were quantified based on the peak areas in the GC-MS chromatograms, following the methodology described by Thabet et al. (2022).

2.4 Estimation of total polyphenolic content

The TPC was quantified using the Folin-Ciocalteu method adapted for a 96-well microplate format (Larrazabal-Fuentes et al., 2019). Initially, the EO sample (500 μg/mL) was combined with 10% (v/v) Folin-Ciocalteu reagent at a ratio of 1: 5 and allowed to stand for 5 min. Subsequently, a sodium carbonate solution was added to the mixture at a volume four times that of the sample and the mixture was shaken for 1 min. After incubation for 1 h at 25 °C, the absorbance was recorded at 765 nm using a microplate reader. A calibration curve was generated using gallic acid dilutions ranging from 0 to 1000 μg/mL. The results were expressed as milligrams of gallic acid equivalents per milliliter of EO.

2.5 Evaluation of antioxidant activities

The antioxidant potential of the EOs was assessed using the ferric reducing antioxidant power (FRAP) assay, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, in conjunction with the determination of the TPC. Vitamin C was employed as the standard reference. The protocols outlined below were adapted for use with 96-well microplates. All assays were conducted in triplicate.

2.5.1 DPPH radical scavenging activity assay

The DPPH radical scavenging capacity of the EOs was evaluated using the methodology of Larrazabal-Fuentes et al. (2019). The percent inhibition (I%) was calculated with the formula: I% = [(Ac - As)/(Ac)] × 100, where Ac is the absorbance of the control and As is the absorbance of the sample. The results were reported as IC_{50} values, representing the concentration of EO (μg/mL) required to inhibit 50% of the DPPH radicals in the solution, determined through linear regression analysis of the percentage of residual DPPH versus sample concentration.

2.5.2 FRAP assay

The FRAP assay was performed as described by Tian et al. (2019). An extract solution (500 μg/mL, 10 μL) was mixed with

freshly prepared FRAP solution (70 µL), and the change in absorbance was measured at 593 nm after a 30-min incubation at 37 °C. Standard solutions of $FeSO_4{\cdot}7H_2O$ (0-500 µg/mL) and vitamin C (0-200 µg/mL) were used to construct the calibration curve. FRAP results were expressed as milligrams of vitamin C equivalent per milliliter of EO.

2.5.3 ABTS scavenging activity

The ABTS scavenging activity of the EO was determined following the procedures of Kıvrak (2014). ABTS radical cation (ABTS+) was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) at room temperature in the dark for 16 h. The ABTS+ solution was then diluted with ethanol to achieve an absorbance of 0.700 ± 0.005 at 734 nm. This solution (160 µL) was mixed with 40 µL of EO (0-20000 µg/mL), and the absorbance was measured at 734 nm after a 30-minute incubation at 30 °C. Vitamin C at various concentrations (0-200 µg/mL) served as the reference. The percentage scavenging of ABTS radicals was calculated using the equation from the DPPH assay. The results were expressed as IC_{50} values, calculated based on the linear regression of the percentage of residual ABTS versus sample concentration.

2.6 Evaluation of antibacterial activity

2.6.1 Microbial strains employed

The antimicrobial efficacy of the EOs was assessed against a panel of bacterial strains, including *S. aureus* CMCC26003, *Listeria* ATCC 19111, *Salmonella* CVCC541, *E. coli* CVCC10141, and *P. multocida* C48-1. These strains were obtained from the Harbin Institute of Veterinary Medicine (Harbin, Heilongjiang, China).

2.6.2 Determination of minimal inhibitory concentrations

The MICs of the EOs were determined using the broth microdilution method, as outlined in the CLSI protocols M60 (CLSI, 2017) and M100 (CLSI, 2018). The procedure involved preparing a stock solution of EO at 25 mg/mL in a mixture of 20% dimethyl sulfoxide (DMSO) and 80% distilled water. Initially, 100 µL of this stock solution was added to the first well of a 96-well plate, followed by serial two-fold dilutions to achieve concentrations ranging from 25 to 0.05 mg/mL (Cui et al., 2018). Subsequently, each bacterial strain was inoculated into LB broth to achieve a McFarland standard of 0.5, diluted 100-fold, and then added to the wells at a volume of 100 µL per well. The MIC was defined as the lowest concentration of EO that inhibited visible growth of the bacterial strains after an incubation period of 16–18 h at 37 °C. Chloramphenicol, at concentrations ranging from 10 to 0.04 mg/mL, was used as a positive control, while a solution of 20% DMSO-80% distilled water served as the negative control. All experiments were conducted in triplicate to ensure accuracy and reproducibility.

2.7 Statistical analysis

Significance was determined at a p-value threshold of < 0.05. Data were processed using GraphPad Prism® version 7.0 and presented as mean ± standard deviation (SD). To ascertain statistically significant differences among the groups, a one-way analysis of variance (ANOVA) was performed.

3 Results and discussion

3.1 Chemical composition of essential oils

The medicinal parts of two Gerbera species, namely the whole plants of *G. piloselloides* and *G. delavayi*, were subjected to hydrodistillation, yielding yellow EOs with distinctive odors. The yields for *G. piloselloides* and *G. delavayi* were 0.14% (w/w) and 0.26% (w/w), respectively. The chemical compositions of these EOs were elucidated using GC/MS. The compositional percentages of the EOs from *G. piloselloides* and *G. delavayi* are presented in Tables 1 and 2, respectively. The total ion chromatograms for the EOs of both species (EOgp and EOgd) are depicted in Figure 1. GC/MS analysis of EOgp identified 24 components, with berkheyaradulene (32.03%), 4-(2', 4', 4'-trimethyl-cyclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one (12.86%), caryophyllene (6.78%), and cycloisolongifolene (5.30%) as the principal constituents. In contrast, EOgd comprised 100 components, with butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (*E*)- (10.50%), cyperene (9.70%), β-panasinsene (7.13%), benzamide, *N*-(1-adamantyl)-2-hydroxy- (6.12%), and benzene, 1-(1,1-dimethylethyl)-4-ethyl- (5.31%) as the predominant compounds.

In a prior phytochemical study, the researchers documented the presence of 17 volatile organic components in *G. piloselloides*, encompassing fatty acids, terpenes, and aromatic compounds. Notably, neryl (*S*) -2-methylbutanoate (35.99%), 4-hydroxy-3-methylacetophenone (8.74%), and n-hexadecanoic acid (7.48%) emerged as the predominant constituents of the plant's essential oil (Luo et al., 2013). Previous studies have identified various volatile organic compounds in specific parts of *G. piloselloides*, such as leaves, caudices, and roots (Tang et al., 2003). However, our study focused on the essential oils extracted from the whole plants of *G. piloselloides* and *G. delavayi*. The observed discrepancies in the identified compounds may be attributed to the varying environmental conditions of the plant collection sites and the inclusion of multiple plant parts in our analysis.

Previous research on Gerbera has revealed the presence of coumarins, sesquiterpenoids, triterpenoids, and cyanogenic glycosides (Liu et al., 2010). Despite the distinct EO profiles of the two Gerbera species, eight compounds, including (-)-aristolene, 4-(2', 4', 4'-trimethyl-cyclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one, berkheyaradulene, cyclosativene, cyperene, naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1*S*-*cis*)-, humulene, and caryophyllene, are common to both, as detailed in Table 3.

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TABLE 1 Constituents of the essential oil from *Gerbera piloselloides*..

No.[a]	Components	Retention time (min)	RI[b]	CAS number	Molecular formula
1	Ethanol	1.58	61	64-17-5	C_2H_6O
2	Ethyl ether	1.677	73	60-29-7	$C_4H_{10}O$
3	Ethyl acetate	2.186	136	141-78-6	$C_4H_8O_2$
4	(+)-alpha-Pinene	9.36	1024	7785-70-8	$C_{10}H_{16}$
5	Adipic acid, di(trans-hex-3-enyl) ester	13.157	1494	No	$C_{18}H_{30}O_4$
6	Thymol	15.686	1807	89-83-8	$C_{10}H_{14}O$
7	1-Ethyl-3-(propen-1-yl)adamantane	16.244	1876	No	$C_{15}H_{24}$
8	(-)-Aristolene	16.413	1897	6831-16-9	$C_{15}H_{24}$
9	(-)-alpha-Gurjunene	16.518	1910	489-40-7	$C_{15}H_{24}$
10	Cycloisolongifolene	16.583	1918	No	$C_{15}H_{24}$
11	cyclosativene	16.874	1954	22469-52-9	$C_{15}H_{24}$
12	alpha-longipinene	16.93	1961	5989-08-2	$C_{15}H_{24}$
13	4-(2', 4', 4'-trimethyl-yciclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one	17.076	1979	No	$C_{14}H_{20}O$
14	Berkheyaradulene	17.181	1992	65372-78-3	$C_{15}H_{24}$
15	Cyperene	17.351	2013	2387-78-2	$C_{15}H_{24}$
16	Longifolene	17.48	2029	61262-67-7	$C_{15}H_{24}$
17	Caryophyllene	17.561	2039	87-44-5	$C_{15}H_{24}$
18	Humulene	18.021	2096	26259-79-0	$C_{15}H_{24}$
19	Carvacryl acetate	18.15	2112	6380-28-5	$C_{12}H_{16}O_2$
20	(+)-DELTA-CADINENE	18.756	2187	483-76-1	$C_{15}H_{24}$
21	2,3,5,6-tetramethyl-Phenol	19.282	2252	527-35-5	$C_{10}H_{14}O$
22	Neryl 2-methylbutanoate	19.403	2267	51117-19-2	$C_{15}H_{26}O_2$
23	Caryophyllenyl alcohol	19.532	2283	No	$C_{15}H_{26}O$
24	Caryophyllene oxide	19.613	2293	1139-30-6	$C_{15}H_{24}O$
25	humulene epoxide ii	19.936	2333	19888-34-7	$C_{15}H_{24}O$
26	Isocaryophillene	20.073	2350	13877-93-5	$C_{15}H_{24}$
	1,1,1,3,5,5,5-Heptamethyltrisiloxane				
27	Total Identified	30.593	3652	1873-88-7	$C_7H_{22}O_2Si_3$
	Monoterpenes			76.61	
	Sesquiterpenes			0.31	
	Phenolic Compounds			55.50	
	Aromatic Compounds			2.73	
	Esters			13.75	
	Other Compounds			1.98	

[a] Compounds listed in order of elution from the Rtx-5MS capillary column.
[b] Retention indices relative to C_8-C_{20} n-alkanes on an Rtx-5MS capillary column.

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TABLE 2 Constituents of the essential oil from *Gerbera delavayi*.

No.[a]	Components	Retention time (min)	RI[b]	CAS number	Molecular formula
1	Ethanol	1.572	60	64-17-5	C_2H_6O
2	Ethyl ether	1.669	72	60-29-7	$C_4H_{10}O$
3	Ethyl Acetate	2.178	135	141-78-6	$C_4H_8O_2$
4	2-Isopropoxyethanol	6.282	643	109-59-1	$C_5H_{12}O_2$
5	3-Ethylidenecycloheptene	9.36	1024	–	C_9H_{14}
6	Sabinene	10.257	1135	3387-41-5	$C_{10}H_{16}$
7	1-(4-Methylphenyl)ethanol	10.354	1147	536-50-5	$C_9H_{12}O$
8	6-methyl-5-Hepten-2-one	10.507	1166	110-93-0	$C_8H_{14}O$
9	2,2-dimethyl-3-octyne	10.62	1180	19482-57-6	$C_{10}H_{18}$
10	Alpha -Phellandrene	10.944	1220	99-83-2	$C_{10}H_{16}$
11	O-Cymene	11.307	1265	527-84-4	$C_{10}H_{14}$
12	D-Limonene	11.404	1277	5989-27-5	$C_{10}H_{16}$
13	Eucalyptol	11.469	1285	470-82-6	$C_{10}H_{18}O$
14	Beta-Ocimene	11.719	1316	13877-91-3	$C_{10}H_{16}$
15	(Z)-linalool oxide (furanoid)	12.196	1375	5989-33-3	$C_{10}H_{18}O_2$
16	(+)-2-Carene	12.713	1439	–	$C_{10}H_{16}$
17	cyclene	12.85	1456	508-32-7	$C_{10}H_{16}$
18	N-methyl-2-pyrolidene	13.376	1521	33838-11-8	C_5H_9N
19	nerol oxide	13.602	1549	1786-08-9	$C_{10}H_{16}O$
20	5-methyl-3-(1-methylethylidene)-1,4-Hexadiene	13.99	1597	–	$C_{10}H_{16}$
21	(-)-Terpinen-4-ol	14.086	1609	20126-76-5	$C_{10}H_{18}O$
22	3-methylene-1,5,5-trimethyl-cyclohexene	14.256	1630	16609-28-2	$C_{10}H_{16}$
23	2-hydroxy-4-methylbenzaldehyde	14.305	1636	698-27-1	$C_8H_8O_2$
24	3-Carene	14.773	1694	13466-78-9	$C_{10}H_{16}$
25	2-isopropyl-4-methyl anisole	14.927	1713	31574-44-4	$C_{11}H_{16}O$
26	Citral	15.347	1765	5392-40-5	$C_{10}H_{16}O$
27	cis-Thujopsene	15.735	1813	470-40-6	$C_{15}H_{24}$
28	1-(4-Hydroxy-3-methylphenyl)ethanone	15.969	1842	876-02-8	$C_9H_{10}O_2$
29	1,5-dimethyl-2,4-bis(1-methylethyl)-benzene	16.276	1880	5186-68-5	$C_{14}H_{22}$
30	1-Isopropyl-4,7-dimethyl-1,2,4a,5,8,8a-hexahydronaphthalene	16.389	1894	5951-61-1	$C_{15}H_{24}$
31	(-)-Aristolene	16.583	1918	6831-16-9	$C_{15}H_{24}$
32	Cedrene-V6	16.623	1923	–	$C_{15}H_{24}$
33	Aciphyllene	16.704	1933	–	$C_{15}H_{24}$
34	cyclosativene	17.011	1971	22469-52-9	$C_{15}H_{24}$
35	alfa.-Copaene	17.116	1984	138874-68-7	$C_{15}H_{24}$
36	4-(2', 4', 4'-trimethyl-yciclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one	17.197	1994	–	$C_{14}H_{20}O$
37	Berkheyaradulene	17.254	2001	65372-78-3	$C_{15}H_{24}$
38	(-)-Cyperene	17.496	2031	2387-78-2	$C_{15}H_{24}$

(Continued)

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TABLE 2 Continued

No.[a]	Components	Retention time (min)	RI[b]	CAS number	Molecular formula
39	Caryophyllene	17.714	2058	87-44-5	$C_{15}H_{24}$
40	1-(1,1-dimethylethyl)-4-ethyl-benzene	17.795	2068	7364-19-4	$C_{12}H_{18}$
41	2,4-diethyl-7,7-dimethylcyclohepta-1,3,5-triene	17.868	2077	–	$C_{13}H_{20}$
42	2,5-Dimethylchroman-4-one	17.9	2081	69687-87-2	$C_{11}H_{12}O_2$
43	beta-maaliene	17.973	2090	489-29-2	$C_{15}H_{24}$
44	Humulene	18.126	2109	6753-98-6	$C_{15}H_{24}$
45	Alloaromadendrene	18.175	2115	25246-27-9	$C_{15}H_{24}$
46	rotundene	18.207	2119	–	$C_{15}H_{24}$
47	beta-Panasinsene	18.433	2147	56684-97-0	$C_{15}H_{24}$
48	Selina-3,7(11)-diene	18.482	2153	6813-21-4	$C_{15}H_{24}$
49	gamma-selinene	18.506	2156	515-17-3	$C_{15}H_{24}$
50	beta-Guaiene	18.538	2160	88-84-6	$C_{15}H_{24}$
51	Alloaromadendrene	18.587	2166	25246-27-9	$C_{15}H_{24}$
52	alpha.-Muurolene	18.651	2174	31983-22-9	$C_{15}H_{24}$
53	(+)-Calarene	18.797	2192	17334-55-3	$C_{15}H_{24}$
54	(+)-DELTA-CADINENE	18.91	2206	483-76-1	$C_{15}H_{24}$
55	Guaia-9,11-diene	18.974	2214	–	$C_{15}H_{24}$
56	Isolongifolene	19.023	2220	1135-66-6	$C_{15}H_{24}$
57	(+)-α-murolene	19.071	2226	17627-24-6	$C_{15}H_{24}$
58	1, 1, 5-Trimethyl-1, 2-dihydronaphthalene	19.128	2233	–	$C_{13}H_{16}$
59	3-Methyl-2-butenoic acid, 4-methoxybenzyl ester	19.241	2247	–	$C_{13}H_{16}O_3$
60	(E)-3,7-Dimethylocta-2,6-dienyl ethyl carbonate	19.338	2259	–	$C_{13}H_{22}O_3$
61	[(2E)-3,7-dimethylocta-2,6-dienyl] butanoate	19.629	2295	106-29-6	$C_{14}H_{24}O_2$
62	Caparratriene	19.694	2303	–	$C_{15}H_{26}$
63	1,3-dimethyl-5-ethylbenzene	19.782	2314	934-74-7	$C_{10}H_{14}$
64	.beta.-Guaiene	19.895	2328	88-84-6	$C_{15}H_{24}$
65	Delta-Selinene	19.968	2337	473-14-3	$C_{15}H_{24}$
66	Alloaromadendrene	20.025	2344	025246-27-9	$C_{15}H_{24}$
67	Alpha-Elemene	20.081	2351	5951-67-7	$C_{15}H_{24}$
68	dehydro-aromadendrene	20.122	2356	–	$C_{15}H_{22}$
69	Xanthurenic acid	20.186	2364	59-00-7	$C_{10}H_7NO_4$
70	4,8a-dimethyl-6-prop-1-en-2-yl-1,3,5,6,7,8-hexahydronaphthalen-2-one	20.316	2380	–	$C_{15}H_{22}O$
71	1,2,3,5,6,7,8,8a-octahydro-1-methyl-6-methylene-4-(1methylethyl)naphthalene	20.356	2385	150320-52-8	$C_{15}H_{24}$
72	4-(2,3,4,6-Tetramethylphenyl)-3-buten-2-one	20.429	2394	–	$C_{14}H_{18}O$
73	Epizonarene	20.501	2403	41702-63-0	$C_{15}H_{24}$
74	Longifolene	20.566	2411	475-20-7	$C_{15}H_{24}$
75	Cedren-13-ol, 8-	20.615	2417	18319-35-2	$C_{15}H_{24}O$

(Continued)

10.3389/fpls.2025.1527525

TABLE 2 Continued

No.[a]	Components	Retention time (min)	RI[b]	CAS number	Molecular formula
76	1,2,3a,6-Tetramethyloctahydrocyclopenta[c]pentalen-3(3ah)-one	20.728	2431	–	$C_{15}H_{24}O$
77	7R,8R-8-Hydroxy-4-isopropylidene-7methylbicyclo[5.3.1]undec-1-ene	20.946	2458	–	$C_{15}H_{24}O$
78	3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2 Naphthalenone	21.019	2467	–	$C_{15}H_{22}O$
79	Longipinocarvone	21.229	2493	–	$C_{15}H_{22}O$
80	Cadina-1(10),6,8-triene	21.326	2505	1460-96-4	$C_{15}H_{22}$
81	2,2,7,7-tetramethyltricyclo[6.2.1.01,6]undec-5-en-4-one	21.6	2539	23747-14-0	$C_{15}H_{22}O$
82	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	21.721	2554	–	$C_{15}H_{24}O_2$
83	Corymbolone	21.923	2579	97094-19-4	$C_{15}H_{24}O_2$
84	2,2,7,7-tetramethyltricyclo[6.2.1.01,6]undec-5-en-4-one	22.02	2591	23747-14-0	$C_{15}H_{22}O$
85	Fukinanolid	22.247	2619	19906-72-0	$C_{15}H_{22}O_2$
86	(2-hydroxy-5-methylphenyl)-(4-methoxyphenyl)methanone	22.279	2623	–	$C_{15}H_{14}O_3$
87	6,10,14-trimethylpentadecan-2-one	22.36	2633	502-69-2	$C_{18}H_{36}O$
88	(2-Hydroxy-5-methylphenyl)(4-methoxyphenyl)methanone	22.602	2663	–	$C_{15}H_{14}O_3$
89	1-Methyl-1-silolanyl heptanoate	22.723	2678	–	$C_{12}H_{24}O_2Si$
90	2,6-ditert-butylnaphthalene	23.418	2764	3905-64-4	$C_{18}H_{24}$
91	n-Hexadecanoic acid	23.644	2792	57-10-3	$C_{16}H_{32}O_2$
92	N-Mesitytricyclo-[3.2.1.0(2.4)]octane-3-carboxamide	24.008	2837	342394-51-8	$C_{18}H_{23}NO$
93	Kaur-15-ene	24.169	2857	5947-50-2	$C_9H_{12}N_2O_5S$
94	2-(4-methoxybenzoyl)-1,6-dimethyl-1,2,3,4-tetrahydropyrrolo[1,2-a]pyrazine	24.202	2861	–	$C_{17}H_{20}N_2O_2$
95	Kaur-16-ene	24.662	2918	562-28-7	$C_{20}H_{32}$
96	N-(1-adamantyl)-2-hydroxybenzamide	25.09	2971	3728-06-1	$C_{17}H_{21}NO_2$
97	2,5-Diethylpyrazine	25.317	2999	013238-84-1	$C_8H_{12}N_2$
98	propoxy(dipropyl)phosphane	25.454	3016	6418-60-6	$C_9H_{21}OP$
99	o-Terphenyl	25.535	3026	84-15-1	$C_{18}H_{14}$
100	N-1-Adamantyl-p-nitrobenzalimine	25.987	3082	–	$C_{17}H_{20}N_2O_2$
101	3-Adamantan-1-yl-3-oxo-propionitrile	26.197	3108	–	$C_{13}H_{14}NO$
102	Hexestrol	26.294	3120	84-16-2	$C_{18}H_{22}O_2$
103	1-(4-Methoxyphenyl)-4,6-dimethyl-2(1H)-pyrimidinone	27.773	3303	74360-11-5	$C_{13}H_{14}N_2O_2$
104	6,6-Diphenylfulvene	27.975	3328	2175-90-8	$C_{18}H_{14}$
105	Triphenylene	28.112	3345	217-59-4	$C_{18}H_{12}$
106	Benz[a]anthracene	28.524	3396	56-55-3	$C_{18}H_{12}$
107	dimethylphenylsilane	28.758	3425	766-77-8	$C_8H_{11}Si$
108	2,6-dimethylocta-2,4,6-triene	29.074	3464	673-84-7	$C_{10}H_{16}$
	Total identified			95.06	

[a]Compounds listed in order of elution from the Rtx-5MS capillary column.
[b]Retention indices relative to C_8-C_{20} n-alkanes on an Rtx-5MS capillary column.

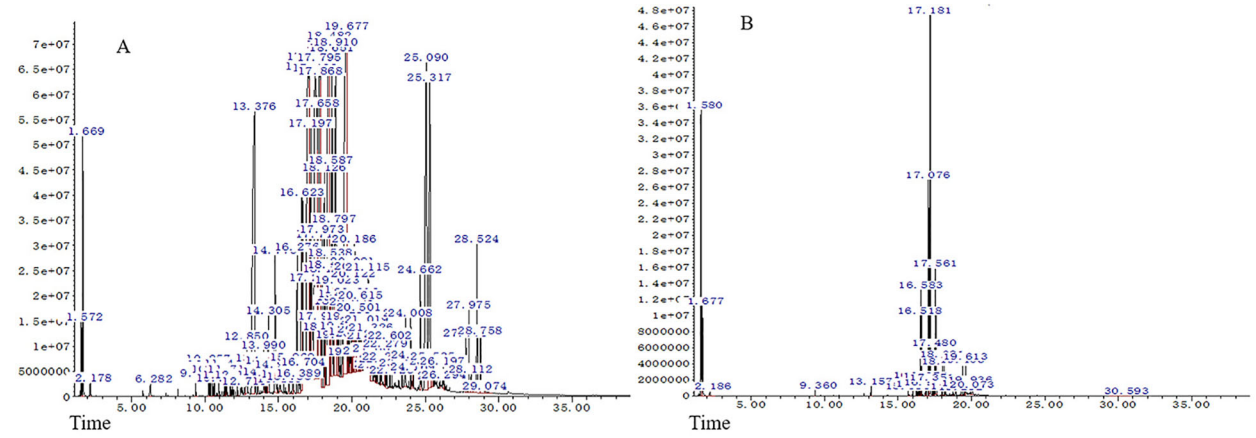

FIGURE 1
GC/MS profiles of the essential oils from *Gerbera piloselloides* **(A)** and *Gerbera delavayi* **(B)**.

Berkheyaradulene is particularly abundant in *G. piloselloides* (10.50%), representing a sesquiterpene hydrocarbon with an unusual carbon skeleton characterized by a bridgehead carbon connected to three rings, also found in other Asteraceae plants (Szöke et al., 2004). Caryophyllene, notable for its cyclobutane ring, a rare occurrence in nature, is often accompanied by isocaryophyllene and α-humulene, its ring-opened isomer (Taherpour et al., 2010). Cyperene, a tetracyclic sesquiterpene, possesses unique properties such as sterilizing, antioxidant, anticarcinogenic, and immune-boosting functions (Skała et al., 2016; Hu et al., 2017). Thymol, with its thyme oil-like aroma, may contribute to the use of *G. piloselloides* in winemaking and meat cooking. Thymol's expectorant properties have been documented, and it also exhibits bactericidal effects, suggesting its potential in treating bronchitis and whooping cough (Zhou et al., 2019). Furthermore, thymol holds promise for applications in the preservatives industry, as an insect repellent, and in the perfume industry (Roufegarinejad et al., 2018; Reyhani et al., 2022; Dadé et al., 2023).

TABLE 3 Common chemical compounds in essential oils of *Gerbera piloselloides* and *Gerbera delavayi*.

Compound	Content (%)	
	Gerbera piloselloides	*Gerbera delavayi*
(-)-Aristolene	0.357	1.611
cyclosativene	0.753	7.831
4-(2', 4', 4'-trimethyl-yciclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one	12.862	1.715
Berkheyaradulene	32.025	1.224
Cyperene	0.701	9.700
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.268	3.654
Humulene	1.761	1.284
Caryophyllene	6.782	0.77
Total (%)	55.509	27.789

3.2 Antioxidant capacity of essential oils

EOs are integral aromatic constituents found in herbs and spices, conferring them with a range of biological activities, including antimicrobial, antifungal, antioxidant, and anti-inflammatory effects (Valdivieso-Ugarte et al., 2019). However, the composition of EOs in these herbs is intricate, lacking a straightforward and precise method for a comprehensive and objective evaluation of the antioxidant capacity of traditional Chinese medicines. Consequently, a variety of antioxidant assays are necessary to profile the total antioxidant potential of natural extracts in this context.

We assessed the antioxidant potential of EOgp and EOgd by evaluating their efficacy in scavenging the stable free radicals ABTS and DPPH. The radical scavenging activities of the EOs are depicted in Figures 2A and 2B, respectively. The concentrations of the EOs required to inhibit each radical by 50% (IC_{50}) are presented in Table 4. Notably, *G. delavayi* exhibited a significantly higher DPPH free radical scavenging ability (IC_{50} 0.7 mg/mL) compared to *G. piloselloides* (IC_{50} 69.5 mg/mL). However, both *G. piloselloides* and *G. delavayi* demonstrated similar ABTS free radical scavenging activity, with IC_{50} values of 81 μg/mL and 105.8 μg/mL, respectively. It is documented that certain compounds with ABTS scavenging capability may not exhibit DPPH scavenging activity, which could account for the observed results (Borah et al., 2019).

The reducing capability of the extracts was determined using a microplate reader to track the conversion of Fe^{3+} to Fe^{2+} in the presence of the extracts. An increase in absorbance is indicative of the extract's reducing power. The influence of antioxidant concentration on FRAP inhibition is summarized in Figure 3. FRAP results were expressed as milligrams of vitamin C equivalent per milliliter of oil, and TPC data are provided in

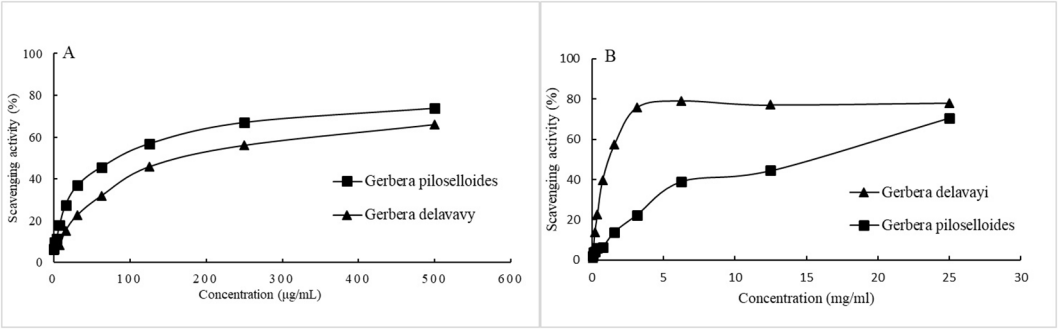

FIGURE 2
Radical scavenging activity of the essential oils from *Gerbera piloselloides* and *Gerbera delavayi* against the ABTS radical **(A)** and DPPH **(B)**. Data are represented as mean standard deviation (SD) of triplicate experiments.

TABLE 4 Antioxidant activities of essential oils from *Gerbera piloselloides* and *Gerbera delavayi*.

Sample	TPC (mg eq gallic acid/ mL oil)	FRAP (mg eq vitaminC/ mL oil)	IC_{50} values	
			DPPH (mg/mL)	ABTS (µg/mL)
Gerbera piloselloides	27.4 ± 2*	7.8 ± 1*	69.5 ± 1***	81 ± 5*
Gerbera delavayi	68.6 ± 4*	19.7 ± 5*	0.7 ± 0.002***	105.8 ± 11**
Vitamin C	N.T.	N.T.	0.003 ± 0.0001***	1.5 ± 0.02***

IC50, Inhibitory Concentration at 50%. Values are mean ± standard deviation (n = 3). Statistically significant differences: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. N.T., Not Tested.

Table 4. *G. delavayi* displayed a superior antioxidant capacity, with 19.7 mg eq vitamin C/mL oil, compared to *G. piloselloides* (7.8 mg eq vitamin C/mL oil). The FRAP antioxidant activities were directly proportional to the TPC, with *G. piloselloides* and *G. delavayi* exhibiting TPC values of 27.4 mg eq gallic acid/mL oil and 68.6 mg eq gallic acid/mL oil, respectively. Studies by other researchers have also linked the antioxidant activity of *Solanum elaeagnifolium* to its TPC (Bouslamti et al., 2022). These findings suggest that TPC compounds contribute significantly to the antioxidant activity of *G. delavayi*.

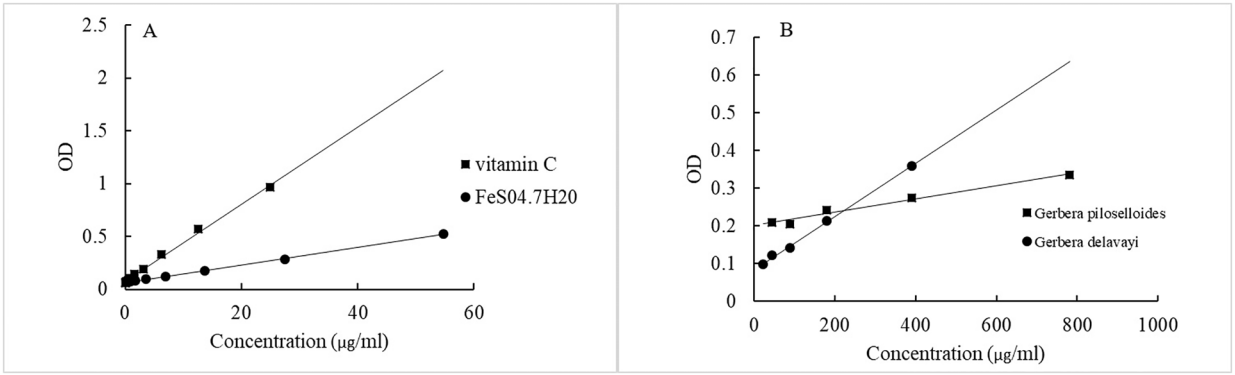

FIGURE 3
Concentration-dependent effects of antioxidants on the inhibition of the FRAP assay. **(A)** shows the correlation coefficients (r^2) for vitamin C (r^2 = 0.996) and $FeSO_4{\cdot}7H_2O$ (r^2 = 0.999); **(B)** depicts the correlation coefficients for *Gerbera piloselloides* (r^2 = 0.978) and $FeSO_4{\cdot}7H_2O$ (r^2 = 0.998).

TABLE 5 Minimum Inhibitory Concentrations (MICs) of essential oils from *Gerbera piloselloides* and *Gerbera delavayi* Franch, and Chloramphenicol against selected strains.

Microbial Strains	MIC (mg/mL) for EO from *Gerbera piloselloides*	MIC (mg/mL) for EO from *Gerbera delavayi*	MIC (mg/mL) for Chloramphenicol
S.aureus CMCC26003	12.5	12.5	5
Listeria ATCC 19111	6.3	6.3	10
Salmonella CVCC541	12.5	12.5	5
Pasteurella multocida C48-1	6.3	12.5	5
E. coli CVCC10141	12.5	12.5	10

3.3 Antimicrobial activity of essential oils

Bacterial infections continue to be a leading cause of mortality worldwide, a situation exacerbated by the persistent emergence of antibiotic resistance (Huemer et al., 2020). EO components derived from medicinal plants are noted for their high biological activity, and the quest for alternative antimicrobial agents to replace antibiotics has become a focal point of contemporary research (Coimbra et al., 2022).

This study assessed the antimicrobial potential of *G. piloselloides* and *G. delavayi* by evaluating their inhibitory effects against *Listeria*, *S. aureus*, *Salmonella*, *P. multocida*, and *E. coli*. The minimum inhibitory concentrations (MICs) of the EOs against these microbial strains are detailed in Table 5. The data reveal that EOgp demonstrated inhibitory activity against *Listeria* ATCC 19111, *S. aureus* CMCC26003, *Salmonella* CVCC541, *P. multocida* C48-1, and *E. coli* CVCC10141 with MICs of 6.3 mg/mL, 12.5 mg/mL, 12.5 mg/mL, 6.3 mg/mL, and 12.5 mg/mL, respectively. Similarly, Eogd exhibited efficacy against the same pathogens with MIC values of 6.3 mg/mL, 12.5 mg/mL, 12.5 mg/mL, 6.3 mg/mL, and 6.3 mg/mL, respectively. Notably, the antimicrobial potency of both EOs against *Listeria* surpassed that of chloramphenicol.

The biological effects of EOs are a consequence of the synergistic interaction of all molecules within the oil, and it is erroneous to attribute these effects to a single compound (Melo et al., 2020). The predominant components identified in both plant EOs were terpenes, natural products that serve diverse roles in various organisms and exhibit a wide array of structural diversity. *Listeria* has long been implicated as a primary agent of foodborne diseases in humans and animals. Cho et al. (2020) reported on the combined activities of gaseous oregano and thyme thymol EOs against *Listeria* monocytogenes. In the study by Said et al. (2016), oxygenated terpenes such as chamazulene-a degradation product, β-thujone, and camphor were identified as the main components of bioactive oils with antibacterial activity against *Listeria* monocytogenes. In the present manuscript, we also observed a high terpenoid content in both EOgp and EOgd, which may be responsible for their significant inhibitory effects against *Listeria*. The presence of these oxygenated terpenes in our extracts aligns with the findings of Said et al. (2016), suggesting that these compounds could be key contributors to the antibacterial properties observed. Our data further support the potential of natural plant-derived EOs as agents for controlling *Listeria* monocytogenes in antibacterial applications. However, it is important to note that while these EOs show promise, their safety profile must be thoroughly investigated before they can be considered for practical use.

4 Conclusion

In the present investigation, we assessed the chemical constituents, as well as the antioxidant and antimicrobial properties, of EOs extracted from the whole plants of *G. piloselloides* (EOgp) and *G. delavayi* (EOgd) via hydrodistillation. Our findings reveal that both EOgd and EOgp exhibit significant antioxidant capabilities and demonstrate differential inhibitory effects against five tested microbial strains. Notably, both essential oils exerted potent antibacterial effects against *Listeria* monocytogenes *in vitro*. These findings contribute to the growing body of evidence supporting the potential of these species as natural sources for the development of therapeutic products. Further research is needed to explore the specific mechanisms of action and safety profiles of these essential oils.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Author contributions

JW: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. WH: Data curation, Formal analysis, Resources, Software, Writing – original draft. JC: Formal analysis, Investigation, Visualization, Writing – review & editing. JH: Methodology, Supervision, Validation, Writing – review & editing. CK: Data curation, Resources,

Visualization, Writing – original draft. ZS: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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