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Detection and analysis of the volatile components in the essential oils of *Chrysanthemum* and *Opisthopappus* species and their hybrid progeny

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Abstract

Chrysanthemum and *Opisthopappus* are genera that include perennial herbaceous floral species, including excellent varieties with strong fragrances resulting from long-term artificial selection. Thus, they are ornamentally and economically important flower resources. In this study, a water distillation method was used to extract essential oils from the inflorescences of *Chrysanthemum* and *Opisthopappus* wild resources and hybrid progeny with high essential oil contents (*Chrysanthemum morifolium* 'xiangjin', *C. morifolium* 'xiangyun', *C. morifolium* 'xinjiboju', *Opisthopappus taihangensis*, *Opisthopappus longilobus*, *Chrysanthemum lavandulifolium*, and *C. morifolium* 'minghuangju'). The essential oil extraction rates were as follows: 1.17‰, 2‰, 1.67‰, 2.17‰, 0.43‰, 1‰, and 1.17‰. On the basis of HS-SPME-GC-MS (Headspace solid phase microextraction-gas chromatography-mass spectrometry), 225 volatile compounds were detected in the seven analyzed essential oil samples. Each essential oil had a relative volatile component content exceeding 0.3. The three most abundant compounds were olefins (46 types), alcohols (34 types), and esters (18 types). The volatile components with relatively high contents included thymol, D-camphor, pinene, eucalyptol, 2-terpineol, terpineol, trans-caryophyllene, and β-elemene. These volatile compounds have strong biological activities and are useful components of medicines and daily-use products. An evaluation of their antibacterial effects demonstrated that the essential oils of *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, and *C.* 'minghuangju' inhibited the growth of *Escherichia coli*. The *C. lavandulifolium* essential oil inhibited the growth of *Pectobacterium carotovorum*. The results of this study will provide researchers with an important theoretical basis for the development and application of *Chrysanthemum* and *Opisthopappus* essential oils.

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INTRODUCTION

Chrysanthemums are perennial floral species in the family Compositae[1]. They produce economically valuable ornamental flowers with multiple uses (e.g., as medicine, food, and tea)[2]. *Opisthopappus* Shih is a genus endemic to China and distributed in the Taihang Mountains. *Opisthopappus taihangensis* and *Opisthopappus longilobus*, which are the two species in this genus, are second-class, critically endangered, and protected plants in China[3]. Both species are resistant to drought, shade, and cold stresses. Their flowers are mostly white, making them an important germplasm resource with ecological, ornamental, and economic value[4−7].

To date, research on *Opisthopappus* plants has mainly focused on the genetic differences and interrelationships among *O. taihangensis* varieties[8], the physiological characteristics underlying drought resistance and transcriptome changes[9,10], genetic diversity[11], tissue culture[3,12,13], metal element contents in the soil in which *O. longilobus* is grown[11], and hybrid breeding involving *Opisthopappus* plants and related species[14]. However, the fragrance and essential oil components of *O. taihangensis* and *O. longilobus* flowers have not been comprehensively identified and characterized. Therefore, the objective of this study was to detect and analyze the fragrance and essential oil components of *Opisthopappus* flowers, with a particular focus on their antibacterial properties, which may be relevant for increasing the economic value of *Opisthopappus* species. The findings of this study may provide researchers and breeders with important information related to the protection, development, and utility of endangered *Opisthopappus* plants.

The commonly used methods for extracting essential oils include steam distillation, solvent extraction (including supercritical CO_2 extraction), and the application of high pressure. Characteristic aromatic and volatile oils have been extracted from flowers, leaves, fruits, stems, and other plant parts. The main volatile components in essential oils are terpenes. The terpenoids of essential oils are primarily monoterpenes and sesquiterpenes. In addition to olefin, essential oils also contain alcohols, aldehydes, ketones, esters, and other naturally occurring terpenoids. The essential oils extracted from aromatic plants are an extremely important raw material in the fragrance industry and are widely exploited in the daily-use chemical industry. Essential oils are also used for the production of food and medicine because of their diverse biological activities[15,16]. The essential oils of chrysanthemum have various uses in many fields (e.g., to enhance the storage of vegetables and meat and food preservation). Unlike pesticides and other harmful compounds, chrysanthemum essential oils are natural and safe for human consumption. Moreover, they have antibacterial and antioxidant properties as well as proven pharmacological effects that can prevent infections[17−19], eliminate inflammation, and remove scars. Therefore, these essential oils have been used in cosmetics and for massage therapy[20]. The compounds in aromatic essential oils with the strongest antibacterial effects are mainly terpenes, phenols, and alcohols, which are commonly produced by aromatic plants[21]. Previous research revealed that aromatic plant essential oils inhibit the activities of pathogenic bacteria by altering the fatty acid outer membrane, degrading the cell membrane, and inducing the leakage of metabolites and ions[22]. *Chrysanthemum indicum* essential oils can be included in moisturizing creams, mosquito repellents, laundry detergents, dishwashing liquids, shower gels, and shampoos to provide these daily-use products with the fragrance and antibacterial and anti-inflammatory properties of *C. indicum*[23]. The predominant volatile aromatic components of the *Chrysanthemum morifolium* 'Tianmeng Mountain Imperial' essential oil include 22 types of hydrocarbons, 9 types of alcohols, 7 types of esters, and 2 types of ketones[24]. Researchers used a water distillation method to extract the essential oils of *C. morifolium* 'Kunlun Snow' plants. A microdilution method used to investigate the inhibitory effect of the *C. morifolium* 'Kunlun Snow' essential oil on cryptococcus fungi revealed that the essential oil treatment can degrade the fungal cell membrane and alter cell membrane permeability[25]. Other researchers extracted essential oils from *C. morifolium* 'Jiugongxiangju' stems and leaves via water distillation. The essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS) and tested for their antibacterial properties. These analyses confirmed that *C. morifolium* 'Jiugongxiangju' essential oils have antibacterial effects[26]. Earlier research that applied GC-MS and GC-olfactometry techniques to identify the aromatic components in seven chrysanthemum essential oils revealed terpenes, esters, alcohols, ketones, acids, and aldehydes as the primary compounds[27]. Moreover, the antibacterial activities of the *C. morifolium* 'Chuju' essential oil and the underlying mechanism were determined on the basis of an SDS-PAGE analysis, an examination of DNA topoisomerase activity, and an oxidative respiration metabolism test. The results of the DNA topoisomerase analysis demonstrated that the *C. morifolium* 'Chuju' essential oil simultaneously inhibits the activities of topoisomerases I and II[28]. A previous investigation analyzed the essential oil components of three *Chrysanthemum* species (*C. coronarium*, *C. fuscatum*, and *C. grandiflorum*) by GC-MS, which revealed the insecticidal activities of the essential oils. Furthermore, essential oils extracted from various chrysanthemum species according to diverse methods can protect crops from pest infestations, with implications for small-scale grain production[29].

On the basis of the available wild resource collection and the findings of our earlier hybridization research, we selected *Chrysanthemum* and *Opisthopappus* wild species, varieties, and hybrid offspring for this study. Essential oils were extracted from *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, *C. lavandulifolium*, and *C.* 'minghuangju' and their compositions were analyzed. Specifically, HS-SPME-GC-MS technology was used to identify the volatile components of the extracted essential oils and to analyze the types of compounds and the components unique to particular plants. The results of this study may be relevant for characterizing chrysanthemum essential oil components with important industrial uses.

RESULTS

Extraction of essential oils

We previously collected wild resources of *O. taihangensis*, *O. longilobus*, and *C. lavandulifolium* as well as the hybrid offspring of *C. lavandulifolium* (i.e., *C.* 'xiangjin' and *C.* 'xiangyun'). We also collected *C.* 'xinjiboju' and *C.* 'minghuangju' plants, which produce flowers with a special aroma suitable for tea (Fig. 1). Regarding the hybrids and wild species with high essential oil contents, relatively large amounts of essential oils have been extracted from the inflorescences of *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, *C. lavandulifolium*, and *C.* 'minghuangju'. The test materials were obtained from

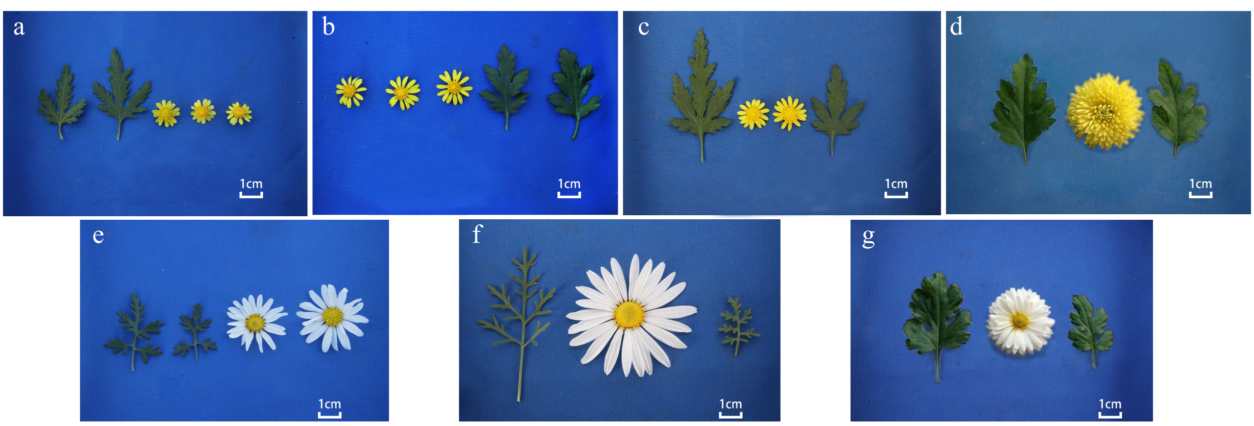

Fig. 1 Plant material used in the extraction of essential oil (a) *C.* 'xiangjin' flowers and leaves; (b) *C.* 'xiangyun' flowers and leaves; (c) *C. lavandulifolium* flowers and leaves; (d) *C.* 'xinjiboju' flower and leaves; (e) *O. taihangensis* flowers and leaves; (f) *O. longilobus* flower and leaves; (g) *C.* 'minghuangju' flower and leaves.

the Shunyi Beilangzhong Experimental Base of the Beijing Academy of Agriculture and Forestry Sciences. By using a water distillation method, we extracted differentially colored essential oils from the seven analyzed samples (Fig. 2). A further analysis revealed the essential oils of *C.* 'xiangyun' and *O. longilobus* were bright blue and black, respectively. The essential oil extraction rate was highest for *O. taihangensis* (2.17‰), followed by *C.* 'xiangyun' (2‰) and *C.* 'xinjiboju' (1.67‰) (Supplemental Table S1, Fig. 3).

Analysis of the volatile components of essential oils

By analyzing the components of the seven essential oils, a total of 225 compounds were identified and quantified. More specifically, 60 olefins (including enones and enols), 34 alcohols, 18 esters, 8 alkanes, 3 types of ketones, and 2 types of aromatic compounds were detected as the main volatile components.

The volatile components of the essential oil extracted from *C.* 'xiangjin' were separated and identified by GC-MS. A total of 62 aromatic compounds were detected; the ion current diagram is presented in Fig. 4a. The analysis of the volatile components of the *C.* 'xiangjin' essential oil (Supplemental Table S2) identified bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)- (24.62%), bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate (17.7%), and thymol (11.06%) as three highly abundant components.

The extracted volatile components of the *C.* 'xiangyun' essential oil were separated and identified by GC-MS. A total of 56 aromatic compounds were detected. The ion current diagram is presented in Fig. 4b, whereas the results of the analysis of the volatile components are provided in Supplemental Table S2. According to the data, D-camphor (bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-) (47.06%) was the component with the highest relative content in the *C.* 'xiangyun' essential oil.

The separation and identification of the volatile components of the *C.* 'xinjiboju' essential oil by GC-MS resulted in the detection of 64 aromatic compounds. The ion current diagram and the results of the analysis of the volatile components are presented in Fig. 4c and Supplemental Table S2, respectively. The data revealed the relatively high contents of pinene ((1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) (3.38%), eucalyptol (5.72%), bicyclo(3.3.1)non-2-ene (10.025%), bicyclo[3.1.1]hept-2-ene-6-one, 2,7,7-trimethyl- (12.48%), and bicyclo[2.2.1] heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (10.36%).

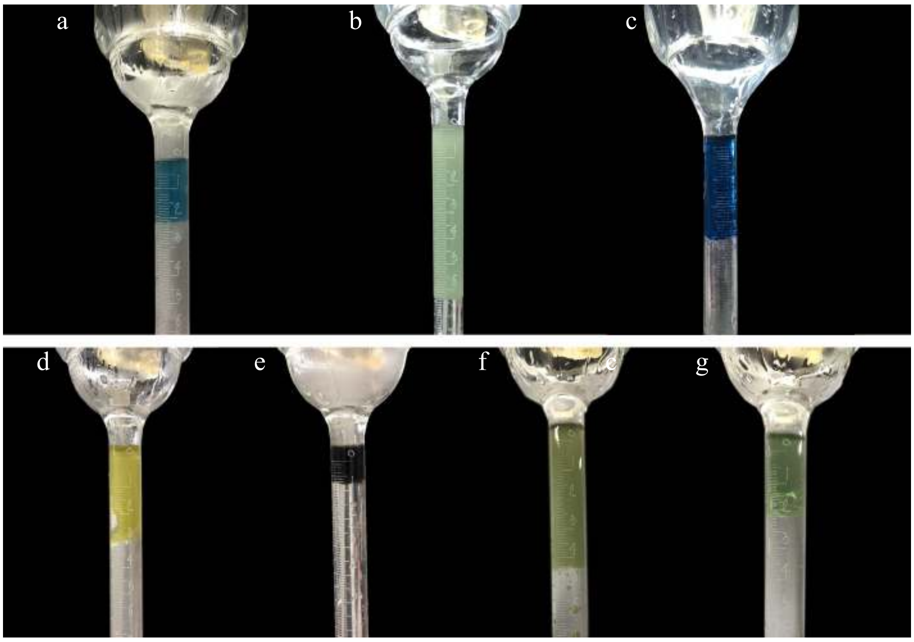

Fig. 2 Appearance of the essential oils extracted from the seven examined materials. (a) *C.* 'xiangyun'; (b) *O. taihangensis*; (c) *C.* 'minghuangju'; (d) *C.* 'xiangjin'; (e) *O. longilobus*; (f) *C.* 'xinjiboju'; (g) *C. lavandulifolium*.

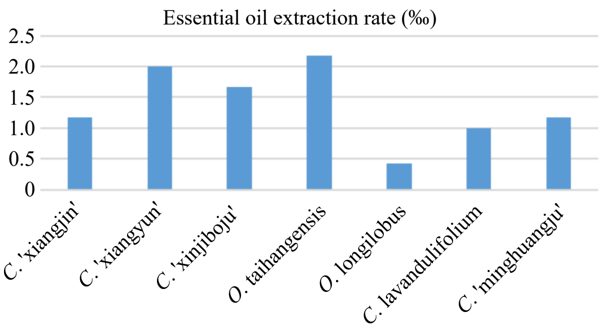

Fig. 3 Extraction rate of various essential oils.

The GC-MS analysis of the volatile components of the *O. taihangensis* essential oil detected 71 aromatic compounds. The ion current diagram is presented in Fig. 4d, whereas the results of the analysis of the volatile components are provided in Supplemental Table S2. The data indicated eucalyptol (5.74%), linalool (1,6-octadien-3-ol, 3,7-dimethyl-) (7.73%), bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1α,4β,5α)]- (15.26%), thujone (8.38%), and 2-naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- (7.41%) were highly abundant in the *O. taihangensis* essential oil.

The volatile components in the *O. longilobus* essential oil were separated and identified by GC-MS, resulting in the

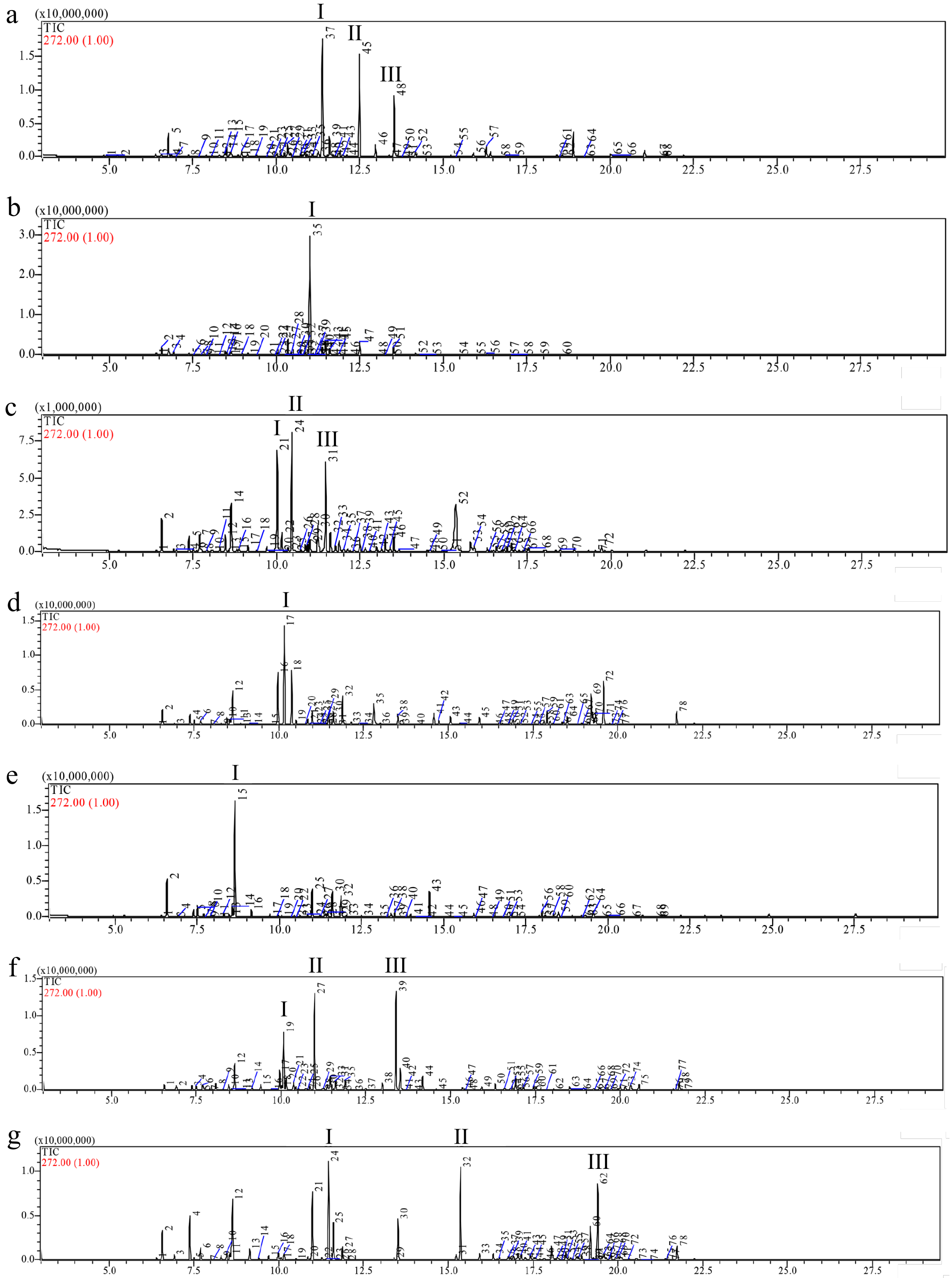

Fig. 4 Ion flow diagram of essential oil of 7 plant materials. (a) Ion current diagram of the *C.* 'xiangjin' essential oil. I: Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-; II: Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate; III: Thymol. (b) Ion current diagram of the *C.* 'xiangyun' essential oil. I: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-. (c) Ion current diagram of the *C.* 'xinjiboju' essential oil. I: Bicyclo(3.3.1)non-2-ene; II: Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-; III: Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-. (d) Ion current diagram of the *O. taihangensis* essential oil. I: Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-. (e) Ion current diagram of the *O. longilobus* essential oil. I: Eucalyptol. (f) Ion current diagram of the *C. lavandulifolium* essential oil. I: Eucalyptol; II: 2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-; III: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-. (g) Ion current diagram of the *C.* 'minghuangju' essential oil. I: Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-; II: Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-; III: Agarospirol.

detection of 66 aromatic compounds. The ion current diagram is presented in Fig. 4e. The results of the analysis of the volatile components are listed in Supplemental Table S2. The *O. longilobus* essential oil contained relatively large amounts of (1R)-(+)-α-pinene (9.06%) and eucalyptol (27.71%).

On the basis of the GC-MS analysis of the volatile components of the essential oil extracted from *C. lavandulifolium*, 74 aromatic compounds were detected. The ion current diagram and the results of the analysis of the volatile components are respectively presented in Fig. 4f and Supplemental Table S2. The data reflected the relatively high contents of eucalyptol (5.06%), 2H-pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl- (9.36%), and D-camphor (16.39%).

The volatile components in the *C.* 'minghuangju' essential oil separated and identified by GC-MS included 63 aromatic compounds. The ion current diagram is presented in Fig. 4g and the results of the analysis of the volatile components are provided in Supplemental Table S2. The data revealed the relatively high contents of (1R)-2,6,6-trimethylbicyclo[3.1.1]-hept-2-ene (3.28%), .beta.-phellandrene (4.55%), eucalyptol (8.02%), D(+)-camphor (7.58%), bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (11.63%), 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (4.44%), bornyl acetate (4.4%), cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.-beta.,4.beta.)]- (10.14%), 3-decanynoic acid (3.99%), and agarospirol (9.12%).

Comparison and analysis of the volatile components of several chrysanthemum essential oils

The three components that were relatively highly abundant in the *C.* 'xiangjin' essential oil were bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)- (24.62%), bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate (17.7%), and thymol (11.06%). The volatile component with the highest relative content in the *C.* 'xiangyun' essential oil was bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- (47.06%). The components with the highest relative contents in the *C.* 'xinjiboju' essential oil were (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (3.38%), eucalyptol (5.72%), bicyclo(3.3.1)non-2-ene (10.025%), bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl- (12.48%), and bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (10.36%). The components with the highest relative contents in the *O. taihangensis* essential oil were eucalyptol (5.74%), 1,6-octadien-3-ol, 3,7-dimethyl- (7.73%), bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]- (15.26%), thujone (8.38%), and 2-naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- (7.41%). The components with the highest relative contents in the *O. longilobus* essential oil were (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (9.06%) and eucalyptol (27.71%). The components with the highest relative contents in the *C. lavandulifolium* essential oil were eucalyptol (5.06%), 2H-pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl- (9.36%), and bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- (16.39%). The components with the highest relative contents in the *C.* 'minghuangju' essential oil were (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (3.28%), .beta.-phellandrene (4.55%), eucalyptol (8.02%), D(+)-camphor (7.58%), bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)- (11.63%), 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (4.44%), bornyl acetate (4.4%), cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- (10.14%), 3-decanynoic acid (3.99%), and agarospirol (9.12%).

A total of 133 aromatic compounds were detected during the comparison of the *C.* 'xiangjin', *C.* 'xiangyun', and *C. lavandulifolium* (wild species) essential oils (Fig. 5a). The following 15 components were common among the three essential oils (11.3% of the total components): pinene; β-phellandrene; β-myrcene; α-phellandrene; o-cumene; γ-terpinene; cis-4-carene; bicyclo[3.1.1]hept-2-ene-6-one, 2,7,7-trimethyl-; (S)-cis-verbenol; isocyclocitral; bicyclo[2.2.1]heptan-3-one, 6,6-dimethyl-2-methylene-; bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetic acid; myrtyl acetate; caryophyllin; and farnesene.

Regarding the comparison of the essential oils of three wild chrysanthemums (*C.* 'minghuangju', *C. lavandulifolium*, and *C.* 'xinjiboju'), a total of 152 aromatic compounds were detected, of which 15 were common to all three essential oils (9.9% of the total components) (Fig. 5b). The analysis of the essential oil contents indicated the aromatic compounds were most similar between the *C. lavandulifolium* and *C.* 'xinjiboju' essential oils.

The comparison of the essential oils extracted from the endangered *O. taihangensis* and *O. longilobus* samples detected 137 aromatic compounds, including 29 compounds that were present in both essential oils (26.9% of the total components) (Fig. 5c).

Verification of the antibacterial effects of chrysanthemum essential oils

The antibacterial effects of the essential oils extracted from *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, *C. lavandulifolium*, and *C.* 'minghuangju' were tested using *Escherichia coli* DH5α and *Pectobacterium carotovorum*

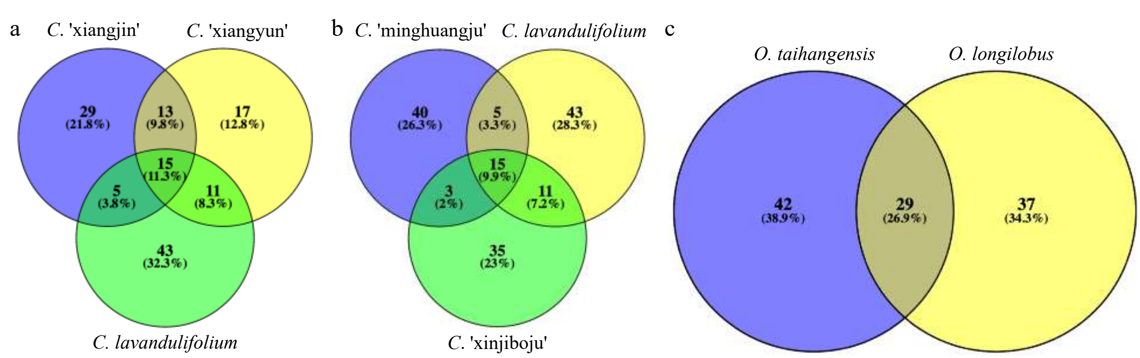

Fig. 5 Comparison of the essential oil components. (a) *C.* 'xiangjin', *C.* 'xiangyun', and *C. lavandulifolium* essential oil comparison group; (b) *C.* 'minghuangju', *C. lavandulifolium*, and *C.* 'xinjiboju' essential oil comparison group; (c) *O. taihangensis* and *O. longilobus* essential oil comparison group.

strain BC1, which are difficult to control. After a 24-h incubation at 37 °C, an examination of the *E. coli* cultures on solid medium in plates revealed growth inhibition zones for the *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, and *C.* 'minghuangju' essential oil treatments. In contrast, there was no growth inhibition zone for the control treatment. The *C. lavandulifolium* essential oil treatment clearly inhibited *P. carotovorum* growth, in contrast to the lack of inhibition for the control treatment. Of the seven analyzed essential oils, those of *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, and *C. lavandulifolium* had the strongest antibacterial effects (Table 1, Fig. 6).

Table 1. Antibacterial effects of seven essential oils.

Essential oil type name	Bacteriostatic ring width (mm)			The average value (mm)
C. 'xiangjin' essential oil	3.25	2.25	3	2.8 ± 0.55
C. 'xiangyun' essential oil	10	4.25	8	7.42 ± 3.17
C. 'xinjiboju' essential oil	3.25	4.25	11	6.17 ± 4.84
O. taihangensis essential oil	3.45	14	15	11.15 ± 7.7
O. longilobus essential oil	2.35	2.15	2	2.17 ± 0.18
C. 'minghuangju' essential oil	2.55	2.25	3	2.6 ± 0.4
C. lavandulifolium essential oil	4.25	3.75	4.50	4.17 ± 0.52

DISCUSSION

The most common chrysanthemum essential oil is yellow or green, In this study, the *C.* 'xiangyun' and *C.* 'minghuangju' essential oils were extracted at a relatively high rate and had a special color. Essential oils vary significantly in terms of color because of the diversity in their phytochemical components (e.g., ochre red myrrh essential oil, red orange essential oil with blue–purple fluorescence under high-intensity light, blue chamomile essential oil, black vetiver essential oil, and light green fragrant bergamot essential oil). The essential oil of *Matricaria recutita*, which belongs to the family Compositae, is similar to the *C.* 'xiangyun' and *C.* 'minghuangju' essential oils extracted in this study, both of which were blue. The chamomile essential oil is blue because of the presence of the organic compound azulene. The chamomile plant itself does not contain azulene, but it does contain a matricin. The matricin is the result of a series of chemical reactions (e.g., dehydration, hydrolysis, and decarboxylation) during the

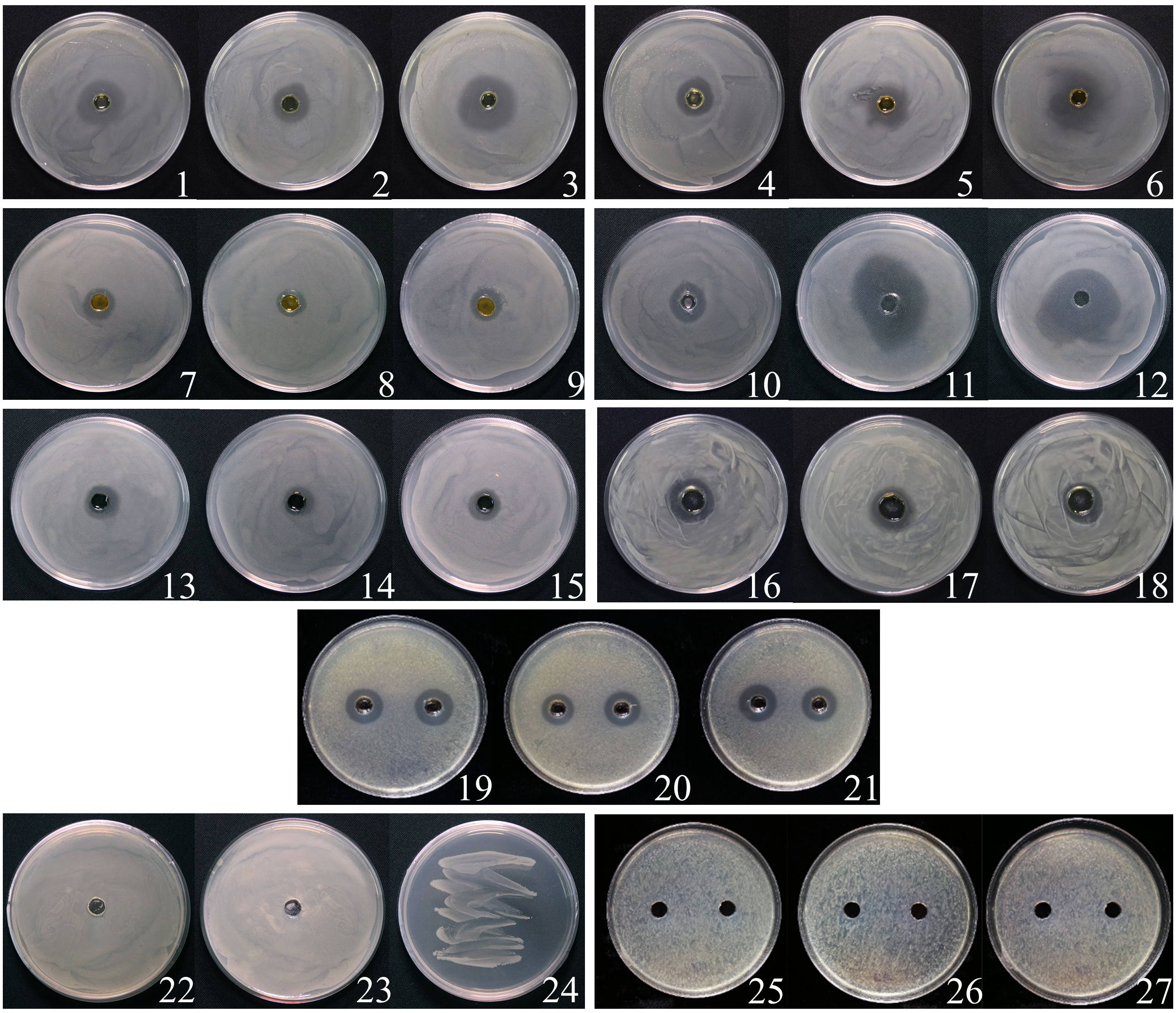

Fig. 6 Antibacterial effects of the essential oils. 1–3: *C.* 'xiangyun' essential oil; 4–6: *C.* 'xinjiboju' essential oil; 7–9: *C.* 'xiangjin' essential oil; 10–12: *O. taihangensis* essential oil; 13–15: *O. longilobus* essential oil; 16–18: *C.* 'minghuangju' essential oil; 19–21: *C. lavandulifolium* essential oil; 22–24: *E. coli* DH5α control; 25–27: *P. carotovorum* control.

extraction process. The matricin are responsible for the blue coloration of the chamomile essential oil. Naturally obtained azulene may produce a variety of colors, including blue, green, blue–purple, or even red–purple. In addition to German chamomile, some yarrow plants also contain matricin. Thus, their essential oil also appears blue because of the presence of the azure hydrocarbon. Moreover, some mugwort plants, such as wormwood, also produce blue essential oils.

Regarding the essential oil odors, we revealed that the 225 compounds detected in seven essential oil samples included caryophyllene (trans-caryophyllene), o-cumene, γ-terpinene, (1S, 3R)-cis-4-carene, β-phellandrene, and α-phellandrene. The relatively abundant compounds included thymol, D-camphor, eucalyptol, borneol, linalool, syringone, (−)-α-cubic benzene, α-epoxy-terpineol acetate, pinene, β-phellandrene, 4-terpene alcohol, borneol acetate, beta-elemene, 3-decenoic acid, and linalool, of which pinene, D-camphor, and eucalyptol were the main essential oil constituents with a pleasant aroma as well as biological activities.

Among the identified compounds, the representative acyclic monoterpenes were myrcene, linalool, and phellandrene; the representative monocyclic monoterpenes were terpineol and terpinene; the representative bicyclic monoterpenes were pinene, camphene alkene, and camphor; and the representative sesquiterpenes were farnesol and caryophyllene. Small aliphatic compounds, including alcohols, aldehydes, ketones, and acids, were also frequently detected in the analyzed essential oils.

Among them, caryophyllene, camphor and other substances are generally the main volatile components of wild chrysanthemum, ground cover chrysanthemum and some wormwood plants[29,30]. Both camphor and camphene have camphor-type aromas, so the odor of *O. longilobus*, *C.* 'xiangyun' and *C. lavandulifolium* essential oil with relatively high contents of camphor and camphene are relatively cool and slightly irritating. The highest relative content in *O. longilobus* essential oil is Eucalyptol. Eucalyptol is a monoterpenoid compound with a camphor-like odor.

Linalool, which was identified as a component of the Taihang chrysanthemum essential oil, produces an aroma that is similar to that of lily of the valley and is one of the most important compounds in the fragrance and flavor industries. Additionally, it decreases the pain responses mediated by various neurotransmitter systems[31] and has antioxidant, anti-inflammatory, anticancer, antispasmodic, and antiparasitic activities[32]. However, a previous study indicated that more than 95% of the linalool produced worldwide is used as an aromatizer and flavor enhancer[33]. Plants with high linalool contents include oregano (90.3%), coriander (73.7%), salvia (70.6%), spinach (61.5%), basil (43.1%), prickly ash (46.1%), and marjoram (41.2%)[34]. The total annual demand for L-linalool is 12,000 tons, but only 5,400 tons are produced worldwide under natural conditions.

Camphor, which was detected as the most abundant component in the *C.* 'xiangyun' essential oil, has a distinctive spicy aroma and taste, although its taste eventually dissipates. Moreover, camphor has a strong inhibitory effect on pests and bacteria. Specifically, it inhibits *E. coli* growth because of its detrimental effects on bacterial metabolism, chemotaxis, and anti-stress-related responses[35].

Eucalyptol, which has the highest relative content in O. longilobus essential oil, is anti-inflammatory and analgesic, reducing pain and inflammation through mechanisms that may involve antioxidant effects[36].

Beta-phellandrene is a common constituent of aromatic plant essential oils. It is a natural bioactive insecticide[37]. Many bioinsecticides contain β-phellandrene as an important active ingredient[38]. Caryophyllene is a bicyclic sesquiterpenoid that can be used to alter the flavor of foods containing clove, nutmeg, and citrus components[39]. Furthermore, γ-terpinene and α-terpinene often coexist and are associated with citrus aromas[40]. Phellandrene is a spice intermediate that is also associated with citrus aromas. It is commonly used as a natural active ingredient in biopesticides. It can also be used as a synthetic raw material for the production of terpene resins and menthol. The antibacterial, antiseptic, and insecticidal components of chrysanthemum essential oils, including β-thumberene, β-phellandrene, and other volatile compounds, are irritating to the skin. Thus, chrysanthemum essential oils should be diluted with other essential oils before they are used.

Essential oils produced by aromatic plants have a variety of functions. For example, most of the compounds that are responsible for the volatilized odors of essential oils also have strong antibacterial effects. Furthermore, in addition to inhibiting the growth of bacteria and fungi, the volatile compounds in essential oils can attract insect pollinators, prevent pest infestations, and inhibit the growth of other plants (i.e., allelopathic effect). As pure natural bacteriostatic agents, plant essential oils leave minimal residues and are volatile, highly degradable, and relatively non-toxic (i.e., environmentally friendly), which is in sharp contrast to many synthetic pesticides and insecticides. Therefore, they have been included in horticultural products, food, medicine, and beauty products. They are also currently the focus of considerable research and development across industries. In this study, we extracted chrysanthemum essential oils and verified their antibacterial effects.

MATERIALS AND METHODS

Plant materials

The experimental materials (*C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, *C. lavandulifolium*, and *C.* 'minghuangju') were collected from the Shunyi Beilangzhong Experimental Base of the Beijing Academy of Agriculture and Forestry Sciences (Beijing, China).

Extraction of essential oils

All water distillers used for extracting essential oils were 10-L desktop essential oil pure water distillers (Luosha Biological Company China, Jiangsu). The collected fresh plant samples were weighed and then added to the distillers. The fresh plant sample weights were as follows: *C.* 'xiangjin': 3,000 g; *C.* 'xiangyun': 1,060 g; *C.* 'xinjiboju': 3,000 g; *O. taihangensis*: 3,000 g; *O. longilobus*: 2,300 g; *C. lavandulifolium*: 3,000 g; and *C.* 'minghuangju': 3,000 g. After adding 6 L deionized water, an electric ceramic stove was used to heat the solution (2,000 W) to boiling, after which the stove was adjusted to 1,200 W and the condensed water supply was turned on for a 2-h distillation. The essential oil content after the extraction was recorded and then the essential oil extraction rate was calculated as follows: essential oil extraction rate (‰) = essential oil weight/material sample weight × 1,000. Additionally, the state of the essential oils was examined. Upon completion of the distillation, the distillate was collected and the pure dew was removed to obtain the essential oil with a strong fragrance at −4 °C and in darkness. The volatile components of the essential oils extracted by water distillation were subsequently analyzed.

Analysis of the volatile components in the essential oils

The Shimadzu GCMS-QP2010 system was used to detect and analyze the essential oils from *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, *C. lavandulifolium*, and *C.* 'minghuangju'. The volatile compounds in the essential oils were detected and analyzed by HS-SPME-GC-MS. The chromatographic column was a DB-5MS quartz capillary column (30 m × 0.25 mm × 0.25 μm). The carrier gas was He (99.999%) and the injection port temperature was 250 °C. Additionally, the split injection mode was used, with a total flow rate of 17.1 ml/min. The split ratio was 10, the ion source temperature was 200 °C, and the interface temperature was 250 °C. The MS analysis was completed using a detector at 1 kV and a mass scan range of 30–500 m/z in the full scan mode. The heating program was 30 min long, with an initial temperature of 50 °C, which was maintained for 2 min, after which it was increased to 220 °C at 8 °C/min and then maintained for 6.75 min. The solvent cut time was 3 min. The solid phase extraction was performed using a 50/30 μm DVB/CAR PDMS fiber. The sample was added to a 15-ml glass bottle and then placed in a water bath at 50 °C. The fiber was inserted into the headspace for a 30-min extraction. The extract was desorbed in the injection port at 250 °C for 3 min.

Essential oil samples were collected after adding 5 μl methanol to 995 μl formulated oil. The resulting 1-ml solution was diluted 10-fold and then added to a 5-μl sample collected from the headspace of the 15-ml glass bottle.

Verification of the antibacterial properties of the essential oils

The standard strains of *E. coli* (DH5α) and *P. carotovorum* (BC1) were used to assess the antibacterial effects of the essential oils. Specifically, bacterial cultures (1 × 10^8 CFU/ml) with no antibiotics were uniformly applied on solid LB medium in plates. A 100-μl aliquot of each essential oil was added to a small hole (1 cm diameter) in the middle of the plates. More specifically, the extracted essential oils of *C.* 'xiangjin', *C.* 'xiangyun', *C.* 'xinjiboju', *O. taihangensis*, *O. longilobus*, and *C.* 'minghuangju' were added to the plates with *E. coli* (DH5α), whereas the essential oil of *C. lavandulifolium* was added to the plates with *P. carotovorum* (BC1). Additionally, the plates with no essential oil added to the hole were used as the controls. After a 24-h incubation at 37 °C, the bacterial growth on each plate was examined.

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Conflict of interest

The authors declare that they have no conflict of interest.

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