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ORIGINAL ARTICLE

Chemical composition and antimicrobial activities of volatile oil extracted from *Chrysanthemum morifolium* Ramat.

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Abstract Volatile oil in *Chrysanthemum morifolium* Ramat (*C. morifolium*) was extracted by the method of water vapor distillation and its chemical components was identified by gas-chromatography coupled with mass spectrometry (GC–MS). The volatile oil are evaluated for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus, Salmonella enteritids, Pseudomonas aeruginosa* and *Bacillus subtilis*. Effects of surfactant, temperature, pH and ultraviolet light on antibacterial activity stability of volatile oil were analyzed too. Total 56 compounds were identified in *C. morifolium* volatile oil. The main constituents in *C. morifolium* volatile oil were monoterpenes and sesquiterpenes compounds, including hydrocarbons, esters, aldehydes, ketones, phenols and organic acids. α-curcumene was the most abundant volatile component (12.55%). The volatile oil showed promising antibacterial activity against 5 selected strains. The inhibitory effect on *P. aeruginosa* exhibited maximum inhibition zone diameter 20.43 mm, and *E. coli* showed 12.29 mm. The volatile oil treated with surfactant Tween 20 showed the strongest antibacterial activity, followed by Tween 80 and the SDS lowest, which showed the lowest. pH also had different effect on antibacterial activity stability of the *C. morifolium* volatile oil. No significant difference effect on antibacterial activity stability of volatile oil was observed with temperature and UV treatment.

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Introduction

Chrysanthemum morifolium Ramat which belongs to the tribe *Anthemideae* in the *Asteraceae* family has been widely cultivated for more than 3000 years in China. The dry capitulum of the genera *Chrysanthemum* plants showed many benefits to human health. Its flowers are frequently taken in the manner of tea drinking, as well as used in Chinese traditional medicine (Matsuda et al. 2002). Furthermore, it's one of the specialties in Zhejiang Province and one of the first batches of medicines approved by Chinese Health Ministry as authentic medicinal and edible plant (Wu et al. 2009).

Volatile oil is the odorous, volatile products of the secondary metabolism of plants by water steam distillation. They are used as fragrances and flavors in the perfume and food industries and recently as well as aromatherapy (Enan 2001; Isman 2006). Many reports had focused on volatile flavor composition and pharmacological effects of *Chrysanthemum* species (Choi and Kim 2011; Haouas et al. 2012; Chang and Kim 2013). Haouas et al. (2012) analyzed the components of three species of *Chrysanthemum* growing in Tunisia (*C. coronarium*, *C. fuscatum*, and *C. grandiflorum*) by GC–MS method. The result showed that the volatile oils obtained from leaves and flowers shared a similar qualitative composition, but the relative proportions of the constituents were quite different. The main common constituents of all the volatile oils were *a*-pinene, myrcene, *a*-humulene, *β*-caryophylene, spathulenol, and caryophyllene oxide. *Chrysanthemum* species are generally

considered to be have a broad spectrum biologically activity, such as antioxidant, anticancer, aldose reductase inhibition;anti-inflammation, curing osteoporosis, antifungal activity and antimicrobial activities (Cheng et al. 2005; Cheon et al. 2009; Lee et al. 2009; Chang et al. 2010).

In the present study, chemical compositions of volatile oil obtained from *C. morifolium* were identified by GC–MS, the effect of surfactant, temperature, pH and UV on the antibacterial activity against several selected bacteria were evaluated too. It is aim to evaluate the valuable chemical materials, provide accurate compositional data as index of *C. morifolium* and provide a scientific basis for comprehensive development and utilization in *C. morifolium*.

Materials and methods

Materials

Chrysanthemum morifolium Ramat. Hemsl was picked in October 2015 from Baoxing County in Sichuan Province, China, and was supplied by Mao Yuan Agricultural Science and Technology Development Co., Ltd. The flowers dried at 40 °C for 12 h and was ground with a micro plant grinding machine to fine powder, then pulverized and sieved through a 0.15 mm sieve, sealed and set aside for further use.

Anhydrous acetone, Anhydrous sodium sulfate, Anhydrous alcohol, Agar powder (all of analytical grade) were purchased from Chengdu Kelong Chemical Reagent Co., Ltd. (Chengdu, China); Peptone, beef extract from Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China).

The antibacterial activities of the volatile oil were tested against Gram negative strain, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* CICC 20612 and Gram positive strain. *Escherichia coli* ATCC 25922, *Salmonella enteritids* CICC 21482, *Pseudomonas aeruginosa* ATCC 27853. The strains were stored at − 80 °C in Microbiology Laboratory, College of Food Science, Sichuan Agricultural University.

Volatile oil extraction

30 g *C. morifolium* powder was placed in the volatile oil extractor, 400 mL distilled water was added, heated to reflux extraction for 9 h, dried with Na_2SO_4 to get *Chrysanthemum* volatile oil. In this study, hydro distillation extraction (HDE) method (Schultz et al. 1997) was used since it does not use organic solvents capable of contaminating the plant volatile oil.

It exactly weighed 0.80 g *C. morifolium* volatile oil and used anhydrous acetone to volume to 10 mL, over 0.45 μm organic membrane, took 1 mL to get on GC–MS determination.

Components analysis of volatile oils by GC–MS analysis

The analysis of the volatile oil was performed using Agilent 7890A-5975C GC–MS (Agilent technologies company in the United States), equipped with a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm) for the separation. The mass-selective detector was operated in electron impact ionization (EI) mode with a mass scan range from m/z 40–550 at 70 eV. Helium (He) was used as the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 250 and 170 °C, respectively. A sample of 1.0 μL of volatile oil was injected manually using a 5:1 split ratio. The temperature program was as follows: initial temperature of 75 °C held for 2 min, followed by the ramping up of the temperature at a rate of 5 °C/min up to 130 °C, which was held for 7 min, 2.5 °C/min up to 170 °C for 10 min, finally 5 °C/min up to 250 °C for 10 min. The temperature of the MSD transfer line was 250 °C. The temperature of the ion source was 230 °C, and that of the MS quadrupole was 280 °C.

The components were identified by comparing their GC retention indices, NIST mass spectral search program (Version 2.0, National Institute of Standards and Technology), and mass spectra with published data. The compounds were tentatively identified on the basis of their retention times and by interpretation of MS fragmentation patterns with those of standard libraries NIST11. Amounts of the detected volatiles were based on comparison of their peak areas. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to compare significant differences (at the 5% significance level, $p < 0.05$) between treatments using the statistical package SPSS 20.0 for Windows.

Antibacterial activity analysis

Antibacterial tests carried out by the Oxford cup method using 200 μL of suspension containing 10^6 CFU/mL of bacteria spread on nutrient agar (NA) medium. Oxford cups (6 mm in diameter) were placed on the inoculated agar, and 100 μL of volatile oil was added into each Oxford cup. Ethanol solution (95%) was used as the negative control, placed for 2 h at 4 °C to completely diffused into the petri plates, then incubated at 37 °C for 24 h. The inhibition zone diameter was measured with a vernier. Tests were performed in triplicate.

Minimum inhibitory concentration (MIC) analysis

Volatile oils were added into the nutrient agar petri plates ranged at a concentration of 1.30, 0.67, 0.325, 0.163 and 0.081%, respectively. Test strain suspension was adjusted to 10^3–10^4 CFU/mL. For each test, a suspension of the tested strain (100 μL) was spread on the petri plates, and incubated at 37 °C for 24 h. The MIC is defined as the lowest concentration of the volatile oil at which the microorganism does not demonstrate visible growth on the plates.

Antibacterial stability analysis

Surfactants

Tween 20, Tween 80, and SDS were used to evaluate antibacterial activities of volatile oil dissolved in surfactants. The volatile oil for antibacterial tests was prepared with a concentration of 10% (v/v) solution filtered by through 0.22 μm filters. Antibacterial activities were measured according to the method described previously, Tween 20, Tween 80, and SDS surfactants as the control respectively.

pH

Volatile oil was diluted to a concentration of 10% (v/v) solution. Solution pH values were adjusted to 2, 4, 7, 9, 11 with 1 mol/L of NaOH or 1 mol/L of HCl respectively, and then solution was adjusted to near neutral pH 15 min later, and filtered by 0.22 μm filter. Antibacterial activities were measured according to the method above, 95% ethanol as a control.

Temperature

Volatile oil was dissolved in 95% ethanol to a concentration of 10% (v/v) solution and filtered by 0.22 μm Organic filter membrane, then placed at 40, 60, 80, 100 and 121 °C for 15 min, respectively. Antibacterial activities were tested according to the method above.

UV irradiation

Volatile oil was dissolved in 95% ethanol to a concentration of 10% (v/v) solution and filtered by 0.22 μm organic filter membrane. The irradiation was done by lamp with power of 8 W. The UV radiation with waves of 253.7 nm was performed from the distance of lamp to the sample of 40 cm. The following times of irradiation 10, 20, 30, 40 and 50 min were used, respectively. Antibacterial activity of volatile oil exposed to UV irradiation was determined with the method previously.

Results and discussions

Identification of chemical composition of *C. morifolium* volatile oil

The chemical composition of the volatile oil was analyzed by GC–MS, and the result is listed in Table 1. As presented in Table 1, fifty-six components were identified, constituting 42.00% of the volatile oil composition of *C. morifolium*. Hydrocarbons represented 32.00% in oil was the most abundant compound, followed by esters (2.03%), organic acids (3.65%), ketones (1.97%), alcohols (1.69%), aldehydes (0.62%), phenols (0.04%). The major components in the volatile oil of *C. morifolium* are monoterpenes and sesquiterpenes, including α-curcumene, α-farnesene, *β*-bisabolene, bisabolol, capric acid, linoleic acid, *n*-heptadecane, nonadecane and *n*-pentacosane. Among them the highest amount is α-curcumene (12.55%), this is consistent with the findings of Chang and Kim (2009). In the report, thirty-six volatile components of *Chrysanthemum* constituted 58.15% of the total volatile composition were characterized tentatively, consisting of 19 hydrocarbons, 7 alcohols, 2 ketones, 2 esters, 4 aldehydes, 1 oxide, and 1 miscellaneous component. The predominant components of *Chrysanthemum* were α-curcumene, and α-sesquiphellandrene. Whereas, α-pinene, 1,8-cineol, and chrysanthenone were the main aroma compounds of in Korea.

Antibacterial activity of *C. morifolium* volatile oil

Chrysanthemum morifolium volatile oil is liquid at room temperature and does not dissolve in water. In the experiments, volatile oil can be dissolved in ethyl acetate, 95% ethanol, isopropyl alcohol, acetone, *n*-octanol, *n*-hexane and *n*-heptane. But the above organic solvents had certain suppression bacterial effect for tested strains. The pre-test results showed that 95% ethanol's inhibition effect on tested strains is relatively weak, so 95% ethanol was used as solvent for volatile oil. The inhibitory effects are presented in Fig. 1.

In Table 2, it had a certain inhibitory effect on the test strains and the inhibition zone diameter varied from 7.64 to 8.51 mm. 10% volatile oil solution diluted by 95% of ethanol had obvious inhibitory effect on *E. coli*, *S. aureus,* S. *enteritidis*, *P. aeruginosa* and *B. subtilis*. Wherein the maximum inhibition was *P. aeruginosa* and inhibition zone diameter was 20.43 mm. The minimum inhibitory effect was *E. coli* and inhibition zone diameter is 12.29 mm,

Table 1 Chemical Composition of the volatile oil from *C. morifolium*

No.	Constituent	Molecular formula	Molecular weight	Composition[a] (%)
Hydrocarbon				32.000
1	1,2,5,5-Tetramethyl-1,3-Cyclopentadiene	C_9H_{14}	122	0.023
2	2,4-Dimethyl Styrene	$C_{10}H_{12}$	132	0.035
3	2-Ethyl-*p*-Xylene	$C_{10}H_{14}$	134	0.023
4	Ionene	$C_{13}H_{18}$	174	0.144
5	α-Cedrene	$C_{15}H_{24}$	204	0.121
6	β-Caryophyllene	$C_{15}H_{24}$	204	0.269
7	β-Farnesene	$C_{15}H_{24}$	204	0.582
8	(−)-Isocaryophyllene	$C_{15}H_{24}$	204	0.278
9	γ-selinene	$C_{15}H_{24}$	204	0.117
10	α-Curcumene	$C_{15}H_{22}$	202	12.545
11	α-farnesene	$C_{15}H_{24}$	204	3.443
12	β-Bisabolene	$C_{15}H_{24}$	204	1.029
13	7-α-Selinene	$C_{15}H_{24}$	204	0.724
14	β-Sesquiphellandrene	$C_{15}H_{24}$	204	0.871
15	3-Octadecene	$C_{18}H_{36}$	252	0.092
16	1,2,3,4-Tetrahydro-1,6,8-Trimethylnaphthalene	$C_{13}H_{18}$	156	0.077
17	1,2,3,4-Tetrahydro-1,5,7-Trimethylnaphthalene	$C_{13}H_{18}$	156	0.061
18	1,1,6-Trimethyl-1,2-Dihydronaphthalene	$C_{13}H_{16}$	154	0.349
19	Cyclopentadecane	$C_{15}H_{30}$	210	0.128
20	*n*-Heptadecane	$C_{17}H_{36}$	240	3.185
21	Octadecane	$C_{18}H_{38}$	254	0.142
22	Nonadecane	$C_{19}H_{40}$	268	4.179
23	*n*-Eicosane	$C_{20}H_{42}$	282	0.103
24	*n*-Heneicosane	$C_{21}H_{44}$	296	0.060
25	2-Methyleicosane	$C_{21}H_{44}$	296	0.117
26	*n*-Docosane	$C_{22}H_{46}$	310	0.491
27	*n*-Tetracosane	$C_{24}H_{50}$	338	0.251
28	*n*-Pentacosane	$C_{25}H_{52}$	352	2.024
29	10-Methylicosane	$C_{21}H_{44}$	296	0.163
30	*n*-Heptacosane	$C_{27}H_{56}$	380	0.374
Esters				2.034
31	Phthalic Acid-Butyl Tetradecyl Ester	$C_{26}H_{42}O_4$	418	0.130
32	Dibutyl Phthalate	$C_{16}H_{22}O_4$	278	0.061
33	Ethyl Palmitate	$C_{18}H_{36}O_2$	284	0.397
34	Methyl Linoleate	$C_{19}H_{34}O_2$	294	0.235
35	Acetic Acid-3,7,11,15-Tetramethyl-Hexadecyl Ester	$C_{22}H_{44}O_2$	340	0.342
36	Ethyl Linoleate	$C_{20}H_{36}O_2$	306	0.404
37	Ethyl Linolenate	$C_{20}H_{34}O_2$	306	0.330
38	Di(2-Ethylhexyl) Phthalate	$C_{24}H_{38}O_4$	390	0.060
39	*L*-Bornyl Acetate	$C_{12}H_{20}O_2$	292	0.075
Aldehydes				0.616
40	Isocyclocitral	$C_{10}H_{16}O$	152	0.565
41	*P*-Isopropylbenzaldehyde	$C_{10}H_{12}O$	148	0.009
Phenols				0.042
42	2,2′-Methylene Bis (6-Tert-Butyl-4-Methyl)Phenol	$C_{23}H_{32}O_2$	340	0.042
Alcohols				1.689
43	(−)-Terpinen-4-ol	$C_{10}H_{18}O$	154	0.033

Table 1 continued

No.	Constituent	Molecular formula	Molecular weight	Composition[a] (%)
44	2-Methyl-5-(1-Methyl Ethenyl)-2-Cyclohexen-1-ol	$C_{10}H_{16}O$	152	0.021
45	Bisabolol	$C_{15}H_{26}O$	222	1.177
46	Isophytol	$C_{20}H_{40}O$	296	0.067
47	Phytol	$C_{20}H_{40}O$	296	0.391
Ketones				1.974
48	Camphor	$C_{10}H_{16}O$	152	0.02
49	Phytone	$C_{18}H_{36}O$	268	0.279
50	2-Heptadecanone	$C_{17}H_{34}O$	254	0.219
51	2-methylene-5-(1-methylethyl)cyclohexanone	$C_{16}H_{28}O_2$	252	0.282
52	1-Tert-Butyl-7-Methoxynaphthalene	$C_{15}H_{18}O$	214	1.174
Organic acids				3.646
53	Capric Acid	$C_{10}H_{20}O_2$	172	1.005
54	Palmitic Acid	$C_{16}H_{32}O_2$	256	0.572
55	Linoleic Acid	$C_{18}H_{32}O_2$	280	2.028
56	*β*-Acetylacrylic Acid	$C_5H_6O_3$	114	0.041
Total				42.000

[a]Percentage of relative peak aera

which may be the result of the inhibitory effect of ethanol and volatile oil synergy.

Through analysis of variance, the sample group showed significant difference compared with the untreated group ($p < 0.01$) in inhibitory effect on 5 tested strains. It showed that volatile oil inhibited 5 tested strains. The inhibition size ranked *P. aeruginosa* > S. enteritidis > *B. subtilis* > *S. aureus* > *E. coli*. The plant volatile oils are tremendous enriched with terpenoids which exert inhibitory action against microorganisms by disrupting their membranes (Burt 2004). In the present work, the main components of *C. morifolium* volatile oil are monoterpenes and sesquiterpenes, including α-curcumene, α-farnesene, β-bisabolene, bisabolol, capric acid, linoleic acid, n-heptadecane, nonadecane and n-pentacosane. The antibacterial activity of the oils could, in part, be associated with α-curcumene, which was previously reported for its antibacterial activity (Schwob et al. 2002). In addition, the components in lower amount such as β-bisabolene and bisabolol, which are already known to exhibit antibacterial activity (Forrer et al. 2013; Kamatou and Viljoen 2010), could also contribute to the antibacterial activity of the oils.

The MIC of volatile oil against 5 tested strains showed that minimum inhibitory concentration of *E. coli* and *S. aureus* was 1.30%, *S. enteritidis* and *B. subtilis* was 0.67%, *P. aeruginosa* was 0.32%. Currently, the antibacterial mechanism of volatile oil is not very sure. Therefore, the further studies need to explore the components of the volatile oil which play a key role in the antibacterial effect. Özcan and Erkmen (2001) analyzed the antimicrobial activity of the essential oil of nine plant spices. The results showed that the essential oil tested varied in their antimicrobial activity. Individual or combinations of plant essential oils may provide an efficacious mixture for the inactivation of pathogenic and spoilage microorganisms, and to achieve adequate shelf-life of foods. Hu et al. (2009) analyzed the volatile oils from the flowers, leaves, barks, roots and fruits of *A. brachypus* were extracted individually by hydro distillation, and their chemical constituents were isolated and characterized by means of GC–MS. The antimicrobial activities of the volatile oils was evaluated against 11 microorganisms (9 strains bacteria and 2 strains yeast) using agar disc diffusion and broth micro-dilution methods. The bacteria, including gram-positive bacteria and gram-negative bacteria, were more sensitive to the oils than yeasts.

Antibacterial activity stability of volatile oil

Volatile oils are particularly prone to quantitative and quantitative change due to environmental factors. In consideration of changes of volatile oil which may influence antibacterial activity. The effect of environmental factors including surfactant, temperature, pH and ultraviolet was showed.

Surfactants

In Fig. 2, volatile oil dissolved in different surfactants showed significant activity against selected strains. The inhibition zone diameter against was *E. coli, S. aureus, S. enteritidis, P. aeruginosa and B. subtilis* were 8.17 to

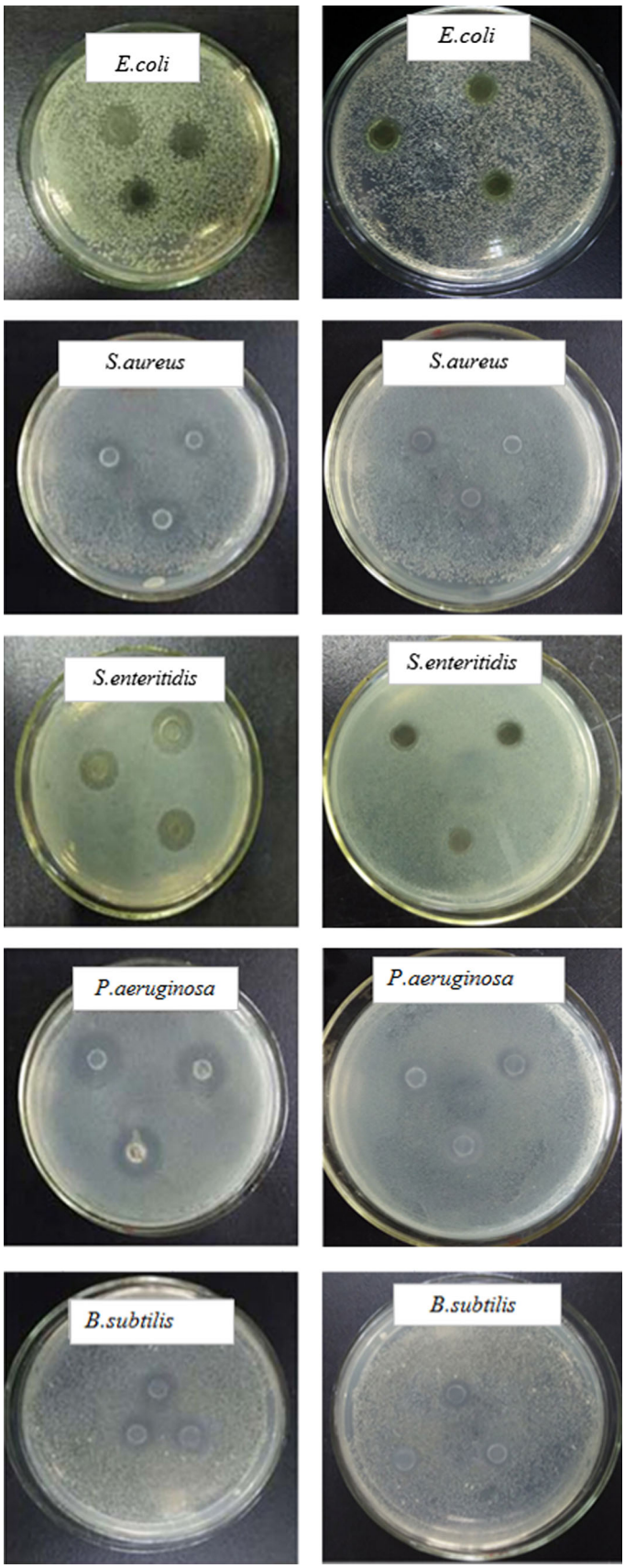

Fig. 1 the inhibitory effect of the *Chrysanthemum morifolium* R. volatile oil on 5 kinds of tested microbes, on the left is the experimental group, the right is the control group (95% ethanol)

Table 2 The inhibitory effect of *C. morifolium*. volatile oil on *E. coli*, *S. aureus*, *S. enteritidis*, *P. aeruginosa, B. subtilis* 5 kinds of test bacteria and the minimum inhibitory concentration

Microorganisms	Inhibition zone diameter (mm)		MIC
	10% Volatile oil of *Chrysanthemum morifolium* R.	Control	Concentration (v/v) (%)
E. coli	12.29 ± 0.49	8.51 ± 0.61	1.30
S. aureus	13.14 ± 0.60	7.64 ± 0.43	1.30
S. enteritidis	15.20 ± 0.64	8.26 ± 0.13	0.67
P. aeruginosa	20.43 ± 0.69	8.38 ± 0.23	0.33
B. subtilis	14.16 ± 0.54	8.34 ± 0.41	0.67

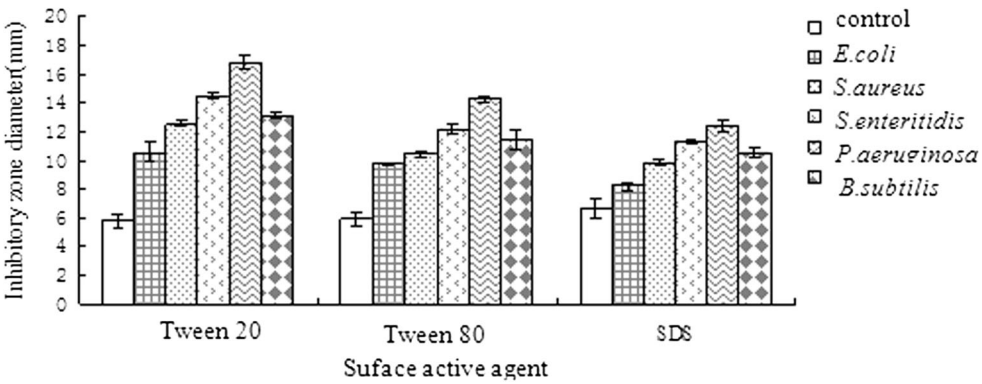

Fig. 2 Effect of different surfactants on the antibacterial activity of *Chrysanthemum morifolium* R. volatile oil

10.57 mm, 9.81 to 12.52 mm, 11.32 to 14.47 mm, 12.34 to 16.77 mm and 10.48 to 13.12 mm respectively. The variance analysis showed that the antibacterial activity of volatile oil dissolved in surfactants against five bacteria strains was significant ($p < 0.01$). Inhibition zone diameters were less than 7 mm in untreated group and there is no inhibitory effect. Comprehensive above, with 10% Tween 20, Tween 80 solution and 5% SDS solution prepared 10% volatile oil solution had inhibitory effect. When volatile oil dissolved in Tween 20 had the strongest antibacterial activity, followed by Tween 80 and 5% SDS solution. This is mainly due to the Tween 20 and Tween 80 as emulsifier allow volatile oil to solubilize effective antibacterial ingredient to the solution. But the volatile oil solution of Tween 80 formulated more viscous, not conducive to the spread which results that volatile oil dissolved in Tween 20 is better than in Tween 80. Volatile oil dissolved in SDS solution had weaker solubilization and emulsification than Tween 20 and Tween 80. Therefore, the *C. morifolium* volatile oil antibacterial active ingredient can not distribute evenly in the solution and the SDS solution had the weakest antibacterial effect.

Temperature

In Table 3, Inhibition zone diameters of *C. morifolium* volatile oil treated at different temperatures on *E. coli* was range from 11.81 to 12.40 mm with untreated group 12.21 mm. *S. aureus* were 12.96 to 13.32 mm with untreated group 13.01 mm, *S. enteritidis* were 15.04 to 15.30 mm with untreated group 15.18 mm. *P. aeruginosa*'s were 19.63 to 20.12 mm with untreated group 19.92 mm. *B. subtilis* were 13.96 to 14.42 mm with untreated group 14.06 mm. No significant differences was detected among 5 tested strains on *C. morifolium* volatile oil treated at different temperature by analysis of variance temperature ($p > 0.05$). LSD multiple comparisons showed that the difference between the treatment group and untreated group was not significant, too. Briefly, heat treatment had no effect on antibacterial stability of *C. morifolium* volatile oil, may be attributed to the main antibacterial ingredient, such as terpene compounds, aldehyde and ketone compounds, acids, alcohols, phenols and other substances were not decomposed. As a result, this result forecast that *C. morifolium* volatile oil may be a potential volatile oil as natural preservatives added in food and it can still play a role in antimicrobial preservative in the course of food processing.

pH

In Table 3, *C. morifolium* volatile oil solution was treated with different pH, *E. coli* inhibition zone diameters was ranged from 11.38 to 12.18 mm with untreated group 12.15 mm. The significantly different among volatile oil's antibacterial activity treated by different pH was show by

Table 3 Effect of different temperature, pH, UV on the antibacterial activity of *C. morifolium* volatile oil inhibition zone diameter:mm

Microorganisms	Temperature					Untreated
	40 °C	60 °C	80 °C	100 °C	121 °C	
E. coli	12.34 ± 0.45	12.40 ± 0.15	12.08 ± 0.30	12.18 ± 0.36	12.01 ± 0.23	12.21 ± 0.25
S. aureus	13.12 ± 0.26	13.32 ± 0.45	13.09 ± 0.27	13.05 ± 0.28	12.96 ± 0.22	13.01 ± 0.32
S. enteritidis	15.23 ± 0.22	15.22 ± 0.21	15.04 ± 0.19	15.30 ± 0.28	15.14 ± 0.07	15.18 ± 0.25
P. aeruginosa	20.06 ± 0.35	19.91 ± 0.20	19.63 ± 0.63	20.12 ± 0.25	20.04 ± 0.30	19.92 ± 0.42
B. subtilis	14.42 ± 0.18	14.06 ± 0.39	13.94 ± 0.31	14.16 ± 0.38	13.96 ± 0.30	14.06 ± 0.36
Microorganisms	pH					Untreated
	2	4	7	9	11	
E. coli	11.38 ± 0.24	11.55 ± 0.24	12.08 ± 0.48	12.18 ± 0.23	11.54 ± 0.19	12.15 ± 0.23
S. aureus	12.22 ± 0.50	12.74 ± 0.26	13.09 ± 0.24	12.84 ± 0.19	12.79 ± 0.19	12.92 ± 0.26
S. enteritidis	14.06 ± 0.19	14.42 ± 0.32	15.13 ± 0.32	14.85 ± 0.29	13.80 ± 0.47	15.22 ± 0.15
P. aeruginosa	19.36 ± 0.44	19.89 ± 0.14	20.11 ± 0.15	20.01 ± 0.23	19.37 ± 0.39	20.08 ± 0.35
B. subtilis	13.22 ± 0.26	13.83 ± 0.31	14.12 ± 0.39	13.91 ± 0.14	13.01 ± 0.21	14.06 ± 0.24
Microorganisms	UV					Untreated
	10 min	20 min	30 min	40 min	50 min	
E. coli	12.09 ± 0.45	11.91 ± 0.35	12.22 ± 0.06	12.03 ± 0.16	11.98 ± 0.27	12.15 ± 0.22
S. aureus	13.08 ± 0.30	13.14 ± 0.26	13.12 ± 0.28	13.18 ± 0.30	13.07 ± 0.22	13.09 ± 0.28
S. enteritidis	15.18 ± 0.39	14.92 ± 0.23	15.09 ± 0.14	14.95 ± 0.23	14.88 ± 0.28	15.21 ± 0.32
P. aeruginosa	19.68 ± 0.65	19.97 ± 0.16	20.10 ± 0.14	19.90 ± 0.20	19.96 ± 0.15	20.04 ± 0.38
B. subtilis	14.02 ± 0.39	13.86 ± 0.35	13.51 ± 0.23	13.68 ± 0.55	13.65 ± 0.47	13.78 ± 0.24

variance analysis ($p < 0.01$). LSD multiple comparison showed that no significant treatment between treated groups pH 7–9 and control groups. While other treated groups and untreated group were significantly different. The inhibition zone diameters on *S. aureus* was ranged from 12.22 to 13.09 mm with untreated group 12.92 mm. Variance analysis showed that the *C. morifolium* volatile oil handled by different pH showed significantly different antibacterial activity ($p < 0.05$). LSD multiple comparison showed that, pH 2 treatment group and untreated group was highly significant, while other treatments and untreated groups were not significantly different. Inhibition zone diameter against *S. enteritidi* were 13.80–15.13 mm with untreated group 15.22 mm. The significantly different antibacterial activity among treated groups was detected too by variance analysis ($p < 0.01$). LSD multiple comparisons showed, treatment group pH 7–9 and untreated group was not significant difference but other treatments and with untreated group were significantly different. *P. aeruginosa*'s inhibition zone diameters was within the range of 19.36–20.11 mm with untreated group 20.08 mm. Variance analysis showed that volatile oil's antibacterial activity handled by different pH was significantly different ($p < 0.05$) and LSD multiple comparison showed treatment groups (pH 4, pH 7 and pH 9) and untreated groups were not significant different. *B. subtilis*'s inhibition zone diameters was within the range of 13.01–14.12 mm while untreated showed 14.06 mm. Variance analysis showed that the antibacterial activity vary significantly by different pH treatment ($p < 0.01$). LSD multiple comparisons showed that under pH 4, pH 7 and pH 9 conditions, the difference between treatment group and untreated group was not significant. However, the other treatments and the untreated group were significantly different. In summary, acid and alkali environment had effect on *C. morifolium* volatile oil to weaken the inhibitory effect. In the near neutral environment, the impact on *C. morifolium* volatile oil inhibitory effect was not obvious.

UV

In Table 3, *C. morifolium* volatile oil antibacterial solution was processed through ultraviolet irradiation at different times. The inhibition zone diameters of *E. coli* of volatile oil ranged from 11.91 to 12.22 mm, while the untreated group was 12.15 mm. Then, the inhibition zone diameters

of *S. aureus*'s ranged from 13.07 to 13.18 mm, and the untreated group was 13.09 mm. Moreover, the inhibition zone diameters of S. enteritidis's ranged from 14.88 to 15.18 mm, and the untreated group was 15.21 mm. In addition, the inhibition zone diameters of *P. aeruginosa* ranged from 19.68 to 20.10 mm, and the untreated group was 20.04 mm. *B. subtilis*'s ranged from 3.51 to 14.02 mm, and the untreated group was 13.78 mm. Result of variance analysis showed that it is no significant differences antibacterial activity of *C. morifolium* volatile oil to 5 tested strains ($p > 0.05$) under the ultraviolet irradiation at different times. LSD multiple comparison indicted that the treatment group and the untreated group were not significant. In summary, the UV light irradiation had little influence on the stability of *C. morifolium* volatile oil antibacterial activity.

Conclusion

The research identified 56 kinds of compounds from *C. morifolium* volatile oil, the main components were monoterpenes and sesquiterpenes compounds, including hydrocarbons, esters, aldehydes, ketones, phenols and organic acids. Among them, α-curcumene had the highest proportion, accounting for 12.55%. *C. morifolium* volatile oil had certain inhibitory effects on 5 tested strains and the inhibitory effects ranked: *P. aeruginosa* > *S. enteritidis* > *B. subtilis* > *S. aureus* > *E. coli*. Wherein it had the maximum inhibitory effect on *P. aeruginosa* and inhibition zone diameter reached 20.43 mm. The minimum inhibitory effect on *E. coli* and its inhibition zone diameter was 12.29 mm. Surfactant had great impact on its antibacterial stability and *C. morifolium* volatile oil had strongest antibacterial activity with Tween 20 as emulsifier and Tween 80 as emulsifier followed and SDS was weakest. The pH has certain influence on the stability antibacterial activity of *C. morifolium* volatile oil. Temperature and UV had the least influence on the stability of antibacterial activity.

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References

Burt S (2004) Essential oils: their antibacterial properties and potential applications in food-a review. Int J Food Microbiol 94:223–253

Chang KM, Kim GH (2009) Comparative chemical composition of domestic and imported *Chrysanthemum indicum* L. flower oils. Food Sci Biotechnol 18(5):1288–1292

Chang KM, Kim GH (2013) Volatile aroma constituents of gukhwa (*Chrysanthemum morifolium* R.). Food Sci Biotechnol 22(3):659–663

Chang KM, Choi EM, Kim GH (2010) Chemical constituents of *Chrysanthemum indicum* L. flower oil and effect on osteoblastic MC3T3-E1 cells. Food Sci Biotechnol 19(3):815–819

Cheng W, Li J, You T, Hu C (2005) Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of *Chrysanthemum indicum* Linné. J Ethnopharmacol 101(1):334–337

Cheon MS, Yoon TS, Lee DY, Choi GY, Moon BC, Lee AY, Choo BK, Kim HK (2009) *Chrysanthemum indicum* Linné extract inhibits the inflammatory response by suppressing NF-κB and MAPKs activation in lipopolysaccaride-induced RAW 264.7 macrophages. J Ethnopharmacol 122:473–477

Choi HS, Kim GH (2011) Volatile flavor composition of gamguk (*Chrysanthemum indicum*) flower essential oils. Food Sci Biotechnol 20(2):319–325

Enan E (2001) Insecticidal activity of essential oils: octopaminergic sites of action. Comp Biochem Physiol 130:325–327

Forrer M, Kulik EM, Filippi A, Waltimo T (2013) The antimicrobial activity of alpha-bisabolol and tea tree oil against *Solobacterium moorei*, a gram-positive bacterium associated with halitosis. Arch Oral Biol 58(1):10–16

Haouas D, Cioni PL, Halima-Kamel MB, Flamini G, Hamouda MHB (2012) Chemical composition and bioactivities of three *Chrysanthemum* essential oils against *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). J Pest Sci 85(3):367–379

Hu HB, Zheng XD, Hu HS, Li Y (2009) Chemical compositions and antimicrobial activities of essential oils extracted from *Acanthopanax brachypus*. Arch Pharm Res 32(5):699–710

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Kamatou GP, Viljoen AM (2010) A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. J Am Oil Chem Soc 87(1):1–7

Lee DY, Choi GY, Yoon TS, Cheon MS (2009) Anti-inflammatory activity of *Chrysanthemum indicum* extract in acute and chronic cutaneous inflammation. J Ethnopharmacol 123:149–154

Matsuda H, Morikawa T, Toguchida I, Harima S, Yoshikawa M (2002) Medicinal flowers. vi. absolute stereostructures of two new flavanone glycosides and a phenylbutanoid glycoside from the flowers of *Chrysanthemum indicum* L.: their inhibitory activities for rat lens aldose reductase. Chem Pharm Bull 50(7):972–975

Özcan M, Erkmen O (2001) Antimicrobial activity of the essential oils of Turkish plant spices. Eur Food Res Technol 212(6):658–660

Schultz TH, Flath RA, Mon TR, Eggling SB, Teranishi R (1997) Isolation of volatile components from a model system. J Agric Food Chem 25:446–449

Schwob I, Bessiere JM, Dherbomez M, Viano J (2002) Composition and antimicrobial activity of the essential oil of *Hypericum coris*. Fitoterapia 73(6):511–513

Wu XN, Yu CH, Bao QN (2009) And the mechanism of anti-inflammatory effects of total flavonoids of *Chrysanthemum morifolium* Ramat. J Clin Pharmacol Ther China 14(9):1000–1003