African Journal of Biotechnology Vol. 9(25), pp. 3817-3821, 21 June, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.140
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Flavonoids and volatiles in *Chrysanthemum morifolium* Ramat flower from Tongxiang County in China

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Accepted 5 May, 2010

Flavonoids and volatiles in a traditional herbal medicine *Chrysanthemum morifolium* Ramat flower were determined by HPLC and GC/MS, respectively. Eight flavonoids and fifty eight volatiles were identified. Luteolin-7-glucoside and quercitrin were the most abundant flavonoids and they amounted for 85.7% of the total detected flavonoids. B-Humulene was the most abundant volatile and ledene oxide-(I) the next abundant one and the two volatiles were 16.3 and 9.0% of total volatiles, respectively. The bioactivities of the partial components in *C. morifolium* Ramat flower were discussed. It is considered that the health benefits of *C. morifolium* Ramat might be related to its abundant flavonoids and volatiles.

Key words: *Chrysanthemum morifolium,* herbal medicine, chemical composition, high performance liquid chromatography (HPLC), gas chromatography tandem with mass spectrometer (GC-MS).

INTRODUCTION

Botanical extracts have long been used to treat disease and plant sourced materials play a major role in primary health care in many developing countries (Aridogan et al., 2002). Flower of *Chrysanthemum morifolium* Ramat has long been used as traditional Chinese medicine and beverage (Ye et al., 2007). Research showed that *C. morifolium* has the functions to reduce myocardial vulnerability and exert antiarrhythmic effects on heart rhythm disorder induced by aconitine or ischemia/ reperfusion (Zhang et al, 2009). *C. morifolium* health benefits of the herbal plants are closely related to its bioactive components. However, little information on the chemical composition of *C. morifolium* Ramat flower has been reported. Revealing the chemical composition of *C. morifolium* Ramat flower is interesting for the studies on the healthy functionalities of this plant.

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Abbreviations: **HPLC,** High performance liquid chromatography; **GC-MS,** gas chromatography tandem with mass spectrometer; **SDE,** successive distillation extraction; **MAPK,** mitogen-activated protein kinase; **CB2,** cannabinoid receptor type-2.

Flavonoids are important bioactives in herbal plants (Gohar et al., 2009; Gone et al., 2009; McNulty et al., 2009; Zhou et al., 2009). Volatiles in medicinal plants play a vital role in healthcare systems of most traditional and modern medicines (Hassanpouraghdam, 2009). In order to reveal the chemical compositions of *C. morifolium* Ramat, a high performance liquid chromatography (HPLC) and a gas chromatography/mass spectrometry (GC/MS) were used to determine the composition and levels of flavonoids and volatiles in ethanol extract of its flowers.

MATERIALS AND METHODS

Equipments and materials

Equipments used in the tests were a Model LC20A HPLC (Shimadzu Co., Kyoto, Japan) and a Model HP6890/5973 GC/MS (Agilent Technologies Inc., CA, USA). A successive distillation extraction (SDE) apparatus described by Liang et al. (2005) was used to extract volatiles.

Dry flower of *C. morifolium* Ramat produced in Tongxiang County, China, was purchased from market, ground using a EUPA TSK-927S grinder (Cankun Co. Ltd, Shanghai, China) and sifted through 2-mm mesh sifter (Lantian Co. Ltd., Hangzhou, China) before extraction. Flavonoid reference compounds were Sigma products (Sigma-Aldrich, St. Louis, USA). The other chemicals used were HPLC grade reagents.

Extraction of *C. morifolium* Ramat flower

The ground flower sample (15 g) was extracted in 300 mL 60% aqueous ethanol in a water bath at 80°C for 200 min. The extract was filtered through cotton wool and then cooled on ice. The cold filtrate was centrifuged at 5000 x g for 15 min. The supernatant was used for soluble solids determination and HPLC analysis of flavonoids.

Determination of soluble solids

20 mL of the above supernatant was placed in a weighted dry glass dish (15 cm in diameter) and dried at 80°C overnight and then at 103°C for 180 mm, cooled in a desiccator and finally weighted. Soluble solids concentration was expressed as mg soluble solids per kg dry flower.

HPLC analysis of flavonoids and their derivatives

The HPLC were carried out as following conditions: injection volume,10 µL; 5-µm TC-C18 column (4.6 × 250 mm, Agilent Technologies Inc, CA, USA), column temperature at 35°C, mobile phase A being acetonitrile/acetic acid/water (3/0.5/96.5,v/v/v) and mobile phase B acetonitrile /acetic acid /water (50/0.5/49.5,v/v/v), linear gradient elution from 72.5% A / 27.5% B (v/v) to 65% A / 35% B (v/v) during 0 - 10 min, from 65% A / 35% B (v/v) to 20% A /80% B (v/v) during 10 - 35 min, from 20% A /80% B (v/v) to 0% A /100% B (v/v) during 35 - 40 min; mobile phase flow rate 1 mL min^{-1}. The eluate was monitored at 360 nm. Flavonoids and their derivatives in the tested sample were determined by comparing the retention times and peak areas with those of authentic reference compounds. The identified compounds were confirmed by internal standard as described by Uesawa and Mohri (2005).

GC/MS analysis of volatiles

Volatiles were extracted in a successive distillation extraction (SDE) apparatus as method by Liang et al. (2005). Five grams of the ground sample and 100 µL internal standard reference ethyl caprate (0.2 µg $µL^{-1}$) were placed in the sample extraction flask with 250 mL of freshly boiled distilled water which was placed in a boiling water bath. 30 mL of ethyl ether was placed in a volatiles collecting flask which was placed in water bath at 50 successive distillation extraction (SDE). The sample was extracted for 60 min, during which the volatile compounds were evaporated and absorbed in the ethyl ether in the volatiles collecting flask. The ethyl ether phase was then transferred into a 50 mL glass tube and dehydrated with 5 g of Na_2SO_4 for 24 h. The dehydrated ethyl ether phase was concentrated to about 1.0 mL under reduced pressure at 42 successive distillation extraction (SDE). The concentrate was used for GC/MS analysis.

GC/MS was carried out based on a modified method by Kim et al. (2007). The column was a 30 m x 0.32 mm (i.d.) HP-INNO wax fused capillary column with a film thickness of 0.5 µm (Agilent Technologies Inc., CA, USA). The column temperature was programmed at 50°C for 5 min, then from 50°C to 210°C at a rate of 3°C min^{-1}, remained at 210°C for 10 min and finally increased from 210 to 230°C at a rate of 3°C min^{-1}. The injector temperature was 250°C and injection volume was 1.0 µL. The flow rate of the helium carrier gas (99.999% purity) was 1 mL min^{-1}. The mass spectrometer was used at ionization voltage of 70 eV and ion source temperature 230°C. The identification of the volatile components was done by comparing their Kovats GC retention indices and mass spectra with those of authentic compounds or reported values. Relative concentrations of detected volatiles were expressed as the ratio of the peak height of the tested volatiles to the peak height of internal reference ethyl caprate.

Data analysis

The tests in the present paper were carried out in duplicate and the mean values of the duplicate tests were presented. Mean values and standard deviations were calculated on software of the SAS System for Windows version 8.0 (SAS Institute Inc, Cary, NC, USA).

RESULTS AND DISCUSSION

Contents of soluble solids, flavonoids and their glycosides

Soluble solids of *C. morifolium* Ramat flower extracted by 60% (v/v) aqueous ethanol solution were 463.30 ± 3.90 mg g^{-1}, which was 50% higher than that of black tea reported by Liang and Xu (2003). It suggests that a high extract yield will be obtained if it is used as material for extracting additive for food stuff and medicines.

Twenty (20) peaks were monitored in the HPLC profile of extract of *C. morifolium* Ramat flower (Figure 1), among which 8 peaks were qualitatively and quantitatively identified based on retention times of 30 HPLC reference compounds used and the other 12 were not identified owing to lack of authentic references. Among the identified flavonoids, there were 4 flavonoid glycosides, that is, vitexin-2-O-rhamnoside, quercetin-3-galactoside, luteolin-7-glucoside, and quercetin-3- glucoside. Luteolin-7-glucoside was the most abundant, followed by quercitrin. These two compounds accounted for 85.7% of total flavonoids that were detected. Vitexin-2-O-rhamnoside, kaempferol and apigenin were the least abundant flavonoids in the sample, being less than 1 mg kg^{-1} (Table 1).

It was reported that luteolin-7-glucoside had anti-asthmatic activity in an ovalbumin-induced lung inflammation via the down-regulation of T helper 2 cytokine transcripts as well as the inhibition of prostaglandin E-2 production (Jin et al., 2009). It also had antioxidant and inflammatory activities (Ha et al., 2006; Silva et al., 2006), hepatoprotective effects (Lima et al., 2006) and inhibitory effect on aortic vascular smooth muscle cell proliferation (Kim et al., 2006). Quercitrin was reported to have anti-oxidant and anti-carcinogenic activities via its inhibition of neoplastic transformation by blocking activation of the MAPK pathway and stimulation of cellular protection signaling (Hanen et al., 2009; Ding et al., 2010). Quercitrin concentration in *C. morifolium* Ramat flower of this study was significantly higher than that detected in *Mesembryanthemum edule* shoot extracts reported by Hanen et al. (2009). This suggests that the health benefits of *C. morifolium* Ramat flower might be related to its abundant luteolin-7-glucoside and quercitrin and *C. morifolium* Ramat flower is a good source of these natural bioactives.

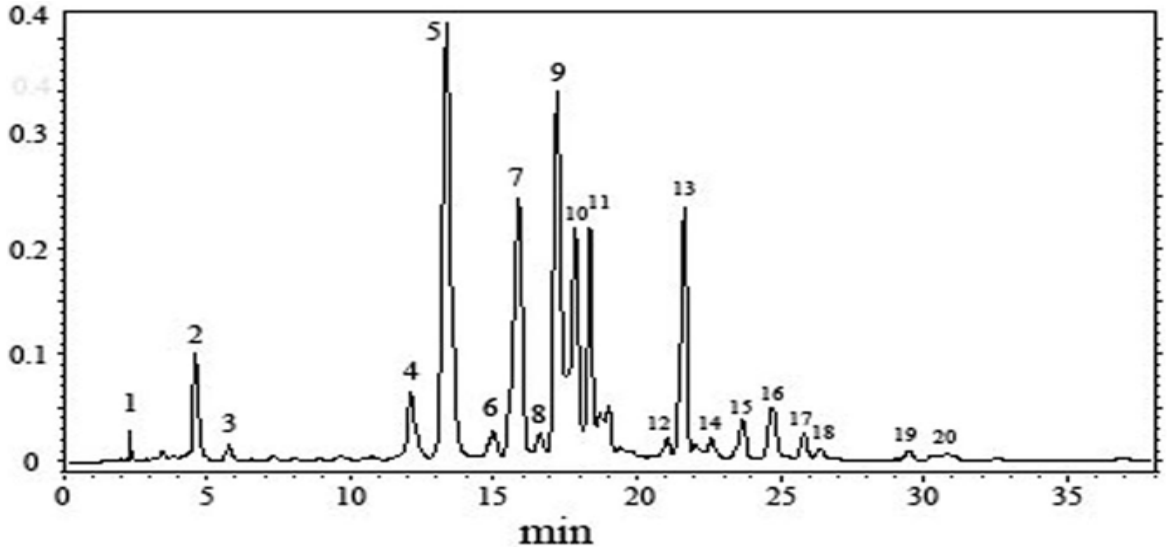

Figure 1. HPLC profile of flavonoids in extract of *C. morifolium* Ramat flower. **Peak 3:** quercetin-3-galactoside; **4:** vitexin-2-O-rhamnoside; **5:** luteolin-7-glucoside; **8:** quercetin-3- glucoside; **9:** quercitrin-12-myricetin; **18:** luteolin; **19:** apigenin; and **20:** kaempferol. The other peaks were not identified owing to lack of authentic compounds.

Table 1. Contents of flavonoids in *C. morifolium* Ramat flower.

Compounds	Content (mg g^{-1})
Vitexin-2-O- rhamnoside	0.10 ± 0.01
Quercetin-3-galactoside	2.46 ± 0.02
Luteolin-7-glucoside	50.59 ± 0.94
Quercetin-3- glucoside	1.33 ± 0.09
Quercitrin	21.38 ± 0.80
Myricetin	2.13 ± 0.08
Luteolin	5.22 ± 0.48
Apigenin	0.70 ± 0.10
Kaempferol	0.14 ± 0.02
Total	83.95 ± 2.77

Contents of volatiles

Fifty eight (58) volatiles were identified in the sample of *C. morifolium* Ramat flower by GC/MS. β-Humulene was the most abundant volatile and its peak height was 96.48 times of that of internal reference ethyl caprate, or 16.3% of total concentration of the 58 detected volatiles (Table 2). Ledene oxide-(I) was the next abundant volatile, being 52.96 times of internal reference or amounting for 9.0% of total volatiles. There were 17 volatiles whose relative concentration was more 10 times than the internal reference, accounting for 72.9% of the detected volatiles, suggesting that they pre-dominated the volatiles in *C. morifolium* Ramat flower.

B-Humulene, who's another name is β-caryophyllene, is notable for having a cyclobutane ring, which is rare in nature. B-Humulene was shown to selectively bind to the cannabinoid receptor type-2 (CB2) and to exert significant cannaB-Humulene, who's another name is β-caryo-phyllene, is notable for having a cyclobutane ring, which is rare in nature. B-Humulene was shown to selectively bind to the cannabinoid receptor type-2 (CB2) and to exert significant cannabimimetic anti-inflammatory effects in mice (Gertsch et al., 2008). Extracts of *Cordia verbenacea*, in which α-humulene, an isomer of β-humulene, was the most important components, had strong antibacterial activity (Michielin et al., 2009). B-Humulene has been described as having a woody-spicy, dry, clove-like tenacious odor and somewhat bitter taste (Galindo-Cuspinera et al., 2002) and it is a major aromatic component of hops and a major contributor to the flavor of beer. It has been extensively used in the perfumery and flavour industries and in manufacture of polymers and adhesives. There has been no study on the bioactivities of ledene oxide-(I). The essential oil of Tibetan medicine from *Dracocephalum heterophyllum* Benth, containing ledene oxides (I) and (II) , had strong anti-bacterial and antioxidant activities (Zhang et al., 2008). The present studies showed that *C. morifolium* Ramat flower will be a good source of β-humulene and ledene oxide-(I) owing to its high concentrations.

Essential oil composition of herbs varied with their sources. The predominant components of *Chrysanthemum indicum* L. flower oils from Korea were α-pinene, 1,8-cineol and chrysanthenone, buEssential oil composition of herbs varied with their sources. The predominant components of *Chrysanthemum indicum* L. flower oils from Korea were α-pinene, 1,8-cineol and chrysanthenone, but camphor, α-curcumene and β-sesquiphellandrene in that from China (Chang and Kim, 2009). The source of the *C. morifolium* flower in the present paper was from

Table 2. Concentrations of volatiles in *C. Morifolium* Ramat flower.

Volatiles	I_k [a]	Relative concentration [b]
β-Humulene	1779	96.48 ± 3.20
Ledene oxide-(I)	1812	52.96 ± 1.76
cis-Z-α-Bisabolene epoxide	1814	36.84 ± 1.01
3,4-Dihydro-2,2-dimethyl-2H-1-benzopyran	1772	36.00 ± 2.32
trans-Limonene oxide	1665	26.52 ± 1.67
2-Methyl-5-(1-methylethenyl)-cyclohexanone	1760	22.51 ± 0.56
2,6-Dimethyl-1,3,6-heptatriene	1785	19.24 ± 0.68
1,6-Dibromo-hexane	1835	18.79 ± 0.87
β-Elemene	1616	16.64 ± 0.09
Bromo-cyclohexane	1737	15.76 ± 1.05
1-(1,5-Dimethyl-4-hexenyl)-4-methylbenzene	1674	15.28 ± 1.32
3,3,6,6-Tetraethyl-tricyclo[3.1.0.0(2,4)]hexane	1726	15.25 ± 1.07
3-Cyclohexene-1-methanol	1791	13.29 ± 0.10
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1813	12.41 ± 1.02
Caryophyllene	1632	11.57 ± 098
1-tert-Butyl-1,5-Cyclooctadiene	1751	11.49 ± 0.87
6-Methyl-5-hepten-2-one	1328	10.53 ± 0.66
Eicosane	2000	9.54 ± 0.54
Caryophyllene oxide	1724	9.45 ± 0.62
Docosane	2209	8.90 ± 0.35
5-(1,5-Dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene	1681	7.80 ± 0.52
Bicyclo[10.1.0]tridec-1-ene	1730	7.54 ± 0.17
Isoaromadendrene epoxide	1807	7.44 ± 0.42
1,5,9,13-Tetradecatetraene	1735	7.01 ± 0 .11
Eudesma-4(14),11-diene	1769	6.54 ± 0.06
1H-Cyclopropa[a]naphthalene	1762	6.53 ± 0.02
Heneicosane	2100	6.35 ± 0 .04
Cedrol	1744	6.29 ± 0.17
(1,1-Dimethylpropyl)benzene	1733	6.08 ± 0.53
Camphene	1773	5.85 ± 0.36
Longifolenaldehyde	1741	5.81 ± 0.20
3-Methyl-2-cyclohexen-1-one	1375	5.19 ± 0.34
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one	1441	5.03 ± 0.23
trans-Z-α-Bisabolene epoxide	1820	5.01 ± 0.33
2,4-bis(1,1-Dimethylethyl)phenol	1694	3.03 ± 0.24
β-Sesquiphellandrene	1698	2.96 ± 0.10
2,3,3-Trimethyl-1-butene	1739	2.92 ± 0.05
Germacrene	1672	2.83 ± 0.12
7,11-Dimethyl-3-methylene-1,6,10-dodecatriene	1658	2.72 ± 0.07
1-Methyl-4-(1-methylethylidene)-cyclohexane	1798	2.29 ± 0 .06
3,5-Dimethyl-2-ethyl-1,3-cyclopentadiene	1567	2.21±0.06
cis-α-Santalol	1843	2.21 ± 0.07
3,4,4-Trimethyl-2-cyclohexen-1-one	1720	2.14 ± 0.10
Spathulenol	1787	2.10 ± 0.12
α-Farnesol	1802	2.10 ± 0.08
Borneol	1457	1.88 ± 0.10
1,2-Benzenedicarboxylic acid, butyl octyl ester	1888	1.75 ± 0.11
9,10-Dehydro-Isolongifolene	1913	1.72 ± 0.02
1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde	1436	1.56 ± 0.13
α.-Farnesene	1689	1.48 ± 0.09
Limonene	1302	1.41 ± 0.03

Table 2. Cont.

1,8-Dimethyl-4-(1-methylethyl)- spiro[4.5]dec-8-en-7-one	1829	1.27 ± 0.01
5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	1225	1.14±0.10
α-Pinene	1285	1.04 ± 0.06
4-Bromo-2-methyl-1-butene	1920	0.95 ± 0.02
4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	1467	0.80 ± 0.01
1-(1,4-Dimethyl-3-cyclohexen-1-yl)- ethanone	1491	0.64 ± 0.03
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-ol acetate	1544	0.60 ± 0.05
Total		591.67 ± 37.46

[a:] I_k, Kovats retention index, $I_k = 100*n + 100*(t_x-t_n)/(t_{n+1}-t_n)$; n, the number of carbon atoms in the alkane; t_x, retention time of aimed component; t_{n+1} and t_n, the retention time of alkane with 'n+1' and 'n' carbon atoms.
[b:]100 µL internal standard reference ethyl caprate (0.2 µg $µL^{-1}$) was extracted with the sample and the relative concentrations of detected volatiles were expressed as the ratio of the peak height of the tested volati.

Tongxiang County, the most important producing area of this flower in China. This study showed that *C. morifolium* Ramat flower is rich in flavonoids luteolin-7-glucoside and quercitrin as well as volatiles β-humulene and ledene oxide-(I). The health benefits of this traditional medicine might be related to these abundant compounds. By revealing its chemical composition, the derived information shall be helpful for further investigation on healthy functionalities of this plant.

ACKNOWLEDGEMENT

This work was financially supported by the Agricultural Ministry of China (Project No. nyhyzx 2007-03-35-1).

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