



Chemical Composition and Antimicrobial Activity of Essential Oils of Stems of *Macleaya cordata* (Willd) R. Br.

CHUNMEI LI and JIANPING YU*

Institute of Biochemistry and Nutrition, Guizhou University, Guiyang 550025, P.R. China

*Corresponding author: Tel: +86 851 3856374; E-mail: Yuyp666666@163.com

Received: 1 April 2014;

Accepted: 27 June 2014;

Published online: 6 November 2014;

AJC-16225

The essential oil from the stems of *Macleaya cordata* R.Br. obtained by hydrodistillation was analyzed by gas chromatography/mass spectrometry. Fifty compounds consisting up to 99.17 % of the essential oil were identified by GC and GC/MS analysis. The essential oil exhibited potential antimicrobial activity against all tested microorganisms, with diameters of inhibition zones ranging from 7.7 ± 0.6 to 16.9 ± 0.8 mm and MIC values from 125 to 500 $\mu\text{g/mL}$. To understand the antibacterial mechanism of the essential oil, we selected the sensitive bacterium *R. solanacurum* as model to observe of the action of essential oils of *M. cordata* on the membrane structure by SEM. The treated cell membranes were damaged severely; resulting in cell atrophy and condenses into clusters, lost the original rod structure. The results presented here indicate that the essential oil of *M. cordata* has antimicrobial properties and is therefore a potential source of antimicrobial agents for many industries in the future.

Keywords: *Macleaya cordata*, Essential oils, Chemical composition, Antimicrobial activity.

INTRODUCTION

The continuing development of bacterial and fungicidal resistance in human and plant pathogens necessitates the discovery and development of new antimicrobials¹⁻⁴. Plant secondary metabolites originate from the interaction between plants and environment during the long period of evolution in plants and play an important role in protecting plants against invasion. Essential oils and their components have been proved to possess many biological activities including antimicrobial, insecticidal and antioxidant activities⁵⁻⁸ and they have attained a special interest and development. Today, essential oils with antioxidant and antimicrobial activities is an ideal resource in the filed of research on food and feed additives⁹⁻¹⁰.

Macleaya cordata (Willd) R. Br., a plant of papaveraceae family, has been used as an important and traditional Chinese medicine for a long time for its wide spectrum of biological activities, e.g. antimicrobial, antifungal, antiinflammatory and antitumor¹¹⁻¹³. This herb is mainly distributed in throughout the south, southeastern and northwest of China. The phytochemical and pharmacological studies conducted on this medicinal herb have successfully isolated several alkaloids¹⁴. Several quaternary benzo[c] phenanthridine alkaloids from *M. cordata* which exhibited cytotoxicity^{15,16}, protease inhibitory¹⁷, antimicrobial¹⁸, molluscicidal¹⁹ and insecticidal activities^{20,21}. antiinflammatory^{22,23}, antitumor activity^{24,25}, antiviral²⁶⁻²⁸.

M. cordata has attracted much attention from investigators depending on its bioactive alkaloids¹⁸, however, the other chemical compositions of *M. cordata* were rarely investigated. The literature data for the chemical composition of the essential oil from *M. cordata* are scarce, according to our best of knowledge, there is only one report on the chemical composition of the essential oil of *M. cordata*²⁹, in the literature and its antimicrobial potentials have not been evaluated so far. The aim of the present study was to analyze the chemical composition of the stems essential oil of *M. cordata* from China as well as to evaluate its antimicrobial activity and mechanism.

EXPERIMENTAL

Analytical grade ethanol, dimethyl sulphoxide, anhydrous sodium sulphate, sodium carbonate and all cultures media were obtained from Green Ltd. (Guiyang, China). Ultra pure water was used for the experiments. The shape of *R. solanacurum* was observed with a scanning electron microscopy (SEM) (HitachiS-3400N). Absorbance was measured using a UV-visible spectrophotometer (TU-1901, China).

Plant material: *M. cordata* were collected in Guizhou Province of China during August 2012 and their roots, stems and leaves were separated, dried in the shade at room temperature and ground (80 mesh). The taxonomical identification of the plant material was done by Prof. Haimin Liao of Guizhou University. An authenticated specimen (GZIBN-20120813) of

the plant was also deposited in the Institute of Biochemistry and Nutrition, Guizhou University.

Isolation of the essential oils: Crushed stems were submitted to hydrodistillation in a Clevenger-type apparatus at 100 °C for 6 h. The distilled essential oils were dried over anhydrous sodium sulphate, filtered and preserved in a sealed brown glass vial at 4 °C until tested and analyzed.

Analysis of essential oil: Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Agilent 6890 N GC/5975MSD-SCAN (Agilent Technologies, Palo Alto, CA, USA) in the electron impact (EI) ionization mode (70 eV) and zebron ZB-5Msi (bonded and cross-linked 5 % phenyl-95 % Dimethyl polysiloxane, 30 m length and 0.25 mm I.D., 0.25 µm film thickness) capillary column (Phenomenex, Torrance, USA). The inlet and GC/MS interface temperatures were kept at 250 and 280 °C, respectively. The temperature of EI 70 eV source was 230 °C with full scan (20-480 *m/z*). The oven temperature was held at 50 °C for 2 min, then programmed to 310 °C at rate of 5 °C/min kept constant at 310 °C for 8 min. The split ratio was 1:40. Helium (99.99 %) was the carrier gas at a flow rate of 1 mL/min. Diluted samples (1/100 in hexane, v/v) were injected manually. The mass spectra of essential oil and extract components were identified by comparing the mass spectra of the analytes with those of authentic standards from the mass spectra of Wiley 275 and Mass Spectra Library (Nist 2005). The relative concentration of each compound in essential oil was quantified according to the peak area integrated by the analysis program^{30,31}.

Antimicrobial activity

Microbial strains: The essential oil of *M. cordata* were individually tested against a set of eight microorganisms. Following microbial strains were used in this research: Three Gram-positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 27217 and *Staphylococcus haemolyticus* ATCC 29213). Three Gram-negative (*Ralstonia solanacearum* GMI 1000, *Agrobacterium tumefaciens* ATCC 11158, *Escherichia coli* CMCC 44102), and one fungus (*Aspergillus niger* CMCC 98003), one yeast (*Candida albicans* ATCC 10231) and microbial were selected for antimicrobial activity assay. Bacterial strains were cultured 24 h at 37 °C in liquid LB medium (yeast extract 5 g/L, peptone 10 g/L, NaCl 5 g/L, pH 7), Fungus and yeast were cultured overnight at 28 °C for 48 h in liquid potato dextrose (PD) medium. The diluted microbial suspension (10⁶ cfu/mL) was ready for assay.

Disc diffusion assay: Determination of antimicrobial activities of the essential oil of *M. cordata* were accomplished by the standard disc diffusion method³². The essential oil was dissolved in dimethyl sulfoxide at an initial concentration of 10 mg/mL and sterilized after being filtered by 0.45 µm Millipore filters, respectively. Bacteria suspensions were spread on these media by sterile swab. Each disc (6 mm in diameter) was injected 10 µL of 10 mg/mL sample, then the discs were placed on the plates one by one. Negative controls were prepared using DMSO. Gentamycin (20 µg/disc) were used as positive controls for bacteria and nystatin (20 µg/disc) for fungi and yeasts. Standard antibiotic discs which were selected for suitable microorganisms, were placed into the same plates as positive controls. An empty disc was used to test if discs were sterile

or not. The inoculated plates were incubated at 37 °C for 24 h for the bacterial strains, at 27 °C for 72 and 48 h for the fungus and yeast. The zone of inhibition against the tested microorganisms were measured and the antimicrobial activity was evaluated.

Determinations of the minimum inhibitory concentration (MIC): The sample were dissolved in DMSO at an initial concentration of 10 mg/mL and filtered by 0.45 µm Millipore filters for sterilization. Serial broth dilution method was carried out as described by Muroi and Kubo³³. Solutions of the essential oil ranging from 1000 to 7.8 µg/mL were prepared by serial two fold dilutions. A suspension of each microorganism ((10⁶ cfu/mL) was obtained by measuring the absorbance of the solution at wavelength 620 nm using a ultraviolet spectrometer. the successive dilutions of the oils samples and 1 mL portions of the culture media were placed in the test tubes; then mixed with 20 mL of the cell suspensions. The microbial growth was examined after bacteria being incubated at 37 °C for 24 h. The same procedure was performed for fungi and yeast incubated for 48 h at 28 °C. Gentamycin for bacteria and nystatin for fungi were used as standard drugs for positive control in the conditions identical to that of the tests materials. The results are expressed in Minimal inhibitory concentration (MIC), the weakest concentration of the samples yielding no visible growth. Microorganism growth was indicated by turbidity. The MICs of the standards (gentamycin, nystatin) were also determined in parallel experiments, to control for the sensitivity of the microorganisms. The bactericidal/fungicidal activities were determined by a sub-cultivation of the samples into normal culture media at appropriate temperature and incubation times.

Observation of the action on *R. solanacearum* cells by scanning electron microscopy: Different volume of LB medium, essential oils solutions and *R. solanacearum* cells were added to 10 mL cultures resulting in final concentration of 100 µg/mL of the essential oils and concentration of 10⁶ cfu/mL *R. solanacearum*. Control experiment was conducted in absence of essential oils. The cultures were incubated at 37 °C with shaking at 150 rpm. After 12 h, bacterial suspension was washed three times with PBS buffer and then fixed with 4 % glutaraldehyde solution at 4 °C for 12 h. After the reaction completed, the fixed bacterias were then washed with 30, 50, 70, 80, 90 and 100 % of ethanol respectively. After gold spraying and freezing-drying in a high vacuum, the shape of bacteria was observed using a scanning electron microscopy.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: Air-dried stems of the plant were subjected to hydrodistillation using a Clevenger-type apparatus and the yellow-coloured oil was obtained in the yield of 0.089 % (w/w). The total ion map of essential oil from the leaves of *M. cordata* was showed in Fig. 1 and 50 compounds consisting up to 99.17 % of the essential oil were identified by GC and GC/MS analysis (Table-1). The major components (relative content ≥ 1 %) are palmitic acid 37.52 %, pentadecanal 8.74 %, butyl phthalate 7.43 %, linoleic acid 5.89 %, (E)-9,17-octadecadienal 3.63 %, nonadecane 3.57 %, myristic acid 3.51 %, octoil 3.42 %,

TABLE-1
PHYTOCHEMICAL COMPOSITIONS OF THE ESSENTIAL OILS FROM THE STEMS OF *M. cordata*

No.	Compounds	Molecular formula	RT ^a	Composition (%)
1	Benzaldehyde	C ₇ H ₆ O	6.89	0.05
2	Hyacinthin	C ₈ H ₈ O	9.08	0.24
3	Nonanal	C ₉ H ₁₈ O	10.68	0.08
4	(E)-2-Nonenal	C ₉ H ₁₆ O	12.27	0.05
5	Caprylic acid	C ₈ H ₁₆ O ₂	13.28	0.13
6	Decanal	C ₁₀ H ₂₂ O	13.53	0.26
7	2-Undecanone	C ₁₁ H ₂₂ O	15.94	0.1
8	2,4-Decadienal	C ₁₀ H ₁₆ O	16.66	0.06
9	4-Vinyl-2-methoxy-phenol	C ₉ H ₁₀ O ₂	16.75	0.17
10	Capric acid	C ₁₀ H ₂₀ O ₂	18.42	0.4
11	Tetradecane	C ₁₄ H ₃₀	18.69	0.14
12	Geranyl acetone	C ₁₃ H ₂₂ O	20.09	0.16
13	2,6-di-butyl-2,5-cyclohexadiene-1,4-dione	C ₁₄ H ₂₀ O ₂	20.54	0.37
14	2,6-di(<i>t</i> -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	C ₁₅ H ₂₄ O ₂	20.7	0.19
15	β-Ionone	C ₁₃ H ₂₀ O	20.99	0.35
16	Pentadecane	C ₁₅ H ₃₂	21.16	0.31
17	Lauric acid	C ₁₂ H ₂₄ O ₂	23.39	1.87
18	Hexadecane	C ₁₆ H ₃₄	23.56	0.51
19	2-Cedrol	C ₁₅ H ₂₆ O	23.96	1.37
20	Butyl phosphate	C ₁₂ H ₂₇ O ₄ P	24.87	0.48
21	1-Heptadecene	C ₁₇ H ₃₄	25.38	0.29
22	2-Pentadecanone	C ₁₅ H ₃₀ O	25.86	0.34
23	Pentadecanal	C ₁₅ H ₃₀ O	26.21	8.74
24	Hexadecanal	C ₁₆ H ₃₂ O	26.44	0.5
25	Myristic acid	C ₁₄ H ₂₈ O ₂	27.78	3.51
26	6,10,14-Trimethyl-pentadecanone	C ₁₈ H ₃₆ O	28.95	0.9
27	Isobutyl phthalate	C ₁₆ H ₂₂ O ₄	29.5	2.13
28	Pentadecylic acid	C ₁₅ H ₂₀ O ₂	29.68	1.94
29	(E)-9,17-Octadecadienal	C ₁₈ H ₃₂ O	29.86	3.63
30	Nonadecane	C ₁₉ H ₄₀	30.02	3.57
31	Farnesyl acetone	C ₁₈ H ₃₀ O	30.46	0.25
32	Methyl palmitae	C ₁₇ H ₃₄ O ₂	30.57	0.47
33	Butyl phthalate	C ₁₆ H ₂₂ O ₄	31.39	7.43
34	Palmitic acid	C ₁₆ H ₃₂ O ₂	31.91	37.52
35	Methyl linoleate	C ₁₉ H ₃₄ O ₂	33.82	0.71
36	Linoleic acid	C ₁₈ H ₃₂ O ₂	34.91	5.89
37	Tricosane	C ₂₃ H ₄₈	37.35	0.49
38	Tetracosane	C ₂₄ H ₅₀	39	0.52
39	Pentacosane	C ₂₅ H ₅₂	40.59	1.28
40	Octoil	C ₂₄ H ₃₈ O ₄	41.41	3.42
41	Hexacosane	C ₂₆ H ₅₄	42.12	0.83
42	Heptacosane	C ₂₇ H ₅₆	43.6	1.02
43	Squalane	C ₃₀ H ₆₂	44.13	0.95
44	Octacosane	C ₂₈ H ₅₈	45.02	1.18
45	Nonacosane	C ₂₉ H ₆₀	46.4	0.8
46	Triacontane	C ₃₀ H ₆₂	47.72	0.77
47	Hentriacontane	C ₃₁ H ₆₄	49.03	0.91
48	(17.a.H,21.B.H)-Hopane	C ₃₀ H ₅₂	49.88	0.82
49	Dotriacontane	C ₃₂ H ₆₆	50.26	0.49
50	Tritriacontane	C ₃₃ H ₆₈	51.48	0.58
	Total	-	-	99.17

^a Retention time

isobutyl phthalate 2.13 %, pentadecylic acid 1.94 %, lauric acid 1.87 %, 2-cedrol 1.37 %, pentacosane 1.28 %, octacosane 1.18 % and heptacosane 1.02 %. comprising 84.50 % of the essential oils. Saturated fatty acids, alkanes, unsaturated fatty acids, aromatic compounds, terpenoids, Other aliphatic compounds in the oil accounted for 46.10, 14.34, 6.60, 16.06, 1.18 and 14.89 %, respectively. The chemical profile of the stem bark essential oil of *M. cordata* in this study was different from that of the previous reports²⁹. Distinct differences in the

yield and composition of essential oils from *M. cordata* may be arise from environment, tested parts, growth season, physiological age of the plant and the method of isolation.

Antimicrobial activity: The antimicrobial activities of the essential oil were estimated against eight tested microorganisms, including three Gram-negative bacteria, three Gram-positive bacteria, one fungus and one yeast. The diameters of the zones of inhibition and the MICs of the essential oil are shown in Table-2. The diameters of inhibition zones

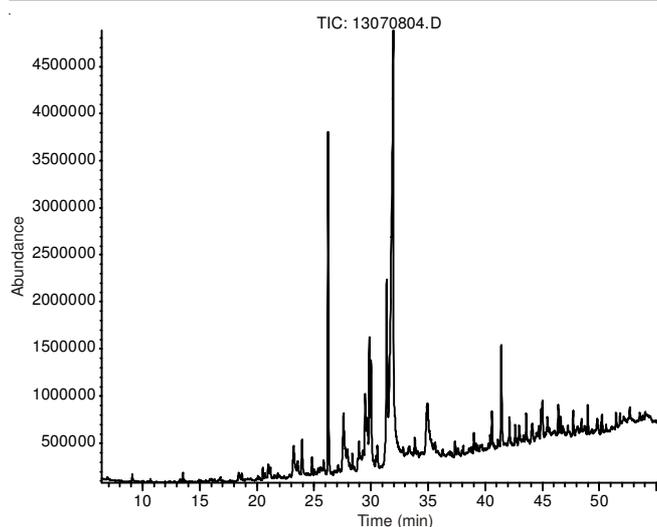


Fig. 1. Total ion map of essential oil from the stems of *M. cordata*

of the oil on test microorganisms ranged from 7.7 ± 0.6 to 15.9 ± 0.8 mm and the MIC values ranged from 125 to 500 $\mu\text{g}/\text{mL}$. Among all tested microorganisms *R. solanacarum* and *S. aureus* were more sensitive to the essential oil, with the MIC values of 125 $\mu\text{g}/\text{mL}$. The diameters of inhibition zones of the essential oil against *R. solanacarum* and *S. aureus* were 15.9 ± 0.8 and 15.1 ± 1.0 mm, respectively. The oil also showed antifungal activities against one fungus and one yeast. The antimicrobial activities of the essential oil against bacteria and fungi were demonstrated to be dose dependent. The inhibition zones of the essential oil for each assay on test bacteria showed a significant correlation with MIC values ($P < 0.05$). Control treatment (DMSO) did not show an inhibitor effect on any of the bacteria.

Action of the essential oil of *M. cordata* on the structures of *R. solanacarum* cells: Though this research found the essential oil of *M. cordata* could inhibit bacterial growth, the mode of action of essential oil on the bacteria is still unknown. In this paper, to understand the antibacterial activity and acting mechanism of the essential oil of *M. cordata*, the sensitive bacteria *R. solanacarum* was selected as the tested microorganism. The action of the essential oil of *M. cordata* on the structures of *R. solanacarum* cells was studied.

The electron micrographs by scanning electron microscopy of *R. solanacarum* cells treated and untreated with the essential oil of *M. cordata* were displayed in Fig. 2. Micrograph by SEM (Fig. 2a) showed the surface of *R. solanacarum* cells untreated with the essential oil of *M. cordata* was smooth and showed typical characters of rod shape, while cells treated with 100 $\mu\text{g}/\text{mL}$ essential oil (Fig. 2b) were damaged severely, resulting in cell atrophy and condenses into clusters, lost the original rod structure. A large number of misshapen and fragmentary bacterial cells can be seen from micrographs. We hypothesized that the acting mechanism of the essential oil from *M. cordata* antibacterial activity are the structure and permeability of cell membrane of treated bacteria changed, so that the substances exuded in the cytoplasm, at last cells dehydrate, atrophy and crack. This hypothesis needs to be further proved.

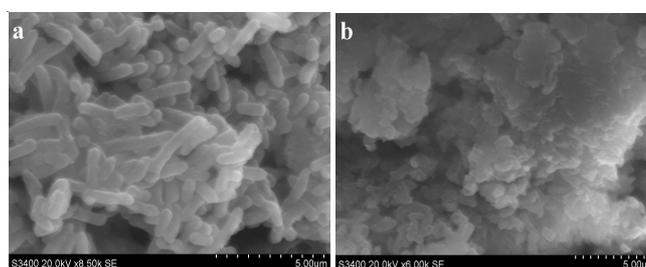


Fig. 2. Action of the essential oil of *M. cordata* on the structures of *R. solanacarum* cells observed by SEM. (a) native *R. solanacarum*. (b) treated with 100 $\mu\text{g}/\text{mL}$ the essential oil

Conclusion

This is the first report on the antimicrobial activities of *M. cordata* essential oil. The essential oil was screened to show a wide spectrum of inhibitory activity against pathogenic bacteria, fungi and yeast. The antimicrobial activity of the stems essential oil of *M. cordata* could be attributed by major compounds. It has also been suggested that minor components interact with the other components, affecting the antimicrobial activities of the oils. It is possible that the activity of the main components is regulated by the other minor molecules.

TABLE-2
ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *M. cordata*^a

Microorganisms	Essential oil		Gentamicin		Nystatin	
	Disc diameter ^b	MIC ^c	Disc diameter	MIC	Disc diameter	MIC
Gram-positive						
<i>B. subtilis</i> ATCC 6633	9.2 ± 0.7	250	26.1 ± 1.1	7.8	-	-
<i>S. aureus</i> ATCC 27217	15.1 ± 1.0	125	26.9 ± 0.7	7.8	-	-
<i>S. haemolyticus</i> ATCC 29213	14.1 ± 1.1	250	25.4 ± 1.1	7.8	-	-
Gram-negative						
<i>A. tumefaciens</i> ATCC 11158	11.5 ± 1.4	500	30.1 ± 1.6	15.6	-	-
<i>R. solanacarum</i> GM11000	15.9 ± 0.8	125	24.7 ± 1.2	7.8	-	-
<i>E. coli</i> CMCC44102	7.7 ± 0.6	500	23.8 ± 0.9	7.8	-	-
Fungi						
<i>A. niger</i> CMCC(B) 98003	12.9 ± 0.8	250	-	-	23.4 ± 1.0	31.3
Yeast						
<i>C. albicans</i> ATCC 10231	11.1 ± 1.2	250	-	-	22.4 ± 1.5	15.6

^aResults are means of three different experiments, ^bDiameter of inhibition zone including disc diameter of 6 (mm), Mean \pm standard deviation for triplicate experiments, ^cMIC, minimum inhibitory concentration (as $\mu\text{g}/\text{mL}$)

To understand the antibacterial mechanism of the essential oil to bacteria, we first selected *R. solanacarum* as model to observe of the action of essential oils of *M. cordata* on the membrane structure of *R. solanacarum*. The SEM micrograph of *R. solanacarum* cells treated with the essential oils showed that big change appeared in the cell membrane and cell atrophy and condenses into clusters, lost the original rod shape. *R. solanacarum* cells treated with the essential oil also showed many fragmentary cells by SEM. According to the results of this study, the essential oil of *M. cordata* may be suggested as a new potential source of natural antimicrobial. This is the first report on the antimicrobial activity of essential oil from *M. cordata*, though further work is now required to evaluate the antimicrobial compounds and mechanism, In addition, other biological activities of the essential oil of *M. cordata* is now required to further develop.

ACKNOWLEDGEMENTS

This work was financially supported by the Natural Science Foundation of Guizhou Province (NY[2011]3094, [2010] 4008, [2010]3072 and [2009]7012-1).

REFERENCES

- D.L. Zink, *Emerg. Infect. Dis.*, **3**, 467 (1997).
- S. Brul and P. Coote, *Int. J. Food Microbiol.*, **50**, 1 (1999).
- K.M. Schuenzel and M.A. Harrison, *J. Food Prot.*, **65**, 1909 (2002).
- V.A. Shanmuganathan, M. Armstrong, A. Buller and A.B. Tullo, *Eye*, **19**, 284 (2005).
- N. Dudareva, F. Negre, D.A. Nagegowda and I. Orlova, *Crit. Rev. Plant Sci.*, **25**, 417 (2006).
- C. Sarikurku, K. Arisoy, B. Tepe, A. Cakir, G. Abali and E. Mete, *Food Chem. Toxicol.*, **47**, 2479 (2009).
- M. Zuzarte, M.J. Gonçalves, C. Cavaleiro, J. Canhoto, L. Vale-Silva, M.J. Silva, E. Pinto and L. Salgueiro, *J. Med. Microbiol.*, **60**, 612 (2011).
- H.J. Yeom, J.S. Kang, J.H. Kim and I.K. Park, *J. Agric. Food Chem.*, **60**, 7194 (2012).
- S. Burt, *Int. J. Food Microbiol.*, **94**, 223 (2004).
- S. Singh, I.P.S. Kapoor, G. Singh, C. Schuff, M.P. Lampasona and C.A.N. Catalan, *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.*, **83**, 357 (2013).
- F. Feng, F.Z. Ye, C.L. Li, W.Y. Liu and N. Xie, *Chinese J. Nat. Med.*, **10**, 0378 (2012).
- L. Ouyang, X.L. Su, D.S. He, Y.Y. Chen, M. Ma, Q.J. Xie and S.Z. Yao, *J. Sep. Sci.*, **33**, 2026 (2010).
- J. Tian, J.P. Yu, Y.H. Ge, L.Y. Qi and C.M. Li, *Pa. Med.*, **32**, 1108 (2010).
- Z.B. Hu, Y. Xu, S.C. Feng and G.J. Fan, *Acta Pharmacol. Sin.*, **14**, 535 (1979).
- F.R. Stermitz, K.A. Larson and D.K. Kim, *J. Med. Chem.*, **16**, 939 (1973).
- J. Vrba, P. Dolezel, J. Vicar and J. Ulrichova, *Toxicol. in Vitro*, **23**, 580 (2009).
- O.V. Iagodina, E.B. Nikol'skaia and M.D. Faddeeva, *Tsitologiya*, **45**, 1032 (2003).
- P. Kosina, J. Gregorova, J. Gruz, J. Vacek, M. Kolar, M. Vogel, W. Roos, K. Naumann, V. Simanek and J. Ulrichova, *Fitoterapia*, **81**, 1006 (2010).
- M. Zhong, G.Y. Li, J.G. Zeng, L. Zhang, K.L. Huang, J.M. She, X. Li and W.Y. Wei, *J. Med. Plants Res.*, **5**, 521 (2011).
- K. Wang, C. Luo and H. Liu, *Afr. J. Agric. Res.*, **7**, 5925 (2012).
- J.Y. Yao, J.Y. Shen, X.L. Li, Y. Xu, G.J. Hao, X.Y. Pan, G.X. Wang and W.L. Yin, *Parasitol. Res.*, **107**, 1035 (2010).
- J. Lenfeld, M. Kroutil, E. Marsalek, J. Slavik, V. Preininger and V. Simanek, *Planta Med.*, **43**, 161 (1981).
- K. Penciková, P. Kollár, V. Müller Závalová, E. Táborská, J. Urbanová and J. Hošek, *Phytomedicine*, **19**, 890 (2012).
- B.C. Jang, J.G. Park, D.K. Song, W.K. Baek, S.K. Yoo, K.H. Jung, G.Y. Park, T.Y. Lee and S.I. Suh, *Toxicol. in vitro*, **23**, 281 (2009).
- H. Park, E. Bergeron, H. Senta, K. Guillemette, S. Beauvais, R. Blouin, J. Sirois and N. Fauchoux, *Biochem. Biophys. Res. Commun.*, **399**, 446 (2010).
- Z.F. Zhang, Y. Guo, L.W. Zhang, J.B. Zhang and X.H. Wei, *Acta Pharm. Sinica B*, **2**, 464 (2012).
- M.L. Colombo and E. Bosisio, *Pharmacol. Res.*, **33**, 127 (1996).
- M.A. Kerry, O. Duval, R.D. Waigh and S.P. Mackay, *J. Pharm. Pharmacol.*, **50**, 1307 (1998).
- L.J. Chen, S.Y. Zhou, H.Z. Shi and J. Yin, *Chinese Agric. Sci. Bull.*, **25**, 94 (2009).
- F. Oke, B. Aslim, S. Ozturk and S. Altundag, *Food Chem.*, **112**, 874 (2009).
- R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured: Carol Stream IL, USA., (2001).
- E.O. Kose, I.G. Deniz, C. Sarikurku, O. Aktas and M. Yavuz, *Food Chem. Toxicol.*, **48**, 2960 (2010).
- H. Muroi and I. Kubo, *J. Appl. Microbiol.*, **80**, 387 (1996).