Journal of Medicinal Plants Research Vol. 5(23), pp. 5605-5610, 23 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Antifungal activity of extracts by supercritical carbon dioxide extraction from roots of *Echinacea angustifolia* and analysis of their constituents using gas chromatography-mass spectrometry (GC-MS)

**Li Dahui, Wang Zaigui and Zhang Yunhua***

College of Life Sciences, Anhui Agricultural University, Changjiang Xilu No.130, Hefei 230036, China.

Accepted 11 August, 2011

**In this work the supercritical fluid extraction with carbon dioxide was applied to obtain the extracts from roots of *Echinacea angustifolia* DC. The extracts were evaluated through the antifungal *in vitro* assay against fungal strain *Botrytis cinerea*. Furthermore, the active compounds from the extracts were separated by macroporous resin and analyzed using gas chromatography-mass spectrometry (GC-MS). The results show that the extracts, obtained using SC-$CO_2$ extraction technique, had strong antifungal activity against *B. cinerea* with $EC_{50}$ and $EC_{90}$ values of 948 and 1869 µg/ml, minimum inhibitory concentration (MIC) of 250 µg/ml, and minimum fungicidal concentration (MFC) of 2000 µg/ml, respectively. After separation with macroporous resin chromatography, nine fractions were collected from the SC-$CO_2$ extracts. At concentration of 2000 µg/ml, fractions 3, 7, and 8 presented highly antifungal activity against *B. cinerea*, with inhibition ratio of 40.59, 73.04 and 53.37%, respectively. Determined by the GC-MS analysis, the composition of fraction 7 consisted of different kinds of volatile oils, most of which were ketones (82.71%) and unsaturated hydrocarbons (9.59%). The present study has provided the new evidence that the Echinacea extracts are involved in the antifungal activity.**

**Key words:** Antifungal activity, *Echinacea angustifolia* DC, gas chromatography-mass spectrometry (GC-MS), SC–$CO_2$ extraction.

## INTRODUCTION

*Echinacea angustifolia* DC., together with *Echinacea purpurea* L. and *Echinacea pallida* L., is an herbaceous perennial and a member of the Asteraceae family with a well-established tradition of medicinal use in North America, Europe and Australia (Wills and stuart, 1999; Speroni et al., 2002). The main active compounds of these *Echinacea* species include alkamides and polyacetylenes, caffeic and ferulic acid derivatives, polysaccharides and glycoproteins, volatile oils and pyrrolizidine alkaloids, such as tussilagine and isotussilagine (Percival, 2000; Luo et al., 2003).

Recently, these *Echinacea* species have been widely used for medicinal purposes, in treating acute upper respiratory infections, urinary tract infections, burns and disorders such as viral infections, cutaneous affections and chronic disease due to a deficiency of immunological responses (Speroni et al., 2002; Barrett, 2003; Luo et al., 2003). Their medical and pharmacological activities, such as anti-inflammatory effects, stimulation of macrophages and natural killer cells, have been well studied (Barrett, 2003). Certain evidences for the antimicrobial activities of *Echinacea* extracts have been provided by observations of protecting immunosuppressed mice against systemic infections with *Listeria monocytogenes* and *Candida albicans* by stimulating macrophage and neutrophil function (See et al., 1997). Additionally, they were reported to increase chemotoxicity in neutrophils and

*Corresponding author. E-mail: zhang_yunhua@yahoo.com.cn. Tel: +86-551-5786-865. Fax: +86-551-5786-201.

**Abbreviations: $EC_{90}$,** 90% effective concentration; **GC-MS,** gas chromatography-mass spectrometry; **$EC_{50}$,** median effective concentration; **MFC,** minimum fungicidal concentration; **MIC,** minimum inhibitory concentration; **PDA,** potato-dextrose-agar; **SC-CO2,** supercritical carbon dioxide; **SFE,** supercritical fluid extraction.

bactericidal activity against *Staphylococcus* and to kill cells infected either with the parasite *Leishmania enriettii* or with yeast *C. albicans* (Percival, 2000). Moreover, it was found that hexane extracts of *Echinacea* variably inhibit growth of yeast strains of *Saccharomyces cerevisiae*, *Candida shehata*, *Candida kefyr*, *C. albicans*, *Candida steatulytica* and *Candida tropicalis* under near UV irradiation (phototoxicity) and to a lower extent without irradiation (Binns et al., 2000).

Organic solvent extraction has been applied for more than decades in the researches of natural products. Although organic solvents, such as ethyl acetate, chloroform, methanol or acetone, are effective, these extraction methods have many disadvantages such as time consuming and labor intensive. Moreover, when the methods are applied, large amounts of solvent and sample are usually required. And the used solvents may represent a potential hazard to human health and environment. Supercritical fluid extraction (SFE) is a relatively recent technique which presents various advantages over traditional methods, for example, the use of low temperatures, reduced energy consumption, and high product quality due to the absence of solvent in solute phase (Chester et al., 1996). Supercritical carbon dioxide (SC-$CO_2$) is a better solvent for SFE due to its particular characteristics such as moderate critical conditions (31.1℃ and 73.8 MPa) and easy availability. It is also non-toxic, inflammable and chemically stable. The applications of SFE are multiple in the areas of cosmetics, essences, foods, and environmental protection (Brunner, 2005). So far, organic solvent extraction techniques have been applied for extraction from the roots and the aerial parts of *Echinacea* species. However, to our knowledge, no investigation using SC-$CO_2$ extraction to obtain bioactive substances from roots of *Echinacea* species, has ever been reported.

Due to the advantage of SC-$CO_2$ extraction, which might provide another effective method looking for the active components from *Echinacea* species, in the present work, the supercritical $CO_2$ extraction was applied to obtain the extracts from roots of *E. angustifolia*. Then the extracts were evaluated through the antifungal *in vitro* assay against a pathogenic fungal strain *Botrytis cinerea*. Where possible, in addition to medicinal uses, the Echinacea extracts might have potential roles, such as an appropriate target to develop into the plant-originated fungicides. Furthermore, the active compounds from the extracts were separated by macroporous resin and analyzed using GC-MS.

## MATERIALS AND METHODS

### Plant material

*E. angustifolia* DC. was collected at the Botanic Garden, City of Linyi, Shandong Province of China, in September 2009. Fungal strain *B. cinerea* Pers. ex Fr. was obtained from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences.

### Instruments and chemicals

HA221-50-06 type of SC-$CO_2$ extraction instrument (Huaan Supercritical Fluid Extraction Instrument Co. Ltd., Nantong, Jiangsu Province, China); $CO_2$ with purity ≧99.5% was provided by Beijing Hepubeifen Gas Industry Co. Ltd., Beijing, China; HYP-150 type of disintegrator (Beijing Huanyatianyuan Mechanical Technologies Co. Ltd., Beijing, China); Agilent 6890 gas chromatography-5973N mass spectrometry (Agilent Technologies Inc., USA); Milli-Q water purification system (Millipore Inc., USA). All reagents were of chromatographic reagent grade (Beijing Chemical Reagents Co. Ltd., Beijing, China).

### Sample preparation and SC-$CO_2$ extraction

After being cleaned, the dried roots of *E. angustifolia* were crushed by the disintegrator and passed through 60 mesh sieve. The obtained sample powder was stored in the desiccator until the extraction was performed. The sample powder (1000 g) was placed inside the SC-$CO_2$ extraction cell and submitted to the extraction. The operating conditions were set as: $CO_2$ flow rate 21 L/h, extraction pressure 30 MPa, extraction temperature 46℃, separation temperature 45℃, separation pressure 5.5 MPa, total extracting time 100 min. Through the SC-$CO_2$ extraction, fat soluble extracts were collected into flasks.

### Antifungal activity assay

The antifungal activity assay was manipulated by the means of plate-based fungal growth inhibition method with certain revision (Wu et al., 2009). The procedure was as follows: (1) Fungal strain *B. cinerea* was cultured on PDA medium plates at 25℃ for five days, then its hyphae discs with 6 mm in diameter were cut off from petri dishes, (2) acetone solution of the SC-$CO_2$ extracts and 0.5 ml Tween-80 were added to 20 ml PDA medium to produce the PDA plates with final concentration of 2000, 1000, 500, 250, and 125 μg/ml, respectively; the same volume of acetone was similarly treated as negative control, (3) each of the hyphae disc above was inoculated on the PDA plates with different concentration of the extracts;, (4) inhibition of the fungal growth was determined by the diameter of hyphal growth at 25℃ until the hyphal growth of the negative control was full of the plates. Inhibition ratio was calculated using the following formulation:

Inhibition ratio (%) = [(diameter of negative control – diameter of experimental hyphae)/ diameter of negative control] × 100 %.

Calibration curves were constructed by plotting the inhibition ratio (Y) against the concentrations (X), to calculate linear regressive equation, $EC_{50}$ and $EC_{90}$ against *B. cinerea*. For each concentration, the antifungal activity was evaluated with three replicates. Software SPSS13.0 was used for Duncan's Multiple Range Test.

### Value of MIC and MFC against *B. cinerea*

According to the fungal growth inhibition method mentioned eralier, the PDA plates containing the SC-$CO_2$ extracts with final concentration of 4000, 2000, 1000, 500, 250, 125, 62.5 and 31.25 μg/ml were prepared, respectively. The same volume of acetone was similarly treated as negative control. Then, 100 μl suspension of the spores ($5\times10^4$ spores/ml) was added into the PDA plates and co-incubated at 25℃ for 48 h. Minimum inhibitory concentration (MIC) was defined as the minimum concentration that totally

**Table 1.** Inhibitory effects of different concentrations of the SC-$CO_2$ extracts against *B. cinerea*. Colony growth diameter was the mean value of three experiments. Different letters are significant different at P<0.05 based on Duncan's multiple range test.

| Test fungus | Concentrations (μg/ml) | Colony growth diameter (mm) | Inhibitory ratio (%) | Regressive equation | $EC_{50}$ (μg/ml) | $EC_{90}$ (μg/ml) | $R^2$ |
|---|---|---|---|---|---|---|---|
| *B. cinerea* | 2000 | 10.22 | 87.55[a] | y = 0.0434 ×+8.8757 | 948 | 1869 | 0.9240 |
| | 1000 | 30.21 | 63.20[b] | | | | |
| | 500 | 46.94 | 42.83[c] | | | | |
| | 250 | 67.64 | 17.61[d] | | | | |
| | 125 | 73.85 | 10.05[e] | | | | |
| | CK | 82.10 | 0.00 | | | | |

inhibited fungal growth at this moment. Based on the MIC, the further co-incubation was continued for 7 days to determine minimum fungicidal concentration (MFC) that totally inhibited fungal growth.

#### Macroporous resin chromatography

To further investigate the bioactive compounds of *E. angustifolia* roots, macroporous resin HP-20 was employed to separate the antifungal components from the SC-$CO_2$ extracts. Prior to the separation experiments, HP-20 resin was washed with ethanol and loaded into a column (4×150 cm). Then HP-20 resin was extensively washed with plenty of distilled water to remove ethanol. Sample solution (30 mg/ml) of SC-$CO_2$ extracts was loaded into the resin column, followed by elution with distilled water, gradient solution of water-acetone (9:1, 7:3, 5:5, 3:7, 1:9, v/v), and acetone at a flow rate of 1.5 ml/min, respectively. The eluate was received in an auto-partial collector (10 min per test tube). After analysis by thin layer chromatography, the eluted fractions with the same mobility were mixed together, and concentrated by rotary evaporation and freeze-dried. Antifungal activity of the eluted fractions was evaluated using the method described previously.

#### GC-MS analysis

The GC-MS analysis was conducted with an Agilent 6890-5973N GC/MS network system, equipped with a splitless injector, and a network mass selective detector. A HP-5MS capillary column was used (30 m × 0.25 mm ID, 0.25 μm film thickness). The carrier gas was ultra-pure helium at constant flow of 1.0 ml/min. The injector temperature was set at 300℃. The initial oven temperature of 150℃ was held for 2 min; the temperature was increased by 2℃/min to 180℃ and held for 2 min; the temperature was increased by 5℃/min to 200℃ and held for 3 min; and the temperature was increased by 8℃/min to final 250℃ and held for 10 min. Injections of 1 μl were made in splitless mode.

The Agilent 5973N mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1718 eV. The MS transfer line temperature was set at 280℃, the source temperature 230℃, and the quadrupole temperature 150℃. Detection was accomplished in selected ion monitoring mode, scanned m/z 63, 79, 210. Compounds were identified by comparing their spectra with those in the Wiley (v.138) library and those of authentic standards.

## RESULTS

To evaluate the antifungal activity of the SC-$CO_2$ extracts from roots of *E. angustifolia* against fungal pathogen *B. cinerea*, the plate-based *in vitro* assay was used. The result showed that negative contols (acetone) haven no inhibitory activities against the fungal growth. Whereas on the PDA plate with the SC-$CO_2$ extracts, the growth of the fungal was inhibited, showing a decreased diameter of hyphae disc. There is a dose-effect relationship between concentration of the SC-$CO_2$ extracts and inhibition ratio, and significant difference ($P<0.05$) between the inhibitory ratios at different concentrations (Table 1). The SC-$CO_2$ extracts could inhibit the fungal growth with $EC_{50}$ and $EC_{90}$ values of 948 and 1869 μg/ml (Table 1), MIC and MFC values of 250 and 2000 μg/ml (Table 2), respectively.

Via macroporous resin HP-20, total of 31 fractions was separated from the SC-$CO_2$ extracts. Preliminary analyzed by thin layer chromatography, those fractions with the same mobility were mixed together, resulting in 9 fractions. And the resultant 9 fractions were collected and evaluated through the antifungal *in vitro* assay against *B. cinerea*, respectively. As showed in the Table 3, there is a significant difference ($P<0.05$) between inhibitory ratios of different fractions. Fractions 3, 7 and 8 showed stronger activity against *B. cinerea*, with inhibitory ratios of 40.59, 73.04 and 53.37%, respectively (Table 3).

Based on the antifungal activity assay, Fraction 7 with the inhibition ratio >70% was selected for GC-MS analysis (Figure 1). Mass spectrometer identification showed that it consisted of different kinds of volatile oils, most of which were ketones (82.71%) and unsaturated hydrocarbons (9.59%), as showed in the Table 4. (R)cyclopentadecanone was found out to be the major composition with relative content of 79.12% (Table 4).

## DISCUSSION

SC-$CO_2$ extraction is a highly effective technique for the researches of natural products. There is an increasing interest in employing SC-$CO_2$ to extract active components in practices (Ling et al., 2006). In present work, the antifungal components were extracted from roots of *E. angustifolia* using the SC-$CO_2$ technique. The

**Table 2.** The results of MIC and MFC. "+"to show *B. cinerea* growth; "—" to show no *B. cinerea* growth.

| Test fungus | Inhibitory effects | Concentrations (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 31.25 | 62.5 | 125 | 250 | 500 | 1000 | 2000 | 4000 |
| *B. cinerea* | MIC | + | + | + | - | - | - | - | - |
| | MFC | + | + | + | + | + | + | - | - |

**Table 3.** Inhibitory action of different fractions via macroporous resin against *B. cinerea*. Negative control's diameter was 71.5 mm. Colony growth diameter was the mean value of three experiments at solvent concentration of 2000 μg/ml. Different letters are significant different at $P<0.05$ based on Duncan's multiple range test.

| Isolation fraction | Isolation ratio (%) | Colony growth diameter (mm) | Inhibitory ratio (%) |
|---|---|---|---|
| 1 | 8.54 | 62.31 | 12.85[a] |
| 2 | 1.59 | 67.80 | 5.17[b] |
| 3 | 26.12 | 42.48 | 40.59[c] |
| 4 | 2.45 | 59.78 | 16.39[d] |
| 5 | 3.84 | 51.66 | 27.75[e] |
| 6 | 1.72 | 61.84 | 13.51[f] |
| 7 | 39.34 | 19.28 | 73.04[g] |
| 8 | 9.55 | 33.34 | 53.37[h] |
| 9 | 6.85 | 54.98 | 23.10[i] |

SC-$CO_2$ extracts had high antifungal activity against *B. cinerea* with $EC_{50}$ and $EC_{90}$ values of 948 and 1869 μg/ml (Table 1), MIC and MFC values of 250 and 2000 μg/ml (Table 2), respectively. After macroporous resin chromatography, 9 fractions with different antifungal activities against *B. cinerea* were separated from the extracts. *B. cinerea* has been known as a fungal pathogen for grey mould, which can cause some fruits (such as tomato and strawberry) to be rotten, and result in serious loss in yields (Wang, 2000). Unfortunately, there are no effective and safe treatments to control the fungal disease. Although chemical fungicides can protect the fruits from the fungal disease at certain degree, pesticide residues would pollute environment and endanger consumers (Reffstrup et al., 2010). The result that the herb was found to contain antifungal components against *B. cinerea*, together with its non-toxic in mice, rats and humans even when administered intravenously at high doses (See et al., 1997), indicated the potential roles for *E. angustifolia* to develop into a botanical fungicide.

To further investigate the bioactive compounds of *E. angustifolia* roots, macroporous resin HP-20 was employed to separate the antifungal components from the SC-$CO_2$ extracts. Separation method by macroporous resin has been widely used in the separation and enrichment of bioactive compounds from many natural products (Zhang et al., 2008). Macroporous resin produces good recovery because of their unique adsorption properties and other advantages, including its simple procedure, high efficiency and easy regeneration. Therefore, the separation method is suitable for large-scale industrial production. In the present work, the antifungal components were separated by macroporous resin chromatography, which indicating that the separation method may be applied to the large-scale separation and purification of bioactive components from *Echinacea* species for their practical application.

The composition of the root extracts from *Echinacea* species, compared to the upper plant extracts, is very different (Percival, 2000). Root parts have more volatile oils and pyrrolizidine alkaloids, such as tussilagine and isotussilagine, than the aerial parts. The main active compounds of the aerial parts are alkamides and polyacetylenes, caffeic and ferulic acid derivatives, polysaccharides (such as 4-O-methyl-glucuronylarabinoxylans, rhamnoarabinogalactans and acidic arabinogalactan) and glycoproteins (Percival, 2000; Luo et al., 2003). Of the caffeic acid derivatives, only cichoric acid, shows immunostimulatory properties, promoting phagocyte activity *in vitro* and *in vivo*, antihyaluronidase activity and has a protective effect on the free-radical-induced degradation of collagen (Pellati et al., 2004). The antioxidant and antimicrobial activities of Echinacea extracts have been shown in various researches. The antioxidant activity could be ascribed to the polyphenolic components (Cervellati et al., 2002), such as flavonoids (Pietta et al., 1998; Burda and Oleszek, 2001), phenolic acids (Velioglu et al., 1998) or phenolic diterpenes (Pietta et al., 1998). The antimicrobial activity, such as antibacterial and antiviral effects, was

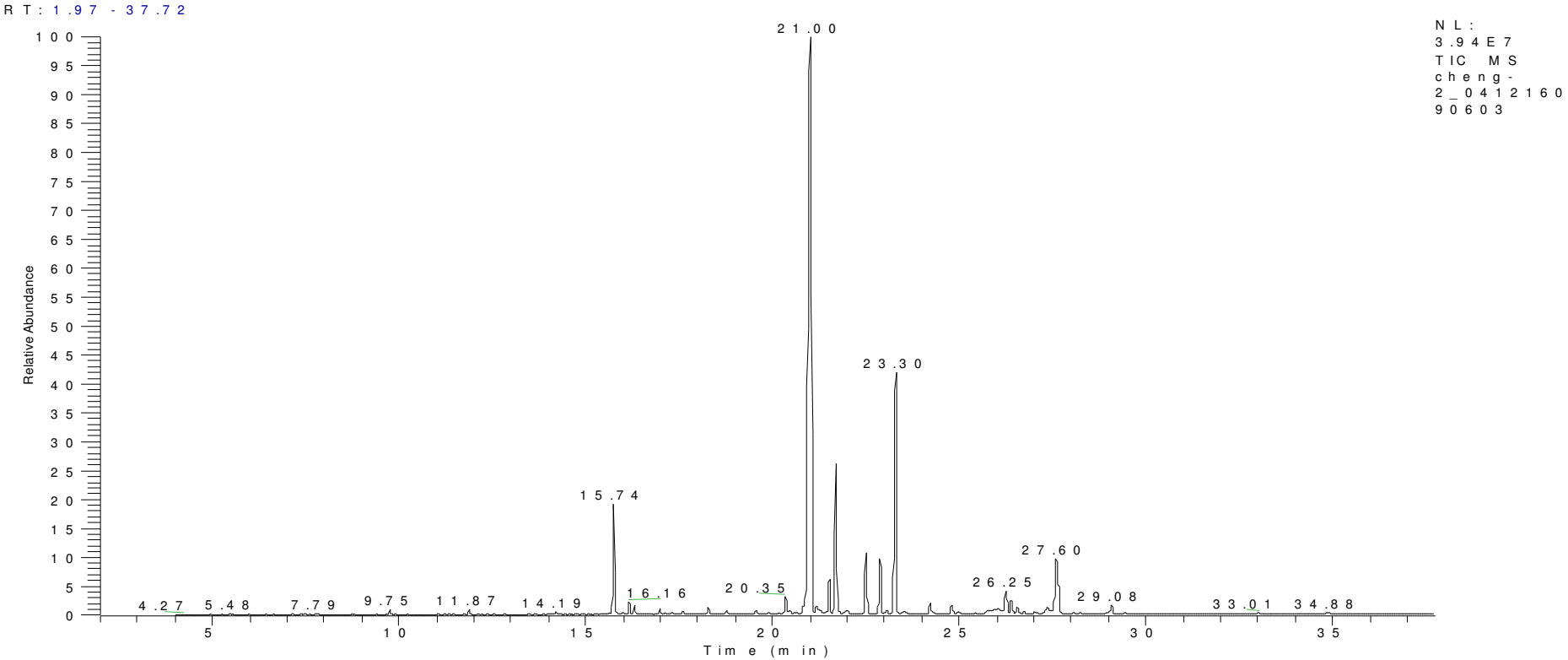


**Figure 1.** Total ioncurrent chromatogram of Fraction 7.

**Table 4.** Composition of the Fraction 7 from *E. angustifolia* roots.

| Peak No. | Retention time (min) | Compounds | Area (%) |
|---|---|---|---|
| 1 | 9.75 | (R)6-undecanone | 0.23 |
| 2 | 11.87 | (R)2-undecanone | 0.17 |
| 3 | 13.49 | (M)8-oxo-2-nonenal | 0.09 |
| 4 | 14.19 | (M)trans-5-dodecenal | 0.18 |
| 5 | 15.74 | (M)trans-9-tetradecenyl | 6.55 |
| 6 | 16.16 | (R)1-pentadecenal | 0.61 |
| 7 | 16.31 | (M)2-tridecanone | 0.59 |
| 8 | 17.13 | (M)2,4-dodecadienal | 0.06 |

**Table 4.** Contd.

| | | | |
|---|---|---|---|
| 7 | 16.31 | (M)2-tridecanone | 0.59 |
| 8 | 17.13 | (M)2,4-dodecadienal | 0.06 |
| 9 | 17.61 | α-methyl phenyl ethyl carbinyl acetate | 0.24 |
| 10 | 18.78 | (M)2-tetradecanone | 0.26 |
| 11 | 20.99 | (R)cyclopentadecanone | 79.12 |
| 12 | 21.51 | (R)2-pentadecanone | 2.34 |
| 13 | 22.51 | (Z)-4-hexadecenal-6-yne | 4.63 |
| 14 | 22.88 | (E)- 6-hexadecenal-4-yne | 4.12 |
| 15 | 24.99 | (E)-cyclododecenal | 0.23 |
| 16 | 26.56 | Methyl-cis-15-tetracosenoate | 0.58 |

related to echinacoside and caffeic acid derivative (Bergeron et al., 2000). In present work, the extract obtained by the SC-$CO_2$ extraction from roots of *E. angustifolia* contained a number of bioactive compounds, some of which showing *in vitro* antimicrobial activity against the tested fungal strain *B. cinerea*. Determined by GC-MS analysis, the composition of the antifungal extract included many kinds of volatile oils (Table 4), which was consistent with the properties of the identified chemical constituents in Echinacea roots (Luo et al., 2003). Two of these chemical constituents, (R)cyclopentadecanone and methyl-cis-15-tetracosenoate, have not previously been reported in the genus *Echinacea*. These chemical constituents in roots of *E. angustifolia* may provide a material basis against fungal pathogen *B. cinerea*. And the present study has provided the new evidence that the Echinacea extracts are involved in the antifungal activity.

## REFERENCES


Barrett B (2003). Medicinal properties of *Echinacea*. Phytomedicine, 10: 66-86.

Bergeron C, Livesey JF, Awang DVC, Arnason JT, Rana JB, Baum R, Letchamo W (2000). A quantitative HPLC method for the quality assurance of Echinacea products on the North American market. Phytochem. Anal., 11: 207-215.

Binns SE, Purgina B, Bergeron C, Smith ML, Ball L, Baum BR, Arnason JT (2000). Light-mediated antifungal activity of Echinacea extracts. Planta Med., 66: 241-244.

Brunner G (2005). Supercritical fluids: technology and application to food processing. J. Food Eng., 67: 21-33.

Burda S, Oleszek W (2001). Antioxidant and antiradical activities of flavonoids. J. Agric. Food Chem., 49: 2774-2779.

Cervellati R, Renzulli C, Guerra MC, Speroni E (2002). Evaluation of antioxidant activity of some natural polyphenolic compounds using the Briggs-Rauscher reaction method. J. Agric. Food Chem., 50: 7504-7509.

Chester T, Pinkston J, Raynie D (1996). Supercritical fluid chromatography and extraction. Anal. Chem., 68: 487-514.

Ling J, Dai L, Ding X (2006). GC-MS analysis of supercritical carbon dioxide extraction from the stroma of *Cordyceps sinensis*. Mycosystema, 25: 138-141.

Luo XB, Chen B, Yao SZ, Zeng JG (2003). Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of *Echinacea purpurea* by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. J. Chromatogr. A., 986: 73-81.

Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F (2004). Analysis of phenolic compounds and radical scavenging activity of *Echinacea spp*. J. Pharm. Biomed. Anal., 35: 289-301.

Percival SS (2000). Use of Echinacea in medicine. Biochem. Pharm., 60: 155-158.

Pietta P, Simonetti P, Mauri P (1998). Antioxidant activity of selected medicinal plants. J. Agric. Food Chem., 46: 4487-4490.

Reffstrup TK, Larsen JC, Meyer O (2010). Risk assessment of mixtures of pesticides: Current approaches and future strategies. Regul. Toxicol. Pharm., 56: 174-192.

See DM, Broumand N, Sahl L, Tilles JG (1997). *In vitro* effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. Immunopharmcology, 35: 229-235.

Speroni E, Govonib P, Guizzardib S, Renzullia C, Guerra MC (2002). Anti-inflammatory and cicatrizing activity of *Echinacea pallida* nutt, root extract. J. Ethnopharm., 79: 265-272.

Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem., 46: 4113-4117.

Wang L (2000). Horticultural plant pathology. Chinese Agriculture Press, Beijing.

Wills RBH, Stuart DL (1999). Alkylamide and cichoric acid levels in *Echinacea purpurea* grown in Australia. Food Chem., 67: 385-388.

Wu Z, Wang Y, Ai Q (2009). Inhibitory effect and antimicrobial mechanism of pyrolin on *Monilinia fructicola* in peach. Sci. Agric. Sin., 42: 2784-2792.

Zhang Y, Jiao J, Liu C (2008). Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography. Food Chem., 107: 1326-1336.