International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 8 (2), pp. 001-009, February, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antioxidant and antibacterial activities of *Camptotheca acuminate* D. seed oil

Lin Wang[1, 2], Zhiwei Yang[1*], Sicen Wang[3], Shuqiu Wang[1] and Junxing Liu[1]*

[1]School of Basic Medical Sciences, Jiamusi University, Jiamusi 15400, P. R. China.
[2] The First Affiliated Hospital of Jiamusi University, Jiamusi 154003, P. R. China.
[3]Research and Engineering Center for Natural Medicine, Xi'an Jiaotong University, Xi'an 710061, P. R. China.

Accepted 21 November, 2020

This study was designed to explore the *in vitro* antioxidant and antibacterial activities of *Camptotheca acuminate* D. seed oil, which were extracted by supercritical fluid extraction (SFE) or petroleum ether extraction methods. The major constituent of the oil were described as (Z,Z,Z)-9,12,15-Octadecatrien-1-ol (54.92%) and 2-[(trimethylsilyl)oxy]-3-[4-[(trimethylsilyl)oxy]phenyl]-trimethylsilyl ester (26.53%) in supercritical fluid and petroleum ether extracts. The oil and the components were subjected to screen for their possible antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene bleaching test. In the DPPH test system, free radical scavenging activities of supercritical fluid extracts and petroleum ether extracts were determined to be 7.55 ± 0.11% and 4.38 ± 0.08% (v/v), respectively. As to the β-carotene bleaching test system, the two values were 15.93 ± 0.11% and 6.87 ± 0.15% (v/v), respectively. The activities of antioxidant and antibacterial in components of petroleum ether were more efficient than in components of supercritical fluid extraction. As to the antimicrobial activities of the essential oil against 8 species bacterium, *C. acuminate* D. seed oil had remarkable antibacterial activity, especially to *staphylococcus aureus* (ATCC 6538). Thus, *C. acuminate* D. seed oil could be judged as a kind of patent drug which has antioxidant and antibacterial activity effectively.

Key words: *Camptotheca acuminate* D. seed oil, antioxidant activities, antibacterial activities.

INTRODUCTION

Camptotheca acuminata Decaisne belongs to the family of Nyssaceae. It is a kind of deciduous and medicinal tree from south China and listed as an endangered species in China (Cheng et al., 2008; Li et al., 2002; (Yang et al, 2011) DOI: 10.1002/qua.23046). The main active ingredients of *C. acuminate* D. extracts are quinoline and indole alkaloids (Wall et al., 1966). The camptothecin (CPT) (a terpenoid quinoline alkaloid) and its analogues are the potent topoisomerase I inhibitors, which have been used as the anticancer drugs to treat ovarian, lung and colorectal cancers or the antiviral agents (Li and Adair, 1994; Oberlies and Kroll, 2004).

Some studies on anticancer activities of *C. acuminata* D. have been reported. However, the antioxidant activity of its seed oil has not yet been studied. The seed oil of *C. acuminata* D. possesses the potential as high-quality edible oil that is beneficial to health and valuable natural antioxidants in cosmetic and pharmaceutical industries.

Industrial seed oils are generally obtained with the aid of mechanical process and organic solvent extraction (mainly hexane). The oil prepared by mechanical separation is of high quality, but in most cases the yield is low. Hexane extraction can achieve almost complete recovery of the oil, but the solvent is quite harmful to human health and environment, embarrassing the use in food, cosmetic and pharmaceutical industries. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO_2) is an alternative method to extract the oils from natural

*Corresponding author. E-mail: yzws-123@163.com. iujunxing0982@163.com, Tel: +086 0454 6166452. Fax: +086 0454 8618355.

products and has received considerable attention (Gomes et al., 2007; Lu et al., 2007; SalgIn, 2007). The oil obtained by SC-CO_2 extraction is of high quality, and the yield is comparable with those of organic solvent extractions (Friedrich et al., 1982; Molero Gómez et al., 1996). In fact, CO_2 extracts have been generally considered as safety in food applications (Gerard and May, 2002), and SFE has been served as a very potential technology in food and pharmaceutical operations (King, 2000).

To the best of our knowledge, the antioxidant and antibacterial activities of *C. acuminate* D. seed oil *in vitro* have not yet been evaluated. Therefore, in this work, the oil will be separately extracted by SFE and petroleum ether extraction methods, and the activities will be evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, β-carotene bleaching test, as well as minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determinations. We anticipate that the investigation will be of value in the development of antioxidant and antibacterial agents.

MATERIALS AND METHODS

Preparation of extract

Isolation of C. acuminata seed oil by petroleum ether

C. acuminate D. seeds were air dried at room temperature and ground, and 100 g were subjected to the distillation with a British-type clevenger apparatus at 100°C for 3 h. Then, the extracts were filtered and concentrated in vacuum at 45°C, yieldin g the seed soil in yellow.

Supercritical carbon dioxide (SC-CO_2) extraction

C. acuminate D. seeds were air dried at room temperature and ground in a grinder with a mesh of 2 mm in diameter. Then, they were passed through a 0.5 mm sieve to obtain a fine powder. The extraction temperature was 45°C and the pressure was 5 .5 MPa. The flow rate was determined using a watch. The flow rate of CO_2 was 10 kg/h; and the extraction time was 2 h. Liquid CO_2 was supplied from a gas cylinder. Before passing into the extraction vessel filled with the samples by pump, the liquid CO_2 was adjusted to the desired pressure and heated to a specified temperature to reach the supercritical state. Finally, the extracts were lyophilized and kept in dark at 4°C until the next step.

Gas chromatography

The sample was diluted by ethyl acetate (1:100) and mixed. The analysis of the essential oil was performed using a VG platform II Gas chromatography-Mass spectroscopy (GC–MS) system equipped with an Rtx-5MS capillary column (30 × 0.25 mm; film thickness 0.25 mm). For the Rtx-5MS detection, the injector temperature was set at 280°C; split injection with a ratio of 100: 1; and the injection volume was 1 µl with a flow controlled by a linear model. Helium was the carrier gas at a flow rate of 1.6 ml/min. Starting from 60°C, the temperature was raised to 280 a t 10°C /min and held for 5 min. Injector and detector MS transfer line temperatures were set at 200 and 280°C, respecti vely, and the sample collection time was 3 min (m/z = 40 to 500).

Antioxidant activity

General description

Antioxidant activity was assessed by DPPH assay and β-carotene bleaching test. All data collected for each assay were the average of the measurements of three independent experiments.

DPPH radical scavenging assay and the oil obtained by SC-CO_2 extraction

We measured the bleaching of purple-colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Wu et al., 2010). An aliquot of the sample (100 µl) was mixed with 1.4 ml of ethanol and then added to 1 ml of 0.004% DPPH (Sigma–Aldrich) in ethanol. The mixture was vigorously shaken and then immediately placed in a UV–Vis spectrophotometer (AWARENESS) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ascorbic acid (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The radical scavenging activities of samples were calculated as the inhibition percentage of DPPH according to the formula:

Inhibition percentage (Ip) = [(AB-AA)/AB] ×100 (Yen and Duh, 1994)

where AB and AA are the absorbance values of the blank sample and the tested samples examined after 70 min, respectively.

β-Carotene-linoleic acid bleaching assay

Antioxidant activity of the samples was determined with β-carotene bleaching test (Wu et al., 2010). Approximately 10 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml chloroform. The carotene–chloroform solution of 0.2 ml was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed by a rotary evaporator (RE-52AA) at 40°C for 5 min, and to th e residue, 50 mL of distilled water was added, slowly with vigorous agitation, to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the sample solution and the absorbance was immediately measured at 470 nm against a blank consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50°C, and the oxidation of the emulsion was moni tored by a spectrophotometer at 470 nm over a period of 60 min. Control samples contained 10 ml of water instead. Butylated hydroxytoluene (BHT) (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as the inhibition percentage relative to the control after 60 min incubation with the following equation:

$$AA = 100(DRC - RS)/DRC$$

Where AA, antioxidant activity; DRC, degradation rate of the control [ln(a/b)/60]; DRS, degradation rate in the presence of the sample [ln(a/b)/60]; a, absorbance at time 0; b, absorbance at 60 min.

Antimicrobial activity

The microorganisms used for testing antimicrobial sensitivity included *Bacillus subtilis* 6633, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 49134, *Escherichia coli* ATCC 11229, *Proteus vulgaris, Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger V. Tiegh*. They were obtained from the Center for Microbiology Research, Jiamusi Medical Research Institute.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) determination of *Camptotheca acuminate* D. seed oil

The MIC and MBC were measured by the broth micro-dilution method (NCCLS, 2002). The essential oils were individually dissolved in sterilized physiological saline solution (0.9% w/v) supplemented with Tween 80 (Sigma) at a final concentration of 0.5% (v/v). Serial doubling dilutions of the oils were prepared in a 96-well microtiter (µL) plate in the range of 0.156 to 4.000% (v/v). Each essential oil dilution (100 µL) was dispensed into the wells of a microtiter plate, and each well was then inoculated with 100 µL of the suspension. The obtained suspensions were mixed with a micro-pipette. The final concentration of each strain was adjusted to 10^5 to 10^6 CFU/mL. All microtiter plates against all microorganisms were incubated at 37°C for 24 h, except for *A. niger* that was incubated at 25°C for 5 days. After incubation, the wells were examined for the microorganism growth, and the MICs were determined. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not show visible growth. The MBCs were confirmed by reinoculating on agar plates with 10 µL of each culture medium from the microplates. The MBCs were defined as the lowest concentration of the essential oil at which incubated microorganisms were completely killed. *Streptomycin* and *Amphotericin B* were served as the positive controls. Each experiment was repeated for three times.

RESULTS AND DISCUSSION

Chemical composition

We identified 50 components in the *C. acuminate* D. seed oil obtained by SC-CO_2 extraction (Table 1). Fifty-three (53) components were identified in the oil obtained by petroleum ether (Table 2). The major components were (Z,Z,Z) - 9, 12, 15 - Octadecatrien- 1- oil (54.92%), octadecanoic acid (13.46%), n-hexadecanoic acid (11.63%) in the seed oil (Table 1) obtained by SC-CO_2. While the major components were [4-[(trimethylsilyl)oxy]phenyl]-2-[(trimethylsilyl)oxy]-3-2-propenoicacid trimethylsilyl ester (26.53%), gamma-sitosterol (23.49%), (Z,Z,Z)-9,12,15-octadecatrienoic acid, and methyl ester (19.19%) in the seed oil obtained by petroleum ether.

Antioxidant activity

The antioxidant activities of the essential *C. acuminate* D. seed oil obtained by SC-CO_2 extraction or petroleum ether were determined by two complementary test systems: DPPH assay and β-carotene bleaching tests. The results of antioxidant activity in these test systems were collected and shown in Figures 1 to 3. In the DPPH test system, free radical - scavenging activity of *C. acuminate* D. seed oil obtained by SC-CO_2 extraction was determined to be 81.39 ± 0.92%; whereas the oil obtained by petroleum ether was 87.13 ± 1.81% (Figure 1). As for the lipid peroxidation inhibitory activity of the essential oil by the β-carotene bleaching test, the results were consistent with the data obtained from the DPPH test (Figure 2). Compared with BHT, the effects of *C. acuminate* D. seed oil obtained by SC-CO_2 extraction or petroleum ether were 80.82 ± 0.32% and 85.47 ± 0.54%, respectively. The concentration of 50% inhibition (IC_{50}) values of BHT, *C. acuminate* D. seed oil obtained by SC-CO_2 or petroleum ether were 3.24 ± 0.12%, 7.55 ± 0.11% and 4.20 ± 0.08%, respectively (Figure 3). It seemed that the antioxidant activities of all the tested samples were mostly related to their concentrations, and the IC_{50} values of these two types of seed oil were both higher than that of the synthetic antioxidant BHT (Figures 2 and 3).

Antimicrobial activity

Minimal inhibitory concentration (MICs) and minimal bactericidal concentration (MBCs) of Camptotheca acuminate D. seed oil

As shown in Tables 3 and 4, the essential oils exhibited inhibitory effects of all the testing organisms. The oil obtained by SC-CO_2 exhibited somewhat higher antimicrobial activity on *S. epidermidis* ATCC 49134 rather than other microorganisms; whereas the oil obtained by petroleum ether showed more potent on *S. aureus* ATCC 6538, *P. aeruginosa* and *C. albicans*. The antimicrobial activities of the seed oil obtained by SC-CO_2 against *B. subtilis* 6633, *P. vulgaris* and *A. niger V. Tiegh* were less than those of other microorganisms; whereas the oil obtained by petroleum ether showed less inhibitory effects on *B. subtilis* 6633 and *P. vulgaris.* The MICs of the oil obtained by SC-CO_2 extraction ranged from 0.625% (v/v) to more than 5.000% (v/v) for all testing microorganisms; while as to petroleum ether, the values ranged from 0.625% (v/v) to more than 5.000% (v/v). The MBCs were similar or even higher than the corresponding MIC values.

The activity components of Camptotheca acuminate D. seed oil

The essential oil of *C. sativum* obtained on hydro-distillation was analyzed by GC–MS. We identified 24 components, representing 92.7% of the total oil. Table 1 summarized the constituents identified by GC–MS analysis, their retention indices and area percentages (concentrations). The oil was dominated by aldehydes and alcohols, which accounted for 55.5 and 36.3%, respectively. The major aldehydes were 2E-decenal (15.9%) and decanal (14.3%), while the alcohols mainly consisted of 2E-decen-1-ol (14.2%) and n-decanol

Table 1. The chemical compositions of *C. acuminate* D. seed oil obtained by supercritical carbon dioxide extraction (SC-CO_2).

No.	RT	Compounds	Molecular formula	MW	Relative
1.	3.246	Nonane	128	C_9H_{20}	0.25
2.	3.652	propyl-cyclohexane,	126	C_9H_{18}	0.10
3.	4.097	Hexanoic acid	116	$C_6H_{12}O_2$	0.19
4.	4.443	(E,E)-2,4-Heptadienal,	110	$C_7H_{10}O$	0.14
5.	4.627	1-ethyl-Cyclohexene,	110	C_8H_{14}	0.05
6.	6.079	Phenylethyl Alcohol	122	$C_8H_{10}O$	0.06
7.	8.510	8-Methylene-3 oxatricyclo[5.2.0.0(2,4)]nonane	136	$C_9H_{12}O$	0.13
8.	8.552	(E,E)-2,4-Decadienal,	152	$C_{10}H_{16}O$	0.09
9.	8.872	2,7-Dimethyl-1,3,7-Octatriene,	136	$C_{10}H_{16}$	0.33
10.	12.737	7-Bromomethyl-Pentadec-7-ene,	302	$C_{16}H_{31}Br$	0.06
11.	14.169	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.07
12.	14.574	Heneicosane	296	$C_{21}H_{44}$	0.09
13.	15.015	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	0.07
14.	15.090	6,10,14-Trimethyl-2-Pentadecanone,	268	$C_{18}H_{36}O$	0.09
15.	15.225	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	0.06
16.	15.417	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	$C_{16}H_{22}O_4$	0.79
17.	15.888	1,2-Benzenedicarboxylic acid, butyl octyl ester	334	$C_{20}H_{30}O_4$	0.09
18.	16.077	9-Hexadecenoic acid	254	$C_{16}H_{30}O_2$	0.14
19.	16.329	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	11.63
20.	16.595	Heneicosane	296	$C_{21}H_{44}$	0.28
21.	17.217	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.07
22.	17.424	1-Octadecanol	270	$C_{18}H_{38}O$	0.14
23.	17.537	2,6,10,15-Tetramethyl-Heptadecane,	296	$C_{21}H_{44}$	0.12
24.	17.618	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester,	292	$C_{19}H_{32}O_2$	0.10
25.	17.714	Phytol	296	$C_{20}H_{40}O$	0.15
26.	18.195	(Z,Z,Z)-9,12,15-Octadecatrien-1-ol,	264	$C_{18}H_{32}O$	54.92
27.	18.265	Octadecanoic acid	284	$C_{18}H_{36}O_2$	13.46
28.	18.917	cis,cis,cis-7,10,13-Hexadecatrienal	234	$C_{16}H_{26}O$	0.55
29.	19.297	Pentacosane	352	$C_{25}H_{52}$	0.15
30.	19.542	11,14,17-Eicosatrienoic acid, methyl ester	320	$C_{21}H_{36}O_2$	0.29
31.	19.882	E-8-Methyl-7-dodecen-1-ol acetate	240	$C_{15}H_{28}O_2$	0.24
32.	20.912	Tetratetracontane	618	$C_{44}H_{90}$	0.56
33.	21.084	2-Mono-Palmitin,	330	$C_{19}H_{38}O_4$	0.99
34.	21.672	Pentatriacontane	492	$C_{35}H_{72}$	0.16
35.	21.975	Octadecanal	268	$C_{18}H_{36}O$	0.07
36.	22.281	(Z,Z)-9,12-Octadecadienoic acid, trimethylsilyl ester	352	$C_{21}H_{40}O_2Si$	0.18
37.	22.350	(all-Z)-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester,	342	$C_{23}H_{34}O_2$	0.14
38.	22.502	E,Z-1,3,12-Nonadecatriene	262	$C_{19}H_{34}$	3.94
39.	22.585	Methyl(Z)-5,11,14,17-eicosatetraenoate	318	$C_{21}H_{34}O_2$	4.30
40.	22.683	1-Mono-Stearin,	358	$C_{21}H_{42}O_4$	0.63
41.	23.251	1-Hentetracontanol	592	$C_{41}H_{84}O$	0.66
42.	23.667	(all-E)- 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22 Tetracosahexaene,	410	$C_{30}H_{50}$	1.39
43.	24.183	6,6-Dimethyl-Bicyclo[3.1.1] hept-2-ene-2-ethanol,	166	$C_{11}H_{18}O$	0.06
44.	24.268	Triacontane	422	$C_{30}H_{62}$	0.66
45.	24.331	17-Pentatriacontene	490	$C_{35}H_{70}$	0.51
46.	24.540	2-Nonadecanone	282	$C_{19}H_{38}O$	0.14
47.	25.074	8-Methyltocol	402	$C_{27}H_{46}O_2$	0.18
48.	26.091	Octadecanal	268	$C_{18}H_{36}O$	0.10
49.	26.519	β-Tocopherol	416	$C_{28}H_{48}O_2$	0.28
50.	26.862	Tetratriacontane	478	$C_{34}H_{70}$	0.15

Table 2. The chemical compositions of *C. acuminate* D. seed oil obtained by petroleum ether.

No.	RT	Compounds	Molecular formula	MW	Relative
1.	3.235	1,3,5,7-Cyclooctatetraene	104	C_8H_8	0.10
2.	5.199	3,6-dimethyl-Decane,	170	$C_{12}H_{26}$	0.06
3.	6.183	1,2,4,5-tetramethyl-Benzene,	134	$C_{10}H_{14}$	0.07
4.	8.071	Pentadecane	212	$C_{15}H_{32}$	0.07
5.	8.301	4,6-dimethyl-Dodecane,	198	$C_{14}H_{30}$	0.15
6.	8.923	Heptadecane	240	$C_{17}H_{36}$	0.04
7.	11.008	Acetamidocyclohexane	141	$C_8H_{15}NO$	0.07
8.	11.120	Cetyl iodide	352	$C_{16}H_{33}I$	0.12
9.	11.158	Heptadecane	240	$C_{17}H_{36}$	0.06
10.	11.393	2,4-bis(1,1-dimethylethyl)-Phenol,	206	$C_{14}H_{22}O$	0.07
11.	11.668	Heneicosane	296	$C_{21}H_{44}$	0.04
12.	13.629	Eicosane	282	$C_{20}H_{42}$	0.08
13.	14.103	Hexadecane	226	$C_{16}H_{34}$	0.04
14.	14.177	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.11
15.	15.008	5-Isopropyl-1-methyl-1-cyclohexene	138	$C_{10}H_{18}$	0.07
16.	15.095	6,10,14-trimethyl-2-Pentadecanone,	268	$C_{18}H_{36}O$	0.06
17.	15.419	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	$C_{16}H_{22}O_4$	0.16
18.	15.600	diethyl-Borinic acid,	86	$C_4H_{11}BO$	0.05
19.	15.878	2,6,10,14-tetramethyl-Hexadecane,	282	$C_{20}H_{42}$	0.06
20.	16.270	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	3.19
21.	17.424	1-Octadecanol	270	$C_{18}H_{38}O$	0.06
22.	17.538	Heneicosane	296	$C_{21}H_{44}$	0.08
23.	17.714	Phytol	296	$C_{20}H_{40}O$	0.13
24.	18.037	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester,	292	$C_{19}H_{32}O_2$	19.19
25.	18.162	(Z,Z)-9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	3.35
26.	18.442	1,54-Dibromotetrapentacontane	914	$C_{54}H_{108}Br_2$	0.60
27.	19.292	Pentacosane	352	$C_{25}H_{52}$	0.11
28.	20.118	Tetracosane	338	$C_{24}H_{50}$	0.08
29.	20.792	trans-9-Octadecen-1-ol	268	$C_{18}H_{36}O$	0.09
30.	20.910	Tetratetracontane	618	$C_{44}H_{90}$	0.37
31.	21.078	2-mono-Palmitin,	330	$C_{19}H_{38}O_4$	0.14
32.	21.673	Pentatriacontane	492	$C_{35}H_{72}$	0.07
33.	21.906	(3.beta.)-Ergost-5-en-3-ol,	400	$C_{28}H_{48}O$	1.98
34.	22.142	3-Fluorobenzoic acid, 4-hexadecyl ester	364	$C_{23}H_{37}FO_2$	0.23
35.	22.342	1-Pent-3-ynylcyclopenta-1,3-diene	132	$C_{10}H_{12}$	0.10
36.	22.441	1,54-dibromo-Tetrapentacontane,	914	$C_{54}H_{108}Br_2$	0.46
37.	22.489	9-Octadecenoic acid, (E,E,E)-1,2,3-propanetriyl ester,	884	$C_{57}H_{104}O_6$	0.31
38.	22.569	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, ethyl ester,	306	$C_{20}H_{34}O_2$	0.34
39.	22.608	2-Nonadecanone	282	$C_{19}H_{38}O$	0.29
40.	22.675	Stigmastane-3,6-dione	428	$C_{29}H_{48}O_2$	0.18
41.	23.250	Pentafluoropropionic acid, heptadecyl ester	402	$C_{20}H_{35}F_5O_2$	0.28
42.	23.483	1-Hexadecanesulfonyl chloride	324	$C_{16}H_{33}ClO_2S$	0.46
43.	23.664	All-trans-Squalene	410	$C_{30}H_{50}$	0.85
44.	24.167	[4-[(trimethylsilyl)oxy]phenyl]-2-[(trimethylsilyl)oxy]-3-2-Propenoic acid, trimethylsilyl ester	396	$C_{18}H_{32}O_4Si_3$	26.53
45.	24.265	.gamma.-Sitosterol	414	$C_{29}H_{50}O$	23.49
46.	24.578	Fucosterol	412	$C_{29}H_{48}O$	1.38
47.	25.034	[3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-dodecahydro -3,8,8,11a-tetramethyl-5H-3,5a-Epoxynaphth[2,1-c]oxepin, ,	278	$C_{18}H_{30}O_2$	1.39
48.	25.318	Betulin	442	$C_{30}H_{50}O_2$	0.43
49.	25.560	Cedran-8-yl acetate	264	$C_{17}H_{28}O_2$	3.32
50.	25.725	Lup-20(29)-en-3-one	424	$C_{30}H_{48}O$	4.30
51.	26.091	cis-1-Chloro-9-octadecene	286	$C_{18}H_{35}Cl$	0.52
52.	26.323	Lupenyl acetate	468	$C_{32}H_{52}O_2$	3.79
53.	26.514	.beta.-Tocopherol	416	$C_{28}H_{48}O_2$	0.41

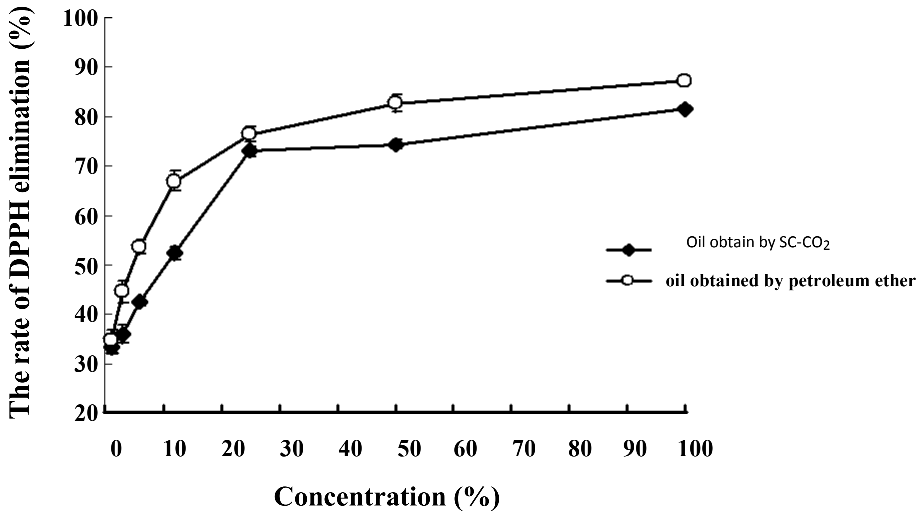

Figure 1. The rate of DPPH elimination. Values of each curve are means ± SD (n, 3). $p < 0.01$.

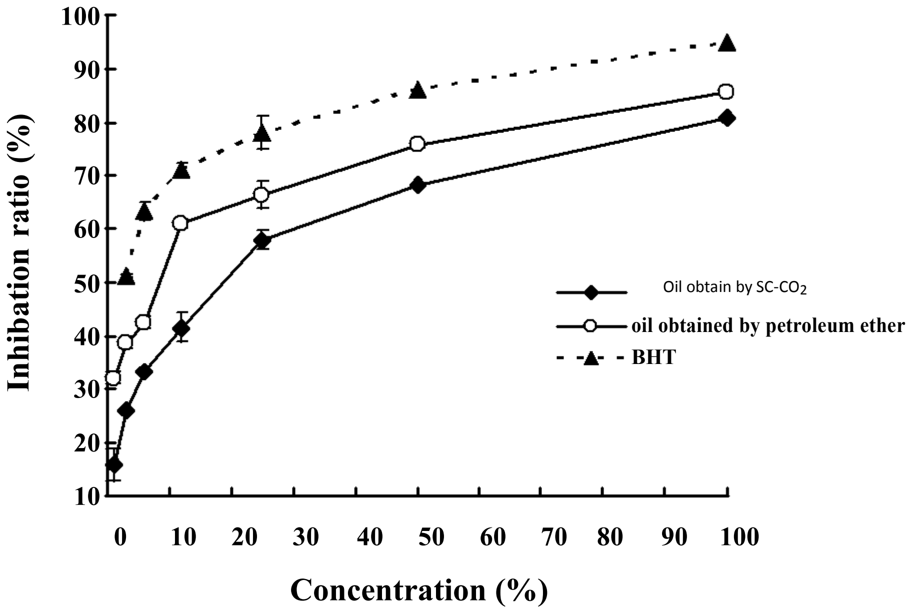

Figure 2. β-Carotene bleaching test. Values of each curve are means ± SD (n, 3). $p < 0.01$.

(13.6%). Other aldehydes in appreciable amounts were 2E-tridecen-1-al (6.75%), 2E-dodecenal (6.23%), dodecanal (4.36%) and undecanal (3.23%). The alcohol undecanol (3.37%) was also in fairly good amount. The monoterpenes apinene (0.04%) and linalool (0.32%) were in trace amounts. However, the chemical composition of the essential oil was different from that observed from Tunisian plant materials (Msaada et al., 2007). Indeed, in the Tunisia study, the predominant aldehyde was 2E-dodecenal, while in our study, it was 2E-decenal. The essential oil was evaluated for antimicrobial activity against pathogenic strains of Gram positive (*S. aureus* and *Bacillus* spp.) and Gram negative (*E. coli*, *P. aeruginosae* , *S. typhi*, *Klebsiella pneumoniae*, and *Proteus mirabilis*) bacteria. It was active against all the bacterial strains except *P. aeruginasae*.

The oil also showed an obvious antifungal activity against *C. albicans* and *P. aeruginosae*, which also been

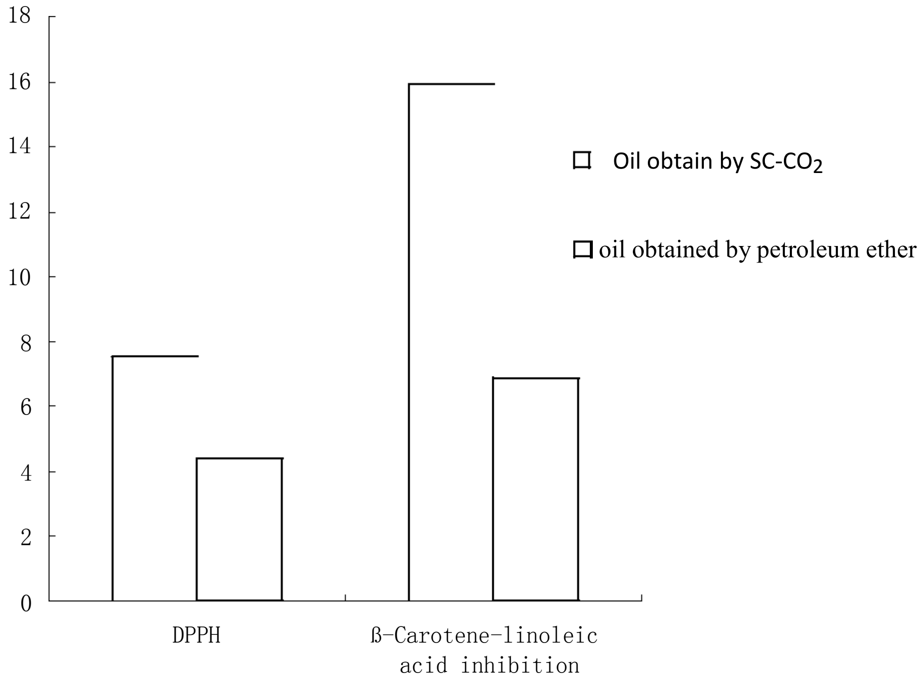

Figure 3. The concentration of 50% inhibition (IC_{50}) values of *C. acuminate* D. seed oil obtained by $SC\text{-}CO_2$ or petroleum ether.

Table 3. The inhibitory effects of C. acuminate D. seed oil obtained by SC-CO2 against all the testing organisms.

Bacterial strain	MIC (%)	MBC (%)
B. subtilis 6633	>5	>5
S. aureus ATCC 6538	1.25	1.25
S. epidermidis ATCC 49134	0.625	2.5
E. coli ATCC 11229	2.5	>5
P. vulgaris	>5	>5
P. aeruginosa	2.5	>5
C. albicans	1.25	5
A. niger V. Tiegh	>5	>5

Table 4. The inhibitory effects of C. acuminate D. seed oil obtained by petroleum ether against all the testing organisms.

Bacterial strain	MIC (%)	MBC (%)
B. subtilis 6633	>5	>5
S. aureus ATCC 6538	0.625	1.25
S. epidermidis ATCC 49134	2.5	2.5
E. coli ATCC 11229	5	>5
P. vulgaris	>5	>5
P. aeruginosa	0.625	1.25
C. albicans	0.625	2.5
A. niger V. Tiegh	1.25	5

observed to be resistant to the essential oils from other plants, such as *Achillea holosericea* (Magiatis et al., 1999) and Stachys species (Skaltsa et al., 2003). This microorganism is less susceptible to the anti-microbial properties of essential oils than others, and its tolerance is thought to result from its outer membrane (Cox and Radolf, 2001). And the ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reason for its lethal action (Cox and Radolf, 2001). This antimicrobial activity against bacteria and fungi has also been demonstrated in essential oils extracted from *C. sativum* seeds (Lo Cantore et al., 2004). Although the concentrations of the oil were generally about 100 times more than those of the standard antibiotics (chloramphenicol), they showed marked antibacterial and antifungal activities, as demonstrated by their zones of inhibition (Tables 3 to 6). This concentration difference between the essential oil and the standard antibiotic can be explained by the fact that the active components in the oil comprise only a fraction of the oil. Therefore, the concentration of the active components could be much lower than the standard antibiotics we used. Importantly, if the active components were isolated and purified, they would probably show higher antimicrobial activities than those observed here. Among the Gram negative bacteria, the oil was very active against *K. pneumoniae* and *P. mirabilis.* The best activity was observed for the Gram positive bacteria. In general, the oil showed greater

Table 5. The antimicrobial activity curve of oil obtained by SC-CO_2.

Concentration/Time	MIC/2	MIC (MBC)	2MIC	Control
0	5600	7000	8400	6200
1	4000	4400	600	10600
2	4400	1700	0	18800
4	2400	600	0	23800
8	6300	0	0	28600
12	9500	0	0	42400
24	20100	0	0	61800
30	21300	0	0	64600

Table 6. The antimicrobial activity curve of oil obtained by petroleum ether.

Concentration/Time	MIC/2	MIC (MBC)	2MIC	Control
0	9300	11100	10400	11500
1	5700	4400	1100	14000
2	5000	3800	300	19300
4	3100	2200	200	24000
8	400	1200	0	28800
12	1000	200	0	53400
24	8800	6900	0	68800
30	10600	11300	0	74800

antibacterial activity than antifungal activity (Tables 3 to 6). Aldehydes and alcohols are known to be active but with different specificity and activity levels, which is related not only to the functional group but also to hydrogen bonding parameters (Skaltsa et al., 2003). As a minor component in this study, linalool has been found to have antimicrobial activity against various microbes, except for *P. aeruginosae* (Carson and Riley, 1995), which is also known to inhibit spore germination and fungal growth. The inhibition of sporelation appeared to arise from respiratory suppression of aerial mycelia (Lahlou and Berrada, 2001).

Conclusions

Our study showed that *C. acuminate D.* seed oils had extraordinary antioxidant and antibacterial activity *in vitro*. Due to its virulence, this seed oil can work as natural antioxidants and antimicrobial, which is a promising alternative to the use of synthetic antioxidants in food supplement or in pharmaceutical and cosmetic industry. But there have been few studies on the activity of *C. acuminate* D. seed oil. In this study, we evaluated its inhibitory activity in several common bacteria and estimated its antioxidant effectiveness by β-carotene bleaching and DPPH tests. Our results further demonstrated that *C. acuminate* D. seed oil had remarkable antioxidant and antibacterial activity, especially *S. aureus* ATCC 6538. The seed oil possesses various biological functions, notably antibacterial and Antioxidant properties that can be widely used as alternative to synthetic antioxidant or antibacterial. Therefore, we hope our study provides a foundation for future research of extracting ingredients from plants or herbs as natural antioxidant and antibacterial.

ACKNOWLEDGEMENT

We are grateful for the financial supports from the Key Research Subject of Jiamusi University (No. Szj2008-016).

REFERENCES

Carson CF, Riley TV (1995). Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. J. Appl. Bacteriol., 78(3): 264-269.

Cheng Y, Li M, Xu T (2008). Potential of poly (amidoamine) dendrimers as drug carriers of camptothecin based on encapsulation studies. Eur. J. Med. Chem., 43(8): 1791-1795.

Cox DL, Radolf JD (2001). Insertion of fluorescent fatty acid probes into the outer membranes of the pathogenic spirochaetes Treponema pallidum and Borrelia burgdorferi. Microbiology, 147(Pt 5): 1161-1169.

Friedrich J, List G, Heakin A (1982). Petroleum-free extraction of oil from soybeans with supercritical CO2 J. Am. Oil Chem. Soc., 59(7): 288-292.

Gerard D, May P (2002). Herb and spice carbon dioxide extracts-versatile, safe ingredients for premium food and health food. Food Tech. p 1-5.
Gomes PB, Mata VG, Rodrigues AE (2007). Production of rose geranium oil using supercritical fluid extraction. J. Supercrit. Fluids, 41(1): 50-60.
King JW (2000). Advances in critical fluid technology for food processing. Food Sci. Tech. Today, 14(4): 186-191.
Lahlou M, Berrada R (2001). Composition and niticidal activity of essential oils of three chemotypes of Rosmarinas officinalis L. Pharmaceutical Biol., I41: 207-210.
Li S, Adair KT (1994). Camptotheca acuminata Decaisne. Xi Shu, a promising anti-tumor and anti-viral tree for the 21st century. Nacogdoches: Stephen F. Austin State University Press.
Li S, Yi Y, Wang Y, Zhang Z, Beasley RS (2002). Camptothecin accumulation and variations in camptotheca. Planta Med., 68(11): 1010-1016.
Lo Cantore P, Iacobellis NS, De Marco A, Capasso F, Senatore F (2004). Antibacterial Activity of Coriandrum sativum L. and Foeniculum vulgare Miller Var. vulgare (Miller) Essential Oils. J. Agric. Food Chem., 52(26): 7862-7866.
Lu ZG, Zheng GC, Yu SM (2007). Composition analysis of groundcherry seed oil by supercritical CO_2 extraction. Food & Machinery, 2: 88-89+113.
Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S (1999). Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia. Planta Med., 65(8): 749-752.
Molero Gómez A, Pereyra López C, Martinez de la Ossa E (1996). Recovery of grape seed oil by liquid and supercritical carbon dioxide extraction: a comparison with conventional solvent extraction. Chem. Eng. J. Biochem. Eng. J., 61(3): 227-231.
Msaada K, Hosni K, Taarit MB, Chahed T, Marzouk B (2007). Variations in the essential oil composition from different parts of Coriandrum sativum L. cultivated in Tunisia. Ital. J. Biochem., 56(1): 47-52.
NCCLS (2002). In National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: proposed standard. P 1-25.
Oberlies NH, Kroll DJ (2004). Camptothecin and Taxol: Historic Achievements in Natural Products Research. J. Nat. Prod., 67(2): 129-135.
SalgIn U (2007). Extraction of jojoba seed oil using supercritical CO2+ethanol mixture in green and high-tech separation process. J. Supercrit. Fluids, 39(3): 330-337.
Skaltsa HD, Demetzos C, Lazari D, Sokovic M (2003). Essential oil analysis and antimicrobial activity of eight Stachys species from Greece. Phytochemistry, 64(3): 743-752.
Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA (1966). Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from Camptotheca acuminata1, 2. J. Am. Chem. Soc., 88(16): 3888-3890.
Wu N, Zu Y, Fu Y, Kong Y, Zhao J, Li X, Li J, Wink M, Efferth T (2010). Antioxidant Activities and Xanthine Oxidase Inhibitory Effects of Extracts and Main Polyphenolic Compounds Obtained from Geranium sibiricum L. J. Agric. Food Chem., 58(8): 4737-4743.
Yang ZW, Wu XM, Zu YG, Yang G, ZHou LJ (DOI: 10.1002/qua.23046). Understanding the chiral recognitions between neuraminidases and inhibitors: studies with DFT, docking and MD methods Int. J. Quantum. Chem.,
Yen GC, Duh PD (1994). Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. J. Agric. Food Chem., 42(3): 629-632.