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# Cytotoxicity activity of extracts and compounds from *Commiphora myrrha* resin against human gynecologic cancer cells

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The purpose of this report is to explore the cytotoxicity effects of extracts and compounds from *Commiphora myrrha* resin on human gynecologic cancer cells. The results showed that AE (85% EtOH extract), and petroleum ether extract (PE) from *C. myrrha* significantly inhibited cell proliferation of A2780, SK-OV-3, and Shikawa with dose-dependent relation *in vitro*. The inhibitory effects of AE and PE on A2708 cell were strongest and the IC<sub>50</sub>s were 15.8 and 26.91 µg/ml, respectively. The IC<sub>50</sub>s of AE and PE on Shikawa cell lines were 20.73 and 26.63 µg/ml respectively. Furthermore, nine compounds were isolated and identified from bio-activity guided separation fraction, and were determined the cytotoxicity activity on A2780, SK-OV-3, SiHa cells and Shikawa cells. Compounds 1- 4, 6 and 7 are isolated from this genus for the first time. The compound 6 and 7 exhibited obvious cytotoxicity effects on A2780, SK-OV-3, and Shikawa cancer cells with dose-dependent relationship. The antiproliferative activity of compound 6 on A2780 cells was most obviously with IC<sub>50</sub> 46.89 µM. The compound 7 with IC<sub>50</sub> 26.93 µM inhibited cell growth of SK-OV-3 cells. The determined compounds have never shown antiproliferative activities on SiHa cells. These findings suggested that extracts and compounds from myrrh could be useful for preventing and treating human gynecologic cancer disease.

**Key words:** *Commiphora myrrha*, cytotoxicity activity, human gynecologic cancer cell lines.

## INTRODUCTION

The *Commiphora* genus belongs to the family Burseraceae comprises over 200 species, which is a native that belong to the seasonally dry tropic of Africa, Arabia, and India (Vollesen, 1989; Su et al., 2009a). Myrrh, a yellow fragrant oleo-gum resin, is a famous traditional herb, which is derived from the damaged bark of *Commiphora* genus (Steyn, 2003). *Commiphora myrrha* (Nees) Engl. is a small tree or a large shrub which found in the dry and arid regions of Ethiopia and Somalia (the largest producers and exporters of myrrh) and to some extent in northern Kenya (Baser et al., 2003). Myrrh

has many medicinal powers and has been used to treat various diseases, such as amenorrhea, ache, dysmenorrhoea, tumors, fever, stomach complaints (for example, for stimulating the appetite and the flow of digestive juices), diseases of gall bladder, chest ailments, snake and scorpion bites, and skin infections in India, China, Rome, Greece and Babylon (El ashry et al., 2003; Massoud, 2001).

Previous investigations have revealed that myrrh contains about 2 to 8% essential oil (myrrhol), 23 to 40% resin (myrrhin), 40 to 60% gum, and 10 to 25% bitter principles. Essential oil of myrrh was reported that the furanosesquiterpenoids were rich in the exudates, and around 20 different compounds of this type have been isolated and identified (Shen et al., 2008; Zhu et al., 2003; Su et al., 2008). Phytochemicals present in this

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plant investigated previously and resulted in a series of metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives isolated and identified from *Commiphora* species (Shen et al., 2007; Hanus et al., 2005; El Ashry et al., 2003). These secondary metabolites and crude extracts of the *Commiphora* species exhibited diverse biological activities, such as cytotoxic, anesthetic, anti-inflammatory, and antimicrobial effects, and so on (Lukas et al., 2005; Nomicos, 2007; Omar, 2005; Massoud et al., 2004; Avlessi et al., 2005). During our previous studies, we discovered that the extract of myrrh exhibited significant antidysmenorrhagic activity (Wang, et al., 2009) and inhibition on isolated uterus contraction and aromatase inhibitory activity (Su et al., 2008), and as well as protection of HUVEC damage induced by H<sub>2</sub>O<sub>2</sub> (Su et al., 2009b).

Recent years, the researchers are paying more attention to the anticancer field of myrrh (Ji et al., 2008; Wu et al., 2009; Li et al., 2009). The effects of two novel oxygenated bisabolane-type sesquiterpenes isolated from *Commiphora discoidetum* on tumor cell line SMMC-7721 were investigated. The cytotoxicity of myrrh oil to human gingival fibroblasts and epithelial cells were measured (Tiptona et al., 2003). However, the activity against on human gynecologic cancer cell lines and their active components of myrrh have been lacking relatively.

The aim of this present study was to evaluate the cytotoxicity effects on human gynecologic cancer cell lines with the extracts from *C. myrrha* (Nees) Engl. Furthermore, nine compounds were obtained and identified through bio-guided separation from active extract, and part compounds were determined the cytotoxicity activity on human ovarian cancer cell lines A2780, SK-OV-3, cervical carcinoma cell line SiHa, and endometrial carcinoma cell line Shikawa.

## MATERIALS AND METHODS

### Collection of plant material

Myrrh was purchased from commercial market in Nanjing city of China. It is thought that, the resin used in this study was collected from Guangdong, China, in April, 2007 and traded in the market as myrrh resin. And identified as resin derived from *Commiphora myrrha* (Nees) Engl. by Prof. Jin-ao Duan (Department of Chinese Medicine, Nanjing University of Chinese Medicine, Nanjing, in China). A voucher specimen (No. NJUTCM - 20070726) was deposited at the Herbarium of the Jiangsu Key laboratory for TCM formulae Research, Nanjing University of Chinese Medicine, R.P. China.

### Preparation of the extracts and isolation of compounds from *C. myrrha* resin

The dried resin (5.0 kg) of *C. myrrha* was crushed and then extracted with 85% EtOH (2 x 50 L) under reflux for 2 h in duplicate, and the solvent was removed under reduced pressure. The residue (AE) (900 g) was dissolved in hot water and partitioned with

petroleum ether, EtOAc, and n-BuOH (5 x 2.5 L, 1 day each), respectively. Successively, the petroleum ether fraction (PE) (113.5 g), EtOAc fraction (EE) (240 g), n-BuOH (BE) (180 g) and the water extract (WE) (60 g) were obtained to assay the cytotoxicity activities. PE was shown to be active in the cytotoxicity assays of against A2780, SK-OV-3, SiHa, and Shikawa and EE were shown analgesic activity in our previous studies. Then PE (100.0 g) and EE (200.0 g) were submitted to a series of separations by silica gel (300 to 400 mesh) column (5 x 100 cm) chromatography to afford compounds 1 (100 mg), 2 (1600 mg), 3 (100 mg), 4 (200 mg), 5 (50 mg), 6 (100 mg), 7 (300 mg), 8 (100 mg) and 9 (100 mg).

### Cell culture

Human ovarian cancer cells (designated A2780 and SK-OV-3), cervical carcinoma cells (designated SiHa), and endometrial carcinoma cells (designated Shikawa) were provided by Nanjing Keygen Biotech (Co., Ltd. Nanjing, China). Cells were routinely cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA), DMEM medium (Gibco), McCoy's 5A medium (SIGMA Chemical Co., St. Louis, MO, USA), supplemented with 10% (v/v) newborn calf serum (Hangzhou Sijiqing Biological Engineering Material Co., Ltd.) (Termed the growth medium) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in incubator. Cultures were dissociated with 0.25% trypsin in phosphate buffered (pH 7.2-7.4) saline (PBS) (Gibco).

### Instrumentation and reagents

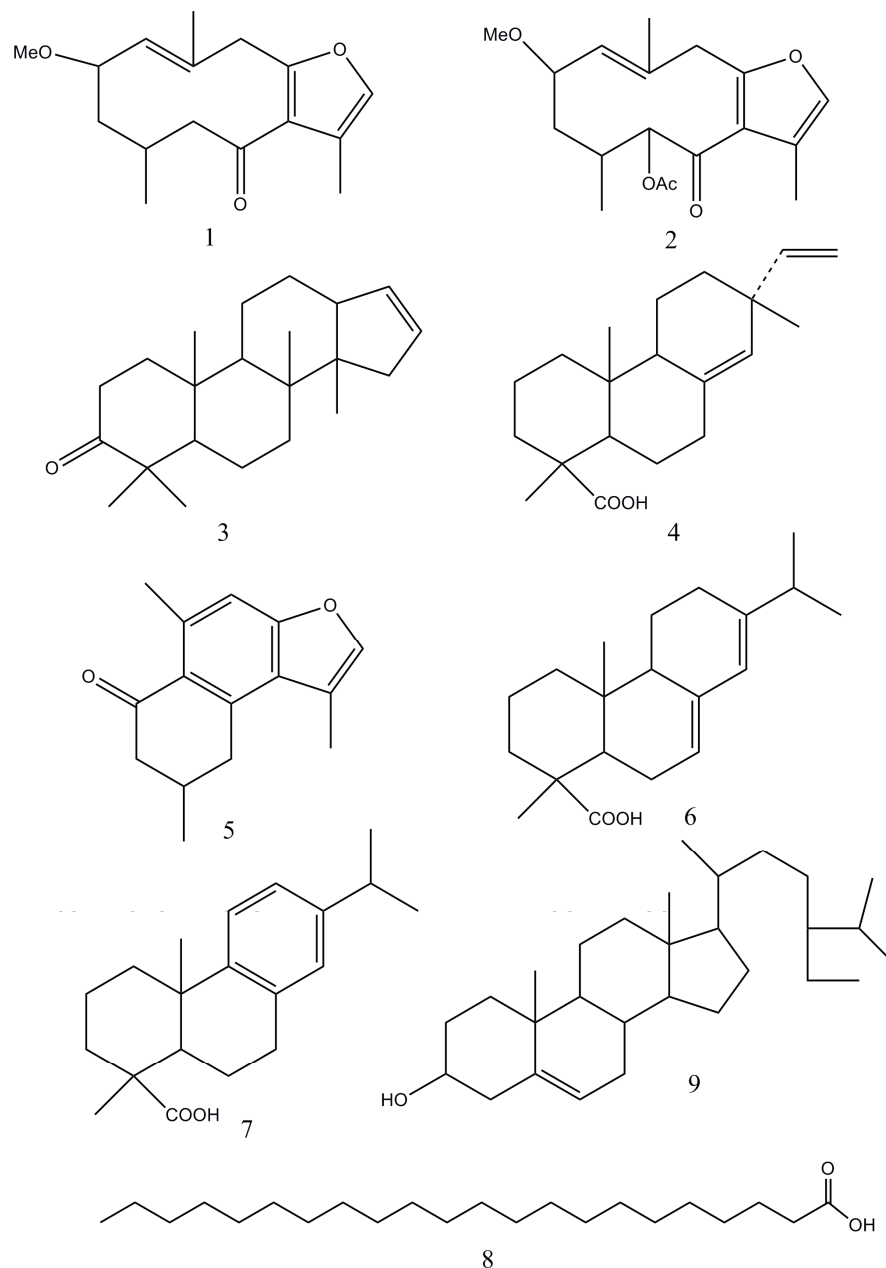
Laminar flow clean bench (Model: SW-CJ-1FD) was purchased by Suzhou Purification Equipment (Co., Ltd., China); XD-101 CO<sub>2</sub> incubator (Sanyo Denki Co. Ltd., America); Microplate Reader (model NO. 550 Serial NO. 16971) obtained from Bio-Rad Laboratories. All the reagents for extraction and isolation are analytical purity. MTT was obtained from Amresco (Co., Ltd., USA); DMSO (Amresco, USA); Paclitaxel injection (NO. 080502) was obtained from Haiyao Pharmaceutical (Co., Ltd., Hainan, China).

### Cytotoxicity activity assay

Cytotoxicity activity was determined by the ability of the cells to metabolically reduce the tetrazolium salt, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT), to a purple formazan dye. This is a sensitive (detecting as few as 10<sup>4</sup> viable cells / ml) and accurate test, with color development strongly correlating with cell numbers. It is widely used as a measure of cytotoxicity (Twentyman et al., 1987; Wei et al., 2009). Briefly, individual cells were made into a concentration of 5 x 10<sup>4</sup> cells/ml cell suspension. The 96-well plates (Costar, Co., Ltd., Beijing, China) were seeded with 100 µl cell suspension (5 x 10<sup>3</sup> cells per well) and incubated overnight at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in incubator.

The drugs were diluted with completed medium to the desired concentration, then added into 96-well plates with 100 µl per well. At the same time, negative control group, solvent control group, positive control group were set up for the experiments. The processed 96-well plates were incubated 72 h at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in incubator.

The processed 96-well plates were treated with 20 µl of MTT (5 mg/ml), and incubated for a further 4 h in incubator. The growth medium was then removed and dissolved in DMSO (150 µl per cell). Shake the plates gently for 10 min and make the mixture blending. The cells were treated for 72 h, after which they were incubated with MTT reagents and the absorbance read at 490 nm. Growth inhibition rate was obtained as percentage over the



**Figure 1.** Chemical structures showing the relative configuration of compounds 1-9 isolated from the active extract of *C. myrrha*.

untreated control.

#### Statistical analysis

The results were expressed as inhibition ratio and  $IC_{50}$  (50% concentration of inhibition) following by multiple comparisons test. The 50% concentration of inhibition ( $IC_{50}$ ) was calculated by regression analysis of IgC-inhibitory rate. In the MTT assay,  $IC_{50}$  is referred to as the drug concentration reduced to half of the absorbance (OD). It is equivalent with the average of the minimum lethal dose of the drug cultured cells. The data were performed with SPSS13.0 software. The  $IC_{50}$  is smaller the anti-proliferation activity

is more significant.

## RESULTS

### Identification of compounds 1-9

Spectral analysis (UV, MS,  $^1H$ NMR,  $^{13}C$ NMR, HMBC, HMQC, mp, TLC) and comparison with literatures data allowed the identification of compounds 1-9 (Figure 1). Compounds 2, 4, 6 and 7 were isolated and elucidated

**Table 1.** *In vitro* cytotoxicity activity of *C. myrrha* extracts and compounds 2, 4, 6 and 7 against human gynecological cancer cell lines.

Substance/extracts	IC <sub>50</sub>			
	A2780	SK-OV-3	Shikawa	SiHa
Control	-	-	-	-
Taxol (μM)	12.84	13.38	13.44	13.98
AE (μg/ml)	15.81	22.08	20.73	57.83
PE (μg/ml)	26.91	38.72	26.63	32.20
EE	-	-	-	-
BE	-	-	-	-
WE	-	-	-	-
C.2	-	-	-	-
C.4	-	-	-	-
C.6 (μM)	46.89	60.86	64.36	-
C.7 (μM)	35.97	26.93	36.18	-

Data are presented as IC<sub>50</sub> values and 95% confidence intervals for human ovarian cancer cells A2780 and SK-OV-3, cervical carcinoma cells SiHa, and endometrial carcinoma cells Shikawa. Taxol was used as positive control. Experiments were performed in triplicate. AE (85% EtOH extract); PE (petroleum ether fraction); EE (EtOAc fraction); BE(n-BuOH fraction); WE(water fraction); C.2 (compound 2); C.4 (compound 4); C.6 (compound 6); C.7 (compound 7).

the chemical structures in our previous experiments (Su et al., 2009). They are namely 2 - methoxy - 5 - acetoxo - furanogermacr - 1(10) - en - 6 - one, sandaracopimaric acid, abietic acid, and dehydroabietic acid, respectively.

Compound 1 was obtained with white column (Pet/EtOAc); mp. 83~84°C; EI-MS *m/z*: 262[M]<sup>+</sup> (100); molecular formula: C<sub>16</sub>H<sub>22</sub>O<sub>3</sub>; <sup>1</sup>H-NMR data (500MHz, CDCl<sub>3</sub>): δH (int, mult, *J* in Hz) 5.21 (1H, *br d*, 8.0, H-1), 4.00 (1H, *dt*, 8.9, 2.5, H-2), 1.74 (1H, *m*, 1.98, H-3), 2.42 (1H, *m*, H-4), 2.52 (2H, *m*, H-5), 3.21 (1H, *m*, H-9), 7.01 (1H, *br s*, H-12), 1.89 (3H, *br s*, H-13), 1.11 (3H, *d*, 7.0, H-14), 1.79 (3H, *d*, 1.2, H-15); <sup>13</sup>C - NMR data (500MHz, CDCl<sub>3</sub>) δ(ppm): 133.5 (C-1), 74.6 (C-2), 37.0 (C-3), 25.3 (C-4), 50.5 (C-5), 202.7 (C-6), 119.7 (C-7), 151.5 (C-8), 38.3 (C-9), 133.5 (C-10), 126.0 (C-11), 137.4 (C-12), 8.3 (C-13), 21.9 (C-14), 18.1 (C-15), 55.5 (-OMe). By comparison of their spectral data with those reported in the literature (Dekebo et al., 2000), this compound was identified as 2-methoxy-8, 12-epoxygermacra-1 (10), 7, 11-triene-6- one.

Compound 3 was a white needle (Pet/EtOAc); mp. 122~123°C; EI-MS *m/z*: 314[M]<sup>+</sup> (30), 94 (100); molecular formula: C<sub>22</sub>H<sub>34</sub>O; <sup>1</sup>H-NMR data (500MHz, CDCl<sub>3</sub>) δH (int, mult, *J* in Hz): 1.45 (m, H-1), 2.43 (m, H-2), 1.40 (m, H-5), 1.48 (m, H-6), 1.33 (m, H-7), 1.60 (m, H-7), 1.55 (m, H-9), 1.60 (m, H-11), 1.72 (m, H-12), 2.75 (m, H-13), 5.66 (m, H-16), 5.57 (m, H-17), 1.06 (s, H-18), 0.95 (s, H-19), 1.09 (s, H-20), 1.05 (s, H-21), 1.01 (*br s*, H-22); <sup>13</sup>C-NMR data (125MHz, CDCl<sub>3</sub>) δ (ppm): 39.8 (C-1), 34.2 (C-2), 218.2 (C-3), 47.6 (C-4), 55.5 (C-5), 19.8 (C-6), 34.8 (C-7), 39.8 (C-8), 50.3 (C-9), 37.1 (C-10), 22.4 (C-11), 23.9 (C-12), 47.8 (C-13), 53.0 (C-14), 39.9 (C-15), 130.1 (C-16), 134.1 (C-17), 17.9 (C-18), 15.8 (C-19), 26.8 (C-20), 21.1 (C-21), 17.1 (C-22). The above data compared

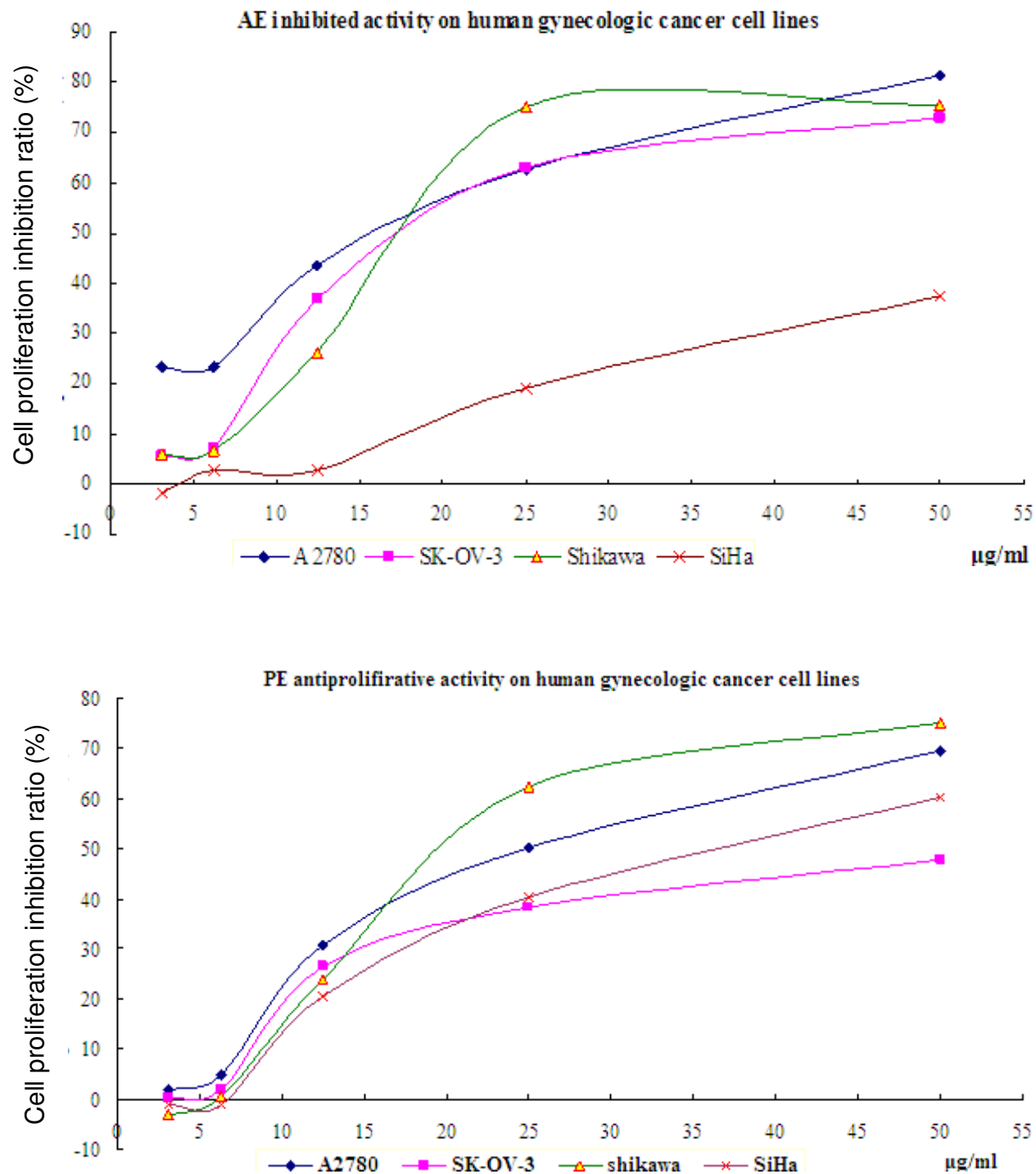
with which in the literature (Provan et al., 1986) and the compound was identified as mansumbinone.

Compound 5 was a white column (EtOAc); EI-MS *m/z*: [M]<sup>+</sup> 228; molecular formula: C<sub>15</sub>H<sub>16</sub>O<sub>2</sub>; <sup>1</sup>H-NMR data (500MHz, CDCl<sub>3</sub>) δH (int, mult, *J* in Hz): 2.34 (1H, *m*, H-3a), 2.73 (1H, *m*, H-3b), 2.34 (1H, *m*), 2.88 (1H, *dd*, *J* = 16.8, 10.4 Hz, H-5a), 3.54 (1H, *dd*, *J* = 16.8, 4.0 Hz, H-5b), 7.14 (1H, *br s*), 7.33 (1H, *br s*), 2.40 (3H, *d*, *J* = 1.2 Hz), 1.18 (3H, *d*, *J* = 6.1 Hz), 2.71 (3H, *br s*). The compound was identified as myrrhone by comparing with the literature (Zhu et al., 2003).

Compound 8 was obtained with white needle (Pet / EtOAc); mp. 81~82°C; EI - MS *m/z*: 340 [M]<sup>+</sup> (100); molecular formula: C<sub>22</sub>H<sub>44</sub>O<sub>2</sub>; By comparison of their spectral data with those reported in the literature, this compound was identified as docosanoic acid. Compound 9 was identified as β-sitosterin. The analysis of thin-layer chromatography was the same with the reference substance of β-sitosterin. Our previous experiments indicated that the structure of compounds 2, 4, 6 and 7 stated that the compounds inhibited HUVEC proliferation. Guided by the results of these previous experiments we tested the cytotoxicity activity of extracts and terpenes type compounds on human gynecologic cancer cell lines.

### Cytotoxicity activity on cancer cells of extracts and compounds from *C. myrrh*

The extracts and terpenes compounds isolated (Figure 1) from resin of *C. myrrh* were evaluated the cytotoxicity effects on four human gynecologic cancer cell lines, A2780, SK-VO-3, shilawa, and SiHa. In the cytotoxicity assay (Table 1 and Figure 2), the AE fraction exhibited



**Figure 2.** The cytotoxicity activity and dose-dependent relationship on four human gynecologic cancer lines of extracts of *C. myrrha*.

significantly inhibiting cell proliferation on the all four cell lines with dose-dependent relationship *in vitro*. At the concentration of 50 µg/ml, with the inhibited ratio of 81.85% on human ovarian cancer cells lines A2780. The  $IC_{50}$ s of AE on A2780, SK-VO-3, shilawa, and SiHa were

15.81, 22.08, 20.73 and 57.83 µg/ml, respectively. The antiproliferative effect of PE on endometrial carcinoma cell lines (Shikawa) was more sensitive than AE. The  $IC_{50}$ s of PE on A2780, SK-VO-3, shilawa, and SiHa were 26.90, 38.71, 26.63 and 32.20 µg/ml, respectively. The

AE and PE extracts of *C. myrrha* have inhibited proliferation of A2780, SK-OV-3, Shikawa, SiHa with dose-dependent *in vitro*. Furthermore, the data stated that the AE was the more sensitive and has stronger antiproliferative effects on A2780 cell lines than of PE, while PE extract was more sensitive and has more significant anticancer activity on Shikawa cell lines. While other fractions showed no obvious bioactivities on the described carcinoma cell lines. The observation cell morphology of four human gynecologic cancer cells also stated that AE was sensitive to A2780 cell growth and PE was sensitive to Shikawa cell growth and with the inhibited ratio of 81.85 and 75.23%, respectively.

The compounds 2, 4, 6 and 7 from PE of *C. myrrha*, in the concentration range of 6.25 - 100  $\mu$ M, were determined the antiproliferative activity on described four cancer cell lines. The results (Table 1) elucidated that the compounds 6 and 7 significantly inhibited cancer cells proliferation of A2780, SK-VO-3, Shikawa, and SiHa cell lines, respectively. The compound 7 obviously inhibited SK-VO-3 cell growth with  $IC_{50}$  of 26.93  $\mu$ M. While the compound 6 significantly inhibited A2780 cell growth with  $IC_{50}$  of 46.89  $\mu$ M. It was clear that the two compounds showed remarkably antiproliferative activity on human ovarian cancer cells with dose - dependent relationship (Figure 3). The cell morphology observation of four human gynecologic cancer cells also stated that compound 6 was more sensitive to A2780 cell growth and compound 7 was more sensitive to SK-VO-3 cell growth and with the inhibited ratio of 79.31 and 85.74%, respectively.

It can be seen from Table 1 and Figure 3 that all compounds showed no inhibiting effects on SiHa cell lines from cervical carcinoma cells. The compounds 6 and 7 showed mild inhibited effects on Shikawa cells with  $IC_{50}$  of 64.36 and 36.18  $\mu$ M, respectively.

## DISCUSSION

This study examined the cytotoxicity activity of extracts and purified components from *C. myrrha*. Myrrh is a traditional medicinal herb, which has been used for treatment of tumor, some kinds of inflammatory, hurt diseases such as traumatic injury, and all kinds of pain in folk (Abdul-Ghani et al., 2009; Lemenih et al., 2004). However, its cytotoxicity activity on human gynecologic cancer cell lines and the bioactive components have never been precisely elucidated in spite of its increasing researches recently.

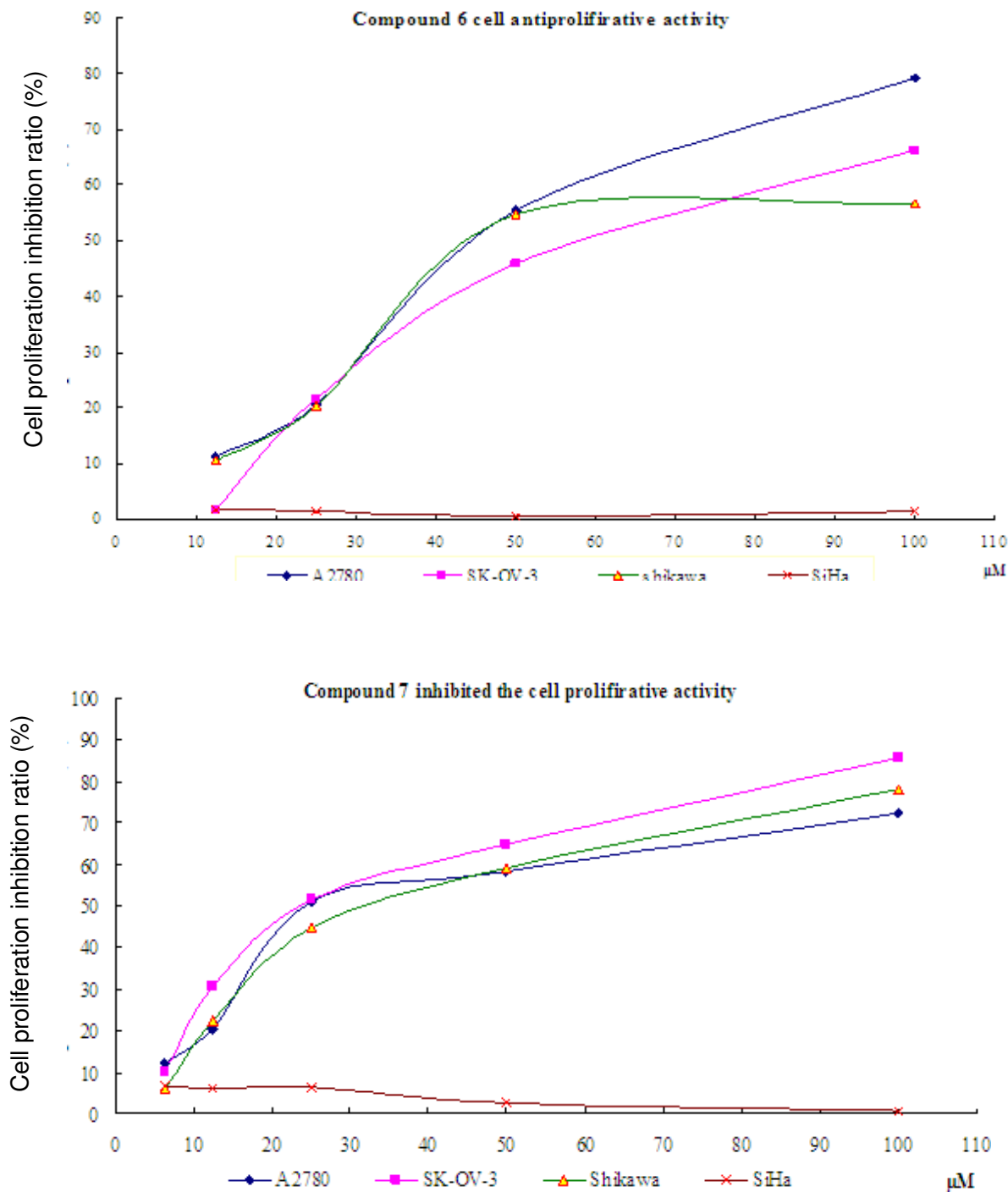
In this present paper, the cytotoxicity effects showed that the fat-soluble extracts and chemical components exhibited significant activities on four human gynecological cancer cell lines. Bio-guided isolation, nine chemical constituents were isolated from the active fraction of *C. myrrha*. The compounds 1-4, 6 and 7 are isolated from this genus for the first time, and they belong to sesquiterpene and diterpene acid type compounds. It

was found that two compounds of diterpene resin acid, namely abietic acid and dehydroabietic acid, significantly inhibited proliferation of human ovarian cancer cell line A2780, and human ovarian cancer cell line SK-VO-3, with  $IC_{50}$  46.89 and 26.93  $\mu$ M, respectively. Actually, it was reported that the water extract of *C. myrrha* resin significantly inhibited cell growth of eight cancer cell lines of A549, LLC, Panc-1, Panc02, MCF-7, MCNeuA, PC-3 and LNCaP, and the inhibition ratio above 75% (Shoemaker et al., 2005). In addition, two germacrane-type sesquiterpenes possessed antiproliferative effects on prostatic cancer cell lines, and inhibited proliferation on colic cancer cell at high concentration (Ji et al., 2008). The cytotoxicity activity and active components of *C. myrrha* on human gynecologic cancer cell lines reported in this paper will provide evidences for the traditional therapy for some certain diseases. However, the environment of anticancer activity experiments *in vitro* is different from *in vivo*, the activity of these compounds and extracts need to be further validated by animal experiments.

The anticancer mechanisms from previous researches mainly were associated with inhibiting growth of cancer cells, kill cancer cells, antiangiogenesis, immune-enhancing properties, and so on (Yang et al., 1998; Yan et al., 2009; Arunasree, 2010). It is known that myrrh possesses properties of activating blood circulation to dissipate blood stasis and antitumor. But the anticancer mechanisms of active components have never elucidated. Myrrh was used in clinic (for example: XIHUANGWAN) for treating liver cancer, cholangiocarcinoma, pancreatic cancer with significant activity. In this paper, we found diterpene resin acid was associated with antiproliferation on human gynecological cancer cell. These results corroborate the results of our previous experiments indicating that the diterpene resin acids compounds exhibited obvious inhibitory effects on HUVEC proliferation and implied that the antiproliferative activity on human gynecological cancer cell lines may be associate with their potential antiangiogenic properties. While the anticancer mechanisms of the components needed to be investigated.

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**Figure 3.** The cytotoxicity activity and dose-dependent relationship on four human gynecologic cancer lines of compound 6 and 7 from active fraction of *C. myrrha*.

Innovation Team of Jiangsu Higher Education.

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