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Research Article

Chemical Components of Volatile Oil from *Curcuma Kwangsiensis* and Its Growth Inhibition on H446 Cells

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Abstract

Objective: The experiment intended to analyze chemical components of volatile oil from *Curcuma kwangsiensis* by GC-MS, and to explore its inhibitory action on the growth of human lung cancer cells.

Methods: Extracted with the steam distillation, chemical compositions of volatile oil from *Curcuma kwangsiensis* were isolated and identified by GC-MS and computer similarity retrieval. Relative percentage contents of each ingredient were determined with peak area normalization. And active ingredients of *Curcuma kwangsiensis* were determined its inhibitory action on the growth of human lung cancer cells.

Results: Twenty compounds were identified from volatile oil from *Curcuma kwangsiensis*. They were almost sesquiterpenes and monoterpenes. The relative percentage content of curzerenone was highest, 4.94 %, followed by eucalyptol, 3.03 %, γ-gurjunenepoxide-(1), 2.03 %, germacrone, 1.8 %, camphor, 1.57 %. The IC50 value of volatile oil from *Curcuma kwangsiensis* acting on H446 cells was 7.55±0.38 μg/mL.

Conclusion: Compounds of volatile oil from *Curcuma kwangsiensis* were almost sesquiterpenes and monoterpenes, and volatile oil from *Curcuma kwangsiensis* had an inhibitory action on the growth of human lung cancer cells, H446 cells.

Introduction

Curcuma Kwangsiensis S. G. Lee et C. F. Liang originates from the rhizome of the Curcuma Genus which is commonly used in traditional Chinese medicine. Pharmacological studies showed that curzerenone [1], curcumol, curdione, β-elemene, germacrone were the active ingredients of volatile oil from rhizoma curcuma. In addition, they had the effect of anti-inflammation, antibiosis [2], antivirus [3], immunoenhancement, anticancer [4,5], anti-pathogen, anti-early pregnancy, and declining enzyme. Particularly, its anticancer effect has attracted much attention and it has been widely used in clinic.

Gas Chromatography-Mass Spectrometry (GC-MS) is a highly selective, sensitive and efficient analytical method, especially suitable for the analysis of volatile compounds. Volatile oil, also known as essential oil, is a kind of important ingredient in traditional Chinese medicine with a wide range of biological activities. The volatile oil is the volatile liquid and its boiling point is generally no more than 300 °C. Therefore, it is suitable for GC-MS analysis.

Accordingly, the rhizome of *curcuma Kwangsiensis* was taken as the object. It was used to extract and identify the volatile oil. In addition, the volatile oil was detected with MTT. Consequently, volatile oil form *curcuma Kwangsiensis* had an inhibitory action on the growth of human lung cancer cells. The study provided a scientific basis for the further development and utilization for Curcuma kwangsiensis.

Materials and Methods

Experimental medicine, reagents and instrumentations

Experimental materials and reagents: In this experiment, the rhizome of *curcuma Kwangsiensis* S. G. Lee et C. F. Liang was collected in Guangxi province, China. They were purchased in Guangzhou Qingping medicine market, identified by Hu-Biao Chen, professor of School of Chinese Medicine, Hong Kong Baptist University.

RPMI1640 Medium was a product of Gibco by life technologies. MTT (3-(4,5-Dimethylthiazol-yl)-2,5-diphenyllapatinibrazolium bromide) was a product of Sigma Chemical Co. (St. Louis, MO, USA). Other routine laboratory reagents were of analytical or HPLC grade and were obtained from commercial sources.

Instrumentations: GCMS-QP2010SE: Shimadzu Company; Volatile Oil Extractors: Conventional Glassware; CO_2 Incubator: Thermo Scientific Forma, USA; XDS-2 Inverted Microscope: Guangzhou

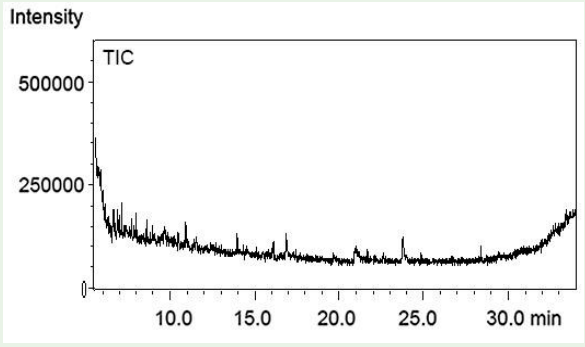


Figure 1: Total ion chromatography of ethyl acetate. The ethyl acetate was the solvent control. The total ion chromatography of ethyl acetate was obtained under the GC-MS Conditions. Temperature programmed gas chromatography and electron impact (EI) ion source were used for the analysis of complex samples that we described in the methods.

Yuxian Optical Instrument Company Limited, Guangzhou Optical Instrument Factory; Eppendorf Research Plus Pipette: Germany; TS-2 Shaker: Haimen Kylin-Bell Lab Apparatus Company Limited.

Methods

Extraction of Volatile Oil and Post-processing

Extraction of volatile oil from *curcuma kwangsiensis* and post-processing: Powder of the rhizome of *Curcuma kwangsiensis* at 50 g and 500 ml water were put into a round-bottomed flask. Subsequently, the soaked sample was distilled in a Clevenger-type apparatus for 5 hours [6].

The volatile oil was centrifuged and the top layer of volatile oil was diluted 50000 times with ethyl acetate.

GC-MS conditions of volatile oil

GC conditions: Chromatographic column: DB-5ms (30 m × 0.25 mm × 0.25 μm); Carrier gas: High purity helium; Flow rate of carrier gas: 3.0 mL/min; injection temperature: 230 °C; The column temperature was initially programmed to and kept at 50.0 °C for 1 minute, increased at 5.0 °C per minute to 140.0 °C, and then increased at 8.0 °C per minute to 230.0 °C, finally increased at 10.0 °C per minute to 280.0 °C and held for 5.0 minutes; Injection mode was split injection. The split ratio: 10:1; Liner velocity: 39.0 cm/s.

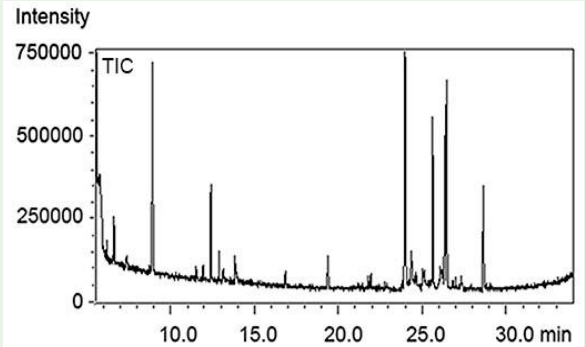


Figure 2: Total ion chromatography of volatile oil from *Curcuma Kwangsiensis*. The total ion chromatography of ethyl acetate was obtained under the GC-MS Conditions. Temperature Programmed Gas Chromatography (TPGC) and Electron Impact (EI) ion source were used for the analysis of complex samples that we described in the methods. Constituents were identified by comparison of authentic compounds with reference spectra in the computer library (NIST 27 and NIST 147) and confirmed by comparison of those authentic compounds with data in literature.

MS conditions: Ion source: Electron impact (EI) ion source; Ion source temperature: 200 °C; Electron energy: 70 eV; Interface temperature: 280 °C; Solvent delay: 5.5 minutes; Mass range m/z: 40-400; Detection voltage: 0.3 Kv. The retrieval spectral library was NIST27 and NIST147.

Active Determination

Cell culture

H446 cells were human lung cancer cells. H446 cells were cultivated in RPMI1640 medium containing 10 % FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) in an incubator with a humidified atmosphere of 5 % CO_2 at 37 °C.

Cell growth inhibitory experiment

H446 cells with exponential growth were plated in 96-well plates with 1.5×104 cells/ mL in a final volume of 190 μL/ well. After 24 hours of incubation, 10 μL volatile oil from *Curcuma Kwangsiensis* at different concentrations was added in the 96-well plates, the final concentrations were 1.5625 μg/mL, 3.125 μg/mL, 6.25 μg/mL, 12.5 μg/mL, 25 μg/mL and 50 μg/mL. The control groups were only cultivated in RPMI1640 medium, each dose setting four parallel holes. After 68 h treatment, 10 μL MTT was added to each well. After 4 hours of incubation, the supernatant was removed. The crystals were solved with 100 μL anhydrous DMSO for each well and oscillated for 10 min. Then optical density was measured by a model 550 microplate reader at 540 nm, with 655 nm as the reference filter. The experiment was performed at least three times. According to the absorbance values, cell growth inhibitory rate and IC50 values were calculated. IC50 was the 50 % inhibitory concentration. It was defined as the anticancer medicine concentration causing 50 % reduction in cell viability and calculated from the cytotoxicity curves (Bliss's software). Cell survival was calculated with the following formula: survival (%) = (mean experimental absorbance / mean control absorbance) × 100 % [7].

Results

Ethyl acetate chromatography as solvent control

The ethyl acetate was the solvent control. Total ion chromatography of ethyl acetate (Figure 1) was obtained with GC-MS analysis under Methods. Compared with the figure 1 and figure 2, the peak of the solvent almost had no interference on the detection of volatile oil from *Curcuma Kwangsiensis*.

Qualitative analysis of volatile oil from *curcuma kwangsiensis*

Total ion chromatography of volatile oil from *Curcuma Kwangsiensis* (Figure 2) was obtained with GC-MS analysis under Methods. The mass spectrograms were obtained after each peak in the ion chromatograms being scanning by mass spectral. Twenty compounds were identified via similarity comparison with compounds of database NIST 27 and NIST 147. For example, germacrone was identified at 25.650 minutes by comparison of authentic compounds with reference spectra in the computer library

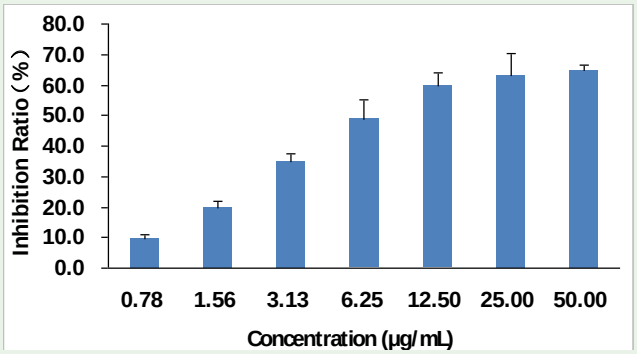


Figure 3: The volatile oil from *Curcuma kwangsiensis* had an inhibitory action on H446 cells. Cells were grown and treated with the volatile oil from *Curcuma kwangsiensis* at 1.5625 *μg*/mL- 50 *μg*/mL for 68 hours. Then, 10 μL of MTT was added to each well. After 4 hours of incubation, the supernatant was removed. The crystals were solved with 100 μL anhydrous DMSO for each well and oscillated for 10 minutes. Then optical density was measured by a model 550 microplate reader at 540 nm, with 655 nm as the reference filter. Cell survival was calculated with the following formula: survival (%) = (mean experimental absorbance / mean control absorbance) × 100 %. IC50 values were results of thrice experiments.

(NIST 27 and NIST 147) and confirmed by comparison of those authentic compounds with data in literature [9,10]. And the relative percentage contents of each chromatographic peak were calculated with peak area normalization, which was presented in Table 1.

Volatile oil from *curcuma kwangsiensis* showed inhibitory action on h446 cells

To explore the potential use of volatile oil from *Curcuma kwangsiensis* as an anticancer agent, we tested its possible *in vitro* toxicity. The toxicity in H446 cells was evaluated using the MTT assay for volatile oil samples. The assessment of cytotoxicity was performed for volatile oil samples in the range of 1.5625 *μg*/mL- 50 *μg*/mL. The volatile oil induced a dose-dependent inhibition of the cell cytotoxicity of the cell line (Figure 3). The value of IC50 was 7.55 ± 0.38 *μg*/mL. The results showed that volatile oil from *Curcuma kwangsiensis* exhibited an inhibitory action on human lung cancer cells, H446 cells.

Discussion

In this research, twenty compounds were identified from volatile oil from *Curcuma kwangsiensis*. In detail, these constituents were 1-heptyn-6-one, camphene, isobornyl acetate, eucalyptol, 5-methylene-9-decen-2-one, camphor, borneol, isoborneol, *α*-terpineol, elemene, α-bisabolene, α-bisabolol, α-cadinol, curzerenone, Cis-Z-α-bisabolene epoxide, trans-Z-α-bisabolene epoxide, germacrone, neocurdione, γ-gurjunenepoxide-(1), 2-methoxy-6-(1-methyl-2-propenyl)-naphthalene.

Twenty-nine compounds were identified from volatile oil from *Curcuma kwangsiensis* by Zhang, et al. [8]. Those ingredients were α-pinene, camphene, *β*-pinene, eucalyptol, 2-nonanol, 3,7-methyl-1,6-octadien-3-ol, camphor, borneol, isoborneol, α-terpineol, 4-vinyl-4-methyl-3(1-propenyl)-1-(propyl)-cyclohexene, 1-vinyl-1-methyl-2,4-Di(1-propenyl)-cyclohexane, caryophyllene, α-caryophyllene, *α*-curcumene, di-epi-*α*-cedrene, 3-(1,5-dimethyl-4-vinyl)-6-methylenecyclohexene, 8S-1,4-cedran-diol, cubebnol, 8,9-dehydro-9-formyl-cycloisolongifolene, 5*α*-androst-1-en-3-one, agarospirol, cedrene, Ar-turmerone, 3,7-dimethyl-10-methylvinyl-3,7-cyclodecadienone, curdione, 3,7,11-trimethyl-6,10-myrcene-1-yn-3-ol, acetyl-4,6,8-trimethylazulene, 6-methoxy-2-(1-3-butylene)-naphthalene.

Twenty-four compounds were identified from volatile oil from *Curcuma kwangsiensis* by Luo, et al. [9]. Those ingredients were myrcene, D-cinene, cineole, camphor, isoborneol, borneol, 1-vinyl-1-methyl-2,4-dipropenylcyclohexane, *β*-elemene, 1-(1,1-methoxyl-ethoxyl)-2-methylbenzene, *γ*-elemene, 4-ethynyl-4-hydroxyl-3,5,5-trimethylcyclohexenone, 3,3,6,6-tetranethyltricyclohexene, 10-methylvinyl-3,7-cyclodecadienone, 1,3,5-triisopropylbenzene, 2,4,6-pentamethylaniline, epi-curzerenone, 3-ethyl-2,5-dimethyl-1,3-hexadiene, 3,4,5,6,7,8-hexahydro naphthalenone, curzerenone, 2,3-dihydrobenzofuran, *β*-turmerone, germacrone, 6-methyl-2-butene naphthalenen, curdione.

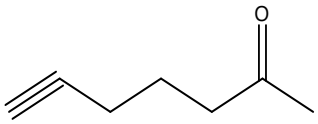
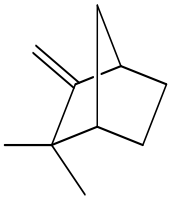
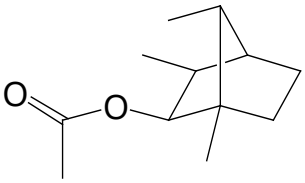
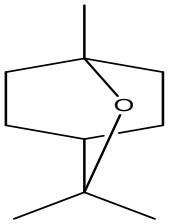
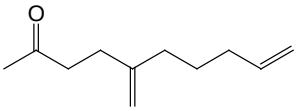
Twenty-six compounds were identified from volatile oil from *Curcuma kwangsiensis* S. G. Lee et C. F. Liang by Yuan, et al. [10]. In detail, these constituents were D- cinene, eucalyptol, 2-nonanol, *α*-terpinene, camphor, isoborneol, *β*-elemene, *β*-caryophyllene, aromadendrene, *β*-selinene, *α*-caryophyllene, selina-4(14), 11-diene, ledene, *α*-cubebene, *α*-farnesene, curzerene, *γ*-elemene, ledol, 10-epi-*γ*-eudesmol, furanodiene, *β*-eudesmol, furandione, germacrone, curdione, curcumenol, curzerenone.

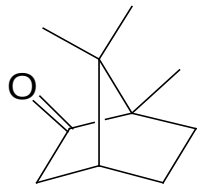
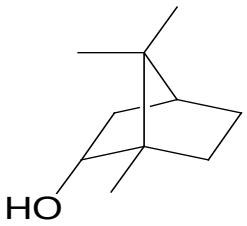
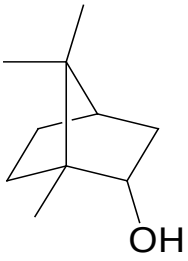
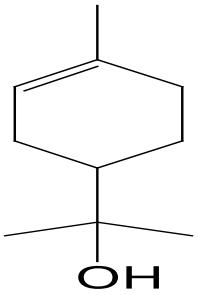
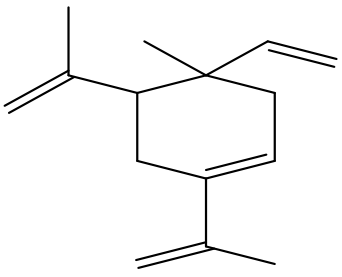
Some same compounds were identified, comparing our results with those of references. For example, comparing with Zhang, et al. [8], the same compounds were camphene, eucalyptol, camphor, borneol, isoborneol, *α*-terpineol. Comparing with Luo, et al. [9], the same compounds were camphor, isoborneol, borneol, curzerenone, germacrone. Comparing with Yuan, et al. [10], the same compounds were *α*-terpinene, camphor, isoborneol, germacrone, curzerenone. There were also differences between each other, which might be due to differences in the method of the experiment or the use of retrieval spectral library.

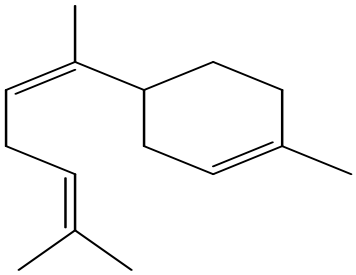
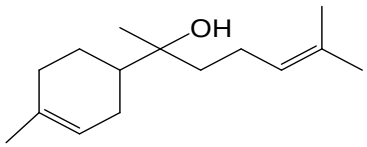
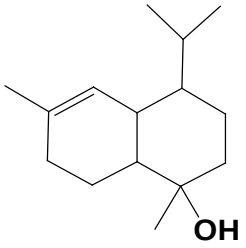
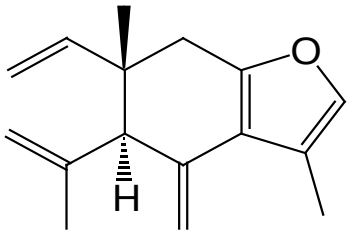
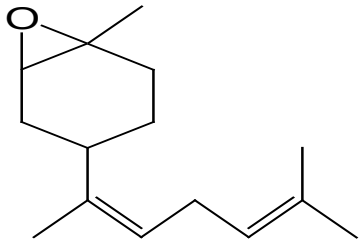
Furthermore, twenty compounds were almost monoterpenes and sesquiterpenes. Sesquiterpenes were curzerenone, germacrone, neocurdione, Cis-Z-*α*-bisabolene epoxide, elemene, *α*-bisabolol, *α*-cadinol, *α*-bisabolene, trans-Z-*α*-bisabolene epoxide. Monocyclic monoterpenes were eucalyptol, *α*-terpineol. Bicyclic monoterpenes were camphor, isoborneol.

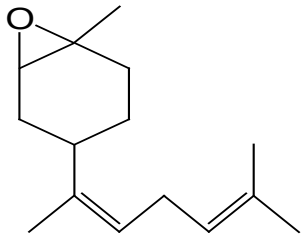
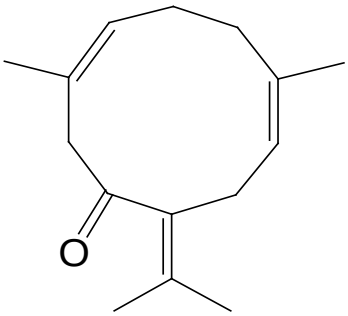
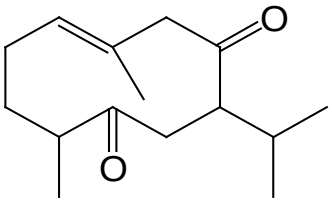
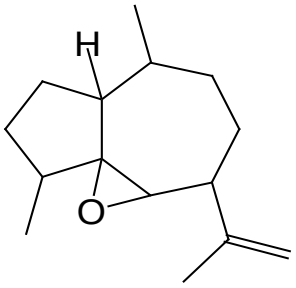
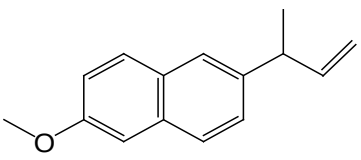
It's showed that the volatile oils from Curcuma possessed potent anticancer activity. It was reported that the rhizome oil of *Curcuma purpurascens* Bl exhibited strong cytotoxicity against HT29 cells, weak cytotoxicity against A549, Ca Ski, and HCT116 cells, and no inhibitory effect against MCF7 cells. It showed mild cytotoxicity against a noncancerous human lung fibroblast cell line (MRC5) [11]. Moreover, essential oil of *Curcuma zedoaria* presented anti-angiogenic activity *in vitro* and *in vivo*, resulting in suppressing melanoma growth and lung metastasis [12]. And Chien-Chang Chen reported that the essential oil obtained from *Curcuma zedoaria Roscoe*, known as zedoary, possessed efficient cytotoxic effects on non-small cell lung carcinoma cells and caused cell apoptosis [13]. In addition, treatment with the essential oil of *Curcuma wenyujin* inhibited the growth of HepG2 cells in a dose-dependent manner, which exhibited antiproliferative effect in HepG2 cells by inducing apoptosis [14].

Table 1: Chemical components and relative percentage contents of volatile oil from *Curcuma Kwangsiensis*.

Number	Retention time (min)	Relative Molecular Mass	Molecular Formula	Compound Name	Structural	Relative Percentage Contents (%)
1	6.200	110	$C_7H_{10}O$	1-Heptyn-6-one		1.10
2	6.625	136	$C_{10}H_{16}$	Camphene		0.93
3	8.842	196	$C_{12}H_{20}O_2$	Isobornyl acetate		0.73
4	8.933	154	$C_{10}H_{18}O$	Eucalyptol		3.03
5	11.542	166	$C_{11}H_{18}O$	5-Methylene-9-Decen-2-one		0.63

6	12.392	152	$C_{10}H_{16}O$	Camphor		1.57
7	12.883	196	$C_{12}H_{20}O_2$	Borneol		0.51
8	13.142	154	$C_{10}H_{18}O$	Isoborneol		0.76
9	13.858	154	$C_{10}H_{18}O$	α-Terpineol		0.97
10	19.383	204	$C_{15}H_{24}$	Elemene		0.51

11	21.775	204	$C_{15}H_{24}$	α-Bisabolene		0.24
12	21.933	204	$C_{15}H_{24}$	α-Bisabolol		0.28
13	21.950	222	$C_{15}H_{26}O$	α-Cadinol		0.28
14	23.975	230	$C_{15}H_{28}O_2$	Curzerenone		4.94
15	24.350	220	$C_{15}H_{24}O$	cis-Z-α-Bisabolene epoxide		0.99

16	24.625	220	$C_{15}H_{24}O$	trans-Z-α-Bisabolene epoxide		0.21
17	25.650	218	$C_{15}H_{22}O$	Germacrone		1.80
18	26.342	236	236	Neocurdione		1.50
19	26.458	220	$C_{15}H_{24}O$	γ-Gurjunenepoxide-(1)		2.03
20	28.642	212	$C_{15}H_{16}O$	2-Methoxy-6-(1-methyl-2-propenyl)-naphthalene		1.07

In order to clarify the anticancer activity of volatile oils extracted from *Curcuma kwangsiensis*, cell growth inhibitory assay was carried out. Indeed, our results exhibited that the oil could effectively inhibit growth of lung cancer H446 cells, with the IC50 values of 7.55±0.38 μg/ml.

Lung cancer, also known as carcinoma of the lung or pulmonary carcinoma, is a malignant lung tumor. It is characterized by uncontrolled cell growth in tissues of the lung. The growth can spread beyond the lung by process of metastasis into nearby tissue or other parts of the body if left untreated. Surgery, radiotherapy and chemotherapy are the common treatments. Small cell lung cancer always responds better to chemotherapy and radiotherapy, whereas non-small-cell lung cancer is sometimes treated with surgery. Lung cancer is the most common cause of cancer-related death in men and women all over the world. Furthermore, it was responsible for 1.56 million deaths annually, as of 2012 [15]. Therefore, it is important to find suitable medicine. In recent years, a lot of studies have proved the efficacy of important oils and their chemical components as source of new bioactive natural products, including anticancer [16,17].

Recently, Zhao, et al. reported that β-elemene could notably inhibit the proliferation of non-small cell lung cancer cells [18]. Moreover, Curzerenone and neocurdione inhibited cell proliferation in human cancer cell lines MCF-7, HCT-116 and Ca Ski in a dose-dependent way [19]. In addition, a recent research showed that treatment of the hepatoma cell lines HepG2 and Bel7402 with germacrone promoted cell apoptosis [20].

This study found that volatile oil from *Curcuma kwangsiensis* had an inhibitory action on H446 cells. This might be elemene, curzerenone, neocurdione, germacrone at work. The study provided an effective strategy to overcome malignant lung tumor.

Volatile oils have not only promising potentials for maintaining and promoting health but also preventing and potentially treating some diseases. However, the widely low water solubility and stability, together with the high volatility and side effects associated with their use have limited their application in medicine. In this study, the side effects of volatile oil from *Curcuma Kwangsiensis* should be further researched. It was reported that polymeric nanoparticulate formulations, extensively studied with significant improvement of the essential oil antimicrobial activity, and lipid carriers, including liposomes, solid lipid nanoparticles, nanostructured lipid particles, and nano- and microemulsions. Furthermore, molecular complexes such as cyclodextrin inclusion complexes also represent a valid strategy to increase water solubility and stability and bioavailability and decrease volatility of essential oils [21]. So we could package the oils in polymeric nanoparticulate formulations, lipid carriers and molecular complexes as drugs for people to take it.

Conclusion

In summary, twenty compounds were identified from volatile oil from *Curcuma kwangsiensis* in this experiment, most of which were sesquiterpenes and monoterpenes. And the volatile oil from *Curcuma kwangsiensis* had an inhibitory action on the growth of H446 cells, which provided a scientific basis for the further development and utilization of *Curcuma kwangsiensis*.

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