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Full Length Research Paper

Study on spectrum-effect relationship between fingerprint of essential oil and of anti-tumor effect from *Curcuma kwangsiensis*

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The objective of this study was to investigate the effective component group represented by fingerprint contributed to pharmacodynamics by grey relational analysis, and provide a reference for traditional Chinese medicine quality control and develop evaluation criteria of pharmacodynamics. The fingerprint of essential oil from *Curcuma kwangsiensis* was established by gas chromatography-mass spectrometry (GC-MS), and the anti-tumor effect of curcuma oil was studied. At the premise of the acquired GC-MS characteristic of fingerprint and pharmacodynamics data to inhibit proliferation of nasopharyngeal carcinoma cell, the relational grade and relational sequence between fingerprint of essential oil and of inhibiting proliferation rate were calculated by grey relational analysis. According to the relational grade, the sequence of contribution to inhibit proliferation to nasopharyngeal carcinoma cell was Curcumol > Curdione > β-Elemene > Germacrone > Curzerenone > α-Caryophyllene > δ-Selinene > β-Caryophyllene > β-Elemenone > a-Pinene > α-Caryophyllene > Eucalyptol > Camphor > 2-Nonanol > Isoborneol > Curzerene > δ-Elemene > Camphene > D-Limonene > Borneol. In conclusion, the study identified the effective components within *C. kwangsiensis* for anti-tumor activities. This provided the spectrum-effect relationship in a type of traditional Chinese medicine, which is important for quality control and evaluation of complementary medicine.

Key words: *Curcuma kwangsiensis*, spectrum-effect relationship, essential oil, gas chromatography-mass spectrometry.

INTRODUCTION

Guangxi *Curcuma kwangsiensis S*.G. Lee et C. F. Liang is one important traditional Chinese medicine with anti-tumor bioactivity (Qin et al., 2010). The essential oil of *C. kwangsiensis* (EOCK) maintained major bioactivities. Many molecules were isolated from the essential oil, including curcumol, β-elemene, curzerenone and curcuma curdione (Yang et al., 2005; He et al., 2010). The present study set out to set up the fingerprint spectrum of EOCK, and then tried to correlate the different spectrum peaks with varied anti-tumor activity on nasopharyngeal carcinoma cell line CNE-2. The study provided the possibility to predict or screen the quality of herbal medicine with a fast approach in high throughput manner.

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MATERIALS AND METHODS

Instruments

The following instrument and reagents were used in present study: 5973N-6890 gas chromatograph-mass spectrometer (Agilent, USA), column HP-5MS capillary column (Agilent, USA); FA1004 electronic balance (Shanghai Balance Instrument Factory JingKe

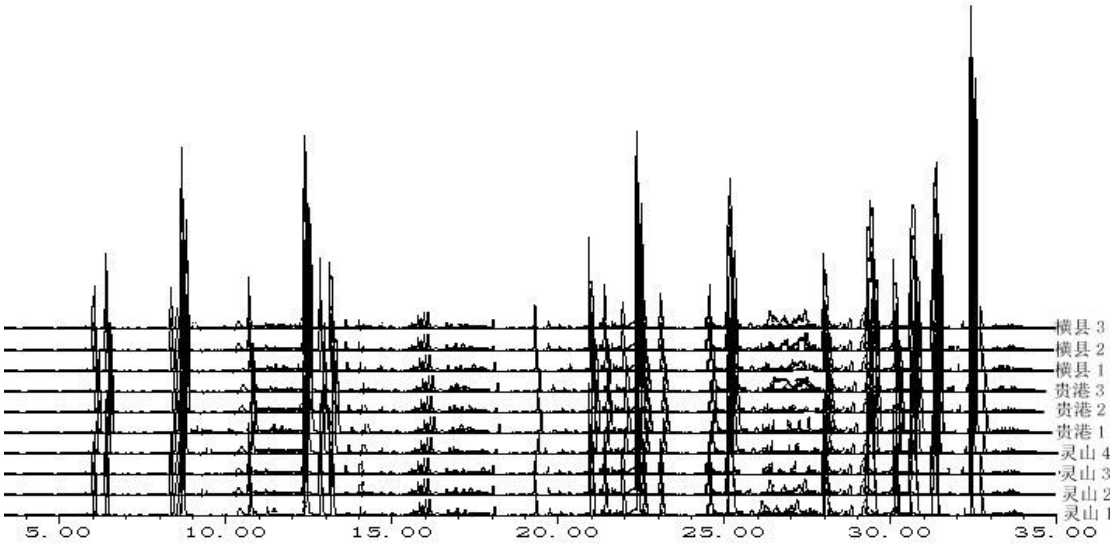

Figure 1. The fingerprint analysis of 10 batches of EOCK samples (x axis is the time).

manufacturing), heater thermostat (Shanghai Electric Instrument Manufacturing Manager), thermo CO_2 incubator (U.S. thermo scientific company), Olympus inverted fluorescence microscope (Olympus Corporation, Japan), laser scanning confocal microscope (Olympus Corporation, Japan), Bio-TEK microplate reader (United States Bio Inc.), electrophoresis tank (U.S. Bio-RAD Company), GENIUS gel imaging system (Tianneng Technology Co., Ltd. Shanghai).

Reagents

Hochest33342 (KGI biotech companies), tetrazolium salt (MTT) (Amresco Inc. USA), dimethyl sulfide (DMSO) (Sigma product), the cell culture medium PRMI1640 (Gibco Company), fetal bovine serum (FBS) (Hangzhou Evergreen Company), an anti-and secondary antibody (Beijing Jinqiao biotechnology companies in the shirt), and the remaining were of analytical grade. CNE-2 cells were passage-based scientific experiments Center security lines.

Plant material

10 batches of the *C. kwangsiensis* samples were collected from Lingshan (4 batches), Guigang (3 batches), and Hengxian (3 batches). The fresh samples were dried and minced before made into powder. The essential oil was extracted according to the method described in Chinese pharmacy book 2005.

GC-MS analysis

The HP-5MS capillary column (30.0 m × 250 μm × 0.25 μm) was used for GC-MS analysis. The flow rate of helium was 1.0 ml/min; the sample volume was 1 μl. The temperature increase program: 65°C/2 min, 65 to 90°C (5°C increase per min, then 3 min at 90°C), 103 to 150°C (8°C increase per min, then 15 min at 150°C), 150 to 280°C (20°C increase per min). The temperature of injector for sample was 250°C; ion source at 230°C with ionization mode EI. The scanning mass range: 45 to 550 amu.

The finger print spectrum of EOCK was determined from the stable and specific peaks among all 10 batches of samples. The 14 peaks of β-elemene was selected for internal control (peak S), with time and area as 1. The relative time (α value) and area (ratio between other peaks and peak S) of other peaks were calculated.

Anti-tumor activity analysis

30 mg EOCK was dissolved in 3 ml ethanol (10 mg/ml), and then diluted with RPMI culture medium containing 12% bovine serum to reach a final concentration of 100 μg/ml. CNE-2 cells were seeded in 96-hole plate and the drugs or control were applied for 48 h. 20 μl MTT solution was added for 4 h and then mixed with 150 μl DMSO after removal of the supernatant. Finally, the light absorbance vale (value A) was measured at 490 nm.

The inhibition rate of cell growth = [(control A-experiment A)/control A] × 100%

Statistical analysis

The data were represented by mean ± SD, and analyzed with SPSS 13.0 software (Chicago, US). ANOVA and t-test were used for statistical comparisons and $p < 0.05$ was considered as statistically significant.

RESULTS

Fingerprint spectrum of EOCK from GC-MS analysis

The GC-MS fingerprint spectrum was shown in Figure 1. The peaks from 1 to 20 were α-Pinene, Camphene, D-Limonene, Eucalyptol, 2-Nonanol, Camphor, Borneo, Isoborneol, α-Caryophyllene, Curzerene, α-Caryophyllene, β-Caryophyllene, δ-Elemene, β -Elemene, δ -Selinene, Curzerenone, Curcumol, Curdione, Germacrone, and β -Elemenone, respectively

Table 1. The relative time and area values of 10 batches of EOCK samples.

Peak	α value	Relative area ratio									
		1	2	3	4	5	6	7	8	9	10
1	0.24	0.26	0.21	0.27	0.27	0.19	0.29	0.21	0.24	0.21	0.23
2	0.25	0.56	0.57	0.56	0.62	0.40	0.60	0.49	0.52	0.66	0.61
3	0.34	0.25	0.22	0.29	0.40	0.20	0.31	0.22	0.33	0.49	0.23
4	0.35	1.58	1.03	1.74	1.20	1.24	1.89	1.39	1.00	0.98	1.11
5	0.42	0.38	0.33	0.37	0.60	0.26	0.40	0.34	0.50	0.33	0.36
6	0.49	1.81	1.13	1.66	1.38	1.18	1.80	1.59	1.00	0.98	1.22
7	0.51	0.51	0.53	0.23	0.62	0.16	0.24	0.45	0.83	0.66	0.57
8	0.54	0.62	0.47	0.61	0.80	0.44	0.67	0.54	0.67	0.82	0.50
9	0.85	0.60	0.46	0.85	0.40	0.60	0.92	0.53	0.33	0.33	0.49
10	0.89	1.69	1.28	1.60	1.20	1.13	1.73	1.49	1.00	1.28	1.38
11	0.89	0.36	0.27	0.38	0.42	0.27	0.41	0.32	0.33	0.30	0.29
12	0.92	0.34	0.26	0.36	0.40	0.26	0.40	0.30	0.33	0.29	0.28
13	0.97	0.37	0.29	0.39	0.60	0.29	0.67	0.34	0.33	0.31	0.30
14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
15	1.13	0.71	0.54	0.76	0.99	0.54	1.11	0.63	0.62	0.61	0.58
16	1.17	1.08	0.82	1.57	1.38	0.82	1.33	0.96	0.94	1.25	0.88
17	0.96	0.62	0.47	0.86	1.02	0.47	1.11	0.55	0.54	0.00	0.00
18	1.21	0.97	0.73	0.61	1.20	0.73	1.33	0.85	0.84	0.49	0.79
19	1.26	1.78	1.49	1.64	2.20	1.49	2.44	1.74	1.71	1.31	1.45
20	1.29	2.42	1.69	2.46	2.04	1.69	2.22	1.97	1.93	1.97	1.97

Table 2. The inhibition rates of 10 batches of EOCK on CNE-2 cell growth.

Batch	1	2	3	4	5	6	7	8	9	10	Mean
Inhibition rate (%)	58.61	45.93	78.36	91.34	45.93	98.57	52.69	51.84	8.88	8.49	54.06

The relative time (α value) and area (ratio between other peaks and peak S) of other peaks were calculated (Table 1).

The anti-tumor activity of EOCK

The inhibition rates of 10 batches of EOCK on CNE-2 cell growth showed great differences among groups (Table 2).

The correlationship analyses between specific peaks and the inhibition rates

To investigate the spectrum based treatment effects, we further analyzed the correlationship analyses between different peaks and the inhibition rates on CNE-2 cell growth (Table 3).

DISCUSSION

The present study differentiated the contribution of different GC-MS peaks in EOCK to the cancer cell growth inhibition effect. The top 5 components were curcumol, curdione, β-elemene, germacrone, and curzerenone, which are the main anti-tumor components in EOCK. Therefore the GC-MS spectrum, especially the specific peaks, could be used as indices of bioactivity of EOCK; as well, the quality analysis of crude herbal medicine samples (Yang et al., 2005; Xiang et al., 2011). In the present study, we found significant differences of cancer cell growth inhibition among samples collected from different areas, and guigang samples showed the highest inhibition rate. This suggested that the different GC-MS peaks could represent the therapeutic effects to some extent, which greatly shortens the time. The fingerprint analysis approach provides fast quality determination in herbal medicine, which is much faster compared to laboratory tests with cancer cells (Liang et al., 2009; Liu et al., 2010). This also implies new directions in production and improvement of herbal medicine. The guided selection of plants with ideal spectrum would finally bring better drugs. Moreover, the understanding of the most bioactive molecules provides more hints for new

Table 3. The different level of correlation of 20 peaks with the inhibition rates.

Peak	Correlationship	Rank of the correlationship
x_1	0.755 ± 0.066	10
x_2	0.698 ± 0.065	18
x_3	0.675 ± 0.055	19
x_4	0.747 ± 0.054	12
x_5	0.722 ± 0.059	14
x_6	0.725 ± 0.054	13
x_7	0.602 ± 0.072	20
x_8	0.712 ± 0.061	15
x_9	0.770 ± 0.064	6
x_{10}	0.709 ± 0.060	16
x_{11}	0.752 ± 0.060	11
x_{12}	0.764 ± 0.063	8
x_{13}	0.706 ± 0.067	17
x_{14}	0.781 ± 0.046	3
x_{15}	0.768 ± 0.049	7
x_{16}	0.774 ± 0.066	5
x_{17}	0.921 ± 0.024	1
x_{18}	0.798 ± 0.064	2
x_{19}	0.780 ± 0.063	4
x_{20}	0.756 ± 0.074	9

drug design.

The medicinal plants have been important for new drug discovery and the current biomedical techniques are developing (Akuodor et al., 2012; Coopoosamy and Naidoo, 2012; Esath-Natheer et al., 2012). Our study further emphasized the natural products in pharmacy and pharmacology research, and the approach we employed here suggested novel directions in identifying active compounds from complex mixture.

In conclusion, the study proved the possibility of drug screening with GC-MS analysis approach in a high through-put manner. The correlationship between therapeutic effects and GC-MS peaks had important implications on new drug discoveries.

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