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Thermo-chemical investigation on the quantity-antibacterial effect relationship of five berberine alkaloids in *Rhizoma Coptidis* on *Escherichia coli* growth

Weijun Kong,^{a,b} Yanling Zhao,^{a*} Limei Shan,^a Xiaohe Xiao,^a Weiying Guo^b

^a*Institute of Chinese Materia Medica, 302 Hospital of People's Liberation Army, Beijing, 100039, PR China*

^b*Pharmacy college, Liaoning Medical University, Liaoning Jinzhou, 121001, PR China*

* **Corresponding author.** PLA Institute of Chinese Materia Medica, 302 Hospital of People's Liberation Army, 100 West 4th Ring Middle Road, Beijing 100039, PR China. Tel.: +86 10 66933324; fax: +86 10 63 879915. E-mail address: zhao2855@263.net (Y.-L. Zhao).

ABSTRACT

The inhibitory effects of five berberine alkaloids from rhizoma of *Rhizoma Coptidis*, a traditional Chinese medicinal herb, on *Escherichia coli* growth were investigated at 37 °C by microcalorimetry. The five alkaloids were: berberine, coptisine, epiberberine, palmatine and jatrorrhizine. The thermogenic power-time curves of *Escherichia coli* growth with and without berberine alkaloids were by the Thermal Activity Monitor (TAM) Air Isothermal Calorimeter, meanwhile the values of growth rate constants k , growth inhibitory ratio I , maximum heat output P_m and generation time t_G were calculated. In accordance with thermo-kinetic model, the relationships of the drugs, such as $I - c$, $k - c$, $P_m - k$ were investigated. c was the concentration of the drugs. Half-inhibitory concentration of the drugs, IC_{50} , was obtained by quantitative analysis. From the view of thermo-kinetics and molecular structure, the relationship between quantity and effects of five berberine alkaloids was discussed. Also, the minimum inhibitory concentration (MIC) and the minimal bacteriocidal concentration (MBC) of the five berberine alkaloids on anti-*Escherichia coli* growth was obtained by tube dilution method. Meanwhile, the action mechanism of antibacterial effect was studied. The efficiency of these five berberine alkaloids on anti-growth of *E. coli* was as follows: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. This work illustrated that microcalorimetry was a useful tool to investigate the antibacterial activity of medicinal herbs and provided a general model to study the quantity and antimicrobial effects relationship of medicinal herbs from the view of thermo-chemistry and molecular structure.

KEY WORDS: *Rhizoma Coptidis*, Berberine alkaloids; *Escherichia coli*, Microcalorimetry, Quantity and antibacterial effect relationship

INTRODUCTION

Rhizoma Coptidis (Huanglian in Chinese) is a traditional Chinese medicinal (TCM) herb, and is officially listed in the Chinese Pharmacopoeia (1). It is known to show antimicrobial activity against *Staphylococcus aureus* (2), *Escherichia coli*, *Bacillus anthracis* and antifungal activity against *Candida albicans* and *Aspergillus niger* (3, 4). Its extract has also strong antimicrobial activities and is used for treating dysentery, cholera, leukemia, diabetes and lung cancer (5). The major active components of the herb are berberine alkaloids (BAs), such as berberine, coptisine, epiberberine, palmatine and jatrorrhizine, which are often used as criteria in the quality control of *Rhizoma Coptidis* products (6, 7). BAs are also active components in large numbers of plant-derived drugs such as antimicrobial from Berberidaceae and Rutaceae family.

In this study, the five BAs were tested against *Escherichia coli* (*E. coli*) by microcalorimetry to provide more references for the antimicrobial activity of *Rhizoma Coptidis*. And some useful informations such as P_m , k , I , IC_{50} which could not be obtained from other methods was acquired from this study. This information was important to present the antibacterial activity of drugs.

E. coli is one of the most common pathogenic bacterium in clinic. It has been used as an important tool to screen the bioactive part of folium of *Isatis indigotica* (8) and to evaluate the quality of *Rhizoma Coptidis* (9).

The microcalorimetric method is one of the important techniques for thermo-chemistry and thermokinetic study. In any living system, the various metabolic

events are all biochemical reactions producing heat. By monitoring the heat effect with a sensitive calorimeter, the microcalorimetric method can directly determine the biological activity of a living system and provide a continuous measurement of heat production, thereby giving much information about the metabolism of organism in both qualitative and quantitative ways. By analysis of thermogenic curves obtained from microcalorimetric measurement, it can reveal many temporal details about the microbial metabolism not observable by other methods (10). The microcalorimetric method has been widely applied in studying metabolism of microorganism and cultured tissue cells (11, 12, 13, 14). As is known that chloramphenicol is a broad-spectrum antibiotic, it can inhibit strongly the growth of *Escherichia coli*, *salmonella* and *Staphylococcus aureus*, etc (15, 16). In this study, chloramphenicol was selected as a standard positive controlling drug to study the antimicrobial activity.

We investigate the relationship between quantity and antibacterial effect of five BAs from *Rhizoma Coptidis* on *E. coli* growth based on thermo-kinetic model by quantitative analysis of the thermo-kinetic data. This work provides a powerful method for studying the pharmacodynamic action of *Rhizoma Coptidis*, which is helpful to discover and search for bioactive components of Traditional Chinese Medicinal compounds.

MATERIALS AND METHODS

The TAM Air Isothermal Calorimeter, manufactured by Thermometric AB Company of Sweden, was used to measure the heat-output of the metabolism of *E. coli*. This isothermal micro-calorimeter is an eight-channel twin instrument. The microcalorimeter is thermostated at the range of 5 - 60 °C, with a limitation of detectability of 2 µw. The experiment was performed as the manufacturer's recommend and the report of Wadso (17). Picolog software (Pico Technology Ltd) was used to process the data.

Apparatus

Chemicals

Rhizoma Coptidis (No.084523), which was accredited by professor Xiao-He Xiao, Institute of Chinese Materia Medica, 302 Hospital of PLA (People's Liberation Army), Beijing, 100039, PR China, was the rhizoma of *Coptis chinensis* Franch, Ranunculaceae, collected from Anguo city, Hebei province, China. was the dried root of *Coptis chinensis* Franch. Berberine, jatrorrhizine, palmatine, coptisine and epiberberine were supplied by National Institute for the Control of Pharmaceutical

and Biological Products. The five BAs were extracted from *Rhizoma Coptidis* and their structures were given in Fig.1.

E. coli strain (*Escherichia coli* CMCC B44103) and the standard chloramphenicol were provided by the Chinese Center for Type Culture Collections, National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100051, China. *E. coli* was grown in a peptone culture medium, which contained 10 g peptone, 6 g beef extract and 5 g NaCl. Medium pH was adjusted to 7.0 - 7.2 with 1 mM NaOH before autoclaving. LB culture medium, which contained 10g peptone, 5 g yeast extract and 5 g NaCl and Medium pH was adjusted to 7.0 - 7.2 with 1 mM NaOH before autoclaving.

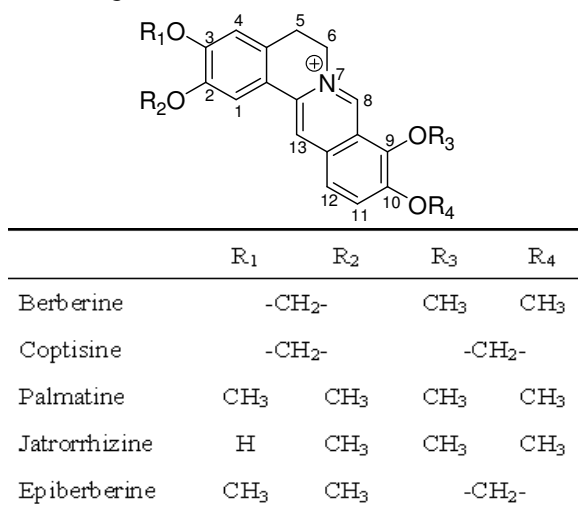


Fig.1 Chemical structures of investigated BAs from *Rhizoma Coptidis*

Experimental procedure

At the beginning of the experiments, *E. coli* was inoculated into LB culture medium, with 2×10⁶ cells per mL. Cells were suspended in the peptone culture medium, and the fresh prepared BAs solutions by using water with different concentrations were added to the cell suspension.

The microcalorimeter was thermostated at 37 °C, and the measurement made using the ampoule method. All the ampoules containing the bacterial suspension of *E. coli* and one of the BAs were sealed up and put into 8-channel calorimeter block. After about 30 min (the temperature of ampoules reached 37 °C), the thermogenic power-time curves were recorded until the recorder returned to the baseline. All data were collected continuously by using the dedicated software package.

Then, the MIC and MBC of the five BAs on *E. coli* growth were determined by tube dilution method. The

berberine alkaloids solution were diluted double decremented continuously and respectively. The different dilutions of BAs were added into LB culture medium respectively, 100 μ L microbial suspension of *E. coli* was inoculated in every tube and was cultivated for 24 h at 37°C to observe the growth of *E. coli*. The MIC is the average concentration of BAs between the least concentration of the tube in which the *E. coli* has non-proliferation and the maximum concentration of the tube in which the *E. coli* proliferates obviously, the MBC was the least concentration of the tube in which the *E. coli* is killed and has non-proliferation (18).

RESULTS

The thermogenic P-t curves and parameters

The heat-production growth curve of *E. coli* could be divided into four phases, i.e. lag phase, first exponential phase, second exponential phase and decline phase. The exponential model of *E. coli* metabolism could be used in the two growth processes:

$$P_t = P_0 \exp(kt) \quad \text{or} \quad \ln P_t = \ln P_0 + kt \quad (1)$$

where P_0 was the heat output power at time 0, and so was P_t at time t . The thermogenic curve formula of the exponential phase of growth was Eq. (1). According to Equation (1), the growth rate constant (k) was obtained by fitting $\ln P_t$ and t to a linear equation (Table 1).

Table 1

Table 1 showed $k = (0.02899 \pm 0.00291) \text{ min}^{-1}$ and all of the correlation coefficients, r , exceeded 0.995, indicating a good reproducibility and relationship.

The thermogenic power-time (P-t) curves of *E. coli* growth in the presence of 0.10 mg/ml of chloramphenicol and five BAs were showed in Fig 2. It was clear that the addition of drugs delays the maximum peak-time (t_m), which also suggests that chloramphenicol and five BAs have inhibitory effect on *E. coli*. At the same time, the maximum power-output (P_m) decreases correspondingly (as can be seen from the heights of the highest peaks in Fig.2). Compared to the control, the sequence of P_m was chloramphenicol < berberine < coptisine < epiberberine < palmatine < jatrorrhizine, which meanted the strength of anti - *E. coli* growth was chloramphenicol > berberine > coptisine > epiberberine > palmatine > jatrorrhizine > control.

The thermogenic P-t curves of *E. coli* in the presence of different BAs were showed in Fig 3. The generation time (t_G) of *E. coli* could be obtained from the formula: $t_G = (\ln 2)/k$. Table 2 showed k , t_G and P_m of *E. coli* growth in the presence of BAs.

Table 2

Growth inhibitory ratio (I)

I was defined as:

$$I \% = [(k_0 - k_c) / k_0] \times 100 \% \quad (2)$$

where k_0 was the growth rate constant at concentration 0, so was k_c at concentration c . Table 2 showed the I of *E. coli* by different drugs. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called the half-inhibitory concentration IC_{50} . IC_{50} can be regarded as the inhibiting concentration of causing a 50% decrease of the *E. coli* growth.

The power-time curves of *E. coli* growth in Fig. 3 for the five BAs were similar and the curves could still be divided into four phases. They had same profiles but different peak-heights. Some similarities and differences could be observed from a qualitative point. The curves demonstrated that the lag phase was prolonged and the highest peak degraded with the increasing concentrations of the five BAs. The similar results were showed in Table 2 that the values of k_2 and P_m decreased and t_G increased with the increasing concentration of five BAs, indicating that the five BAs bona fide inhibited the growth of *E. coli*. But, with the differences of k_2 , P_m and t_G values, the five BAs had different antibacterial activity.

I - c relationship

I - c relationship could be obtained by fitting I and c to a linear equation. IC_{50} could be obtained from the linear equation.

$$\text{For berberine : } I \% = 6.7512 c + 45.7032, R = 0.9297$$

$$IC_{50} = 0.06 \text{ mg/mL } (0.05 - 0.35 \text{ mg/mL})$$

$$\text{For coptisine: } I \% = 2.6143 c + 45.4013, R = 0.9496$$

$$IC_{50} = 0.08 \text{ mg/mL } (0.05 - 0.35 \text{ mg/mL})$$

$$\text{For epiberberine: } I \% = 7.3429 c + 46.4711, R = 0.9948$$

$$IC_{50} = 0.22 \text{ mg/mL } (0.05 - 0.35 \text{ mg/mL})$$

$$\text{For palmatine: } I \% = 6.8253 c + 30.8902, R = 0.9944$$

$$IC_{50} = 2.80 \text{ mg/mL } (0.50 - 3.50 \text{ mg/mL})$$

$$\text{For jatrorrhizine: } I \% = 2.6799 c + 7.2141, R = 0.9881$$

$$IC_{50} = 13.14 \text{ mg/mL } (4.5 - 13.5 \text{ mg/mL})$$

P_m - k relationship

P_m - k relationship could be obtained by fitting P_m and k to a linear equation.

$$\text{For berberine : } P_m = 0.0341k + 0.9394, R = 0.9714$$

$$(0.05 - 0.35 \text{ mg/mL})$$

$$\text{For coptisine: } P_m = 0.0634 k + 1.0846, R = 0.9922$$

$$(0.05 - 0.35 \text{ mg/mL})$$

$$\text{For epiberberine: } P_m = 0.0548 k + 1.0216, R = 0.9761$$

$$(0.05 - 0.35 \text{ mg/mL})$$

$$\text{For palmatine: } P_m = 0.0398 k + 1.4112, R = 0.9707$$

$$(0.50 - 3.50 \text{ mg/mL})$$

$$\text{For jatrorrhizine: } P_m = 0.0316 k + 1.4011, R = 0.9743$$

$$(4.50 - 13.5 \text{ mg/mL})$$

k-c relationship

k-c relationship could be obtained by fitting *k* and *c* to a linear equation. *k* decreased with the drug concentrations increased.

For berberine: $k = 0.0033 - 0.0002 c$, $R = -0.9287$

(0.05-0.35 mg/mL)

For coptisine: $k = 0.0034 - 0.0004 c$, $R = -0.9508$

(0.05-0.35 mg/mL)

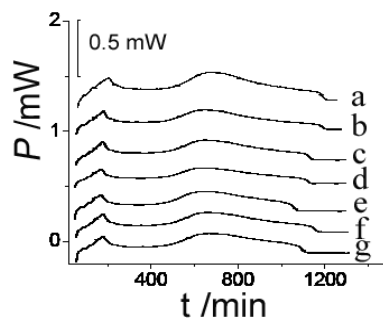


Fig.2 The power-time (*P-t*) curves for growth of *Escherichia coli* at 37 °C without and with drugs.

For epiberberine: $k = 0.0091 - 0.0008 c$, $R = -0.9947$

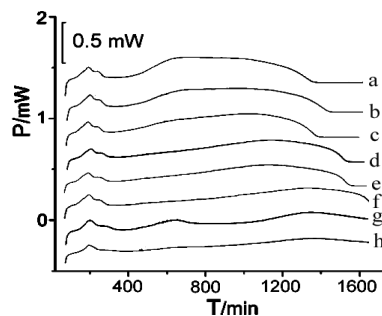
(0.05-0.35 mg/mL)

For palmatine: $k = 0.0079 - 0.0015 c$, $R = -0.9844$

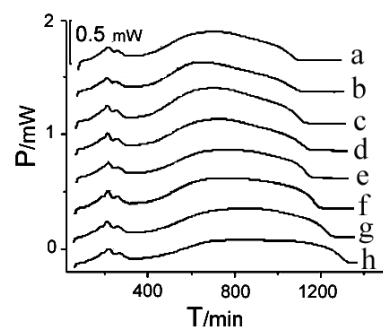
(0.50-3.50 mg/mL)

For jatrorrhizine: $k = 0.0307 - 0.0021 c$, $R = -0.9870$

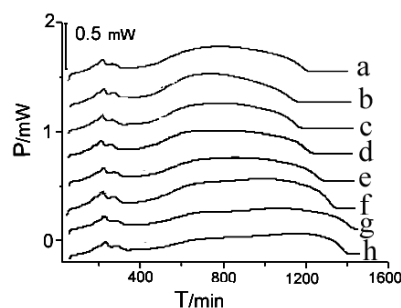
(4.50-13.5 mg/mL)



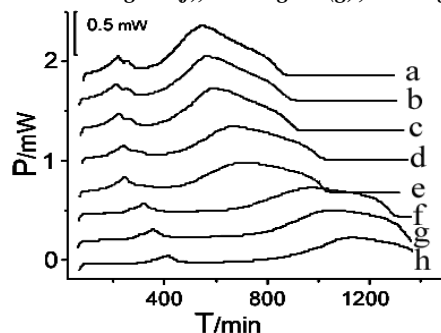
(A) The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).



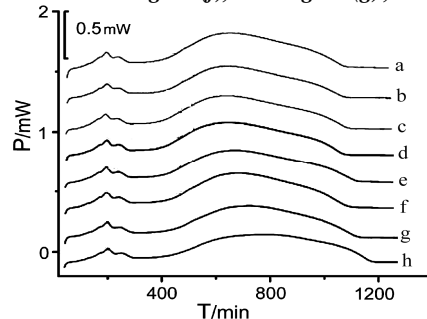
(B) The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).



(C) The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h).



(D) The concentrations of palmatine: 0 mg/ml (a), 0.5 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g), 3.50 mg/ml (h).



(E) The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g), 13.5 mg/ml (h).

Fig 3. The power-time curves of *E. coli* growth in the presence of different concentrations of berberine (A), coptisine(B), epiberberine (C), palmatine (D), jatrorrhizine(E).

E. coli was cultured in peptone culture medium supplemented with different concentrations of five BAs respectively, and monitored of TAM air at 37 °C. (A) The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (B) The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (C) The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (D) The concentrations of palmatine: 0 mg/ml (a), 0.50 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g), 3.50 mg/ml (h). (E) The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g), 13.5 mg/ml (h)

Table 1 - Growth rate constant (*k*) of *E. coli* growth at 37 °C

Experiment	1	2	3	4	5	6	7	8
<i>k</i> /min ⁻¹	0.0304	0.0308	0.0284	0.0285	0.0287	0.0279	0.0279	0.0293
<i>R</i>	0.9976	0.9957	0.9984	0.9969	0.9985	0.9978	0.9959	0.9973

^Rcoefficient correlation

The growth rate constant (*k*) of *E. coli* growth without drugs at 37 °C was obtained by microcalorimetry. The data in the table indicated a good reproducibility and relationship of the results.

Table 2 - The values of *k*, generation time *t_G*, inhibitory ratio *I* and maximum power-output *P_m* of *E. coli* growth in the presence of different concentrations of five BAs

BAs	C/mg.mL ⁻¹	<i>k</i> /min ⁻¹	<i>R</i> ^a	<i>t_G</i> /min	<i>I</i> /%	<i>P_m</i> /mW
Berberine	0	0.030	0.999	23.1	0	1.744
	0.05 ^b	0.019	0.992	36.5	36.7	1.161
	0.10 ^b	0.017	0.992	40.8	43.3	1.134
	0.15 ^b	0.015	0.995	46.2	50.0	1.124
	0.20 ^b	0.013	0.994	53.3	56.7	1.102
	0.25 ^b	0.011	0.992	63.0	63.3	1.057
Coptisine	0.30 ^b	0.009	0.995	77.0	70.0	1.000
	0.35 ^b	0.007	0.996	99.0	76.6	0.954
	0.05 ^b	0.022	0.996	31.5	26.7	1.401
	0.01 ^b	0.020	0.996	34.7	33.3	1.396
	0.15 ^b	0.017	0.995	40.8	43.3	1.269
	0.20 ^b	0.013	0.994	53.3	56.7	1.221
Epiberberine	0.25 ^b	0.010	0.995	69.3	66.7	1.168
	0.30 ^b	0.008	0.995	86.6	73.3	1.128
	0.35 ^b	0.006	0.996	115.5	80.0	1.102
	0.05 ^b	0.027	0.995	25.7	10.0	1.521
	0.10 ^b	0.024	0.995	26.6	20.0	1.461
	0.15 ^b	0.022	0.996	29.0	26.7	1.413
Palmatine	0.20 ^b	0.018	0.995	38.5	40.0	1.335
	0.25 ^b	0.014	0.996	49.5	53.3	1.271
	0.30 ^b	0.011	0.996	63.0	63.3	1.202
	0.35 ^b	0.010	0.995	69.3	66.7	1.123
	0.50 ^b	0.028	0.996	24.8	6.70	1.606
	1.00 ^b	0.027	0.996	25.7	10.0	1.583
Jatrorrhizine	1.50 ^b	0.025	0.997	27.7	16.7	1.567
	2.00 ^b	0.022	0.997	29.0	26.7	1.542
	2.50 ^b	0.020	0.996	34.7	33.3	1.515
	3.00 ^b	0.016	0.997	43.3	46.7	1.460
	3.50 ^b	0.010	0.996	69.3	66.7	1.414
	4.50 ^b	0.029	0.995	23.9	3.3	1.662

6.00 ^b	0.028	0.994	24.8	6.7	1.651
7.50 ^b	0.026	0.993	26.7	13.3	1.633
9.00 ^b	0.023	0.994	27.7	23.3	1.591
10.5 ^b	0.020	0.993	30.1	33.3	1.547
12.0 ^b	0.017	0.994	40.8	43.3	1.485
13.5 ^b	0.013	0.994	53.3	56.7	1.443

^a Correlation coefficient. ; ^b Average of three times experiments.

E. coli was cultured in peptone culture medium supplemented in the presence of different concentrations of five BAs respectively, and monitored by TAM air at 37 °C. (A) The concentrations of berberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (B) The concentrations of coptisine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (C) The concentrations of epiberberine: 0 mg/ml (a), 0.05 mg/ml (b), 0.10 mg/ml (c), 0.15 mg/ml (d), 0.20 mg/ml (e), 0.25 mg/ml (f), 0.30 mg/ml (g), 0.35 mg/ml (h). (D) The concentrations of palmatine: 0 mg/ml (a), 0.5 mg/ml (b), 1.00 mg/ml (c), 1.50 mg/ml (d), 2.00 mg/ml (e), 2.50 mg/ml (f), 3.00 mg/ml (g), 3.50 mg/ml (h). (E) The concentrations of jatrorrhizine: 0 mg/ml (a), 4.50 mg/ml (b), 6.00 mg/ml (c), 7.50 mg/ml (d), 9.00 mg/ml (e), 10.5 mg/ml (f), 12.0 mg/ml (g), 13.5 mg/ml (h).

Table 3 - The values of k and P_m of *E. coli* growth in the presence of the five BAs at different concentrations

BAs	k /min ⁻¹	R	RSD%	P_m /mW	R	RSD%
Control	0.029			1.733		
	0.031	0.998	0.12	1.730	0.992	0.30
	0.031			1.727		
Berberine	0.009			1.096		
	0.010	0.998	0.10	1.103	0.997	0.47
	0.011			1.094		
Coptisine	0.015			1.212		
	0.017	0.991	0.36	1.218	0.992	0.42
	0.010			1.220		
Epiberberine	0.020			1.341		
	0.021	0.993	0.26	1.335	0.993	0.42
	0.016			1.343		
Palmatine	0.024			1.551		
	0.019	0.995	0.25	1.543	0.991	0.40
	0.022			1.546		
Jatrorrhizine	0.025			1.602		
	0.028	0.998	0.30	1.597	0.995	0.50
	0.022			1.592		

E. coli was cultured in peptone culture medium supplemented in the presence of five BAs respectively, and monitored by TAM air at 37 °C. The concentration was 0.20 mg/ml for berberine, 0.20 mg/ml for coptisine, 0.20 mg/ml for epiberberine 2.00 mg/ml for palmatine and 9.00 mg/ml for jatrorrhizine.

MIC and MBC

The MIC of the five BAs were 8.6 µM for berberine, 15.79 µM for coptisine, 20.8 µM for epiberberine, 30.32 µM for palmatine and 56.35 µM for jatrorrhizine. And the MBC of the five BAs were 21.4 µM for berberine, 32.8 µM for coptisine, 43.9 µM for epiberberine, 92.3 µM for palmatine and 114.2 µM for jatrorrhizine.

Reliability and stability of microcalorimetry

The “P-t” curves of *E. coli* in Fig.3 demonstrated that the lag phase prolonged and k and P_m decreased with the increasing concentrations of BAs. Table 2 showed

that the values of k and P_m decreased and t_G increased with the increasing concentration of OAs, indicating that four BAs bona fide inhibited the growth of *E. coli*. In order to evaluate the reliability and stability of microcalorimetry, triplicate experiments using these five BAs have been performed under the above-mentioned conditions. Table.3 showed the values of k and P_m of *E. coli* growth with the five BAs at different concentrations.

Table 3 .

IC50 value -IC50 is used to represent the sensitivity of

bacteria to drugs. The smaller IC₅₀ is, the stronger antibacterial activity the drugs possess. The sequence of the five IC₅₀ was: berberine < coptisine < epiberberine < palmatine < jatrorrhizine. Accordingly, the efficiency of these five BAs on anti-growth of *E. coli* was as follows: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. Berberine had the strongest activity of anti - *E. coli* growth. Jatrorrhizine and epiberberine had poor anti - *E. coli* activity. Jatrorrhizine with the IC₅₀ of 13.14 mg/mL had the poorest activity of anti - *E. coli* growth.

k value

The value of *k* can be thought as one of the characteristic constants to illustrate the growth of bacterium. The change of *k* can be used to estimate the antibacterial strength of drugs when different drugs were added or drug concentration changes at the same conditions. The smaller the absolute value of slope rate of *k*-*c* line is, the stronger antibacterial effect the drug has. The order of the absolute values of *k*-*c* linear relationship of these five BAs is: berberine < coptisine < epiberberine < palmatine < jatrorrhizine. Accordingly, the sequence of antibacterial activity of the five BAs was: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. Berberine has the strongest inhibitive effects on *E. coli* growth.

The MIC and MBC for every drug also illustrated that the efficiency of these five BAs on anti-growth of *E. coli* was: berberine > coptisine > epiberberine > palmatine > jatrorrhizine. The sequence of anti-growth of *E. coli* was identical to the sequence from the values of IC₅₀ and *k*, illustrating that microcalorimetry was accurate and useful for investigating the antibacterial activity of five BAs on *E. coli* growth.

Possible mechanism of action

The thermogenic curves of *E. coli* growth affected by various BAs from *Rhizoma Coptidis* indicated that all tested drugs had inhibitory effects on the tested bacteria. The lag phase of bacteria increased with the increasing concentrations of all tested BAs. Berberine, palmatine and coptisine showed stronger inhibitory effects on *E. coli* than the other two BAs. All BAs belong to berberines of benzyltetrahydroisoquinolines. There are different substituted groups at C₂, C₃, C₉ and C₁₀ of phenyl ring (see Fig.1). The functional groups methylenedioxy at C₂ and C₃ on phenyl ring improve antimicrobial activity more strongly than methoxyl at C₂ and C₃ on phenyl ring. However, the effect of bacteriostasis is not significant with methylenedioxy or methoxyl at C₉ and C₁₀ on phenyl ring. Combined with the results of test, the analysis of

BAs function suggested the possibility that the functional groups methylenedioxy at C₂ and C₃ would be the principal groups which induce the action of bacteriostasis among the herbs which contain berberines. From the molecular structure of the tested BAs, we could find that the five BAs have different functional groups on benzyltetrahydroisoquinolines ring. So, the number, position and type of functional groups on benzyltetrahydroisoquinolines ring have important influence on the antibacterial activities of the five BAs. The five BAs have different antimicrobial effect on *E. coli* growth, which is due to the efficiency of a detoxification mechanism with the DNA-helicase activity of bacterial being inhibited. The berberines alkaloids can be connected to the vestibule which is shaped by DNA and Topoisomerase to form a DNA-drugs-Topoisomerase ternary complex. The complex affects the duplication of DNA and thus bacterial growth is inhibited (19). The thermo-kinetics informations provided from the thermo-chemical studies illustrated that berberine alkaloids inhibited the microbial growth by prohibiting the DNA synthesis of microbial.

DISCUSSION

Our experiments selected microcalorimetry as a tool to investigate the antibacterial activity of medicinal herbs (20, 21). Tab.3 showed that the reliability and stability of this method was good. Compared with cup-plate method and nephelometry, microcalorimetry not only supplies a new point of view for the evaluation of bioactivity of drugs but provides more information about the bacterial growth. By using it, the energy changes of four growing periods of *E. coli*, which represented the regularity of microbial population growth such as the lag phase, logarithmic phase, stationary phase and decline phase could be distinguished from the heat production curve. Values of *P_m* and *k* for power-time curve are determined simultaneously which could describe the heat growing production and metabolic process of microbes dynamically and precisely. In this study, we have investigated the antibacterial action of the five BAs in *Rhizoma Coptidis* on *E. coli* growth based on biothermo-kinetics, providing more references and insights for studying the mechanism of action of these five natural products, the relationship between drug and bacterium metabolism. In this study, we investigated the quantity-antibacterial effect relationship of five BAs on *E. coli* growth quantitatively from the view of thermodynamics and molecular structure. Furthermore, the action mechanism of antibacterial effect was studied from the molecular

biology and cellular level. Also, the MIC of the BAs was also investigated. This work also provided a thermokinetic model to study the quantity-antibacterial effect relationship of drugs on microbial growth. All these were helpful for searching and discovering more pharmaco-dynamic actions and components of *Rhizoma Coptidis*.

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