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Investigation of anti-microbial activity of Catechin on *E.coli* growth by microcalorimetry

Abstract Catechin is a major monomer of Chinese *Rhubarb*. Chinese *Rhubarb* has been claimed to have a therapeutic value as a bacteriostatic drug. It is also known that total rhubarb anthraquinone mixture and its individual components such as emodin is the effective components. However, the catechins component in Chinese *Rhubarb* such as catechin has not been characterized. In the present study, the power-time curves of *E. coli* growth were obtained and the action on them by addition of catechin in different concentrations *in vitro* were studied by microcalorimetry. The results suggest *E. coli* growth is inhibited by catechin in a dose-dependent manner starting from the concentration of 0.05 to 1.6mg.mL$^{-1}$. The correspondence analysis reveals $k_2$ and $P_2$ are the significant parameters to evaluate the antimicrobial effect. Microcalorimetry is a useful tool to evaluate the antimicrobial effect with its sensitive and significant quantitative information.

Key words

*Anti-microbial activity, Catechin, Microcalorimetry*

1. Introduction

As a member of the polyphenol family, catechin exists in Chinese *Rhubarb*, which is a traditional Chinese medicine and has been applied extensively. According to recent studies, Chinese *Rhubarb* possesses pharmacological values of anticancer(*Yu* et al. 2008), a suppressor of renal(*Wang* et al. 2009, *Yan* et al.2008) and antiinflammatory(*Mi* et al. 2006). Studies also shows that *Rhubarb* has the anti-microbial effect (*Kong* et al. 2009). Furthermore, lots of research is concerned the anti-microbial effect of anthraquin and derivatives such as emodin on *candida albicans* and *S.aureu* (*Wu* et al. 2006, *Kong* et al. 2009). However, it is still unknown whether the catechins components in *Rhubarb* such as catechin exert similar effects.
In addition, the effect of catechin and its analogs which occupy approximately 10 percent of Rhubarb on E. coli growth have not been reported. So it will be very interesting to know how microbial activities change under the action of catechin.

Microcalorimetry is a non-specific technique that can be used to measure very small heat effect associated with chemical or biochemical reactions and applied in chemical and pharmaceutical industry as well as in science research due to its high precision and sensitivity (Liu et al. 2000, Xie et al. 1998). It can provide real-time quantitative data that has proven to be indispensable for a wide variety of applications: from determining stability of inanimate materials to metabolic activity of complex living system, under “normal” and modified environmental condition (Zheng et al. 2006). In recent years, it has been used to measure the overall metabolism of microorganism, cell, and tissue cultures as well as more complex biological system. Studies also shows that the interaction between drugs and a wide range of organisms such as fundamental studies of bacterial growth can be detected by microcalorimetry (Fan et al. 2008). In addition, conventional microbiological methods of drug analysis are not only time consuming, but also difficult to interpret objectively at best semi-quantitative. Microcalorimetry, on the other hand, can provide rapid quantitative data about the overall effect of drugs.

E. coli is one of the human pathogenic microorganisms that belong to the class of Gram negative. A previous study also shows that some components of Rhubarb display a significant antibacterial activity against E. coli (Yoshiyuki et al. 2004, Su et al. 2008). Consequently, it is a good choice for studying the effects of catechin on E. coli growth. This may help us to understand the general effects that catechin or other catechins components may have on other microorganisms.

In this paper, the thermal activity monitor (TAM) air isothermal microcalorimeter has been applied to investigate the effect of catechin on E. coli growth. Heat production in a cell suspension was measured and quantitative data reflected the dynamic changes of the growth process of E. coli under the action of catechin. Simultaneously, Correspondence analysis and Similarity analysis help to elucidate the effects of catechin on the biological processes. What is more important
is that the work may provide a useful idea to investigate the antimicrobial effect of catechin and other polyphenol family in *Rhubarb*.

2. Material and methods

2.1. Materials Catechin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing 100051, P.R. China). It was extracted from Chinese *Rhubarb* and its structure was given in Fig. 1. *E. coli* was provided by China Center of Type Culture Collection (Wuhan University, Wuhan 430072, P.R. China). It was grown in peptone culture medium, which was a solution per 1,000ml (pH 7.2-7.5) Containing NaCl 5 g, peptone 10 g, and beef extract 6g and was sterilized in high pressure steam at 121°C for 30min. Lactose broth (LB) Culture medium containing 5g NaCl, 10g peptone, and 5g yeast extract/L was also prepared freshly. The sterilized culture medium did not display the phenomenon of releasing heat according to microcalorimetry.

2.2. Instrument The TAM air isothermal microcalorimeter was used to determine the metabolic power-time curves of *E. coli*. This microcalorimeter was eight-channel twin instrument and thermostated at the range of 5-60°C, with a limit of detectibility of 2μw. The software supplied to TAM air was used to monitor and record the heat flow over the Peltier module when the baseline drift was less than 20μw over 24h. For more details of the instrument, see the report of Zheng (Zheng et al. 2006).

2.3. Methods

2.3.1. Preparation of sample Catechin was collected and dissolved in sterilized distilled water to 20mg.mL⁻¹. At the beginning of the experiment, *E. coli* was seeded at an initial density of 2×10⁶ cells.mL⁻¹. *E. coli* was then incubated with 5ml fresh LB culture medium in a 20mL glass ampoule, the freshly prepared catechin solutions with various concentrations were also added to the cell suspension. The experiments above were all carried with aseptic technique.

2.3.2. Microcalorimetric study The power-time curves of *E. coli* growth were detected by microcalorimetry with ampoule method. All the ampoules containing the cell suspension of *E. coli* and catechin solution with a serial of concentration were...
sealed up and put into eight channel calorimeter block. When the curve recorder had returned to the baseline and stabilized, *E. coli* growth was ended. If necessary, further calibration should be done after a stable baseline is obtained. All data were recorded continuously and the calorimeter was thermostated at 37°C in the whole experiments.

2.3.3. Similarity analysis To draw ideas from similarity analysis on the HPLC chromatography fingerprint of traditional Chinese medicine from different source (Chen et al. 2008), the power-time curves of *E. coli* growth affected by different concentration of catechin should be evaluated by their similarities, which come from the calculation on the correlative coefficient of original data. In this paper, the correlation coefficient of similarity among the power-time curves of *E. coli* affected with and without catechin was calculated by using cosin method.

2.3.4. Correspondence analysis Correspondence analysis (Kong et al. 2009) is an important multivariate statistical method for studying the relationship between investigated factor and some variables. This method can transform some multivariate variables into two principal components (PC) variables $Z_1$ and $Z_2$, which have the equal contribution to the total data set. Then the PC variable aggregations ($Z_1$ and $Z_2$) of each valid point are calculated, the loads of investigated factor and some variables are reflected on the same factor axis, and the relationship between them can be easily analyzed and explained. So the internal change regularity of investigated factor can be directly represented. In this part, CA was used on the many quantitative parameters taken from the power-time curves of *Escherichia coli* growth with different concentration catechin by using the software of SAS 8.0.

3. Result

3.1. Power-time curves of *E. coli* growth

The growth power-time curve of *E. coli* in medium at 37°C was shown in Fig.2. It was a typical growth curve of this bacterial species and could be divided into stage1(A-D) and stage2 (D-F). At the same time, five phases included a lag phase(A-B), a first exponential growth phase(B-C), a stationary phase(C-D), a second exponential growth phase(D-E), and a decline phase(E-F). Fig.3 showed a serial of
power-time curves for *E. coli* growth in the presence of different concentrations of catechin solution at the same temperature and culture conditions. The power-time curves could still be divided into five phases, in which all were similar to those in Fig.1, except for the lag phase, which was significantly different. During the first and second exponential growth phases, catechin had the ability to inhibit bacterial growth in a dose-concentration manner. The first and second exponential growth phases became longer with increasing catechin concentration and the maximum heat output was gradually reduced when the catechin was present in the culture medium. As could be seen from the profiles of these curves, the growth of *E. coli* was influenced by this compound solution.

3.2. Thermo-kinetic parameters for *E. coli* growth

In the exponential phase of growth, the growth rate constants for *Escherichia coli* can be fitted by means of the following equation:

\[ P_t = P_0 \exp (kt) \text{ or } \ln P_0 + kt \]

Where \( P_0 \) represents the heat-output power at time \( t=0 \) and \( P_t \) represents the power at time \( t \). By use of this equation, the growth rate constants \((k_1, k_2)\) of the first and the second exponential phase for the growth of *E.coli* at 37°C without any substance were calculated and given in Table.1. From Table 1, it was apparent that among all of the coefficients, R were greater than 0.9995, indicating a good reproducibility and correlationship. These growth rate constant were inversely proportional to the catechin concentrations and were shown in Table.2.

To show the results in a quantitative fashion, the maximum power output in the first exponential \( P_1 \) and the second exponential phase \( P_2 \), the appearance time of the maximum power output in the first exponential phase \( t_1 \) and second exponential phase \( t_2 \) were shown in Table.2, where it can be seen that as the catechin concentration increased, \( P_1, P_2 \) decreased and \( t_1, t_2 \) prolonged respectively. Table.2 showed an integral of power versus time obtained the value of the heat output \( Q_1, Q_2 \) ( in stage1, stage2 )and the total heat output \( Q_t \) obtained from the power-time curves of *E. coli* growth affected by different concentration of catechin solution.
3.3 Relationship between quantitative thermokinetic parameter and concentration of catechin

The 3D histograms of the relationship between quantitative thermo-kinetic parameters in Table.2 and concentration of catechin were shown in Figure 4. Considering the trends of 3D histograms, it could be concluded that the value of $k_1$, $k_2$ and $P_1$, $P_2$ decreased with the concentration increasing, $t_1$, $t_2$ increased with the concentration increasing versus control, but $Q_1$, $Q_2$ and $Q_3$ had some changes irregularly and the extent of change were not in the same manner. It was essential to find an objective date analysis to evaluate the action of catechin on *E. coli*.

3.4. Data analysis

3.4.1. Similarity analysis

It was interesting to evaluate the power-time curves of *E. coli* growth treated by catechin by their similarities. Thus, the correlation coefficient of the curves of *E.coli* growth without any substance and the curves of *Escherichia coli* growth affected by different concentrations of catechin were calculated and listed as 0.9976, 0.9978, 0.9786, 0.9678, 0.9465, 0.8769 and 0.7865. This result showed that curves of *E. coli* growth affected by catechin altered. The diminishing trend of correlation coefficient also showed that the change enhanced with increasing the concentration of catechin. All these showed that catechin in different concentrations affected *E. coli* growth at varied level.

3.4.2. Result of CA

In order to further show the tendency and the internal change rule of concentration on the effect, correspondence analysis for the quantitative parameters $k_1$, $t_1$, $P_1$, $k_2$, $t_2$, $P_2$, $Q_1$, $Q_2$ taken from power–time curves and the different concentration of catechin were performed. Because $Q_1$ was the summation of $Q_1$ and $Q_2$, it was deleted for the normal running of this software of CA. The result of CA showed that the two PCs ($Z_1$ and $Z_2$) contained 99.86 of the information of the original eight parameters and the equation of $Z_1$ and $Z_2$ were:

$$Z_1 = 0.3091k_1 + 0.1311t_1 + 0.1633P_1 + 0.6265k_2 - 0.0300t_2 + 0.3998P_2 + 0.1115Q_1 + 0.1501Q_2$$

$$Z_2 = 0.0284k_1 - 0.0054t_1 + 0.0126P_1 - 0.1271k_2 - 0.0000t_2 - 0.0544P_2 - 0.0045Q_1 + 0.0233Q_2$$

The absolute values of the coefficient before these parameters represented the contribution proportion of the parameters to $Z_1$ and $Z_2$. It could be concluded from the
equation that $k_2$ and $P_2$ were the significant parameters to evaluate the effect of catechin on *E. coli* growth.

For analyzing the relationship between concentration of emodin and eight parameters, the scatter plot for CA was constructed and shown in Fig. 5. In this procedure, the data point of control with concentration 0μg.ml⁻¹ of catechin was set as zero, and the other data points of group with different concentration of catechin were scattered in different quadrants. It could be seen from this figure that the distance between each data point and original point was lengthened with the increase of the concentration of catechin and the potency was definitely dose-dependent. The general results from the change of $k_2$, $P_2$ in Tab. 2 and the scatter plot for CA in Fig. 5 both illustrated that catechin at different concentration had disparity effect on *E. coli* growth. Starting from a low concentration catechina had a notable action and the action was enhanced with the catechin concentration increased.

4. Discussion

Chinese *Rhubarb* contains various types of catechins, it is also know that *Rhubarb* presents the effect of antibacterial and emodin and other anthraquinone components have the active effect. In fact, catechins components occupy approximately 10 percent of *Rhubarb* and its monomer catechin(C) has the typical and basic structure that contains benzene ring and hydroxy group. The basic structure may be the active constituent of antioxidant activity (N. Ihara et al. 2005, Pamela et al. 2001). However, it is unknown whether catechin and its analogues have the inhibiting effect on bacteria such as *E. coli*. Some methods such as micro-dilution, anti-microbial circle methods have more or less disadvantages in evaluating qualitatively and quantitatively the antimicrobial activity.

In this study, microcalorimetry was used due to its sufficiently sensitivity and accuracy especially when the concentration of catechin was low. From the power-time curves of *E. coli*, the result that the time of the exponential phases of *E. coli* was prolonged with increasing concentration of catechin indicated that *E. coli* took longer time to produce a sufficient number of cells for a detectable signal. At the same time, nine quantitative parameters were obtained to estimate the anti-microbial activity of
catechin. By the power-time curves and quantitative parameters, it could be seen that starting from a low concentration of 0.05mg.mL$^{-1}$, catechin had the inhibition effect on *E.coli* growth and was dose-dependent. The CA of multiple parameters showed that parameters $k_2$ and $p_2$ might be the main two parameters and played more important role in evaluating the inhibitory effect of *E.coli* growth. The action of the drugs on the bacteria depended on the structure of the drugs, the inhibitory effect of catechin on *E. coli* may be related with the hydroxy group which could transform into carbonyl group through dehydrogenation and carbonyl group could cross link with phospholipid on the cellular membrane and result in the further damage of membrane and physiological functions. This would explain why *E. coli* growth was inhibited in the presence of catechin. The analogues such as ECG, EGCG, EGC contain the similar structure so that they perhaps had the similar effect. Further work on the analogues and the actual action mechanism of these compounds on microbes was in progress in our laboratory.

In summary, our work showed that catechin could inhibit the growth of *E. coli* and was dose-dependent. Microcalorimetry was adaptable to detect the interaction of drug and microbes. This work also indicated the possible and promising prospect of microcalorimetry combined with appropriate analysis method could provide more important information for study the influence of drug and other compounds on different microbes.

**Acknowledgements**

The authors are grateful for the support from the National Basic Research of China (973 project: 2007CB512607), the State Youth Science Foundation(30625042), National Natural Science Foundation of China(No. 30600824) and the Education Department Science Foundation of Liaoning Province (L2010334).

**References**


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Table 1  Rate constants for the growth of *E.coli* at 37°C

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
<th>No.6</th>
<th>Mean value</th>
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<tr>
<td>$k_1$(min$^{-1}$)$^a$</td>
<td>0.02338</td>
<td>0.02345</td>
<td>0.02334</td>
<td>0.02335</td>
<td>0.02342</td>
<td>0.02332</td>
<td>0.02337±0.00005$^b$</td>
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<tr>
<td>$R$</td>
<td>0.9989</td>
<td>0.9986</td>
<td>0.9985</td>
<td>0.9979</td>
<td>0.9979</td>
<td>0.9983</td>
<td>0.99835±0.00039</td>
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<tr>
<td>$k_2$(min$^{-1}$)$^c$</td>
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<td>0.00504</td>
<td>0.00512</td>
<td>0.00502</td>
<td>0.00509</td>
<td>0.00506</td>
<td>0.005065±0.00003</td>
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<tr>
<td>$R$</td>
<td>0.9945</td>
<td>0.9985</td>
<td>0.9983</td>
<td>0.9978</td>
<td>0.9958</td>
<td>0.9974</td>
<td>0.99705±0.00157</td>
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</table>

*a* the growth rate constant of the first exponential phase  
*b* Mean± S.E  
*c* the growth rate constant of the second exponential phase
Table 2 Quantitative thermo-kinetic parameters for *E.coli* growth treated by different concentrations of catechin

<table>
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<tr>
<th>c (mg.mL(^{-1}))</th>
<th>(k_1) (min(^{-1}))</th>
<th>(t_1) (min)</th>
<th>(P_1) (mW)</th>
<th>(k_2) (min(^{-1}))</th>
<th>(t_2) (min)</th>
<th>(P_2) (mW)</th>
<th>(Q_1) (J)</th>
<th>(Q_2) (J)</th>
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<td>0.00504</td>
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Figure captions

Fig.1 Chemical structure of catechin

Fig.2 The power-time curve of *Escherichia coli* growth at 37°C without any substance. It was a typical metabolic curve and could be divided into stage1 (A-D) and stage2(D-F) and five phases, i.e., a lag phase (A-B), the first exponential growth phase (B-C), a stationary phase (C-D), the second exponential growth phase (D-E), and a decline phase (E-F).

Fig.3 The power-time curves of *Escherichia coli* growth at 37°C affected by different concentrations of catechin. The concentrations of catechin were (a) 0, (b) 0.05, (c) 0.1, (d) 0.2, (e) 0.4, (f) 0.8, (g) 1.6 mg.mL\(^{-1}\).

Fig.4 Relationships between quantitative thermo-kinetic parameters and concentration (0.05-1.6mg.mL\(^{-1}\)) of catechin. (1) the growth rate constants \(k_1\) and \(k_2\), (2) the maximum power output \(P_1\) and \(P_2\), (3) the appearance times of the maximum power output \(t_1\) and \(t_2\), and (4) the heat output \(Q_1\), \(Q_2\), and \(Q_t\).

Fig.5 Scatter plot for CA. This scatter plot was obtained by correspondence analysis on the relationship between concentration of catechin and the eight parameters from the power-time curves of *E.Coli* growth by using software of SAS 8.0.
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