

Screening and analysis of bioactive compounds with biofingerprinting chromatogram analysis of traditional Chinese medicines targeting DNA by microdialysis/HPLC

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Abstract

Biofingerprinting chromatogram analysis, which is defined as the comparison of fingerprinting chromatograms of the extract of traditional Chinese medicines (TCMs) before and after the interaction with biological systems (DNA, protein, cell, etc.), was proposed for screening and analysis of the multiple bioactive compounds in TCMs. A method of microdialysis sampling combined with high performance liquid chromatography (HPLC) was applied to the study of DNA-binding property for the extracts of TCMs. Seven compounds were found to bind to calf thymus DNA (ct-DNA) from the TCMs of *Coptis chinensis Franch* (Coptis), but only three ones from *Phellodendron amurense Rupr.* (Phellodendron) and none from *Sophora flavescens Ait.* (Sophora) to bind to ct-DNA, respectively. Three of them were identified as berberine, palmatine and jatrorrhizine and their association constants (K) to ct-DNA were determined by microdialysis/HPLC. Competitive binding behaviors of them to ct-DNA were also investigated.

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1. Introduction

Screening and analysis of bioactive compounds is always a challenge for research of the traditional Chinese medicines (TCMs). Among the up to hundreds or even thousand of components contained in the extracts of TCMs, only a few of them are responsible for the pharmaceutical and/or toxic effects [1]. Many approaches have been developed for the screening of the bioactive compounds in TCMs or other natural products [2–10]. A conventional procedure for discovery of bioactive components is the extraction of compounds from TCMs followed by pharmacological screening of the purified compounds. The way to increase the probability of success is still controversial, and more and more works were reduced to

trial-and-error experiments. High-throughput screening methods using receptors and specific enzymes as targets have been extensively used in the field of screening candidates from synthetic compound libraries [2,3]. However, they are only partially applicable to TCMs because the purified compounds are still required and the pharmaceutical activity of TCMs is mostly resulted from combination effect of a group of compounds. An effective approach based on the concept of pharmacokinetics was introduced by Homma et al. [4,5] to discover active compounds from TCMs, on the hypothesis that the active compounds should appear in blood and urine with appropriate blood concentrations and urinary excretion rates after the administration of extract of the TCMs. Thus the active compounds could be distinguished from the comparison of the analysis results of blood and urine after the administration with the blank. However, the proposed procedures are laborious and time-consuming. HPLC separation is an efficient way for TCMs study due

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to its high performance and selectivity. Although retention based on interactions of the solutes with stationary phase can be obtained, information on their bioactivities is still unknown due to the lack of correlation with their retentions in conventional HPLC. To overcome this problem, some combined techniques of HPLC with other approaches such as microfractionation and bioassays were developed [6]. In addition, affinity chromatography with immobilized biomolecules as the stationary phases was introduced to screen and analyze the bioactive components from TCMs. This method has been applied to probe the interaction properties of bioactive compounds in TCMs with plasma proteins such as the human serum albumin (HSA) immobilized stationary phase [7,8]. In addition, liposome-immobilized silica as stationary phase was used by Mao et al. [9] to separate permeable components in TCMs. Generally, the separations of TCMs with these stationary phases were low efficiency, and for some kinds of TCMs even not satisfactory.

Microdialysis plays a unique role in the neurochemical, pharmacological, physiological and behavioral studies, and has attracted much attention in the fields of pharmaceutical, biological and analytical chemistry [11]. In this technique, relatively “clean” samples can be collected for following assay, due to the exclusion of large molecules by the microdialysis membrane. Microdialysis combined with HPLC was developed to study the binding behaviors of drugs [12–16] and metal ions [17] to proteins *in vitro*, respectively.

In this work, the biofingerprinting chromatogram analysis, which is defined as the comparison of fingerprinting chromatograms before and after the interaction with biological systems (DNA, protein, cell, etc.), is proposed for the screening and analysis of multiple bioactive compounds in the extract of TCMs. We applied microdialysis/HPLC method for studying the binding behaviors of three kinds of TCMs to ct-DNA, which is the molecular target of many antimicrobial, antiviral and antitumour active drugs [18]. Biological interaction property of the components in the extract of TCMs can be obtained with biofingerprinting chromatogram analysis. Multiple bioactive components interacted with DNA can be simultaneously discerned. It paved the way for screening and analysis of the bioactive compounds in TCMs, and may extend to the study of the interaction of the extract of TCMs with other biopolymers.

2. Experimental

2.1. Reagents and chemicals

The calf thymus DNA (ct-DNA) purchased from Sigma (St. Louis, MO, USA) was deproteinized and dissolved in a BPES buffer at pH 7.0 (6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM EDTA, 185 mM NaCl). The DNA-phosphate concentration was spectrophotometrically determined by using molar extinction coefficient of 6600 M⁻¹ cm⁻¹ at 260 nm against BPES buffer. The standards of berberine chloride,

jatrorrhizine chloride and palmatine chloride were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Acetonitrile was of HPLC grade. Distilled water was further purified by Milli-Q system (Millipore, Milford, MA, USA); other chemicals were of analytical grade.

2.2. Preparation of the extracts of traditional Chinese medicines

The traditional Chinese medicines of Coptis (root), Phellodendron (bark) and Sophora (root) were purchased from a local store (Dalian, China). 15 g Coptis was crushed into powder in a grinder, and immersed in 150 mL 30% ethanol overnight, then heated to reflux for 1.5 h. The extraction was repeated twice. After combining them together the extract was filtered through a 0.45 μm membrane and was vaporized to dry, then dissolved in 25 mL BPES buffer and stored for further experiments. Extraction of Phellodendron and Sophora was performed with the procedures as for Coptis except that Sophora was extracted by 50% ethanol.

2.3. Microdialysis sampling

The microdialysis system comprises a Cole-Parmer 74900 microdialysis pump (Cole-Parmer, Chicago, USA) and a homemade microdialysis probe with a cellulose membrane (Spectrum, LA, USA) at a length of 10 mm and a molecular weight cut-off of 18,000 Da. The perfusion solution is BPES buffer and the perfusion rate is 1 μL/min. The microsyringe was filled with the perfusion solution before sampling.

The analyte-DNA or the extracts of TCMs-DNA mixture were incubated at 37.0 °C in a water bath over 10 min. Then the probe was placed into the solution for microdialysis sampling. After proceeding for 20 min to reach an equilibration, the “interaction microdialysate” was collected for 40 min. The collected microdialysate was handled for HPLC analysis. The “blank microdialysate” was also collected by exactly the same procedure as the “interaction microdialysate” with the absence of DNA in sample solution. The “interaction microdialysate” and “blank microdialysate” represent the TCMs solutions after and before the interaction with DNA, respectively.

2.4. HPLC analysis

The HPLC system consisted of two LC-10ATvp pumps (Shimadzu, Kyoto, Japan), a Rheodyne-type injector valve with a 10 μL loop, an SPD-10Avp UV detector (Shimadzu, Kyoto, Japan), a WatersTM 996 photodiode array detector (Waters, Milford, MA, USA), a WDL-95 chromatographic workstation (National Chromatographic R.&A. Center, Dalian, China) and a Millennium 32 workstation (Waters, Milford, MA, USA). The 250 mm × 4.6 mm I.D. column packed with 5 μm Hypersil-BDS was from Elite Analytical Instruments Co. (Dalian, China). The mobile phase was

acetonitrile/20 mM Britton-Robinson buffer (1350 μ L H₃PO₄, 1150 μ L HAc, 1.236 g H₃BO₃ in 1000 mL water, pH 3.0) containing 5 mM sodium heptyl sulfate.

The integration of the peak areas including the poorly resolved peaks was performed by the chromatograms data manipulation software exploited by National Chromatographic R.&A. Center of China.

2.5. Absorption spectroscopy

Absorption spectra were measured using a Jasco-550 spectrophotometer (JASCO, Kyoto, Japan). Titrations of the analyte with DNA, covering a wide range of DNA-phosphate/analyte ratios (P/A), were performed by adding aliquots of a concentrated DNA solution to an analyte solution by keeping concentration at 5 μ M. The analyte-DNA mixtures were incubated at 37.0 in a water bath for 30 min prior to measurements.

2.6. Recovery of microdialysis

The recovery of microdialysis sampling (*R*), also called the microdialysate extraction fraction, is defined as the concentration ratio of the free analyte in microdialysate (*C_d*) to that in analyte-DNA solution. The microdialysate was collected by microdialysis sampling in a standard solution with BPES buffer of pH 7.0 and then analyzed by HPLC. The recovery of individual analyte was calculated from the ratio of peak areas for analyte in microdialysate to that in standard solution.

3. Results and discussion

3.1. Biofingerprinting chromatogram analysis for the extracts of *Coptis*, *Phellodendron* and *Sophora*

Microdialysate from the extract solution of TCMs itself and the mixed solution of the extract with DNA were collected respectively, according to the procedure described in the experimental section and were analyzed by HPLC under identical conditions. The interaction property of compounds in the extract of TCMs with DNA can be deduced from the comparison of the two obtained chromatograms in a graph, i.e., biofingerprinting chromatogram analysis. The components extracted from TCMs can be simply divided into two groups: some have an interaction with DNA and were labeled as “interactive components”, which can be characterized with association constants between them with DNA, while others do not. Among the interactive compounds, those bound to DNA will be confined in the semipermeable membrane of the microdialysis probe and thus cannot be collected in the microdialysate. Accordingly, the peak areas of the interactive components after the interaction with DNA have a decrease in the fingerprinting chromatogram; while for those not responsible for DNA binding, there is almost no

change in their peak areas before and after the interaction for all the molecules can pass through the probe membrane. Therefore, from the biofingerprinting chromatogram analysis, the DNA-binding compounds in the TCMs extract can be easily distinguished from those not binding. This method is advantageous for the needlessness of the purification of the component.

The TCMs of *Coptis*, *Phellodendron* and *Sophora* were chosen as model samples for biofingerprinting chromatogram analysis, as many reports have showed that the abundant alkaloids in these TCMs are the most promising category to have interactions with DNA [19]. *Coptis* has been commonly used with the effects of clearing heat, drying up dampness, purging toxicosis and detoxification in clinic in China. It is known to contain protoberberine alkaloids such as berberine, palmatine, coptisine, epiberberine, jatrorrhizine, columbamine and berberastin. Another TCM *Phellodendron*, used in clinical treatment some cases in combination with *Coptis*, is reported as an anti-stomachic, anti-inflammatory and anti-pyretic agent with the major constituents of protoberberine alkaloids [20,21]. Fig. 1a and b show the biofingerprinting chromatograms at the detection wavelength of 345 nm for the extract of *Coptis* and *Phellodendron*. Under the photodiode array (PDA) detection mode all the chromatograms with wavelength ranging from 200 to 800 nm can be simultaneously obtained. The fingerprinting chromatograms are different at each detection wavelength, thus the information obtained would be different in some cases, i.e., interaction might be found at a wavelength but not very obvious at another. A chromatogram with the least interfering of the inactive peaks to the active ones is propitious to the screening. But for the cases of *Coptis* and *Phellodendron*, the interaction information obtained from the fingerprinting chromatograms differed not much at each detection wavelength. 345 nm was chosen for the smooth baselines and the maximal absorption of their main peaks. The integration of the peak areas showed that seven peaks in *Coptis* and three in *Phellodendron* decreased after the interaction with DNA, and obvious reduction of the height of the peaks can also be observed in the chromatograms, which indicated that they have interaction with DNA. Peak identification was performed by the comparison with retention time and the UV-vis spectrum with standards. Internal standard with an appropriate concentration was used as to identify components in the extract of the TCM. At the same time, a single sample of the standard with the same UV-vis spectrum scanned by the PDA detection further confirmed the identification. Among the seven active components in *Coptis*, three were identified as jatrorrhizine, palmatine and berberine with their molecular structures shown in Fig. 2. The identified active substances in *Phellodendron* were the same three compounds. Berberine and palmatine have been reported as DNA binding compounds [22–28]. Since all of them are protoberberine alkaloids and have similar structures, it is not surprising that jatrorrhizine can also bind to DNA. The other four active compounds in *Coptis* with the retention times of 18.25 (\pm 0.12), 21.40

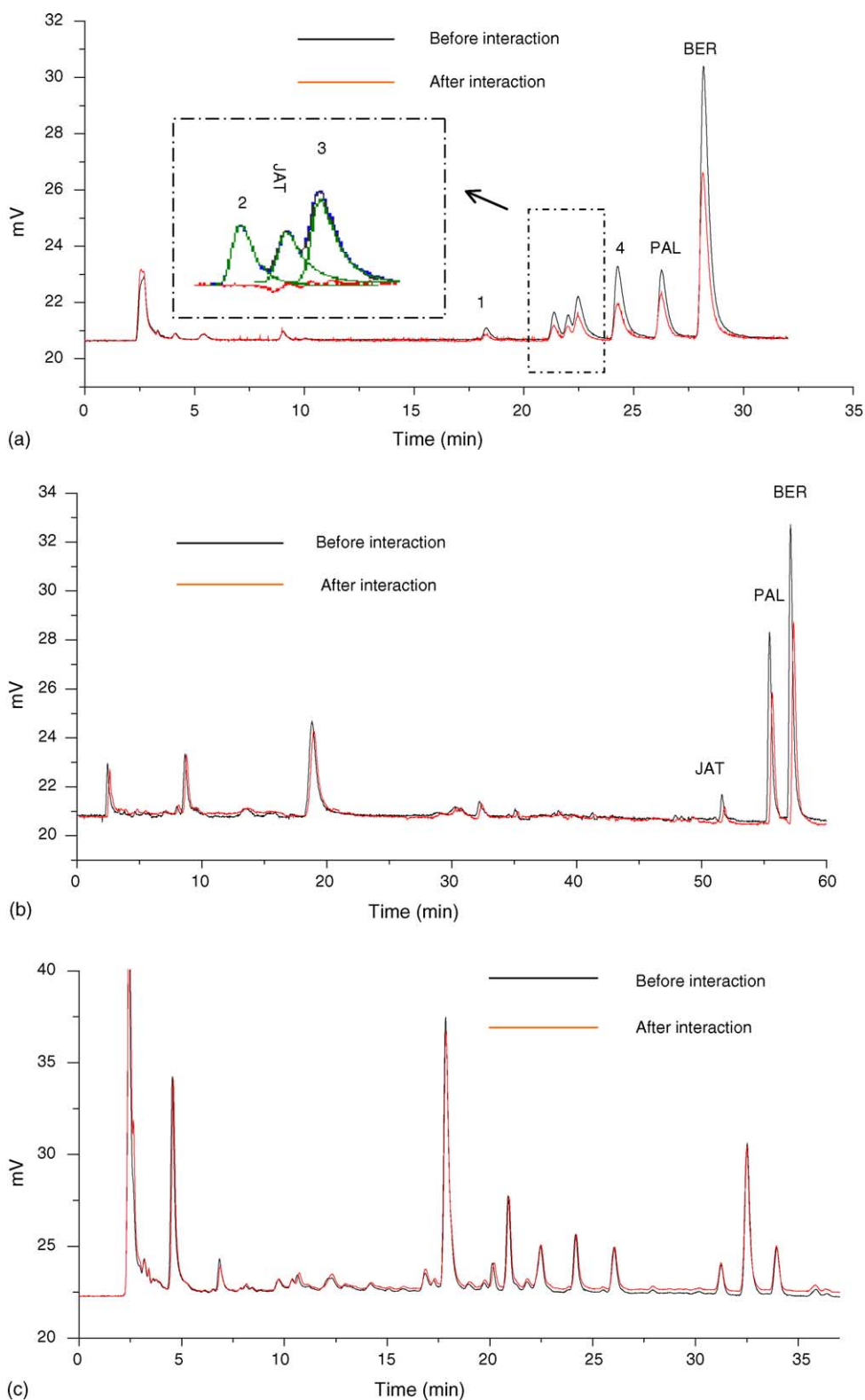


Fig. 1. Biofingerprinting chromatograms for the extracts of the TCMs Coptis (a), Phellodendron (b) and Sophora (c). Chromatograms for 10 μ L microdialysate from the extract of a TCM itself and the mixed solution of its extract with ct-DNA at a final concentration of 103 μ M phosphate are indicated as black and red curves, respectively. Chromatographic conditions: column, 250 mm \times 4.6 mm I.D. packed with 5 μ m Hypersil-BDS; mobile phase, CH₃CN/20 mM Britton-Robinson buffer (1350 μ L H₃PO₄, 1150 μ L HAC, 1.236 g H₃BO₃ in 1000 mL water, pH 3.0) containing 5 mM sodium heptenylsulfonate; ambient temperature; flow rate, 1 mL/min; linear gradient elution, (a) 0–40 min for 20–45% CH₃CN, (b) 0–25 min for 11% CH₃CN, 25–60 min for 15–40% CH₃CN, (c) 0–40 min for 10–30% CH₃CN; detection wavelength, 345 nm for (a) and (b), 260 nm for (c). Peak identifications: (JAT) jatrorrhizine; (PAL) palmatine; (BER) berberine. Inset: is the simulated chromatogram of the unresolved peaks.

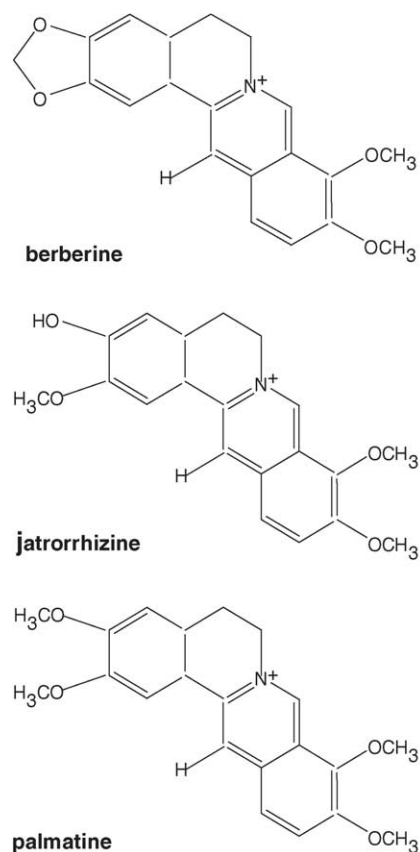


Fig. 2. Molecular structures of berberine, palmatine and jatrorrhizine.

(± 0.10), 22.50 (± 0.09) and 24.33 (± 0.03) min were not identified due to the lack of the standards. Another TCM Sophora used for the treatment of acute dysentery, gastrointestinal hemorrhage, and eczema was also analyzed by the fingerprinting chromatograms. It is known to contain a number of quinolizidine alkaloids and a series of flavonoids. No DNA binding compound was discovered in the biofingerprinting chromatograms at the detection wavelength from 200 to 800 nm. Fig. 1c shows typical fingerprinting chromatograms at 260 nm.

TCMs were often used without purification in the traditional Chinese clinic therapy. Due to the presence of abundant components contained in it, a TCM may display different pharmaceutical effects under various conditions. For Coptis and Phellodendron, the alkaloids binding to DNA may contribute to the effects of clearing heat or anti-inflammatory because these symptoms sometimes were caused by bacteria and virus.

The binding degree of any component can be defined as:

$$\text{Binding degree} = \frac{A_b - A_a}{A_b} \times 100\%$$

Where A_a and A_b are the peak areas of a compound after and before the interaction with DNA in the biofingerprinting chromatograms, respectively. The binding degrees of compounds show their binding abilities in the environment of the

Table 1

Competitive binding degrees of active compounds in the extract of Coptis and Phellodendron

Peak	Solute	Binding degree (%)
Extract of Coptis		
1	Not identified	23.0
2	Not identified	45.8
JAT	Jatrorrhizine	39.6
3	Not identified	41.5
4	Not identified	49.9
PAL	Palmatine	36.2
BER	Berberine	48.2
Extract of Phellodendron		
JAT	Jatrorrhizine	50.8
PAL	Palmatine	30.2
BER	Berberine	29.1

Table 2

Calibration curves of jatrorrhizine, palmatine and berberine

Solute	Calibration curve ^a	<i>r</i>
Jatrorrhizine	$A = 13.60C - 206$	0.999
Palmatine	$A = 12.99C + 14.1$	0.999
Berberine	$A = 9.555C - 286$	0.999

^a *C* is the concentration of alkaloids (μM); and *A* for the corresponding peak areas.

interaction mixture. To test the robustness of this method, a single compound berberine was analyzed by the method and its binding degrees with ct-DNA were obtained. The in-day RSD of the binding degrees was 0.64% and the day-to-day RSD was 1.62% ($n = 5$), which was acceptable for the evaluation of the binding ability of the active compounds.

Table 1 shows the binding degrees of the active compounds in Coptis and Phellodendron. It was observed that the binding degrees of berberine in the extract of Coptis and Phellodendron were 48.2 and 29.1%, respectively, despite of its similar concentrations in the two samples (0.014 mM in the former, 0.015 mM in the latter, calculated according to the calibration curve of berberine described below). As there are many other components present in those extracted samples with DNA binding abilities or not, a synergistic or antagonistic effect may happen to the binding behaviors of berberine to DNA, which may account for great discrepancies of its binding degree in different extracted samples. This phenomenon is common in the interaction of TCMs complex with the biomolecules target and is also one of the major differences of TCMs from synthetical drugs. Learning of the binding degrees of the components in TCMs may help to elu-

Table 3

Recovery of jatrorrhizine, palmatine and berberine with microdialysis sampling

Solute	Recovery (%)	RSD (%) ($n = 3$)
Jatrorrhizine	78.2	1.5
Palmatine	63.2	2.3
Berberine	82.3	1.8

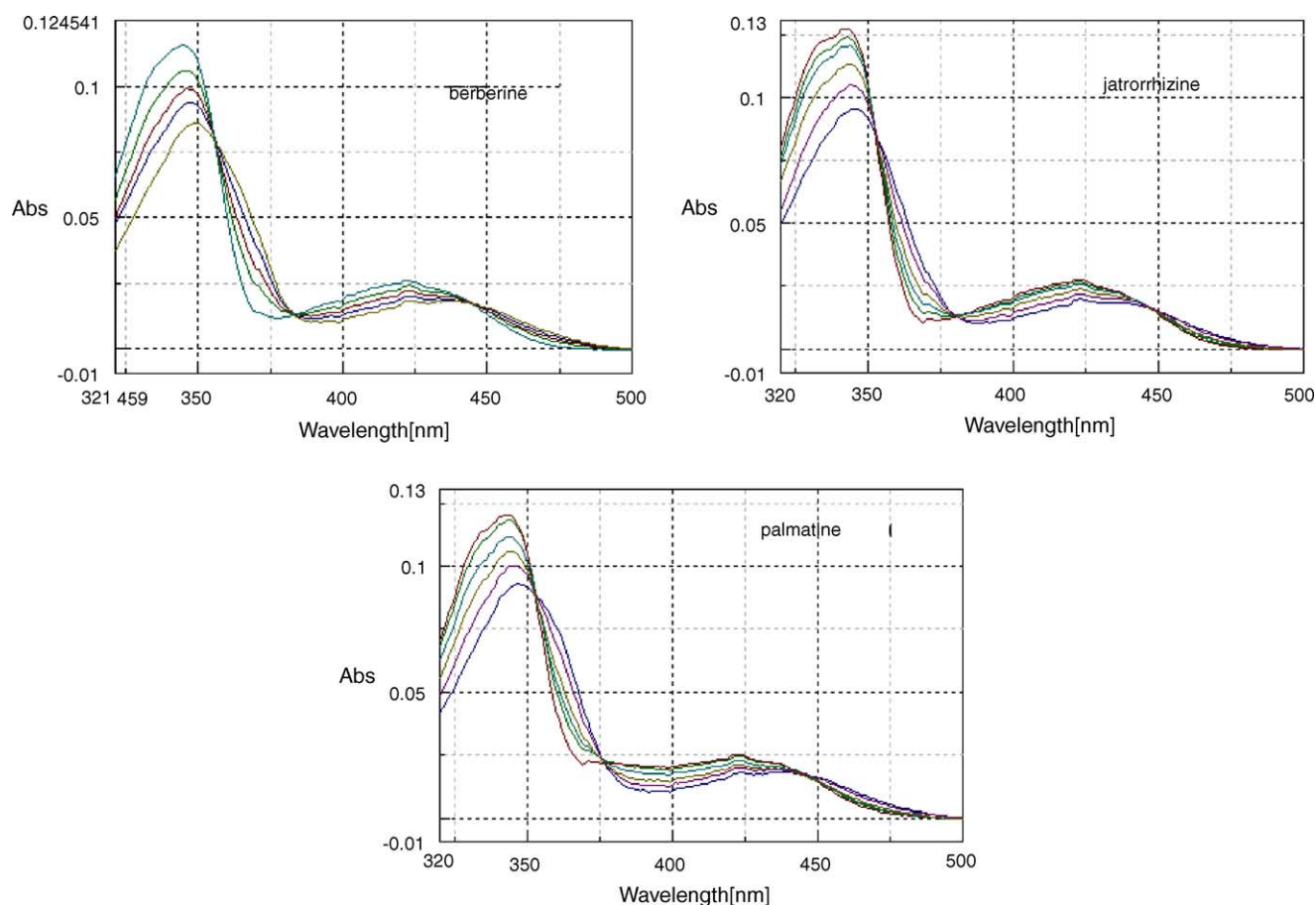


Fig. 3. Ct-DNA titrations of alkaloids in BPES buffer pH 7.0. To 3 mL of analyte solution were added by aliquots of a concentrated ct-DNA solution. Spectra are referenced against DNA solutions of exactly the same DNA concentration and were adjusted to a common baseline at 500 nm. The phosphate-DNA/analyte ratio increased from 0 to 20 (top to bottom curves, at 420 nm).

cidate the molecular mechanism in pharmaceutical activity of TCMs.

Additional evidence regarding the binding of berberine, palmatine and jatrorrhizine to DNA comes from spectrometric measurements. Representative UV–vis absorption spectra of the three compounds and their complexes with DNA are shown in Fig. 3. Addition of increasingly higher concentrations of ct-DNA resulted in bathochromic and hypochromic changes of the absorption maxima, i.e., analyte interaction with DNA caused a decrease in the absorption peaks towards longer wavelength. Two clear isosbestic points were observed

on spectrum of each analyte. All these spectrometric changes suggest the binding of the compounds to DNA.

Compared to the traditional pharmacological screening and HTS, some superiorities of the biofingerprinting chromatograms analysis in the screening of bioactive compounds from complex natural products is noticeable. It avoids the most time-consuming step to purify each compound, which is indispensable for the former methods. It combines the high separation performance and the biological identification. This method is applicable to almost all the targets employed in HTS such as enzymes, receptors, DNA and cells.

Table 4
Binding parameters for alkaloid-DNA interaction determined by UV and microdialysis/HPLC method

Compound	n_1 (nucleotides)	K_1 (10^4 M^{-1})	r	n_2 (nucleotides)	K_2 (10^4 M^{-1})	r	Method
Jatrorrhizine	1.90	3.97	-0.98	0.12	2.90	-0.99	Microdialysis
	0.42	3.84	-0.97	–	–	–	UV
Palmatine	3.51	1.44	-0.98	1.40	1.22	-0.99	Microdialysis
	2.28	1.30	-0.96	–	–	–	UV
Berberine	2.20	3.42	-0.97	0.08	2.17	-0.98	Microdialysis
	0.73	3.08	-0.97	–	–	–	UV

3.2. Determination of the association constants of berberine, jatrorrhizine and palmatine by microdialysis/HPLC method

During the determination of the association constant of an analyte, the final concentration of the DNA-phosphate was maintained at 33.3 μM . Then the analytes berberine, palmatine or jatrorrhizine was added at the final concentration varied from 2.08 to 66.6 μM . The mixtures were incubated at 37 $^{\circ}\text{C}$ for 30 min following with the microdialysis sampling. Then the obtained microdialysates were further analyzed by HPLC. Thus the concentration of the analyte in the microdialysate can be obtained from the peak area of the chromatogram according to the calibration curves as shown in Table 2.

The concentrations of free analyte (C_f) in the analyte-DNA solution can be calculated as follows:

$$C_f = \frac{C_d}{R},$$

where C_d is the concentration of analyte in the microdialysate and R is the recovery of the analyte of the microdialysis, which was obtained by the procedure described in the experimental section. The recoveries for the three alkaloids of berberine, jatrorrhizine and palmatine are presented in Table 3, and the concentration of binding analytes can be easily obtained by subtracting C_f from the total.

The data was expressed in the form of Scatchard plots [29], which were analyzed according to the formula of McGhee and Von Hippel [30]:

$$\frac{r}{C_f} = K - nKr$$

where r is the ratio of concentration of the binding analyte to that of the nucleotides. In this equation the association constant for binding to an isolated site (K) is given by the intercept on the ordinate of a Scatchard plot (r/c axis) and n , the apparent site-size, represents the number of nucleotides occupied by a single analyte molecule [30].

Scatchard analysis of all the three analytes shows biphasic curves as in Fig. 4 and the obtained constants are listed in Table 4, which suggests that there are two types of interaction sites in ct-DNA for the alkaloids to bind: one is the primary binding site with higher affinity; the other is the secondary binding site with lower affinity. Several binding modes of protoberberine alkaloids to DNA have been reported. A partial intercalation of berberine into ct-DNA was first suggested [27] on the basis of NMR investigation. However, a fluorescence spectral study [24] of berberine with ct-DNA suggested a groove binding rather than an intercalation process. Then, results obtained by the competition dialysis method [22] indicated that berberine bind only to poly(dA)-[poly(dT)]₂ triplex DNA but not to duplex DNA. In addition, model of mix-mode of intercalation and groove binding was

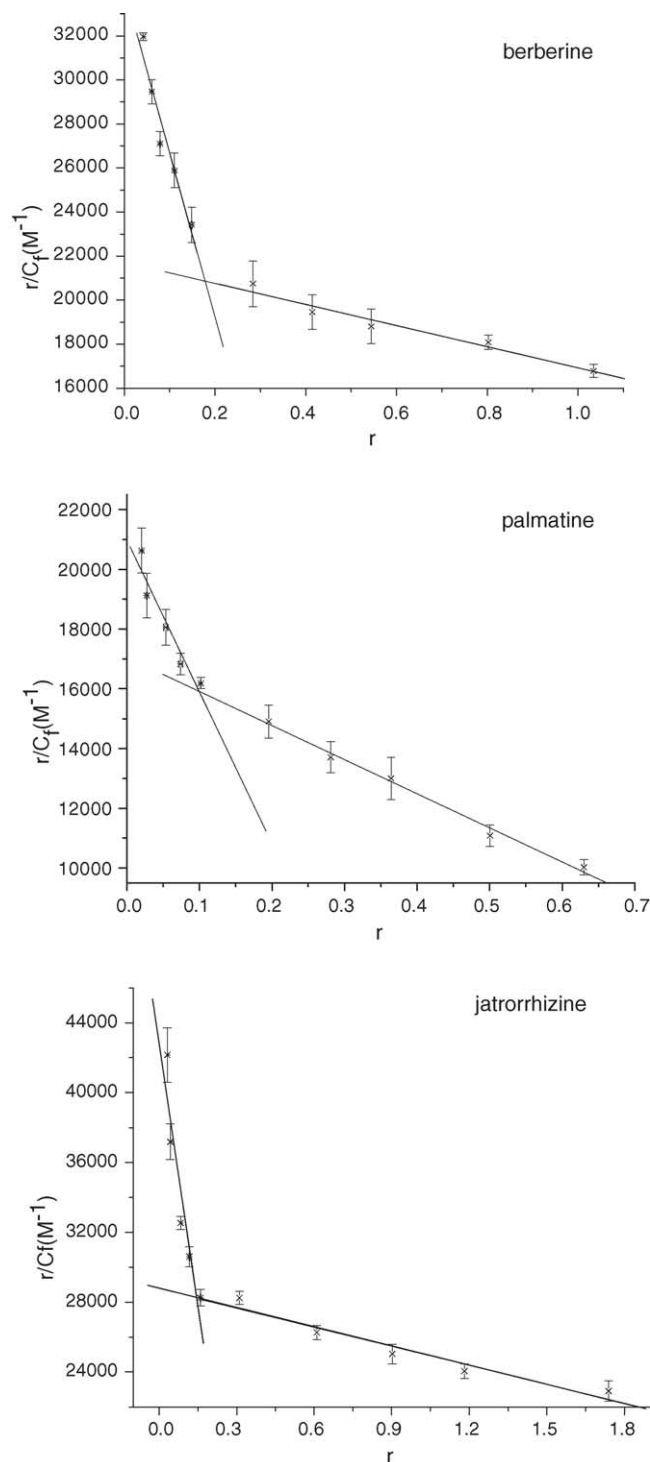


Fig. 4. Scatchard plots for interaction of berberine–DNA, palmatine–DNA and jatrorrhizine–DNA.

proposed [28]. A common agreement of all these studies was that drugs binds in a single type of binding site of DNA. It has been proved by Tajmir-Riahi et al. [31–33] that drugs can bind to DNA on two or three types of sites: A–T base pairs, G–C base pairs or backbone PO₂ groups, but the case of protoberberine alkaloids was not reported. The overall asso-

ciation constants of the three portoberberines to DNA were also determined with the method of UV absorption spectroscopy according to the work of Neault and Tajmir-Riahi [32] and listed in Table 4. As seen in the table, all the three compounds have the moderate affinity to ct-DNA with association constants of 10^4 M^{-1} . Either the primary, secondary or the overall binding constant of berberine is larger than that of palmatine, i.e., berberine has a stronger affinity to ct-DNA than palmatine, which is consistent with the results in other system [23].

3.3. Competitive binding of jatrorrhizine, palmatine and berberine to calf thymus DNA

In the mixed solution of the TCMs extracts with a large biomolecules such as DNA or protein, complex interactions between components, such as synergistic, or antagonistic effects are responsible for the bioactivity or toxicity of TCMs. It is important to know the role a compound plays in the total bioactivity and toxicity under the existence of the other components and how the compounds affect each other during the interaction with the target molecule. To be aware of the binding parameters of each component is the first step for the studies of collective binding of drugs mixture to the target. The mostly used spectrum-based methods in drug-biopolymer interaction study such as UV-vis and fluorescence are not equal to the task, for the spectra is the total contribution of all the components and that of single one can not be well distinguished. Due to the high separation ability of HPLC, microdialysis/HPLC provides a good method for the binding of multi-drugs to biopolymers.

Competitive binding of jatrorrhizine, palmatine and berberine to ct-DNA were studied in pairs by the microdialysis/HPLC method. Since it is relative simple and easy to control, we focused on the binding of analytes to the primary sites of DNA. In the competitive binding experiment, the final concentrations of analyte used as solute and DNA were kept at constant and the DNA-phosphate/analyte ratios (P/A) were larger than 16, ensuring the binding were mainly on their primary binding site of DNA. Increasing concentrations of competing agent were added and followed by microdialysis sampling and HPLC analysis. Then the solute and the competing agent were exchanged and analyzed with the same procedure.

As can be seen in Fig. 5, the competing curves of all the three analyte pairs, palmatine–jatrorrhizine, berberine–palmatine and berberine–jatrorrhizine, display the similar characters. The binding degree of the solute was decreased as the competing agent was added; i.e. the analyte was displaced by the competing agent. The results suggest that each pair of analytes have the same type of primary binding site on DNA. Therefore, it can be concluded that all the three compounds have the same type of the primary binding sites on ct-DNA.

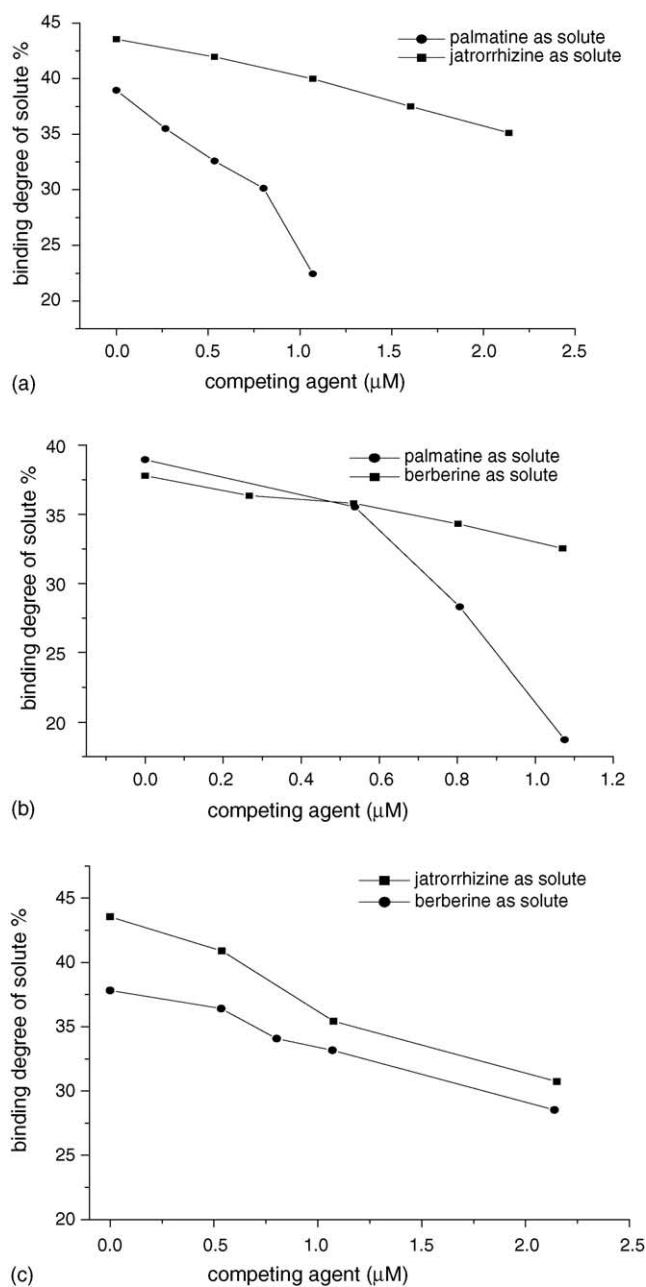


Fig. 5. Competitive binding study on the primary binding site of ct-DNA for pairs of (a) jatrorrhizine and palmatine, (b) berberine and palmatine and (c) jatrorrhizine and berberine.

4. Conclusions

The biofingerprinting chromatogram analysis based on the microdialysis/HPLC method provides a new strategy for the screening and analysis of the bioactive substances in TCMs or other natural products. From the biofingerprinting chromatogram analysis for a traditional Chinese medicine, the biological information can be derived as follows: (1) those bioactive compounds binding to biomolecular target (e.g., ct-DNA here) can be distinguished quickly and easily from the large number of components in TCMs; (2) under the

competitive binding environment, the binding degree of multiple compounds in the extract of TCM to the target can be obtained. This strategy has been applied to the study of DNA-binding property for the TCM extracts of *Coptis*, *Phellodendron* and *Sophora*. Seven compounds were found as DNA-binding agents in *Coptis* from the biofingerprinting chromatogram analysis, and only three compounds from *Phellodendron* and no compound *Sophora* were found to bind to ct-DNA. Three compounds with binding activity to ct-DNA were identified as berberine, palmatine and jatrorrhizine. Scatchard analysis of those three compounds by the method of microdialysis/HPLC indicates that each of them can bind to two type-independent binding sites on ct-DNA. Competitive binding study suggests that all of three compounds have the same type of primary binding site on ct-DNA.

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