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Prediction and Screening of Biologically Active Compounds in Honeysuckle (*Lonicera japonica*) by Biopartitioning Micellar Chromatography

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A new *in vitro* method, based on the retention data in biopartioning micellar chromatography, is validated for prediction and screening of bioactive components of honeysuckle (*Lonicera japonica*). Relationships between the capacity factors of honeysuckle extracts and chemical drugs with related curative effects are studied. Finally,statistical analysis and predictive model were obtained. The results reveal that anti-inflammatory, antipyretic and analgesic effects are the main efficacy of honeysuckle, antibiotic and antiviral effects take second place, effect of lowering blood pressure, high blood fat and blood sugar is the weakest. Besides, with further relevant Matlab programming function, the probability of the components associated with different effects can be obtained by calculating the honeysuckle capacity factors, so as to identify which peak represents the active ingredient in the chromatogram and which is the main pharmacological action. The use of biopartioning micellar chromatography is simple,reproducible and can provide key information for predicting and screening bioactive constituents of honeysuckle extracts.

Keywords: Predict and screen, Biopartioning Micellar Chromatography, Honeysuckle (*Lonicera japonica*), Bioactive compounds.

INTRODUCTION

Traditional Chinese medicines (TCM) usually contain hundreds or even thousands of components, a single ingredient can be obtained through current means of chemical extraction and isolation. However,before the animal experiments, these single components can not be identified as effective composition. Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of polioxyethylene (23) laurylether (Brij35) under adequate experimental conditions and can be useful to mimic the drug partitioning process in biological system. It is well-known that drug must permeate a series of sequential biomembranes before reaching a target location where they can express desired pharmacological activity[1]. Based on this physicochemical property, biopartioning micellar chromatography has been developed as a promising tool to predict absorption processes *in vivo*. It has been demonstrated that the use of retention data obtained in a chromatographic system constituted by micellar mobile phases(Brij35) and C18 reversed stationary phase under adequate experimental conditions is helpful in describing the biological behavior of different kinds of drugs[2-10]. The retention of compounds in this chromatographic system depends on its interactions with modified reversed stationary phase and micelles in the mobile phase. These interactions are governed by hydrophobic, electronic and steric properties of compounds. The usefulness of biopartioning micellar chromatography in constructing good models could be attributed to the fact that the characteristics of the biopartioning micellar chromatography (BMC) systems are similar to biological barriers and extracellular fluids[11]. It has been studied comprehensively in the chemical drugs, protein-drug binding and has great significance in guiding the drug design and the development of new drugs[12]. Moreover, biological activity of different oral drugs were studied by our research group[13-16].

With strong separation ability, biopartioning micellar chromatography is suitable for the screening of bioactive compounds in complex matrices, such as traditional Chinese medicines, *etc*. However, there is little information available in literature about screening bioactive ingredients of traditional Chinese medicines in conjunction with related chemical drugs of similar curative effects. Honeysuckle is the flowers of *Lonicera japonica* thunb, which has been planted widely in China. It is one of the most famous traditional Chinese medicine herbs and usually used to cure common cold and

fever[17]. Honeysuckle contains chlorogenic acid (CA), luteolin-7-*O*-glucoside, volatile oil, flavone, saponins, polysaccharides and polyphenolic compound[18]. As one of the major bioactive compounds rich in honeysuckle, chlorogenic acid can significantly suppress the N-nitrosating reaction and inhibit hepatic glucose 6-phosphatase which is a significant factor in the abnormal diabetic state[19] and also serves as antioxidant, anti-inflammatory, antitumor, antimutagenic and anticarcinogenic agent[20-23]. Honeysuckle has shown a wide spectrum of biological and pharmacological activities such as antibacterial, antiviral[24], antioxidant[25].

In this work, an integration approach of biopartioning micellar chromatography with mathematical analysis was established and applied for the prediction and screening of bioactive components in honeysuckle extracts. The models were established by software with retention values in biopartioning micellar chromatography. The experimental results reveal that anti-inflammatory, antipyretic and analgesic effects are the main efficacy of honeysuckle, the antibiotic and antiviral effects take second place, effect of lowering blood pressure (BP), high blood fat and blood sugar is the weakest. Besides, with further relevant Matlab programming function, the probability of the components associated with different effects can be obtained by calculating the honeysuckle capacity factors, so as to identify which peak represents the active ingredient in the chromatogram and which is the main pharmacological action. This study confirmed that application of biopartioning micellar chromatography in screening active ingredients of traditional Chinese medicines is practicable. It paved the way for screening and analysis of the bioactive compounds in traditional Chinese medicines and may extend to the study of the interaction of the extract of traditional Chinese medicines with other biopolymers. This paper provides a model for rapid screening of active ingredients from traditional Chinese medicines ----honeysuckle. The screening and analyzing of the bioactive components in traditional Chinese medicines are very important not only for the quality control of the crude herb substances but also for elucidating the therapeutic mechanisms of traditional Chinese medicines, which is the key to modernize Chinese herbal medicine.

EXPERIMENTAL

Antibiotics and antiviral drugs: Ofloxacin, lomefloxacinhydrochloride, cefdinir, cefprozil, tetracycline, cefalexin, thiamphenicol, gatifloxacin, ciprofloxacin, ganciclovir, lamivudine, cefuroxime axetil, clindamycin phosphate for injection, clarithromycin sustained release tablet, amoxicillin, streptomycin sulphate, Zidovudine.

Antihypertensive drugs: Captopril, perindopril, propranolol hydrochloride, metoprolol tatrate, diltiazem hydrochloride, valsartan, imidapril, benazepril hydrochloride, pravastatin, metformin, atenolol, losartan potassium, alfuzos in hydrochloride, bisoprololfumarate, fluvastatin, prazosin hydrochloride, Simvastatin.

Antipyretic analgesics and antiinflammation drugs: Ibuprofen, aminopyrine, nimesulide, tramadol hydrochloride, methylprednisolone sodium succinate, benorilate, celecoxib.

All the drugs were donated by the pharmaceutical and pharmaceutical chemistry laboratories of West China School of Pharmacy, Sichuan University (Chengdu, China). Chlorogenic acid (purity 99.9 %) was purchased from the Institute for the Control of Pharmaceutical and Biological Products of China (Chengdu, China). Besides, the Flos Lonicerae Japonicae was purchased from Changchunteng Pharmacy (Chengdu, China).

General procedure

Preparation of honeysuckle samples: Extracting solution of honeysuckle was prepared using three kinds of methods to get as much ingredient as possible.

Reflux extraction: 10 g of sample was placed into a 250 mL round bottomed flask and 120 mL of 75 % ethanol was added and then decocted to boil keeping for 90 min with 80 °C thermostat water bath. The process was repeated twice. The combined alcoholic extract was filtered through two layers filter paper, then concentrated under the reduced pressure with a rotary evaporator.

Ultrasonic extraction: Ultrasonic-assisted extraction was performed in a sonication cleaning bath operated at a frequency of 40 kHz and an ultrasonic input power of 250 W with a volume of 4 L. The available range for extraction temperature was from 0 to 110 °C. Extraction process was carried out as follows: 10 g of honeysuckle powder was placed in a 250 mL Erlenmeyer flask and mixed with 120 mL of 75 % aqueous ethanol solution, after which the flask was placed in the constant temperature bath at 60 °C for 5-6 h, then extraction carried out for 45 min. Upon extraction, the mixture was filtered through a Buchner funnel under vacuum, after which the filtrates were collected in a volumetric flask. the filtrate was concentrated with a rotary evaporator evaporation.

Water decoction: 10 g of dried honeysuckle powder was weighted accurately to a beaker, 120 mL deionized water was added,put the beaker on the electric furnace, then decocted to boil keeping for 1 h. The process was repeated twice. The combined water extract was filtered through two layers filter paper, then concentrated under the reduced pressure with a rotary evaporator.

All the above extracts were then centrifuged at 12,000 rpm for 3 min. The supernatant was filtered through a 0.45 μm nylon filter then stored in the refrigerator at 4 °C.

Preparation of solutions: The mobile phase consisted of 0.04 mol/L polyoxyethylene (23) laurylether (Brij35, Acros, NJ, USA) with 0.01 mol/L sodium dihydrogenphosphate (analytical-reagent grade, Kelong, Chengdu, China) and was adjusted to pH 7.4, which is the plasmatic pH value by sodium hydroxide. In order to reproduce the osmotic pressure of biological fluids, Sodium chloride (9.20 g/L, analytical-reagent grade, Kelong, Chengdu, China) was added to the micellar mobile phase. Sodium chloride concentration was close to physiological concentration of biological fluids.

Stock standard solutions were prepared by dissolving 10 mg of the compound in 10 mL of mobile phase solution or deionized water.

Detection method: The retention of drugs was measured using a chromatograph with an LC-6A pump, an LC-6A UV detector and a CTO-6A column thermostat (Shimadzu, Japan). Data was collected and processed on a Alltech computer installed with chromstation software. The solutions were injected

into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 µL loop. The HPLC column was a Diamonsil TM C18 column (5 µm, 4.6 × 250 mm i.d.) with a phenomenex security Guard TMC18 guard cartridge. The UV detection of honeysuckle extracts was monitored from 210 to 300 nm. Water was from a Millipore (Billerica, MA, USA) synergy TM 185 system and was degassed before HPLC. The mobile phases injected into the chromatograph was filtered through 0.45 µm micro porous membrane. The mobile phase flow rate was 1 mL/min. All the assays were carried out at 37 °C for simulating human body temperature. The retention data in biopartioning micellar chromatography were calculated as capacity factors, $k_{BMC} = (t_r-t_0)/t_0$, where t_r is the retention time of the test compound and t_0 is the column dead time, which is the first fluctuation of baseline, determined by injecting water. The k_{BMC} values used in this study were the average value of triplicate. The retention data were highly reproducible.

RESULTS AND DISCUSSION

Screening and analysis data: Besides chlorogenic acid, flos lonicerae may also contain many different unknown components with different physicochemical properties. In this study, components of honeysuckle were extracted as much as possible by different methods, as we commented previously. Detect different ingredients in 200-400 nm ultraviolet absorption wavelength range. Considering the random error and systematic error, such as concentration of mobile phase, experimental conditions and the tiny difference of temperature, *etc*. The time of pure water, chlorogenic acid reference substance and different acid, alkaline and amphoteric drugs (benazepril, propranolol, prazosin) were investigated and inter-days and intra-days precision of their retention values were all less than 2 %, which indicate that repeatability is good for all the retention values tested. Therefore, the retention values of each peak in every honeysuckle chromatogram with a difference more than 3 % are considered different ingredients. Table-1 shows the biopartioning micellar chromatography retention data of the honeysuckle extract, which were calculated as capacity factors, $k_{BMC} = (t_r-t_0)/t_0$.

Table-2 shows the calculation results of drugs with similar pharmacological effects of honeysuckle.

Chlorogenic acid (CA) and flavonoids represented by galuteolin are the major bioactive constituent in honeysuckle[26]. The maximum absorption wavelength of CA is 324-327 nm[27-29]. Literature reported at wavelength of 355 nm, three main ingredients (CA, galuteolin and hyperosid) in honeysuckle show strong UV absorption[30]. Simultaneous determination of CA and galuteolin in honeysuckle at 350 nm was reported by Zhang *et al.*[31]. Thus, chose the chromatogram with the minimum wavelength of 210 nm, the highest wavelength (390

TABLE-1
CAPACITY FACTOR OF HONEYSUCKLE EXTRACT- k_{BMC} OF HONEYSUCKLE EXTRACT IN BMC SYSTEM

k_1	k_2	k_3	k_4	k_5	k_6	k_7	K_8	k_9	k_{10}
0.005	0.051	0.200	0.210	0.272	0.277	0.527	0.680	0.718	3. 008
k_{11}	k_{12}	k_{13}	k_{14}	k_{15}	k_{16}	k_{17}	k_{18}	k_{19}	k_{20}
2.420	3.108	3.188	3.231	3.282	3.355	3.359	4.273	4.353	2.23
k_{21}	k_{22}	k_{23}	k_{24}	k_{25}	k_{26}	k_{27}	K_{28}	k_{29}	k_{30}
2.420	3.108	3.188	3.231	3.282	3.355	3.359	4.273	4.353	4.562
k_{31}	k_{32}	k_{33}	k_{34}	k_{35}	k_{36}	k_{37}	K_{38}	k_{39}	k_{40}
4.583	4.665	4.909	5.610	5.956	7.324	7.749	8.242	8.828	9.202
k_{41}	k_{42}	k_{43}	k_{44}	k_{45}	k_{46}	–	–	–	–
9.393	10.152	10.938	12.20	13.214	14.253	–	–	–	–

TABLE-2
CAPACITY FACTOR OF THE RELATED WESTERN MEDICINE

Antibiotics and antiviral drugs	k_1	Antihypertensive drugs	k_2	Antipyretic analgesicsand antiinflammation drugs	k_3
Ofloxacin	2.082	Captopril	10.89	Ibuprofen	1.515
Lomefloxacin Hydrochloride	0.565	Perindopril	1.525	Aminopyrine	3.740
Cefdinir	0.924	Propranolole hydrochloride	17.56	Nimesulide	1.115
Cefprozil	0.544	Metoprolol tartrate	2.659	Tramadol hydrochloride	7.318
Cefalexin	0.415	Melbine	0.384	Methylprednisolone sodium succinate	7.814
Tetracycline	1.486	Diltiazem hydrochloride	3.050	Benorilate	1.231
Thiamphenicol	2.483	Valsartan	0.266	Celecoxib	1.879
Gatifloxacin	1.047	Imidapril	3.384		
Ciprofloxacin	1.242	Benazepril hydrochloride	2.060		
Ganciclovir	0.470	Pravastatin	4.483		
Acyclovir	0.620	Atenolol	0.591		
Zidovudine	2.271	Losartan potassium	15.461		
Lamivudine	0.881	Alfuzosin hydrochloride	13.089		
Cefuroxime axetil	19.94	Bisoprolol fumarate	6.664		
Clindamycin phosphate for injection	1.828	Fluvastatin	16.063		
Clarithromycin sustained release tablet	0.457	Prazosin hydrochloride	18.516		
Streptomycin sulphate	0.557	Simvastatin	10.513		
Amoxicillin	1.085				

nm) and maximum absorption wavelength (254, 325 and 350 nm) of the principal components to show the components of honeysuckle extracts detected at different wavelength representatively (Figs. 1-5).

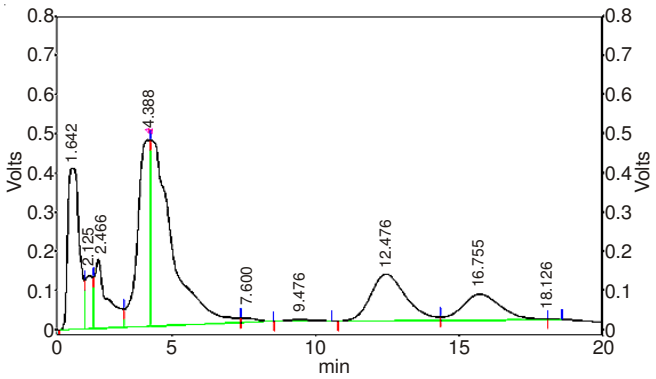


Fig. 1. Decoction of flos lonicerae peaks (210 nm)

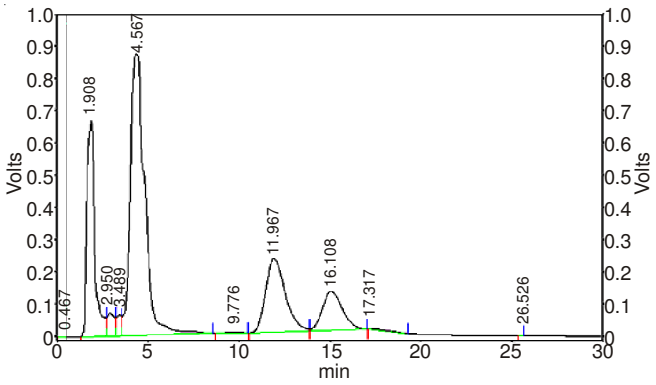


Fig. 2. Decoction of flos lonicerae peaks (254)

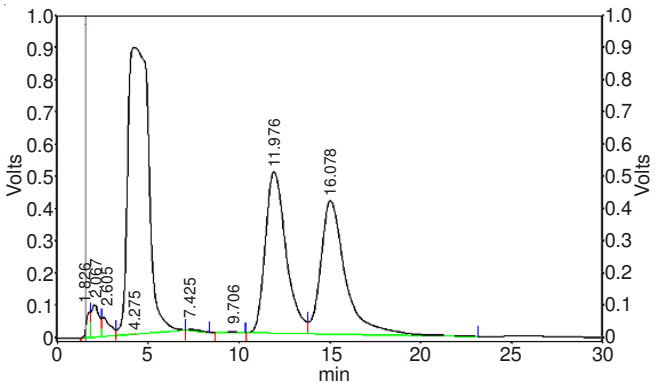


Fig. 3. Decoction of flos lonicerae peaks (325)

Software and data processing: SPSS 15 for windows program, Matlab 6 of the Math Works Incorporation and Excel 2003 of Microsoft office software were used to accomplish the statistical analysis.

Analysis of variance method (ANOVA): Analysis of variance, also known as ANOVA, is perhaps the most power

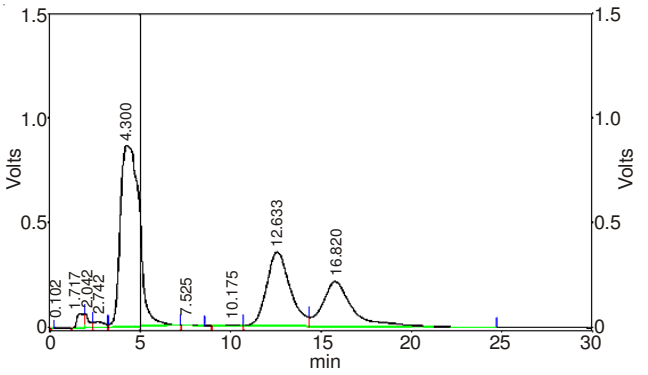


Fig. 4. Decoction of flos lonicerae peaks (350 nm)

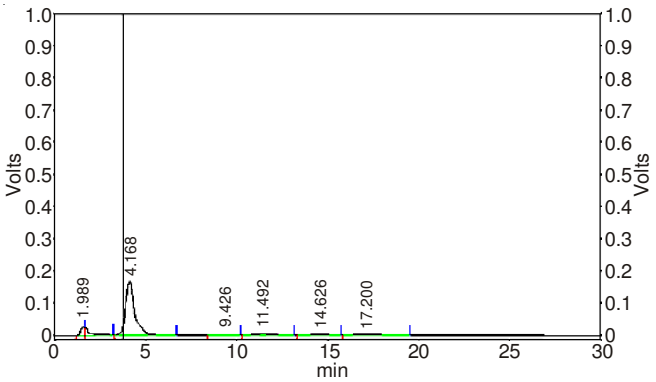


Fig. 5. Decoction of flos lonicerae peaks (390)

ful statistical tool. ANOVA is a general method of analyzing data from designed experiments, whose objective is to compare two or more group means. The analysis of variance is used widely in the biological, social and physical sciences.

Table-3 contains the results of the statistical analysis through variance analysis.it shows that significance level is $\alpha = 0.05$, $P = 0.008 < 0.05$, $F_{0.95}(3,85) = 3.3$, because $F = 4.16 > 3.3$, thus, on the significant level ($\alpha = 0.05$), levels of experimental data between groups have significant differences. We can then determine there are differences among the capacity factors of honeysuckle extract and related drugs.

Make scatter plot with the absolute values of the differences between the RSD of honeysuckle extract and the three kind of chemical drugs respectively, as shown in the Fig. 6. If the ordinate is more close to zero that indicate pharmacological effect of the drug is more similar with the component of honeysuckle extract. From Fig. 6, we can preliminary draw a conclusion that honeysuckle mainly shows antipyretic, analgesic and antiinflammatory effects.

Compare the mean of the capacity factors between honeysuckle extract and the three kind of related drugs respectively, through the analysis, we got the following conclusions shown in Table-4. The behavior in biopartioning micellar chromatography system of honeysuckle extract is more similar with antipyretic analgesic antiinflammatory drugs',which is

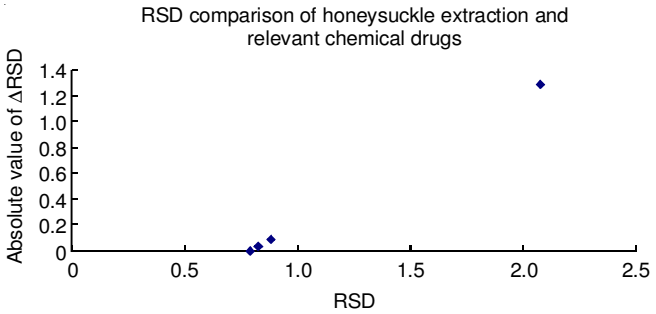
TABLE-3
ANALYSIS OF VARIANCE

Error sources	Square sum	Freedom degree	Mean square	F	Sig.
$A_{\text{between groups}}$	$S_A = 260.590$	$f_A = 3$	$V_A = 86.863$	$F = 4.16$	0.008
$e_{\text{within groups}}$	$S_e = 1774.841$	$f_e = 85$	$V_e = 20.880$	–	–
Total	$S_T = 2035.431$	$f_T = 88$	–	–	–

TABLE-4
MULTIPLE VARIANCE COMPARISON BETWEEN HONEYSUCKLE AND RELATED CHEMICAL DRUGS

(I) x	(J) x	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
		Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
1	2	1.932	1.267	0.511	-1.681	5.545
	3	-3.387	1.293	0.084	-7.076	0.302
	4	0.577	1.851	0.992	-4.704	5.857

1 In the (I) x represents honeysuckle extract; 2,3,4 in the (J) x represent antibacterial antiviral drugs, antihypertensive drugs, antipyretic analgesic anti-inflammatory drug respectively



Item	1-Honeysuckle extract	2-Antipyretic analgesics and anti-inflammation drugs	3-Antihy-pertensive drugs	4-Antibiotic sand Antiviral drugs
RSD	0.793	0.826	0.882	2.076
|ΔRSD|	0	0.033	0.089	1.283

|ΔRSD| represents the absolute value of the subtraction results between honeysuckle and each kind of drugs, respectively. The point on the Fig. 1 from left to right represent 1-honeysuckle extract, 2-antipyretic analgesics and antiinflammation drugs, 3-antihypertensive drugs, 4-antibiotics and antiviral drugs, respectively

Fig. 6. RSD comparison of honeysuckle extraction and relevant chemical drugs

consistent with the initial judgment. However, the result is also not so precise while the sig. is bigger than 0.05.

Since the above results are just simply preliminary judgment, in order to achieve a more reliable result, a detailed statistical model was established to make further analysis.

Programming method: Matlab 6 of the Math Works Incorporation was used to perform the statistical analysis of programming.

Pharmacological ingredients were classified according to the capacity factors, firstly, take advantage of the drug data to establish mathematical model to simulate the probability of each factor belong to corresponding category. Set up a collection for the capacity factor of a kind of drugs as $\{a_1,a_2,..,a_n\}$, b is for unspecified capacity factor. we can assume that for each known factor corresponding to an average (a_i), there's a normal distribution $N(a_i,\sigma_i^2)$ with a standard σ (to be provided). Probability prediction function of all experiment data can be obtained by adding up probability density function of all the distribution, written simply as f.

$$f(x)=\frac{1}{n}\sum_{i=1}^{n}\frac{1}{\sqrt{2\pi\sigma_i}}e^{-\frac{(x-a_i)^2}{2\sigma_i^2}}$$ where the $\frac{1}{n}$ is to ensure the integration of the entire function f(x) equals to 1. To estimate the probability of capacity factor b belongs to which category of drugs, we only need to observe the condition of the function f(x) in the vicinity of b value. Therefore, the integral value p of f(x) in the interval P[b-r/2, b + r/2] is considered, where b is the midpoint between the width of r. Three different integration p_1, p_2, p_3 can be concluded by using the three kinds of chemical drugs data. Moreover, compare the three values (p_1, p_2, p_3), then we can see which is the most possible category that the capacity factor b belongs to.

The above process can be realized by programming with Matlab. In the process of actual calculation, we assume that all σ_i get the same value σ. Observing a set of data and the experimental result,we can get that σ = 0.12, r = 0.015. In addition, if for a certain factor, the three integral values p_1, p_2, p_3 are less than 5 %, then we don't think this factor is likely to belong to any kind of the three known drugs. The specific calculation results all keep three decimal places (Teble-5).

Because the extraction methods are mainly for chlorogenic acid and flavonoids, which are considered as the principal components, component of flos lonicerae may haven't been fully extracted. Briji35 is a kind of nonionic surfactant, which is lipophilic and small polar with strong eluting ability. Liposoluble constituents in Flos lonicerae extracts may be eluted in a short time. The data is far from comprehensive, since the category and quantity of the experimental drugs are limited, the results with mathematical statistical analysis are less precise and perfect. However, this study provides a creative idea, which is combining efficacy of chemical drugs and Chinese traditional herbs with metabolism model *in vitro* and mathematical statistical analysis to predict and screen the bioactive constituents.

A new approach for assigning bioactivity to individual components in extracts from honeysuckle (*Lonicera japonica*) is demonstrated. We can get ingredients with different efficacy by screening active component of Chinese herbs and prescription through predicting model *in vitro*, thereby, further deduce the effective part of herbs. Separate effective component and verify the associated pharmacological activity purposefully with these components, avoiding interference of a large number of inactive ingredients, instead of a lot of isolation and purification for traditional Chinese medicines, provide a new, fast and effective method for material foundation of efficacy of Chinese herbs and compound prescription of Chinese medicine.

Conclusion

The aim of this paper is to provide a method to predict and identify active ingredients in honeysuckle by biopartioning micellar chromatography. It reflects the interactions of drugs and cell membrane to some extent, the retention behavior of

TABLE-5
CAPACITY FACTOR OF EXTRACT OF FLOS LONICERAE AND ITS RELATED CHEMICAL DRUGS PROBABILITY

Capacity factor k	Most possible category	Relative probability	Second possible category	Relative probability	Least possible category	Relative probability
0.005	**2**	0.020	**1**	0.0008	**3**	1.29E-19
0.051	**2**	0.043	**1**	0.003	**3**	4.26E-18
0.104	2	0.091	1	0.011	3	1.84E-16
0.200	2	0.229	1	0.078	3	1.15E-13
0.210	2	0.244	1	0.092	3	2.13E-13
0.272	2	0.327	1	0.232	3	9.25E-12
0.277	2	0.332	1	0.247	3	1.23E-11
0.527	1	1.116	2	0.285	3	2.92E-06
0.680	1	0.646	2	0.158	3	0.0007
0.718	1	0.524	2	0.116	3	0.002
0.778	1	0.430	2	0.059	3	0.010
0.973	1	0.599	3	0.283	2	0.001
1.362	3	0.529	1	0.240	2	0.078
1.473	3	0.515	1	0.216	2	0.178
1.512	3	0.511	1	0.201	2	0.194
1.525	3	0.504	2	0.195	1	0.195
1.542	3	0.490	2	0.194	1	0.185
1.745	3	0.331	1	0.167	2	0.043
1.789	3	0.394	1	0.192	2	0.033
2.034	1	0.239	3	0.207	2	0.191
2.230	1	0.281	2	0.072	3	0.007
2.420	1	0.250	2	0.029	3	1.83E-05
3.108	2	0.188	3	4.55E-07	1	2.39E-07
3.188	2	0.153	3	1.20E-05	1	6.04E-09
3.231	2	0.150	3	5.90E-05	1	6.83E-10
3.282	2	0.166	3	0.0003	1	4.56E-11
3.355	2	0.197	3	0.003	1	6.61E-13
3.359	2	0.198	3	0.003	1	5.12E-13
4.273	**2**	0.042	**3**	2.56E-05	**1**	1.05E-49
4.353	2	0.108	3	1.08E-06	1	4.25E-54
4.562	2	0.158	3	3.29E-11	1	1.53E-66
4.583	2	0.138	3	9.71E-12	1	7.10E-68
4.665	2	0.062	3	6.48E-14	1	3.79E-73
4.909	**2**	0.0004	**3**	1.30E-21	**1**	4.23E-90
5.610	**2**	3.77E-18	**3**	5.42E-45	**1**	9.78E-149
5.956	**2**	5.724E-09	**3**	5.56E-29	**1**	3.64E-183
7.324	3	0.474	2	5.34E-08	1	0
7.749	3	0.411	2	3.64E-19	1	0
8.241	**3**	0.001	**2**	6.22E-39	**1**	0
8.828	**3**	1.47E-16	**2**	3.41E-44	**1**	0
9.202	**2**	2.39E-27	**3**	4.58E-30	**1**	0
9.393	**2**	2.47E-20	**3**	1.26E-38	**1**	0
10.152	**2**	0.002	**3**	2.05E-83	**1**	0
10.938	2	0.179	3	4.1E-148	1	0
12.197	**2**	1.92E-13	**3**	1.9E-290	**1**	0
13.214	2	0.115	2	0	1	0
14.253	**2**	8.30E-22	**2**	0	**1**	0

1. Represents antibacterial antiviral drugs, 2. represents blood pressure (fat) blood sugar medicine; 3. represents antipyretic analgesic anti-inflammatory drugs;the bold Numbers to the corresponding capacity factor k probably do not belong to any of the three kinds of medicine

the active ingredients in biopartioning micellar chromatography system has significant correlation with pharmacological activities. Screening traditional Chinese medicines active ingredients through combining the related biological activity parameters of the curative effect of chemical drugs, provide a scientific basis for the application of traditional Chinese medicines in the future. This study provides key information for traditional Chinese medicines quality control and more reflective of the quality control of the efficacy of the traditional Chinese medicines. Screening of effective components of Chinese herbal medicine has a huge promotion for study on effects of traditional Chinese medicines compounds, conducive to the development and modernization of traditional Chinese medicines. However, the experimental data is still scarce, further studies are still necessary to get a more precise model.

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REFERENCES

1. Z.G. He, J. Sun, X. Wu, R. Lu, J.F. Liu and Y.J. Wang, *Curr. Drug Metab*., **9**, 152 (2008).
2. L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *Anal. Chem*., **70**, 28 (1998).
3. M. Cuenca-Benito, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *J. Chromatogr. A*, **814**, 121 (1998).
4. J.M. Sanchis-Mallols, R.M. Villanueva-Camanas, S. Sagrado and M.J. Medina-Hernandez, *Chromatographia*, **46**, 605 (1997).
5. M. Molero-Monfort, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *Biomed. Chromatogr*., **13**, 394 (1999).
6. C. Quinones-Torrelo, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *J. Med. Chem*., **42**, 3154 (1999).
7. Y. Martin-Biosca, M. Molero-Monfort, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *Biomed. Chromatogr*., **13**, 478 (1999).
8. L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci*., **740**, 59 (2000).
9. J.J. Martŷìnez-Pla, S. Sagrado, R.M. Villanueva-Camañas and M.J. Medina-Hernández, *J. Chromatogr. B: Biomed. Sci. Appl.*, **757**, 89 (2001).
10. M. Molero-Monfort, Y. Martin-Biosca, S. Sagrado, R.M. Villanueva-Camanas and M.J. Medina-Hernandez, J. Chromatogr. A, 870, 1 (2000).
11. M. Molero-Monfort, L. Escuder-Gilabert, R.M. Villanueva-Camañas, S. Sagrado and M.J. Medina-Hernández, *J. Chromatogr. B: Biomed. Sci. Appl.*, **753**, 225 (2001).
12. M. Hadjmohammadi and M. Salary, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **912**, 50 (2013).
13. S.-R. Wang, Y. Chen, L.-P. Wu, W.-J. Miao, M.-J. Xiong, C. Chen, Z.-R. Zhong and L.-M. Ye, *J. Pharm. Biomed. Anal.*, **46**, 243 (2008).
14. S.R. Wang, C. Chen, M.J. Xiong, L.P. Wu and L.M. Ye, *J. Chromatogr. Sci*., **48**, 134 (2010).
15. L. Wu, L. Ye, C. Chen, J. Wu and Y. Chen, *Biomed. Chromatogr*., **22**, 606 (2008).
16. Y. Chen, Li-ping Wu, Cong Chen, Li-ming Ye, *Biomed. Chromatogr*., **24**, 195 (2010).
17. Pharmacopoeia Commission of People's Republic of China, Pharmacopoeia of People's Republic of China, The Chemical Industry Press, Beijing (2005).
18. W.C. Chang and F.L. Hsu, *Prostaglandins Leukot. Essent. Fatty Acids*, **45**, 307 (1992).
19. H. Hemmerle, H.J. Burger, P. Below, G. Schubert, R. Rippel, P.W. Schindler, E. Paulus and A.W. Herling, *J. Med. Chem*., **40**, 137 (1997).
20. F. Pellati, S. Benvenuti, L. Magro, M. Melegari and F. Soragni, *J. Pharm. Biomed. Anal*., **35**, 289 (2004).
21. A.S. Moreira, V. Spitzer, E.E.S. Schapoval and E.P. Schenkel, *Phytother. Res*., **14**, 638 (2000).
22. T. Nakamura, Y. Nakazawa, S. Onizuka, Saori Satoh, A. Chiba, K. Sekihashi, A. Miura, N. Yasugahira and Y.F. Sasaki, *Mutat. Res. Genet. Toxicol. Environ. Mutagen*., **388**, 7 (1997).
23. Y.F. Sasaki, A. Chiba, M. Murakami, K. Sekihashi, M. Tanaka, M. Takahoko, S. Moribayashi, C. Kudou, Y. Hara, Y. Nakazawa, T. Nakamura and S. Onizuka, *Mutat. Res. Genet. Toxicol. Environ. Mutagen*., **371**, 203 (1996).
24. P.J. Houghton, Z. Boxu and Z. Xisheng, *Phytother. Res*., **73**, 84 (1993).
25. S.Y. Kim, J.H. Kim, S.K. Kim, M.J. Oh and M.Y. Jung, *J. Am. Oil Chem. Soc*., **71**, 633 (1994).
26. H. Xin, J. Feng, R.-M. Cheng and N. Xin, *Chin. J. Exp. Trad. Med. Formulae*, (2011).
27. F. Liao and X. Yao, *J. Jiangxi Chem. Ind*., **4**, 43 (2011).
28. L. Wu and Z. Zhang, *J. Food Sci*., **26**, 130 (2005).
29. Y. Wei, X. Liu and J. He, *J. Shandong Inst. Light Ind*., **4**, 26 (2007).
30. P. Li, H. Jin and J. Jia, *J. Tianjin Medical J*., **37**, 519 (2009).
31. Y. Zhang, J. Li, T. Chen and F. Li, *J. Tianjin Univ. Tradit. Chin. Med*., **30**, 107 (2011).