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Spectrum-effect relationship between hplc fingerprint and anti-inflammatory activity of n-butanol parts of *Tetrastigma planicaule* (Hook) Gagnep

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Abstract: The present study aimed to evaluate the spectrum-effect relationships between high-performance liquid chromatography fingerprints and anti-inflammatory effects of Tetrastigma planicaule(Hook.)Gagnep. Chemical fingerprints of ten batches of Tetrastigma planicaule from various sources were obtained by HPLC. The anti-inflammatory activity was investigated by a model of ear swelling in mice caused by xylene and a model of cotton pellet granuloma. Hierarchical cluster analysis (HCA) results showed that all the samples were clustered into four categories, which was basically consistent with the principal component analysis (PCA) results. The results of the joint grey relational analysis (GRA) and partial least squares regression analysis (PLSR) showed that peaks 1, 2 and 12 were positively correlated with the anti-acute inflammatory effect (ear swelling) in mice, and peaks 3, 5, 6 and 11 were positively correlated with the anti-chronic inflammatory effect (cotton pellet granuloma) in mice. The anti-inflammatory effect of Tetrastigma planicaule is the result of the synergistic effect of multiple components, which provides a basis for further exploring the anti-inflammatory substances and quality evaluation of the herb.

Keywords: *Tetrastigma planicaule* (Hook) Gagnep, n-butanol part, fingerprints, anti-inflammatory effect, spectrum-effect relationship.

INTRODUCTION

Tetrastigma planicaule (Hook) Gagnep, a traditional Chinese medicine derived from the vine stem of Tetrastigma planicaule, a genus of climbing vine in the grapevine family, which is widely distributed in southwest and southern China, the Zhuang and Yao ethnic minorities in China regular used it as a medicinal plant (Flora of China, 2004). Studies have shown that Tetrastigma planicaule can invigorate and relieve tendons, expelling wind and removing damness, and has a good therapeutic effect on bruises and painful rheumatism (Huang *et al*., 2021). Modern pharmacological investigations revealed that Tetrastigma planicaule has a broad range of physiological activities, such as antibacterial, anticancer, antioxidant (Qiu *et al*., 2020; Qiu *et al*., 2018). However, previous studies have been completed mainly in the fields of pharmacognosy, chemical composition and fingerprint (Zhang *et al*., 2019; Li *et al*., 2019; Zhen *et al*., 2017), the mechanism and dose-effect relationship of its pharmacodynamic substances have not been fully determined, which is not conducive to safe clinical use and further development and utilization, in-depth investigation is still required for its pharmacodynamic material basis and quality evaluation.

Inflammation is a defense response of the immune system in response to tissue damage caused by external factors or inflammation-causing factors. The common drugs used by Western medical practitioners to treat inflammation are NSAIDs, glucocorticoids, and biologics. However, there are some defects in the treatment of these drugs. Excessive use of glucocorticoids can reduce the immune defences of the patient's organism, Symptoms such as indigestion and abdominal pain may occur during the use of NSAIDs, biologics have adverse effects such as gastrointestinal reactions and immunosuppression (Ronchetti *et al*., 2018; Harirforoosh *et al*., 2013; Moore *et al*., 2020). In recent years, the combination of Chinese and Western medicine has achieved good results in the treatment of inflammation-related diseases (Xing *et al*., 2020; Tang *et al*., 2019; Feng *et al*., 2019). Traditional Chinese medicine (TCM) has peaceful medicinal properties, few toxic side effects, diverse chemical components, high pharmacological activity and can exert a variety of effects with rarely adverse reactions, which has substantial benefit to be developed and utilized. The application of TCM in anti-inflammatory is a hotspot of research at home and abroad, which is an important contribution to the development of new anti-inflammatory TCMs and the investigation of the material basis and mechanism of their performs. Related research clarifies, the genus climbing vine has good anti-inflammatory activity (Li *et al*., 2020; Hu *et al*., 2021; Yao *et al*., 2017). But there are few reports on the anti-inflammatory effects

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of Tetrastigma planicaule belonging to this genus; the plant has massive scope for research.

Chromatographic fingerprint is an effective means for quality control of TCM, as it can reflect the complex chemical information contained in a sample more comprehensively (Zhang *et al*., 2019). By combining fingerprints with pharmacological effects through relevant statistical methods, the effective components of TCM can be screened by analysing the correlation between the chromatographic peaks and their efficacy, thus providing a more scientific and comprehensive evaluation of the effective substances and quality of TCM (Cai *et al*., 2017). In recent years, this method has been applied to evaluate the quality of TCM as an efficient strategy (Shen *et al*., 2018; Lü *et al*., 2017).

In the present study, according to the screening of anti-inflammatory drug-effect parts in prophase, it was concluded that the anti-inflammatory activity of the n-butanol part was better, so this part was selected for the spectrum-effect relationship study. High-performance liquid chromatography (HPLC) was firstly applied to establish the chromatographic fingerprints of Tetrastigma planicaule samples from various origins. Chemometric methods such as similarity analysis (SA), hierarchical cluster analysis (HCA) and principal component analysis (PCA) were subsequently used to analyze the similarities and chemical differences among these samples. The anti-inflammatory activities of extracts were evaluated by the model of xylene-induced ear swelling and cotton pellet granuloma in mice. The correlation between the fingerprint and the anti-inflammatory activity was further calculated by Grey relational analysis (GRA) and partial least squares regression (PLSR). The aim of this work is expected to provide a basis for in-depth exploration of the pharmacodynamic substances and quality evaluation of Tetrastigma planicaule.

MATERIALS AND METHODS

Drugs and reagents

10 batches of Tetrastigma planicaule samples were collected from different parts of Guangxi, The species were authenticated by Ma Li Fei (Deputy Director of Guangxi Yixin Pharmaceuticals) as shown in table 1. Protocatechuic acid was purchased from China Academy of Food and Drug Administration, xylene was purchased from Tianjin Fuyu Fine Chemical Company Limited, dexamethasone was purchased from Zhejiang Xianju Pharmaceutical Co., water was purified by a Millipore China Ltd Simplicity type ultrapure water system, the methanol and acetonitrile were chromatographic purity and the rest of the reagents were analytical purity.

Animals

Male Kunming mice (18-22 g) were purchased from Hunan Slaughter Jingda Laboratory Animal Co., Ltd, production license: SCXK (Xiang 2016-002). They were acclimatized to the housing facilities for 3d before the experiments.

HPLC conditions

The chromatographic separation was performed using an AgiLent ZORBAX SB-C18 column (4.60x250mm, 5μm) at a column temperature of 30°C. The eluent consisted of methanol (A)-0.1% phosphoric acid water (B) with the following gradient elution variations: 0~30min, 5%A; 30~40min, 5%~8%A; 40~45min, 8%~13%A; 45~75min, 13%~27%A; 75~95min, 27%~37%A; 95~115min, 37%~40%A). The compounds were monitored at 260 nm, with a sample injection volume of 10μL. The flow rate was set at 1.0mL/min.

Preparation of standard solutions

The protocatechuic acid was accurately weighed and dissolved in methanol, and then diluted with 50% methanol (v/v) to obtain final concentrations of 20μg/mL. All standard solutions are stored at 4°C before analysis.

Preparation of drug samples for administration to mouse

Tetrastigma planicaule samples were sieved through a 24-mesh (inner diameter 0.85mm) sieve. Extraction was carried out with 10 times the amount of 70% ethanol as extraction solvent and refluxed until the colour remained constant. The total extracts were combined and concentrated. The extraction was then carried out with petroleum ether, ethyl acetate and n-butanol in that order. The n-butanol extracts were combined and concentrated to give the n-butanol fraction. All sample solutions were stored at 4°C before analysis.

Preparation of sample solutions

0.2 g of accurately weighed extracts (n-butanol part) were ultrasonically extracted with 20 mL of 60% methanol for 40 min, and then centrifuged for 10 min at 13000 rpm. The supernatant was collected and passed through a 0.22μm filter membrane, before injection into HPLC. All sample solutions were stored at 4°C before analysis.

Validation of methodology

On the basis of the established HPLC method, a randomly selected batch of Tetrastigma planicaule samples (S1) was performed to sample preparation as previously described. One sample from the batch was analysed in 6 injections to evaluate its precision. Six samples from the batch were analysed consecutively to determine their repeatability. Stability studies were carried out by analysing the samples at different times of the day (0, 3, 6, 19, 21 and 24hours).

Similarity evaluation of HPLC fingerprints

The n-butanol part samples of each origins listed in Table 1 and the reference standard solution were sampled and analysed, the chromatograms were recorded, and the fingerprint data of the 10 batches of n-butanol part extracts were analysed by the HPLC similarity evaluation system (Version 2012A) for chromatographic fingerprints of TCMs by the Chinese Pharmacopoeia Committee. The reference atlas was set to Nanning origin (S1), and the superimposed and control fingerprint profiles (R) were generated for 10 batches of Tetrastigma planicaule samples by the multi-point correction method and median method. Similarity between the reference fingerprint and various chromatograms was determined by the software.

Hierarchical clustering analysis (HCA)

Hierarchical cluster analysis (HCA) is a multivariate analysis method that classifies specimens into clusters based on a measure of distance or similarity between samples and generates a tree-like spectrum for visual analysis. It is often used as a statistical tool to assess similarities and differences within and between groups (Caesar *et al*., 2018). In this part, the HCA of different batches of Tetrastigma planicaule was performed using SPSS statistical analysis software (SPSS for Windows 22.0, SPSS Inc., USA) based on the between-groups linkage method and squared Euclidean distance (Li *et al*., 2020).

Principal component analysis (PCA)

Principal component analysis is a data analysis method that filters out some of the important variables from the original multiple variables by means of mathematical dimensionality reduction, and these variables can reflect the information of the original multiple indicators in a comprehensive manner (Ljubicic *et al*., 2021). In this study, the fingerprint data were performed to principal component analysis based on hierarchical cluster analysis using SPSS 22.0 software. The peak areas of the 13 common peaks of the n-butanol fraction extract of Tetrastigma planicaule from 10 different origins were first standardized, and then principal component analysis was performed on them.

Anti-inflammatory experiments

Xylene induced ear swelling test in mice

The anti-inflammatory activity against acute inflammation was determined using the xylene-induced ear swelling method in mice (Singsai *et al*., 2020). Male Kunming mice weighing 18-22 g were randomly grouped into 8 mice per group to obtain a model group, a positive control (dexamethasone) group, and a high-dose group of n-butanol extract from ten different origins. The model group was given an equal volume of distilled water, the dexamethasone group at 0.6mg per kg administered by gavage dexamethasone and each dosing group was administered by gavage (ig) at 0.4mL per 10g once daily for one week. On the seventh day, 45 min after dosing, xylene was applied evenly to the front and back of the right ear of mice at 20μL each, and the left ear was set up as the control. 15 min later, the mice were removed from the cervical vertebrae and executed, and the ear discs were punched with an 8mm punch in the same area of both ears. The auricular swelling and inhibition rates in mice were evaluated as follows:

Swelling = weight of the ear piece in the right ear - weight of the ear piece in the left ear. Inhibition rate = [(auricular swelling in the model group - auricular swelling in the administration group)/auricular swelling in the model group] × 100%

Cotton pellet granuloma test in mice

The anti-inflammatory activity against chronic inflammation was determined using the cotton pellet granuloma test in mice (Wilches *et al*., 2021). Cotton balls were weighed 10 mg, soaked in 75% ethanol and then rolled into cotton balls and dried at 60^{o}C. Male Kunming mice weighing 18-22 g were randomly grouped into 8 mice per group to obtain a model group, a positive control (dexamethasone) group, and a high-dose group of n-butanol extract from ten different origins.

Mice were anesthetized by intraperitoneal injection of 5% chloral hydrate (0.08mL per 10g), and small incisions were made under the axillae on both sides, and sterilized cotton balls were implanted under the skin and sutured respectively. Day after surgery, the mice were administered by gavage (ig) with an equal volume of distilled water in the model group, the dexamethasone group at 0.6mg per kg administered by gavage dexamethasone and each dosing group was administered by gavage (ig) at 0.4mL per 10g once daily for one week. On the seventh day, one hour after administration, mice were executed, granuloma cotton balls were peeled off, fatty tissue was removed, baked at 60 ^{0}C for six hours until dry, weighed after cooling, and the swelling was calculated by the difference in mass of the cotton balls before and after the experiment. The swelling inhibition rate was calculated according to the formula for the xylene inflammation test.

Grey relational analysis (GRA)

GRA is a commonly used analytical method in spectroscopic studies and has been widely used in the spectroscopic studies of HPLC fingerprinting (Yan *et al*., 2018; Li *et al*., 2020). In this study, the swelling rate of mouse ear and the weight of mouse cotton ball granuloma were used as the two reference sequences and the peak areas of the characteristic peaks of n-butanol sites of the alcoholic extracts of ten batches of Tetrastigma planicaule from different origins were used as the comparative sequences. The transformed potency data were recorded as Y(k) and the fingerprint peak areas were recorded as Xi(k). The absolute difference sequence was calculated as $\Delta i\ (k) = |Y(k)-Xi(k)|$. For the reference series Y(k) there are several comparison series Xi(k), the minimum and maximum values in the absolute difference of all comparison series are noted as Δ(min) and Δ(max) respectively, Δi(k) denotes the absolute difference between the comparison series and the reference series, ρ is the discrimination coefficient, taken as 0.5, and the correlation coefficient ξi(k) = [Δ(min) + ρ × Δ (max)] / [Δi (k) + ρ × Δ (max)]. In this experiment the reference sequence was Δ (min) = 0 and Δ (max) = 3.2797 for mouse ear swelling rate and Δ (min) = 0 and Δ (max) = 3.0481 for mouse cotton ball granuloma weight. The correlation r is the average of the correlation coefficients of each category, n indicates the number of data in the comparison series, and is calculated as

$$r = \frac{1.000}{n}\sum_{k=1}^{n}\xi(k)$$

Partial least squares regression analysis (PLSR)

PLSR is a statistical method combining linear regression analysis, typical correlation analysis and principal component analysis. By establishing the regression equation and comparing the regression coefficients and VIP values, it can accurately reflect the comprehensive contribution of each common peaks to the potency (Naguib *et al*., 2020; Moneeb *et al*., 2015). In this paper, SIMCA 14.0 software was used to analyze the relationship between HPLC fingerprint and drug efficacy of the n-butanol fraction of Tetrastigma planicaule by partial least squares regression. The independent variables were set as 13 common peak areas in the spectrum, and the inhibition of ear swelling and granulomatous inflammation by n-butanol extracts of the different origins of Tetrastigma planicaule were used as dependent variables respectively. The VIP value (the larger the value, the stronger the correlation) is used to reflect the correlation between the two.

Ethical approval

All animal experiments were conducted in accordance with the guidelines of the China Animal Care and Use Committee and approved by the Experimental Animal Welfare Ethics Committee of Guangxi University of Traditional Chinese Medicine (SYXK Gui 2019-0001). Each animal was used only once. Ninety-six mice were used in both anti-inflammatory experiments.

STATISTICAL ANALYSIS

The similarity evaluation was carried out using the Chinese medicine chromatographic fingerprint similarity evaluation system version 2012.1, Statistical package for social sciences (SPSS) version 22.0 software and SIMCA 14.0 software for the statistical analysis of spectral-effect relationships, level of significance (p-value) of 0.05 was selected.

RESULTS

Fingerprint similarity

The results of the similarity between the samples of different origins and the reference sample (S1) are illustrated in table 1. Most of the samples had a similarity greater than 0.900, i.e. the overall quality of the n-butanol part of the extract was generally stable across origins and the few origins with lower similarity may be due to the influence of the growing environment, planting pattern, season, harvesting and processing.

HPLC fingerprints of Tetrastigma planicaule samples

The method validation results are shown in table 2. The relative standard deviation (RSD) for precision, reproducibility and stability were all less than 3%, indicating that the established HPLC fingerprint method was valid. The HPLC fingerprints of the 10 batches of Tetrastigma planicaule samples obtained under optimized HPLC conditions are presented in fig. 1A, and the reference standard fingerprint was generated in fig. 1B. 13 peaks with well separated consecutive peaks were designated as "common peaks", indicating the similarity between the different samples.

In comparison with the control fingerprint (fig. 1C), the peak 5 with a retention time of 21.12 min was identified as the reference peak (S) of protocatechuic acid. The relative retention time RSD of the common peaks ranged from 0.15% to 0.44% and the relative peak areas RSD ranged from 20.56% to 78.44%. That is, the composition of the samples was basically the same between different producing areas. However, the partial peak areas of samples of different origins differed, which indicates that there were some differences in the composition content of the samples from different origins.

Results of HCA

The results of the HCA analysis are shown in fig. 2. Using 5 as the criterion for analysis, the 10 batches of Tetrastigma planicaule samples can be divided into four categories, Nanning (S1), Beihai (S3), Wuzhou (S4), Yulin (S6), Baise (S8), Hechi (S9) and Liuzhou (S10) as one category, Guilin (S2) as one category, Qinzhou (S5) as one category and Hezhou (S7) as one category. That is, the 10 batches of Tetrastigma planicaule samples were not identical, probably due to the different growing environment, temperature and season.

Results of PCA

When using 1 as the screening principle, the principal components with eigenvalues greater than 1 were 1-4, with a cumulative variance contribution of 90.955%. The first four factors were selected for analysis and are shown in fig. 3. The results show that Nanning, Beihai, Wuzhou, Yulin, Baise, Hechi and Liuzhou are in one category, Guilin in one category, Qinzhou in one category and Hezhou in one category. The results are consistent with the cluster analysis.

Table 1: Similarity between different batches of *Tetrastigma planicaule* and reference samples (S1)

Sample number	Sources	Similarity
S1	Nanning, Guangxi, China	1
S2	Guilin, Guangxi, China	0.915
S3	Beihai, Guangxi, China	0.987
S4	Wuzhou, Guangxi, China	0.991
S5	Qinzhou, Guangxi, China	0.748
S6	Yulin, Guangxi, China	0.992
S7	Hezhou, Guangxi, China	0.900
S8	Baise, Guangxi, China	0.972
S9	Hechi, Guangxi, China	0.989
S10	Liuzhou, Guangxi, China	0.990

Table 2: Results of precision, stability and repeatability test on the fingerprint of *Tetrastigma planicaule* samples

Common Peaks	Precision (n = 6)	Stability (n = 6)	Repeatability (n = 6)
	RSD (%)	RSD (%)	RSD (%)
P1	0.61	2.95	0.21
P2	0.45	0.28	0.13
P3	0.71	0.43	0.09
P4	2.85	1.64	0.07
P5	0.00	0.00	0.00
P6	0.82	0.34	0.05
P7	1.14	0.47	0.11
P8	0.40	0.44	0.12
P9	0.36	0.32	0.13
P10	0.90	1.87	0.14
P11	0.95	0.70	0.12
P12	1.23	1.66	0.13
P13	0.46	0.93	0.15

RSD: relative standard deviation.

Table 3: Principal component analysis component rotation matrix of butanol extract from *Tetrastigma planicaule*

Peak	Principal Components			
	1	2	3	4
P1	-0.464	0.058	-0.779	0.329
P2	0.701	-0.131	0.468	-0.153
P3	0.933	-0.194	0.158	-0.025
P4	0.854	0.288	0.208	-0.328
P5	-0.185	0.779	0.490	-0.181
P6	0.815	-0.282	0.287	-0.112
P7	-0.530	-0.651	-0.100	0.496
P8	-0.270	0.905	-0.072	0.119
P9	0.159	0.162	0.164	-0.931
P10	0.038	0.862	-0.320	0.203
P11	0.540	-0.515	0.568	0.162
P12	-0.108	0.377	-0.168	0.881
P13	-0.446	-0.013	-0.821	0.217

The magnitude of the combined influence of the principal components depends on the absolute value of the corresponding loadings. As can be seen from table 3, the loadings of peaks 2, 3, 4 and 6 corresponding to principal component 1 are higher, the loadings of peaks 5, 8 and 10 corresponding to principal component 2 are higher, The loadings of peaks 1 and 13 corresponding to principal component 3 are the highest, and the loadings of peaks 9 and 12 corresponding to principal component 4 are the highest, with peak 5 referring to the protocatechuic acid, and the quality of the *Tetrastigma planicaule* may be influenced by the content of this component.

Table 4: Effects of n-butanol fraction of *Tetrastigma planicaule* from ten different habitats on xylene-induced ear swelling and swelling inhibition rate in mice (n=8)

Group	Dip dosing (246.56g/kg raw)	Swelling degree (x±s, mg)	Inhibition rate %
model	\	7.5±3.2	\
positivity	6mg/kg	4.2±1.9*	44.00
Nanning	4.38g/kg	4.7±1.1*	37.33
Guilin	5.28g/kg	4.3±2.0*	42.67
Beihai	7.67g/kg	2.7±2.0**	64.00
Wuzhou	6.68g/kg	4.0±2.2*	46.67
Qinzhou	9.96g/kg	3.0±1.7**	60.00
Yulin	17.8g/kg	2.8±1.5**	62.67
Hezhou	6.68g/kg	4.0±1.5*	46.67
Baise	11.17g/kg	4.4±1.7*	41.33
Hechi	9.96g/kg	4.1±2.4*	45.33
Liuzhou	5.28g/kg	4.3±2.0*	42.67

Comparison with model group *: P<0.05, **: P<0.01

Table 5: Effects of n-butanol fraction of *Tetrastigma planicaule* from ten different habitats on cotton ball granuloma in mice (n=8)

Group	Dip dosing (246.56g/kg raw)	Dry weight of granulation tissue (x±s, mg)	Inhibition rate %
model	\	13.9±1.2	\
positivity	6mg/kg	9.8±1.6**	29.50
Nanning	4.38g/kg	12.3±1.7*	11.51
Guilin	5.28g/kg	10.9±2.0**	21.58
Beihai	7.67g/kg	10.6±2.3**	23.74
Wuzhou	6.68g/kg	12.2±1.8*	12.23
Qinzhou	8.90g/kg	10.7±1.8**	23.02
Yulin	17.8g/kg	12.2±1.8*	12.23
Hezhou	12.65g/kg	12.1±1.5*	12.95
Baise	11.17g/kg	12.6±1.1*	9.35
Hechi	9.96g/kg	12.0±1.6*	13.67
Liuzhou	10.40g/kg	10.9±1.9**	21.58

Comparison with model group *: P<0.05, **: P<0.01

Table 6: Correlation degree between mouse ear swelling rate and fingerprint peak area of n-butanol fraction of ten batches of *Tetrastigma planicaule*

Peak	Relevance	Peak	Relevance
P1	0.6797	P8	0.8688
P2	0.9150	P9	0.9421
P3	0.9328	P10	0.8495
P4	0.9430	P11	0.9119
P5	0.8717	P12	0.7555
P6	0.9354	P13	0.7750
P7	0.5274		

Table 7: Correlation between weight of cotton ball granuloma in mice and peak area of fingerprint of n-butanol fraction of ten batches of *Tetrastigma planicaule*

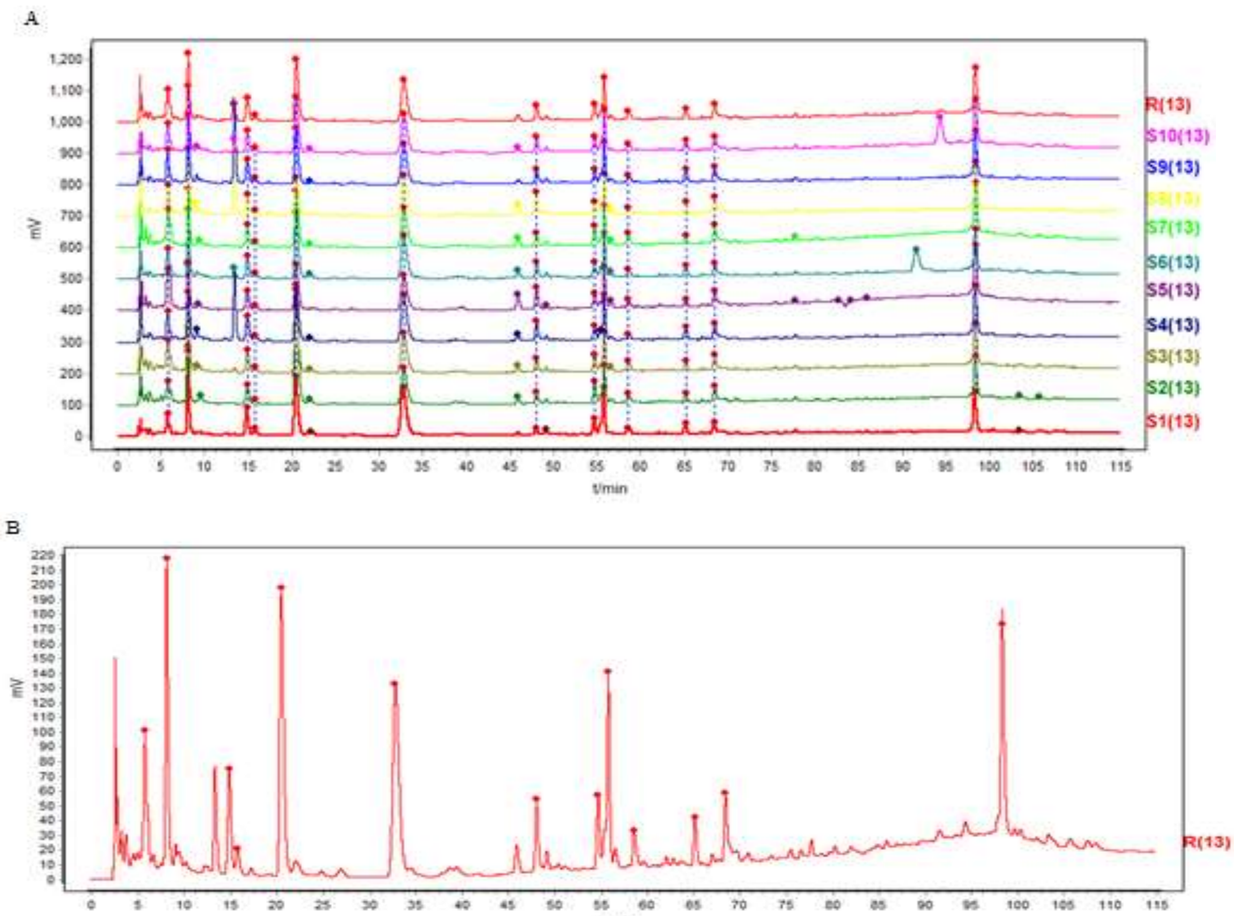
Peak	Relevance	Peak	Relevance
P1	0.6968	P8	0.9030
P2	0.9126	P9	0.9614
P3	0.9527	P10	0.8759
P4	0.9144	P11	0.9381
P5	0.9032	P12	0.7915
P6	0.9495	P13	0.8140
P7	0.6356		

Table 8: Partial regression coefficients of the characteristic peak areas of the n-butanol part of the fingerprint profile of *Tetrastigma planicaule* and the swelling of the auricle in mice

Characteristic peaks	Partial regression coefficient	Characteristic peaks	Partial regression coefficient
X1	-0.60287	X8	0.104089
X2	-0.59043	X9	-0.05217
X3	0.127294	X10	-0.27867
X4	0.254347	X11	0.21697
X5	0.022245	X12	0.461839
X6	-0.07028	X13	-0.0574
X7	-0.20734		

Table 9: Partial regression coefficient between characteristic peak area of n-butanol part fingerprint of *Tetrastigma planicaule* and Granuloma weight in mice

Characteristic peaks	Partial regression coefficient	Characteristic peaks	Partial regression coefficient
X1	-0.08793	X8	-0.05779
X2	-0.04269	X9	-0.01688
X3	0.130709	X10	-0.1204
X4	-0.02671	X11	0.237228
X5	-0.20464	X12	0.105147
X6	0.216302	X13	0.000843
X7	0.086988		



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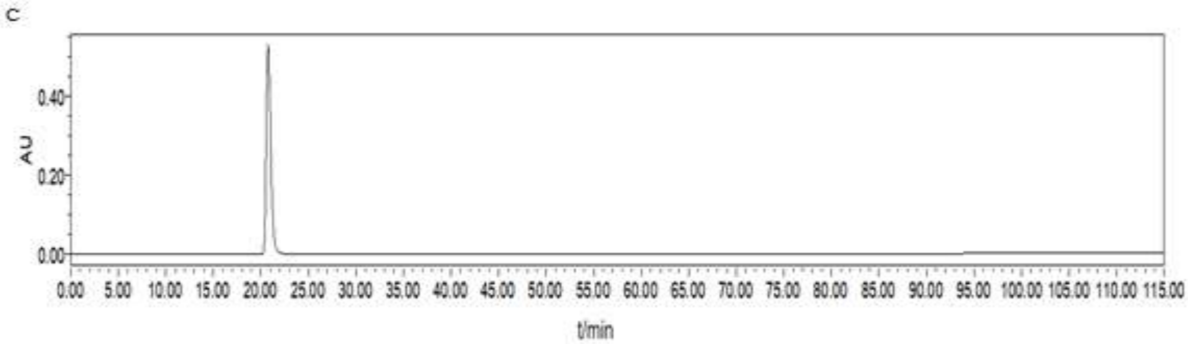

Fig. 1: HPLC results of *Tetrastigma planicaule* samples (A, Superimposed peaks of n-butanol extracts from ten different origins; B, reference standard fingerprint; C, control fingerprint)

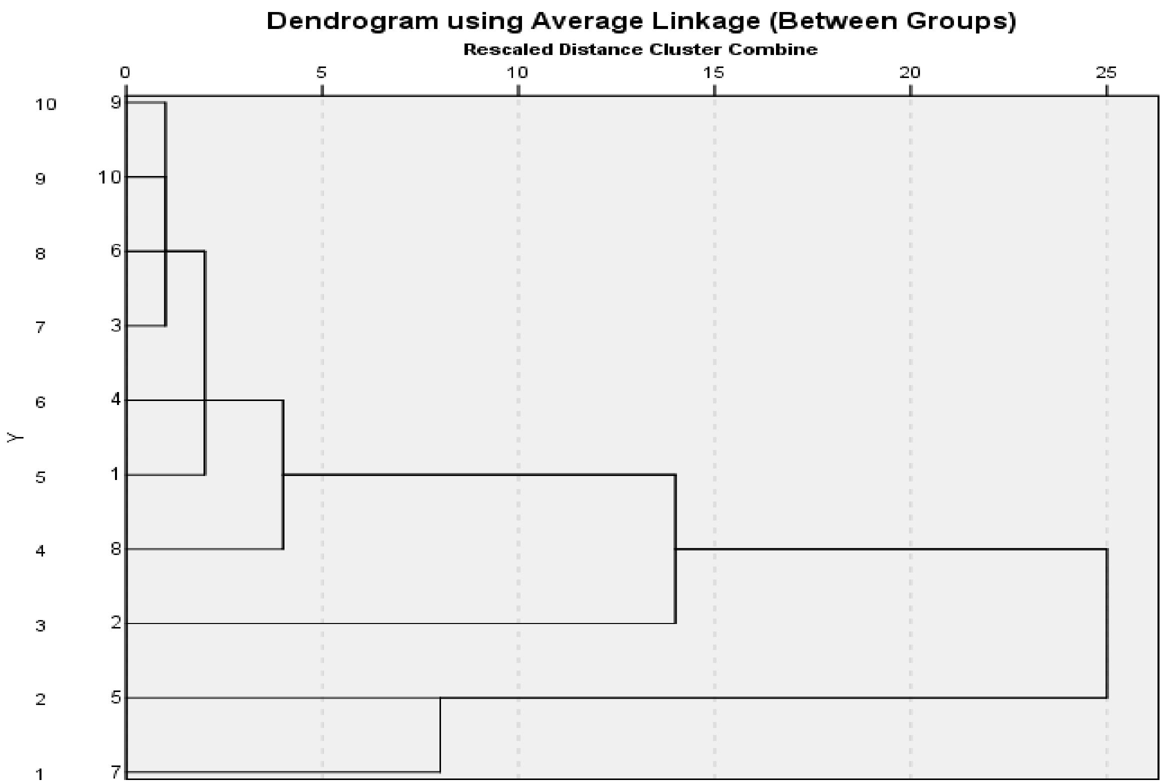

Fig. 2: Cluster analysis diagram of n-butanol extract samples from ten batches of *Tetrastigma planicaule* (The vertical coordinate is the origin)

Anti-inflammatory activity of different origins of Tetra stigma planicaule against ear swelling in mice

The results of the Xylene induced ear swelling test in mice showed that the groups with highly significant differences ($P<0.01$) and significant changes in ear swelling were Yulin, Beihai and Qinzhou Tetrastigma planicaule n-butanol part extract groups, and the groups with significant differences ($P<0.05$) in ear swelling were Nanning, Hechi, Wuzhou, Baise, Guilin, Liuzhou and Hezhou Tetrastigma planicaule n-butanol part extract groups. The groups with significant inhibitory effects were Yulin, Beihai and Qinzhou Tetrastigma planicaule n-butanol part extract groups, the results of which are shown in table 4.

Anti-inflammatory activity of different sources of Tetra stigma planicaule against granuloma in mice

The results of cotton ball granuloma test in mice showed that there was a highly significant difference ($P<0.01$) in Guilin, Beihai, Qinzhou and Liuzhou Tetrastigma planicaule n-butanol part extract groups, and a significant difference ($P<0.05$) in Nanning, Wuzhou, Yulin, Hezhou, Beihai and Hechi Tetrastigma planicaule n-butanol part extract groups on mice granuloma.

The most significant inhibitory effect was found in the n-butanol part extract groups of Guilin, Beihai, Qinzhou and Liuzhou, the results of which are shown in table 5.

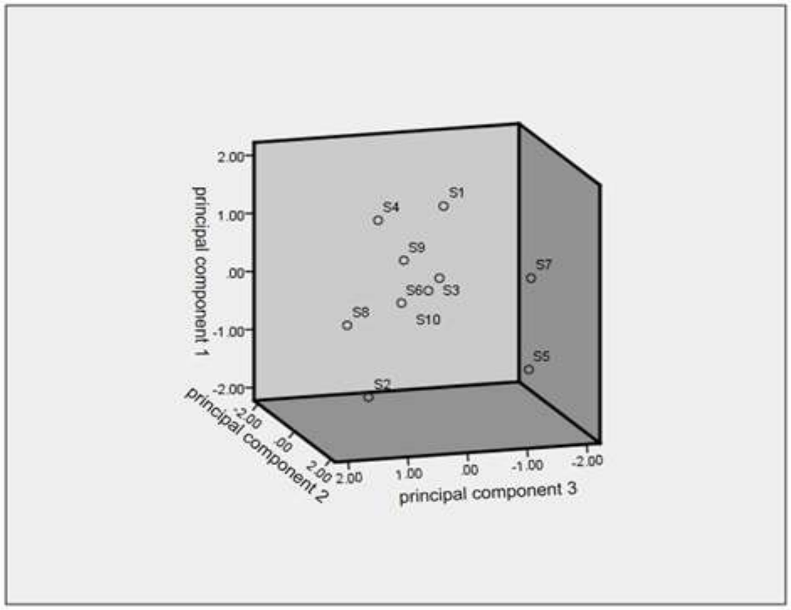

Fig. 3: 3D score of the principal components of the n-butanol part of the extract from ten different batches of *Tetrastigma planicaule*

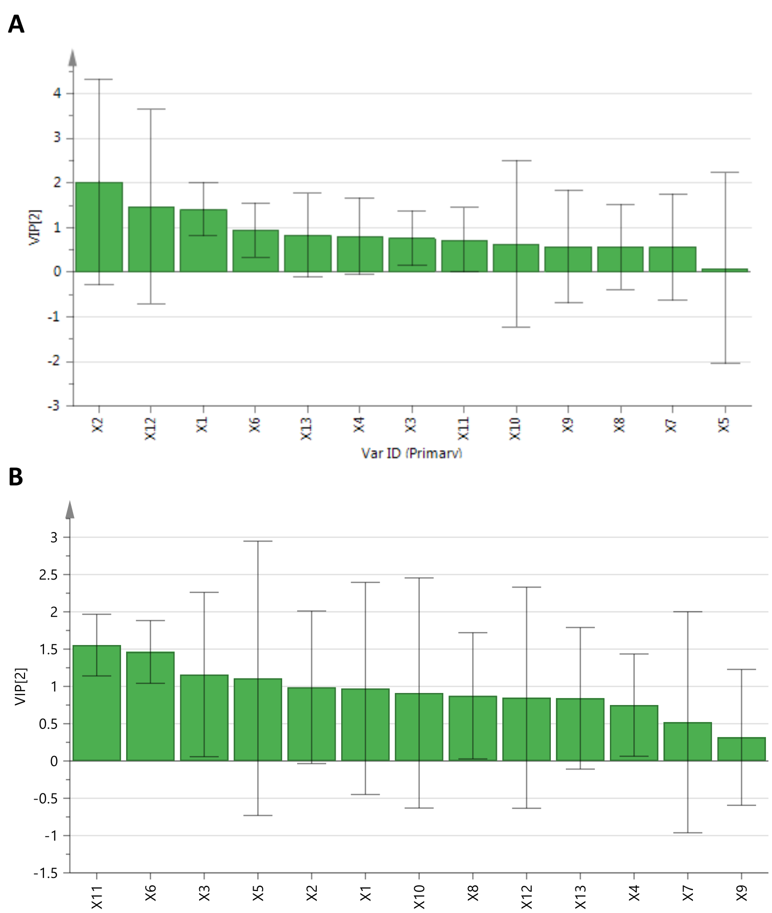

Fig. 4: VIP diagram of the characteristic peak areas of the n-butanol site fingerprints and anti-inflammatory activity of *Tetrastigma planicaule* (A: inhibition of ear swelling in mice B: inhibition of granuloma in mice)

GRA analysis

In this experiment, the inhibition rate of ear swelling and granuloma weight of mice were used as reference sequences respectively and the peak areas of the common peaks in the fingerprint were used as the control sequences. The correlation results are shown in tables 6 and 7. The contribution of the peaks to the anti-inflammatory effect on mouse ear swelling was, in descending order, peak 4>peak 9>peak 6>peak 3>peak 2>peak 11>peak 5>peak 8>peak 10>peak 13>peak 12>peak 1>peak 7. The corresponding correlations of the peaks were all greater than 0.6 except for peak 7, of which the correlations of peaks 2~6 and 8~11 were above 0.8.

The contribution of the peaks to the anti-inflammatory effect on mice cotton ball granuloma was, in descending order, peak 9>peak 3>peak 6>peak 11 >peak 4>peak 2>peak 5>peak 8>peak 10>peak 13>peak 12>peak 1>peak 7, with all peaks having correlations greater than 0.6, of which peaks 2-6, 8, 10, 11 and 13 had correlations above 0.8.

PLSR analysis of fingerprint and inhibition of ear swelling in mice

By using SIMCA-P14.0 software, the regression analysis of HPLC fingerprint characteristic peak areas and mouse ear swelling degree was carried out, as presented in table 8 and fig. 4A. It can be seen that X1, X2, and X12 had VIP values above 1, and X2 > X12 > X1. X1, X2, and X12 were positively correlated with the anti-inflammatory effect, that is, these chromatographic peaks had a great influence on the anti-inflammatory effect of mice. When their contents increased, the anti-acute inflammatory ability of the n-butanol part of *Tetrastigma planicaule* was significantly enhanced.

PLSR analysis of fingerprint and net weight of murine granulomas

The results of the regression analysis of the HPLC fingerprint peak areas with the net weight of mouse granulomas are shown in table 9 and fig. 4B. The anti-inflammatory effect of these peaks on mice was significant, and when their contents increased, the anti-chronic inflammatory ability of the n-butanol part of *Tetrastigma planicaule* was significantly enhanced, with peak 5 referring to protocatechuic acid.

DISCUSSION

In this experiment, the factors affecting the separation of peaks such as column, mobile phase, flow rate and column temperature were investigated in advance and full wavelength scanning was performed on the basis of this. The optimum chromatographic conditions for the HPLC fingerprint were obtained by comparing the separation of peaks, the number of peaks and the baseline smoothness of the chromatographic results.

The common peaks and pharmacodynamic data were analyzed by GRA. The results showed that the correlation between all peaks except peak 7 and the potency index of ear swelling was greater than 0.6, and the correlation between peaks 2-6 and 8-11 and the potency index of ear swelling was greater than 0.8, the correlation between all peaks and the potency index of cotton ball granuloma was greater than 0.6, and the correlation between peaks 2-6, 8, 10, 11 and 13 and the potency index of cotton ball granuloma was greater than 0.8, the ri greater than 0.6 indicates that the chemical component represented by the peak is correlated with the potency index, and the ri greater than 0.8 indicates that the chemical component represented by the peak is more correlated with inflammation. The results of PLSR showed that peaks 1, 2 and 12 were positively correlated with the anti-acute inflammatory effect in mice, and peaks 3, 5, 6 and 11 were positively correlated with the anti-chronic inflammatory effect in mice. The results of GRA and PLSR showed that peaks 1, 2 and 12 had an anti-inflammatory effect on acute inflammation (ear swelling) and peaks 3, 5, 6 and 11 had an anti-inflammatory effect on chronic inflammation (cotton ball granuloma) in mice.

CONCLUSION

In this work, fingerprints of 10 batches of n-butanol part of *Tetrastigma planicaule* were successfully established, and 13 common peaks were identified, among which peak No. 5 was identified as protocatechuic acid. Through GRA and PLSR analysis, the fingerprint was correlated with the efficacy data of ear swelling and cotton ball granuloma. The correlation degree and contribution of each component to the efficacy were preliminarily analyzed, and the anti-inflammatory spectrum-effect relationship of n-butanol fraction of Tetrastigma planicaule was established. This study will provide a scientific basis for the study of anti-inflammatory substances and further development and utilization of this herb.

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