A mathematical model for quality evaluation of total saponins of *Panax japonicas* based on hypolipidemic activity

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**Abstract**

**Objectives**: The chemical finger printing-based methods for evaluating TCMs quality can report partial of TCMs quality without linking to effective constituents. In this study, a mathematical model was established for the quality evaluation of total saponins of *Panax japonicus* (TSPJ), a folk medicine in China and Japan for treating diseases, through coupling the dynamic changes of chemical constitutions with corresponding activities.

**Methods**: High-performance liquid chromatography (HPLC) fingerprints were applied to establish the chromatographic database of TSPJ. The associated hypolipidemic activity database was determined by TG assay using HepG2 cell model. Correlation analyses of two databases were performed by partial least squares (PLS) for calculating regression coefficients, and the interval value of YZL value (the ratio of positive and negative peak-to-peak area coefficient) closely related to hypolipidemic activity was refined by the formula of Norminv function to value the quality of TSPJ.

**Results**: In this study, the chromatographic data of 16 common peaks were obtained from 20 batches of TSPJ. After the estimate by this mathematical evaluation model, seven peaks were positively correlated with hypolipidemic activity, and nine peaks were negatively correlated with hypolipidemic activity. When the YZL value was less than 0.7861, the quality of sample was inferior, while YZL value was more than 6.6992, and the quality of samples was superior. The quality of another ten batches of TSPJ was further assessed to verify this method.

**Conclusion**: These results indicated that the established model could be usefully applied to evaluate the quality of TSPJ in the hypolipidemic activity.

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**1. Introduction**

The rhizomes of *Panax japonicus* C. A. Meyer are widely used as a folk medicine in China and Japan for treatment of diseases such as hyperlipidemia and diabetes, etc. (Commission & Pharmacopoeia, 2005; He et al., 2012; Wen, Zhang, Chen, Dong, & Xiao, 2008). It also has been used as a substitute for the roots of Asia ginseng (*Panax ginseng* C.A. Meyer). Multiple pharmacological activities have been investigated recently on the saponins isolated from *P. japonicus*, such as anti-inflammatory and analgesic, immuno-enhancement, and anti-obesity effects (Yuan et al., 2009; Deng et al., 2017; He et al., 2012; Liu et al., 2016; Wu, Chen, & Zhang, 2016). In our previous study, it was proved that the total saponins of *P. japonicus* (TSPJ) had significant hypolipidemic effects (Yang & Chen, 2010). With the influences of various factors including sources, seasons, transportation and processing methods of traditional Chinese medicine (TCM), the contents of chemical constituents of *P. japonicus* are generally different, which caused the quality fluctuations of TSPJ, resulting in the changes of hypolipidemic activity (Hu et al., 2010). Hence, a digitalized mathematical evaluation model is needed to overcome those shortages in assessing quality of TSPJ based on chemical compositions.
The fluctuations of chemical compositions are common in TCM because it essentially comes from Chinese herbal medicines or natural minerals that possess the complicated characteristics of inhomogeneity and multiple components. To establish a quality evaluation method based on dynamic changes of chemical compositions in accordance with the characteristics and pharmacological activities of TCM has been a challenging problem for a long time. To some extent, the voices that criticize TCM as unscientific and unrepeateable are based on the shortage of quality evaluation. Many great efforts have been done in the field of the quality evaluation of TCM. At the beginning, traditional experiences and organoleptic methods were used for the authenticity and assessment of TCM by the senses such as touch, sight, smell and taste. Microscopic identification and physicochemical identification were then applied in the field according to the characteristic features of herbal materials and ingredients. Afterwards, content determination became a popular method through analyzing single or several ingredients by chromatography. To make up for the deficiency of content determination, fingerprint analysis approach was established based on the connection of qualities and curative effects. All the above methods have made great contributions to TCM evaluation methods (Guo, Yang, Zhou, & Li, 2010). If quality evaluation was based on the direct correlation between the dynamic changes of chemical compositions and the corresponding activities, the corresponding method would be more objective and useful. Until now, most of the reports on quality evaluation of TCM in term of components-efficiency correlation were only used to demonstrate whether the peak of each component was correlated the activity. The results cannot be used to authentically evaluate the quality of TCM samples. Moreover, the digital utilization directly promoted the development of TCM field. At present, the standard of content determination can be expressed digitally in the quality evaluation of TCM. However, the current methods only evaluate the quality of TCM by the content determination of single or multiple components, which limit to reflect the characteristics of the multiple active ingredients and the combination usage of Chinese herbal medicine. Hence, digital quality evaluation methods are worth being studied based on dynamic changes of chemical composition groups of TCM directly correlated with the pharmacological effects of TCM. Our research group has been exploring digital models for the quality evaluation of TCM in recent years. It should be pointed out that plenty of work should be done to establish and enhance digital models for the quality control of TCM, including in exploring and verifying different types of TCM samples (medicinal materials, processed products, extracts, etc.) and samples with different activities.

In this study, a digitalized evaluation model was explored for the quality control of total saponins of TSPJ through interacting dynamic changes of chemical constitutions with corresponding activities. Correlation analyses of the chromatographic database and the hypolipidemic activity database were performed by partial least squares (PLS) for calculating regression coefficients, and the interval of the multiple active ingredients and the combination usage of Chinese herbal medicine. Hence, digital quality evaluation methods were worth being studied based on dynamic changes of chemical composition groups of TCM directly correlated with the pharmacological effects of TCM. Our research group has been exploring digital models for the quality evaluation of TCM in recent years. It should be pointed out that plenty of work should be done to establish and enhance digital models for the quality control of TCM, including in exploring and verifying different types of TCM samples (medicinal materials, processed products, extracts, etc.) and samples with different activities.

2. Materials and methods

2.1. Materials

Acetonitrile (HPLC grade) was ordered from the TEDIA Company (Fairfield, OH, USA). HepG2 cell line was obtained from the Chinese Academy of Medical Sciences for Basic Medical Research Institute (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Grand Island, NY, USA). DMSO and trypsin were ordered from Sigma-Aldrich (St. Louis, MO, USA). Triglycerides (TG) Assay Kit was purchased from Solarbio Life Sciences (Beijing, China). Other chemicals were of reagent grade. Panax japonicus were obtained from Enshi (Hubei province, China) and identified by Prof. Ping Chen from Wuhan Polytechnic University, China.

2.2. Establishment of chemical constitutions database

Chemical chromatographic fingerprint analysis was determined by Agilent 1100 HPLC system coupled with a diode-array detector (Santa Clara, CA, USA). The mobile phase consisted of water containing 0.1% phosphoric acid (A) and acetonitrile (B). Each TSPJ sample of 10 μL was injected and separated on a Phenomenex ODS-BP column (250 mm × 4.6 mm, 4 μm) at 30 °C as the following gradient elution program: 0–30 min, 17%–30% B; 30–60 min, 30%–38% B; 60–70 min, 38%–50% B; 70–78 min, 50% B. The flow rate and detection wavelength were set up as 1.0 mL/min and 203 nm, respectively. TSPJ samples of 6 mg/mL were prepared in 40% acetonitrile and filtered by 0.45 μm filter before injection. HPLC fingerprint validation was assessed from sample repeatability, precision and stability using the seventh batch of TSPJ samples. Six replicates of TSPJ samples were analyzed in a single day to validate the repeatability. To access the precision, the intra- and inter-day variations were determined by analyzing six replicates within the same day and within three successive days, respectively. The stability was performed by determining the same sample at different time points (0, 3, 6, 12, 18 and 24 h).

2.3. Establishment of hypolipidemic activity database

Twenty-five micrograms of TSPJ were dissolved in 10 mL DMEM and filtered through 0.22 μm Millipore filter. The solution was further diluted in DMEM to obtain the final concentration of 250 μg/mL for the following experiments. HepG2 cells were suspended in media at a density of 1.5 × 10⁶ cells/mL and subsequently plated in a 24-well plate. In the control group, 10% BSA medium was added to each well, and 1 mmol/L oleic acid medium was used to the other groups for modeling. After 24 h, the culture medium was replaced with medium containing different batches TSPJ of 10% BSA. Subsequently, HepG2 cells were cultured for 24 h and lysed. The content of triglyceride (TG) was then calculated as follows (Niu, Lv, Li, Zhao, & Sun, 2010):

Concentration of TG (mmol/L) = ODs/ODb × 2.258 mmol/L

where ODs is the absorbance of each sample, and ODb is the absorbance of blank sample.

HepG2 cell hypolipidemic rate (%) = (TGm − TGs)/TGm × 100%

where TGm is the TG concentration of model sample, and TGs is the TG concentration of TSPJ sample.

2.4. Establishment of quality evaluation model for TSPJ

2.4.1. Spectrum-effect correlation analysis

Partial least square (PLS) method was used to analyze the correlation between chemical constitutions database total saponin chemical database and hypolipidemic activity database (Zhou, 44
The independent variable (X) was designated as the 16 common peaks areas of the 20 batches of samples, and the dependent variable (Y) was assigned as the hypolipidemic activity. Data preprocessing was performed by scaling all variables to a zero-mean normalization. After the normalization, the average of variables in each group was 0 and the variance was 1. Leave-one-out cross-validation (LOO-CV) was used to investigate the effect of component numbers on the root mean square error of cross-validation (RMSECV). When the RMSECV value was minimal, the component number was considered to be the most appropriate one. RMSECV was calculated as Eq. (1). The standardized regression coefficients were further obtained by PLS using Matlab 17.0 for associated models.

\[
\text{RMSECV}(f) = \sqrt{\frac{\sum_{j=1}^{n}(y_{cvj} - y_j)^2}{n}}
\]

where \(y_j\) is the real value of cell hypolipidemic activity; \(y_{cvj}\) is the predicted value of hypolipidemic activity calculated by PLS; \(n\) is the number of samples.

2.4.2. Determination of evaluation parameters

(i) Calculations of A, B, C, and YZL values

The products of common peak areas and the absolute values of corresponding regression coefficients were calculated in each batch of spectrum. The sums of the products of positive coefficients and corresponding peak areas were obtained as A value. The sums of the products of negative coefficients and corresponding peak areas were defined as B value. Then, the sum and the ratio of A and B were further calculated as C value and YZL value, respectively. The YZL value had positive correlation to the activity of sample, which directly reflected its quality. All the above defined values were carried out as follows Eq. (2):

\[
\begin{align*}
A_m &= \sum_{i=1}^{n} SiRi (Ri > 0), \\
B_m &= \sum_{i=1}^{n} SiRi (Ri < 0), \\
C_m &= A_m + B_m, \\
\text{YZL}_m &= A_m/B_m
\end{align*}
\]

where \(m\) is the number of samples; \(i\) is the number of common peaks; \(Si\) is the area of common peak \(i\) for sample \(m\); \(Ri\) is the regression coefficient of common peak \(i\); \(A_m, B_m, C_m\) and \(\text{YZL}_m\) are the A, B, C and YZL values of sample \(m\), respectively.

(ii) Calculations of YZL interval values

Norminv function is typically applied for returning the inverse of a normal cumulative distribution function of specified average and standard deviation, namely returning the interval point with a given probability normal distribution. Hence, Norminv function (Eq. (3)) was used for calculating the YZL interval value to validate the quality of TSPJ.

\[
\text{YZL interval value} = \text{Norminv}(p, m, s)
\]

where \(p\) is the probability of superior or inferior in the 20 batches of TSPJ; \(m\) is the mean of the YZL value; \(s\) is the standard deviation of the YZL value.

2.5. Verification of evaluation mode for TSPJ

Another ten batches of TSPJ samples labeled from No. 21 to No. 30 were processed to establish chemical constitutions database and the corresponding hypolipidemic activity database. Then the evaluation parameters (A, B, C and YZL values) were further calculated to the evaluation mode we created.

3. Results

3.1. Establishment of chemical constitutions database

Of all batches of TSPJ samples prepared, 1–20 batches of samples were used for the establishment of digital mode and the other was applied for the validation of the model. Samples were characterized to collect chemical fingerprints using HPLC and RSD values of sample repeatability, precision and stability analyses were all less than 2%. Chemical fingerprints data were further processed to create a fingerprint mutual mode with 16 characteristic common peaks (Fig. 1) by the Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2004 A edition, Chinese Pharmacopoeia Commission, Beijing, China). The area data of 16 common peaks from 20 batches of TSPJ samples were analyzed using Agilent Chemstation (Santa Clara, CA, USA) (Tables S1 and S2).

3.2. Establishment of hypolipidemic activity database

The hypolipidemic effects of TSPJ from 1 to 20 batches of samples were determined by HepG2 model in vitro (Table 1). The results indicated that the concentrations of TG in 17 batches of TSPJ samples were significantly increased. The rates of hypolipidemic effect of No. 11 batch and No. 12 batch were 37.473% and 51.030%, respectively. However, three batches (No. 5, No. 6 and No. 16) of TSPJ samples showed no significant differences in hypolipidemic activity compared with the model control.

3.3. Establishment of quality evaluation mode for TSPJ

3.3.1. Spectrum-effect correlation analysis

The results of the principal component analysis showed that there was no outlier in all batches of TSPJ samples, which indicated that all these samples could be used for the following modeling. The independent variable (X) and dependent variables (Y) were scaled to a zero-mean normalization, and transformation results were displayed in Tables S3–S5. As shown in Fig. 2, the results indicated the model was at the most stable status when the principal component number was three. A series of standardized regression coefficients were obtained and showed that seven peaks (No. 1, No. 2, No. 3, No. 5, No. 8, No. 12 and No. 13) were positively associated with the hypolipidemic activity (Table S6). The other peaks were negatively associated with the hypolipidemic activity (Table S6).

3.3.2. Determination of evaluation parameters

(i) Calculations of A, B, C and YZL values

The A, B, C and YZL values of each TSPJ sample were shown in Table 2. Then we obtained the ranked order of values as follows:

- A value: 11 > 16 > 10 > 1 > 12 > 17 > 19 > 8 > 9 > 3 > 7 > 4 > 14 > 2 > 8 > 15 > 13 > 20 > 6 > 5
- B value: 6 > 18 > 20 > 17 > 19 > 2 > 8 > 7 > 15 > 2 > 13 > 9 > 4 > 14 > 5 > 16 > 10 > 1 > 11 > 12
- C value: 17 > 18 > 19 > 3 > 7 > 9 > 2 > 4 > 8 > 15 > 20 > 16 > 14 > 10 > 1 > 11 > 6 > 5 > 12 > 13

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Hypolipidemic rate: \( \text{YZL} - 3.43 \leq \frac{\mu_2 - 3.43}{2.55} \) = 0.10

\( \text{YZL} - 3.43 \geq \frac{\mu_1 - 3.43}{2.55} \) = 0.15

where \( \mu_1 = 6.6992 \) and \( \mu_2 = 0.7861 \).

The results indicated that the TSPJ sample was superior when the YZL value ≥ 6.6992, and the sample was inferior when the YZL value ≤ 0.7861. The samples scored between two YZL above were categorized as qualified ones. Based on the classification of the YZL value, all the 20 batches of TSPJ were at the range of the activity determination results of each sample. Hence, the above results are the digital evaluation model for the evaluation of TSPJ in terms of hypolipidemic activity.

3.4. Verification of evaluation mode for TSPJ

Ten batches of TSPJ samples were conducted and analyzed as the protocol mentioned before. The related results were shown in Tables S7 and S8 and Table 3. The rates of hypolipidemic effect of No. 21 batch and No. 26 batch were 40.76% and 31.50%, respectively. Sixteen common peak areas of the ten verification batches of TSPJ were 40.76% and 31.50%, respectively. Hence, the YZL interval value was calculated as Eq. 4: YZL \( \sim N (3.43, 2.55^2) \).

Table 1

| No. | TG/(nmol L\(^{-1}\)) | Rate of hypolipidemic effect (%) |
|-----|---------------------|---------------------------------|
| Control | 1.565 ± 0.297 |                        |
| Model | 3.495 ± 0.243** |                        |
| 1 | 2.856 ± 0.139** | 18.28 |
| 2 | 2.622 ± 0.271 | 24.99 |
| 3 | 2.678 ± 0.137** | 23.38 |
| 4 | 2.655 ± 0.191 | 24.02 |
| 5 | 3.390 ± 0.214 | 3.00 |
| 6 | 3.218 ± 0.294 | 7.94 |
| 7 | 2.716 ± 0.261 | 22.29 |
| 8 | 2.619 ± 0.344** | 25.08 |
| 9 | 2.733 ± 0.157 | 21.82 |
| 10 | 2.877 ± 0.218** | 17.68 |
| 11 | 2.185 ± 0.163** | 37.47 |
| 12 | 1.711 ± 0.242 | 51.03 |
| 13 | 2.795 ± 0.435 | 20.03 |
| 14 | 2.616 ± 0.285 | 25.16 |
| 15 | 2.910 ± 0.269** | 16.73 |
| 16 | 2.637 ± 0.305** | 24.56 |
| 17 | 2.646 ± 0.277 | 24.28 |
| 18 | 2.701 ± 0.145 | 22.72 |
| 19 | 2.661 ± 0.272 | 23.87 |
| 20 | 3.314 ± 0.192 | 5.19 |

**P < 0.01 vs control group

**P < 0.01 vs model group

Fig. 1. Chromatographic profile of total saponins of P. japonicus with 16 characteristic common peaks.

Fig. 2. Root mean square error of cross-validation (RMSECV) of principal components.
TSPJ samples were analyzed by Agilent data processing software. The A, B, C, and YZL values were calculated and shown in Table 4, and the ranked order of values as follows:

| Batch | A Value | B Value | C Value | YZL Value |
|-------|---------|---------|---------|------------|
| 21    | 1280.7237 | 188.7580 | 6.7850 |
| 22    | 482.5742 | 795.8440 | 0.6064 |
| 23    | 870.4162 | 551.0680 | 1.5385 |
| 24    | 892.5839 | 491.4533 | 1.5795 |
| 25    | 460.3963 | 602.2511 | 0.6651 |
| 26    | 1311.1069 | 226.7119 | 5.7831 |
| 27    | 900.1291 | 585.0808 | 1.5385 |
| 28    | 941.5882 | 606.7956 | 1.5517 |
| 29    | 922.1608 | 544.5453 | 1.6935 |
| 30    | 1015.4963 | 628.2678 | 1.6084 |

4. Discussion

4.1. A new approach to evaluate TCMs is needed

Traditional Chinese medicine has been used for centuries and is still widely used today. It becomes an urgent that how to identify and evaluate the quality of TCM because of the complexities of chemical components and contents. At present, fingerprint approaches have now been widely applied in TCM for these purposes, such as HPLC, MS, GC–MS, etc. However, neither method can evaluate the quality digitally through connecting with the dynamic changes of chemical compositions with special pharmacological activities. Hence, valid method is therefore essential and should be further developed to evaluate the quality of TCM in an effective and comprehensive way to address the inherent holistic nature of TCM. In this study, a digitalized evaluation mathematic model was explored for the quality evaluation of total saponins of *P. japonicus*.

4.2. Characteristics of established evaluation model

The model has the following characteristics: (1) It can be used to evaluate the quality of samples with various independent variables. (2) This digital mode made the definition of rational fluctuation range in terms of chemical composition group or library of medicinal materials directly related to corresponding pharmacological activities. (3) The quality evaluation of medicinal materials was characterized by digitization mode through disclosing the correlation pharmacological activities and samples with various independent variables. (4) This model can be expanded to evaluate the quality of TCMs in many fields including processed products, TCM prescriptions, crude drugs, and the processes of extraction, separation, purification and refining of medicinal materials. Also, it can be used in other fields that need to be accessed in the superior and inferior correlation between samples with multiple functional independent variables and dependent variables. In conclusion, it is innovative to evaluate the quality of TCMs by our established digital model.

4.3. Current issues in established digital model for quality control of TCM

In this research, we have performed the preliminarily explore the establishment of a quality assessment method for total saponins of *Panax japonicas* associated with the hypolipidemic activity. However, there are still some following issues to be further studied and strengthened. First, to comprehensively evaluate the quality of medicinal materials or prescriptions, it is necessary to establish a database of multiple activity indexes in the pattern of “point–line–surface”. Second, the current chemical constitutions/libraries database is still too narrow since it only includes the information on the “common peak”. It would be more accurate and useful if changing the “common peak” mode to the “polymorphic component” or “holographic component” mode (Lucio-Gutiérrez et al., 2012). Third, the YZL interval value can only be used to determine the quality of TCM samples macroscopically, but not for delicately evaluating samples whose activity fluctuates in a very narrow range. Fourth, medicinal materials with the chemical components that cannot be detected by associated detectors are out of the evaluation range by this digital model. Hence, there is still some work that needs to be studied step by step in the follow-up research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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