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Optimal extraction bioactive components of tetramethylpyrazine in Chinese herbal medicine jointly using back propagation neural network and genetic algorithm in R language

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Abstract: A combinational approach of back propagation neural network (BPNN) and genetic algorithm (GA) was proposed in the present study to optimize the extraction technology of tetramethylpyrazine (TMP) in *Ligusticum wallichii Franchat*. Based on the single factor test, the orthogonal experiment design method of four factors and three levels was adopted, and the concentration of TMP was measured by high performance liquid chromatography (HPLC). Subsequently, BPNN model was trained for a predictive computational model of the performance indices via experimental data, and GA was exploited to find the optimization con ditions for extraction technology of TMP. Meanwhile, both the model and algorithm were implemented in R language. Ethanol concentration of 80%, extraction time of 1.5h, extraction temperature of 55℃ and liquid-solid ratio of 8:1 were derived as optimal conditions with a maximum content of TMP of 2.04 mg/g, which was confirmed with the relative error 2.63% through the validation of the experiments. This mathematical model could be used to analyze and predict the extraction technology of TMP in *Ligusticum wallichii Franchat* and provide a new reference for screening optimization of Chinese medicine effective parts and components.

Keywords: R language, BPNN, GA, TMP, orthogonal experiment, optimization.

INTRODUCTION

Ligusticum wallichii Franchat (Chuanxiong in Chinese), a well-known traditional Chinese medicine (TCM) in China, has drawn considerable attention due to its functions of invigorating the circulation of *qi*, dispelling wind and relieving pain (Shang, 2013). Recently, the modern clinic has witnessed that it plays an important role in the treatments of headache and cardiovascular system diseases (Zhu *et al*., 2016). The detailed studies have confirmed that there are water-soluble and fat-soluble active ingredients in *Ligusticum wallichii Franchat* (Jin *et al*., 2013), such as phthalide compound, phenolic acids, alkaloids. Meanwhile, previous works have reported that tetramethylpyrazine (TMP) in *Ligusticum wallichii Franchat* alkaloids could dilate small arteries and veins (Zhao, 2015), inhibit the platelet aggregation (Sheu *et al*., 2000) and activation of ischemic tissue (Shang *et al*., 2013) and increase blood flow to the cerebral microcirculation (Shen *et al*., 2010). These properties give rise to the reduction of ischemic brain damage and the improvement of nervous system dysfunction (Tan, 2009, Wang *et al*., 2010). Therefore it was widely used in the treatment of ischemic stroke (Kao *et al*., 2013, Zhang *et al*., 2016).

Traditional extraction methods (Chen *et al*., 2007, Yang *et al*., 2013) which didn't consider a combination of extraction factors simultaneously. Hence, we chose the orthogonal design under various factors concurrently to extract active ingredients in TCM. Based on the single factor analysis, extraction process variables, such as ethanol concentration, extraction time, extraction temperature and liquid-solid ratio, were chosen. Then the orthogonal design test was presented using above mentioned single factors to extract TMP. High performance liquid chromatography (HPLC) method was adapted to establish the content determination for TMP. However, after orthogonal analysis, there are complicated relationships between the final extraction conditions and corresponding extraction results. In other words, common analysis method, such as orthogonal analysis, couldn' t cater to the needs of the data processing and analyzing. It is necessary to find a novel, rapid and concise mathematical model or data analysis method for the optimization process.

Due to the efficient and powerful ability to predict and fit the observation data of unknown systems, back propagation neural network (BPNN) (Zipser and Andersen, 1988), which is one of the artificial neural network (ANN) model (Walpita, 1987), has been proven to be an attractive approach for system analysis and prediction in many fields (Catalogna M *et al*., 2012, Dawson and Wilby, 1998, Meng *et al*., 2013), such as medical diagnosis (Alshayea, 2011), computer vision (Kıvanç *et al*., 2007), DNA sequences classification (Zhang *et al*., 2008), formation damage (Mollakhorshidi and Arabjamaloei, 2016), etc. Meanwhile, genetic algorithm (GA), firstly introduced by Goldberg DE

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(Goldberg, 1989), has been shown to possess an efficient, and global randomized searching algorithm for handling both constrained and unconstrained optimization problems, such as molecular structure optimization (Habershon and Zewail, 2006), quality control (Qi, 2006), selection of optimal mathematical models (Ciglarič and Kidrič, 2006), etc. Additionally, it is demonstrated that R language has a professional and powerful ability in the fields of data analysis and data mining (Musunuru, 2013). Therefore, BPNN model is considered to establish the mathematical model for the extraction conditions and the experimental results. The trained network is examined through a separate set of date called the test set to monitor its performance and validity. Where after, GA is developed to evaluate the global maximum TMP and the corresponding conditions under the frame work of this extraction problem.

MATERIALS AND METHODS

Chemicals and reagents

HPLC-grade methyl alcohol was acquired from Merck Co. (Darmstadt, Germany) and water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA). Glacial acetic acid and ethyl alcohol (95%, v/v) were purchased from Hangzhou Chemical Reagent Co., Ltd (China). TMP standard (purity >98%), whose chemical structure was in fig. 1, was obtained from China institute of pharmaceutical and biological products.

The herbal drug, *Ligusticum wallichii Franchat*, was from Huqingyutang Pharmacy (Zhejiang province, China, batch number: 150701) and was identified by Shenwu Huang, the professor of Zhejiang Chinese Medical University. The crude slices of *Ligusticum wallichii Franchat* were of the stipulated quality standards in Chinese Pharmacopoeia (2010 edition).

Apparatus and chromatographic conditions

A Shimadzu LC-10ATVP (Kyoto, Japan) was equipped with a LC-10ATvp infusion pump, a CTO-10Avp column oven, a 7725i manual filling valve and a G1315D ultraviolet-visible detector was used for the chromatographic analysis. All separations were performed on a phenomenex GEMINI C_{18} (150 mm×4.6mm, 5μm). The mobile phase was composed of 0.05% glacial acetic acid aqueous solution (A) and methyl alcohol (B), which was applied in the isocratic elution as follows: 0-15 min, 65% A and 35% B. The flow rate was 0.8 ml/min and the column temperature was set at 25°C. The injection volume was 20μl. The detection wavelength was set at 290 nm, where the TMP had their maximum response of ultraviolet (UV) spectrum. The theoretical plate number of TMP was no less than 4000. This work applied R software 3.1.1 with the toolbox of neural network model and genetic algorithm for data statistics and analysis.

Preparation of standard solution

The standard stock solution of TMP (0.64mg/mL) was prepared in a 25mL volumetric flask with methanol and stored at 4°C. The calibration curve was carried out via a series of standard solutions, which was prepared by diluting the stock solution to appropriate and different concentration range. The concentrations of working solutions for calibration curve were 2, 4, 8, 16, 32, 64μg/mL, denoted by No. 1, 2, 3, 4, 5, 6 working solution, respectively.

Preparation of Ligusticum wallichii Franchat. extract for HPLC quantification

For the preparation of sample solution, 5.00g powdered drug was precisely weighed and extracted by 50mL ethyl alcohol by heating under reflux for 90 min twice. Then, the mixture was filtered and transferred quantitatively to a 100mL measuring flask with ethyl alcohol. After diluted to 100mL, the solution was put through 0.45μm syringe filter and subsequently detected under the established chromatographic conditions.

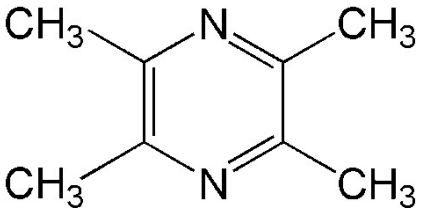

Fig. 1: Chemical structure of TMP in *Ligusticum wallichii Franchat*

Validation of the method

Calibration curve, limit of detection and quantification

The above six working solutions made calibration curve of TMP. Peak area (Y) and its corresponding standard concentration (X) made a linear regression curve. The limit of detection and quantification under the given chromatographic condition were calculated at a signal-to-noise (S/N) ratio of 3 for a limit of detection (LOD), and 10 for a limit of quantification (LOQ), respectively.

Precision

For this part, within-day and between-day precision had been used. To determine the within-day precision, the No.4 working solution was examined five times on the same day. To determine the between-day precision, the same working solution was analyzed on other five consecutive days. The relative standard deviation (RSD) was taken as a measure of precision.

Stability

The stability of sample solution was measured after 0, 2, 4, 6, 8, 12, 24h at room temperature under the selected chromatographic conditions. The RSD values of sample concentrations were used for calculation.

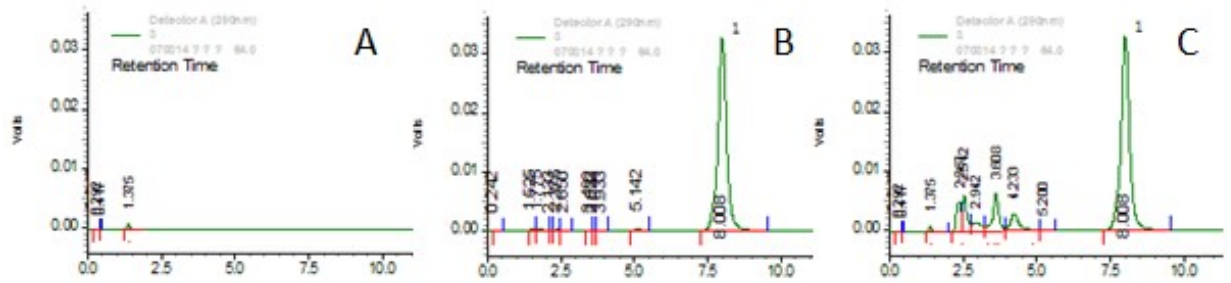

Fig. 2: Chromatograms of blank solution (A), standard solution of TMP (B) and sample solution (C). Peak identification: 1, TMP.

Table 1: Experimental factors and levels

Factor Level	A Ethanol concentration (%)	B Extraction time (h)	C Extraction temperature (°C)	D Liquid-solid ratio
1	60	0.5	55	8∶1
2	70	1	75	10∶1
3	80	1.5	95	12∶1

Table 2: The recovery test of TMP

Sample content (μg)	Added amount (μg)	Measured amount (μg)	Recovery (%)	Average (%)	RSD (%)
26.29	27.60	52.73	95.80	97.51	1.53
25.68	27.60	53.21	99.75		
27.07	27.60	53.77	96.74		
28.71	27.60	55.30	96.34		
25.92	27.60	52.93	97.86		
25.90	27.60	53.10	98.55		

Table 3: Design of $L_9(3^4)$ orthogonal test (n=3)

Number	A	B	C	D	The content of TMP (mg/g)
1	1	1	1	1	2.65
2	1	2	2	2	2.21
3	1	3	3	3	2.07
4	2	1	2	3	2.18
5	2	2	3	1	2.45
6	2	3	1	2	2.72
7	3	1	3	2	2.29
8	3	2	1	3	2.59
9	3	3	2	1	2.85

Recovery

To evaluate recovery, *Ligusticum wallichii Franchat* sample solutions were spiked with standard solution, whose quantities were closed to the samples. The recovery was researched by analyzing six individual samples. Their peak areas were taken into the calibration curve to calculate the recovery.

Research of orthogonal extraction technology

The content of TMP in the sample solution was regarded as an evaluating indicator. According to the L_9 (3^4) orthogonal table, ethanol concentration (A), extraction time (B), extraction temperature (C), and liquid-solid ratio (D) were considered to be the most influencing factors of this extraction issue according to the preliminary experiment. The corresponding factors and levels were shown in table 1.

BPNN model in R language

This paper adopted a three-layer BPNN model to train the experimental data under the R language environment. Four input nodes, A, B, C, D and one output, the content of TMP, were consisted in the network. The number of neurons in the hidden layer was increased from 0 to 8. All data were randomized into two groups: training data (8 data), and testing data (1 date). That was called a method

Table 4: Hidden layer neuron test training results

Hidden layer neuron (size)	Fitting error (%)
0	1.15
1	9.50
2	0.07
3	0.59
4	0.08
5	0.09
6	0.05
7	0.02
8	0.07

Table 5: The training results of the average fitting error and prediction error with different neurons in nine groups of experimental data (%)

Hidden layer neuron Error	0	1	2	3	4	5	6	7	8
Average fitting error	1.08	9.48	0.11	0.08	0.05	0.04	0.03	0.13	0.03
Average predicted error	3.17	10.69	2.71	2.45	4.24	4.16	5.87	3.32	4.55

Table 6: The results of predicted values and experimental values about content of TMP (mg/g)

Group	1	2	3	4	5	6	7	8	9
Predicted values	2.42	2.35	1.86	2.17	2.43	2.73	2.32	2.58	2.85
Experimental values	2.65	2.21	2.07	2.18	2.45	2.72	2.29	2.59	2.85

Table 7: Verification experiment under the optimum process

Number	The content of TMP (mg/g)	Average (mg/g)
1	2.93	2.96
2	2.95	
3	3.01	

of leave-one-out cross validation (Kearns and Ron, 1999).

GA to optimize the target in R language

GA was applied to search the extraction conditions and to optimize the output of BPNN based on emulation of natural evolutionary processes.

Validation of optimum extraction technology

Three samples of 10.0g *Ligusticum wallichii Franchat* powder were performed to extract TMP under the conditions, which were determined by GA. TMP was detected to verify the optimum extraction process.

STATISTICAL ANALYSIS

The data (% etc) were calculated using IBM SPSS Statistics for Windows software (Version 20, IBM Corp, Armonk, NY, USA).

RESULTS

Chromatographic separation and conditions

The specificity of the method was determined by comparing the chromatograms of the blank solution, a standard solution of TMP and sample solution of *Ligusticum wallichii Franchat*. It was inferred from the characteristic chromatograms in fig. 2 that TMP had a good separation, whose separating degree was more than 1.5 and no interfering peaks were found in the chromatogram. The retention time (t_R) of TMP was 8.0 min.

Calibration curve, limits of detection and quantification

The linear regression of TMP was obtained by using the peak area (Y) against the TMP standard concentrations (X). The regression equation for the calibration curve was Y=10203X+25155 (R^2= 0.9994),

Whose concentration range was 2-64μg/mL. The results of LOD and LOQ were 0.5, 1.2μg/mL, respectively.

Precision

The relative standard deviation (RSD) of within-day and between-day precision was 0.99 and 1.39%, respectively, which suggested that the developed HPLC method was feasible.

Stability

The RSD of the stability of sample solution was 2.05% at room temperature by determination of HPLC at the time of 0, 2, 4, 6, 8, 12, 24 h.

Recovery

The recovery of the HPLC method was 95.0~99.75% (n=6) for TMP, and the average value was 97.51% (RSD%=1.53%). The specific results were illustrated in table 2.

Experimental data of orthogonal extraction process

Four factors and three levels were designed in the L_9 (3^4) orthogonal table to extract TMP. The nine copies with the same weight of 10.0 g *Ligusticum wallichii Franchat* powdered drug were precisely weighed and reflux extracted. The extracting solution was vacuum filtrated. Thereafter, the residue was extracted three times. All the extracting solutions were put into the 500mL volumetric flask. The content of TMP in nine sample solutions was measured by HPLC orderly. The final results were presented in table 3, where the integers 1, 2, 3 were exploited in table 1.

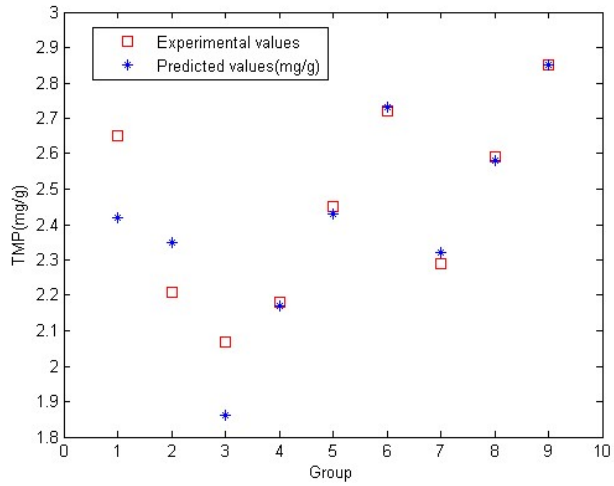

Fig. 3: Comparison of the predicted values versus the experimental values

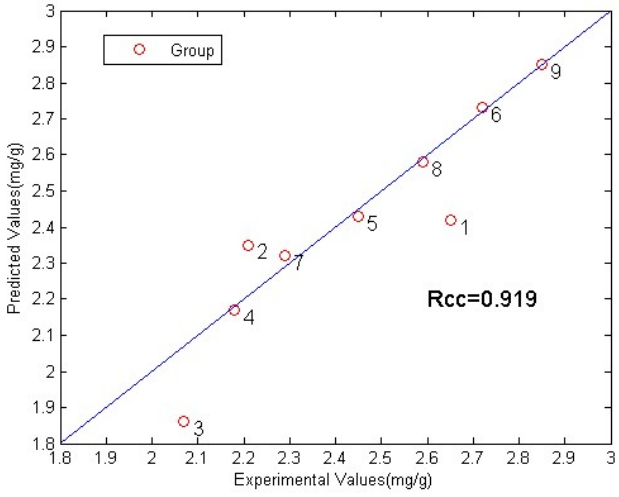

Fig. 4: Correlation between predicted values and experimental values

The results of BPNN model

Set the initial random right (rang) as 0.1, the parameter weight decay (decay) as 5×10^{-4}, the maximum iterations (maxit) as 500, and other parameters as the default values. Firstly, a total of 9 samples of experimental test responded to convergence for avoiding the over-learning with BPNN. The number of hidden layers(Karsoliya, 2012) ranged from 0 to 8 and optimum performance of the model was determined by fitting error, as shown in table 4. Secondly, all data were randomized into 2 groups: a set of 8 training data and a set of 1 testing data. The training and testing performances were determined by the average fitting error and the average prediction error, as displayed in table 5.

It followed from table 4 that the average fitting error increased first and then decreased with the increase of a number of hidden layer neurons. It could be regarded as the distinguished phenomenon of over fitting in statistics. Therefore, 0 and 6 hidden layer neurons were selected as the preliminary choices. Compared with the results of cross validation in table 5, if the number of hidden layer neuron was six, then its average prediction error was worse than that with the number 0. From the above arguments, this paper finally chose 0 hidden layer neuron.

Plot of experimental data versus model predicted values

Using the trained BPNN, the results of predicted values were evaluated, which were shown along with experimental data in table 6. Their comparison was displayed in fig. 3, which unveiled that the model exhibited good fitting results. Moreover, the mean relative error (MRE) and root mean squared error (RMSE) was 3.17%, 1.31%, respectively. Specifically, the relative error (RE) of each group was 8.68%, 6.33%, 10.14%, 0.46%, 0.82%, 0.37%, 1.31%, 0.39%, 0%, respectively, lower than 5%, except for groups 1 and 3. The correlation analysis was given in fig. 4. The correlation coefficient (R_{cc}) was 0.919, which reflected the accuracy of our predicted results. Higher R_{cc} indicated higher agreement between the predicted and the experimental values.

The result of GA to optimize the target

Now, GA was applied to optimize the extraction conditions for the best output of the network by R language. The population size was set as 900. The adjacent maximum algebra was set as 100. The biggest immutable algebra was set as 10. And other parameters were set to the default values. Consequently, the optimum results were obtained as follows: ethanol concentration 80%, extraction time 1.5h, extraction temperature 55℃, and liquid-solid ratio 8:1. The optimum predicted value (the content of TMP) was 3.04mg/g.

Validation of optimum values

It was necessary to validate the accuracy of the optimum process obtained by GA model. According to the above extraction process conditions, the triplicate of 10.0g *Ligusticum wallichii Franchat* powdered drug was precisely weighed and reflux extracted. In the end, the

content of TMP was measured accurately. The results of verification experiment under the optimum process were revealed in table 7.

The average content of TMP was 2.96mg and the network predicted value was 3.04mg/g. Their relative error was 2.63%. The measured value of the optimized extraction process had a higher matching with the predicted value that explained the model had a good predictability.

DISCUSSION

TMP has been proved to have the good physiological activity and medicinal value in the clinic (Fan *et al*., 2006, Zhang *et al*., 2014). It was a liposoluble constituent in volatile oil of *Ligusticum wallichii Franchat*. The quality control of *Ligusticum wallichii Franchat* and its preparations were often analyzed by TMP as the representative ingredient. In the preliminary experiments, we chose a number of factors that might affect the extraction of TMP, such as ethanol concentration, extraction time, extraction temperature, liquid-solid ratio, extraction frequency, immersion time, ultrasonic time, etc. Finally, we found that four factors (ethanol concentration, extraction time, extraction temperature and liquid-solid ratio) had a marked impact on the extraction of TMP. Further, the preliminary results indicated the extraction of TMP was a significant impact on the level of ethanol concentration in 60~80%, the level of extraction time in 0.5~1.5 h, the level of extraction temperature in 55~95℃, and the level of liquid-solid ratio in 8:1~12:1. We also utilized the ultraviolet and visible spectrophotometer (UV) and HPLC to measure the content of TMP in the preliminary experiments. By comparison, the determination result by HPLC was more precise than UV. Thus this study was adopted ethanol to extract TMP and determined it by HPLC.

ANN was a complex network system which was composed of a large number of simple processing units (neurons). It reflected many basic characteristics of human brain function that's a highly complex nonlinear dynamic learning system. Currently, ANN had been successful in many research fields such as pattern recognition (Lu *et al*., 2012), signal processing (Suah *et al*., 2003), knowledge engineering (Yao and Islam, 2008), expert system(Kim *et al*., 1995), robot control(Becerra *et al*., 2003) and optimum combination (Bashiri and Geranmayeh, 2011) *et al*., BPNN, which was trained by the error back-propagation algorithm, was one of the most widely used ANN models in many areas around the world. The experimental data had processed since the BPNN model was established. As was known to all, the values of three parameters (MRE, RMSE and RE) were smaller (≤ 5%) or closed to zero and Rcc was closed to one (Altunkaynak and Wang, 2010), that was deem the degree of deviation was tiny between predicted and experimental values. In other words, it had a statistical significance in statistics.

Of course, the BPNN model also had its own drawbacks such as falling into local extremum. Therefore, the neural network was combined with other methods in recent years, and the typical method was GA. GA were based on evolution theory and natural genetic principles. According to the fitness function (evaluation function) and a series of genetic operations, GA would convert numerical parameters to computer code to finally search the method of optimal solution by the optimization of generation after generation. In other words, GA was a kind of optimization procedures which were good at exploring a large and complex space in an intelligent way to find values close to the global optimum (Song *et al*., 2016).

R was one of the most popular data analysis and visualization platform whose language, R language, could programme the codes to process the data, map, forecase, etc.. Thereby, it would meet the needs of researchers for scientific research. The combination of two models could make the data processing, analysis and prediction become more accurate under the environment of R language.

However, the application of BPNN combined with GA was rarely reported in the effective material extraction of traditional chinese medicine under the environment of R language. In this research, the extraction and optimization of TMP were studied by using R language combined with BPNN and GA under the orthogonal test. At the same time, we also utilized orthogonal analysis to get the optimal extraction condition whose results were same with mathematical model of the above mentioned. At last, the content of TMP was determined by HPLC that the predicted value of the model was close to the measured value. Their relative error was 2.43%, which showed that the model had a good predictability.

CONCLUSION

In this paper, a mathematic model, BPNN combined GA, which under the environment of R language was easy to implement. This mathematic model was established and used to analyse and predict the relationship between the extraction conditions and the content of TMP. This approach had a simple code of the statements, the clear and understandable results. Besides, it was faster, more convenient and powerful than orthogonal analysis. what's more, it would provide a new and heuristic way for TCM researchers to deal with the data.

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REFERENCES

Alshayea Q (2011). Artificial neural networks in medical diagnosis. *International Journal of Computer Science Issues,* **8**(2): 197-228.

Altunkaynak A and Wang KH (2010). Triple diagram models for prediction of suspended solid concentration in Lake Okeechobee, Florida. *Journal of Hydrology,* **387**(3): 165-175.

Bashiri M and Geranmayeh AF (2011). Tuning the parameters of an artificial neural network using central composite design and genetic algorithm. *Scientia Iranica,* **18**(6): 1600-1608.

Becerra JA, Santos J and Duro RJ (2003). Multimodule artificial neural network architectures for autonomous robot control through behavior modulation. *Artificial Neural Nets Problem Solving Methods,* **2687**: 169-176.

Catalogna M, Cohen E, Fishman S, Halpern Z and Nevo UEBJ (2012). Artificial neural networks based controller for glucose monitoring during clamp test. *Plos One,* **7**(8): e44587.

Chen J, Yang B, Li W, Wang X, Lee FS and Yang H (2007). Application of accelerated solvent extraction technique for analysis of active components in traditional Chinese medicinal herbs. *Se Pu.,* **25**(5): 628.

Ciglaric I and Kidric A (2006). Computer-aided derivation of the optimal mathematical models to study gear-pair dynamic by using genetic programming. *Structural and Multidisciplinary Optimization,* **32**(2): 153-160.

Dawson CW and Wilby R (1998). An artificial neural network approach to rainfall-runoff modelling. *Hydrological Sciences Journal,* **43**(1): 47-66.

Fan LH, Wang KZ, Cheng B, Wang CS and Dang XQ (2006). Anti-apoptotic and neuroprotective effects of Tetramethylpyrazine following spinal cord ischemia in rabbits. *BMC Neuroscience,* **7**(1): 48.

Goldberg DE (1989). *Genetic algorithm in search, optimization, and machine learning*: Addison-wesley Publishing Company,Inc.

Habershon S and Zewail AH (2006). Determining molecular structures and conformations directly from electron diffraction using a genetic algorithm. *Chemphyschem,* **7**(2): 353-362.

Jin YQ, Hong YL, Li JR, Li X, Wang XX and GHL (2013). Research progress on chemical constituents and pharmacological effects of *Ligusticum wallichii Franchat*. *Pharmacy and Clinics of Chinese Materia Medica,* **4**(5): 44-48.

Kao TK, Chang CY, Ou YC, Chen WY, Kuan YH, Pan HC, Liao SL, Li GZ and Chen CJ (2013). Tetramethylpyrazine reduces cellular inflammatory response following permanent focal cerebral ischemia in rats. *Experimental Neurology,* **247**(3): 188-201.

Karsoliya S (2012). Approximating number of hidden layer neurons in multiple hidden layer BPNN architecture. *International Journal of Engineering Trends and Technology,* **3**(6): 714-717.

Kearns M and Ron D (1999). Algorithmic stability and sanity-check bounds for leave-one-out cross-validation. *Neural Computation,* **11**(6): 1427-1453.

Kim KH, Park JK, Hwang KJ and Kim SH (1995). Implementation of hybrid short-term load forecasting system using artificial neural networks and fuzzy expert systems. *IEEE Transactions on Power Systems,* **10**(3): 1534-1539.

Kıvanç KC, Boyacı İH, Köksel H and Küsmenoğlu İ (2007). A classification system for beans using computer vision system and artificial neural networks. *Journal of Food Engineering,* **78**(3): 897-904.

Lu HF, Jiang W, Ghiassi M, Lee S and Nitin M (2012). Classification of camellia (theaceae) species using leaf architecture variations and pattern recognition techniques. *Plos One,* **7**(1): e29704.

Meng H, Wang J, Xiong Z, Xu F, Zhao G. Wang Y (2013). Quantitative design of regulatory elements based on high-precision strength prediction using artificial neural network. *PloS One,* **8**(4): e60288.

Mollakhorshidi A and Arabjamaloei R (2016). Estimation of formation damage in overbalanced drilling using artificial neural networks and developing a simple correlation. *Oil Gas European Magazine,* **132**(6): 95-97.

Musunuru K (2013). Data mining and analysis by R language for business research: A case study on stress and its influence on health. *International Journal of Business Analytics and Intelligence,* **1**(2): 14-21.

Qi S (2006). Optimization of multi-objective for manufacturing quality control based on genetic algorithm. *Transactions of the Chinese Society for Agricultural Machinery,* **37**(6): 110-182.

Shang Y H TJF and Hou M *et al*. (2013). Progress on the protective effect of compounds from natural medicines on cerebral ischemia. *Chinese Journal of Natural Medicines,* **11**(6): 588-595.

Shang YH, Tian JF, Hou M and Xu XY (2013). Progress on the protective effect of compounds from natural medicines on cerebral ischemia. *Chinese Journal of Natural Medicines,* **11**(6): 588-595.

Shen Y, Zhuang P, Lin BQ, Zhang WY and Chiou GC (2010). Effect of Tetramethylpyrazine on RPE degeneration, choroidal blood flow and oxidative stress of RPE cells. *International Journal of Ophthalmology,* **3**(3): 205-210.

Sheu JR, Kan YC, Hung WC, Lin CH and Yen MH (2000). The antiplatelet activity of tetramethylpyrazine is mediated through activation of NO synthase. *Life Sciences,* **67**(8): 937-947.

Song JC, Xue GX and Kang YN (2016). A novel method for optimum global positioning system satellite selection based on a modified genetic algorithm. *Plos*

One, **11**(3): e0150005.

Suah FBM, Ahmad M and Taib MN (2003). Applications of artificial neural network on signal processing of optical fibre pH sensor based on bromophenol blue doped with sol–gel film. *Sensors and Actuators B: Chemical,* **90**(1-3): 182-188.

Tan Z (2009). Erratum: Neural protection by naturopathic compounds-an example of tetramethylpyrazine from retina to brain. *Journal of Ocular Biology, Diseases, and Informatics,* **2**(3): 137-144.

Walpita LM (1987). Nonlinear electroabsorption cell for artificial neural networks. *Applied Optics,* **26**(13): 2631.

Wang SL, Lin Y and Tang ZY (2010). Research progress on the protection of tetramethylpyrazine in different kinds of damage of nervous system and its mechanisms. *Chinese Pharmacological Bulletin,* **26**(4): 438-442.

Yang H, Ding L, Li JB, Li XB and Wu ZQ (2013). Extraction of Trace Germanium from Traditional Chinese Medicine Ganoderma Lucidum by Ultrasonic Wave. *Food Research and Development,* **34**(20): 12-14.

Yao X and Islam MM (2008). Evolving artificial neural network ensembles. *IEEE Computational Intelligence Magazine,* **3**(1): 31-42.

Zhang AB, Sikes DS, Muster C and Li SQ (2008). Inferring species membership using DNA sequences with back-propagation neural networks. *Systematic Biology,* **57**(2): 202-215.

Zhang CB, Teng FM, Tu J and Zhang D (2014). Ultrasound-Enhanced Protective Effect of Tetramethylpyrazine against Cerebral Ischemia/ Reperfusion Injury. *Plos One,* **9**(11): e113673.

Zhang Z, Zhang G, Sun Y, Szeto SS, Law HC, Quan Q, Li G, Yu P, Sho E and Siu MK (2016). Tetramethylpyrazine nitrone, a multifunctional neuroprotective agent for ischemic stroke therapy. *Scientific Reports,* **6**: 37148.

Zhao JE (2015). Pharmacological effects of the effective component of Ligustrazine in *Ligusticum wallichii Franchat*. *Strait Pharmaceutical Journal,* **27**(8): 145-146.

Zhu Y, Liu WW, Gu N and Shi AW (2016). Studies on the active components of *Ligusticum wallichii Franchat* and its protective effect on cardiovascular system. *Lishizhen Medicine and Materia Medica Reasearch,* **27**(7): 1701-1704.

Zipser D and Andersen RA (1988). A back-propagation programmed network that simulates response properties of a subset of posterior parietal neurons. *Nature.,* **331**(6158): 679-684.