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A novel quality evaluation method for magnolia bark using electronic nose and colorimeter data with multiple statistical algorithms

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Abstract—The bark of the magnolia tree, which includes both the aromatic and medicinal varieties known as magnolia officinalis and magnolia officinalis, is often used in Chinese patent medicines and clinical prescriptions. The chemical composition of Hou Po is a key indicator of its quality, which is in turn connected to its therapeutic efficacy; the site of origin plays a pivotal role in determining its quality. An innovative approach to quickly, correctly, and fully determining where Hou Po came from and what its key chemical components are is the goal of this research. Methods: The magnolol and honokiol components were analysed using high performance liquid chromatography, while the magnocurarine and magno- fluorine components were analysed using ultra-performance liquid chromatography. We found out what was in the water-soluble extracts by using the cold soak technique. The colorimeter and E-nose were used to identify the Hou Po samples' smell and colour, respectively.
In order to determine where Hou-Po came from and what chemicals were in its water-soluble extracts, we used a number of statistical techniques to build discriminant models that relied on E-nose and colorimeter data. With a classification accuracy of 99.53%, the Random Forest classifier in conjunction with the ten-fold cross-validation approach outperformed the other models tested. For each of the five chemical components, the correlation coefficient between experimental and predicted values was more than 0.96. This research concludes that the electronic nose and colorimeter show promise as quantitative and qualitative tools for assessing the quality of Chinese herbal remedies.

Introduction

Magnolia officinalis REHD. & WILS. and Magnolia officinalis REHD. & WILS. are the two species of the Magnoliaceae family from which the Chinese word "hou po" (dry bark) is derived. Gingko biloba (Rehde & Wilkinson)1 For hundreds of years, people have turned to Hou Po, a traditional Chinese herbal medicine (CHM), for relief from a variety of gastrointestinal issues. A large number of clinical prescriptions and patent medications in China use it as an active ingredient. Traditional macroscopic identification based on subjective observations and linguistic descriptions is now the most utilized approach for assessing Hou Po quality in the market. Two kinds of chemical components are officially specified in the 2015 edition of the People's Republic of China Pharmacopeia.1 Clinical usage of Hou Po is hindered by the inadequacy of current criteria for assessing and monitoring its quality.1
The quality of Hou Po is influenced by several things, but one of the most essential is where it is made. The provinces of Hubei, Sichuan, Zhejiang, Guangxi, and Fujian are among those in China where you may find Hou Po grown in a mix of natural and artificial settings. Distinct origins provide unique growth conditions, which in turn

affect the final product's quality. Provinces like Hubei and Sichuan are known for producing high-quality Hou Po. Specifically, geo-authentic medicinal substance is acknowledged for Hou Po samples from the Hubei city of Enshi. Research has shown that shown that the chemical composition, purity, and clinical effectiveness of samples originating from various locations vary.2 Because even highly qualified specialists have a hard time telling them apart based on macroscopic identification and detecting one or two chemical components, Hou Po pieces from different origin sites may be readily mistaken in the marketplace and for therapeutic purposes.
There are only two ingredients listed in the 2015 edition of the People's Republic of China Pharmacopoeia: magno-lol and honokiol, with a combined level of no less than 2%. Because Hou Po's chemical makeup is diverse and varied, this may directly contribute to ambiguity. Determining the whole chemical profile of Hou Po—which includes volatile oils, lignans, alkaloids, and phenylethanolic glycosides—is insufficient.3 Hou Po gets its distinctive scent from volatile oils. Hou Po samples with a more robust scent are likewise thought to be of higher grade based on the trustworthy experience of macroscopic identification.4 The main components of lignans, magnolol and honokiol, have many biological effects, including antibacterial, anti-inflammatory, anti-neoplastic, and antioxidant properties.5—8 According to other research, alkaloids in CHMs often have substantial

the actions of pharmaceuticals.9, 10 Two of Hou Po's alkaloids, magnocurarine and magnocorine, have antifungal and antiplatelet activities, respectively.12 Consequently, while assessing the quality of Hou Po, it is important to consider this sort of component. Clinical trials have shown that a water decoction of Hou Po can improve gastrointestinal motility disorder and limit the development of human mesangial cells.13, 14 It is in the water-based extract that the phenylethanolic glycosides are most abundant. All things considered, these four aspects are very relevant to Hou Po's quality and should be considered accordingly. It is, therefore, critically necessary to create a new approach of assessing Hou Po's quality.
Experts in traditional Chinese medicine say that color and smell are two of the most important ways to identify things at a macro level.4 But there has been a lack of specific scientific experimental evidence and just nebulous descriptions of these elements up to now. We have developed a colorimeter and an electronic nose (E-nose) to assess the color and odor of CHMs, respectively. As far as experimental data go, these two methods are fast, practical, and dependable. In contrast to other contemporary methods for determining CHM quality, such as gas chromatography (GC), mass spectrometry (MS), and high performance liquid chromatography (HPLC), the E-nose and colorimeter necessitate minimal sample volume, do not use organic reagents, and necessitate only basic sample pretreatments. Much more essential than just providing the contents of one or more components, they provide a complete picture in terms of color and scent. Food, agriculture, medicine, the environment, and the military have all seen increased usage of the E-nose and colorimeter in recent years.24 They have also been used to differentiate between CHMs of the same family25 and distinct varieties26, as well as between CHM samples from various locations27, with varying processing specifications28, and harvested at different times 29 based on odor or color traits.
The current research is focused on creating a new way to quickly, precisely, and thoroughly assess the quality of Hou Po. In order to distinguish the Hou Po samples originating from various locations, we will utilize the E-nose and colorimeter to detect their aroma and color. The next step is to create discriminant models that use various techniques to determine the chemical component contents of the Hou Po samples and their origin.

Materials and methods

Chemicals and reagents

The following sources were used: honokiol (batch number T28O6B5149), magnolol (batch number KS0912CB14), and magnocurarine (batch number M25J9S66499) as reference standards; magno⇘orine (batch number 3536) as a standard from Shanghai Standard Technology; analytical grade methanol (from Beijing Chemical Works, Beijing, China); and acetonitrile and methanol (from Fisher Scientific, Fair Lawn, NJ) as HPLC

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grade and analytical grade, respectively. A super water purification technology was used to obtain ultrapure water.

The following provinces contributed to the 246 batches of Hou Po: Zhe-jiang (51 batches), Sichuan (88 batches), and Hubei (107 batches). Professor Yaojun Yang of the Beijing University of Chinese Medicine's Department of Chinese Materia Medica (Beijing, China) verified the authenticity of all samples.

E-nose analysis

In this investigation, the scents of the Hou Po samples were analyzed using the a-Fox3000 E-nose system (Alpha M.O.S., Toulouse, France). An HS-100 autosampler, a detector unit with a set of twelve chemical sensors of the metal oxide semiconductor type, and pattern recognition software (Alpha Soft V11) were the three primary components of the system. The twelve metal oxide sensors were assigned the numbers S1 through S12. The sensors were LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, T30/1, P10/1, P10/2, P40/1, T70/2, and PA/2, in that order. Relative change in resistance (DR/ R0) was used to represent the sensor responses. After grinding the Hou Po samples into a powder and passing them through a 50-mesh filter, 0.3 g was precisely weighed and sealed in a

ten milliliter vial. A vial containing 1000 mL of headspace air was fed into the detector unit for examination by the sensor array after being incubated at 40 ◦C for 240 s at a rotating speed of 250 r/s. The injection rate was 1000 mL/s and the temperature was set at 50 ◦C.
A constant flow of 150 mL/min of clean air was maintained through the sensor chambers during the measurement procedure, serving as the carrier gas. The sensor data was captured for 120 seconds, with 1 second intervals. To make sure the sensor response values were back to their baseline before smelling the next sample, the 600-second purge time was enough. We measured each sample six times.
All twelve sensors typically respond in the same way for a Hou Po sample, as seen in Fig. 1. The signal from a single sensor to a Hou Po sample as a function of time is shown by each curve. The reaction intensity is shown vertically on the x-axis, while the horizontal axis represents the time up to 120 s. The study's analysis index was determined by taking the greatest response from each sensor.

Colorimeter analysis

In order to identify the color properties of the Hou Po samples, a Hitachi U-3010 ultravioletevisible spectrophotometer was used. A custom-built button-shaped glass color measurement plate, color analysis software, a white calibration plate, an integrating sphere, and other components made up the equipment. The uniform color space system employed in this work is the CIE 1976 L*a*b* (Fig. 2). The axes L*, a*, and b* are orthogonal in this color space. A greater number for the L* value suggests a brighter light.

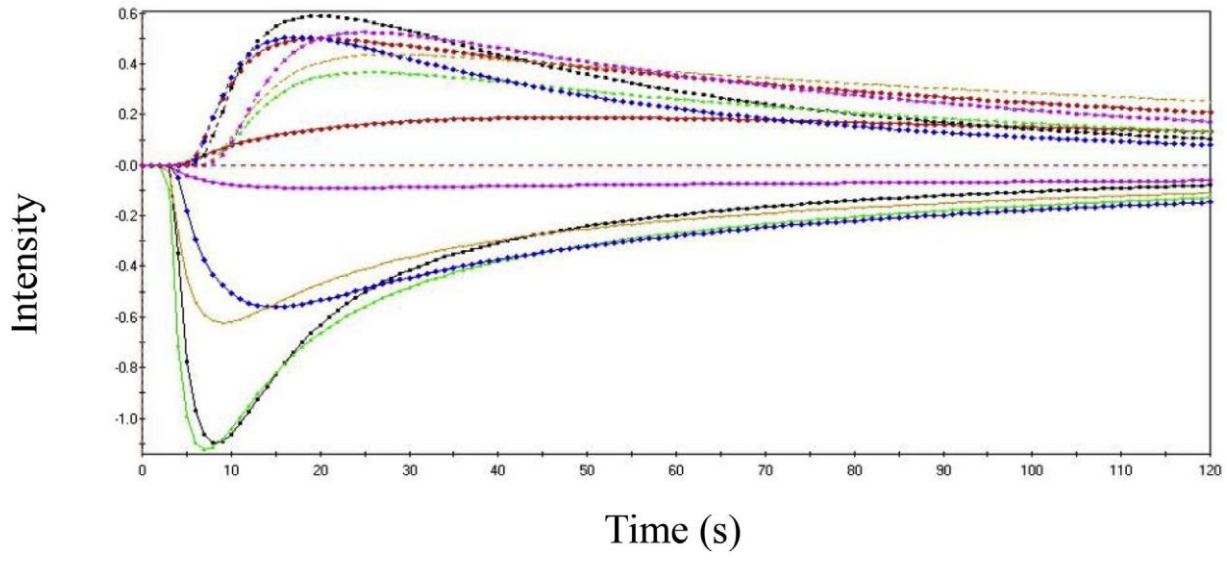

Fig. 1. A typical response of 12 sensors measuring of a Hou Po sample.

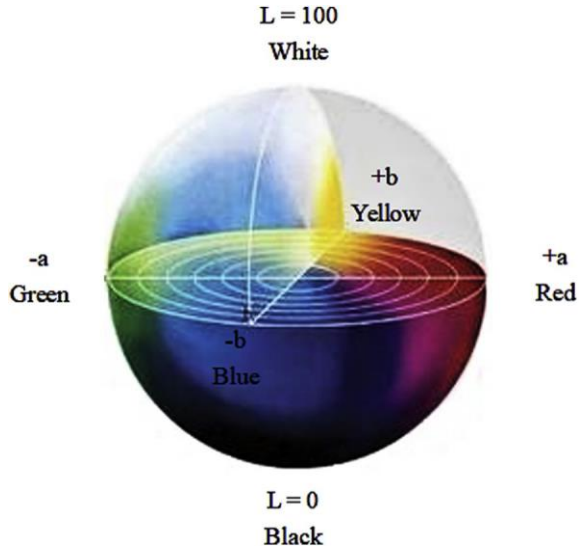

Fig. 2. CIE 1976 L*a*b* uniform color space system.

example color that is lighter in hue and deeper in shade for values that are lower. With a* indicating red, -a* green, b* yellow, and -b* blue, the corresponding tone directions may be represented by the a* and b* values correspondingly. The visible color difference between two color points is shown by the term DE*ab, which is defined as the distance between two points in the color space divided by the product of (DL*)2, (Da*)2, (Db*)2}1/2.

For color determination, the Hou Po samples were crushed into a powder and passed through a 50-mesh filter. They were then deposited onto a glass color measurement plate fashioned like a button. Here are the measuring conditions: The settings for the experiment were as follows: starting wavelength was 780 nm, ending wavelength was 380 nm, slit width was 1 nm, light source was D65, field of vision was set at 10◦, and the scanning at a rate of 600 nm/min. We measured each sample six times.

Determination of magnolol and honokiol

Magnolol and honokiol concentrations were determined by high-performance liquid chromatography (HPLC) as follows: After soaking at room temperature for 24 hours, 0.2 g of 50-mesh sample powder was added to 25 mL of methanol in a conical flask. Just after filtration, 5 mL of filtrate was carefully measured and transferred to a 25 mL volumetric flask. The flask

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was then filled with methanol until it reached a volume of 25 mL, and the mixture was mixed. To prepare the sample solution for high-performance liquid chromatography (HPLC) analysis, this mixture was passed through a 0.22-mm filter membrane.
Using a mobile phase consisting of methanol (78%) and water (22%), a flow rate of 1 mL/min, a column oven temperature of 30 ◦C, an injection, and an Agilent ZORBAX SB-C18 column (4.6 250 mm, 5 mm) from Agilent Technologies Inc. in Santa Clara, CA, the HPLC Agilent system was assembled.

has a detection wavelength of 294 nm and a volume of 5 mL.
To get concentrations of 0.162 and 0.3 mg/mL, respectively, of honokiol and magnolol, the standards were weighed and dissolved in methanol. Analyzing the standard solutions throughout a range of concentrations for honokiol and magnolol, respectively, from 0.162 to 0.000405 and 0.3 to 0.0015 mg/mL, was done to test the linearity of this approach.

Magnocurrine and magnesium determination

We used ultra-performance liquid chromatography (UPLC) to identify the primary alkaloid components of Hou Po, magnocurarine and magnofactorine. After grinding, the Hou Po samples were passed through a 50-mesh filter for analysis. After that, exactly 0.2 g of powder was measured and added to a 50 mL conical flask containing 25 mL of methanol. The flask was then allowed to soak at room temperature for 24 hours. Once the filtration process was complete, the sample solution for UPLC analysis was isolated by passing it through a 0.22-mm filter membrane. This investigation made use of a Waters Acquity UPLC BEH-C18 column (2.1 50 mm, 1.7 mm), an Empower 3 workstation, and a PDA detector from Waters Corp. in Milford, MA. The mobile phase consisted of (A) phosphoric acid/water (0.2:100, v/v) and (B) acetonitrile. The elution was programmed as follows: 0e2 min (9%e11% B), 2e5 min (11%e12% B), and 5e7 min (12%e15% B). The flow rate was 0.4 mL/min. The column oven temperature was 35 ◦C. The injection volume was 0.5 mL. The detection wavelength for magnocurarine was 282 nm, and for magno⇘orine it was 268 nm.
With careful weighing, the magnocurarine and magno* fluorine standards were dissolved in methanol to concentrations of 0.512 and

0.508 mg/mL, correspondingly. Analyzing the reference solutions across a range of 0.0512 to 0.000512 mg/mL for magnocurarine and from 0.0508 to 0.0001016 mg/mL for magnoЬorine allowed us to test the linearity of this approach.

Determination of water-soluble extracts

After combining 4 grams of 50-mesh sample powder with 100 milliliters of water in a 250 milliliter conical flask, the mixture was allowed to soak for 24 hours at room temperature. Following filtering, precisely 20 mL of filtrate was added to an evaporation pan that had been dried to a consistent weight. The pan was then steamed in a water bath until completely dry. Afterwards, the evaporation pan was dried in a dryer at 105 ◦C for three hours, and subsequently, it was chilled in a desiccator for thirty minutes. At last, the evaporation pan was weighed with great precision. One way to measure the concentration of water-soluble extracts was to compare the pre- and post-weight of the evaporation pan.

Statistical analysis

First, the E-nose and colorimeter results were processed using ten classifiers from the Weka software (https://www.cs.waikato.ac.nz/ml/weka/). These classifiers include Bayes Net, Naive Bayes Net, Naive Bayes Updateable, LibSVM, Logistic Analysis, Multiple Layer Perception, RBF Network, NB Tree, Random Forest, and Random Tree. The goal was to create discriminant models that could distinguish the origin places of the Hou Po samples. We used the classification accuracy as our metric to assess these models. To get the classification accuracy,

we used both the external test set verification technique and the ten-fold cross-validation approach. The external test set verification approach used a test set that included 30% of the whole data set. If the classification accuracy was below 80%, the findings would not be taken into consideration.

To create discriminant models for predicting the quantities of associated chemical components: hon-okiol, magnolol, magnocurarine, magnoorine, and water-soluble extracts, the data set was analyzed using the Random Forest classifier from the Weka program. Use the following metrics: root mean squared error (RMSE), mean absolute error (MAE), and correlation coefficient (CC) to assess the quality of the existing models

accuracy in classification. Classifiers LibSVM, Logistic Analysis, Multiple Layer Perception, NB Tree, Random Forest, and Random Tree demonstrated the required degree of feasibility and veracity in identifying the origin places of Hou Po using E-nose and colorimeter data, as shown in the classification accuracies (Table 1). With a maximum classification accuracy of 99.53%, the Random Forest classifier in conjunction with the ten-fold cross-validation approach provided the best classification. In order to proceed with the studies, the Random Forest classifier was paired with the ten-fold cross-validation approach.

(RMSE), relative absolute error (RAE), and root relative squared
error (RRSE) were used as the index. The ten-fold cross-validation method was applied to obtain the prediction Results

Classification results of discriminant models from different originplaces using ten classifiers

Ten different types of classifier were used to obtain precise

Table 1
Classification accuracies of origin place discriminant models from ten classifiers.

Classifiers	Classification accuracy (%)

Prediction results of chemical components discriminant models based on the Random Forest classifier combined with the ten-foldcross-validation method

The water-soluble extracts, honokiol, magnolol, magnocurarine, and magnoflorine were analyzed using the Random Forest classifier. In Table 2 you can see the accuracy of the predictions. With CC, MAE, RMSE, RAE, and RRSE values of 0.9781, 0.1690, 0.3311, 14.6798, and 21.7669, respectively, for honokiol, it was determined that the selected method—the Random Forest classifier coupled with ten-fold cross-validation—could forecast the concentrations of the five component chemicals of Hou Po. For a more natural comparison between expected and experimental results, see Fig. 3. As the data points go closer to the line indicating complete agreement in the scatter plots, the anticipated and experimental values become much more comparable. The most highly correlated of these five component prediction models was the one for water-soluble extracts. Magnocurarine and magnoflowerine models were quite similar.

When it came to determining the Hou Po samples' origins and chemical makeup, the existing models based on their smell and color offered a quick and dependable way to accomplish so.

Table 2
Prediction accuracies of discriminant models for the five chemical components of
Hou Po based on the Random Forest classifier combined with the ten-fold cross-validation.

Chemical components	Values				
	CC	MAE	RMSE	RAE (%)	RRSE (%)
Honokiol	0.9781	0.1690	0.3311	14.6798	21.7669
Magnolol	0.9737	0.2017	0.3666	18.8258	24.4372
Magnocurarine	0.9633	0.0109	0.0193	21.0594	28.3004
Magnoflorine	0.9663	0.0119	0.0209	19.4218	26.9892
Water-soluble extracts	0.9818	0.3770	0.5914	15.4062	20.2298

Abbreviations: CC: correlation coefficient; MAE: mean absolute error; RMSE: root mean squared error; RAE: relative absolute error; RRSE: root relative squared error.

accuracy.

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	Ten-fold cross-validation	External test set verification
Bayes Net	77.24	77.88
Naive Bayes Net	72.63	72.69
Naive Bayes Updateable	72.63	72.69
LibSVM	91.53	90.07
Logistic Analysis	92.68	92.55
Multiple Layer Perception	99.05	97.29
RBF Network	79.61	76.52
NB Tree	97.36	95.94
Random Forest	99.53	99.32
Random Tree	97.36	94.81

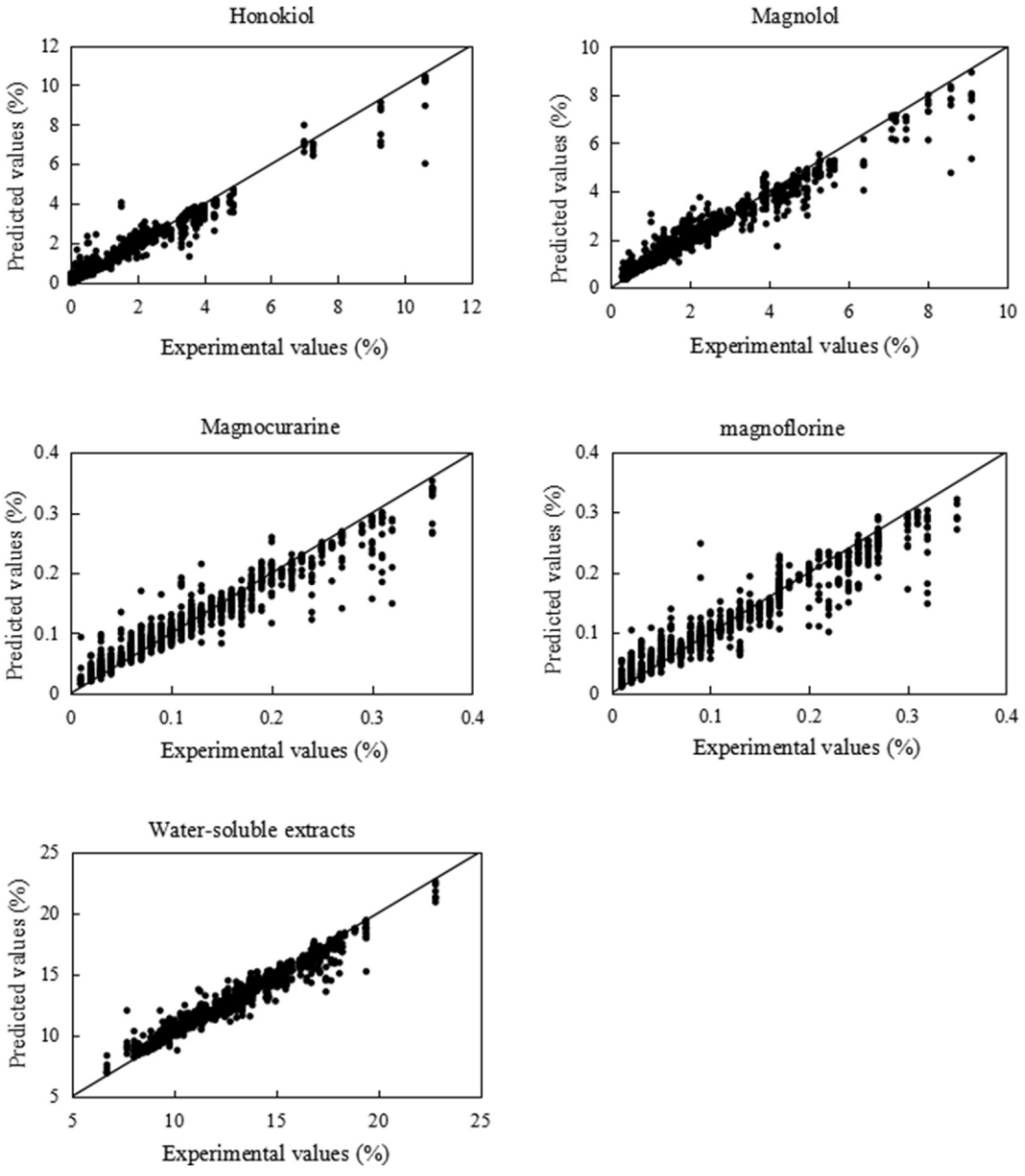

Fig. 3. Comparison between the predicted and experimental values of five chemical components of Hou Po.

Discussion

The classic technique of CHM authentication, macroscopic identification, has the benefits of being quick, easy, and effective.4,30 This approach is a crucial part of assessing the quality of CHM in the People's Republic of China Pharmacopoeia. The method's shortcomings, however, cannot be disregarded: it is very subjective and relies heavily on the assessors' own experiences. We have explored the potential of the E-nose and the colorimeter to measure the odor and color properties of CHMs, in an effort to circumvent these limitations. By combining these two methodologies with suitable statistical tools, the experimental findings demonstrate that a system based on instrumental sensory analysis can be set up to assess the quality of CHMs in a thorough manner.31, 32 Both the scientific rationale for macroscopic identification and a novel approach to creating an easy, quick, and systematic approach of assessing CHM quality. The exterior manifestations of the internal chemical components of CHM, including its smell and color, are extensive. Further study is needed to understand the link between the instrumental sensory features and interior chemical components of CHMs, since there is less research on the material basis of their odor and color characteristics.

Not only does a CHM's chemical makeup determine its therapeutic efficacy, but it also

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serves as a key quality indicator. Plants' chemical make-up varies depending on a variety of environmental and geographical variables at their point of origin, including but not limited to: height, terrain, soil, water quality, weather, sunlight, and rainfall. Consequently, CHM quality is strongly correlated with its area of origin and chemical components. Here, we use the E-nose and colorimeter to reliably identify where Hou Po come from and to forecast what kinds of chemicals are in them. rapidly. Further investigation into the relationship between quality, provenance, and chemical components is required, however E-nose and colorimeter technology do provide new ways to assess Hou Po's quality. Aside from instrumental sensory analysis, a discriminant model should be developed to predict the concentrations of additional chemical components, as well as to identify variations, specifications, and grades of Hou Po. This will help to augment and enhance the current method of quality assessment.Volatile oils, lignans, alkaloids, and phenylethanolic glycosides are the four primary types of compounds found in Hou Po. Only the contents of the last three chemical components have been predicted in this investigation using Hou Po's odor- and color fingerprints. The fragrant scent of Hou Po comes from its volatile components, which the E-nose may interact with to measure. In this way, they link the E-nose reaction to the scent of Hou Po. This further highlights the need of doing more investigations to identify the specific volatile components responsible for the E-nose reactions and how they relate to the Hou Po odor. Further investigation into the link between these factors and Hou Po quality is required. When utilizing the E-nose to assess the quality of Hou Po, volatile components are crucial. Detailed descriptions of future study findings pertaining to volatile components will be provided in subsequent studies.

Conclusions

Three Chinese provinces—Zhejiang, Sichuan, and Hubei—provided 246 batches of Hou-Po samples for this investigation. Specific procedures were used to determine the amounts of various chemical components, such as honokiol, magnolol, magnocurarine, magnolorine, and water-soluble extracts. Using the E-nose and the colorimeter, we were able to identify the smell and color features of the samples, which helped us design a quick and dependable method to thoroughly assess the quality of the Hou Po. Using a variety of statistical methods on this data, we were able to construct multiple discriminant models that could identify the locations of Hou Po's origin and provide predictions about the chemical components it contains. Based on the findings, it is feasible to accurately determine the source locations and forecast the associated chemical components by analyzing the smell and color of samples in conjunction with suitable statistical algorithms. The most accurate model was the one that used Random Forest classifier in conjunction with ten-fold cross-validation. Both the E-nose and the colorimeter have showed promise in this research as tools for quantitative and qualitative evaluation of CHM quality. Researchers are now working on a more sensitive and selective sensor for the volatile components of CHM, as well as more efficient feature extraction approaches, with the goal of improving the discriminant models.

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