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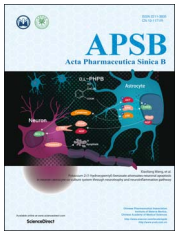

ORIGINAL ARTICLE

A novel quantified bitterness evaluation model for traditional Chinese herbs based on an animal ethology principle

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Abstract Traditional Chinese herbs (TCH) are currently gaining attention in disease prevention and health care plans. However, their general bitter taste hinders their use. Despite the development of a variety of taste evaluation methods, it is still a major challenge to establish a quantitative detection technique that is objective, authentic and sensitive. Based on the two-bottle preference test (TBP), we proposed a novel quantitative strategy using a standardized animal test and a unified quantitative benchmark. To reduce the difference of results, the methodology of TBP was optimized. The relationship between the concentration of quinine and animal preference index (PI) was obtained. Then the PI of TCH was measured through TBP, and bitterness results were converted into a unified numerical system using the relationship of concentration and PI. To verify the authenticity and sensitivity of quantified results, human sensory testing and electronic tongue testing were applied. The quantified results showed a good discrimination ability. For example, the bitterness of Coptidis Rhizoma was equal to 0.0579 mg/mL quinine, and Nelumbinis Folium was equal to 0.0001 mg/mL. The validation results proved that the new

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assessment method for TCH was objective and reliable. In conclusion, this study provides an option for the quantification of bitterness and the evaluation of taste masking effects.

1. Introduction

The popularity of herbal medicines is currently rising worldwide. However, the unpleasant taste of these substances is a common issue and is to some degree impeding the progress of ongoing efforts to give herbal medicines wider applications[1]. Among the offensive tastes, bitterness is one of the most prominent and can severely influence the compliance of patients, especially among infants and children[2,3]. To develop effective taste masking strategies, it is essential to evaluate the bitterness objectively[4]. Our predecessors created a simple classification system for the bitter traditional Chinese herbs (TCH) that depended on sensory evaluation and included the following categories: strongly bitter, moderately bitter, slightly bitter, and not bitter. However, their evaluation methods are not exact, and they became increasingly unable to meet the demands of modern accurate quantification.

After decades of experimenting, several taste evaluation methods have been established[5]. The classic one is the human sensory test. It reflects genuine human perception, but is constrained by experience, identification ability, and individual differences. At the same time, the toxic or potentially hazardous substances in TCH make it difficult to conduct the experiments ethically. In general, this method is inconvenient, time consuming and has a low throughput[6]. To overcome these deficiencies, animal behavioral assays coupled with statistical methods, such as the animal preference test[7] and the conditioned taste aversion tests[8], were explored to evaluate the animals' degree of distaste. However, the assessment was comparative. It could not reflect the overall level of distaste, and animal individual differences could not be ignored. A third method is the chemical evaluation method, in which the dissolution profile of bitter compounds could directly represent the bitterness intensity[9–11]. However, it was unsuitable for TCH because of the complexity and uncertainty of the bitter ingredients[12]. The last method is a modern bionics evaluation represented by the electronic tongue (e-tongue)[13]. This method of detection is sensitive and fast, and even tiny differences can be resolved. However, it requires the sample to be controlled at a lower concentration, and the consistency of the evaluation results between the electronic tongue and real perception is still controversial. Through the analysis of these methods, it was found that they have become more precise over time; whereas there has been no investigations of improved precision in human bitterness detection. It seemed clear that further studies were needed to balance sensitive discrimination and genuine taste perception.

Animals tend to pursue their own interests and avoid risks. Accordingly, the two-bottle preference test (TBP) was designed to assess the unpleasant taste of food or beverage[14], and the preference index (PI) was used as an evaluation indicator[15]. The experiment was performed by observing the preferences and dislikes in the animals' drinking habits. Rats are frequently chosen as test subjects, because rodents have bitter taste receptors that are highly homologous to those of humans and thus have a similar sense of taste[16]. Additionally, the individual differences existing among the rats can be decreased by observing them in large numbers and using a statistical approach. In addition, the bitterness of TCH needs to be fully and integrally characterized, as their components are complex. Therefore, a TBP at an integral level is suitable for TCH. However, there still are lots of challenges since the TBP has not been previously reported with TCH. Since TBP results are relative, and TCH are numerous, there needs to be a unified index and evaluation standard. To achieve the requirement of TCH, the standardization of an animal model, the determination of an evaluation index, the development of a quantification method, and the reliability of quantified results for realistic and sensitive discrimination in the TBP should be optimized.

In this study, the methodology of the animal model was optimized first. Quinine, which is recognized as a standard substance in the study of bitterness perception[17], was selected as the quantified standard. Then, according to the classifications in the *Chinese Pharmacopoeia*[18], the PI of 12 types of TCH, without other tastes, from different bitterness levels was obtained by the optimized method. After that, the bitterness of TCH was quantified based on the standard concentration–PI equation of quinine and the PI of TCH was expressed as the amount of quinine. Finally, the quantified result was verified by human sensory test to get a real sense *in vivo* and e-tongue for sensitive discrimination *in vitro*. At the end of the study, the bitterness of TCH was converted to a united standard based on its biological assessment. This standard could provide a method for an exemplification of the sensory evaluation of natural medicines, and could be a potential tool for taste masking study of bitter TCH preparations.

2. Materials and methods

2.1. Ethics statement

This study was conducted in strict accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. The protocol and experimental designs were approved by the Ethical Committee of Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (Approval ID: 2014KL-016). All animals remained healthy throughout the experiment, and all possible steps were taken to avoid the animals' suffering at any stage of the experiment. Volunteers were given written informed consent regarding the purpose of the study and their right to keep information confidential. Informed written consent was obtained from all participants.

2.2. Chemicals and animals

TCH were purchased from Rongle Pharmacy (Chengdu, China) and identified by Professor Runchun Xu of Chengdu University of

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