

A Novel Quantitative Prediction Approach for Astringency Level of Herbs Based on an Electronic Tongue

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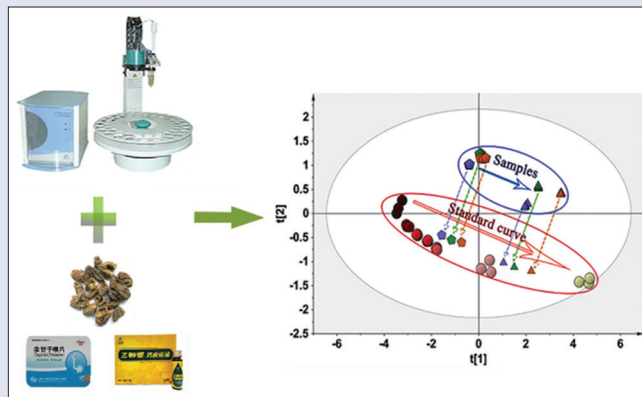
ABSTRACT

Background: The current astringency evaluation for herbs has become dissatisfied with the requirement of pharmaceutical process. It needed a new method to accurately assess astringency. **Methods:** First, quinine, sucrose, citric acid, sodium chloride, monosodium glutamate, and tannic acid (TA) were analyzed by electronic tongue (e-tongue) to determine the approximate region of astringency in partial least square (PLS) map. Second, different concentrations of TA were detected to define the standard curve of astringency. Meanwhile, coordinate-concentration relationship could be obtained by fitting the PLS abscissa of standard curve and corresponding concentration. Third, *Chebulae Fructus* (CF), Yuganzi throat tablets (YGZTT), and Sanlejiang oral liquid (SLJOL) were tested to define the region in PLS map. Finally, the astringent intensities of samples were calculated combining with the standard coordinate-concentration relationship and expressed by concentrations of TA. Then, Euclidean distance (E_d) analysis and human sensory test were processed to verify the results. **Results:** The fitting equation between concentration and abscissa of TA was $Y = 0.00498 \times e^{(-X/0.51035)} + 0.10905$ ($r = 0.999$). The astringency of 1, 0.1 mg/mL CF was predicted at 0.28, 0.12 mg/mL TA; 2, 0.2 mg/mL YGZTTs was predicted at 0.18, 0.11 mg/mL TA; 0.002, 0.0002 mg/mL SLJOL was predicted at 0.15, 0.10 mg/mL TA. The validation results showed that the predicted astringency of e-tongue was basically consistent to human sensory and was more accuracy than E_d analysis. **Conclusion:** The study indicated the established method was objective and feasible. It provided a new quantitative method for astringency of herbs.

Key words: Astringency, electronic tongue, Euclidean distance analysis, human sensory test, quantitation

SUMMARY

- The astringency of *Chebulae Fructus*, Yuganzi throat tablets, and Sanlejiang oral liquid was predicted by electronic tongue
- Euclidean distance analysis and human sensory test verified the results
- A new strategy which was objective, simple, and sensitive to compare astringent intensity of herbs and preparations was provided.



Abbreviations used: CF: *Chebulae Fructus*; E-tongue: Electronic tongue; Ed: Euclidean distance; PLS: Partial least square; PCA: Principal component analysis; SLJOL: Sanlejiang oral liquid; TA: Tannic acid; VAS: Visual analog scale; YGZTT: Yuganzi throat tablets.

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INTRODUCTION

Astringency is generally recognized as a complex group of sensations involving roughness, dryness of oral surfaces and tightening, puckering sensations of the mucosa and muscles around the mouth.^[1-3] It is a tactile sensation and not produced through taste buds.^[2,4,5] Polyphenols are mainly responsible for the astringency of many beverages and fruits (especially unripe), such as tea,^[6] coffee,^[6] wine,^[7] aronia berry juice,^[8] and persimmon.^[9] Meanwhile, they also exist in many herbs.^[10,11] Astringency is not only a tactile sensation but also a special flavor. Whether astringency expresses a flavor or an unpleasant sensation depends on two aspects. The first one is the astringent intensity of samples; the other is the sensitivity of human feelings. The former is objective while the latter is varying. Thus, it is essential to define the degree of astringency.

Currently, the main methods for astringent measurement are human sensory evaluation^[12] and salivary proteins–polyphenols interactions.^[13,14]

Sensory analysis is an experimental method of behavioral psychology based on the comparison of difference among samples. It is highly empirical, cumbersome, and time-consuming. Moreover, it exists errors as astringency perception varies greatly among individuals.^[15] It is mainly used for qualitative classification and semi-quantification. To solve the quantitative problem, gelatin index^[16] based on salivary

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proteins–polyphenols interaction is proposed to estimate astringency. It represents the ability of tannins to precipitate a highly diverse family of collagen-derived proteins or gelatins. However, that method is only an estimation of astringency. Its inaccuracies derive from variations in both the extent of tannin hydrolysis and the variable composition of gelatins among the different commercial products.^[17] Therefore, gelatin index is inconsistent with astringency scores sometimes. Thus, a more objective evaluation method of astringency should be established.

By imitating the human way of sensing and perceiving, electronic tongue (e-tongue) emerged. It generates an electrical signal through sensor array, and data are processed by a computer pattern recognition system so that the unique taste information of sample can be reflected.^[18] E-tongue is more rapid and impartial than human-based tasting panels. It is also highly sensitive, cost-efficient, and reproducible.^[19] It has been used in the research of preliminarily discrimination of astringency.^[20,21] Hayashi *et al.*^[22] confirmed that the result of e-tongue was consistent with human sense in the astringent evaluation of black tea. Apetrei *et al.*^[23] found that electrochemical signals have been successfully employed to estimate chemical parameters related to the polyphenol content or the pH such as tannins content. Current e-tongue research could distinguish and identify the basic taste but is difficult to quantify the difference of samples. Although some researchers have proposed to use Euclidean distance (E_d) to characterize the relative difference in the bitter masking evaluation, it is unsuitable for the evaluation of taste intensity of plentiful samples.^[24,25]

As far as we known, the application of e-tongue in astringency mainly focused on tea and red wine, less involved in herbs and preparations. Moreover, whether e-tongue could be used in quantification of astringency was also a challenge. Therefore, we hoped to establish a method to quantify astringency of herbs based on positioning standard material in this paper it was shown in [Figure 1]. First, quinine, sucrose, citric acid, sodium chloride, and monosodium glutamate which, respectively, represent bitter, sweet, sour, salty, and umami were analyzed by e-tongue to define the basic taste distribution.^[26] Tannic acid (TA) which has been used as a model astringent^[27] in many studies was measured to determine the approximate region of astringency through partial least square (PLS). Second, different concentrations of TA were detected to define the standard curve of astringency. Meanwhile, coordinate-concentration relationship could be obtained by fitting the PLS abscissa of standard curve and corresponding concentration. Third, *Chebulae Fructus* (CF), Yuganzi throat tablets (YGZTT), and Sanlejiang oral liquid (SLJOL) were selected as the research objects and tested to define region in PLS of e-tongue. YGZTT consists of *Phyllanthi Fructus*, borneol, and menthol. SLJOL contains *Phyllanthi Fructus*, CF, and other accessories. Finally, the astringent intensities of samples were calculated combining with the astringent coordinate-concentration relationship and expressed by the concentration of TA. Moreover, E_d analysis which was commonly used to evaluate taste and human sensory test were processed to verify the results. It indicated that the predicted astringency of e-tongue was basically consistent to human sensory. Therefore, the established method to quantify astringency was objective and feasible. It provided a new quantitative method for astringency of herbs. Moreover, predicted results of this method were more accuracy than E_d analysis. Thus, it provided a new idea for astringent evaluation of herbs to use the method established in this paper to replace E_d analysis.

Materials and reagents

Citric acid (No. 20120331), sucrose (No. 20081113), sodium chloride (No. 20130330), TA (No. 2015113001), and casein (No. 20071221) were purchased from Chengdu Kelong Chemical Reagent Co., Ltd. (Chengdu, China). Monosodium glutamate (No. H31A6R2860)

and quinine sulfate (No. YM0317BA14) were purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals used were of analytical grade and available locally.

CF was purchased from Beijing Tongrentang Pharmacy and identified by Prof. Xianming Lu of Chengdu University of Traditional Chinese Medicine as the fruit of *Terminalia chebula Retz.* YGZTT (No. 150102) was purchased from Chengdu Huasun Group Co., Ltd. SLJOL (No. 1403003) was purchased from Chengdu Sanlejiang Pharmaceutical Group.

METHODS

Electronic-tongue test

Analysis method

The samples were measured by a sensor-based system, ASTREE II e-tongue system (Alpha M.O.S., Toulouse, France) equipped with seven liquid cross-selective sensors (ZZ, AB, GA, BB, CA, DA, and JE). The response intensity of each sensor was measured with an Ag/AgCl reference electrode. The potentiometric differences between each coated sensor and the reference electrode contribute to the intensity value of the measured samples.^[28] The acquisition time was fixed at 120 s.^[29] Sensors were rinsed with distilled water between each measurement. Measured data were recorded and analyzed by AlphaSoft Software (Alpha MOS, Toulouse, France). Each sample was replicated 10 times, and only the 8th to 10th datasets were taken into account for the statistical treatment.

Preparation of samples

To study the applicability of the quantified method on different astringency level, samples with different concentration were prepared.^[30] One gram CF was accurately weighed and immersed in 1000 mL of purified water for 0.5 h and then decocted for 1 h. Solution was filtered and diluted with water to 1, 0.1 mg/mL (quantified by crude drug). 0.1 g YGZTT was accurately weighed and dissolved in purified water and diluted to 2, 0.2 mg/mL. 0.1 mL SLJOL was weighed and dissolved in purified water and diluted to 0.002, 0.0002 mL/mL. TA was dissolved in purified water and diluted to 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/mL. Each solution was then filtered through 0.45 μ m nylon membrane filters.

Quantitative determination of astringency

Locating of astringency

Quinine, sucrose, citric acid, sodium chloride, monosodium glutamate, and TA were weighed out and, respectively, dissolved in purified water to 0.5 mg/mL. Five gustatory standard substances and TA solution were detected by e-tongue. PLS was used to determine the approximate distribution region of astringency.

Standard curve of astringency

4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/mL TA solutions were measured by e-tongue to establish coordinate-concentration relationship. Standard curve of astringency could be gotten by fitting the PLS abscissa and respective concentration.

Determination of samples

Sample solutions were measured by e-tongue.

Quantification of astringency

Data obtained by e-tongue were first normalized by SPSS 22.0 package (SPSS Inc., Chicago, IL, USA). SIMCA-P 11.0 version (Umetrics AB, Umea, Sweden) was used to carry on PLS and principal component analysis (PCA). Then, Origin 9.0 (OriginLab, Hampton, Massachusetts,

USA) was used to fit the concentration and the abscissa space of TA in PLS. At last, PLS abscissa space of samples was substituted into the equation of standard curve, and the relevant concentration of TA could be converted. Astringency of samples can be uniformly quantified by TA.

Validation based on Euclidean distance analysis

E_d was the true distance between two points in the dimension space. It was commonly used to predict the taste masking efficiency of formulations by e-tongue.^[25,26,31] It could be calculated by the center coordinates of each sample on the PCA map.

$$E_d = \sqrt{\sum_{i=1}^k (x_i - y_i)^2}$$

Where k represented the number of variables of each sample, x_i represented the value of variable i of the first sample, and y_i represented the value of variable i of the second sample. The shorter the E_d , the similar the astringent intensity. In this paper, E_d represented the distance between each sample and TA at 4 mg/mL. It indicated the stronger astringency while E_d was smaller.

Validation based on human sensory test

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). Participants were received "written informed consent" on the study's purpose and of their right to keep information confidential. Written consent was obtained from all participants or their guardians.

Results of e-tongue were validated by human sensory test through the visual analog scale (VAS). In the test, all volunteers were asked to score the "astringency" using the 100 mm VAS by placing a mark along a 100 mm line.^[32] Sixteen well-trained and healthy volunteers (half men and women, age 20–26 years) participated in the sensory evaluation. Volunteers were selected from graduate students at Chengdu University of Traditional Chinese Medicine. During training sessions, volunteers were trained with different concentrations of TA solutions (0.0625, 0.125, 0.25, 0.5, 1, 2, 4 mg/mL) to accustom them to evaluation scales and intensity of astringency. After that, samples were evaluated. A drop of approximately 10 mL of each solution was applied on the upper surface of tongue. Then, the test solution was spit out. Moreover, the astringency level of solutions was scored. Between each test interval, the mouth was rinsed well to no astringency with distilled water. Volunteers were given a break between each session.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 package (SPSS Inc., Chicago, IL, USA). Data were reported as mean \pm standard deviation (SD) and as individual values in the figures.

RESULTS

Quantitative astringency

Figure 2a shows the response patterns of 0.5 mg/mL TA. Seven sensors were sensitive and selective. Sensor JE showed a high sensitivity to astringent taste, while sensor DA was contrary. As shown in Figure 2b, the relative standard deviation values of all samples were <3%. It suggested that the assay variation of the sensors was minimal and that reproducible results could be generated.

A PLS map of e-tongue of citric acid (sour), sucrose (sweet), sodium chloride (salt), monosodium glutamate (umami), quinine (bitter), these five basic taste substances, and TA (astringent) is shown in Figure 3a. The cluster of each sample was small, indicating good reproducibility of the analysis. Moreover, a clear discrimination between different samples was observed, determining the location of astringency. The PLS map of samples is shown in Figure 3b. Standard fitting curve of TA is shown in Figure 3c. The fitting equation between concentration and PLS abscissa space of standard curve of TA was $Y = 0.00498 \times e^{(-X/0.51035)} + 0.10905$ ($r = 0.999$); fitting curve is shown in Figure 3c. The unified quantitative

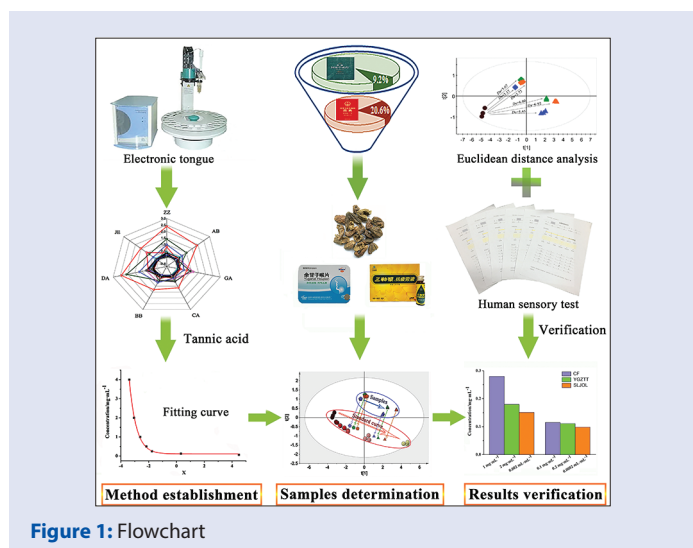


Figure 1: Flowchart

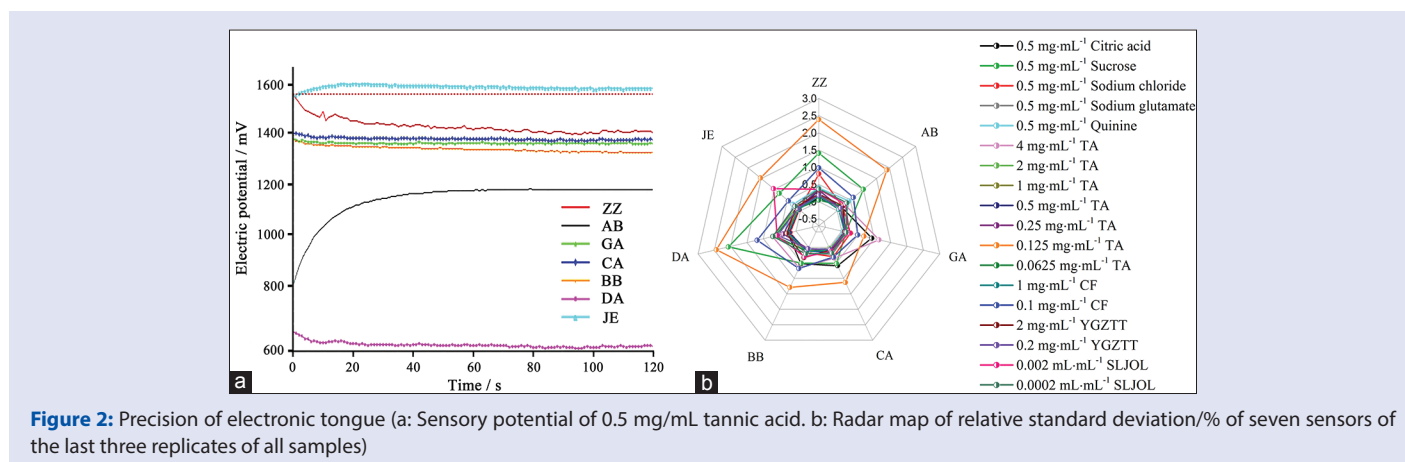


Figure 2: Precision of electronic tongue (a: Sensory potential of 0.5 mg/mL tannic acid. b: Radar map of relative standard deviation/% of seven sensors of the last three replicates of all samples)

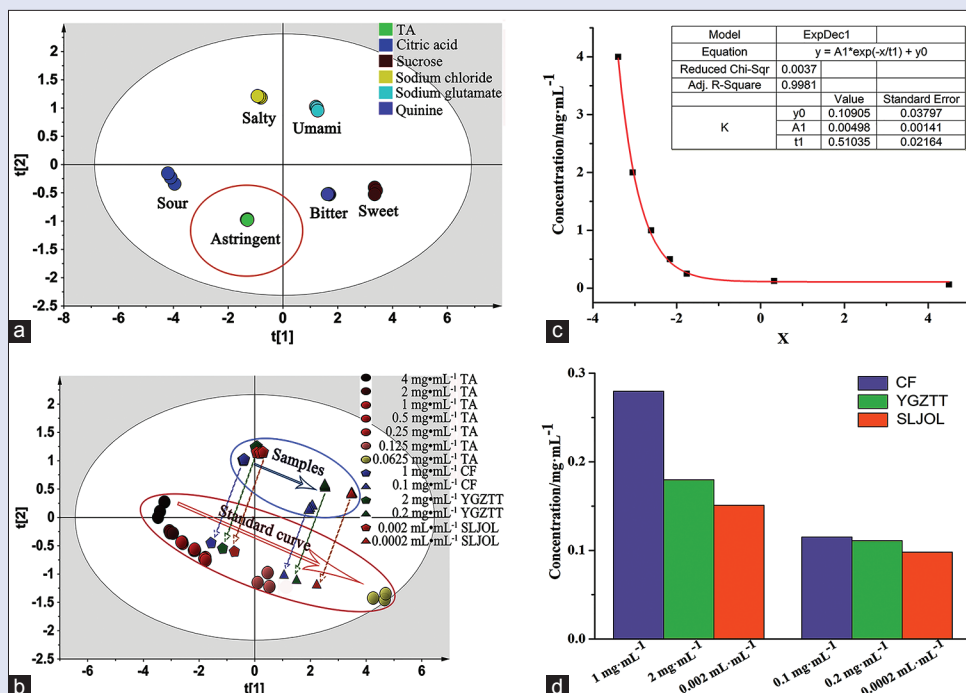


Figure 3: Results of electronic tongue (a: Location of astringency. b: Standard curve of tannic acid, and partial least square map of samples. c: Fitting curve between concentration of tannic acid and partial least square abscissa space. d: Predicted astringent intensity of samples expressed by tannic acid)

results of samples are shown in Figure 3d. Astringent intensity of samples was expressed by corresponding concentration of TA.

Verification results

The results of E_d analysis and human sensory test are shown in Figure 4. To verify the validity of results, correlations were determined with Pearson's coefficients. The correlation coefficient (r) of fitting equation [Figure 5a] between predicted astringency of samples and E_d was 0.7790, and r of fitting equation [Figure 5b] between predicted astringency and human sensory scores was 0.9603. It showed that the predicted astringency of e-tongue was basically consistent to human sensory. Therefore, the established method to quantify astringency was objective and feasible. Moreover, predicted results of this method were more accuracy than E_d analysis as the correlation with human sensory scores was higher.

DISCUSSION AND CONCLUSION

In this paper, method of quantifying astringency of herbs was studied. After verification, a new method to quantify the astringent intensity which was objective, simple, and sensitive was successfully established. It provided a new strategy for quantitative evaluation and comparison of astringent intensity of herbs. Moreover, it may also be feasible for quality control evaluation of herbs containing complex and plenty components by comparing the taste intensity.

Astringency, contributing significantly to the appetitive or aversive character of herbs, was the key factor of affecting taste of medicine and patients' compliance. We counted the "Chinese Pharmacopoeia" of 2015 edition.^[33] It was found that 514 traditional drugs are recorded, and astringent medicine had 106, accounting for 20.6% of total traditional drugs. "National Chinese Traditional Patent Medicine"^[34] was counted containing 7260 kinds of Chinese patent medicine, and 667 astringent drugs, accounting for about 9.2%. However, the current assessment of astringency was semi-quantified, such as very astringent, astringent, and

subastringent. Giving the universality and particularity of astringency in herbs and preparations to consideration, it was necessary to carry out the precise evaluation of astringency of more herbs.

In our previous experiment, tannin content determination^[35] and animal preference test were processed to assess astringency. However, the results were unsatisfied. At first, the correlation between tannin content of herbs and astringent intensity was not good. It suggested that chemical content could not reflect astringency completely. It may be caused by the types and content of polyphenols in the different herbs and preparations.^[36] Polyphenols consisted of combined polyphenol and free polyphenol. The relative molecular mass of combined polyphenol was bigger than free polyphenol, and phenolic hydroxyl of combined polyphenol was more than free.^[37] Therefore, the astringent intensity of combined polyphenol was stronger as it had a higher binding ability with saliva proteins,^[38] for instance, epigallocatechin gallate > epigallocatechin > epicatechin gallate > epicatechin > catechin.^[39,40] Astringent taste was a comprehensive expression of all components. Tannin content could not reflect the real astringency. After that, animal preference test was tried. The correlation between tannin content of herbs and astringent intensity was good, but the sensitivity of rats for lower astringent samples was shortened. E-tongue could reflect the whole and real astringency of substance. Moreover, it was accurate, rapid, and sensitive. Therefore, e-tongue was a better evaluation method for astringency at present.

Bionics technology was adopted in this paper to quantify the astringency. Although it may be some progressive in the objective evaluation of astringency, astringency was a feeling and consciousness produced by the brain. Therefore, it was important to establish an evaluation method to reflect true feelings of brain. An idea was proposed using brain functional test technique to evaluate astringent perception.^[41] Brain functional imaging technology was a real-time, on-line, noninvasive brain imaging method. It was closely linked to brain structure and function and could provide reliable evidence about the basic cognitive

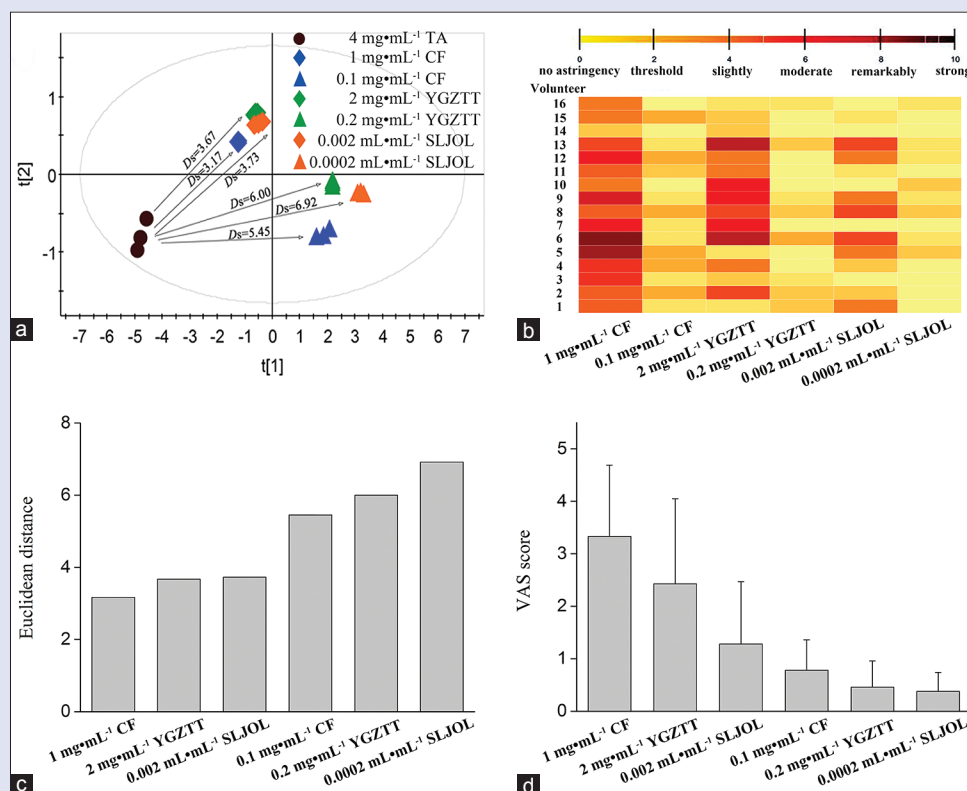


Figure 4: Results of verification (a: Euclidean distance in principal component analysis map. b: Evaluation standard and results of human sensory test. c: Euclidean distance analysis for samples. d: Visual analog scales of human sensory test)

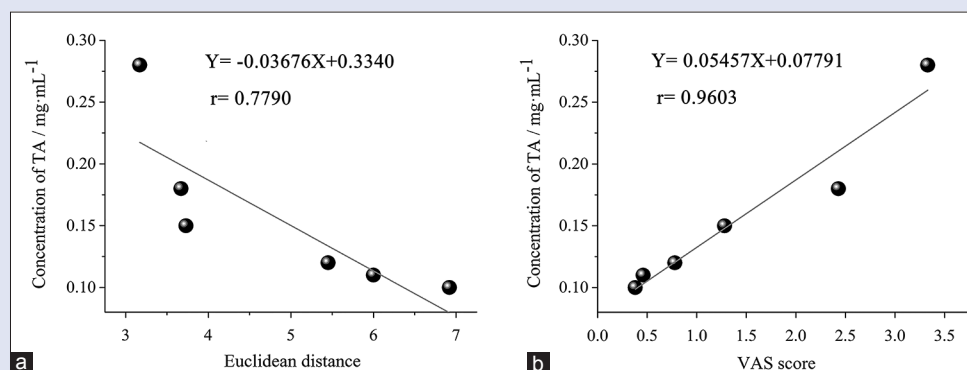


Figure 5: Results of correlation analysis (a: Correlation analysis between Euclidean distance and predicted astringent intensity of samples. b: Correlation analysis between visual analog scale and predicted astringent intensity of samples)

process. It had been widely applied to acupuncture study and become an important means to study the effect of acupoint.^[42] Combined with our research, the intensity of astringency could be shown as the different depth of color of the body feeling in cerebral cortex. Combined with the color quantization method,^[43] true feelings of astringency of people could be converted into a digital express. It would be researched in our further study.

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Conflicts of interest

There are no conflicts of interest.

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