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Article

A novel integrated ESID strategy of critical property-flavor quality attributes of Huangqi Shengmai Yin ☆

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ABSTRACT

There is still a gap between traditional Chinese medicine (TCM) and Western medicine, and it is crucial to build a bridge between them. In this study, taking Huangqi Shengmai Yin as a demonstration, we proposed a novel integrated efficacy-sweetness information discovery (ESID) strategy. Firstly, it was inferred that Huangqi Shengmai Yin possessed the attribute of "sweet", and its sweetness level was determined by intelligent sensory technology. Secondly, the potential property and flavor substances of Huangqi Shengmai Yin were screened based on our T1R2/T1R3-HEMT biosensor combined with UPLC-MS analysis. Finally, the efficacy of the substances was validated through in vitro and in vivo experiments. The results indicated that the sensory taste of Huangqi Shengmai Yin was perceived as sweet, with the sweetness level classified as "slightly sweet". Five potential property and flavor substances of Huangqi Shengmai Yin were discovered, including calycosin, calycosin-7-O-β-D-glucoside, atractylenolide I, ruscogenin and schisantherin A. Surprisingly, it was confirmed that these attributes had strong binding abilities with T1R2 and T1R3, with dissociation constants less than 10^{-8} M. Through in vivo experiments conducted on zebrafish, it was further demonstrated that these attributes had the effects of improving vascular injury and enhancing immunity. In summary, this novel strategy guides the connection between TCM and Western medicine. And it provides a guideline for other TCM research.

1. Introduction

It is a gap between traditional Chinese medicine (TCM) and Western medicine. Based on the physiological cause, Western medicine generally prescribes treatments for specific diseases. However, TCM focuses on symptoms. And plants and animal-based ingredients are often used in TCM products [1]. TCM is a vast treasure trove [2,3]. Chinese government has announced an ambitious plan to modernize the millennia-old practice [4,5].

It is worth mentioning that artemisinin from *Artemisia annua* L. was discovered to be effective in treating malaria [6]. Arsenic has been found to be effective in treating acute promyelocytic leukemia (APL) [7]. Huperzine A from *Huperzia serrata* has been discovered to be used for treating memory dysfunction [8], and taxol from *Taxus brevifolia* has been found to be effective in treating breast cancer [9]. Therefore, it is crucial to fully tap into the treasure trove of TCM and establish a method that serves as a bridge between TCM and Western medicine.

The theory of medicinal properties of TCM is not only a core component, but also an important basis for guiding the compatibility in clinical practice. Among them, the theory of five flavors is one of its core components, which includes sour, bitter, sweet, pungent, and salty flavors. These flavors reflect the therapeutic attributes when applied in clinical application. Therefore, it is of significant importance to establish a bridging method between TCM and Western medicine based on the concept of medicinal properties [10].

Cardiovascular disease is the leading cause of death globally. In recent years, the incidence of cardiovascular disease has been relatively high among the middle-aged and elderly population in China [11–15]. As one of the most common cardiovascular diseases, coronary heart disease (CHD) has a high prevalence rate and should not be ignored.

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The treatment of CHD is one of the most urgent challenges in current clinical practice.

Huangqi Shengmai Yin is derived from the ancient formula Shengmai San, with annual sales exceeding one hundred million yuan. It is composed of Astragali Radix, Codonopsis Radix, Ophiopogonis Radix, Schisandrae Chinensis Fructus and Schisandrae Sphenantherae Fructus, all of which have a sweet taste and medicinal properties. According to TCM theory, the main functions of the sweet taste are nourishing, harmonizing, and alleviating, and nourishing mainly refers to replenishing qi, yang, blood, and yin aspects. The combination of these five herbal medicines works together to exert the effects of tonifying qi and nourishing yin, as well as nourishing heart and replenishing lungs. It is clinically used for patients with CHD who have both qi and yin deficiency, as well as symptoms of palpitations and shortness of breath. However, the material basis of Huangqi Shengmai Yin is not clear.

Intelligent sensory technologies such as electronic tongues are commonly used for the quantification of sensory tastes [16–18]. The combination of sweet-tasting substances in TCM and sweet taste receptors is a prerequisite for their functional effects. Taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3), as sweet taste receptors, are widely distributed in various parts of the body, including the oral cavity, heart, and other areas [19,20]. Biological sensor technology, with its advantages of high sensitivity and accuracy, can be used for the detection of critical quality attributes in TCM [21–24]. UHPLC-LTQ-Orbitrap MS^n, with its characteristics of high resolution and high selectivity, is widely applied in the identification research of chemical components in TCM and complex formulations [25,26].

In this study, taking Huangqi Shengmai Yin as a demonstration, we proposed a novel integrated efficacy-sweetness information discovery (ESID) strategy, which attempted to bridge the gap between TCM and Western medicine. It was used to discover potential property and flavor substances in TCM, and the efficacy of the ingredients was verified through in vitro and in vivo experiments.

2. Material and methods

2.1. Materials

The sweetness of Huangqi Shengmai Yin was characterized by ASTREE II electronic tongue (Alpha MOS, France). The potential property and flavor substances of Huangqi Shengmai Yin were identified by Ultimate 3000 Ultra-high performance liquid chromatograph, LTQ-Orbitrap XL mass spectrometer (with electrospray ion source and Xcalibur 2.1 Chemical workstation, ThermoFisher Scientific USA), CV200 vacuum concentrator (Beijing JM Technology Co., Ltd) and CHI-660E electrochemical workstation (Chenhua Instrument, China). The SQP electronic analytical balance (Sartorius Scientific Instruments (Beijing) Co., Ltd), WMF-3690 inverted fluorescence microscope (Shanghai Wumo Optical Instrument Co., Ltd.) and GZX-50B light incubator (Beijing Runhengao Instrument Equipment Co., Ltd) were used.

Hydrochloric acid was purchased from Shenzhen Bolinda Technology Co., Ltd. (China). T1R2 and T1R3 were provided by CrystalO Biopharma Technology Co., Ltd. (China). AlGaAs/GaAs HEMT chips were provided by Semiconductor Research, Chinese Academy of Sciences. Phosphate-buffered solution (PBS, pH 7.4), 3-Mercaptopropionic acid (3-MPA), N–Hydroxysuccinimide (NHS) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Beijing Zhongnong Boxin Technology Co., Ltd. (China). The reference substances included L-arginine (CAS No. 74-79-3, 98.0% mass fraction), sucrose (CAS No. 57-50-1, 98.0% mass fraction), calycosin (CAS No. 20575-57-9, 98.0% mass fraction), calycosin-7-O-β-D-glucoside (CAS No. 20633-67-4, 98.0% mass fraction), ruscogenin (CAS No. 472-11-7, 98.0% mass fraction), schisantherin A (CAS No. 58546-56-8, 98.0% mass fraction), atractylenolide I (CAS No. 73069-13-3, 98.0% mass fraction), and schisandrin B (CAS No. 61281-37-6, 98.0% mass fraction). All reference substances were purchased from Shanghai Shidande Standard Technical Service Co., Ltd. (China). Huangqi Shengmai Yin, which is composed of Astragali Radix, Codonopsis Radix, Ophiopogonis Radix, Schisandrae Chinensis Fructus and Schisandrae Sphenantherae Fructus, was obtained from Jiangxi Nanchang Jisheng Pharmaceutical Co., Ltd. (China). MS methanol and MS acetonitrile were purchased from Thermo Fisher Scientific Co., Ltd. (America). Ultrapure water was purchased from Guangzhou Watsons Food and Beverage Co., Ltd. (China). Pronase E, Vatalanib (PTK787) 2HCl (Shanghai Macklin Biochemical Co., Ltd), Vinorelbine (Shanghai yuanye Bio-Technology Co., Ltd), Aspirin Enteric-coated Tablets (CSPC OUYI Pharmaceutical Co., Ltd.), Buzhong Yiqi Wan (Tong Ren Tang Technologies Co., Ltd.), DMSO (Damao Chemical Reagent Factory in Tianjin, China), Sodium chloride, Calcium chloride, Potassium chloride and Magnesium sulfate (Beijing InnoChem Science & Technology Co., Ltd), and 12-well cell culture plates (Beijing LABLEAD Co., Ltd.) were used.

Tg (fli1a: EGFP) and Tg (mpx: EGFP) zebrafish embryos were purchased from Nanjing Eze-Rinka Co., Ltd. All zebrafish were reared in a professional culture system environment. Particularly, they met the requirements of HJ1069-2019 issued by the Ministry of Ecology and Environment of the People's Republic of China..

2.2. Methods

2.2.1. The discovery of the sweet taste of Huangqi Shengmai Yin based on electronic tongue

Firstly, Huangqi Shengmai Yin was centrifuged at 5000 r·min^{-1} for 5 min, and the supernatant was collected as the test solution. A series of sucrose solutions such as unsweetened, slightly sweet (40 mg·mL^{-1}), moderately sweet (80 mg·mL^{-1}), sweet (but still acceptable, 150 mg·mL^{-1}), and very sweet (almost unbearable, 290 mg·mL^{-1}) were prepared. Then, the ASTREE II electronic tongue with 7 sensors was used to measure the test solutions, which collected data once per second. The measurement time was set to 120 s and the same sample was measured for 9 times. The response values of the 7 sensors and the relative standard deviation (RSD) of the response values were calculated. Noteworthily, the sensors were pre-equilibrated and calibrated with 0.01 mol·L^{-1} hydrochloric acid solution. The sensors were washed twice for 10 s each time after the test. Moreover, a 20 mL sample of Huangqi Shengmai Yin was used continuously 5 times to evaluate the precision, and 5 parallel samples were measured to evaluate the repeatability.

2.2.2. The discovery of the potential property and flavor substances of Huangqi Shengmai Yin based on our T1R2/T1R3-HEMT biosensor combined with UPLC-MS analysis

2.2.2.1. The interaction between T1R2/T1R3 protein and Huangqi Shengmai Yin. AlGaAs/GaAs HEMT semiconductor material was used as the biosensor chip. A transparent quartz glass tube was attached to the chip surface as the sample pool. A 3-MPA aqueous solution was added to the sample pool to generate Au–S bonds on the HEMT device surface, forming a self-assembled monolayer. Then, 20 mmol·L^{-1} EDC and 50 mmol·L^{-1} NHS were mixed in equal volumes to be activated for 15 min. Finally, 100 μg·mL^{-1} T1R2 and T1R3 proteins were incubated on the chip at 4 °C.

Huangqi Shengmai Yin with nine different concentrations ranging from 10 pg·mL^{-1} to 1 mg·mL^{-1} were combined with the T1R2/T1R3-HEMT biosensor respectively. Ultrapure water was used as the control. Each sample was measured three times. Furthermore, the dissociation constant (K_D) value between the sample and biosensor was calculated according to the following equations [27].

$$[A_b] + [A_g] \leftrightarrow [A_b - A_g] \tag{1}$$

$$K = K_A = \frac{1}{K_D} = \frac{[A_b - A_g]}{[A_b]\,[A_g]} \tag{2}$$

$$\frac{[A_g]}{\Delta I} = \frac{[A_g]}{\Delta I_{max}} + \frac{K_D}{\Delta I_{max}} \tag{3}$$

In the equations, $[A_b]$ is the concentration of the unbound antibody (T1R2, T1R3) immobilized on the sensor surface. $[A_g]$ is the component concentration (recorded as C). $[A_b - A_g]$ is the concentration of the antibody-component complex on the sensor surface. K_A is the association constant, and K_D is the dissociation constant. ΔI is the current change, and ΔI_{max} is the saturated current change.

2.2.2.2. Identification of potential property and flavor substances of Huangqi Shengmai Yin based on UHPLC-LTQ-Orbitrap MS^n. The sample of Huangqi Shengmai Yin was purified by filtration through a microporous membrane with 0.22 μm. Furthermore, the potential property and flavor substances of Huangqi Shengmai Yin interacting with T1R2 were eluted with PBS as a nonspecific eluent with 1 mM·L^{-1} sucrose as a specific eluent. The former was used to wash out unconjugated components, and the latter was used to wash out components that bind to T1R2. Similarly, the potential property and flavor substances of Huangqi Shengmai Yin interacting with T1R3 were eluted with PBS as a nonspecific eluent, and 1 mM·L^{-1} L-arginine as a specific eluent. Moreover, the components of Huangqi Shengmai Yin and the potential property and flavor substances were identified using UHPLC-LTQ-Orbitrap MS^n with an AQUITY HSS T3 column (2.1 mm × 100 mm, 1.8 μm). The mixed control solution of calycosin, calycosin-7-O-*β*-d-glucoside, ruscogenin, schisantherin A, atractylenolide I and schisandrin B with 0.07, 0.05, 0.08, 0.08, 0.07, 0.09 mg·mL^{-1} was purified by filtration through a microporous membrane with 0.22 μm.

The chromatographic and mass spectrometric conditions were as follows: the mobile phase was composed of 0.1% formic acid aqueous solution (A) and acetonitrile (B). The gradient elution program was as follows: 0–2 min, 2% B; 2–8 min, 2%–25% B; 8–21 min, 25%–55% B; 21–26 min, 55%–75% B; 26–27 min, 75%–95% B; 27–28 min, 95% B; 28–29 min, 95–2% B; 29–30 min, 2% B. The flow rate was 0.3 mL·min^{-1}, the column temperature was 30 °C, and the injection volume was 2 μL. The ion source temperature was 350 °C. The ionization source voltage was 4.0 kV, the capillary voltage was 35 V, and the tube lens voltage was 110 V in positive ion mode. The ionization source voltage was −3.0 kV, the capillary voltage was −35 V, the tube lens voltage was 110 V, and the collision voltage was 6–10 V in negative ion mode. The sheath gas flow rate was 40 arb, the auxiliary gas flow rate was 20 arb and the drying gas flow rate was 15 L·min^{-1}. The sample primary mass spectrometry spectrum was scanned with full scan. The mass scan range of *m/z* was 100–1200 and detection resolution was 30,000. The secondary mass spectrometry data was scanned using a data-dependent scan and collision-induced dissociation fragmentation mode.

2.2.3. Efficacy verification of potential property and flavor substances of Huangqi Shengmai Yin based on in vivo and in vitro experiments

2.2.3.1. Interaction between T1R2/T1R3 and potential property and flavor substances of Huangqi Shengmai Yin. The potential property and flavor substances of Huangqi Shengmai Yin were dissolved in 0.5% DMSO. The samples with eleven different concentrations ranging from 0.1 pmol·L^{-1} to 1 mmol·L^{-1} were combined with the T1R2/T1R3-HEMT biosensor respectively. The K_D value for the interaction between T1R2/T1R3 proteins and potential property and flavor substances was calculated, respectively.

2.2.3.2. The pro-angiogenic effect of potential property and flavor substances of Huangqi Shengmai Yin based on the zebrafish vascular injury model. PTK787 was able to interdict the formation of intersegmental vessels in zebrafish. Transgenic zebrafish Tg (fli1a: EGFP) was used to assess the influence of Huangqi Shengmai Yin and its potential property and flavor substances on vessel angiogenesis. Huangqi Shengmai Yin was diluted to 100 μL·mL^{-1} with fresh fish water (5.00 mmol·L^{-1} NaCl, 0.17 mmol·L^{-1} KCl, 0.40 mmol·L^{-1} $CaCl_2$ and 0.16 mmol·L^{-1} $MgSO_4$) for later use. The zebrafish embryos developed to 24 h post fertilization (hpf) were removed from the membrane with 1 mg·mL^{-1} protease E. Then, they were randomly selected and placed in 12-well plates with 10 embryos per well. The control group, model group, commercially available drug group and drug treatment group were set up [28,29]. The control group was treated with fresh fish water containing 0.1% DMSO. The model group was treated with 0.2 μg·mL^{-1} PTK787. The commercial drug control group was treated with 0.2 μg·mL^{-1} PTK787 and 0.2 mg·mL^{-1} aspirin. The drug treatment groups were separately treated with the potential property and flavor substances of Huangqi Shengmai Yin, along with 0.2 μg·mL^{-1} PTK787 for modeling. The final concentration of DMSO was 0.1%. Moreover, 3 wells were set in each group with 10 embryos and 2 mL of liquid. The above groups were exposed at 28.5 °C with a light/dark cycle of 14 h light and 10 h dark for 24 h. Finally, the development of intersegmental vessels (ISVs) of zebrafish was observed and the length of ISVs was calculated.

2.2.3.3. The immune enhancement effect of potential property and flavor substances of Huangqi Shengmai Yin based on the zebrafish immunocompromised model. In TCM, the qi of cereal essence is transformed by the spleen and stomach after digestion and absorption of food. Then, it becomes the main source of qi in the spleen and the whole body. Spleen qi deficiency is a common immune disorder described in TCM literature. Dysfunctions of the immune system are caused by qi imbalance, and immune deficiency is one of the symptoms of spleen qi deficiency [30–33]. Vinorelbine could induce immune injury in zebrafish. Tg (mpx: EGFP) was used to assess the influence of Huangqi Shengmai Yin and its potential property and flavor substances on immune enhancement. The zebrafish embryos at 48 hpf were randomly divided into 12-well plates with 10 embryos per well. The control group, model group, commercially available drug group, and drug treatment group were set up [34,35]. The control group was treated with fresh fish water containing 0.1% DMSO. The model group was treated with 100 μg·mL^{-1} vinorelbine. The commercial drug control group was treated with 100 μg·mL^{-1} vinorelbine and 0.2 mg·mL^{-1} Buzhong Yiqi Wan. The drug treatment groups were separately treated with the potential property and flavor substances of Huangqi Shengmai Yin, along with 100 μg·mL^{-1} vinorelbine for modeling. The final concentration of DMSO was 0.1%. Moreover, 3 wells were set in each group with 10 embryos and 2 mL of liquid. The above groups were exposed at 28.5 °C with a light/dark cycle of 14 h light and 10 h dark for 24 h. Finally, the number of neutrophils in the tail region of zebrafish embryos was counted.

2.2.4. Statistical analysis

The zebrafish data was analyzed using GraphPad Prism 8 software. All data were presented as means ± SD. Statistical evaluation was performed by one-way ANOVA analysis. $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. The discovery of the sweet taste of Huangqi Shengmai Yin based on electronic tongue

According to the 7 electronic tongue sensor responses, the changes in the RSD values were shown in Fig. 1a1 and a2. As the testing time and test number increased, the RSD values of the 7 sensors were observed to gradually decrease. The response values were stabilized between 101 and 120 s, with a RSD value less than 1%. And between the 5th and 8th tests, the RSD value was less than 2%. Therefore, the detection time was determined to be 120 s, with an effective range of response values between 101 and 120 s. And the number of tests was 8, with the effective range of response values between the 5th and 8th tests. The RSD values of the precision and repeatability were shown in Fig. 1a3 and a4, with a RSD value less than 10%. The instrument precision and method repeatability were good.

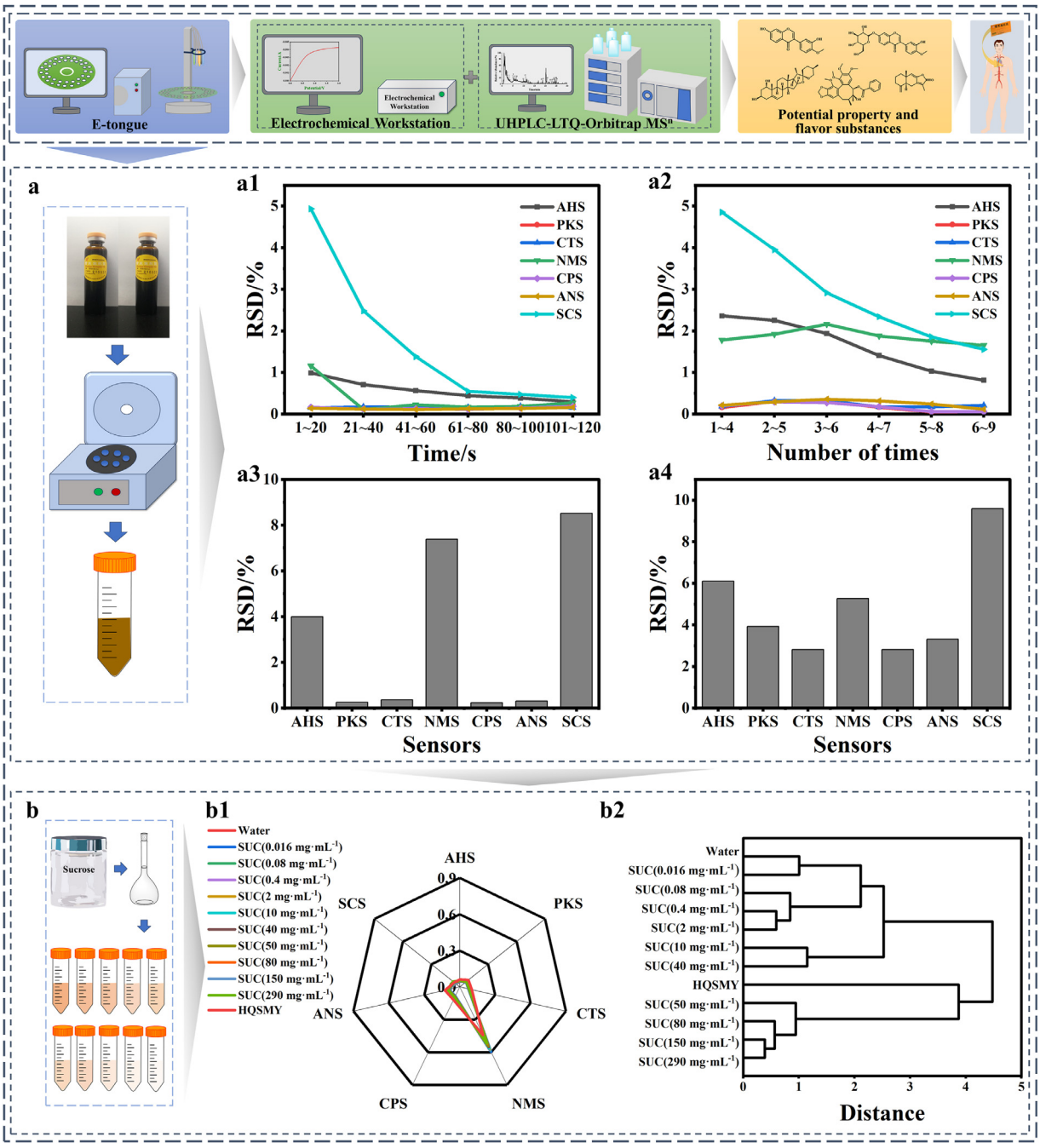

Fig. 1. Sweetness evaluation of Huangqi Shengmai Yin based on electronic tongue. (a) Methodological investigation of E-tongue: (a1) RSD of the response values of the electronic tongue sensors with testing time; (a2) RSD of the response values of the electronic tongue sensors with testing times; (a3) precision of response value of electronic tongue sensors; (a4) repeatability of response value of electronic tongue sensors. (b) Sweetness evaluation of Huangqi Shengmai Yin: (b1) radar map of response value of electronic tongue sensor for sucrose and Huangqi Shengmai Yin; (b2) HCA of response values of electronic tongue sensor for sucrose and Huangqi Shengmai Yin.

Moreover, the response values of the electronic tongue sensors of Huangqi Shengmai Yin and sucrose were standardized, and the radar charts were shown in Fig. 1b1. Among them, the sweet taste sensor (ANS) and the umami taste sensor (NMS) demonstrated higher response values. The results indicated that Huangqi Shengmai Yin possessed the property of sensory sweetness. Further analysis of the sensor response values was conducted using hierarchical cluster analysis (HCA), and the result was depicted in Fig. 1b2. It could be inferred that the sweetness level of Huangqi Shengmai Yin fell between 40~50 mg·mL^{-1} sucrose, and the taste was slightly sweet.

3.2. The discovery of the potential property and flavor substances of Huangqi Shengmai Yin based on T1R2/T1R3-HEMT biosensor combined with UPLC-MS analysis

3.2.1. The interaction between T1R2/T1R3 protein and Huangqi Shengmai Yin

Firstly, Fig. 2a1, a4 showed the drain–source current (I_{DS}-V_{DS}) results for different concentrations of Huangqi Shengmai Yin. The small change in current was observed after adding a blank solution (H_2O). It indicated that the solvent had little influence on the test results. Based on the

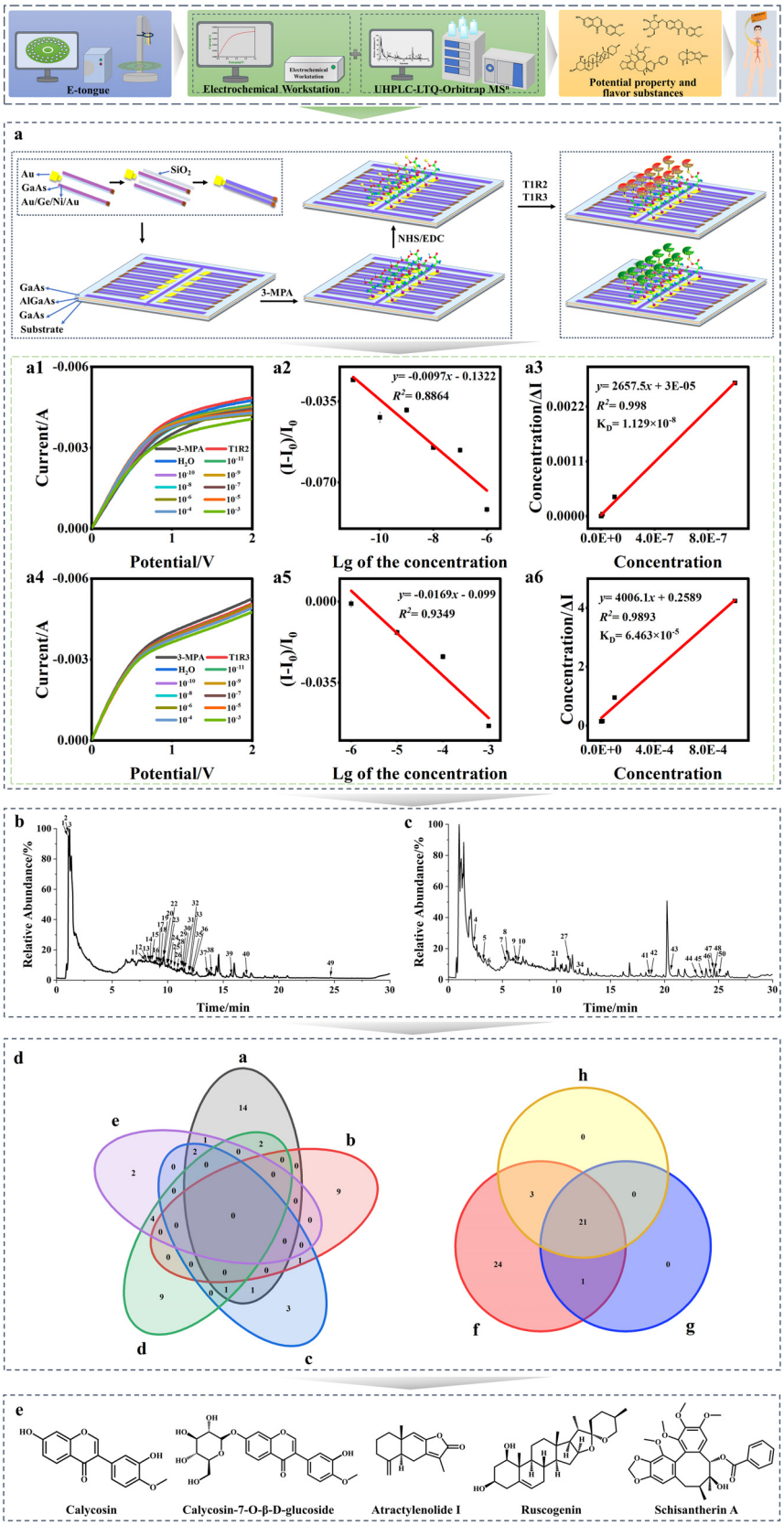

Fig. 2. Screening of potential property and flavor substances of Huangqi Shengmai Yin. (a) The interaction between T1R2/T1R3 protein and Huangqi Shengmai Yin: (a1, a4) I_{DS}-V_{DS} signal change; (a2, a5) linear range and linear fitting results; (a3, a6) dissociation constant results in linear concentration range. (b) Total ion chromatogram (TIC) by negative ion mode of Huangqi Shengmai Yin. (c) Total ion chromatogram (TIC) by positive ion mode of Huangqi Shengmai Yin. (d) Venn diagram of chemical compositions (a: Astragali Radix; b: Codonopsis Radix; c: Ophiopogonis Radix; d: Schisandrae Chinensis Fructus; e: Schisandrae Sphenantherae Fructus; f: Huangqi Shengmai Yin; g: Sucrose eluate; h: Arginine eluate). (e) The structures of the five potential property and flavor substances of Huangqi Shengmai Yin.

logarithm of Huangqi Shengmai Yin sample concentration (LgC) as the x-axis and the relative change of current (I-I_0) /I_0 as the y-axis, linear fitting was performed as shown in Fig. 2a2, a5. The results indicated a good linear relationship between (I-I_0) /I_0 and LgC for T1R2 within the range of LgC from −11 to −6. Similarly, the range of LgC was from −6 to −3 for T1R3.

Based on the Huangqi Shengmai Yin sample concentration ([A_g]) as the x-axis and the ratio of concentration ([A_g]) to the change in current (I-I_0, ΔI) as the y-axis, linear fitting was performed as shown in Fig. 2a3, a6. The K_D values were calculated according to Eqs. 1–3, and the results indicated that the K_D value of Huangqi Shengmai Yin sample interacting with the T1R2 protein was 1.129×10^{-8} g·mL^{-1}. And the K_D value of Huangqi Shengmai Yin sample interacting with the T1R3 protein was 6.463×10^{-5} g·mL^{-1}.

3.2.2. Identification of potential property and flavor substances of Huangqi Shengmai Yin based on UHPLC-LTQ-Orbitrap MS^n

The data of the solution of Huangqi Shengmai Yin were collected by UHPLC-LTQ-Orbitrap MS^n technique in positive and negative ion modes, and the chromatograms were shown in Fig. 2b and c. A total of 50 chemical constituents were in Supplementary Materials Table S1. 25 components binding to T1R2 and T1R3 were identified and discovered, including calycosin, calycosin-7-O-β-d-glucoside, atractylenolide I, ruscogenin, and schisantherin A, as listed in Supplementary Materials Table S2 and Fig. 2d. Some of their fragmentation patterns were as follows:

The quasi-molecular ion peak for the compound with a retention time of 8.53 min was m/z 283.06033, which was presumed to be $C_{16}H_{12}O_5$, with an error of 0.813 ppm. The fragments m/z 255.04002, 268.32452, and others were produced in its secondary mass spectrum. The fragment m/z 268.32452 was produced by m/z 283.06033 removing one molecule of CH_3, and m/z 255.04002 was produced by m/z 283.06033 removing one molecule of CO. Combined with the literature report [36], the compound was identified as calycosin ($C_{16}H_{12}O_5$).

The quasi-molecular ion peak for the compound with a retention time of 9.91 min was m/z 447.12808, and its molecular formula was presumed to be $C_{22}H_{22}O_{10}$, with an error of −1.103 ppm. The fragments m/z 270.13382, 285.11362, and others were generated in its secondary mass spectrum. The fragment m/z 285.11362 was produced by m/z 447.12808 removing one molecule of glucose. Then in the removal of one molecule of CH_3, the fragment m/z 270.13382 was obtained. Combined with the literature report [37], the compound was identified as calycosin-7-O-β-d-glucoside ($C_{22}H_{22}O_{10}$).

The quasi-molecular ion peak for the compound with a retention time of 20.81 min was m/z 231.13815, which was presumed to be $C_{15}H_{18}O_2$, with an error of 0.838 ppm. The fragments m/z 156.93694, 184.96791 and 213.04501 were produced in its secondary mass spectra. The fragment m/z 184.96791 was produced by the m/z 231.13815 removing one molecule of HCOOH, and m/z 156.93694 was produced by m/z 231.13815 removing one molecule of HCOOH and one molecule of C_2H_4. Combined with the literature report [38], the compound was identified as atractylenolide 1 ($C_{15}H_{18}O_2$).

The quasi-molecular ion peak for the compound with a retention time of 23.54 min was m/z 401.19586, which was presumed to be $C_{23}H_{28}O_6$, with an error of −0.013 ppm. The fragments m/z 370.19778, 386.31177, and others were produced in its secondary mass spectrum. The fragment m/z 386.31177 was produced by the m/z 401.19586 removing of one molecule of CH_3, and m/z 370.19778 was produced by the removal of one molecule of CH_3O from m/z 401.19586. Combined with the literature report [39], the compound was identified as schisandrin B ($C_{23}H_{28}O_6$).

The quasi-molecular ion peak for the compound with a retention time of 24.38 min was m/z 431.31705, which was presumed to be $C_{27}H_{42}O_4$, with an error of 3.394 ppm. The fragments m/z 367.25256, 413.29555 and others were produced in its secondary mass spectrum. The fragment m/z 413.29555 was produced by m/z 431.31705 removing one molecule of H_2O, and m/z 367.25256 was produced by m/z 431.31705 removing two molecules of H_2O and one molecule of CO. Combined with the literature report [40], the compound was identified as ruscogenin ($C_{27}H_{42}O_4$).

The quasi-molecular ion peak for the compound with a retention time of 24.52 min is m/z 537.20978, which was presumed to be $C_{30}H_{32}O_9$, with an error of −3.963 ppm. The fragments m/z 371.25568, 415.27136, and others were produced in its secondary mass spectrum. The fragment m/z 415.27136 was produced by the m/z 537.20978 removing one molecule of benzoic acid ($C_7H_6O_2$), and m/z 371.25568 was produced by m/z 537.20978 by the removal of one molecule of $C_7H_6O_2$ and one molecule of CH_3CHO. Combined with the literature report [41], the compound was identified as schisantherin A ($C_{30}H_{32}O_9$).

The quasi-molecular ion peak for the compound with a retention time of 25.03 min was m/z 537.20923, which was presumed to be $C_{28}H_{34}O_9$, with an error of −0.510 ppm. The fragments m/z 371.18774, 385.36475, and 415.23523 were produced as fragment ions in its secondary mass spectrum. The fragment m/z 415.23523 was produced by m/z 537.20923 removing one molecule of C_4H_6COOH. The fragment m/z 385.36475 was produced by m/z 537.20923 removing one molecule of C_4H_6COOH and one molecule of CH_2O. The fragment m/z 371.18774 was produced by m/z 537.20923 removing one molecule of C_4H_6COOH and one molecule of C_2H_4O. Combined with a literature report [42], the compound was identified as schisantherin C ($C_{28}H_{34}O_9$).

Huangqi Shengmai Yin evolved from Shengmai San, which is a combination of Astragali Radix, Codonopsis Radix, Ophiopogonis Radix, Schisandrae Chinensis Fructus and Schisandrae Sphenantherae Fructus. A total of 21 components were present in both the sucrose and arginine eluates. Therefore, they were considered to be of relative importance. Among them, Astragali Radix had 6 unique constituents, Codonopsis Radix had 2, Ophiopogonis Radix had 3, Schisandrae Chinensis Fructus had 3, and Schisandrae Sphenantherae Fructus had 1; thus particular attention should be paid to Astragali Radix. Further, for Astragali Radix, using the content of the components as an indicator and combing with the pharmacopeia, calycosin and calycosin-7-O-β-d-glucoside were selected as the active ingredients [43]. For Codonopsis Radix, using the content of the components as an indicator, atractylenolide I was selected as the active ingredient. For Ophiopogonis Radix, according to the content determination method specified in the pharmacopeia, ruscogenin was selected as the active ingredient [44]. Due to the small dose of Schisandrae Chinensis Fructus and Schisandrae Sphenantherae Fructus, and both of them were plants of the Magnoliaceae, we focused on the common components and chose schisantherin A as the active ingredient.

3.3. Efficacy verification of potential property and flavor substances of Huangqi Shengmai Yin based on in vivo and in vitro experiments

3.3.1. Interaction between T1R2/T1R3 and potential property and flavor substances of Huangqi Shengmai Yin

Based on the order of low to high concentration, calycosin, calycosin-7-O-β-d-glucoside, atractylenolide I, ruscogenin, and schisantherin A solutions were used to act with T1R2 and T1R3 proteins respectively. The results were shown in Fig. 3. The results indicated that the K_D value for interaction of calycosin with T1R2 was 1.704×10^{-12} M, and with T1R3 was 6.407×10^{-10} M. The K_D value for interaction of calycosin-7-O-β-d-glucoside with T1R2 was 7.924×10^{-10} M, and with T1R3 was 6.051×10^{-13} M. The K_D value for interaction of atractylenolide I with T1R2 was 1.057×10^{-9} M, and with T1R3 was 8.818×10^{-11} M. The K_D value for interaction of ruscogenin with T1R2 was 5.255×10^{-8} M, and with T1R3 was 2.165×10^{-10} M. The K_D value for interaction of schisantherin A with T1R2 was 3.854×10^{-10} M, and with T1R3 was 9.017×10^{-10} M. To sum up, all five critical quality attributes were found to have strong binding ability with the sweet taste proteins T1R2 and T1R3. Among them, calycosin had the strongest binding capacity with T1R2, and calycosin-7-O-β-d-glucoside had the strongest interaction with T1R3. Both of these compounds were derived from the Astra-

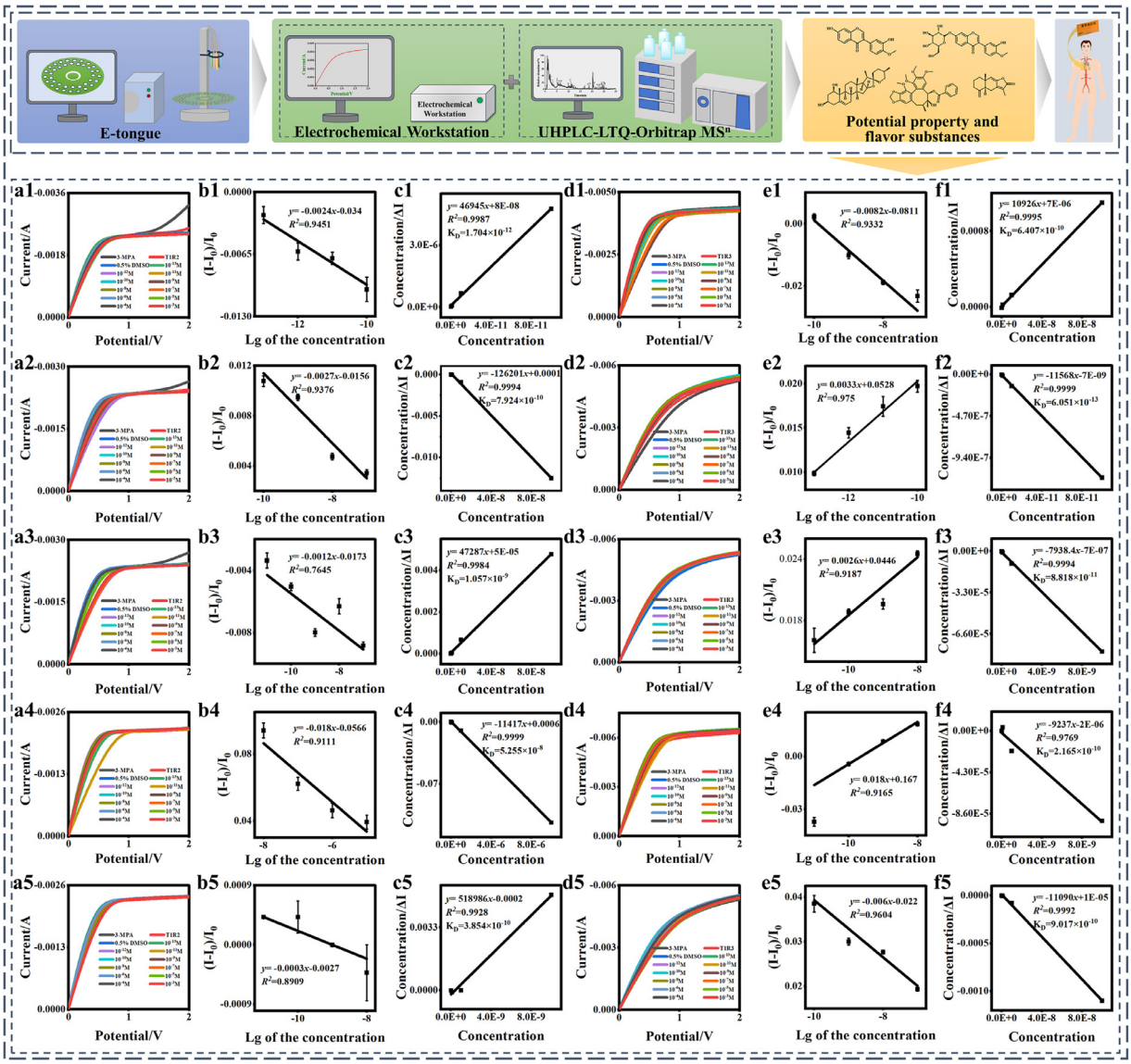

Fig. 3. Interactions between T1R2 and T1R3 proteins and potential property and flavor substances of Huangqi Shengmai Yin. (a1–a5, d1–d5) I_{DS}-V_{DS} signal change; (b1–b5, e1–e5) linear range and linear fitting results; (c1–c5, f1–f5) dissociation constant results in linear concentration range, where (a1–f1) Calycosin; (a2–f2) Calycosin-7-O-β-d-glucoside; (a3–f3) Atractylenolide I; (a4–f4) Ruscogenin; (a5–f5) Schisantherin A.

gali Radix, which was an important component of the Huangqi Shengmai Yin.

3.3.2. *The pro-angiogenic effect of potential property and flavor substances of Huangqi Shengmai Yin based on the zebrafish vascular injury model*

The intersegmental vessels of transgenic zebrafish Tg (fli1a: EGFP) exhibit green fluorescence. The microscopic images of different treatment groups were shown in Fig. 4a2, and the measurements of the total length of ISVs were shown in Fig 4a1. The data were normally distributed. The results showed that 0.2 μg·mL^{-1} PTK787 caused zebrafish intersegmental vascular damage but was not teratogenic. It could significantly reduce the total length of ISVs compared with the control group, indicating that the modeling was successful. Compared with the model group, 2.5 μL·mL^{-1} Huangqi Shengmai Yin was able to reverse the vascular damage caused by PTK787 and exerted angiogenesis-promoting effects. Remarkably, 25 μM·L^{-1} calycosin, calycosin-7-O-β-d-glucoside, ruscogenin, schisantherin A, and 50 μM·L^{-1} atractylenolide I could significantly promote ISVs. The ameliorative effect on the vascular injury of calycosin was stronger at a low dose, and ruscogenin was stronger at medium and high doses. Huangqi Shengmai Yin, calycosin-7-O-β-d-glucoside, atractylenolide I, and schisantherin A showed dose-dependent effects.

3.3.3. *The immune enhancement effect of potential property and flavor substances of Huangqi Shengmai Yin based on the zebrafish immunocompromised model*

The neutrophils of transgenic zebrafish Tg (mpx: EGFP) exhibits green fluorescence. The microscopic images of different treatment groups were shown in Fig. 4b2, and the measurements of the numbers of neutrophils were shown in Fig. 4b1. The data were normally distributed. The results indicated that 100 μg·mL^{-1} vinorelbine significantly reduced the number of neutrophils in the zebrafish tail, indicating the successful construction of the model. Compared to the model group, 2.5 μL·mL^{-1} Huangqi Shengmai Yin, 25 μM·L^{-1} atractylenolide I, 50 μM·L^{-1} calycosin, 100 μM·L^{-1} calycosin-7-O-β-d-glucoside, ruscogenin and schisantherin A exhibited an immune-enhancing effect. Surprisingly, atractylenolide I exhibited significant immune-enhancing effects at a low dose, which was hypothesized to play a key role in the qi tonic effect exerted by Huangqi Shengmai Yin.

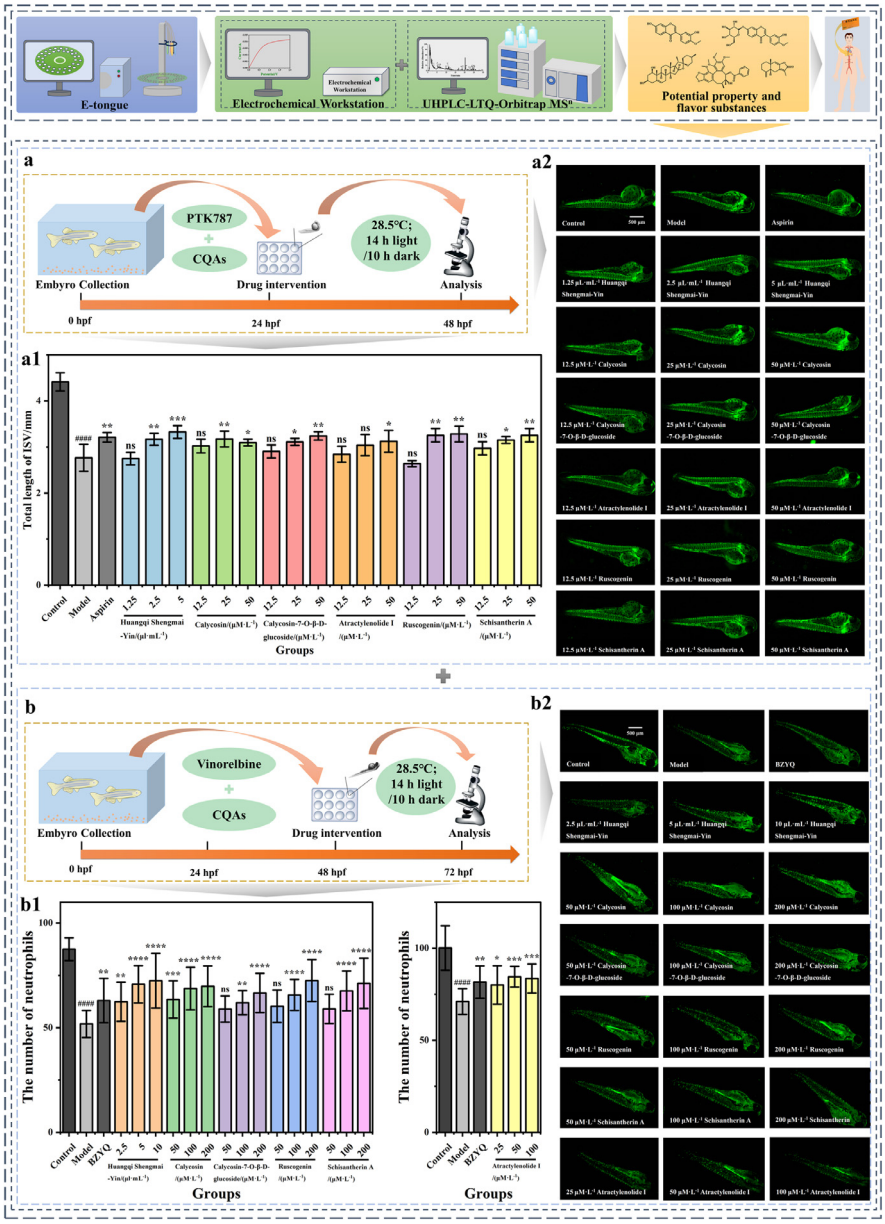

Fig. 4. Efficacy of validation. (a) Promoting effects of Huangqi Shengmai Yin and five potential property and flavor substances with different concentrations on ISVs of zebrafish: (a1) effects of Huangqi Shengmai Yin and five potential property and flavor substances on the ISVs growth; (a2) fluorescence microscopic image of fli1a: EGFP zebrafish. (b) The immune enhancement effects of Huangqi Shengmai Yin and five potential property and flavor substances with different concentrations on zebrafish: (b1) effects of Huangqi Shengmai Yin and five potential property and flavor substances on the number of neutrophils; (b2) fluorescence microscopic image of mpx: EGFP zebrafish. Data are shown as mean ± SEM. $^{####}P < 0.0001$ vs. the control group. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, vs. the model group.

4. Conclusion

In this study, we proposed a novel integrated ESID strategy for the discovery of potential property and flavor substances. Based on this strategy, an attempt was made to bridge the dialogue between TCM and Western medicine, with Huangqi Shengmai Yin as a demonstration. Firstly, the sensory sweetness of Huangqi Shengmai Yin was discovered and determined through intelligent sensory technology. Secondly, the chemical components in the production of Huangqi Shengmai Yin in the real world were analyzed and identified for the first time based on UHPLC-LTQ-Orbitrap MS^n. A total of 50 chemical components such as calycosin and ruscogenin were identified. Surprisingly, we discovered potential property and flavor substances such as calycosin, calycosin-7-O-β-d-glucoside, atractylenolide I, ruscogenin and schisantherin A, and verified them through in vivo and in vitro experiments. In summary, the process of discovering potential property and flavor substances in TCM can be accelerated through this strategy. It provides references for the discovery of other potential property and flavor substances in TCM.

CRediT authorship contribution statement

Yanyu Han: Methodology, Writing – original draft. **Xiaoyan Hu:** Formal analysis. **Mingshuang Li:** Formal analysis. **Xiaojun Zhao:** Formal analysis. **Xiaomeng Wang:** Formal analysis. **Mingyue Yang:** Formal analysis. **Jing Zhao:** Formal analysis. **Xiaomeng Zhang:** Writing – original draft. **Jing Wang:** Writing – original draft. **Xingyue Huan:** Writing – original draft. **Xinyu Guo:** Writing – original draft. **Wuzhen Qi:** Writing – review & editing. **Nan Li:** Writing – review & edit-

ing. **Zhijian Zhong:** Resources. **Xuhai Liu:** Resources. **Boyi Li:** Formal analysis. **Zhisheng Wu:** Formal analysis, Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fmre.2024.04.012.

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