Isolation and identification of key bitter substances in Chrysanthemum morifolium (Chuju) and their interaction mechanism with bitter taste receptors based on HPLC-Q-TOF-MS and molecular docking

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Abstract

Chrysanthemum morifolium（Chuju）, a plant with both medicinal and edible value, faces limitations in its widespread utilization in food and medicine due to its bitter taste. This study aimed to isolate the key components contributing to the bitterness in Chuju by solvent extraction, silica gel column chromatography, and High-performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (HPLC-Q-TOF-MS) guided by sensory evaluation, sensory evaluation combines artificial sensory evaluation and electronic tongue evaluation. And use the Dose-Over-Threshold (DoT) factors to evaluate the relative contribution of these bitter compounds. Finally selected three bitter compounds of the high contribution to bitterness, respectively Vitexin, Orientin and Kaempferol. The DoT factor in the artificial sensory evaluation is 8.77, 2,73 and 0.40. In the electronic tongue, the DoT factor is 136.62, 1.51 and 26.88. Subsequently, using molecular docking to verify the combination of bitter compounds and bitterness receptors between TAS2R14, and get the mechanism of interaction between molecules. Ultimately, the docking scores of the three bitter compounds are all greater than -5, demonstrating their tight binding to the bitterness receptor. Therefore, this study identified the key bitter substances in Chuju and could provide a reference for the wide application and bitter reduction of Chuju in the future.

1 Introduction

Chrysanthemum morifolium (Chuju) is a perennial herb belonging to the Compositae family, primarily cultivated in Chuzhou, Anhui Province. It has been traditionally cultivated for over 2000 years in China for both ornamental and medicinal purposes (Wang et al., 2024). The Chrysanthemum species have long been praised in traditional Chinese medicine history, and continue to be used as valuable drugs, food additives or herbal teas (Hadizadeh et al., 2022). Chrysanthemum flowers are rich in bioactive compounds such as flavonoids, terpenoids, phenolic acids, which exhibit strong properties including antioxidant, antibacterial, anti-inflammatory, anticancer, hepatoprotective, and nephroprotective activities (Sharma et al., 2023). Thus, Chrysanthemum tea has long been used to help improve the function of the cardiovascular system, prevent oxidative damage, enhance healthy immune function, inhibit inflammation and promote detoxification in both modern and traditional pharmaceutical sciences.

However, the bitterness brought by these bioactive components has greatly limited the application of Chuju in food and medicine. Now about chrysanthemum research mostly focused on its chemical composition and biological activity, or the differences in chemical composition among different varieties and regions of chrysanthemums. There is no relevant research on which specific compounds cause the bitter taste of chrysanthemums. Nie et al. identified 18 flavonoids and caffeoylquinic acid compounds by combining UPLC-ESI-Q-TOF MS, GC-MS and principal component analysis (PCA). The identification and quality control methods for chrysanthemums have been established (Nie et al., 2019). Han et al. identified different components in five varieties of chrysanthemums through UPLC-Q-TOF and PCA, and ultimately confirmed 19 components, among which 11 had anti-inflammatory effects (Han et al., 2015). For instance, Ma et al. extracted Huangshan Gongju using organic solvents of different concentrations. The results showed that a high concentration of organic solvents (50–70%) was conducive to obtaining Gongju extract with high phenol content and high antioxidant activity (Ma et al., 2021).

Bitter substances are characterized by a low recognition threshold, making them the most easily detected among the five basic taste sensations. These substances can significantly impact the taste of food, leading to strong unpleasant sensations for consumers (Zheng et al., 2006). Therefore, in order to expand the application scope of Chuju, it is necessary to figure out what the key bitter substances in Chuju. Bitter compounds in food mainly include polyphenols, alkaloids, terpenoids, bitter peptides and Maillard reaction products (Luo et al., 2025). Currently, bitter compounds are predominantly found in plant-based foods and bitter Chinese medicinal herbs, coffee (Gao et al., 2023), tea (Chen et al., 2025), citrus (Jeffries et al., 2025) and Angelica Dahurica (Yu et al., 2020). When identifying bitter substances, these substances all adopt a combination of comprehensive chemical analysis and bitter taste sensory analysis to determine the key bitter compounds.

Molecular docking is often used to study the mechanism of action between ligands and receptors and verify the binding of certain active ingredients to core targets. At the same time, in terms of reducing the bitterness of food, molecular docking is often used to search for inhibitors of bitter taste receptors, thereby achieving the reduction of bitterness in food at the molecular level. Wei et al. used molecular docking techniques revealed interactions between the identified umami peptides and T1R1/T1R3 umami receptors as well as TAS2R14 bitter receptors, highlighting critical binding residues. It was also found that LT-5 has the effect of reducing quinine concentration, thereby enhancing the bitter taste masking effect (Wei et al., 2025). Hong et al. used molecular docking to validate the binding of the active ingredients to the core targets. Confirmed the bitter almond-licorice could be used to treat COVID-19 by inhibiting inflammatory responses and regulating cellular stress (Hong et al., 2023). At present, 26 human bitter receptors have been reported, and among them, TAS2R14 is one of the most broadly tuned bitterness receptors (Nowak et al., 2018).

This study focused on the preparation of Chuju bitter extract through extraction, purification, and sensory evaluation. Taste dilution analysis (TDA) was used for the sensory evaluation of the bitterness intensity of each fraction of a sample (Yang et al., 2021). The bitter extract underwent identification using HPLC-Q-TOF-MS. Express the contribution of bitter compounds to the bitterness of Chuju with DoT (Guo et al., 2024). Finally, molecular docking was used to verify the binding degree between bitter compounds and bitter receptors as well as the intermolecular interaction mechanism. The objective was to elucidate the specific origin of bitter substances in Chuju, establish a foundational understanding of the molecular basis of bitterness in Chuju, propose novel approaches for reducing bitterness in food and medicine, and broaden the applications of Chuju.

2 Materials and methods

2.1 Materials and instruments

Dried chrysanthemum was purchased from Anhui Jutai Chuju Botanical Technology Co., Ltd (Anhui, China). Analytical grade petroleum ether (60–90 ℃), ethyl acetate (99%), n-butyl alcohol, column chromatography silica gel ware purchased from Shanghai Maclean's Biochemical Co., Ltd (Shanghai, China). Deionized water and ethanol (absolute) were utilized for sensory analysis. Berberine hydrochloride was purchased from Xi'an Mischel Biotechnology Co., Ltd (Xi'an, China).

The liquid chromatograph (Vanquish type) and mass spectrometer (Q Exactive Focus type) are from Thermo; Electronic balance; vacuum rotary evaporator (Shanghai Lichen Bangxi Instrument Technology Co., Ltd.); Electronic tongue (Taste Analysis System of INSENT, Japan, model: TS-5000Z),500 ml separating funnel; 30×300mm chromatography column.

2.2 Experimental Methods

2.2.1 Raw material pretreatment

The ethanol extraction method for bitter compounds followed (Ke et al., 2021), with modifications. Dried Chuju powder were ground and sieved (60 mesh). The powder was macerated in 70% ethanol (1:10, w/v) at 25 ℃ for 48 h, vacuum filtered, followed by ultrasonic extraction of the residue in 70% ethanol (1:10, w/v) at 30 ℃ for 30 min. Both filtrates were combined. The combined extract was concentrated under reduced pressure (55 ℃), yielding the Chuju crude extract. The obtained Chuju extract was stored in a refrigerator at 4 ℃.

2.2.2 The extraction method of bitter substances from Chuju

The solvent extraction method was performed according to (Ke et al., 2021) with modifications. The ethanol extract (20mL) was diluted with 200mL of 2.5% ethanol (aq.) and sequentially extract with 200mL of petroleum ether (60–90°C), ethyl acetate, and n-butanol. Each solvent was used for three extractions, and the respective layers were combined. All organic layer and the residual aqueous phase were concentrated separately under reduced pressure (70 ℃), reconstituted in

deionized water, and re-concentrated, yielding four fractions: petroleum ether (F□), ethyl acetate (F□), n-butanol (F□), and aqueous (F□).

2.2.3 Separation of bitter substances in Chuju

According to the method of (Ke et al., 2021) separation was carried out by silica gel column chromatography using a gradient elution system. The most bitter part (from Section 2.2.2) is adsorbed on silica gel: (1) dissolved in petroleum ether; (2) Mix an appropriate amount of silica gel (60 A, 200–425 mesh) with petroleum ether to obtain 150mL of silica gel filler, load it into a column (30×300mm) and compact it; (3) Add the sample, with the sample thickness slightly higher than that of the upper layer of quartz sand by 2cm. Petroleum ether/ethyl acetate gradient elution (total volume: 450 mL) : gradient from 10:0 to 0:10 (v/v) in 10% increments at 2 column volumes (BV)/h. Fractions (20 mL each) were collected, concentrated under reduced pressure (70°C), and reconstituted in 5% ethanol (50 mL, aqueous) for solubility enhancement, yielding 11 subfractions (F□-1 to F□-11).

2.2.4 Sensory Analysis of Bitter Compounds

2.2.4.1 Panel Selection and Training

A sensory panel was established according to ISO 3972: 1991. 45 healthy assessors (25 males, 14 females; aged 20–25 years) with no history of taste disorders were selected. Training comprised:

Bitterness intensity calibration: Assessors rated five concentrations of berberine hydrochloride (0.005, 0.01, 0.025, 0.05, 0.1 g/L) using a 5-point scale, as shown at Table 1. Sessions were conducted at 22–25 ℃. Samples (1 mL) were pipetted onto the tongue root, leave for 10s, then spit out and rinse with water. A 2-min interval was enforced between samples to prevent fatigue. All sample were evaluated twice.

Table 1
Bitter value of qualitative description, grade and quantitative range

A description of the taste of bitterness	Grade	Concentration range
Imperceptible bitter	1	[0-0.005)
Slightly bitter	2	[0.005–0.01)
Moderate of bitterness	3	[0.01–0.025)
Acceptable of bitterness	4	[0.025–0.05)
Extreme bitterness	5	[0.05–0.1)

2.2.4.2 Sensory Evaluation Methods

(1) Taste Dilution Analysis (TDA)

Chuju extracts were dissolved in 5% ethanol aqueous solution (v/v) as the initial stock solution. Serial dilutions were prepared by successive 1:1 (v/v) dilution of the stock solution with 5% ethanol aqueous solution. The serial dilutions of dilutions were then presented to a trained sensory panel (6 males and 6 females) in order of increasing concentration, and each dilution was judged in a triangle test. The dilution at which a difference in taste between the diluted fraction and two blanks (5% ethanol aqueous solution) could be detected was defined as the taste dilution factor (TD). The TD factor of each component was calculated based on the average value of the individual TD values from all panelists, and the TD factors between panelists differed by no more than one dilution step. The interval between sample evaluations was 2 min, and each sample was evaluated twice (Yang et al., 2021).

(2) Ranking Test with Balanced Incomplete Block design (BIBD)

This sensory analysis method complies with ISO 29842: 2011, IDT. The samples should be submitted in accordance with the sample submission method of 15 quaternary components for 10 samples as stipulated in the ISO 29842: 2011 (Table 2). The samples were encoded with three random numbers. The evaluators were asked to rank and rate the bitterness of their four samples, with “1” indicating “the most bitter” and “4” indicating “the least bitter”. There was a 2 min interval between each sample, and the evaluators rinsed their mouths with clean water. There was a total of 45 evaluators for this method, aged between 20 and 25, and students without sensory defects who had undergone sensory evaluation for 2 years (Frangopoulos et al., 2025).

Table 2
BIBD Sample Submission Form

Block	Sample									
	1	2	3	4	5	6	7	8	9	10
1	×	×	×	×						
2	×	×			×	×				
3	×		×				×	×		
4	×								×	×
5	×				×		×		×	
6	×					×		×		×
7		×	×			×			×	
8		×		×			×			×
9		×			×			×		×
10		×					×	×	×	
11			×	×	×			×		
12			×		×				×	×
13			×			×	×			×
14				×	×	×	×			
15				×		×		×	×	

The Friedman rank sum test was performed on the BIBD sequential data, and the calculation is as follows:

$$F = \frac{12}{\lambda pt(k+1)} \sum_{i=1}^{t} Ri^2 - \frac{3(k+1)pr^2}{\lambda} \qquad (1)$$

When the F value is greater than X^2, it indicates a significant difference among the samples, and further multiple comparisons between the rank sum of each sample are required. The LSD_α is calculated as follows:

$$LSD_\alpha = t_{\alpha(\infty)} \sqrt{p(k+1)(rk-r+\lambda)/6} \qquad (2)$$

In the formula:

t = Number of samples;

k = Block capacity;

r = Number of Repetitions;

b = Number of blocks;

λ = Number of times each sample is evaluated;

p = Repetition number of the basic design table;

Ri = sum of rank of sample I;

α = 0.05.

2.2.5 Electronic Tongue Detection

The taste characteristics of the samples were detected using a TS-5000Z electronic tongue (Intelligent Sensor Technology Co., Ltd., Tokyo, Japan). This instrument consists of 6 lipid membrane taste sensors and three reference electrodes, designed to collect taste data for umami (AAE), saltiness (CT0), sourness (CA0), bitterness (C00), sweetness (GL1) and astringency (AE1). The reference solution was prepared using a tasteless sample containing 30 mM of potassium chloride and 0.3 mM of tartaric acid. The taste profiles were measured using the electronic tongue system in accordance with a previously reported method, with minor modifications (Chen et al., 2025), and the numbers of sour and bitter fractions were recorded. The electronic tongue was cleaned and calibrated prior to use, following this procedure: sensor calibration in solution for 30 s, sample measurement for 30 s, and cleaning (2 cycles × 3 s each).

2.2.6 Identification of Bitter substances in Chuju

2.2.6.1 HPLC-Q-TOF-MS identification of separated components

The qualitative analysis of sour and bitter fractions in Amomum villosum extract was performed on a Waters ACQUITY UPLSTM system (Waters Technology Co., Ltd., Beverly, MA, USA) combined with a quadrupole time-of-flight mass spectrometer (Waters Technology Co., Ltd., Beverly, Massachusetts, USA).The chromatographic and mass spectrometry conditions were modified in accordance with the method of (Chen et al., 2025). Separation was achieved using an ACQUITY UPLC® HSS T3 column (2.1 mm × 100 mm, 1.8 μm, Waters) with a mobile phase consisting of A (0.1% formic acid) and B (formic acid in acetonitrile). The sample solution (2 μL) was prepared for component analysis. The column temperature was set at 40 ◦C with a flow rate of 0.3 mL/min. The optimized gradient elution program was as follows: 8% B at 0 ~ 1 min; 8%~98% B at 1 ~ 8 min; 98% B at 8 ~ 10 min; 98%~8% B at 10 ~ 10.1 min; 8% B at 10.1 ~ 12 min.

For mass spectrometry, Thermo Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, USA), the mass spectrometry system was operated in both positive and negative ion modes, with ESI as the ion source. The specified parameters are set as follows: Positive ion spray voltage, 3500 V; negative ion spray voltage, -2500 V; sheath gas, 40 arbs; auxiliary gas ,10 arbs; capillary temperature, 325 ℃. performing a full scan at a resolution of 70,000, with a primary ion scan range of m/z 100 ~ 1000. HCD is used for secondary fragmentation, with a collision energy of 30 eV, and the secondary resolution is 17,500. The first three ions are fragmented during signal acquisition, and dynamic exclusion is employed to remove unnecessary MS/MS information.

2.2.6.2 The contribution degree of key bitter compounds in Chuju

In order to determine the contribution of the screened compounds to the bitterness in Chuzhou chrysanthemum after being compared with the BitterDB database and identified as bitter compounds, the DoT factor was introduced (Guo et al., 2024). The Dose over threshold factor (DoT factor) was selected to represent the contribution degree of bitter compounds to the bitterness in Chuzhou chrysanthemum. When the DoT value is greater than or equal to 1, it indicates that the bitter compound has a significant impact on the overall sensory characteristics (Yan et al., 2023). The DoT factor is the ratio of

the quantitative data of the compound to the threshold of bitterness recognition. The bitterness recognition threshold was obtained from the BitterDB (https://bitterdb.agri.huji.ac.il) database.

2.2.7 Molecular docking

2.2.7.1 Software and databases

The software and databases used in this experiment are free for academic use. PDB (https://www.rcsb.org/) was used to download the 3D structure of the protein. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to download the 3D structure of the ligands. For some compounds that have no 3D structure but only 2D structure. Chem3D (http://www.cambridgesoft.com/software/ChemOffice) was used to solve compounds that only have 2D structures but no 3D structures. Python3.12(https://www.python.org/downloads/) was download and used for language purposes. Sail Vina (https://github.com/beikwx/sail vina) was used for Molecular docking of receptor and ligand interactions. PyMol (https://pymol.org/2/) was used for the visualization of docking results. Protein-Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) was used for view details on the interaction between the compounds and the receptor.

2.2.7.2 Preparation of receptor file

Download the 3D structure of bitter receptor TAS2R14(PDBID: 8VY7, 2.68 Å) in the PDB database. The downloaded 3D structure is opened in PyMol, all molecules except proteins, such as water molecules and pro-ligands, etc., are deleted, and finally the processed bitter receptors are saved with the .pdb extension.

2.2.7.3 Preparation of ligand file

The 3D structures of the key bitter compounds of Chuzhou chrysanthemum identified were downloaded through PubChem. The downloaded 3D structures are opened in PyMol and saved with the .pdb extension to obtain a ligand database.

2.2.7.4 Docking

Docking with Sail Vina, an auxiliary tool for AutoDock Vina. Convert the prepared ligands and receptors in the .pdb format to the .pdbqt format in sail vina. The docking active center is X:137.420; Y:126.679; Z:13.077. The final docking result can be extracted from the docking score through the tool button. The results are visualized in PyMol, and the specific forms of receptor-ligand interactions can be seen in PLIP.

2.2.8 Statistical analysis

Sensory data were collected and elaborated by Friedman test at the significant differences at $P < 0.05$ using IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA). The spectra and radar diagrams for electronic tongue detection were both drawn by Origin 2024 (OriginLab Corporation, Northampton, USA). The interaction diagram of molecular docking was drawn by PyMol (Schrödinger, Inc., New York, USA).

3 Results and discussion

3.1 Results of sensory-guided fractionation

3.1.1 Bitter intensity of solvent extract

After sequential extraction with petroleum ether, ethyl acetate, and n-butyl alcohol, the residual aqueous phase (FIV) showed no detectable bitterness, confirming complete removal of bitter compounds through solvent partitioning. To quantify bitterness intensity, taste dilution (TD) factors were determined through sensory evaluation.

Fraction F□ exhibited the highest TD factor (32), followed by F□ and F□ (both TD = 16), while F□ showed the lowest value (TD = 8). Sensory panel rankings indicated bitterness intensity as F□>F□=F□>F□. The electronic tongue analysis yielded a distinct bitterness ranking (F□>F□>F□>F□) that differed from sensory evaluation results (Table 3 and Fig. 1). This difference possibly due to the sensory evaluation being a comprehensive result of overall the taste, whereas the results of the electronic tongue were limited by the types of sensors used (Miao et al. 2024). Importantly, both methods consistently identified F□ as the most bitter fraction, warranting its selection for further bitter compound identification.

Table 3
The bitterness intensity and TD factor of the solvent extract

Solvent extract	Bitterness	TD factor
F□	6.73	32
F□	1.99	16
F□	-0.93	16
F□	4.63	8

3.1.2 Isolation of F□

The bitter compounds in F□ were fractionated by silica-gel chromatography, yielding eleven subfractions (F□-1 to F□-11). The bitterness intensity of all 11 fractions was assessed using both electronic tongue analysis and BIBD ranking tests. Since the sample size of 11 components is too large for manual sensory evaluation, it is easy to cause sensory fatigue of the evaluators and have an impact on the final results. Therefore, based on the results of the electronic tongue, the least bitter component, namely F□-11, is removed. The final result of the electronic tongue indicated that the most bitter component was F□-10, and the most bitter component was evaluated by artificial senses as F□-4, the results are shown in Table 4 and Fig. 2.

Table 4
BIBD sequential test results and electronic tongue detection data

Sample	F□-1^{d}	F□-2^{bcd}	F□-3^{cd}	F□-4^{a}	F□-5^{bcd}	F□-6^{abc}	F□-7^{ab}	F□-8^{bcd}	F□-9^{bcd}	F□-10^{abcd}
Rank sum(Ri)	57	48	51	32	44	38	37	49	50	44
Bitterness	7.19	7.41	3.82	6.63	6.53	4.95	7.49	7.85	7.68	8.11
X^2	16.92									
F-value	20.56									

According to the Friedman test, the F-value is 20.56. In the X^2 distribution table, when the significance level was 0.05 and df was 9, X^2 = 16.92. F = 20.56 > 16.92, indicating that there was a significant difference in bitterness among the 10 samples. When comparing multiple samples, $LSD_{0.05}$=13.9. The significant differences among samples were labeled according to the rank and comparative letter labeling method of BIBD, and the results are shown in Table 4. The rank sum of F□-4 is the smallest and its bitterness is strongest, but there is no significant difference compared with F□-6, F□-7 and F□-10. The bitterness of sample F□-1 is the smallest. Finally, the results of BIBD and the electronic tongue were combined to identify F□-4 and F□-10.

3.2. Identification of key bitter compounds

For more detailed structural elucidation, the molecular masses of the bitter compounds were determined by HPLC-Q-TOF-MS in the positive/negative ionization mode. Figure 3 and Fig. 4 shows the typical total ion chromatography (TIC) of the suspected bitter substances from FI-4 and FI-10. A total of 19 compounds were identified in FI-4, including 6 in positive ion

mode and 13 in negative ion mode. FI-10 identified 16 compounds, including 6 in positive ion mode and 10 in negative ion mode. A total of 28 compounds were identified from the two components (Table 5). The molecular structures and secondary mass spectra of these 28 compounds are shown in Attached Fig. 1.

Table 5
General Table of Compounds for identification

Fractions	$R_{t/min}$	Formula	Experimental mass	MS/MS (m/z)	compound	Class
F□-4	0.68	C_5H_5N	79.04	(-)94.03,124.04	Pyridine	Others
	0.75	$C_4H_6O_4$	118.03	(-)73.03,116.99	Succinic acid	Organic acid
	0.92	$C_5H_{10}O_3$	118.06	(-)73.03,116.99	5-hydroxypentanoic acid	Others
	1.63	$C_6H_{13}NO_2$	131.09	(-)130.09	L-norleucine	Amino acids
	5.32	$C_{15}H_{10}O_6$	286.05	(-)285.04	Kaempferol	Flavonoids
	6.68	$C_{18}H_{30}O_3$	294.22	(-)275.20,293.21	13-HOTrE(r)	Others
	6.95	$C_5H_{11}NO_2$	117.08	(-)116.93	Trimethyl glycine	Others
	7.23	$C_{21}H_{20}O_{11}$	448.10	(-)269.04	Orientin	Flavonoids
	7.84	$C_{17}H_{14}O_6$	314.08	(-)91.03	3-hydroxy-5-methoxy-6,7-methylenedioxyflavanone	Flavonoids
	8.31	$C_{17}H_{16}O_4$	284.10	(-)283.26	Homopterocarpin	Flavonoids
	8.66	$C_8H_6O_4$	116.03	(-)149.01,165.04	3,4-methylenedioxybenzoic acid	Ketones, Aldehydes, Acids
	10.07	$C_{10}H_{10}O_4$	194.06	(-)223.03	5-hydroxyconiferaldehyde	Others
	11.45	$C_5H_{11}NO_2$	117.08	(-)116.93	4-methylaminobutyrate	Others
	3.36	C_8H_8O	120.06	(+)65.04,80.05,109.05	Phenylacetaldehyde	Others
	4.59	$C_{21}H_{20}O_{10}$	432.11	(+)271.06	Vitexin	Flavonoids
	6.35	$C_{15}H_{22}O_2$	234.11	(+)81.07,93.07,105.07, 107.09,119.09,147.12	Artemisinic	Terpenoids
	6.91	$C_{16}H_{19}NO_3$	273.14	(+)57.07,67.05, 91.05,128.06	Norgalantamine	Alkaloids
	7.35	$C_{15}H_{26}O_2$	238.19	(+)67.05,69.07,81.04, 95.09,1099.10	Geranyl 3-methylbutanoate	Others
	7.80	$C_{16}H_{14}O_5$	286.08	(+)57.07,67.06,68.06,	Sakuranetin	Flavonoids
F□-10	0.75	$C_4H_6O_4$	118.03	(-)73.03,116.99	Succinic acid	Organic acid
	0.88	$C_6H_{12}O_6$	180.06	(-)59.01,71.01,89.02	Fructose	Saccharides
	3.56	$C_7H_6O_3$	138.03	(-)136.89	3-hydroxybenzoic acid	Others

Fractions	$R_{t/min}$	Formula	Experimental mass	MS/MS (m/z)	compound	Class
	4.05	$C_{11}H_8N_2S$	200.04	(-)138.02,182.01	Camalexin	Alkaloids
	4.32	$C_{21}H_{22}O_{11}$	450.12	(-)151.00,287.03	Eriodictyol-7-glucoside	Flavonoids
	5.32	$C_{15}H_{10}O_6$	286.05	(-)285.04	Kaempferol	Flavonoids
	6.95	$C_5H_{11}NO_2$	117.08	(-)116.93	Trimethyl glycine	Others
	7.23	$C_{21}H_{20}O_{11}$	448.10	(-)269.04	Orientin	Flavonoids
	8.80	$C_{21}H_{30}O_3$	330.22	(-)149.10,311.25	Pregn-4-ene-3	Others
	11.45	$C_5H_{11}NO_2$	117.08	(-)116.93	4-methylaminobutyrate	Others
	1.83	$C_8H_9NO_3$	167.06	(+)65.04,80.05,109.05	Pyridoxal	Vitamin
	3.36	C_8H_8O	120.06	(+)65.04,80.05,109.05	Phenylacetaldehyde	Others
	4.59	$C_{21}H_{20}O_{10}$	432.11	(+)271.06	Vitexin	Flavonoids
	6.88	$C_6H_{13}NO_2$	131.09	(+)57.94,59.93,115.96, 116.97,118.97	Propiobetaine	Others
	9.41	$C_{20}H_{42}O_5$	362.30	(+)57.07,71.09,89.06	Tetraethylene glycol monododecyl ether	Others
	11.20	$C_6H_{13}NO_2$	131.09	(+)69.07,86.10	L-isoleucine	Amino acids

3.2.1 Identification of F□-4 by HPLC-Q-TOF-MS

Among the 6 compounds identified by the TIC positive ion mode of F□-4, combined with the information from the BitterDB database, only compound No. 2 was ultimately recorded as a bitter compound. No. 2 was eluted at 4.59 min, and a pseudo-molecular ion $[M + H]^+$ was found at m/z 432.11, it is speculated that the molecular formula is $C_{21}H_{20}O_{10}$. The ion fragment obtained through secondary MS spectrometry is m/z 271.06, losing a molecule with a mass unit of 162. The molecule with a mass unit of 162 might be a glucose group. By comparing the Massbank database, it is finally determined that the compound is vitexin (Fig. 5. A).

Combined with the BitterDB database, vitexin was determined as a bitter compound. To determine the contribution degree of bitterness of this compound, the DoT factor was used for representation. The bitterness threshold was obtained from the literature and the database, as shown in Table 6. The DoT factor of vitexin was calculated to be 8.77, with a high contribution and being a key bitter compound.

Table 6
The DoT factor of bitter compounds

Compound	Fractions	Threshold(mM)	DoT
Vitexin	F□-4	0.001	8.77
	F□-10		136.62
Kaempferol	F□-4	0.01748	0.40
	F□-10		25.88
Orientin	F□-4	0.003	2.73
	F□-10		1.51
L-isoleucine	F□-4	11.00	0.45

For the remaining five compounds, based on the above determination process, finally No. 1 (Rt = 3.36) is phenylacetaldehyde. No.3 (Rt = 6.35) is artemisinic; No. 4 (Rt = 6.91) is norgalantamine; No. 5 (Rt = 7.35) is Geranyl 3-methylbutanoate; No. 6 (Rt = 7.80) is sakuranetin. None of these compounds have been recorded as bitter substances in the BitterDB database or literature, so no further analysis will be conducted subsequently.

Among the 13 compounds obtained by TIC in the F□-4 negative ion mode, No. 1 (Rt = 0.675) is pyridine; No. 2 (Rt = 0.75) is succinic acid; No. 3 (Rt = 0.92) is 5-hydroxypentanoic acid; No. 4 (Rt = 1.63) is L-n-leucine; No. 5 (Rt = 5.32) is kaempferol; No.6 (Rt = 6.68) is (13S)-13-hydroxyoctadeca-9,11,15-trienoic acid; No. 7 (Rt = 6.95) is trimethyl glycine; No. 8 (Rt = 7.23) is orientin; No. 9 (Rt = 7.84) is a 3-hydroxy-5-methoxy-6,7-methylenedioxyflavanone; Number 10 (Rt = 8.31) is homopterocarpin; No. 11 (Rt = 8.66) is 3,4-methylenedioxybenzoic acid; No. 12 (Rt = 10.07) is a 5-hydroxyconiferaldehyde; No. 13 (Rt = 11.45) is 4-methylaminobutyrate. These compounds, in combination with the BitterDB database and literature retrieval, were ultimately identified as bitter substances in compounds No. 5 and No. 8. The bitterness threshold was obtained from the database and literature queries, and the DoT factor was calculated, as shown in Table 6.

The ionic peak of compound No. 5 is m/z 286.0477$[M-H]^-$. It is speculated that the possible molecular formula is $C_{15}H_{10}O_6$. Only m/z 285.04 was detected by the secondary mass spectrometry, ions at m/z 284 and 285 are the key fragment ions of kaempferol derivatives (Pu et al., 2017), combined with the literature and Massbank database (Fig. 5. B), it is kaempferol. Therefore, compound No. 5 is determined to be kaempferol. The bitterness contribution degree of kaempferol, the DoT value is shown in Table 3, which is 0.4. Thus, it can be seen that among the components of F□-4, the bitterness contribution degree of kaempferol is not high.

The ion peak $[M-H]^-$ of compound No. 8 is m/z 448.1, and it is speculated that the molecular formula is $C_{21}H_{20}O_{11}$. The ion fragment obtained by secondary mass spectrometry is m/z 269.04. Combined with the secondary mass spectrometry in the Massbank database (Fig. 5. C), it is speculated that it might be due to the cleavage of the carbon glycosidic bond. The release of neutral glucose molecules yields $[M-H]^-$ of apigenin at m/z 269. Therefore, compound No. 8 is identified as orientin. The DoT factor is shown in Table 6.

3.2.2 Identification of F□-10 by HPLC-Q-TOF-MS

Among the 5 compounds identified by the TIC negative ion mode of F□-10, combined with the information from the BitterDB database, only compound No. 5 was ultimately recorded as a bitter compound. It was eluted at 11.2 min, and a pseudo-molecular ion $[M + H]^+$ was found at m/z 131.17, it is speculated that the molecular formula is $C_6H_{20}NO_2$. The ion fragment obtained through secondary MS spectrometry is m/z 86.10 and m/z 69.07, representing the corresponding fragments $[C_5H_9]^+$ and $[C_5H_{12}N]^+$ respectively, fragment $[C_5H_9]^+$ was formed by the loss of iminogroups from fragment $[C_5H_{12}N]^+$, suggesting the presence of amino groups in this compound. m/z 86.1 shows a relatively high abundance in secondary MS

and may be a marker ion of isoleucine. By comparing the reference literature and the Massbank database (Fig. 5. D), it is finally determined that the compound is L-isoleucine. The DoT factor is shown in Table 6. The results show that the value is less than 1, indicating that the contribution of L- isoleucine to bitterness is not high and it is not a key bitter compound.

Among the remaining four compounds, No. 1 (Rt = 1.83) is pyridoxal; No. 2 (Rt = 3.36) is phenylacetaldehyde; No. 3 (Rt = 4.59) is vitexin, which is consistent with the analysis in F□-4 earlier; No. 4 (Rt = 6.88) is propiobetaine; No. 5 (Rt = 9.41) is tetraethylene glycol monododecyl ether. None of these four compounds has been reported to be bitter compounds, so they will not be analyzed here.

Ten compounds were identified under the F□-10 negative ion mode. No. 1 (Rt = 0.75) was succinic acid. No. 2 (Rt = 0.88) is fructose; No. 3 (Rt = 3.56) is 3-hydroxybenzoic acid; No. 4 (Rt = 4.05) is camalexin. No. 5 (Rt = 4.32) is eriodictyol-7-glucoside; No. 6 (Rt = 5.32) is kaempferol; No. 7 (Rt = 6.95) is betaine; No. 8 (Rt = 7.23) is orientin; No. 9 (Rt = 8.80) is 11α -hydroxyprogesterone; No. 10 (Rt = 11.45) is N-methyl-4. Combining the BitterDB database and literature references, it was finally determined that No. 6 and No. 8 were bitter substances, namely kaempferol and orientin, and the analysis results were consistent with F□-4.

Based on the identification results of F□-4 and F□-10, three bitter compounds were finally screened out as possibly the key bitter substances of Chuju, namely vitexin, kaempferol and orientin.

3.3 Molecular docking between bitterness compounds and TAS2R14

In order to verify the binding mode and binding affinity between the 3 screened bitter compounds and the bitter receptor, molecular docking was further carried out. TAS2R14 was selected for molecular docking. A final docking fraction greater than − 5 indicates a tight binding between the bitter compound and the bitter receptor. The common bitter compound quinine was docked with the bitter taste receptor TAS2R14, and the docking fraction indicated the rationality of the selection of the active center of the bitter taste receptor. Quinine's final docking score was − 8.1

The binding effect and interaction between vitexin and receptor are shown in Fig. 6. The docking fraction of vitexin to the bitter taste receptor is -8.6. It can be seen from the interaction diagram that vitexin forms hydrogen bonds with GLY-229, SER-232, GLY-283 and SER-194 on the receptor, and is connected with ALA-226, PHE-198 and TYR-107 through hydrophobic interactions. Therefore, the connection between vitexin and the bitter taste receptor is tight.

The binding effect and interaction between kaempferol and receptor are shown in Fig. 7. The docking fraction of kaempferol to the bitter taste receptor is -8.9. It can be seen from the interaction diagram that a hydrogen bond is formed between kaempferol and GLY-104 on the receptor, and it is connected to PHE-198 and TYR-107 through hydrophobic interactions. Therefore, the connection between kaempferol and the bitter taste receptor is tight.

The binding effect and interaction between orientin and receptor are shown in Fig. 8. The docking fraction of orientin to the bitter taste receptor is -8.9. It can be seen from the interaction diagram that a hydrogen bond is formed between orientin and GLY-104, LEU-282, LEU-280 and SER-232on the receptor, and it is connected to PHE-198, ILE-111 and TYR-107 through hydrophobic interactions. Therefore, the connection between orientin and the bitter taste receptor is tight.

4 Conclusion

In this study, we identified three key bitter substances in Chuzhou chrysanthemum: vitexin, orientin and kaempferol. We obtained the interaction mode between bitter compounds and bitter receptors through molecular docking. However, due to the complex composition of Chuzhou chrysanthemum and the possible interactions among its components, which may affect the final outcome. Meanwhile, further precise quantitative research should be conducted and compared with consumers' sensory evaluations to verify the correlation between the screened bitter compounds and bitterness. Therefore, the experiment still needs further study.

Declarations

Compliance with ethical standards

Since the research involved tasting and food quality evaluation, the academic committee of the School of Biological and Food Engineering of Chuzhou University approved the study and exempted it from review. According to the FDA, CFR Code of Federal Regulations Title 21, food quality and consumer acceptance studies are exempt from review (US Food and Drug Administration 2017).

Author Contribution

Author 1 (Fang Shangmei, First Author): Conceptualization, Methodology, Software, Experiment, Data Analysis, Writing Original Draft

Author 2 (Zhang Jiaheng): Methodology, Software, Experiment, Data Analysis;

Author 3 (Fang Zhiao): Methodology, Experiment;

Author 4 (Zhang Xinru): Experiment

Author 5 (Long Men, Corresponding Author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing Review & Editing.

Author 6 (Zhan Ge, Corresponding Author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing Review & Editing.

Data availability statement

All relevant data are within the paper.

Conflict of interest statement

We declare that we have no conflict of interest.

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Figures

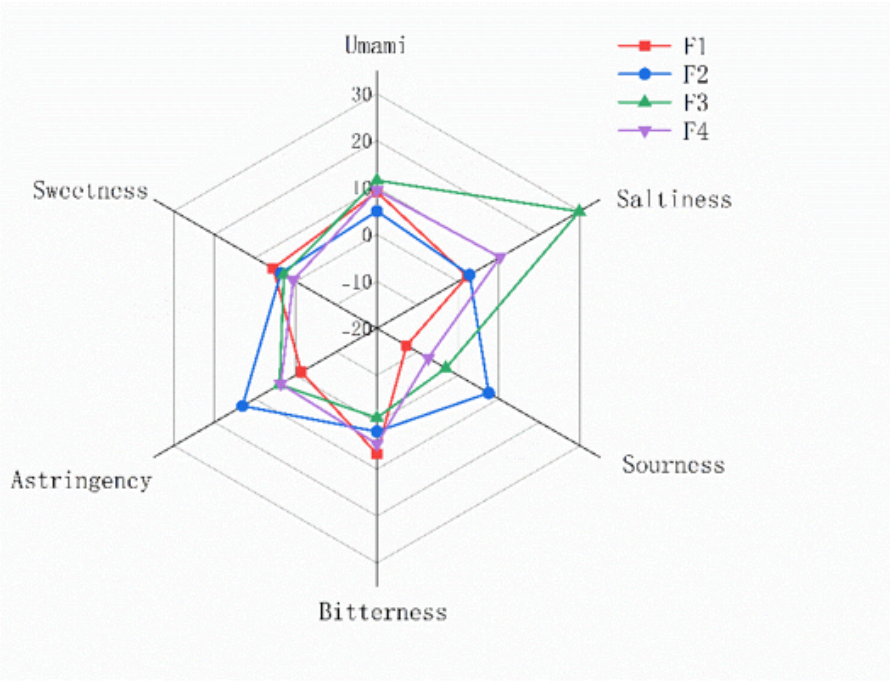

Figure 1

Electron tongue result diagram of solvent-extract

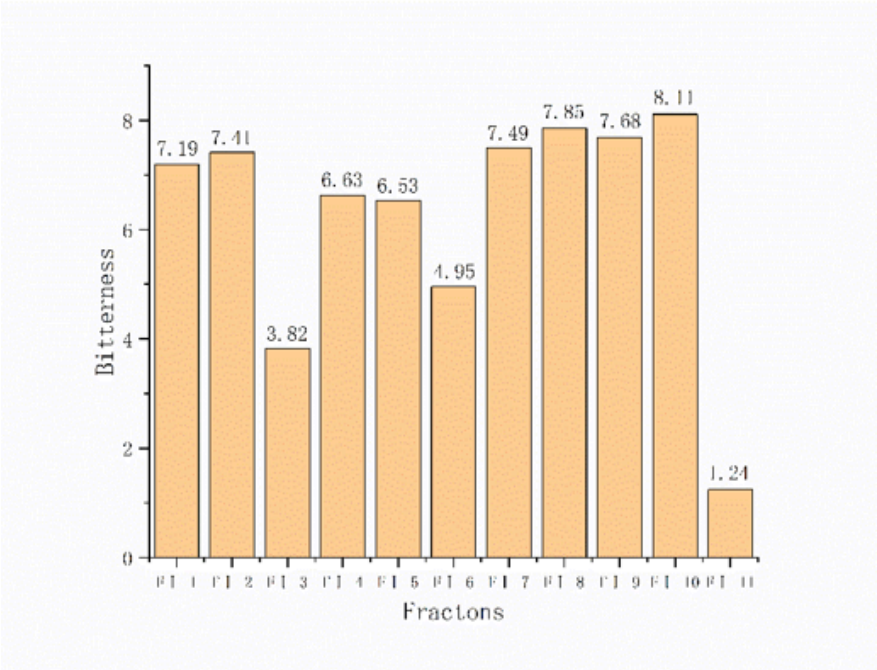

Figure 2

The result diagram of the electronic tongue of component F

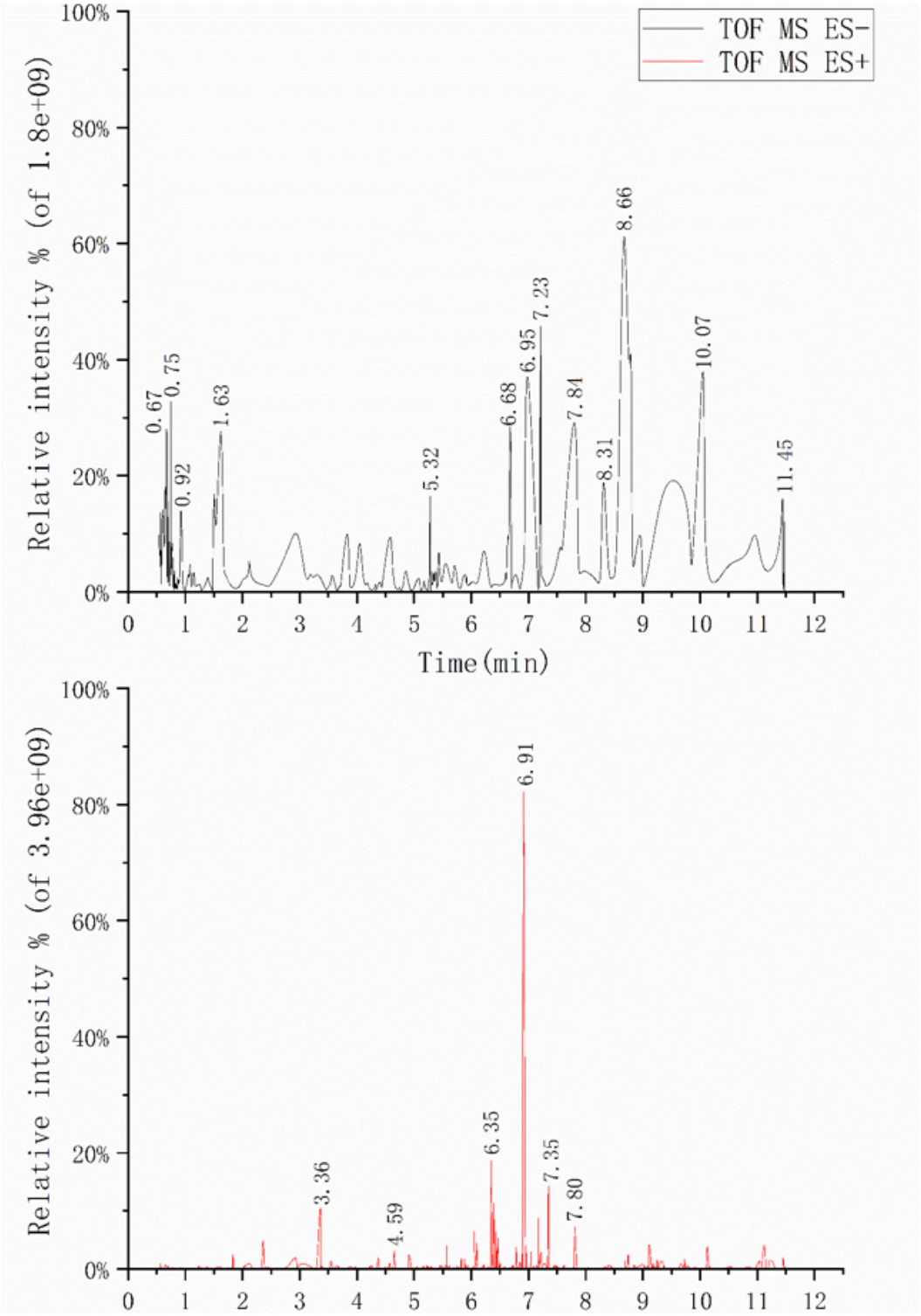

Figure 3

The TIC diagram of F□-4, with black representing the negative ion mode and red representing the positive ion mode

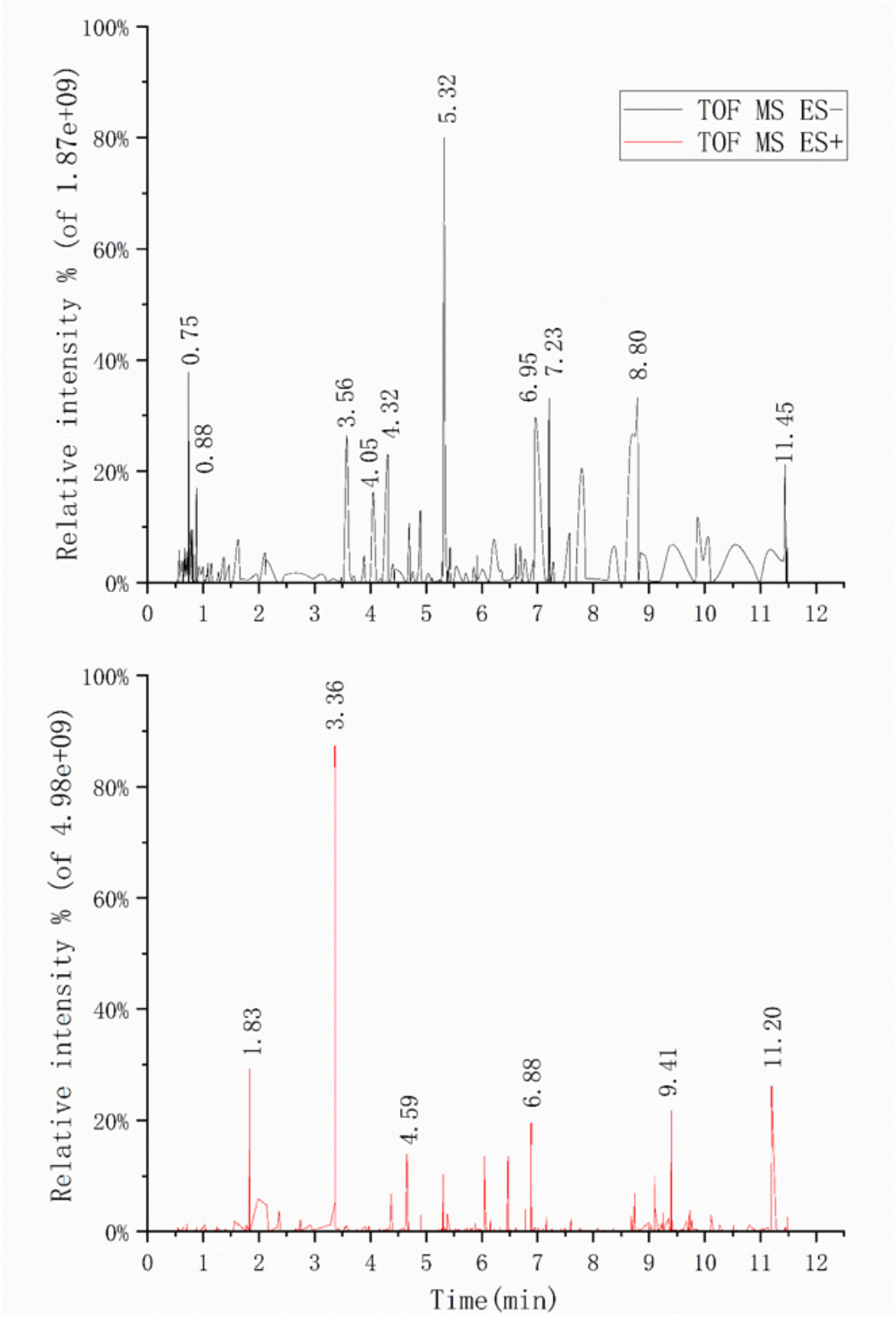

Figure 4

The TIC diagram of F□-10, with black representing the negative ion mode and red representing the positive ion mode

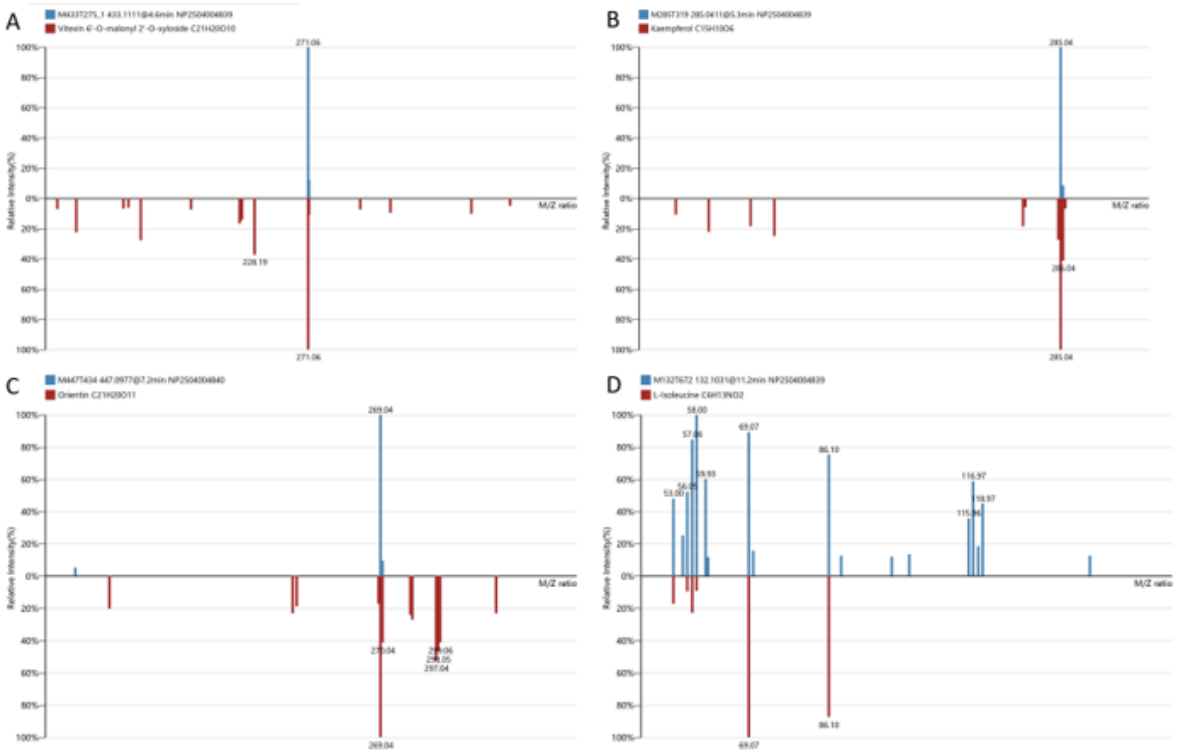

Figure 5

The secondary mass spectrum of the identified compound; A: Vitexin; B: Kaempferol; C: Orientin; D: L-isoleucine

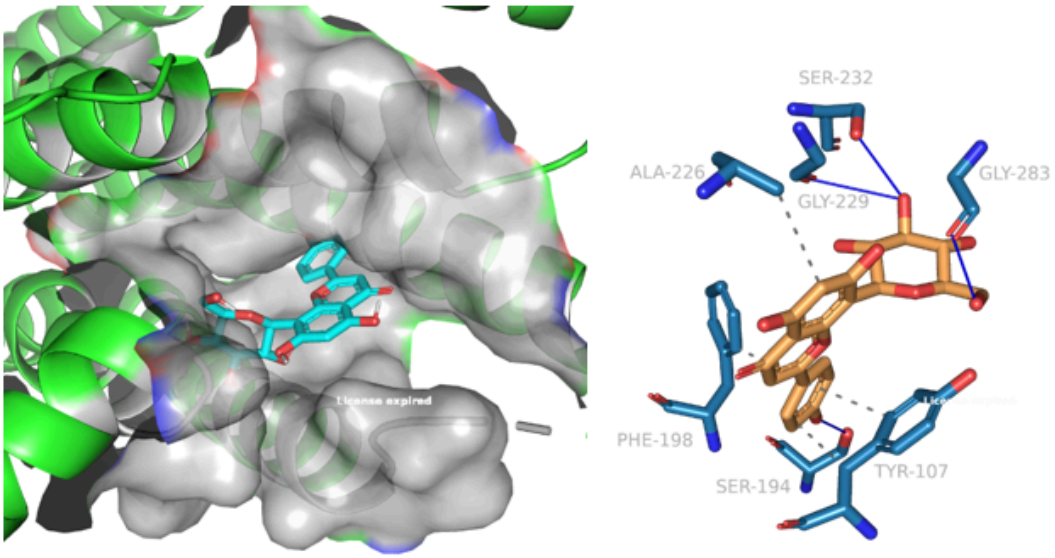

Figure 6

The binding effect of vitexin and TAS2R14 and the intermolecular interaction

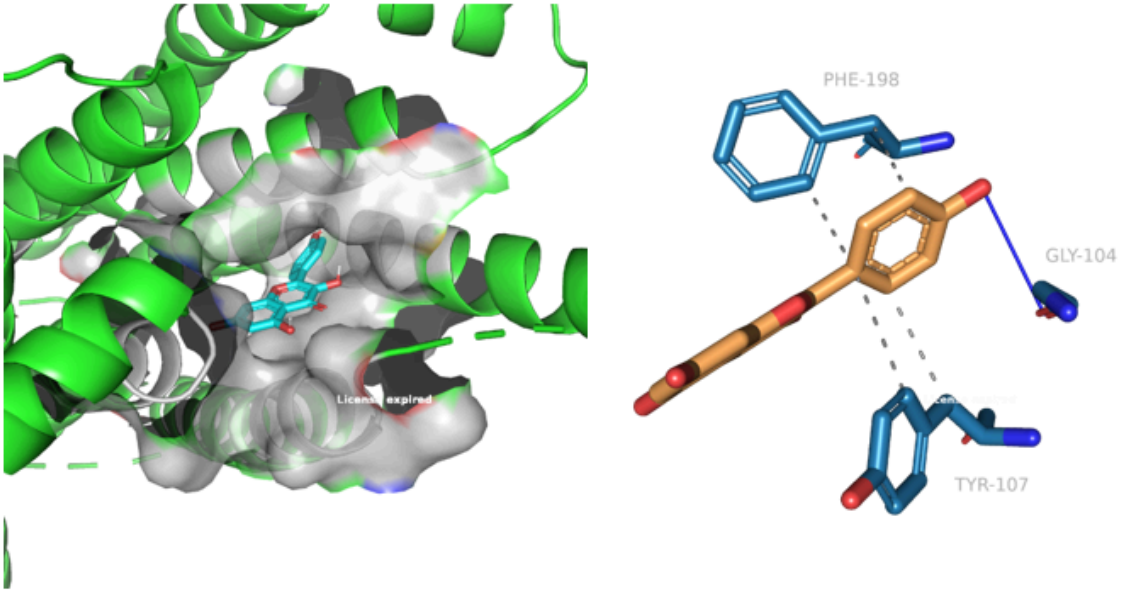

Figure 7

The binding effect of kaempferol and TAS2R14 and the intermolecular interaction

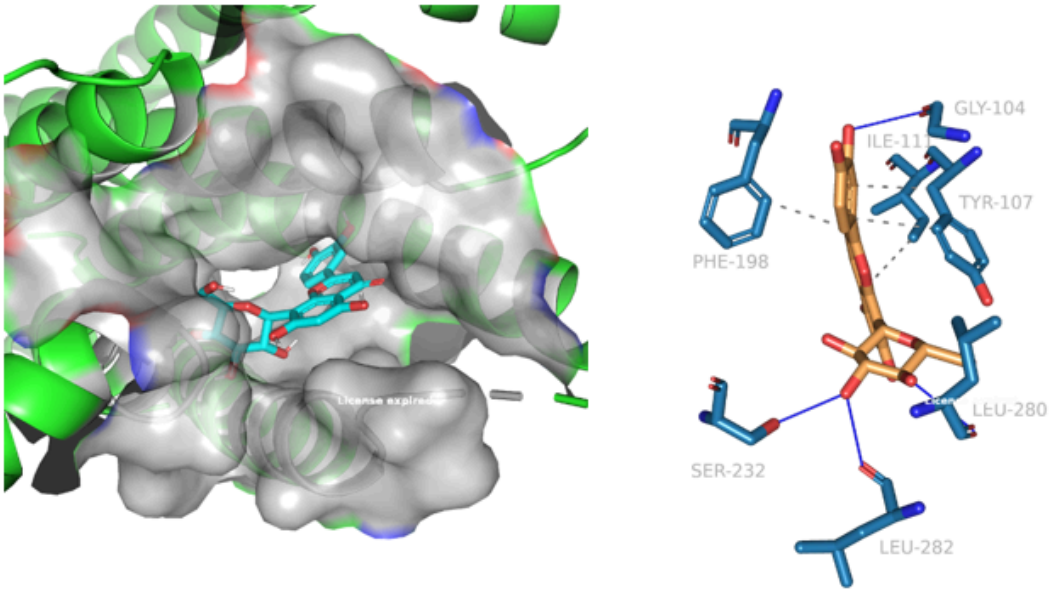

Figure 8

The binding effect of orientin and TAS2R14 and the intermolecular interaction

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