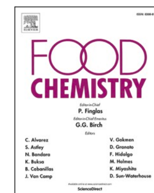
Food Chemistry 388 (2022) 132981

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Sensory-guided isolation and identification of new sweet-tasting dammarane-type saponins from Jiaogulan (*Gynostemma pentaphyllum*) herbal tea

Hong-Xia Zhang[a,b,1], Zhong-Ze Wang[a,c,1], Zhi-Zhi Du[a,*]

[a] Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[b] Key Laboratory for Highly-Efficient Utilization of Forest Biomass Resources in the Southwest China, National Forestry and Grassland Administration, College of Chemical Engineering, Southwest Forestry University, Kunming 650224, China

[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Gynostemma pentaphyllum
Gypenosides
Natural sweetener
Sensory evaluation
Sweetness intensity

ABSTRACT

The purpose of this study was to elucidate the chemical basis for the sweet property produced by *Gynostemma pentaphyllum* and find new natural high-potency (HP) sweeteners. Sixteen new compounds (gypenosides YN **1**–**16**) were obtained by sensory-guided isolation and identification, in which fifteen of them were sweet-tasting constituents with sweetness intensities 10–100 times higher than that of sucrose evaluated by human sensory panel test. Their structures were established by 1D and 2D nuclear magnetic resonance spectra, mass spectroscopy, infrared spectroscopy, UV–visible spectroscopy, and chemical method. Gypenoside YN **4** was the sweetest compound with a concentration of 15.504 ± 1.343 mg/kg, while gypenoside YN **12** has the highest concentration (1397.674 ± 12.948 mg/kg), as shown by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). Structure-activity relationship analysis implied that the compounds' sweetness intensity was associated with side-chain substitutions at C-20 or the number of glucosyl groups at C-3. These new plant-derived natural products may be potential natural sweeteners.

1. Introduction

The gustation of humans, which is the ability to perceive food taste, plays an essential role in food selection, preferences, and intake (Ben Shoshan-Galeczki & Niv, 2020). Sweet, umami, bitter, salty and sour are the five basic tastes that humans can detect (Zhang, Sun, Gu, & Du, 2017). Among them, the sweet taste is an indicator of nutrient-rich food and so is innately attractive to humans with the most pleasant gustatory sensations. In contrast the bitter taste is a warning of toxic ingredients within food, which is linked to food rejection due to unpleasant gustatory sensations (Temussi, 2009). Even babies of a few days old can show their pleasure in response to sweetness but aversion to bitterness (Lindemann, 2001).

With the improvement of people's standard of living, sugar sweeteners such as sucrose, glucose, and fructose have become increasingly widely used in the food and pharmaceutical industries (Goel, Gajula, Gupta, & Rai, 2021), resulting in excessive intake of sugar sweeteners. This has caused some health concerns, as health problems, such as dental caries, hyperglycemia, cardiovascular diseases, and obesity have increased annually (Acevedo, Ramirez-Sarmiento, & Agosin, 2018; Vos et al., 2016). These health issues have motivated people to look for natural non-sugar sweeteners, leading to the discovery and application of steviosides and mogrosides (Soejarto, Addo, & Kinghorn, 2019).

Gynostemma pentaphyllum (Thunb.) Makino, which belongs to Cucurbitaceae family, is commonly known as "Jiaogulan" in China, also called "Southern ginseng". It is a perennial liana plant which is mainly distributed in China, Korea, Japan, and other Southeast Asian countries. It is a popular folk medicine which has been attributed with anticancer, hypolipidemic, hypoglycemic and immunomodulation properties (Zhai et al., 2021). It also has been widely used as herbal tea, vegetables, and

* Corresponding author at: Department of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China.

E-mail address: duzhizhi@mail.kib.ac.cn (Z.-Z. Du).

[1] These authors have contributed equally in this work.

https://doi.org/10.1016/j.foodchem.2022.132981
Received 11 November 2021; Received in revised form 17 March 2022; Accepted 12 April 2022
Available online 15 April 2022
0308-8146/© 2022 Elsevier Ltd. All rights reserved.

function food for centuries (Yang et al., 2013). It was first recorded in the book “Herbs for Famine” of Ming Dynasty in ancient China, in which it is listed as a vegetable.

In 1976, reports of panaxadiol and 2α-hydroxypanaxadiol from a hydrolysate of the saponin-rich fraction of *G. pentaphyllum*, of which the panaxadiol is the aglycone of characteristic saponins from *Panax* species, promoted the interest of domestic and international scholars into phytochemical investigation of *G. pentaphyllum* (Nagai, Izawa, Nagumo, Sakurai, & Inoue, 1981). The dammarane-type saponins from “Jiaogulan”, known as gypenosides, are one of its main functional constituents. So far, more than 300 gypenosides have been isolated and identified from *G. pentaphyllum* and its processed samples (Nguyen, Ha, Yang, Pham, & Oh, 2021). In-depth study of the chemical basis of “Jiaogulan”, has scientifically verified its medicinal efficacy. In recent years, products of *G. pentaphyllum* have become more popular in European and North American countries for their pharmacological activity (Nguyen et al., 2021), such as lowering serum lipid and cholesterol (Weng et al., 2021; Wu et al., 2011), hypoglycaemic (Gao et al., 2016), anti-inflammatory (Wang, Wang et al., 2020), anticancer (Liu, Li, Duan, Xie, & Piao, 2021), hepatoprotective activities (Jia et al., 2018), and neuroprotection (Wang, Zhao et al., 2020; Zhai et al., 2021).

Literature research combined with ethnobotanical investigation showed that *G. pentaphyllum* has sweet and bitter taste varieties (Lu et al., 2013; Wu et al., 2011). The sweet variety has been used as a dietary sweetener in Japan (Nagai et al., 1981). Scholars have carried out systematic and in-depth research on the medicinal substances and health care effects of *G. pentaphyllum*. However, there is scant research into the chemical basis of its tastes and flavors. In previous investigations, the contents of ginsenosides Rb1, Rb3, Rd, F2, and 20(S)-panaxadiol in the sweet taste “Jiaogulan” variant were obviously higher than those in the bitter variant, according to ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) and high-performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD) (Yang et al., 2013). Their homology in the ribosomal ITS-1 region shares only 69.01% (Wu et al., 2011). Kinghorn, Chin, Pan, and Jia (2010) reported that gypenoside XX was a sweet compound that could be used as a natural sweetener, but did not report data regarding its sweetness intensity. Overall, the compounds responsible for the sweet-taste of *G. pentaphyllum* remain unclear.

Sensory-guided isolation strategy can quickly locate the critical taste compounds from a complex extraction (Frank, Ottinger, & Hofmann, 2001), avoiding more time-consuming methods or substances being separated with no taste activity by blind separation (Shi et al., 2009). This method was successfully applied to elucidate the new taste activity substances of a variety of food materials, such as the bitter compound of Huangjiu (Lu et al., 2021), the sweet taste glycosides of *Myriopteron extensum* (Sun et al., 2016, 2018), the sweet taste saponins of “Tugancao” (Zhang et al., 2017), and the sweetness compounds of wine (Cretin, Waffo-Teguo, Dubourdieu, & Marchal, 2019). Therefore we employed this strategy to locate and identify the sweet taste constituents of “Jiaogulan”.

According to our research team’s preliminary sensory evaluation of “Jiaogulan” from several areas, the sample from Yunnan province of China showed a pleasant sweet taste, and was therefore selected for further investigation. Aiming to identify the sweet-tasting compounds to find new natural high-potency (HP) and low-calory sweeteners, we carried out sensory-guided fractionation and purification of this plant extract. As a result, we obtained sixteen new gypenosides involving three structure types (type I-III) according to the side chain at C-20 of aglycone. Fifteen of them showed a sweet taste, with sweetness intensities ranging from 10 to 100 times greater than that of sucrose. One of them showed a bitter taste. Preliminary structure–activity relationship research showed that the sweet intensity of the type III compounds is higher than that of the other two types of compounds when sugar chains of C-3 and C-20 are the same. The contents of these sweet compounds were quantitatively analyzed by ultra-performance liquid chromatography-electron spray ionization-mass spectrometer/mass spectrometer (UPLC-MS/MS).

2. Material and methods

2.1. Chemicals, instruments and procedures

The materials used in extraction and purification were: acetonitrile (HPLC grade) (Shanghai Xingke, Ltd., Shanghai, China), chloroform (Rionlon, Tianjin, China), dioxane (Sinopharm chemical reagent Co. Ltd., Shanghai, China), sodium dicarbonate (Damao, Tianjin, China). Methanol (HPLC grade) and water (LC-MS grade) were purchased from Fisher Scientific (Fisher Scientific, Pittsburgh, Pennsylvania, USA). The materials used in the absolute configurations of sugar units were: hydrochloric acid (Xilong Chemical Co. Ltd., Guangdong, China), *N*-trimethylsilylimidazole (Sangon Biotech, Shanghai, China), L-cysteine methyl hydrochloride (Sigma, Shanghai, China), *n*-hexane (Damao, Tianjin, China), and D-(+)-glucose (D-Glc), D-xylose (D-Xyl), L-rhamnose (L-Rha), purchased from J & K Scientific Ltd (Guangzhou, China).

Spectra of nuclear magnetic resonance (NMR) were recorded by a Bruker Avance III spectrometer (Bruker, Bremen, Germany). The mass spectra were recorded by a Shimadzu UPLC-Q-TOF-MS (Shimadzu, Tokyo, Japan). The optical rotation was tested on an Autopol VI polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). A UV-2401PC spectrophotometer produced by Shimadzu (Shimadzu, Tokyo, Japan) was used to obtain ultraviolet (UV) spectra. Infrared (IR) spectra using KBr pellets were scanned on a NICOLET iS10 spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Silica gel (200 ~ 300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), Sephadex gel LH-20 (GE Healthcare Biosciences AB, Uppsala, Sweden), macroporous resins column chromatography HP-20 (Yunkai, Tianjin, China), reversed phase (RP) C-18 silica gel (40~63 μm, Merck, Darmstadt, Germany) were employed to conduct column chromatography. A semi-preparative HPLC, Newstyle liquid chromatography (LC) system (Hanbon, Jiangsu, China) equipped with a UV–vis detector (NU3000) and a series of YMC-Pack ODS columns were used to purify the compounds. Thin-lay chromatography (TLC) was performed on silica gel GF_{254} (Qingdao Marine Chemical Co., Ltd., Qingdao, China). Spots were detected under UV light and visualized by heating after spraying with 5% vanillin-H_2SO_4 reagent (vanillin: Shanghai Ryon Biological Technology CO., Ltd., Shanghai, China; H_2SO_4: Damao, Tianjin, China). All samples intended for tasting were dried by a VirTis freezing dryer (SP Scientific, Warminster, Pennsylvania, USA) to eliminate the residual solvents.

2.2. Plant materials

The aerial parts of *Gynostemma pentaphyllum* were collected from Yuxi City in Yunnan province of China and identified by Dr. Wen-Yun Chen in Kunming Institute of Botany. Its voucher specimen (JGL-YN-S-001) was kept in Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction, sensory-guided fractionation and purification

The powdered aerial parts of *G. pentaphyllum* (4.3 kg) were extracted with 95% ethanol at 50 °C for 72 h. The filtrates were concentrated under vacuum to produce a thick, dark extract (257 g), which was partitioned by silica gel column chromatography (CC) eluted with petroleum ether-acetone solvent system (10:1, 7:1, 3:1, 1:1, 0:1; v/v) and methanol to produce eight fractions (Fr. 1 ~ Fr.8), respectively. Fraction 8 (200 g), which showed a sweet taste in taste evaluation, was conducted on macroporous resins HP-20 CC (2 kg), eluting by MeOH-H_2O gradient (0:100, 30:70, 50:50, 70:30, 100:0, v/v) to produce six fractions (Fr.8a ~ Fr.8f), according to TLC analyses. Fr.8c ~Fr.8e were sweet-activity

parts according to the sensory test.

Fr.8c (10 g) was separated by a RP C-18 CC system (φ 4.0 × 45 cm), gradient eluted with water, MeOH-H_2O (30%, 50%, 70% and 100% MeOH, to 8 fractions (Fr.8c-1 ~ Fr.8c-8). Fr.8c-2 (4.58 g) was further divided to seven fractions (Fr.8c-2-a ~ Fr.8c-2-g) by RP C-18 CC system (φ 2.0 × 45 cm, MeOH-H_2O 30%, 40%, 50%, 60%, and 100% MeOH). Compounds **3** (239 mg, t_R = 11.30 min) and **9** (357 mg, t_R = 15.00 min) were obtained by semi-preparative HPLC (ODS-AQ column, 250 × 10 mm) with 69% MeOH-H_2O from Fr.8c-2-d, and **4** (24 mg, t_R = 16.4 min) and **7** (13 mg, t_R = 20.6 min) were purified by semi-preparative HPLC (ODS-AQ column, 250 × 10 mm) with 62% MeOH-H_2O from Fr.8c-2-e. Fr.8c-4 (1.08 g) was separated over a RP C-18 CC (φ 2.0 × 45 cm), eluted with MeOH-H_2O (50%, 52%, 54%, 56%, 58%) and 100% MeOH, to yield six fractions (Fr.8c-4-a ~ Fr.8c-4-f). Fr.8c-4-e (0.14 g) was purified by semi-preparative HPLC on an ODS-AQ column (250 × 10 mm, MeOH/H_2O = 70/30) to gain **5** (39 mg, t_R = 25.3 min) and **14** (11 mg, t_R = 13.5 min). Fr.8c-5 (0.38 g) was subjected to RP C-18 CC (φ 2.0 × 45 cm), eluted with MeOH-H_2O (50%, 58%, 60%) and 100% MeOH, to produce five subfractions (Fr.8c-5-a ~ Fr.8c-5-e). Fr.8c-5-d (0.10 g) was separated by semi-preparative HPLC on a YMC-Pack CN column (250 × 10 mm, MeOH/H_2O = 53/47) to obtain **1** (47 mg, t_R = 10.6 min). Fr.8c-8 (1.05 g) was submitted to a RP C-18 CC (φ 2.0 × 45 cm), eluted with MeOH-H_2O (45%, 47%, 49%, 51%, 70%) and 100% MeOH, to give four fractions (Fr.8c-8-a ~ Fr.8c-8-d). Fr.8c-8-c (0.43 g) was further separated by semi-preparative HPLC on an ODS-AQ column (250 × 10 mm, 3.0 mL/min, MeOH/H_2O = 68/32) to yield **2** (216 mg, t_R = 20.6 min). Compound **8** (136 mg, t_R = 16.05 min) was divided from Fr.8c-8-d (0.20 g) by semi-preparative HPLC (ODS-AQ column, 250 × 10 mm, 3.0 mL/min, MeOH/H_2O = 70/30).

Fr.8d (30 g) was chromatographed on RP C-18 CC (φ 4.0 × 25 cm), eluted with water, MeOH-H_2O (30%, 50%, 60%, 70%) and 100% MeOH, to yield nine fractions (Fr.8d-1 ~ Fr.8d-9). Fr.8d-7 (1.70 g) was submitted to a silica gel CC, eluted by chloroform–methanol (8:2, 7:3, 6:4), to obtain nine fractions (Fr.8d-7-a ~ Fr.8d-7-i). Fr.8d-7-b (0.04 g) was divided by semi-preparative HPLC on an ODS-AQ column (250 × 10 mm, 3.0 mL/min, MeOH/H_2O = 60/40) to produce **6** (6 mg, t_R = 25.3 min). Compound **15** (13 mg, t_R = 18. min) was obtained by a semi-preparative HPLC (ODS-AQ column, 250 × 10 mm, 3.0 mL/min, MeOH/H_2O = 60/40) from Fr.8d-7-e (0.14 g). Fr.8d-7-g (0.51 g) was eluted with MeOH-H_2O (55%, 60%, 65%, 70%) and 100% MeOH on RP C-18 CC (φ 2.0 × 25 cm), to yield three subfractions (Fr.8d-7-g-1 ~ Fr.8d-7-g-3). Fr.8d-7-g-1 (0.25 g) and Fr.8d-7-g-3 (0.07 g) were further separated by semipreparative HPLC (ODS-AQ column, 250 × 10 mm, 3.0 mL/min) with 70% MeOH-H_2O to yield **13** (195 mg, t_R = 13.2 min) and **16** (12 mg, t_R = 14.6 min), respectively. Compound **11** (68 mg) was obtained from Fr.8d-8 (0.37 g) by recrystallization in methanol.

Fr.8e (38.93 g) was eluted with $CHCl_3$-MeOH (8:1, 8:2), $CHCl_3$-MeOH-H_2O (7:3:0.5) on a silica gel CC, successively, to produce seven fractions (Fr.8e-1 ~ Fr.8e-7). Fr.8e-2 (12.0 g) was separated again using a silica gel CC ($CHCl_3$-MeOH-H_2O system 3:1:0.1) to yield nine fractions (Fr.8e-2-a ~ Fr.8e-2-i), based on TLC analysis. Fr.8e-2-d (2.1 g) was isolated by RP C-18 CC (φ 2.0 × 25 cm), eluted with MeOH-H_2O (70%, 75%, 78%) and 100% MeOH, to produce three fractions (Fr.8e-2-d-1 ~ Fr.8e-2-d-3). Compound **10** (54 mg, t_R = 6.8 min) was obtained from Fr.8e-2-d-2 (1.03 g) by semi-preparative HPLC (ODS-A column, 250 × 10 mm, 3.0 mL/min, MeCN/H_2O = 50/50). Fr.8e-2-f (1.3 g) was subjected to RP C-18 CC (φ 2.0 × 25 cm), eluted with MeOH-H_2O (65%, 70%, 72%, 75%) and 100% MeOH, to produce four fractions (Fr.8e-2-f-1 ~ Fr.8e-2-f-4). Compound **12** (75 mg, t_R = 5.30 min) was obtained from Fr.8e-2-f-3 (0.55 g) by semi-preparative HPLC (ODS-A column, 250 × 10 mm, 3.0 mL/min, MeCN/H_2O = 50/50).

Gypenosides YN **1**, white amorphous powder, $[\alpha]_D^{19.3}$ − 13.39 (*c* 0.102, methanol); UV (MeOH) λ_{max} (nm) (log ε): 202.00 (3.14); IR (KBr) ν_{max} cm^{-1}: 3428, 2973, 2926, 1631, 1450; HR-ESI-MS 1093.5805 $[M - H]^-$ (calcd. for $C_{53}H_{89}O_{23}$ *m/z* 1093.5800 $[M - H]^-$); NMR data, see table S1 and S2.

Gypenosides YN **2**, white amorphous powder, $[\alpha]_D^{24.8}$ − 11.93 (*c* 0.110, pyridine); UV (MeOH) λ_{max}(nm) (log ε): 195.50 (3.37); IR (KBr) ν_{max} cm^{-1}: 3417, 2939, 2882, 1633, 1450; HR-ESI-MS 1255.6323 $[M - H]^-$ (calcd. for $C_{59}H_{99}O_{28}$ *m/z* 1255.6328 $[M - H]^-$); NMR data, were shown in table S1 and S2.

Gypenosides YN **3**, white amorphous powder, $[\alpha]_D^{19.7}$ − 12.41 (*c* 0.108, methanol); UV (MeOH) λ_{max}(nm) (log ε): 202.00 (3.15); IR (KBr) ν_{max} cm^{-1}: 3406, 2928, 1635, 1450, 1416; HR-ESI-MS 708.3394 $[M-2H]^{2-}$ (calcd. for $C_{65}H_{109}O_{33}$ *m/z* 708.3392 $[M-2H]^{2-}$); NMR data, see table S1 and S2.

Gypenosides YN **4**, white amorphous powder, $[\alpha]_D^{23.4}$ − 10.98 (*c* 0.106, methanol); UV (MeOH) λ_{max}(nm) (log ε): 197.00(3.74); IR (KBr) ν_{max} cm^{-1}: 3418, 2927, 1667, 1631, 1453; HR-ESI-MS 1415.6706 $[M - H]^-$ (calcd. for $C_{65}H_{107}O_{33}$ *m/z* 1415.6700 $[M - H]^-$); NMR data, see table S1 and S2.

Gypenosides YN **5**, white amorphous powder, $[\alpha]_D^{23.5}$ + 2.22 (*c* 0.108, methanol); UV (MeOH) λ_{max}(nm) (log ε): 196.50 (3.51); IR (KBr) ν_{max} cm^{-1}: 3418, 2969, 2939, 1632, 1458; HR-ESI-MS 1093.5801 $[M - H]^-$ (calcd. for $C_{53}H_{89}O_{23}$ *m/z* 1093.5801$[M - H]^-$); NMR data were shown in S1 and S2.

Gypenosides YN **6**, white amorphous powder, $[\alpha]_D^{19.5}$ − 2.96 (*c* 0.106, methanol); UV (MeOH) λ_{max}(nm) (log ε): 202.00 (3.39); IR (KBr) ν_{max} cm^{-1}: 3853, 3727, 3425, 2926, 2068, 1632, 1458; HR-ESI-MS 553.2940 $[M-2H]^{2-}$ (calcd. for $C_{54}H_{90}O_{23}$ *m/z* 553.2942 $[M-2H]^{2-}$); NMR data, see table S1 and S2.

Gypenosides YN **7**, white amorphous powder, $[\alpha]_D^{24.8}$ + 6.14 (*c* 0.104, methanol); UV (MeOH) λ_{max}(nm) (log ε): 195.50 (3.63); IR (KBr) ν_{max} cm^{-1}:3419, 2968, 2927, 2880, 1636, 1454; HR-ESI-MS 1139.5859 $[M - H]^-$ (calcd. for $C_{54}H_{91}O_{25}$ *m/z* 1139.5859 $[M - H]^-$); NMR data, see table S1 and S2.

Gypenosides YN **8**, white amorphous powder, $[\alpha]_D^{24.8}$ − 12.26 (*c* 0.107, pyridine); UV (MeOH) λ_{max}(nm) (log ε): 195.50 (3.65); IR (KBr) ν_{max} cm^{-1}: 3417, 2970, 2935, 1633, 1454; HR-ESI-MS 1255.6326$[M - H]^-$ (calcd. for $C_{59}H_{99}O_{28}$ *m/z* 1255.6328 $[M - H]^-$); NMR data were shown in table S1 and S2.

Gypenosides YN **9**, white amorphous powder, $[\alpha]_D^{24.9}$ − 2.93 (*c* 0.124, methanol); UV (MeOH) λ_{max}(nm) (log ε): 195.50 (3.76); IR (KBr) ν_{max} cm^{-1}: 3421, 2969, 2931, 1636, 1455; HR-ESI-MS 1417.6858 $[M - H]^-$ (calcd. for $C_{65}H_{109}O_{33}$ *m/z* 1417.6857 $[M - H]^-$); NMR data, see table S1 and S3.

Gypenosides YN **10**, white amorphous powder, $[\alpha]_D^{27.4}$ − 3.9 (*c* 0.107, methanol); UV (MeOH) λ_{max}(nm) (log ε): 196.0 (3.94); IR (KBr) ν_{max} cm^{-1}: 3403, 2967, 2941, 1629, 1451, 1049; HR-ESI-MS 1077.5852 $[M - H]^-$ (calcd. for $C_{53}H_{89}O_{22}$ *m/z* 1077.5851 $[M - H]^-$); NMR data, see table S1 and S3.

Gypenosides YN **11**, white amorphous powder, $[\alpha]_D^{27.0}$ − 32.62 (*c* 0.100, pyridine); UV (MeOH) λ_{max}(nm) (log ε): 202.50 (3.51); IR (KBr) ν_{max} cm^{-1}: 3424, 2975, 2924, 2029, 1639, 1550, 1453; HR-ESI-MS 1239.6373 $[M - H]^-$ (calcd. for $C_{59}H_{99}O_{27}$ *m/z*1239.6379 $[M - H]^-$); NMR data were exhibited in table S1 and S3.

Gypenosides YN **12**, white amorphous powder, $[\alpha]_D^{27.0}$ − 4.33 (*c* 0.108, methanol); UV (MeOH) λ_{max}(nm) (log ε): 196.5 (3.80); IR (KBr) ν_{max} cm^{-1}: 3392, 2926, 1739, 1633, 1445; HR-ESI-MS 1281.6489 $[M - H]^-$ (calcd. for $C_{61}H_{101}O_{28}$ *m/z* 1281.6485 $[M - H]^-$); NMR data, see table S1 and S3.

Gypenosides YN **13**, white amorphous powder, $[\alpha]_D^{19.8}$ − 23.64 (*c* 0.110, methanol); UV (MeOH) λ_{max}(nm) (log ε): 202.50 (3.50); IR (KBr) ν_{max} cm^{-1}: 3441, 2970, 2925, 1638, 1453; HR-ESI-MS 700.3419 $[M-2H]^{2-}$ (calcd. for $C_{65}H_{109}O_{32}$ *m/z* 700.3417 $[M-2H]^{2-}$); NMR data, see table S1 and S3.

Gypenosides YN **14**, white amorphous powder, $[\alpha]_D^{24.9}$ − 4.33 (*c* 0.108, methanol); UV (MeOH) λ_{max}(nm) (log ε): 195.50 (3.76); IR (KBr)

ν_{max} cm^{-1}: 3425, 2925, 1632, 1450; HR-ESI-MS 1431.7014 [M − H]$^-$ (calcd. for $C_{66}H_{111}O_{33}$ *m/z* 1431.7013 [M − H]$^-$); NMR data were presented in table S1 and S3.

Gypenosides YN **15**, white amorphous powder, $[\alpha]_D^{19.8}$ + 8.33 (*c* 0.108, methanol); UV (MeOH) λ_{max}(nm) (log ε): 202.50 (3.32); IR (KBr) ν_{max} cm^{-1}:3440, 2924, 2856, 1632; HR-ESI-MS 1123.5902 [M − H]$^-$ (calcd. for $C_{54}H_{91}O_{24}$ *m/z* 1123.5906 [M − H]$^-$); NMR data, see table S1 and S3.

Gypenosides YN **16**, white amorphous powder, $[\alpha]_D^{29.4}$ − 15.29 (*c* 0.107, pyridine); UV (MeOH) λ_{max}(nm) (log ε): 202.50 (3.46); IR (KBr) ν_{max} cm^{-1}: 3440, 2970, 2926, 1634, 1550, 1453; HR-ESI-MS 692.8447 [M−2H]$^{2-}$ (calcd. for $C_{65}H_{109}O_{31}$ *m/z* 692.8443 [M−2H]$^{2-}$); NMR data were shown in table S1 and S3.

2.4. Acidic hydrolysis of compounds and absolute configuration determination of sugar units in compounds

Each compound was accurately weighed as 2 mg, placed in a round-bottom flask, and was acid hydrolyzed to obtain sugar residues as previously described (Liang et al., 2011; Zhang et al., 2017). Anhydrous pyridine (1 mL) and L-cysteine methyl ester hydrochloride (2 mg) were added into each residue and then stirred at 60 °C for 2 h to gain the mixture solution. Then, the *N*-trimethylsilylimidazole (0.2 mL) was mixed into the mixture solution to react at 60 °C for 2 h. The final reaction solution was partitioned by *n*-hexane/H_2O (1/1, 3 mL each). After concentration, the *n*-hexane part was analyzed by Agilent 7890A gas chromatography (GC) with a flame ionization detector (FID) and an HP-5 capillary column, 50 m × 0.32 mm × 0.52 μm (Agilent Technologies, Santa Clara, CA, U.S.A.). The carrier gas was helium (99.999%); injector and FID temperature always held at 250 °C. By comparison of the retention time of derivatives of the gypenoside hydrolyzates with derivatives of L-rhamnose (13.0 min), D-xylose (14.8 min), and D-glucose (19.9 min) standards, the absolute configurations of sugar units were determined.

2.5. Sensory evaluations of fractions and isolated gypenosides

Our research team's evaluation panel was created as previously described (Jia & Yang, 2009; Zhang et al., 2017). They evaluated the taste character and sweetness intensity of fractions and pure gypenosides. This panel consists of seven sweet gustation sensitive panelists (three men and four women, 24 to 45 ages, all Chinese).

The water solutions of crude extract and fractions were made at 0.04 mg/mL by distilled water. Sensory panelists evaluated their taste characters to find the sweet fractions and sub-fractions.

The stock solution of each compound with a concentration of 1 mg/mL was made with distilled water. Stock solution was diluted to obtain a descending series of lower concentration solutions (0.50, 0.25, 0.20, 0.125, 0.10, 0.04, and 0.02 mg/mL). The sucrose solutions with concentrations of 10 mg/mL and 20 mg/mL were prepared. Sixteen compounds, Fr.8 and all sub-fractions from Fr. 8 are soluble in distilled water at room temperature. Saturated solutions of Fr. 1 ~ Fr. 7 with poor solubility were used for evaluation after filtering. The panelists tasted the gypenoside solutions and compared their sweetness with the sucrose solution to determine which solutions had a similar taste to that of the sucrose solution. They needed to rinse their mouths with water in between samples and rest for some time after tasting several cups of samples. The ratio between the concentration of the sucrose solution and that of the gypenosides solution was considered the relative sweetness intensity.

2.6. Quantitation of gypenosides by UPLC–MS/MS

Standard solutions of obtained compounds were prepared in 70% methanol–water by accurately weighing at concentrations of 1.0, 2.0, and 5.0 μg/mL for compounds **1**–**5**, **10**–**13,** and **15** and 5.0, 10.0, and 25.0 μg/mL for compound **8** and 50.0, 100.0, and 250.0 μg/mL for compound **9** to make working curves. The fraction 8, which includes all of the obtained compounds, was accurately weighed into a vial (2 mL) to prepare the sample solution at 2.0 mg/mL. All standard and sample solutions were filtrated through a 0.45 μm filter membrane.

Chromatographic analysis was performed in a Waters Acquity UPLC system (Waters Corp., Milford, MA) on the column BEH C18 (50 mm × 2.1 mm, 1.7 μm, Waters Corp., Milford, MA) with the flow rate of 0.3 mL/min, using a linear gradient composed of water (mobile phase A) and acetonitrile (mobile phase B): 0–6 min, 10–20% B; 6–50 min, 20–40% B; 50–51 min, 40–10% B; 51–55 min, 10% B, and each injection volume was 10 μL.

MS analysis was operated using a Xevo Triple Quadrupole MS (Waters Corporation, Milford, MA, USA) equipped with an ESI source set in negative ionization mode. The parameters were set as follows: capillary voltage, 2.4 kV; cone voltage, 40 V; desolvation temperature, 200 °C; desolvation gas flow, 550 L/h; collision gas, argon; collision energy, 55 eV; desolvation gas, nitrogen; cone gas flow, 150 L/h; source temperature, 150 °C. The quantitation analysis data was collected using the multiple reaction monitoring (MRM) modes (Sun et al., 2022) by simultaneously screening parent and daughter ions. The MassLynx software (version 4.1, Waters, Milford, MA, USA) was used to control the instruments and data acquisition and processing.

2.7. Statistical analysis

Statistical analysis was performed using Microsoft Excel 2019. The data are shown as mean value ± standard deviation (SD) with triplicates.

3. Results and discussion

3.1. Sweet taste-guided isolation and structure determination of compounds 1 ~ 16

The 95% ethanol extraction of *G. pentaphyllum* was fractionated by silica gel CC to yield eight fractions (Fr. 1 ~ Fr. 8). The sweet taste active portion Fr. 8 by sweet sensory evaluation was further fractionated by macroporous resins column chromatography to obtain six subfractions (Fr.8a ~ Fr.8f), of which the sweet taste active subfractions (Fr.8c ~ Fr.8e) were subjected to further isolation and purification on silica gel column, RP C-18 CC, and semipreparative HPLC to yield fifteen sweet-tasting gypenoside (named as *gypenosides* **YN 1**–**9**, **11**–**16**), one bitter-tasting gypenoside (named as *gypenosides* **YN 10**). All sixteen compounds obtained as a white amorphous powder are new natural products (Figs. 1–3), and they could be divided into three types according to the side chain at C-20 of aglycone. Their structures were elucidated as dammarane-type tetracyclic triterpenoids based on their experimental spectroscopic data from one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra, mass spectroscopy (MS), infrared spectroscopy (IR), and UV–visible spectroscopy (UV). The data of heteronuclear single-quantum correlation spectroscopy (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC), ^{1}H–^{1}H correlation spectroscopy (COSY), rotating frame nuclear overhauser effect spectroscopy (ROESY) spectra, and heteronuclear single quantum coherence-total correlation spectroscopy (HSQC-TOCSY) analysis ascertain the assignments of the sugar units and aglycone. Fig. 4 exhibits the selected HMBC, ROESY, and ^{1}H–^{1}H COSY correlations of each representative compound of the three types (**3**, **6**, **and 12**). The Supporting Information has exhibited all the detailed structure determinations of the sixteen compounds.

3.2. Sensory evaluations of extraction, fractions and isolated gypenosides

The sweetness intensities of gypenosides YN **1**–**16** were tested by

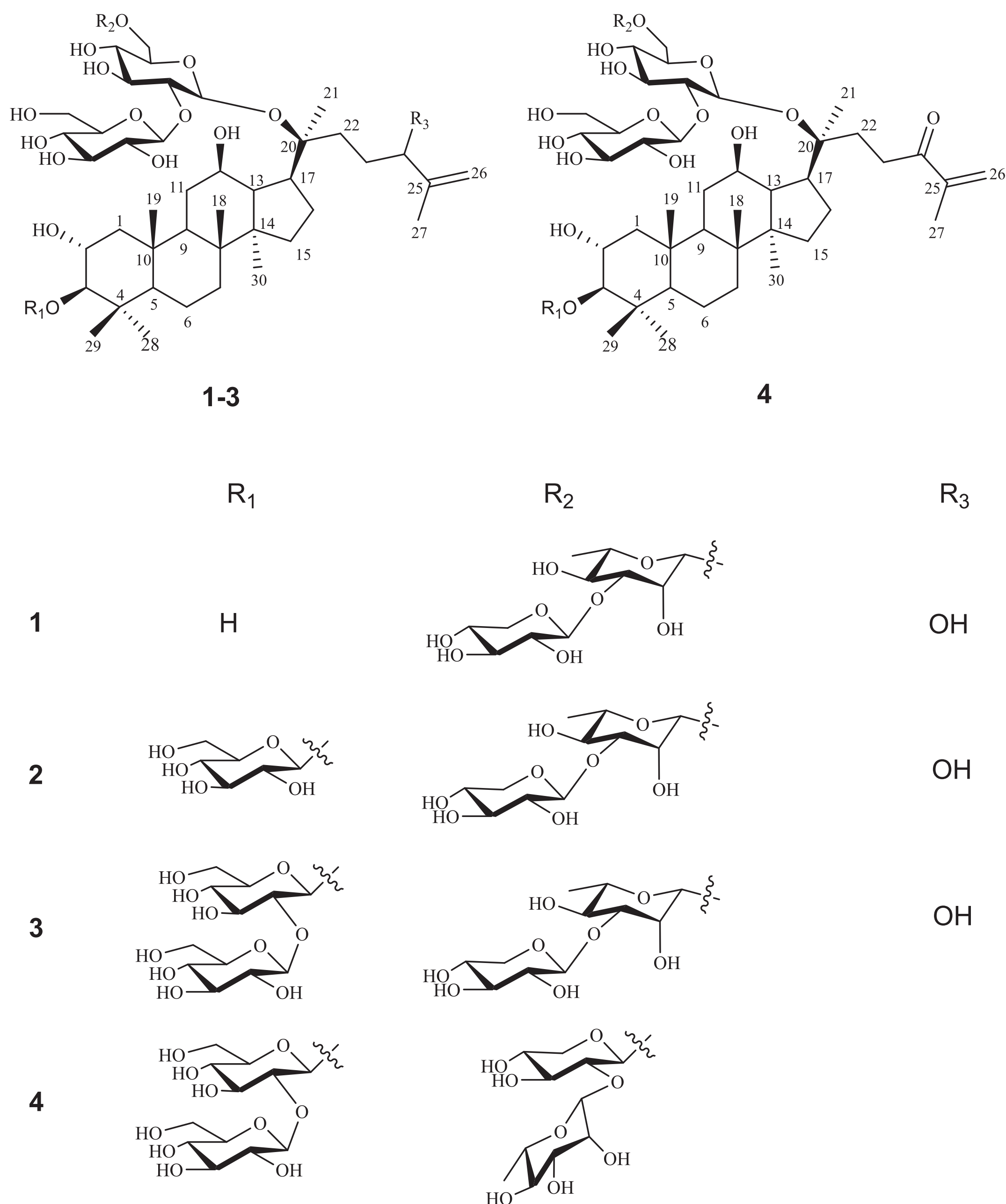


Fig. 1. The structures of compounds **1**–**4**.

seven tasters (see Table 1). It indicated that fifteen of them (**1**–**9**, and **11**–**16**) have a sweet taste, and one compound (**10**) has a bitter taste. Among them, compound **4** has the highest sweet intensity (100 times more than that of sucrose) and the lowest taste thresholds of 0.03 mg/mL, followed by **12**, **13**, **14**, and **16** with moderate sweet intensities and sweetness threshold. Ten gypenosides, **1**–**3**, **5**–**9**, **11**, and **15,** have the lower sweet intensities and the higher sweetness thresholds. Of these compounds, **1**, **4**, **7**–**9** have better gustatory sensations, no after-taste or no bitter taste, and **2**, **3**, **5**, **16** have a slightly astringent taste after-taste, and **6**, **11**–**15** have a slightly bitter after-taste.

During the research process, the sensory evaluation method was used to discover the sweet "Jiaogulan" varieties from many samples. It is a method that can quickly locate fractions containing sweet substances, but at the same time, it is more time-consuming and labor-intensive than mechanized methods. Currently, the electronic tongue, an instrumental method, has been successfully applied to the qualitative and quantitative study of food taste characteristics (Veloso, Dias, Rodrigues, Pereira, & Peres, 2016). Replacing sensory evaluation during sweet variety discovery and sensory-guided separation with an electronic tongue will provide rapid, reproducible results, with continuous operation, especially when used in industrial production it will be cost effective (Phat, Moon, & Lee, 2016; Veloso et al., 2016), and avoid limitations on the number of samples a trained sensory panel can assess per day (Veloso et al., 2016). However, gustatory property evaluation of the isolated compounds is more advantageous for sensory evaluation, and remains the only method by which to provide integrated, direct measurements of peoples' perceived intensities of target attributes (Phat et al., 2016).

3.3. Quantitation analysis of gypenosides by UPLC–MS/MS

The quantities of compounds **1**–**5**, **8**–**13,** and **15** were analyzed by UPLC–MS/MS using the MRM mode. The test data is shown in Table 2. Compound **4** had the highest sweetness, although its concentration was slightly lower, analyzed quantitatively as 15.504 ± 1.343 mg/kg.

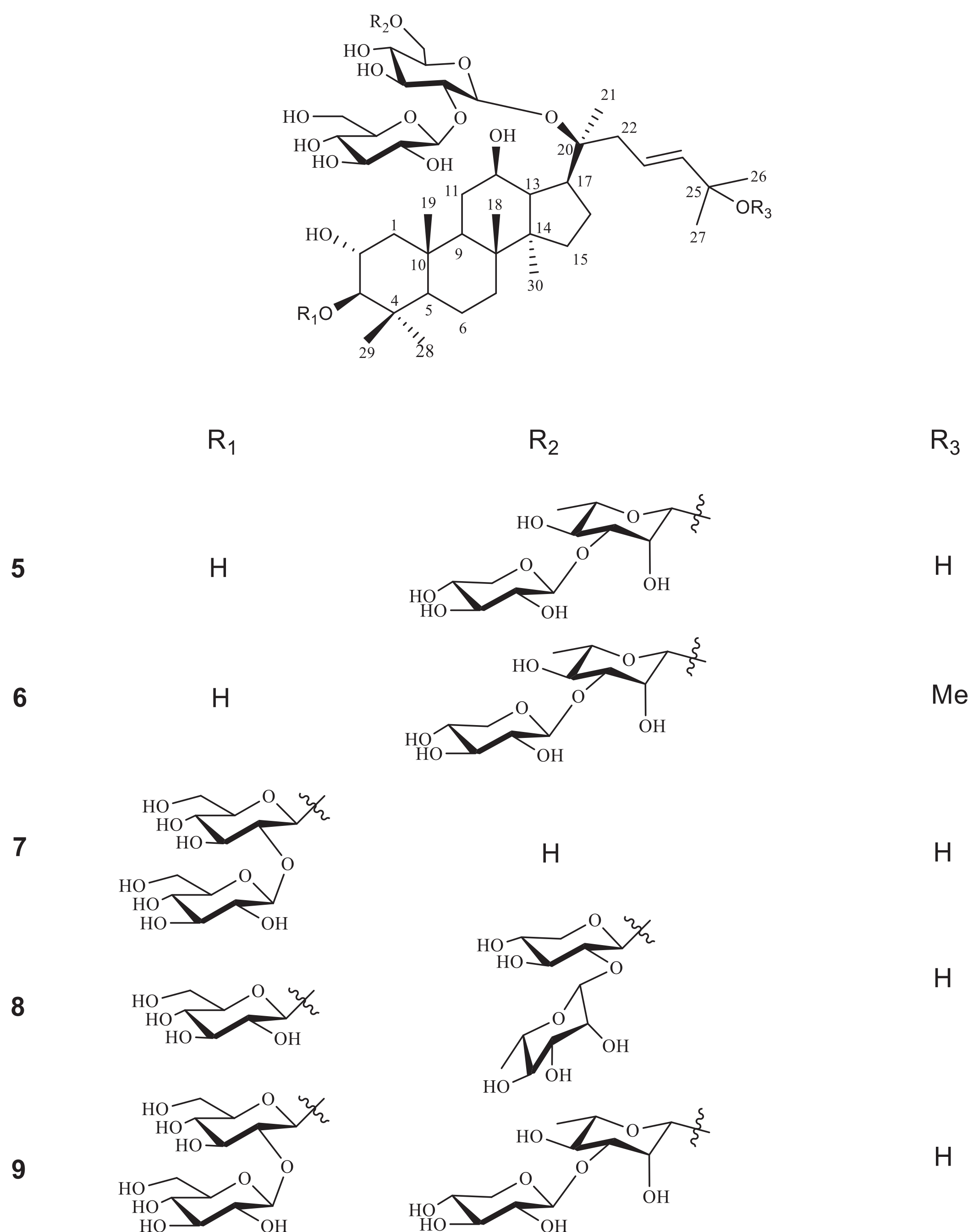


Fig. 2. The structures of compounds **5**–**9**.

Compound **12** was the most abundant substance analyzed quantitatively, reaching 1397.674 ± 12.948 mg/kg. As its sweetness is 50 times higher than that of sucrose, it can provide a preliminarily explanation for the especially sweet taste of this sweet variety. The content of compound **13**, whose sweetness is also 50 times higher than that of sucrose, was quantified as 69.767 ± 2.326 mg/kg. Compounds **6**, **7**, **14**, and **16** were used up in the process of structure elucidation and taste activity evaluation due to the small amount of isolated compounds, resulting in no standard substances to make quantitative curves, so these four compounds were not assayed. According to UPLC analysis, compounds **1** and **5**, **2** and **8**, **3** and **9** could not be separated from each other for the same molecular, parent ions, and daughter ions. Therefore compounds **1**, **2**, and **3** were randomly selected as standard substances to quantify the contents for each of these three pairs of compounds, respectively.

3.4. Structure–activity relationship discussions of isolated gypenosides

Sixteen isolated gypenosides could be divided into three types according to the side chain at C-20 of aglycone. Compounds **1**–**4** belong to type I, of which the side chain at C-20 is 4-methyl-4-pentenyl group and its derivatives. Compounds **5**–**9** belong to type II, of which the side chain is 4-methyl-2-pentenyl and its derivatives. Compounds **10**–**16** belong to type III, of which the side chain is 4-methyl-3-pentenyl. The other structural differences of the sixteen compounds include the sequence and the number of sugar chains linked at C-3 and C-20, the side chain at C-20, and the hydroxylation of C-2. When the sugar chains at C-3 and C-20 are the same, it could be inferred that compounds belonging to type

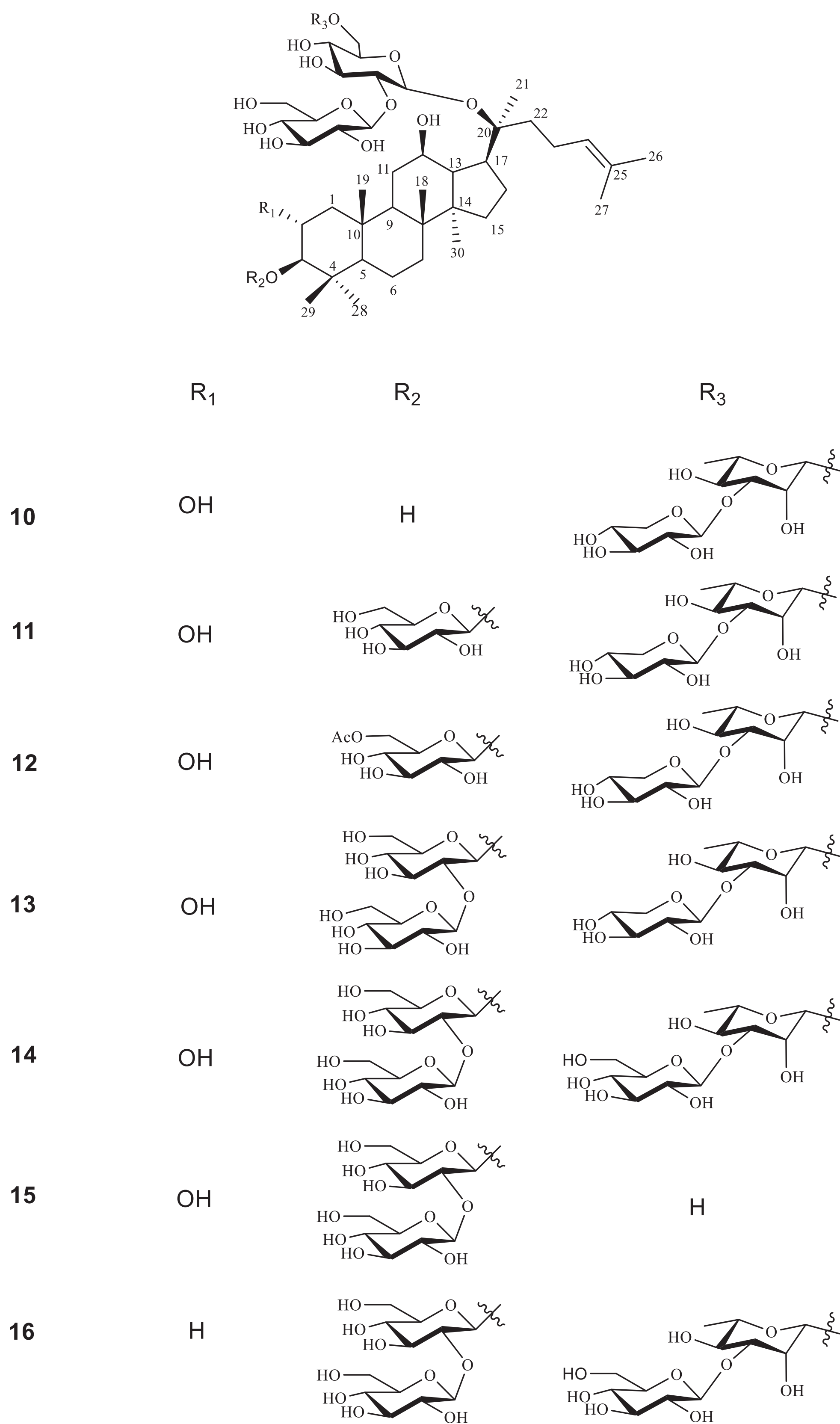


Fig. 3. The structures of compounds **10**–**16**.

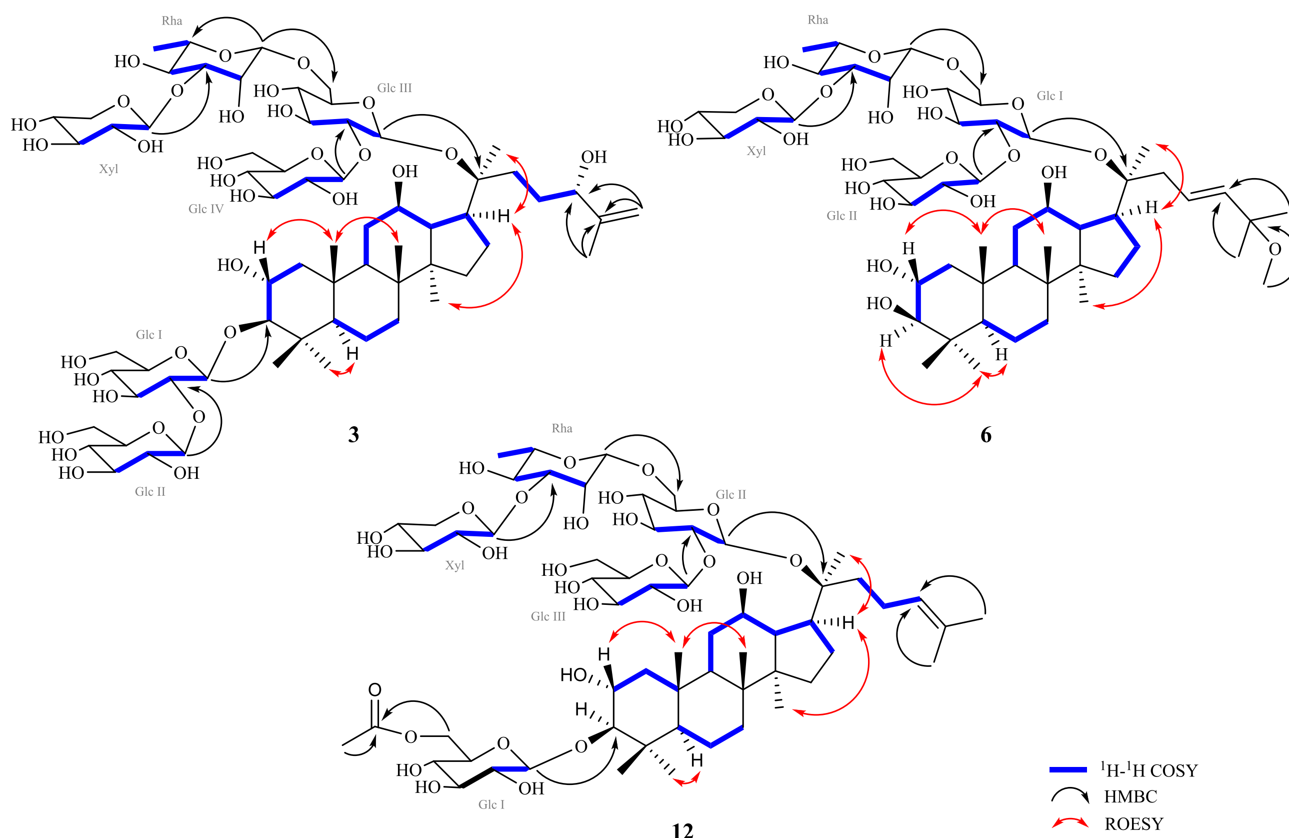


Fig. 4. Selected HMBC, ^{1}H–^{1}H COSY, and ROESY correlations of **3**, **6**, **12** (The sixteen isolated compounds are divided into three types according to the side chain at C-20, and the three compounds selected here are the representatives of the three types.).

Table 1
The sensory evaluation of compounds **1**–**16**.

Compounds	Type	R$_1$	R$_2$	R$_3$	Taste characters	Sweet intensity[a]	Sweet taste threshold (mg/mL)[b]
1	I	-H	-α-L-Rha^3-β-D-Xyl	–OH	Sweet, better gustatory sensation	20	0.17
2	I	-β-D-Glc	-α-L-Rha^3-β-D-Xyl	–OH	Sweet, astringent taste after-taste	20	0.17
3	I	-β-D-Glc^2-β-D-Glc	-α-L-Rha^3-β-D-Xyl	-OH	Sweet, astringent taste after-taste	20	0.17
4	I	-β-D-Glc^2-β-D-Glc	-β-D-Xyl^2-α-L-Rha	=O	Sweet, better gustatory sensation	100	0.03
5	II	-H	-α-L-Rha^3-β-D-Xyl	-H	Sweet, astringent taste after-taste	10	0.1
6	II	-H	-α-L-Rha^3-β-D-Xyl	–CH_3	Sweet, a slightly bitter after-taste	30	0.07
7	II	-β-D-Glc^2-β-D-Glc	-H	-H	Sweet, better gustatory sensation	20	0.07
8	II	-β-D-Glc	-β-D-Xyl^2-α-L-Rha	-H	Sweet, better gustatory sensation	10	0.3
9	II	-β-D-Glc^2-β-D-Glc	-α-L-Rha^3-β-D-Xyl	-H	Sweet, better gustatory sensation	10	0.3
10	III	–OH	-H	-α-L-Rha^3-β-D-Xyl	Bitter	—	—
11	III	–OH	-β-D-Glc	-α-L-Rha^3-β-D-Xyl	Sweet, a slightly bitter after-taste	30	0.1
12	III	–OH	-β-D-Glc^6-acetyl	-α-L-Rha^3-β-D-Xyl	Sweet, a slightly bitter after-taste	50	0.1
13	III	–OH	-β-D-Glc^2-β-D-Glc	-α-L-Rha^3-β-D-Xyl	Sweet, a slightly bitter after-taste	50	0.10
14	III	–OH	-β-D-Glc^2-β-D-Glc	-α-L-Rha^3-β-D-Glc	Sweet, a slightly bitter after-taste	80	0.03
15	III	–OH	-β-D-Glc^2-β-D-Glc	-H	Sweet, a slightly bitter after-taste	10	0.07
16	III	-H	-β-D-Glc^2-β-D-Glc	-α-L-Rha^3-β-D-Xyl	Sweet, astringent taste after-taste	50	0.07

[a] Sweet intensity relative to the sucrose solution (1%, w/w).
[b] Minimum concentration that human can percept.

III have higher sweet intensity than the other two types (**3**, **9**, **13**, and **16**; **2**, **8**, and **11**). In type I, the amount of glucosyl group has almost no effect on sweet intensity (**1**, **2**, and **3**), this phenomenon is also reflected in Type II (**5**, **8**, and **9**). Compound **4** has the highest sweet intensity (100 times that of sucrose solution), which may be due to differences in the order of the sugar chains connected to the sixth position of Glc III, or may be related to the oxidation of the 24-position hydroxyl group to a carbonyl group. In contrast, the sweet intensity increases in type III as the amount of glucosyl group of C-3 (**10**, **11**, **12**, and **13**), especially compound **10**, with C-3 free hydroxyl, which is a bitter compound with no sweet taste. In addition, because xylosyl group of sugar chain at C-20 is replaced by glucosyl, the sweetness of compound **14** is significantly increased (**13**, **14**, and **16**). Acetylation may also increase sweetness (**11** and **12**).

In summary, we speculate that sweetness intensity may be affected by oxidation, glycosylation and acetylation in the chemical structures of gypenosides. This phenomenon may be caused by changes in the position or number of binding sites and binding energy between gypenosides and human taste receptors after oxidation, glycosylation, and acetylation, which would provide more opportunities for the hydrogen bonding which is responsible for sweetness signaling (Mayank & Jaitak, 2015). This speculation would be worth elucidating in future research.

Table 2
Parameters of MRM mode to acquire data and content of compounds **1**–**5**, **8**–**13**, and **15** in the aerial parts of Jiaogulan.

Compd.	Trace ion (*m/z*)		RT (min)	Cone voltage	Collision energy	Dwell (secs)	Regression equation (n = 3)	R^2	Area	Content in aerial parts of Jiaogulan (mg/kg) (Mean ± SD, n = 3)
	parent	daughter								
1 + **5**	1093.25	337.00	21.34	70.0	65.0	0.003	y = 23532x + 349.14	0.9997	78043.971 ± 911.823	76.744 ± 0.000
2 + **8**	1255.20	337.04	15.27	40.0	30.0	0.003	y = 2143.5x - 165.91	0.9997	3207.196 ± 156.784	101.550 ± 0.000
3 + **9**	1417.25	337.00	9.00	60.0	55.0	0.003	y = 438.48x - 1783.9	0.9994	3207.196 ± 16.957	186.047 ± 0.000
4	1415.30	337.00	12.18	70.0	60.0	0.003	y = 14548x - 402.11	0.9998	76886.977 ± 667.026	15.504 ± 1.343
10	1077.25	337.00	37.61	50.0	45.0	0.003	y = 98126x - 5646.8	1.0000	693364.084 ± 3451.011	166.667 ± 0.343
11	1239.30	337.00	30.75	70.0	50.0	0.003	y = 66994x - 6115.3	1.0000	76886.977 ± 2512.974	27.132 ± 1.343
12	1281.25	1221.33	37.07	40.0	35.0	0.003	y = 2723.1x - 240.15	0.9998	159863.339 ± 1458.450	1397.674 ± 12.948
13	1401.25	337.00	22.09	80.0	65.0	0.003	y = 12670x + 318.92	1.0000	39178.426 ± 1134.742	69.767 ± 2.326
15	1123.25	961.20	22.43	70.0	60.0	0.003	y = 1295.6x - 17.212	1.0000	6342.653 ± 35.647	113.953 ± 0.000

4. Conclusions

Food choices and preferences are generally influenced by palatability. For the use of “Jiaogulan”, as an herbal tea, its sweet varieties are significantly more popular with consumers. This paper used gustatory-guided isolation to study the compound responsible for the sweet taste in a variety of “Jiaogulan” from Yunnan province of China. This led to the first discovery of fifteen sweet-tasting compounds and one bitter-tasting compound. All of them are new dammarane-type tetracyclic triterpenoids. These compounds are the main constituents responsible for the sweet tastes of *G. pentaphyllum*. They are characterized by a pleasant sweet taste without any bitter taste or by only a subtle bitter after-taste. This study clarified the chemical basis for the sweet taste of this plant. The newly discovered compounds are potential natural sweeteners and thereby provide new lead substances for the study of natural non-sugar sweeteners. Compound **4** is especially promising for use as a sweetener, due to its high sweetness intensity. Compound **12** has the highest content of the sweet-tasting compounds. The results of this study provide leading molecules for the development of new natural non-sugar sweet substances. It also enriches the natural sweet compound library, and provides a theoretical basis and technical support for discovering natural sweeteners from this extraordinary sweet “Jiaogulan” variety.

In addition, “Jiaogulan” herbal tea is produced by manufacturing processes like green tea, including plucking, pan firing or steaming, rolling, and drying. Whether differences of these sweetness compounds (including structure and content) emerge during high-temperature processing should be studied in the further. Further research is also needed into the differences in molecular mechanisms between this sweet variety from Yunnan province of China and other sweet varieties or bitter varieties elsewhere.

CRediT authorship contribution statement

Hong-Xia Zhang: Validation, Investigation, Writing – original draft. **Zhong-Ze Wang:** Investigation, Formal analysis, Writing – original draft. **Zhi-Zhi Du:** Supervision, Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was sponsored by grants from Yunnan Provincial Scientific Research Project of “FangGuanFu” (2019-1-N-25318000002120) and Research Project (E16H781261) funded by Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, China. We thank Dr. Wen-Yun Chen for identification of the plant specimens. The authors sincerely appreciate the language editing and polishing by Dr. Fiona Worthy. The authors would like to thank the efforts of all panelists participating in the sensory tests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132981.

References

Acevedo, W., Ramirez-Sarmiento, C. A., & Agosin, E. (2018). Identifying the interactions between natural, non-caloric sweeteners and the human sweet receptor by molecular docking. *Food Chemistry, 264*, 164–171. https://doi.org/10.1016/j.foodchem.2018.04.113

Ben Shoshan-Galeczki, Y., & Niv, M. Y. (2020). Structure-based screening for discovery of sweet compounds. *Food Chemistry, 315*, Article 126286. https://doi.org/10.1016/j.foodchem.2020.126286

Cretin, B. N., Waffo-Teguo, P., Dubourdieu, D., & Marchal, A. (2019). Taste-guided isolation of sweet-tasting compounds from grape seeds, structural elucidation and identification in wines. *Food Chemistry, 272*, 388–395. https://doi.org/10.1016/j.foodchem.2018.08.070

Frank, O., Ottinger, H., & Hofmann, T. (2001). Characterization of an intense bitter-tasting 1H,4H-quinolizinium-7-olate by application of the taste dilution analysis, a novel bioassay for the screening and identification of taste-active compounds in foods. *Journal of Agricultural and Food Chemistry, 49*(1), 231–238. https://doi.org/10.1021/jf0010073

Gao, D., Zhao, M., Qi, X., Liu, Y., Li, N., Liu, Z., & Bian, Y. (2016). Hypoglycemic effect of *Gynostemma pentaphyllum* saponins by enhancing the Nrf2 signaling pathway in STZ-inducing diabetic rats. *Archives of Pharmacal Research, 39*(2), 221–230. https://doi.org/10.1007/s12272-014-0441-2

Goel, A., Gajula, K., Gupta, R., & Rai, B. (2021). In-silico screening of database for finding potential sweet molecules: A combined data and structure based modeling approach. *Food Chemistry, 343*, Article 128538. https://doi.org/10.1016/j.foodchem.2020.128538

Jia, N., Lin, X., Ma, S., Ge, S., Mu, S., Yang, C., … Zhao, J. (2018). Amelioration of hepatic steatosis is associated with modulation of gut microbiota and suppression of hepatic miR-34a in *Gynostemma pentaphylla* (Thunb.) Makino treated mice. *Nutrition & Metabolism, 15*, 86. https://doi.org/10.1186/s12986-018-0323-6

Jia, Z., & Yang, X. (2009). A minor, sweet cucurbitane glycoside from *Siraitia grosvenorii*. *Natural Product Communications, 4*(6), 769–772. https://doi.org/10.1177/1934578x0900400606

Kinghorn, A. D., Chin, Y. W., Pan, L., & Jia, Z. (2010). Natural Products as Sweeteners and Sweetness Modifiers. In H.-.-W. Liu, & L. Mander (Eds.), *Comprehensive Natural*

Products II (pp. 269–315). Oxford: Elsevier. https://doi.org/10.1016/B978-008045382-8.00077-0.

Liang, D., Hao, Z. Y., Zhang, G. J., Zhang, Q. J., Chen, R. Y., & Yu, D. Q. (2011). Cytotoxic Triterpenoid Saponins from *Lysimachia clethroides*. *Journal of Natural Products, 74*(10), 2128–2136. https://doi.org/10.1021/np2004038

Lindemann, B. (2001). Receptors and transduction in taste. *Nature, 413*, 219–225. https://doi.org/10.1038/35093032

Liu, H., Li, X., Duan, Y., Xie, J. B., & Piao, X. L. (2021). Mechanism of gypenosides of *Gynostemma pentaphyllum* inducing apoptosis of renal cell carcinoma by PI3K/AKT/mTOR pathway. *Journal of Ethnopharmacology, 271*, Article 113907. https://doi.org/10.1016/j.jep.2021.113907

Lu, Z., Xie, G., Wu, D., Yang, L., Jin, Z., Hu, Z., & Lu, J. (2021). Isolation and identification of the bitter compound from Huangjiu. *Food Chemistry, 349*, Article 129133. https://doi.org/10.1016/j.foodchem.2021.129133

Lu, J. G., Zhu, L., Lo, K. Y. W., Leung, A. K. M., Ho, A. H. M., Zhang, H. Y., … Jiang, Z. H. (2013). Chemical differentiation of two taste variants of *Gynostemma pentaphyllum* by using UPLC-Q-TOF-MS and HPLC-ELSD. *Journal of Agricultural and Food Chemistry, 61*(1), 90–97. https://doi.org/10.1021/jf304154d

Mayank, & Jaitak, V. (2015). Interaction model of steviol glycosides from Stevia rebaudiana (Bertoni) with sweet taste receptors: A computational approach. *Phytochemistry, 116*, 12–20. https://doi.org/10.1016/j.phytochem.2015.05.006

Nagai, M., Izawa, K., Nagumo, S., Sakurai, N., & Inoue, T. (1981). Chem Pharm Bull-Two glycosides of a novel dammarane alcohol from *Gynostemma pentaphyllum*. *Chemical and Pharmaceutical Bulletin, 29*(3), 779–783. https://doi.org/10.1248/cpb.29.779

Nguyen, N. H., Ha, T. K. Q., Yang, J. L., Pham, H. T. T., & Oh, W. K. (2021). Triterpenoids from the genus *Gynostemma*: Chemistry and pharmacological activities. *Journal of Ethnopharmacology, 268*, Article 113574. https://doi.org/10.1016/j.jep.2020.113574

Phat, C., Moon, B., & Lee, C. (2016). Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system. *Food Chemistry, 192*, 1068–1077. https://doi.org/10.1016/j.foodchem.2015.07.113

Shi, S.-Y., Zhang, Y.-P., Jiang, X.-Y., Chen, X.-Q., Huang, K.-L., Zhou, H.-H., & Jiang, X.-Y. (2009). Coupling HPLC to on-line, post-column (bio)chemical assays for high-resolution screening of bioactive compounds from complex mixtures. *TRAC Trends in Analytical Chemistry, 28*(7), 865–877. https://doi.org/10.1016/j.trac.2009.03.009

Soejarto, D. D., Addo, E. M., & Kinghorn, A. D. (2019). Highly sweet compounds of plant origin: From ethnobotanical observations to wide utilization. *Journal of Ethnopharmacology, 243*, Article 112056. https://doi.org/10.1016/j.jep.2019.112056

Sun, G., Dai, Q., Zhang, H. X., Li, Z. J., & Du, Z. Z. (2016). New Sweet-Tasting C21-Pregnane Glycosides from Pericarps of *Myriopteron extensum*. *Journal of Agricultural and Food Chemistry, 64*(49), 9381–9389. https://doi.org/10.1021/acs.jafc.6b04121

Sun, G., Wang, P., Chen, W., Hu, X., Chen, F., & Zhu, Y. (2022). Simultaneous quantitation of acrylamide, 5-hydroxymethylfurfural, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine using UPLC-MS/MS. *Food Chemistry, 375*, Article 131726. https://doi.org/10.1016/j.foodchem.2021.131726

Sun, G., Zhang, H. X., Ma, Y. P., Li, M. X., & Du, Z. Z. (2018). New Sweet-Tasting C21 Pregnane Glycosides from the Roots of *Myriopteron extensum*. *Journal of Agricultural and Food Chemistry, 66*(29), 7735–7739. https://doi.org/10.1021/acs.jafc.8b02348

Temussi, P. A. (2009). Sweet, bitter and umami receptors: A complex relationship. *Trends Biochem Sci, 34*(6), 296–302. https://doi.org/10.1016/j.tibs.2009.02.005

Veloso, A. C. A., Dias, L. G., Rodrigues, N., Pereira, J. A., & Peres, A. M. (2016). Sensory intensity assessment of olive oils using an electronic tongue. *Talanta, 146*, 585–593. https://doi.org/10.1016/j.talanta.2015.08.071

Vos, M. B., Kaar, J. L., Welsh, J. A., Van Horn, L. V., Feig, D. I., Anderson, C. A., … Johnson, R. K. (2016). Added sugars and cardiovascular disease risk in children: A scientific statement from the American Heart Association. *Circulation, 134*(134), 1–18. https://doi.org/10.1161/CIR.0000000000000439

Wang, Z., Wang, Z., Huang, W., Suo, J., Chen, X., Ding, K., … Zhang, H. (2020). Antioxidant and anti-inflammatory activities of an anti-diabetic polysaccharide extracted from *Gynostemma pentaphyllum* herb. *International Journal of Biological Macromolecules, 145*, 484–491. https://doi.org/10.1016/j.ijbiomac.2019.12.213

Wang, J., Zhao, M., Cheng, X., Han, Y., Zhao, T., Fan, M., … Yang, J. L. (2020). Dammarane-Type Saponins from *Gynostemma pentaphyllum* Prevent Hypoxia-Induced Neural Injury through Activation of ERK, Akt, and CREB Pathways. *Journal of Agricultural and Food Chemistry, 68*(1), 193–205. https://doi.org/10.1021/acs.jafc.9b06659

Weng, X., Lou, Y. Y., Wang, Y. S., Huang, Y. P., Zhang, J., Yin, Z. Q., & Pan, K. (2021). New dammarane-type glycosides from *Gynostemma pentaphyllum* and their lipid-lowering activity. *Bioorganic Chemistry, 111*, Article 104843. https://doi.org/10.1016/j.bioorg.2021.104843

Wu, P. K., Tai, W. C. S., Choi, R. C. Y., Tsim, K. W. K., Zhou, H., Liu, X., … Hsiao, W. L. W. (2011). Chemical and DNA authentication of taste variants of *Gynostemma pentaphyllum* herbal tea. *Food Chemistry, 128*(1), 70–80. https://doi.org/10.1016/j.foodchem.2011.02.078

Yang, F., Shi, H., Zhang, X., Yang, H., Zhou, Q., & Yu, L. L. (2013). Two new saponins from tetraploid jiaogulan (*Gynostemma pentaphyllum*), and their anti-inflammatory and alpha-glucosidase inhibitory activities. *Food Chemistry, 141*(4), 3606–3613. https://doi.org/10.1016/j.foodchem.2013.06.015

Zhai, X. F., Zu, M. L., Wang, Y. R., Cui, W. Y., Duan, Y., Yang, C., & Piao, X. L. (2021). Protective effects of four new saponins from *Gynostemma pentaphyllum* against hydrogen peroxide-induced neurotoxicity in SH-SY5Y cells. *Bioorganic Chemistry, 106*, Article 104470. https://doi.org/10.1016/j.bioorg.2020.104470

Zhang, H. X., Sun, G., Gu, J. L., & Du, Z. Z. (2017). New Sweet-Tasting Oleanane-Type Triterpenoid Saponins from “Tugancao” (*Derris eriocarpa* How). *Journal of Agricultural and Food Chemistry, 65*(11), 2357–2363. https://doi.org/10.1021/acs.jafc.7b00137