



# Isolation and identification of sweet substances and sweet aftertaste substances in the fruit of *Phyllanthus emblica*

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## Abstract

Low-calorie sweeteners are widely used to replace or reduce sugar in foods and beverages, and the taste and texture of existing sweeteners are far from that of real sugar. The use of sweetness positive aliasing modifiers is an ideal alternative that can reduce the amount of sugar used while maintaining the original sugar taste. However, the currently available sweetness aliasing agents are synthetic compounds, while natural sweetness aliasing agents have not been reported. Studies have shown that there are potential sweetness modifiers in plants with a “Sweetback effect”. The sensory activity fractionation of the extract of the fruit of *Phyllanthus emblica* was performed to locate the key chemical compounds in *P. emblica* for sweetness and sweetness aftertaste. The chemical structures of the isolated compounds were analyzed by spectroscopic methods such as ESI-MS and NMR (<sup>1</sup>H NMR, <sup>13</sup>C NMR). Fifteen compounds were identified, namely, 2-Furoic acid (1), quercetin (2), p-hydroxybenzaldehyde (3), methyl gallate (4), ethyl 3,4,5-trimethoxybenzoate (5), syringic acid, (6), gallic acid (7), vanillin (8), protocatechuic acid (9), myricetin (10), kaempferol (11), trans-cinnamic acid (12), naringenin (13), oleanolic acid (14), and rutin (15). Sensory analysis of these chemical components showed that 11 of them (1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 15) were compounds with a sweet taste and the other four (7, 9, 10, 11) were compounds with a sweet aftertaste. Experiments on the sweetness regulating properties of sucrose revealed that the sweet aftertaste component in *P. emblica* has sweetness inverse variant regulator properties and naringenin has sweetness forward variant regulator properties.

**Keywords** *Phyllanthus emblica* · Sensory evaluation · Sweetness · Sweet aftertaste · Sweetness regulator

## Introduction

*Phyllanthus emblica* L. (amla), a medicinal plant in the family *Phyllanthaceae*, has generally been cultivated in tropical and subtropical countries, including China, India, Sri Lanka, and Thailand [1]. Its fruit is slightly astringent in the mouth and sweet in the aftertaste, hence gaining its name [2]. It has been reported that *P. emblica* can be used alone or in combination with other traditional Chinese medicines for the treatment of many infectious and non-infectious diseases [3], as well as for skin whitening [4], hypoglycemic and antidiabetic [5], hepatoprotective [6], antiviral [7], immunomodulatory and anti-cancer [8], anti-inflammatory and antioxidant [9], neuroprotective [10], antibacterial [11, 12],

treatment of Respiratory diseases [3], and many other activities. The flavor of the fruit of *P. emblica* is distinguished, with a distinctly bitter and astringent taste when chewed in the mouth, and after careful chewing, the bitterness gradually disappears, followed by a refreshing aroma and a distinctly sweet taste.

The desire for sweetness is innate in humans, and the preference for sweetness causes people to consume more free sugars, but high free sugar intake is an important risk factor for dental caries, obesity, cardiovascular disease, diabetes, and other metabolic diseases [13–18]. Sweeteners are widely used in the food industry to provide sugar reduction and sugar control for consumers who need to reduce and control sugar. Although many natural sweeteners have been isolated from plants [19, 20], only a few have been developed into commercially available sweeteners, such as rebaudioside A, stevioside, steviol glycosides, mogrol glycosides (Luo Han Guo sweetener), and mogrosin V [21, 22]. However, the demand for sweeteners in the food and beverage industry is particularly high, and the production

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of natural sweeteners isolated from plants is very limited, so we need to discover and develop new natural sweeteners.

In search of sweetness allosteric modulators, Senomyx, USA, designed a high-throughput screening model of the sweetness receptor T1R cell line, screened tens of thousands of compounds, and reported a series of novel sweetness positive allosteric modulating PAMs (SE-1, SE-2, SE-3, SE-4) that do not have sweetness per se but can exponentially and selectively increase the sweetness of specific substrates (sucralose and sucrose) and reduce the amount of sugar used [23–25]. It is believed that the “water-taste effect” or “Sweetback effect” is an aftertaste reaction after the sweet taste receptor binds a substance and the water carries it away. There are two binding sites on the sweet taste receptor, a high concentration of low-affinity sites, a low concentration of high-affinity sites, when the high concentration of binding in the low-affinity sites is bitter, inhibiting the sweet taste receptor activity, after drinking water, closely bound to the low-affinity sites, immediately produce the feeling of sweet water, sweet water effect of the substance itself is no sweet taste, there is a sweet water effect on the existence of the regulator [26]. It can be assumed that there is a high probability that sweet taste aliasing agents are present in plants that alter taste perception. The fruit of *P. emblica* tastes sour and astringent at first, but becomes sweet after a long time, and if you drink water, the sweetness is more intense. Therefore, we chose *P. emblica* as the subject for the study of its sweet taste components.

In this study, we investigated the sweet, astringent, and aftertaste substances of *P. emblica*. Utilizing the sensomics approach, compounds with taste activity are isolated by activity-guided fractionation and structurally characterized by mass spectrometry and nuclear magnetic resonance spectroscopy (NMR) experiments, and human sensory analysis to identify the major taste-presenting compounds.

## Materials and methods

### Materials

**Chemicals:** The following materials were used: AR grade methanol (Damao, Tianjin, China), AR chloroform (Xilong Chemical Co. Ltd, China), HPLC methanol (Merck, Shanghai, China), sulfuric acid (Xilong Chemical Co. Ltd., Guangdong, China), acetic acid (Jige, Tianjin, China), D<sub>2</sub>O, CD<sub>3</sub>OD, CDCl<sub>3</sub>, and DMSO-d<sub>6</sub> (Aladdin, Shanghai, China), and water (Wahaha Group Co., Ltd., Hangzhou, China). Edible grade alcohol was purchased from Dingguo Technology Development Co., Inc (Kunming, China).

Column chromatography (CC) was done using silica gel (200–300 mesh, Qingdao Marine Chemical Co. Ltd., China), Sephadex gel LH-20 (Shanghai Mackin Biochemical

Co. Ltd., China). TLC was performed on silica gel GF254 (Qingdao Marine Chemical Co. Ltd., China), and spots were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in ethanol. HPLC analyses and separations were performed on an Agilent HP-1260 system (Agilent Technologies, Santa Clara, CA) equipped with a quaternary gradient pump (G1311C) and a multiwavelength detector (G1314F VWD).

### Extraction of plant material

The air-dried and powdered pericarps of *P. emblica* (6 kg) were extracted with 95% edible grade alcohol three times at room temperature (30L × 3 days × 4times), respectively. The extracts obtained from the above three times were combined, filtered, and concentrated under reduced pressure until the alcohol was absent, and then freeze-dried twice to obtain 750 g of crude product extract. 500 g of the crude extract was dissolved in 7.5 L of distilled water, separated by a D101 macroporous resin column, and eluted with water, 30, 50, 75, and 90% aqueous ethanol, and the alcohol eluted fractions A (100 g), B (90 g), C (70 g), and D (10 g) were collected, concentrated under reduced pressure, and freeze-dried twice (to remove a small amount of solvent). Fractions B and C were determined to be sweet by sensory evaluation.

### Sensory-guided separation of fraction B

The fraction B (80 g) was separated on a silica gel column and elution with a gradient of methanol/chloroform (20:1, 10:1, 5:1, 2:1, 0:1) as eluent to obtain six fractions (B-I–B-VI) by thin-layer chromatography. These fractions were separately vacuum concentrated to remove the solvent, dissolved in water, and lyophilized twice for sensory analysis. According to the sensory analysis, B-II had the highest sweetness and was also sweet after eating. B-II was further isolated and purified, and crystals were precipitated from B-VI, which was identified by NMR as 2-furoic acid. B-II was further isolated and purified.

Compound 1 2-furoic acid. ESI-HR-MS m/z:113 [M + H]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ: 7.66 (1H, s, H-5), 7.26 (1H, d, J = 3.6 Hz, H-3), 6.49 (1H, t, J = 1.6 Hz, H-4). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>) δ: 163.9 (C=O), 147.5 (C-5), 143.9 (C-2), 120.2 (C-3), 112.3 (C-4). These data were consistent with those of reference [27].

### Sensory-guided fraction of fraction B-II

The fraction B-II (20 g) was dissolved in methanol, separated by gel column LH-20, and gradient elution with methanol/water (100:0–0:100) as eluent, and six fractions (B-II-1–B-II-6) were obtained by thin-layer chromatography analysis, and these fractions were, respectively, concentrated under

reduced pressure to remove the solvent, after which they were dissolved in water and freeze-dried twice for sensory evaluation. According to the sensory analysis, it was found that B-II-3 had the highest sweetness and the other flavors were not obvious, while B-II-5, was mainly sour and bitter, but it would return to the sweetness after eating. B-II-3 and B-II-5 were further separated, and B-II-1 was the pure compound, which was identified as quercetin by NMR structure.

Compound 2 quercetin, ESI-HR-MS ( $m/z$ ): 303 ( $M + H$ )<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.63 (1H, s, H-6'), 7.52 (1H, d,  $J = 8.0$  Hz, H-2'), 6.77 (1H, d,  $J = 8.0$  Hz, H-3'), 6.28 (1H, d,  $J = 1.4$  Hz, H-6), 6.08 (1H, d,  $J = 1.4$  Hz, H-8); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 175.9 (C=O), 164.2 (C-7), 161.1 (C-5), 156.8 (C-9), 147.3 (C-4'), 146.5 (C-5'), 144.8 (C-2), 138.8 (C-3), 122.7 (C-1'), 120.2 (C-2'), 114.8 (C-3'), 115.5 (C-6'), 103.1 (C-10), 97.8 (C-6), 92.9 (C-8). These data were consistent with those of reference [28].

### Identification of key taste compounds in fraction B-II-3

The fraction B-II-3 (3.5 g) with the sweet odor was separated by HPLC, and eluted isocratically with methanol/water (60:40, V:V) at a flow rate of 1 ml/min to obtain a total of six fractions (B-II-3-a–B-II-3-f). These fractions were separated from the solvent under vacuum at 40 °C and lyophilized twice for sensory evaluation. A total of four pure compounds were isolated and then structurally identified by NMR, and the compounds isolated were B-II-3-b (p-hydroxybenzaldehyde), B-II-3-c (ethyl 3,4,5-trimethoxybenzoate), B-II-3-e (syringic acid), and B-II-3-f (methyl gallate).

Compound 3 p-hydroxybenzaldehyde: ESI-HR-MS ( $m/z$ ): 123 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 10.87 (1H, s, C=O), 7.90 (2H, d,  $J = 8.6$  Hz, H-2, 6), 7.27 (2H, d,  $J = 8.6$  Hz, H-3, 5); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 195.1 (C=O), 167.6 (C-4), 135.8 (C-2, 6), 132.5 (C-1), 119.4 (C-3, 5). These data were consistent with those of reference [29].

Compound 4 methyl gallate: ESI-HR-MS ( $m/z$ ): 185 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 600 MHz): 6.90 (2H, s, H-2,6), 3.65 (3H, s, O-CH<sub>3</sub>); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 150 MHz): 168.8 (COOH), 144.4 (C-3,5), 138.2 (C-4), 121.4 (C-1), 119.7 (C-2,6), 52.3 (O-CH<sub>3</sub>). These data were consistent with those of reference [30].

Compound 5 ethyl 3,4,5-trimethoxybenzoate. ESI-HR-MS ( $m/z$ ): 263 [ $M + Na$ ]<sup>+</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta$ : 7.28 (2H, s, H-2, 6), 4.35 (2H, q,  $J = 7.1$  Hz, –OCH<sub>2</sub>CH<sub>3</sub>), 1.38 (3H, t,  $J = 7.1$  Hz, –OCH<sub>2</sub>CH<sub>3</sub>), 3.89 (9H, s, 3 × O-CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$ : 125.7 (C-1), 106.9 (C-2, 6), 153.1 (C-3, 5), 142.3 (C-4), 166.5 (C-7), 61.4 (–OCH<sub>2</sub>CH<sub>3</sub>), 14.6 (–OCH<sub>2</sub>CH<sub>3</sub>), 56.4 (3, 5-OCH<sub>3</sub>),

61.1 (4-OCH<sub>3</sub>). These data were consistent with those of reference [31].

Compound 6 syringic acid: ESI-HR-MS  $m/z$ : 199 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.22 (2H, s, H-2, 6), 3.82 (6H, s, 3, 5-OCH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 172.6 (COOH), 150.9 (C-3, 5), 143.9 (C-4), 124.4 (C-1), 110.4 (C-2, 6), 58.8 (3, 5-OCH<sub>3</sub>). These data were consistent with those of reference [32].

### Identification of key taste compounds in fraction B-II-5

The fraction B-II-5 (2.9 g) with the sweet taste was separated by HPLC, and eluted isocratically with methanol/water (60:40, V: V) at a flow rate of 1 ml/min to obtain a total of eight fractions (B-II-5-a–B-II-5-h), which were concentrated at 40 °C under reduced pressure and dissolved in water for freeze-drying, followed by sensory evaluation. A total of five pure compounds were isolated and structurally identified by NMR, and the compounds isolated were B-II-5-b (gallic acid), B-II-5-c (vanillin), B-II-5-d (protocatechuic acid), B-II-5-h (myricetin), and B-II-5-f (kaempferol).

Compound 7 gallic acid. ESI-HR-MS  $m/z$ : 171 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.06 (2H, s, H-2, H-6). <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 169.7 (C=O), 145.5 (C-3, C-5), 138.7 (C-4), 122.8 (C-1), 109.3 (C-2, C-6). These data were consistent with those of reference [30].

Compound 8 vanillin, ESI-HR-MS  $m/z$ : 153 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 Hz)  $\delta$ : 10.27 (1H, s, CHO), 7.43 (1H, d,  $J = 6.3$  Hz, H-6), 7.26 (1H, s, H-2), 7.04 (1H, d,  $J = 8.5$  Hz, H-5), 5.30 (1H, s, 4-OH), 3.97 (3H, s, 3-OCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 Hz)  $\delta$ : 130.0 (C-1), 108.9 (C-2), 147.3 (C-3), 151.8 (C-4), 114.5 (C-5), 127.7 (C-6), 191.4 (CHO), 56.3 (3-OCH<sub>3</sub>). These data were consistent with those of reference [33].

Compound 9 protocatechuic acid, ESI-HR-MS  $m/z$ : 155 [ $M - H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.57 (1H, d,  $J = 1.8$  Hz, H-2), 7.49 (1H, dd,  $J = 7.9, 1.8$  Hz, H-6), 6.97 (1H, d,  $J = 7.9$  Hz, H-5); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 171.0 (C=O), 149.2 (C-4), 143.6 (C-3), 123.6 (C-6), 122.8 (C-1), 117.1 (C-5), 115.6 (C-2). These data were consistent with those of reference [34].

Compound 10 myricetin, ESI-HR-MS:  $m/z$ : 319 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.34 (2H, s, H-2', 6'), 6.38 (1H, d,  $J = 1.6$  Hz, H-6), 6.18 (1H, d,  $J = 1.6$  Hz, H-8); <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$ : 177.4 (C=O), 165.7 (C-7), 162.6 (C-5), 158.3 (C-9), 148.1 (C-2), 146.9 (C-3', 5'), 137.5 (C-4'), 137.1 (C-3), 123.2 (C-1'), 108.7 (C-2', 6'), 104.6 (C-10), 99.4 (C-6), 94.5 (C-8). These data were consistent with those of reference [35].

Compound 11 kaempferol, ESI-HR-MS  $m/z$ : 287 [ $M + H$ ]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.96 (2H, d,  $J = 8.2$  Hz, H-2', H-6'), 6.88

(2H,d,J=8.2 Hz,H-3',H-5'), 6.42 (1H,d,J=1.7 Hz,H-8), 6.20 (1H,d,J=1.7 Hz,H-6); <sup>13</sup>C-NMR (150 MHz,CD<sub>3</sub>OD) δ: 147.1 (C-2), 135.4 (C-3), 176.2 (C-4), 160.1 (C-5), 97.9 (C-6), 163.8 (C-7), 93.5 (C-8), 156.2 (C-9), 103.2 (C-10), 121.5 (C-1'), 129.4 (C-2'), 115.3 (C-3'), 159.1 (C-4'), 115.5 (C-5'), 129.4 (C-6'). These data were consistent with those of reference [28].

### Sensory-guided separation of fraction C

Fraction C (70 g) was separated on a silica gel column with a gradient elution of methanol/chloroform (20:1, 10:1, 5:1, 2:1, 0:1) as eluent to obtain four fractions (C-I–C-IV) by thin-layer chromatography. These fractions were separately vacuum concentrated to remove the solvent, dissolved in water, and lyophilized twice for sensory analysis. According to the sensory analysis, C-IV had the highest sweetness and was also sweet after eating. C-IV was further isolated and purified.

### Sensory-guided separation of fraction C-IV

Fraction C-IV (18 g) was separated on a gel column and eluted with a gradient of methanol/water (100:0–0:100) to obtain four fractions(C-IV-1–C-IV-4), one of which was the compound trans-cinnamic acid, which was subjected to sensory evaluation and had a sweet taste but also other flavors; the other fractions were concentrated under reduced pressure to remove the solvent, and then, the result was suspended in water, freeze-dried twice, and subjected to sensory evaluation. C-IV-3 had the strongest sweetness and had a sweet taste and was further separated. Fraction C-IV-3(6 g) was separated on a gel column LH-20 and eluted with a gradient of methanol/water (100:0–0:100), and five fractions were obtained by thin-layer chromatography. These fractions were concentrated under reduced pressure to remove the solvent, and then, the result was suspended in water, freeze-dried twice, and subjected to sensory evaluation. The fractions with the strongest sweetness identified after sensory evaluation were repeatedly subjected to gel column chromatography until the most critical sweet chemical components were separated. The isolated sweet components were structurally identified by MS and NMR, and the chemical components obtained after structural identification was trans-cinnamic acid (12), naringin (13), oleanolic acid (14), and rutin (15).

Compound 12 trans-cinnamic acid, ESI-HR-MS m/z:149[M+H]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ: 7.64 (1H, d, J=16.0 Hz, H-7), 7.59 (2H, m, H-2, 6), 7.39 (3H, m, H-3–5), 6.49 (1H, d, J=16.0 Hz, H-8); <sup>13</sup>C-NMR (150 MHz,CDCl<sub>3</sub>) δ: 171.2 (C=O), 145.9 (C-7), 136.3 (C-1), 131.4 (C-4), 130.1 (C-2, 6), 129.3 (C-3, 5), 120.4 (C-8). These data were consistent with those of reference [36].

Compound 13 naringenin, ESI-HR-MS m/z: 273 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz,CD<sub>3</sub>OD) δ: 7.31 (1H, d, J=8.3 Hz, H-3', 5'), 6.81 (1H, d, J=8.3 Hz, H-2', 6'), 5.89 (1H, s, H-6), 5.88 (1H, s, H-8), 5.34 (1H, dd, J=13.2, 2.6 Hz, H-2), 3.11 (1H, dd, J=17.1, 13.2 Hz, H-3α), 2.69 (1H, dd, J=17.1, 2.6 Hz, H-3β). <sup>13</sup>C-NMR (150 MHz,CD<sub>3</sub>OD) δ: 197.9 (C=O), 168.5 (C-7), 165.6 (C-5), 159.2 (C-4'), 131.2 (C-1'), 129.2 (C-2', 6'), 116.5 (C-3', 5'), 103.5 (C-10), 97.2 (C-6), 96.3 (C-8), 80.6 (C-2), 44.2 (C-3). These data were consistent with those of reference [37].

Compound 14 oleanolic acid, ESI-HR-MS, m/z:457[M+H]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz,CDCl<sub>3</sub>)δ:0.74 (3H,s,H-26), 0.77(3H,s,H-24), 0.90 (3H,s,H-23), 0.91(6H,s,H-26,29), 0.92 (3H,s,H-30), 0.98 (3H,s,H-25), 1.06 (3H,s,H-27), 1.22–1.91 (m,CH<sub>2</sub>,CH), 3.22 (1H,dd,H-3), 5.28 (1H,t,J=3.2 Hz,H-12); <sup>13</sup>C-NMR (150 MHz,CDCl<sub>3</sub>)δ: 182.6 (C-28),142.5(C-7), 121.5 (C-12), 77.9 (C-3), 54.0 (C-5), 46.5 (C-9), 45.4 (C-19), 44.7 (C-17), 40.4 (C-14), 39.8 (C-18), 38.1 (C-8), 37.6 (C-4), 37.2 (C-1), 35.9 (C-10), 32.7 (C-21), 31.9 (C-20), 31.4 (C-22), 31.3 (C-7), 29.6 (C-20), 26.9 (C-23), 26.5 (C-15), 26.0 (C-2), 24.8 (C-27), 22.5 (C-30), 22.3 (C-16), 21.7 (C-11), 17.1 (C-6), 16.0 (C-26), 14.4 (C-25), 14.2 (C-24). These data were consistent with those of reference [38].

Compound 15 Rutin, ESI-HR-MS m/z 611 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (600 MHz,DMSO-d<sub>6</sub>)δ: 12.59 (1H,s,5-OH), 10.86 (1H,s,7-OH), 9.71 (1H,s,4'-OH), 9.22 (1H,s,3'-OH), 7.56 (1H,dd,J=8.2,2.3 Hz,H-6'), 7.53 (1H,d,J=2.3 Hz,H-2'), 6.90 (H, d, J=8.2 Hz,H-5'), 6.41 (1H,d,J=1.9 Hz,H-8), 6.20 (1H,d,J=1.9 Hz,H-6), 5.34 (1H,d,J=7.3 Hz,H-1''), 4.34 (1H,s,H-1'''), 3.06–3.65 (10H,H-2''-H-6'',H-2'''-H-5'''), 2.50 (1H,s,5-OH)0.0.99 (3H,d,J=6.2 Hz,6'''-CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz,DMSO-d<sub>6</sub>)δ: 177.8 (C-4), 164.5 (C-7), 161.7 (C-5), 157.1 (C-2), 156.9 (C-9), 148.9 (C-4'), 144.1 (C-3'), 133.7 (C-3), 122.0 (C-6'), 121.6 (C-1'), 116.7 (C-5'), 115.7 (C-2'), 104.4 (C-10), 101.6 (C-1''), 101.2 (C-1'''), 99.1 (C-6), 94.0 (C-8), 76.8 (C-5''), 76.3 (C-3''), 74.5 (C-2''), 72.2 (C-4'''), 70.9 (C-4''), 70.8 (C-2'''), 70.4 (C-5'''), 68.8 (C-3'''), 67.4 (C-6''), 18.2 (C-6'''-CH<sub>3</sub>). These data were consistent with those of reference [39].

### Analysis of taste modulation properties of sucrose by each taste active ingredient

Preparation of sucrose solution: purified water was used as the solvent to configure sucrose solution with 6% sucrose content. Ten equal parts of the prepared sucrose aqueous solution were taken out into small paper cups, and to these, 10 parts of sucrose aqueous solution were added protocatechuic acid, eugenol, p-hydroxybenzaldehyde, trans-cinnamic acid, 2-furoic acid, rutin, vanillin, naringenin, quercetin, and gallic acid. The prepared solutions were taken to the members of the sensory panel for evaluation of the taste

enhancement. A 5-point scale was used to score each solution (0: non-detectable; 5: strong sensation). The target sensory profile was sweetness. A total of 15 major taste-presenting substances were obtained in Euphorbia, but only ten other compounds were investigated, because methyl gallate, ethyl 3,4,5-trimethoxybenzoate, oleanolic acid, myricetin, and kaempferol were obtained in small amounts, which were not sufficient for these five compounds to be studied for taste modulating properties.

### Sensory evaluation of compounds

Sensory evaluation panels of three females and three males (23 to 25 years old) consisting of a total of six members were trained. These panel members were trained until they were familiar with the five basic taste sensations; sodium chloride at a mass fraction of 0.35%, sucrose at 1%, citric acid at 0.08%, caffeine at 0.08%, and alum at 0.25% were used as standard control solutions for salty, sweet, sour, bitter, and astringent tastes; and the solution to be tested was dissolved in distilled water to prepare a 1% concentration solution. The members of the evaluation team gave a score of 0–5 to the solution to be tested by comparing the taste sensation of the solution to be tested with the standard control solution, with no taste recorded as 0 and significant taste recorded as 5.

### Taste dilution analysis

Each sample was dissolved in water to make a stock solution. Dilution solutions of the samples were presented in an order of ascending concentrations (0.007, 0.015, 0.031, 0.062, 0.125, 0.250, and 0.500 mg/mL). The experiment was performed by the three-point determination method, the solution to be tested was compared with two blank controls (distilled water), and if the group members could just identify the difference in taste, the dilution multiple at this point was the dilution value (TD). Record the evaluation results of each evaluator and take their average, and the evaluation process shall not discuss and communicate with each other.

## Results and discussion

### Sensory-guided separation of *P. emblica*

To identify the main flavor compounds in *P. emblica* (Fig. 1), a freshly prepared suspension of *P. emblica* was subjected to taste analysis. Thus, a trained sensory panel was asked to evaluate the intensity of bitter, astringent, sweet, salty, sour, and aftertaste attributes on a linear intensity scale ranging from 0 (undetectable) to 5 (detectable). Sourness and bitterness were rated the highest with values 1.4 and 1.5, respectively. Followed by sweetness (1.3), astringency (1.2), and

sweet aftertaste (1.2) which were relatively higher, and salty (0.1) was considered less intense. To isolate and characterize the sweet and sweet aftertaste compounds, *P. emblica* was separated by different column chromatography methods for extraction.

### Sensory-guided separation of *P. emblica* extract

After extracting the powdered *P. emblica* with 95% edible grade alcohol, it was then separated and purified using macroporous adsorption resin D101, sequentially using different concentrations of alcohol as eluent, rotary evaporation to remove the solvent, and then lyophilized to obtain 30% ethanol eluted fraction (fraction A), 50% ethanol eluted fraction (fraction B), 75% ethanol eluted fraction (fraction C), and 90% ethanol eluted fraction (fraction D). Given fractions were dissolved according to their natural concentrations and given to a trained panel for sensory analysis. Fraction B (Table 1) exhibited the highest astringency (1.9) and sourness (1.9) and also the highest aftertaste (1.2) for fraction B, followed by relatively high bitterness intensity (1.6) and sweetness intensity (1.9) for fraction C. The sour intensity (1.1) and astringency intensity (0.8) of component A were lower than those of fractions B and C. Therefore, further separation of fractions B and C was performed to isolate the key taste-presenting compounds.

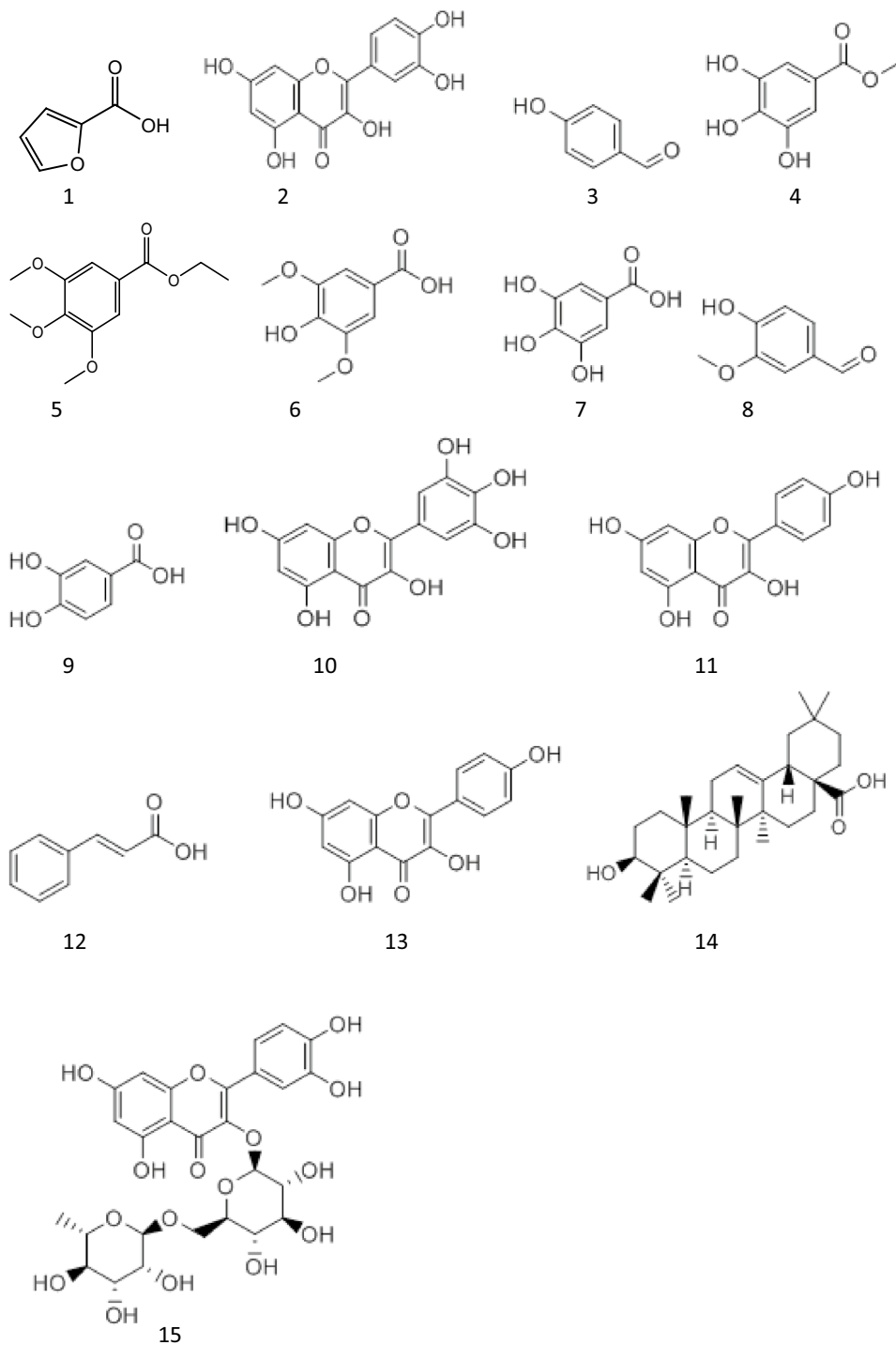
### Sensory-guided separation of *P. emblica* extract fraction B

The fraction B was purified by silica gel column chromatography to obtain six fractions (B-I–B-VI), which were freeze-dried twice to solvent-free after rotary evaporation of the solvent from these fractions to dryness. The freeze-dried powder was then placed in an aqueous solution and evaluated by comparative taste analysis (Fig. 2). After sensory evaluation, B-II had the highest sweetness (2), sourness (1.9), and astringency (1.2), and was also sweet after eating. The B-II was further isolated and purified.

### Sensory-guided separation of fraction B-II

To determine the sweetness components of B-II, fraction B-II was separated using gel LH-20 chromatography to obtain six subfractions (B-II-1–B-II-6). These fractions were collected separately, the solvent was removed from these fractions, freeze-dried twice, and sensory evaluations were performed. Fraction B-II-3 had the strongest sweetness (1.6) and the other flavors were not strong. b-II-5 had the highest bitterness (1.5), sourness (1.2), and astringency (1.0), with sweet aftertaste (1.4) after eating (Fig. 3). To further locate the compounds with a sweet taste and sweet aftertaste, fraction B-II-3, which exhibited the greatest sweet

**Fig. 1** Chemical structures of the main taste compounds identified in *P. emblica*



taste intensity, and fraction B-II-5, which had sweet after-taste, were further separated.

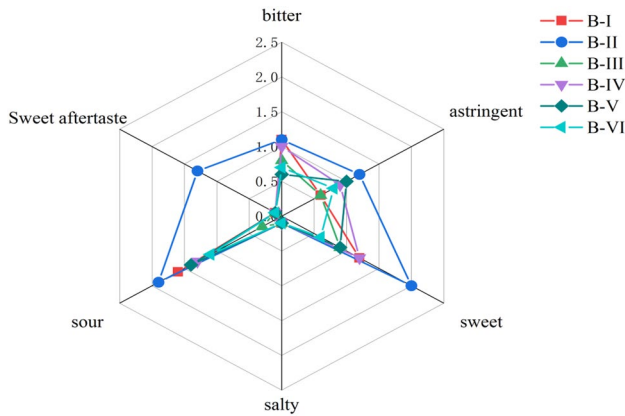
### Sensory-guided separation of fraction B-II-3

To further determine the sweet components in B-II-3, fraction B-II-3 was separated by high-performance liquid chromatography (HPLC) to obtain six fractions

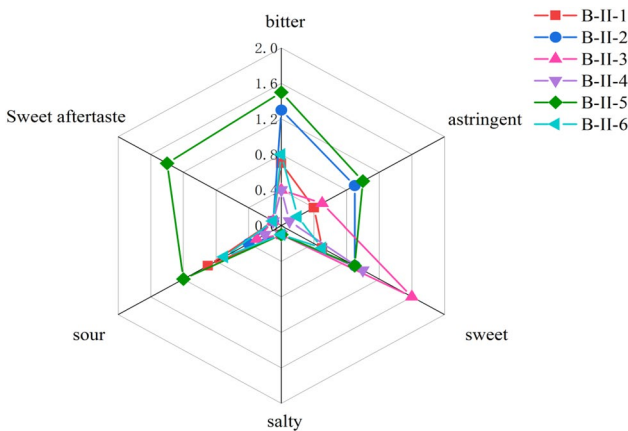
(B-II-3-a–B-II-3-f) (Fig. 4). These fractions were removed from the solvent, lyophilized, and subjected to sensory analysis. Fraction B-II-3-b (0.8) was more intensely sweet, followed by B-II-3-c (0.7), B-II-3-e (0.7), and B-II-3-f (0.6) (Fig. 5). Compounds B-II-3-b were identified p-hydroxybenzaldehyde, B-II-3-c as methyl gallate, B-II-3-e as ethyl 3,4,5-trimethoxybenzoate, and B-II-3-f

**Table 1** Sensory evaluation of *P. emblica* and extracts

Taste intensity of	Phyllanthus emblica Linn	A	B	C	D
Bitter	1.5	1.9	1.8	1.6	1.3
Astringent	1.2	0.8	1.9	1.0	0.6
Sweet	1.3	1.0	1.3	1.9	1.5
Salty	0.1	0.1	0.1	0.1	0.1
Sour	1.4	1.1	1.9	1.2	0.8
Sweet aftertaste	1.0	0.1	1.2	0.1	0.1

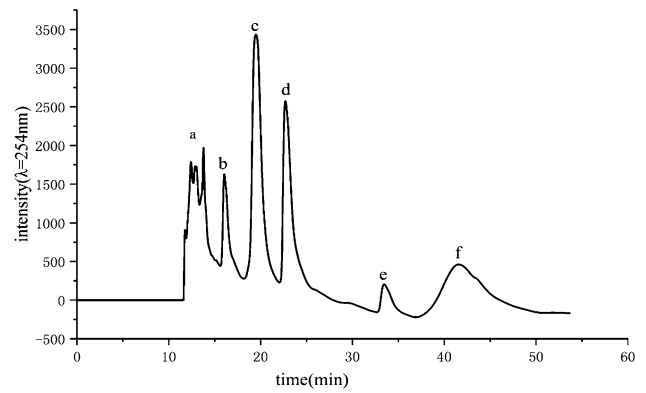


**Fig. 2** Sensory analysis of silica gel column fractions of extract B

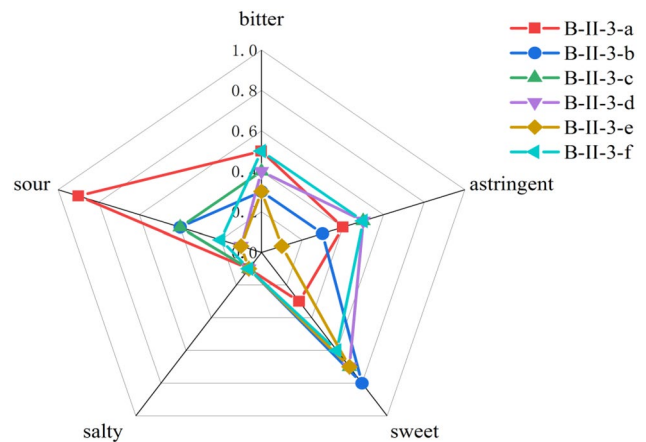


**Fig. 3** Sensory analysis of silica gel column fractions of extract B-II

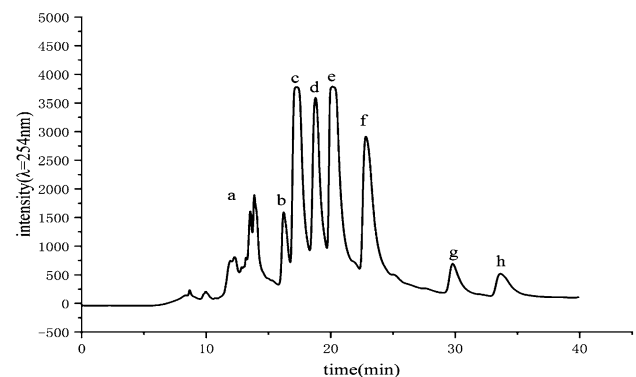
as syringic acid by 1D NMR experiments and mass spectrometry. After the sensory evaluation of these chemical components, it was found that p-hydroxybenzaldehyde, methyl gallate, and ethyl 3,4,5-trimethoxybenzoate all had a slightly sweet taste, and the sweetness was not strong. In contrast, eugenol has both sweet, sour, and bitter flavors.



**Fig. 4** Chromatogram of fraction B-II-3 using RP-HPLC



**Fig. 5** Sensory evaluation of fraction B-II-3 purified using RP-HPLC



**Fig. 6** Chromatogram of fraction B-II-5 using RP-HPLC

### Sensory-guided separation of fraction B-II-5

To determine the sweet aftertaste components of B-II-5, further separation of B-II-5 was carried out using the HPLC phase to obtain eight fractions (B-II-5-a–B-II-5-h) (Fig. 6).

These fractions were removed from the solvent, lyophilized, and subjected to sensory analysis. These components also exhibited strong astringency, sourness, and bitterness, with the strongest astringency being B-II-5-b (1.5), the strongest sourness being B-II-5-b (0.9), the strongest bitterness being B-II-5-h (1.1), and the strongest aftertaste being B-II-5-b (1.5) (Fig. 7). Mass spectrometry and NMR were used to structurally identify these chemical components, which were B-II-5-b (gallic acid), B-II-5-g (vanillin), B-II-5-d (protocatechuic acid), B-II-5-h (myricetin), and B-II-5-f (kaempferol). After sensory evaluation, gallic acid and protocatechuic acid were found to have a sour and astringent taste in the mouth first, followed by a sweet aftertaste. Vanillin has a slightly sweet taste and is accompanied by an astringent taste. Myricetin and kaempferol both have a sweet taste and are also sweet after eating.

### Sensory-guided isolation of *P. emblica* extract fraction C

To further determine the sweet components in fraction C, fraction C was separated and purified by silica gel column chromatography to obtain four fractions (C-I–C-IV), and the solvents of these fractions were spin evaporated dry and then freeze-dried twice to be solvent-free. The freeze-dried powder was then placed in an aqueous solution and evaluated by comparative taste analysis. The sensory evaluation was followed by C-IV sweetness (1.5) with the highest astringency (0.5) (Fig. 8). The further isolation and identification of C-IV were followed.

### Identification of taste compounds in fraction C-IV

To further localize the substances with a sweet taste, gel column chromatography was used to repeatedly separate the sweet taste fractions from C-IV. These fractions were removed from the solvent, lyophilized, and subjected to

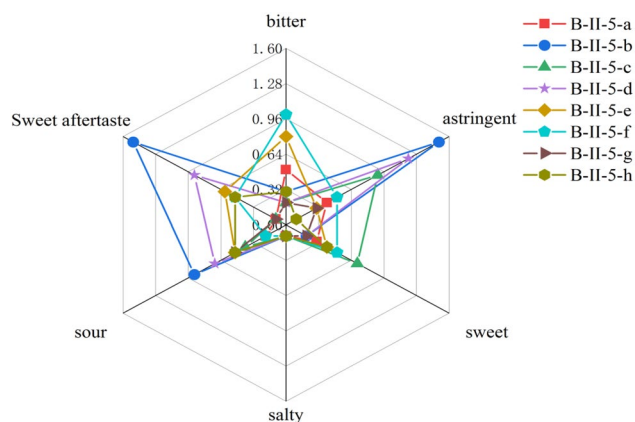


Fig. 7 Sensory evaluation of fraction B-II-5 purified using RP-HPLC

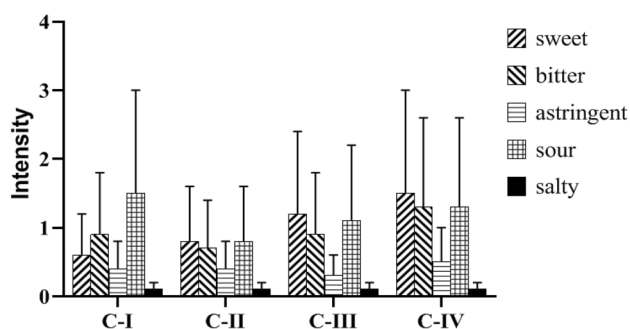


Fig. 8 Sensory analysis of fractions C

sensory analysis. Four chemical components with a sweet taste were finally obtained, namely trans-cinnamic acid (12), naringin (13), oleanolic acid (14), and rutin (15). Sensory analysis of these pure compounds was subsequently performed (Fig. 9). The most intense sweetness was trans-cinnamic acid (1.0); followed by naringin (0.7), but naringin also had the most intense bitterness (0.8), astringency (0.7), and acidity (0.6). In contrast, oleanolic acid (0.6) and rutin (0.5) only had a slightly sweet taste.

### Sensory activity of sweet and aftertaste sweetening compounds

To analyze the sensory activity of the previously identified substances, the taste threshold of each compound was determined (Table 2). The highest sweetness threshold is for naringin (15 mg/ml), which has only a very low sweetness; the other compounds 1, 2, 3, 6, 8, 12, and 15 have relatively low sweetness thresholds (1.88–0.63 mg/ml) and have a more pronounced sweetness, but these chemical compounds have some other flavors besides the sweetness. Gallic acid had the highest intensity of sweet aftertaste with the lowest threshold (0.32 mg/ml); it also had an astringent taste with

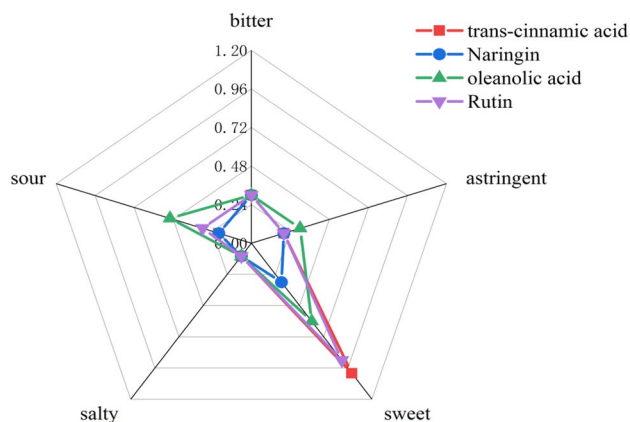


Fig. 9 Sensory analysis of fractions C-IV



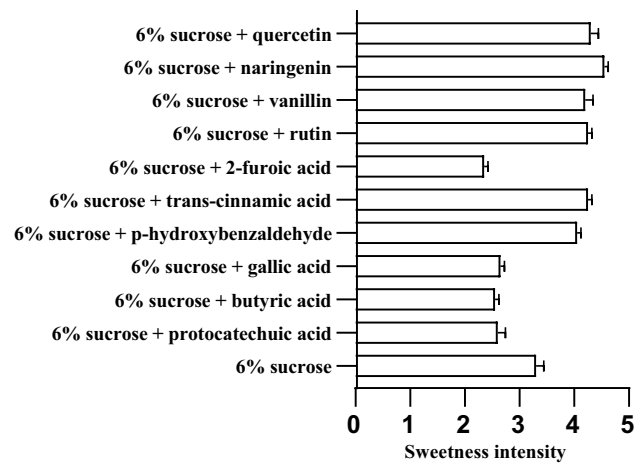
**Table 2** Sensory evaluation of compounds 1–15

Compound	Taste quality	Taste threshold (mg/ml)
2-Furoic acid (1)	Sweet	1.88
Quercetin (2)	Sweet	0.63
p-Hydroxybenzaldehyde (3)	Sweet	1.25
Methyl gallate (4)	Sweet	–
Ethyl 3,4,5-trimethoxybenzoate (5)	Sweet	–
Syringic acid (6)	Sweet	1.88
Gallic acid (7)	astringent	1.25
	sweet aftertaste	0.32
Vanillin (8)	sweet	1.88
Protocatechuic acid (9)	astringent	0.94
	Sweet aftertaste	1.72
Myricetin (10)	Sweet aftertaste	4
Kaempferol (11)	Sweet aftertaste	10
Trans-cinnamic acid (12)	Sweet	0.94
Naringenin (13)	Sweet	15
Oleanolic acid (14)	Sweet	–
Rutin (15)	Sweet	0.94

a threshold of (1.23 mg/ml). Protocatechuic acid had the lowest threshold for astringency (0.94 mg/ml) and also had a sweet aftertaste. Quercetin had the lowest threshold for sweet (0.63 mg/ml).

### Taste modulation properties of sucrose solutions by taste-presenting substances

A total of ten compounds, namely protocatechuic acid, syringic acid, p-hydroxybenzaldehyde, trans-cinnamic acid, 2-furoic acid, rutin, vanillin, naringenin, quercetin, and gallic acid, were added to 6% sucrose solution. As shown in Fig. 10, the score of 6% sucrose solution was  $3.3 \pm 0.2$ . It can be seen from the figure that 2-furoic acid, gallic acid, syringic acid, and 2-furoic acid significantly reduced the sweetness of sucrose solution and had the effect of inhibiting sweetness. In contrast, the sweetness of aqueous sucrose solutions with the addition of p-hydroxybenzaldehyde, trans-cinnamic acid, rutin, vanillin, naringenin, and quercetin increased significantly. The aqueous sucrose solution with naringenin was sweeter, with a sweetness score of  $4.5 \pm 0.1$ . Naringenin itself had only a particularly light sweetness and was not significantly sweet, but it was added to the aqueous sucrose solution to increase the sweetness of the sucrose solution, and it can be used as a sweetness enhancer. However, other substances, such as p-hydroxybenzaldehyde, trans-cinnamic acid, rutin, vanillin, and quercetin, have their sweetness and are more obvious sweetness, and it is presumed that these substances can sweeten the sucrose solution because of their sweetness and superimposed effect.

**Fig. 10** Taste modulation properties of each compound on sucrose solution

### Conclusions

In conclusion, the sensory isolation and structural identification of the extracts of *P. emblica* powder identified 11 (1,2,3,4,5,6,8,12,13,14,15) of *P. emblica* as compounds with a sweet taste and the other four (7,9,10,11) as compounds with a sweet aftertaste. Quantitative studies are currently underway to demonstrate their relative contribution to the overall sweetness of *P. emblica* powder. A study of the taste modulating properties of sucrose revealed that 2-furoic acid, gallic acid, syringic acid, and protocatechuic acid have sweetness inhibiting effects. The sweet aftertaste components of *P. emblica* have sweetness inverse variant modulator properties. And p-hydroxybenzaldehyde, trans-cinnamic acid, rutin, vanillin, naringenin, and quercetin have sweetness-enhancing effects. P-hydroxybenzaldehyde, trans-cinnamic acid, rutin, vanillin, and quercetin itself have strong sweetness, these substances can make sweetness enhancement is their sweetness and the superposition effect. While naringenin itself only has a low sweetness, but can increase the sweetness, there is potential as a sweetness enhancer.

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### Declarations

**Conflict of interest** The authors declare that we have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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