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Norsesquiterpenoids and triterpenoids from strawberry cv. Falandi

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ABSTRACT

Falandi is a common strawberry (*Fragaria* × *ananassa* Duch.) cultivar in southern China. Further study of the chemical constituents in Falandi fruit led to the isolation of nine norsesquiterpenoids and three triterpenoids. Falandioside D (**1**) and falandins A (**2**) and B (**3**) were new norsesquiterpenoids, and the others excluding tormentic acid (**11**) were found in strawberry for the first time. Compounds **1** and **11** exhibited potent α-glucosidase inhibitory activity with the half maximal inhibitory concentration (IC_{50}) values of 565.0 and 27.4 μM in comparison to acarbose (619.9 μM). Compounds **3**, **7** (blumenol C glucoside), and **11** showed cytotoxicity against human nasopharyngeal carcinoma cell line CNE1 with the IC_{50} values of 57.6, 56.4, and 36.0 μM, respectively. Among new compounds, **1** showed 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation scavenging capacity (IC_{50} = 36.2 μM). These results suggested that non-phenolic constituents were also involved in the antidiabetic, antitumour, and antioxidant effects of strawberry fruit.

1. Introduction

Strawberry (*Fragaria* × *ananassa*, Rosaceae) fruit provides a variety of nutrients such as potassium, manganese, vitamins C and E, folic acid, and carotenoids. It is also a rich source of phenolic constituents which include flavonoids (mainly anthocyanins, with flavonols and flavanols providing a minor contribution), phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), hydrolysable tannins (ellagitannins and gallotannins), and lignans (Basu, Nguyen, Betts, & Lyons, 2014; Giampieri et al., 2012; Smeds, Eklund, & Willför, 2012). However, to our knowledge, very few non-phenolic compounds were isolated from strawberry fruit. Roscher, Herderich, Steffen, Schreier, and Schwab (1996) obtained a flavor compound, 2,5-dimethyl-4-hydroxy-3[2H]-furanone, and its 6′-*O*-malonylglucoside from the fruit of *F.* × *ananassa* cv. Senga Sengana. Hirai et al. (2000) obtained three antifungal triterpenoids, euscaphic acid, tormentic acid, and myrianthic acid from unripe fruit of *F.* × *ananassa* cv. Houkouwase which had been wounded and inoculated with the conidia of *Colletotrichum musae*.

Strawberry cv. Falandi is widely cultivated in southern China. In a previous study, twenty-two phenolic compounds were isolated from the fruit of this cultivar (Yang, Xie, Jiang, & Wei, 2016). This study was focused on the isolation and structure determination of non-phenolic compounds present in the fruit under the guidance of a HPLC refractive index (RI) detector and thin layer chromatography (TLC) as well as the evaluation of their biological activities.

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2. Materials and methods

2.1. General procedures

TLC was performed on pre-coated silica gel $HSGF_{254}$ plates (Jiangyou Silica Gel Development Co., Yantai, China), and visualized by spraying 10% sulfuric acid in ethanol (v/v) followed by heating. HPLC was conducted using a LC-6AD pump (Shimadzu, Kyoto, Japan) connected to a RID-10A detector (Shimadzu) and the columns used were 250 mm × 4.6 mm inner diameter (i.d.) for analysis and 250 mm × 20 mm i.d. for preparation, YMC-pack ODS-A, particle size 5 μm (YMC, Kyoto, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured using a Bruker Ascend-500 spectrometer (Bruker BioSpin Gmbh, Rheinstetten, Germany) in deuteromethanol (CD_3OD) with residual peaks as references. Heteronuclear single bond coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear overhauser effect spectroscopy (NOESY) spectra were obtained using the same NMR spectrometer as required. CNE1 cell line was provided by Guangzhou Jinan Biomedicine Research and Development Center (Guangzhou, China). α-Glucosidase (from yeast *Saccharomyces cerevisiae*) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma-Aldrich (St. Louis, MO, USA). *p*-Nitrophenyl-α-D-glucoside (*p*-NPG) and acarbose were from J&K Scientific Ltd. (Beijing, China). L-Ascorbic acid was from Shanghai Boao Biotech Co. (Shanghai, China). Other reagents and apparatuses used for medium pressure liquid chromatography (MPLC), optical density (OD), high resolution electrospray ionization mass spectrometry (HR-ESI-MS), ESI-MS, optical rotation (α_D), and UV spectra were the same as previously described (Yang et al., 2016).

2.2. Plant material

Fresh ripe fruits of *F.* × *ananassa* cv. Falandi were collected from a commercial production farm (flatland, east latitude 113.3466°, north longitude 23.2994°, altitude 42.5 m) in Taihe town, Baiyun District, Guangzhou in March of 2013. After calyxes were manually removed, each edible receptacle was sliced into five or six pieces.

2.3. Extraction and isolation

The fresh receptacle slices (82.0 kg) were immersed in 95% ethanol (50 L × 3) thrice in a stainless steel bucket with lid at room temperature (18–29 °C) for 2 d per time during which period manual stirring was conducted from time to time. The filtrated solution was condensed under reduced pressure to give an extract (3.7 kg). The extract was dissolved in water to a total volume of 6 L and then partitioned with petroleum ether (boiling point 60–90 °C, 4 L × 3) for depigmentation. The aqueous layer was sequentially partitioned with ethyl acetate (4 L × 4) and *n*-butanol (4 L × 4) to give ethyl acetate-soluble (68.6 g) and *n*-butanol-soluble (360.0 g) fractions. The later fraction was dissolved in water and passed through an Amberlite XAD-7HP macroporous resin column which was eluted with water and then 90% ethanol to yield an ethanol eluate (86.6 g). The eluate and ethyl acetate-soluble fraction were combined (155.2 g) and subjected to silica gel (3000 g) column (745 mm × 120 mm i.d.) chromatography which was eluted with chloroform ($CHCl_3$)/methanol (MeOH) (v/v, 10:0, 10 L → 9:1, 24 L → 85:15, 19 L → 8:2, 12 L → 7:3, 26 L → 6:4, 15 L → 0:10, 15 L) to provide fractions A–F according to their TLC profiles. Fraction B (14.8 g) was separated by MPLC which was eluted with MeOH/H_2O (v/v, 2:8 → 3:7 → 4:6 → 5:5 → 6:4 → 7:3 → 8:2, 3 L each) to furnish fractions B1–B24. Fraction B4 (210 mg) was separated by Sephadex LH-20 column chromatography (CC) which was eluted with MeOH and purified by HPLC using acetonitrile (MeCN)/H_2O/acetic acid (AcOH) (v/v/v, 12:88:0.1) as mobile phase at the flow rate of 5 mL/min to yield compounds **2** [retention time (t_R) 56.9 min, 2.5 mg] and **3** (t_R 79.3 min, 1.5 mg). Fraction B5 (1.9 g) was sequentially separated by MPLC and Sephadex LH-20 CC and purified by HPLC using MeCN/H_2O/AcOH (v/v/v, 18:82:0.1) as mobile phase at 5 mL/min to give compound **5** (t_R 63.5 min, 3.7 mg). Fraction B8 (305 mg) was separated by Sephadex LH-20 CC and purified by HPLC using MeCN/H_2O/AcOH (v/v/v, 18:82:0.1) as mobile phase at 5 mL/min to yield compounds **6** (t_R 51.0 min, 3.0 mg) and **7** (t_R 45.0 min, 3.0 mg). Fraction B20 (50 mg) was separated by Sephadex LH-20 CC and purified by HPLC using MeOH/H_2O (v/v, 7:3) as mobile phase at 5 mL/min to provide compound **10** (t_R 39.0 min, 5.0 mg). Fraction B22 (265 mg) was separated by silica gel CC and purified by HPLC using MeOH/H_2O (v/v, 68:32) as mobile phase at 5 mL/min to give compound **11** (t_R 59.0 min, 5.0 mg). Fraction D (17.0 g) was subjected to silica gel CC which was eluted with $CHCl_3$/MeOH (v/v, 9:1 → 8:2 → 7:3 → 6:4) to yield fractions D1–D4. Fraction D2 (11 g) was separated by MPLC which was eluted with MeOH/H_2O (v/v, 1:9 → 2:8 → 3:7 → 4:6 → 5:5 → 6:4 → 7:3 → 8:2, 2 L each) to furnish fractions D2-1–D2-21. Fraction D2-2 (960 mg) was separated by MPLC which was eluted with MeOH/H_2O (v/v, 1:9 → 2:8 → 3:7 → 4:6, 1 L each) and purified by silica gel CC to afford compounds **4** (16.0 mg), **8** (3.0 mg), and **9** (5.0 mg). Fraction D2-8 (992 mg) was separated by MPLC which was eluted with MeOH/H_2O (v/v, 3:7 → 4:6 → 5:5, 1 L each) to give compound **1** (8.0 mg). Fraction D2-14 (46 mg) was separated by silica gel CC which was eluted with $CHCl_3$/MeOH (v/v, 8:2) to yield compound **12** (12.0 mg). Each fraction was analyzed by TLC and HPLC for the comparison of compound(s) it contained. The same compound could usually be found in adjacent fractions. In this study, only a small amount of each compound was purified by column chromatography and/or HPLC for structure determination.

2.4. α-Glucosidase inhibition assay

This assay was conducted in 96-well microtiter plates following the procedures as previously described (Yang et al., 2016). In brief, test compounds were dissolved in dimethyl sulfoxide (DMSO) to six serial concentrations. α-Glucosidase and *p*-NPG were dissolved in 67 mM sodium phosphate buffer (PH 6.8) to 0.5 U/mL and 5 mM, respectively. Four kinds of solutions were made. Test solution contained 112 μL of buffer, 20 μL of enzyme, and 8 μL of a compound. Test blank solution contained 132 μL of buffer and 8 μL of a compound. Negative control solution contained 112 μL of buffer, 20 μL of enzyme, and 8 μL of DMSO. Negative blank solution contained 132 μL of buffer and 8 μL of DMSO. Acarbose was used as a positive reference. The plates were carefully shaken to thoroughly mix the solutions and kept at 37 °C for 15 min. After 20 μL of *p*-NPG was added to each well, the plates were incubated at 37 °C for 15 min again. Then, 80 μL of 0.2 M sodium carbonate in the buffer was added to terminate the reaction. The amount of *p*-nitrophenol hydrolyzed from *p*-NPG by α-glucosidase was quantified by measuring its OD value at 405 nm.

$$\text{Inhibition rate } (\%) = [1 - (OD_{test} - OD_{test\ blank})/(OD_{negative\ blank} - OD_{blank})] \times 100$$

2.5. Cytotoxicity assay

Cytotoxicity was determined by MTT colorimetric assay following the procedures as previously described (Zhou, Xie, Wu, & Wei, 2013). In brief, CNE1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum in a humidified

atmosphere with 5% CO_2 at 37 °C throughout the assay. Test compounds were dissolved in DMSO to six serial concentrations. One hundred microliter of cells in medium at the density of 5×10^4 cells/mL in the exponential growth phase was pipetted into 96-well plates and incubated for 24 h, during which period the cells formed a monolayer on the flat bottom. After the supernatant per well was removed, 100 μL of fresh medium and 100 μL of a compound were added and mixed thoroughly. Control wells received 200 μL of fresh medium containing 0.5% DMSO without a compound. Doxorubicin hydrochloride, a conventional drug used in cancer chemotherapy, was used as a positive reference. After 72 h of incubation, 20 μL of 5 mg/mL MTT in DMSO was added and shaken for 15 min. Four hours later, the supernatant per well was removed and 150 μL of DMSO was added, and the plate was vortex shaken for 15 min to dissolve blue formazan crystals. The OD value of each well was measured at 570 nm.

$$\text{Inhibition rate } (\%) = (1 - \text{OD}_{\text{treat}}/\text{OD}_{\text{control}}) \times 100\%.$$

2.6. Antioxidant activity evaluation

Antioxidant activity was evaluated by ABTS radical cation decolourisation assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay according to the procedures as previously described (Yang et al., 2016). L-Ascorbic acid was used as a positive reference.

2.7. Statistical analysis

Each concentration of test compounds was done in triplicate. IC_{50} values were calculated and expressed as means ± standard deviations (SD). The values were statistically analyzed by one-way analysis of variance (ANOVA) in SPPS 16.0 software package, and $p < 0.05$ indicated statistically significant difference.

3. Results and discussion

The ethanol extract of strawberry cv. Falandi fresh fruits were sequentially separated by solvent partition, column chromatography, and MPLC, and purified by HPLC under the guidance of RI detector to provide twelve pure compounds (Fig. 1). All the compounds were measured for ^1H and ^{13}C NMR and ESI-MS spectroscopic data. The compounds with chiral center(s) were measured for optical rotation values. In addition, new compounds were measured for UV, HR-ESI-MS, and two-dimensional NMR spectra.

3.1. Spectroscopic data of compounds **1**–**3** and **12**

3.1.1. Falandioside D (**1**)

10-Methoxy-2,7-dimethyl-10-oxodeca-2*E*,4*E*-dienoic acid glucoside. White amorphous powder; $[\alpha]_D^{20}$ −3.1 (*c* 1.01, MeOH); UV (MeOH) λ_{max} nm (log ε) 264 (3.88); ESI-MS *m/z* 441 $[M+Na]^+$ and 417 $[M-H]^-$; HR-ESI-MS *m/z* 441.1719 $[M+Na]^+$ (calcd for $C_{19}H_{30}NaO_{10}^+$, 441.1731, error −2.8 ppm); ^1H NMR (500 MHz, CD_3OD) δ 7.17 (1H, d, *J* = 11.4 Hz, H-3), 6.47 (1H, dd, *J* = 15.0, 11.4 Hz, H-4), 6.09 (1H, dt, *J* = 15.0, 7.4 Hz, H-5), 2.44 (1H, ddd, *J* = 13.8, 7.4, 6.3 Hz, H-6), 2.14 (1H, dt, *J* = 13.8, 8.4 Hz, H-6), 1.95 (1H, m, H-7), 4.03 (1H, ddd, *J* = 7.5, 5.2, 4.2 Hz, H-8), 2.68 (1H, dd, *J* = 15.6, 7.5 Hz, H-9), 2.59 (1H, dd, *J* = 15.6, 4.2 Hz, H-9), 1.91 (3H, d, *J* = 0.8 Hz, H_3-11), 0.96 (3H, d, *J* = 6.8 Hz, H_3-12), 3.68 (3H, s, OCH_3), 4.33 (1H, d, *J* = 7.8 Hz, H-1′), 3.15 (1H, dd, *J* = 9.1, 7.8 Hz, H-2′), 3.33 (1H, dd, *J* = 9.1, 8.6 Hz, H-3′), 3.26 (1H, dd, *J* = 8.6, 5.7 Hz, H-4′), 3.22 (1H, dd, *J* = 5.7, 2.4 Hz, H-5′), 3.81 (1H, dd, *J* = 11.9, 2.4 Hz, H-6′), and 3.63 (1H, dd, *J* = 11.9, 5.7 Hz, H-6′); ^{13}C NMR (125 MHz, CD_3OD) δ 171.5 (C-1), 126.9 (C-2), 139.7 (C-3), 129.0 (C-4), 142.1 (C-5), 36.9 (C-6), 38.5 (C-7), 81.4 (C-8), 38.8 (C-9), 174.8 (C-10), 12.8 (C-11), 15.3 (C-12), 52.2 (OCH_3), 104.6 (C-1′), 75.3 (C-2′), 78.1 (C-3′), 71.7 (C-4′), 77.4 (C-5′), and 63.0 (C-6′).

3.1.2. Falandin A (**2**)

3,6-Dihydroxy-5,11-epoxy-7*E*-megastigmaen-9-one. White amorphous powder; $[\alpha]_D^{20}$ −20.0 (*c* 0.24, MeOH); UV (MeOH) λ_{max} nm (log ε) 256 (3.84); ESI-MS *m/z* 279 $[M+K]^+$ and 479 $[2M-H]^-$; HR-ESI-MS *m/z* 263.1252 $[M+Na]^+$ (calcd for $C_{13}H_{20}NaO_4^+$, 263.1254, error −0.6 ppm); ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data in CD_3OD, see Table 1.

3.1.3. Falandin B (**3**)

3,6-Dihydroxy-5,11-epoxy-7*Z*-megastigmaen-9-one. White amorphous powder; $[\alpha]_D^{20}$ −12.1 (*c* 0.38, MeOH); UV (MeOH) λ_{max} nm (log ε) 258 (3.98); ESI-MS *m/z* 263 $[M+Na]^+$; HR-ESI-MS *m/z* 263.1251 $[M+Na]^+$ (calcd for $C_{13}H_{20}NaO_4^+$, 263.1254, error −1.1 ppm); ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data in CD_3OD, see Table 1.

3.1.4. Suavissimoside R1 (**12**)

White amorphous powder; ESI-MS *m/z* 703 $[M+Na]^+$ and 679 $[M-H]^-$; ^1H NMR (500 MHz, CD_3OD) δ 4.13 (1H, m, H-2), 2.89 (1H, d, *J* = 8.4 Hz, H-3), 5.32 (1H, t, *J* = 4.2 Hz, H-12), 2.61 (1H, td, *J* = 13.7, 4.8 Hz, H-16), 2.52 (1H, s, H-18), 1.41 (3H, s, H_3-24), 0.98 (3H, s, H_3-25), 0.81 (3H, s, H_3-26), 1.34 (3H, s, H_3-27), 1.20 (3H, s, H_3-29), 0.93 (3H, d, J = 6.7 Hz, H_3-30), 5.30 (1H, d, *J* = 8.0 Hz, H-1′), 3.80 (1H, br d, *J* = 11.8 Hz, H-6′), and 3.68 (1H, dd, *J* = 11.8, 4.3 Hz, H-6′); ^{13}C NMR (125 MHz, CD_3OD) δ 49.0 (C-1), 69.3 (C-2), 84.4 (C-3), 50.6 (C-4), 57.7 (C-5), 21.5 (C-6), 34.4 (C-7), 41.2 (C-8), 47.9 (C-9), 39.6 (C-10), 25.0 (C-11), 129.6 (C-12), 139.7 (C-13), 42.9 (C-14), 29.6 (C-15), 26.5 (C-16), 49.8 (C-17), 55.0 (C-18), 73.8 (C-19), 42.8 (C-20), 27.2 (C-21), 38.3 (C-22), 182.6 (C-23), 25.0 (C-24), 16.6 (C-25), 17.5 (C-26), 24.5 (C-27), 178.5 (C-28), 27.1 (C-29), 16.6 (C-30), 95.8 (C-1′), 73.6 (C-2′), 78.6 (C-3′), 71.1 (C-4′), 78.3 (C-5′), and 62.4 (C-6′). This was the first report of its NMR data measured in CD_3OD.

3.2. Structure determination

Compound **1** was obtained as white amorphous powder with negative optical rotation. It was determined to have the molecular formula $C_{19}H_{30}O_{10}$ based on its HR-ESI-MS and NMR data. The ^1H NMR spectrum showed signals readily recognized for three olefinic protons (H-3, H-4, and H-5), a methoxyl (δ 3.68, 3H, s), and two methyls (H_3-11 and H_3-12). The ^{13}C NMR spectrum showed signals of two carboxyls (C-1 and C-10), four olefinic carbons (C-2, C-3, C-4, and C-5), a methoxyl carbon (δ 52.2), two methyl carbons (C-11 and C-12), and an oxygenated methine carbon at δ 81.4 (C-8). Moreover, the ^1H and ^{13}C NMR spectra exhibited signals typical of a β-glucosyl moiety with anomeric proton and carbon at δ 4.33 (1H, d, *J* = 7.8 Hz, H-1′) and 104.6 (C-1′) (Xu, Xie, & Wei, 2014). Analysis of the correlations in the HSQC spectrum clarified the direct connections of protons to carbons. In the HMBC spectrum, the correlations from H-3 to C-1, C-4, C-5, and C-11, H-8 to C-6, C-10, and C-12, and the methoxyl protons to C-10 were observed, which led us to determine the aglycone of **1** to be monomethyl glansreginate (Ito, Okuda, Fukuda, Hatano, & Yoshida, 2007). Further, the HMBC correlations from H-1′ to C-8 and H-8 to C-1′ ascertained the connection of β-glucosyl moiety to C-8 via an oxygen bridge. Based on the aforementioned evidence, compound **1** was identified as 10-methoxy-2,7-dimethyl-10-oxodeca-2*E*,4*E*-dienoic acid glucoside, and trivially named falandioside D.

Compound **2** was assigned the molecular formula $C_{13}H_{20}O_4$ based on its positive ion mode HR-ESI-MS peak at *m/z* 263.1252

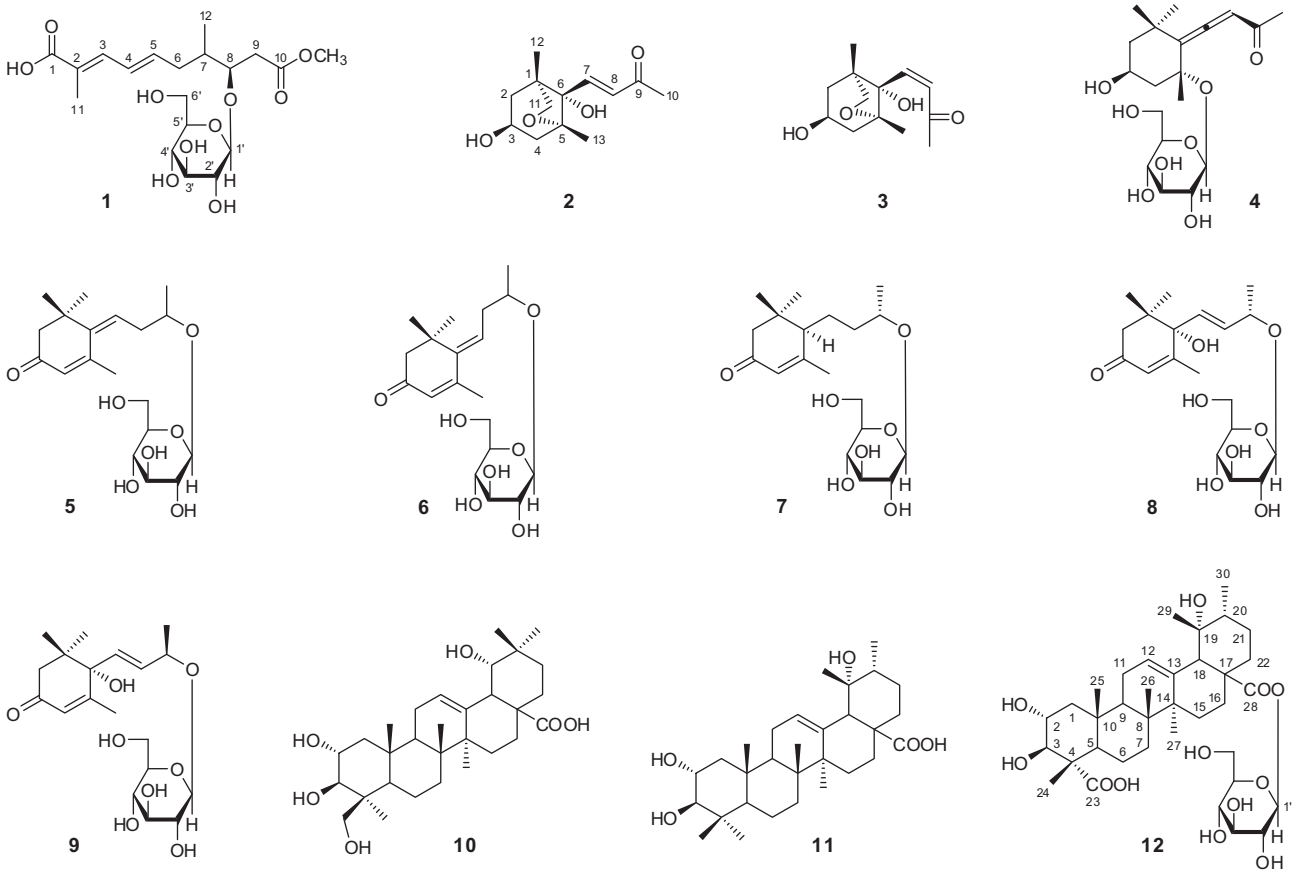

Fig. 1. Structures of compounds **1**–**12**.

Table 1
^{1}H and ^{13}C NMR data of compounds **2** and **3** in CD_3OD.

	2		**3**	
H/C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1		49.8		49.7
2 eq	1.86 (dd, 13.5, 6.6)	44.5	1.85 (dd, 13.0, 6.8)	44.5
2ax	1.67 (dd, 13.5, 12.2)		1.66 (dd, 13.0, 9.8)	
3	4.11 (m)	66.0	4.13 (m)	66.0
4 eq	2.04 (dd, 13.6, 6.6)	46.0	2.04 (dd, 12.6, 5.5)	46.0
4ax	1.74 (dd, 13.6, 10.6)		1.74 (dd, 12.6, 10.7)	
5		87.8		87.8
6		83.3		83.3
7	6.46 (d, 16.0)	132.4	6.42 (d, 12.5)	132.3
8	6.64 (d, 16.0)	138.1	7.92 (d, 12.5)	133.5
9		214.7		210.0
10	2.29 (3H, br s)	14.1	2.05 (3H, br s)	14.0
11	3.81 (dd, 7.3, 1.6)	77.3	3.81 (d, 6.8)	77.3
	3.72 (d, 7.3)		3.71 (d, 6.8)	
12	0.90 (3H, s)	16.3	0.92 (3H, s)	16.4
13	1.12 (3H, s)	19.6	1.14 (3H, s)	19.7

$[M+Na]^+$. The ^{1}H and ^{13}C NMR spectra (Table 1) showed a total of thirteen carbon signals, including a carbonyl at δ 214.7 (C-9), three methyls at δ 2.29 (3H, br s, H_3-10), 0.90 (3H, s, H_3-12), and 1.12 (3H, s, H_3-13), a hydroxymethyl at δ 3.81 (1H, dd, J = 7.5, 1.6 Hz, Ha-11) and 3.72 (1H, d, J = 7.3 Hz, Hb-11), and a *trans*-olefinic bond with protons at δ 6.46 (1H, d, J = 16.0 Hz, H-7) and 6.64 (1H, d, J = 16.0 Hz, H-8) and carbons at δ 132.4 (C-7) and 138.1 (C-8). These data were indicative of a megastigmane (Zhang et al., 2010). In the HMBC spectrum, the correlations from Ha-11 to C-12, Hb-11 to C-5 at δ 87.8 and C-6 at δ 83.3 suggested the presence of a 5,11-epoxy (Abe & Yamauchi, 2000). Moreover, the HMBC correlations from δ 1.86 (1H, dd, J = 13.5, 6.6 Hz, Heq-2), 1.67 (1H, dd, J = 13.5, 12.2 Hz, Hax-2), 2.04 (1H, dd, J = 13.6, 6.6 Hz, Heq-4), and 1.74 (1H, dd, J = 13.6, 10.6 Hz, Hax-4) to C-3 at δ 66.0, H-7 to C-1 at δ 49.8 and C-5, and H-8 to C-6 and C-10 at δ 14.1 clarified the connections of two hydroxyl groups to C-3 and C-6. Further, mutual correlations in the NOESY spectrum between H-7 and Hax-2/Hax-4, H-3 and Heq-2/Heq-4 led us to assign the relative configurations of **2** to be as shown. Consequently, compound **2** was identified as 3,6-dihydroxy-5,11-epoxy-7*E*-megastigmaen-9-one, and trivially named falandin A.

Compound **3** was established to have the same molecular formula as compound **2**. Comparison of their ^{1}H and ^{13}C NMR data (Table 1) revealed that the differences lay in the δ values of H-8 and C-8 as well as the J values between H-7 and H-8. The J value of 12.5 Hz in **3** was characteristic of a *cis* olefinic configuration (Hiradate, Morita, Sugie, Fujii, & Harada, 2004). Therefore, compound **3** was determined as 3,6-dihydroxy-5,11-epoxy-7*Z*-megastigmaen-9-one, and trivially named falandin B.

The known compounds were identified by analysis of their ^{1}H and ^{13}C NMR and ESI-MS data and comparison of their data with the reported values. They were citroside A (**4**) (Zhang et al., 2010), 4,6*Z*-megastigmadien-3-one-9-glucoside (**5**) (Khan et al., 2003), 4,6*E*-megastigmadien-3-one-9-glucoside (**6**) (Khan et al., 2003), blumenol C glucoside (**7**) (Takeda et al., 1997), corchoionoside C (**8**) (Yoshikawa et al., 1997), (6*S*,9*R*)-roseoside (**9**) (Yoshikawa et al., 1997), sericic acid (**10**) (Yeo, Park, & Kim, 1998), tormentic acid (**11**) (Jin et al., 2004), and suavissimoside R1 (**12**) (Gao et al., 1985).

3.3. α-Glucosidase inhibitory activity

Compounds **1**–**12** were evaluated for *in vitro* α-glucosidase inhibitory activity. As shown in Table 2, compound **11** exhibited much

Table 2
α-Glucosidase inhibitory and cytotoxic activities of compounds **1**–**12**.

Compound	(IC_{50}, μM)	
	α-Glucosidase inhibition	Cytotoxicity
1	564.98 ± 10.37[a]	>100
3	>750	57.63 ± 3.07[a]
7	>750	56.40 ± 4.95[a]
11	27.43 ± 2.15[b]	36.02 ± 0.48[b]
2, **4**–**6**, **8**–**10**, **12**	>750	>100
Acarbose	619.94 ± 118.34[a]	
Doxorubicin hydrochloride		0.26 ± 0.02[c]

Values represent means ± SD ($n = 3$).
In each row different small letters indicate significant difference ($p < 0.05$).

Table 3
Antioxidant activity of compounds **1**–**3**.

Compound	ABTS (IC_{50}, μM)	DPPH (IC_{50}, μM)	FRAP (mmol/g)
1	36.18 ± 0.64[a]	102.92 ± 3.87[b]	1.49 ± 0.01[c]
2	>100	>100	1.24 ± 0.01
3	>100	>100	1.09 ± 0.00
L-Ascorbic acid	13.27 ± 0.28[a]	35.23 ± 1.62[b]	10.83 ± 0.08[c]

Values represent means ± SD ($n = 3$).
In each row different small letters indicate significant difference ($p < 0.05$).

more potent activity with the IC_{50} value of 27.4 μM than the positive control, acarbose (619.9 μM). The inhibitory activity of compound **1** was comparable to acarbose. However, the IC_{50} values of other compounds were over 750 μM.

3.4. Cytotoxicity

Compounds **1**–**12** were evaluated for *in vitro* cytotoxicity against human nasopharyngeal carcinoma cell line CNE1 by MTT assay. Among them, compounds **3**, **7**, and **11** demonstrated weak activity with the IC_{50} values of 57.6, 56.4, and 36.0 μM, respectively, in comparison to doxorubicin hydrochloride (0.26 μM), while the others were inactive (IC_{50} > 100 μM) (Table 2).

3.5. Antioxidant activity

Three new compounds were evaluated for antioxidant activity using three different assays (Table 3). Compound **1** showed moderate ABTS radical cation scavenging activity with the IC_{50} value of 36.2 μM in comparison to L-ascorbic acid (13.3 μM), while it was inactive in scavenging DPPH radicals (IC_{50} > 100 μM) and in FRAP. Compounds **2** and **3** were inactive in three assays.

It was noteworthy that other biological activities of compounds **8**, **9**, and **11** had been reported. Corchoionoside C (**8**) showed anti-inflammatory activity in rat HAPI cells via inhibition of lipopolysaccharide (LPS)-induced inducible nitric oxide synthase mRNA expression at 50 μM treated 30 min before LPS challenge measured after 6 h by reverse transcription polymerase chain reaction analysis (Hara et al., 2011). It also exhibited inhibitory effect on the generation of osteoclast in receptor activator nuclear factor κB ligand and macrophage colony stimulating factor-stimulated mice bone marrow-derived cells at 200 μM after 4 d by tartrate-resistant acid phosphatase assay (Cuong et al., 2009). Corchoionoside C (**8**) and (6*S*,9*R*)-roseoside (**9**) could inhibit the release of leukotriene in Institute of Cancer Research mice bone marrow-derived cultured mast cells at 100 mM after 6 h by ELISA (Yajima, Oono, Nakagawa, Nukada, & Yabuta, 2009). Villar, Payá, Hortigüela, and Cortes (1986) found tormentic acid (**11**) to be an hypoglycemic agent.

4. Conclusions

This study revealed twelve non-phenolic compounds present in the fruit of strawberry cv. Falandi. Some of these compounds showed diverse biological activities such as α-glucosidase inhibition, cytotoxicity, antioxidation, anti-inflammation, inhibiting osteoclast generation and leukotriene release, and hypoglycermic action, which suggested that non-phenolic compounds were also involved in the health promoting effects of strawberry fruit.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.02.036.

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