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^{1}H NMR spectroscopy-guided isolation of new sucrose esters from *Physalis alkekengi* var. *franchetii* and their antibacterial activity

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ABSTRACT

Ten new sucrose esters, physakengoses A–J (**1**—**10**), were isolated from the aerial parts of *Physalis alkekengi* var. *franchetii* under the guidance of ^{1}H NMR spectroscopy. Their structures were determined by spectroscopic analyses (HRESIMS, 1D and 2D NMR, and ESIMS) and chemical methods. These new compounds were tested for antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Among them, compounds **2** and **5**–**8** showed potent inhibitory effects against test strains with MIC values ranging from 3.5 to 14.9 μg/mL. These findings may indicate that the *P. alkekengi* var. *franchetii* has potential application as an ingredient in pharmaceuticals.

Chemical compounds

Ethyl ethanoate (PubChem CID: 8857)
Methylene chloride (PubChem CID: 6344)
Methanol (PubChem CID: 887)
Methyl cyanide (PubChem CID: 6342)

1. Introduction

Physalis alkekengi var. *franchetii* (Solanaceae), a medicinal and edible plant, is widely distributed and cultivated in Europe and Asia [1]. The aerial parts of *P. alkekengi* var. *franchetii* have been used as a traditional Chinese medicine for treatment of sore throat, cough, eczema, hepatitis, urinary problems and tumors [2]. Previous investigations of *P. alkekengi* var. *franchetii* were focused on physalins, which exhibited anti-inflammatory, cytotoxic, antioxidant and antibacterial activities [2–4]. As a part of continuous chemical studies on the genus *Physalis* [5,6], we throw our sight to sucrose esters. The presence of sucrose esters are restricted in their distribution, previously being reported from the Solanaceae genera *Physalis*, *Nicotiana*, *Petunia*, *Solanum*, and *Lycopersicon*, as well as from the Asteraceae, Cannaceae, and Polygalaceae families [7]. Sucrose esters, structurally featured in sucrose and long fatty acid ester, have exhibited potent antibacterial and anti-inflammatory activities [8–10]. Since ^{1}H NMR-guided isolation showed the potential to facilitate the current efforts on distinct natural product discovery [11,12], it was applied to help us rapidly target and isolate sucrose esters from the aerial parts of *P. alkekengi* var. *franchetii*. Herein we describe the ^{1}H NMR-guided isolation and identification of ten new sucrose esters (Fig. 1) and their *in vitro* antibacterial activities.

2. Experimental

2.1. General

The optical rotation values were recorded on a Jasco P-1020 polarimeter and IR data were detected on a Bruker Tensor 27 spectrometer. 1D and 2D NMR experiments were carried out in methanol-d_4 on a Bruker Avance III NMR instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C), and TMS was set as the internal standard. Agilent UPLC-Q-TOF (6520B) was used to acquire HRESIMS data. HPLC analysis was performed on an Agilent 1260 Series instrument equipped with a DAD detector and a Shim-pack VP-ODS column (4.6 × 250 mm, i.d.). Silica gel (200–300 and 100–200 mesh, Qingdao Marine Chemical Co., Ltd.), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and ODS (40–63 μm, Fuji) were used for column chromatography (CC). All chemical reagents used were analytical grade (Jiangsu Hanbon Science and Technology Co., Ltd., Nanjing, China).

2.2. Plant material

The aerial parts of *P. alkekengi* var. *franchetii* were collected in October 2014 from Fuyang city, Anhui province, People's Republic of China, and identified by *Prof*. Mian Zhang. A voucher specimen (No. PAF-20150422) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

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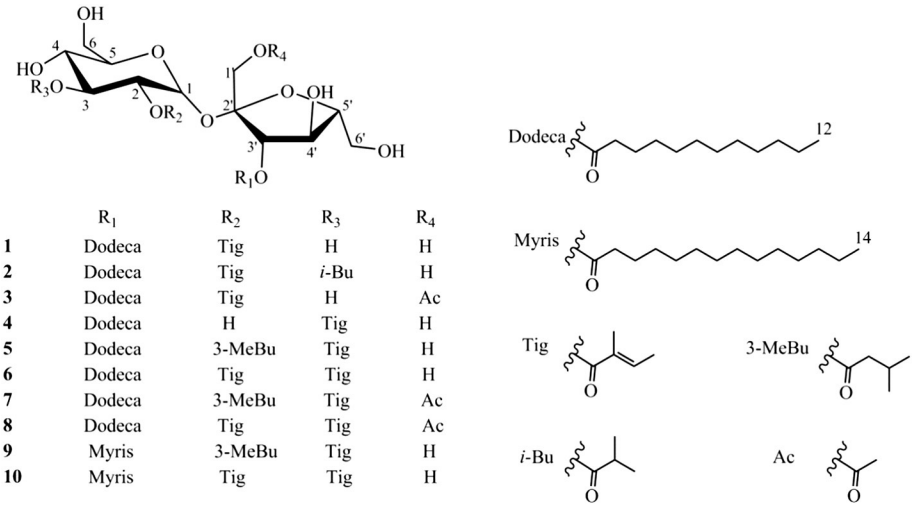

	R_1	R_2	R_3	R_4
1	Dodeca	Tig	H	H
2	Dodeca	Tig	*i*-Bu	H
3	Dodeca	Tig	H	Ac
4	Dodeca	H	Tig	H
5	Dodeca	3-MeBu	Tig	H
6	Dodeca	Tig	Tig	H
7	Dodeca	3-MeBu	Tig	Ac
8	Dodeca	Tig	Tig	Ac
9	Myris	3-MeBu	Tig	H
10	Myris	Tig	Tig	H

Fig. 1. Structures of compounds **1**–**10**.

2.3. Extraction and isolation

The plant materials (3.0 kg) were extracted with 95% EtOH (3 × 10 L) under reflux. After removing the solvents under reduced pressure, the viscous residue (240.0 g) was obtained. The residue was dissolved in distilled water (2 L), followed by successive partition with petroleum ether (PE), dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc). The PE, CH_2Cl_2, EtOAc and H_2O fractions were separately analyzed by ^{1}H NMR spectroscopy, and the characteristic ^{1}H NMR signals for sucrose esters were detected from PE fraction. Therefore, the PE fraction (80.0 g) was further chromatographed by using a silica gel column with a gradient of petroleum ether-acetone (100:1–1:1, v/v) to give four fractions, Frs. A–D. Subsequently, Frs. A–D were subjected to ^{1}H NMR spectroscopy, and the signals for sucrose esters were observed particularly in Fr. D. Thus, Fr. D (25.0 g) was further separated by an MCI gel column using MeOH-H_2O (30:10–90:10, v/v) as eluent to yield six major subfractions (Frs. D1–D6). Fr. D2 (8.0 g) was applied to an ODS column eluted with solvent system of MeOH-H_2O (70:30–90:10, v/v) to afford Frs. D2a–D2e. Fr. D2b was purified by preparative HPLC using MeOH-H_2O (80:20, v/v) as the mobile phase to give compounds **1** (8.0 mg, t_R = 8.5 min) and **5** (17.0 mg, t_R = 16.7 min). Compounds **2** (4.0 mg, t_R = 10.9 min) and **7** (20.0 mg, t_R = 12.5 min) were obtained from Fr. D2c by recycling-preparative HPLC eluted with MeCN-H_2O (60:40, v/v). The Fr. D2d was subjected to recycling-preparative HPLC with MeCN-H_2O (65:35, v/v) to furnish compounds **6** (24.0 mg, t_R = 11.9 min) and **8** (4.0 mg, t_R = 8.5 min). Compounds **4** (5.0 mg, t_R = 6.3 min) and **3** (11.0 mg, t_R = 8.4 min) were obtained by preparative HPLC with MeOH-H_2O (80:20, v/v) from Fr. D2e. Fr D3 (6.0 g) was chromatographed by an ODS column eluted with MeOH-H_2O (70:30–90:10, v/v) to get four major subfractions (Frs. D3a–D3d). Compounds **9** (10.0 mg, t_R = 15.7 min) and **10** (15.0 mg, t_R = 12.9 min) were obtained from Fr. D3b and Fr. D3c by recycling-preparative HPLC with MeOH-H_2O (85:15, v/v), respectively.

2.4. Data of the isolated compounds

Physakengose A (**1**): amorphous solid; $[a]_D^{25}$ + 25.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3364, 2924, 2852, 1721, 1658, 1634, 1469, 1424, 1382 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 3); HRESIMS *m/z* 629.3142 $[M + Na]^+$ (calcd for $C_{29}H_{50}NaO_{13}$, 629.3144).

Physakengose B (**2**): amorphous solid; $[a]_D^{25}$ + 42.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3417, 2923, 2852, 1742, 1634, 1469, 1384, 1265, 1160 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 3); HRESIMS *m/z* 699.3559 $[M + Na]^+$ (calcd for $C_{33}H_{56}NaO_{14}$, 699.3562).

Physakengose C (**3**): amorphous solid; $[a]_D^{25}$ + 34.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3418, 2923, 2852, 1747, 1634, 1468, 1383, 1262, 1162 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 3); HRESIMS *m/z* 671.3250 $[M + Na]^+$ (calcd for $C_{31}H_{52}NaO_{14}$, 671.3249).

Physakengose D (**4**): amorphous solid; $[a]_D^{25}$ + 21.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3366, 2923, 2852, 1721, 1658, 1634, 1469, 1383, 1264 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 3); HRESIMS *m/z* 629.3143 $[M + Na]^+$ (calcd for $C_{29}H_{50}NaO_{13}$, 629.3144).

Physakengose E (**5**): amorphous solid; $[a]_D^{25}$ + 32.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3409, 2923, 2853, 1741, 1657, 1468, 1384, 1265, 1155 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 1 and 3); HRESIMS *m/z* 713.3722 $[M + Na]^+$ (calcd for $C_{34}H_{58}NaO_{14}$, 713.3719).

Physakengose F (**6**): amorphous solid; $[a]_D^{25}$ + 35.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3418, 2923, 2852, 1719, 1656, 1469, 1383, 1272, 1156 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 2 and 3); HRESIMS *m/z* 711.3560 $[M + Na]^+$ (calcd for $C_{34}H_{56}NaO_{14}$, 711.3562).

Physakengose G (**7**): amorphous solid; $[a]_D^{25}$ + 25.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3416, 2923, 2852, 1746, 1634, 1468, 1382, 1261, 1049 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 2 and 3); HRESIMS *m/z* 755.3820 $[M + Na]^+$ (calcd for $C_{36}H_{60}NaO_{15}$, 755.3824).

Physakengose H (**8**): amorphous solid; $[a]_D^{25}$ + 36.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3366, 2922, 2851, 1743, 1633, 1469, 1384, 1262, 1075 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 2 and 3); HRESIMS *m/z* 753.3667 $[M + Na]^+$ (calcd for $C_{36}H_{58}NaO_{15}$, 753.3668).

Physakengose I (**9**): amorphous solid; $[a]_D^{25}$ + 22.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3396, 2924, 2853,1726, 1650, 1467, 1385, 1266, 1154 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 2 and 3); HRESIMS *m/z* 741.4031 $[M + Na]^+$ (calcd for $C_{36}H_{62}NaO_{14}$, 741.4032).

Physakengose J (**10**): amorphous solid; $[a]_D^{25}$ + 37.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3365, 2923, 2853, 1719, 1657, 1468, 1383, 1273, 1155 cm^{-1}; ^{1}H NMR and ^{13}C NMR data (see Tables 2 and 3); HRESIMS *m/z* 739.3886 $[M + Na]^+$ (calcd for $C_{36}H_{60}NaO_{14}$, 739.3875).

2.5. Alkaline hydrolysis

Compounds **1**–**10** (2.0 mg each), dissolved in 2 M aqueous NH_4OH (2 mL), were heated at 50 °C for 4 h. The reaction mixtures were neutralized with 2 M formic acid to pH 3 and extracted with EtOAc (3 × 3 mL). Then, the aqueous phase was repeatedly dried with the method reported in the literature [8]. Co-TLC analysis ($CHCl_3$: HAc:

Table 1
^{1}H NMR data (500 MHz, CD_3OD) of Compounds **1**–**5**.

Position	**1**	**2**	**3**	**4**	**5**
	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)
1	5.54, d (3.7)	5.57, d (3.5)	5.59, d (3.5)	5.45, d (3.6)	5.57, d (3.4)
2	4.62, dd (10.1, 3.7)	4.76, dd (10.4, 3.5)	4.66, dd (10.1, 3.5)	3.63, m	4.83[a]
3	3.83[a]	5.41, t (9.7)	3.83[a]	5.24, t (9.7)	5.41, t (9.7)
4	3.46, t (9.5)	3.69, t (9.7)	3.48, t (9.3)	3.61,t (9.7)	3.67, t (9.7)
5	3.86, m	3.93, m	3.85, m	3.91, m	3.95, m
6a	3.83[a]	3.84, d (11.7)	3.83[a]	3.82[a]	3.84, d (11.8)
6b	3.76, m	3.78, m	3.76, m	3.79, m	3.78, m
1′	3.47, d (11.7)	3.46, d (11.7)	4.04, d (11.5)	3.64, d (12.0)	3.55, d (11.7)
1b′	3.31[a]	3.32[a]	3.89, d (11.5)	3.55, d (12.0)	3.44, d (11.7)
3′	5.39, d (8.4)	5.39, d (8.4)	5.25, d (8.4)	5.37, d (8.1)	5.39, d (8.4)
4′	4.29, t (8.4)	4.30, t (8.4)	4.28, t (8.4)	4.30, t (8.1)	4.31, t (8.4)
5′	3.85, m	3.88, m	3.84, m	3.90, m	3.87, m
6a′	3.78, m	3.78, m	3.78, m	3.79, m	3.78, m
6b′	3.78, m	3.78, m	3.78, m	3.79, m	3.78, m
3′-*O*-			Dodeca[b]		
2a	2.43, t (7.4)	2.55, m	2.44, t (7.4)	2.50, dt (15.3, 8.0)	2.55, dt (15.6, 7.5)
2b	2.43, t (7.4)	2.47, m	2.44, t (7.4)	2.43, dt (15.3, 8.0)	2.46, dt (15.6, 7.5)
3	1.66, m	1.68, m	1.66, m	1.68, m	1.69, m
4–11	1.33, m	1.31, m	1.30, m	1.30, m	1.30, m
12	0.90, t (6.8)	0.90, t (6.8)	0.90, t (6.8)	0.90, t (6.9)	0.90, t (6.6)
2-*O*-	Tig[c]	Tig[c]	Tig[c]		3-MeBu[d]
2a					2.13, d (7.0)
2b					2.12, d (7.0)
3	6.94, dq like (1.1, 7.0)	6.85, m	6.95, q (7.0)		1.96, m
4	1.83, d (7.3)	1.78, d (7.0)	1.82, d (7.0)		0.87, d (6.7)
5	1.85, br s	1.80, br s	1.85, br s		0.87, d (6.7)
3-*O*-		*i*-Bu[e]		Tig[c]	Tig[c]
2		2.57, m			
3		1.06, d (7.0)		6.93, dq like (1.3, 7.0)	6.87, q (7.0)
4		1.11, d (7.0)		1.81, d (7.0)	1.79, d (7.0)
5				1.87, br s	1.82, br s
1′-*O*-			Ac		
2			2.05, s		

[a] Overlapped with other signals.
[b] Dodeca = dodecanoyl.
[c] Tig = tigloyl.
[d] 3-MeBu = 3-methylbutanoyl.
[e] *i*-Bu = isobutyryl.

Table 2
^{1}H NMR data (500 MHz, CD_3OD) of compounds **6**–**10**.

Position	**6**	**7**	**8**	**9**	**10**
	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)	δ_H (multi, *J* in Hz)
1	5.60, d (3.5)	5.57, d (3.1)	5.63, d (3.5)	5.57, d (3.5)	5.59, d (3.5)
2	4.78[a]	4.86, dd (10.3, 3.4)	4.83[a]	4.80[a]	4.78[a]
3	5.47, t (9.7)	5.41, t (9.7)	5.47, t (9.7)	5.42, t (9.7)	5.47, t (9.7)
4	3.72, t (9.7)	3.69, t (9.7)	3.72, t (9.7)	3.68, t (9.7)	3.71, t (9.7)
5	3.94, m	3.92, m	3.93, m	3.95, m	3.94, m
6a	3.83, dd (12.2, 1.9)	3.84, m	3.85, dd (12.5, 2.1)	3.84, dd (12.6, 1.8)	3.84, dd (12.1, 2.1)
6b	3.78, m	3.78, m	3.79, m	3.79, m	3.78, m
1a′	3.47, d (11.8)	4.09, d (11.6)	4.04, d (11.6)	3.56, d (11.8)	3.47, d (11.7)
1b′	3.34, d (11.8)	4.01, d (11.6)	3.94, d (11.6)	3.46, d (11.8)	3.33, d (11.7)
3′	5.40, d (8.4)	5.31, d (8.2)	5.27, d (8.4)	5.38, d (8.4)	5.40, d (8.4)
4′	4.31, t (8.4)	4.31, t (8.2)	4.30, t (8.4)	4.31, t (8.4)	4.31, t (8.4)
5′	3.87, m	3.88, m	3.87, m	3.88, m	3.87, m
6a′	3.78, m	3.78, m	3.79, m	3.79, m	3.78, m
6b′	3.78, m	3.78, m	3.79, m	3.79, m	3.78, m
3′-*O*-	Dodeca[b]	Dodeca[b]	Dodeca[b]	Myris[c]	Myris[c]
2a	2.56, dt (16.1, 7.8)	2.56, dt (15.5, 7.5)	2.56, dt (16.0, 7.8)	2.55, dt (15.6, 7.5)	2.56, dt (15.5, 7.8)
2b	2.46, dt (16.1, 7.8)	2.47, dt (15.5, 7.5)	2.46, dt (16.0, 7.8)	2.47, dt (15.6, 7.5)	2.46, dt (15.5, 7.8)
3	1.69, m	1.68, m	1.69, m	1.70, m	1.68, m
4–11	1.30, m	1.30, m	1.30, m	1.30, m	1.30, m
12	0.90, t (6.9)	0.91, t (6.9)	0.90, t (6.8)	1.30, m	1.30, m
13				1.30, m	1.30, m
14				0.91, t (6.8)	0.90, t (6.9)
2-*O*-	Tig[d]	3-MeBu[e]	Tig[d]	3-MeBu[e]	Tig[d]
2a		2.18, d (6.9)		2.13, d (6.9)	
2b		2.17, d (6.9)		2.12, d (6.9)	
3	6.81, m	1.96, m	6.81, dq like (1.2, 7.0)	1.96, m	6.81, m
4	1.77, d (7.2)	0.87, d (6.6)	1.77, d (7.0)	0.87, d (6.7)	1.77, d (7.2)
5	1.75, br s	0.87, d (6.6)	1.75, br s	0.87, d (6.7)	1.75, br s
3-*O*-	Tig[d]	Tig[d]	Tig[d]	Tig[d]	Tig[d]
3	6.81, m	6.87, q (7.1)	6.81, dq like (1.2, 7.0)	6.87, dq like (1.1, 7.1)	6.81, m
4	1.77, d (7.2)	1.79, d (7.1)	1.77, d (7.0)	1.79, d (7.1)	1.77, d (7.2)
5	1.79, br s	1.82, br s	1.79, br s	1.82, br s	1.79, br s
1′-*O*-		Ac	Ac		
2		2.09, s	2.06, s		

[a] Overlapped with other signals.
[b] Dodeca = dodecanoyl.
[c] Myris = myristyl.
[d] Tig = tigloyl.
[e] 3-MeBu = 3-methylbutanoyl.

H_2O = 3: 3.5: 0.5, R_f = 0.41) in comparison with authentic sucrose, indicated the presence of sucrose.

2.6. Antimicrobial assay

Gram-positive *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus subtilis* ATCC 6633 (*B. subtilis*) and Gram-negative *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) and *Escherichia coli* ATCC 25922 (*E. coli*) were used to test antibacterial activity by disk diffusion assay [13]. Each paper disk (6 mm diameter) permeating with 10 μL of test sample (100 μg/mL methanol) was dried and placed on Mueller Hinton agar plate containing bacterial inoculum. Then the bacterial strains were incubated at 37 °C for 24 h. For evaluating antibacterial potential, diameter (in mm) of the growth inhibition zone was recorded. The broth microdilution method was used to measure the minimal inhibition concentration (MIC) according to the protocols of the National Committee for Clinical and Laboratory Standards [14]. Following inoculation of the test bacteria on nutrient agar for 18–24 h, a colony of approximately 1 mm in diameter was collected by using a sterile loop and was dissolved into Mueller Hinton broth. Each bacterial strain was diluted to 10^5 cfu/mL before antimicrobial assay. Stock solutions were firstly resolved in DMSO and diluted to varieties of concentrations (0–50.0 μg/mL, DMSO < 1%) with broth afterwards. After incubation at 37 °C for 24 h, the optical density was recorded at 578 nm. MIC value was defined as the minimum concentration of compound at which the growth of microorganism was half inhibited. Streptomycin and penicillin were used as positive controls for Gram-positive bacteria and Gram-negative bacteria, respectively. All of the antimicrobial assays were performed in triplicate.

3. Results and discussion

The application of ^{1}H NMR spectroscopy-guided isolation prompted us to narrow the scope of targeted sucrose esters to the Fr. D of PE layer

Table 3
^{13}C NMR data (125 MHz, CD_3OD) of compounds **1**–**10** (δ in ppm).

Position	1	2	3	4	5	6	7	8	9	10
1	90.9	91.1	91.1	93.4	91.1	91.1	91.3	91.4	91.1	91.1
2	74.5	72.5	74.4	71.5	72.1	72.7	71.9	72.5	72.1	72.7
3	72.4	73.5	72.4	76.9	73.8	73.8	73.7	73.7	73.8	73.8
4	71.5	69.0	71.4	69.3	69.4	69.2	69.3	69.1	69.4	69.2
5	74.2	74.4	74.4	74.6	74.3	74.5	74.6	74.7	74.4	74.5
6	62.3	61.8	62.2	61.9	61.9	61.9	61.9	61.9	61.9	61.9
1′	64.4	64.1	66.2	65.1	64.4	64.1	65.6	65.9	64.4	64.1
2′	105.0	105.1	103.1	104.6	105.1	105.1	103.4	103.3	105.1	105.1
3′	78.6	78.5	79.3	79.7	78.9	78.5	79.1	79.3	78.8	78.5
4′	73.4	73.3	73.0	73.8	73.5	73.5	73.2	73.2	73.5	73.5
5′	84.1	84.1	84.1	84.1	84.0	84.1	84.3	84.2	84.0	84.1
6′	63.0	63.1	62.9	63.0	63.0	63.1	63.0	63.0	63.0	63.1
3′-*O*-	Dodeca[a]	Dodeca[a]	Dodeca[a]	Dodeca[a]	Dodeca[a]	Dodeca[a]	Dodeca[a]	Dodeca[a]	Myris[b]	Myris[b]
1	174.9	175.1	174.8	175.0	175.2	175.1	175.1	175.1	175.2	175.1
2	34.9	34.9	34.8	35.1	34.9	34.9	34.8	34.8	34.9	34.9
3	26.1	26.1	26.0	26.0	26.1	26.1	26.0	26.0	26.1	26.1
4–11	23.7–33.0	23.7–33.1	23.7–33.1	23.7–33.1	23.7–33.0	23.7–33.1	23.7–33.1	23.7–33.1	23.7–33.1	23.7–33.1
12	14.4	14.4	14.4	14.4	14.4	14.5	14.4	14.4	23.7–	23.7–
13									33.1	33.1
14									23.7–33.1	23.7–33.1
									14.4	14.4
2-*O*-	Tig[c]	Tig[c]	Tig[c]		3-MeBu[d]	Tig[c]	3-MeBu[d]	Tig[c]	3-MeBu[d]	Tig[c]
1	169.1	168.4	169.1		173.7	168.5	173.7	168.5	173.7	168.5
2	129.5	129.0	129.3		44.3	129.0	44.3	128.9	44.3	129.0
3	139.6	140.4	139.9		26.6	140.2	26.7	140.4	26.7	140.2
4	14.5	14.4	14.5		22.7	14.4	22.7	14.3	22.7	14.5
5	12.1	12.0	12.1		22.7	12.0	22.7	12.0	22.7	12.2
3-*O*-		*i*-Bu[e]		Tig[c]	Tig[c]	Tig[c]	Tig[c]	Tig[c]	Tig[c]	Tig[c]
1		177.9		169.7	168.7	168.8	168.6	168.8	168.7	168.8
2		35.2		129.9	129.6	129.6	129.6	129.6	129.6	129.6
3		19.6		138.4	138.9	138.7	138.9	138.7	138.9	138.7
4		19.1		14.4	14.4	14.3	14.3	14.5	14.4	14.3
5				12.3	12.2	12.1	12.2	12.2	12.2	12.0
1′-*O*-			Ac				Ac	Ac		
1			171.8				172.0	171.8		
2			20.6				20.6	20.6		

[a] Dodeca = dodecanoyl.
[b] Myris = myristyl.
[c] Tig = tigloyl.
[d] 3-MeBu = 3-methylbutanoyl.
[e] *i*-Bu = isobutyryl.

rapidly. In detail, the ^{1}H NMR spectrum of Fr. D (Fig. S70) showed methylene groups of fatty acid ester in the range δ_H 1.2–1.4, terminal methyl group of fatty acid ester in the range δ_H 0.8–1.0, sugar moiety signals in the range δ_H 3.4–4.5 and anomeric protons of the sugar moiety in the range δ_H 5.3–5.8. By comparison of these characteristic ^{1}H signals with those reported in the literature [15], we assumed that sucrose esters exist in the Fr. D. As expected, the further isolation of Fr. D led to ten new sucrose esters (**1**–**10**).

Physakengose A (**1**) was obtained as an amorphous solid. The molecular formula of $C_{29}H_{50}O_{13}$ was determined by its molecular ion in the HRESIMS at *m/z* 629.3142 $[M + Na]^+$ (calcd for $C_{29}H_{50}NaO_{13}$, 629.3144). The IR spectrum exhibited strong absorptions at 3364 and 1721 cm^{-1}, which attributed to hydroxyl and ester functions, respectively. The NMR data of **1** revealed that it contained signals for sucrose and long chain fatty acid ester moieties [15]. The presence of a sucrose unit was deduced from the analysis of the NMR spectra (Tables 1 and 3), which showed the anomeric CH signals of the glucopyranose (δ_H 5.54, d, J = 3.7 Hz and δ_C 90.9; CH-1) and that of the anomeric carbon of the fructofuranose (δ_C 105.0, C-2′). Alkaline hydrolysis and comparison of the spectroscopic data with those reported in the literature also confirmed the existence of sucrose [16]. ESIMS, ^{1}H and ^{13}C, TOCSY and HMBC spectra allowed identification of the acyl groups as dodecanoyl (*m/z* 183) and tigloyl (*m/z* 83). The positions of these groups were established by the HMBC correlations from H-2 (δ_H 4.62) to C-1 (δ_C 169.1) of tigloyl group, as well as that of H-3′ (δ_H 5.39) to C-1 (δ_C 174.9) of dodecanoyl group (Fig. 2). Moreover, the ESIMS fragments at *m/z* 245 and 345 (Fig. S7) corresponded to tigloylated glucopyranosyl and dodecanoylated fructofuranosyl units, respectively, thus confirming physakengose A (**1**) as 3′-*O*-dodecanoyl-2-*O*-tigloylsucrose.

Physakengose B (**2**) gave the molecular formula $C_{33}H_{56}O_{14}$ on the basis of its $[M + Na]^+$ HRESIMS ion peak at *m/z* 699.3559 (calcd for $C_{33}H_{56}NaO_{14}$, 699.3562). The NMR data (Tables 1 and 3) of **2**, similar to those of **1**, also showed characteristic signals for sucrose and fatty acid ester. The main differences were the presence of signals belonging to an additional isobutyryl unit [δ_H 2.57 (1H, m), 1.06 (3H, d, J = 7.0 Hz)

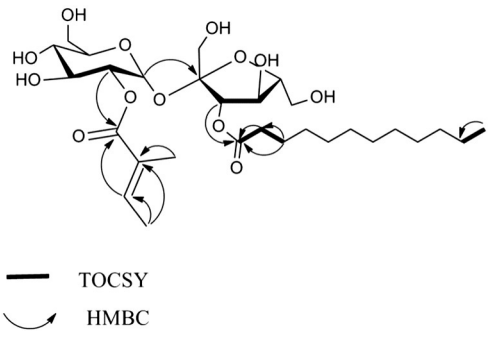

Fig. 2. Selected HMBC and TOCSY correlations of compound **1**.

and 1.11 (3H, d, $J = 7.0$ Hz); δ_C 177.9, 35.2, 19.6, 19.1] and the downfield shift of H-3 (δ_H 5.41) relative to that (δ_H 3.83) in **1**. The isobutyryl unit was located at C-3 determined by the HMBC correlation from H-3 to its carbonyl carbon (δ_C 177.9). The 3-isobutyryl-2-tigloyl glucopyranosyl moiety was further confirmed by the fragment ion at *m/z* 315 (Fig. S14). Consequently, the structure of compound **2** was assigned as 3′-*O*-dodecanoyl-3-*O*-isobutyryl-2-*O*-tigloylsucrose.

The molecular formula of physakengose C (**3**) was determined to be $C_{31}H_{52}O_{14}$ with the $[M + Na]^+$ ion at *m/z* 671.3250 in the HRESIMS. As was the case with **2**, the ^1H and ^{13}C NMR data (Tables 1 and 3) of compound **3** closely resemble that of **1**, except for the presence of signals attributable to an acetyl unit [δ_H 2.05 (3H, s); δ_C 171.8, 20.6], and the downfield shift of H_2-1′ (δ_H 3.89, 4.04) relative to those (δ_H 3.31, 3.47) in **1**. The HMBC correlation from H_2-1′ (δ_H 3.89, 4.04) to acetoxy carbonyl (δ_C 171.8) indicated that the acetyl unit was located at the C-1′. Therefore, the structure of compound **3** was elucidated as 1′-*O*-acetyl-3′-*O*-dodecanoyl-2-*O*-tigloylsucrose.

Physakengose D (**4**) owned the same molecular formula $C_{29}H_{50}O_{13}$ as that of compound **1** with the analysis of its HRESIMS spectrum. A comparison of the spectroscopic data (Tables 1 and 3) with those of **1** revealed an overall similarity. The difference between them was determined by the HMBC spectrum; the correlation between H-3 (δ_H 5.24) of the glucopyranose to the carbonyl carbon of tigloyl unit (δ_C 169.7) suggested the tigloyl unit was attached to C-3. Accordingly, the structure of compound **4** was assigned as 3′-*O*-dodecanoyl-3-*O*-tigloylsucrose.

Physakengose E (**5**) was isolated as an amorphous solid, and its molecular formula was determined as $C_{34}H_{58}O_{14}$ by its HRESIMS spectrum. The NMR data (Tables 1 and 3) showed signals belonging to sucrose, long fatty acid ester and tigloyl units as in compound **4**. In addition, proton and carbon signals ascribing to 3-methylbutanoyl group [δ_H 2.13 (1H, d, $J = 7.0$ Hz), 2.12 (1H, d, $J = 7.0$ Hz), 1.96 (1H, m) and 0.87 (6H, d, $J = 6.7$ Hz); δ_C 173.7, 44.3, 26.6, 22.7, 22.7] were observed in **5** and assigned at C-2 based on HMBC correlation between H-2 (δ_H 4.83) and its carbonyl carbon (δ_C 173.7). The peak at *m/z* 329 (Fig. S34) also corroborated the difference on the pyranose moiety substitution and the structure of **5** was established as 3′-*O*-dodecanoyl-2-*O*-3-methylbutanoyl-3-*O*-tigloylsucrose.

Physakengose F (**6**), designated with the molecular formula of $C_{34}H_{56}O_{14}$ in accordance with its HRESIMS spectrum. Although the ^1H and ^{13}C NMR data (Tables 2 and 3) of compound **6** were analogous to those of **1**, the presence of signals attributable to an additional tigloyl unit [δ_H 6.81 (1H, m), 1.77 (3H, d, $J = 7.2$ Hz) and 1.79 (3H, br s); δ_C 168.8, 129.6, 138.7, 14.3, 12.1] were obviously observed in **6**. The position of the second tigloyl unit at C-3 was established by the correlation between its carbonyl carbon (δ_C 168.8) and H-3 (δ_H 5.47) of the glucopyranose observed in the HMBC spectrum. Thus, the structure of compound **6** was identified as 3′-*O*-dodecanoyl-2, 3-di-*O*-tigloylsucrose.

On the basis of HRESIMS and NMR experiments, the molecular formulas of physakengoses G (**7**) and H (**8**) were found to be $C_{36}H_{60}O_{15}$ and $C_{36}H_{58}O_{15}$, respectively. Their ^1H and ^{13}C NMR spectra (Tables 2 and 3) were similar to those of **5** and **6**, except for the presence of signals belonging to additional acetyl groups (δ_H 2.09, δ_C 172.0, 20.6; δ_H 2.06, δ_C 171.8, 20.6). The further analysis of their HMBC spectra, together with ESIMS fragments at *m/z* 329 and 387 for **7** (Fig. S48) and *m/z* 327 and 387 for **8** (Fig. S54), allowed us to formulate **7** as 1′-*O*-acetyl-3′-*O*-dodecanoyl-2-*O*-3-methylbutanoyl-3-*O*-tigloylsucrose and **8** as 1′-*O*-acetyl-3′-*O*-dodecanoyl-2,3-di-*O*-tigloylsucrose.

The molecular ions at *m/z* 741.4031 and 739.3886 ($[M + Na]^+$) in the HRESIMS spectra of compounds **9** and **10** confirmed their molecular formulas as $C_{36}H_{62}O_{14}$ and $C_{36}H_{60}O_{14}$, respectively, and indicated they both contained an additional C_2H_4 than compounds **5** and **6**. The ^1H and ^{13}C NMR data (Tables 2 and 3) of **9** and **10** were almost superimposable with those of **5** and **6**. A comprehensive study on the 1D and 2D NMR spectra of compounds **9** and **10** indicated that these two compounds each had two more methylene groups in its fatty acid chain, which were further confirmed by the ESIMS fragment ion at *m/z* 211 (Figs. S61, S68) corresponding to a myristyl unit. Accordingly, the structures of compounds **9** and **10** were established as depicted.

Table 4
Minimum Inhibitory Concentration (MIC) of compounds **1**–**10** (μg/mL).[a]

Compounds	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	>50.00	>50.00	>50.00	>50.00
2	9.71 ± 2.83	8.89 ± 1.63	14.91 ± 2.56	>50.00
3	33.26 ± 6.90	16.78 ± 2.05	>50.00	>50.00
4	33.71 ± 4.33	>50.00	34.08 ± 2.25	>50.00
5	9.81 ± 1.48	5.95 ± 0.85	13.12 ± 2.42	>50.00
6	5.32 ± 1.47	3.50 ± 1.49	5.79 ± 1.15	>50.00
7	6.57 ± 0.86	8.78 ± 1.67	4.51 ± 3.02	>50.00
8	5.78 ± 0.96	3.57 ± 1.02	3.21 ± 0.95	>50.00
9	6.63 ± 0.93	43.55 ± 2.96	15.04 ± 2.24	>50.00
10	4.70 ± 0.96	18.48 ± 3.69	6.71 ± 1.50	>50.00
Penicillin[b]	0.06 ± 0.03	0.13 ± 0.05		
Streptomycin[b]			0.41 ± 0.15	0.45 ± 0.15

[a] Values are represented as the means ± SD based on three independent experiments.
[b] Penicillin and Streptomycin were used as positive controls.

Previous investigations have suggested that sucrose esters from *Physalis* species and the ethanol extracts of *P. alkekengi* var. *franchetii* inhibited the growth of Gram-positive and Gram-negative bacteria [17,18]. Thus, the ten isolates were evaluated for antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*. As shown in Table 4, all compounds had no activity against *E. coli*, but exhibited various degrees of antibacterial activity against other three bacteria. The antibacterial data against Gram-positive bacteria of the sucrose esters provided clear evidence for the first time that the C-2 and C-3 substitution at the glucopyranose is a subtle but critical parameter, variations of which would remarkable spoil antibacterial activity. To elaborate, compounds **2** and **5**–**10**, which were disubstituted at C-2 and C-3, showed antibacterial activity against *S. aureus* and *B. subtilis*. Contrastively, compounds **1**, **3** and **4**, with only one substituted group at C-2 or C-3, displayed weaker or no antibacterial activity. Notably, compounds **6** and **8** showed potent antibacterial activity to the test bacteria (*S. aureus*, *B. subtilis* and *P. aeruginosa*), with MIC values below 6 μg/mL. These results indicated that the di-tigloyl substitution may play an important role in the antibacterial activity.

In conclusion, this study reported the ^1H NMR Spectroscopy-guided isolation of ten new sucrose esters and evaluation for their *in vitro* antibacterial activity. The activity results showed that compounds **2** and **5**–**8** possessed potent activity which provided a scientific basis for the supplement of the active ingredients in *P. alkekengi* var. *francheti* as well as for the development of novel agents against bacteria.

Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

HRESIMS, ^1H NMR, ^{13}C NMR, HSQC, HMBC, TOCSY and ESIMS spectra of **1**-**10** and the ^1H NMR spectra of PE, Fr. D, CH_2Cl_2, EtOAc and H_2O fractions of *Physalis alkekengi* var. *franchetii* are available in Supporting information. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.fitote.2016.09.007.

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