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Steroidal saponins and sapogenins from fenugreek and their inhibitory activity against α-glucosidase

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ABSTRACT

The seed of *Trigonella foenum-graecum* L. (fenugreek) has been reported to be rich in saponins, especially the dioscin or diosgenin, which are natural anti-diabetic agents with relatively low toxicity. Thus, the present study was to purify the saponins and sapogenins from fenugreek and to evaluate their α-glucosidase inhibitory activity *in vitro*. As a result, 33 steroidal saponins and sapogenins were isolated, including six undescribed ones and 27 previously known molecules. Among them, compounds **10**, **12**, **17**, **22** and **29** were five 25*R* and 25*S* isomer mixtures of spirostanol saponins or sapogenins. The structures of compound **1**–**6** were established by 1D and 2D NMR spectroscopic analyses, high-resolution mass spectrometry, and chemical evidence. Compared to the positive control, sapogenins **26**, **27**, **14** and saponins **18** and **23** considerably inhibited α-glucosidase at IC_{50} values of 15.16, 8.98, 7.26, 5.49 and 14.01 μM, respectively. These results support the therapeutic potential of fenugreek in the treatment of diabetes with saponins and sapogenins as the active constituents.

1. Introduction

Natural plants and their ingredients have been an important source for the discovery of the active constituents for medicinal purposes. Numerous medicinal plants or their bioactive constituents have displayed modulatory effects to diseases in traditional medicine for many years [1]. Fenugreek (*Trigonella foenum-graecum* L.) belonging to the family Fabaceae. It is widespread throughout China, India, and North African countries, and is cultivated to be used in both the food industry and medicine [2]. In traditional Chinese or Ayurvedic systems of medicine, the seeds of fenugreek can alleviate the severity of many disorders, such as hypertension, hyperlipidemia, and immune diseases [3]. Modern pharmaceutical studies have also demonstrated that fenugreek possessed various biological activities, including anti-oxidative [4], anti-inflammatory [5], anti-diabetic [6], anti-hyperlipidemic [7], anti-obesity [8], anti-tumor [2] and promotion of sexual health effect [9].

Modern phytochemical studies demonstrate that galactomannans, saponins, alkaloids, flavonoids, polyphenol, and stilbenes are abundantly present in fenugreek [9]. Saponins have the highest chemical content in fenugreek, which are natural anti-diabetic agents with relatively low toxicity. Some saponins such as dioscin from the fenugreek has been reported to show significantly decreased blood lipid and glucose levels in a mice model induced by high-fat diet [10]. Meanwhile, the main sapogenin diosgenin, a naturally occurring aglycone of fenugreek, has also been reported to exhibit an insulin-like antihyperglycemic effect on streptozotocin-induced diabetic rats [11] and also improves glucose metabolism in the liver [12].

To date, nearly 15 sapogenins and 50 saponins have been reported from fenugreek [13]. However, only several high content compounds, such as dioscin and diosgenin have been evaluated for their anti-diabetic activity. In nature, most sapogenins possess the more favorable chemical properties due to the lack of the sugar chain, therefore, they have better bioactivities and bioavailability than their former saponins [14]. Furthermore, some sapogenins may show certain biological effects that were not exhibited by former saponins [15]. Hydrolysis of saponins is a common method to convert saponins to sapogenins, which may also offer a whole new range of natural or synthetic 'metabolites' with superior biological activity [16].

To the best of our knowledge, the anti-diabetic related activities of constituents from fenugreek have not yet to be systematically investigated. Furthermore, our preliminary biological test has demonstrated that the resultant hydrolysate exhibited moderate inhibitory activity against α-glucosidase. Hence, our present study focused on the chemical composition of fenugreek, including not only major saponins but also sapogenins by hydrolysis. In addition, all the isolated compounds were evaluated for their inhibitory activity against α-glucosidase *in vitro*. As a result, 3 undescribed steroid saponins, 3 undescribed

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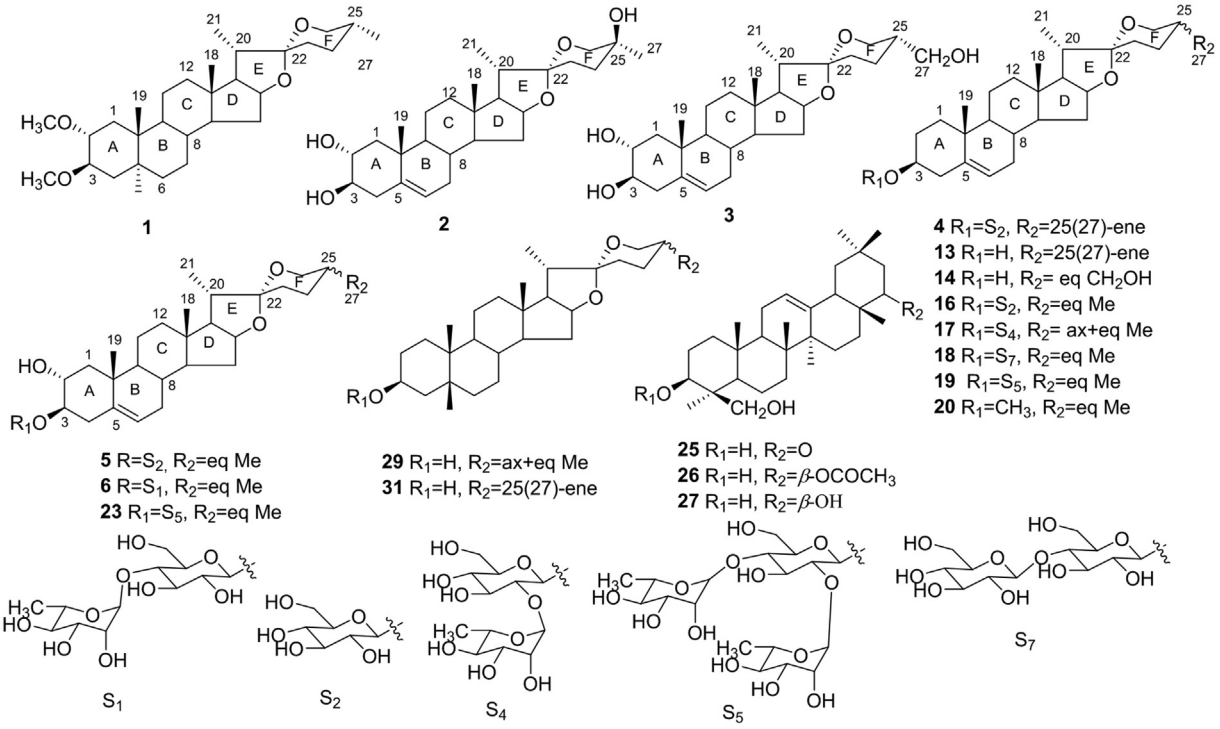

Fig. 1. Chemical structures of some compounds.

steroid sapogenins and 25 known compounds were obtained (shown in Supporting information). Herein, the isolation and structure elucidation of these compounds and their anti-diabetic ability *in vitro* are now reported.

2. Experimental

2.1. General experiment procedure

Optical rotations were recorded using an Anton Paar MCP 200 polarimeter (Anton Paar GmbH, Ostfildern, Germany). HRESIMS data were carried out on a Bruker micrOTOF-Q mass spectrometer (Billerica, MA, USA). NMR spectra were obtained from a Bruker AVANCE-400/-600 spectrometer (Karlsruhe, Germany) with tetramethylsilane (TMS) as an internal standard. HPLC was performed on a Prep HPLC (Beijing CXTH3000 system) using a YMC C_{18} reversed-phase column (10 × 250 mm, 5 μm; YMC-Pack, Japan) which employing a P3000 pump and equipped with a UV3000 spectrophotometric detector. Column chromatography (CC) was carried out on silica gel (100–200 and 200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), ODS (50 mm, YMC Co., Ltd., Kyoto, Japan) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden).

2.2. Plant material

The 75% ethyl alcohol extract of fenugreek (500 g) was purchased from Xian Runxue biotechnology company in August 2016 (Shanxi, China).

2.3. Extraction and isolation

The fenugreek extract (300 g) was applied to a HPD-100 macroporous resin column (HPD-100 macroporous resin, 2 kg) eluted with H_2O, 20% EtOH, 70% EtOH and 95% EtOH, respectively. The 95% EtOH solution was concentrated under reduced pressure to give a crude saponin (50 g), which was further separated by silica gel column chromatography eluting with CH_2Cl_2-MeOH (100:0–0:100, v/v) to yield eight fractions (Fr. A - H). Fraction B was applied to a ODS gel column (70% MeOH in H_2O) and following separated by Sephadex LH-20 and preparative HPLC (v = 3 mL/min) to give compounds **28** (7 mg, 76% MeOH-H_2O, t_R 47.5 min), **15** (48 mg, 78% MeOH-H_2O, t_R 66.2 min), **4** (8 mg, 80% MeOH-H_2O, t_R 33.2 min), **16** (54 mg, 80% MeOH-H_2O, t_R 41.7 min), **32** (18 mg, 82% MeOH-H_2O, t_R 80.9 min). Fraction C was purified via ODS (70% MeOH in H_2O) and further purified by preparative HPLC (v = 3 mL/min) to afford compounds **8** (9 mg, 76% MeOH-H_2O, t_R 64.6 min), **9** (24 mg, 76% MeOH-H_2O, t_R 42.3 min), **5** (9 mg, 76% MeOH-H_2O, t_R 53.0 min), **6** (22 mg, 76% MeOH-H_2O, t_R 51.6 min). Fraction E was purified by ODS gel (70% MeOH in H_2O) and further purified by preparative HPLC (v = 3 mL/min) to afford compounds **17** (17 mg, 81% MeOH-H_2O, t_R 39.8 min), **18** (30 mg, 81% MeOH-H_2O, t_R 43.9 min), **33** (8 mg, 59% MeOH-H_2O, t_R 53.0 min), **21** (15 mg, 74% MeOH-H_2O, t_R 43.5 min). Fraction F was purified by ODS gel (70% MeOH in H_2O) and further purified by TLC and preparative HPLC (v = 3 mL/min) to yield compounds **22** (8 mg, 78% MeOH-H_2O, t_R 39.0 min), **10** (10 mg, 78% MeOH-H_2O, t_R 51.8 min) and **19** (20 mg). Fraction G was decolorized by ODS (70% MeOH in H_2O) and further purified by preparative HPLC to give **23** (40 mg, 78% MeOH-H_2O, t_R 45.4 min) and **24** (60 mg, 74% MeOH-H_2O, t_R 100.3 min).

Saponins of fenugreek (100 g) were obtained by macroporous resin column eluted with 70% EtOH. According to the hydrolysis method reported [17], hydrolyzate of saponins (30 g) were obtained. Elution was stepwise with a petroleum ether-ethyl acetate gradient (from petroleum ether to ethyl acetate, 50:1 to 1:1) to afford nine fractions a-j. Fr. b (1 g) was applied to TLC to yield compound **1** (8 mg) and **20** (4 mg). Fr. d (8 g) was applied to ODS, Sephadex LH-20, TLC and semipreparative HPLC (v = 3 mL/min) to yield **29** (90 mg, 87% MeOH-H_2O, t_R 46.7 min), **30** (8 mg, 86% MeOH-H_2O, t_R 58.7 min) and **31** (10 mg). Compounds **12** (100 mg) and **13** (5 mg) were obtained from Fr. e by ODS, Sephadex LH-20, TLC and recrystallization. Fr. f (1 g) was purified via ODS column (MeOH-H_2O (100:100–100:0, v/v)) and semipreparative HPLC (v = 3 mL/min) to afford **26** (3.5 mg, 83% MeOH-H_2O, t_R 40.8 min). Fr. g (3 g) was purified via ODS column with MeOH-H_2O (100:100–100:0, v/v), recrystallization and

Table 1
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data for the steroid moieties of compounds **1–6** in chloroform-*d* (compound **1**) and pyridine-d_5 (compounds **2–6**).

position	**1**		**2**		**3**		**4**		**5**		**6**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	0.83, m 2.10, m	42.2	1.46, m 2.41, m	47.0	1.45, m 2.40, m	47.0	0.97, m 1.70, m	37.8	1.31, m 2.33, m	46.2	1.31, m 2.33, m	46.2
2	3.25, m	80.8	4.17, m	73.0	4.16, m	73.0	1.75, m 2.15, m	30.6	4.08, m	70.6	4.06, m	70.8
3	3.10, m	83.9	3.84, m	77.2	3.85, m	77.2	3.99, m	79.0	3.85, m	85.7	3.83, m	85.4
4	1.22, m 1.79, m	32.6	2.74, m	41.3	2.71, m	41.3	1.10, m 1.70, m	40.9	2.58, m 2.72, m	38.1	2.58, m 2.74, m	37.9
5	1.11, m	44.2		141.7		141.6		141.3		140.5		140.4
6	1.24, m 1.34, m	28.0	5.44, d (5.1)	121.7	5.43, d (4.4)	121.7	5.33, d (4.9)	122.1	5.31, d (4.2)	122.2	5.33, d (4.9)	122.3
7	0.90, m 1.71, m	32.1	overlap	32.7	overlap	32.6	1.43, m -	32.5	1.49, m 1.83, m	32.5	1.48, m	32.6
8	1.52, m	34.4	overlap	31.6	overlap	31.6	1.53, m	32.0	1.50, m	31.5	overlap	31.5
9	0.71, m	54.3	1.07, m	50.8	1.05, m	50.8	0.89, m	50.6	0.96, m	50.5	0.98, m	50.6
10		36.8		38.9		38.9		37.4		38.3		38.4
11	1.30, m 1.58, m	21.1	overlap	21.7	1.53, m	21.7	- 1.43, m	21.5	overlap	21.6	1.45, m 1.54, m	21.6
12	1.15, m 1.74, m	39.9	1.13, m 1.72, m	40.2	1.11, m 1.70, m	40.3	2.48, m 2.74, m	39.7	1.08, m 1.66, m	40.1	1.09, m 1.68, m	40.2
13		40.6		40.9		40.9		40.2		40.8		40.8
14	1.09, m	56.1	1.07, m	57.0	1.06, m	57.0	1.07, m	57.0	1.04, m	56.9	1.05, m	56.9
15	1.25, m 1.99, m	31.8	overlap	32.5	overlap	32.7	1.87, m 2.02, m	32.6	1.40, m 2.00, m	32.6	overlap	32.5
16	4.41, m	80.8	4.54, dd (7.5, 14.7)	81.7	4.57, dd (7.5, 14.7)	81.5	4.54, m	81.8	4.53, m	81.4	4.55, m	81.5
17	1.77, m	62.2	1.83, m	63.2	1.82, m	63.3	1.81, m	63.3	1.79, m	63.2	1.80, m	63.2
18	0.77, s	16.5	0.86, s	16.8	0.86, s	16.8	0.84, s	16.7	0.80, s	16.7	0.83, s	16.7
19	0.84, s	13.3	1.11, s	21.1	1.10, s	21.1	0.92, s	19.8	0.97, s	20.8	0.94, s	20.8
20	1.87, m	41.6	2.04, m	41.8	1.98, m	42.5	1.97, m	42.2	1.94, m	42.3	1.96, m	42.3
21	0.98, d (7.0)	14.5	1.18, d (6.9)	15.3	1.16, d (6.9)	15.4	1.10, d (6.9)	15.4	1.13, d (6.9)	15.4	1.14, d (6.9)	15.4
22		109.2		109.7		110.1		109.8		109.6		109.6
23	1.62, m 1.68, m	31.4	overlap	30.6	overlap	31.9	2.26, m 2.73, m	29.4	1.56, m 1.64, m	32.2	overlap	32.2
24	1.46, m	28.8	1.89, m 2.41, m	36.0	1.81, m	24.5	1.78, m -	33.6	overlap	29.6	overlap	29.6
25	1.65, m	30.3		66.8	2.07, m	39.6		144.8	overlap	30.9	overlap	31.0
26	3.38, m 3.49, dd (4.4, 10.9)	66.8	3.63, dd (1.9, 10.9) 4.15, dd (10.1)	70.4	3.66, m 3.73, m	64.8	4.05, d (12.0) 4.47, d (12.2)	64.5	3.49, m 3.57, dd (3.1, overlap)	67.2	3.50, m 3.59, m	67.2
27	0.80, d (6.3)	17.1	1.58, s	25.4	3.90, t (11.1) 4.16, m	64.5	4.79, s 4.83, s	109.1	0.68, d (5.6)	17.7	0.70, d (5.5)	17.7
2-OCH_3	3.44, s	57.5										
3-OCH_3	3.44, s	57.0										

δ in ppm, *J* values are in parentheses and reported in Hz. The assignments were based on HSQC and HMBC experiments.
[a]Overlapped with other signals.

semipreparative HPLC (v = 3 mL/min) to afford **25** (126 mg, 80% MeOH-H_2O, t_R 47.9 min) and **27** (11 mg, 81% MeOH-H_2O, t_R 47.0 min). Fr. h was applied to ODS and semipreparative HPLC (v = 3 mL/min) to yield **24** (35 mg, 74% MeOH-H_2O, t_R 100 min), **7** (7 mg, 74% MeOH-H_2O, t_R 107.9 min) and **14** (13 mg, 75% MeOH-H_2O, t_R 49.8 min). Fr. i was subjected to Sephadex LH-20 and semipreparative HPLC (v = 3 mL/min) to give **2** (4 mg, 66% MeOH-H_2O, t_R 44.8 min) and **3** (5 mg, 66% MeOH-H_2O, t_R 51.4 min). For the detailed isolation and purification protocol, the flow chart was given in Fig. 1 of Supporting information.

Compound **1:** white amorphous powder, $[\alpha]_{20}^{D}$ −81.50 (*c* 0.2, Acetone). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in chloroform-*d* see Table 1; HRESIMS *m/z* 483.3430 $[M + Na]^+$ (calculated for $C_{29}H_{48}O_4Na$ 483.3440).

Compound **2:** white amorphous powder, $[\alpha]_{20}^{D}$ −69.50 (*c* 0.25, MeOH). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in pyridine-d_5 see Table 1; HRESIMS *m/z* 447.3109 $[M + H]^+$ (calculated for $C_{27}H_{43}O_5$ 447.3105).

Compound **3:** white amorphous powder, $[\alpha]_{20}^{D}$ −57.30 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in pyridine-d_5 see Table 1; HRESIMS *m/z* 447.3101 $[M + H]^+$ (calculated for $C_{27}H_{43}O_5$ 447.3105).

Compound **4:** white amorphous powder, $[\alpha]_{20}^{D}$ −48.80 (*c* 0.25, MeOH). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in pyridine-d_5 see Tables 1 and 2; HRESIMS *m/z* 597.3395 $[M + Na]^+$ (calculated for $C_{33}H_{50}O_8Na$ 597.3398).

Compound **5:** white amorphous powder, $[\alpha]_{20}^{D}$ −74.00 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in pyridine-d_5 see Tables 1and 2; HRESIMS *m/z* 615.3463 $[M + Na]^+$ (calculated for $C_{33}H_{52}O_9Na$ 615.3504).

Compound **6:** white amorphous powder, $[\alpha]_{20}^{D}$ −117.50 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in pyridine-d_5 see Tables 1 and 2; HRESIMS *m/z* 761.4082 $[M + Na]^+$ (calculated for $C_{39}H_{62}O_{13}Na$ 761.4083).

*2.4. Acid hydrolysis of compounds **4–6** and HPLC analysis for sugar residues [18]*

Compounds **4**, **5** and **6** (each 2.0 mg) were dissolved in 4 M HCl (2 mL) and heated at 90 °C for 3 h. After evaporation to dryness under reduced pressure, the reaction mixture was extracted by EtOAc for 3 times. The water layer was evaporated to dryness. The dried residue, D-glucose (standard sugar, 1.0 mg) and L-rhamnose (standard sugar, 1.0 mg) were dissolved in pyridine (1 mL) and treated with L-cysteine

Table 2
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data for the sugar moieties of compounds **4**–**6** in pyridine-d_5.

Position	**4**		**5**		**6**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
Sugar	3-*O*-Glc		3-*O*-Glc		3-*O*-Glc	
1′	5.05, d (7.7)	103.0	5.07, d (7.8)	103.8	5.02, d (7.8)	103.4
2′	4.07, t (8.1)	75.8	4.08, m	75.3	4.02, m	75.6
3′	4.33, m	78.9	4.29, m	78.8	4.25, m	77.0
4′	4.28, m	72.1	4.23, m	72.0	4.48, m	78.6
5′	4.07, m	78.5	4.03, m	79.0	3.80, m	77.7
6′	4.41, dd (5.2, 11.6)4.58, m	63.3	4.35, m4.57, m	63.0	4.13, dd (3.5, 12.2)4.28, m	61.6
Sugar					Rha (1 → 4)	
1″					5.90, s	103.1
2″					4.59, m	73.2
3″					4.70, m	73.0
4″					4.37, m	74.4
5″					5.02, d (7.8)	70.5
6″					1.75, d (6.2)	18.9

δ in ppm, *J* values are in parentheses and reported in Hz. The assignments were based on HSQC and HMBC experiments.

methyl ester hydrochloride (3.0 mg) stirred at 60℃ for 1 h. Then *o*-tolyl isothiocyanate (5 μL) was added to the mixture, and was heated at 60 ˚C for another 1 h. The supernatants were analyzed by HPLC [mobile phase: 25% CH_3CN-H_2O (0.1% formic acid of water); flow rate: 1 mL/min; detection: UV (250 nm)]. The configurations of D-glucose and L-rhamnose for compounds **4**–**6** were determined by comparison of the retention times with those of standard D-glucose and L-rhamnose (Shanghai Macklin, China) giving a single peak at 16.87 and 29.07 min, respectively.

2.5. Activities assay

The inhibitory activity against α-glycosidase was determined as previously reported after a slight modification [19]. Briefly, different concentrations of compounds (20 μL) were added to α-glycosidase solution (30 μL, 1.5 U/mL, pH 6.8–7.0). After preincubation for 5 min at 37 ℃, 150 μL pNPG (6 mg/mL) and 800 μL PBS (0.1 M) were added to the solution. After incubation for 30 min at 37 ℃, 2 mL Na_2CO_3 (1 M) were added to the solution. Acarbose and 1% DMSO (20 μL) were used as the positive group and blank control group, respectively. The reaction was monitored by change of absorbance at 405 nm using a microplate reader. The inhibitory activity against PTP1B was assayed as previous report [19].

3. Results and discussion

Compound **1** was isolated as a white, amorphous powder with a molecular formula $C_{29}H_{48}O_4$, which was deduced by the positive HRESIMS (*m/z* 483.3430 $[M + Na]^+$, calcd 483.3440). Its characteristic spirostanol skeleton was indicated by two methyl signals at δ_H 0.77 (3H, s) and 0.84 (3H, s), two methyl doublet signals at δ_C 0.80 (3H, d, J = 6.3 Hz) and 0.98 (3H, d, J = 7.0 Hz) in its ^{1}H NMR spectrum, as well as a quaternary carbon at δ_C 109.2 in ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR data (Tables 1) of **1** were very similar to those of gitogenin [20] except for two methoxyl protons at δ_H 3.44 (6H, s), which revealed the same B-F rings and a different A ring. The two methoxyl protons δ_H 3.44 (6H, s) were assigned at C-2 and C-3 of ring A, which confirmed by HMBC (Fig. 2) correlations between δ_H 3.44 (H-OCH_3) and δ_C 80.9 (C-2)/83.9 (C-3), δ_H 3.25 (H-2) and δ_C 83.9 (C-3), δ_H 3.10 (H-3) and δ_C 80.8 (C-2). The α-oriented of the proton at C-5 was assigned using the chemical shifts of δ_C 44.2 (C-5) and 13.3 (C-19). The α-oriented and β-oriented of the proton at C-2 and C-3 were comfirmed by the NOESY correlations of H-19 (δ_H 0.84)/H-2 (δ_H 3.25) and H-3 (δ_H 3.10)/H-5 (δ_H 1.11), respectively. In addition, the correlations of H-16 (δ_H 4.41)/H-17 (δ_H 1.77) (α-oriented) and H-14 (δ_H 1.09)/H-16 (δ_H 4.41) (α-oriented) were also observed in NOESY spectrum (Fig. 2). The methyl group at C-25 was assigned as α-oriented using the chemical shifts of C-23 (δ_C 31.4), C-24 (δ_C 28.8), C-25 (δ_C 30.3), and C-27 (δ_C 17.1) by referring to reported data for gitogenin. Thus, **1** was elucidated to be 2α,3β-dimethoxy gitogenin.

Compound **2** was isolated as a white amorphous powder with a molecular formula of $C_{27}H_{42}O_5$, which was indicated by the positive HRESIMS data (*m/z* 447.3109 $[M + H]^+$, calcd 447.3105). The ^{1}H and ^{13}C NMR data (Table 1) indicated that the structure of **2** was like those of yuccagenin [21], except for the difference on ring F. The additional OH group at C-25 was evidenced by the H-27 methyl signal becoming a singlet and shifting downfield from δ_H 0.79 (1H, d, J = 5.6 Hz) in yuccagenin to δ_H 1.58 (1H, s) in **2**, which was further confirmed by the HMBC (Fig. 2) correlations between the proton signals at δ_H 1.58 (H-27) with the carbon signal at δ_C 36.0 (C-24), 66.8 (C-25) and 70.4 (C-26). Comparison of the chemical shift of C-25 (δ_C 66.8) of compound **2** with the corresponding values reported for (25*R*)-isonuatigenin and (25*S*)-isonuatigenin (δ_C 81.6 and 65.1, respectively) indicated an axial OH group [22]. In addition, the relative stereochemistry of **2** was elucidated through the NOESY experiments: δ_H 4.17 (H-2)/δ_H 1.11 (CH_3-19) (β-oriented), δ_H 4.54 (H-16)/δ_H 1.83 (H-17) (α-oriented), δ_H 1.58 (CH_3-27)/δ_H 3.63 (H-26α) (α-oriented), δ_H 0.86 (H-18)/δ_H 2.04 (CH_3-20) (β-oriented). Thus, compound **2** was characterized as (25*S*)-2α,3β,25-trihydroxyspirost-5-en.

Compound **3** was obtained as a white powder, the molecular formula deduced to be $C_{27}H_{42}O_5$ from the HRESIMS data (*m/z* 447.3101 $[M + H]^+$, calcd 447.3105). The ^{1}H and ^{13}C NMR chemical shifts arising from rings A–E of **3** were in good agreement with those of yuccagenin [21], suggesting the same partial structure, with α- and β-orientation of the 2- and 3-OH groups, as is the case in yuccagenin. The ^{1}H NMR spectrum of **3** (Table 1) exhibited two methyl singlets δ_H 0.86 (3H, s), δ_H 1.10 (3H, s) and one methyl doublet signal δ_H1.16 (3H, d, J = 6.9 Hz). The lack of C-27 methyl group and the presence of an oxymethyl group were easily recognizable in the spectral data. With respect to the F-ring, the presence of an oxymethylene signal (δ_H 3.90 and 4.16, δ_C 64.5) and the disappearance of the characteristic methyl signals (C-27) implied hydroxylation at C-27, which was also confirmed by the HMBC (Fig. 2) correlations between δ_H 4.16 (H-27) and δ_C 24.5 (C-24), δ_H 3.73 (H-26) and δ_C 64.5 (C-27). The ^{1}H and ^{13}C NMR data of ring F in **3** were also like those of nuatigenin [23], which indicated the same (*R*)-configuration of C-25. In addition, the correlations of δ_H 4.16 (H-2)/δ_H 1.10 (CH_3-19) (β-oriented), δ_H 4.57 (H-16)/δ_H 1.82 (H-17) (α-oriented), δ_H 2.07 (H-25)/δ_H 3.73 (H-26β) (β-oriented) and δ_H 0.86 (H-18)/δ_H 1.98 (CH_3-20) (β-oriented) were observed in NOESY spectrum. Accordingly, the structure of **3** was elucidated as (25*R*)-2α,3β,27-trihydroxyspirost-5-en.

Compound **4** was isolated as a white, amorphous powder. The positive HRESIMS (*m/z* 597.3395 $[M + Na]^+$, calcd for $C_{33}H_{50}NaO_8$, 597.3398) indicated that the molecular formula of **4** is $C_{33}H_{50}O_8$. In the ^{1}H NMR spectrum of **4**, three methyl signals at δ_H 0.84 (3H, s), 0.92 (3H, s), 1.10 (3H, d, J = 6.9 Hz), one olefinic protons at δ_H 5.33 (1H, d, J = 4.9 Hz), two geminal protons at 4.05 (1H, d, J = 12.0 Hz), 4.47 (1H, d, J = 12.0 Hz), and one anomeric proton at δ_H 5.05 (1H, d, J = 7.7 Hz) were exhibited. Five characteristic carbon signals of F-ring at δ_C 109.8 (C-22), 29.4 (C-23), 33.6 (C-24), 144.8 (C-25), 64.5 (C-26) and 109.1 (C-27), indicate the presence of a spirostanol skeleton. Comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) with those of compound **16** [24] revealed that the signals were similar except for the appearance of the signals for an exocyclic olefin (δ_H 4.79 and 4.83, δ_C 144.8 and 109.1) and the disappearance of the signals for a methyl (CH_3-27) and a methine (CH-25). Thus, compound **4** was proposed to be a 25,27-dehydro derivative of compound **16**, and this was confirmed by the HMBC (Fig. 2) correlations of the H-26 (δ_H 4.47) with the

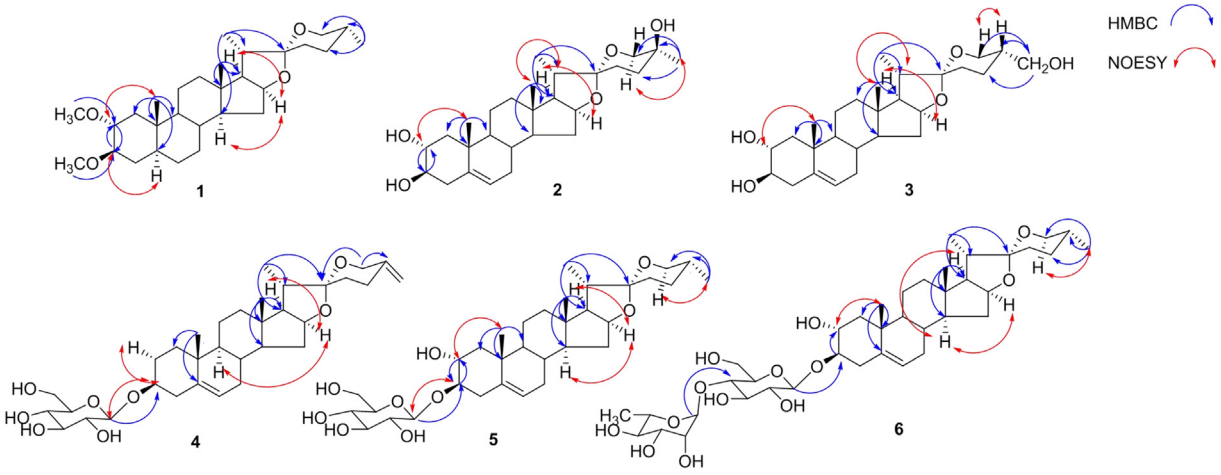

Fig. 2. The key HMBC and NOESY correlations of compounds **1**–**6**.

exomethylene signal δ_C 144.8 (C-25). With the aid of acid hydrolysis and appropriate derivatization of **3**, the monosaccharides were characterized as D-glucose (t_R: 17.188 min) (see Supporting information). The ^1H and ^{13}C NMR of the sugar units in **4** were assigned using HMQC and HMBC. The HMBC correlation between δ_H 5.05 (1H, d, J = 7.7 Hz) and δ_C 79.0 (C-3) proved the β-D-Glc to be located at C-3 of the aglycone. In addition, the correlations of δ_H 3.99 (H-3)/δ_H 2.15 (H-2α) (α-oriented), δ_H 5.05 (H-1′)/δ_H 3.99 (H-3) (α-oriented), δ_H 4.54 (H-16)/δ_H 1.81 (H-17) (α-oriented) and δ_H 4.54 (H-16)/δ_H 0.89 (H-9) (α-oriented) were observed in NOESY spectrum. Accordingly, the structure of compound **4** was elucidated as sceptrumgenin 3-*O*-β-D-glucopyranoside.

Compound **5** was obtained as a white, amorphous powder with the molecular formula of $C_{33}H_{52}O_9$ based on HRESIMS (m/z 615.3463 $[M + Na]^+$, calcd for 615.3504). The ^1H NMR spectral data (Tables 1 and 2) for the aglycone moiety of **5** showed four methyl groups at δ_H 0.80 (3H, s), 0.97 (3H, s), 1.13 (3H, d, J = 6.9 Hz), 0.68 (3H, d, J = 5.6 Hz) and one olefinic proton at δ_H 5.31 (1H, d, J = 4.2 Hz). The ^{13}C NMR spectral data gave four methyl carbons at δ_C16.7 (C-18), 20.8 (C-19), 15.4 (C-21), 17.7 (C-27), two olefinic carbon signal at δ_C 122.2 (C-6) and 140.5 (C-5), and a quaternary carbon at δ_C 109.6 (C-22), such results suggest a steroidal aglycone of the (25*R*)-spirost-5-ene type. Furthermore, the HSQC signals between δ_H 4.08 (H-2) and δ_C 70.6 (C-2), δ_H 3.85 (H-3) and δ_C 85.7 (C-3), revealed two secondary alcoholic functions at C-2 and C-3, respectively. These data allowed for the identification of the aglycone as yuccagenin [21]. Acid hydrolysis of **5** liberated glucose, the absolute configurations of sugar moieties was determined to D-glucose by HPLC analysis of the derivatives (t_R: 17.574 min) (see Supporting information). The chemical shifts, the absolute values of the coupling constants, as well as extensive 2D NMR spectroscopic data of **5** displayed the β-configuration for the glucose units [δ_H 5.07 (1H, d, J = 7.8 Hz, H-1′), δ_C 103.8 (C-1′)]. The HMBC (Fig. 2) correlations between δ_H 5.07 (H-1′) with δ_C 85.7 (C-3) confirmed the linkage of the glucose units. In addition, the correlations of δ_H 5.07 (H-1′)/δ_H 3.85 (H-3) (α-oriented), δ_H 4.08 (H-2)/δ_H 0.97 (CH_3-19) (β-oriented), δ_H 4.53 (H-16)/δ_H 1.04 (H-14) (α-oriented), δ_H 4.53 (H-16)/δ_H 1.79 (H-17) (α-oriented) and δ_H 3.49 (H-26α)/δ_H 0.68 (H-27) (α-oriented) were observed in NOESY spectrum. Therefore, the structure of compound **5** was established as yuccagenin 3-*O*-β-D-glucopyranoside.

Compound **6** was isolated as a white, amorphous powder. Its positive HRESIMS showed an ion peak at m/z 761.4082 ($[M + Na]^+$ calcd. 761.4083), indicating a molecular formula of $C_{39}H_{62}O_{13}$. Comparison of NMR (Tables 1 and 2) spectroscopic data of compound **6** with those of **5** indicated that **6** had the same aglycone as **5**, except for the signals of the sugar moiety. The ^1H NMR spectrum of compound **6** gave two anomeric protons at δ_H 5.02 (1H, d, J = 7.8 Hz), and 5.90 (1H, s), which were correlated with δ_C 103.4 and 103.1 in HSQC spectral data, respectively. Acid hydrolysis of **6** yielded glucose and rhamnose. The absolute configuration of glucose and rhamnose were identified as D- and L-, respectively, according to the HPLC analysis (D-glucose, t_R: 17.602 min; L-rhamnose, t_R: 29.075 min) (see Supporting information). The chemical shifts, the absolute values of the coupling constants, as well as extensive 2D NMR spectroscopic data, indicated the β-configuration for the glucose units [δ_H 5.02 (1H, d, J = 7.8 Hz, H-1′); δ_C 103.4 (C-1′)] and an α-configuration for the rhamnosyl unit [δ_H 5.90 (1H, s, H-1″); δ_C 103.1 (C-1″)]. The HMBC (Fig. 2) correlations between δ_H 5.02 (H-1′) and δ_C 85.4 (C-3) proves the *O*-heterosidic linkage between glucose and C-3. Another HMBC correlation between δ_H 5.90 (H-1″) and δ_C 78.6 (C-4′), suggested the (1 → 4) linkage between rhamnose and glucose. In addition, the correlations of δ_H 4.06 (H-2)/δ_H 0.94 (CH_3-19) (β-oriented), δ_H 1.05 (H-14)/δ_H 4.55 (CH_3-16) (α-oriented), δ_H 1.05 (H-14)/δ_H 1.80 (H-17) (α-oriented) and δ_H 3.50 (H-26)/δ_H 0.70 (CH_3-27) (α-oriented) were observed in NOESY spectrum. Thus, the structure of compound **6** was thus established as yuccagenin 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

The known compounds were identified as gitogenin (**7**), (25*R*)-5α-spirostane-2α,3β-diol 3-*O*-β-D-glucopyranoside (**8**), Fenugreek saponinI(**9**), (25*R/S*)-5α-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**10**), (25*R*)-5α-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**11**), diosgenin/yamogenin (**12**), sceptrumgenin (**13**), isonarthogenin (**14**), (25*R*)-5-en-spirostane-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**15**), diosgenin-3-*O*-β-D-glucopyranoside (**16**), (25*R/S*)-5-en-spirostane-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**17**), (25*R*)-5-en-spirostane-3β-ol 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (**18**), dioscin (**19**), 3β-methoxy diosgenin (**20**), (25*R*)-5-en-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**21**), (25*R/S*)-5-en-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**22**), (25*R*)-5-en-spirostane-2α,3β-diol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**23**), yuccagenin (**24**), soyasapogenol E (**25**), 22β-acetoxyolean-12-ene-3β, 24-diol (**26**), soyasapogenol B (**27**), 3-*O*-β-D-glucuronopyranosyl soyasapogenol B methyl ester (**28**), sarsasapogeninn/smilagenin (**29**), sarsasapogenin (**30**), macranthogenin (**31**), (25*S*)-5β-spirostan-3β-ol 3-*O*-β-D-glucopyranoside (**32**), spongipregnoloside A (**33**) by the comparison of their NMR data with the previous reports.

Type 2 diabetes is a biological metabolic disorder which is characterized by the high blood glucose levels (hyperglycemia), and has become a major global public health concern [25]. α-glucosidase inhibitors can decrease the fasting and postprandial blood glucose levels by effectively delaying intestinal glucose absorption [26]. To

Table 3
The inhibitory activity against α-glucosidase of extracts and isolated compounds.

compound	α-glucosidase IC_{50}	compound	α-glucosidase IC_{50}
1	> 100[a]	**19**	96.33 ± 6.25[a]
2	88.28 ± 6.74[a]	**20**	21.16 ± 2.73[a]
3	> 100[a]	**21**	> 100[a]
4	> 100[a]	**22**	> 100[a]
5	> 100[a]	**23**	14.01 ± 0.89[a]
6	66.36 ± 4.87[a]	**24**	> 100[a]
7	> 100[a]	**25**	33.03 ± 5.39[a]
8	> 100[a]	**26**	15.16 ± 2.84[a]
9	> 100[a]	**27**	8.98 ± 1.25[a]
10	> 100[a]	**28**	> 100[a]
11	> 100[a]	**29**	26.52 ± 6.73[a]
12	> 100[a]	**30**	> 100[a]
13	58.78 ± 7.48[a]	**31**	44.00 ± 10.35[a]
14	7.26 ± 0.87[a]	**32**	> 100[a]
15	> 100[a]	**33**	> 100[a]
16	> 100[a]	75% EtOH extract	> 100[b]
17	49.21 ± 5.24[a]	hydrolyzate of saponins	33.53 ± 5.42[b]
18	5.49 ± 0.84[a]	Acarbose	5.23 ± 0.87[b]

[a] μM.
[b] μg/mL.

investigate the hypoglycemic effects of fenugreek, all purified compounds and crude extract were evaluated for their α-glucosidase inhibitory activities. As shown in Table 3, the acid hydrolyzed products of fenugreek exhibited more potent inhibition of α-glucosidase than fenugreek extract, with an IC_{50} value 33.53 μg/mL. The different inhibitions against α-glucosidase were possibly associated with their different structural types. Sapogenins **26**, **27**, **14** and saponins **18** and **23** considerably inhibited α-glucosidase at IC_{50} values of 15.18, 8.98, 7.26, 5.49 and 14.01 μM, respectively, compared with the IC_{50} of positive control at 5.23 μM. Among all the tested compounds, Sapogenins obtained from acid hydrolyzed products exhibited higher inhibitory activity than the saponins as a whole, because of the lack of the sugar chains. Meanwhile, the three pentacyclic triterpene (**25**, **26** and **27**) showed better inhibitory activity aganist α-glucosidase, compared with steroidal sapogenins. Compounds **1**–**6**, along with compounds **13**, **14**, **17**, **18, 19, 20, 23, 25, 26, 27, 29** and **31** were also evaluated their inhibitory activity against PTP1B, However, none of those compounds exhibited PTP1B inhibitory activity (IC_{50} > 100) while Na_3VO_4 was used as a positive control (IC_{50} = 29.8 μM).

4. Conclusion

In summary, the phytochemical investigation of the seed of fenugreek led to the isolation and characterization of 33 steroidal sapogenins and saponins, including 3 undescribed steroidal saponins, and 3 undescribed steroidal sapogenins. The chemical structures of the new compounds were elucidated on the basis of extensive spectroscopic analyses (1D, 2D-NMR), HRESIMS data analysis and chemical evidence. All the compounds were evaluated for their α-glucosidase inhibitory activity. Sapogenins obtained from acid hydrolyzed products were demonstrated to have greater inhibitory activity than the saponins as a whole.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2020.108690.

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