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Pharmacological Activities of *Agave seemanniana* and Isolation of Three Steroidal Saponins

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Authors' contributions

Author SMAK designed the study, wrote the protocol. Author FRM managed the interpretation of the chemistry results and reviewed the final form of the manuscript. Author SAM wrote the first draft of the manuscript and with FSES carried out the practical work of the study. Authors FSES and JE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To investigate the analgesic, anti-inflammatory and ulceroprotective properties of *Agave seemanniana* methanolic leaf extract (MEAS) and its saponin containing fraction (SFAS), in animal models compared to standard drugs. In addition to separation and characterization of the major saponin fraction of *Agave seemanniana* leaves.
Place and Duration of Study: The study was carried out at the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt, between June 2011 and March 2013.
Methodology: The analgesic activity was assessed using the hot plate method and aspirin being a positive standard, the anti-inflammatory activity was investigated using the standard carrageenan-induced paw edema method against indomethacin as standard and the ethanol induced ulcer was used to record the ulcer protective effect of methanolic leaf extract of *Agave seemanniana* (MEAS) and saponin fraction of *Agave seemanniana*

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(SFAS). Separation and characterization techniques were used for the phytochemical study of the saponin content of the extract.
Results: The results of analgesic activity of MEAS and SFAS using the hot plate test, revealed significant increase in the reaction time at dose of 100mg/kg b.wt. The same dose of the two test drugs demonstrated significant ulcer protective activity. The results of anti-inflammatory activity revealed that the reduction of the carrageenan-induced paw edema was significant at a dose of 100mg/kg b.wt. Furthermore, SFAS contained mainly saponins of pregnane and furostanol types. The structures of three isolated and characterized saponins indicated that these saponins had a common oligosaccharide moiety linked to the aglycone C-3 position and identified as α-L- rhamnopyranosyl–(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl- (1→4)-β-D-galactopyranosyl.
Conclusion: This study contributes to the search for potent and locally available plant materials as analgesic, anti-inflammatory and ulcer protective drugs known for their high saponin content.

Keywords: Agave seemanniana; Analgesic; Anti-inflammatory; Ulceroprotective.

1. INTRODUCTION

The genus *Agave* belongs to the Asparagaceae family which is well known for its content of steroidal saponin processing several nucleuses as spirostane nucleus from *A. macroacantha* [1] and from *A. utahensis* [2-3], furostane nucleus from *A. utahensis* [3] and *A. sisalana* [4] and a cholestane nucleus from *A. fourcroydes* [5] and *A. Americana* [6].The saponin fraction of several *Agave* species exhibited different pharmacological activities as analgesic, anti-inflammatory, ulcero-protective and cytotoxic activities [5,7-10]. In continuation of our studies on bioactive saponins from *Agave* species [8] we report on the analgesic, anti-inflammatory and ulcero-protective activities of the methanolic extract of *A. seemanniana* leaves (MEAS) as well as the saponin-containing fraction (SFAS) derived from this extract. Furthermore, the isolation and structure elucidation of three steroidal saponins 1-3 isolated for the first time from SFAS are also described.

2. Methodology

2.1 Instruments and Materials

Optical rotations were measured using a Kruss optronic polarimeter P8000. IR spectra were recorded on a JASCO FT/ IR-6100 spectrophotometer. NMR spectra were recorded on a JEOL α 400, 399.65 MHz for ^{1}H NMR and 100.40 MHz for ^{13}CNMR. The spectra were run in CD_3OD or C_5D_5N and chemical shifts were given in ppm with TMS as internal standard. ESI-MS spectra were measured on Thermo-FINNGAN, LCQ Advantage MAX mass spectrometer. Diaion HP-20 (Mitsubishi chemicals) and silica gel G_{60} (Merck) were used for column chromatography. Preparative TLC was carried out on silica gel plates (0.25 mm thick, Merck). The plates were visualized by spraying with 20% H2SO4 in MeOH followed by heating. HPLC was performed on JASCO system 800 instrument using Devlosil Lop-ODS and the solvent mixture MeCN/H2O (27.5-72.5) Gradient, detector UV 8000, Capcell Pak C18 column (4.6mm i.d. x 250 mm, 5µm), detector UV 8000. PC was carried out using Whatman paper No1 and spots were visualized by spraying with aniline phthalate reagent.

2.2 Plant Material

Agave seemanniana Asparagaceae was obtained from Orman public Garden, Giza, Egypt in January 2009 and identification was confirmed by Dr. Therese Labib senior specialist for plant identification. A voucher specimen (no. SA-3- *Agave seemanniana*) has been deposited in the Herbarium of Pharmacognosy department, Faculty of Pharmacy, Helwan University.

2.3 Extraction, Preparation of MEAS and SFAS and Isolation of Saponins

The air dried leaves of *A. seemanniana* (1.5 kg) were extracted three times with MeOH. The solvent was distilled off the combined MeOH extract .A part of the remaining residue (12 g) was freeze- dried (MEAS) and kept until use for pharmacological work. The remaining part of the residue (16 g) was dissolved in H_2O and the aqueous solution was passed through a column packed with porous polymer gel Diaion HP-20. Elution was carried out with distilled H_2O followed by 25%, 50%, 75% and finally 100% MeOH. The 100% and the 75% eluates were combined and a part of the residue obtained after evaporating the solvent (8.5 g) was freeze-dried (SFAS) and kept until use for pharmacological work. The rest part of the residue (6.1 g) was chromatographed on silica gel column eluted with CH_2CL_2/MeOH mixture. Fourty Fractions, 100ml each were collected. Fractions 1-8 eluted with 14% MeOH were combined (2.1g) and the combined fraction was repeatedly chromatographed using preparative TLC silica gel and solvent system CH_2CL_2:MeOH:H_2O (65:30:5) to yield 1 (12.4 mg). Fractions (9–35) eluted with the same solvent mixture were combined (3.2 g) and a part of the combined fraction was subjected to repeated HPLC to give 2 (20mg) and a mixture of 2 and 3 (18 mg).

2.4 General Method for Acid Hydrolysis

Each saponin (2.0 mg) dissolved in dioxane (50 µl) and 2N HCl (1:1) was heated at 95°C for 30 minutes. Dioxane was evaporated and the residue was diluted with water and extracted with ethyl acetate. The remaining aqueous layer was concentrated and the monosaccharide content was detected by PC using n-BuOH/ AcOH/H_2O system (4:1:5 v/v, upper layer). Sugar components were identified by comparison with standard samples after spraying with aniline phthalate.

2.5 Animals and Drugs

Adolescent male albino rats weighing 120-150 g and adult albino mice of both sexes weighing 18-20 g were used. The used drugs carrageenan (Sigma- Aldrich, Egypt), Aspirin® (ADCO, Egypt), indomethacin (Merck, Germany) and ranitidine® (GSK, Egypt) were administered orally. Both MEAS and SFAS were suspended in distilled water or saline and the control animals received the same amount of the vehicle. They were obtained from animal house colony of the national research center, Dokki, Giza, Egypt.

2.6 Analgesic Activity (Hot Plate Test)

The method described by Woolfe & McDonald (1944) [17] and Laviola & Alleva (1990) [18] was applied. Analgesia was assayed by means of the hot plate method. Groups each of six mice were used. One group that was used as control received distilled water; four groups were given orally the test drugs at dose levels of 100 and 200 mg/ kg b.wt, respectively. The last group received aspirin at dose of 100 mg/ kg b.wt.. The animals were dropped gently on

the hot plate at 55ºC. The reaction time was recorded after 0, 30, 60 and 90 minutes after administration of the test and standard drugs.

2.7 Anti-inflammatory Activity (Carrageenan-Induced Paw Edema)

The method reported by Winter et al (1962) [19] was used. Groups of 6 rats were used. The test drugs were orally given a dose of 100 mg/ kg b.wt. and indomethacin, as standard material was given a dose of 20 mg/ kg b.wt . After 1 hr, 0.05 ml of 1% carrageenan was injected into the sub-plantar tissue of the right hind paw. The volume of the hind paw was measured prior to carrageenan injection and the volume of the oedema was measured after 1,2,3,4 hours. Swelling in treated animals was calculated as a percentage inhibition in comparison with controls injected by equal volume of saline into the other hind paw.

2.8 Ulcer-protective Activity

Six groups of six rats were fasted for 18 hrs. The first group served as control, received orally distilled water. The second to the fifth groups received the test drugs at the dose level of 100 and 200 mg/kg b.wt. While the last group received ranitidine at 50 mg/kg b. wt. After 30 minutes absolute ethanol (1.5 ml/kg) was orally given then 1 hr later animals were decapitated. The stomach was removed and opened along the greater curvature after being rinsed with saline, then examined with a magnifying lens (10x). The percentage of lesions or erosions was calculated.

2.9 Statistical Analysis

For the statistical analysis, results are expressed as mean ± standard error of the mean (S.E.M). Data were subjected to analysis using Kruskal-Wallis non parametric one way ANOVA .Values of *(P = .05)* were considered statistically significant.

3. RESULTS AND DISCUSION

3.1. Pharmacology

3.1.1 Nalgesic activity

The results indicated that both MEAS and SFAS at doses 100 and 200 mg/ kg b.wt. showed significant *(P = .05)* increase in the reaction time compared to the control. (Fig. 1)

3.1.2 Anti-inflammatory

The observed significant *(P = .05)* suppressive activity of the test drugs was seen at 100 mg/kg b. wt. The superior anti-inflammatory potency of MEAS compared to that of SFAS was also shown. These findings are consistent with the view that other active phyto-constituent(s) besides saponins may account for the higher anti-inflammatory action of MEAS. (Fig. 2)

3.1.3 Ulceroprotective activity

The protective activity of MEAS and SFAS on ethanol induced ulcer in rats using orally administrated absolute ethanol (1.5 ml/kg) was studied .The results showed that this effect was significant *(P =.05)* at the two dose levels 100 and 200 mg/kg b. wt. for SFAS while in

case of MEAS, significant *(P = .05)* activity was observed at 200 mg/kg b. wt. Ranitidine, the standard drug used, demonstrated significant *(P = .05)* activity at 50 mg/kg b. wt. (Table 1)

Table1. Effect of MEAS and SFAS on ethanol-induced ulcer

Treatment	Ulcer number (mean ± SE)	Ulcer severity (mean ± SE)
Control ulcer	3.66±.09	8.66±.98
Ranitidine 50 mg/Kg	0.67±.01*	1.11±.03*
MEAS 100 mg/kg	2.0±.04	1.50±.06
MEAS 200 mg/kg	0.33±.01*	0.33±.06*
SFAS 100 mg/kg	0.66±.03*	1.33±.07*
SFAS 200 mg/kg	0.66±.02*	1.16±.06*

*Each value represents the mean of 6 rats ± SE of the mean. Statistical analysis was carried out using Kruskal- Wallis non parametric one way ANOVA. *Statistically significant from the control normal (P = .05).*

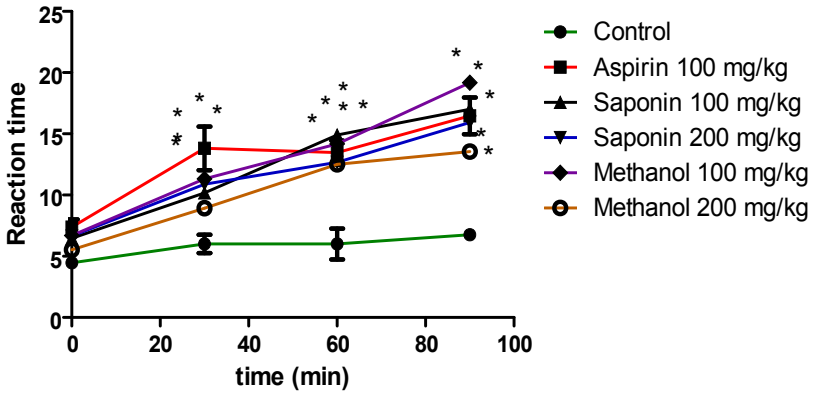


Fig. 1. Analgesic activity of MEAS and SFAS using the hot plate test

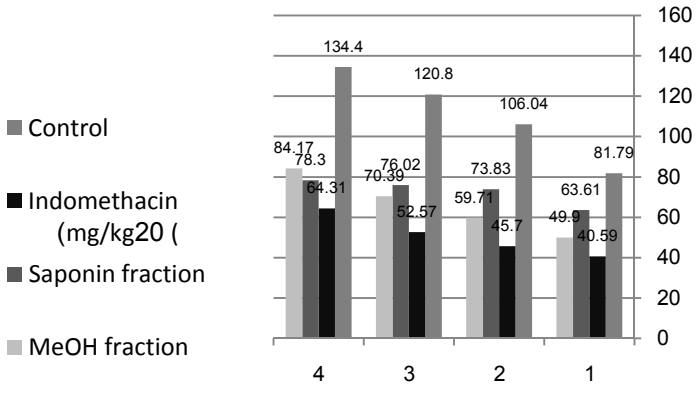


Fig. 2. Anti- inflammatory activities of MEAS and SFAS using the carregenan edema method, in term of edema (% change from base line)

3.2 Chemistry

Compound 1 showed absorptions due to α, β-unsaturated ketone (1643 cm^{-1}) and hydroxyl groups (3398 cm^{-1}) in its IR spectrum. The ^{1}H NMR spectrum of 1 in CD_3OD (Table 2) exhibited two tertiary methyl proton signals at δ 0.86 (6H,*s*) and a keto-methyl proton signal at δ 2.23 (3H,*s*) as well as an olefinic proton signal at δ 6.88 (1H,*m*). The ^{13}C NMR spectrum of 1 in CD_3OD (Table 3) confirmed the presence of α, β-unsaturated carbonyl functionality by demonstrating signals at δ 198.2 (C=O), 155.1 (C) and 146.2 (CH). The spectrum also showed five anomeric carbon signals (Table 3). From the above evidences, 1 was suggested to be a steroidal saponin of pregnane type with 16-ene-20-one structure. Compound 1 exhibited a quasi-molecular ion peak at *m/z* 1103 $[M+Na]^+$ in its ESI-MS, consistent with a molecular formula $C_{50}H_{80}O_{25}$. Of the 50 carbon signals in the ^{13}C NMR spectrum, 21 were assigned to the aglycone moiety and 29 carbons were accounted for five sugar units assignable to three hexoses, one deoxyhexose and one pentose. Acid hydrolysis of 1 afforded the sugar components D-galactose, D-glucose, D-xylose and L-rhamnose. The assignments of A and B ring carbons of the aglycone part of 1 were made possible by comparison with those reported for plant sapogenin , spirostan-5α,3β-ol [11] after considering the glycosylation shift at C-3 position (δ 76.9). The assignments of C and D ring carbons were established by comparison with the corresponding ones of other pregnanes with 16-ene-20-one structure [12,13]. The pentasaccharide nature of 1 was confirmed by the presence of five anomeric proton signals (Table 2) in the ^{1}H NMR spectrum. The β-anomeric configurations of the galactose, glucose and xylose units were determined from their large $^{3}J_{H\text{-}1,\ H\text{-}2}$ values (7.0-7.2Hz). The α-anomeric configuration of the rhamnose unit was determined by the δ values of C-3 and C-5. Inspection of the ^{13}C chemical shift values of the pentasaccharide moiety attached to aglycone C-3 of 1 in comparison with those reported for methyl pyranosides [11], revealed the presence of 4-substituted β- D- galactopyranose (Gal), 2, 3-disubstituted β- D -glucopyranose (GlcI), 4-substituted β- D- glucopyranose (GlcII), a terminal β- D -xylopyranose (Xyl) and a terminal α- L -rhamnopyranose (Rha) units. These values were similar to those of the oligosaccharide moiety linked to C-3 position of compound 4 (Fig. 3c) [1]. The only difference is the presence of an additional six carbon signals typical for a terminal α- L - rhamnopyranose unit linked to C-4 of the β- D - glucopyranose unit Glc II for compound 1. This conclusion was deduced from the observed downfield position of C-4 at δ 78.1 and upfield position of C-3 and C-5 at δ 76.1 and 74.0, respectively, for Glc II with respect to the correspondings in 4. Accordingly, the structure of 1 (Figure 3A) was formulated as 3β-hydroxy-5α-pregn-16-en-20-one-3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. This is the first reported occurrence of steroidal saponin of pregnane type in plants of genus *Agave*.

Compound 2 displayed a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 1397 in its ESI-MS in agreement with a molecular formula $C_{63}H_{106}O_{32}$. The structure of 2 was suggested to be a methoxy furostanol saponin by ^{1}H NMR (CD_3OD) [δ 3.27 (3H, *s*)] and ^{13}C NMR (CD_3OD) [δ 112.6] spectra (Tables 2 & 3). The ^{1}H NMR spectrum also showed four steroid methyl signals at δ 0.97 (*d*, *J*=6.0 Hz), 0.94 (*d*, *J*=6.0 Hz), 0.84 (*s*) and 0.79 (*s*). The structure of the aglycone part of 2 was established as 22 -methoxy (25R) -5α-furostan-3β, 26 diol based on the close similarity between the δ values of A and B ring carbon signals for 2 and those of 1, also between the δ values of the rest of aglycone carbon signals and the corresponding literature values of the structurally related methoxy furostanol saponin 26-*O*-β-D-glucopyranosyl- 22 - methoxy (25R) - 5α-furost-5-en-3 β, 26 diol 3-*O*–L–rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl -(1→6)] - β-D-glucopyranoside [14]. The ^{1}H NMR spectrum of 2 displayed in the sugar area six anomeric proton signals seen in (Table 2). Acid hydrolysis of

2 afforded the sugar components identical to those obtained after acid hydrolysis of 1. Comparison of the δ values of carbon signals of 2 with those of 1, clearly revealed close resemblance with regard to the oligosaccharide chain attached to aglycone C-3 position(δ 77.1), indicating the presence of the same pentasaccharide moiety at this position. The additional six carbon signals for 2 (δ 103.2, 73.8, 78.8, 70.4, 76.8 and 61.5) were assigned to a terminal β-D-glucopyranose unit (Glc III) linked to aglycone C-26 position (δ 76.5). Therefore, compound 2 (Figure 3B) was assigned the structure of 26-*O*-β-D-glucopyranosyl-22 -methoxy- (25R)-5 α- furostan-3β, 26-diol 3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound 3 was obtained after HPLC as inseparable mixture with compound 2 in the ratio of 1:2. Acid hydrolysis of 2 and 3 afforded sugar components identical to those obtained after acid hydrolysis of compounds 1 and 2. Inspection of the ^{13}C NMR spectrum of 2 and 3 in pyridine-d_5 (Table 3) revealed that the methoxy group at C-22 (δ 47.4) in compound 2 was replaced by a hydroxyl function in compound 3. Both compounds have one set of carbon signals with regard to the oligosaccharide and aglycone moieties except to those due to C-23 and C-22. Comparison of the chemical shift values of these signals in 2 with the corresponding ones of 3 showed that in 3 C-23 was deshielded by 6.3 ppm and C-22 was shielded by 2.0 ppm, as would be expected from a demethylation effect. The 25R configurations of 2 and 3 were proven by the difference of the δ values of the germinal H_2-26 protons ($\Delta\ \delta_H$= 0.4ppm) [15]. The ^{1}H NMR spectrum of 2 and 3 in pyridine-d_5 showed one set of signal for the 3-*O*-sugar chain which contained five anomeric proton signals assigned as shown in (Table 2). The spectrum also displayed two further anomeric proton signals at δ 4.82 (d, *J*=7.5Hz) and 4.78 (d, *J*=7.5Hz) assigned to 26-*O*- β-D-glucopyranose units for 2 and 3, respectively. Interconversion between hydroxyl and methoxy groups at C-22 of oligofurostanoides, has been previously reported in literature [16] .Thus, the structure of 3 was formulated as 26-O-β-D-glucopyranosyl 22 - hydroxy - (25R)-5 α- furostan- 3 β, 26- diol 3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

3.3 Characteristics of the Compounds

Compound 1: Amorphous solid $[\alpha]^{26}_D$ = - 67.4 (c = 0.02, MeOH). IR ν_{max} cm^{-1}: 1643, 3398. ESI-MS m/z: 1103 $[M+Na]^+$, 868, 722. ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3
Compound 2: Amorphous solid $[\alpha]^{26}_D$ = -31,22 (c = 0.01, MeOH). ESI-MS *m/z*: 1397 $[M+Na]^+$, 1108, 799, 668, 593. ^{1}H NMR: see Table 2. ^{13}C NMR: see Table 3.

Table 2. ^{1}H NMR data for compounds 1-3

H	1 (CD_3OD)	2 (CD_3OD)	2 (C_5D_5N)	3 (C_5D_5N)
16	6.88(*m*)	4.33(*m*)		
18	0.86(*s*)	0.79(*s*)	0.81	0.81
19	0.86(*s*)	0.84(*s)*	0.68	0.68
21	2.23(*s*)	*0.*97*(d*,6.0)	1.17(*d*,6.0)1.05(*d*,6.0)	1.30(*d*,6.0)
26			3.52(*dd*,11,5.0),3.92	3.52(*dd*,11,5.0),3.92
27		0.94(*d*,6.0)	1.05(*d*,6.0)	1.05(*d*,6.0)
OCH3		3.27(*s*)	3.26(*s*)	
Gal H-1	4.36(*d*,7.0)	4.36(*d*,7.0)	4.85(*d*,7.7)	4.85(*d*,7.7)
Glc I H-1	4.58(*d*,7.2)	4.57(*d*,7.0)	5.10(*d*,7.0)	5.10(*d*,7.0)
Glc II H-1	4.89(*d*,7.0)	4.86(*d*,7.0)	5.46(*d*,6.5)	5.46(*d*,6.5)
Xyl H-1	4.59(*d*,7.2)	4.58(*d*,7.0)	5.15(d,7.0)	5.15(*d*,7.0)
Rha H-1	5.15(*brs*)	5.15(*brs*)	6.04(*brs*)	6.04(brs)
Rha-CH3	1.25(*d*,6.0)	1.24(*d*,6.0)	1.61(*d*,5.8)	1.61(*d*,5.8)
Glc III H-1		4.23(*d*,8.0)	4.82(*d*,7.5)	4.78(*d*,7.5)

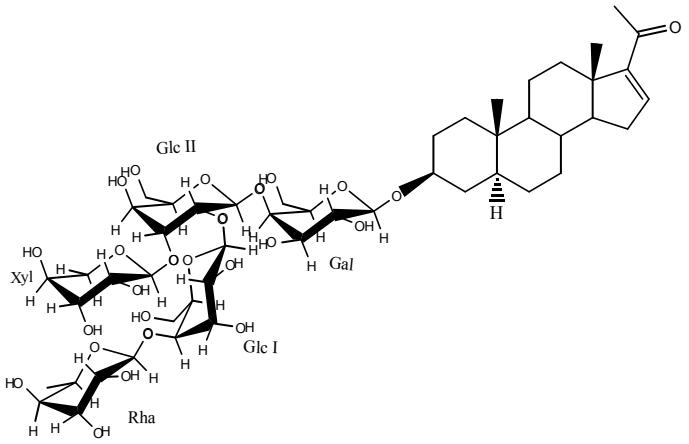
**Data in parentheses represent the multiplicity and coupling constant values*

Table 3. ^{13}C NMR data of compounds 1,2 & 3

Carbon No	1 (CD_3OD)	2 (CD_3OD)	2 (C_5D_5N)	3 (C_5D_5N)	*4 (C_5D_5N)
Aglycone					
1	36.7	36.9	37.4	37.2	
2	29.0	30.1	30.0	30.0	
3	76.9	77.1	77.6	77.6	
4	35.5	35.2	34.9	34.9	
5	44.9	44.7	44.8	44.8	
6	28.5	28.6	29.0	29.0	
7	33.8	31.4	32.5	32.5	
8	34.8	35.21	35.3	35.3	
9	54.9	54.5	54.6	54.6	
10	35.6	35.5	35.9	35.9	
11	20.8	20.8	21.3	21.3	
12	36.7	39.7	40.1	40.1	
13	46.2	40.8	41.2	41.2	
14	56.4	56.2	56.5	56.5	
15	31.9	32.1	32.2	32.2	
16	146.2	81.1	81.5	81.2	
17	155.1	65.8	64.4	64.0	
18	16.6	16.1	16.6	16.8	
19	11.4	11.5	12.4	12.4	
20	198.2	40.0	40.6	40.8	
21	25.8	15.5	16.3	16.5	
22		112.6	112.8	110.8	
23		33.7	30.8	37.1	
24		29.1	28.3	28.3	
25		34.0	34.5	34.5	
26		76.5	75.3	75.3	

27		16.6	17.6	17.6	
OMe			47.4		
3-O-sugar					
Gal					
1	101.3	101.3	102.6	102.6	102.0
2	72.6	72.6	73.2	73.2	72.8
3	74.0	74.0	75.3	75.3	75.2
4	79.6	79.6	79.7	79.7	79.6
5	74.2	74.2	76.4	76.4	76.8
6	59.7	59.7	60.8	60.8	60.4
Glc I					
1	103.3	103.3	104.7	104.7	104.9
2	82.8	82.7	81.0	81.0	81.0
3	86.6	86.6	87.3	87.3	86.3
4	70.8	70.8	70.4	70.4	70.1
5	77.1	76.9	77.7	77.7	77.2
6	61.3	61.3	63.0	63.0	62.6
Glc II					
1	102.7	102.7	104.4	104.4	104.6
2	75.1	75.0	75.3	75.3	75.9
3	76.1	76.1	75.4	75.4	77.4
4	78.1	78.1	78.5	78.5	70.7
5	74.0	74.0	75.3	75.3	78.4
6	61.7	61.7	62.4	62.4	62.1
Xyl					
1	103.6	103.6	104.9	104.9	104.7
2	74.0	74.0	75.3	75.3	74.7
3	78.8	78.8	78.5	78.5	78.2
4	69.5	69.5	70.7	70.7	70.4
5	65.8	65.8	67.3	67.3	67.0
Rha					
1	101.4	101.4	102.8	102.8	
2	70.9	70.9	72.4	72.4	
3	71.8	71.8	72.6	72.6	
4	74.0	74.0	74.2	74.2	
5	68.8	68.8	69.8	69.8	
6	16.6	16.6	18.6	18.6	
26-O-sugar					
Glc III					
1		103.2	105.1	105.1	
2		73.8	75.0	75.0	
3		78.8	78.4	78.4	
4		70.4	71.9	71.8	
5		76.8	78.7	78.7	
6		61.5	63.0	63.0	

*[1]



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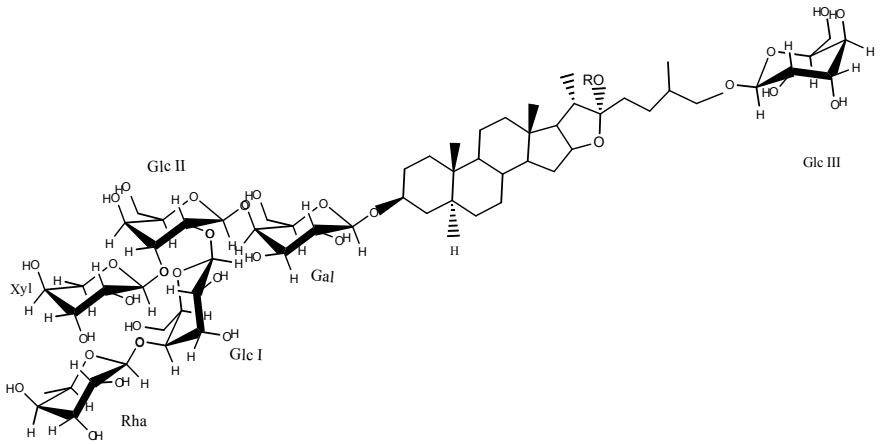
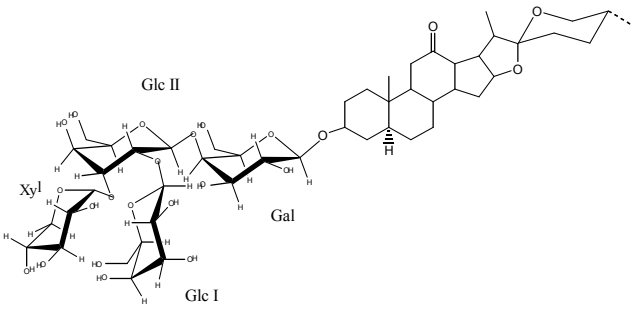


B

	R
2	CH_3
3	H



C

Fig. 3. Structures of saponins , (A) compound 1: 3β-hydroxy-5α-pregn-16-en-20-one-3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside., (B) compound 2: 26-*O*-β-D-glucopyranosyl- 22 -methoxy- (25R)-5 α- furostan-3β, 26-diol 3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. (B) compound 3: 26-O-β-D-glucopyranosyl 22 - hydroxy - (25R)-5 α- furostan- 3 β, 26- diol 3-*O*–α–L–rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D–xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, (C) reference compound 4 [1].

4. CONCLUSION

The present study revealed the analgesic, anti-inflammatory and ulceroprotective activities of the methanolic extract of *A. seemanniana* leaves and its saponin containing fraction. We state that both the total methanol extract MEAS and total saponins fraction SFAS posses nearly similar activities. It is worth noting that a number of plant extracts contained steroidal saponins, have been also reported to exhibit analgesic and anti-inflammatory activities when tested in animal models e.g. *Discorea membranacea* [20] and *Smilax corbularia* [21].

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23: (Revised 1985) U.S. Department of Health, Education and Welfare. as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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