JOURNAL OF AGRICULTURE AND BASIC SCIENCES
ISSN Online: 2518-4210
http://www.jabsjournal.com/

Full Length Research Article

Anti-inflammatory Steroidal Saponins from *Panicum turgidum*

Ahmed A. Zaki[a,b*], Longxin Qiu[b,c], Zulfiqar Ali[b], Shabana I. Khan[b], Ikhlas A. Khan[b]
[a]Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.
[b]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.
[c]School of Life Sciences, Longyan University, Longyan 364012, P.R.China
*Correspondence should be addressed to, Ahmed A. Zaki; ahmedawadzaki@yahoo.co.uk

Abstract

The bio-guided fractionation and phytochemical investigation of *Panicum turgidum* extract resulted in the isolation of three steroidal saponins, identified as yamogenin-3β-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**1**), yamogenin-3*β*-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**2**), and diosgenin-17*α*-hydroxy-3β-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**3**). Their structures were established on the basis of spectroscopic methods including 1D and 2D NMR and HRESIMS. The isolated compounds were tested for anti-inflammatory activities by iNOS inhibition assay. Compound **2** showed potent activity with IC_{50} 2.1 µM, followed by compounds **3** and **1** with IC_{50} 8.6 and 16 µM, respectively.

Keywords: Steroidal saponins, *Panicum turgidum*, anti-inflammatory.
Received: 10-08-2016, Accepted: 21-09-2016

Introduction

Inflammation is a kind of body response to protect against harmful agents and help the process of normal tissue renewal. The persistence of an inflammatory response may lead to chronic diseases, including arthritis and atherosclerosis (Jang *et al*., 2013). Macrophages play an important role in the production of pro-inflammatory mediators such as nitric oxide (NO) in response to a wide variety of activation signals including the bacterial lipopolysaccharide (LPS). Therefore, the assessment of anti-inflammatory activity by measuring the inhibition of inducible nitric oxide synthase (iNOS) activity in LPS-induced mouse macrophages (RAW264.7) is a widely accepted assay. Moreover, the identification of anti-inflammatory agents from natural products which are valuable sources of pharmacologically active secondary metabolites, is vitally important.

Plants of the genus *Panicum* are wild grasses and serve as food for grazing animals and supplementary food resource for desert inhabitants in the Middle East. *Panicum turgidum* is used traditionally in Central Sahara, Algeria in treatment of constipation and skin diseases (Hammiche and Maiza, 2006). Additionally, some of the *Panicum* species have anti-inflammatory and antipyretic activities (Zaki *et al*., 2016). These biological activities were attributed mainly to the presence of steroidal saponins which are typical constituents of the genus (Lee *et al*., 2009). However, the scientific reports on *Panicum turgidum* are scarce and its chemistry is not investigated.

Herein, we report the anti-inflammatory activity guided fractionation of methanolic extract of *P. turgidum* aerial parts, isolation and identification of pure compounds, and their anti-inflammatory activity by measuring the inhibition of iNOS activity in LPS-induced mouse macrophages.

Experimental Procedures

The 1D- and 2D-NMR experiments were recorded on a Bruker DRX-500 MHz spectrometer using pyridine-d_5 as the solvent, with solvent peaks used as the internal standard. Mass spectra were obtained on an Agilent Technologies 6200 series mass spectrometer. UV spectra were recorded on a Varian Cary 50 Bio UV-visible spectrophotometer. IR spectra were recorded on an Agilent Technologies Cary 630 FTIR. Reversed-phase HPLC [Waters Alliance 2695, equipped with photodiode array detector, and Luna C_{18} column (150 × 4.6 mm, 5 µm particle size; Phenomenex, Inc.)] was used for determination of the absolute configuration of the sugars. Column chromatography was performed over flash silica gel (32-63µ, Dynamic adsorbents Inc.), reversed-phase C-18 (Polar bond, J. T. Baker). Analytical TLC was performed on silica gel F_{254} aluminum sheet (20 x 20 cm, Fluka) or Silica 60 RP-18 F_{254}S aluminum sheet (20 x 20 cm, Merck). The detection was performed at UV-254 nm.

Spots were visualized by spraying with 1% vanillin (Sigma) in conc. H_2SO_4-EtOH (10:90) following then heating with a heat gun. Isolation and purification procedures were done with Analytical grade solvents (Fischer chemicals).

Plant material

Aerial parts of *Panicum turgidum* Forssk. were collected from the eastern desert, Egypt on April, 2014 and identified by Dr. Yasser A. El-Amier, Botany Department, Ecology, Faculty of Science, Mansoura University, Mansoura 35516, Egypt. Voucher specimen (PT-1-PD) was kept in Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University.

Extraction and isolation

Air dried and powdered aerial parts of *P. turgidum* Forssk. (500 g) were extracted by percolation with methanol at room temperature (3L x 3 x 24h). The three extracts were combined and evaporated to dryness under reduced pressure. The crude residue (24.8 g) was suspended in distilled water and defatted with hexanes and then fractionated with $CHCl_3$ and EtOAc, successively. The EtOAc and aqueous extracts (8.5 g) were combined and subjected to VLC (30 cm × 3.5 cm) over RP_{18} silica and eluted with gradients of H_2O-MeOH (1:0, 1 L), (4:1, 1 L), (7:3, 1 L), (3:2, 2 L), (1:1, 2 L), (2:3, 2 L), (3:7, 2 L), (1:4, 2 L), and (0:1, 2 L) to obtain 15 fractions. Fraction 15 (2.25 g) was subjected to silica gel column (3.5 x 85 cm) and eluted gradually with EtOAc/$CHCl_3$/MeOH/H_2O (10:6:4:1) to give five fractions. Repeated column chromatography of these fractions resulted in the isolation of two compounds; **1** (50 mg), and **2** (86 mg). Fraction 14 (2.3 g) was applied to silica gel column (4 x 90 cm) and eluted with EtOAc/$CHCl_3$/MeOH/H_2O (10:6:4:1 then 6:4: 4:1) to get 16 fractions (Fr. 14A-Fr. 14P). Column chromatography of fraction Fr. 14B (80 mg) over silica (1.7 cm x 75 cm) with EtOAc/$CHCl_3$/MeOH/H_2O (6:4:4:1) yielded compound **3** (40 mg). The absolute configuration of sugars was determined following a previously reported procedure. (Zaki *et al*., 2016)

Anti-inflammatory Activity

The inhibition of iNOS activity was determined in LPS-induced mouse macrophages (RAW264.7) as described earlier (Zhao *et al*., 2014). In brief, cells were seeded at a density of (100,000 cells/well) in 96-well plates and cultured for 24 h before treating with the test sample and lipopolysaccharide (LPS, Sigma-Aldrich, St Louis, MO, USA) (5 µg/mL) for 24 h. The activity of iNOS was determined by measuring the nitrite concentration in the cell supernatant using Griess reagent (Sigma-Aldrich, St Louis, MO, USA). The inhibition of nitrite production by the test sample was calculated in comparison to the vehicle control and IC_{50} values were computed from concentration response curves. Parthenolide was used as the positive control for inhibition of iNOS.

Results and discussion

The anti-inflammatory activities of methanolic extract and fractions were investigated through a cell-based assay that determined the inhibition of iNOS activity resulting into a decrease in intracellular nitric oxide levels. The total extract and hexanes, chloroform, ethyl acetate, and aqueous fractions showed iNOS inhibition with IC_{50} of 78, 34, 34, 33 and 32 µM, respectively. The most active fractions ethyl acetate and aqueous were combined together and subjected to repeated column chromatography. Three steroidal saponins **1**-**3** (Figure 1) were isolated and identified through the analyses of their NMR spectroscopic and MS spectrometric data as well as by comparing their NMR data with those previously reported in the literature.

Compound **1** had a molecular formula of $C_{45}H_{72}O_{16}$, determined by means of positive HR-ESI-MS ($[M+Na]^+$ at *m/z* 891.4597). The ^1H and ^{13}C NMR spectra exhibited resonances for three anomeric carbons at δ_C 101.1 (δ_H 4.96, Glc-1), 103.7 (δ_H 6.34, Rha I-1) and 102.9 (δ_H 5.82, Rha II-1). Together with HMQC and HMBC experiments the glycone part was deduced as *α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside linked to C-3 of yamogenin aglycone. The NMR data of **1** are consistent with those previously reported (Pires *et al*., 2002).

Compound **2**, which had the molecular formula of $C_{51}H_{82}O_{20}$, was determined by means of negative HR-ESI-MS showing ion peak at *m/z* 1049.5120 $[M+Cl]^-$. The ^{13}C NMR spectrum exhibited 51 resonances including those for four sugar units. The ^1H-NMR spectrum revealed the presence of 3 methyls for 6-deoxysugars (three rhamnose units) at δ_H 1.79 (3H) and 1.61 (6H), and 4 anomeric protons attributed to three α-L-rhamnose and one β-D-glucose. The ^1H-NMR spectrum of **2** showed one olefinic proton at δ_H 5.37 (δ_C 122.3) attributed to C-6, two singlets of three protons at δ_H 0.81 (δ_C 15.3) and 1.02 (δ_C 18.9) and two doublets of three protons each at δ_H 1.12 (J = 6 Hz H-21) and 1.05 (J = 6.0 Hz, H-27). The stereochemistry of C-25 was deduced from the chemical shift of C-25 of ring F where C-27 methyl protons resonance at δ_H> 1.00, supported the axial orientation of C-27 methyl (Agrawal, 2005). The spirostane skeleton was further confirmed by the resonance of C-22 at δ_C 110.3 (Agrawal *et al*., 1985), presence of two secondary

alcoholic functions at δ_H 3.86 (δ_C 78.2, C-3) and 4.55 (δ_C 80.7, C-16) and olefinic bond between C-5 and C-6 (δ_C 141.2, 122.3). The ^{1}H and ^{13}C spectroscopic data of **2** together with 2D NMR analysis (COSY, HMQC, and HMBC experiments), were in full agreement with (25S)-spirost-5-en-3β-ol (yamogenin) as the aglycone of compound **2**. The HMQC and HMBC experiments allowed the assignments of all the carbon resonances and correlations and therefore the identification of the sugars as one glucopyranoside at C-3 with one α-L-rhamnopyranoside glycosylated at C-2´ and one (α-L-rhamnose-(1→4)-rhamnopyranoside) glycosylated at C-4´. These glycosylation positions of the sugar units were confirmed by means of HMBC experiment in which correlations were observed between H-1 Glc (δ_H 4.95) and C-3 (δ_C 78.2) of the aglycone indicated that glucose was linked to C-3 of the aglycone, correlation between H-1 Rha I (δ_H 5.79) and C-2 Glc (δ_C 78.5), cross peak between H-1 Rha II (δ_H 6.25) and C-4 Glc (δ_C 78.1) and correlation between H-1 Rha III (δ_H 6.35) and C-4 Rha II (δ_C 81.6). Compound **2** was concluded to be yamogenin-3*β*-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**2**). The NMR data of **2** are in full agreement with previously reported results (Yoshikawa *et al.*, 2007)

Compound **3** had a molecular formula $C_{45}H_{72}O_{17}$, which was deduced from HR-ESI-MS data showing an ion peak at *m/z* 907.4498 $[M+Na]^+$. The ^{1}H-NMR spectrum of the aglycone part of **2** showed one olefinic proton at δ_H 5.35 (δ_C 122.7) attributed to C-6, two angular methyl signals at δ_H 0.93, 1.05 (each 3H, s), two secondary methyl signals δ_H 1.25 (3H, *d*, *J* = 8 Hz) and 0.68 (3H, *d*, *J* = 8 Hz). The ^{13}C NMR spectrum of **3** showed four signals at lower field than 100 ppm; signals at δ_C100.6, 102.4, 103.2 were due to anomeric carbons, and the signal at δ_C 110.8 was assignable to C-22 of the spirostane skeleton. The down-field shift of C-27 (δ_C 18.1) and up-field shift of H-27 to less than 1.0 (δ_H 0.68) were consistent with (25R)-spirostanes (Chen *et al*., 2011). The olefinic proton at δ_H 5.35 (δ_C 122.7) and oxygenated quaternary carbon observed at δ_C 90.4, together with the HMBC correlation between H-21 (δ_H 1.25, *d*) and δ_C 90.4, supported the aglycone of **3** to be diosgenin-17α-OH with sugars attached to its C-3 position (Gajdus *et al*., 2014; Lee *et al*., 2009). The sugars were assigned as one glucopyranosyl and two rhamnopyranosyl units from their NMR data (δ_H 5.85 *s*, 6.38 *s*, 1.59*d*, *J* = 6.4 (3H), 1.73*d*, *J* = 6.2 (3H); δ_C 102.4, 103.2, 18.8, 18.9). The HMBC correlation of anomeric proton H-1´ (δ_H 4.96 *d*, *J* = 7.3 Hz) with C-3 and H-3 (δ_H 3.88 *m*) with the anomeric carbon C-1´ (δ_C 100.6) indicated the attachment of glucopyranose to C-3, correlation between H-1´´ (δ_H 5.85, *s*) and C-2´ (δ_C 79.2) prove the (1→2) linkage between glucose and rhamnose, the correlation between H-1´´´ (δ_H 6.38 s) C-4´ (δ_C 78.5) indicated (1→4) linkage between glucose and the second rhamnose unit. Therefore, the structure of **3** was determined as diosgenin-17*α*-hydroxy-3β-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside. The NMR data were consistent with those previously published (Gajdus *et al.*, 2014). The ^{13}C NMR data of compounds **1**-**3** are listed in Table 1. The isolated compounds (**1**-**3**) were evaluated for anti-inflammatory activity and the results (Table 2) showed that **2** exhibited potent iNOS inhibition (IC_{50} 2.1 µM) compared to **1** and **3** (**(**IC_{50} 16.0, 8.6 µM, respectively). These results suggest that the anti-inflammatory activity of *P. turgidum* is due to the steroidal saponins.

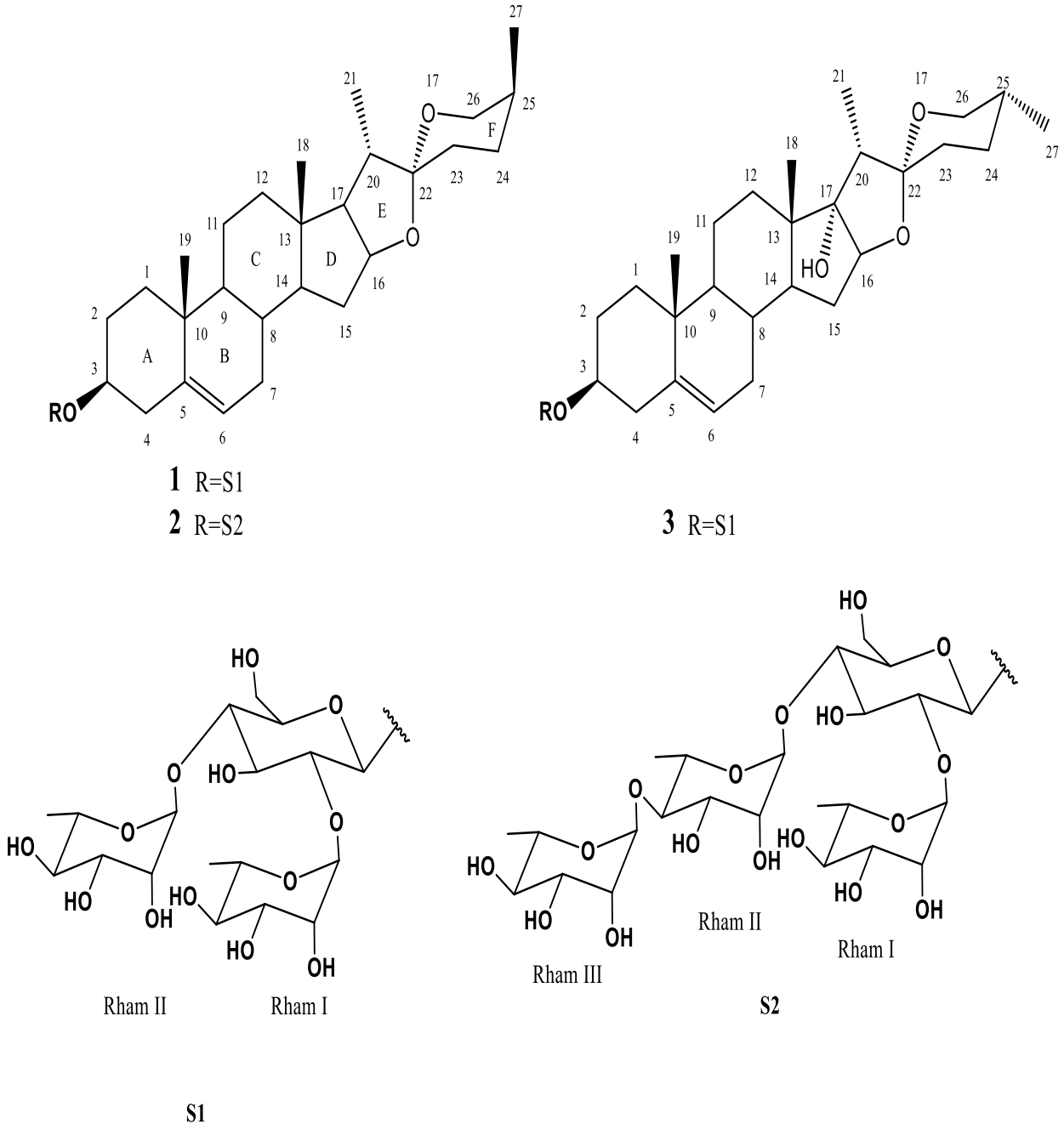

Figure 1: Chemical structures of compounds **1**-**3**.

Table 1: ^{13}C NMR data of compounds **1-3** (pyridine-d_5, 125 MHz).

#	**1**	**2**	**3**
1	38.04	37.9	38.0
2	31.07	31.0	32.2
3	78.9	78.2	78.2
4	39.9	37.6	39.9
5	141.7	141.2	141.7
6	122.8	122.3	122.7
7	32.6	32.6	32.5
8	33.2	32.7	32.7
9	51.2	50.7	50.6
10	38.4	39.4	38.5
11	22.0	21.5	21.3
12	40.8	40.9	32.5
13	41.4	40.2	45.5
14	57.5	57.0	53.9
15	33.1	32.1	32.8
16	82.1	80.7	90.4
17	63.5	63.1	90.7
18	15.8	15.3	18.2
19	20.4	18.8	20.3
20	43.4	42.9	45.7
21	17.3	16.7	10.6
22	110.7	110.3	110.8
23	32.7	26.8	31.1
24	28.4	27.9	29.7
25	31.5	30.5	31.3
26	66.0	65.5	67.1
27	18.3	16.8	18.0
3-*O*-Glc			
1′	101.1	100.1	100.6
2′	79.01	78.5	79.2
3′	78.7	78.2	78.3
4′	79.4	78.1	78.5
5′	77.8	77.4	77.2
6′	62.2	61.6	61.7
2′-Rha			
1′′	103.7	102.7	103.2
2′′	73.4	72.9	72.8
3′′	73.7	73.0	73.1
4′′	74.8	74.5	74.2
5′′	70.49	70.3	69.9
6′′	19.5	19.1	18.9
4′-Rha			
1′′′	102.9	102.7	102.4
2′′′	73.6	73.3	72.8
3′′′	73.7	73.6	73.2
4′′′	74.92	81.6	74.4
5′′′	71.33	68.8	70.8
6′′′	19.42	19.3	18.8
4′′′-Rha			
1′′′′		103.7	
2′′′′		73.2	
3′′′′		73.3	
4′′′′		74.3	
5′′′′		70.8	
6′′′′		19.9	

Table 2. Inhibition of Inducible Nitric Oxide Synthase (iNOS) by compounds **1**-**3**.

Compound	IC_{50}*
1	16
2	2.1
3	8.6
Parthenolide[a]	0.32

*IC_{50} values are expressed in µM, [a] positive control.

References

Agrawal, P., D. Jain, R. Gupta and R. Thakur. (1985). Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. *Phytochem.* 24: 2479-2496.

Agrawal, P.K. (2005). Determining ring-F configuration in spirostane-type steroidal sapogenins by ^{1}H NMR. *Indian J. Chem.*, 44: 1092-1094.

Chen, P.-Y., C.-H. Chen, C.-C. Kuo, T.-H. Lee, Y. H. Kuo and C.-K. Lee. (2011). Cytotoxic steroidal saponins from *Agave sisalana*. *Planta Medica.* 77: 929.

Gajdus, J., Z. Kaczyński, A. Kawiak, E. Lojkowska, J. Stefanowicz-Hajduk, J.R. Ochocka and P. Stepnowski. (2014). Isolation and identification of cytotoxic compounds from the rhizomes of *Paris quadrifolia* L. *Pharmacognosy Mag.*, 10: S324.

Hammiche, V. and K. Maiza. (2006). Traditional medicine in Central Sahara: pharmacopoeia of Tassili N'ajjer. *J. Ethnopharmacology.* 105 (3): 358-367.

Jang, M., S.-W. Jeong, S.K. Cho, K.-S. Ahn, B.-K. Kim. and J.-C. Kim. (2013). Anti inflammatory effects of 4 medicinal plant extracts in lipopolysaccharide-induced RAW 264.7 cells. *Food Sci. Biotech.,* 22: 213-220.

Lee, S.T., R.B. Mitchell, Z. Wang, C. Heiss, D.R. Gardner. and P. Azadi. (2009). Isolation, Characterization, and Quantification of Steroidal Saponins in Switchgrass (*Panicum virgatum* L.). *J. Agric. Food Chem.* 57: 2599-2604.

Pires, V.S., A.T. Taketa, G. Gosmann, E.P. Schenkel. (2002). Saponins and sapogenins from *Brachiaria decumbens* Stapf. *J. Brazilian Chem. Soc.*, 13: 135-139.

Yoshikawa, M., F. Xu, T. Morikawa, Y. Pongpiriyadacha, S. Nakamura, Y. Asao, A. Kumahara and H. Matsuda. (2007). Medicinal flowers. XII. 1) New spirostane type steroid saponins with antidiabetogenic activity from *Borassus flabellifer*. *Chemical and Pharmaceutical Bulletin.*, 55: 308-316.

Zaki, A.A., Z. Ali, Y.A. El-Amier, I.A. Khan. (2016). A New Neolignan from *Panicum turgidum*. *Natural Product Communications.*, 11: 987 -988.

Zhao, J., S.L. Khan, M. Wang, Y. Vasquez, M.H. Yang, B. Avula, Y.-H. Wang, C. Avonto, T.J. Smillie. and I.A. Khan. (2014). Octulosonic acid derivatives from roman chamomile (*Chamaemelum Nobile*) with activities against inflammation and metabolic disorder. *J. Nat. Products.*, 77: 509-515.