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Detailed characterization of saponins isolated from *Zygophyllum Propinqueem* Decne

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Abstract

Zygophyllum propinquum Decne (syn. Z. *coccineum*, family: Zygophyllaceae) is a low shrub, perennial herb, or desert succulent undershrub and has several important biological activities. The major secondary metabolites of this plant are a class of ursane-type triterpene saponins. Saponins derive their name from stable foam formation in water. These saponins have peculiar properties like, bitterness, fish poisoning, haemolysis, complex formation with cholesterol. Saponins are consisting of two main parts, one is the aglycone part while the other one is the glycone part. The glycone part is further consisting of sugar moieties. Current studies were conducted to isolate specifically biologically important saponins. Saponins were isolated successfully using standard procedures and characterized successfully using different spectroscopic techniques including Fourier Transform-Infrared Spectroscopy, Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy. Two saponins were isolated from the whole plant of *Zygophyllum propinquum* Decne with the help of repeated column chromatography and HPLC. The purified saponins were hydrolyzed with H_2SO_4-dioxane resulting in lactone formation. All the compounds (saponins and lactone) were characterized with the help of FAB-MS and 1D and 2D-NMR techniques. Their structures were confirmed to be 3-*O*-*β*-D-glucopyranosyl-(1→6)- *β*-D-2-*O*-sulfo-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-glucuronopyranosyl] ester (1), (3*β*-*O*-2-O-sulfo-*β*-D-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-2-*O*-sulfonylglucuronopyranosyl] ester (2), and 3*β*-Hydroxy urs-28,20 *β*-olide (3).

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Introduction

Zygophyllum propinquum Decne (syn. Z. *coccineum*, family: Zygophyllaceae) is found in various parts of the world including Sindh and Baluchistan provinces of Pakistan (Ghafoor, 1974). It has numerous pharmacological activities (Saber and El-Moghazy, 1960; Eskander and Won, 1995; Pollmann *et al.*, 1997; Gibbons and Oriowo, 2001). Morphologically, *Z. propinquum* appears as a low shrub, perennial herb, or desert succulent undershrub, is up to 75 cm high, and is characterized by numerous stems and erect, young, green branches (Soliman, 1939; Reed, 1997).

Z. propinquum is a saponin-rich plant. Phytochemical investigations have revealed that the major secondary metabolites in *Z. propinquum* are a class of quinovic acid compounds belonging to the ursane-type triterpene saponins, including zygophylloside S, together with a known flavonoid glycoside and a sterol glycoside (Nasir and Ali, 1970-1979; Ahmad *et al.*, 1990; Ahmad *et al.*, 1992; Ahmad *et al.*, 1993; Ghazala, 1992; Elgamal *et al.*, 1995; Amin *et al.*, 2011). Saponins derive their name from stable foam formation in water. Plants producing saponins consist of about 90 families with about 472 different plant species (Hostettmann and Marston, 1995; Fenwick *et al.*, 1991). These saponins have peculiar properties like bitterness, fish poisoning, haemolysis, complex formation with cholesterol (Cui *et al.*, 1999; van Setten *et al.*, 1998; Chludil *et al.*, 2002; Kofler, 1927), etc. Saponins are made up of glycone and aglycone parts. The glycone part is made up of sugar moieties. Saponins are mainly classified as triterpenoidal, steroidal and steroidal alkaloid types. The aglycone part of saponin is also called genin or sapogenin. Saponins are extremely widely distributed in the plant kingdom. The saponins constitute an important group of natural products of wide spectra of biological activity. The aims of the current study are to isolate biologically important saponins from *Zygophyllum propinquum* Decne and characterize them with the help of available different spectroscopic techniques. These studies would help the researchers to follow the same techniques for the isolation of saponins from different plant species and evaluate these valuable compounds further for their efficacies against different ailments.

Materials and methods

For the isolation of saponins, we followed our previously published methodology (Qaisar *et al.*, 2011). The *Zygophyllum propinquurn* Decne whole plant (20 Kg) was washed with distilled water and air dried. The dried plant material was ground, extracted with methanol and then concentrated resulting in a sticky residue (800 g). First, it was partitioned between water and ethyl acetate and then the aqueous portion was further partitioned with butanol three times. The combined butanolic fractions were concentrated and subjected to column chromatography. The solvent mixture used throughout was chloroform-methanol (80:20). Mixtures of saponins were obtained in different fractions which were further subjected to column chromatography with reverse phase silica and finally purified with preparative-thin layer chromatography using butanol, acetic acid and water (12:3:7) as mobile phase. Final purification was made with HPLC using RP-18 column.

Results and discussion

Saponin **1** (Fig. 1) gave a quasi-molecular ion peak at m/z 1035 $[M-H]^-$ and prominent peaks due to loss of hexoses at m/z 873 (M-Glc) and m/z 711 $[M-Glc-Glc]^-$ in the negative FABMS. The peak at m/z 711 revealed the presence of hexose-SO_3H intact with the saponin. A detailed discussion regarding FAB-MS has already been made in our previously published paper (Qaisar *et al.*, 2011).

The NMR spectral data of saponin **1** revealed the feature of a triterpenic acid with a double bond and three sugar units, one of which was linked to the carboxylic group (δ H 3.26, δ C 94.2) *via* an ester linkage. The remaining sugars were attached to the oxygen at C-3. The ^{1}H-NMR spectrum of **1** displayed signals for six tertiary Me groups at δ 0.95,0.81, 0.89, 0.91 0.74, 1.29 and one secondary Me group at δ 0.97 (d, *J*=7.0 Hz) in the aglycon moiety, indicating a

pentacyclic triterpene skeleton of the ursane or taraxastane series. One of the Me signals appeared at a comparatively low field (δ1.29, s) suggesting that it could be attached to an olefinic carbon. In addition, it was possible to observe an olefinic proton signal at δ5.41 (1H dd, *J*=9.0, 1.5 Hz) and a signal typical of H-3_{ax} 3.18 dd (*J*=11.4, 4.5 Hz) due to the presence of *β*-OH group at C-3 position (Table 1).

Table 1. NMR data of the isolated compounds.

	Compound a		Compound b		Compound c		
Carbon #	Chemical Shift δ	DEPT	Chemical shift δ	DEPT	Chemical shift δ	DEPT	δH^1 JHH in Hz
1	39.9	CH_2	39.9	CH_2	38.8	CH_2	0.84, 1.54m
2	25.5	CH_2	25.8	CH_2	25.2	CH_2	1.54m
3	78.94	CH	78.8	CH	78.9	CH	3.18dd (4.5, 11.4 Hz)
4	Not appeared	C	44	C	41.1	C	
5	58.6	CH	57.9	CH	55.4	CH	0.66 dd (2.0, 11.4Hz)
6	18.6	CH_2	18.5	CH_2	18.2	CH_2	1.39m, 1.25m
7	34.3	CH_2	34.3	CH_2	33.9	CH_2	1.35, 1.45
8	43.3	C	43.1	C	40.5	C	
9	53.3	CH	53.4	CH	50.4	CH	1.24dd
10	39.2	C	Not appeared	C	37.19	C	
11	20.5	CH_2	20.5	CH_2	20.9	CH_2	1.61m
12	28.2*	CH_2	28.1*	CH_2	27	CH_2	1.9m
13	39.6	CH	39.6	CH	42	CH	1.52m
14	41.6	C	41.7	C	42.08	C	
15	28.3*	CH_2	28.2*	CH_2	27.3	CH_2	0.85m
16	31.5	CH_2	31.6	CH_2	32.6	CH_2	1.67, dt (4.6, 5.8Hz)
17	51.9	C	51.3	C	51	C	
18	51.5	CH	51.9	CH	49.4	CH	1.05dd (11.6, 5.5Hz)
19	41.6	CH	41.5	CH	42	CH	1.21m
20	145.8	C	145.7	C	84.08	C	
21	119.7	CH	119.8	CH	27.4	CH	2.01ddd (4.4, 8.9, 3.4 Hz), 1.11ddd(2.7,4.4,13.4Hz)
22	27.4	CH_2	27.3	CH_2	27.5	CH_2	1.19m
23	29.7	CH_3	29.5	CH_3	27.9	CH_3	0.81
24	18.1	CH_3	18	CH3	16.2	CH_3	0.95
25	18.5	CH_3	18.4	CH_3	16.3	CH_3	0.89 s
26	18	CH_3	18	CH_3	15.3	CH_3	0.74 s
27	17.4	CH_3	17.3	CH_3	14.2	CH3	0.74 s
28	178.1	C	178.8	C	177.1	C	
29	23.8d	CH_3	23.7	CH_3 (d)	18.7	CH_3	0.97d(7.0 Hz)
30	25.5s(1.65)	CH_3	25.6	CH_3	23.9	CH_3	1.29 s
	GlucA		GlucA′				
1	105		105.1′				
2	82.5		82.5′				
3	82.4		82.3′				
4	74.4		74.1′				
5	71.1		71.0′				
6	183		183.1′				
	GLC″		GlucA″				
1	94		94.94″				
2	77.5		70.4″				
3	78.9		73.2″				
4	74.1		77.5″				
5	81.1		78.5″				
6	62		182.4″				
	GLC‴						
1	94.2						
2	74.1						
3	78.3						
4	71.4						
5	78.9						
6	63						

Full assignment of the proton and carbon signals of the aglycon part of **1** was secured by proton NMR, COSY and HMQC spectra. The proton and carbon signals due to A, B, C and D rings indicated a 3*β*-hydroxy-12,13 dihydro-ursane skeleton carrying a COOR group at C-28 and glycosylated at C-3. After the acid hydrolysis of saponin **1**, the aglycone of the saponin was lactonized to compound **3** (Fig. 1) (discussed later in detail). The position of glycosylation of the aglycone gave a signal at δ 3.18 (dd *J*=11.4, 4.5 Hz) due to H-3. The lactonized carbon (C-20) signal at δ84.0 in ^{13}C-NMR spectra revealed that the saponin is glycosylated at C-3 and C-28 positions. This suggested that the double bond in the saponin may be at C20-C21 position.

The position of the double bond at C20- C21 can be proved by the presence of one proton signal at δ5.41 connected to olefinic carbons (δ C 145.8 and 119.7) supporting the structure C=CH. The CH_3 signal at δ1.64 (s, Me-30) indicated a vinylic-Me and a signal at δ0.97 (d, *J*=7.0 Hz) was assigned to Me-29.

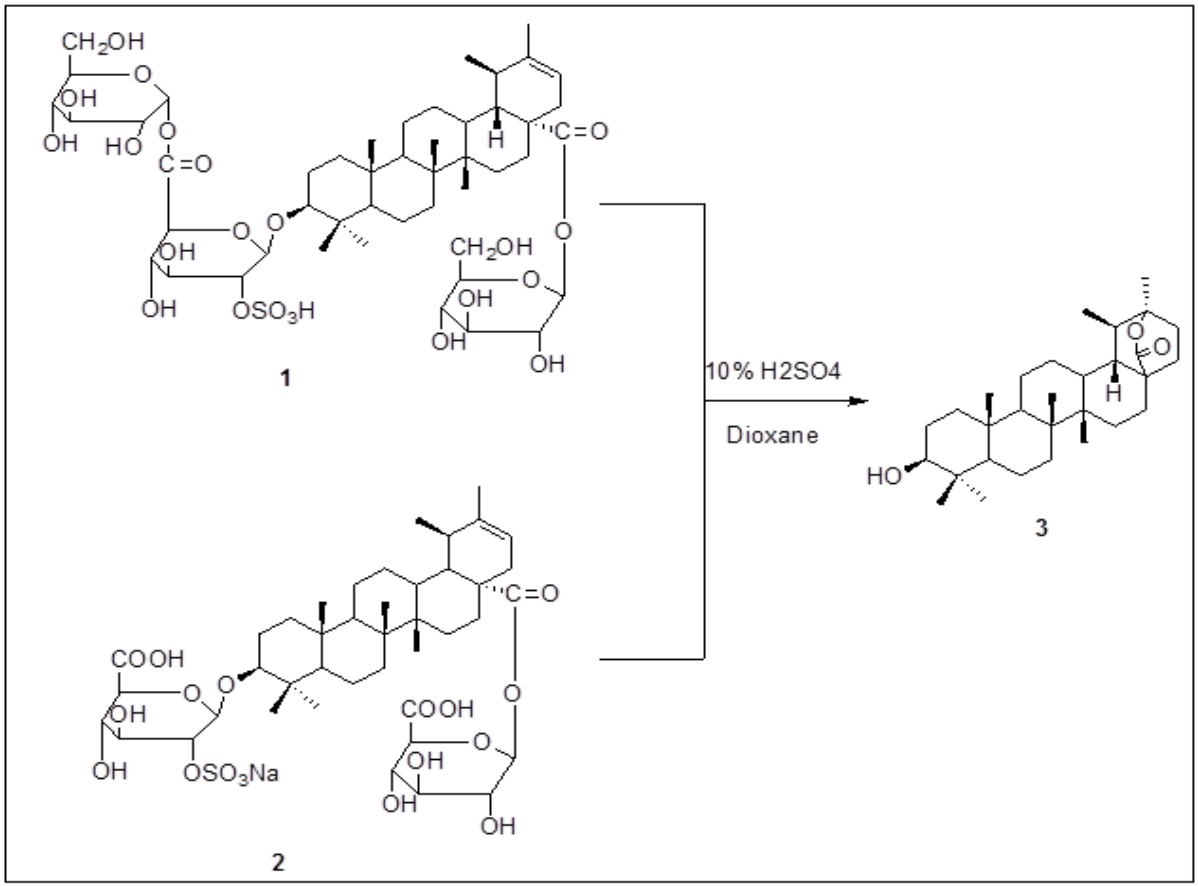

Fig. 1. Saponins and their hydrolyzed product (lactone).

The position of the double bond was confirmed by the data reported in the literature (Fenwick *et al.*, 1991; Cui *et al.*, 1999) and also by COSY interaction between H-21and H-22. The value of the coupling constant of H-18 (*J*=11.6 Hz) and H-19 (δ 1.21, m) indicated that both protons were in axial position. Therefore the Me at C-19 should be in *β*-position and ring D/E should have a *cis* connection. The structure of saponin **1** was further confirmed by HMBC, HMQC & HOHAHA spectroscopy. The ^{13}C-NMR spectra of **1** revealed the presence of 48 carbon signals. The anomeric signals at δ105.0, 94.0 and 94.2 confirmed the presence of three sugar residues attached to the aglycon at C-28 and C-3 positions. The anomeric proton can easily be correlated by HMQC spectra. The three sugars attached to the aglycone were confirmed by acid hydrolysis and FABMS negative ion spectra. Key correlation peaks in HMBC spectra between H-1′ of glucuronic acid at δ4.62 ppm and C-3 (δ C = 78.94) of the aglycon confirmed that glucuronic acid is

attached at C-3 position as shown in the structure. Glucuronic acid further attached to glucose by an ester linkage to C-1′ ′ of glucose was suggested by HMBC correlation. The downfield shift of H-2′ of glucuronic acid and C-2′ of glucuronic acid signals indicated that SO_3H group is at position 2 of glucuronic acid. The HOHAHA and COSY spectra also indicated that the proton that appeared at δ4.62 ppm attached to C-2 at δ82.5 was correlated with the anomeric proton of glucuronic acid. Esterified sugar substituent at C-28 was confirmed with the help of HMBC and HMQC. The FAB-MS spectra showed the sequence of sugar and the presence of SO_3H in glucuronic acid. The HMQC and HOHAHA spectra suggested that the anomeric carbons at δ105.0 were connected to C-3 and 94.2 to C-28. The anomeric proton showed further correlation with carbon at δ27.4 which was assigned to C-22.

All the above data strongly suggest the structure for the saponin **1** to be 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-2-*O*-sulfo-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-glucuronopyranosyl] ester (Qaisar *et al.*, 2011).

The saponin **2** (Fig. 1) gave a quasi-molecular ion peak at m/z 909 $[M-H]^-$ along with peaks at 732 [M-GlucA], 455 [M-H-GlucA- GlucA-SO_3Na] in the negative FABMS. The presence of a peak at m/z 732 revealed the presence of a hexose intact in saponin. The peak at m/z 455 revealed the presence of another hexose –SO_3Na intact with saponin (Qaisar *et al.*, 2011).

The NMR spectral data of saponin **2** revealed the feature of triterpenic acid with a double bond and two sugar units which were linked to the carboxylic group (C-28) *via* an ester linkage. The remaining sugar was attached to C-3 position of aglycon moiety. The proton NMR data of **1** and **2** displayed similar signals for six tertiary Me groups (δ0.96, 0.81,0.88,0.91,0.74,1.28) and one secondary Me at δ0.97 (d, *J*= 7.0) in the aglycon moiety indicating a pentacyclic triterpene skeleton of ursane or taraxastane series. The presence of a signal for a double bond was observed for the olefinic proton at δ5.44 (1H, dd, *J*= 8.9, 1.6 Hz). The signal at δ3.1 (dd, *J* = 11.3, 4.4 Hz) also revealed the *β*-hydroxy group at C-3 position (see Table 1). The full assignment of the proton and carbon signal of aglycon was achieved by proton NMR, COSY and HMQC spectra. The ^{1}HNMR and ^{13}CNMR signals revealed the presence of a COOR group at C-28 and a glycosylation linkage at C-3 position. The ^{13}CNMR spectra of **2** revealed the presence of 42 carbon signals. The anomeric signals at δ94.94 and 105.1 confirmed the presence of two sugar residues attached to the aglycon at C-28 and C-3 positions. The anomeric signals can be correlated by HMQC spectra. The acid hydrolysis and (-)-FABMS confirmed that two sugars were attached to the aglycon. Correlation in the HMBC spectra between H-1′ of glucuronic acid at δ4.60 and δ78.8 at C-3 of the aglycon confirmed that glucuronic acid is attached at C-3 position. The downfield shift of H-2′ of glucuronic acid and C-2′ of glucuronic acid, which is in close agreement with compound **1**, showed the presence of SO3Na at position C-2′ of the glucuronic acid (Ahmad *et al.*, 2005). The HOHAHA and COSY spectra also indicated that the proton appeared at 4.63 ppm (attached to C-2, δ82.6) and showed coupling with the anomeric proton of glucuronic acid. Esterified sugar substituted at C-28 was confirmed with the help of HMQC. The sugars were indicated as glucuronic acid.

Based on the above spectral evidence, the compound was identified as 3*β*-*O*-2-O-sulfo-*β*-D-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-2-*O*-sulfonylglucuronopyranosyl] ester **2** (Fig. 1).

Compound **3** (Fig. 1) was isolated by hydrolyzing 20 mg each of saponin **1** and **2** separately with 10% H_2SO_4-dioxane and refluxed for 3 hours at 100 °C. The reaction mixture was diluted with H_2O and extracted with $CHC1_3$ to give compound **3**. The aqueous layer was neutralized with $KHCO_3$, and sugars were identified by TLC ($CHCl_3$-MeOH-AcOH-H_2O, 8:3:5:2), spraying with sugar reagent. The ElMS of compound **3** showed a very weak molecular ion

peak at 456. The peak matching of this peak resulted in an exact value of 456.3608 which corresponds to the molecular formula $C_{30}H_{48}O_3$. The IR spectrum showed an absorption band at 3600 cm^{-1} (hydroxyl) and 1735 cm^{-1}(δ-lactone).

The ^{1}HNMR exhibited signals for seven methyl groups. One of the methyl signals appeared as a doublet at δ0.97 (d, *J*=7.0 Hz, H-29) which showed that it was a secondary methyl, while the rest were tertiary and appeared as singlets at δ0.74 (H-27), 0.81, 0.81 (H-24), 0.89 (H-25), 0.91 (H-26), 0.95 (H-23) and 1.29 (H-30), indicating a pentacyclic triterpenoid skeleton. A doublet of a doublet at δ3.18 (*J*= 11.4, 4.5 Hz) was assigned to H-3α. The carbonyl carbon appeared at δ177.1 (C-28) in the ^{13}C-NMR spectrum which was a characteristic peak of lactone carbonyl carbon. The broadband showed all 30 carbons. The DEPT experiment revealed the presence of seven methyl, nine methylene, seven methine and seven quaternary carbon atoms. The chemical shift values and ^{1}H/^{13}C connectivities of various carbon atoms are shown in Table 3 and were in agreement with the data reported in the literature (Errington and Jeffereies, 1988).

The above data strongly suggest the proposed structure of the compound under discussion to be lactone 3*β*-Hydroxy urs-28,20 *β*-olide **3** (Fig. 1). This study, on lactone, in turn, further confirmed the proposed structures for compounds **1** and **2** to be the exact structures with the same basic skeleton.

Conclusions

Saponins 3-*O*-*β*-D-glucopyranosyl-(1→6)- *β*-D-2-*O*-sulfo-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-glucuronopyranosyl] ester **(1)**, (3*β*-*O*-2-O-sulfo-*β*-D-glucuronopyranosylurs-20(21)-en28 oic acid 28-*O*-[*β*-D-2-*O*-sulfonylglucuronopyranosyl] ester **(2/)** were isolated successfully from *Zygophyllum propinquum* Decne and characterized accordingly using different spectroscopic techniques. A lactone formed during acid hydrolysis of these saponins was not only identified itself with the help of spectroscopic techniques, but it also helped, in turn, in the exact identification of the two saponins (described above). The research described here will help the researchers in the field to isolate and identify easily the medicinally important same type of saponins.

Competing interests

There are no competing interests.

References

Amin E, El-Hawary SS, Fathy MM, Mohammad R, Ali Z, Tabanca N, Wadge DE, Becnel JJ, Khan IA. 2011. Triterpenoidal saponins: bioactive secondary metabolites from *Zygophyllum coccineum*. Planta Medica **77**, 488-491. https://doi.org/10.1055/s-0030-1250463

Ahmad VU, Ghazala, Uddin S. 1992. A triterpenoid saponin from *Zygophyllum propinquum*. Phytochemistry **31,** 1051-1054. https://doi.org/10.1016/0031-9422(92)80073-N

Ahmad VU, Ghazala, Uddin S, Ali MS. 1993. Saponins from *Zygophyllum propinquum*. Phytochemistry **33,** 453-455. https://doi.org/10.1016/0031-9422(93)85538-3.

Ahmad VU, Ghazala, Uddin S, Bano S. 1990. Saponins from *Zygophyllum propinquum*, Journal of Natural Products **53,** 1193-1197. https://doi.org/10.1021/np50071a010.

Ahmad VU, Iqbal S, Kousar F, Badar S, Arshad S, Tareen RB. 2005. Two new saponins from *Zygophyllum atriplicoides*, Chemical and Pharmaceutical Bulletin **53**, 1126-1130. https://doi.org/10.1248/cpb.53.1126.

Chludil HD, Seldes AM, Maier MS. 2002. Antifungal steroidal glycosides from the Patagonian starfish Anasterias minuta: structure-activity correlations, Journal of Natural Products **65**, 153-157. https://doi.org/10.1021/np010332x.

Cui M, Sun W, Song F, Liu Z, Liu S. 1999. Multi-stage mass spectrometric studies of triterpenoid saponins in crude extracts from *Acanthopanax senticosus* harms, Rapid Communication in Mass Spectrometry **13,** 873-879. https://doi.org/10.1002/(SICI)1097-0231(19990530)13:10<873::AID-RCM571>3.0.CO;2-2

Errington SG, Jefferies PR. 1988. Triterpenoid sapogenins of *Pittosporum phillyraeoides*, Phytochemistry **27,** 543-545. https://doi.org/10.1016/0031-9422(88)83138-8.

Elgamal MHA, Shaker KH, Pöllmann K, Seifert K. 1995. Triterpenoid saponins from *Zygophyllum* species, Phytochemistry **40,** 1233-1236. https://doi.org/10.1016/0031-9422(95)00436-B.

Eskander EF, Won JH. 1995. Hypoglycaemic and hyperinsulinemic effects of some Egyptian herbs used for treatment of diabetes mellitus (Type II) in rats, Egyptian Journal of Pharmaceutical Sciences **36,** 331-342.

Fenwick GR, Price KR, Tsukamoto C, Okubo K. 1991. Toxic Substances in Crop Plants, eds. J.P.F. D'Mello, C. M. Duffus, H. Duffus, The Royal Society of Chemistry, Cambridge.

Ghafoor A. Zygophyllaceae. 1974. In: Nasir E, Ali SI, editors. Flora of West Pakistan. Karachi: University of Karachi, p 1-35.

Ghazala. 1992. Studies on chemical constituents of *Zygophyllum propinquum* (*Z. coccineum*). PhD thesis, HEJ Research Institute of Chemistry, University of Karachi, Pakistan.

Gibbons S, Oriowo MA. 2001. Antihypertensive effect of an aqueous extract of Zygophyllum coccineum L. in rats, Phytotherapy Research **15,** 452-455. https://doi.org/10.1002/ptr.836.

Hostettmann K, Marston A. 1995. Saponins. Cambridge University Press, Cambridge.

Kofler L. 1927. Die Saponine, Julius Springer Verlag, Vienna. 7-40 p. https://doi.org/10.1007/978-3-7091-9826-1_3.

Nasir E, Ali S. (Eds.). 1970-1979. Flora of West Pakistan. No. 1-131. Islamabad, Karachi.

Pöllmann K, Gagel S, Elgamal MHA, Shaker CH, Seifert K. 1997. Triterpenoid saponins from the roots of *Zygophyllum* species, Phytochemistry **44,** 485-489. https://doi.org/10.1016/s0031-9422(96)00552-3.

Qaisar M, Uddin G, Ahmad VU, Rauf A. 2011. Mass Fragmentation Pattern of New Zygophyllosides from *Zygophyllum propinquum* Decne, Middle-East Journal of Scientific Research **8,** 526-529.

Reed JD. 1997. Ecological biochemistry of secondary plant compounds in herbivore nutrition. Session 11-Constraints on Animal Production from forages and grasslands. http://www.internationalgrasslands.org/files/igc/publications/1997/iii-221.pdf.

Saber AH, El-Moghazy Shoaib AM. 1960. Zygophyllum coccineum. V. Chemistry of leaf and Stem. Journal of Pharmaceutical Science of the U.A.R., **1**, 1-6.

Soliman G. 1939. Quinovic acid from *Zygophyllum coccineum* L., Journal of Chemical Society 1760-1761. https://doi.org/10.1039/JR9390001760.

Van Setten DC, ten Hove GJ, Wiertz EJHJ, Kamerling JP, Van de Werken G. 1998. Multiple stage tendom mass spectrometry for structural characterization of saponins, Analytical Chemistry **70,** 4401-4409. https://doi.org/10.1021/ac980365q.