Phytochemistry Letters 13 (2015) 256–261

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Triterpenoids and triterpenoid saponins from the aerial parts of *Fagonia indica* Burm

Rabia Farheen[a], Bina Shaheen Siddiqui[b,*], Iffat Mahmood[a], Shabana Usman Simjee[b,c], Saba Majeed[b]

[a] Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal, Karachi 75270, Pakistan
[b] HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 29 June 2015
Accepted 1 July 2015
Available online xxx

Keywords:
Fagonia indica
Leaves
Twigs and fruits
Triterpenes
Triterpenoid saponins
Cytotoxicity

ABSTRACT

As part of our search of new bioactive compounds from indigenous medicinal plants, phytochemical investigation of the aerial parts of *Fagonia indica* Burm led to the isolation of seven compounds including two new compounds, namely, indicacin (**1**) and fagonicin (**2**), and five known compounds (**3**–**7**) from the methanol extract. Compounds **6** and **7** are hitherto unreported from this plant. The structures of the new compounds were elucidated from their spectral data, mainly HREIMS, 1D NMR (^{1}H, ^{13}C NMR, and DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC), and by comparison with the literature data. The new compounds **1** and **2** were assayed for their cytotoxicity against human colorectal cancer cell line H-29. Compound **1** exhibited 51.40% cytotoxicity at 6.25 μM/mL dose whereas compound **2** demonstrated 39.3% cytotoxicity at the same dose.

1. Introduction

Fagonia indica Burm belongs to the family Zygophyllaceae (Pullaiah, 2006). It is commonly known as 'sachchi booti' in Pakistan. It is a small genus of erect or prostrates, more or less woody herbs and under shrubs (Manjunath, 1956). All species of *Fagonia* are shrub, sherbets or herbs, rarely higher than (60–100) cm, and up to about 100 cm wide. The genus *Fagonia* is confined to warm and arid areas of all continents except Australia (Beier, 2005). It is found mostly in Indo-Pakistan subcontinent westwards to North and East tropical Africa in arid and semi arid region (Shinwari and Shah, 2003). In Pakistan it is represented by 8 genera and 22 species (Ghafoor, 1974). The plant has been used by local people in the treatment of fever, asthma, vomiting, dysentery, urinary discharge, leucoderma, biliousness and typhoid and as blood purifier and plant ash is given to children suffering from anemia. The fruit of this plant is rich in ascorbic acid (Khare 2007). The twigs are commonly applied as tooth brushes and bark in the scabies (Shinwari and Shah, 2003). Aerial parts of *Fagonia indica Burm* are used as a remedy for tumors, and leaf and twigs are used for cancer (Graham et al., 2000).

Several triterpenes and saponins have been reported from this genus which include nahagenin, betulic acid (Rahman et al., 1982), hederagenin and ursolic acid (Rahman et al., 1984), 3β, 23-dihydroxy-23,28-di-*O*-β-D-glucopyranosyl-taraxer-20-en-28-oic acid, 23,28-di-*O*-3β-*O*-D-glucopyranosyl-23-hydroxy taraxer-20-en-28 oic acid (Ansari et al., 1987), 21, 22α-epoxy-23-*O*-β-D-glucopyranosyl-nahagenin (Ansari et al., 1988), 3-sulfate esters of 3β,27-dihydroxyolean-12-en-28-oic acid and 3β-27-dihydroxy-28-carboxy-*O*-β-D-glucopyranosylolean-12-en (Perrone et al., 2007). In the present article, we report on the isolation and structure elucidation of two new compounds, a triterpenoid glycoside (**1**), a triterpene (**2**) and five known compounds (**3**–**7**) from the methanol extract of the aerial parts of *Fagonia indica* Burm. Compounds **5** and **6** are reported for the first time from this plant.

2. Results and discussion

Aerials parts of *Fagonia indica* were repeatedly extracted with MeOH by soaking the plant material in methanol at room temperature. The methanol extract was shaken out with ethyl acetate and water. After usual workup the residue obtained from ethyl acetate fraction was subjected to various chromatographic techniques to afford compounds **5**–**7**. The aqueous phase was treated with *n*-butanol and the butanol fraction afforded

* Corresponding author. Fax: +92 21 99261713.
E-mail address: siddiqui_bina@yahoo.com (B.S. Siddiqui).

http://dx.doi.org/10.1016/j.phytol.2015.07.001
1874-3900/

compounds **1–4** after repeated reverse phase column and thick layer chromatography (Fig. 1).

Known compounds were identified as 3β-*O*-β-D-glucopyranosyl-20-en-23-hydroxytaraxer-28-oic acid (**3**) and 3β-hydroxy-23-*O*-β-D-glucopyranosyl-28-carboxy-*O*-β-D-glucopyranosyl-taraxer-20-en **(4)** (Akbar et al., 1987), β-amyrin (**5**) (Mahato and Kundu, 1994), lupeol (**6**) (Mahato and Kundu, 1994; Burns et al., 2000), and β-sitosterol (**7**) (Zhang et al., 2006) respectively, by comparison of their mass, ^{1}H and ^{13}C NMR spectral data with the reported data.

The new compounds (**1**) and (**2**) were assayed for their cytotoxicity against human colorectal cancer cell line H-29 and showed 51.40% and 48.3% inhibition at 6.25 and 12.5 μM/mL concentration respectively.

Compound **1** was isolated as white amorphous powder. Its molecular formula was determined as $C_{36}H_{56}O_{10}$ on the basis of HRFABMS (negative mode) peak of $[M-H]^+$ at *m/z* 647.3802 $[M-H]^+$ (calc. 647.3780 for $C_{36}H_{55}O_{10}$) from the HRFABMS and HREIMS at *m/z* 468.6736 ($C_{30}H_{44}O_4$; M^+-glucose) and ^{13}C NMR spectral data. Its IR spectrum showed absorptions for hydroxyl (3425 cm^{-1}), carbonyl (1730 cm^{-1}), carboxyl (1710) and olefin (1658 cm^{-1}). The ^{1}H NMR spectrum showed five methyl singlets at δ 0.74, 0.81, 0.85, 1.15 and 1.26 connected to carbons at δ 16.4, 18.1, 16.2, 26.7 and 28.3 respectively in the HSQC spectrum, a triplet for H-12 of pentacyclic triterpenes at δ_H 5.20 (1H, t, J = 3.6 Hz) connected to C-12 at δ_C 122.8 and a double doublet at δ_H 2.81 (dd, J = 13.0, 4.5) for H-18 connected to C-18 at δ_C 42.7. These data were suggestive of an oleanane type skeleton of **1.** Further, two

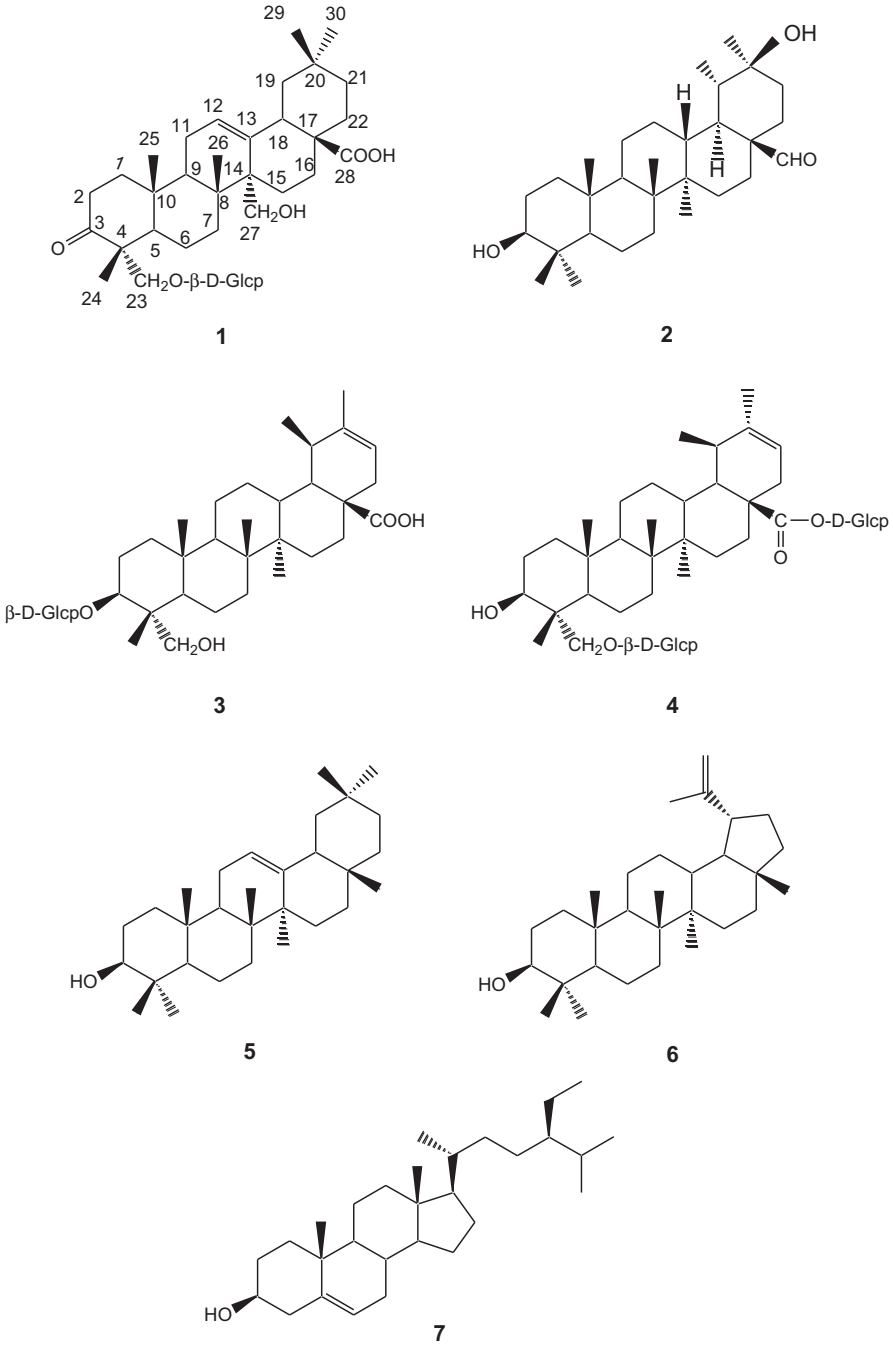

Fig. 1. Structures of 7 compounds isolated from *Fagonia indica*.

pairs of downfield methylene protons at δ_H 3.40, 3.62 (d, J = 10.8 Hz, H-23a/b) and δ_H 3.24, 3.55 (d, J = 11.0 Hz, H-27a/b) showing HSQC correlation with C-23 (δ 66.4) and C-27 (δ 76.4) revealed that two methyls of the primary skeleton have been functionalized to a hydroxymethylene group (C-27) and a oxymethylene (C-23). In addition, the hydroxyl group at C-3 was not present and instead a carbonyl group was indicated at δ_C 211.7. The location of a hydroxyl group at C-23 was deduced by the chemical shifts of C-23 and C-24 (Table 1) (Mahato and Kundu, 1994) and the location of hydroxyl group at C-27 and of the carbonyl group at C-3 was assigned by HMBC correlations of H-27 with C-13 and C-14 and of both H-23 and H-24 with C-3. The ^{1}H and ^{13}C NMR data (Table 1) further revealed a glucose moiety by an anomeric proton at δ_H 4.45 (1H, d, J = 7.5 Hz, H-1′) connected to the anomeric carbon at δ_C 102.8 (C-1′) in the HSQC spectrum, oxygen bearing methylene protons at δ 3.79 (1H, dd, J = 11.7, 4.2 Hz, H-6′a) and δ 3.92 (1H, dd J = 11.7, 2.7 Hz, H-6′b) connected to a CH_2 carbon at δ 62.4 (C-6′) and four methine protons at δ 4.14, 3.85, 4.22 and 3.20 (each 1H, m) connected to CH carbons at δ 81.1, 75.4, 77.4, 76.1 in the HSQC spectrum. Both H-23a and H-23b showed ^3J connectivity in the HMBC spectrum with the anomeric carbon at δ_C 102.8 (C-1′) and an up field quaternary carbon at δ 48.0 (C-4). Hence glucose was placed at C-23. The connectivity of H-1′ with H-2′; of H-2′ with H-3′; of H-3′ with H-4′; of H-4′ with H-5′; and of H-5′ with H-6′ in the ^{1}H–^{1}H COSY spectrum led to assign each proton of the sugar moiety. The NMR profile of sugar protons and carbons showed that glucose was β-D-pyranose. The NOESY correlations between H-5, H-9 and H-23a/b and between H-9 and H-27a/b supported the assignment of H-23 and H-27, and their respective carbons through HSQC interactions. Thus the structure of indicacin was determined as 3-oxo-12-en-23-*O*-β-D-glucopyranosyl-27-hydroxyolean-28-oic acid. The sugar moiety was further confirmed as D-glucose through acid hydrolysis of **1** followed by GC analysis of the ester derivative and comparison with authentic sample.

Compound **2** was obtained as amorphous powder. Its molecular mass was determined on the basis of HREIMS at *m/z* 458.7222 which was in accordance with its molecular formula $C_{30}H_{50}O_3$. Analysis of its NMR data indicated that it is a pentacyclic triterpene of ursane skeleton (Seebacher et al., 2003). Thus ^{13}C NMR (broad band, DEPT 90° and 135°) showed 30 carbons including 7 methyls, 10 methylenes, 7 methines and 6 quarternary carbons. The ^{1}H NMR spectrum revealed the presence of six methyl singlets at δ 0.73, 0.80, 0.87, 0.94, 0.96 and 1.12 and one methyl doublet at δ 1.22 (J = 6.0 Hz). Accordingly, it was indicated that **2** is a taraxarane or ursane triterpenoid. The presence of only one methyl doublet further indicated a substituent at either C-19 or C-20. The molecular formula showed index of hydrogen deficiency as six, justifying a pentacyclic triterpenoid skeleton and one aldehyde group as indicated by the IR (ν_{max} 2730, 1715 cm^{-1}) as well as ^{1}H and ^{13}C NMR spectra (δ_H 9.51, s, δ_C 206.7). Two downfield carbons at δ 78.9 (CH) and δ 73.2 (C) (Table 2) showed the presence of two hydroxyl groups indicated by the IR spectrum (ν_{max} 3384 cm^{-1}). The secondary hydroxyl group was placed at C-3 on biogenetic consideration and its β-disposition was decided from the double doublet of H-3 (δ 3.14, J = 11.2, 4.8 Hz). The aldehyde proton (δ 9.51) showed HMBC interaction with C-17 (δ 61.5) and C-18 (δ 49.3), thus indicating the aldehyde group to be located at C-28. In order to decide between the ursane or taraxarane skeleton and location of second OH group at C-19 or C-20, the carbon-13 NMR data of C-13, C-18, C-29 and C-30 of **2** were compared with several examples of similar structures in the literature and were found closer to those of taraxarane skeleton. In both 19- and 20-hydroxy ursane, C-13 appears downfield at δ 50.6 (Misra and Laatsch, 2000) and δ 49.4 (Kuo et al., 2014) respectively while in taraxarane having a hydroxyl or oxygen substituent at C-20, C-13 shift appears at δ 38.9 (Susunaga et al., 2001) and 39.3 (Zhao et al., 2006) which were closer to that of **2** (δ 38.1) thus suggesting it to be a 20-hydroxy taraxarane triterpene. The NOESY interactions between H-18 and H-27, H-29 as well as between H-13 and H-19, H-26 further supported that ring-D and -E are *trans*-fused. Chemical shifts for C-20 (δ 73.2) and C-30 (δ 31.2) of **2** compare well with 20*S* ($\delta_{C\text{-}20}$:73.6; $\delta_{C\text{-}30}$: 30.3) geometry as against 20*R* ($\delta_{C\text{-}20}$: 75.3; $\delta_{C\text{-}20}$:27.4) (Susunaga et al., 2001) geometry suggesting that the hydroxyl group at C-20 is β and hence **2** has *S* configuration at C-20. Similar analogies were noted for C-18 shifts which are in agreement with a 20*S*-hydroxytaraxastane. A relatively downfield shift of C-17 may be attributed to hydrogen bonding between OH at C-20 and CHO at C-17 and a δ effect of OH on C-17 (Eggert et al., 1976). The configuration of various centers and assignment of methyl groups was determined through NOESY interactions between H-24, H-25 and H-26; of H-23 with H-3 and H-27 as well as of CHO with C-26. Hence the structure of (**2**) was defined as 3β, 20*S*-dihydroxytaraxastane-28-al.

The NMR data of compounds **3** and **4** (Table 2) matched well with those reported in literature (Ansari et al., 1987). However this report presents a complete assignment of all the protons and

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compound **1** in MeOH-d4 (δ, ppm; J, Hz).

Position	δ_C	δ_H	Position	δ_C	δ_H
1/CH_2	39.2	1.26 (m), 1.47 (m)	19/CH_2	43.2	1.60 (m), 0.85 (m)
2/CH_2	33.7	2.13 (m), 2.61 (m)	20/C	30.7	–
3/C	211.7	–	21/CH_2	30.6	1.46 (m), 1.15 (m)
4/C	48.0	–	22/CH_2	30.2	2.01 (m), 1.50 (m)
5/CH	49.0	1.66 (m)	23/CH_2	66.4	3.40 (d, 10.8), 3.62 (d, 10.8)
6/CH_2	19.2	1.43 (m), 1.61 (m)	24/CH_3	16.4	0.74 (s)
7/CH_2	33.5	1.25 (m), 1.27 (m)	25/CH_3	16.2	0.85 (s)
8/C	39.0	–	26/CH_3	18.1	0.81 (s)
9/CH	46.0	1.47 (m)	27/CH_2	76.4	3.24 (d, 11.0), 3.55 (d, 11.0)
10/C	37.9	–	28/C	177.1	–
11/CH_2	24.4	1.88 (m), 1.73 (m)	29/CH_3	28.3	1.26 (s)
12/CH	122.8	5.20 (t, 3.6)	30/CH_3	26.7	1.15 (s)
13/C	146.0	–	Glucose:		
14/C	40.4	–	1′/CH	102.8	4.45 (d, 7.5)
15/CH_2	27.0	1.15 (m), 1.54 (m)	2′/CH	81.1	4.14 (m)
16/CH_2	24.4	1.88 (m), 1.01 (m)	3′/CH	75.4	3.85 (m)
17/C	47.0	–	4′/CH	77.4	4.22 (m)
18/CH	42.7	2.81 (dd, 13.0, 4.5)	5′/CH	76.1	3.20 (m)
			6′/CH_2	62.4	3.79 (dd, 11.7, 4.2), 3.92 (dd, 11.7, 2.7)

Assignments based on HSQC, HMBC and DEPT experiments.

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **2** in $CHCl_3$ (δ, ppm: *J*, Hz).

Position	δ_C	δ_H	Position	δ_C	δ_H
1/CH_2	38.6	0.87 (m), 1.68 (d, 3.2)	16/CH_2	27.3	1.61 (m), 1.53 (m)
2/CH_2	28.1	0.94 (m), 0.96 (m)	17/C	61.5	–
3/CH	78.9	3.14 (dd, 11.2, 4.8)	18/CH	49.3	2.18 (dd, 10.8)
4/C	38.8	–	19/CH	46.8	1.68 (m)
5/CH	55.1	0.67 (m)	20/CH	73.2	
6/CH_2	18.2	1.34 (m), 1.48 (m)	21/CH_2	29.5	1.22 (m) 1.40 (m)
7/CH_2	33.0	1.24 (m), 1.58 (m)	22/CH_2	34.5	2.01 (m), 1.50 (m)
8/C	41.2	–	23/CH_3	27.9	0.94
9/CH	50.3	2.19 (m)	24/CH_3	15.3	0.73 (s)
10/C	37.0	–	25/CH_3	16.1	0.80 (s)
11/CH_2	21.2	1.21 (m), 1.47 (m)	26/CH_3	16.2	0.87 (s)
12/CH	29.2	0.94 (m), 1.23 (m)	27/CH_3	14.5	0.96
13/C	38.1	2.02 (m)	28/CH	206.7	9.51
14/C	43.4	–	29/CH_3	25.0	1.22 (d, 6.0)
15/CH_2	29.0	0.95 (m), 1.22 (m)	30/CH_3	31.2	1.12 (s)

Assignments based on HSQC, HMBC and DEPT experiments.

carbons on the basis of 2D NMR analysis. Compounds **5, 6** and **7** were identified by matching their NMR data with the reported values as β-amyrin (Mahato and Kundu, 1994), lupeol (Burns et al., 2000) and β-sitosterol (Zhang et al., 2006).

3. Experimental

3.1. General experiment

Infrared spectra were measured on VECTOR 22 spectrophotometer; ν in cm^{-1}. Specific rotations were determined using JASCO P-200 polarimeter. NMR spectra were measured on Avance AV-300, and AV-500 and AV-600 spectrometers operating at 300, 500 and 600 MHz for ^{1}H NMR and 75, 125 and 150 MHz respectively for ^{13}C NMR spectra. EI and HREIMS were recorded on MSRoute mass spectrometer and Jeol-JMS-HX-110 instruments respectively. Column chromatography (CC) was performed with silica gel 60 PF_{254} (*E. Merck*, 70–230 mesh size). Aluminum cards precoated (0.5 mm thickness) with silica gel SiF_{254} (*E. Merck*) and *RP-18* F_{254} *S* (*E. Merck*) were used for analytical chromatography. Sephadex LH-20, (Sigma–Aldrich) and silica gel RP-18 (Merck) were used for column chromatography of more polar fractions. For TLC of polar compounds RP-18 F_{254} (*E. Merck*, 0.25 mm) precoatd TLC glass plates were used. HPLC was carried out using preparative recycling HPLC (JAIGEL-ODS-M80).

3.2. Cancer cell line, chemicals and spectral measurements

For anti-cancer activity, human colorectal cancer cell line HT 29 was purchased from American Type Tissue Culture Collection (ATCC, USA). The cells were cultured in DMEM (Sigma Chemical, MO, USA) supplemented with 1% penicillin and streptomycin, 1%, amphoterecin B, 1% sodium pyruvate, 1% L-glutamine and 10% fetal bovine serum [FBS (Sigma Chemical, MO, USA)] in a humidified atmosphere at 37 °C containing 5% CO_2. Gas chromatography was carried out using flame ionization detector (FID) on the less polar capillary column OPTIMA®-5-Accent (60m × 0.32 mm ID with 0.25 μm film thickness of 5% phenyl/95% methyl silicone) installed on a Shimadzu GC-10 for GC-FID analysis. The analysis was performed with an initial temperature 50 °C for 2 min then ramped at a rate of 7 °C/min. to a final temperature of 260 °C with holding time of 30 min. Injector with a splitting ratio of 1:8 was set at 235 °C and FID at 400 °C. Carrier and make up gas was nitrogen with a flow of 12.334 mL/min. at a pressure of 90 kPa. For GC-MS experiments Agilent 6890 gas chromatograph, equipped with ZB-5MS (30m × 0.32 ID and 0.25 μm film thickness) was combined with a Jeol, JMS-600H mass Spectrometer operating in EI mode with ion source at 250 °C and electron energy at 70 eV. Carrier gas was helium at a pressure of 17.2 psi. All fractions were dissolved in chloroform and injection volume was adjusted between 1.0 and 5.0 mL depending upon the detector response.

3.3. Plant material

The aerial parts of *Fagonia indica* Burm were collected from Hyderabad, Sindh region during the month of August to September. The plant was authenticated by Afshen Ather, Curator of the Centre for Plant Conservation, Department of Botany, University of Karachi and a voucher specimen (no. 86583) was deposited in the Herbarium of the same department.

3.4. Extraction and isolation

The aerial parts (5 kg) of *Fagonia indica* were repeatedly (×3) extracted with methanol (12 L each time) at room temperature. The extract obtained on evaporation of solvent under reduced pressure was partitioned between EtOAc and water. The EtOAc layer was treated with anhyd. Na_2SO_4, charcoaled and concentrated. A part (2 g) of this residue (60 g) on silica gel CC and elution with petrol-EtOAc (9:1) afforded a mixture of compounds which were purified on repeated silica gel CC using mixture of petrol-EtOAc to afford **5** (9.5:0.5; 32.3 mg), **6** (9.25: 0.75; 25.6 mg) and **7** (9:1; 15 mg).

The main aqueous phase was concentrated under reduced pressure and treated with *n*-BuOH. The *n*-butanolic phase was washed, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a gummy residue (125 g). This was separated by reverse phase column chromatography (LH-20; H_2O, H_2O-MeOH gradient system, 1:1 to 2:8) to afford 3 major fractions (LH-1, LH-2 and LH-3). LH-1 (0.50 g) was further purified by reverse phase column chromatography (RP-18 silica gel; H_2O-MeOH; 1:1) and yielded 10 fractions. Fraction 1 (0.37 g) was reloaded on RP-18 column to give 15 fractions. Fraction no. 12 afforded an amorphous solid pure on TLC. This was identified as **3** (28.0 mg). Fraction LH-2 (0.58 g) afforded 36 fractions. These were combined on the basis of TLC to give 10 fractions. Fraction 10 was subjected to preparative recycling HPLC (JAIGEL-ODS-M80; Serial 208,550, JAL LC-908W) using MeOH-H_2O (60:40) as mobile phase; flow rate (3 mL/min) to afford one pure compound characterized as a new compound **2** (18.0 mg). LH-3 (0.24 g) gave 10 fractions. These fractions were combined on the basis of TLC to give 3 fractions. Fraction 2 was further purified on RP-18 column (H_2O: MeOH; 1:1). It afforded a pure compound characterized as a new constituent **1** (12.5 mg). Fraction 3 was purified on RP-18 TLC cards using MeOH-H_2O to afford **4** (8:2; 5.8 mg).

3.5. 3-Oxo-12-en-23-O-β-D-glucopyranosyl-27-hydroxyolean-28-oic acid (*1*) (=indicacin)

Colorless amorphous powder; $[\alpha]_D^{24}$ + 11.5° (*c*, 0.3, MeOH); HRFABMS *m/z*: 647.3802 $[M-H]^+$ (calc. 647.3780 for $C_{36}H_{55}O_{10}$). HREIMS *m/z*: 468.6736 ($C_{30}H_{44}O_4$; M^+-$C_6H_{12}O_6$; calcd for $C_{30}H_{44}O_4$, 468.6752); IR (KBr) ν_{max} cm^{-1}: 3425, 3093, 2984, 1730, 1710, 1658. ^{1}H and ^{13}C NMR data are given in Table 1.

3.6. 3β, 20S-Dihydroxytaraxastane28-al (*2*) (=fagonicin)

Colorless amorphous powder; $[\alpha]_D^{24}$ + 34.5° (*c* = 0.3, MeOH); HREIMS *m/z*: 458.7222 ($C_{30}H_{50}O_3$; M^+; calcd. for $C_{30}H_{50}O_3$ 458.7232); IR ν_{max} ($CHCl_3$) cm^{-1}: 3384, 2933, 2730, 1715. ^{1}H and ^{13}C NMR data are given in Table 2.

3.7. 3β-O-β-D-Glucopyranosyl-20-en-23-hydroxytaraxer-28-oic acid (**3**)

Colorless amorphous powder; $[\alpha]_D$ ^{24} +2^{00} (*c*, 0.3, MeOH); HREIMS *m/z* (rel.int.): 634.8428 ($C_{36}H_{58}O_9$; M^+; calcd for $C_{36}H_{58}O_9^+$, 634.8488), 456.7060 ($C_{30}H_{48}O_3$; M^+-$C_6H_{12}O_6$ calcd for $C_{30}H_{48}O_3^+$, 456.7074) (5.0), 248.1766 ($C_{16}H_{24}O_2$) (30.3), 203.1811 ($C_{15}H_{23}$) (65.2), 133.1027 ($C_{10}H_{13}$) (62.3), 55.0774 (C_4H_7) (33.2). ^{1}H and ^{13}C NMR data are given in Table 3.

3.8. 3β-Hydroxy-23-O-β-D-glucopyranosyl-28-carboxy-O-β-D-glucopyranosyl-taraxer-20-en (**4**)

Colorless amorphous powder; $[\alpha]_{578}$ ^{24} + 1° (*c*, 0.4; MeOH); HREIMS *m/z* (rel.int.): 796.9970 ($C_{42}H_{68}O_{14}$; M^+; calcd for $C_{42}H_{68}O_{14}^+$, 796.9908), 436.6764 ($C_{30}H_{44}O_2$; M^+-2($C_6H_{12}O_6$; calcd for $C_{30}H_{44}O_2^+$, 436.6764) (28), 248.1766 ($C_{16}H_{24}O_2$) (23.3), 203.1811 ($C_{15}H_{23}$) (57.2), 133.1027 ($C_{10}H_{13}$) (45.3), 55.0774 (C_4H_7) (10.0); ^{1}H and ^{13}C NMR data are given in Table 3.

Table 3
NMR data of compounds **3** (^{1}H 500 and ^{13}C 125 MHz) and **4** (^{1}H 300 and ^{13}C 75 MHz) in MeOH-d4 (δ, ppm; J, Hz).

Position	3		4	
	δ_C	δ_H	δ_C	δ_H
1/CH_2	39.5	1.00 (m), 1.59 (m)	39.4	1.06 (m), 1.06 (m)
2/CH_2	27.4	1.59 (m), 1.59 (m)	27.5	1.60 (m), 1.60 (m)
3/CH	73.1	3.82 (dd,11.2, 4.5)	78.6	3.49 (dd, 11.2, 4.5)
4/C	42.0	–	42.1	–
5/CH	48.9	1.28 (d,11.0)	48.5	1.31 (d, 11.0)
6/CH_2	19.1	1.48 (m), 1.34 (m)	19.0	1.47 (m), 1.31 (m)
7/ CH_2	35.0	1.48 (m), 1.30 (m)	34.8	1.47 (m), 1.31 (m)
8/C	43.1	–	43.1	–
9/CH	51.8	1.45 (dd, 9.5,4.1)	51.5	1.47 (dd, 9.5, 4.1)
10/C	38.0	–	38.0	–
11/CH_2	22.9	1.30 (m), 1.21 (m)	22.8	1.60 (m), 1.21 (m)
12/CH_2	28.9	1.20 (m), 1.59 (m)	28.7	1.20 (m), 1.68 (m)
13/CH	40.3	2.30 (ddd)	40.4	2.30 (ddd)
14/C	43.4	–	43.5	–
15/CH_2	30.5	1.12 (m), 1.48 (m)	30.2	1.12(m), 1.55(m)
16/CH_2	35.1	1.48 (m), 1.88 (m)	33.7	1.43(m), 2.03(m)
17/C	50.9	–	50.4	–
18/CH	50.2	1.21 (d, 10.8)	50.2	1.21 (d, 10.8)
19/CH	38.6	2.07 (m)	38.5	2.07 (m)
20/C	143.6	–	144.0	–
21/CH	118.8	5.22 (br.d, 6.6)	118.1	5.22 (br.d, 6.6)
22/CH_2	39.8	1.59 (br. d,15.9), 2.27 (dd,15.9,7.2)	38.3	1.78 (br. d, 15.9) 2.27 (dd,15.9,7.2)
23/CH_2	75.9	3.70 (d,10.0), 3.42 (d,10.0)	75.6	3.66 (d, 11.5), 3.44 (d,11.5)
24/CH_3	12.7	0.70 (s)	12.6	0.69 (s)
25/CH_3	17.3	0.88 (s)	17.3	0.87 (s)
26/CH_3	16.9	0.97 (s)	16.6	0.94 (s)
27/CH_3	15.5	0.98 (s)	15.5	0.99 (s)
28/C	170.3	–	175.8	–
29/CH_3	24.1	1.00 (d, 6.6)	23.8	1.00 (d, 6.6)
30/CH_3	22.2	1.59 (s)	22.0	1.60 (s)
Glu-1				
1′/CH	102.9	4.44 (d, 8.0)	102.9	4.44 (d, 7.5)
2′/CH	81.2	4.15 (dd, 8.5,7.5)	81.1	4.15 (m)
3′/CH	75.6	3.83 (m)	75.7	3.85 (m)
4′/CH	76.2	3.44 (m)	76.1	3.44 (m)
5′/CH	77.2	4.22 (m)	77.1	4.23 (m)
6′/CH_2	62.5	3.89 (dd,11.7,4.2) 3.79 (dd,11.7,2.7)	62.4	3.78 (dd,11.7,4.2) 3.88 (dd,11.7,2.7)
Glu-2				
1″/CH	–	–	95.7	5.39 (d, 8.1)
2″/CH	–	–	71.1	3.33 (m)
3″/CH	–	–	74.2	3.25 (m)
4″/CH	–	–	78.3	3.38 (m)
5″/CH	–	–	72.7	3.83 (m)
6″/CH_2	–	–	62.4	3.78 (dd,12.0,5.4) 3.69 (dd,12.0,3.0)

Assignments based on HSQC, HMBC and DEPT experiments.

3.9. Acid hydrolysis of compound **1**

Compound **1** (2 mg) was refluxed with HCl (0.5 N, 2 mL) for 2 h. The hydrolyzed product was extracted with CH_2Cl_2. The residue obtained on usual work up of the aqueous layer was dissolved in pyridine (1 mL) and a solution of L-cysteine ethyl ester hydrochloride (0.1 M) in pyridine (2 mL) was added. The reaction mixture was heated at 60 °C for 1 h. Acetic anhydride (1 mL) was then added and the mixture was heated for another 1 h. Acetylated thiazolidine derivative, obtained after drying the mixture in vacuum was subjected to GC under following conditions: capillary column SPB-5 (60 m × 0.32 mm, 0.25 lm); carrier gas N2; injection temperature 250 °C; detection temperature 260 °C; column temperature 150 °C (1 min), 10 °C/min to 260 °C (60 min). The configuration was determined by comparing the retention time with acetylated thiazolidine derivatives prepared in a similar manner from standard sugar (t_R D-glucose 24.04 min).

3.10. Determination of growth inhibition activity

3.10.1. Compounds preparation

The compounds (each 1 mg/mL) were taken in sterile 0.01 N NaOH to obtain the stock solutions. The working solutions were prepared from the stock solution by diluting it in the Dulbecco modified Eagle medium (DMEM). Five different working concentrations (Table 4) were used to treat the HT 29 cells for MTT assay. The cells treated with only NaOH were used as control. Final concentration of NaOH used in the assay was 0.01 N.

3.10.2. Microscopy

Cells were grown on 25 cm^3 flasks and treated with test fractions mentioned above or 0.01N of NaOH for 24 h. The Nikon inverted microscope was used at 10× and 20× magnifications to examine the effect of fractions of *F. indica* on growth inhibition of HT 29 cells. MTT assay was used further to validate our observation.

3.10.3. MTT assay to evaluate growth inhibition

The effect of compounds **1** and **2** on growth inhibition of colorectal cancer cells was evaluated by the MTT assay which calorimetrically quantifies insoluble purple formazan compound produce by viable cells. Briefly, monolayer of the cells was trypsinized, re-suspended in medium containing 10% FBS and 2×10^3 cells/100 μL were plated in 96-wells plate and kept in a humidified atmosphere at 37 °C containing 5% CO_2. After 24 h when monolayer was established media was aspirated and 100 μL

Table 4
Growth inhibitory effect indicacin (**1**) and fagonicin (**2**) isolated from *F. indica* on HT 29 cell line at different concentrations.

Compounds	Concentration (μM/mL)	% Growth inhibition
Indicacin	6.25	51.40404
	12.50	48.31951
	25.0	47.09734
	50.0	29.75411
Fagonicin	6.25	39.3569
	12.50	48.37771
	25.0	37.55274
	50.0	34.35181
Control (NaOH)	6.25	0.758
	12.50	0.741
	25.0	0.816
	50.0	0.827

Average of three independent determinations values are mean ± SD.

of various concentrations of fractions and test compounds of *F. indica* in media containing 1% FBS were added. Maximum NaOH concentration used was 0.01 N. Cells treated with 0.01% NaOH in media (containing 1% FBS) were used as untreated controls. After 24 h, sample solution in the wells was flicked off and 100 μL of 0.5 mg/mL MTT dye (Promega, USA) was added to each well and incubated in 37 °C, 5% CO_2 for 3 h. After incubation, supernatant was removed and 100 μL of DMSO was added to each well to solubilize formazan. The absorbance was recorded on 96-wells plate ELISA reader at 490 nm. The percentage of viable cells following treatment was normalized to untreated controls. Assays were performed at least three times in triplicates. The growth inhibition (%) was calculated using the following formula:

$$\text{Growth inhibition}(\%) = 100 - \left[\frac{\text{test reading}}{\text{control reading}} \times 100\right]$$

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.07.001

References

Ansari, A.A., Kenne, L., Rahman, A., 1987. Isolation and characterization of two saponins from *Fagonia indica*. Phytochemistry 26, 1487–1490.

Ansari, A.A., Kenne, L., Rahman, A., Wehler, T., 1988. Isolation and characterization of a saponin from *Fagonia indica*. Phytochemistry 27, 3979–3982.

Beier, B.A., 2005. A revision of the desert shrub *Fagonia* (Zygophyllaceae). Syst. Biodivers. 3, 221–263.

Burns, D., Reynolds, W.F., Buchanan, G., Reese, P.B., Enriquez, R.G., 2000. Assignment of ^{1}H and ^{13}C spectra and investigation of hindered side-chain rotation in lupeol derivatives. Magn. Reson. Chem. 38 (7), 488–493.

Eggert, H., VanAntwerp, C.L., Bhacca, N.S., Djerassi, C., 1976. Carbon-13 nuclear magnetic spectra of hydroxyl steroids. J. Org. Chem. 41, 71–78.

Ghafoor, A., 1974. Zygophyllaceae. In: Nasir, E., Ali, S.I. (Eds.), Flora of West Pakistan, No. 76. National Herbarium, Agricultural Research Council, Rawalpindi, Pakistan.

Graham, J.G., Quinn, M.L., Fabricant, D.S., Farnsworth, N.R., 2000. Plants used against cancer- an extension of the work of Jonathan Hartwell. J. Ethnopharmacol. 73, 347–377.

Khare, C.P., 2007. Indian Medicinal Plants, an Illustrated Dictionary. Springer Verlag, Berlin/Heidelberg, Germany p. 259.

Kuo, P.-C., Chen, G.-F., Yang, M.-L., Lin, Y.-H., Peng, C.-C., 2014. Chemical constituents from the fruits of *Forsythia suspensa* and their antimicrobial activity. BioMed Res. Int.. http://dx.doi.org/10.1155/2014/304830.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids—a compilation and some salient features. Phytochemistry 37, 1517–1575.

Manjunath, B.L., 1956. The Wealth of India, vol. 4. Council of Scientific and Industrial Research, New Dehli p. 01.

Misra, L., Laatsch, H., 2000. Triterpenoids, essential oil and photo-oxidative 28-13-lactonization of oleanolic acid from *Lantana camara*. Phytochemistry 54, 969–974.

Perrone, A., Masullo, M., Bassarello, C., Hamed, A.I., Belisario, M.A., Pizza, C., Piacente, S., 2007. Sulfated triterpene derivatives from *Fagonia arabica*. J. Nat. Prod. 4, 584–588.

Rahman, A., Ansari, A.A., Steven, A., Clardy, J., 1982. The isolation and structure of nahagenin. Heterocycles 19, 217–220.

Rahman, A., Ansari, A.A., Lennart, K., 1984. Hederagenine, ursolic acid and pinatol from *Fagonia indica*. J. Nat. Prod. 47, 186–187.

Shinwari, M.I., Shah, M., 2003. Medicinal Plants of Margalla Hills National Park Islamabad. Pakistan Museum of Natural History, Islamabad p. 72.

Seebacher, W., Simic, N., Weis, R., Saf, R., Kunert, O., 2003. Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. Magn. Reson. Chem. 41, 636–638.

Susunaga, G.S., Siani, A.C., Pizzolati, M.G., Yunes, R.A., Monache, F.D., 2001. Triterpenes from the resin of *Protium heptaphylum*. Fitoterapia 72, 709–711.

Zhang, X., Cambrai, A., Miesch, M., Roussi, S., Raul, F., Aoude-Werner, D., Marchioni, E., 2006. Separation of Δ^5-and Δ^7-phytosterols by adsorption chromatography and semipreparative reversed phase high-performance liquid chromatography for quantitative analysis of phytosterols in foods. J. Agric. Food Chem. 54, 1196–1202.

Zhao, M., Zhang, H., Fu, L., Li, N., Bai, J., Sakai, J., Wang, L., Tang, W., Hasegawa, T., Ogura, H., Kataoka, T., Oka, S., Kiuch, M., Hirose, K., Ando, M., 2006. Taraxasterane- and ursane-type triterpenes from nerium oleander and their biological activities. J. Nat. Prod. 69, 1164–1167.