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A novel acylated flavonol tetraglycoside and rare oleanane saponins with a unique acetal-linked dicarboxylic acid substituent from the xero-halophyte *Bassia indica*

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ABSTRACT

In recent years, the scientific interest and particularly the economic significance of halophytic plants has been highly demanding due to the medicinal and nutraceutical potential of its bioactive compounds. A xero-halophyte *Bassia indica* is deemed to be a very cheap source of natural entities without chemical or biological investigation. In this context, a new acylated flavonol tetraglycoside, kaempferol-3-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-[*β*-D-galactopyranosyl-(1→3)-2-*O*-*trans*-feruloyl-*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**14**), together with rare occurring flavonol triglycoside, isorhamnetin-3-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**15**), were isolated from the aqueous methanol extract of the aerial parts of *B. indica*. The study also reported an optimal separation and characterization of a new *seco*-glycosidic oleanane saponin with 2′*R*,3′*S* stereocenters, identified as (2′*R*,3′*S*)-3-*O*-[2′-hydroxy-3′-(2″-*O*-glycolyl)-oxo-propionic acid-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosyl-olean-12-en-3*β*-ol-28-oic acid (**17**), in addition to its derivative, 3-*O*-[2′-(2″-*O*-glycolyl)-glyoxylyl-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosyl-olean-12-en-3*β*-ol-28-oic acid (**16**). The structures of all isolated compounds were elucidated based on 1D, 2D NMR, and HR-MS analysis, as well as comparing with similar derivatives published in the literature. Furthermore, thirteen known compounds were isolated and identified as *β*-sitosterol (**1**), vanillic acid (**2**), *o*-hydroxybenzoic acid (**3**), *p*-hydroxybenzoic acid (**4**), 6,7-dihydroxycoumarin (**5**), methyl caffeate (**6**), caffeic acid (**7**), quercetin (**8**), uracil (**9**), thymidine (**10**), tachioside (**11**), isorhamnetin-3-*O*-*β*-D-glucopyranoside (**12**), kaempferol-3-*O*-rutinoside (**13**). The anticholinesterase activity of all isolated compounds was evaluated.

1. Introduction

Natural product research continues to play a vital role in paving the way toward discovery of new sources of bioactive lead compounds throughout history and has been applied in many fields of medicine and pharmaceutical industry [1]. Up to date, there are many examples of natural compounds that have been developed into effective drugs for various diseases such as infectious diseases, Alzheimer, diabetes, and cancer, *etc.* [2].

Halophytes are plants able to grow in dry and saline habitats. They are regarded as a potential economic source of nutrients, bioactive constituents, in addition to the ability to display substantial health benefits [3]. The halophyte family Chenopodiaceae is comprising about 1400 species in 105 genera with several species growing in the Egyptian ecosystem [4,5]. Several chemical compounds related to diverse chemical classes were identified in Chenopodiaceae including alkaloids [6], saponins [7], flavonoid glycosides [8], ecdysteroids [9,10], sesquiterpenoids [11], lignanamides [12], phenolic acids [13,14], and coumarins [15]. Additionally, various members of this family have been implied to contain various compounds that have shown several biological activities such as antioxidant, anticholinesterase [16], anti-inflammatory [17], analgesic [18], antihyperlipidemic [19], antihypertensive [20], antitumor [21], hepatoprotective [22], antidiabetic [23], and tyrosinase inhibitory activity [24].

Bassia indica (Wight) A.J.Scott. (synonym: *Kochia indica*) is a xero-halophyte herb belongs to the family Chenopodiaceae and widely

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distributed in Egypt. It has been reported to display antitumor, antioxidant, and cardiotonic activities [25]. However, the detailed chemical composition and biological activities of *B. indica* have not been fully investigated. The plant *B. indica* is commonly eradicated by rural people which lead to waste of natural resources. Therefore, the utilization of such halophyte extracts with high nutritional and pharmaceutical value for their chemical and biological characterization represents a powerful tool in drug discovery with much less cost.

In continuation of our ongoing projects for the discovery of effective substances from halophytic plants distributed in the Egyptian ecosystem, a study was designed aiming at isolation, identification, and characterization of compounds from *B. indica* growing in Egyptian desert lands in order to valorize such kind of plants and to shed more light toward the utilization of halophytes as a very cheap and promising source of novel bioactive compounds.

The current study reports on the isolation and identification of 17 compounds, including one new acylated flavonol tetraglycoside, a rarely occurring flavonol triglycoside, and two rare occurring *seco*-glycosidic oleanane saponins. The anticholinesterase activity of the isolated compounds was also determined.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a JASCO P-2200 polarimeter. NMR spectra were recorded on a DRX 600 spectrometer (Bruker Daltonics, USA). Tetramethylsilane (TMS) was used as reference. Chemical shift and coupling constant were recorded in δ (ppm) and J (Hz), respectively. HR-ESI-MS data for compounds were recorded on a quadrupole time-of-flight mass spectrometer (Agilent QTOF-LC-MS, Agilent Technologies, USA). Fractionation and separation of compounds was performed on column chromatography packed with silica gel (75–150 μm, Merck, Darmstadt, Germany), RP_{18} (38–63 μm, Wako Pure Chemical Corporation, Osaka, Japan), Sephadex LH-20 (Sigma Aldrich, St. Louis, MO, USA), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), and Biotage selekt equipped with RP_{18} flash column chromatography (Biotage Selekt, Sfär C_{18} Duo column, 30 μm, 30 g). Final purification of the isolates was further achieved by using medium pressure liquid chromatography (Pure C-850 Flash prep®, Buchi, Switzerland) with UV-ELSD detection, normal flash columns (40 μm), RP_{18} flash columns (20 μm), Preparative column (Zorbax Extend-C_{18} PrepHT, 21.2×150 mm, 5 μm, Agilent, USA). Moreover, some isolates were purified on preparative NP-TLC (Wako Pure Chemical Corporation, Osaka, Japan) and preparative RP-TLC (Merck, Darmstadt, Germany). TLC investigation was carried out on plates precoated with silica gel GF254 and RP_{18}F254 (Merck, Darmstadt, Germany), then chromatograms were visualized under UV light at (254 and 365 nm) and sprayed with 10% MeOH-H_2SO_4 and/or Liebermann–Burchard reagents, followed by heating for 5 min at 105 °C. Acetylthiocholine iodide (ACTI) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Galantamine hydrobromide and AChE from *Electrophorus electricus* (electriceel), 518 U/mg, was purchased from Sigma (St. Louis, MO, USA). Ellman's reagent, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), was obtained from Wako company (Osaka, Japan).

2.2. Plant material

The flowering aerial parts of *B. indica* were collected from desert areas south of Egypt (Cairo-Assiut desert road) in September 2019. The plant was identified by Prof. Ibrahim A. El-Garf, Department of Botany, Faculty of Science, Cairo University, Egypt. A voucher specimen (BIC-2019) was prepared and deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Egypt.

2.3. Extraction and isolation

The dried aerial parts of *B. indica* (1 Kg) were extracted with 80% MeOH till exhaustion followed by evaporation to give a semi-solid dark brown residue (130 g, 13%). The obtained crude residue was suspended in distilled water (500 mL) and subjected to fractionation successively with *n*-hexane (500 mL × 6), EtOAc (500 mL × 6), and *n*-BuOH (500 mL × 4) to yield 32, 4, and 22 g, respectively.

The EtOAc fraction (3 g) was chromatographed over silica gel for CC (75–150 μm, 2 × 65 cm) and eluted with *n*-hexane containing increasing proportions of EtOAc and MeOH to obtain seven fractions (fractions 1 to 7).

Fraction 1 (136 mg) was chromatographed over Sephadex LH-20 column (30 g, MeOH; 150 mL) to afford compound **1** (23 mg). Fraction 2 (220.4 mg) was chromatographed over Sephadex LH-20 column (50 g, MeOH; 300 mL) to yield three subfractions (2–1, 2–2, and 2–3). Subfraction 2–1 (112 mg) was further purified over MPLC with silica gel flash column (2 columns; each 4 g) eluted with CH_2Cl_2-MeOH (9.5:0.5 *v/v*) as a mobile phase to obtain compounds **2** (6 mg), **3** (12 mg), **4** (8 mg), and **5** (10 mg), respectively. Subfraction 2–2 (62.2 mg) was further purified over Sephadex LH-20 column (30 g, 100% MeOH) to afford compound **6** (19 mg) and compound **7** (8.5 mg). Subfraction 2–3 (38.3 mg) was purified on preparative RP-TLC (H_2O-MeOH, 100 mL, 1:1 *v/v*) to afford compound **8** (3.3 mg).

Fraction 5 (1954 mg) was chromatographed on MPLC with RP-C18 flash column (40 μm, 12 g) using gradient elution system of H_2O-MeOH (9:1 to 0:1) as a mobile phase and a flow rate 10 ml/min to afford five major subfractions (5–1 to 5–5). Subfraction 5–1 (100 mg) was purified over Sephadex LH-20 column (30 g, MeOH; 150 mL) to afford **9** (10 mg). Subfraction 5–2 (162 mg) was purified over Sephadex LH-20 column (30 g, MeOH; 150 mL) then subjected to further purification over NP-TLC using CH_2Cl_2-MeOH as an eluent (9:1, 100 ml) to obtain compound **10** (6.2 mg) and compound **11** (4.5 mg) which was further purified over silica gel CC (1 g, CH_2Cl_2-MeOH, 8.5:1.5). Subfraction 5–5 (1380 mg) was subjected to purification over Sephadex LH-20 column (100 g, MeOH; 500 mL) to yield compound **12** (9 mg), while the rest of fraction (1100 mg) was chromatographed over MPLC with RP-C18 flash column (40 μM, 12 g) eluted gradually with H_2O-MeOH (9:1 to 0:1) to obtain four subfractions (6–1 to 6–4). Subfraction 6–3 (66 mg) was purified over Sephadex LH-20 column (20 g, MeOH; 100 mL) to obtain compound **13** (7.5 mg).

The *n*-BuOH fraction (21 g) was subjected to fractionation on open column packed with Diaion HP-20 (100 g, 3 × 70 cm) flushed with five mobile phases of H_2O-MeOH (1:0, 4:1, 1:1, 1:4, 0:1, each 10 L) to afford 5 fractions (Bu1 to Bu-5).

Fraction Bu3 (1 g) was chromatographed over MPLC system with flash column RP-C18 (40 μm, 12 g) eluted with H_2O-MeOH (8.5:1.5 to 7:3) with flow rate 10 ml/min to obtain four subfractions Bu3–1 to Bu3–4. Subfraction Bu3–2 (180 mg) was further purified over MPLC system with flash column RP-C18 (40 μM, 4 g) eluted with an isocratic system of H_2O-MeOH (4:1) followed by final purification over preparative HPLC column (Zorbax Extend-C18 PrepHT, 21.2×150 mm, 5 μm) connected to MPLC system using H_2O-MeOH (4:1) with flow rate 5 ml/min as an eluent to obtain compounds **14** (4.3 mg) and **15** (4.1 mg).

Fraction Bu4 (3.5 g) was fractionated over Biotage using RP-C18 flash column (30 μm, 30 g, 25 mL/min) flushed with H_2O-ACN (98:2 to1:1) to afford eleven subfractions (Bu4–1 to Bu4–11). Subfraction Bu4–10 (402 mg) was chromatographed over MPLC system connected to two flash columns RP-C18 (40 μm, each 4 g) using H_2O-ACN (4:1 to 1:1) to obtain three subfractions (Bu4–10-1 to Bu4–10-3). Subfractions Bu4–10-2 (200 mg) was purified over an open column packed with reversed phase silica (38–63 μm, 1×50 cm) and eluted with H_2O-ACN (7:3) to obtain **16** (11.5 mg) and **17** (12 mg).

*2.3.1. Kaempferol-3-O-β-D-glucopyranosyl-(1→6)-O-[β-D-galactopyranosyl-(1→3)-2-O-trans-feruloyl-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**14**)*

Yellow needles; $[\alpha]_D^{25}$ -30.06 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 1093.3028 [M-H]$^-$, (calcd. for $C_{49}H_{57}O_{28}$, 1093.3036).

*2.3.2. 3-O-[2′-(2″-O-glycolyl)-glyoxylyl-β-D-glucuronopyranosyl]-28-O-β-D-glucopyranosyl-olean-12-en-3β-ol-28-oic acid (**16**)*

White powder; ^{1}H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 925.4428 [M-H]$^-$ (calcd. for $C_{46}H_{70}O_{19}$ 925.4433).

*2.3.3. (2′R,3′S)-3-O-[2′-hydroxy-3′-(2″-O-glycolyl)-oxo-propionic acid-β-D-glucuronopyranosyl]-28-O-β-D-glucopyranosyl-olean-12-en-3β-ol-28-oic acid (**17**)*

White powder; ^{1}H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 955.4538 [M-H]$^-$ (calcd. for $C_{47}H_{71}O_{20}$ 955.4539.

2.4. Acid hydrolysis

One milligram of each compound **14**, **15, 16**, and **17** was subjected to acid hydrolysis by 2 M HCl in water (0.5 mL) and refluxed at 95 °C for 2 h. Neutralization of the reaction mixture was performed by addition of NH_4OH, then EtOAc was added four times to extract the aglycone, hence sugars were remained in the aqueous solution. The aqueous solution was then lyophilized and freeze dried to obtain a residue. The residue was then dissolved in a few drops of MeOH and used for identification of the sugar moiety by comparison with authentic sugar samples.

2.5. Anticholinesterase assay

The isolated compounds (**1**–**17**) were evaluated for their AChE inhibitory effect by the *in vitro* spectrophotometric method of Ellman's assay in 96-well plate [26]. AChE has the ability to hydrolyze the substrate acetylthiocholine iodide resulting in the product thiocholine which is then reacts with Ellman's reagent [5,5-dithiobis-(2-nitrobenzoic acid), DTNB] to produce a yellow colored 5-thio-2-nitrobenzoate which can be detected at 405 nm. Galantamine was used as a positive control. In a 96-well plate, 25 μL of 15 mM ACTI, 125 μL of 3 mM DTNB in buffer B (50 mM Tris HCl, PH = 8, 0.1 M NaCl, 0.02 M MgCl2·6H2O), 50 μL of buffer A (50 mM Tris-HCl, PH 8, 0.1% BSA) and 25 μL of tested compounds in concentration ranges from 1 to 250 μg/mL (dissolved in 25% DMSO) were allowed to react followed by measuring the absorbance with a microplate reader (Biotek, Winooski, VT, USA) at 405 nm. Moreover, 25 μL of AChE (0.25 U/mL in buffer A) was added and the absorbance was detected again. A solution of 25% DMSO was used as a negative control. To exclude any increase in absorbance due to the colored compounds or spontaneous hydrolysis of the substrate, the absorbance before addition of the enzyme was subtracted from the absorbance after adding the enzyme.

3. Results and discussion

Phytochemical investigation of the aqueous methanol extract prepared from the dried aerial parts of *B. indica* resulted in isolation of new acylated flavonol tetraglycoside (**14**), alongside with a rare flavonol triglycoside (**15**) and two rare triterpene *seco*-glycosidic oleanane saponins having a unique acetal-linked dicarboxylic acid substitution (**16**, **17**), in addition to 13 known compounds. The isolated compounds were categorized into steroid, phenolic acids, phenolic glycoside, dihydroxycoumarin, nucleic acids, flavonol aglycone, flavonol glycosides, and oleanane saponins. The structures of isolated compounds (Fig. 1) were elucidated based on different spectroscopic measurements including ^{1}H, ^{13}C, APT, HSQC, HMBC, COSY NMR and HR-MS analysis.

3.1. Determination of isolated compounds

Structures of the known compounds were determined by comparison of their NMR data with those reported in the literature and they were identified as *β*-sitosterol (**1**) [27], vanillic acid (**2**) [28], *o*-hydroxybenzoic acid (**3**) [29], *p*-hydroxybenzoic acid (**4**) [30], 6,7-dihydroxycoumarin (**5**) [31], methyl caffeate (**6**) [32], caffeic acid (**7**) [33], quercetin (**8**) [34], uracil (**9**) [35], thymidine (**10**) [36], tachioside (**11**) [37], isorhamnetin-3-*O*-*β*-D-glucoside (**12**) [38], kaempferol-3-*O*-rutinoside (**13**) [39].

Compound **14** was isolated as yellow needles. The optical rotation of **14** was $[\alpha]_D^{25}$ -30.06 (*c* 0.02, MeOH). In HR-ESI-MS (Fig. S1), the molecular formula of **14** was deduced to be $C_{49}H_{58}O_{28}$ based upon the

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **14** (in DMSO-d_6).

No.	δ_H (*J* in Hz)	δ_C	No.	δ_H (*J* in Hz)	δ_C	No.	δ_H	δ_C
• Aglycone			• α-L-rhamnopyranoside			• Feruloyl moiety		
1	–		1‴	5.11 brs	99.8	1⁗′	–	126.2
2	–	156.9	2‴	4.84 t (8.6)	72.3	2⁗′	7.14 brs	111.7
3	–	133.2	3‴	3.68 m	84.5	3⁗′	–	148.3
4	–	177.8	4‴	3.04 m	72.3	4⁗′	–	149.5
5	–	161.7	5‴	3.17 m	68.6	5⁗′	6.72 d (8.3)	115.7
6	6.17 d (1.6)	99.1	6‴	0.93 d (6.2)	18.1	6⁗′	6.96 brd	122.9
7	–	164.4	• β-D-glucopyranoside			7⁗′	6.34 d (15.6)	115.9
8	6.34 d (1.6)	94.1	1⁗′	4.30 d (7.6)	100.9	8⁗′	7.43 d (15.6)	144.8
9	–	156.8	2⁗′	3.20* m	70.8	9⁗′	–	166.2
10	–	104.4	3⁗′	3.40 m	68.9	O-Me	3.78 s	56.1
1′	–	121.5	4⁗′	3.51* m	76.9			
2′, 6′	7.91 d (8.7)	131.3	5⁗′	3.28 m	75.9			
3′, 5′	6.89 d (8.7)	115.7	6⁗′	3.51–3.70 m	61.1			
4′	–	160.2	• β-D-galactopyranoside					
• β-D-glucopyranoside			1⁗″′	4.27 d (7.6)	103.5			
1″	5.59 d (7.6)	97.9	2⁗″′	3.03–3.33* m	70.7			
2″	3.51 m	80.1	3⁗″′	3.33–3.40* m	77.4			
3″	3.67 m	76.6	4⁗″′	3.18 m	68.7			
4″	3.74 m	70.4	5⁗″′	3.70* m	76.8			
5″	3.01 m	73.7	6⁗″′	3.77 m	61.5			
				3.83 m				
6″	3.57 m	66.5						
	3.18 m							

Chemical shifts are recorded in ppm, coupling constants (*J*) are given in Hz. The assignments were determined based on HSQC, HMBC, and ^{1}H—^{1}H COSY experiments. (*) refers to overlapped signals.

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **16** and **17** (in D_2O-Pyridine-d_5).

	16		17	
No.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	0.85 m	38.3	1.16 m	39.2
2	1.64 m, 1.93 m	25.2	1.73 m, 1.92 m	25.7
3	3.11 dd (3.5, 11.6)	89.6	3.10 dd (3.5, 11.6)	90.0
4	–	37.7	–	38.7
5	0.46 brd	54.9	0.45 d (11.7)	55.3
6	–	17.5	–	17.9
7	1.15 m	32.9	1.14 m	36.3
8	–	41.1	–	41.6
9	–	48.6	–	47.4
10	–	35.8	–	38.1
11	1.55 m	22.8	1.59 m	23.2
12	5.14 brs	122.1	5.14 brs	122.6
13	–	143.2	–	143.6
14	–	45.4	–	45.8
15	1.25 m	29.6	1.24 m	29.0
16	1.82 m	22.3	1.83 m	22.8
17	___	45.4	–	46.9
18	2.82 m	40.6	2.83 m	41.2
19	1.22 m	46.4	1.22 m	46.4
20	–	31.5	–	32.5
21	1.05 m	32.0	1.05 m	30.1
22	1.53 m	32.2	1.53 m	36.2
23	1.01 s	27.2	1.01 s	27.6
24	0.77 s	15.9	0.77 s	16.3
25	0.59 s	14.5	0.59 s	14.9
26	0.72 s	16.4	0.72 s	16.8
27	0.75 s	25.1	0.75 s	25.6
28	–	177.3	___	177.8
29	0.94 s	32.2	0.94 s	32.6
30	0.71 s	22.7	0.71 s	23.1
	3-*O*-*β*-D-glucuronic acid		**3-*O*-*β*-D-glucuronic acid**	
1′	4.67 d (7.5)	104.5	4.68 d (7.5)	105.1
2′	3.84 m	73.6	3.84 m	74.1
3′	3.97 m	84.4	4.03 dd (9.5, 11.5)	83.5
4′	3.82 m	72.1	3.82 m	71.6
5′	4.05 m	76.9	4.04 m	76.7
6′	–	175.2	–	174.4
	Substitution at C-3 of glucuronic acid		**Substitution at C-3 of glucuronic acid**	
1″	–	174.4	–	177.6
2″	5.43 s	101.4	4.58 d (2.5)	74.1
3″	4.56 d (14.8) 4.45 d (15.2)	66.4	5.50 d (2.5)	104.9
4″	–	177.1	4.70 d (15.5) 4.40 d (15.4)	68.12
5″	–	–	–	177.9
	28-*O*-*β*-D-glucopyranoside		**28-*O*-*β*-D-glucopyranoside**	
1‴	5.74 d (8.1)	94.2	5.73 d (8.2)	94.7
2‴	3.80 m	71.1	3.80 m	71.2
3‴	3.90 m	69.4	3.90 m	69.9
4‴	3.78 m	77.0	3.78 m	77.3
5‴	3.69 m	77.0	3.69 m	77.6
6‴	4.07 m 3.97 dd (6.6, 13.8)	60.6	4.03 m 3.95 m	61.1

Chemical shifts are recorded in ppm, coupling constants (*J*) are given in Hz. The assignments were determined based on HSQC, HMBC, and ^{1}H—^{1}H COSY experiments.

molecular ion peak at *m/z* 1093.3028 $[M-H]^-$, (calcd. for $C_{49}H_{57}O_{28}$ 1093.3036). The ^{1}H, ^{13}C, HSQC, HMBC, ^{1}H—^{1}H COSY NMR spectra confirmed the presence of flavonol moiety, four sugar moieties, in addition to feruloyl moiety. The ^{1}H NMR spectrum (Table 1, Fig. S2) showed several characteristic signals, one broad singlet aromatic proton at δ_H 7.14, six doublet aromatic protons at δ_H 6.17 d (J = 1.6 Hz), 6.34 d (J = 1.6 Hz), 7.91 d (J = 8.7 Hz), 6.89 d (J = 8.7 Hz), 6.72 d (J = 8.3 Hz), 6.96 brd (J = 8.5 Hz), two doublet olefinic protons at δ_H 6.34 d (J = 15.6 Hz), 7.43 d (J = 15.6 Hz), one singlet aromatic methoxyl proton at δ_H 3.78 s, and one doublet aliphatic methyl signal at δ_H 0.93 d (J = 6.2 Hz). Sugar moieties were identified by the presence of four anomeric signals resonated at δ_H 5.59 d (J = 7.6 Hz), 5.11 brs, 4.30 d (J = 7.6 Hz), 4.27 d (J = 7.6 Hz), in addition to signals at δ_H 3.05–4.84 for sugar protons.

The ^{13}C NMR (Table 1 and Fig. S3) of **14** exhibited 49 carbon resonances that categorized into two methyls (one methoxy and one methyl of rhamnose), 3 methylenes, 29 methines, and 13 quaternary carbon signals, as confirmed with the aid of HSQC experiment (Fig. S4). According to HSQC data, the four anomeric protons were detected in ^{1}H NMR spectrum and found to be correlated with corresponding carbons 5.59 d / 97.9, 5.11 brs / 99.8, 4.30 d / 100.8, 4.27 d / 103.5. Furthermore, one doublet proton at δ_H 0.93, δ_C 18.1 indicated a rhamnose moiety. The ^{1}H and ^{13}C NMR spectral data as well as comparison with previously published data exhibited a kaempferol glycoside [40,41]. The ^{13}C NMR also exhibited a methoxylated carbon signal at δ_C 56.1, alongside with glycoside carbon signals at δ_C 61.2–84.5 ppm. The presence of feruloyl moiety with *trans*-geometry was deduced basically from tow olefinic doublets δ_H 6.34 and δ_H 7.43 which were correlated with carbons at δ_C 115.9 and 144.8 respectively.

The HMBC correlations (Fig. 2 and Fig. S5) of anomeric proton at δ_H 5.59 and C-3 (δ_C 133.2) confirmed the glycosidic linkage at C-3 of the aglycone. Other HMBC and HSQC correlations revealed that the *β*-D-glucopyranosyl sugar was the primary *O*-linked sugar at C-3. HMBC cross peaks of anomeric protons at δ_H 5.11 and δ_H 4.30 with C-2″ (δ_C 80.1) and C-6″ (δ_C 66.5), respectively. The deshielded signal at δ_C 84.5 of C-3‴ of rhamnose moiety suggested that another sugar monomer is linked there, which was then confirmed by HMBC correlations of H-1⁗ (δ_H 4.27) with C-3‴. Moreover, a feruloyl moiety was proved to be attached at H-2‴ of rhamnose moiety as HMBC correlation was detected between downfielded triplet proton observed at δ_H 4.84 and carbonyl group of the feruloyl moiety at δ_C 166.2. Additionally, long range HMBC correlation between δ_H 6.34 (H-7⁗) and carbonyl group (C-9⁗) at δ_C 166.2 clearly confirmed the feruloyl moiety.

The sugar moieties were determined through the total acid hydrolysis followed by examinations over TLC with authentic sugar samples that indicated the presence of glucose, rhamnose, and galactose. Hence, the absolute configuration of sugars has been determined as *β*- for glucose and galactose and *α*-for rhamnose [42,43]. Accordingly, from the above mentioned data, **14** was identified as kaempferol-3-*O*-*β*-D-glucopyranosyl-(1→6)-O-[*β*-D-galactopyranosyl-(1→3)-2-*O*-*trans*-feruloyl-*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside.

Compound **15** was obtained as yellow powder. It is a rare flavonol triglycoside as it has been reported previously only from *Allium neapolitanum* [44]. Hence, **15** was isolated and elucidated through comparing the published NMR data [44] with our data, as well as HR-ESI-MS analysis (Fig. S7), and its molecular formula deduced to be $C_{34}H_{42}O_{21}$ based upon the molecular ion peak in negative ion mode at *m/z* 785.2136 $[M-H]^-$, (calcd. for $C_{34}H_{41}O_2$ 785.2140). Consequently, **15** was identified as isorhamnetin-3-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside.

Compound **16** was obtained as white powder. Its molecular formula was established by HR-ESI-MS (Fig. S13) to be $C_{46}H_{69}O_{19}$ from the $[M-H]^-$ ion at *m/z* 925.4428 (calcd. for $C_{46}H_{70}O_{19}$ 925.4433). The ^{1}H NMR (Table 2 and Fig. S14), ^{13}C NMR (Table 2 and Fig. S15), and HSQC (Fig. S16) spectra of **16** showed characteristic pattern for oleanolic acid aglycone, seven tertiary methyl signals at δ_H 0.59 (H-25), 0.71 (H−30), 0.72 (H-26), 0.75 (H-27), 0.77 (H-24), 0.94 (H-29), 1.01 (H−23), olefinic proton at δ_H 5.14 (brs, H-12), in addition to two olefinic carbons at δ_C 122.1, δ_C 143.2, and carbonyl signal at δ_C 177.3, which are characteristic for triterpene oleanane aglycone [45,46]. The ^{13}C NMR spectrum exhibited seven methyls, twelve methylene, sixteen methine, eleven quaternary carbons. The HSQC correlations of δ_H 5.74 (d, J = 8.1 Hz) and δ_H 4.67 (d, J = 7.5 Hz) with δ_C 94.2 and δ_C 104.5 confirmed the presence of two sugar monomers. HMBC cross peaks (Fig. 3 and Fig. S17) of anomeric protons at δ_H 5.74 with δ_C 177.3 (C-28), δ_H 4.67 with δ_C 175.2 (C-6′) and δ_C 89.6 (C-3) revealed that two sugars, *β*-D-glucose and *β*-D-glucuronic acid, were linked to C-28 and C-3,

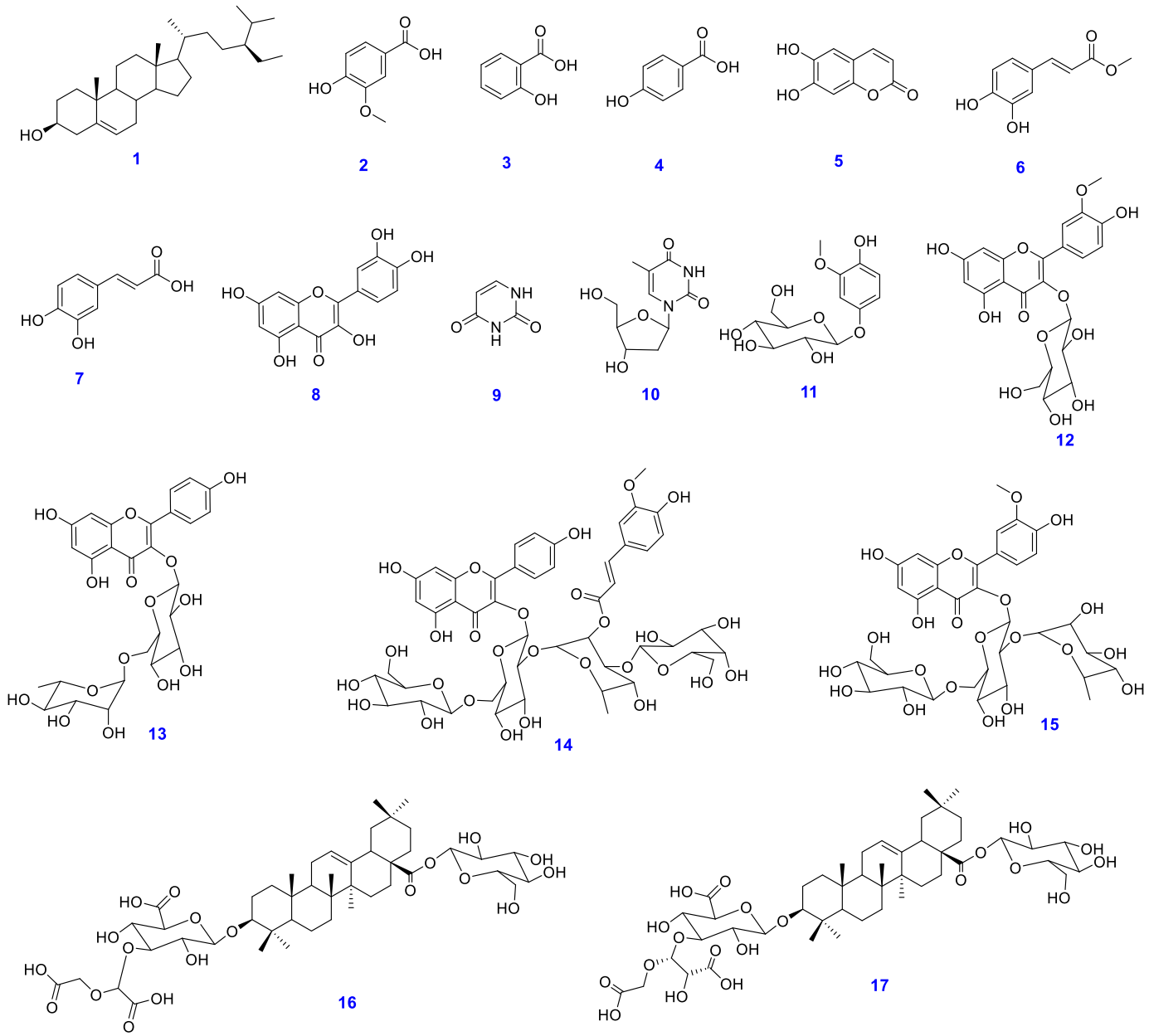

Fig. 1. Chemical structure of isolated compounds (1–17).

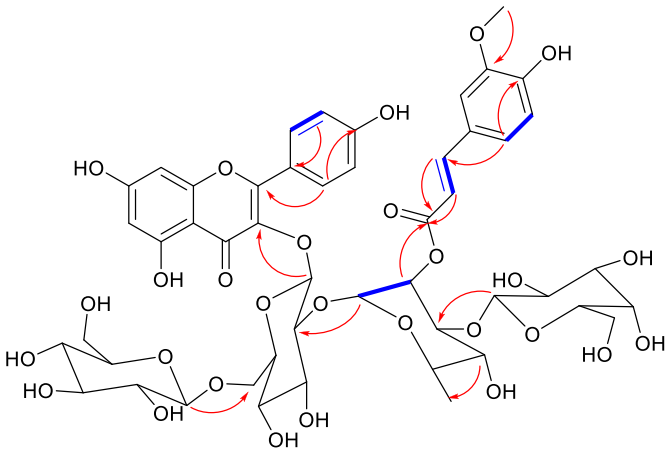

Fig. 2. Key HMBC and COSY correlations of compound 14.

respectively. The deshielded signal at δ_C 84.4 of C-3′ indicated that another side chain is linked there, which was then proved by HMBC correlations of δ_H 5.43 (H-2″) with δ_C 84.4 (C-3′) and δ_C 66.4 (C-3″). The suggested *seco*-glycosidic dicarboxylic substitution at C-3′ was further confirmed by HMBC correlations of two protons at δ_H 4.56 (d, J = 14.8 Hz), δ_H 4.45 (d, J = 15.2) with δ_C 101.4 (C-2″) and 177.1 (C-4″).

Finally, from the above-mentioned data, **16** was identified as 3-*O*-[2′-(2″-*O*-glycolyl)-glyoxylyl-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosyl-olean-12-en-3β-ol-28-oic acid. It was found in a good agreement with the isolated one in literature [47] and herein we report **16** as a rarely occurring *seco*-glycosidic oleanane saponin and the hypothesis of biosynthetic pathway of this compound is supported in supplementary data (Fig. S25).

Compound **17** was obtained as white powder. The HR-ESI-MS spectrum (Fig. S19) of **17** displayed a $[M\text{-}H]^-$ ion at m/z 955.4538 (calcd. for $C_{47}H_{71}O_{20}$ 955.4539), hence the molecular formula established to be $C_{47}H_{72}O_{20}$. The ^1H and APT NMR (Table 2) spectra of **17** displayed seven methyl signals at δ_H 0.59 (H-25), 0.71 (H-30), 0.72 (H-

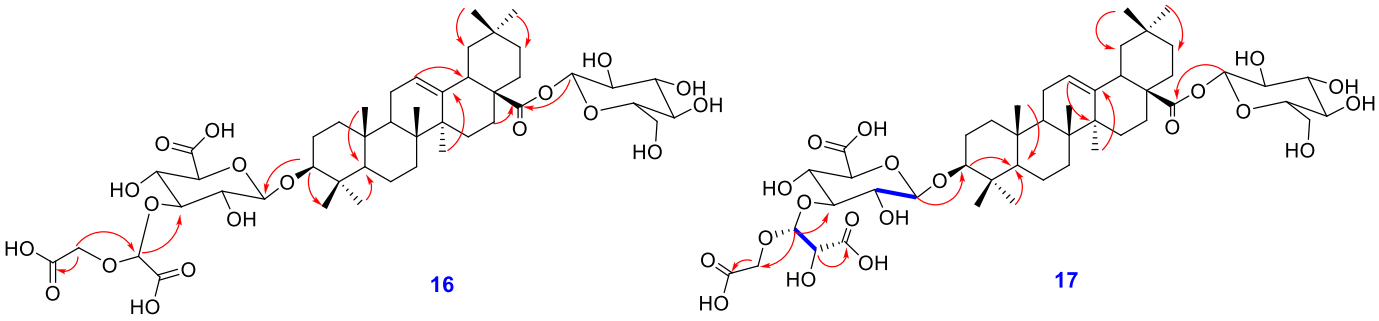

Fig. 3. Key HMBC and COSY correlations for 16 and 17.

26), 0.75 (H-27), 0.77 (H-24), 0.94 (H-29), 1.01 (H-23), one signal at δ_H 3.10 (dd, $J = 3.5, 11.5$ Hz, H-3), one olefinic proton at δ_H 5.14 (brs, H-12), two olefinic carbons at δ_C 122.6 and 143.6, and carbonyl signal at δ_C 177.8 indicating a triterpene oleanane aglycone [45,46,48]. The signals at δ_H 4.58 (d, $J = 2.5$ Hz), δ_H 5.50 (d, $J = 2.5$ Hz), δ_H 4.70 (d, $J = 15.5$ Hz), and δ_H 4.40 (d, $J = 15.4$) revealed the presence of oxo-propionic acid and glycolyl moieties. The APT NMR and HSQC data exhibited the presence of 47 carbon signals including seven methyls, twelve methylene, seventeen methine, eleven quaternary carbons. The HSQC correlations (Fig. S22) of δ_H 5.73 (d, $J = 8.2$ Hz) and δ_H 4.68 (d, $J = 7.5$ Hz) with δ_C 94.7 and δ_C 105.1 revealed the presence of two anomeric carbons for two sugar monomers, which is in a good agreement with the published data for very related saponin compounds in the literature [48]. The deshielded carbon signal at δ_C 83.5 of C-3′ of glucuronic acid suggested that another side chain is linked there, where the HMBC correlations (Fig. 3 and Fig. S23) of δ_H 5.50 with δ_C 83.5 (C-3′) confirmed the acetal-linked dicarboxylic acid substitution at C-3′. Moreover, the *seco*-glycosidic acetal-linked substitution moiety was proved by other HMBC correlations of H-2″ at δ_H 4.58 with δ_C 177.6 (C-1″) and δ_C 104.9 (C-3″), in addition to the correlations of protons at δ_H 4.70, δ_H 4.40 (H-4"a and H-4"b) with δ_C 177.9 (C-5″) and δ_C 104.9 (C-3″). The two stereocenters of the 2″ and 3″ positions of *seco*-glycosidic substitution were assigned to be *R* and *S*, respectively, based on comparison of the detailed NMR data with the related dimethyl ester derivative previously published in the literature [49]. Based upon all the above-mentioned evidences, **17** was elucidated as (2′*R*,3′*S*)-3-*O*-[2′-hydroxy-3′-(2″-*O*-glycolyl)-oxo-propionic acid-*β*-D-glucuronopyranosyl]-28-*O*-*β*-D-glucopyranosyl-olean-12-en-3*β*-ol-28-oic acid.

3.2. Anticholinesterase activity

Acetylcholine is the main neurotransmitter, responsible for cholinergic signaling in the brain. Therefore, phytochemical constituents which inhibit acetylcholinesterase (AChE) and restore acetylcholine levels are highly targeted for treating symptoms of Alzheimer's disease (AD). In this context, the isolated compounds (**1–17**) were tested *in vitro* for their AChE inhibitory activity (Table 3).

Among the isolates, compounds **5**, **8**, **13**, and **17** exhibited significant inhibitory activity toward acetylcholinesterase enzyme with IC_{50} 3.6, 18, 27.9, 29.6 μg/mL, respectively, compared to the standard drug galantamine (12.5 μg/mL). On the other hand, compounds **10**, **1**, **16**, **2**, and **12** exerted marked inhibitory activity with IC_{50} 45.71, 55.8, 63.11, 88.3, 93.2 μg/mL, respectively.

It was reported previously that phenolic acids and flavonoids are capable to reduce the oxidative stress and consequently they are considered as potential lead compounds for development of cholinesterase inhibitor drugs that can be used for treatment of AD [50]. Hence, the plant phenolic constituents can play an important role in the improvement of cognitive symptoms and help to protect against neurodegenerative disorders. Additionally, the phenolic acids have some benefits over the well-known inhibitors as they are commonly distributed in variety of foods and their daily intake with food has no adverse effects [51]. As a result, the phenolic derived phytoconstituents should be considered for the dietary prevention of AD.

Table 3
Anticholinesterase activity (IC_{50}) of isolated compounds (**1–17**) from *B. indica*[a]

Compound	IC_{50} (μg/mL)
1	55.8 ± 3.8
2	88.3 ± 1.10
3	> 250 μg (48.5%)*
4	203.2 ± 4.5
5	3.6 ± 0.07
6	NA[b]
7	112.9 ± 3.08
8	18 ± 1.3
9	NA
10	45.7 ± 0.57
11	>250 (26%)*
12	93.2 ± 1.2
13	27.9 ± 0.8
14	>250 (40.6%)*
15	>250 (35%)*
16	63.1 ± 1.5
17	29.6 ± 1.7
Positive control (Galantamine)	12.5 ± 0.6

[a] The results are presented as the mean ± SD of values ($n = 3$). *Inhibitory activity expressed at a concentration 250 μg/mL. [b]NA means there is no activity obtained at tested concentration.

As a comprehensive interpretation in this study, flavonoids as quercetin (**8**) and similar flavonoids in aglycone form showed marked inhibitory activity against AChE. Also, some flavonoid glycosides have been reported for their activity against AChE. In our study, flavonoid glycosides (**12** and **13**) exerted marked inhibitory activity, whereas the activity is dramatically reduced with the presence of more sugar residues in the molecule as in compounds **14** and **15**. The activity of phenolic acids toward AChE was dependent mainly on the number and/or position of OH groups and propenoic acid side chain. For instance, the isolated compound **2** showed a relatively high activity in comparison with **3** and **4**. Also, compound **5** with catechol and cyclized pyrone moieties showed potent inhibitory activity against AChE. In the literature, it was reported that the presence of propenoic (CH=CH-COOH) group (caffeic acid) has a significant effect on the activity toward AChE compared with CH_2-CH_2-COOH (hydrocaffeic acid) or COOH groups (protocatechuic acid). Besides, the methylation of caffeic acid will lead to a marked decline in the inhibitory activity [52].

4. Conclusion

In summary, natural plants have long been regarded as useful source of lead compounds for fighting various chronic diseases including AD.

Owing to the lack of efficacy and severe side effects associated with the current therapies for AD treatment, there is an urgent need for newer molecules that are capable of inhibiting the acetylcholinesterase enzyme for the treatment of AD.

The xero-halophyte *B. indica* is a very cheap source of novel bioactive constituents. In the present study, the phytochemical investigation of *B. indica* resulted in isolation and identification of seventeen compounds including new acylated flavonol tetraglycoside, rare flavonol triglycoside, and two rarely occurring *seco*-glycosidic triterpene oleanane saponins, in addition to other flavonoid compounds and phenolic acid derivatives. Additionally, the isolated compounds in this study showed promising therapeutic activity toward the acetylcholinesterase enzyme. Thus, the plant could be utilized as a potential source of lead drugs for medicinal purposes with economic values.

To the best of our knowledge, this is the first report concerning a detailed phytochemical and biological characterization of the halophytic herb *B. indica* and the outcomes from this study will inspire natural product researchers to valorize and study other halophytes of the family Chenopodiaceae.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

^{1}H, APT, and ^{13}C NMR spectra of compounds 14–17 and their HSQC, HMBC, and COSY correlations are available as Supporting information. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2021.104907.

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