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New pregnane glycosides from *Caralluma hexagona* Lavranos and their *in vitro* α-glucosidase and pancreatic lipase inhibitory effects

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ABSTRACT

Three new pregnane glycosides in addition to four known compounds were isolated from the methylene chloride fraction of *Caralluma hexagona* Lavranos using bioassay-guided fractionation. The new compounds were identified as 12,20-di-*O*-benzoyl-3β,8β,12β,14β,20-pentahydroxy-(20*R*)-pregn-5-ene-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-digitaloside **(1)**, 3β,8β,14β,20-tetrahydroxy (20*R*)-pregn-5-ene-3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-digitaloside-20-*O*-β–D-glucopyranoside **(2)**, 3β,8β,14β,20-tetrahydroxy-(20*R*)-pregn-5-ene-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-digitaloside-20-*O*-β–D-glucopyranoside **(3)**, along with the known compounds luteolin 4`-*O*-neohesperidoside **(4)**, apigenin-8-*C*-neohesperoside **(5)**, β-sitosterol **(6)** and β-sitosterol glucoside **(7)**. Preliminary studies of the crude methanolic extract and methylene chloride fraction showed inhibitory effects against α-glucosidase and pancreatic lipase. Among the isolated compounds, compound **5** showed the most potent α-glucosidase inhibition with IC_{50} value of 0.82 ± 2.50 μM compared to acarbose (0.81 ± 0.86 μM). Whereas compound **1** showed the highest inhibitory activity on pancreatic lipase with an IC_{50} value of 23.59 ± 2.49 μM compared to orlistat (7.41 ± 2.26 μM).

1. Introduction

Caralluma is one of the prominent genera of family Asclepiadaceae, distributed in dry regions of tropical Asia, south Mediterranean, near east and in the north, central and east Africa (Albers and Meve, 2002). Certain species of *Caralluma* are edible and form part of the traditional medicine system of many countries (Adnan et al., 2014). The anti-diabetic and anti-obesity activities of certain members of genus *Caralluma* were confirmed by several authors (Habibuddin et al., 2008; Ambadasu et al., 2013; Bellamakondi et al., 2014; Sudhakara et al., 2014; Abdel-Sattar et al., 2017; Vitalone et al., 2017). In addition, the antioxidant, anticancer, antiulcer, neuroprotective, antihypertensive, hepatoprotective activities were also reported (Abdallah et al., 2013; Ahmad et al., 2015; Al-Mehdar et al., 2015; Poodineh et al., 2015; Atlas, 2016; Al-Naqeb, 2017). *Caralluma fimbriata, C. tuberculata* and *C. quadrangula* are among the most common species used as antidiabetic and antiobesity. *Caralluma fimbriata* with its major constituents identified as pregnane glycosides was recently introduced into the US market as a dietary supplement for weight loss with no history of adverse effects (Sudhakara et al., 2014). The key and characteristic phytochemicals in the genus *Caralluma* are mainly pregnane-type steroidal aglycones and glycosides, in addition to other constituents such as flavone glycosides, megastigmane glycosides, triterpenes and sterols (Bader et al., 2003; Abdel-Sattar et al., 2008; Waheed et al., 2011; Abdelaziz M Dawidar et al., 2012). The isolation of the pregnane glycoside russelioside B from *Caralluma quadrangula* with potential activity in controlling weight gain associated with high-fat feeding in rats (Abdel-Sattar et al., 2018) and stemmoside C from *Solenostemma argel* with *in vitro* lipase inhibitory activity (El-Shiekh et al., 2019) encouraged the authors to investigate the unexplored species *C. hexagona* from Yemen.

Caralluma hexagona Lavranos is distributed in Yemen, Saudi Arabia and Oman (Albers and Meve, 2002). In the present study, total extracts and fractions, in addition to compounds isolated from the most active fraction of the aerial parts of *C. hexagona* were tested *in vitro* for their inhibitory activity on α-glucosidase and pancreatic lipase.

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Table 1
Inhibitory effects of total extracts, fractions and isolated compounds from the aerial parts of *C. hexagona* on enzymes related to carbohydrate and fat metabolism.

Sample	α-Glucosidase inhibition (%)		Lipase inhibition (%)	
Extract/ fraction	**% Inhibition 2 mg/mL**	**IC_{50} mg / mL**	**% Inhibition 100 µg/mL**	**IC_{50} µg / mL**
W	28.44 ± 3.25	> 2	4.67 ± 1.98	> 100
M	42.59 ± 1.95	2.66 ± 0.22	27.13 ± 2.23	> 100
MC	**94.66 ± 2.80**	**0.78 ± 0.02**	48.82 ± 0.50	> 100
D2	NO	NO	5.06 ± 6.00	> 100
D3	24.95 ± 2.04	> 2	NO	NO
Acarbose	**60.29 ± 0.91**	**0.71 ± 0.03**		
Orlistat			**55.88 ± 1.01**	**4.30 ± 0.92**
Compounds/ standards	**% Inhibition 1 mM**	**IC_{50} mM**	**% Inhibition 100 µM**	**IC_{50} µM**
1	11.95 ± 0.56	> 1	63.70 ± 2.60	23.59 ± 2.49
2	3.62 ± 3.19	> 1	4.35 ± 2.30	> 100 µM
3	19.26 ± 2.62	> 1	3.62 ± 3.47	> 100 µM
4	41.64 ± 8.90	> 1	12.09 ± 2.60	> 100 µM
5	55.18 ± 0.13	0.82 ± 2.50	11.22 ± 1.87	> 100 µM
6	7.19 ± 4.31	> 1	8.80 ± 4.15	> 100 µM
7	43.17 ± 4.22	> 1	35.62 ± 4.17	> 100 µM
Acarbose	55.13 ± 0.49	0.81 ± 0.86		
Orlistat			55.59 ± 0.98	7.41 ± 2.26

W; Water extract; M; MeOH extract; MC; Methylene chloride fraction; D2; MeOH : H_2O (1:1) diaion fraction; D3; MeOH diaion fraction; NO; No inhibition.

2. Results and discussion

2.1. Isolation and identification of the isolated compounds from C. hexagona

Using a bio-guided fractionation approach, the different extracts of *C. hexagona* (water, methanol, a methylene chloride were subjected to *in vitro* inhibitory assay using *α*-glucosidase and lipase enzymes. The methylene chloride fraction showed the highest activity with 94.66 % and 48.82 % inhibition against *α*-glucosidase and lipase enzymes, respectively (Table 1). Therefore, the methylene chloride fraction was subjected to bioassay-guided isolation, leading to the isolation of seven compounds. The compounds were three new pregnane glycosides (**1-3,** Fig. 1), in addition to two known flavone glycosides and two known sterols. The known compounds were identified as luteolin 4`-*O*-neohesperidoside **(4)** (Rizwani et al., 1990), apigenin-8-*C*-neohesperoside **(5)** (Scharbert et al., 2004), *β*-sitosterol **(6)** (Yahya et al., 2011) and *β*-sitosterol glucoside **(7)** (Luo et al., 2009) by comparison with available authentic samples (Co-TLC and IR) and reported spectral data. The structures of the new compounds were determined as follows.

Compound **1** was obtained as a white amorphous powder (34 mg), the HRESI-MS analysis showed a adduct ion peak at 919.4105 in a positive mode (calc. for $C_{48}H_{64}O_{16}Na$, 919.4194) which is assigned for the molecular formula $C_{48}H_{64}O_{16}$, with 17 degrees of unsaturation. IR spectrum of this compound showed absorption bands due to hydroxyl (3434 cm^{-1}) and ester (1719, 1452 and 1277 cm^{-1}) groups. In addition, ^{13}C-NMR spectra showed the presence of four methyl, one methoxyl, 26 methines, eight methylene groups and nine quaternary carbon atoms with a total of 48 carbons, 21 of these carbons were ascribed for the C_{21} steroidal moiety (Tables 2 and 3). The ^{1}H NMR spectra also provided important information about the nature of the aglycone, two angular methyl groups appeared as singlet signals at δ_H 1.26 and 1.09 (δ_C 10.85 & 18.20) assigned for the angular methyl CH_3-18 and CH_3-19, respectively, which can be assigned to pregnane type of steroids (Deepak et al., 1989). This class of steroids has a two-carbon atom side-chain at C-17 (C-20 and C-21), the protons at C-21 usually appear as a methyl group which appear as doublet in the region of 1.0–1.5 ppm in the case of the presence of $CHOHCH_3$ moiety (Herz et al., 1997)., Compound **1** revealed the presence of two doublet methyl signals at δ_H 1.15 (2 × 3 H) assigned for CH_3-21 and CH_3-6 of a 6-deoxy sugar. Positive chemical tests for steroids and deoxy sugars, in addition to the NMR data (Tables 2 and 3), indicated that compound **1** was composed of two hexoses, two benzoyl groups, and a pregnenepentol aglycone. A double bond located at C-5/C-6 was deduced from the broad singlet signal at δ_H 5.29 which showed correlation with C-5 (δ_C 138.74), C-10 (δ_C 37.24) and C-8 (δ_C 73.86) in the HMBC spectrum (Fig. 2). The previous correlation (HMBC) suggested the presence of OH group at C-8 (Abdel-Sattar et al., 2001, 2002; Cioffi et al., 2006). The presence of OH group at C-8 was further confirmed by the upfield shifted value of C-6 (δ_C 119.20) relative to the non-hydroxylated C-8 (δ_C 121.80) (Cioffi et al., 2006; Abdallah et al., 2013). The other oxygenated quaternary carbon at δ_C 86.26 was assigned to C-14 in accordance with many reported data (Abdel-Sattar et al., 2002; Abdallah et al., 2013). The signals at δ_H 3.42 (1H, *m*), 4.94 (1H, *b.s*) and 5.20 (1H, *br.q*) were correlated with the oxygenated methine carbons at δ_C 77.84, 78.38 and 74.14, in the HMQC spectrum, and assigned for the protons H-3, H-12, and H-20, respectively. ^{1}H- and ^{13}C-NMR spectra showed the presence of two acyl groups identified as two benzoyl moieties (δ_H: 7.28–8.01 and δ_C 128.97–166.19) (Abdel-Sattar et al., 2007). The acylation positions at C-12 (δ_C 78.38) and C-20 (δ_C 74.14) were confirmed from the downfield shift of their corresponding protons and carbons. Further, the results of HMBC correlations (Fig. 2) showed correlations between the signal of carbonyl carbons of benzoyl groups and H-12 (4.94 Hz) and H-20 (5.20 Hz), respectively, of the aglycone moiety (Abdallah et al., 2013). For the absolute configuration of C-20, Kimura and his co-authors (1982) compared ^{13}C NMR spectral data of 20*R* and 20*S* pregnane epimers and found that there are significant differences in the ^{13}C chemical shifts values of C-16 and C-20 in the two sets of epimers. This was explained by the fact that the free rotation of the side chain in the 20-hydroxy-C/D-*cis*-pregnane type steroids was assumed to be restricted by steric hindrance among the C-18 methyl, C-21 methyl, and C-20 hydroxyl groups based on a CPK model (the space-filling model). Therefore, the C-21 methyl group in 20*S* alcohols of C/D-cis pregnane-type steroids is close to the plane of the D-ring, resulting in upfield shifts of the C-20 resonances (steric shift) (Kimura et al., 1982). Therefore, the configuration of C-20 of compound **1** was deduced to be *R*-configuration as explained by Al-Massarani et al. (2012) and by comparison to the closely related glycosides (Panda et al., 2003; Kunert et al., 2009; Al-Massarani et al., 2012; Abdallah et al., 2013; Elsebai and Mohamed, 2015; Tsoukalas et al., 2016). From the previous data, the aglycone moiety was identified as 12, 20-di-*O*-benzoyl-3*β*, 8*β*, 12*β*, 14*β*, 20-pentahydroxy-(20*R*)-pregn-5-ene and confirmed by comparison with the closely related pregnane glycosides (Abdel-Sattar et al., 2001, 2002; Abdallah et al., 2013). Spectral data of compound **1** (Table 3) showed that it contained two anomeric carbons signals at δ_C 101.68 & 103.75 correlated with anomeric protons at δ_H 4.22 (*d*. *J* = 6.8) & 4.34 (*d*. *J* = 8) respectively, which indicated that there were two sugar units in the compound **1** (Table 3). The sugar moieties were detected in the acid hydrolysate (Elsebai and Mohamed, 2015), one was identified as *β*-D-glucose by comparison of an authentic sample, in addition to another nonpolar sugar relative to glucose (6-deoxy sugar). The methyl signal at δ_H 1.15 (*d*, 6, H-6) was correlated to carbon signals δ_C 17.48 (HMQC) and a methoxyl singlet at δ_H 3.39 was correlated to carbon signal at δ_C 58.25 (HMQC) which suggested the presence of a 6-deoxy-3-*O*-methyl-D-galactopyranose (digtalose) as second sugar moiety. The sugar sequence of compound **1** was confirmed by the HMBC spectrum, which showed correlations between H-1` of digitalose at $\delta_H$ 4.22 and C-3 at $\delta_C$ 77.84 of the aglycone, and between H-3 ($\delta_H$ 3.42) and C-1` (δ_C 101.68) of digitalose. Similarly, the correlations between H-1`` of glucose (δ_H 4.34) and C-4` of digitalose (δ_C 74.70). The glycosylation site was proved at C-3 as a result of the downfield shift of C-3 (δ_C 77.84) and the upfield shifts of C-2 (δ_C 29.27) and C-4 (δ_C 38.58). Thus, the sequence and linkage sites of the sugar units were established as *β*-D-glucopyranosyl-(1→4)-*β*-D-digitalopyranose-aglycone. By comparing the second sugar data with the reported ones (Halim and Khalil, 1996; Abdel-Sattar

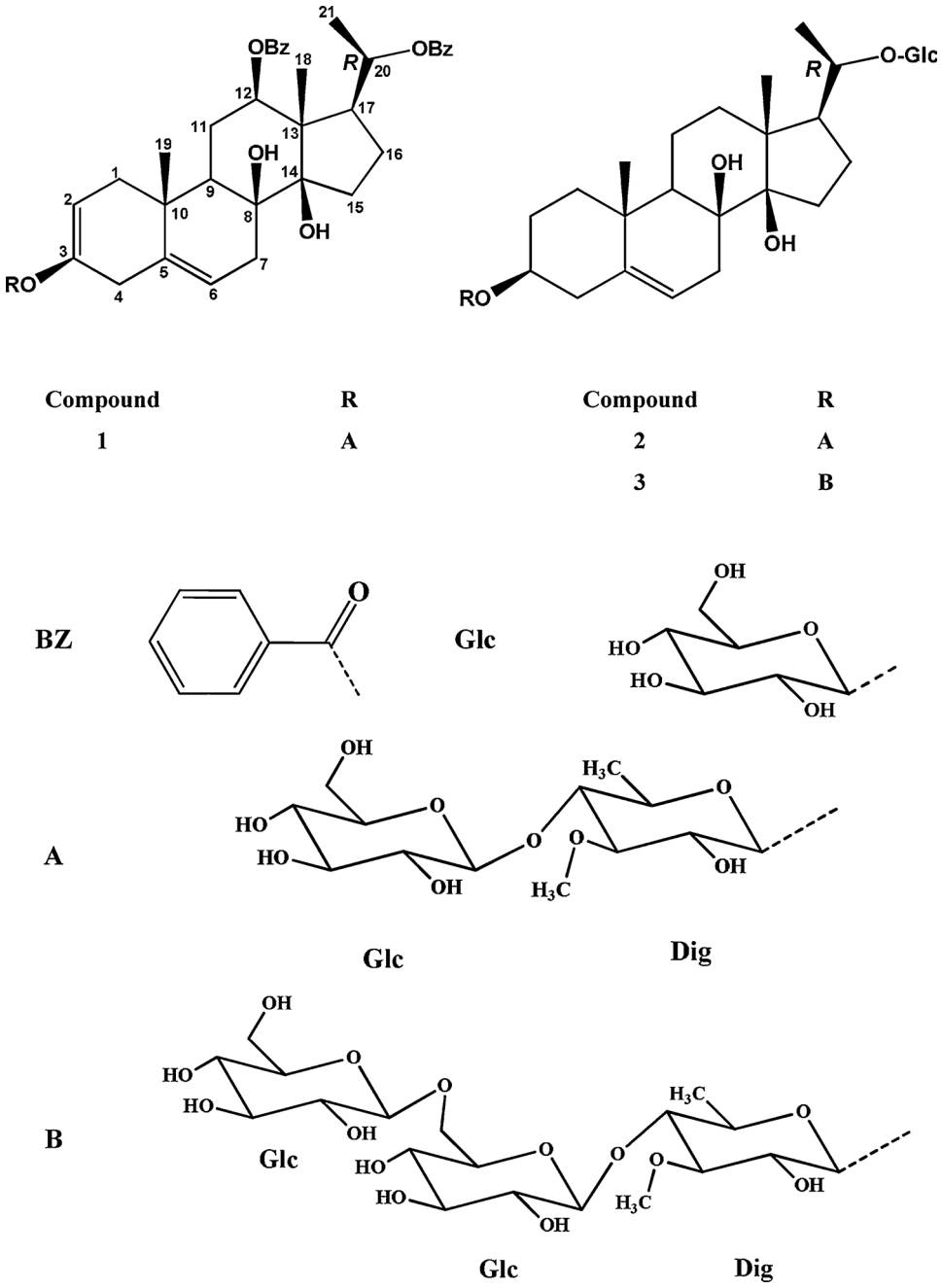

Fig. 1. Structures of compounds 1, 2 and 3.

et al., 2001), with digitalose attached at C-3 of the aglycone. The *β*-configuration of all sugar moieties was deduced from the coupling constants of their anomeric protons. The intensive spectral analysis of compound **1** through ^{1}H-, ^{13}C-NMR, HMQC, HMBC, and COSY experiments allowed unambiguous assignment of all protons and carbons (Tables 2 and 3). From the aforementioned data and by comparison with closely related pregnane glycosides, compound **1** was identified as 12, 20-di-*O*-benzoyl-3*β*, 8*β*, 12*β*,14*β*, 20-pentahydroxy-(20*R*)-pregn-5-ene 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-digitalopyranoside (Fig. 1).

Compound **2** was isolated as an amorphous powder. Based on the HRESI-MS data, the molecular formula of **2** was determined to be $C_{40}H_{66}O_{18}$ (857.4169 $[M + Na]^{+}$, calc. 857.4249). The NMR data of the aglycone was close to those of compound **1** except for the absence of the hydroxylation at C-12, which was confirmed from the presence of upfield shifted CH_2 group at δ_C 40.50 (C12). Therefore, the aglycone moiety was identified as 3*β*,8*β*,14*β*,20-tetrahydroxy-(20*R*)-pregn-5-ene, which was confirmed by comparison with closely related pregnane glycosides (Al-Massarani et al., 2012; Dawidar et al., 2012). The analysis of ^{1}H and ^{13}C NMR spectra of this compound showed the presence of three sugar moieties attached to the aglycone with two glycosylation sites (Tables 2 and 3) and showed the absence of acyl groups. The first glycosylation site was proved to be at C-3 similar to the sugar chain of compound **1** and the second glycosylation site was found to be C-20. This was proved from the downfield shift (+10 ppm) of the C-20 signal relative to data of non-substituted glycosides (Abdul-Aziz Al-Yahya et al., 2000) and also from the observed long-range correlation between C-20 (δ_C 77.79) and H-1``` of the sugar unit with anomeric proton at δ_H 4.42 in the HMBC spectrum (Fig. 3). The sugar units were identified as *β*-D-glucopyranosyl-(1→4)-*β*-D-digitalopyranose at C-3 and a *β*-D-glucose at C-20 by comparison to the spectral data of compound **1**. From the above-mentioned data, compound **2** was identified as 3*β*,8*β*,14*β*,20-tetrahydroxy-(20 *R*)-pregn-5-ene-3-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-

Table 2
^{1}H- and ^{13}C NMR (400 MHz, 100 MHz) of aglycone of compounds 1, 2 and 3.

3		2		1		Position
CD_3OD		**CD_3OD**		**DMSO**		
δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	
38.70	1.92 (1Ha, m) 1.09 (1Hb, m)	38.76	1.82 (1Ha, m) 1.09 (1Hb, m)	38.70	0.95 (1Ha, m) 2.17 (1Hb, m)	**1**
28.85	1.92 (1Ha, m) 1.73 (1Hb, m)	28.91	1.71 (1Ha, m) 1.89 (1Hb, m)	29.27	1.50 (1Ha, m) 1.74 (1Ha, b.d,, 10.4)	**2**
78.94	3.57 *m*	79.05	3.58 *m*	77.84	3.42 *m*	**3**
38.16	2.49 (1Ha, *dd*, 3.6,12.4) 2.35 (1Hb, *t*, 12.4)	38.22	2.36 (1Ha, *m*) 2.46 (1Hb, *m*)	38.58	1.74 (1Ha, *b.d*, 10.4) 2.38 (1Hb, *b.d*, 10.4)	**4**
138.81	–	138.80	–	138.74	–	**5**
118.86	5.36 *b.s*	118.93	5.36 *b.s*	119.20	5.29 *b.s*	**6**
35.04	2.17 *b.s*	35.10	2.17 *b.s*	35.15	2.08 (2H, *b.s*)	**7**
73.69	–	73.91	–	73.86	–	**8**
46.92	1.44 *m*	46.98	1.43 *m*	44.34	1,50 *m*	**9**
36.97	–	37.03	–	37.24	–	**10**
19.00	1.92 (1Ha, m) 1.82 (1Hb, m)	18.68	1.92 (1Ha, m) 1.82 (1Hb, m)	24.67	1.58 (1Ha, m) 1.97 (1Hb, m)	**11**
40.44	1.44 *m*	40.50	1.43 *m*	78.38	4.94 *b.s*	**12**
47.13	–	47.19	–	53.36	–	**13**
85.35	–	85.41	–	86.26	–	**14**
18.62	1.99 (1Ha, m) 1.92 (1Hb, m)	19.07	1.62 (2H, *m*)	34.37	1.66 (1Ha, m) 1.85 (1Hb, m)	**15**
34.82	1.62 *m*	34.88	1.62 (1Ha, *m*) 2.00 (1Hb, *m*)	24.55	1.92 (2H, *m*)	**16**
56.83	1.65 *m*	56.89	1.65 *m*	49.85	2.17 *m*	**17**
15.83	1.31 (3H, *s*)	15.89	1.31 (3H, *s*)	10.85	1.26 (3H, *s*)	**18**
17.27	1.21 (3H, *s*)	17.32	1.21 (3H, *s*)	18.20	1.09 (3H, s)	**19**
77.73	4.00 *br.q*	77.79	4.00 *br.q*	74.14	5.20 *br.q*	**20**
18.62	1.31 (3H, *d*, 6)	19.96	1.31 (3H, *d*, 6)	19.78	1.15 (3H, *d*, 6)	**21**
				Benzoyl at C-12		
				166.19	–	**1**
				130.71	–	**2**
				129.66	8.01 (2H, *d*, *J* = 7.6)	**3,7**
				129.25	7.57 (2H, *m*)	**4,6**
				133.83	7.70 *m*	**5**
				Benzoyl at C-20		
				165.42	–	**1**
				130.71	–	**2**
				128.97	7.28 (2H, *m*)	**3,7**
				129.31	7.71 (2H, *m*)	**4,6**
				133.45	7.57 *m*	**5**

digitaloside-20-*O*-*β*–D-glucopyranoside (Fig. 1).

Compound **3** was obtained as amorphous powder, Based on the HRESI-MS data, the molecular formula of **3** (Fig. 1)was determined to be $C_{46}H_{76}O_{23}$ (995.4678 $[M-H]^-$, calc. 996.4777). The NMR spectral analysis of compound **3** revealed similarity with spectral data of compound **2,** except for the presence of additional sugar moiety attached to the sugar chain at C-3.

This was confirmed from the presence of four anomeric protons and carbons indicating a tetrasaccharide. The sequential assignments of proton and carbon resonances of the sugar moieties and their connectivity to each other or to the aglycone part were determined from HMQC, HMBC, and ^{1}H–^{1}H COSY experiments. The terminal sugar in the sugar chain at C-3 was identified as glucose from the C–H long-range correlation (Fig. 4) observed between H-1```` of the terminal D-glucose (δ_H 4.42) and C-6`` of the middle D-glucose (68.90) in the HMBC spectrum and also confirmed by comparing these data with those reported by Halim and Khalil (1996). From the above-mentioned data, compound **3** was identified as 3*β*,8*β*,14*β*,20-tetrahydroxy-(20*R*)-pregn-5-ene-3-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-digitaloside-20-*O*-*β*–D-glucopyranoside (Fig. 1).

2.2. α-Glucosidase and lipase inhibition activity

The inhibitory capacity of total extracts, fractions and isolated compounds of *C. hexagona* against *α*-glucosidase and lipase enzymes are presented in Table 1. The methanolic extract inhibited *α*-glucosidase with IC_{50} value of 2.66 ± 0.22 mg/mL and inhibited lipase with 27.13 ± 2.23 % at 100 µg/mL, whereas the aqueous extract inhibited both enzymes at the same concentrations by 28.44 ± 3.25 % and 4.67 ± 1.98 % for *α*-glucosidase and lipase, respectively. Among the fractions of the methanolic extract, methylene chloride fraction showed the most potent *α*-glucosidase and lipase inhibitory activities with IC_{50} of 0.78 ± 0.02 mg/mL for *α*-glucosidase. However, fractions eluted from the diaion column showed the lowest or no inhibitory activities against both enzymes. The 50 % inhibition percentage of isolated compounds ranged from 3.62 ± 3.19–55.18 ± 0.13 at concentration 1 mM and 3.62 ± 3.47–63.70 ± 2.60 at concentration 100 µM on *α*-glucosidase and lipase enzymes, respectively. The most potent inhibitory activity against *α*-glucosidase was found for the flavonoid glycoside; compound 5 with an IC_{50} 0.82 ± 2.50 mM compared to standard acarbose (0.81 ± 0.86 mM), apigenin-*C*-glycoside was previously reported to have *α*-glucosidase inhibitory activity(Xiao et al., 2016). Whereas compound 1 showed the highest significant inhibitory activity against lipase with IC_{50} value 23.59 ± 2.49 µM compared to standard orlistat (7.41 ± 2.26 µM), Pregnane glycosides were previously reported as anti-obesity agents through modulating food intake, improving lipid profile or by reducing intestinal fat absorption *via* inhibition of pancreatic lipase (Komarnytsky et al., 2013; Abdel-Sattar

Table 3
^{1}H- and ^{13}C NMR (400 MHz, 100 MHz) of sugar moieties of compounds 1, 2 and 3.

3		2		1		Position
CD_3OD		**CD_3OD**		**DMSO**		
δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	
Dig		**Dig**		**Dig**		
101.48	4.37 (*d*. *J* = 7.6)	101.57	4.37 (*d*. *J* = 7.6)	101.68	4.22 (*d*. *J* = 6.8)	**1**
70.44	3.62 *m*	70.06	3.62 *m*	69.89	3.35 *m*	**2**
84.24	3.26 *m*	84.36	3.26 *m*	84.46	3.14 *m*	**3**
73.48	4.21 (*d*, *J* = 1.6)	73.45	4.21 (*d*, *J* = 1.6)	74.70	4.00 *b.s*	**4**
69.94	3.63 *m*	70.07	3.63 *m*	69.50	3.55 *m*	**5**
16.18	1.31 (3H, *d*,6)	15.99	1.31 (3H, *d*, 6)	17.48	1.15 (3H, *d*, 6)	**6**
57.26	3.53, *s*	57.11	3.53 *s*	58.25	3.39 *b.s*	**OCH_3**
Glc		**Glc**		**Glc**		
102.78	4.62 (*d*. *J* = 7.6)	102.78	4.62 (*d*. *J* = 7.6)	103.75	4.34 (*d*. *J* = 8).	**1**
73.86	3.23 *m*	73.95	3.23 *m*	74.91	3.00 *m*	**2**
76.50	3.33 *m*	76.43	3.33 *m*	77.19	3.14 *m*	**3**
70.19	3.31 *m*	70.24	3.31 *m*	70.75	3.05 *m*	**4**
75.97	3.27 *m*	74.55	3.22 *m*	77.56	3.05 *m*	**5**
68.90	4.16 (1Ha, *d*. *J* = 11.5.) 3.79 (1Hb, *dd*. 11.5, 6.5)	61.63	3.87 (1H, *t*, 11.8) 3.67 (1H, *dd*, 11.8, 5.3)	61.86	3.66 *m* 3.38 *m*	**6**
Glc						
103.63	4.42(*d*. *J* = 7.6)					**1**
73.90	3.20 *m*					**2**
76.62	3.33 *m*					**3**
70.02	3.33 *m*					**4**
76.36	3.26 *m*					**5**
61.33	3.88 (1Ha, *t*, 10.9) 3.67 (1Hb, *dd*, 10.9, 5.2)					**6**
Glc at C-20		**Glc at C-20**				
103.14	4.42 (*d*. *J* = 7.6)	103.20	4.42 (*d*. *J* = 7.6)			**1**
74.37	3.24 *m*	76.56	3.24 *m*			**2**
76.56	3.31 *m*	76.80	3.31 *m*			**3**
70.44	3.30 *m*	70.46	3.30 *m*			**4**
77.16	3.37 *m*	77.22	3.37 *m*			**5**
62.86	3.62 (2H, *b.s*)	61.34	3.62 (2H, *m*)			**6**

et al., 2018; El-Shiekh et al., 2019). In the current study, compound **1** showed the highest activity, whereas **2** and **3** exhibited the weakest activity, which may be attributed to the presence of acyl groups in compound **1**.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P2100 polarimeter (Japan). Mass spectra were measured on a SHIMADZU LCMS-IT-TOF spectrometer. The NMR experiments for all compounds were performed on a Bruker AscendTM 400/R NMR spectrometer operating at a proton NMR frequency of 400 MHz and ^{13}C-NMR (100 MHz). The NMR spectra were recorded in a suitable solvent ($CDCl_3$, DMSO-*d6* or CD_3OD) using TMS as internal standard and chemical shifts were given in δ ppm value. Infra-red spectrophotometer, Schimadzu IR-435, PU-9712 was used for recording IR spectra using KBr discs. Analytical thin-layer chromatography (TLC) was carried out on Merck TLC plates (250 µm thickness, KGF Silica gel 60 and KGF RP-18 Silica gel 60) and spots were visualized in the visible, under UV light (254 and 365 nm) before and after spraying with aluminum chloride and by spraying the dried plates with *p*-anisaldehyde/H_2SO_4 followed by heating at 110 °C. Column chromatography (CC): silica gel (Silica gel, 35–70 µm and 63–200 µm; Fluka, Sigma-Aldrich Chemicals, Germany), RP-C18 (silica gel, 40 − 63 µm; Merck) and Diaion HP-20 AG (Mitsubishi Chemical Industries Co. Ltd, Japan).

3.2. Plant material

The fresh aerial parts of *Caralluma hexagona* Lavranos were collected from Bani Habash Mountain, Almahweet, Yemen, in June 2017 and was chopped into small pieces, dried in shade. The plant was kindly authenticated by Dr. Abdul Wali A. Al Khulaidi, Associate Professor of plant geography, flora and vegetation, member of the center of middle east plants, Al Baha University, Baljurashi, Saudi Arabia. Voucher samples (# 10.7.2019.1) were kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

3.3. Preparation of aqueous extract for in vitro assay

Part of the powdered aerial parts of *C. hexagona* (10 g) was extracted using a decoction method with H_2O (2 × 75 mL, 10 min each) to give 1.83 g of light green residue for further *in vitro* biological studies.

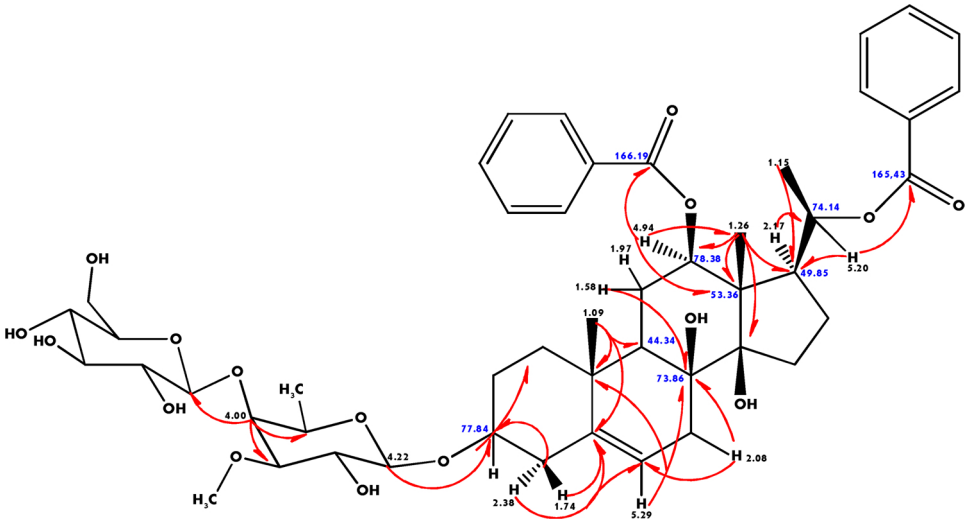

Fig. 2. Some selected HMBC correlations for compound 1.

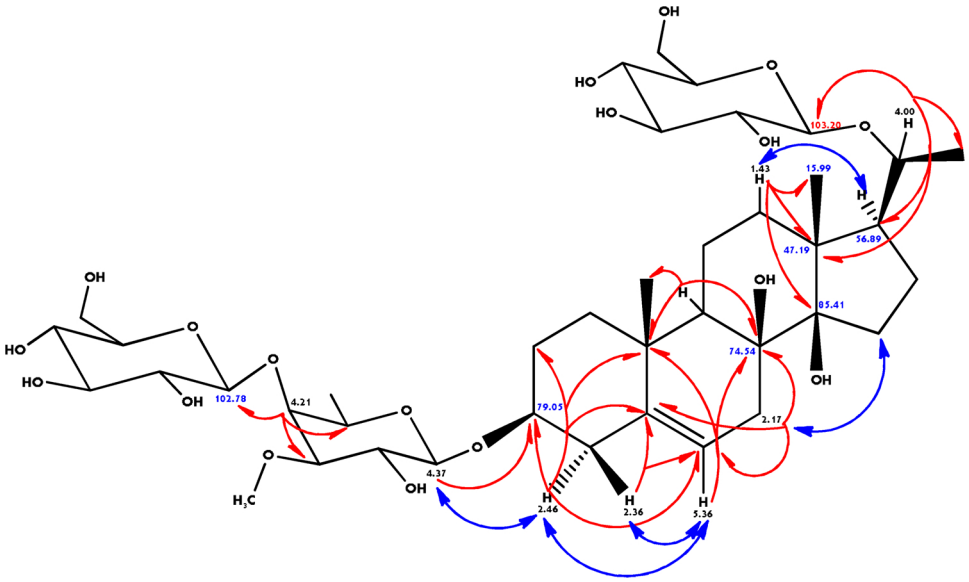

Fig. 3. Some selected HMBC (→) and NOESY (↔) correlations for compound 2.

3.4. Extraction and isolation

The powdered aerial part of *C. hexagona* (2.40 kg) was extracted with methanol (2 × 5 L) by maceration at room temperature for seven days. The methanolic extract was evaporated under reduced pressure to yield a light green solid residue (300 g). The methanolic extract was subjected to fractionation according to the scheme presented in Fig. 5. Part of the methanolic extract (220 g) was suspended in distilled water: MeOH (4: 1 v/v) and partitioned with methylene chloride (4 × 500 mL) to afford 70 g of CH_2Cl_2 fraction. The remaining water was passed through a diaion HP-20 column (600 g, 5 × 100 cm) and eluted with H_2O (3 L), H_2O: MeOH (1:1 v/v) (3 L), and MeOH (3 L). On the evaporation of the three fractions, 101, 26 and 23 g of fractions were obtained, respectively. Part of the methylene chloride fraction (30 g) was chromatographed over open column chromatography using normal silica gel 60 (600 g, 63–200 µm, 5 × 100 cm) with methylene chloride and methanol with an increasing percentage of methanol (5–100 %) as eluent. Fifty-five fractions (200 mL, each) were collected and monitored by TLC after spraying with *p*-anisaldehyde sulfuric acid spray reagent followed by heating at 110 C°. Similar fractions were pooled together to give eight fractions (F1 to F8). Fraction F3 (6. 5 g) was rechromatographed over silica gel column (120 g, 63–200 µm, 3.5 × 50 cm), using *n*-hexane: ethyl acetate (90:10 v/v), as an eluent, which yielded compound **6** (220 mg). Compound **7** (350 mg) was precipitated by methanol as a white powder from fraction F4 (2.0 g). Fraction F5 (5.8 g) was rechromatographed over Silica gel column (120 g, 63–200 µm, 3.5 × 50 cm), eluted using CH_2Cl_2 : MeOH (95: 5 v/v, 1 L), CH_2Cl_2: MeOH (90 : 10 v/v, 1 L) and CH_2Cl_2 : MeOH (85 : 15 v/v, 1 L), to give subfractions F5-1 (0.3 g), F5-2 (1.5 g), F5-3 (0.7 g), F5-4 (0.6 g), F5-5 (0.42 g), F5-6 (0.75 g) and F5-7 (0.56 g). Fraction F5-4 was rechromatographed over silica gel column (20 g, 35 − 70 µm, 1 × 60 cm), using *n*-hexane: ethyl acetate: methanol (3: 5: 2 v/v/v), as an eluent yielding compound **1** (34 mg). Fraction F6 (6 g) was rechromatographed over silica gel column (120 g, 63–200 µm, 3.5 × 50 cm), and was eluted using CH_2Cl_2 : MeOH (90: 10 v/v, 2 L), CH_2Cl_2:MeOH (85: 15 v/v, 1 L) and CH_2Cl_2 : MeOH (80: 20 v/v, 1 L) and MeOH 100 % (0.5 L) to give subfractions F6-1 (0.95 g), F6-2 (2.10 g), F6-3 (1.35 g), F6-4 (0.65 g) and F6-5 (0.73 g). Fraction F6-4 was rechromatographed using RP- 18 silica gel column (30 g, 40 − 63 µm, 2.5 × 25 cm), using MeOH: H_2O (1: 1 v/v), as an eluent to give three compounds; Compound **2** (60 mg), compound **4** (9 mg) and compound **5** (14 mg). Finally, part of fraction F8 (0.70 g) was rechromatographed over RP- 18 silica gel column (30 g, 40 − 63 µm, 2.5 × 25 cm), using MeOH: H_2O (1:1 v/v), as an eluent to yield compound **3** (29 mg).

Compound (1)

White amorphous powder $[\alpha]_{25}^{D}$ +15.3 (c. 0.1, MeOH); IR υ_{max} (KBr, cm^{-1}): 3434, 2930, 1719, 1452, 1277, 1115, 1069, 1022 and 714; HR-ESIMS *m/z* 919.4105 $[M + Na]^+$ (calc. for $C_{48}H_{64}O_{16}Na$,

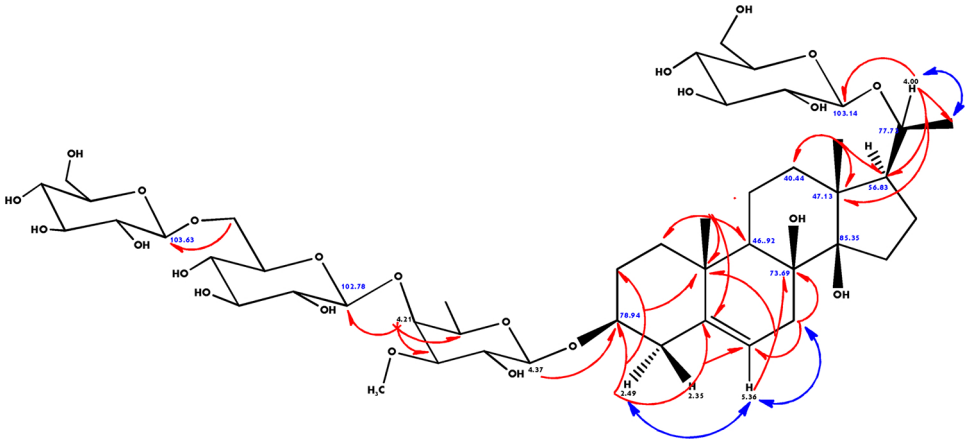

Fig. 4. Some selected HMBC (→) and NOESY (↔) correlations for compound 3.

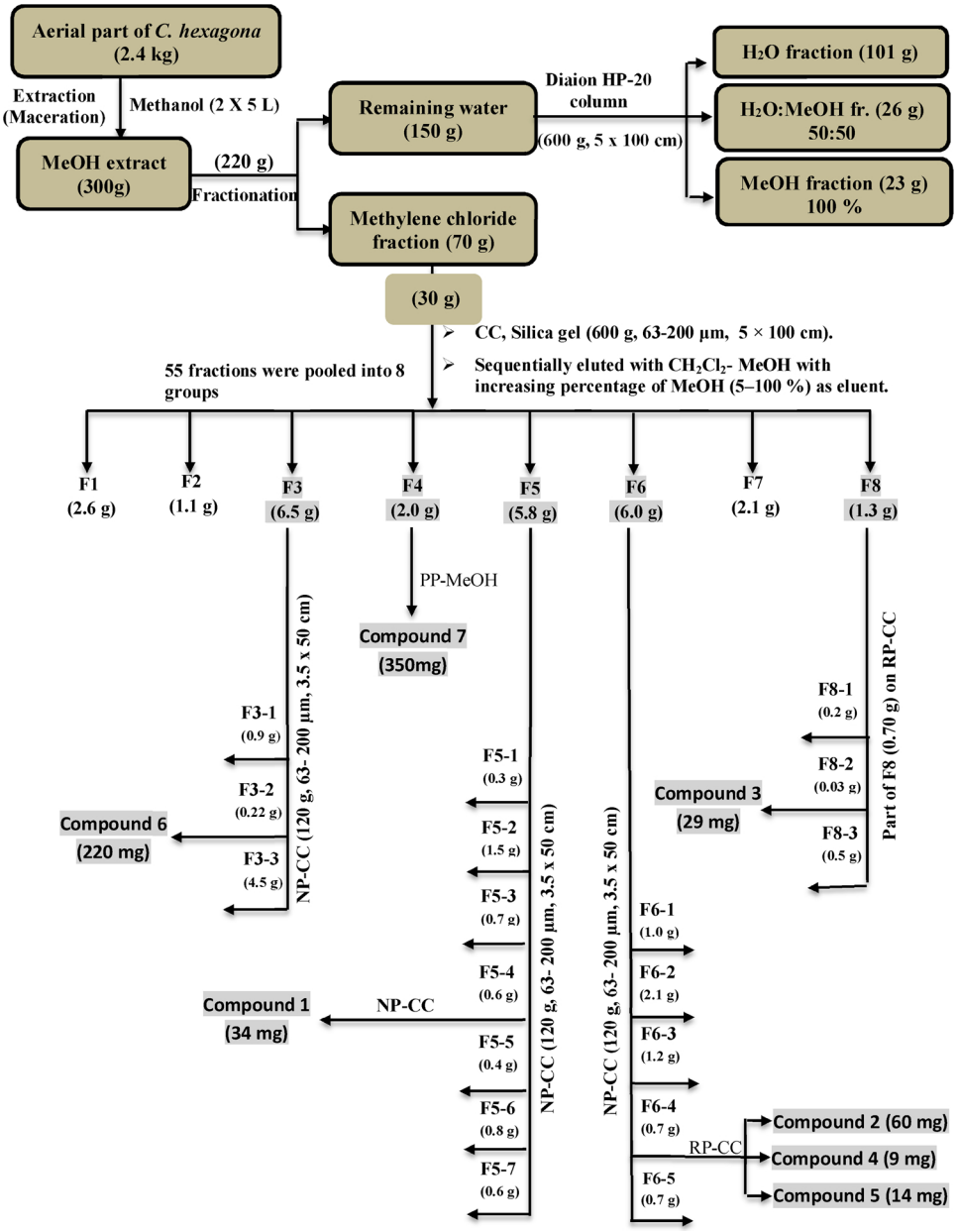

Fig. 5. Scheme for the chromatographic isolation of the bioactive compounds from methylene chloride fraction of *C. hexagona*.

919.4194) in positive mode; Table 2 for ^{1}H, ^{13}C NMR (400 MHz, 100 MHz, DMSO-d_6) of aglycone moiety; and Table 3 for ^{1}H, ^{13}C NMR of sugar moieties.

Compound (2)

Amorphous powder; $[\alpha]_{25}^{D}$ +15.5 (*c*. 0.1, MeOH); HR-ESIMS *m/z* 857.4169 $[M + Na]^+$ (calc. for $C_{40}H_{66}O_{18}Na^+$, 857. 4249) in positive mode; see Table 2 for ^{1}H, ^{13}C NMR (400 MHz, 100 MHz, CD_3OD) of aglycone moiety; and Table 3 for ^{1}H, ^{13}C NMR of sugar moieties.

Compound (3)

Amorphous powder; $[\alpha]_{25}^{D}$ -31.4 (*c*. 0.1, MeOH); HR-ESIMS *m/z* 995.4678 $[M-H]^-$ (calc. for $C_{46}H_{76}O_{23}H^-$, 996.4777) in positive mode; see Table 2 for ^{1}H, ^{13}C NMR (400 MHz, 100 MHz, DMSO-d_6) of aglycone moiety; and Table 3 for ^{1}H, ^{13}C NMR of sugar moieties.

3.5. Acid hydrolysis

Compounds **1**, **2** and **3** (10 mg, each) were refluxed, separately with 1 % HCI (10 mL) in a boiling water bath for 5 h. thereafter, 10 mL H_2O was added and concentrated to 5 mL, and the reaction mixture was kept in a boiling H_2O bath for 30 min and extracted with CH_2Cl_2. The aqueous phase of the hydrolysate was neutralized with 2 N Na_2CO_3 solution, the mixture filtered and the filtrates were concentrated under reduced pressure, tested for carbohydrates by TLC using EtOAc-MeOH-H_2O – HOAc (65: 15: 15:20) as a solvent system, and sprayed with freshly prepared aniline phthalate reagent.

3.6. In vitro enzyme inhibitory assay

3.6.1. Chemicals and drugs

Lipase (Type II: from Porcine pancreas), α-glucosidase from *Saccharomyces cerevisiae* (19.3 U / mg solid, G5003-100UN), orlistat was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). *p*-nitrophenyl-α-D-glucopyranoside (L10070), *p*-nitrophenyl-palmitate (L10896) and acarbose (J61737) were purchased from Alfa Aesar.

3.6.2. Inhibition of α-glucosidase

α-Glucosidase inhibitory activity was measured colorimetrically using the substrate *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) according to an assay method reported by Gutiérrez-Grijalva et al. (2019) with slight modification. Briefly, in 96-microwell plates, 25 µL of extracts, fractions or compounds previously diluted in 5 % DMSO were incubated for 10 min at 37 °C with 50 µL of α-glucosidase from *Saccharomyces cerevisiae* (0.6 U / mL) in phosphate buffer (0.1 M, pH 7). Then, 25 µL of 3 mM *p*NPG as a substrate in phosphate buffer (pH 7) was added, and the mixture was incubated again for 5 min at 37 °C. Enzyme activity was determined by measuring the release of *p*- nitrophenol from the *p*NPG substrate at 405 nm using a microplate reader (Tecan, USA). This assay was carried out using acarbose (purity, 95 %) as a positive control and 5 % DMSO as a negative control (blank). The percentage of inhibition of α-glucosidase was calculated according to the equation:

% Inhibition = [(A blank – A sample) / A blank] x 100

Where **A** blank is the absorbance of the control (blank, without inhibitor), and **A** sample is the absorbance in the presence of the inhibitor.

The percentage inhibitory activity of α-glucosidase was presented at final concentration 2 mg/mL for the total extracts and fractions and at final concentration 1 mM for the isolated compounds then serial dilutions were prepared to calculate the IC_{50} for the most active samples.

3.6.3. Inhibition of pancreatic lipase

The inhibition of pancreatic lipase activity was performed using *p*-nitrophenyl palmitate (*p*-NPP) as substrate and porcine pancreatic lipase (PPL) which was described by (Kordel and Schmid, 1991) with slight modification. 25 µL of extracts, fractions or compounds were pre-incubated with 50 µL of PPL (1 mg/mL) for 10 min in a Tris – HCl buffer (100 mM, pH 8) at 37 °C before assaying the PPL activity. The reaction was then started by adding 10 µL of solution *p*-NPP (10 mM, in isopropanol), the volume was diluted to 200 µL using the Tris – HCl buffer. After incubation at 37 °C for 20 min, the absorbance was read at 405 nm. This assay was also carried out with orlistat as a positive control and 5 % DMSO as blank. The percentage of inhibition of lipase was calculated according to the equation:

% Inhibition = [(A blank – A sample) / A blank] x 100

The results were presented as percentage of lipase inhibition for all extracts and fractions at 100 µg/mL, or compounds at 100 µM and were also presented as the IC_{50} for the highest inhibitor using serial dilutions of 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µM comparing with the orlistat as a standard. Absorbance was measured on TECAN microplate reader (CO. USA).

4. Conclusion

A survey of literature revealed that many species of *Caralluma* can control diabetes and obesity due to their pregnane glycosides content. In the current study, the methylene chloride fraction showed the highest inhibitory activity against both α-glucosidase and pancreatic lipase enzymes in comparison with diaion eluted fractions due to its pregnane content. These results suggested that *C. hexagona* could possibly offer a complimentary safe approach to the treatment and management of obesity by blocking the digestion and absorption of dietary lipids and carbohydrates. However, further *in-vivo* studies with experimental animal models are needed to confirm this activity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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