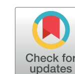
Biomedicine & Pharmacotherapy 127 (2020) 110143

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

New calogenin pregnane glycoside derivative from *Huernia saudi- arabica* and its Lipase and α-Glucosidase Inhibitory Activities



Abeer Mohamed El Sayed*,[1], Essam AbdElSattar, Mohammed Nabil Khalil

Pharmacognosy Department, College of Pharmacy, Cairo University, Kasr El-Einy Street, 11562, Cairo, Egypt

ARTICLE INFO

Keywords:
Docking
Huernia saudi-arabica
Obesity
α-Glucosidase
Pancreatic lipase
Pregnane glycoside

ABSTRACT

As ongoing investigation of *Huernia saudi-arabica* D.V.Field (Asclepiadaceae), a new steroidal pregnane glycoside (Huernioside A) was isolated from dichloromethane fraction (DCM); it was identified as 3*β*, 11, 14*β*, 20(*R*)-tetrahydroxy-pregna-5,9(11)-diene-3-*O*-*β*-D-thevetopyranosyl-(1-4)-*β*-D-cymaropyranoside(HCP) through analysis of 1D, 2D NMR besides ESI-MS data. The alcoholic extract of the aerial part (ALE), DCM and HCP showed inhibitory potential against pancreatic lipase compared to orilstat. Among the tested samples, the ALE and HCP exhibited a promising pancreatic lipase inhibitory commotion through IC_{50} values of 0.61 ± 0.15, 1.23 ± 0.07 mg/ml (equivalent to 88.8 μM), respectively. HCP was prevailed to have a mixed mode of inhibition as exposed by enzyme kinetic studies. Hydrophobic interactions were the major forces involved in ligand enzyme interactions. In contrast, moderate α-glucosidase inhibitory activities were evidenced for ALE and HCP (% inhibition: 24.8 ± 1.8 and 26.6 ± 2.5, respectively) compared to acarbose. This investigation is the first to report on the possible *in vitro* anti-obesity and anti-diabetic impact of *H. saudi-arabica*.

1. Introduction

Metabolic syndrome is defined as a combination of metabolic complaints including hyperglycemia, hypertension, obesity, high-serum triglycerides and low level of high-density lipoprotein [1]. Pancreatic lipase and α-glucosidase are key targets for nutraceuticals and drugs alleviating metabolic syndrome [2]. Pancreatic lipase has a key responsibility in absorption of lipid through triglycerides hydrolysis to glycerol and free fatty acids. While breaking down of starch and disaccharides into glucose for intestinal uptake is the main role of α-glucosidase enzyme, inhibition of these enzymes can hold back triglyceride and carbohydrate absorption, thus causing a reduction in the rate of glucose absorption into the blood. A therapeutic approach for managing obesity and diabetes is inhibition of these enzyme activities in digestive organs [3–5].

Phytochemicals and/or plant extracts that can constrain the pancreatic lipase and *α*-glucosidase enzymes are regarded as valuable agents to control serum levels of sugar besides fat accompanied by minimal side effects when compared to orlistat and acarbose [6,7]. Moreover, they have the aptitude to persuade body weight decrease and avert diet induced obesity [8,9]. Lately, inhibitors of pancreatic lipase are highly evaluated because of their role in hydrolysis of over 80% of the total dietary fat [10]. The inhibition results in delay or reduction in lipid absorption and therefore protects the pancreas, which will reinstate regular insulin production from the *β* cells [11]. Type II diabetes occurs due to dysfunction of insulin-producing pancreatic *β* cells, whose destruction could be instigated by the extreme accretion of lipids in the pancreas [12,13]. *In vitro* and *in vivo* studies of antidiabetic activities of pregnanes phytochemicals have been previously reported[14,15].

Pregnanes and pregnane glycosides are stated in numerous members of the subfamily Asclepiadaceae [15,16]. About 70 species of *Huernia* (Asclepiadaceae) are distributed in the tropical part of the world, South Africa, Ethiopia and Saudi Arabia [17]. Several members of the genus *Huernia* are famine-food plants and have promising ethnopharmacological uses [18]. For example, the wound healing activity of *Huernia Sp.Nov.aff.Boleana* was verified [19] as well as its antidiabetic activity [14]. *Huernia hystrix* was studied as acetylcholinesterase inhibitor, having antioxidant, antiinflammatory, and antimicrobial effects [18]. A previous study by the authors on *Huernia saudi- arabica* have reported the isolation of pregnane glycoside ester which showed a potent anti-schistosomal effect [20]. Pregnane glycosides attracted recent attention because of their health benefits as anti-obesity agents [15,21,22]. Hypoglycemic effect of non-acetylated and acetylated pregnane glycosides was previously reported in different

* Corresponding author.
E-mail addresses: galbd_abeer@yahoo.com, abeer.ali@pharma.cu.edu.eg (A.M. El Sayed), essam_abdelsattar@yahoo.com (E. AbdElSattar), Mohamed.Nabil@pharma.cu.edu.eg (M.N. Khalil).

[1] Website: http://scholar.cu.edu.eg/?q=galbd/.

https://doi.org/10.1016/j.biopha.2020.110143
Received 23 December 2019; Received in revised form 30 March 2020; Accepted 30 March 2020
0753-3322/ © 2020 Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

Caralluma species [15,23]. Therefore, the aim of the present study is to test the possible inhibitory activity of *Huernia saudi -arabica* extracts and their pregnane glycoside on lipase and α-glucosidase enzyme.

In the present study, the authors have stated, for the first time, the inhibitory effects of the alcoholic extract (ALE), dichloromethane soluble (DCM) fraction as well as the new steroidal pregnane glycoside, huernioside A (HCP) isolated from *H. saudi-arabica* on pancreatic lipase and α-glucosidase enzymes. Enzyme kinetics and docking studies were performed to investigate the lipase inhibitory activity.

2. Materials and Methods

2.1. General experimental procedures

Vacuum-liquid (VLC) and column (CC) chromatography were carried out on silica gel 60 (Merck, Kieselgel 60, 70–320 μm), silica gel 60 (Merck, Kieselgel 60, 230–400 μm); respectively. LH-20 Sephadex (25–100 μm, GE Healthcare), reversed phase (RP-18), precoated silica gel F254, RP-18 and cellulose thin layer chromatographic (TLC) plates (20 × 20 cm) were purchased from Sigma-Aldrich (Chemicals-Germany). The following solvent systems were used for developing the chromatograms S1: n-hexane: ethyl acetate (6:4), S2: n-hexane: chloroform (7:3), S3: methanol: water 7:3 and methanol: water 4:6v/v. All solvents were of analytical grade. *p*-anisaldehyde-sulfuric acid was used for visualization of triterpenoids and/or steroids on TLC plates. A Bruker micro TOF mass spectrometer was used for recording mass spectra (ESI-MS). Jasco FT/IR-460 plus (Tokyo, Japan) was utilized to monitor IR spectra as KBr discs. Melting point was determined using Afon® DMP100 Melting Point. Bruker high performance digital FT-NMR spectrophotometer (Karlsruhe, Germany) ^{1}H-NMR 400 (^{1}H) and ^{13}C-NMR 100 (^{13}C) at MHz spectra were detected on a working in $CHCl_3$-d1 as a solvent and chemical shifts were specified in δ (ppm) virtual to tetramethylsilane as interior average.

Reference compounds including D-thevetose and D-cymarose were used to identify the monosaccharides in the glycoside hydrolysate. Sugar identification, including its absolute configuration, was performed as previously reported [20,23]. Lipase was obtained from porcine pancreas (Sigma, Germany), 4-nitrophenyl palmitate was purchased from Alfa Aesar,(Germany). Methanol was of analytical grade. α-Glucosidase was obtained from *Saccharomyces cerevisiae* (Sigma, Germany), 4-nitrophenyl-α-glucoside was obtained from Alfa Aesar, (Germany). Tris base was obtained from (Sigma, Germany). Orlistat and acarbose were purchased from Sigma Aldrich (St Louis, MO, USA). All supplementary chemicals used were of a methodical grade.

2.2. Plant materials

In March, 2011, the plant was collected from southwest Saudi Arabia in rocky regions of El Taif. Wadi Thee-Gazal in the Al-Shafa region (SW Arabia 2000 m (AMSL) Above Medium Sea Level) is the richest area in vegetation in Al-Taif province, including many species of genus Huernia. Authentication of the plants was carried by Prof Dr. Nahid Wally, Faculty of Science, King Abdelaziz University, Jeddah, Saudi Arabia. In the herbarium of the college of pharmacy, King Abdul-Aziz University (Girls section) Jeddah, Saudi Arabia, a voucher specimen was deposited (# 11677-A).

The aerial parts (500 g) of *H.saudi-arabica* were dried and percolated in ethanol at extent temperature to give a dark green semisolid deposit 15 g following evaporation of the solvent. Alcohol extract (ALE) 10 g was suspended in water and defatted with petroleum ether, then successive extraction was conducted using Dichloromethane (DCM) and n-butanolto yield 3, 4 and 3.8 g of solid residue, respectively. TLC of the different soluble fraction revealed that the DCM is the richest soluble fraction in the phytoconstituents. Consequently, it was subjected to further investigation.

2.3. Fractionation and isolation of the components of the dichloromethane soluble fraction

Four grams were chromatographed on a VLC column packed with silica gel H (210 g, 12.5 x 7 cm) using gradient elution with chloroform, and chloroform- methanol mixtures. Aliquots of 200 ml each, were gathered and examined by TLC. Alike fractions were collected together to get two main fractions (I-II). Fraction II (1.57 g), eluted with 20% methanol/chloroform, was additionally subjected to re-chromatography on top of RP-18 silica gel column through (7:3) methanol-water as eluent which leads to isolation of HCP compound (20 mg).

2.4. Acid hydrolysis

Two mg of HCP were hydrolyzed according to the procedure reported by Ma et al., [24]. The hydrolsate contained the aglycone (Δ 9(11) calogenin pregnane), cymarose and thevetose as determined by TLC comparison with authentic samples [25–27]. Moreover, the comparison of chemical shift values of ^{13}CNMR to those reported in literature gave unequivocal evidence about the configuration of the sugars. The absolute configurations of D-cymarose, and D-thevetose were determined as per the method published by Hara et al. [28]. This determination was conformed the observation that D-cymarose is common in *Huernia* species. *Huernia and Caralluma* species were characterized with the presence of cymarose and thevetose and others sugars.

2.5. HCP compound

It is a white amorphous powder; 30.72μM, 65.1%; m.p.170-172 °C; IR (KBr)ν_{max}: 3370 and 1448 cm^{-1}; ESI/MS m/z 657 $[M^{+}Na\text{-}H_2O]^{+}$ in positive mode and m/z 651 [M-H]- in negative (calc. for $C_{35}H_{56}O_{11}$.651); as shown in Table S1 for^{1}H- NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz,$CDCl_3$) assignments.

2.6. Assay for Lipase Inhibitory activity

Inhibitory action in opposition to pancreatic lipase was deliberate subsequent to the previously published method of Pöhnlein et al., [29]. Enzyme assay was composed of 220 μl final volume. First, lipase enzyme (50 μl) of 2.5 mg/ml solution in buffer tris-HCl (200 mM, pH 7.5) was incubated with tris buffer (-158 μl) and the inhibitor (10 μl, methanolic solution) or methanol was used in case of blank assay. Incubation lasted for 5 minutes at room temperature. The reaction started by addition of *p*-nitrophenyl palmitate (2 μl of 10 mM solution in MeOH) and lasted for 30 min at 37 °C. The color of the developed product was measured using microplate reader (Tecan Infinite f50, Switzerland) at 405 nm. Blank assays were performed to nullify the inherent color of the extracts.

2.7. Assay for α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined by a colorimetric assay utilizing a well-established protocol with some modifications [30]. Enzyme assay was composed of 220 μl. It was performed in 96-well microplate. First, 100 μl of the substarte (NPG, 6 mM) is mixed with 10 μl tested inhibitor. Then, the response started by the adding of the enzyme (100 μl of 0.3 U/ml) after incubation at 37 °C for 15-20 min. The color of the developed product was measured using microplate reader at 405 nm. Blank assays were performed to nullify the inherent color of the extracts. The percent of inhibition was estimated as follows:

% of inhibition = (AN –At)/AN X 100

Where AN is absorbance of normal enzyme assay with no inhibitor; At is absorbance of enzyme assay containing tested extract or compound.

2.8. Mode of inhibition

To detect the mode of the inhibition of the tested compound HCP against lipase enzyme Line weaver-Burk plots were utilized. Enzyme reactions were performed in absence and presence of the inhibitor (8.8 and 4.4μM) at different substrate concentrations (10, 5, 2.5, 1.25 μM) of *p*-nitrophenyl palmitate. Ki was deliberate by means of Graph Pad Prism software utilizing the equation applied for assorted type of inhibition.

V max App = V max /(1 + I/(Alpha * Ki))

Km App = Km * (1 + I/Ki)/(1 + I/(Alpha * Ki))

Y = V max App * X/ (Km App + X)

The factor I is the concentration of inhibitor. The factors Alpha, Vmax, Km and Ki were determined by Prism which fits one best-fit value for the whole set of information.

2.9. Molecular docking of orlistat and compound HCP

Molecular Operating Environment software (MOE), Chemical Computing Group Inc., Montreal, Canada) was used for performing molecular docking. Pancreatic lipase crystal l structure was downloaded from PDB (1lpb) (http://www.rcsb.org/). Hydrogens, connectors and atoms were added following the standard procedure of MOE. Binding pocket was located and confirmed to contain the amino acid Ser152. Compound HCP was drawn in chemsketch software where its SMILES code was exported to MOE. SMILES structural code of orilstat was imported from the Pubchem database (PubChem CID: 3034010). Both ligands were subjected to energy minimization. All compounds were stored in a single database file to be used in docking estimation. Docking was performed between target dummies and ligands in the database file with the following defaults: i) triangle matcher was utilized as the placement methodology, ii)London dG was utilized as Scoring methodology and was adjusted to the default values.

2.10. Data presentation and statistical analysis

The entire data were presented from a bare minimum of three experiments. Analysis of concentration mortality data was conducted to estimate the IC_{50} value and dose-response using the Graph Pad Prism 6 software.

3. Results

3.1. Identification of pregnane from H. saudi-arabica

Compound HCP (named huernioside A) was isolated as white amorphous powder (20 mg (30.72μM), mp170-172 °C with $C_{35}H_{56}O_{11}$ the molecular formula, as prevailed from its ^{13}C-NMR in addition to ESI/MS *m/z* 657 $[M^{+}Na\text{-}H_2O]^{+}$ in positive mode and 651[M-H]- in negative mode (Figs. S1–7). It exhibits an activist Libermann-Burchard as well as keller-kiliani reactions demonstrating the incidence of steroidal skeleton with a 2-deoxy sugar [31]. IR scale showed assimilation bands owing to the presence of hydroxyl (3370 cm^{-1}) and the absence of the carbonyl groups. The ^{1}H and^{13}C-NMR data (Figs. S1–2) revealed the presence of two anomeric protons (δ_H4.88, 4.32) and carbon signals at δ_c95.7, 104.2 correspondingly, indicating the presence a diglycoside consisting of methoxy sugars of 6-deoxyhexose and 2,6dideoxyhexose (cymarose and thevetose) units (Table S1, Fig. S1). The inner sugar unit was recognized as cymarose, whereas the terminal one was identified as thevetose (6-deoxy-3-*O*-methyl-D-glucose) when compared to data in literature [25,32]. Identity of the deoxy sugars was confirmed from the signals at doublet signals at δ_H 1.31, 1.33 (every 3H, *d*, $J = 6.2$ Hz) which were correlated among the ^{13}C-NMR signals at δ_c18.3, 17.8 respectively, and assigned to the secondary methyl (C-6`), (C-6``). In addition, the $^{1}$H-NMR methyl singlet's at $\delta_H$ 3.45, 3.67 (3 H) was correlated with carbon signals at $\delta_c$ 57.81, 60.68 and credited to the two $OCH_3$ groups of the sugars moieties attached to C-$3_{Cym}$ and C-$3_{Thev}$, correspondingly. The axial orientation of H-2``,H-3`` and H-4`` feature for thevetose was consistent with the splitting pattern and coupling constant of H-3`` (*t*, $J = 8.0$ Hz). Cymarose unit was glycosylated at C-4 as revealed by a downfield shift observed for C-4_{Cym} 82.6 ppm. The ^{13}C-NMR spectrum of HCP (Fig. S2) exhibits the glycosylation site at C-3 which was deduced from a downfield shift of C-3 and the upfield shifts of C-2 and C-4. A long-range correlation between C-3 (δ_c77.4) and H-1` ($\delta_H$4.88) in the HMBC spectrum prevailed the attachment of the cymarose to C-3. The recognition of the sugar part and its connection sits (H-1`$_{cym}$-C-3, H-1``$_{Theve}$-C-4`$_{Cym}$) was confirmed as the C-1` of the inner cymarose-I unit typically resonate up field ($\delta_c$95.7) when connected to C-3 of the aglycone in disparity to C-1`` of thevetose (δ_c104.2) linked to C4` of cymarose. The sequence of sugar units was also defined by HMBC spectrum which prevailed correlation between H-1``$_{Thev}$ (δ_H 4.32, *d*, $J = 7.8$ Hz) and C-4`$_{Cym}$(δ_c 82.6). Therefore, the sugar chain was established to be *β*-D-theveopyranosyl-(1-4)-*β*-D-cymaropyranoside. The *β* pattern of the anomeric protons was proved from their large *J* H1, H2 coupling constant 7.8 and 9.3 Hz respectively (Table S1).

The structure of the aglycone moiety of HCP was deduced to be calogenin from careful examination of its1D-, 2D-NMR data and comparison to literature [33–35]. ^{1}H-NMR spectrum (Fig. S1 & Table S1) showed two singlets and one doublet at δ_H 1.00, 1.60 and 1.33 (*d*, $J = 6.0$) this indicated the presence of methyl groups CH_3-19, CH_3-18 and CH_3-21groups respectively. ^{1}H-NMR spectrum also showed an olefinic proton at δ_H5.38 (H-6) of a double bond located at C-5/C-6 on the basis of long-range HMBC correlations between H-6 and C-4 and C-7 which is in agreement with literature [32]. Moreover, ^{13}C-NMR showed a second double bond located at C-9/C-11 at δ_c 113.2 and 175.6 as two quaternary carbons, respectively, with a hydroxyl group at C-11. The position of OH at C-11 was evidenced from the downfield of C-11 (175.6) which showed a long range correlation with H-12 (δ_H 1.92) relative to the previously reported value [22]. In HSQC spectrum signals at δ_H 3.54 (1H, m) and 3.59 (1H, br q) were correlated with the oxygenated methine carbons at 77.4 and 77.2 ppm, and recognized for protons H-3 and H-20 correspondingly. The oxygenated quaternary carbons at δ_c 175.5 and 113.2 were predicted to C-11 and C-14 which is in agreement with previously reported data [32,34]. Configuration of C-20 was left unassigned in many of the previously identified pregnane glycosides [25,32]. On the other hand, a cautious examination was carried out to determine the C-20 configuration through comparing the ^{13}C-NMR of the 20R and 20S pregnane compounds [35]. As there was notable changes in the ^{13}C chemical shits values for C-16 and C-20 between the two sets of epimers. The reported ^{13}C shifts values for C-20 and C-16 in case of 20*R* and *S* epimers were approximately at 71.0, 65.0 ppm for C-20 and 27.0, 19.0 ppm for C-16 respectively [35]. Likewise, it was found that the ^{13}C chemical shifts values for C-16 and C-20 at δ_c 20.3 and 77.2 respectively, pinpoint of an *R*-configuration for C-20 [34]. As explained from the fact that the free rotation in the 20-hydroxy-C/D-*Cis*-pregnane type steroids was implicit to be limited by steric hindrance amongst C-18 methyl, C-21 methyl and C-20 hydroxyl groups on the origin of the space-filling model, which proves that HCP is of the 20*R* epimer pregnane steroids. Fusion of rings C and D is *Cis* in accordance to previously reported data [34]. After a careful analysis of the NMR spectra (COSY, HSQC, HMBC) (Figs. S3–5) and assessment of reported values the genin was identified as Δ 9(11) calogenin [25,34,36]. The vicinal H-17 to H-20 coupling constants was observed as small values in the ^{1}H-NMR spectra. Cross-peaks were observed between H-17 and H-20, CH_3-18 and H-20 in NOESY spectrum. The compound prevailed H-17 and H-20 in the *α*-configuration (δ_H 2.67, br d, 2.7 Hz) and (δ_H3.55, m) respectively as revealed from the NOESY spectrum when compared with the allied compounds [34]. Elevated strength cross peaks from a long-range coupling by H-18 with H-17 and

H-12 (axial) were assigned. Consequently, HCP evidenced the presence of four hydroxyl groups located at 3*β*, 11, 14*β* and 20 based on spectra data and reported data [34]. Based on these findings, the aglycone of the HCP compound was determined to be 20*R* –pregn-5,9-dien-3*β*, 11, 14*β*, 20-tetraol ($C_{21}H_{32}O_4$). From the above detailed information, the HCP compound was recognized as huernioside A: 3*β*-11, 14*β*, 20(*R*)-tetrehydroxy-pregna-5,9(11)-diene-3-*O*-*β*-D-theveopyranosyl-(1-4)-*β*-D-cymaropyranoside as reported in the current study for the first time in nature.

3.2. Lipase Inhibitory activity

Among the tested extracts, ALE showed the highest inhibitory activity against lipase, the lowest IC_{50}, followed by the isolated compound HCP and finally the DCM fraction (Table S2 & Fig. S8). HCP had a higher inhibitory activity with IC_{50} (1.23 ± 0.07) at 88.8μM than its fraction, around half of the IC_{50} of the DCM (2.66 ± 0.20). This can be explained by the presence of other antagonizing constituents. However, the ALE IC_{50} (0.61 ± 0.15) was more active than the compound, half the IC_{50} of HCP. This could indicate the presence of other constituents with lipase inhibitory activity in the extract and/or possible synergistic interaction. Few pregnane diterpenoids were tested for lipase inhibitory activity; such as stemmoside C, a pregnane glycoside isolated from *Solenostemma argel* (Argel) which showed lipase inhibitory inhibitory activity [37].

3.3. α-glucosidase inhibitory activity

The tested ALE, DCM and the HCP compound had a weak *α*-glucosidase activity (Table S3). Therefore, the percentage of inhibition of the highest attainable concentration of each of them was determined in the enzyme assay conditions. Dichloromethane fraction showed the highest activity; however, it was less than 50% inhibition. Similar to the current findings, pregnane glycosides form *Gymnema sylvestre* exhibited such a poor inhibitory activity [38]. However, other diterpenes of ent-kaurane [39,40] or taxane skeletons [41,42] have potent activities.

3.4. Mode of inhibtion

Mode of inhibition of the HCP compound was demonstrated to exhibit mixed mode of inhibition. Presence of the inhibitor has affected the k_m value and V_{max} (Fig. S9, Table S4) which indicates an impact on the affinity of substrate to the enzyme and the enzyme reaction rate. Similar mode of inhibition was observed with polyphenolic constituents [43], the aflavin-3,3’-digallate [44] methanolic extract of some plant extracts [45,46].

3.5. Molecular docking of orlistat and compound HCP

Similar to many docking studies performed for orlistat, the formamido oxygen or the carbonyl of the lactone ring could accept H-bond from serine amino acid (Ser152) in the active site (Fig. S10 a, b) [47,48]. The long tridecanyl side chain contributes to the hydrophobic interaction between orlistat and the hydrophobic amino acids lining the active pocket of lipase. The binding score for orlistat was determined to be (-12.1) which was lower than that of HCP, *viz*. (-12.05). This was further confirmed by the lower IC_{50} of orlistat. The binding interaction between HCP and lipase consisted entirely of hydrophobobic interactions to the hydrophobic amino acids lining the active site (Fig. S10 c, d). However, a possible interaction with serine (152) is could occur as confirmed by the appropriate distance (Fig. S8c). In both cases, the aglycone part was essential for binding to the receptor (Fig. S10 c, d). Such a weak interaction of HCP with the active site explained the much higher IC_{50} compared to orlistat (88 folds). Nevertheless, such hydrophobic interaction is essential for the ligand-receptor recognition [49,50]. However, the kinetic study has shown that HCP has a mixed mode of inhibition which may allude that HCP might have another binding site to the protein. This will need a more detailed study.

4. Discussion

The present work contributes to the ongoing phytochemical and biological characterization of the interesting succulent species of *Huernia* [20]. Despite their utilization as famine food and in folk medicine [14,18], their chemical profiling was poorly studied. A new pregnane glycoside was isolated and identified utilizing modern spectroscopical techniques. Moreover, the potential of the alcoholic extract and HCP as possible lipase inhibitors was highlighted. HCP showed a promising activity with a moderate IC_{50} value. So far, phenolics, saponins, alkaloids, polysaccharides and aromatic terpenoids were studied [10,51]. In contrast to other investigated phytochemicals, HCP has two merits. First, its pregnane nucleus is essential for hydrophobic interaction to the receptor. Such an interaction is essential in the ligand-receptor recognition [49,50]. Therefore, pregnane scaffold can be used as lead nucleus for designing more active derivatives. Second, its mixed mode of inhibition could be inciting to investigate other possible inhibitor-enzyme interacting sites. Overall, pregnane derivatives need more studies to elucidate their pancreatic lipase inhibitory activity. Consequently, their nucleus can be modified to develop more potent derivatives. The most significant enzyme accountable for digestion of dietary fat, slowing down the declaration of fat into adipose tissue and repression of weight gain is pancreatic lipase which has beneficial effects to overweight and obesity [52]. Reviews have discovered that plants loaded in phytoconstituents as steroidal saponins and polyphenol can inhibit pancreatic lipase and reduce weight gain in high-fat diets [32]. Therefore, the ability of natural products in inhibiting lipase and α-glucosidase might provide a substitute therapy for the management of obesity [53].

5. Conclusion

The authors highlight that the new steroidal pregnane glycoside (Huernioside A); 3*β*,11, 14*β*,20(*R*)-tetrahydroxypregna-5,9(11)-diene-3-*O*-*β*-D-thevetopyranosyl-(1-4)-*β*-D-cymaroopyranoside could be the future therapeutic agents for inhibition of pancreatic lipase.

6. Contribution of all authors

Dr Abeer M.El Sayed contributes to idea of paper, study design, collection of materials, methodology, isolation and identification of the new isolated compound, writing the paper and revising it. Dr. Essam Abdel Satter contributes to identification of the new isolated compound, revising the manuscript. Dr Mohamed N. Khalil contributes to do the enzyme assays, writing the paper and revising it.

7. Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

Authors would like to thank Dr Nahid Wally, College of Science, King Abd El Aziz University, Jeddah, Saudi Arabia who kindly identified *Huernia-saudi arabia* plant.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110143.

References

[1] N. Chatsumpun, B. Sritularak, K. Likhitwitayawuid, New Biflavonoids with α-glucosidase and pancreatic lipase inhibitory activities from Boesenbergia rotunda, Molecules (Basel, Switzerland) 22 (11) (2017) 1862.
[2] K. Sakulnarmrat, I. Konczak, Composition of native Australian herbs polyphenolic-rich fractions and in vitro inhibitory activities against key enzymes relevant to metabolic syndrome, Food Chemistry 134 (2) (2012) 1011–1019.
[3] S.A. Tucci, E.J. Boyland, J.C. Halford, The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents, Diabetes Metab Syndr Obes 3 (2010) 125–143.
[4] W.-C. Hung, X.-H. Ling, C.-C. Chang, H.-F. Hsu, S.-W. Wang, Y.-C. Lee, C. Luo, Y.-T. Lee, J.-Y. Houng, Inhibitory effects of Siegesbeckia orientalis extracts on advanced glycation end product formation and key enzymes related to metabolic syndrome, Molecules (Basel, Switzerland) 22 (10) (2017) 1785.
[5] M.S. Costamagna, I.C. Zampini, M.R. Alberto, S. Cuello, S. Torres, J. Pérez, C. Quispe, G. Schmeda-Hirschmann, M.I. Isla, Polyphenols rich fraction from Geoffroea decorticans fruits flour affects key enzymes involved in metabolic syndrome, oxidative stress and inflammatory process, Food chemistry 190 (2016) 392–402.
[6] A.L. de la Garza, F.I. Milagro, N. Boque, J. Campión, J.A. Martínez, Natural Inhibitors of Pancreatic Lipase as New Players in Obesity Treatment, Planta Med 77 (08) (2011) 773–785.
[7] M. Marrelli, M.R. Loizzo, M. Nicoletti, F. Menichini, F. Conforti, Inhibition of Key Enzymes Linked to Obesity by Preparations From Mediterranean Dietary Plants: Effects on α-Amylase and Pancreatic Lipase Activities, Plant Foods for Human Nutrition 68 (4) (2013) 340–346.
[8] J.O. Unuofin, G.A. Otunola, A.J. Afolayan, In vitro α-amylase, α-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of Kedrostis africana (L.) Cogn, Heliyon 4 (9) (2018) e00810-e00810.
[9] G.A. Mohamed, S.R.M. Ibrahim, E.S. Elkhayat, R.S. El Dine, Natural anti-obesity agents, Bulletin of Faculty of Pharmacy, Cairo University 52 (2) (2014) 269–284.
[10] E. Bialecka-Florjanczyk, A.U. Fabiszewska, J. Krzyczkowska, A. Kurylowicz, Synthetic and Natural Lipase Inhibitors, Mini reviews in medicinal chemistry 18 (8) (2018) 672–683.
[11] M.H. Yang, Y.-W. Chin, K.D. Yoon, J. Kim, Phenolic compounds with pancreatic lipase inhibitory activity from Korean yam (Dioscorea opposita), Journal of enzyme inhibition and medicinal chemistry 29 (1) (2014) 1–6.
[12] O.T. Hardy, M.P. Czech, S. Corvera, What causes the insulin resistance underlying obesity? Curr Opin Endocrinol Diabetes Obes 19 (2) (2012) 81–87.
[13] A.E. Brandon, B.M. Liao, B. Diakanastasis, B.L. Parker, K. Raddatz, S.A. McManus, L. O'Reilly, E. Kimber, A.G. van der Kraan, D. Hancock, D.C. Henstridge, P.J. Meikle, G.J. Cooney, D.E. James, S. Reibe, M.A. Febbraio, T.J. Biden, C. Schmitz-Peiffer, Protein Kinase C Epsilon Deletion in Adipose Tissue, but Not in Liver, Improves Glucose Tolerance, Cell Metabolism 29 (1) (2019) 183–191 e7.
[14] S.O. Alzahrani, A.M. Alwagdani, A.M. Alotaibi, G. Hamaidi, M. Al-Remawi, Y. Gouda, K. Mohamed, Study of the antidiabetic activity of Huernia Sp Nov. aff. boleana growing in high altitude areas of southwest Saudi Arabia, Annals Biol Sci 3 (2015) 15–20.
[15] E. Abdel-Sattar, E.T. Mehanna, S.H. El-Ghaiesh, H.M. Mohammad, H.A. Elgendy, S.A. Zaitone, Pharmacological Action of a Pregnane Glycoside, Russelioside B, in Dietary Obese Rats: Impact on Weight Gain and Energy Expenditure, Frontiers in pharmacology 9 (2018).
[16] R. Xu, Y. Yang, Y. Zhang, F. Ren, J. Xu, N. Yu, Y. Zhao, New pregnane glycosides from Gymnema sylvestre, Molecules (Basel, Switzerland) 20 (2) (2015) 3050–3066.
[17] N.M. Waly, S.A. Al-Rehaily, Taxonomic Studies on some species of genus Huernia R. Br.(Asclepiadaceae) growing in AL-Taif Province, Saudi Arabia, Taeckholmia 33 (2016).
[18] S.O. Amoo, J.F. Finnie, J. Van Staden, Acetylcholinesterase Inhibition, Antioxidant, Antiinflammatory, Antimicrobial and Phytochemical Properties of Huernia hystrix, Phytotherapy Research 26 (5) (2012) 639–645.
[19] F. Hamam, A. Eldalo, Q. Abdallah, I. Al-Deeb, S. Alzahrani, A. Alwagdani, A. Alotaibi, A.-R. Nasr, Y. Gouda, K. Mohamed, Pharmacological activities of a novel plant species, Huernia Sp. Nov. aff. Boleana growing in the high mountains of southwest Saudi Arabia, Molecular medicine reports 17 (4) (2018) 6059–6067.
[20] A.M. El Sayed, M.M. Basyoni, S.H. ElGayed, A.A. El-Badry, E. Abdel-Sattar, Pregnane glycoside from Huernia saudi-arabica as latent schistosomicidal mediator, Natural product research (2018) 1–6.
[21] B.L. Graf, I. Raskin, W.T. Cefalu, D.M. Ribnicky, Plant-derived therapeutics for the treatment of metabolic syndrome, Current opinion in investigational drugs (London, England: 2000) 11 (10) (2010) 1107.
[22] S. Liu, Z. Chen, J. Wu, L. Wang, H. Wang, W. Zhao, Appetite suppressing pregnane glycosides from the roots of Cynanchum auriculatum, Phytochemistry 93 (2013) 144–153.
[23] E. Abdel-Sattar, S.A. El-Maraghy, R.S. El-Dine, S.M. Rizk, Antihyperglycemic activity of Caralluma quadrangula in streptozotocin-induced diabetic rats, *Bulletin of Faculty of Pharmacy*, Cairo University 55 (2) (2017) 269–272.
[24] X.-X. Ma, F.-T. Jiang, Q.-X. Yang, X.-H. Liu, Y.-J. Zhang, C.-R. Yang, New pregnane glycosides from the roots of Cynanchum otophyllum, Steroids 72 (11–12) (2007) 778–786.
[25] R.S. Pawar, Y.J. Shukla, I.A. Khan, New calogenin glycosides from Hoodia gordonii, Steroids 72 (13) (2007) 881–891.
[26] F. Gao, Y.-C. Yao, S.-B. Cai, T.-R. Zhao, X.-Y. Yang, J. Fan, X.-N. Li, J.-X. Cao, G.-G. Cheng, Novel immunosuppressive pregnane glycosides from the leaves of Epigynum auritum, Fitoterapia 118 (2017) 107–111.
[27] Z. Wang, M. Jiang, A. Khan, S. Cai, X. Li, J. Liu, G. Kai, T. Zhao, G. Cheng, J. Cao, Epigynumgenane-type pregnane glycosides from Epigynum cochinchinensis and their immunosuppressive activity, Phytochemistry 168 (2019) 112127.
[28] S. Hara, H. Okabe, K. Mihashi, Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4 (R)-carboxylates, Chemical and Pharmaceutical Bulletin 35 (2) (1987) 501–506.
[29] M. Pöhnlein, T. Finkbeiner, C. Syldatk, R. Hausmann, Development of a microtiter plate-based assay for the detection of lipase-catalyzed transesterifications in organic solvents, Biotechnol Lett 37 (3) (2015) 705–710.
[30] L. Ting, X. Zhang, Y. Song, J. Liu, A microplate-based screening method for alpha-glucosidase inhibitors, Chinese Journal of Clinical Pharmacology and Therapeutics 10 (10) (2005) 1128–1134.
[31] X. Li, H. Sun, Y. Ye, F. Chen, Y. Pan, C-21 steroidal glycosides from the roots of Cynanchum chekiangense and their immunosuppressive activities, Steroids 71 (1) (2006) 61–66.
[32] A. Braca, A. Bader, I. Morelli, R. Scarpato, G. Turchi, C. Pizza, N. De Tommasi, New pregnane glycosides from *Caralluma negevensis*, Tetrahedron 58 (29) (2002) 5837–5848.
[33] M. Abdul-Aziz Al-Yahya, E. Abdel-Sattar, E. Guittet, Pregnane glycosides from Caralluma russeliana, Journal of natural products 63 (10) (2000) 1451–1453.
[34] S.M. Al-Massarani, S. Bertrand, A. Nievergelt, A.M. El-Shafae, T.A. Al-Howiriny, N.M. Al-Musayeib, M. Cuendet, J.-L. Wolfender, Acylated pregnane glycosides from *Caralluma sinaica*, Phytochemistry 79 (2012) 129–140.
[35] M. Kimura, K. Hayashi, H. Narita, H. Mitsuhashi, Studies on the Constituents of Asclepiadaceae Plants. LI. Oxidation at the 18-Methyl Group of C/D-cis-Pregnane Type Steroids and ^{13}C-Nuclear Magnetic Resonance Spectra of 18-Oxygenated Pregnanes and Related Compounds, CHEMICAL & PHARMACEUTICAL BULLETIN 30 (11) (1982) 3932–3941.
[36] S.-X. Qiu, L.-Z. Lin, G.A. Cordell, M. Ramesh, B.R. Kumar, M. Radhakrishna, G.K. Mohan, B.M. Reddy, Y.N. Rao, B. Srinivas, N.S. Thomas, A.V.N. Appa Rao, Acylated C-21 steroidal bisdesmosidic glycosides from Caraluma umbellata, Phytochemistry 46 (2) (1997) 333–340.
[37] R. El-shiekh, D. Al-Mahdy, M. Hifnawy, E. Abdel-Sattar, In-vitro screening of selected traditional medicinal plants for their anti-obesity and anti-oxidant activities, South African journal of botany 123 (2019) 43–50.
[38] R. Xu, Y. Yang, Y. Zhang, F. Ren, J. Xu, N. Yu, Y. Zhao, New pregnane glycosides from Gymnema sylvestre, Molecules (Basel, Switzerland) 20 (2) (2015) 3050–3066.
[39] D.-X. Yan, C.-A. Geng, T.-H. Yang, X.-Y. Huang, T.-Z. Li, Z. Gao, Y.-B. Ma, H. Peng, X.-M. Zhang, J.-J. Chen, LC-MS guided isolation of diterpenoids from Sapium insigne with α-glucosidase inhibitory activities, Fitoterapia 128 (2018) 57–65.
[40] Z.T. Deng, C.A. Geng, T.H. Yang, C.L. Xiang, J.J. Chen, Chepraecoxins A-G, ent-Kaurane Diterpenoids with alpha-Glucosidase Inhibitory Activities from Chelonopsis praecox, Fitoterapia 132 (2019) 60–67.
[41] P.H. Dang, H.X. Nguyen, T.T.T. Duong, T.K.T. Tran, P.T. Nguyen, T.K.T. Vu, H.C. Vuong, N.H.T. Phan, M.T.T. Nguyen, N.T. Nguyen, S. Awale, α-Glucosidase Inhibitory and Cytotoxic Taxane Diterpenoids from the Stem Bark of Taxus wallichiana, Journal of Natural Products 80 (4) (2017) 1087–1095.
[42] V.K. Bajpai, Y.-H. Park, M. Na, S.C. Kang, α-Glucosidase and tyrosinase inhibitory effects of an abietane type diterpenoid taxoquinone from Metasequoia glyptostroboides, BMC Complementary and Alternative Medicine 15 (1) (2015) 84.
[43] A.I. Martinez-Gonzalez, E. Alvarez-Parrilla, A.G. Diaz-Sanchez, L.A. de la Rosa, J.A. Nunez-Gastelum, A.A. Vazquez-Flores, G.A. Gonzalez-Aguilar, In vitro Inhibition of Pancreatic Lipase by Polyphenols:A Kinetic, Fluorescence Spectroscopy and Molecular Docking Study, Food technology and biotechnology 55 (4) (2017) 519–530.
[44] S. Glisan, S. Sae-Tan, K. Grove, N. Yennawar, J. Lambert, Inhibition of digestive enzymes by tea polyphenols: enzymological and in silico studies (1045.34), The FASEB Journal 28 (1_supplement) (2014) 1045.34.
[45] S.L. Ong, S.H. Mah, H.Y. Lai, Porcine Pancreatic Lipase Inhibitory Agent Isolated from Medicinal Herb and Inhibition Kinetics of Extracts from Eleusine indica (L.) Gaertner, Journal of Pharmaceutics 2016 (2016) 9.
[46] A. Gholamhoseinian, B. Shahouzehi, F. Sharifi-Far, Inhibitory effect of some plant extracts on pancreatic lipase, International Journal of Pharmacology 6 (1) (2010) 18–24.
[47] G.K. Veeramachaneni, K.K. Raj, L.M. Chalasani, S.K. Annamraju, B. Js, V.R. Talluri, Shape based virtual screening and molecular docking towards designing novel pancreatic lipase inhibitors, Bioinformation 11 (12) (2015) 535–542.
[48] B. Ahmed, U. Ali Ashfaq, M. Usman Mirza, Medicinal plant phytochemicals and their inhibitory activities against pancreatic lipase: molecular docking combined with molecular dynamics simulation approach, Natural product research 32 (10) (2018) 1123–1129.
[49] J.S. Wright 3rd, G.J. Lyon, E.A. George, T.W. Muir, R.P. Novick, Hydrophobic interactions drive ligand-receptor recognition for activation and inhibition of staphylococcal quorum sensing, Proceedings of the National Academy of Sciences of the United States of America 101 (46) (2004) 16168–16173.
[50] L.-T. Cheng, Z. Wang, P. Setny, J. Dzubiella, B. Li, J.A. McCammon, Interfaces and hydrophobic interactions in receptor-ligand systems: A level-set variational implicit solvent approach, The Journal of Chemical Physics 131 (14) (2009) 144102.
[51] N.A. Lunagariya, N.K. Patel, S.C. Jagtap, K.K. Bhutani, Inhibitors of pancreatic lipase: state of the art and clinical perspectives, EXCLI journal 13 (2014) 897–921.
[52] A. Dechakhamphu, N. Wongchum, Screening for anti-pancreatic lipase properties of 28 traditional Thai medicinal herbs, Asian Pacific Journal of Tropical Biomedicine 5 (12) (2015) 1042–1045.
[53] G.A. Mohamed, S.R. Ibrahim, E.S. Elkhayat, R.S. El Dine, Natural anti-obesity agents, *Bulletin of Faculty of Pharmacy*, Cairo University 52 (2) (2014) 269–284.