Potential protein antiglycation, antiproliferation, and in silico study on the antidiabetic enzymes of bioactive metabolites from Adonis microcarpa DC and their ADMET properties

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ABSTRACT
Adonis microcarpa DC has not been comprehensively studied for its phytochemical and biological properties. The phytochemical investigation of the above-ground parts led to the isolation of adonitol (1), lup-20(29)-en-3β-ol (2), 3β-3-hydroxyolean-12-en-28-oate (3), strophanthidin-3-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxoside (4), strophanthidin-3-O-β-glucopyranosyl-(1→4)-β-boiviopyranoside (5) and gluco-(1→6)-strophanthidin-3-O-β-glucopyranosyl-(1→4)-β-boiviopyranoside (6). Their structures were assigned based on extensive 1D- and 2D-nuclear magnetic resonance spectral analyses. All the compounds are isolated for the first time from this species. Compounds 2 and 3 revealed high potency as antiglycated agents. The docking study introduced α-amylase as a preferable candidate for inhibition compared to α-glucosidase, with a slight superiority of 3. Aldose reductase was inhibited by 2 in a noncompetitive manner, while 3 probably was inactive toward it. Molecular docking suggested the activity of 2 and 3 as the possible inhibitors of α-glucosidase and α-amylase, while aldose reductase is an additional target of 2 by an allosteric effect. In silico physicochemical properties, such as absorption, distribution, metabolism, excretion, and toxicity parameters, of compounds were also predicted. Compounds 1, 2, and 3 were favorable in an acceptable prediction. The antiproliferative potentials on six human cancer cell lines in addition to a normal human lung fibroblast (WI-38) were carried out. Cell viability was evaluated and both triterpenoids have not exerted any cytotoxicity on the tested normal cell line. Interestingly, compound 3 rather than 2 introduced a considerable antiproliferative effect against the tested colon cancer cell lines.

INTRODUCTION
Plant active metabolites are under intensive examinations globally to supplement the drugs with low indices of toxicity (Abd-Alla et al., 2014, 2016; Favre et al., 2020; Shang et al., 2019). The secondary plant metabolites were reported to have the highest potentiality to provide the future drugs for the management of various diseases including inflammations, hypercholesterolemia, hyperlipidemia, and immunosuppressive disorders, as well as diabetes (Aly et al., 2017; Shang et al., 2019).

Type 2 diabetes mellitus is described as a metabolic disorder characterized by several factors, including hyperglycemia and hyperlipidemia, resulting in increased reactive oxygen species and the formation of advanced glycation end products (AGEs) (Choudhury et al., 2018; Franco et al., 2017; Justino et al., 2018). At present, many treatments involving the use of antidiabetic agents derived from medicinal plants have been recommended. Their antidiabetic and inhibitory potential against enzymatic glycation of α-amylase and α-glucosidase, as well as nonenzymatic glycation and lipid peroxidation with low indices of toxicity, were reported (Choudhury et al., 2018; Justino et al., 2018).

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The genus *Adonis* L. (Ranunculaceae) comprises 32 annual or perennial herbaceous species (Hosseini et al., 2019; Shang et al., 2019). Due to the beauty of the flower, the plants of genus *Adonis* were used historically for ornamental purposes in some countries (Shang et al., 2019). *Adonis* species have long been used in European and Chinese folk medicine due to their cardiac-enhancing effects. To date, more than 130 chemical components have been isolated and identified from the genus *Adonis* (Shang et al., 2019). Except for cardiac glycosides, some flavones, carotenoids, and coumarins in the genus were also isolated and identified. They had very wide pharmacological activities, including anti-inflammatory, antioxidant, neuroprotective, and antiallergic properties (Shang et al., 2019). Little was published on other structural types such as terpenoids and more attention should be paid to these compounds. *Adonis microcarpa* DC within the 32 species has not been comprehensively studied. During our continued search for bioactive compounds with hypoglycemic properties from medicinal plants (Abd-Alla et al., 2014, 2016; Aly et al., 2016, 2017; Shaaban et al., 2018; Shalaby et al., 2014), we describe accordingly herein the isolation and structural determination of a pentahydric alcohol adonitol (1) and two pentacyclic triterpenoids lup-20(29)-en-3β-ol (2) and 3β-3-hydroxyolean-12-en-28-oate (3) as well as three strophanthidin glycosides. Compound 1 was identified as a pentahydric sugar alcohol and is also known as xylitol or ribitol.

Xylitol, the nonnutritive sweetener, was reported recently (Deo et al., 2020) to reduce AGEs. The latter activity of this isolated compound encouraged us to examine the antiglycation properties of the major isolated pentacyclic triterpenoids: compounds 2 and 3. Alongside, the authors have evaluated their antiproliferative effects against six carcinoma cell lines. Their exerted cytotoxicity on the human lung fibroblast, the WI-38 cell line, was also examined as a model of a normal cell line. Inhibition of the major enzymes such as α-amylase and α-glucosidase is considered an essential approach to hyperglycemia treatment associated with type 2 diabetes (Oboh et al., 2014). Previous in vitro investigations evidenced the capability of compound 2 to inhibit α-amylase and α-glucosidase as well as aldose reductase taking into consideration that the compound was less potent against the α-amylase enzyme (Rama et al., 2014). On the other hand, compound 3 was found to be stronger against α-glucosidase than α-amylase (Zhang et al., 2017). Moreover, it was capable of inhibiting the later enzyme (Wang et al., 2010). The molecular docking study of compounds 2 and 3 using three enzymes was carried out to further confirm their efficiency as hypoglycemic agents.

**EXPERIMENTAL METHODS**

**Plant materials**

The above-ground parts of *A. microcarpa* DC (Ranunculaceae) at the flowering stages were harvested from the Northern Coast of Egypt, near Marsa Matrouh. The plant was identified by Dr. Mohamed El-Gebali, Taxonomy Specialist, El-Orman Botanical Garden at Giza. Authentic reference material is available at the Department of Chemistry of Natural Compounds. The plant was ground to powder substance after drying.

**Phytochemical investigation**

**General instrumental procedures**

The ultraviolet lamp (λ max = 254 and 330 nm, Shimadzu), a product of Hanovia Lamps, was used for localization of spots on chromatograms. Koller’s heating stage microscope was used for the measurement of the melting point. UV: Shimadzu® UV-visible spectrophotometer. nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance® DRX-400 Spectrophotometer and Varian-400 Spectrophotometers. EI-MS spectra were taken on HP MS-5988. δ values are reported as ppm relative to TMS in the convenient solvent. The X-ray instrument ENRAF Nonius FR 590 Kappa CCD single crystal diffractometer was used. α-Radiation (l = 0.71073 Å) was used with an X-ray tube, operated at 20 mA/50 kV. The two-dimensional CCD detector measured the intensity of diffraction from the crystal. A microscope displayed the crystal on the PC monitor.

**Solvent systems, spray reagents, and chromatographic techniques**

High analytical grade chemicals from Sigma-Aldrich (St Louis, MO), Merck (Darmstadt, Germany), and Fluka (Buchs, Switzerland) were used. All solvents used for extraction and chromatographic separation were of analytical grade. Column chromatography (CC) was carried out using silica gel (Si) 60 (E. Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Thin-layer chromatography (TLC) was carried out on silica gel 60 F 254 precoated aluminum plates (0.2 mm; Merck), and the solvent systems Si; EtOAc/C<sub>2</sub>H<sub>5</sub> (0.5: 9.5), S; EtOAc/C<sub>2</sub>H<sub>5</sub> (2: 9), S; EtOAc/MeOH/H<sub>2</sub>O/HOAc (16: 2.0: 0.5: 0.5), and S; CHCl<sub>3</sub>/MeOH (7:3) were used. R<sub>f</sub>; p-anisaldehyde-sulfuric acid, R<sub>f</sub>; sulfuric acid/methanol (30%), followed by heating at 105°C for 1–2 minutes for terpenes, and R<sub>f</sub>; Kedde’s reagent (2% of 3,5-dinitrobenzoic acid in ethanol mixed equally with 1N ethanolic potassium hydroxide) were used as spray reagents.

**Chemical characterization of *A. microcarpa* DC above-ground parts**

**Quantitative estimation of cardiac glycosides**

This was carried out using the UV-spectrophotometric method based on the analytical data using Kedde’s reagent according to Solich et al. (1992) with some modifications.

**Preparation of sample**

The above-ground parts of *A. microcarpa* DC (10 g) were extracted with 100 ml of methanol (80%) under shaking at room temperature for 48 hours, followed by filtration through a centered glass funnel. Five ml of the extract was added to 15 ml of distilled water and the resulting solution was treated with 2 ml of freshly prepared lead acetate (12.5%). The volume was adjusted to 25 ml with distilled water and then filtered. The filtrate (10 ml) was placed in a 25 ml volumetric flask and 5 ml of 4.7% disodium hydrogen phosphate solution was added, followed by filtration and the volume adjusted to 25 ml by distilled water.

**Determination of the total cardiac glycosides**

The calibration curve of olinotiside based on Kedde’s reagent was prepared as follows: olinotiside (25 mg) was
dissolved in spectroscopic methanol (125 ml). A definite volume corresponding to 0.025, 0.050, and 0.10 mg was completed to 25 ml with spectroscopic methanol. Freshly prepared Kedde’s reagent (2 ml) was added to the prepared solution of olitoriside (2 ml). The mixture was kept at room temperature for 5 minutes, whereby a pink color developed. The absorption maximum was measured at λ_max 560 nm, against a blank of Kedde’s reagent (2 ml) mixed with methanol (2 ml). The average of three measurements was taken for each concentration. A volume (2 ml) of the purified extract of A. microcarpa was mixed with methanolic 3,5-dinitrobenzoic acid (2%, 1 ml) and the methanolic potassium hydroxide (1N, 1 ml). The absorption maximum was measured after 5 minutes at λ_max 560 nm. An average of three measurements was taken for the extract.

**Extraction, isolation, and purification of compounds**

The air-dried powdered above-ground parts (900 g) were exhaustively extracted with 80% aqueous methanol (3 × 1.2 l) at room temperature. The combined methanolic extract was concentrated to 2 l at reduced pressure at 50°C and left overnight at room temperature. The precipitate formed was filtered off, then dissolved in the least amount of methanol, and left overnight at room temperature. A white precipitate was formed, which was separated by filtration and recrystallized from methanol to give rise to 25 mg of pure compound 1.

The mother liquor was dried under a vacuum at 50°C to give rise to 27 g of oily brownish residue. The residue was resolved on CC using Si 60 (300 g, 80 × 1.5 cm) as an adsorbent and eluted first with dichloromethane, followed by a gradual increase of methanol proportions. The fractionation was monitored using TLC and similar fractions (each 50 ml) were combined and freed from the solvent. The chromatogram was visualized using UV (254/365 nm) and consequent spraying with Rf and Rf, respectively.

Fraction I was chromatographed on Si CC (90 × 1.5 cm) and eluted first with toluene/EtOAc (9:1 v/v) to give rise to crude compound 2, which was purified by crystallization from methanol/ethyl acetate mixture to give 34 mg yield. Compound 3 (60 mg) was eluted with toluene/EtOAc (9:1 v/v) and crystallized from ethyl acetate.

Fraction II was subjected to Sephadex LH-20 (118 g, 75 × 2 cm, MeOH) CC to give rise to 158 mg of a crude mixture of compounds 4 and 5. The mixture was subjected to RP-18 CC (15 × 0.5 cm) connected with a prostatic pump with a flow rate of 5 ml/minute and eluted with 50% MeOH/MeOH. The fractionation was monitored by TLC, where similar fractions were collected to give rise to two subfractions A and B. Subfraction A was crystallized from MeOH/EtOAc to give 13 mg of pure compound 4. Subfraction B (18 mg) was separated as a mixture of two compounds (ca 2:1): 4 and 5, respectively.

Fraction III was rechromatographed on Si CC (250 g, 75 × 2 cm) CC and eluted with dichloromethane/methanol (85:15) to give rise to 11 mg of compound 6, which was crystallized from methanol/diethyl ether.

The chemical structures of the separated compounds were identified by the application of spectroscopic methods of 1H and 13C NMR (Fig. 1 and 2).

**Adonitol (1)**

M.p. 85°C–90°C, 1H NMR dimethyl sulfoxide ([DMSO]_d_6): 4.54 (s, 3H), 4.48 and 4.31 (5 OH); 3.59–3.49 (m, 2 CH_2); 3.42–3.34 (m, 3 CH) ppm and 13C NMR: 72.75 (3 CH); 63.02 (2 CH_2) ppm. The structure was confirmed by X-ray crystallography (Fig. 2).

**Lup-20(29)-en-3β-ol (2)**

C_{19}H_{30}O white crystalline needles; m.p.: 210°C–212°C; Rf: 0.84 (2:9, EtOAc/C,H_3); C_{19}H_{30}O. 1H NMR (CDCl_3, 300 MHz): 4.70 and 4.52 (2H, m, CH_2-29), 3.22 (1H, dd, H-3), 2.42 (1H, m, H-19a), 1.92 (1H, m, H-21a), 1.72 (3H, s, CH_3-20), 1.46 (1H, m, H-21β), 1.05 (3H, s, CH_3-26), 0.97 (3H, s, CH_3-23), 0.98 (3H, s, CH_2-27), 0.90 (3H, s, CH-25), 0.86 (3H, s, CH3-28), 0.82 (3H, s, CH2-24); 13C NMR (CDCl_3, 75 MHz): 150.8 (C-20), 109.4 (C-29), 79.2 (C-3), 55.6 (C-5), 50.1 (C-9), 48.0 (C-18), 48.0 (C-19), 42.8 (C-17), 42.5 (C-14), 41.2 (C-8), 40.2 (C-22), 39.0 (C-4), 38.7 (C-1), 38.1 (C-13), 37.3 (C-10), 35.4 (C-16), 34.2 (C-7), 29.2 (C-21), 27.6 (CH2-23), 27.7 (C-2-15), 25.6 (C-12), 21.6 (C-11), 19.2 (CH3-30), 18.2 (C-6), 17.5 (CH2-28), 16.0 (2CH2-25/26), 15.3 (CH2-24), 14.2 (CH2-27).

**3β-3-Hydroxyolean-12-en-28-oate (3)**

1H NMR (CDCl_3, 400 MHz): δ 1.53–1.55 (1H, m, C1-H), 1.22–1.24 (1H, m, C2-H), 3.37 (1H, dd, C3-H), 0.72 (1H, bs, C5-H), 1.32–1.35 and 1.46–1.490 (1H, m, C6-H), 1.35–1.40 (1H, m, C7-H), 1.52–1.56 (1H, m, C-9), 1.90–1.92 (1H, m, C11-H), 2.81 (1H, dd, C18-H), 1.54–1.62 (1H, m, C19-H), 1.32–1.42 (1H, m, C21-H), 1.51–1.55 and 1.72–1.77 (1H, m, C22-H), 1.4 (3H, s, C23-CH3), 0.96 (3H, s, C24-CH3), 0.89 (3H, s, C25-CH3), 0.84 (3H, s, C26-CH3), 1.30 (3H, s, C27-CH3), 0.94 (3H, s, C29-CH3), 0.98 (3H, s, C30-CH3) ppm. 13C NMR (CDCl_3, 100 MHz): δ 38.4 (C-3), 27.1 (C-2), 78.6 (C-4), 38.4 (C-5), 55.5 (C-5), 18.2 (C-6), 32.6 (C-7), 9.6 (C-8), 42.8 (C-9), 37.1 (C-10), 23.5 (C-11), 124.3 (C-12), 41.5 (C-14), 72.7 (C-15), 32.1 (C-16), 46.3 (C-17), 41.4 (C-18), 46.4 (C-19), 30.61 (C-20), 33.5 (C-21), 311.7 (C-22), 28.2 (CH2-23), 15.6 (CH2-24), 15.4 (CH2-25), 16.7 (CH2-26), 25.7 (CH2-27), 183.1 (CH1-28), 183.9 (C-28), 33.3 (C-29), 23.6 (CH3-30).

**Strophanthidin glycosides (4–6)**

The 1H and 13C NMR resonance assignments of strophanthidin-3-O-β-D-gluco-25-ol (1→4)-β-D-glucopyranoside (4), strophanthidin-3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (5) and gluco-(1→6)-strophanthidin-3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (6) are listed in Table 1.

**Biological activities**

All chemicals served in the antiglycation assay were of analytical grade (Sigma, Serva, and Lonza). Cell culture assay was carried out depending on aseptic conditions using sterile plastics (Greiner) and reagents (Lonzha and Sigma) of tissue culture grade.
Antiglycation assay

The assay was carried out according to Matsuura et al. (2002) following the modified method of Shaaban et al. (2018). Compounds’ stock solutions and rutin, the glycated inhibitor standard, were prepared as 20× of the required tested concentration in DMSO before starting the assay. Determining the effective concentration for 50% glycation inhibition (EC_{50}) was recorded from the nonlinear regression analysis.

Antiproliferation assay

Cultures of six cancer and normal lung cell lines

Six human cancer cell lines were used to examine the ability of compounds 2 and 3 to inhibit the growth of incubated cancer cells. The cancer cell line panel was human type from the American Type and Culture Collection (ATCC), except Huh cells: two breast: MCF-7 (ATCC® HTB-22™) and MDA-MB-231 (ATCC® CRM-HTB-26™), two colorectal: Caco-2 (ATCC® HTB-37™) and HCT-116 (ATCC® CCL-247™), and two liver: Huh-7 (Vacsera, Egypt) and HepG2 (ATCC® HB-8065™). In addition, the lung WI-38 (ATCC® CCL-75™) cell line was the selected model to examine the cytotoxicity effects of the compounds on normal cells.

In vitro screening of the antiproliferation properties of compounds 2 and 3

Compounds 2 and 3 together with the standards and DMSO vehicle were incubated separately, with each cell line to get their GI_{50}. All cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum (FBS) except MCF-7 cells. Moreover, 1% non-essential amino acid (NEAA), 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate were also added to the culture growth media. The basic medium of MCF-7 culture was Roswell Park Memorial Institute RPMI 1640, while the same supplements as above that were supplied lacked 1% NEAA. The routine maintenance of each cell type as well as the prepared stocks of the tested compounds followed our experience in the drug discovery field (Galal et al., 2018; Shaaban et al., 2018). In brief, 40 mM stock solution in DMSO was separately prepared from every compound while being stored under dry condition. Simultaneous

Figure 1. Structure of isolated compounds from aqueous methanolic extract of the above-ground parts of A. microcarpa DC (Ranunculaceae).
dilutions from the stocks were continued into the culture growth medium free of FBS before the incubation with the cancer cell type. The sulforhodamine-B (SRB) assay (Vichai and Kirtikara, 2006) was followed but differed in the SRB concentration (0.01%). Colchicine (BDH Laboratory Supplies) and Adricin (doxorubicin-hydrochloride) from the Company of United EIMC were the positive controls against cancer cells, while cisplatin (Mylan, Saint Priest, France) was the examined standard against the WI-38 cell.

**Statistical analysis**

Experimental results were analyzed in duplicate and expressed as means ± standard error of the mean (SEM). The concentrations of getting 50% of the activity (EC$_{50}$, CC$_{50}$, and GI$_{50}$) were calculated using the nonlinear regression fitting model of GraphPad Prism 6.0 Software.

**In silico study on the antidiabetic enzymes**

The files corresponding to the 3D structure of the enzymes used in docking studies were downloaded from the Protein Data Bank: α-glucosidase, PDB code: 5NN8 (Roig-Zamboni et al., 2017); α-amylase, PDB code: 2QV4 (Maurus et al., 2008); and aldose reductase, PDB code: 4YS1 (Rechlin et al., 2017). The PDB files of proteins were prepared using VEGA ZZ 3.0.5 (Pedretti et al., 2003) by deleting the native ligand, water, and unwanted co-crystallized compounds. The native ligands, compound 2 and compound 3, were designed by ChemBio3D Ultra 14.0, followed by VEGA ZZ 3.0.5, for performing energy minimization applying the Molecular Orbital PACkage protocol. The docking process was conducted by automated docking software AutoDock Vina 1.0.3 (Trott and Olson, 2010). The grid box was adjusted for α-glucosidase in dimensions of 30 × 30 × 30 Å and the space coordinates were $X = -14.84$, $Y = -38.55$, and $Z = 93.4$. Three-dimensional positioning of the grid box for α-amylase was $X = 10.77$, $Y = 44.50$, and $Z = 27.11$ and the size was 26 × 26 × 26 Å, respectively. The geometrical shape of the interaction site in the case of aldose reductase was rectangular with dimensions equal to 17.2, 16.3, and 27.2 for $X$, $Y$, and $Z$, respectively. It was centered at $X = 69.1$, $Y = -7.21$, and $Z = 47.12$ in the space. The PyMOL 2.3.2 software was used for visualization (Schrodinger, 2015).

**In silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction of the isolated compounds**

**In silico** physicochemical properties (ADMET,) of the isolated compounds were predicted using the Swiss ADME website (Daina et al., 2017) and ADMET SAR website (Cheng et al., 2012).

**RESULTS AND DISCUSSION**

**Chemical characterization**

Phytochemical analysis of *A. microcarpa* DC above-ground parts led to the isolation of pure bioactive compounds 1–6 (Fig. 1). All the compounds are isolated for the first time from this species. The three strophanthidin glycosides 4–6 were previously isolated from *Adonis mongolica* (Thieme and Lamzhav, 1976).

The structure of compound 1 was confirmed by X-ray crystallography (Fig. 2) as the pentahydric alcohol adonitol. Adonitol is also known as xylitol or ribitol and can be found in nature in many vegetables, fruits, oats, and mushrooms and is produced in small quantities in the human organism. It was previously isolated from many *Adonis* spp. such as *Adonis coerulea*, *Adonis leiosepala*, and *A. mongolica* (Evdokimov, 1979; Zhang et al., 1991).

The $^1$H NMR spectrum of compound 2 showed the seven sharp singlets assigned to the protons of tertiary methyl groups. The two multiplets that could be ascribed to a methylene group at C-29 appeared. Compounds 2 and 3 were identified and their structures were confirmed by comparison with published data as pentacyclic triterpenoid lup-20(29)-en-3β-ol (Menezes-de-Oliveira et al., 2011) and 3β-3-hydroxyolean-12-en-28-oate 3 (Mahato and Kundu, 1994).

The aglycone part of compounds 4–6 was identified as strophanthidin based on the carbon signals of the α,β-
unsaturated-γ-lactone ring. The presence of a quaternary carbon signal suggested the presence of a hydroxyl group at C-5. Also, the presence of a hydroxyl group at C-14 was established by the presence of a quaternary carbon signal. Finally, the presence of a methyl group signal (C-18) and aldehyde group signal (C-19) confirms the strophanthidin structure of the three compounds. The other carbon signals were found to be at the expected chemical shifts (Table 1). Some proton signals (Table 1) were assigned based on the corresponding carbon signals in the heteronuclear multiple quantum coherence (HMOC) spectrum. Compound 4 was identified as strophanthidin-3-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxoside (Fig. 1) from its spectroscopic one- and two-dimensional NMR and comparing its spectroscopic published data (Mahato and Kundu, 1994; Yoshikawa et al., 1998). The position of the digitoxose at C-3 of strophanthidin in compound 4 was confirmed by the cross-peaks C-3/H-1′ and C-1′/H-3. Also, the attachment of the glucose with the digitoxose at C-4 was confirmed by the cross-peaks C-4/H-1″ and C-1″/H-4″ in its heteronuclear Table 1. 1H (400 MHz) and 13C (100 MHz) NMR data of strophanthidin glycosides (4–6).

| Position | δH (400 MHz) | Position | δC (100 MHz) |
|----------|--------------|----------|--------------|
| Glycone moiety | (4) | (6) | (4) and (5) | (6) | Sugar moiety | (4) | (6) | (4) |
| 1 | a | 2.04 | a | 2.14 | 25.92 | 24.18 | 25.32 | 1′ | a | 5.01 | 4.88 | 99.10 |
| | β | 1.40 | β | 1.32 | | | | | | | | |
| 2 | a | 1.67 | a | 1.60 | 25.20 | 25.26 | 26.04 | 2′ | a | 1.80 | a | 1.72 | 37.15 |
| | β | 2.05 | β | 1.96 | | | | | | | | |
| 3 | a | 4.26 | a | 4.18 | 76.39 | 74.94 | 76.460 | 3′ | β | 4.22 | 4.13 | 66.60 |
| | a | 1.69 | a | 1.58 | 36.85 | 35.72 | 37.02 | 4′ | a | 3.52 | 3.47 | 76.38 |
| | β | 2.18 | β | 2.18 | | | | | | | |
| 5 | | | | | 75.26 | 75.32 | 75.37 | | 5′ | a | 4.15 | 4.06 | 70.37 |
| 6 | a | 1.72 | a | 1.63 | 37.19 | 39.04 | 37.34 | 6′ | a | 0.98 | 1.25 | 17.24 |
| | β | 2.19 | β | 2.10 | | | | | | | | |
| 7 | a | 1.77 | a | 2.13 | 18.94 | 19.03 | 19.03 | | | | | |
| | β | 1.69 | | | | | | | | | | |
| 8 | β | 2.04 | β | 1.96 | 42.56 | 42.72 | 42.72 | | | | | |
| 9 | a | 1.49 | a | 1.71 | 40.50 | 40.34 | 40.48 | | | | | |
| 10 | | | | | 54.78 | 56.22 | 56.33 | 1″ | a | 4.40 | 4.32 | 102.44 |
| 11 | a | 1.65 | a | 1.51 | 23.27 | 23.42 | 23.42 | 2″ | β | 3.34 | 3.23 | 75.92 |
| | β | 1.37 | β | 1.22 | | | | | | | | |
| 12 | a | 1.57 | a | 1.46 | 40.40 | 40.16 | 40.58 | 4″ | β | 3.39 | 3.30 | 72.01 |
| | β | 1.61 | β | 1.50 | | | | | | | | |
| 13 | | | | | 50.82 | 50.79 | 50.32 | 5″ | a | 3.47 | 3.47 | 78.04 |
| 14 | | | | | 86.14 | 86.02 | 86.08 | | β | 3.75 | β | 3.76 |
| 15 | a | 2.12 | | | 32.42 | 32.52 | 32.52 | | | | | |
| | β | 1.80 | | | | | | | | | | |
| 16 | a | 1.96 | a | 2.18 | 27.94 | 28.04 | 28.04 | | | | | |
| | β | 1.96 | β | 1.90 | | | | | | | glyc-1″ | 4.40 |
| 17 | a | 2.90 | a | 2.90 | 51.92 | 51.87 | 51.89 | 2″ | | | | |
| 18 | 0.92 | 0.86 | 16.18 | 16.27 | 16.29 | 3″ | | | | | |
| 19 | 9.14 | 10.05 | 210.02 | 210.03 | 210.02 | 4″ | | | | | |
| 20 | β | – | β | – | 178.32 | 178.33 | 178.33 | 5″ | | | | |
| 21 | a | 5.14 | a | 5.04 | 75.23 | 75.40 | 75.38 | 6″ | a | 3.88 | | |
| | β | 4.50 | β | 4.93 | | | | | | | | |
| 22 | | | | | 5.98 | 6.00 | 117.94 | 118.00 | 117.98 | | | |
| 23 | – | – | – | – | 177.32 | 178.27 | 177.34 | | | | | |

dig = digitoxose; Biov = Biovinose; glc = glucose; Glycone-1 = digitoxose for compound 4 and Biovinose for compound 6; Glycone-2 = glucose for each of compounds 4 and 6; Biovinose = J1,2a = 9.6; J1,2b = 2.3; J2a,2b = 12.6; J2a,3 = J2b,3 = J3,4 = 3.0; J4,5 = 1.5; J5,6 = 6.6 Hz. Glucose: J1,2 = 7.6; J2,3 = J4,5 = J5,6 = 9.6; J5,6 = 6.0; J6a,6b = 6.5; J6a,6b = 12.1 Hz.
multiple bond correlation (HMBC) spectrum. Compound 5 was obtained as a mixture with compound 4 (ca 1:2, resp.). The mixture showed a positive test for cardiac glycosides using Kedde’s reagent. Both compounds 4 and 5 have the same aglycone but the first sugar of each compound has C-4 of different stereochemistry that was confirmed by 1H, 13C NMR and distortionless enhancement by polarization transfer (DEPT) spectra (Islam et al., 2013; Mahato and Kundu, 1994; Yoshikawa et al., 1998). The chemical structures of compounds 4 and 5 were unambiguously assigned based on one-dimensional (1D) and two-dimensional (2D) NMR of 1H, 13C, DEPT, HMQC, and HMBC (Lei et al., 1996; Mahato and Kundu, 1994). Compound 5 was identified as strophanthin-3-O-β-glucopyranosyl-(1→4)-β-boiviopyranoside. Also, gluco-(1→6) strophanthin 3-O-β-glycopyranosyl-(1→4)-β-boiviopyranoside 6 (Thieme and Lamzhav, 1976) was identified and confirmed by comparison with data in previous literature.

Previous studies have reported other compounds identified in Adonis spp., e.g., k-strophanthin B, cymarin, adonitoxin, acetyladonitoxin, vernadigin, strophenodigen, and strophanthin (Shang et al., 2019). Cardiac glycosides are important active compounds of the genus Adonis. Therefore, a quantitative estimation of the total cardiac glycosides content of the investigated species A. microcarpa DC would be necessary. This was carried out using the UV-spectrophotometric method based on the analytical data using Kedde’s reagent and its percentage was found to be 0.52.

**Biological activities**

The present study was extended to investigate the antiglycation activities and antiproliferative properties against six cancer cell lines of the major triterpenoids isolated from A. microcarpa DC (Ranunculaceae).

The capability of several medicinal plants to inhibit many of the embroiled enzymes implicated in the pathogenesis of diabetes has been evidenced (Choudhury et al., 2018). Elevation of glucose levels in the blood of diabetic patients most likely results in the higher detection of glycated hemoglobin (HbA1c) (Choudhury et al., 2018). A minor amount of HbA1c was detected in normal blood indices. However, its percentage is accelerated by the increment of serum glucose (Leow, 2016). Generally, amino groups of proteins (e.g., HbA1c) and their metabolites, peptides and amino acids are nonenzymatic glycosylate by reduced sugars eventually resulted in forming insoluble complexes known as AGEs. Hence, the accumulation of AGEs becomes responsible for a versatile complication in diabetes mellitus and the etiology of aging disorders (Choudhury et al., 2018) as well. Fortunately, testing the capacity of a compound to directly suppress the glycated proteins is available by the *in vitro* antiglycation assay (Matsuura et al., 2002).

Compound 2 which is a dietary triterpene, previously isolated from Zanthoxylum conspersipunctatum, was reported to have diverse biological effects, including anti-inflammatory, antidiabetic, and antitumor (Ahmed et al., 2011; Choudhury et al., 2018). In our study, compound 2 was completely able to inhibit AGEs formation while EC₅₀ (26.5 μM) was interestingly comparable to rutin (EC₅₀ = 26.0 μM). In a separate *in vitro* study by our team, we did not record any toxicity against Vero cells or hTERT-RPE1 at its recorded bioactive concentration range (Abd-Alla et al., 2021). Besides, our results in the present study against the fibroblast normal lung cells (WI-38) did not record any toxicity up to 50 μM, the highest applied concentration (Table 3).

On the other hand, the activity of oleanolic acid as an antiglycation agent was previously evidenced (Choudhury et al., 2018). Compound 3 (3β-3-hydroxylan-12-en-28-oate) was able to completely suppress the formed glycation down to 25 μM (Table 2). It exerted cytotoxicity against the normal lung cell line displaying EC₅₀ = 57.44 ± 2.0 while 25 μM was the maximum nontoxic concentration (Table 3).

| Table 2. Evaluation of the antiglycation capacities of compounds 2 and 3 isolated from *A. microcarpa* DC. above-ground parts. |
|---|---|
| **Compound** | **EC₅₀ ± SEM** |
| 2 (Lup-20(29)-en-3β-ol) | 26.5 ± 2.4 μM |
| 3 (3β-3-Hydroxyolean-12-en-28-oate) | <25.0 μM |
| Standard (Rutin) | 26.0 ± 1.1 μM |

The values are expressed as mean ± SEM while compound 3 inhibited 100% of the glycation formation in all examined concentrations (200–25 μM).

EC₅₀ = The effective concentration that inhibited 50% glycation.

| Table 3. Cytotoxicity of compounds 2 and 3 on normal lung cell line (WI-38). |
|---|---|---|
| **Compound** | **CC₅₀ (μM)** | **MNTC (μM)** |
| 2 (Lup-20(29)-en-3β-ol) | N | >50.0 |
| 3 (3β-3-Hydroxyolean-12-en-28-oate) | 57.44 ± 2.0 | 25.0 |
| Cisplatin (μg/ml) | 2.26 ± 0.035 | 0.31 |

MNTC = Maximum nontoxic concentration; N = The indicated compound did not show any cytotoxicity at the screening concentration (50 μM), so no further test was carried out.

CC₅₀ = Concentration caused 50% cytotoxicity to the normal WI-38 cell line. The tested compounds were first, screened at 50 μM, while only, the cytotoxic compound was subjected to further examination to determine their defined CC₅₀. The values were determined by 48 hours SRB assay. Data given are derived from at least two independent experiments, expressed as mean ± SEM.
Table 4. Antiproliferative properties of compounds 2 and 3 isolated from *Adonis microcarpa* DC above-ground parts.

| Compound | HepG2 (µM)* | Huh-7 (µM)* | MCF-7 (µM) | MB-MDA-231 (µM)* | Caco-2 (µM) | HCT-116 (µM) |
|----------|-------------|-------------|------------|-----------------|-------------|--------------|
| Compound 2 | 52.56 ± 3.99 | 65 ± 4.12 | 45 ± 3.10 | 75 ± 10.11 | 42 ± 3.99 | 60 ± 5.34 |
| Compound 3 | 52.82 ± 1.42 | 61.01 ± 5.01 | 23.24 ± 0.99 | 59.56 ± 1.56 | 18.14 ± 1.98 | 20.68 ± 4.26 |
| Colchicine (µg/ml) | 0.16 ± 0.022 | 0.244 ± 0.06 | 0.12 ± 0.006 | 0.65 ± 0.06 | 0.02 ± 0.001 | – |
| Adrinic (µg/ml) | 1.00 ± 0.097 | 1.25 ± 0.075 | 0.85 ± 0.114 | 1.37 ± 0.13 | 0.21 ± 0.014 | 1.43 ± 0.13 |

Compound 2 = Lup-20(29)-en-3β-ol; Compound 3 = 3β-3-Hydroxyolean-12-en-28-oate; – = not determined; *GI₅₀ = Concentration that caused 50% growth inhibition in the incubated cancer cells.

Values were determined by 48 hours SRB assay. Data given were derived from at least two independent experiments and expressed as mean ± SEM.

**Compound 2** could fight diabetes mellitus directly by inhibiting the enzymes (Nazaruk & Borzym-Kluczyk, 2015) as indicated in the docking study in the present work and indirectly by suppressing the formed AGEs. Compound 2 introduced its antiproliferative activity against six examined cell lines at higher concentrations while GI₅₀ > 40 µM. Our data agreed with previous investigations in that lupeol has a higher therapeutic concentration margin and is reported as a nontoxic compound (Saleem, 2009). The antiproliferative properties of 3β-3-hydroxyolean-12-en-28-oate 3 against the same examined cancer cell lines were relatively ameliorated against three of the examined cancer cells compared to lup-20(29)-en-3β-ol 2. It gave moderate activities with GI₅₀ near 20 µM (Table 4). The normal lung cells were subjected separately to the effect of compounds 2 (lup-20(29)-en-3β-ol) and 3 (3β-3-hydroxyolean-12-en-28-oate) to evaluate their exerted cytotoxicity on the normal cell line (WI-38). Compound 2 did not show any toxicity on the tested model of normal cells as shown in Table 3. 3β-3-hydroxyolean-12-en-28-oate 3 was toxic but at a considerable concentration (CC₅₀ was 57.44 µM).

In the current study, compound 3 was relatively nontoxic and has anticancer and antiglycation properties. Oleanolic acid was reported to promote insulin secretion from pancreatic β-cells (de Melo et al., 2010). A previous study reported this compound to exhibit lower IC₅₀ values against the targeted enzymes of treating diabetes mellitus than the standard antidiabetic drug, acarbose (Choudhury et al., 2018). It has also been reported to improve blood glucose tolerance and ameliorates visceral obesity (de Melo et al., 2010). The compound was a potent inhibitor of α-glucosidase rather than α-amylase. The compound was suggested as possibly a better alternative to the antidiabetic drug acarbose and this is in line with our present data. Our study additionally showed that the molecular docking study of compound 3 using aldose reductase enzyme and redocking of the co-crystallized ligand was carried out to check its efficiency as hypoglycemic agents were confirmed (Figs. 3–5).

This compound has been reported as an active agent with antioxidant and anti-inflammatory activities. The relationship between the antioxidant and antiglycation properties was confirmed in many articles (Favre et al., 2020; Wöllnerhansen et al., 2020). In parallel with our findings, the compound has high inhibitory action of key enzymes linked to type 2 diabetes such as α-amylase and α-glucosidase (Choudhury et al., 2018; Mohammed et al., 2017). The physiochemical examination of the aqueous methanolic extract of the above-ground parts of *A. microcarpa* DC (Ranunculaceae) also led to the isolation of three strophanthinid glycosides: strophanthidin-3-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxoside 4, strophanthidin-3-O-β-glucopyranosyl-(1→4)-β-boiviopyranoside 5, and gluco-(1→6) strophanthidin 3-O-β-glucopyranosyl-(1→4)-β-boiviopyranoside 6. The structures were assigned based on extensive 1D and 2D NMR experiments. Many strophanthidin as well as strophanthidin glucopyranosides have displayed selective cytotoxicity toward malignant tumor cell lines including HSC-2, HSC-3, HSC-4, and HL-60 cells with a CC₅₀ range of 0.012–2.8 mM. Studies also indicated that they may trigger caspase-3-independent apoptotic cell death in HL-60 and HSC-2 cells. The CC₅₀ values of the positive control melphalan were 8.7, 25, 32, and 1.4 mM in HSC-2, HSC-3, HSC-4, and HL-60 cells, respectively (Kubo et al., 2012). Amurensiosides A, B, D, and E were moderately cytotoxic to HSC-2 cells with IC₅₀ values of 66, 26, 47, and 58 mg/ml, respectively; the activity of the positive control melphalan was 13 mg/ml (Kuroda et al., 2010). Many cardenolide glycosides have shown cytotoxic activities against HL-60 promyelocytic and HSC-2 cells (Kubo et al., 2015). Cymarilic acid, a compound isolated from the methanol extract of *Adonis amurensis*, has shown no significant cytotoxicity against tumor cell line A549 (ED₅₀ > 5 mg/ml) but was found to be strongly inhibitory toward tube formation induced by human umbilical vein endothelial cells (Shang et al., 2019). Cymarin and cymarol (0.031 and 0.021 mg/ml) exhibited potent cytotoxicity against a human solid tumor cell line A549 (human lung carcinoma) while being inactive on murine leukemic cells (L1210, 5 mg/ml) (Shang et al., 2019). The antiglycation properties of compounds 2 and 3 persuaded us to search for the possible biological target. Among a group of enzymes, α-glucosidase and α-amylase were selected as they are known to be inhibited by rutin (Dubey et al., 2017) which is the reference compound in the antiglycation assay (Table 2). The investigation of binding orientations of 2 and 3 that suffer from a shortage of hydrogen bond mediators was interesting to identify the impact of the hydrophobic interaction on stabilizing these compounds, which were proved biologically active.

**Molecular docking**

To produce dependable results, the validation of the process was planned by at least one of two theoretical techniques. The first one was applied for the three enzymes, and it was by redocking of the cocrystallized ligand which was carried out to check its efficiency as a hypoglycemic agent (Fig. 5).

The second theoretical technique was by docking rutin into α-glucosidase (Fig. 3) and α-amylase (Fig. 4). The redocking was approached by designing the native ligands from the 2D form and transformed them into the 3D structure which was followed
Figure 3. The docking trials toward α-glucosidase: (a) native ligand (orange stick) in comparison to cocrystallized ligand (black stick), (b) rutin (blue stick), (c) compound 2 (light blue stick), and (d) compound 3 (yellow stick).

Figure 4. The molecular docking trial of α-amylase: (a) native ligand (orange stick) in comparison to cocrystallized ligand (black stick), (b) rutin (blue stick), (c) compound 2 (light blue stick) and, (d) compound 3 (yellow stick).
by energy minimization to pick up the best conformer. Designing the 3D structure of the native ligands and docking them rather than extraction and redocking of the ready-designed cocrystallized native ligand was preferable. It allowed us to validate the whole process, i.e., the energy minimization of the compounds, the docking algorithm, and the grid box parameters.

For α-glucosidase, the redocking produced a binding mode of slight deviation from the cocrystallized one while it retained the same number of hydrogen bonds which was eight (Fig. 3). The theoretical affinity was not high (~6.5 kcal/mol) which was not expected. Rutin showed good affinity toward the enzyme that complies with the biological assay and the published data (Dubey et al., 2017). The affinity was ~8.7 kcal/mol and it formed five hydrogen bonds with the pocket (Fig. 3). The theoretical affinities of the compounds were moderate, ~7.8 and 7.6 kcal/mol for 2 and 3, respectively. Compound 2 has only one hydrogen bond donor with no acceptor within the structure. The suggestion of being stabilized inside the enzyme by hydrophobic interaction towards the surrounding amino acids, Phe525, Trp481, and Asp282, was likely. Differently, 3β-3-hydroxyolean-12-en-28-oate 3 could form three hydrogen bonds as a maximum with the amino acid backbone. This position in which it formed the hydrogen bonds appeared less stable (~7.3 kcal/mol) which may make the hydrophobic interaction the significant contributor in stabilizing enzyme-compound 3 complex (Fig. 3). The allosteric binding possibility was tested by the blind docking approach and was found more probable for compound 3 than compound 2.

Compounds 2 and 3 showed higher stability inside α-amylase which suggests this enzyme is responsible for their hypoglycemic activity. The redocked native ligand showed affinity toward the pocket of ~7.6 kcal/mol with fewer hydrogen bonds (eight) while it was twelve for the cocrystallized ligand (Fig. 4). The experimental efficacy of rutin was endorsed by docking, which showed an affinity of ~8.7 kcal/mol and formed five hydrogen bonds (Fig. 4). Compound 2 appeared to form a stable complex with the enzyme by probable hydrophobic interaction with Trp59, Trp62, and His305. These amino acids may also be involved in this stabilization of compound 3 which demonstrated an affinity of ~10.3 kcal/mol without forming any hydrogen bonds with the protein. The pose which developed three hydrogen bonds had a good affinity as well (~8.5 kcal/mol). Although the last affinity was inferior to the most stable pose, this binding mode represented a possibility (Fig. 4).

Aldose reductase is another possible target edited to the antiglycation activity of our compounds. The mechanism of inhibition of this enzyme is noncompetitive by binding the inhibitor with a binding site adjacent to the site of nicotinamide adenine dinucleotide phosphate (NADP) binding (Eisenmann et al., 2009; Rechlin et al., 2017; Zheng et al., 2012). The validation process showed excellent accuracy as the redocking of NADP inside the active site was typical to the crystallized ligand with high affinity (~10.7 kcal/mol) (Fig. 5). The grid box was adjusted to contain the active and allosteric sites. The docking trials of compounds 2 and 3 provided us with a good idea about their suggested inhibitory effect toward aldose reductase. The pentacyclic triterpenoid lup-20(29)-en-3β-ol 2 gave results that supported the published data (Ramu et al., 2014) and its binding poses were only limited to the allosteric site. The affinity of the picked pose was considerably high (~9 kcal/mol); consequently, the ability of the enzyme to bind the substrate NADP may be impacted (Fig. 5). On the other
Table 5. *In silico* ADMET predictions of the isolated compounds.

| Compound number | MW$^a$ | Rotatable bonds | H-bond acceptors | H-bond donors | MR$^b$ | TPSA$^c$ | Lipinski violations | Consensus log P$^d$ | Log S (ESOL class)$^e$ | Absorption | Distribution | Metabolism |
|-----------------|--------|-----------------|------------------|---------------|---------|----------|---------------------|----------------|---------------------|------------|-------------|------------|
| 1               | 152.15 | 4               | 5                | 5             | 31.96   | 101.15   | 0                   | -1.75          | Highly soluble      | low low low  low | -0.14884    | CYP1A2 inhibition |
| 2               | MW1    | 1               | 1                | 1             | MR2     | TPSA3    | 1                  | 7.31            | Poorly soluble     | low low low  low | 1.016751    | CYP2C19 inhibition |
| 3               | 152.15 | 1               | 3                | 2             | 31.96   | 101.15   | 1                  | 6.06            | Poorly soluble     | low low low  low | 1.036938    | CYP2C9 inhibition |
| 4               | MW1    | 1               | 14               | 7             | MR2     | Soluble  | 2                  | -0.26           | Soluble            | low low low  low | 0.622995    | CYP2D6 inhibition |
| 5               | 152.15 | 1               | 14               | 7             | 31.96   | Soluble  | 3                  | -0.26           | Soluble            | low low low  low | 0.622995    | CYP3A4 inhibition |
| 6               | MW1    | 1               | 19               | 10            | MR2     | Soluble  | 3                  | -2.03           | Soluble            | low low low  low | 0.566022    | CYP inhibitory promiscuity |

a. Cytochrome P450: substrate and inhibitor

CYP1A2 inhibition
CYP2C19 inhibition
CYP2C9 inhibition
CYP2D6 inhibition
CYP3A4 inhibition
CYP inhibitory promiscuity

b. Pharmacokinetic transporters

BRCP inhibitor
BSEP inhibitor
OATP1B1 inhibitor
OATP1B3 inhibitor
OATP2B1 inhibitor
OCT1 inhibitor

Excretion

MATE1 inhibitor
hand, compound 3 showed a low affinity toward the enzyme (−7.4 kcal/mol). The binding mode in which the compound was inserted deeply in the inhibitory site of the enzyme and consequently the tremendous steric effect toward the bound substrate appeared less possible (affinity: −5.5 kcal/mol) (Fig. 5). However, the reported activity of compound 3 on aldose reductase was suggested to be suppressive during the transcription stage (Wang et al., 2010).

Adonitol or xylitol is a five-carbon polyol (Fig. 1) that is produced from d-xylose. Xylitol has been used since the early 1960s in the diet of diabetic patients and, most recently, as a sweetener in products aimed at improved oral health (Wölnerhanssen et al., 2020). It is characterized by the same sweetness and bulk as sucrose with one-third fewer calories. It has no unpleasant aftertaste and insulin is not required for its metabolism (Deo et al., 2020). Considerable effort has been made to seek and identify inhibitors of the glycated proteins to alleviate the severity of diabetes complications (Deo et al., 2020; Favre et al., 2020; Wölnerhanssen et al., 2020). The present study explores the chemical composition of the aqueous methanolic extract of the above-ground parts of A. microcarpa DC (Ranunculaceae) and its total cardiac glycosides content. Also, it explores the potential of the two pentacyclic triterpenoids (compounds 2 and 3) as promising future antiglycation agents (Table 2).

According to the molecular modeling study, a slight superiority of compound 3 was recorded. Aldose reductase (Fig. 5) could be inhibited by the triterpenoid 2 in a noncompetitive manner while the triterpenoid 3 was probably inactive toward it. Also, this study has investigated the antiproliferative potential on six human cancer cell lines in addition to a normal human lung fibroblast (WI-38) and both compounds were not cytotoxic on the examined normal cell line.
In silico ADMET parameters

In silico physicochemical properties, ADMET parameters, of the isolated compounds are predicted and summarized in Table 5. The predicted compounds 1, 2, and 3 were found to be favorable in an acceptable prediction.

Our study showed the importance of A. microcarpa and hence investigation of sustainable usage practices is necessary for this species. In China, the above-ground parts of another Adonis species, Adonis vernalis, are harvested from the wild as a raw material for the pharmaceutical industry (Shang et al., 2019). With the abundant use of this species as well as slow plant growth, this resource has rapidly decreased and is close to extinction (Lange, 2000). Since 1982, A. vernalis has been protected in several countries and its trade was banned in many East European countries.

CONCLUSION

The present study provides insight into A. microcarpa above-ground parts and lays a solid foundation for further development of this plant. Furthermore, it can be concluded that A. microcarpa above-ground parts are a rich pool of pentacyclic triterpenoids (2 and 3). They are potent natural sources that possess the potential to be developed into a natural supplement to aid the management process of hyperglycemia-associated type 2 diabetes and as inhibitors of the glycated proteins. Additionally, compound 3 rather than 2 could be considered a potent antiproliferative agent against the tested colon cancer cell lines. Also, the in silico approach has predicted the toxicity of A. microcarpa. Further clinical evaluation of activity should be conducted after addressing the problem of the rapidly decreasing resources.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

ETHICAL APPROVALS

This study does not involve experimentation on animal or human subjects.

CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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