Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Cytotoxic, α-Glucosidase, and Antioxidant Activities of Flavonoid Glycosides Isolated from Flowers of *Lotus lanuginosus* Vent. (Fabaceae)

Mohamed Habib Oueslati[1,2], Jalloul Bouajila[3], Arbi Guetat[4,5], Fraj Al-Gamdi[6], Fayçal Hichri[7]

[1]Department of Chemistry, Northern Border University, Faculty of Science, Arar, Saudi Arabia, [2]University of Carthage, Faculty of Science Bizerte, Laboratory of Heteroorganic Compounds and Nanostructured Materials, Zarzouna, Tunisia, [3]University of Toulouse, Faculty of Pharmacy of Toulouse, Laboratory of IMRCP UMR CNRS, Toulouse F-31062, France, [4]Department of Biological Sciences, Northern Border University, Faculty of Science, Arar, Saudi Arabia, [5]Department of Biology, University of Carthage, Laboratory of Nanobiotechnology and Medicinal Plants, National Institute of Applied Science and Technology (INSAT), BP, Tunis Cedex, Tunisia, [6]Department of Biological Sciences, King Abdulaziz University, Faculty of Science, Jeddah, [7]Department of Chemistry, King Khalid University College of Science for Girls in Abha, Abha, Saudi Arabia

Submitted: 26-08-2019 Revised: 16-09-2019 Published: 31-03-2020

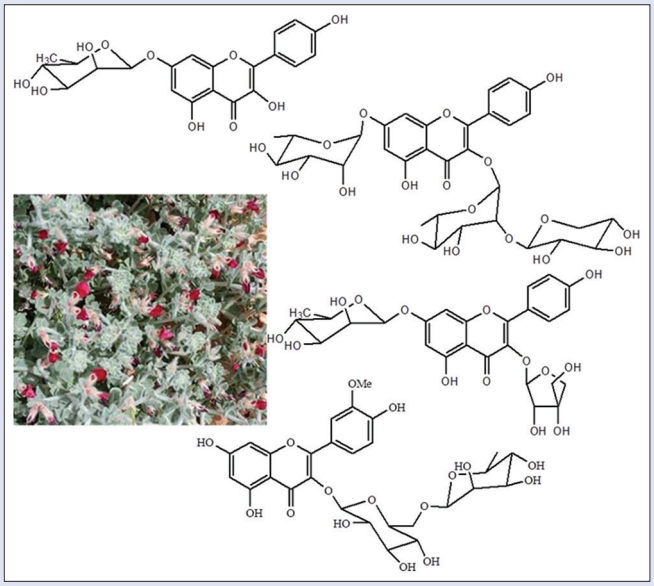
ABSTRACT

Background: *Lotus* taxa (Fabaceae) were well known as an important source of polyphenols and flavonoids. *Lotus lanuginosus* Vent. is a widespread plant in the northern region of Saudi Arabia. Phytochemical and biological activities screening focusing of the species are rare. **Objective:** The objectives of this study were to isolate secondary metabolites from the flowers of *L. lanuginosus* Vent., elucidate their structures, and evaluate their biological activities. **Materials and Methods:** Chromatography methods of the n-butanolic extract of fresh flowers of *L. lanuginosus* led five products (1–5). Their structures were characterized based on spectroscopic evidence (ultraviolet, infrared, high-resolution mass spectrometry, and nuclear magnetic resonance) and compared to the spectral data signaled in the literature. Cytotoxic activities of the isolated compounds were evaluated against HCT-116 and MCF-7 cancer cell lines. α-glucosidase and antioxidant activities were also performed. **Results:** Five compounds were isolated and characterized for the first time in *L. lanuginosus* Vent.: Lotaustralin (1), kaempferol-7-O-α-L-rhamnopyranoside (2), kaempferol-3-*O*-apiofuranosyl-7-O-rhamnopyranosyl (3), isorhamnetin-3-O-rutinoside (4), and kaempferol-3-O-(2″-β-D-xylopyranosyl)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (5). The results of cytotoxic activities against HCT-116 and MCF-7 cancer cell lines of the isolated compounds were presented. Among them, compounds 2 and 3 showed a moderate activity on HCT-116 cancer cells with half maximal inhibitory concentration values ranging between 8 and 31.5 μM. Moreover, compounds (2–5) displayed significant antioxidant and moderate α-glucosidase activities. **Discussion and Conclusions:** All the isolated products (1–5) are signaled for the first time in *L. lanuginosus* Vent. Among flavonoids glycosides, compound 2 showed the best cytotoxic activity. By the end of the present study, *L. lanuginosus* have been characterized as a highly cyanogenic plant and as an important source of polyphenols and flavonoids.

Key words: Antioxidant activity, cytotoxic activity, flavonoids glycosides, α-glucosidase inhibition

SUMMARY

- One Cyanogenic glucoside (1) and four flavonoid glucoside derivatives (2, 3, 4 and 5) were isolated from the flowers of *Lotus lanuginosus*. All compounds were isolated for the first time from this species. Compounds 2 and 3 showed moderate activity against HCT116 cancer cells. Moreover, compounds (2, 3, 4 and 5) displayed significant antioxidant and moderate α-Glucosidase activities. This species could be used as renewable source of polyphenols and flavonoids and as bioactive alternatives in pharmaceutical bioassays.



Abbreviations used: ABTS: 2,2′-azino-Bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; HCT-116: Human colon cancer cell line; MCF-7: Breast cancer cell line; NMR: Nuclear magnetic resonance; HRMS: Electrospray-high-resolution mass spectrometry; UV: Ultraviolet; MTT: 3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide; PNPG: P-nitrophenyl-α-D-glucopyranoside

Correspondence:

Dr. Mohamed Habib Oueslati,
Department of Chemistry, College of Science,
Northern Border University, Arar, Saudi Arabia.
E-mail: oueshabib@yahoo.fr

DOI: 10.4103/pm.pm_232_19

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Oueslati MH, Bouajila J, Guetat A, Al-Gamdi F, Hichri F. Cytotoxic, α-glucosidase, and antioxidant activities of flavonoid glycosides isolated from flowers of *Lotus lanuginosus* Vent. (Fabaceae). Phcog Mag 2020;16:S22-7.

INTRODUCTION

Family Fabaceae (Leguminosae) is one of the most important families of the flowering plants, which consists approximately with about 18,000 species.[1,2] The genus *Lotus* contains about 120 species mainly distributed around the Mediterranean region. In Saudi Arabia, botanical families of Fabaceae, Lamiaceae, Asteraceae, and Apiaceae are well represented in the flora of the country.[3,4] Collenette[5] and Chaudhary[6] reported that the genus *Lotus* L. (Fabaceae) in Saudi Arabia is present with eight species. *Lotus peregrinus, Lotus cytisoides,* and *Lotus lanuginosus* have been characterized as highly cyanogenic plants.[7,8] The *Lotus species* were well known as an important source of polyphenols and flavonoids.[9-12] However, these previous phytochemical investigation studies mainly focused on the extraction and isolation of flavones, isoflavones, and flavanols in *Lotus* species. Four kaempferol glycosides isolated from *Lotus edulis*, having catalytic inhibitors of wheat germ topoisomerase.[13] Flavonol diglucosides of the aglycones kaempferol, quercetin, and isorhamnetin were previously isolated from *Lotus polyphyllos, Lotus hebranicus,* and *Lotus tenuis*.[14-16] *L. creticus* revealed that they are good sources of isoflavonoid derivatives. As a part of our work on *L. lanuginosus,* we have previously reported the isolation of kaempferol-7-O-α-rhamnoside used for the synthesis of gold nanoparticle.[17] However, in the present work, we described the isolation and the structure elucidation of the major compounds isolated from *L. lanuginosus* flower extracts. The evaluation of the biological potentialities such as cytotoxic, anti-α-glucosidase, and antioxidant activities will be the second part of the present investigation.

MATERIALS AND METHODS

General experimental procedures

The one-dimensional and two-dimensional-nuclear magnetic resonance (NMR) spectra of the compounds were recorded in deuterated methanol (CD_3OD) using a JEOL JNM ECX 500 NMR spectrometer and TMS as an internal standard. The coupling constants and the chemical shifts were expressed, respectively, in Hertz and δ parts per million (ppm). The infrared spectra were recorded using an infrared spectrometer (Thermo Scientific Nicolet iS5). The ultraviolet (UV) spectra were recorded on a UV–visible spectrophotometer (Jasco V-670). The electrospray-high-resolution mass spectrometry (HRMS) spectra were obtained using an ultra-performance liquid chromatography Xevo G2 Q time-of-flight system (Waters).

Plant material

In May 2018, *L. lanuginosus* Vent. was collected during the full-bloom stage from the wild in Wadi Elmarâa, a tributary of Wady Arar (Wadi of the Anizah tribe: 30°55' 13" N, 41°0'3"E) in the northern region of Saudi Arabia. The plant specimen was identified in the Department of Biology, College of Sciences, Northern Border University. A voucher specimen (LOLA 910) was deposited in the Herbarium of the College of Science.

Extraction and isolation

Two kilograms of fresh flowers was macerated in a closed glass container with methanol at room temperature for 1 week. The obtained extract was filtered and then evaporated under reduced pressure. The crude extract (28.5 g) was dissolved in water (1 L) and then separated by decantation using n-hexane, $CHCl_3$, and n-BuOH, leading, respectively, 4.67 g, 5.46 g, and 13.47 g of dry extracts.

The n-BuOH dry extract (13.47 g) was chromatographed over Si gel column (80 cm × 6 cm, 70–230-mesh, i.d.), using mobile phase EtOAc/MeOH. After the thin-layer chromatography (TLC) monitoring, the column chromatographic fractions were labeled into five fractions (A_1–A_5). Fraction A_1 (3.68 g) was reduced into four subfractions (B_1–B_4) by column chromatography on silica gel, using CH_3Cl/MeOH as eluent. Fraction B_2 (2.26 g) was repeatedly separated on a silica gel column using CH_3Cl/MeOH (85/15) as eluent to obtain 1 (1.56 g) and 2 (367 mg). Fraction A_2 (2.86 g) was separated into four subfractions (C_1–C_4) using silica gel column chromatography (CH_2Cl_2/MeOH, 98:2). Subfraction C_3 was rechromatographed over a RP-C_{18} column with H_2O-MeCN (75/25) as eluent to obtain 3 (1.22 g) and 4 (184 mg). Fraction A_4 (4.12 g) was precipitated in ethyl acetate at 0°C for 24 h to give an impure yellow precipitate (2.56 g). The impure solid was purified by flash column chromatography eluted with CH_3Cl/MeOH (70:30) to give a major yellow powder 5 (2.15 g).

Acid hydrolysis of compounds 2–5

Twenty-five milligrams of samples were added to 10% HCl (5 mL) and refluxed for 4 h. The resulting reaction mixtures were partitioned with EtOAc. After neutralization of the aqueous fraction by Amberlite IRA-400 (Sigma-Aldrich), it was concentrated to dryness using rotary evaporator (Buchi R-210), to furnish a monosaccharide residue. The sugars were detected by TLC RP-18 analysis [Acetonitrile (0.1% formic acid)/water (0.1% formic acid) (75:25)], detection (aniline phthalate) by comparison with authentic samples β-D-glucopyranose, β-D-xylopyranose, and α-L-rhamnopyranose (Sigma-Aldrich).

Cytotoxic activity assay

Tested cell lines

In the present study, two cancer cell lines were used: HCT-116 (colorectal carcinoma) and MCF-7 (breast cancer). 10% fetal calf serum and 2 mM L-glutamine was used as a complete growth medium to conserve the cell lines in Roswell Park Memorial Institute. For incubation, cell lines were maintained at 37°C with a humidified atmosphere of 5% CO_2. All the tests were performed when the cells had reached 80% confluence, and trypan blue exclusion assay was referred for testing the viability of cell lines.[18]

Measuring cell viability (3-4,5-dimethylthiazol-2, 5-diphenyltetrazolium bromide assay)

The cytotoxicity of the isolated compounds against HCT-116 (colorectal carcinoma) and MCF-7 (breast cancer) was evaluated spectrophotometrically through 3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) assay. The assay was performed in a 96-well microtiter plate.[19] The concentration of cell lines was fixed on 10×10^3 and 12×10^3 cells/well, respectively, for HCT-116 and MCF-7. The adherent cells were incubated overnight (37°C) in a 5% enriched CO_2 atmosphere. For the cells in the exponential growth phase, further incubation (37°C) was maintained for 72 h and the concentrations of the tested compounds varied between 2 and 100 µM. After removing the medium, incubated cells (37°C) were treated with 50 µL of MTT solution (3 mg/mL in phosphate-buffered saline) during 20–40 min. The dissolving of the cell mitochondria and the precipitation of violet formazan crystals was proceeded through adding 80 µL of 100% dimethyl sulfoxide. The measurement of the obtained reaction was taken, spectrophotometrically, at 540 nm. Doxorubicin, a conventional anticancer drug, was used as a positive control. The measuring of the cell viability was calculated using the Formula I:

I: % inhibition = $100 * [(Abs_{control} - Abs_{blank\ control})/(Abs_{sample} - Abs_{blank\ sample})]$.

Where $Abs_{control}$ is the absorbance measured of the total cell activity as described without any inhibition, $Abs_{blank\ control}$ is the absorbance of MTT substrate, and Abs_{sample} is the absorbance of each inhibitor sample. The

experiment was tested in three replications, and the anticancer effect of the isolated compounds was expressed as half maximal inhibitory concentration (IC_{50}) values calculated from the mean values of percentage.

In vitro antioxidant assays

The *in vitro* antioxidant assay of the isolated compounds was tested using 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH•)- and $ABTS^{•+}$-scavenging tests:

2,2′-diphenyl-1-picrylhydrazyl radical method assay

The used method was developed by Bondet *et al.*[20] with the point of view to determine the antioxidant activity of the isolated compounds in a like manner using a stable free DPPH radical. The assay is based on the measurement of the scavenging capacity of antioxidants toward it. Subsequently, 500 µl of compounds (at different concentrations: 20–250 µg/mL) was dissolved in 500 µl of 0.1 mM methanolic DPPH solution. The reaction was kept at room temperature in the dark for 30 min. The optical density (517 nm) was then measured. Methanol solution was used as a negative control and DPPH as a positive control. The following equation (II) was referred for the calculation of the percentage of DPPH inhibition:

II (%) = [(AB − AA)/AB] ×100.

Where:

AB: the optical density value (517 nm) of the control.

AA: The optical density value (517 nm) of the compound.

IC_{50} values were presented for DPPH method assay. IC_{50} is the concentration of the sample required to inhibit 50% of radical. The experiment was tested in three replications, and IC_{50} values were reported as means ± standard deviation.

ABTS assay

The used standard method was described by Besbes *et al.*[21] ABTS assay is based on the ability of the isolated compounds to scavenge the stable ABTS radical (ABTS•+). The stock solutions included 7 mM ABTS solution and 2.45 mM potassium persulfate solution. It has been stored in the dark at room temperature for 12 h. Before the experiment, an absorbance value equal to 0.7 ± 0.2 at 750 nm of the stock solution was optimized through several dilutions. To test the antioxidant ABTS assay of the isolated compounds, 3000 µl mixture was prepared and the optical density was measured after 30 min at 750 nm. 150 µl of isolated compounds at different concentration (20–250 µg/ml) was dissolved in 2850 µl ABTS•+ solution to obtain the abovementioned 3000 µl mixture. The ABTS assay was calculated using the equation II, and all calculations were performed in triplicate.

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of isolated compounds was performed as described by Tao *et al.*[22] P-nitrophenyl-α-D-glucopyranoside (PNPG) was used as a substrate for this experiment. The mixture was prepared in methanol; 250 µL of the isolated compounds (10–1000 µM), 0.3 U/mL of α glucosidase (in phosphate buffer, pH 6.9) and 2.5-mM PNPG was respectively added. The negative control was prepared by dissolving enzyme and substrate in MeOH. As a positive control, the acarbose was used beside the isolated compound and the mixture was prepared in MeOH. Absorbance was measured at 405 nm and the measurement was conducted in triplicate. The percentage of the inhibition (%) reflects the glucosidase inhibitory activity and was calculated by the formula (III):

(III): α-glucosidase inhibition activity (%) = $[(A_{control} - A_{sample})/A_{control}]$ ×100.

Where $A_{control}$ is the absorbance (405 nm) in the presence of isolated compound and A_{sample} is the absorbance (405 nm) of control.

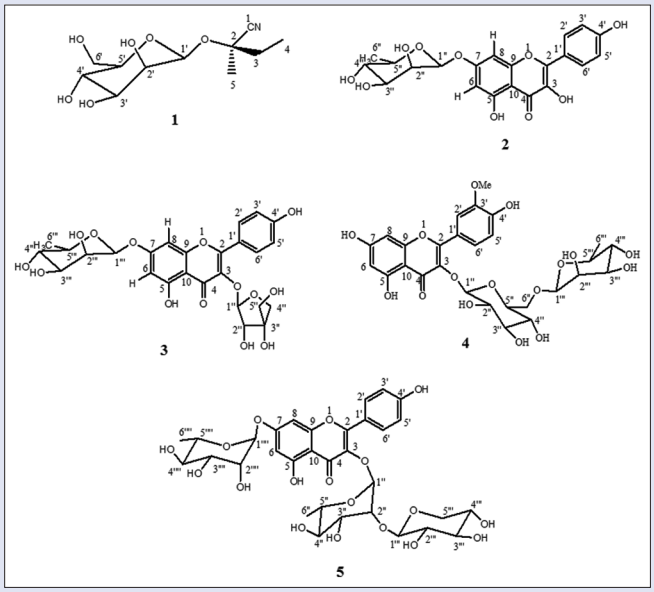


Figure 1: Structure of five compounds isolated from the flowers of *Lotus lanuginosus* Vent.

RESULTS

Structural elucidation of compounds 1–5

The n-butanolic extract obtained from the plant was fractionated by a repeatedly chromatographic procedures with normal-phase and reversed-phase silica gel, layer chromatography (TLC) to isolate and characterize for the first time five compounds in *L. lanuginosus*: lotaustralin (1),[23] kaempferol-7-O-α-L-rhamnopyranoside (2),[24] kaempferol-3-*O*-apiofuranosyl-7-*O*-rhamnopyranosyl (3),[13] isorhamnetin 3-O-rutinoside (4),[25] and kaempferol-3-O-(2”-β-D-xylo pyranosyl)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (5)[26] [Figure 1].

Lotaustralin (1): White amorphous powder: ^{1}H NMR (500 MHz, CD_3OD) δ_H 1.8 (m, H_3), 1.72 (m, H_4), 8.2 (s, H_5), 6.84 (d, J = 1.5, H_6), 4.84 (d, J = 8, $H_{1'}$), 5.23 (m, $H_{2'}$), 5.1 (m, $H_{3'}$), 3.75 (d, *m*, $H_{4''}$). 5.0 (*m*, $H_{5'}$). 4.17 (m, $H_{6\alpha}$), 4.10 (m, $H_{6'\beta}$), 3.75 (d, *m*, $H_{4''}$). ^{13}C NMR (125 MHz, CD_3OD) δ_C 119.2/C_1, 77.2/C_2, 34.1/C_3, 25.2/C_4, 8.2/C_5, 98.1/$C_{1'}$, 72.2/$C_{2''}$, 72.1/$C_{3''}$, 70.1/C $_{4''}$, 69.8/C-$_{5''}$, 61.8/$C_{6''}$. HRMS: 357.2042 $[M+Na]^+$ (calculated for $C_{20}H_{30}O_4$).

kaempferol-7-O-α-L-rhamnopyranoside (2): Yellow amorphous powder: ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.86 ($H_{2'/6'}$, d, J = 8.5,), 6.94 ($H_{3'/5'}$, d, J = 8.5), 6.72 (H8, d, J = 1.5,), 6.45 (H_6, d, J = 1.5), 7-O-rham [5.58 ($H_{1''}$, d, J = 1.5), 4.02 ($H_{2''}$, dd, $J1$ = 1.5, $J2$ = 3.6), 3.86 ($H_{3''}$, dd, $J1$ = 3.6, $J2$ = 9.5), 3.55 ($H_{4''}$, m), 3.61 ($H_{5''}$, m), 1.26 ($H_{6''}$, d, J = 6.0)]. ^{13}C NMR (125MHz, CD_3OD): δ_C 161.5/C_2, 137.6/C_3), 178.2/C_4, 160.5/C_5, 101.1/C_6, 161.2/C_7, 95.2/C_8, 156.7/C_9), 105.3/C_{10}, 120.7/$C_{1'}$, 131.6/$C_{2'/6'}$, 116.2/$C_{3'/5'}$, 158.4/$C_{4'}$, 7-O-rham [99.5/$C_{1''}$, 71.3/$C_{2''}$, 70.5/$C_{3''}$, 72.2/$C_{4''}$, 69.6/$C_{5''}$, 16.4/$C_{6''}$.

kaempferol-3-*O*-apiofuranosyl-7-*O*-rhamnopyranosyl (3): Yellow amorphous powder: ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.88 (d, J = 8.5, $H_{2'/6'}$), 6.91 (d, J = 8.5, $H_{3'/5'}$), 6.66 (d, J = 2, H_8), 6.42 (d, J = 2, H_6), 3-*O*-apiofuranosyl [5.72 (d, J = 2, $H_{1''}$), 4.31 (d, J = 2, $H_{2''}$), 3.64 (d, J = 10, $H_{4''\alpha}$), 3.61 (d, $H_{4''\beta}$), 3.65 (d, J = 11.5, $H_{5''\alpha}$), 3.53 (d, J = 11.5, $H_{5''\beta}$)]. 7-O-rham [5.40 (d, J = 2, $H_{1'''}$), 4.06 (br s, $H_{2'''}$), 3.86 (m, $H_{3'''}$), 3.50 (m, $H_{4'''}$), 3.61 (m, $H_{5'''}$), 1.28 (d, J = 6.0, $H_{6'''}$)]. ^{13}C NMR (125MHz, CD_3OD): δ_C 160.2/C_2, 135.2/C_3, 178.2/C_4, 161.4/C_5, 99.1/C_6, 162.0/C_7, 94.2/C_8, 156.5/C_9, 105.9/C_{10}, 120.9/$C_{1'}$, 130.6/$C_{2'/6'}$, 114.7 $C_{3'/5'}$, 158.4/$C_{4'}$, 3-*O*-apiofuranosyl [109.6/$C_{1''}$, 77.8/$C_{2''}$, 79.7/$C_{3''}$), 75.2/$C_{4''}$, 63.5/$C_{5''}$, 7-O-rham [99.1/$C_{1'''}$, 70.2/$C_{2'''}$, 70.7/$C_{3'''}$), 72.2/$C_{4'''}$), 69.8/$C_{5'''}$), 16.7/$C_{6'''}$).

Isorhamnetin 3-O-rutinoside (4): Yellow amorphous powder: ^{1}H NMR (500 MHz, CD_3OD) δH in ppm): 7.97 (d, *J* = 2.0, $H_{2'}$), 7.66 (dd, *J*1 = 8.5, J_2 = 2.0, $H_{6'}$), 6.94 (d, *J* = 8.5, $H_{5'}$), 6.44 (d, *J* = 2.0, H_8), 6.23 (d, *J* = 2.0, H_6), 5.27 (d, *J* = 7.5, $H_{1''}$); 4.55 (d, *J* = 2.0, $H_{1'''}$), 4.2 (d, *J* = 6.9, $H_{1''''}$), 3.98 (s, 3′-O-Me) 3.84 (dd, *J*1 = 2, J_2 = 12, $H_{6''a}$), 3.63 (m, $H_{2'''}$), 3.51 (m, $H_{4'''}$), 3.48 (m, $H_{5''}$), 3.46 (dd, *J*1 = 2, J_2 = 12 $H_{6''b}$), 3.43 (m, $H_{5'''}$), 3.38 (m, $H_{3''}$), 3.29 (m, $H_{4''}$), 3.27 (m, $H_{3''}$), 3.27 (m, $H_{3''}$), 1.12 (d, *J* = 6, $H_{6''''}$). ^{13}C NMR (125MHz, CD_3OD, δ_C 177.9/C_4, 164.7/C_7, 161.6/C_5, 157.4/C_2, 157.1/C_9, 149.4/$C_{4'}$, 146.9/$C_{3'}$, 134.0/C_3, 122/$C_{6'}$, 121.6/$C_{1'}$, 114.7/$C_{5'}$, 113.1/$C_{2'}$, 104.3/C_{10}, 102.9/$C_{1''}$, 101.1/$C_{1'''}$, 101.5/$C_{1''}$, 98.5/C_6, 93.5/C_8, 76.77/$C_{5''}$,76.0/$C_{3''}$, 74.5/$C_{2''}$, 72.4/$C_{4''}$, 70.8/$C_{4'''}$, 70.7/$C_{2'''}$, 70.2/$C_{3'''}$, 68.3/$C_{5'''}$, 67.1/$C_{6'''}$, 55.3 (3′-OMe), 16.4.1/$C_{6''''}$.

kaempferol-3-O-(2”-α-L-arabinopyranosyl)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (5): Yellow amorphous powder: ^{1}H NMR (500 MHz, CD_3OD), δH: 7.85 (d, *J* = 8.5, $H_{2'/6'}$), 6.98 (d, *J* = 8.5, $H_{3'/5'}$), 6.77 (d, *J* = 1.5, H_8), 6.50 (d, *J* = 1.5, H_6), 3-O-Rahm [5.47 (d, 1.5, $H_{1''}$), 4.22 (dd, *J* = 3.5, 1.5, $H_{2''}$), 3.86 (dd, J_1 = 3.5, J_2 = 9.5, $H_{3''}$), 3.41 (m, $H_{4''}$), 3.71 (m, $H_{5''}$), 1.03 (d, *J* = 6.0, $H_{6''}$)]. 2”-O–arabinopyranosyl [4.31 (d, *J* = 7.5, $H_{1'''}$), 3.21 (dd, *J* = 9, 7.5, $H_{2'''}$), 3.33 (m, $H_{3'''}$), 3.42 (m, $H_{4'''}$), 3.70 (1H, dd, *J*1 = 11.5, *J*2 = 5.0, $H_{5'''a}$), 3.09 (dd, *J*1 = 11.5, *J*2 = 10.5 $H_{5'''b}$)]. 7-O-rham [5.58 (d, *J* = 1.5, $H_{1''''}$), 4.02 (dd, *J*1 = 1.5, *J*2 = 3.5, $H_{2''''}$), 3.86 (dd, J_1 = 3.5, J_2 = 9.5, $H_{3''''}$), 3.50 (m, $H_{4''''}$), 3.61 (m, $H_{5''''}$), 1.28 (d, *J* = 6.0, $H_{6''''}$)]. ^{13}C NMR (125MHz, CD_3OD). δ_C 160.5/C_2, 135.6/C_3, 178.5/C_4, 161.5/C_5, 99.1/C_6, 162.2/C_7, 94.2/C_8, 156.7/C_9, 106.3/C_{10}. 120.9/$C_{1'}$, 130.6/$C_{2'/6'}$, 115.2/$C_{3'/5'}$, 158.4 $C_{4'}$, 3-O-Rahm [101.8/$C_{1''}$, 81.2/$C_{2''}$, 70.5/$C_{3''}$, 72.2/$C_{4''}$, 70.3/$C_{5''}$), 16.3/$C_{6''}$; 2”-O–arabinopyranosyl [106.39/$C_{1'''}$, 73.8/$C_{2'''}$, 76.4/$C_{3'''}$, 69.5/$C_{4'''}$, 65.7/$C_{5'''}$, 7-O-rham [98.5/$C_{1''''}$, 70.3/$C_{2''''}$, 70.5/$C_{3''''}$, 72.2/$C_{4''''}$), 69.9/$C_{5''''}$), 16.6/$C_{6''''}$.

Cytotoxic activities

The cytotoxic activities *(in vitro)* of five isolated compounds (1–5) were performed using two cancer cell lines (HCT-116 and MCF-7). The conventional anticancer drugs in clinical trials, doxorubicin, were used as a positive control. Figure 2 shows the different cytotoxic activities of the tested isolated compounds. Among them, compound 2 showed the most potent activities against HCT-116 with 8.2 μM (±1.3) as IC_{50} value and a moderate activity against MCF-7 (IC_{50} = 74.0 μM ± 9). Compound 3 was found to be with a relatively moderate activity against the two tested cancer cell lines, and the values of IC_{50} were 46.3 μM (±9) and 80 (±9), respectively, for HCT-116 and MCF-7 cell lines. For the rest of the compounds (1, 4, and 5), the IC_{50} exceeded the value of 100 μM and were with low cytotoxic activities against the two tested cell lines [Figure 2a and b].

Antioxidant activity

The results of the DPPH- and ABTS-scavenging activities of the isolated compounds (2–5), obtained from the flowers of the studied plant, are shown in Figure 3. Gallic acid was used as a positive control. As shown in Figure 3, the isolated compounds 2 and 4 exhibited more antioxidant activity both for DPPH- and ABTS-scavenging assay. Gallic acid is far away more potent than kaempferol-3-O-(2”-β-D-xylopyranosyl)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (5) and kaempferol-3-O-apiofuranosyl-7-O-rhamnopyranosyl (3).

α-glucosidase inhibitory activity

In the present study, the five isolated compounds (1–5) were screened for their inhibitory activities *(in vitro)* against the α-glucosidase enzymes. As shown in Figure 4, all flavonoids glycosides (2, 3 and 5) exhibited moderate α-glucosidase activity with the IC_{50} ranging from 276 to

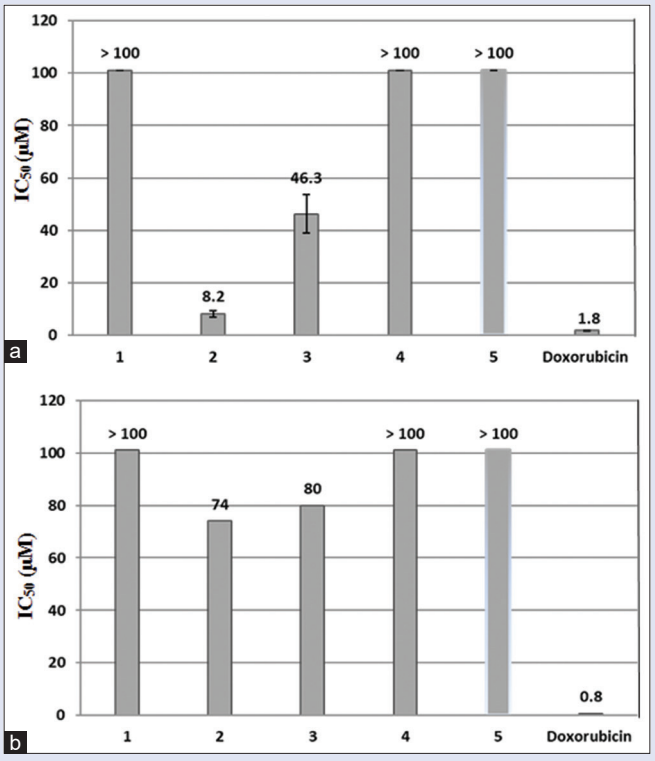


Figure 2: *In vitro* cytotoxic activities against two cancer cell lines HCT-116 (a) and MCF-7 (b) of compounds (1–5) isolated from flowers of *Lotus lanuginosus*

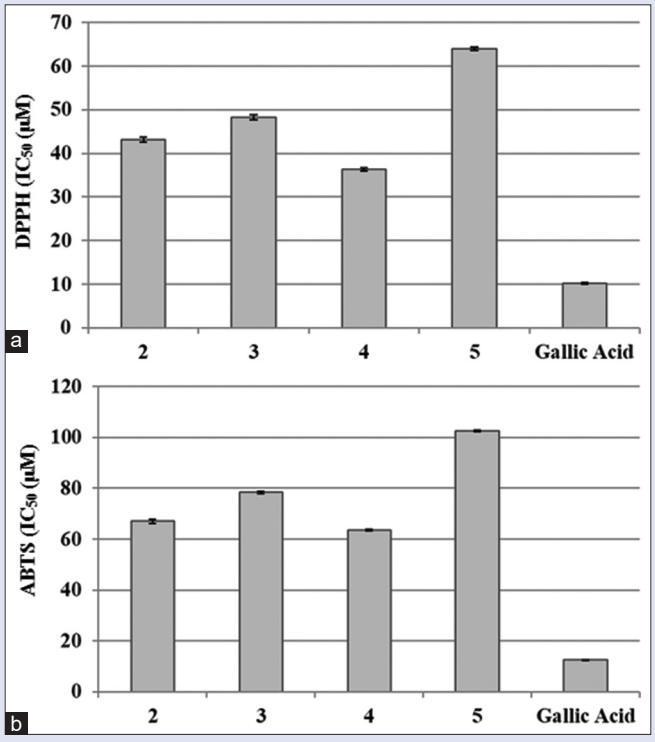


Figure 3: Antioxidant activities of compounds (2–5) isolated from flowers of *Lotus lanuginosus*. (a) 2,2′-diphenyl-1-picrylhydrazyl radical radical-scavenging activity; (b) ABTS radical cation decolorization assay

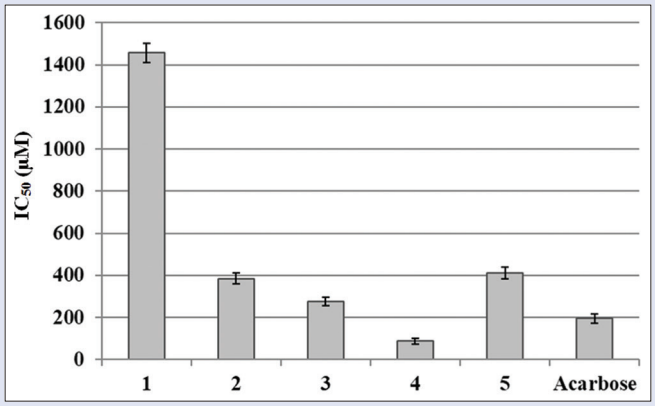


Figure 4: α-glucosidase inhibitory activity of five compounds isolated from flowers of *Lotus lanuginosus*. Acarbose, an alpha-glucosidase inhibitor, was used as a positive control

412 µM. The isolated compound 4 exhibited a high effect with an IC_{50} value less than 100 µM (87.24 ± 12.6). Compared to the positive control, isorhamnetin 3-O-rutinoside (4) showed IC_{50} two times less than acarbose (acarbose IC_{50} = 195.21 ± 21.46).

DISCUSSION

Chromatographic separation of the butanolic extract of *L. lanuginosus* flowers resulted in the isolation of five compounds. Among them, four compounds were flavonoid glycosides and only one cyanogenic glucoside. The five characterized compounds were identified for the first time from the plant. Previous studies reported that compounds 3 and 5 were isolated from *L. edulis*[13] and *Lotus lalambensis,*[27] respectively.

The cytotoxic activities against HCT-116 and MCF-7 cancer cell lines showed compound 2 showed the most potent activity against HCT-116 cancer cell lines with IC_{50} =8.2 µM. Moreover, the compound showed a moderate activity against MCF-7 cancer cell lines. However, the compound 3 showed moderate activity on both cancer cell lines [Figure 2]. Previous studies showed that flavonoid aglycones exhibited higher anticancer potential than their glycosides in cell level.[28,29] Regarding the structure of compound 1, the type of substituted monosaccharide and its position in kaempferol seem to decrease the cytotoxic activity of these compounds.

The results of DPPH radical-scavenging activity and ABTS radical cation decolorization assay of flavonoid glycosides 2-5 revealed that compounds 2, 3, and 5 exhibited a moderate antioxidant activity. Among the four tested compounds, 4 showed a greater antioxidant activity with IC_{50} = 36 and 62 µg/mL, respectively, for DDPH and ABTS assays. The high antioxidant effect of the compound could be attributed to the hydroxyl group fixed in C-7 position. A previous study demonstrated that isorhamnetin 3-O-rutinoside was isolated from several plant taxa which revealed a high antioxidant activity.[30,31]

Our results of α-glucosidase activity showed that the better activity was recorded in the case of compound 4. In comparison with 2, 3, and 5 which demonstrated a moderate α-glucosidase activity, the presence of hydroxyl at C-7 in compound 4 could be the factor of increasing the inhibitory activity.[32] On the other hand, the nature of the sugar seems to have an impact on the inhibitory action; since that, compound 3 was more active than the two other compounds: 2 and 5. These results are in agreement with other studies showing that the glycosylation of flavonoids decreased α-glucosidase activity.[33] Lotaustralin (1) present the lowest α-glucosidase activity with IC_{50} value of 1456 µM. Laura *et al.*[34] reported that cyanogenic glucosides showed low α-glucosidase activity.

CONCLUSIONS

To the best of our knowledge, this is the first report on mainly active α-glucosidase inhibitors, cytotoxic and antioxidant metabolites present in the *L. lanuginosus*. Five compounds (1–5) were isolated. Compound 4 showed the most α-glucosidase inhibitory activity, which was superior to a control. On the other hand, compounds 2 and 3 exhibited a moderate activity against cancer cell lines (HCT-116 and MCF-7). Moreover, compound 4 exhibited the most potent antioxidant (DPPH) and ABTS activities.

Financial support and sponsorship

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through General Research Project under grant number (Project Number RG.P1/90/40).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wink M, Mohamed GI. Volution of chemical defense traits in the leguminosae: Mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the rbcL gene. Biochem Syst Ecol 2003;31:897-917.
2. Mabberley DJ. Mabberley'S Plant-Book. 3rd ed. Cambridge: Cambridge University Press; 2007.
3. Oueslati MH, Bouajila J, Belkacem MA, Harrath AH, Alwasel SH, Ben Jannet H. Cytotoxicity of new secondary metabolites, fatty acids and tocols composition of seeds of *Ducrosia anethifolia* (DC.) Boiss. Nat Prod Res 2019;33:708-14.
4. Guetat A, Boulila A, Boussaid M. Phytochemical profile and biological activities of *Deverra tortuosa* (Desf.) DC.: A desert aromatic shrub widespread in Northern region of Saudi Arabia. Nat Prod Res 2019;33:2708-13.
5. Collenette IS. Wildflowers of Saudi Arabia national commission for wildlife conservation and development (NCWCD). National government publication; 1st ed Saudi Arabia; Riyadh; 1999. p. 799.
6. Chaudhary SA. Flora of the kingdom of Saudi Arabia. Vol. 2. Riyadh, KSA: Ministry of Agriculture and Water, National Agriculture and Water Research Center; 2001. p. 92.
7. Band L, Heyn CC, Plitmann U. Distribution of cyanogenesis in *Lotus* (Leguminosae). Taxon 1981;3:601-8.
8. Rizk AM. The Phytochemistry of the Flora of Qatar. Doha: Scientific and Applied Research Center, University of Qata; 1986. p. 85-90.
9. Deng J, Chen S, Yin X, Wang K, Liu Y, Li S, *et al.* Systematic qualitative and quantitative assessment of anthocyanins, flavones and flavonols in the petals of 108 *Lotus* (*Nelumbo nucifera*) cultivars. Food Chem 2013;139:307-12.
10. Huang B, Ban XQ, He JS, Tong J, Tian J. Hepatoprotective and antioxidant activity of ethanolic extracts of edible *Lotus* (*Nelumbo nucifera* Gaertn.) leaves. Food Chem 2010;120:873-8.
11. Mukherjee PK, Mukherjee D, Maji AK, Rai S, Heinrich M. The sacred *Lotus* (*Nelumbo nucifera*) – Phytochemical and therapeutic profile. J Pharm Pharmacol 2009;61:407-22.
12. Valls J, Millán S, Martí MP, Borràs E, Arola L. Advanced separation methods of food anthocyanins, isoflavones and flavanols. J Chromatogr A 2009;1216:7143-72.
13. Spanou C, Bourou G, Dervishi A, Aligiannis N, Angelis A, Komiotis D, *et al.* Antioxidant and chemopreventive properties of polyphenolic compounds derived from Greek legume plant extracts. J Agric Food Chem 2008;56:6967-76.
14. Abdel-Kader MS, Amer ME, Tang S, Kingston DG. Two new isoflavone derivatives from the roots of an Egyptian collection of *Lotus polyphyllos*. Nat Prod Res 2006;20:922-6.
15. Yang SS, Gao F, Mabry TJ, Amer ME, Abdel-Kader MS, Mahmoud ZF, *et al.* Flavonoids from *Lotus creticus*. Phytochemistry 1989;28:1749-50.
16. Mahmoud ZF, Amer ME, Abdel-Kader MS, Abdel-Salam NA. A coumestan from *Lotus creticus*. Phytochemistry 1990;29:355-6.
17. Oueslati MH, Ben Tahar L, Harrath AH. Catalytic, antioxidant and anticancer activities of gold nanoparticles synthesized by kaempferol glucoside from Lotus leguminosae. Arab J Chem 2018. [In press]. [Doi: 10.1016/j.arabjc. 2018.09.00].

18. Louis KS, Siegel AC. Cell viability analysis using trypan blue: Manual and automated methods. Methods Mol Biol 2011;740:7-12.
19. Bekir J, Mars M, Vicendo P, Fterrich A, Bouajila J. Chemical composition and antioxidant, anti-inflammatory, and antiproliferation activities of pomegranate (*Punica granatum*) flowers. J Med Food 2013;16:544-50.
20. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT Food Sci Technol 1997;30:609-15.
21. Besbes HM, Mosbah H, Mssada K, Jannet BH, Aouni M, Selmi B. Acetylcholinesterase inhibitory and antioxidant properties of roots extracts from the Tunisian *Scabiosa* arenaria Forssk. Ind Crops Prod 2015;67:62-9.
22. Tao Y, Zhang Y, Cheng Y, Wang Y. Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. Biomed Chromatogr 2013;27:148-55.
23. Akgul Y, Ferreira D, Abourashed EA, Khan IA. Lotaustralin from *Rhodiola rosea* roots. Fitoterapia 2004;75:612-4.
24. Liu Q, Liu M, Mabry TJ, Dixon RA. Flavonol glycosides from *Cephalocereus senilis*. Phytochemistry 1994;36:229-31.
25. Dehaghani ZA, Asghari G, Dinani MS. Isolation and Identification of nicotiflorin and narcissin from the aerial parts of *Peucedanum aucheri* Boiss. JASTA 2017;7:45-51.
26. Lin HY, Chang ST. Kaempferol glycosides from the twigs of *Cinnamomum osmophloeum* and their nitric oxide production inhibitory activities. Carbohydr Res 2012;364:49-53.
27. El-Youssef HM, Murphy BT, Amer ME, Al-Rehaily AJ, Abdel-Kader MS, Kingston DG. Two new flavonol glycosides from the aerial parts of growing in Saudi Arabia. Nat Prod Sci 2008;14:86-9.
28. de Araújo ME, Moreira Franco YE, Alberto TG, Sobreiro MA, Conrado MA, Priolli DG, *et al.* Enzymatic de-glycosylation of rutin improves its antioxidant and antiproliferative activities. Food Chem 2013;141:266-73.
29. Fawzy GA, Al-Taweel, AM, Baky NA, Marzouk MS. Cytotoxic and renoprotective flavonoid glycosides from *Horwoodia dicksoniae*. Afr J Pharm Pharmacol 2012;6:1166-75.
30. Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, *et al.* Chemical characterization and biological effects of Sicilian *Opuntia ficus indica* (L.) mill. Fruit juice: Antioxidant and antiulcerogenic activity. J Agric Food Chem 2003;51:4903-8.
31. Okoth DA, Chenia HY, Koorbanally NA. Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (*Anacardiaceae*). Phytochemistry Lett 2013;6:476-81.
32. Fang H, Peng Z, Hao-Yue W, Gang-Xiu C, Zhong-Wen X, Guan-Hu B. Inhibition of flavonoid glycosides from Lu'an Guapian tea on a-glucosidase and a-amylase: Molecular docking and interaction mechanism. Food Funct 2018;9:4173-83.
33. Xiao J. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Crit Rev Food Sci Nutr 2017;57:1874-905.
34. Laura FB, Araceli PV, Mariana TP, Robert B, Edelmira L, Rachel M. A-glucosidase inhibitors from *Vauquelinia corymbosa*. Molecules 2015;20:15330-42.