SUPPLEMENTARY MATERIAL

Polyphenolics From *Gymnocarpos decandrus* Forssk roots and their biological activities

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Abstract

Phytochemical investigation of *Gymnocarpos decandrus* roots lead to the isolation, characterization and evaluation of four compounds: the bis-coumarin daphnoretin, two biflavonoids: wikstrol A and wikstol B in addition to β-sitosterol glucoside. Their structures were established via spectroscopic data. The crude root extract showed a significant antimicrobial activity against *Bacillus subtilis*. In addition, Coagulation activity of the same extract and daphnoretin were investigated via measuring their effect on prothrombin time (PT) and activated partial thromboplastin time (aPTT) assay in citrated plasma collected from healthy regular blood donors and they were found to prolong the PT and aPTT assays ($p<0.05$). The three polyphenolics were described for the first time from the Caryophllaceae family. Furthermore, this is the first phytochemical and biological study to be carried on *G. Decandrus* roots.

key words:

Gymnocarpos decandrus, daphnoretin, wikstrol A, wikstrol B, biflavonoids

1. General experimental procedures:

All the solvents used were of analytical grade. Silica gel (230–400 mesh), precoated TLC plates silica gel 60 (GF-254) were purchased from Merck, Germany. The 1D and 2D NMR analysis were obtained using a Bruker avance III (Switzerland) 400 MHz. Residual peaks of the deuterated solvents were used to reference the spectrum. EIMS was obtained on a Delsi-Nermag R30-10. UV-Visible spectra were carried on a Helios α thermo spectronic, England, supported with software Vision 32®.

1.1. Plant material, Extraction and isolation of the compounds

The plant material was collected from Omayed biosphere and kindly identified by prof. Dr. Loutfy Boulos, National Research Center, Dokki, Cairo, Egypt. A voucher specimen were deposited at department of pharmacognosy (GD108), faculty of pharmacy, Alexandria university, Egypt.

Air dried roots (400 gm) were extracted with 70 % ethyl alcohol to yield (14 gm), the extract was concentrated then fractionated with methylene chloride followed by butanol. The CH_2Cl_2 fraction was chromatographed on a silica gel column and elution was carried using CH_2Cl_2 and increasing concentration of ethyl acetate. Fraction eluted with 10% EtOAc afforded compound 1 (30 mg) and compound 2 (30 mg). While compound 3 (15 mg), and compound 4 (18 mg) were isolated from the ethyl acetate fraction using also silica gel column chromatography.

1.2. Antimicrobial screening using Agar Well Diffusion method :

Test organisms

Microorganisms used in this study represent pathogenic species commonly associated with nosocomial infections. The bacteria were maintained in the microbiology Laboratory at Faculty of pharmacy, University of Alexandria and consisted of two Gram-positive strains, *Bacillus subtilis (*ATCC19659) and *Staphyllococcus aureus* (ATCC6538P), and two Gram-negative strains, *Escherischia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC9027).in addition to the pathogenic yeast *Candida albicans* (ATCC2091) .

Preparation of the agar plate:

The sterile nutrient agar was poured aseptically as 40 ml portions into sterile Petri dishes then incubated after solidification at 37° C for 18 h.

Preparation of the inoculum:

Each tested organism was subcultured in 3 ml sterile nutrient broth and the resultant microbial growth was firstly compared with 0.5 'McFarland Opacity Standard' which was equivalent to approximately 10^8 CFU/ml and properly diluted; if necessary, to achieve the same turbidity of the standard.

Procedure of the test:

Sterile cotton swabs were used to streak all over the surface of the nutrient agar .The plates were then left to dry at room temperature for few minutes.

A sterile cork porer (8 mm in diameter) was used to prepare wells in the solid nutrient agar plates. (60 mg/ml of the alcoholic root extract and 5 mg/ml of compound 1 both dissolved in 75 % DMSO) and another well with same volume of 75 % DMSO as a vehicle control. Allow a period of free diffusion for 2 h, then incubate at 37° C for 18-24 h.

Reading and interpretation of Results:

After incubation, the diameters of inhibition zones around the wells were measured, to the nearest mm, in three different directions using a ruler and the average diameter was recorded and compared to that of the control.

1.3. Anti coagulant assay

Preparation of plasma

Blood samples were collected from healthy volunteers using a disposable polypropylene syringe and then anti-coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 × g for 15 min and plasma was separated , pooled and stored at 4°C until its use. (Lei etal 2015).

Prothrombin time PT assay (effect on extrinsic pathway)

In a test tube, 1 ml of pooled plasma was added to 1 ml plant extract and incubated for 5 min at 37°C. Then, 0.2 ml of Liquiplastin reagent (Tulip Diagnostic Pvt Ltd, India) pre-warmed at 37°C was added to 0.1 ml of the plant/plasma mixture. Coagulation time was recorded for each sample using an

automatic coagulative instrument (Dade Behring BFT II Coagulation Analayzer). The steps were repeated three times for each sample and average of the test value was noted.

Activated partial thromboplastin time aPTT assay (effect on intrinsic pathway)

The plasma/ extract mixture was prepared as in PT assay then incubated for 1 min at 37°C . 0.2 ml of Liquicelin phospholipid preparation derived from rabbit brain and ellagic acid was added. Then the mixture was incubated for 3 min at 37°C , 0.1 ml of prewarmed calcium chloride solution was added and coagulation time was recorded for each sample using an automatic coagulative instrument (Dade Behring BFT II Coagulation Analayzer). The steps were repeated three times for each sample and average of the test value was noted.

Phosphate buffer was used instead of the extracts for the negative control and 10 mg/ml of EDTA for the positive control.

2. Spectral data

Table S1. NMR of compound 1 in DMSO-d6

	Compound 1	
C/H	***δ* C**	***δ* H**
2	160.2	
3	136.2	
4	131.5	7.85 (1H, s)
4a	110.7	
5	109.9	7.19 (1H, s, H-5)
6	146.2	
7	150.9	
8	103.3	6.86 (1H,s)
8a	147.9	
2′	160.3	
3′	114.9	6.37 (1H, d,J=9.2 Hz)
4′	144.6	8.02 (1H, d, J=9.2 Hz)
4′a	114.0	
5′	130.5	7.71 (1H, d, J=8.4 Hz)
6′	114.4	7.10 (1H, dd, J=8.4,2.3 Hz)
7′	157.5	
8′	104.5	7.16 (1H, d, J=2.3)
8′a	155.5	
OMe-6	56.5	3.78 (3H, s,)

OH		10.3

Table S 2. NMR of compounds 3-4 in CD_3OD

	3		**4**	
	***δ*C**	***δ* H**	***δ* C**	***δ* H**
2	81.4	4.09 (d, J=7.65)	81.0	4.6 4(1H, dd, J = 10.2, 2.2 Hz)
3	67.5	3.88 (m)	67.3	3.7(m)
4	27.7	2.85 (dd, J= 16,7 Hz) 2.44 (dd, J= 16,7 Hz)	25.2	2.44 (2H, m)
5	154.6	-	156.2	-
6	95.2	5.95 (1H,s)	95.1	6.01 (s)
7	153.4	-	152.3	-
8	98.3		98.8	-
9	154.6	-	155.4	-
10	100.2	-	99.2	-
1̀	130.1	-	130.1	-
2̀,6̀	128.1	7.08 (2 H, d, J=8.4 Hz)	127.1	6.66(2 H, d, J=8.6 Hz)
3̀,5̀	114.4	6.64 (2 H, d, J=8.4 Hz)	114.6	6.57(2 H, d, J=8.6 Hz)
4̀	156.4	-	157.1	-
Unit II			Unit II	
2	163.8	-	163.2	-
3	114.6		111.8	-
4	182.3	-	182.3	-
5	161.9		159.7	-
6	98.3	6.13 (d, J= 2 Hz)	98.9	6.05 (2 H, d, J= 1.8 Hz)
7	164.1	-	163.4	-
8	93.1	6.25 (d, J= 2 Hz)	93.6	6.30 (2 H, d, J= 1.8 Hz)
9	158.0	-	158.3	
10	103.0	-	103.3	-
1̀	124.6	-	124.3	-
2̀,6̀	130.0	7.23 (2 H, d, J=9.1 Hz)	130.2	7.36 (2 H, d, J=8.9 Hz)
3̀,5̀	114.2	6.68 (2 H, d, J=9.1 Hz)	114.8	6.78 (2 H, d, J=8.9 Hz)
4̀	159.2	-	159.7	
3-OH		4.90 (1 H, br s)		4.90 (1 H, br s)

Figure S1: Structure of daphnoretin

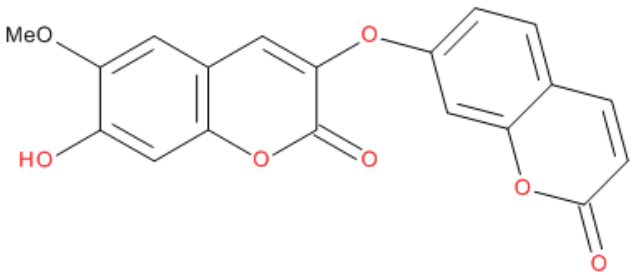
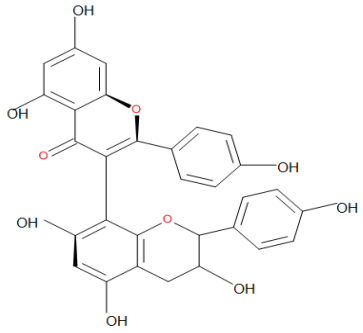
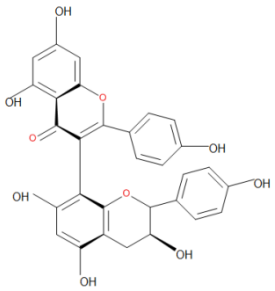


Figure S2:structures of wikstrol A and B



Wikstrol A



Wikstrol B