Short Communication

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 Caryophyllaceae family. Furthermore, this is the first phytochemical and biological study to be carried on G. decandrus roots.

Keywords

Gymnocarpos decandrus; daphnoretin; wikstrol A; wikstol B; biflavonoids

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1. Introduction

Gymnocarpos decandrus Forssk. 1775 (f. caryophyllaceae) is a perennial shrublet widely distributed all over Egypt, specially in Sinai peninsula and the Mediterranean coastal strip’s slope. A limited number of phytochemical studies have been carried out only on the aerial parts and revealed the presence of sterols, triterpenes, flavonoids, catechin derivatives, triterpenoidal saponin glycosides, flavonol glycosides, Ferulic acid, rosmarinic acids and oleuropin (Bechlem et al. 2017; Sallam and Galala 2017; and Zitouni 2017). Essential oil of G. decandrus were found to be composed of hexadecanoic acid, dodecanoic acid, vinyl guaiacol, and tetradecanoic acid (Sallam and Galala 2017). The aerial parts alcoholic extract of G. decandrus have been reported to exhibit analgesic, antipyretic, α-amylase inhibitory, anti-inflammatory, diuretic, antioxidant, antimicrobial and local anaesthetic activities (Sallam and Galala 2017; Zitouni 2017). Moreover, the aqueous extract of the aerial part showed activity against melanoma cell lines (Sathiyamoorthy et al. 1999).

On the other hand, no information is available on the constituents of the plant roots except for the qualitative phytochemical analysis carried by Zitouni (2017), who reported the presence of coumarin, tannins, flavonoids, saponins and anthocyanin in roots extract.

The diversity of the chemical constituents present in G. decandrus aerial parts extract has encouraged us in this present study, to carry out phytochemical separation and bioassay for testing antimicrobial and anticoagulant activity of the plant roots extract.

2. Results and discussion

2.1. Structure elucidation of components isolated from G. decandrus

2.1.1. Compound 1

EIMS of 1 showed a peak at m/z 352 indicating a molecular weight of C_{19}H_{12}O_{7}, melting point (242°C) 13C-NMR and APT spectra resolved 18 carbon signals, which were classified by chemical shifts and HSQC spectrum as two carbonyl groups [δ_C 157.6 (C-2), δ_C 160.2 (C-2)], eight sp^2 quaternary carbons, eight sp^2 methines and one sp^3 methoxyl. 1HNMR showed a set of ortho-coupled aromatic signals at δ_H 6.39 (1H, d, J = 9.2 Hz) and δ_H 8.02 (1H, d, J = 9.2 Hz) characteristic of the H-3 and H-4 coumarin ring, a singlet and an ABX-type coupling system δ_H 7.71 (1H, d, J = 8.4 Hz), δ_H 7.16 (1H, d, J = 2.3) and δ_H 7.71 (1H, d, J = 8.4 Hz) assigned to H-5’, H-6’ and H-8’ suggesting a C-7’ link biscoumarin ether. Thorough study of the 2D spectral data (HMBC, COSY) suggested a C-3, C-7 ether linkage biscoumarin with a methoxyl substitution at C-6. Compound 1 was thus identified as daphnoretin (Venditti et al. 2019).

2.1.2. Compound 3

The 13C NMR spectrum of 3 (Table S1) showed signals for 30 carbons of which, only one carbonyl group was evident at δ_C 182. The 1H-NMR showed a pair of para-disubstituted aromatic rings with diastereotopic benzylic protons at δ_H 7.23 (2H, d, J = 8.4 Hz), δ_H 6.68 (2H, d, J = 8.4 Hz), δ_H 7.08 (2H, d, J = 9.1 Hz) and δ_H 6.64 (2H, d, J = 9.
Two attached protons with a meta relationship ($J_{6,8} = 2\text{ Hz}$) appeared at $\delta_H 6.1$ and $\delta_H 6.3$. Further, one proton signal appeared at $\delta_H 5.95$ (1H, s, H-6'). (Cândido et al. 2016) Resonances for two methine groups (doublet at $\delta_H 3.88$ and multiplet at $\delta_H 4.05$) revealing a skeletal type of a flavone “apigenin” attached to a flavanol nucleus ‘8-hydroxy afzelechin’. The suggested structure was further confirmed by thorough study of the 2D (COSY and HMBC) along with UV spectra. The point of attachment of both flavonoidal skeleton were found to be C-C connected biflavonoids with $3 \rightarrow 8$ linkage and its identity confirmed to be wikstrol B (Baba et al. 1994).

### 2.1.3. Compound 4

The $^{13}$C NMR spectrum of 4 (Table S2) showed signals for 30 carbons, of which only one carbonyl carbon at $\delta_C 182$. The $^1$H NMR showed signals assignable to two pairs of 4-oxyphenyl groups The first moiety’s hydrogens resonate at $[\delta_H 6.78$ and $\delta_H 7.36$ (each 2H, d, $J = 8.9\text{Hz}$), their carbons resonate at $\delta_C 114.7$ and $\delta_C 130.2$ respectively, in addition to 2 quaternary carbons $\delta_C 124$, and $\delta_C 159$. Similarly, a second moiety could be derived. NMR also revealed the presence of a $2,8$ (or $2,6$)-disubstituted $3,5,7$-trioxy-3,4-dihydrobenzopyran ring ($\delta_H 6.01$ (1H, s), $\delta_H 4.64$ (1H, d, $J = 10.2$, $2.2\text{ Hz}$), $\delta_H 3.7$ (1H, m) and $\delta_H 2.5$ (2H, m). The biflavonoid 4 was identified as wikstrol A, an isomer of Wikstrol B with axial chirality of C-8/C-3–previously isolated from Wikstroemia sikokiana (Baba et al. 1994).

Several bioactivities have been reported for naturally-occurring biflavonoids, including anticancer, anti-inflammatory, antibacterial and anticlotting. However, the plant species that produce biflavonoids as abundant secondary metabolites are not widely distributed (Gontijo et al. 2017).

Compound 2 was identified via NMR studies to be $\beta$-sitosterol glucoside (Khan and Hossain 2015).

### 2.2. Antimicrobial activity of the root extract and daphnoretin

Alcoholic extract of G. decandrus roots was tested against two Gram-positive strains, *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative strains, *Escherichia coli*, *Pseudomonas aeruginosa* in addition to *Candida albicans*, the extract showed inhibitory zone against *B. subtilis* but the activity was not due to daphnoretin as the later had no activity against any of the tested organisms.
2.3. Anticoagulant activity of the extract and daphnoretin

The search for novel anticoagulant derived from natural substances is in high demand nowadays. Currently, most clinical anticoagulant agents are coumarins, such as warfarin and acenocoumarol. The APTT assay was used to determine the effects of extract on intrinsic factors such as II, V and XII and/or common pathways. PT evaluates the extrinsic and/or common pathway of the coagulation cascade. In this study we show that the extract prolonged clotting time via both intrinsic and extrinsic pathways (Table 1). Daphnoretin had moderate effect on PT and slight effect on APTT which agrees well with the previous observation that coumarins usually interfere with the intrinsic coagulation process but usually not with the extrinsic process (Lei et al. 2015).

It is worth mentioning that this is the first report on anticoagulant activity of daphnoretin.

3. Conclusion

Our investigation on the chemical constituents of the extract from G. decandrus roots led to the obtainment of four compounds daphnoretin, wikstrol A, wikstrol B and β-sitosterol glucoside. The crude root extract showed a significant antimicrobial activity against Bacillus subtilis. Besides, the extract and daphnoretin was found to possess anticoagulant activity via measuring its effect on the PT and aPTT assays.

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Disclosure statement

No potential conflict of interest was reported by the author.

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