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SHORT COMMUNICATION

Validation of Antidiabetic Potential of Gymnocarpos decandrus Forssk

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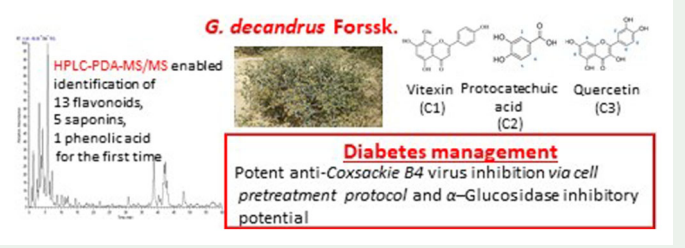
ABSTRACT

Gymnocarpos decandrus Forssk. is a well-known grazing wild plant. This study targets scientific validation of its claimed antidiabetic activity and exploring its bioactive metabolites. Chromatographic purification of *G. decandrus* ethanol extract (GDEE) allowed isolation of vitexin (**C1**), protocatechuic acid (**C2**) and quercetin (**C3**). HPLC-PDA-MS/MS enabled identification of nineteen metabolites; 13 flavonoids, 5 saponins, and 1 phenolic acid in *G. decandrus* and four in the genus *Gymnocarpos* for the first time. The antidiabetic potential was evaluated *via* testing the *Coxsackie B4* virus and α-glucosidase inhibitory potentials. C3 exhibited its potent antiviral activity through blocking of the virus attachment (96.28%, SI 4.41) and virus inactivation before adsorption (91.47%, SI 4.78). GDEE and C1–C3 showed dose dependent α-glucosidase inhibitory activity with IC_{50} of 733.9, 293.3, 118.1 and 69.1 μg/mL, respectively. Our study represents the sole complete map for *G. decandrus* secondary metabolites and presents it as promising drug for diabetes management.



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1. Introduction

One of the most common chronic diseases in nearly all countries is diabetes mellitus (DM). It continues to increase in significance and number. The prevalence of diabetes

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in Egypt is increasing in the last decades (Meo et al. 2019). Egypt will have at least 8.6 million adults with DM by the year 2030 (Shaw et al. 2010). DM is the 11th most important cause of premature mortality in Egypt. It is responsible for 2.5% of all years of life lost. Similarly, it is the sixth most important cause of disability burden in Egypt (National Information Center for Health and Population 2004). *Coxsackie B4* virus is a serotype of *Enterovirus B*, which can trigger an autoimmune reaction resulting in destruction of the insulin-producing beta cells, which is one of several different etiologies of DM (Ylipaasto et al. 2004). Unfortunately, there are no any specific medications or vaccines until now for prevention or treatment of such infections (De Beeck and Eizirik 2016). Phenolics from natural origin have shown promising *Coxsackie B4* inhibitory potentials (Özçelik et al. 2011; Okba et al. 2017).

Gymnocarpos decandrus Forssk. (Caryophyllaceae) is widely distributed in the middle east area and north Africa. (Petrusson and Thulin 1996). Traditionally, it is used as a food for grazing animals (Bhatt and Santo 2017). Recently, it was reported that it exhibited potent analgesic, diuretic, antitumor, antimicrobial, anticoagulant and α-amylase inhibitory activities (Sathiyamoorthy et al. 1999; Bouaziz et al. 2009; Sallam and Galala 2017; Fathy 2019). Although several studies have reported its pharmacological activities yet only few studies were traced on the phytoconstituents of its aerial parts (Sallam and Galala 2017) and roots (Fathy 2019).

The current study targets evaluation of *G. decandrus* Forssk. potential in the treatment of diabetes *via* testing the antiviral (*Coxsackie B4*) and α-glucosidase inhibitory potentials of its crude extract and isolated phytochemicals. Furthermore, HPLC-PDA-MS/MS profiling for the crude extract secondary metabolites was also achieved.

2. Results and discussion

2.1. Chromatographic purification of GDEE

The chromatographic fractionation of GDEE afforded the isolation of vitexin **C1** (Kim et al. 2005), protocatechuic acid **C2** (Nguyen et al. 2015) and quercetin **C3** (Huang et al. 2013). They were identified by comparing their physicochemical characters and spectroscopic data with those reported in literature. Co-TLC with authentic reference compounds has confirmed their identification. Their structures are shown in supporting information Figure S1 and their ^{1}H, ^{13}C-NMR data are presented in supporting information Table S1. Vitexin (**C1**) and protocatechuic acid (**C2**) were reported for the first time in *G. decandrus* aerial parts.

2.2. Secondary metabolites profiling

HPLC-PDA-MS/MS method was adopted to provide a complete map for profiling of *G. decandrus* Forssk secondary metabolites so as to explore more phytoconstituents with the proposed antidiabetic activity. HPLC leads tentative identification of 19 compounds including flavonoids, phenolic acid and saponins (supporting information Table S2). Identification was based on the MS spectra, molecular and fragment ions of each compound. The base peak chromatogram is shown in supporting information

Figure S2. MS/MS spectra of the identified compounds were represented in supporting information Figures S3–S21.

2.2.1. Flavonoids

Thirteen flavonoids; 9 flavonols, 1 flavone, 2 isoflavones and 1 flavan-3-ol were detected. The fragmentation pattern of the observed flavonoids was matched with that reported for *O*-flavonoid glycosides (Cuyckens and Claeys 2004; Hossain et al. 2010), where the loss of the sugar moieties (162 Da for hexoses, 132 Da for pentoses and 146 Da for monodeoxyhexoses) was the most common fragments observed. Nine flavonol glycosides were identified. Six pks. (2,4,7–9 and 15) of them were glycosides of isorhamnetin aglycone (*m/z* 315), while the other three pks. (3,13 and 17) were of quercetin aglycone (*m/z* 301). ***Saponins:*** Five triterpenoidal saponins of oleanane type (pks. 5,6,10,12 and 14) were tentatively identified. Tetrahydroxy oleanane (pks. 5, 6 and 10) aglycones at *m/z* 503 in addition to trihydroxy oleanane (pks. 12 and 14) aglycones were observed at *m/z* 487 (Martha Pérez Gutiérrez 2016). ***Phenolic acid:*** fragmentation pattern was in agreement with that of hydroxygallic acid (Fathoni et al. 2017).

To the best of our knowledge, this study provides the first secondary metabolite profile of *G. decandrus* Forssk. Further isolation and full characterisation of these metabolites is highly recommended.

2.3. Cytotoxicity

The effect of the GDEE along with C1–C3 on the proliferation and viability of the mammalian cells were tested prior to the determination of their antiviral activity (supporting information Table S3). GDEE and C1–C3 were found to be non-cytotoxic according to the reported limits of cytotoxicity (Magadula and Suleimani 2010). The CC_{50} for GDEE was 441.38 μg/mL and for the isolated compounds ranged from 22.04 to 23.86 μg/mL. The maximum nontoxic concentrations (MNTC) on Vero cells were estimated as 78.12 and 7.812 μg/mL for GDEE and the isolated compounds, respectively. These concentrations were used while testing the antiviral potency.

2.4. Antiviral activity

Epidemiological data showed an increased DM (type 1) incidence after epidemics due to enteroviruses. The enteroviral RNA has been detected in 50% of DM (type 1) patients' blood at the time of disease onset (Yin et al. 2002). Enteroviral infection is one of the environmental risk factors causing DM (type 1) (Hyöty et al. 1998; Dotta et al. 2007). *Coxsackies B4* has been isolated from DM (type 1) patients (Yoon et al. 1979), and some of these isolates have been reported to cause DM in mice (See and Tilles 1995).

The antiviral activity was investigated using three different protocols; virus pretreatment, cell pretreatment and post infection treatment protocols, and were represented by *CoxB4* inhibition percentage, IC_{50} and SI. The results varied significantly according to the used protocol and the tested samples (Table 1). The observed antiviral activity was found to be dose dependent at concentration range 19–78 μg/mL for GDEE and

Table 1. Antiviral activity of GDEE and the isolated compounds **C1–C3**.

Tested items	Measured parameters	Protocol A	Protocol B	Protocol C
GDEE	O.D[a] ± S.E.	0.093 ± 0.0037	0.213 ± 0.0073	0.109 ± 0.0035
	Antiviral %	1.55	77.95	1.50
	IC_{50} µg/mL	–	60.73	–
	SI	–	7.27	–
C1	O.D[a] ± S.E.	0.196 ± 0.0037	0.191 ± 0.0052	0.139 ± 0.0015
(vitexin)	Antiviral %	81.65	62.17	19.78
	IC_{50} µg/mL	5.7	6.31	–
	SI	3.86	3.49	–
C2 (protocatechuic acid)	O.D[a] ± S.E.	0.133 ± 0.0055	0.232 ± 0.0023	0.159 ± 0.0041
	Antiviral %	32.55	91.18	2.26
	IC_{50} µg/mL	10.73	5.25	–
	SI	–	4.84	–
C3	O.D[a] ± S.E.	0.209 ± 0.0050	0.239 ± 0.0024	0.119 ± 0.0009
(quercetin)	Antiviral %	91.47	96.28	36.16
	IC_{50} µg/mL	4.91	5.41	–
	SI	4.78	4.41	–

[a]Mean of six determinations; S.E.: standard error; O.D.: optical density at MNTC; GDEE: *G. decandrus* ethanol extract; IC_{50}: 50% inhibition concentration; SI: selectivity index; protocol A: virus pretreatment; protocol B: cell pretreatment; protocol C: post infection treatment.

1.9–7.8 µg/mL for the isolated compounds. GDEE caused significant inhibition using antiviral protocol B (77.95%), unlike protocol A (1.55%) and protocol C (1.50%) (Table 1). C3 was the most active one where it exhibited potent inhibitory potential against *CoxB4* by 96.28% (protocol B) followed by 91.47% (protocol A). Thus, quercetin exhibited its potent antiviral activity through blocking the attachment of the virus to the cell surface and inactivation of the virus before adsorption on the host cells. C1 and C2 exhibited high potency through cell pretreatment protocol B. protocatechuic acid blocks the viral attachment by 91.18% followed by vitexin 62.17%. On the other hand, all tested samples have negligible activity on *CoxB4* replication after Vero cell infection (protocol C) except for quercetin which exerted mild activity (36.16%). As SI values of more than 3 indicate potentially safe antiviral activity (Chattopadhyay et al., 2009). Subsequently, GDEE and the isolated compounds are considered as potentially safe antiviral drugs.

2.5. α-Glucosidase inhibitory activity

The inhibitory activity was represented by percent inhibition (supporting information Figure S22) and IC_{50} (supporting information Table S4). The observed activity was found to be dose dependent. GDEE and C1–C3 exhibited significant α-glucosidase inhibitory activity of IC_{50} 733.90, 293.30, 118.17 and 69.18 µg/mL, respectively. C3 showed the highest activity followed by C1, and C2 with IC_{50} of 0.23, 0.68, and 0.77 mM, respectively. Our findings are in agreement with the reported α-glucosidase inhibitory potency of these phenolics (Kawabata et al., 2003; Adefegha et al. 2015; Jhong et al. 2015).

This observed potent antidiabetic activity was attributed to the detected flavonoids and saponins since their antidiabetic potency is well documented (Shimizu et al. 2000; Babu et al. 2013; Elekofehinti 2015; Vinayagam and Xu 2015). The herein newly explored *G. decandrus* Forssk *CoxB4* virus and α-glucosidase inhibitory potentials, in addition to its previously reported α-amylase inhibitory activity (Sallam and Galala 2017) suggested further detailed *in vivo* and preclinical studies to be performed on such valuable plant as a new candidate for DM management.

To the best of our knowledge, this is the first study on *CoxB4* virus and α-glucosidase inhibitory effects on *G. decandrus* Forssk crude extract and isolated bio-phytochemicals.

3. Conclusion

G. decandrus Forssk. aerial parts ethanol extract as well as its isolated bio-phytochemicals were able to inhibit *CoxB4* virus and α-glucosidase enzyme significantly. Thus, it could be used as a natural drug for controlling diabetes mellitus *via* different mechanisms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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