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SHORT COMMUNICATION

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Bioassay-guided fractionation and identification of antidiabetic compounds from the rind of *Punica Granatum* Var. nana

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ABSTRACT

This study aimed to evaluate the antidiabetic potential of the rind of *Punica granatum* var. nana. Acute oral toxicity test revealed the safety profile of its ethanolic extract. The extract was administered at 200 mg/kg b.wt to streptozotocin-induced diabetic rats. Serum diagnostic markers of diabetes (insulin, glucose and glycated hemoglobin), inflammatory mediators (tumor necrosis factor-α, interleukin-6, and nitric oxide), and oxidative stress (total antioxidant capacity and reduced glutathione and malondialdehyde) were assayed. The ethanolic extract was further fractionated and assessed for the aforementioned bioactivities at two different doses (100 and 200 mg/kg b.wt). The results revealed that the ethyl acetate fraction of rind exhibited the highest activities. Using different chromatographic techniques, four compounds were isolated and identified as rutin, gallic acid, nictoflorin, and tulipanin. In conclusion: The ethyl acetate fraction of the rind of *Punica granatum* var. nana may provide a potential therapeutic approach for hyperglycemia.

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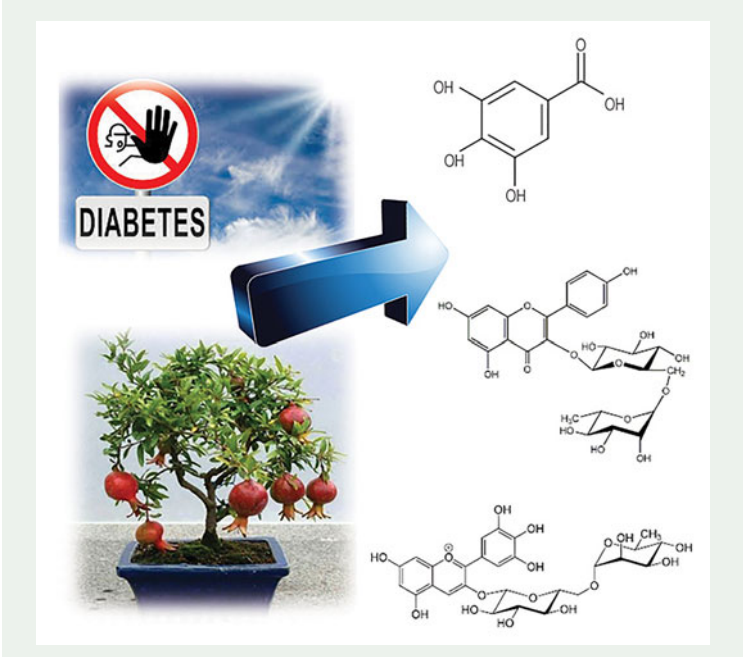

1. Introduction

Antidiabetic medications are often associated with undesirable side effects or diminution in response after prolonged use (Alhadramy 2016). As a consequence, there is an increase of interest towards natural medicine as a way to treat various types of diseases with fewer side effects versus synthetic pharmaceutical drugs. Pomegranate (family *Punicaceae*), includes only one genus *Punica* and two species (*Punica protopunica* Balf. and *Punica granatum* L.). *Punica granatum* L. variety nana (dwarf pomegranate) is cultivated in Egypt as an ornamental tree (Khalifa and Loutfy 2006). There is evidence that the rind and the fruit of *Punica granatum* L. have anti-diabetic activity (Khalil 2004; Wu and Tian 2019) and several phytoconstituents had been isolated (Al-Mosawe and Al-Saadi 2012; Middha et al. 2013; Al-Rawahi 2014; Rafiq et al. 2016). However, nothing was traced concerning the phytochemical composition or biological activity of the rind of *Punica granatum* var. nana. Hence, the present study was designed to evaluate the antidiabetic, antioxidant and anti-inflammatory activities of the rind of *Punica granatum* var. nana and isolate the active constituents responsible for these activities.

2. Results and discussion

The result of the oral acute toxicity (OECD 2001) revealed no signs of toxicity at the fixed sole dose of 2000 mg/kg for 14 days. The STZ- induced diabetic animals received 200 mg/kg of ethanolic extract of *P. granatum* var. nana showed significant increase serum level of insulin and reduce the blood levels of glucose and glycated Hb, indicating the pronounced antidiabetic potential of the phyto-constituents present in this extract.

In addition, this ethanolic extract ameliorates the STZ- induced oxidative stress and inflammation as evidence from the significant increase in the TAC and GSH, and decrease MDA, TNF-α and IL-6 levels (Supplementary material, Table S1).

Therefore, the ethanolic extract of *P. granatum* var. nana was then subjected to successive fractionation using petroleum ether, methylene chloride and ethyl acetate. The ethyl acetate fraction at a doses of 100 and 200 mg/kg b.wt showed higher potent dose- dependent ameliorative **antidiabetic** (Supplementary material, Figure S1 A–C), **anti-inflammatory** (Supplementary material, Figure S1 D–F) as well as, **antioxidant** (Supplementary material, Figure S1 G–I) activities than other fractions (Supplementary material, Figure S1 A–I).

In type 2 diabetes (T2D) model induced by Streptozotocin-nicotinamide, the pancreatic β-cells is selectively destructed by STZ, which is partially protected by NA to attain the development of moderate hyperglycaemia (Masiello 2006) which led to the oxidative insult of β-cells (Broniowska et al. 2014). The significant increase in pro-inflammatory cytokines represented by IL-6 and TNF-α refracts the enhancement of inflammation, which elicits the increase in production of free radicals resulted from increased lipid peroxidation represented by MDA (El Hawary et al. 2016). In addition, the increase in the inducible nitric oxide synthase in β-cells, which leads to increase in NO production. Moreover, significant depletion in serum antioxidant capacity represented by decrease in TAC and GSH activities.

Four compounds (R1–R4) (Supplementary material, Figure S2) have been isolated from the ethyl acetate fraction of the rind of *Punica granatum* var. nana, and identified as quercetin 3-*O*-rutinoside (rutin) (R1), gallic acid (R2), kaempferol 3-*O*-rutinoside (nictoflorin) (R3) and delphinidin 3-*O*-rutinoside (tulipanin) (R4). These compounds were identified by UV, EI/MS and NMR (^{1}H and ^{13}C) spectra and confirmed by comparing with authentic standards and published data (Mabry et al. 1996; Al-Mosawe and Al-saadi 2012; Li-Li et al. 2014). Rutin, gallic acid and tulipanin could contribute in the anti-diabetic activity (Niture et al. 2014; Kato et al. 2015; Stephen et al. 2015). Meanwhile, the anti-oxidant and anti-inflammatory activity of the rind could probably due to kaempferol 3-*O*-rutinoside (Wang et al. 2015), delphinidin 3-*O*-rutinoside (Lazzé et al. 2003) and gallic acid (BenSaad et al. 2017) existence. To the best of our knowledge, assessment of the antidiabetic, anti-inflammatory and antioxidant activities, as well as, phytochemical study of *Punica granatum* var. nana rind was considered the first. Gallic acid was firstly isolated from *Punica granatum* rind and was isolated for the first time from the species.

3. Conclusion

From the current study, we could conclude that the ethyl acetate fraction of the ethanolic extract of rind of *P. granatum* L. var. nana, exhibited a profound biological effect, not limited to anti-diabetic effect but also extended to anti-inflammatory and anti-oxidant activities. Therefore, this extract could be used in pharmaceutical preparations after clinical trials to guarantee the safety of the product.

Disclosure statement

The authors declare no conflict of interest.

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