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α -Glucosidase and glycation inhibitory activities of Rumex lunaria leaf extract: a promising plant against hyperglycaemia-related damage

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SHORT COMMUNICATION

α -Glucosidase and glycation inhibitory activities of *Rumex lunaria* leaf extract: a promising plant against hyperglycaemia-related damage

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ABSTRACT

Rumex lunaria L. is a Canarian medicinal plant, belonging to Polygonaceae. The potential antidiabetic activity of the methanolic extract of the leaves was investigated. For this purpose, the inhibition of α -glucosidase and albumin glycation by the extract was studied. Further, the anti-radical activity and the phytochemical composition were detected. The reduction of α -glucosidase activity was significant from 3 μ g/mL, while the BSA glycation inhibition started from 100 μ g/mL. Moreover, the extract exhibited a significant free-radical scavenger activity. Its phytochemical characterization showed the presence of carotenoids, phenolic and flavonoid compounds, whereas anthraquinones were not detected. C-flavonoid glycosides were identified and quercetin-O-hexoside-O-deoxyhexoside was the most detected (22.67 ± 0.02 mg/g). The findings indicate that the methanolic *R. lunaria* leaf extract has significant anti- α -glucosidase, anti-radical and anti-glycation activities. This research is the first showing the potential antidiabetic activity of *R. lunaria*.

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AGEs; α -glucosidase; BSA assay; ORAC assay; ROS; glycosides

1. Introduction

Rumex lunaria L. (Polygonaceae), popularly known as 'vinagrera' or 'calcosa', is a native plant of Canary Islands, also naturalized in Italy. *Rumex* species are known to produce several types of secondary metabolites, such as flavonoid glycosides, anthraquinones, naphthalenes, and phenolic acids (Hasan et al. 1995; Desta et al. 2016). In the traditional medicine, leaves and roots of *R. lunaria* have been used to prepare infusions or cataplasms useful as vulnerary, emollient, astringent and anti-inflammatory agents (Vasas et al. 2015). Various *R.* species such as *nervosus*, *abyssinicus* and *patientia* are also traditionally used in the treatment of diabetes (Gunes et al. 1999; Vasas et al. 2015). Further, the capacity of *R. patientia* seed extracts to decrease blood glucose level of STZ-induced diabetic rats has been reported (Degirmenci et al. 2005; Sedaghat et al. 2011).

Diabetes is a metabolic disorder mainly characterized by chronic increase of blood glucose levels. To explain the association between hyperglycaemia and vascular complications, one of the most important mechanisms proposed is the formation of advanced glycation end products (AGEs), which are triggered by endogenous processes related to hyperglycaemia and oxidative stress (Madonna et al. 2018). On this basis, the recognition of plant-derived products capable of reducing glycaemia and AGEs formation represents a great challenge in medicine. Thus, the main aim of this research was the evaluation of anti-radical, anti- α -glucosidase and anti-glycation activities of the methanolic extract of *R. lunaria* leaves.

2. Results and discussion

The extract of *R. lunaria* leaves was evaluated with BSA assay to determine its ability to reduce AGEs formation. The extract (0.1–1.0 mg/mL) showed a concentration-dependent anti-glycation effect (Figure S1). The highest concentration reduced BSA glycation by $61.2 \pm 0.6\%$ after 5 days of incubation, while 50 mM aminoguanidine, the positive control, inhibited glycation only by $51.2 \pm 3.7\%$. Furthermore, the inhibition was maintained after 7 and 11 days of incubation, albeit the effect progressively decreased (Figure S1). These results are in agreement with previous data on the anti-glycation activity of other *R.* species; in detail, *R. vesicarius* leaves juice prevented in vitro glucose induced haemoglobin glycation (Tiwari et al. 2013). It has been suggested that the anti-glycation activity of extracts depends on the phenolic content; more precisely, authors have found a positive correlation between in vitro inhibition of AGEs formation and phenolic content of various tropical plant extracts (Ramkissonoon et al. 2013). In agreement with this observation, the total phenolic content (TPC) of *R. lunaria* extract was high (244.7 ± 6.2 mg GAE/g), while the flavonoid content was relatively low (25.9 ± 0.7 mg QE/g, Table S1). Thus, it is likely that phenols exert an inhibitory effect on glycation by delaying the oxidation of glycated proteins (Yeh et al. 2017). Since it is known that AGEs formation is accelerated by oxidative stress, the antioxidant activity of *R. lunaria* extract was investigated by means of ORAC assay. The extract exhibited a trolox equivalent antioxidant capacity (TEAC) of 1524.7 ± 124.7 μ mol/g (Table S1), which is a high value also in relation to other *R.* species (Vasas et al. 2015). Further, the high level of phenols detected in the extract also

Table 1. Retention time (RT), significant ions of the mass spectrum (*m/z*) and amount of each compound detected in the methanolic extract of leaves from *R. lunaria*.

RTmin	Compound	[M-H]	Fragments	Quantity, mg/g
7.1	Galloyl catechin	441	289 245 203	6.02 ± 0.02
7.6	Quercetin-6-C-hexoside	447	357 327 299 255 175	14.9 ± 0.07
8.0	Kaempferol-6-C-hexoside	431	341 311 283 239 163	8.11 ± 0.03
8.3	Quercetin-8-C-hexoside	447	357 327 299 255 175	14.26 ± 0.07
9.2	Quercetin-O-hexoside-O-deoxyhexoside	609	463 301 271 243	22.67 ± 0.02
9.4	Quercetin-hexoside-deoxyhexoside	609	301 271 243	4.16 ± 0.02
9.7	Quercetin-O-deoxyhexoside	447	301	1.76 ± 0.01
32.8	β-carotene*	535	—	0.79 ± 0.02

*Compared with reference standard compound.

supports its radical scavenger activity since several authors demonstrated a positive correlation between TPC and antioxidant activity (Giusti et al. 2017; Simo et al. 2018).

The ability of *R. lunaria* extract to inhibit the activity of α-glucosidase, an intestinal enzyme which allows the absorption of glucose, was also evaluated. The extract, in the range of 1 µg/mL - 10 µg/mL, induced a concentration-dependent inhibition of α-glucosidase; furthermore, with the highest concentration the enzymatic activity was completely abolished (Figure S2). Further, the inhibition of α-glucosidase activity was significant from 3 µg/mL, which is a very low concentration using plant extracts. A previous research indicated that also *R. vesicarius* leaves, used as vegetables, inhibited the rat intestinal α-glucosidase activity with an IC₅₀ of 1.1 ± 0.4 mg/mL expressed as acarbose equivalents (Tiwari et al. 2013).

The ¹H-NMR spectrum of *R. lunaria* extract supports the presence of sugar residues, and two anomeric proton signals suggest the presence of O-glycosides (Figure S3). Further, signals support the presence of fatty acids as well as aromatic protons ascribable to flavones. The LC-DAD-MS analysis allowed the detection of flavonoid and carotenoid derivatives in the extract (Table 1). Galloyl catechin, two pairs of isomeric flavonoid C-glycosides, two pairs of kaempferol C-hexosides and two pairs of quercetin glycosylated with a hexoside and deoxyhexoside were detected (Figure S4), with also six peaks presenting UV spectrum ascribable to carotenoids. Previously, some authors reported the presence of C-glycosyl flavonoids in other species of *R.* (Rao et al. 2011). On the other hand, *R.* species are generally described having anthraquinones which were detected also in *R. lunaria* leaf extracts (Navarro et al. 2012). Nevertheless, in the present research these compounds were not identified. In agreement with this observation, other authors showed no obvious association between anthraquinones detection and *R.* species (Getie et al. 2003; Desta et al. 2016).

3. Conclusion

This research for the first time shows the in vitro anti-glycation, anti-oxidant and anti-α-glucosidase properties of the methanolic extract of leaves from *R. lunaria*. Overall, the present data suggest that *R. lunaria* extract could be developed as an effective and economical support to treat hyperglycaemia-correlated pathologies. However, in vivo studies are necessary to define its pharmacological use.

Disclosure statement

No conflict of interest was reported by the authors.

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