PROOF COVER SHEET

Author(s):	Guglielmina Froldi, Diana Luján González, Francesca Rosteghin, Margherita Grison, Stefania Sut, Stefano Dall'Acqua, and Candelaria del Carmen Sánchez-Mateo
Article title:	α-Glucosidase and glycation inhibitory activities of *Rumex lunaria* leaf extract: a promising plant against hyperglycaemia-related damage
Article no:	GNPL_A_1569655
Enclosures:	1) Query sheet 2) Article proofs

Dear Author,

1. Please check these proofs carefully. It is the responsibility of the corresponding author to check these and approve or amend them. A second proof is not normally provided. Taylor & Francis cannot be held responsible for uncorrected errors, even if introduced during the production process. Once your corrections have been added to the article, it will be considered ready for publication.

Please limit changes at this stage to the correction of errors. You should not make trivial changes, improve prose style, add new material, or delete existing material at this stage. You may be charged if your corrections are excessive (we would not expect corrections to exceed 30 changes).

For detailed guidance on how to check your proofs, please paste this address into a new browser window:
http://journalauthors.tandf.co.uk/production/checkingproofs.asp

Your PDF proof file has been enabled so that you can comment on the proof directly using Adobe Acrobat. If you wish to do this, please save the file to your hard disk first. For further information on marking corrections using Acrobat, please paste this address into a new browser window: http://journalauthors.tandf.co.uk/production/acrobat.asp

2. Please review the table of contributors below and confirm that the first and last names are structured correctly and that the authors are listed in the correct order of contribution. This check is to ensure that your name will appear correctly online and when the article is indexed.

Sequence	Prefix	Given name(s)	Surname	Suffix
1		Guglielmina	Froldi	
2		Diana Luján	González	

3		Francesca	Rosteghin	
4		Margherita	Grison	
5		Stefania	Sut	
6		Stefano	Dall'Acqua	
7		Candelaria del Carmen	Sánchez-Mateo	

Queries are marked in the margins of the proofs, and you can also click the hyperlinks below.

General points:

1. **Permissions:** You have warranted that you have secured the necessary written permission from the appropriate copyright owner for the reproduction of any text, illustration, or other material in your article. Please see http://journalauthors.tandf.co.uk/permissions/usingThirdPartyMaterial.asp.

2. **Third-party content:** If there is third-party content in your article, please check that the rightsholder details for re-use are shown correctly.

3. **Affiliation:** The corresponding author is responsible for ensuring that address and email details are correct for all the co-authors. Affiliations given in the article should be the affiliation at the time the research was conducted. Please see http://journalauthors.tandf.co.uk/preparation/writing.asp.

4. **Funding:** Was your research for this article funded by a funding agency? If so, please insert `This work was supported by <insert the name of the funding agency in full>', followed by the grant number in square brackets `[grant number xxxx]'.
5. **Supplemental data and underlying research materials:** Do you wish to include the location of the underlying research materials (e.g. data, samples or models) for your article? If so, please insert this sentence before the reference section: `The underlying research materials for this article can be accessed at <full link>/ description of location [author to complete]'. If your article includes supplemental data, the link will also be provided in this paragraph. See <http://journalauthors.tandf.co.uk/preparation/multimedia.asp> for further explanation of supplemental data and underlying research materials.

6. The **PubMed** (http://www.ncbi.nlm.nih.gov/pubmed) and **CrossRef databases** (www.crossref.org/) have been used to validate the references. Changes resulting from mismatches are tracked in red font.

AUTHOR QUERIES

Q1: The ORCID details of the authors have been validated against ORCID registry. please check the ORCID ID details of the authors.

How to make corrections to your proofs using Adobe Acrobat/Reader

Taylor & Francis offers you a choice of options to help you make corrections to your proofs. Your PDF proof file has been enabled so that you can mark up the proof directly using Adobe Acrobat/Reader. This is the simplest and best way for you to ensure that your corrections will be incorporated. If you wish to do this, please follow these instructions:

1. Save the file to your hard disk.

2. Check which version of Adobe Acrobat/Reader you have on your computer. You can do this by clicking on the "Help" tab, and then "About".

If Adobe Reader is not installed, you can get the latest version free from http://get.adobe.com/reader/.

3. If you have Adobe Acrobat/Reader 10 or a later version, click on the "Comment" link at the right-hand side to view the Comments pane.

4. You can then select any text and mark it up for deletion or replacement, or insert new text as needed. Please note that these will clearly be displayed in the Comments pane and secondary annotation is not needed to draw attention to your corrections. If you need to include new sections of text, it is also possible to add a comment to the proofs. To do this, use the Sticky Note tool in the task bar. Please also see our FAQs here: http://journalauthors.tandf.co.uk/production/index.asp.

5. Make sure that you save the file when you close the document before uploading it to CATS using the "Upload File" button on the online correction form. If you have more than one file, please zip them together and then upload the zip file.
If you prefer, you can make your corrections using the CATS online correction form.

Troubleshooting

Acrobat help: http://helpx.adobe.com/acrobat.html
Reader help: http://helpx.adobe.com/reader.html

Please note that full user guides for earlier versions of these programs are available from the Adobe Help pages by clicking on the link "Previous versions" under the "Help and tutorials" heading from the relevant link above. Commenting functionality is available from Adobe Reader 8.0 onwards and from Adobe Acrobat 7.0 onwards.

Firefox users: Firefox's inbuilt PDF Viewer is set to the default; please see the following for instructions on how to use this and download the PDF to your hard drive: http://support.mozilla.org/en-US/kb/view-pdf-files-firefox-without-downloading-them#w_using-a-pdf-reader-plugin

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1569655

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

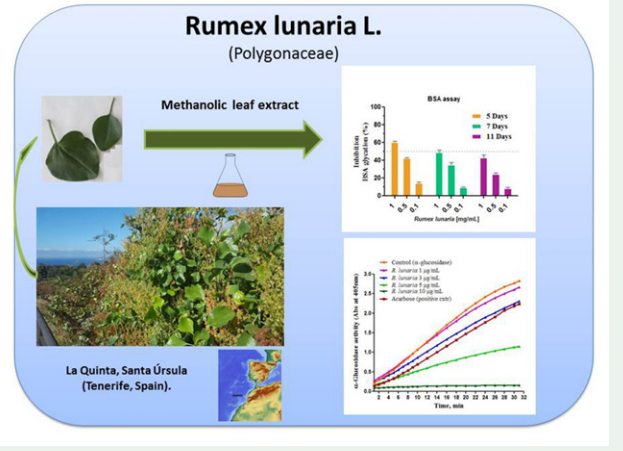
α-Glucosidase and glycation inhibitory activities of *Rumex lunaria* leaf extract: a promising plant against hyperglycaemia-related damage

Q1 Guglielmina Froldi[a], Diana Luján González[a], Francesca Rosteghin[a], Margherita Grison[a], Stefania Sut[b], Stefano Dall'Acqua[a] and Candelaria del Carmen Sánchez-Mateo[c]

[a]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy; [b]Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padova, Italy; [c]Departamento de Medicina Física y Farmacología, Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain

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 μg/mL, while the BSA glycation inhibition started from 100 μg/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*.



ARTICLE HISTORY
Received 26 September 2018
Accepted 7 January 2019

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

CONTACT Guglielmina Froldi g.froldi@unipd.it

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1569655

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 ± 0.6% after 5 days of incubation, while 50 mM aminoguanidine, the positive control, inhibited glycation only by 51.2 ± 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 (Ramkissoon 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 μmol/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 IC_{50} of 1.1 ± 0.4 mg/mL expressed as acarbose equivalents (Tiwari et al. 2013).

The ^{1}H-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.

Funding

This work was supported by the University of Padova (DOR 2017). DLG is grateful to the Traineeship Erasmus+.

ORCID

Guglielmina Froldi http://orcid.org/0000-0002-5936-1789

References

Degirmenci I, Ustuner MC, Kalender Y, Kalender S, Gunes HV. 2005. The effects of acarbose and *Rumex patientia* L. on ultrastructural and biochemical changes of pancreatic B cells in streptozotocin-induced diabetic rats. J Ethnopharmacol. 97(3):555–559.

Desta KT, Lee WS, Lee SJ, Kim Y-H, Kim G-S, Lee SJ, Kim ST, El-Aty AMA, Warda M, Shin H-C, et al. 2016. Antioxidant activities and liquid chromatography with electrospray ionization tandem mass spectrometry characterization and quantification of the polyphenolic contents of *Rumex nervosus* Vahl leaves and stems. J Sep Science. 39(8):1433–1441.

Getie M, Gebre-Mariam T, Rietz R, Höhne C, Huschka C, Schmidtke M, Abate A, Neubert RHH. 2003. Evaluation of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea viscosa*, *Rumex nervosus* and *Rumex abyssinicus*. Fitoterapia. 74(1–2):139–143.

Giusti F, Caprioli G, Ricciutelli M, Vittori S, Sagratini G. 2017. Determination of fourteen polyphenols in pulses by high performance liquid chromatography-diode array detection (HPLC-DAD) and correlation study with antioxidant activity and colour. Food Chem. 221:689–697.

Gunes HV, Degirmenci I, Aydin M, Bozan B, Aral E, Tunalier Z, Ustuner C, Ercakir M, Baser KHC, Basaran A. 1999. The effects of *Rumex patientia* L. and *Urtica dioica* L. on some blood and urine parameters, and liver and kidney histology in diabetic rats. Turkish J Med Sci. 29: 227–232.

Hasan A, Ahmed I, Jay M, Voirin B. 1995. Flavonoid glycosides and an anthraquinone from *Rumex chalepensis*. Phytochemistry. 39(5):1211–1213.

Madonna R, Pieragostino D, Balistreri CR, Rossi C, Geng Y-J, Del Boccio P, De Caterina R. 2018. Diabetic macroangiopathy: Pathogenetic insights and novel therapeutic approaches with focus on high glucose-mediated vascular damage. Vascul Pharmacol. 107:27–34.

Navarro E, Rodríguez B, Jiménez J, Navarro R, Alonso S. 2012. Identificación y aislamiento de compuestos de *Rumex lunaria* L. Endemismo canario con actividad farmacológica. Canar Médica y Quirúrgica. 27:53–59.

Ramkissoon JS, Mahomoodally MF, Ahmed N, Subratty AH. 2013. Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. Asian Pac J Trop Med. 6(7):561–569.

Rao KNV, Ch S, Banji D, Sandhya S, Mahesh V. 2011. A study on the nutraceuticals from the genus *Rumex*. Hygeia, J Drugs Med. 3:76–88.

Sedaghat R, Roghani M, Ahmadi M, Ahmadi F. 2011. Antihyperglycemic and antihyperlipidemic effect of *Rumex patientia* seed preparation in streptozotocin-diabetic rats. Pathophysiology. 18(2):111–115.

Simo MK, Nguepi MD, Sameza ML, Toghueo RK, Fekam FB, Froldi G. 2018. Cameroonian medicinal plants belonging to Annonaceae family: radical scavenging and antifungal activities. Nat Prod Res. 32(17):2092–2095.

Tiwari AK, Jyothi AL, Tejeswini VB, Madhusudana K, Kumar DA, Zehra A, Agawane SB. 2013. Mitigation of starch and glucose-induced postprandial glycemic excursion in rats by antioxidant-rich green-leafy vegetables' juice. Pharmacogn Mag. 9(Suppl 1):S66–S73.

Vasas A, Orbán-Gyapai O, Hohmann J. 2015. The genus *Rumex*: review of traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 175:198–228.

Yeh WJ, Hsia SM, Lee WH, Wu CH. 2017. Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings. J Food Drug Anal. 25(1):84–92.