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Inhibition of α-amylase, α-glucosidase and pancreatic lipase by phenolic compounds of *Rumex maderensis* (Madeira sorrel). Influence of simulated gastrointestinal digestion on hyperglycaemia-related damage linked with aldose reductase activity and protein glycation

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ABSTRACT

In this work, we report the *in vitro* inhibitory potential of *Rumex maderensis* methanolic extracts (leaves, flowers, and stems) towards key digestive enzymes linked to type-2 diabetes and obesity (α-amylase, α-glucosidase, pancreatic lipase). The inhibitory activity towards aldose reductase and glycation of bovine serum albumin (BSA) is also reported; in these latter assays, the effect of simulated digestion on the bioactivities was evaluated. The inhibitory activities of *R. maderensis* extracts were statistically compared with the inhibition produced by reference compounds for each assay. The analysed extracts exhibited significant inhibitory activities, which decreased after the gastrointestinal digestion, possibly due to some loss of phenolics that took place during the digestion process. The most important results were observed during the BSA-glycation assay, in which the analysed extracts presented higher potency than a reference compound: aminoguanidine (AMG). This research is the first showing the potential anti-diabetic activity of *R. maderensis*.

1. Introduction

Type-2 diabetes (T2DM) is a metabolic disorder characterized by chronic increased blood glucose levels (hyperglycaemia) (Aghajanyan, Nikoyan, & Trchounian, 2018; S.; Kumar, Narwal, Kumar, & Prakash, 2011). This condition affects more than 90% of diabetic cases and is expected to reach more than 360 million people in 2030 (Ahmed, Mughal, Younas, & Ikram, 2013; You, Chen, Wang, Luo, & Jiang, 2011). Persistent hyperglycaemia induces the development of diabetic complications (retinopathy, nephropathy, cardiovascular diseases, etc) through the formation of advanced glycation end-products (AGEs), accumulation of sorbitol through the overactivation of the polyol pathway, and increase the generation of free radicals beyond normal physiological control (Froldi et al., 2019; Jang et al., 2008; S.; Kumar et al., 2011).

Increased consumption of green-leafy vegetables is associated with a lower risk of T2DM development (D. A. Kumar et al., 2015; Tiwari et al., 2013). This therapeutic effect is due to the presence of phenolic compounds, capable of maintaining glucose homeostasis, with least side effects of the current therapy (Aghajanyan et al., 2018; Tiwari et al., 2013). Hence, a dietary strategy that mitigate post-prandial hyperglycaemia may help prevent or delay the progression of T2DM (Podsę;dek, Majewska, & Kucharska, 2017; Savran et al., 2016).

The genus *Rumex* contains more than 200 species that have been widely used in folk medicine for the treatment of various diseases and ailments, including T2DM (Aghajanyan et al., 2018; J. M.; Kim, Lee, Jang, & Kim, 2006; Savran et al., 2016). Further, the *in vivo* capacity of *Rumex* species to decrease blood glucose and lipids in animal models has been documented (Aghajanyan et al., 2018; Ahmed et al., 2013; Khatri, Bahadur, Chhetri, & Poudel, 2018; Kwon et al., 2018; Tiwari et al., 2013). *Rumex maderensis* Lowe (family Polygonaceae), locally known as "azedas" (Madeira sorrel), is a wild perennial herb/shrub that grows spontaneously on Madeira archipelago (Portugal) (Press & Short, 1994). Leaves are consumed as soups or salads, but also used in traditional medicine (diuretic, blood depurative, skin diseases) (Press & Short, 1994; Rivera & Obón, 1995). A previous study (Spínola, Llorent-Martínez, & Castilho, 2018) reported flavonoids and phenolic acids as the main components of *R. maderensis* and responsible for its relevant free radical scavenging properties.

Before exerting any physiological effect, phenolic compounds must

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first survive the passage though the gastrointestinal tract (Gunathilake, Ranaweera, & Rupasinghe, 2018). Hence, it is important to evaluate their metabolic fate and the effect of the digestion process on their potential bioactivities (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007).

To our best knowledge, no studies regarding the therapeutic value of *R. maderensis* on T2DM have been conducted so far. As a follow-up investigation, in this work the inhibitory activity of this species on digestive enzymes (α-amylase, α-glucosidase, lipase), aldose reductase and protein glycation was evaluated, using *in vitro* models. Second, the impact of simulated gastrointestinal digestion on the potential bioactivities of *R. maderensis* was determined.

2. Experimental

2.1. Chemicals and reagents

Soluble starch (p.a.), sodium azide (> 99%), potassium iodate (99.5%) were acquired from Merck (Darmstadt, Germany). Acarbose, aminoguanidine hydrochloride (AMG, ≥ 98%), bovine serum albumin (BSA, ≥ 98%), intestinal acetone powder from rat (source of α-glucosidase), α-amylase from porcine pancreas (type VI-B), lipase (type II; from porcine pancreas), ammonium sulfate (≥99%), DL-glyceraldehyde (≥98%), β-mercaptoethanol (≥99%), sodium carbonate (100%), *p*-nitrophenyl-α-D-glucopyranoside (*α-p*NPG), *p*-nitrophenyl butyrate (*p*NPB), orlistat and D-(−)-ribose (≥99%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Human aldose reductase (HAR) was purchased from Prozomix (Northumberland, UK) and β-nicotinamide adenine dinucleotide reduced tetrasodium salt hydrate (NADPH, ≥ 97%) from Calbiochem (MA, USA). 1-Deoxynojirimycin (1-DNJ; 95–99%) were obtained from Biopurify phytochemicals LTD (Chengdu, China). Quercetin dihydrate (> 99%) was acquired from Riedel-de Haen.

2.2. Sample preparation and extraction of phenolic compounds

Plant material of *R. maderensis* was collected in May 2014 at Curral das Freiras (Madeira Island, Portugal). The preparation of samples and extraction of phenolic compounds was described in detail previously (Spínola et al., 2018). Briefly, the lyophilized material (leaves, flowers and stems in separate) was mixed with methanol (1:25 solid material:solvent ratio), ultrasonicated (60 min), filtered (Whatman No.1 filter papers) and concentrated to dryness under reduced pressure in a rotary evaporator (at 40 °C). The resulting dry extracts (DE) were kept in 5 mL capped flasks at 4 °C until analysis.

Other batches of freeze-dried *R. maderensis* were further submitted to *in vitro* gastrointestinal digestion, using a static model that simulated, sequentially, mouth, stomach and small intestine digestion. The procedure was explained in detail previously (Spínola et al., 2018). At the end of the simulation, samples were centrifuged, and the supernatant was recovered, filtered, lyophilized and submitted to extraction, as mentioned above. The resulting extracts were stored at 4 °C pending its use.

2.3. Analysis of phenolic compounds by HPLC

Identification and quantification of phenolic compounds of *R. maderensis* (before and after *in vitro* gastrointestinal digestion) was carried out by HPLC analysis. Briefly, separation was carried out on a Phenomenex Gemini C18 column (5 μm, 250 × 3.0 mm i.d.) using a mobile phase composed by acetonitrile (A) and water/formic acid (0.1%, v/v) at a flow rate of 0.4 mL min^{-1}. Gradient elution: 20% B (0 min), 25% B (10–20 min), 50% B (40 min), 100% B (42–47 min), 20% A (49–55 min); injection volume: 5 μL; column temperature: 30 °C. Mass spectrometry conditions: dry and nebulizer gas (N_2): flow rate 10 mL min^{-1} and pressure 50 psi; nebulizer temperature and voltage: 365 °C and +4500 V, respectively; scanning range: m/z 100–1000.

Apigenin, caffeic acid, cyanidin-3-*O*-glucoside (C3G), catechin and quercetin were the standards used to the relative quantification of flavones, hydroxycinnamic acids (HCAs), anthocyanins, flavan-3-ols and flavonols, respectively. TIPC (total individual phenolic content) was defined as the sum of the concentrations of quantified phenolic compounds. For more details please check the reference Spínola et al. (2018).

2.4. In vitro enzymes inhibition assays

α-Amylase, rat α-glucosidase, pancreatic lipase and aldose reductase inhibition assays were performed as described before. The inhibitory activity of analysed samples was expressed as the IC_{50} values (mg mL^{-1}), determined from the least-squares regression line of the logarithmic concentrations plotted against percentage inhibition. This value corresponds to the concentration of the samples able to reduce the enzyme activity by 50% with reference to the control.

2.5. BSA glycation inhibition assay

Inhibition of AGEs formation was measured with adaptations from a previous protocol (Spínola, Pinto, Llorent-Martínez, Tomás, & Castilho, 2019). In a black 96 well-plate, 100 μL of BSA solution (10 mg mL^{-1}), 100 μL of ribose solution (0.5 M) and 40 μL of sample extracts (serial dilutions) were combined. After incubation (24 h at 37 °C), plates were analysed at an excitation wavelength of 355 nm and emission wavelength of 460 nm (Victor3 microtiter reader, Perkin–Elmer). Aminoguanidine (AMG) and quercetin standard were used as positive controls.

2.6. Statistical analysis

Statistical analysis was performed using SPSS Statistics software v.20 (IBM SPSS Statistics for Windows, IBM Corp., USA). Data of all analysis, in triplicate, are expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed to determine whether there are any statistically significant differences among parameters experimentally determined, followed by Tukey's HSD post-hoc test. A 5% significance level was considered for all tests. Pearson correlation coefficients (*r*) were determined to corroborate relationships between selected parameters.

3. Results and discussion

The phenolic composition of studied *R. maderensis* methanolic extracts was previously reported by our research group (Spínola et al., 2018). Flowers and leaves showed the highest TIPC and stems the lowest (Fig. 1A).

Simulated digestion had great impact on the phenolic composition of *R. maderensis* (Fig. 1B) (a decrease of about 50%)(Spínola et al., 2018). TIPC of flowers was the most affect (−55.8%), followed by leaves (−52.0%) and stems (−45%).

3.1. In vitro inhibition of digestive enzymes

α-Amylase and α-glucosidase are key enzymes responsible for the digestion of dietary carbohydrates into glucose (Shiwani, Singh, & Wang, 2012; Tiwari et al., 2013; N.-N.; Wu et al., 2018). Inhibition of these enzymes is used as a therapeutic measure in diabetic patients since it retards carbohydrate digestion and subsequently reduces the rate of glucose absorption in the gut (D. A. Kumar et al., 2015; Moradi-Afrapoli et al., 2012; Savran et al., 2016). The suppression of postprandial hyperglycaemia delays the progression of micro- and macrovascular complications and AGEs formation (Akkarachiyasit, Charoenlertkul, Yibchok-Anun, & Adisakwattana, 2010; Jang et al.,

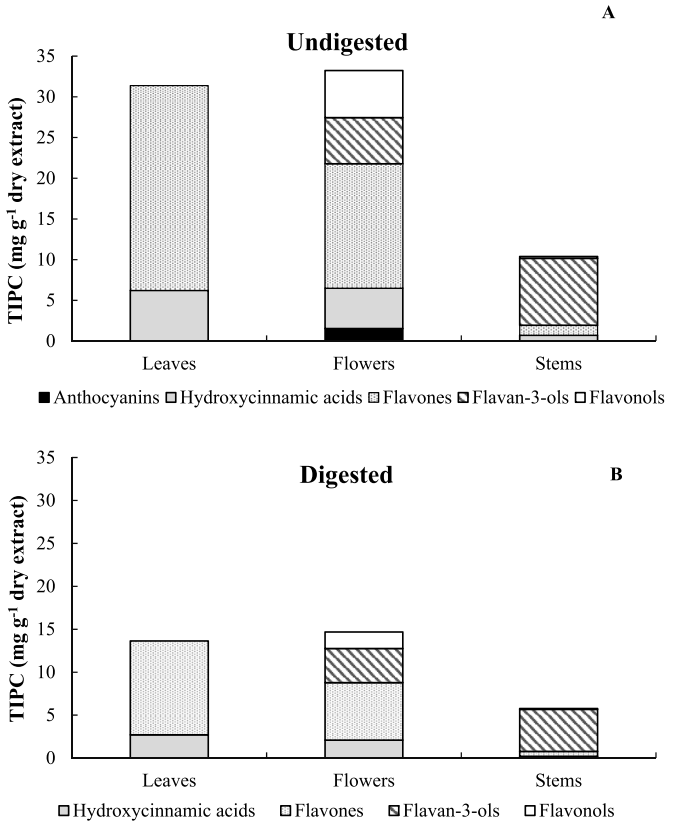

Fig. 1. Total individual phenolic content (TIPC) of *R. maderensis* methanolic extracts before (Undigested) and after total *in vitro* gastrointestinal digestion (Digested). TIPC for each chemical family were calculated using data from Spínola et al. (2018).

2008). Synthetic inhibitors of carbohydrate-hydrolysing enzymes (acarbose, voglibose) are used as hypoglycaemic agents (Ahmed et al., 2013; S.; Kumar et al., 2011). However, their prolonged use has some disadvantages including drug resistance, side effects, and even toxicity (Aghajanyan et al., 2018; Khatri et al., 2018).

Obesity is an important risk factor for cardiovascular disease and hyperglycaemia since it induces insulin resistance in glucose-recipient organs (Kwon et al., 2018; Sugiyama et al., 2007). Pancreatic lipase is responsible for the hydrolysis of dietary lipids and its inhibition results in reduced fat absorption, contributing to weight loss (Conforti et al., 2012). Orlistat, a clinically used pancreatic lipase inhibitor, limits the absorption of dietary fat but presents some undesirable effects (Conforti et al., 2012; Sugiyama et al., 2007). Hence, natural digestive enzyme inhibitors from plant foods arise as an alternative strategy for the prevention and management of T2DM and obesity (Ahmed et al., 2013; Conforti et al., 2012; Podsę;dek et al., 2017).

In this work, extracts displayed a moderate α-amylase inhibitory capacity (Table 1). Flowers showed the strongest activity, while leaves the lowest. All samples presented higher IC_{50} values than commercial drug acarbose ($p < 0.05$), an expected observation since they are complex mixtures were active compounds account for a small part of the whole sample.

Flowers extract was also the most active against α-glucosidase activity ($p < 0.05$) (Table 1). Pure compounds acarbose and 1-DNJ, a strong α-glucosidase inhibitor (Yin, Zhang, Feng, Zhang, & Kang, 2014), presented the best inhibitory effects.

For the pancreatic lipase assay, stems showed the best inhibitory activity among the tested extracts (Table 1). In this case, orlistat (positive control) presented the lowest IC_{50} value ($p < 0.05$).

Several *Rumex* species have successfully inhibited digestive enzymes

Table 1
In vitro inhibitory activities of *R. maderensis* extracts towards key digestive enzymes linked to type-2 diabetes and obesity. Results are expressed as the IC_{50} value (mg mL^{-1}). Data represent the mean ± standard deviation ($n = 3$).

	α-Amylase	α-Glucosidase	Lipase
Leaves	3.03 ± 0.12[a]	4.47 ± 0.13[a]	6.59 ± 0.32[a]
Flowers	0.94 ± 0.05[c]	1.23 ± 0.03[b]	5.17 ± 0.25[b]
Stems	1.50 ± 0.08[b]	4.31 ± 0.22[a]	4.27 ± 0.18[c]
Reference Compounds			
Acarbose	0.02 ± 0.01[d]	0.12 ± 0.01[c]	–
1-DNJ	–	0.01 ± 0.01[d]	–
Orlistat	–	–	0.47 ± 0.02[d]

N.I.: no inhibition. 1-DNJ: 1-Deoxynojirimycin. Means in the same column not sharing the same letter are significantly different at $p < 0.05$ probability level.

(Ahmed et al., 2013; Conforti et al., 2012; Froldi et al., 2019; Khatri et al., 2018; Savran et al., 2016; Shiwani et al., 2012) by *in vitro* assays, but this is the first report regarding *R. maderensis*. The juice of *R. vesicarius* L. Chooka reduced starch-induced postprandial glycaemic excursion in rats due to its strong α-amylase and α-glucosidase inhibitory activities (Tiwari et al., 2013).

Poor correlations were observed between TIPC and inhibition of digestive enzymes (Table 2). The same pattern was observed previously (Conforti et al., 2012; D. A.; Kumar et al., 2015). The enzyme inhibitory activity of plant extracts seems not only to rely on total amounts of phenolic compounds but depend on the type of molecules (Khatri et al., 2018).

According to literature, the catabolic activity of α-glucosidase is affected by a wide range of phenolic classes (S. Kumar et al., 2011; Yin et al., 2014). This finding corroborates the present data, where a great variety of phenolic compounds (anthocyanins, HCAs and flavonols) in the flowers extract (Fig. 1) seemed responsible for the inhibition of α-glucosidase (Table 1). In the present study, anthocyanins and flavonols were the main contributors for inhibition of α-glucosidase (Table 2). Anthocyanins have been reported to be effective α-glucosidase inhibitors (Esatbeyoglu, Rodríguez-Werner, Schlosser, Winterhalter, & Rimbach, 2017; Podsę;dek et al., 2017; N.-N.; Wu et al., 2018; You et al., 2011). C3G, the only anthocyanin identified in *R. maderensis* flowers (Spínola et al., 2018), was more active than cyanidin and cyanidin-3,5-diglucoside towards inhibition of α-glucosidase (Akkarachiyasit et al., 2010). However, anthocyanins alone were not responsible for the α-glucosidase inhibition of *R. maderensis*. Other components present in the analysed extracts might also contributed for the inhibitory activities, as suggested by other authors (Esatbeyoglu et al., 2017; N.-N.; Wu et al., 2018). Indeed, flavonol glycosides have been shown to inhibit α-glucosidase (Froldi et al., 2019; Moradi-Afrapoli et al., 2012; Tadera, Minami, Takamatsu, & Matsuoka, 2006). Within the flavonoids family, flavonols seem to be the strongest inhibitors of α-glucosidase due to the 3-OH in conjugation with a 4-oxo function in the C-ring (Tadera et al., 2006).

The activities of α-amylase and pancreatic lipase seemed mainly affected by flavan-3-ols (Table 2). Catechin and its oligomers (proanthocyanidins or condensed tannins) were the main flavan-3-ols of flowers and stems of *R. maderensis* (Spínola et al., 2018). Previous works (Huang et al., 2013; Kusano et al., 2011; Sugiyama et al., 2007) have reported the potent inhibitory activity of proanthocyanidins towards α-amylase and pancreatic lipase. The effectiveness of proanthocyanidins seems to increase according to the degree of polymerization (Sugiyama et al., 2007). In fact, a proanthocyanidin trimer from *R. maderensis* extracts had a higher contribution for pancreatic lipase inhibition ($r = 0.993$) than dimer ($r = 0.913$) and monomer ($r = 0.831$) of catechin (Spínola et al., 2018).

Table 2
Correlation coefficients (*r*) observed among phenolic composition of *R. maderensis* extracts and evaluated *in vitro* bioactivities.

Parameters	α-Amylase	α-Glucosidase	Lipase	Aldose reductase	BSA glycation
TIPC	0.187	−0.525	0.580	0.134	−0.972
Anthocyanins	−0.707	−0.989	0.043	−0.744	−0.352
Hydroxycinnamic acids	0.463	−0.527	0.966	0.415	−0.998
Flavonols	−0.803	−0.990	0.010	−0.866	0.840
Flavan-3-ols	−0.948	−0.254	−0.968	−0.910	−0.321
Flavones	0.630	−0.057	0.998	0.589	−0.957

3.2. *In vitro* inhibition of aldose reductase (AR)

It has been suggested that AR, the key enzyme of the polyol pathway, plays a central role on the pathogenesis of diabetic cataract, neuropathy, nephropathy, etc (J. M. Kim, Jang, Lee, Lee, & Kim, 2008; J. M. Kim et al., 2006). In normal conditions, this enzyme has low substrate affinity to glucose, and primarily reduces toxic aldehydes and carbonyls. But, in hyperglycaemia conditions, the excessive glucose is converted into sorbitol by this enzyme in various tissues and organs of the human body (eyes, kidneys, myelin, sheath, etc.). The accumulation of intracellular sorbitol will subsequently lead to tissue abnormalities due to osmotic stress (Matsuda, Morikawa, IwaoToguchida, & Yoshikawa, 2002). Synthetic AR inhibitors (Zopolrestat, Epalrestat, Sorbinil, etc) have been developed and can reduce the hyperglycemia-induced polyol pathway, contributing to the treatment and prevention of diabetic complications (H. M. Kim et al., 2010). However, several side effects and limited efficacy have reduced their usage. Therefore, natural AR inhibitors are of significance in the treatment and prevention of diabetic complications due to less toxicity (H. M. Kim et al., 2010; J. M. Kim et al., 2006).

R. maderensis extracts were potent aldose reductase inhibitors, albeit they showed weaker inhibition effect than quercetin ($p < 0.05$), used as reference compound (Table 3). In this case, flowers showed the strongest inhibition and leaves the lowest ($p < 0.05$).

Similar to previous enzymes assays (section 3.1.), a poor correlation was obtained between the phenolic contents and aldose reductase activity (Table 2). Flavan-3-ols were the main contributors for the reported inhibitions.

Leaves of *R. crispus* showed a strong inhibitory activity against AR ($IC_{50} = 0.05\ mg\ mL^{-1}$)(H. M. Kim et al., 2010). Quercetin-3-*O*-rhamnoside and quercetin aglycone isolated from different parts of *R. japonicus* displayed significant activities on AR (J. M. Kim et al., 2008, 2006). Flavones were considered stronger AR inhibitors than flavan-3-ols (Matsuda, Morikawa, Toguchida, & Yoshikawa, 2002), but in the present study their effect was not significant (Table 2). Considering that dimers and trimers of (+)-catechin were the main flavan-3-ols of *R. maderensis* (Spínola et al., 2018), the obtained data (Table 3) suggest that polymerization enhance the effectiveness of (+)-catechin derivatives against AR.

After simulated digestion, extracts exhibited higher IC_{50} values (Table 3), meaning that after the digestion process extracts displayed reduced inhibitory activities against AR (a 63.11–88.38% decrease). This is probably linked with the reduced amounts of phenolic compounds present in the digested extracts (Fig. 1B). During human digestion, dietary phenolic compounds are exposed to different physical (temperature and agitation), chemical (pH) and biochemical (enzymes) conditions, which influence their bioavailability. This environment results in several changes (hydrolysis, oxidation, epimerization) in their chemical structure and properties and/or degradation, so their bioactivities are affected (Bermúdez-Soto et al., 2007; Gunathilake et al., 2018). Although in lower potency, the digested extracts still displayed inhibitory activity suggesting their potential effects in the human body after passage though the gastrointestinal tract.

3.3. *In vitro* inhibition of BSA glycation

Chronic hyperglycaemia and oxidative stress in diabetic patients induce the non-enzymatic formation of AGEs, which are implicated in diabetic microvascular, renal, atherosclerosis and other complications (Froldi et al., 2019; J. M.; Kim et al., 2006; Tiwari et al., 2013). These harmful adducts are generated *via* the reaction between amino groups of proteins and reducing sugars (Maillard reaction) and their accumulation results in structural and functional alterations in plasma and extracellular matrix proteins (Gugliucci, Bastos, Schulze, & Souza, 2009; Jang et al., 2008; Tsuji-Naito, Saeki, & Hamano, 2009). Glycation initially progresses to form the fast and highly reversible Schiff base intermediate. Then, the products of the Amadori rearrangement involving Maillard reactions (3-deoxyglucosone, glyoxal, and methylglyoxal) lead to the formation of AGEs through several complex reactions (dehydration, oxidation, cyclisation) (Tsuji-Naito et al., 2009; Yeh, Hsia, Lee, & Wu, 2017). The toxic effects of these molecules include cross-linking of proteins and interaction with specific receptors, which leads to enhanced formation of reactive oxygen species (ROS) and inflammation through activation of pro-inflammatory mediators (Jang et al., 2008; Yeh et al., 2017). Therefore, inhibiting protein glycation has been considered as an effective strategy to slow down the development/progression of diabetic complications (Tiwari et al., 2013; Yeh et al., 2017).

The analysed extracts were able to prevent glycation of BSA induced by ribose. In this assay, the order of potency was leaves > flowers >

Table 3
In vitro inhibitory activities of *R. maderensis* extracts towards aldose reductase and glycation of BSA, before (Undigested) and after complete simulated gastrointestinal digestion (Digested). Results are expressed as the IC_{50} value (mg mL^{-1}). Data represent the mean ± standard deviation ($n = 3$).

	Aldose reductase		BSA-glycation	
	Undigested	Digested	Undigested	Digested
Leaves	1.59 ± 0.05^{d}	4.31 ± 0.10^{b}	0.93 ± 0.03^{f}	3.43 ± 0.18^{c}
Flowers	0.74 ± 0.04^{f}	3.10 ± 0.21^{c}	1.21 ± 0.05^{e}	3.50 ± 0.12^{c}
Stems	1.01 ± 0.04^{e}	8.69 ± 0.52^{a}	2.54 ± 0.03^{d}	11.89 ± 0.82^{a}
Reference Compounds				
Aminoguanidine	–	–	9.56 ± 0.36^{b}	–
Quercetin	0.10 ± 0.01^{g}	–	0.11 ± 0.01^{g}	–

Means in the assay not sharing the same letter are significantly different at $p < 0.05$ probability level.

stems (Table 3). These results are in agreement with previous works on the anti-glycation activity of other *Rumex* species (Froldi et al., 2019; Jang et al., 2008; J. M. Kim et al., 2006; D. A. Kumar et al., 2015; Tiwari et al., 2013). AMG, an anti-glycation experimental agent (Gugliucci et al., 2009), was less effective than the tested extracts of *R. maderensis* (Table 3). Due to its toxicity, AMG is currently used as positive control in glycation studies (Bains, Gugliucci, & Caccavello, 2017). Quercetin standard showed the strongest inhibitory activity among tested samples (Table 3) and it has been suggested as a better and a more potent anti-glycating agent than AMG (Ashraf et al., 2015). Five phenolic compounds isolated from *R. japonicus* fruits displayed stronger inhibitory activities against protein glycation than AMG (IC_{50} = 961 μM). Among these, isoquercitrin and (+)-catechin were the most potent compounds ($IC_{50} \leq 20.7$ μM) (Jang et al., 2008). The anti-glycation activity of *R. lunaria* leaves extract is possibly attributed to quercetin glycosides (79.47% of phenolic content)(Froldi et al., 2019).

After simulated digestion, the potency of extracts against inhibition of AGEs formation decreased (a 65.42–78.63% reduction) in comparison with native capacity (Table 3). Digested leaves and flowers presented the most effective inhibitory activity. Only stems were less active than AMG. The lower anti-glycation activity of the digested samples agrees with the degradation of phenolic compounds that was observed during the digestion procedure (Fig. 1B). Additionally, when exposed to mild-alkaline conditions, a fraction of phenolic compounds undergo structural transformations that result in metabolites with different chemical structures that, usually, possess lower biological properties. The pH variations during digestion may affect racemization of compounds creating enantiomers with different biological effects (Bermúdez-Soto et al., 2007). As a result, phenolic compounds could not react effectively, and their bioactivities are impaired. Interference of phenolic compounds with other constituents of the sample (proteins, carbohydrates, fibers) may also cause changes in their biological effects (Gunathilake et al., 2018). Nevertheless, digested extracts still retained some of their inhibitory effects (higher than AMG) and this mechanism may help to provide a protective effect against hyperglycemia-mediated protein damage after passage through the alimentary tract. Previous findings (Bains et al., 2017) suggested that AGEs could also be originated during digestion (in the intestinal lumen). Even if phenolic compounds are not absorbed by the gastrointestinal mucosa, they might exert their protective effects in the gastrointestinal tract, by preventing the formation of AGEs and limiting the circulating AGEs load.

Strong correlations were observed between prevention of protein glycation and TIPC, HCAs and flavones ($r \geq -0.957$) (Table 2). In this assay, the total amounts of phenolic compounds seemed more important than specific classes of molecules. The anti-glycation activity of HCAs (in particular caffeic and chlorogenic acids) and flavones (apigenin and luteolin derivatives) have been reported (Bains et al., 2017; Gugliucci et al., 2009; Tsuji-Naito et al., 2009). These phytochemicals exert their beneficial effects thought inhibition of AGEs-precursors formation and/or free-radicals or metals scavenging.

Oxidative stress has been implicated in the pathogenesis of diabetic complications due to the increased free radicals generation that damage biomolecules and disturb normal cellular and metabolic functions (D. A. Kumar et al., 2015; Moradi-Afrapoli et al., 2012; Shiwani et al., 2012; Tiwari et al., 2013). Glycation is a major source of ROS and carbonyl species and, at the same time, favored by hyperglycaemia-mediated oxidative stress (Gugliucci et al., 2009; C.-H.; Wu & Yen, 2005; Yeh et al., 2017). The anti-glycation properties of phenolic compounds is associated with their structural features and capacity to delay the oxidation of glycated proteins (Yeh et al., 2017). In our previous work (Spínola et al., 2018), *R. maderensis* extracts (before and after gastrointestinal digestion) displayed relevant free-radical scavenging activities ($ABTS^{\bullet+}$, $DPPH^{\bullet}$, $NO^{\bullet}$, and $O_2^{-\bullet}$ assays). A good correlation was obtained between the antioxidant activities and protein glycation assays ($r \geq 0.813$). Given the link mentioned above, we hypothesized that prevention of AGEs formation by *R. maderensis* extracts was, at least partly, due to the antioxidant functions of their phenolic constituents.

4. Conclusions

In this work, *R. maderensis* extracts exhibited important inhibitory capacity toward key enzymes linked to type-2 diabetes and obesity, although lower than reference compounds concerning digestive enzymes and aldose reductase. However, the analysed extracts exhibited higher inhibitory potency towards BSA-glycation than the reference compound AMG. In addition, although the digestion process diminished the inhibitory activity, leaves and flowers extracts were still more active against BSA-glycation than AMG. Therefore, *R. maderensis* present interesting properties for nutraceuticals production or used directly as a dietary intervention to prevent hyperglycaemia-related complications. However, additional biological tests are necessary to fully evaluate its therapeutic efficiency.

Declaration of competing interest

None.

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