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Tomato plant leaves: From by-products to the management of enzymes in chronic diseases

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ABSTRACT

Lycopersicon esculentum Mill. is one of the world's most important crops and its enormous by-products, such as leaves, are an excellent source of bioactive compounds with potential application in several industries. In this work, extracts from tomato plant leaves (cultivars: Caramba, Valentine, Negro, Abuela, Río Alto, Anairis, Rosa and Yack) were examined, for the first time, to establish their phytochemical fingerprint and capacity to inhibit key enzymes involved in Alzheimerís disease (AD) (acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and 5-lipoxygenase (LOX)) and in *diabetes mellitus* (DM) (α-glucosidase and α-amylase). The hydromethanol extracts were shown to contain high amounts of phenolics (3774–9252 $\mu g\,g^{-1}$ of dry extract), quercetin-3-*O*-rutinoside, quercetin-3-*O*-pentosyl-rutinoside, and chlorogenic and neochlorogenic acids being the major ones. The acetone extracts rich in pigments, namely chlorophylls (46.40 ± 3.81–$136.4 \pm 7.94\ mg\,g^{-1}$ of dry extract), as well as alkaloids extracts rich in tomatine (224 ± 4.39–$745 \pm 4.96\ mg\,g^{-1}$ of dry extract), were also analysed. Moreover, hydromethanol extracts exhibited inhibitory activity against AChE ($IC_{50} = 3806 \pm 113$–$9911 \pm 50.5\ \mu g\,mL^{-1}$), BuChE ($IC_{50} = 133.1 \pm 2.68$–$369.1 \pm 17.2\ \mu g\,mL^{-1}$), LOX ($IC_{25} = 35.2 \pm 0.49$–$533 \pm 8.9\ \mu g\,mL^{-1}$), α-glucosidase ($IC_{50} = 1.14 \pm 0.04$–$6.48 \pm 0.13\ mg\,mL^{-1}$) and α-amylase ($IC_{50} = 1.11 \pm 0.02$–$1.78 \pm 0.03\ mg\,mL^{-1}$). On the contrary, the alkaloid extracts were only effective against BuChE ($IC_{50} = 82.4 \pm 1.34$–$172 \pm 10.2\ \mu g\,mL^{-1}$). Chemical fingerprint and biological activities varied according to the analysed cultivar. Overall, our results suggest that *L. esculentum* leaves are promising by-products and valuable sources of bioactive compounds for AD and DM management.

1. Introduction

Alzheimer's disease (AD) constitutes more than 60–80% of cases of dementia and is the fourth leading cause of death (Natarajan et al., 2009). Biochemically, AD is described by a reduction of the levels of the neurotransmitter acetylcholine (ACh), which is hydrolysed primarily by acetylcholinesterase (AChE) and secondly by butyrylcholinesterase (BuChE). By inhibiting these enzymes, ACh levels increase and a slowdown of the disease progression occurs (Custódio et al., 2015; Ferreres et al., 2015). In fact, drugs based on cholinesterase inhibitory properties are amongst the most prescribed ones (Mocan et al., 2016; Sarikurkcu et al., 2014). Additionaly, a marked increase in the expression and activity of several other enzymatic systems has also been documented to occur during neurodegenerative processes. In particular, lipoxygenases have been suggested to be involved in AD pathomechanism and other aging-related events (recently reviewed by Czapski et al., 2016). Lipoxygenases play an important role in inflammation, since they are involved in the biosynthesis of inflammatory lipid mediators, such as leukotrienes and prostagladins, their inhibition being considered as one of the targets for the prevention of diseases whose development is linked to oxidative stress and inflammation. Chronic inflammation in the brain is a pathological feature of Alzheimer's disease (AD) (Dzoyem and Eloff, 2015).

Diabetes mellitus (DM) affects more than 300 million people worldwide and is expected to be the 7th leading cause of death in 2030 (Custódio et al., 2015). Type 2 DM accounts for 90% of cases of diabetes, being characterized by a fast increase of blood glucose levels, due to the hydrolysis of starch by α-amylase and absorption of glucose in the small intestine by α-glucosidase. According to numerous studies, the inhibition of both α-glucosidase and α-amylase enzymes plays a key role in the management of diabetic complications (Figueiredo-González et al., 2016; Lordan et al., 2013; Oboh et al., 2012; Savran et al., 2016; Uysal et al., 2016).

Recent epidemiological, clinical and biological evidences support a link between DM and AD since elevated glucose levels and diabetes might be associated with cognitive dysfunction, the most prevalent cause of which is AD (Barbagallo and Dominguez,

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2014; Sridhar et al., 2015). Although the nature of the association between both diseases is yet not fully understood, common preventive and therapeutic agents could be beneficial in their prevention and treatment. In this context, efforts should target the search for novel, effective and safe inhibitors from natural sources against key enzymes related with these diseases (Zengin, 2016).

Agricultural industry residues and wastes constitute a significant proportion of worldwide agricultural productivity. Currently, the trend in the agribusiness sector is to recover, to evaluate, and to find better uses for all their by-products, such as, peels, seeds, stems, and leaves (Silva-Beltrán et al., 2015a). Tomato is the third most important vegetable grown in the world (Larbat et al., 2014). Despite little information available in the literature concerning non-edible plant organs, they constitute valuable by-products for the food, cosmetic and pharmaceutical industry (Taveira et al., 2012).

Tomato plant (*Lycopersicon esculentum* Mill., Solanaceae) leaves exhibit an interesting, yet complex, mixture of bioactive substances, such as phenolics (Ferreres et al., 2011; Larbat et al., 2014; Silva-Beltrán et al., 2015b; Taveira et al., 2012), alkaloids (Ferreres et al., 2011; Silva-Beltrán et al., 2015a; Taveira et al., 2014, 2012) and carotenoids (Gupta et al., 2015; Silva-Beltrán et al., 2015a). These compounds could be a more acceptable resource of enzyme inhibitors, since some phenolic compounds have already been addressed to several remarkable biological activities, including the capacity to inhibit cholinesterases (ChEs) (Custódio et al., 2015; Ferreres et al., 2015; Vinholes et al., 2011), 5-lipoxygenase (LOX) (Bekir et al., 2013) and/or α-glucosidase and α-amylase (Lordan et al., 2013; Oboh et al., 2012). Moreover, previous findings have also shown some alkaloids to be potent inhibitors of both AChE and BuChE (Santos et al., 2012; Taveira et al., 2014) and of LOX (Ratheesh et al., 2010), as well as to inhibit carbohydrate hydrolysing enzymes (Kumar et al., 2011). Oboh et al. (2015) suggested a relationship between carotenoid contents and bioactivities against cholinesterases.

The rationale of this work is the expansion of the knowledge on tomato leaves, as well as the valorization of by-products, by providing new natural drugs for potential use in the treatment of high-prevalence diseases, namely AD and DM. Thus, the neuroprotective and antidiabetic effectiveness of the leaves of eight *L. esculentum* cultivars was evaluated using cell-free models as easy and fast tool for a first approach. As far we know, this study represents the first assessment of the antidiabetic potential of tomato leaf extracts. In addition, it is the first time that the neuroprotective effect of the selected cultivars is studied. Only one previous work reported the neuroprotective activity of alkaloids extract of the leaves of other tomato cultivars against human neuroblastoma SH-SY5Y cells, which were used as the source of AChE and BChE (Taveira et al., 2014). In addition, it is also characterized for the first time the chemical fingerprint of the leaves of eight *L. esculentum* cultivars. The activities of tomato leaves could be linked to their constituents and might support their use as a cheap and natural treatment/management of AD and DM. Moreover, the sustainable use of tomato leaves, either for the isolation of bioactive compounds, or for the preparation of therapeutically useful extracts for pharmaceuticals and/or food applications, will provide economic value to this by-products from the agricultural industry.

2. Materials and methods

2.1. Chemicals and standards

Acarbose, galanthamine, α-glucosidase (=maltase from *Saccharomyces cerevisiae*), α-amylase (from porcine pancreas), 4-nitrophenyl α-D-glucopyranoside (PNP-G), dinitrosalicylic acid, soluble starch, AChE (from electric eel), acetylthiocholine iodide (ATCI), BuChE (from equine serum), *S*-butyrylthiocholine iodide (BTCI), bovine serum albumin (BSA), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), Trizma® hydrochloride (Tris–HCl), lipoxydase (from glycine max), *tert*-butyl methyl ether (TBME), linoleic acid and the reference compounds β-carotene (≥95.0%), lutein, chlorophyll *a* and chlorophyll *b* (≥90.0%), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Quercetin-3-*O*-rutinoside (95.0%), quercetin (99.0%), tomatine, tomatidine, and chlorogenic acid were from Extrasynthèse (Genay, France) and neochlorogenic acid (98.0%) was from Chengdu Biopurify Phytochemicals LTD (Chengdu, China). Neoxanthin and violaxanthin (95.0%) were purchased from Carote-Nature (Lupsinggen, Switzerland).

Methanol, acetone and glacial acetic acid were acquired to Chem-Lab NV (Zedelgem, Belgium). Water, methanol and acetonitrile of HPLC grade, potassium sodium tartrate tetrahydrate, trisodium phosphate, potassium dihydrogen phosphate, sodium hydroxide, sodium chloride, and ammonia solution 25% were obtained from Merck (Darmstadt, Germany). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and triethylammonium phosphate 1 M (TEAP) were purchased from Fluka (Steinheim, Germany) and Ion-exchange (sulphonic acid bonded silica with H^+ counterion (SCX) was from Supelco (Bellefonte, USA).

2.2. Plant materials and preparation of the extracts

The selected cultivars in this work were Caramba, Valentine, Negro, Abuela, Río Alto, Anairis, Rosa and Yack, which are among the most consumed in Galicia (Spain).

The leaves from eight cultivars of *L. esculentum* were collected in June, from tomato plants grown in a greenhouse in Galicia, Spain. The plant material was washed with distilled water, and lyophilized for later use.

For each cultivar, an hydromethanol extract (HME) was prepared as follows: the powdered leaves (ca 400 mg) were sonicated with 20 mL of methanol:water (50:50, v/v) (30 min), followed by stirring maceration (120 min, 300 rpm) at room temperature and further sonication (30 min). The extract was filtered under vacuum, concentrated to dryness and stored at −20 °C, protected from light. The same procedure was followed with acetone to obtain the acetonic extract (AcE).

The method for recovering alkaloids was based in our previous work (Taveira et al., 2012), with minor modifications. Briefly, powdered leaves (ca 200 mg) were sonicated with 4 mL of 5% acetic acid (30 min), followed by stirring maceration (120 min, 300 rpm) at room temperature and further sonication (30 min). Then, the extract was centrifuged and supernatant was directly loaded into a SCX cartridge, previously activated with 10 mL of methanol followed by 10 mL of 5% acetic acid. The sorbent was washed with 20 mL of 5% methanol and then, the retained compounds were eluted with 20 mL of 2.5% ammonium in methanol. The resulting alkaloids-purified extract (AE) was concentrated to dryness and stored at −20 °C protected from light.

All extractions were performed in triplicate.

2.3. HPLC-DAD analysis

Identification of compounds was performed according to the procedures previously described by our group (Fernandes et al., 2016; Ferreres et al., 2011; Taveira et al., 2012).

HME and AE were analysed on an analytical HPLC unit (Gilson), using a reversed-phase ACE 3 C-18-AR column (150 × 4.6 mm, 3 μm particle size, Advanced Chromatography Technologies, Aberdeen, Scotland). For HME the solvent system used was a gradient of 1% acetic acid (A) and MeOH (B), starting with 20% B and installing a gradient to obtain 60% B at 20 min, 90% B at 35 min and 100% B at 37 min, at a solvent flow rate of 0.8 mL min^{-1}; for AE the solvent