Journal of King Saud University – Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Phenolic compounds from *Actinidia deliciosa* leaves: Caco-2 permeability, enzyme inhibitory activity and cell protein profile studies

Joana Henriques [a,b], Pedro Luis Falé [a,b], Rita Pacheco [b,c], Maria Helena Florêncio [a,b], Maria Luísa Serralheiro [a,b,*]

[a] *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[b] *Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[c] *Área Departamental de Engenharia Química. Instituto Superior de Engenharia de Lisboa, Av. Conselheiro Emídio Navarro, 1959-007 Lisboa, Portugal*

ARTICLE INFO

Article history:
Received 3 March 2017
Accepted 25 July 2017
Available online xxxx

Keywords:
Actinidia deliciosa leaves
Rutin
Quercitrin
Acetylcholinesterase
Transketolase
Polyphenols permeation
Caco-2 cells

ABSTRACT

Chemical compounds from leaves of fruit-producing trees, a waste from agricultural activity can be isolated and used as a source of natural bioactive chemicals. Boiling water was used as an extractant of bioactive compounds from *Actinidia deliciosa* leaves and co-extracted fibres were removed with ethanol precipitation. Rutin and quercitrin were the main flavonoids identified and quantified by RP-HPLC-DAD. No cytotoxicity was detected for any of the extracts towards Caco- 2 cell line. A permeation of approx. 14% of extract components through the cells monolayer was determined. The cell protein profile of Caco-2 cells was modified when in the presence of the fibre-free extract and transketolase was the protein over-expressed in the presence of polyphenols. Acetylcholinesterase inhibitory activity was also studied, IC_{50} of 0.56 mg/mL was obtained with the fibre-free extract. *A. deliciosa* leaves are a good source of phenolic compounds and, therefore, some advantage may be taken of this agricultural residue, due to their biological activity.

1. Introduction

Leaves from plants used in agriculture in fruit production generate a huge quantity of industrial wastes that have been thought, nowadays, as a good source of bioactive compounds, mainly polyphenolics (Santana-Méridas et al., 2012; Manach et al., 2004). Polyphenolics are a diverse type of chemical compounds with several biological activities, among which antioxidant (Talukder et al., 2016), anti-inflammatory (Li et al., 2014), anti-acetylcholinesterase (Falé et al., 2009; Neagu et al., 2016), antidiabetic (Asgar, 2013), antihypercholesteroliemia (Falé et al., 2014; Lee et al., 2013) can be referred. *Actinidia deliciosa* plantation has been increasing in Europe during the last decade (CONFAGRI, 2012; AREFLH, 2014). The generation of wastes from tree pruning is a general problem for the agriculture sector. Therefore, in those cases where the leaves are a source of bioactive compounds, instead of being discarded, they could be used with advantage. There are several processes to take advantage of compounds present in the plant leaves. Water, the solvent traditionally used to prepare medicinal decoctions and infusions, is able to extract several phenolic compounds (Falé et al., 2013a, 2013b; Falé et al., 2014; Henriques et al., 2017; Lizcano et al., 2010). Mucilage, a soluble fiber, polysaccharide-rich material, is co-extracted in these procedures (Prabakaran et al., 2011) and precipitation with ethanol has been used to separate this type of polymers from phenolic compounds (Ghanem et al., 2010). Taking advantage of phenolic compounds requires the evaluation of their toxicity and permeability through the intestinal cells. Caco-2 cells, a cell line derived from colorectal adenocarcinoma, are recommended by FDA to evaluate the permeation of drugs (Awortwe et al., 2014). These cells have the ability to grow in a monolayer, developing all the enzymatic machinery similar to the cells present in the small intestine (Engle et al., 1998). Several studies indicate that some polyphenols can permeate this barrier although in a low amount (Borrás-Linares et al., 2015; Falé et al., 2014). In the present work, the biological activity of *A. deliciosa* leaves will be studied, *in vitro*,

* Corresponding author at: Faculdade de Ciências, Universidade de Lisboa, Edifício C8, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail address: mlserralheiro@fc.ul.pt (M.L. Serralheiro).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jksus.2017.07.007
1018-3647/

using acetylcholinesterase (AChE, acetylcholine acetylhydrolase, E. C. 3.1.1.7) inhibition as model. This enzyme is located in the synapsis (Colovic et al., 2013) and neuromuscular junctions (Rotundo, 2003). AChE catalyses the hydrolysis of the neurotransmitter acetylcholine, ending the neuronal impulse. The inhibition of this activity is used in the symptomatic approach to the of Alzheimer's disease treatment (Colovic et al., 2013; Mehta et al., 2012) and to accelerate the gastrointestinal transit in severe constipation (Bharucha et al., 2013). To inhibit this enzyme but most of them have secondary effects (Colovic et al., 2013) so, the search for new and natural enzyme inhibitors is a matter of importance. It has been demonstrated that polyphenols are able to modify the cell protein expression (Pollio et al., 2016) what may explain some of the biological activities found for these compounds. The objective of the present work was to prepare an extract with bioactive compounds from an agricultural waste, with no cell toxicity and biological activity, which can be used in the development of new added-value products. The novelty of this work resides in the detection of several phenolic compounds in *A. deliciosa* leaves with biological activity and effect on cell proteome that have not been previously reported for these leaves.

2. Material and methods

2.1. General experimental procedure

All chemicals were analytical grade. MTT (thiazolyl tetrazolium bromide), bovine serum albumin, rutin, acetylcholinesterase (AChE), acetylthiocholine (AChI) and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma (Barcelona, Spain). Trifluoroacetic acid, quercitrin and methanol were obtained from Merck (VWR, International, Darmstadt, Germany). Ethanol was obtained from Carlo Erba (Peypin, France). Roswell Park Memorial Institute (RPMI) medium, Hank's balanced salt solution (HBSS), glutamine, Pen-Strep (penicillin and streptomycin mixture), and fetal bovine serum (FBS) were bought from Lonza (Verviers, Belgium). For the one-dimensional electrophoresis gel GE Healthcare Amersham™ ECL™ was used in a 4–12% concentration. The marker protein sample buffer (5X SDS PAGE) and staining solution, BlueSafe, were purchased from NZYTech®.

2.2. Plant material

Fully expanded leaves from *Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson (Actinidiaceae) were collected in December 2016 from plants cultivated at the region of Beira Baixa, Portugal (lat. 39° 46′ 47,783″ N; long. 7° 48′ 20,171″ W, 244 m alt). Fresh leaves were washed and immediately used for extraction as decoction, 48 h after collection.

2.3. Preparation of plant extracts

Aqueous plant extracts were prepared as decoction by using 10 g of grounded leaves in 100 mL of distilled water, boiling for 10 min. The decoctions were filtered through number 1 Whatman grade paper and lyophilized. The yield of extraction was 114 mg of extract/g of plant. For the phenolic compounds isolation, the precipitation of fibers was developed as described in Ghanem et al. (2010), with small modifications (Henriques et al., 2017).

2.4. Reverse Phase High Performance Liquid Chromatography (RP-HPLC-DAD)

The chromatographic analysis was carried out using reverse phase liquid chromatography with a diode array detector (RP-HPLC-DAD) as described in Falé et al. (2013b). The quantification of rutin and quercitrin was carried out using the RP-HPLC-DAD by injecting standard solutions and preparing a calibration curve under the experimental conditions (Falé et al., 2013b). The results were analysed using Microsoft® Excel 2007.

2.5. Cytotoxicity studies in Caco-2 cells

This study was carried out as described in Falé et al. (2013a, 2013b) using the MTT test.

2.6. Permeation studies in Caco-2 cell line

Caco-2 cells (ATCC#HTB-37) a human colorectal adenocarcinoma epithelial cell line, were cultured as described in Falé et al. (2014). Non-purified extract and the fibres-free extract were introduced in the apical chamber and samples were withdrawn after 6 h from the apical and basolateral compartment. These samples were immediately analysed for the phenolic compounds by RP-HPLC-DAD as described in Falé et al. (2013b) and the cells were frozen at −80 °C for protein analysis. The permeation was measured as % of the amount introduced in the apical chamber and as apparent permeability coefficients (Papp). These were determined as indicated in Falé et al. (2014).

2.7. Polyacrylamide gel Electrophoresis (SDS–PAGE)

The cells (previously stored at −80 °C) that were under contact with the extracts and the control were disrupted by sonication during 10 min and then centrifuged at 4°C, 12,000*g* for 10 min. The supernatant was removed (150 μL) and proteins were quantified using the Bradford method (Bio-Rad®). A volume containing approximately 20 μg protein was used to obtain protein by precipitation with two volumes of acetone and left in the cold (4 °C) for 12 h. The samples were centrifuged at 4 °C, 12,000*g* for 10 min and then dried under nitrogen current. Electrophoresis was performed on a horizontal homogeneous precast gel 4–12% acrylamide (45 × 80 × 1.4 mm) using a GE Healthcare Life Sciences system and following the instructions provided. The precipitated protein from each sample, blank, cells under the influence of extract and mucilage-free extract, were dissolved with sample buffer and water in a ratio of 1/4. In each gel, well was added 20 μL of the sample (20 μg protein). Twenty microliters of molecular weight marker and sample buffer were also applied. Electrophoretic separation was carried out at 160 V for 80 min. The gel was stained with BlueSafe™ for one hour and photographed using ImageQuant LAS 500 system, GE Healthcare Life Sciences. The gel bands were treated and quantified using ImageJ software.

2.8. Protein identification by mass spectrometry

The protein identification was carried out as a service at Mass-Spectrometry Unit, ITQB-UNL, Oeiras, Portugal.

2.9. Acetylcholinesterase inhibition

Acetylcholinesterase inhibition was measured as described by Falé et al. (2013b) using the extract and the fibre-free extract.

2.10. Data analysis

The software used was Microsoft® Excel 2010, and the results were expressed as mean ± standard deviation. Additional analysis of variance (ANOVA) was performed with $\alpha = 0.05$ using the software developed by Microsoft®.