Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Chemical characterization and anti-inflammatory activity of luteolin glycosides isolated from lemongrass

Vera Francisco [a,b], *Artur Figueirinha* [b,c], *Gustavo Costa* [b,d], *Joana Liberal* [a,b], *Maria Celeste Lopes* [a,d], *Carmen García-Rodríguez* [e], *Carlos F.G.C. Geraldes* [a,f,g], *Maria T. Cruz* [a,d], *Maria T. Batista* [b,d,*]

[a] *Center for Neurosciences and Cell Biology, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal*

[b] *Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal*

[c] *Department of Environment, Polytechnic Institute of Viseu, Campus Politécnico de Repeses, 3504-510 Viseu, Portugal*

[d] *Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal*

[e] *Instituto de Biología y Genética Molecular, Universidad de Valladolid – CSIC, C/Sanz y Forés 3, 47003 Valladolid, Spain*

[f] *Coimbra Chemistry Center, University of Coimbra, 3004-535 Coimbra, Portugal*

[g] *Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, 3001-401 Coimbra, Portugal*

ARTICLE INFO

Article history:
Received 13 April 2014
Received in revised form 3 July 2014
Accepted 7 July 2014
Available online

Keywords:
Cymbopogon citratus
Lemongrass
Flavonoids
Luteolin
Glycosylation
Anti-inflammatory

ABSTRACT

Flavonoids from lemongrass – *Cymbopogon citratus* (DC.) Stapf – leaves infusion, a commonly consumed beverage for the treatment of inflammatory-related conditions, were investigated in this work. Luteolin *O*-, *C*- and *O*,*C*-glycosides were isolated and identified by nuclear magnetic resonance, being the cassiaoccidentalin B structure fully characterized for the first time in lemongrass. The anti-inflammatory activity of luteolin and its glycosides was evaluated in lipopolysaccharide-stimulated macrophages. Luteolin glycosides demonstrated less cytotoxicity than luteolin itself. Although glycosylation decreases luteolin anti-inflammatory properties, being higher to *C*-glycosylation, an inhibitory effect on inflammatory mediator production (nitric oxide and IL-1β) was verified for the luteolin 7-*O*-*β*-glucopyranoside, without cytotoxic effects. Therefore, luteolin glycosides from lemongrass infusion are evidenced as a less toxic alternative to current anti-inflammatory drugs with promising use in pharmaceutical and food supplement industries. Additionally, this work establishes structure–activity relationships, which constitutes valuable information in the design of anti-inflammatory luteolin glycosides devoid of toxicity.

© 2014 Elsevier Ltd. All rights reserved.

* *Corresponding author.* Tel.: +351 239 488 497; fax: +351 239 488 503.
E-mail address: mtpmb@ff.uc.pt (M.T. Batista).

http://dx.doi.org/10.1016/j.jff.2014.07.003
1756-4646/© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Cymbopogon citratus (DC.) Stapf, Poaceae, is an aromatic herb known as lemongrass, native from India and tropical Asia and currently it grows worldwide. Given its subtle citrus flavor, the fresh and dried leaves of lemongrass are common ingredients of Asian cuisine in teas, soups and curries, being also suitable for fish, seafood and poultry. In African and Latin American countries, this herb is highly consumed as an aromatic and pleasant-tasting herbal drink. Furthermore, the infusion of its aerial parts has widespread use in folk medicine to treat digestive illness, inflammation, diabetes, nervous disorders and fever (Shah et al., 2011). In recent years, our group has been involved in the identification of functional ingredients from lemongrass. We demonstrated that mono- and polymeric flavonoids, such as luteolin and apigenin glycosides and proanthocyanidins, respectively, strongly contributed to antioxidant (Figueirinha, Paranhos, Pérez-Alonso, Santos-Buelga, & Batista, 2008) and anti-inflammatory properties (Figueirinha, Cruz, Francisco, Lopes, & Batista, 2010; Francisco et al., 2011, 2013) of an essential oil-free infusion from lemongrass. However, the activities and molecular mechanisms of the purified phenolic compounds from lemongrass that may be responsible for anti-inflammatory properties have not been reported yet. In the current work, we have now focused our attention on the chemical characterization of isolated monomeric flavonoids and their anti-inflammatory activities for future potential applications as functional food ingredients.

Chronic inflammation is one of the leading causes of mortality in the Western world since it underlies the pathogenesis of diabetes, cardiovascular diseases, atherosclerosis, Alzheimer and cancer (Liu & Zeng, 2012; Osborn & Olefsky, 2012; Price et al., 2012). Actually, non-steroid anti-inflammatory drugs (NSAIDs) are widely used in clinics, but their toxic effects prompted the search for new and safe anti-inflammatory agents. Epidemiological studies evidenced a direct correlation between the dietary intake of flavonoids, characterized by a diphenylpropane structure (C6–C3–C6), and their health promoting effects (Heiss, Keen, & Kelm, 2010). Moreover, numerous experimental data have demonstrated that flavonoids, such as luteolin, have a wide range of biological activities, including antioxidant, antimicrobial, anticancer, anti-allergic and anti-inflammatory (López-Lázaro, 2009). These data highlight the valuable therapeutic potential of luteolin and luteolin derivatives, which have been previously described as lemongrass constituents by us (Figueirinha et al., 2008).

In the present work, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was used for a full characterization of flavonoids from lemongrass leaf infusion. Additionally, the anti-inflammatory activity of lemongrass isolated compounds was investigated in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages by measuring the production of pro-inflammatory mediators that raise and maintain inflammation, such as nitric oxide (NO) and cytokines, namely interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α (Fortin, McDonald, Fülöp, & Lesur, 2010; Kopf, Bachmann, & Marsland, 2010). Structure–activity relationships of luteolin glycosides were also disclosed.

2. Materials and methods

2.1. Chemicals

Dulbecco's Modified Eagle Medium, penicillin, streptomycin, LPS from *Escherichia coli* (serotype 026:B6) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide were obtained from Sigma–Aldrich Química (St. Louis, MO, USA). Fetal bovine serum was from Gibco (Paisley, UK) and Trizol® reagent was from Invitrogen (Carlsbad, CA, USA). Luteolin, luteolin 7-*O*-*β*-glucopyranoside, luteolin 6-*C*-*β*-glucopyranoside and luteolin 8-*C*-*β*-glucopyranoside were from Extrasynthese (Genay, France). iScript™ select cDNA synthesis kit and SYBR-Green were purchased to BioRad (Hercules, CA, USA). Primers were from MWG Biotech (Ebersberg, Germany).

2.2. Plant material

Dry leaves of *C. citratus* (DC.) Stapf, known as lemongrass, were purchased from ERVITAL® (Mezio, Castro Daire, Portugal) in July 2004 and kept at –20 °C until use. The plant was cultivated in the region of Mezio, Castro D'Aire (Portugal). A voucher specimen was deposited in the Herbarium of Aromatic and Medicinal Plants of the Faculty of Pharmacy – University of Coimbra (A. Figueirinha 0109). The identity of the plant was confirmed by J. Paiva (Botany Department, University of Coimbra, Portugal).

2.3. Extraction and isolation of flavonoids

An essential oil-free infusion of lemongrass was prepared and fractionated by column chromatography as previously described (Figueirinha et al., 2008). All the fractionation process was monitored by high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) for polyphenols. The flavonoid-rich fraction was used as the starting point to isolate luteolin 7-*O*-*β*-glucopyranoside, luteolin 6-*C*-*β*-glucopyranoside and luteolin 2″-*O*-rhamnosyl-*C*-(6-deoxy-ribo-hexos-3-ulosyl), commonly known as cassiaoccidentalin B. Freeze-dried flavonoid-rich fraction was dissolved in methanol and applied on chromatography cellulose paper sheets (46 × 57 cm) Whatman® 3MM (Maldstone, England), and eluted in a saturated chamber with 15% acetic acid. Each isolated spot was detected by UV (366 nm) observation and removed from the paper by 50% methanol extraction and purified on polyamide. Structural elucidation of each isolated flavonoid was achieved by ^{1}H NMR.

2.4. ^{1}H NMR analysis

^{1}H NMR spectra as well as 2D NMR data were obtained on a Varian VNMRS-600 NMR (^{1}H NMR at 600 MHz) spectrometer, at 25 °C using Methanol-d4 (CD_3OD) as solvent and pre-saturation technique to suppress the water signal. The 1D spectra were acquired in phase sensitive mode with spectral bandwidth of 5411.3 Hz (8.5 ppm–0.5 ppm) and relaxation time of 2 seconds. Each sample data were acquired using 8–16 accumulations with 16k points. Bi-dimensional spectra (2D) (gCOSY) e (TOCSY) were acquired in sensitive phase States–Haberkorn type with

Download English Version:

https://daneshyari.com/en/article/7624932

Download Persian Version:

https://daneshyari.com/article/7624932

Daneshyari.com