Chemico-Biological Interactions 225 (2015) 47–53

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Spectroscopic studies on the *in vitro* antioxidant capacity of isopentyl ferulate

Keylla C. Machado [a], George Laylson S. Oliveira [b], Élida B.V. de Sousa [c], Iwyson Henrique F. Costa [b], Katia C. Machado [b], Damião Pergentino de Sousa [c], Prabodh Satyal [d], Rivelilson Mendes de Freitas [a,b,*]

[a] Post-Graduation Program in Pharmacology, Federal University of Piauí, CEP 64.049-550 Teresina, Piauí, Brazil
[b] Laboratory of Research in Experimental Neurochemistry of Post-Graduation Program in Pharmaceutical Sciences, Federal University of Piauí, CEP 64.049-550 Teresina, Piauí, Brazil
[c] Department of Pharmaceutical Science, Federal University of Paraíba, CEP 58.051-900 João Pessoa, Paraíba, Brazil
[d] Department of Chemistry, University of Alabama in Huntsville, AL 35899, USA

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form 1 November 2014
Accepted 13 November 2014
Available online 21 November 2014

Keywords:
Antioxidant
Free radicals
Hydroxyl
Nitric oxide
Phenylpropanoids

ABSTRACT

Essential oils have played a prominent role in research on natural products, due to the high level of bioactive constituents, which include those derived from phenylpropanoids or terpenoids. This study aimed to evaluate the antioxidant capacity of isopentyl ferulate (IF) employing *in vitro* experimental models for elimination of the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$), hydroxyl ($OH^{\cdot}$) and nitric oxide (NO), as well as its capacity to electron transfer by reducing potential and inhibition of lipid peroxidation by TBARS (thiobarbituric acid reactive substances) method. In all *in vitro* antioxidants protocols, isopentyl ferulate showed to be potent in a concentration of 54.4 nM, presenting a percentage inhibition of 91.29 ± 0.57, 92.63 ± 0.28, 83.62 ± 0.18, 77.07 ± 0.72 and 79.51 ± 0.32% for $DPPH^{\cdot}$, $ABTS^{\cdot+}$, hydroxyl, nitric oxide and TBARS level, respectively. The increase of absorbance at 700 nm in the concentrations of 3.4, 6.8, 13.6, 27.2 and 54.4 nM shows the reducing potential of IF. Similar results were obtained with Trolox (559 nM), a hydrophilic synthetic analogue of α-tocopherol, which is widely used as a standard antioxidant. The present study demonstrated that isopentyl ferulate has an antioxidant activity *in vitro* experimental models, suggesting that this compound could enhance the development of a new product with antioxidant properties. However, further *in vivo* studies are needed to assign possible implications in the treatment of diseases related with free radicals.

1. Introduction

The chemicals substances that have one or more unpaired electrons are considered free radicals, which have as main characteristic that facility to donate their electrons to other molecules causing chain reactions and oxidative damage [1,2]. Free radicals and related molecules are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS) derived and a wide variety of these radicals are produced during normal metabolism in biological systems, which are counterbalanced by cellular antioxidant mechanisms. However, the imbalance by excess ROS and RNS and decreased antioxidant defense systems at cellular level cause oxidative stress, which can induced to damage by peroxidation of cellular structures, protein oxidation, DNA damage and inhibition of electron transport chain in mitochondria [3,4].

The free radicals formation during physiological processes results in the development of antioxidant defense mechanisms. Antioxidants are able to inhibit or prevent oxidation processes of vital molecules in cellular processes and can be either produced in the human body or can be absorbed from the diet [5]. Thus, it is necessary to search for new antioxidant compounds as therapeutic agents against diseases in which oxidative stress is involved in pathophysiology [6]. The use of natural antioxidants in the treatment and prophylaxis of diseases induced by free radicals has certain advantages. Most of these agents derived from natural products can produce few side effects because of its low toxicity compared to other drugs [7]. In perspective, compounds obtained from medicinal plants are relevant, and among them, the essential oils have been highlighted.

Among the groups of substances of natural origin with promising antioxidant capacity, essential oils have played a prominent

* Corresponding author at: Programa de Pós-graduação em Ciências Farmacêuticas/Universidade Federal do Piauí, Ininga, Teresina CEP 64.049-550, Piauí, Brazil. Tel./fax: +55 86 3215 5870.
E-mail address: rivelilson@pq.cnpq.br (R.M. de Freitas).

http://dx.doi.org/10.1016/j.cbi.2014.11.008
0009-2797/

role in scientific research due to its high level of bioactive components. The majority of them are derived of phenylpropanoids or terpenoids [8]. The phenylpropanoids are a diverse group of compounds derived from the carbon skeleton of phenylalanine that are involved in plant defense [9]. Among these compounds, stands out the ferulic acid that presents several biological properties as antibacterial [10], antiviral [11], neuroprotective [12] analgesic [13], spasmolytic [14], anti-inflammatory [15] and mainly antioxidant [16,17]. Furthermore, the development of their derivatives which comprise similar chemical structure are promising, especially with regard to pharmacological effects [18,19].

Considering the relevance of oxidative stress in the development of various diseases, there is a growing interest in the search for new compounds with antioxidant capacity. Thus, the present study has the objective of providing information on the antioxidant potential of synthetic compound called of isopentyl ferulate, a new ester derivative of ferulic acid. The *in vitro* antioxidant capacity was assessed by the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$), hydroxyl ($OH^{\cdot}$) and nitric oxide (NO), as well as its ability to transfer electrons by reducer potential and inhibit lipid peroxidation by TBARS method. In this study, a possible mechanism of antioxidant reactions of isopentyl ferulate (IF) has been discussed for the first time in the literature.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Trolox, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium nitroprusside (SNP), 2,2′-azobis-2-amidinopropane dihydrochloride (AAPH), 2-deoxyribose and potassium ferricyanide were from Sigma–Aldrich Co. (St. Louis, MO, USA). All chemicals and solvents used were analytical grade, and obtained from Sigma–Aldrich.

2.2. Obtaining compound

Isopentyl ferulate (3-(4-hydroxy-3-methoxyphenyl) isopentanoyl of propeonate; (Fig. 1) is a compound derived from ferulic acid, has a refractive index of 1.544 ± 0.02, surface tension of 40.3 ± 3.0 dyn/cm and density of 1.104 ± 0.06 g/cm^3. The ester was prepared as described by Khatkar et al. [20].

Process for the esterification of ferulic acid: in a stirred mixture of ferulic acid (5 mmol) in isoamyl alcohol (200 mL) was added to concentrated sulfuric acid (0.067 mL, 1.25 mmol) and the reaction mixture was subjected at reflux for 3 h in a 500 mL flask. After cooling to 25 °C, ethyl acetate was added and the solution was washed with water and brine. The ethyl acetate fraction was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on a silica gel column using 20% ethyl acetate in hexane to give isopentyl ferulate (yield 55%) [21].

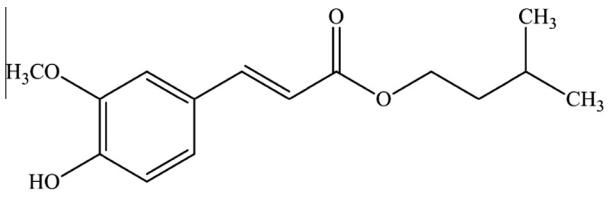

Fig. 1. Chemical structure of isopentyl ferulate.

2.3. Evaluation of antioxidant capacity of isopentyl ferulate by DPPH· radical scavenging

For evaluation of antioxidant capacity against the DPPH· radical, the methodology described by Silva et al. [22] was used with some modifications. Briefly, a reaction mixture containing the IF (3.4, 6.8, 13.6, 27.2 and 54.4 nM) with 2.7 ml of DPPH· stock solution (100 μM) was stirred vigorously and incubated at room temperature in the dark for 30 min. The antioxidant evaluation was performed in triplicate and the absorbance values were expressed as percentage inhibition of DPPH· by the following equation:

$$\%\ \text{Inhibition of DPPH}^{\cdot} = \{(A_{\text{control}} - A_{\text{reaction mixture}}) \times 100\}/A_{\text{control}}$$

in which A_{control} is the absorbance of the ethanolic solution of DPPH· and $A_{\text{reaction mixture}}$ is the absorbance of the reaction mixture containing the DPPH· radical and the concentrations of IF. The effective concentration (EC_{50}) of the IF required for 50% inhibition of DPPH· radical at 517 nm was determined. The same experimental procedure was used with the positive control Trolox (559 nM).

2.4. Evaluation of antioxidant capacity of isopentyl ferulate by $ABTS^{\cdot+}$ radical scavenging

For the determination of antioxidant capacity against the $ABTS^{\cdot+}$ radical, the methodology described by Re et al. [23] was used with some modifications. The $ABTS^{\cdot+}$ radical cation was initially formed from the reaction of 5 ml of a 7 mM ABTS in 88 μL of a 2.45 mM solution of potassium persulfate ($K_2S_2O_8$), which was incubated at room temperature in the absence of light for 16 h [24]. Elapsed this time, a solution of $ABTS^{\cdot+}$ was diluted in ethanol to obtain a solution with absorbance of 0.70 ± 0.05 at 734 nm. In the dark and at room temperature, different aliquots of IF were transferred (3.4, 6.8, 13.6, 27.2 and 54.4 nM) to a tube with 1960 μl of the $ABTS^{\cdot+}$ radical. The experiment was performed in triplicate and the absorbance readings were performed at the time of 6 min in a spectrophotometer (734 nm). The results were expressed as percentage of inhibition of the $ABTS^{\cdot+}$ radical by the following equation:

$$\%\ \text{Inhibition of ABTS}^{\cdot+} = \{(A_{\text{control}} - A_{\text{reaction mixture}}) \times 100\}/A_{\text{control}}$$

in which A_{control} is the initial absorbance of ethanolic solution of $ABTS^{\cdot+}$ and $A_{\text{reaction mixture}}$ is the absorbance of the reaction mixture containing $ABTS^{\cdot+}$ radical and the concentrations of IF. The effective concentration (EC_{50}) of IF needed to inhibit 50% of $ABTS^{\cdot+}$ radical at 517 nm was determined. The same experimental procedure was used with the positive control Trolox (559 nM).

2.5. Evaluation of antioxidant capacity of isopentyl ferulate by hydroxyl radical ($OH^{\cdot}$) scavenging

For evaluation of antioxidant capacity against the hydroxyl radical generated by Fenton reaction, the methodology described by Lopes et al. [25] was used with modifications. Briefly, various concentrations of IF (3.4, 6.8, 13.6, 27.2 and 54.4 nM) was added to the reactional medium (Fenton reaction) containing $FeSO_4$ (6 mM), 2-deoxyribose (5 mM), H_2O_2 (100 mM) and phosphate buffer (20 mM, pH 7.4). The reaction mixture was performed for 30 min at ambient temperature and terminated by the addition of phosphoric acid (4%, w/w) followed by 1% TBA (50 mM, NaOH aqueous solution). Then, the reaction mixture was heated for 15 min at 95 °C, cooled and the absorbance measured (532 nm).

The results were expressed as percentage of 2-deoxyribose degradation by the following equation: 2-deoxyribose degradation % = $\{(A_{\text{control}} - A_{\text{reaction mixture}}) \times 100\}/A_{\text{control}}$, in which, A_{control} is the initial absorbance of reaction medium generator of the