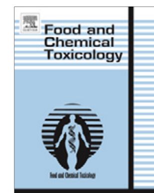
Food and Chemical Toxicology 49 (2011) 1167–1173

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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



Evaluation of antioxidant and antigenotoxic activity of two flavonoids from *Rhamnus alaternus* L. (Rhamnaceae): Kaempferol 3-O-β-isorhamninoside and rhamnocitrin 3-O-β-isorhamninoside

Wissem Bhouri [a,b], Mohamed Ben Sghaier [a,b], Soumaya Kilani [a,b], Ines Bouhlel [a,b], Marie-Geneviève Dijoux-Franca [c], Kamel Ghedira [b], Leila Chekir Ghedira [a,b,*]

[a] *Laboratoire de Biologie Cellulaire et Moléculaire, Faculté de Medecine Dentaire Monastir, Rue Avicenne, Monastir 5000, Tunisia*
[b] *Unité de pharmacognosie, 99/UR/07, Faculté de Pharmacie, Monastir Rue, Avicenne 5000, Tunisia*
[c] *Laboratoire de Botanique, Pharmacognosie et Phytothérapie, UMR CNRS 5557 Ecologie Microbienne, Faculté de Pharmacie Université Claude Bernard, Bâtiment Nétien-8 Avenue Rockefeller 69373, Lyon cedex 08, France*

ARTICLE INFO

Article history:
Received 28 October 2010
Accepted 15 February 2011
Available online 19 February 2011

Keywords:
K3O-ir
R3O-ir
Genotoxicity
Antigenotoxicity
Antioxidant capacity

ABSTRACT

The antioxidant activity of kaempferol 3-O-β-isorhamninoside (K3O-ir) and rhamnocitrin 3-O-β-isorhamninoside (R3O-ir), isolated from the leaves of *Rhamnus alaternus* L., was determined by the ability of each compound to inhibit NBT photoreduction and to scavenge the free radical $ABTS^{+\cdot}$. Genotoxic and antigenotoxic activities were assessed using the SOS chromotest.

At a concentration of 150 μg/assay the two compounds showed the most potent inhibitory activity against superoxide anion by respectively 80.4% and 85.6%. K3O-ir was a very potent radical scavenger with an IC_{50} value of 18.75 μg/ml. Moreover, these two compounds exhibit an inhibitory activity against genotoxicity induced by nitrofurantoine and aflatoxine B1 using the SOS chromotest bacterial assay system in the presence of *Escherichia coli* PQ37 strain.

In this study, we have also evaluated correlation between antigenotoxic and antioxidant effects of K3O-ir and R3O-ir. The highest correlation was showed with R3O-ir ($r = 0.999$).
© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Medicinal and spice plants, which are well known for their pharmacological activity, contain many substances that exhibit radical-scavenging properties. Among these substances, polyphenolic compounds are abundant in foods of plant origin. The application of such bioactive plant components may increase the stability of foods and, at the same time, improve their health properties associated with anti-cancer, antiallergic and anti-inflammatory activities of polyphenols in the human body (Moure et al., 2001; Rice-Evans et al., 1996). Polyphenols are believed to possess the ideal chemical structure for scavenging free radicals. It has been demonstrated *in vitro* assays that polyphenols are more active than vitamins E and C, commonaly used antioxidants (Rice-Evans et al., 1997).

Probably the most important natural polyphenols are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kähkönen et al., 1999).

The genus *Rhamnus* (Rhamnaceae), which is encountered both in temperate and in tropical countries, includes well-known medicinal species possessing various biological properties (Mai et al., 2001). Generally, *Rhamnus* species contain anthraquinones such as emodin (Wei et al., 1992) or chrysophanol (Alemayu et al., 1993; Abegaz and Peter, 1995) , their reduced forms or their glycosides (Abegaz and Peter, 1995), while some others contain flavonoids (Coskun et al., 1990; Lin and Wei, 1994; Marzouk et al., 1999). *Rhamnus alaternus* (Rhamnaceae) is a small tree located principally in the north of Tunisia, where it is known as "Oud El-khir". Traditionally, it has been used as a digestive, diuretic, laxative, hypotensive and for the treatment of hepatic and dermatological complications (Boukef, 2001). Previous studies have shown potent antioxidant, free radical scavenging, antimutagenic and antigenotoxic activities of crude extracts from *R. alaternus* (Ben Ammar et al., 2007a,b; Ben Ammar et al., 2008a,b; Chevolleau et al., 1992; Ben Ammar et al., 2005).

In our studies on the elucidation of the antioxidant, genotoxic and antigenotoxic effects of flavonoids, two triglycoside flavonoids isolated from the leaves of *R. alaternus* i.e. the K3O-ir and R3O-ir were tested.

* Corresponding author at: Laboratoire de Biologie Cellulaire et Moléculaire, Faculté de Medecine Dentaire Monastir, Rue Avicenne, Monastir 5000, Tunisia.
E-mail address: leila.chekir@laposte.net (L.C. Ghedira).

0278-6915/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2011.02.011

2. Materials and methods

2.1. Chemicals

Ampicillin; 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); the mutagens aflatoxin B1(AFB1) and nitrofurantoine (NF).

Potassium persulfate ($K_2S_2O_8$), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Trolox) from Aldrich (Steinheim, Germany). 3-Nitrotetrazolium blue chloride (NBT), Riboflavine, superoxide dismutase (SOD) and Dimethyl sulfoxide (DMSO) was obtained from Fluka (Steinheim, Germany). S9 Fraction was purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA).

2.2. Extraction method

Dried and powdered leaves (100 g) of *R. alaternus* were first defatted with petroleum ether (1 L), then extracted with chloroform (1 L), ethyl acetate (1 L), and methanol (1 L) using a Soxhlet apparatus (6 h). Four different extracts were obtained. They were concentrated to dryness and kept at 4 °C in the absence of light. Among these extracts, only the Soxhlet methanolic extract was fractioned and purified in this study.

Additionally, in order to obtain a total oligomer flavonoid (TOF) enriched extract, the powdered leaves were macerated in water:acetone mixture (1:2) for 24 h, under continuous stirring. The extract was filtered and the acetone was evaporated under low pressure, to obtain an aqueous phase. The phlobaphenes were removed by precipitation with an excess of NaCl at 5 °C for 24 h. The supernatant was extracted with ethyl acetate, concentrated and precipitated in an excess of chloroform. The precipitate was then separated and TOF extract yielded.

2.3. Fractionation and isolation methods

K3O-ir was directly obtained by fractionation of the TOF extract on a silica gel column with EtOAc:MeOH:H_2O (100:15:13) solvent system as eluent.

The methanolic extract (6 g) was fractionated by vacuum liquid chromatography (VLC) on a silica gel column eluted with CH_2Cl_2:MeOH with a gradual increase in of the MeOH content and eight fractions were collected. Fractions 5, 6 and 7 were rechromatographed over a silica gel column using an EtOAc:MeOH:H_2O (100:15:13) solvent system, to give seven subfractions. The subfraction 5 was rechromatographed on a C18 gel column using an H_2O:MeOH (70:30–0:100) gradient solvent system to afford R3O-ir.

K3O-ir and R3O-ir were identified by analysis of the negative fast atom bombardment mass spectra (FAB-MS), 1H NMR (Nuclear magnetic resonance) spectroscopy and 13C NMR spectroscopy Fig. 1 (Ben Ammar et al., 2009).

2.4. Bacterial tester strain

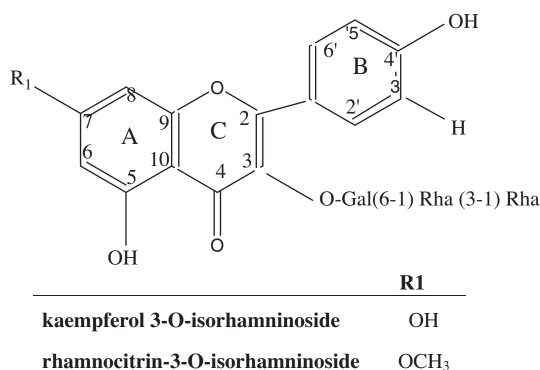
Escherichia coli PQ 37 strain was kindly provided by Prof. M. Quillardet (Institut Pasteur, Paris, France). The complete genotype, as well as strain construction details can be found in Quillardet and Hofnung (1985). Frozen permanent copies of the tester strain were prepared and stored at −80 °C.

2.5. SOS chromotest

The SOS chromotest was employed to determine the effect of K3O-ir and R3O-ir on the genotoxicity of B[a]P: indirect acting mutagen, and nitrofurantoine (direct acting mutagen) induced genotoxicity. The SOS chromotest with *Escherichia coli* PQ37 strain was performed according to the procedure described by Quillardet and Hofnung (1985). Three doses of each compound (1, 5, and 10 μg/assay) were prepared and tested in triplicate with and without an exogenous metabolic activation system (S9). Positive controls were prepared by exposure of the bacteria to either AFB1 or NF. After 2 h of incubation at 37 °C, with shaking, two sets of 300 μl samples were used for assaying β-galactosidase (β-gal) and alkaline phosphatase (AP) activities. In this assay, the β-galactosidase synthesis (lacZ gene) is dependent on sfiA activation and is used to measure the induction of SOS repair system. The activity of the constitutive enzyme alkaline phosphatase was used as a measure of protein synthesis and toxicity. Enzyme activities were assessed spectrophotometrically. The SOS induction factor (IF) in treated cells was obtained by comparing β-galactosidase/alkaline phosphatase ratio in treated and untreated cells (Kevekordes et al., 1999). For the evaluation of the protective effect of K3O-ir and R3O-ir on the induction of the SOS response by NF (in the absence of the S9 activation preparation) and AFB1 (in the presence of the S9 activation preparation), 10 μl of NF solution (10 μg/assay) or AFB1 solution (5 μg/assay) were added into tubes with 10 μl of the compound tested concentrations. Antigenotoxicity was expressed as percentage inhibition of genotoxicity induced by either NF or AFB1 according to the formula:

$$(\%)\text{Inhibition} = 100 - (\text{IF}_1 - \text{IF}_0)/(\text{IF}_2 - \text{IF}_0) \times 100$$

where IF_1 is the induction factor in the presence of the tested compound and the genotoxin, IF_2 the induction factor in the absence of the tested compound and in the presence of the genotoxin, and IF_0 is the induction factor of the negative control. Data were collected as a mean ± S.D. of three experiments.



	R1
kaempferol 3-O-isorhamninoside	OH
rhamnocitrin-3-O-isorhamninoside	OCH_3

Fig. 1. Chemical structures of compounds.

2.6. Radical-scavenging activity

An improved ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] radical cation decolorization assay was used. It involves the direct production of the blue/green $ABTS^{+\cdot}$ chromophore through the reaction between ABTS and potassium persulfate. The addition of antioxidants to the performed radical cation reduces it to ABTS, to an extent and on a timescale, depending on the antioxidant activity, the concentration of the antioxidant, and the duration of the reaction (Re et al., 1999). ABTS was dissolved in water to a final concentration of 7 mM. $ABTS^{+\cdot}$ was produced by the reaction of ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{+\cdot}$ solution was diluted with ethanol to an absorbance of 0.7(±0.02) at 734 nm. In order to measure the antioxidant activity of tested compounds, 10 μl of samples at various concentrations (0.05, 0.1, 0.15, 0.2 mg/ml) were added to 990 μl of diluted $ABTS^{+\cdot}$ and the absorbance recorded every minute. The kinetic reaction stopped when the absorbance at 734 nm was stable. Each concentration was analyzed in triplicate. The percentage decrease in absorbance at 734 nm was calculated for each point; the antioxidant capacity of the test compound was expressed in percent inhibition (%), and IC_{50} value was calculated from regression analysis. The antioxidant activity of the tested compounds was compared to that of a standard powerful antioxidant the Trolox. The results are also reported as the Trolox equivalent antioxidant capacity (TEAC), which is the molar concentration of the Trolox giving the same percentage decrease in absorbance of the $ABTS^{+\cdot}$ radical as 0.2 mg/mL of each of the tested compounds, at a specific time point (Van den Berg et al., 2000).

2.7. Superoxide mediated reduction of nitro blue tetrazolium by photochemically reduced riboflavin

The test implements two principal reactions (Liochev and Fridovich, 1995):

(a) 2 NBTH·NBT + $NBTH_2$ (Formazan).
(b) NBTH + O_2 NBT + $O_2^{\cdot}$ (a quasi-equilibrium).

When the riboflavin is photochemically activated, it reacts with the NBT to give NBTH (Beauchamp and Fridovich, 1971) that leads to formazan according to reaction (a). In the presence of oxygen, concentrations of radical species are controlled by the quasi equilibrium (b). Thus, superoxide anions appear indirectly when the test is performed under aerobic conditions. In the presence of an antioxidant that can donate an electron to NBT, the purple color typical of the formazan decays, a change that can be followed spectrophotometrically at 560 nm.

The protocol is described as follow:

Reduction of NBT was carried out at room temperature (22 °C) under fluorescent lighting (20 watt, 20 cm). The standard incubation mixture (3.5 ml) contained 6 μM riboflavin, in 16 mM phosphate buffer (pH 7.8) and 85 μM NBT. Tested compounds were dissolved in 0.2% of DMSO. After 5 min incubation, the reaction was stopped by switching off the light and the addition of 0.05 ml SOD (1 mg/ml). For each compound concentration, control sample containing 0.05 ml SOD solution, which was added before exposure to fluorescent lighting, was analyzed to rule out the possible direct reduction of NBT, by tested compounds and other reducing agents. For estimation of the superoxide-driven reduction of NBT the absorbance of a control sample was subtracted from that of standard reaction mixture.

Download English Version:

https://daneshyari.com/en/article/5854338

Download Persian Version:

https://daneshyari.com/article/5854338

Daneshyari.com