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In vitro antioxidant and antigenotoxic potentials of myricetin-3-*o*-galactoside and myricetin-3-*o*-rhamnoside from *Myrtus communis*: Modulation of expression of genes involved in cell defence system using cDNA microarray

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Abstract

Antioxidant activity of myricetin-3-*o*-galactoside and myricetin-3-*o*-rhamnoside, isolated from the leaves of *Myrtus communis*, was determined by the ability of each compound to inhibit xanthine oxidase activity, lipid peroxidation and to scavenge the free radical 1,1-diphenyl-2-picrylhydrazyl. Antimutagenic activity was assessed using the SOS chromotest and the Comet assay. The IC_{50} values of lipid peroxidation by myricetin-3-*o*-galactoside and myricetin-3-*o*-rhamnoside are respectively 160 μg/ml and 220 μg/ml. At a concentration of 100 μg/ml, the two compounds showed the most potent inhibitory effect of xanthine oxidase activity by respectively, 57% and 59%. Myricetin-3-*o*-rhamnoside was a very potent radical scavenger with an IC_{50} value of 1.4 μg/ml. Moreover, these two compounds induced an inhibitory activity against nifuroxazide, aflatoxine B1 and H_2O_2 induced mutagenicity. The protective effect exhibited by these molecules was also determined by analysis of gene expression as response to an oxidative stress using a cDNA micro-array. Myricetin-3-*o*-galactoside and myricetin-3-*o*-rhamnoside modulated the expression patterns of cellular genes involved in oxidative stress, respectively (GPX1, TXN, AOE372, SEPW1, SHC1) and (TXNRD1, TXN, SOD1 AOE372, SEPW1), in DNA damaging repair, respectively (XPC, LIG4, RPA3, PCNA, DDIT3, POLD1, XRCC5, MPG) and (TDG, PCNA, LIG4, XRCC5, DDIT3, MSH2, ERCC5, RPA3, POLD1), and in apoptosis (PARP).

Keywords: Myricetin-3-*o*-galactoside; Myricetin-3-*o*-rhamnoside; Antimutagenicity; Antioxidant capacity; cDNA array

1. Introduction

The univalent reduction of molecular oxygen results in reactive oxygen species (ROS). ROS include free radicals such as superoxide ions ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$) as well as non free radical species such as hydrogen peroxide (H_2O_2) (Halliwell and Gutteridge, 1998). Although the generation of ROS is an essential defence mechanism in some instances. In excessive concentrations or in the wrong location, it can damage proteins, carbohydrates, polyunsaturated fatty acids and DNA and may thus lead to oxidative stress and to a variety of degenerative processes and diseases (Alho and Leionen, 1999). These include immunodeficiency syndrome, heart diseases,

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neurological disorders, inflammations and cancers (Alho and Leionen, 1999; Duh, 1998; Frilich and Riederer, 1995). DNA damages may include strand breaks and base adducts, yet the truly relevant mechanisms need further research. Reactions deleterious for the DNA may proceed concomitantly. The SOS chromotest using *E. coli* PQ37, described by Quillardet and Hofnung (1985) was currently used for evaluating the genotoxicity and/or anti-genotoxicity of a wide variety of compounds (Quillardet and Hofnung, 1985).

Besides, all aerobic organisms, including human being, have antioxidant and antigenotoxic defences that protect against oxidative and genotoxic damage. Numerous damage removal and repair enzymes are present to remove or repair damaged molecules (Halliwell and Gutteridge, 1998). As enzymatic defence mechanisms, superoxide dismutase (SOD), which catalyses dismutation of superoxide anions to hydrogen peroxide, catalase (CAT), which converts H_2O_2 intramolecular oxygen and water, and selenodependent glutathione peroxidase (GPX), which catalyses the degradation of H_2O_2 and hydroperoxides, originating from unsaturated fatty acids at the expense of reduced glutathione, are important. The expression levels of genes involved in defence system against free radical and their genotoxic effects can be quantified using cDNA microarray containing probes related to genes of interest. This method is powerful and very sensitive (Schena et al., 1995 and Watson et al., 1998). Recently, microarrays have been largely used in studies of ageing (Hazane et al., 2005). Studies of gene expression may help to determine the network of genes involved in the oxidative process.

In view of several drawbacks of synthetic compounds for the human organism, examination of preparations of plant origin for this purpose has received more attention. At present, about 4000 compounds of polyphenolic structures are known. They exert a wide variety of biological actions including anticarcinogenic, antimutagenic and antioxidative activities (Yagi et al., 2002; Hayder et al., 2003, 2004, 2005; Kilani et al., 2005a,b,c and Ben Ammar et al., 2005).

M. communis (Myrtaceae) is a perennial shrub, widely distributed in the Mediterranean area. In folk medicine, the leaves are used as a mouthwash for the treatment of candidiasis (Bezanger-Bequese et al., 1975). The essential oil obtained from the leaves is used mainly in the treatment of lung disorders (Bezanger-Bequese et al., 1975). The effects of aqueous, enriched total oligomers flavonoids (TOF), hexane, chloroform, ethyl acetate, methanol extracts and essential oil from leaves of *M. communis*, on genotoxicity induced by both Aflatoxin B1 (AFB1) and nifuroxazide, were investigated in a bacterial assay system, i.e. the SOS chromotest with *E. coli* PQ 37. These extracts exhibited an important free radical scavenging activity towards the 1,1-diphenyl-2-picrylhydrazyl (DPPH·) free radical (Hayder et al., 2003, 2004). These results suggested deepening our study in order to purify and test molecules obtained from the most active extract detected previously (Hayder et al., 2004).

In our studies on the elucidation of the antioxidant and antigenotoxic effects of flavonoids, (myricetin-3-*o*-galactoside and myricetin-3-*o*-rhamnoside), isolated from *M. communis* leaves, we used a cDNA microarray, containing 82 probes including gene families involved in cell defence to oxidative stress and DNA repair (Hazane et al., 2005). Thus, we analyzed gene expression levels *via* expression of their transcripts. But, only 39 transcripts were detected.

2. Material and methods

2.1. Plant materials

M. communis var *italica* was collected from the National Park of Boukornine in the north east of Tunisia, in November 2003. Identification was carried out by Prof. Chaieb (Department of Botany, Faculty of Sciences. University of Sfax), according to the flora of Tunisia (Pottier-Alaptit, 1997). A voucher specimen has been kept in our laboratory for future reference (M.C.11-98). The leaves were shade dried, powdered and stored in a tightly closed container for further use.

2.2. Extraction method

The powdered leaves were extracted with boiling water for 15–20 min. After filtration, the extract was freeze–dried (aqueous extract). The aqueous extract (45 g) was dissolved in 100 ml of water and successively partitioned with chloroform (3 × 100 ml), ethyl acetate (3 × 100 ml) and butanol (3 × 100 ml) to provide three different extracts after concentration to dryness. The yields of the extracts were respectively 0.91%, 11.7% and 27.33%.

Myricetin-3-*o*-galactoside (Fig. 1) and myricetin-3-*o*-rhamnoside (Myricetin) (Fig. 2) were isolated from ethyl acetate fraction. Ethyl acetate extract (2 g) was subjected to sephadex LH-20 gel column chromatography with MeOH–H_2O (90:10) as eluent. Fractions 11–15 were combined (243 mg) and were rechromatographied through a C-18 silica gel column (1 cm i.d. × 5 cm) eluted with a gradient of H_2O–MeOH (100:0 → 90:10 → 80:20 → 70:30 → 60:40 → 50:50 → 40:60 → 20:80 → 0:100). One portion of

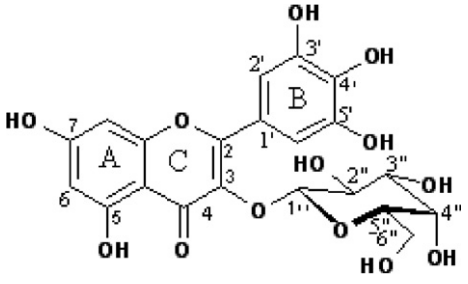

Fig. 1. Myricetin-3-*o*-galactoside.