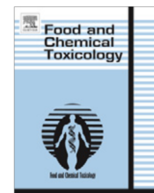
Food and Chemical Toxicology 50 (2012) 4191–4198

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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



In vitro protective effects of *Thymus quinquecostatus* Celak extracts on *t*-BHP-induced cell damage through antioxidant activity

Yon-Suk Kim [a], Seung-Jae Lee [a], Jin-Woo Hwang [a], Eun-Kyung Kim [b,d], Seong-Eun Kim [a], Ee-Hwa Kim [c], Sang-Ho Moon [d], Byung-Tae Jeon [d], Pyo-Jam Park [a,d,*]

[a] *Department of Biotechnology, Konkuk University, Chungju, Chungbuk 380-701, Republic of Korea*
[b] *Division of Food and Bioscience, Konkuk University, Chungju, Chungbuk 380-701, Republic of Korea*
[c] *Department of Acupoint and Meridian, College of Oriental Medicine, Semyung University, Jecheon, Chungbuk 390-711, Republic of Korea*
[d] *Nokyong Research Center, Konkuk University, Chungju, Chungbuk 380-701, Republic of Korea*

ARTICLE INFO

Article history:
Received 7 May 2012
Accepted 8 August 2012
Available online 17 August 2012

Keywords:
Thymus quinquecostatus Celak
Antioxidant activity
Free radical
Scavenging activity
t-BHP

ABSTRACT

The purpose of this study was to evaluate the antioxidative activities of water and 70% ethanolic extracts from the *Thymus quinquecostatus* Celak (TQC) for natural antioxidant source. The antioxidant activities were compared with other natural and synthetic antioxidants. The levels of total polyphenols and flavonoids were also determined. The extracts were found to have different levels of antioxidant properties in a few kind of assay. The results showed that higher radical scavenging activity, reducing power and antioxidant capacity in FRAP than those of BHT as a positive control. In addition, the extracts from the TQC leaf and stem showed stronger antioxidant activity than that of vitamin C, α-tocopherol in ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. Cytoprotective and anti-apoptotic effect of water extracts from TQC was also prevented *t*-BHP-induced toxicity in Chang liver cells. Therefore, these results indicate that TQC extracts have antioxidant properties through its ability to enhance the cell viability, reduction of production of ROS, inhibition of oxidative damage, mitochondria dysfunction and ultimately inhibition of cell apoptosis. Based on the results described above, it is suggested that TQC has the potential to protect liver on *t*-BHP-induced cell damage and should be considered as a prospective functional food.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Free radicals and reactive oxygen species (ROS) are mainly produced by mitochondrial oxidative metabolism and cytochrome P450 systems in hepatocytes. However, when the ROS production is greater than the detoxification capacity of the cell, excessive ROS causes extensive damage to DNA, proteins, and lipids, and acts as a mediator of pro-inflammatory, abnormal proliferative, and carcinogenic events (Simonian and Coyle, 1996; Mehendale et al., 1994). Antioxidants protect other molecules from oxidation when they are exposed to free radicals and reactive oxygen species, which have been implicated in the of many diseases and in food deterioration and spoilage (Koleva et al., 2000). Synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have been widely used as antioxidants in the food industry. However the safety of these synthetic antioxidants has been questioned. Therefore, there is an increasing interest in natural antioxidants, which might help prevent oxidative damage (Silva et al., 2005). The antioxidant phytochemicals from plants, particularly flavonoids and polyphenols, have been reported to inhibit the propagation of free radical reactions, protecting the human body from various diseases (Kinsella et al., 1993). *Thymus quinquecostatus* Celak (TQC) is a kind of aromatic herbs that belong to the Lamiaceae family. Two varieties of *T. quinquecostatus* (*T. quinquecostatus* var. japonica and *T. quinquecostatus* Celak) are found in Korea. These plants have been used as food additive, flavoring property cause of unique fragrance (Baik et al., 2009) and diaphoretics, antiflatulents, antitussives and perfumes in folk medicine. However, recent studies are nothing presence of antioxidant activity in the TQC. There are just reported to have anti-tumor effect (Sun et al., 2005), immunological effects *in vivo* (Sun et al., 2003) in TQC. *tert*-butyl hydroperoxide (*t*-BHP) is an organic lipid hydroperoxide analog, which is commonly used as a pro-oxidant for evaluating mechanisms involving oxidative stress in cells and tissues (Rush et al., 1985; Khadidja et al., 2008). Therefore, this study was mainly focused on the antioxidant effects of TQC through the contents of total polyphenols, flavonoid and the antioxidant properties in several kinds of assays, protective effect of TQC extracts against the ROS/oxidative stress in *t*-BHP induced cell damage. Thus, it could

* Corresponding author at: Department of Biotechnology, Konkuk University, Chungju, Chungbuk 380-701, Republic of Korea. Tel.: +82 43 840 3588; fax: +82 43 852 3616.
E-mail address: parkpj@kku.ac.kr (P.-J. Park).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.08.015

be a potential application of TQC as natural antioxidant supplementary foods in the food industry.

2. Materials and methods

2.1. Materials

tert-Butyl hydroperoxide (*t*-BHP), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 2′,7-dichlorodihydrofluorescin diacetate (DCFH-DA), 2,4,6-tripyridyl-s-triazine (TPTZ), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-azobis(2-amidinopropane) hydrochloride (AAPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (4-pyridyl-1-oxide)-*N*-tert-butylnitrone (4-POBN), linoleic acid, ammonium thiocyanate, 2,6-di-*tert*-butyl-4-methylphenol (BHT), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) and catechin, Potassium ferricyanide, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrous and ferric chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). In addition, the TQC was obtained at Jayang Farm (Chungju, Korea). All other reagents were of the highest grade available commercially.

2.1.1. Preparation of extracts from the TQC

The TQC was extracted with water and 70% ethanol. The 70% ethanolic extracts were prepared by two times with 70% ethanol under reflux for 1 day and filtered two times with whatman No. 41 and No. 5 at room temperature (R.T.). The filtrate was evaporated by an evaporator (EYELA, Tokyo, Japan) at 40 °C and then lyophilized in a freeze-dryer (Samwon, Busan, Korea) to obtain freeze-dried extract. The water extracts were decocted for approximately 2 h one time, filtered and then lyophilized as ethanol extracts. The dried extract was weighed, and the extract yield was then calculated and expressed as the percentage of the weight of the crude extract to the raw material (10 g).

2.1.2. Identification of the main compound

The comparative HPLC analysis of the crude extracts, authentic 2-isopropyl-5-methyl phenol (thymol) and 5-isopropyl-2 methyl phenol (carvacrol) confirmed that the chromatogram of the active peak matches that of thymol, carvacrol. In order to determine their contents, HPLC analysis was carried out according to the following conditions. HPLC (Shimadzu, Kyoto, Japan) and Alltima HP C18 column (250 × 4.6 mm) were used with methanol: water: acetic acid (60:40:2) as a mobile phase. The flow rate was 1.0 mL/min and compounds were detected at 280 nm. The retention times for thymol and carvacrol were 32.2 min and 33.9 min, respectively.

2.2. Determination of total polypenol contents

Total phenolic contents of TQC extracts were determined using Folin–Ciocalteu assay (Aline et al., 2005). Briefly, 10 mg of TQC extracts were individually dissolved in 10 mL of D.W. Then, 0.1 mL of these solutions was mixed with 50 μL of 50% Folin–Ciocalteau reagent, and 150 μL of 20% sodium carbonate (Na_2CO_3). After incubation at R.T. for 30 min, the absorbance of the reaction mixtures were measured at 760 nm by a spectrophotometer (SECOMAM, Ales, France). Gallic acid was used as a standard, and the total polyphenol contents of TQC extracts were expressed in milligram gallic acid equivalents (mg GAE/g extract).

2.3. Determination of total flavonoid contents

Total flavonoid contents were determined by the aluminum colorimetric method (Quettier-Deleu et al., 2000), using catechin as a standard. Briefly, the test samples were individually dissolved in D.W. Then, the sample solution (150 μL) was mixed with 150 μL of 2% $AlCl_3$. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 510 nm by using a spectrophotometer. The total flavonoid content was expressed as catechin equivalents in milligram per gram extract (mg CE/g extract).

2.4. Radical scavenging activity by ESR measurement

2.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using an ESR spectrometer (JES-FA machine; JOEL, Tokyo, Japan) according to the technique described by Kim et al. (2008). Sixty microlitres of each sample (or ethanol itself as control) was added to 60 μL of DPPH (60 μM) in ethanol. After 10 s of vigorous mixing, the solutions were transferred to Teflon capillary tubes and fitted into the cavity of the ESR spectrometer. The spin adducts were determined by the ESR spectrometer exactly 2 min later under the measurement conditions.

2.4.2. Alkyl radical scavenging activity

Alkyl radicals were generated by AAPH. The PBS (pH 7.4) reaction mixtures containing 40 mM AAPH, 40 mM 4-POBN and indicated concentrations of tested samples were incubated at 37 °C in a water bath for 30 min and then transferred to 100 μL Teflon capillary tube and measured the spin adduct.

2.4.3. Hydroxyl radical scavenging activity

Hydroxyl radicals were generated via the Fenton reaction, and reacted rapidly with nitrone spin trap DMPO. The resultant DMPO-OH adducts were detected using an ESR spectrometer. Reaction mixtures containing 100 μL of 0.3 M DMPO, 100 μL of 10 mM $FeSO_4$, and 100 μL of 10 mM H_2O_2 were mixed with the test samples, and then transferred to a Teflon capillary tube and determined the spin adduct.

2.5. ABTS radical scavenging activity

The total antioxidant activities of the water and 70% ethanolic extracts from TQC were measured by the $ABTS^{\cdot}$ radical cation decolorization assay involving the preformed $ABTS^{\cdot}$ radical cation (Re et al., 1999; Erkan et al., 2008). ABTS radical cation ($ABTS^{\cdot}$) was produced by the 7 mM ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at R.T. for 14 h before use. To determine the scavenging activity, 0.9 mL of ABTS reagent was mixed with 0.1 mL of extracts and the absorbance was measured at 734 nm after 6 min of reaction at R.T. The antioxidant activities of TQC extracts were expressed by trolox equivalents antioxidant capacity (TEAC), as mM trolox equivalents/mg extract.

2.6. FRAP (ferric reducing antioxidant power) assay

For obtaining the antioxidant capacity, the FRAP (ferric reducing antioxidant power) method was conducted according to Benzie and Strain (1996). To conduct the assay, a 3 mL aliquot of a FRAP reagent, a mixture of 0.3 M acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride (10:1:1 v/v/v), were combined with 1 mL of TQC extract. To determine the antioxidant capacity of the samples, the absorbance values were compared with those obtained from the standard curves of $FeSO_4$ (0–10 mM). The antioxidant capacity values were expressed as mM $FeSO_4$ equivalent in mg extract (mM $FeSO_4$ eq./mg extract).

2.7. Reducing power

The reducing power of the TQC extracts was determined according to the method described by Chung et al. (2005). A 0.25 mL aliquot of each extracts (0.1–1.0 mg/mL), BHT (0.1–1.0 mg/mL) were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. 0.25 mL of 10% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 13,000*g* for 5 min. The supernatant (0.5 mL) was mixed with 0.5 mL distilled water and 0.1% $FeCl_3$ (0.1 mL), and then the absorbance was measured at 700 nm against blank sample. BHT was used as a positive control. A higher absorbance of the reaction mixture indicated greater reducing power.

2.8. Lipid peroxidation inhibition assay in linoleic acid system

2.8.1. Ferric thiocyanate (FTC) method

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The method was followed of Chang et al. (2002). A reaction solution, containing extracts (4 mL, 1.0 mg/mL), 2.51% linoleic acid emulsion (4 mL), phosphate buffer (8 mL, 0.05 M, pH 7.0) and D.W (3.9 mL) was placed in a glass vial with a screw cap and mixed with a vortex mixer. The reaction mixture was incubated at 40 °C in the dark and the degree of oxidation was measured according to the thiocyanate method. The peroxide value was determined by recording the absorbance at 500 nm every 2 days until the absorbance of the control reached a maximum.

2.8.2. Thiobarbituric acid (TBA) method

The samples prepared for FTC method were used for this assay. To 1 mL of the sample solution in a 10 mL tube, was added 2 mL of 20% aqueous trichloroacetic acid and 2 mL of 0.67% aqueous thiobarbituric acid by kikuzaki and Nakatani (1993). The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance of the final day of TBA assay.

2.9. Cell viability

The cell viability was estimated by MTT assay, which is a test of normal metabolic status of cells based on the assessment of mitochondrial activities (Je et al., 2009). Chang liver cells were seeded in 96-well plate at a concentration of 4.0×10^5 cells/mL. After 20 h, the cells were treated with different concentrations of various ethanol extracts from TQC, and incubated in a humidified incubator at 37 °C for 1 h. Then, 80 μM *t*-BHP was added as final concentration, and incubated for 24 h. Thereafter, a 100 μL of MTT stock solution (0.5 mg/mL) was added and incubated for 4 h. Then, the supernatants were aspirated and the formazan crystals in each well were dissolved in 150 μL of DMSO. Absorbance was measured by spectroflurometer (SpectraMax M2/M2e, CA, USA) at a wavelength of 540 nm. The optical density of the formazan formed in the control cells was taken as 100%.

Download English Version:

https://daneshyari.com/en/article/5852376

Download Persian Version:

https://daneshyari.com/article/5852376

Daneshyari.com