Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 861–868
Food Chemistry
www.elsevier.com/locate/foodchem

# Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts

Jelena Kukić [a], Višnja Popović [a], Silvana Petrović [a,*], Pavel Mucaji [b], Ana Ćirić [c], Dejan Stojković [c], Marina Soković [c]

[a] *Institute of Pharmacognosy, Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia*
[b] *Department of Pharmacognosy and Botany, Pharmaceutical Faculty, Comenius University, Odbojarov 10, 83232, Bratislava, Slovak Republic*
[c] *Mycological Laboratory, Department for Plant Physiology, Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia*

Received 11 July 2007; received in revised form 27 July 2007; accepted 3 September 2007

**Abstract**

The whole, fresh involucral bracts of cardoon, *Cynara cardunculus* L. (Compositae), were extracted with EtOH and an aqueous suspension of the obtained EtOH extract was partitioned successively with $CHCl_3$, EtOAc and *n*-BuOH, leaving a residual water extract. All obtained extracts were evaluated for their antioxidant and antimicrobial properties. The antioxidant potential was evaluated using following *in vitro* methods: FRAP (ferric reducing antioxidant power) assay, and scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Antimicrobial activity was estimated using a microdilution technique against food-borne, mycotoxin producers and human pathogenic bacteria and micromycetes. The following bacteria were tested: *Salmonella typhimurium*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, as well as micromycetes: *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium ochrochloron*, *Penicillium funiculosum*, *Trichoderma viride*, *Fusarium tricinctum* and *Alternaria alternata*. Results showed that all extracts possessed concentration-dependent antioxidant activity. In biological assays, *C. cardunculus* extracts showed antimicrobial activity comparable with standard antibiotics.


*Keywords:* *Cynara cardunculus*; Involucral bracts; Antioxidant activity; FRAP; DPPH; Antimicrobial activity

## 1. Introduction

Cardoon or wild artichoke (*Cynara cardunculus* L., Compositae) is a perennial plant, which shares a recent common ancestor with the modern cultivated "globe" artichoke, *C. scolymus* L. Both plants have their origin in edible *Cynara* cultivars used by early farmers in the Mediterranean region (Kelly & Pepper, 1996). Traditional applications of *C. cardunculus* consider the usage of the blanched leaves, fleshy leaf petioles and the receptacle in soups, stews and salads (do Amaral Franco, 1976; Fernandez, Curt, & Aguado, 2006; Grieve, 1971). There are reports of usage of its petioles and roots if properly prepared (Kelly & Pepper, 1996). Flowers of *C. cardunculus* are rich in proteases, namely cardosins A and B, due which aqueous extracts of its flowers have been used for

*Abbreviations*: ATCC, American type of culture collection; *n*-BuOH, *n*-butanol; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; $CHCl_3$, chloroform; DMSO, dimethylsulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DSM, Deutsche Sammlung von Mikroorganismen; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; EtOH, ethanol; FC reagent, Folin–Ciocalteu reagent; FRAP assay, ferric reducing antioxidant power assay; IAM, Institute of Applied Microbiology, University of Tokyo, Japan; MA, malt agar; MBC, minimum bactericidal concentration; MeOH, methanol; MFC, minimum fungicidal concentration; MH, Müller–Hinton; MIC, minimum inhibitory concentration; NP/PEG reagent, natural products-polyethylene glycolreagent; TAA, total antioxidant activity; TPTZ, 2,4,6-tris(2-pyridyl)-*s*-triazine.

* Corresponding author. Tel.: +381 11 39 70 379/722; fax: +381 11 39 72 840.
*E-mail address:* silvana@pharmacy.bg.ac.yu (S. Petrović).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.005

centuries in the Iberian Peninsula for manufacturing of ovine and/or caprine milk cheeses (Fernandez et al., 2006; Silva & Malcata, 2005). Cardoon is traditionally used as a diuretic, choleretic, cardiotonic and an antihemorrhodial (Koubaa, Damak, McKillop, & Simmonds, 1999) agent. Cardoon leaves are used for their cholagogue, choleretic and choliokinetic actions, for treatment of dyspepsia and as antidiabetics (Koubaa et al., 1999; Paris & Moyse, 1971).

Previous chemical investigations have shown the presence of saponins, sesquiterpene lactones, flavones, sterols, coumarins and lignans in leaves and seeds of *C. cardunculus* (Koubaa & Damak, 2003; Pinelli et al., 2007; Ševčikova, Glatz, & Slanina, 2002; Valentao et al., 2002). In involucral bracts of the investigated species were identified sterols, triterpenoid saponins, coumarins, flavonoids and caffeic acid derivatives (Mučaji, Grančai, Nagy, Višňovská, & Ubik, 2000).

The antioxidant activity of a lyophilized aqueous extract of cardoon leaves and against superoxide radical is reported (Valentao et al., 2002). Mono- and dicaffeoylquinic acids which are present in cardoon extracts showed anti-HIV integrase activity (Slanina et al., 2001). Triterpenoid saponins, isolated from involucral bracts of *C. cardunculus*, reduce the chemically induced mutagenesis *in vitro* (Križkova, Mučaji, Nagy, & Krajčovič, 2004) and possess anticomplement activity (Mučaji, Bukovsky, Grančai, & Nagy, 2003). Recent study showed that *C. cardunculus* leaf extract prevents the age-associated loss of vasomotor function (Rossoni, Grande, Galli, & Visioli, 2005).

The objectives of this study were to investigate antioxidant and antimicrobial activity of various extracts from *C. cardunculus* involucral bracts, as well as activity of some compounds previously isolated therein.

## 2. Materials and methods

### 2.1. Chemicals

Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma Chemical Co. (St. Louis, USA), L-ascorbic acid from Lachema (Neratovice, Czech Republic); Müeller–Hinton agar (MH), malt agar (MA) from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia), streptomycin (Streptomicin-sulfat, ampoules 1 g) and miconazole from Galenika, a.d. (Belgrade, Serbia). Standard compounds **1–9**, namely: apigenin (**1**), cynarasaponins A + H (**2**), luteolin 7-glucoside (**3**), apigenin 7-rutinoside (**4**), luteolin (**5**), chlorogenic acid (**6**), β-sitosterol (**7**), cynarasaponins B + K (**8**) and apigenin 7-glucoside (**9**), were isolated previously from *C. cardunculus* involucral bracts at the Department of Pharmacognosy and Botany, Pharmaceutical Faculty, Comenius University, in Bratislava.

### 2.2. Plant material

The whole involucral bracts of *C. cardunculus* were collected from plants grown at the Medicinal Plants Garden in Bratislava. A voucher specimen was deposited at the Pharmaceutical Faculty, Comenius University, Bratislava.

### 2.3. Extraction

The whole, fresh involucral bracts were cut into pieces and repeatedly extracted with EtOH (96%, v/v) at room temperature. Aqueous suspension of the concentrated EtOH extract was partitioned successively with $CHCl_3$, EtOAc and *n*-BuOH, leaving a residual water extract. All obtained extracts, including the residual water extract, were evaporated to dryness and used for all investigations.

### 2.4. Determination of total phenolics content

Total phenolics content was determined with the Folin–Ciocalteu (FC) reagent as previously described (Velioglu, Mazza, Gao, & Oomah, 1998). 100 µl of the extract dissolved in methanol were mixed with 750 µl of FC reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 750 µl of $Na_2CO_3$ (60 g/l) solution were added to the mixture. After 90 min, the absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents (mg of gallic acid/mg dry weight extract).

### 2.5. Antioxidant activity

#### 2.5.1. Thin-layer chromatography

Each extract and previously isolated compounds (**1–9**) were dissolved in appropriate solvent, applied to silica gel plates (Merck, Darmstadt, Germany), and developed using different solvent systems: EtOAc/HCOOH/glacial AcOH/water (100:11:11:26, v/v/v/v), toluene/EtOAc/HCOOH (5:4:1, v/v/v), and toluene/EtOAc (7:3, v/v) systems. Components were detected by spraying with NP/PEG reagent (flavonoids, phenolic acids) and with vanillin-sulphuric acid (VS) reagent (saponins and sterols) (Wagner & Bladt, 1996). The DPPH test, performed directly on TLC plates (0.2% DPPH in MeOH (w/v) used as spray reagent), revealed contributions to the antioxidant activity of different compounds separately (Cuendet, Hostettmann, & Potterat, 1997).

#### 2.5.2. FRAP assay

Total antioxidant activity (TAA) was investigated using the ferric reducing antioxidant power (FRAP) assay, which is based upon reduction of $Fe^{3+}$-TPTZ complex under acidic conditions. Increase in absorbance of blue-coloured ferrous form ($Fe^{2+}$-TPTZ complex) is measured at 593 nm. FRAP reagent was freshly prepared by mixing 25 ml of acetate buffer (300 mM, pH 3.6), 2.5 ml of TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml of $FeCl_3$

(20 mM) in water solution. 100 μl of each extract dissolved in appropriate solvent were added to 4.5 ml of FRAP reagent, stirred and incubated for 30 min; absorbance was measured at 593 nm, using FRAP working solution as blank. A calibration curve of ferrous sulfate (100–1000 μM) was used, and results were expressed in μmol $Fe^{2+}$/mg dry weight of extract. The relative activity of the samples was compared to L-ascorbic acid (Pellegrini et al., 2003).

#### 2.5.3. DPPH radical assay

Extracts were dissolved in appropriate solvents, mixed with 1 ml of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in MeOH, and final volume was adjusted to 5 ml. Mixtures were virgously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using MeOH as blank. 1 ml of 0.5 mM DPPH diluted in 4 ml of MeOH was used as control. Neutralisation of DPPH radical was calculated using the equation: $S(\%) = 100 \times (A_0 - A_s)/A_0$, where $A_0$ is the absorbance of the control (containing all reagents except the test compound), and $A_s$ is the absorbance of the tested sample. The $SC_{50}$ value represented the concentration of the extract that caused 50% of neutralisation (Cuendet et al., 1997). Results were compared with the activity of L-ascorbic acid.

### 2.6. Bioassays

#### 2.6.1. Test on antibacterial activity

In order to obtain quantitative data for extracts and previously isolated compounds (**1–9**), the modified microdilution technique was used (Daouk, Dagher, & Sattout, 1995; Hanel & Raether, 1998). The following bacteria were tested: *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 29213). The organisms tested were obtained from the Department for Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

The bacterial suspension was adjusted with sterile saline to a concentration of approximately $1.0 \times 10^7$ cells/ml. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid Müller–Hinton (MH) agar (Institute of Immunology and Virology, Torlak, Belgrade, Serbia) to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique, using 96-well microtitre plates. The bacterial inocula applied contained approximately $1.0 \times 10^5$ cells in a final volume of 100 μl/well. The extracts and compounds tested were dissolved in DMSO (0.1–1.0 mg/ml) and added to broth medium with bacterial inocula. The microplates were incubated for 24 h at 37 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations which completely inhibited bacterial growth (MICs). The minimum bactericidal concentrations (MBCs) were determined by serial subcultivation of 2 μl in microtitre plates containing 100 μl of broth per well and further incubation for 24 h at 37 °C. The lowest concentration with no visible growth was defined as the MBC, indicating ⩾99.5% killing of the original inoculum. DMSO was used as a negative control, while streptomycin was used as a positive control (0.5–2.0 μg/ml). Dilutions of the inocula were also cultured on solid MH to verify the absence of contamination and to check their validity.

#### 2.6.2. Test on antifungal activity

Antifungal activity of the extracts and previously isolated compounds (**1–9**) was investigated using the modified microdilution technique (Daouk et al., 1995; Hanel & Raether, 1998). For the bioassays, eight fungi were tested: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus flavus* (ATCC 9643), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), *Trichoderma viride* (IAM 5061), *Fusarium tricinctum* (CBS 514478) and *Alternaria alternata* (DSM 2006). The organisms tested were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological research "Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar (MA) and the cultures were stored at +4 °C and subcultured once a month (Booth, 1971).

The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately $1.0 \times 10^5$ in a final volume of 100 μl/well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The compounds and extracts investigated were dissolved in DMSO (0.1–1.0 mg/ml) and added to broth malt medium with fungal inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μl in microtitre plates containing 100 μl of malt broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating ⩾99.5% killing of the original inoculum. DMSO was used as a negative control, while miconazole was used as a positive control (0.1–5.0 μg/ml).

### 2.7. Statistical analysis

The results of the experiments were analyzed by two factorial analysis of variance (ANOVA). The Package programme Statistica (release 4.5, Copyright StatSoft, Inc. 1993) was used for statistical evaluation. Antioxidant activity and determination of total phenolics content were run

in triplicates. Experiments on antimicrobial activity were replicated twice on the same occasions. All analyses were done in triplicate for each replicate ($n = 2 \times 3$).

## 3. Results

Total phenolic contents were 0.203, 0.062, 0.050, 0.046 and 0.026 mg of gallic acid equivalent/mg dry weight for EtOAc, *n*-BuOH, EtOH, water and $CHCl_3$ extracts of *C. cardunculus* whole involucral bracts, respectively (Table 1).

Total antioxidant activities (TAA) of the investigated extracts were 0.38, 0.36, 0.35, 0.34 and 0.12 μmol $Fe^{2+}$/mg dry weight for EtOAc, *n*-BuOH, EtOH, water and $CHCl_3$ extracts, respectively. L-Ascorbic acid, used as a standard, had a TAA of 7.41 μmol $Fe^{2+}$/mg (Table 1). Scavenging of DPPH radical was concentration-dependent. EtOAc extract expressed the strongest activity ($SC_{50}$ = 21.50 μg/ml), while *n*-BuOH, EtOH and water extracts showed moderate activities ($SC_{50}$ = 127.10, 157.00 and 173.15 μg/ml, respectively). The $CHCl_3$ extract did not reach 50% of DPPH· neutralisation at the highest concentration applied (Table 1).

The TLC-DPPH· test showed that phenolic compounds were the main antioxidant components in the investigated extracts. The most prominent anti-DPPH zones were revealed only few seconds after spraying with DPPH· reagent, in chromatograms of EtOAc, *n*-BuOH and EtOH extracts. According to applied standards, the main "scavengers" were apigenin (**1**), luteolin (**5**), apigenin 7-glucoside (**9**), and luteolin 7-glucoside (**3**) previously isolated from EtOAc extract (Grančai, Nagy, Suchý, & Ubik, 1993), as well as apigenin 7-rutinoside (**4**) and chlorogenic acid (**6**) from the *n*-BuOH extract (Grančai, Mučaji, Nagy, & Ubik, 1996; Mučaji et al., 2000). Cynarasaponins (**2**, **8**), previously isolated from *n*-BuOH extract (Mučaji, Grančai, Nagy, Budéšínský, & Ubik, 1999; Mučaji, Grančai, Nagy, Budéšínský, & Ubik, 2001), and β-sitosterol (**7**) from $CHCl_3$ extract (Grančai, Nagy, Suchý, & Ubik, 1992), did not express any scavenging activity.

The results of testing of antibacterial activity of *C. cardunculus* extracts showed that the EtOAc extract was the most effective (with MICs of 1.0–1.5 mg/ml and MBCs 1.5–2.0 mg/ml), followed by the EtOH, $CHCl_3$, water and *n*-BuOH extracts. *S. typhimurium* was found to be the most resistant species, with MICs of 1.5–2.0 mg/ml and MBCs of 2.0–2.5 mg/ml. *E. coli* was the most sensitive, with MICs of 1.0–1.5 mg/ml and MBCs of 1.5–2.0 mg/ml. Commercial streptomycin showed higher antibacterial potency than did the extracts tested (Table 2). Considering the antifungal potential of the investigated *C. cardunculus* extracts, the EtOAc extract was also the most effective, with values of MICs and MFCs in the equal range of 1.0–1.5 mg/ml (Table 3). Miconazole showed stronger antifungal activity than did the extracts tested. As for the standard compounds, the uppermost antibacterial, as well as the highest antifungal activity was observed for luteolin (**5**) with MICs and MBCs

Table 1
Antioxidant activity and total phenolics content of *Cynara cardunculus* extracts

| Extract | FRAP value[a] | DPPH· scavenging[b] | Total phenolics content[c] |
|---|---|---|---|
| EtOAc | 0.38 ± 0.01 | 21.50 ± 1.87 | 0.203 ± 0.018 |
| BuOH | 0.36 ± 0.01 | 127.10 ± 0.88 | 0.062 ± 0.019 |
| EtOH | 0.35 ± 0.01 | 157.00 ± 0.16 | 0.050 ± 0.010 |
| $H_2O$ | 0.34 ± 0.01 | 173.15 ± 0.65 | 0.046 ± 0.007 |
| $CHCl_3$ | 0.12 ± 0.02 | – | 0.026 ± 0.002 |
| L-Ascorbic acid | 7.41 ± 0.05 | 4.09 ± 0.08 | – |

[a] In μmol $Fe^{2+}$/mg dry weight of extract.
[b] $SC_{50}$, μg/ml.
[c] mg of gallic acid equivalents/mg dry weight of extract.

Table 2
Minimum inhibitory and bactericidal concentrations (MICs and MBCs) of *Cynara cardunculus* extracts (mg/ml)

| Bacteria | | Extracts | | | | | Streptomycin |
|---|---|---|---|---|---|---|---|
| | | BuOH | EtOH | EtOAc | $CHCl_3$ | $H_2O$ | |
| *S. typhimurium* | MIC | 2.0 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.0 | 2.0 ± 0.2 | 2.0 ± 0.1 | 0.0010 ± 0.0002 |
| | MBC | 2.5 ± 0.3 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.5 ± 0.3 | 2.0 ± 0.1 | 0.0010 ± 0.0002 |
| *E. coli* | MIC | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.5 ± 0.0 | 1.0 ± 0.0 | 0.0005 ± 0.0001 |
| | MBC | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.0 | 2.0 ± 0.2 | 1.5 ± 0.1 | 0.0010 ± 0.0002 |
| *S. epidermidis* | MIC | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.1 | 1.5 ± 0.0 | 1.5 ± 0.0 | 0.0010 ± 0.0000 |
| | MBC | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 0.0010 ± 0.0000 |
| *S. aureus* | MIC | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 0.0010 ± 0.0002 |
| | MBC | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.2 | 0.0010 ± 0.0003 |
| *B. subtilis* | MIC | 2.0 ± 0.2 | 2.0 ± 0.2 | 1.0 ± 0.0 | 1.5 ± 0.0 | 1.0 ± 0.0 | 0.0005 ± 0.0000 |
| | MBC | 2.5 ± 0.0 | 2.0 ± 0.0 | 1.0 ± 0.0 | 2.0 ± 0.2 | 1.0 ± 0.0 | 0.0005 ± 0.0002 |

Table 3
Minimum inhibitory and fungicidal concentrations (MICs and MFCs) of *Cynara cardunculus* extracts (mg/ml)

| Fungal species | | Extracts | | | | | Miconazole |
|---|---|---|---|---|---|---|---|
| | | BuOH | EtOH | EtOAc | $CHCl_3$ | $H_2O$ | |
| *A. flavus* | MIC | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.0005 ± 0.0000 |
| | MFC | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.5 ± 0.0 | 2.0 ± 0.0 | 0.0020 ± 0.0002 |
| *A. niger* | MIC | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.0015 ± 0.0003 |
| | MFC | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.0 | 1.5 ± 0.0 | 2.0 ± 0.2 | 0.0040 ± 0.0002 |
| *A. ochraceus* | MIC | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.0015 ± 0.0002 |
| | MFC | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.0 ± 0.0 | 0.0040 ± 0.0004 |
| *P. funiculosum* | MIC | 1.5 ± 0.1 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.0020 ± 0.0000 |
| | MFC | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.0050 ± 0.0000 |
| *P. ochrachloron* | MIC | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.0020 ± 0.0002 |
| | MFC | 1.5 ± 0.2 | 1.5 ± 0.1 | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.0050 ± 0.0000 |
| *T. viride* | MIC | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.0020 ± 0.0000 |
| | MFC | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.0 | 0.0020 ± 0.0000 |
| *F. tricinctum* | MIC | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.2 | 0.0002 ± 0.0000 |
| | MFC | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.5 ± 0.0 | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.0010 ± 0.0002 |
| *A. alternata* | MIC | 1.5 ± 0.2 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.0 | 0.0002 ± 0.0000 |
| | MFC | 1.5 ± 0.0 | 1.5 ± 0.1 | 1.0 ± 0.0 | 1.5 ± 0.0 | 1.0 ± 0.0 | 0.0010 ± 0.0002 |

ranging from 0.05 to 0.10 mg/ml, and MICs and MFCs ranging from 0.03 to 0.10 mg/ml (Tables 4 and 5).

## 4. Discussion

Many studies report the polyphenolic composition of cultivated and wild artichokes. The major class of polyphenols in *C. scolymus* is caffeic acid derivatives (Mulinacci et al., 2004) which, in heads, mainly occurs as esters with quinic acid; leaves and heads of globe artichoke have also been found to be rich in mono- and dicaffeoylquinic compounds and flavonoids (Alamanni & Cossu, 2003; Fratianni, Tucci, De Palma, Pepe, & Nazzaro, 2007; Pinelli et al., 2007; Schutz, Kammerer, Carle, & Schieber, 2004; Wang et al., 2003). As for cardoon, *C. cardunculus*, there are reports on phenolic composition of its leaves: caffeoylquinic acids and glycosides of luteolin and apigenin were identified using HPLC (Pinelli et al., 2007; Valentao et al., 2002). In the involucral bracts of this plant, various compounds were also identified: β-sitosterol, sitosteryl-3β-glucoside, sitosteryl-3β-acetate, taraxasterole and taraxasteryl-3β-acetate (Grančai et al., 1992), apigenin, apigenin 7-glucoside, luteolin and luteolin 7-glucoside (Grančai et al., 1993), apigenin 7-rutinoside, luteolin 7-rutinoside (Grančai et al., 1996), and apigenin 7-methylglucuronide (Mučaji et al., 2000), scopolin and scopoletin (Grančai, Nagy, Mučaji, Suchý, & Ubik, 1994a), cynarin (Grančai, Nagy, Suchý, & Novomeský, 1994b) and chlorogenic acid (Mučaji et al., 2000), cynarasaponins A and H, and their methyl derivatives (Mučaji et al., 1999), and cynarasaponins B and K (Mučaji et al., 2001).

As previously shown, apigenin, luteolin and their glycosides are powerful antioxidants (Kwon, Kim, Kim, Kim, & Kim, 2002; Müller, Vasconcelos, Coelho, & Biavatti, 2005). The antioxidant effectiveness of apigenin was determined in models, such as the *in vitro* lipoprotein oxidation model (Vinson, Dabbagh, Serry, & Jang, 1995). The antioxidant properties of luteolin 7-glucoside and of the respective aglycone, luteolin, have already been observed against low-density lipoprotein oxidation (Brown & Rice-Evans, 1998), DPPH free radical scavenging activity and $ABTS^{\cdot+}$ radical cation-scavenging effects (Wang et al., 1998).

Chlorogenic acid is one of the most abundant phenolic acids in various plant extracts and also the most active antioxidant constituent. It has been shown that the antioxidant activities of 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid are almost the same as those of chlorogenic acid when assayed for scavenging activity on superoxide anion radicals and inhibitory effect against oxidation of methyl linoleate (Takeoka & Dao, 2003). 3,4-Di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, along with chlorogenic acid, inhibited lipid peroxidation and exhibited neuroprotective activities (Nakajima, Shimazawa, Mishima, & Hara, 2007).

β-Sitosterol generally showed low antioxidant activity, compared to different phenolics, such as flavonoids, caffeic and chlorogenic acid, but it exhibited a higher lipid peroxidation inhibition rate (Yokota et al., 2006). Antioxidant activity of β-sitosterol, determined by the oxidative stability instrument (OSI), was considerable (Weng & Wang, 2000), and even much stronger than that of α-tocopherol (Jiang & Wang, 2006). It seems that β-sitosterol, which inhibits active oxygen produced by neutrophyls, exerts its antioxidative action through a preventive action, such as stabilization of the cell membrane. Caffeic acid derivatives and polyphenols that capture hydroxyl and superoxyde anion radicals act as radical-scavengers, while β-sitosterol exerts a preventive action by inhibiting the excess production of active oxygen by various cells (Yokota et al., 2006).

Table 4
Minimum inhibitory and bactericidal concentrations (MICs and MBCs) of the compounds tested[a] (mg/ml)

| Bacteria | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Streptomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. typhimurium* | MIC | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.05 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.0010 ± 0.0002 |
| | MBC | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.05 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.0010 ± 0.0002 |
| *E. coli* | MIC | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.0005 ± 0.0001 |
| | MBC | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.0010 ± 0.0002 |
| *S. epidermidis* | MIC | 0.15 ± 0.02 | 0.15 ± 0.00 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.10 ± 0.01 | 0.15 ± 0.00 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.15 ± 0.02 | 0.0010 ± 0.0000 |
| | MBC | 0.20 ± 0.00 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.01 | 0.10 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.0010 ± 0.0000 |
| *S. aureus* | MIC | 0.15 ± 0.00 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.01 | 0.05 ± 0.00 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.01 | 0.0010 ± 0.0002 |
| | MBC | 0.20 ± 0.02 | 0.20 ± 0.00 | 0.20 ± 0.02 | 0.20 ± 0.01 | 0.05 ± 0.00 | 0.20 ± 0.02 | 0.20 ± 0.00 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.0010 ± 0.0003 |
| *B. subtilis* | MIC | 0.15 ± 0.02 | 0.15 ± 0.00 | 0.15 ± 0.00 | 0.15 ± 0.02 | 0.05 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.0005 ± 0.0000 |
| | MBC | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.02 | 0.20 ± 0.00 | 0.10 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.00 | 0.15 ± 0.02 | 0.0005 ± 0.0002 |

[a] Apigenin (**1**), cynarasaponins A + H (**2**), luteolin 7-glucoside (**3**), apigenin 7-rutinoside (**4**), luteolin (**5**), chlorogenic acid (**6**), β-sitosterol (**7**), cynarasaponins B + K (**8**) and apigenin 7-glucoside (**9**).

Table 5
Minimum inhibitory and fungicidal concentrations (MICs and MFCs) of the compounds tested[a] (mg/ml)

| Fungal species | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Miconazole |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. flavus* | MIC | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0005 ± 0.0000 |
| | MFC | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0020 ± 0.0002 |
| *A. niger* | MIC | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0015 ± 0.0003 |
| | MFC | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0040 ± 0.0002 |
| *A. ochraceus* | MIC | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.01 | 0.05 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.0015 ± 0.0002 |
| | MFC | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0040 ± 0.0004 |
| *P. funiculosum* | MIC | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.01 | 0.0020 ± 0.0000 |
| | MFC | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.00 | 0.10 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.0050 ± 0.0000 |
| *P. ochrachloron* | MIC | 0.05 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.01 | 0.0020 ± 0.0002 |
| | MFC | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.10 ± 0.01 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.01 | 0.0050 ± 0.0000 |
| *T. viride* | MIC | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.01 | 0.05 ± 0.02 | 0.0020 ± 0.0000 |
| | MFC | 0.05 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.10 ± 0.01 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0020 ± 0.0000 |
| *F. tricinctum* | MIC | 0.05 ± 0.02 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.00 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.0002 ± 0.0000 |
| | MFC | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.00 | 0.10 ± 0.02 | 0.0010 ± 0.0002 |
| *A. alternata* | MIC | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0002 ± 0.0000 |
| | MFC | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.0010 ± 0.0002 |

[a] Apigenin (**1**), cynarasaponins A + H (**2**), luteolin 7-glucoside (**3**), apigenin 7-rutinoside (**4**), luteolin (**5**), chlorogenic acid (**6**), β-sitosterol (**7**), cynarasaponins B + K (**8**) and apigenin 7-glucoside (**9**).

Results of our experiments are consistent with previous data reported (Alamanni & Cossu, 2003). As main antioxidant compounds in investigated *C. cardunculus* extracts, we identified flavones: apigenin and luteolin, and their glycosides, as well as chlorogenic acid. The highest antioxidant activity of the EtOAc extract could be explained, among other causes, by the presence of apigenin and luteolin in significantly larger amounts than in other extracts.

Our experiments showed substantial antimicrobial activities of *C. cardunculus* involucral bract extracts, with MICs, MBCs and MFCs of 1.00–2.50 mg/ml. The EtOAc extract was again the most effective.

Mossi and Echeverrigaray (1999) found that $CH_2Cl_2$ *C. scolymus* leaf extract, in concentrations of 5 mg/ml, completely inhibited the growth, with a bactericidal effect on *Staphylococcus aureus*, *Bacillus cereus* and *B. subtilis*. Zhu, Zhang, and Lo (2004) investigated antimicrobial activities of different extracts of *C. scolymus* leaf and showed that the *n*-BuOH fraction was the most active, followed by the $CHCl_3$ and EtOAc fractions. Similar investigations were done with successive $CHCl_3$, EtOH, and EtOAc partitions of extracts of *C. scolymus* leaf, head, and stem. The MIC values for fungi were at or below 2.5 mg/ml and for bacteria were at or above 2.5 mg/ml (Zhu, Zhang, Lo, & Lu, 2005).

The results of our experiment show that all standard compounds, previously isolated from involucral bracts of *C. cardunculus*, possess antimicrobial activity against all tested strains of bacteria and fungi (MICs, MBCs and MFCs in a range of 0.03–0.10 mg/ml). Among them, luteolin showed the best activity.

Similar results were also previously observed with compounds isolated from *C. scolymus* leaves. Among them, chlorogenic acid, cynarin, luteolin 7-rutinoside, and cynaroside exhibited relatively higher activities than did other compounds and were more effective against fungi than against bacteria, with MICs ranging from 0.05 to 0.20 mg/ml (Zhu et al., 2004). Antimicrobial activities of apigenin, apigenin 7-glucoside, luteolin and other flavones have also been previously reported (Aljančić et al., 1999; Tshikalange, Meyer, & Hussein, 2005).

Results obtained herein on antioxidant and antimicrobial activity of different extracts of *C. cardunculus* involucral bracts support the traditional medicinal use of this plant and provide grounds for further establishing its use as a functional food.

## Acknowledgement

This research was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia (Grants Nos. 143012 and 143041).

## References

Alamanni, M. C., & Cossu, M. (2003). Antioxidant activity of the extracts of the edible part of artichoke (*Cynara scolimus* L.) var spinoso sardo. *Italian Journal of Food Science, 15*, 187–195.

Aljančić, I., Vajs, V., Menković, N., Karadžić, I., Juranić, N., Milosavljević, S., et al. (1999). Flavones and sesquiterpene lactones from Achillea atrata subsp. multifida: antimicrobial activity. *Journal of Natural Products, 62*, 909–911.

Booth, C. (1971). Fungal culture media. In J. R. Norris & D. W. Ribbons (Eds.), *Methods in microbiology* (pp. 49–94). London and New York: Academic Press.

Brown, J. E., & Rice-Evans, C. A. (1998). Luteolin-rich artichoke extract protects low-density lipoprotein from oxidation *in vitro*. *Free Radical Research, 29*, 247–255.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Daouk, R. K., Dagher, S. M., & Sattout, J. E. (1995). Antifungal activity of the essential oil of *Origanum syriacum* L. *Journal of Food Protection, 58*, 1147–1149.

do Amaral Franco, J. (1976). *Cynara* L.. In T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, & S. M. Walters, et al. (Eds.). *Flora Europaea* (Vol. 4). Cambridge: Cambridge Unversity Press.

Fernandez, J., Curt, M. D., & Aguado, P. L. (2006). Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Industrial Crops and Products, 24*, 222–229.

Fratianni, F., Tucci, M., De Palma, M., Pepe, R., & Nazzaro, F. (2007). Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chemistry, 104*, 1282–1286.

Grančai, D., Nagy, M., Suchý, V., & Ubik, K. (1992). Constituents of *Cynara cardunculus* L. I. Sterols and pentacyclic triterpens. *Farmaceutický Obzor, 61*, 577–580.

Grančai, D., Nagy, M., Suchý, V., & Ubik, K. (1993). Constituents of *Cynara cardunculus* L. II. Flavonoids. *Farmaceutický Obzor, 62*, 31–33.

Grančai, D., Nagy, M., Mučaji, P., Suchý, V., & Ubik, K. (1994a). Constituents of *Cynara cardunculus* L. III. Coumarins. *Farmaceutický obzor, 63*, 447–449.

Grančai, D., Nagy, M., Suchý, V., & Novomeský, P. (1994b). Cynarin from the fresh flower buds of *Cynara cardunculus*. *Fitoterapia, 65*, 282.

Grančai, D., Mučaji, P., Nagy, M., & Ubik, K. (1996). Constituents of *Cynara cardunculus* L. IV. Flavonoid glycosides. *Farmaceutický obzor, 65*, 255–256.

Grieve, M. (1971). *A modern herbal*. New York: Dover Publications.

Hanel, H., & Raether, W. (1998). A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses, 31*, 148–154.

Jiang, A., & Wang, C. (2006). Antioxidant properties of natural components from *Salvia plebeia* on oxidative stability of ascidian oil. *Process Biochemistry, 41*, 1111–1116.

Kelly, M., & Pepper, A. (1996). Controlling *Cynara cardunclus* (Artichoke Thistle, Cardoon, etc.). In J. E. Lovich, J. Randall, & M. D. Kelly (Eds.). *Proceedings of the california exotic pest plant council symposium* (Vol. 2, pp. 97–101). San Diego: California Exotic Pest Plant Council.

Koubaa, I., Damak, M., McKillop, A., & Simmonds, M. (1999). Constituents of *Cynara cardunculus*. *Fitoterapia, 70*, 212–213.

Koubaa, I., & Damak, M. (2003). A new dilignan from *Cynara cardunculus*. *Fitoterapia, 74*, 18–22.

Križkova, L., Mučaji, P., Nagy, M., & Krajčovič, J. (2004). Triterpenoid cynarasaponins from *Cynara cardunculus* L. reduce chemically induced mutagenesis *in vitro*. *Phytomedicine, 11*, 673–678.

Kwon, Y. S., Kim, E. Y., Kim, W. J., Kim, W. K., & Kim, C. M. (2002). Antioxidant constituents from *Setaria viridis*. *Archives of Pharmacal Research, 25*, 300–305.

Mossi, A. J., & Echeverrigaray, S. (1999). Identification and characterization of antimicrobial components in leaf extracts of globe artichoke (*Cynara scolymus* L.). *Acta Horticulturae, 501*, 111–114.

Mučaji, P., Grančai, D., Nagy, M., Budéšínský, M., & Ubik, K. (1999). Triterpenoid saponins from *Cynara cardunculus* L. *Pharmazie, 54*, 714–716.

Mučaji, P., Grančai, D., Nagy, M., Višňovská, Z., & Ubik, K. (2000). Apigenin-7-methylglucuronide from *Cynara cardunculus* L. *Česká a Slovenská Farmacie, 49*, 75–77.

Mučaji, P., Grančai, D., Nagy, M., Buděšínský, M., & Ubik, K. (2001). Monodesmosidic saponins from *Cynara cardunculus* L. *Česká a Slovenská Farmacie, 50*, 277–279.

Mučaji, P., Bukovsky, M., Grančai, D., & Nagy, M. (2003). Anticomplement activity of saponins from *Cynara cardunculus* L. *Česká a Slovenská Farmacie, 52*, 306–309.

Mulinacci, N., Prucher, D., Peruzzi, M., Romani, A., Pinelli, P., Giaccherini, C., et al. (2004). Commercial and laboratory extracts from artichoke leaves: estimation of caffeoyl esters and flavonoidic compounds content. *Journal of Pharmaceutical and Biomedical Analysis, 34*, 349–357.

Müller, S. D., Vasconcelos, S. B., Coelho, M., & Biavatti, M. W. (2005). LC and UV determination of flavonoids from Passifora alata medicinal extracts and leaves. *Journal of Pharmaceutical and Biomedical Analysis, 37*, 399–403.

Nakajima, Y., Shimazawa, M., Mishima, S., & Hara, H. (2007). Water extract of propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. *Life Sciences, 80*, 370–377.

Paris, R., & Moyse, H. (1971). *Précis de matière médicale* (tome III). Paris: Masson et Cie.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. *Journal of Nutrition, 133*, 2812–2818.

Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., & Romani, A. (2007). Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chemistry, 105*(4), 1695–1701.

Rossoni, G., Grande, S., Galli, C., & Visioli, F. (2005). Wild artichoke prevents age associated loss of vasomotor function. *Journal of Agricultural and Food Chemistry, 53*, 10291–10296.

Schutz, K., Kammerer, D., Carle, R., & Schieber, A. (2004). Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-ESI/MSn. *Journal of Agricultural and Food Chemistry, 52*, 4090–4096.

Silva, S. V., & Malcata, F. X. (2005). Studies pertaining to coagulant and proteolytic activities of plant proteases from *Cynara cardunculus*. *Food Chemistry, 89*, 19–26.

Slanina, J., Taborska, E., Bochorakowa, H., Humpa, O., Robinson, W. E., & Schram, K. H. (2001). New and facile method of preparation of the anti-HIV agent 1, 3-dicaffeoylquinic acid. *Tetrahedron Letters, 42*, 3383–3385.

Ševčikova, P., Glatz, Z., & Slanina, J. (2002). Analysis of artichoke extracts (*Cynara cardunculus* L.) by means of micellar electrokinetics capillary chromatography. *Electrophoresis, 23*, 249–252.

Takeoka, G. R., & Dao, L. T. (2003). Antioxidant constituents of almond [*Prunus dulcis* (Mill.) D.A. Webb] hulls. *Journal of Agricultural and Food Chemistry, 51*, 496–501.

Tshikalange, T. E., Meyer, J. J. M., & Hussein, A. A. (2005). Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. *Journal of Ethnopharmacology, 96*, 515–519.

Valentao, P., Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M., & Bastos, M. L. (2002). Antioxidative properties of cardoon (*Cynara cardunculus* L.) infuzion against superoxide radical, hydroxyl radical and hypochlorous acid. *Journal of Agricultural and Food Chemistry, 50*, 4989–4993.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry, 43*, 2800–2802.

Wagner, H., & Bladt, S. (1996). *Plant drug analysis. A thin layer chromatography atlas* (2nd ed.). Berlin, Heidelberg: Springer-Verlag.

Wang, M., Li, J., Rangarajan, M., Shao, Y., La Voie, E. J., Huang, T.-C., et al. (1998). Antioxidative phenolic compounds from sage (*Salvia officinalis*). *Journal of Agricultural and Food Chemistry, 46*, 4869–4873.

Wang, M., Simon, J. E., Fabiola Aviles, I., He, K., Zheng, Q.-Y., & Tadmor, Y. (2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry, 51*, 601–608.

Weng, X. C., & Wang, W. (2000). Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food Chemistry, 71*, 489–493.

Yokota, J., Takuma, D., Hamada, A., Onogawa, M., Yoshioka, S., Kusunose, M., et al. (2006). Scavenging of reactive oxygen species by *Eriobotrya japonica* seed extract. *Biological & Pharmaceutical Bulletin, 29*, 467–471.

Zhu, X., Zhang, H., & Lo, R. (2004). Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *Journal of Agricultural and Food Chemistry, 52*, 7272–7278.

Zhu, X., Zhang, H., Lo, R., & Lu, Y. (2005). Antimicrobial activities of *Cynara scolymus* L. leaf, head, and stem extracts. *Journal of Food Science, 70*, M149–M152.