Antioxidant and Antimicrobial Activity of *Cynara cardunculus* Extracts

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Abstract

The whole, fresh involucral bracts of cardoon, *Cynara cardunculus* L. (Compositae), were extracted with EtOH and aqueous suspension of obtained EtOH extract was partitioned successively with $CHCl_3$, EtOAc and *n*-BuOH, leaving residual water extract. All obtained extracts were evaluated on 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 microdilution technique against food-borne, mycotoxin producers and human pathogenic bacteria and micromycetes. 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 possess 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; Grieve, 1971; Fernandez, Curt, & Aguado, 2006). 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 centuries in the Iberian Peninsula for manufacturing of ovine and/or caprine milk cheeses (Silva & Malcata, 2005; Fernandez et al., 2006). Cardoon is traditionally used as a diuretic, choleretic, cardiotonic and an antihemorrhodial (Koubaa, Damak, McKillop, & Simmonds, 1999). Cardoon leaves are used for their cholagogue, choleretic and choliokinetic actions, for treatment of dyspepsia and as antidiabetics (Paris & Moyse, 1971; Koubaa et al., 1999).

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

The antioxidant activity of 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, Taborska, Bochorakowa, Humpa, Robinson, & Schram, 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 from therein.

2. Material and methods

2.1. Chemicals

Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma Chemical Co. (St. Louis, U.S.A.); L-ascorbic acid from Lachema (Neratovice, Czech Republic); Müeller-Hinton agar (MH), Malt agar (MA) from 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 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 in 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 residual water extract. All obtained extracts, including residual water extract, were evaporated till dryness and used for all investigations.

2.4. Determination of total phenolics content

Total phenolics content was determined using Folin-Ciocalteu (FC) reagent as previously described (Velioglu, Mazza, Gao, & Oomah, 1998). 100 µl of the extract dissolved in methanol was 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 was 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 weigh 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 on 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) system. 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). 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 Ferric Reducing Antioxidant Power (FRAP) assay, which is based upon reduction of Fe^{3+}-TPTZ complex in acidic conditions. Increase in absorbance of blue colored ferrous form (Fe^{2+}-TPTZ complex) is measured at 593 nm. FRAP reagent was freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml $FeCl_3$ (20 mM) water solution. 100 µl of each extract dissolved in appropriate solvent was added in 4.5 ml of FRAP reagent, stirred and incubated for 30 min absorbance was measured at 593 nm, using FRAP working solution as blank. Calibration curve of ferrous sulfate (100-1000 µM) was used, and results were expressed in µmol Fe^{2+}/mg dry weight

125 extract. The relative activity of the samples was compared to L-ascorbic acid (Pellegrini
126 et al., 2003).
127
128 *2.5.3. DPPH radical assay*
129 Extracts were dissolved in appropriate solvents, mixed with 1 ml of 0.5 mM 2,2-
130 diphenyl-1-picrylhydrazyl radical (DPPH) in MeOH, and final volume adjusted up to 5
131 ml. Mixtures were virgously shaken and left 30 min in dark. Absorbance was measured at
132 517 nm using MeOH as blank. 1 ml of 0.5 mM DPPH diluted in 4 ml of MeOH was used
133 as control. Neutralisation of DPPH radical was calculated using the equation:
134 $S(\%)=100\times(A_0-A_s)/A_0$, where A_0 is the absorbance of the control (containing all reagents
135 except the test compound), and A_s is the absorbance of the tested sample. The SC_{50}
136 value represented the concentration of the extract that caused 50% of neutralisation
137 (Cuendet et al., 1997). Results were compared with the activity of L-ascorbic acid.
138
139 *2.6. Bioassays*
140 *2.6.1. Test on antibacterial activity*
141 In order to obtain quantitative data for extracts and previously isolated compounds (**1-**
142 **9**), the modified microdilution technique was used (Hanel & Raether, 1988; Daouk,
143 Dagher, & Sattout, 1995). The following bacteria were tested: *Salmonella typhimurium*
144 (ATCC 13311), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907),
145 *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 29213).
146 The organisms tested were obtained from Department for Plant Physiology, Institute for
147 Biological Research "Siniša Stanković", Belgrade, Serbia.
148 The bacterial suspension was adjusted with sterile saline to a concentration of
149 approximately 1.0 $\times 10^7$ cell/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 1.0×10^5 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 in 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 a 2 µl into 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 modified microdilution technique (Hanel & Raether, 1988; Daouk et al., 1995). 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*

174 *viride* (IAM 5061), *Fusarium tricinctum* (CBS 514478) and *Alternaria alternata* (DSM
175 2006). The organisms tested were obtained from the Mycological Laboratory,
176 Department of Plant Physiology, Institute for Biological research "Siniša Stanković",
177 Belgrade, Serbia. The micromycetes were maintained on malt agar (MA) the cultures
178 were stored at +4 °C and subcultured once a month (Booth, 1971).

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

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

196

197 *2.7. Statistical analysis*

The results of the experiments were analyzed by two factorial analysis of variance (ANOVA). The Package program 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 same occasions. All analyses were run in triplicate for each replicate ($n = 2 \times 3$).

3. Results

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

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

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, 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 *n*-BuOH extract (Grančai, Mučaji, Nagy, & Ubik, 1996; Mučaji et al., 2000). Cynarasaponins (**2**, **8**) previously isolated from *n*-BuOH extract (Mučaji, Grančai, Nagy, Buděšínský, & Ubik, 1999; Mučaji, Grančai, Nagy, Buděšínský, & 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 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 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 drug streptomycin showed higher antibacterial potency than extracts tested (Table 2). Considering antifungal potential of investigated *C. cardunculus* extracts, EtOAc extract was also the most effective one with values of MICs and MFCs in equal range of 1.0-1.5 mg/ml (Table 3). Miconazole showed stronger antifungal activity then extracts tested. As for the standard compounds, the uppermost antibacterial, as well as the highest antifungal activity was observed by luteolin (**5**) with MICs and MBCs ranged from 0.05-0.10 mg/ml, and MICs and MFCs ranged from 0.03-0.10 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* are caffeic acid derivatives (Mulinacci et al., 2004) which, in heads, mainly occur as esters with quinic acid; leaves and heads of globe artichoke have been also found to be rich in mono- and dicaffeoylquinic compounds and flavonoids (Alamanni & Cossu, 2003; Wang, Simon, Fabiola Aviles, He,

248 Zheng, & Tadmor, 2003; Schutz, Kammerer, Carle & Schieber, 2004; Fratianni, Tucci,
249 De Palma, Pepe, & Nazzaro, 2007; Pinelli et al., 2007). As for cardoon, *C. cardunculus*,
250 there are reports on phenolic composition of their leaves: caffeoylquinic acids and
251 glycosides of luteolin and apigenin were identified using HPLC (Valentao et al., 2002;
252 Pinelli et al., 2007). In the involucral bracts of this plant various compounds were also
253 identified: β-sitosterol, sitosteryl-3β-glucoside, sitosteryl-3β-acetate, taraxasterole and
254 taraxasteryl-3β-acetate (Grančai et al., 1992), apigenin, apigenin 7-glucoside, luteolin
255 and luteolin 7-glucoside (Grančai et al., 1993), apigenin 7-rutinoside, luteolin 7-
256 rutinoside (Grančai et al., 1996), and apigenin 7-methylglucuronide (Mučaji, et al.,
257 2000), scopolin and scopoletin (Grančai, Nagy, Mučaji, Suchý, & Ubik, 1994a), cynarin
258 (Grančai, Nagy, Suchý, & Novomeský, 1994b) and chlorogenic acid (Mučaji et al.,
259 2000), cynarasaponins A and H, and their methyl derivatives (Mučaji et al., 1999), and
260 cynarasaponins B and K (Mučaji et al., 2001).
261 As previously showed, apigenin, luteolin and their glycosides are powerful antioxidants
262 (Kwon, Kim, Kim, Kim, & Kim, 2002; Müller, Vasconcelos, Coelho, & Biavatti, 2005).
263 The antioxidant effectiveness of apigenin was determined in models such as the *in vitro*
264 lipoprotein oxidation model (Vinson, Dabbagh, Serry, & Jang, 1995). The antioxidant
265 properties of luteolin 7-glucoside and of the respective aglycon, luteolin, have already
266 been observed against low-density lipoprotein oxidation (Brown & Rice-Evans, 1998),
267 DPPH free radical scavenging activity and $ABTS^{\bullet+}$ radical cation scavenging effect
268 (Wang et al., 1998).
269 Chlorogenic acid is one of the most abundant phenolic acids in various plant extracts
270 and also the most active antioxidant constituent. It has been shown that the antioxidant
271 activities of 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid are almost the same as
272 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 exhibit neuroprotective activities (Nakajima, Shimazawa, Mishima, & Hara, 2007).

β-Sitosterol generally showed low antioxidant activity, comparing 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 was suggested 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).

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, by presence of apigenin and luteolin in significantly larger amount than in other extracts.

Our experiments presented substantial antimicrobial activity of *C. cardunculus* involucral bracts extracts with MICs, MBCs and MFCs of 1.00-2.50 mg/ml. EtOAc extract was again the most effective.

297 Mossi and Echeverrigaray (1999) found that CH_2Cl_2 *C. scolymus* leaf extract, in
298 concentrations of 5 mg/ml, completely inhibited the growth with a bactericidal effect on
299 *Staphylococcus aureus*, *Bacillus cereus* and *B. subtilis*. Zhu, Zhang, and Lo (2004)
300 investigated antimicrobial activity of different extracts of *C. scolymus* leaf and showed
301 that the *n*-BuOH fraction was the most active one, followed by $CHCl_3$ and EtOAc
302 fractions. Similar investigations were done with successive $CHCl_3$, EtOH, and EtOAc
303 partitions of extracts of *C. scolymus* leaf, head, and stem. The MIC values for fungi were
304 at or below 2.5 mg/ml and for bacteria were at or above 2.5 mg/ml (Zhu, Zhang, Lo, &
305 Lu, 2005).
306 The results of our experiment showed that all standard compounds, previously
307 isolated from involucral bracts of *C. cardunculus*, possess antimicrobial activity against
308 all tested strains of bacteria and fungi (MICs, MBCs and MFCs in a range of 0.03-0.10
309 mg/ml). Among them, luteolin showed the best activity.
310 Similar results were also previously observed with compounds isolated from *C.*
311 *scolymus* leaves. Among them, chlorogenic acid, cynarin, luteolin 7-rutinoside, and
312 cynaroside exhibited a relatively higher activity than other compounds and were more
313 effective against fungi than against bacteria, with MICs ranged from 0.05-0.20 mg/ml
314 (Zhu et al., 2004). Antimicrobial activity of apigenin, apigenin 7-glucoside, luteolin and
315 other flavones has been also previously reported (Aljančić et al., 1999; Tshikalange,
316 Meyer, & Hussein, 2005).
317 Herein obtained results on antioxidant and antimicrobial activity of different extracts of
318 *C. cardunculus* involucral bracts supported the traditional medicinal use of this plant and
319 provided grounds for its further establishing as a functional food.

ABBREVIATIONS USED: 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 glycol reagent; TAA, total antioxidant activity; TPTZ, 2,4,6-tris(2-pyridyl)-*s*-triazine.

ACKNOWLEDGMENT. This research was supported by the Ministry of Science and Environmental Protection of Republic of Serbia (Grants № 143012 and 143041).

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468 M152.

469 **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	-

470 [a] in μmol Fe^{2+}/mg dry weigh extract, [b] SC_{50}, μg/ml, [c] mg of gallic acid equivalent/mg
471 dry weigh 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

476 **Table 3.** Minimum inhibitory and fungicidal concentrations (MICs and MFCs) of *Cynara*

477 *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

Table 4. Minimum inhibitory and bactericidal concentrations (MICs and MBCs) of the compounds tested* (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

* 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* (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

* 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**)