

Antioxidative potential of *Chenopodium botrys* L. (Amaranthaceae)

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Abstract

Evaluation of the antioxidant potential of methanol extract of *Chenopodium botrys* L. (Amaranthaceae) collected from six different locations in Republic of Macedonia was performed. Several methods were used for testing the antioxidative activity: 1) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2) ferric reduction power assay (FRAP), 3) inhibition of H₂O₂ activity, 4) non-site-specific hydroxyl radical-catalyzed 2-deoxy-D-ribose degradation (NSSOH) and 5) site-specific hydroxyl radical-catalyzed 2-deoxy-D-ribose degradation (SSOH). The IC₅₀ values ranged from 0.26-3.10 mg/mL, 3.01-12.71 mg/mL and 2.60-12.29 mg/mL, for DPPH, NSSOH and SSOH assays, respectively. The H₂O₂ inhibition activity and the ferric reducing power capacity were from 28.84-46.56% and 26.14-43.40%, respectively. The obtained data establish the antioxidant potency in concentration-dependent manner. Additionally, total phenols (TPC) and total flavonoid content (TFC) were determined. The estimated values ranged from 27.77-71.25 mg GAE/g DW and from 7.35-16.33 mg QE/g DW, respectively.

Keywords: *Chenopodium botrys*, total flavonoids, total phenols, antioxidative activity, DPPH, NSSOH assay, SSOH assay, FRAP, H₂O₂ inhibition

Introduction

Oxidative stress plays an important role in the etiology of chronic diseases such as cancer, atherosclerosis and diabetes as well as age-related neurological degenerative disorders. In the last couple of decades, the misperception of safety chronic consumption of synthetic antioxidants has increased the interest in phytochemicals as biological antioxidants which are defined as substances present in low concentrations that can reduce or prevent oxida-

tion of the easily oxidisable substrate (Shikov et al., 2006; Benzie and Strain, 1996). Among enormous variety of secondary metabolites, polyphenols have a wide range of biological activities: anti-inflammatory, antiallergenic, antioxidant and antitumor, and have an impact on improving the immune system, as well (Pasko et al., 2008). Numerous studies have demonstrated the importance of naturally occurring dietary polyphenols in promoting of cardiovascular health and emphasized the significant role these compounds play in limiting the effects of cellular aging (Khura et al., 2013). Also, they pointed to the impact of

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phenolic compounds on carcinogenic cells which is crucial for their antitumor potential (Boivin et al., 2009). Anticancer activity is partly attributed to the antioxidative effects of phenolic compounds and polyphenols as they are defined as reducing agents, free radical scavengers, hydrogen donors and inhibitors of pro-oxidative enzymes (Gawlik-Dziki, 2012; Cai et al., 2004). Since, recent studies are increasingly directed towards finding new antioxidant compounds, that requires a lot of information and additional research related to antioxidative potential of natural components (Nikolova et al., 2011).

The genus *Chenopodium* comprises numerous members of perennial and annual species, which occur everywhere in the world. They could grow as aromatic or non-aromatic herbaceous plants or as shrubs and small trees (Fuentes-Bazan et al., 2012). *C. botrys* L. (syn. *Dysphania botrys* (L.) Mosyakin and Clemants), known as Jerusalem Oak Goosefoot or Feathered Geranium, commonly grows in Europe and is native to Mediterranean region, but it also could be found in Asia, India, Himalayas, Turkey, Cyprus, Africa, Australia and North and South America (Seidemann, 2005). The plant is known as traditional medicine for different ailments in respiratory tract, especially for the treatment of catarrh and humoral asthma, when it is used as a substitute for *C. ambrosioides* (Yadav et al., 2007). Concerning literature data, *C. botrys* contains flavonoids, alkaloids, terpenes and essential oil (Kokanova-Nedialkova et al., 2009). Regarding this, pharmacological analyses confirm medical potential of *C. botrys* in the development of new drugs. Various isomers of specific monoterpene compound, ascaridole, were identified in *C. botrys* oil which was associated with the activity against various tumor cell lines *in vitro* (Morteza-Semnani, 2015). Additionally, in the aerial parts of *C. botrys*, the flavon chrysoeriol as well as flavonoids: salvigenin, sinensetin, hispidulin, quercetin and their derivatives were identified, which were related to a potential antioxidant activity (Kokanova-Nedialkova et al., 2009).

In the flora of the Republic of Macedonia (RM), 15 species of the genus *Chenopodium* occur naturally, including *C. botrys* (Micevski, 1995). Dried over ground flowering parts of the plant (*herba*) are utilized by local people for preparing infusions or liquid extracts with diuret-

ic, antispasmodic, carminative and antidiarrheal properties (Maksimovic et al., 2005). Despite its usage, chemical composition and biological activity of this plant are poorly known. Therefore, the aim of this study was determination of the total phenolic and total flavonoid content as well as evaluation of the antioxidant capacity of *C. botrys* collected from different localities of the Republic of Macedonia (RM).

Experimental

Plant material

The aerial parts of *C. botrys* were collected in the period from July to September in 2012, 2013 and 2014 at six localities in RM (Table 1). Plant identity was verified and a herbarium voucher specimen (NoCB1/12; NoCB1/13 and NoCB1/14) was deposited at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, University 'Ss. Cyril and Methodius', Skopje, R. Macedonia. Collected plant material was air-dried and preserved in paper bags until performing the analysis, when it was properly milled and homogenised.

Chemicals and standards

Folin-Ciocalteu reagent, Gallic acid, Aluminum chloride and Quercetin were purchased from Merck (Darmstadt, Germany), Sodium carbonate, Sodium nitrite, Ascorbic acid, Butylated hydroxyanisole (BHA) and Sodium hydroxide from Alkaloid (Skopje, R. Macedonia), while Methanol was purchased from Carlo Erba (Milano, Italy).

Extract preparation

2.5 g of dried and fine grounded plant material was extracted in ultrasonic bath (50/60 Hz, 720 W) at room temperature, twice with 15 mL of 100% methanol (HPLC grade), each time for 15 min. After filtration, the volume was made up to 25 mL with methanol. Obtained methanol extracts (0.1 g/mL) were used for determination of total phenols and total flavonoids and for evaluation of the

Table 1: Plant material of *C. botrys*

No.	Sample	Locality	Geographical position in RM	Year of harvesting
1.	CB1/12	Kozuf Mtn.	Southeast	2012
2.	CB2/13	Pretor	Southwest	2013
3.	CB3/14	Strumica	Southeast	2014
4.	CB4/14	Zletovo	East	2014
5.	CB5/13	Radovis	East	2013
6.	CB6/14	Stip	East	2014

antioxidant activity.

Determination of total phenolic content (TPC)

TPC was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton et al., (1999) with slight modifications. Folin-Ciocalteu reagent (2.5 mL, 1:10 v/v diluted with distilled water) was added to the test sample (0.1 mL methanol extract), and stirred for 5 min at room temperature. Subsequently, 3 mL of 7% sodium carbonate was added and made up to 10 mL with distilled water. This mixture was incubated at room temperature in dark place for 1 h. After incubation, absorbance of blue color was measured at 765 nm using a Agilent 8453 UV-Vis spectrophotometer. The total phenolic content was estimated as mg of gallic acid equivalents (GAE) per gram of dried weight of plant material (mg GAE/g DW) using an equation obtained from standard gallic acid calibration curve.

Determination of total flavonoid content (TFC)

TFC was determined using the Aluminum chloride assay described by Lallianrawna et al., (2013). An aliquot of the test sample (0.1 mL methanol extract) was mixed with 0.1 mL of 5.0% sodium nitrite and allowed to stand for 5 min. Then, 0.15 mL of 10.0% aluminum chloride were added to the mixture, kept at room temperature for six more minutes and followed by the addition of 0.5 mL of 1 M sodium hydroxide. The total volume was made up to 2.5 mL with distilled water. The resulting solution was mixed well and the absorbance was measured immediately at 510 nm using an Agilent 8453 UV-Vis spectrophotometer. The blank was prepared by replacing the extracts with an equal volume of deionized water. The TFC was expressed as mg of quercetin equivalents (QE) per gram of dried weight of plant material (mg QE/g DW) using an equation obtained from standard quercetin calibration curve.

Free radical scavenging activity (DPPH assay)

The scavenging activity of DPPH free radicals of *C. botrys* extracts was done according the method reported by Kurin et al., (2012) with minor modifications. 200 μ L of different concentrations of tested samples (100, 50 and 20 mg/mL) were transferred in a cuvette and 4 mL of 100 μ M ethanol solution of DPPH radical were added. Mixtures were shaken vigorously for one min and left to stand 10 min in dark place at room temperature. The reduction of the DPPH free radicals was measured by reading the absorbance at 517 nm on final concentrations of extracts in the reaction mixture (1.0, 2.5 and 5.0 mg/mL). Methanol was used as control blank. Quercetin and BHA were used as standards, with final concentrations in the reaction mixture of 0.0005, 0.001 and 0.0025 mg/mL. The antioxidant capacity of methanol extracts was expressed as IC₅₀ val-

ues (extract concentration providing 50% of radical scavenging activity) that were calculated by interpolation from the graph obtained by plotting the percentage of inhibition against extract concentration.

Ferric reducing antioxidant power (FRAP)

The ferric reducing power of *C. botrys* methanol extracts was determined by using the Potassium ferricyanide/ferric chloride assay reported by Shahat et al. with minor modifications [23]. Different concentrations of extracts (100, 50 and 20 mg/mL) were added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL of 10% trichloroacetic acid was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 1% FeCl₃. After 30 min, the absorbance was measured at 700 nm on the final concentrations of extracts in the reaction mixture (2.8 mg/mL). Quercetin and ascorbic acid were used as standards with final concentrations in the reaction mixture of 0.007 mg/mL. The ferric reducing antioxidant power was expressed in percentage (%) after construction of FeSO₄ x 7H₂O calibration curve. Higher absorbance of the reaction mixture indicated greater reducing power.

H₂O₂ inhibition effect

The H₂O₂ inhibition effect of *C. botrys* extracts was determined according to the method reported by Serteser et al., (2009). One mL of extract (100 mg/mL), 3.4 mL of 0.1 M phosphate buffer (pH 7.4) and 0.6 mL of 43 mM H₂O₂ were mixed and after 60 minutes the absorbance of the mixture with final concentrations of extracts (20 mg/mL) in the reaction mixture was measured at 230 nm. Controls without H₂O₂ were prepared for each sample concentration. To determine the H₂O₂ mM concentration that did not involve the reaction, a linear regression equation was used. 3.4 mL of phosphate buffer were added to 0.6 mL of 10, 15, 25, 43 and 50 mM H₂O₂ and the absorbance was measured at 230 nm. Linear regression equations were obtained by the diagram of concentration against absorbance. Quercetin was used as standard solution. The percentage of inhibition was calculated from the H₂O₂ concentration of control and H₂O₂ concentration of sample using the following equation:

$$\text{H}_2\text{O}_2 \text{ inhibition capacity (\%)} = \left[1 - \left(\frac{\text{H}_2\text{O}_2 \text{ conc. of sample}}{\text{H}_2\text{O}_2 \text{ conc. of control}} \right) \right] \times 100$$

Nonsite-Specific Hydroxyl Radical-Mediated 2-Deoxy-D-Ribose Degradation (NSSOH)

Inhibition of nonsite-specific hydroxyl radical-mediated degradation was carried out according to Halliwell et

al., (1987) with slight adaptations. The reaction mixture contained 100 μL of methanol extract, 500 μL 2-Deoxy-D-ribose (5.6 mM, in KH_2PO_4 -NaOH buffer, pH 7.4), 200 μL premixed (1:1 v/v) FeCl_3 (100 μM) and EDTA (104 μM), 100 μL H_2O_2 (1.0 mM) and 100 μL ascorbic acid (1.0 mM). After vortexing and 30 min incubation at 50 $^\circ\text{C}$, trichloroacetic acid (1 mL, 2.8%) and thiobarbituric acid (1 mL, 1.0%) were added. The mixture was vortexed and heated at 50 $^\circ\text{C}$ for 30 more minutes. The extent of oxidation was estimated from the solution absorbance of the final concentrations of extracts in the reaction mixture (0.6, 1.6 and 3.3 mg/mL) at 532 nm. Quercetin and BHA were used as standards with final concentrations in the reaction mixture of 0.6, 1.6 and 3.3 $\mu\text{g}/\text{mL}$. IC_{50} values were estimated using linear regression of the linear part of the curve.

Site-Specific Hydroxyl Radical-Mediated 2-Deoxy-D-ribose Degradation (SSOH)

Inhibition of site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation was measured according to Halliwell et al., (1987) with slight adaptations. The reaction mixture contained 100 μL of methanol extract, 500 μL 2-Deoxy-D-ribose (5.6 mM in KH_2PO_4 -NaOH buffer, pH 7.4), 100 μL FeCl_3 (100 μM), 100 μL KH_2PO_4 -NaOH buffer (pH 7.4), 100 μL H_2O_2 (1.0 mM) and 100 μL ascorbic acid (1.0 mM). After vortexing and 30 min incubation at 50 $^\circ\text{C}$, trichloroacetic acid (1 mL, 2.8%) and thiobarbituric acid (1 mL, 1.0%) were added. The mixture was vortexed and heated at 50 $^\circ\text{C}$ for 30 min. The extent of oxidation was estimated from the solution absorbance of the final concentrations of extracts in the reaction mixture (0.6, 1.6 and 3.3 mg/mL) at 532 nm. Quercetin and BHA were used as standards with final concentrations in the reaction mixture of 0.6, 1.6 and 3.3 $\mu\text{g}/\text{mL}$. IC_{50} values were estimated using linear regression of the linear part of the curve.

Statistical analysis

Data obtained from determination of TPC and TFC were expressed as mean values \pm SD as all experiments were carried out in triplicate. Statistical analyses were performed by statistical package STATGRAPH 21.0. The p value less than 0.05 was considered to be statistically significant.

Results and discussion

Total phenolic and total flavonoids content

The *C. botrys* samples analysed in this study showed presence of total phenolic content in range of 27.77 to 71.25 mg of gallic acid equivalents per gram of dried weight of plant material (mg GAE/g DW), for samples from Stip (CB6/14) and Pretor (CB2/13), respectively (Table 2).

Table 2. Total phenolic and flavonoid content in *C. botrys*

Sample	Locality	Total phenolics (mg GAE/g DW \pm SD)	Total flavonoids (mg QE/g DW \pm SD)
CB1/12	Kozuf	47.08 \pm 2.95	16.33 \pm 0.84
CB2/13	Pretor	71.25 \pm 4.70	9.34 \pm 1.68
CB3/14	Strumica	51.28 \pm 0.41	7.69 \pm 0.36
CB4/14	Zletovo	35.61 \pm 0.10	8.52 \pm 1.81
CB5/13	Radovis	28.42 \pm 5.63	7.35 \pm 1.25
CB6/14	Stip	27.77 \pm 0.31	11.91 \pm 3.20

The quantitative analysis of total flavonoid content in different samples of *C. botrys* revealed values of TFC from 7.35 to 16.33 mg quercetin equivalents per gram of dried weight of plant material (mg QE/g DW) in samples from Radovis (CB5/13) and Kozuf (CB1/12), respectively (Table 2).

In the available literature, there is no data about the *C. botrys* TPC and TFC, thus only few articles could be found related to the total phenolic and total flavonoid content of other *Chenopodium* species. Few authors reported that *C. album* herb extracts contained 3.36 mg/g DW of free polyphenols (Nowak et al., 2015), while leaves of *C. album* comprised 44.2 μg GAE/mg of total phenolics and 9.53 μg CE/mg of flavonoids (catechins) (Dasgupta and De, 2007).

DPPH radical scavenging activity

The methanol extract of *C. botrys* showed DPPH radical scavenging activity with IC_{50} values from 0.26-3.10 mg/mL (Table 3). The radical scavenging ability of methanol extracts was compared to the scavenging activity of two reference substances, quercetin and BHA. The estimated IC_{50} values were incomparably lower (1.0 $\mu\text{g}/\text{mL}$ and 6.0 $\mu\text{g}/\text{mL}$, respectively). The highest DPPH scavenging activity showed the methanol extract prepared from samples from Strumica (CB3/14).

Non-site-specific hydroxyl radical-catalyzed 2-deoxy-D-ribose degradation (NSSOH)

In the method with 2-deoxy-D-ribose as a substrate, hydroxyl radicals ($\text{OH}\cdot$) are generated in conditions when EDTA-chelated iron (III) ions are incubated at physiological pH with reducing agent and H_2O_2 . These hydroxyl radicals are capable to transfer on substrate and to fragment it into thiobarbituric acid reactive substances (TBARS). An extract that inhibits the development of TBARS in this system can be considered capable for scavenging hydroxyl radicals, protecting the carbohydrates from oxidative degradation (Shikov et al., 2006). *C. botrys* methanol extract has shown capability of inhibiting the formation of TBARS in a concentration-dependent manner with IC_{50} values from 3.01 to 12.71 mg/mL. According the estimated values, extracts from samples collected from Strumica (CB3/14) and

Table 3: Antioxidant activity of methanol extracts of *C. botrys*

Sample	DPPH assay IC ₅₀ (mg/mL)	NSSOH assay IC ₅₀ (mg/mL)	SSOH assay IC ₅₀ (mg/mL)	FRAP assay (%)	H ₂ O ₂ inhibition Capacity assay (%)
CB1/12	1.99	6.83	4.81	26.98	36.20
CB2/13	0.95	3.01	2.60	43.37	36.22
CB3/14	0.26	6.05	4.06	43.40	36.73
CB4/14	2.10	10.27	9.97	30.28	28.84
CB5/13	1.76	12.37	10.38	26.14	31.89
CB6/14	3.10	12.71	12.29	30.82	46.56
Quercetin	1.00*	0.29	6.39*	21.37**	64.89**
BHA	6.00*	0.29	7.10*	-	-
Ascorbic acid	-	-	-	27.71**	-

*µg/mL

** initial concentration of ascorbic acid and quercetin 0.25 mg/mL

Pretor (CB2/13) have shown better radical scavenging activity compared to samples from other localities (Table 3). The antioxidant activity of quercetin and BHA was significantly higher in comparison to the activity of the tested samples (IC₅₀ values were 0.29 mg/mL for both standards).

Site-specific hydroxyl radical-catalyzed 2-deoxy-D-ribose degradation (SSOH)

In the site-specific hydroxyl radical-mediated 2-deoxy-D-ribose assay, an extract that inhibits the formation of TBARS in this system does not scavenge hydroxyl radicals but prevents their formation by chelating and deactivating iron (III) ions. Thus, the experiment is performed without EDTA (Shikov et al., 2006). The methanol extract of *C. botrys* was capable for preventing hydroxyl radical mediated oxidative degradation of 2-deoxy-D-ribose at physiological pH, with IC₅₀ values from 2.60 to 12.29 mg/mL. The extracts from the samples of *C. botrys* collected from Strumica (CB3/14) and Pretor (CB2/13) have shown the most prominent activity (Table 3). The antioxidant activity of quercetin and BHA was significantly higher, with IC₅₀ values 6.39 µg/mL and 7.10 µg/mL, respectively.

FRAP assay

Ferric reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The initial concentration of *C. botrys* methanol extract (100 mg/mL) showed ferric reducing power from 26.14-43.40% (Table 3). The extract of *C. botrys* collected from Pretor (CB2/13) and Strumica (CB3/14) have shown better activity than other samples. The reducing power of methanol extracts was compared to the activity of quercetin and ascorbic acid. These substances with initial concentration of 0.25 mg/mL showed 21.37% and 27.71% of reducing potential of ferric ions, respectively.

H₂O₂-inhibition activity

Reactive oxygen hydroxyl radical can be formed by Fenton reaction, in the presence of metal ions and H₂O₂. Indirect neutralization of resulting hydroxyl radical by H₂O₂-inhibition activity is a significant method for the determination of antioxidant activity. Scavenging of H₂O₂ by extracts may be attributed to their phenolic compounds, which can donate electrons to H₂O₂, thus neutralizing it to water (Ebrahimzadeh et al., 2010). The estimated percentages of inhibition showed that methanol extract of *C. botrys* was capable of scavenging hydrogen peroxide in a concentration dependent manner. H₂O₂-inhibition activity ranged from 28.84 to 46.56%. The extracts with initial concentration of 100 mg/mL prepared from *C. botrys* samples from Strumica (CB3/14) and Pretor (CB2/13) showed the highest H₂O₂ inhibition (36.73% and 46.56%, respectively) but lower potential than quercetin (64.89 %) with the initial concentration of 0.25 mg/mL (Table 3).

In the present study, the evaluation of the antioxidant activity of methanol extracts of *C. botrys* was tested by methods that involve different biochemical mechanisms: DPPH radical scavenging activity, ferric reducing power determination (FRAP), H₂O₂-inhibition activity and inhibition of non-site-specific and site-specific hydroxyl radical-catalyzed 2-deoxy-D-ribose degradation. All examined extracts showed activity in each performed assay in concentration-dependent manner, with better antioxidant activity in higher concentrations. The methanol extracts of *C. botrys* from Strumica (CB3/14) and Pretor (CB2/13) have shown better activity in all assays, as they have lower IC₅₀ values in DPPH, NSSOH and SSOH assays, and higher inhibition percentage in FRAP and H₂O₂-inhibition activity assays (Fig. 1).

Comparing the obtained results for the antioxidant activity to additionally determined TPC and TFC, positive correlation can be found for the most samples of *C. botrys*, especially for samples collected from Pretor and Strumica, as they contained the highest TPC (71.25 and 51.28 mg GAE/g DW, in CB2/13 and CB3/14, respectively). However, the content of TFC cannot be correlated, as the sam-

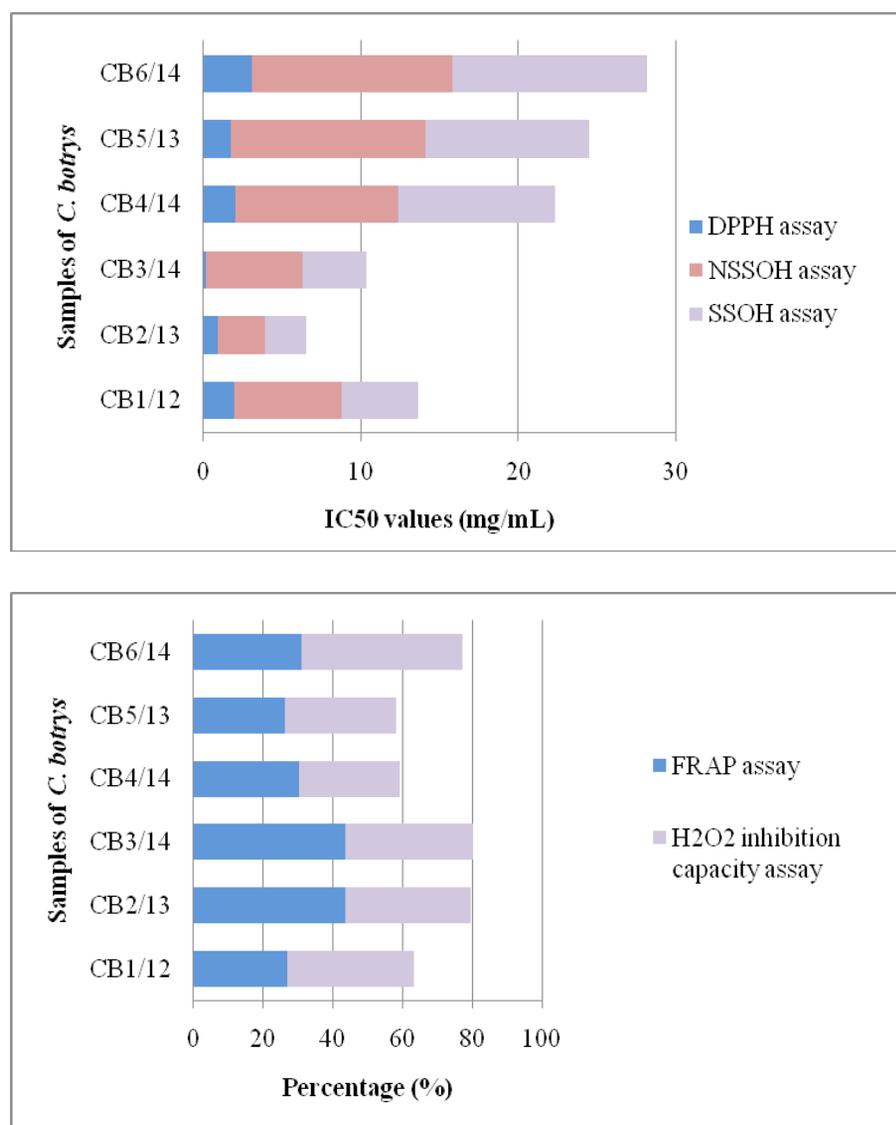


Fig. 1. Comparison of antioxidative activity of methanol extract of *C. botrys* obtained by different antioxidative assays.

ples that contained the highest amounts of TFC did not act as the best antioxidant agents.

Positive correlation of antioxidant activity with higher TPC was previously reported. Thus, for *Marrubium peregrinum* L. (Lamiaceae), high content of phenolic compounds as well as significant linear correlation between their concentration values and antioxidant activity indicated that these compounds contributed to the strong antioxidant activity (Stankovic, 2011). Total phenolic and flavonoid contents showed positive correlations with the DPPH radical scavenging activity ($p < 0.01$) and negative correlations with IC_{50} ($p < 0.01$) for methanol extracts of four *Ulva* species (Farasat et al., 2014). The extracts of *Inula viscosa* showed significant antioxidant activity as the plant contained considerable levels of phenols. The ethanol extract (0.3 mg/mL) showed a remarkable antioxidant activity in three systems of assays (against 2,2-diphenyl-1-pic-

rylhydrazyl, total antioxidant capacity and iron-reducing capacity), which was probably due to its high content of polyphenols (274.39 ± 6.94 mg GAE/g dry extract) (Chahmi et al., 2015).

Since phenols are generally responsible for antioxidant activity of plant extracts, in almost all cases, the antioxidant activity followed the total phenolic content. That was proven by estimation of the correlation coefficient between the total phenolic content and antioxidant activity by Kopjar et al., (2009). The correlation coefficients between the parameters of interest ranged from 0.5749 to 0.9604. A positive relationship between TEAC (Trolox equivalent antioxidant capacity), ABTS⁺ (2,2'-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid radical) and FRAP values and total phenolic content, measured by HPLC, was found in many representative herbs within Lamiaceae and Asteraceae families, as well (Wojdylo et al., 2007). Finally, con-

tribution of the phenolics (catechin gallate, catechin, vanillic acid, kaempferol, ferulic acid, quercetin, resorcinol and 4-methylresorcinol) to the TAC (total antioxidant capacity) value indicated that phenolics (resorcinol) remarkably forwarded to the antioxidant capacity of the water-soluble extract of *Chenopodium pallidicaule*. This plant is considered as a potential source of natural antioxidants and other bioactive compounds which can be important for human health (Penarrieta et al., 2008).

Conclusion

Methanol extract of several specimens of *Chenopodium botrys* (Amaranthaceae), collected from different regions of the territory of Republic of Macedonia, showed scavenging activity against DPPH radical as well as antioxidant activity in non-site specific and site-specific hydroxyl radical catalyzed 2-deoxy-D-ribose degradation. The extracts exhibited inhibition of ferric reduction power (FRAP assay) and in H₂O₂ activity as well. In comparison to the antioxidant activity of the reference substances (quercetin, BHA and ascorbic acid) the antioxidant activity of *C. botrys* methanol extracts was consider as very poor, despite the relatively high content of total phenols and total flavonoids.

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Резиме

Антиоксидативен потенцијал на *Chenopodium botrys* L. (Amaranthaceae)

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Клучни зборови: *Chenopodium botrys*, вкупни флавоноиди, вкупни полифеноли, антиоксидативна активност, DPPH, NSSOH, SSOH, FRAP, инхибиција на H₂O₂.

Направена е евалуација на антиоксидативниот потенцијал на метанолен екстракт од *Chenopodium botrys* L. (Amaranthaceae) собран од шест различни локации во Република Македонија. За таа цел користени се неколку методи за тестирање на антиоксидативната активност: 1) тест за определување на способноста за неутрализација на слободни радикали (DPPH); 2) тест за определување на фери редуирачки антиоксидативен капацитет (FRAP); 3) тест за определување на неутрализирачкиот капацитет врз H₂O₂; 4) неспецифичен хидроксирадикал посредуван 2-деокси-D-рибоза тест (NSSOH); и 5) специфичен хидроксирадикал посредуван 2-деокси-D-рибоза тест (SSOH). Вредностите за IC₅₀ се движат од 0,26-3,10 mg/mL, 3,01-12,71 mg/mL и 2,60-12,29 mg/mL, за DPPH, NSSOH и SSOH тестовите, соодветно. Инхибиторната активност на H₂O₂ и фери-редуирачката моќност се движат од 28,84-46,56% и 26,14-43,40%, соодветно. Добиените податоци покажуваат концентрациски зависен антиоксидативен потенцијал. Дополнитено, беше утврдена и содржината на вкупните полифеноли (TPC) и вкупните флавоноиди (TFC). Определените вредности се: 27,77-71,25 mg GAE/g на сува маса за вкупни полифеноли и 7,35-16,33 mg QE/g на сува маса за вкупни флавоноиди.