CAPACITY OF EXTRACTS OF SWEET CHESTNUT CONCERNING TO REMOVE LIPID PEROXIDATION

KAPACITET EKSTRAKTA PITOMOG KESTENA ZA SUZBIJANJE LIPIDNE PEROKSIDACIJE

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ABSTRACT

Permanent attention of researchers has been focused on the use of natural antioxidants to inhibit lipid peroxidation, or to protect the damage provoked by free radicals. Extracts of sweet chestnut, Lovran's Marrone cultivar and grafted Italian ″Marrone″ cultivar obtained in two years (2006. and 2007.), were investigated for their capacity to remove lipid peroxidation in liposomes exposed to hydroxyl radical, and for their total phenolics and flavonoids content. Selected tissues of sweet chestnut (Castanea sativa Mill.) fruit, leaf, catkin, spiny burs, young and old bark of chestnut tree have been extracted under the same conditions using 50% ethanol and afterwards dry extracts of examined samples have been gained. All extracts, excluding those with low content of both, total phenolics and flavonoids, showed the ability to protect liposomes from lipid peroxidation. We find practical importance of results in the fact that extract of spiny burs could potentially have beneficial effects in diet of hypertensive patients.

Key words: extracts of sweet chestnut, Castanea sativa Mill., membrane fluidity, flavonoid, phenolic compounds

SAŽETAK

Pozornost istraživača plijeni mogućnost korištenja prirodnih antioksidansa u inhibiciji lipidne peroksidacije, ili za zaštitu od oštećenja prouzročenih slobodnim radikalima. U dvogodišnjem razdoblju (2006. i 2007.) istraživan je kapacitet ekstrakta pitomog kestena, kultivara Lovranski marun i cijepljeni Talijanski marun za suzbijanje lipidne peroksidacije na liposomima izloženim hidroksi radikalima, kao i sadržaj fenolnih tvari i flavonoida. Dijelovi ploda kestena (Castanea sativa Mill.), list, resa, ježevica, mlada i stara kora drveta ekstrahirani su pod istim uvjetima korištenjem 50% etanola, a nakon toga je iz istraživanih uzoraka dobiven suhi ekstrakt. Svi ekstrakti, osim onih s malim sadržajem ukupnih fenola i flavonoida, pokazuju sposobnost zaštite liposoma od lipidne peroksidacije. Praktični značaj rezultata istraživanja vidimo u mogućnosti korištenja ekstrakta ježevica u dijeti pacijenata oboljelih od hipertenzije.

Ključne reči: ekstrakti pitomog kestena, Castanea sativa Mill., membranska fluidnost, flavonoidi, fenolne komponente

INTRODUCTION

Reactive Oxygen Species (ROS) can induce lipid peroxidation which can break down membrane integrity. Lipid peroxidation is known to correlate with the decrement of membrane fluidity [7] hanges in the cell membrane fluidity are known to affect properties and functions of the cell membrane including cell growth, signal transduction, membrane permeability, transport systems, receptor functions or enzyme activities [9]. There are indications that the sum of deleterious free radical reactions are going on continuously throughout the cells and tissues constitutes the aging process or is a major contributor to it [11]. To control the level of ROS and to protect cells under stress conditions, plant tissues contain several enzymes scavenging ROS (superoxide dismutase, catalase, peroxidases) and a network of low molecular mass antioxidants (ascorbate, glutathione, phenolic compounds, tocopherols) [3].

If oxygen metabolites are indeed involved in many pathological processes, we should be able to prevent these processes by addition of antioxidants or radical scavengers [8] and a prolong life. Meanwhile, the difficulty is that the body acts to maintain flexible and responsive intracellular redox poise, enabling a swift genetic response to stress. In other words, the body as a whole, prevents the intracellular redox state from being 'swamped' by antioxidant supplements [15, 10].

Natural and synthetic phenolic compounds are prototypic chain-breaking antioxidants [13, 16]. Phenolic components can scavenge both hydroxy radicals and lipid radicals suggesting that they act to reduce toxicity by interfering with lipid peroxidation cascade [18]. Their effectiveness of antioxidant protection has been found to be related to their incorporation rate into cells and to their orientation in biomembranes [14, 23]. The protective effect of phenolic compounds against lipoperoxidative damage depends on the hydrogen-donating capacity of a hydroxyl group in each molecule [2].

More parts and tissues of Castanea sativa Mill. (sweet chestnut) are exploited. The wood is used for timber, paper, or fuel; fruits are consumed roasted or boiled and as sweetened chestnut spread or jam [4]. So far, a little is known about antioxidant potential of this plant and possible application in diet and therapy. To find new natural sources of active extracts we investigated capacity to control of lipid peroxidation of different parts of plant Castanea sativa Mill.

MATERIAL AND METHODS

Chemical reagents

Folin-Ciocalteu reagent was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid and (+) catechin hydrate (Fluka A.G., grad, Gerrmany) have been used. 7-DS (2-(5-carboxypentyl)-2-undecyl-4,4-dimethyloxazolidine-3-oxyl) was purchased from Molecular Probes, (Junction City, OR, USA). These chemicals were of analytical reagent grade. All other used chemicals and solvents used were of the highest analytical grade, and they were obtained from commercial providers.

Sample preparation

In the area of the The Una – Sana canton (B&H) four chestnut-producing areas (Bužim, Velika Kladuša, Cazin and Bosanska Krupa) have been chosen. Out of the three most predominant cultivars: sweet chestnut, Italian Marrone and Lovran Marrone. Scan research has been done for sweet chestnut, while for the other two cultivars only separate plant parts are represent. The fruits were harvested in the chestnut ripening season, from the middle of September to the end of October. The results for the seed reference for miled seed without burs. Separate parts and tissues of seed have investigated, just like: peeled chestnut (hand-peeled), the brown seed shell and the red internal fruit peel. In addition, leaves, catkin, spiny burs and bark of tree have been analyzed.

After drying at room temperature, the samples have been milled for further analysis by laboratory homogenizator. The mean particle diameter of samples was determined according to [19] The plant material has been extracted by 50% ethanol. To 50.0 g of sample 250 ml of 50% ethanol was added (ratio sample: solvent was 1:5; w/v). The extraction by ultrasound was performed (30 min). An ultrasonic bathroom Branson model b-220 Smith-Kline Company (50/60 Hz, 125 W) was used. After mass checking, and solvent addition, the liquid extract was obtained by filtration through Whatman Grade No. 4 filter paper. The aliquot of liquid extract was taken and the solvent was completely removed by evaporation under vacuum, at temperature of 40°C. In this way, the dry extract of investigated chestnut samples was obtained. The yield of dry extract was calculated. Dry extract samples were kept at 4°C.

Analysis of total phenolics compounds

The content of total phenolics compounds has been determined in dry extracts by the Folin - Ciocalteu procedure [21]. The absorbance was measured at wavelength of 765 nm. The content of total phenolics compounds in investigated plant methanol extracts has been expressed as g of gallic acid equivalents (GAE) per 100 g of the dry extract sample (%; w/w), i.e. %GAE.

Analysis of total flavonoids

Table 1: Mean particle size and yield of dry extract of *Castanea sativa* Mill. (2006.). Values are presented as means ± S.D.
Tablica 1: Srednji promjer čestica i prinos suhog ekstrakta *Castanea sativa* Mill. (2006.). Vrijednosti su predstavljene kao srednja vrednost ± standardna devijacija

Sample	Mean particle size (mm)	Yield of dry extract % (w/w)
Sweet chestnut		
Seed	0.27 ± 0.007	7.09 ± 0.091
Brown seed shell	0.40 ± 0.021	3.30 ± 0.083
Red seed coat	0.24 ± 0.009	6.79 ± 0.076
Peeled chestnut	0.26 ± 0.009	12.79 ± 0.092
Leaf	0.18 ± 0.008	4.94 ± 0.038
Catkin	0.24 ± 0.023	10.04 ± 0.046
Old chestnut bark	0.27 ± 0.005	3.40 ± 0.089
Young chesnut bark	0.26 ± 0.012	7.84 ± 0.021
Spiny burs	0.28 ± 0.008	1.82 ± 0.066
Lovran's Marrone cultivar		
Seed	0.27 ± 0.008	8.28 ± 0.033
Brown seed shell	0.31 ± 0.009	7.13 ± 0.034
Red seed coat	0.18 ± 0.015	13.32 ± 0.074
Peeled chestnut	0.26 ± 0.007	5.51 ± 0.054
Leaf	0.31 ± 0.007	7.03 ± 0.063
Grafted Italian ″Marrone″ cultivar		
Leaf	0.23 ± 0.012	6.18 ± 0.045
Catkin	0.34 ± 0.007	10.58 ± 0.078

The total flavonoids content was analysed by aluminium chloride colorimetric assay [17]. It have been expressed as g of catehin equivalents (CE) per 100 g of the dry extract sample (%; w/w), i.e. %CE. Analysis was performed on Hewlett Packard 8452 spectrophotometer.

Lipid peroxidation induced by Fenton reaction

The systems of liposome and erythrocyte ghost have been used extensively as biological models for in vitro lipid peroxidation studies. Cellular membranes, which contain abundant phospholipids, such as phosphatidylcholine (lecithin), are major targets of free radicals which induce lipid peroxidation and thereby cause malfunctioning of membranes by altering membrane fluidity and membrane bound enzyme and receptor functions [12].

For the preparation of liposomes, a chloroform solution of L-α-phosphatidylcholine (purchased from Sigma-Aldrich) was evaporated under vacuum to dry condition. A phosphate buffer (Na_2HPO_4 1.2 g/L, NaH_2PO_4 0.43 g/L, pH 7.4) was added to obtain concentration of 125 mM of lipids, and the suspension was vortexed for 5 min. Extracts dissolved in the buffer were added to the final concentration of 0.2 mg/mL (final concentration of lipids was 100 mM). In the control sample, aliquot of buffer was added. Samples were exposed to radical-generating mixture consisting of 0.5 mM H_2O_2 and 0.075 mM $FeSO_4$, for 20 minutes after which 10 aliquots of buffer were added in order to stop the reaction. The samples were centrifuged for 10 minutes/10000 rpm and supernatant was discarded. The liposomes were again dissolved in the buffer, and the solution was applied to dried 7-DS (previously dissolved in methanol), and vortexed. Control samples were treated in the same manner, but without exposure to the radical-generating system. The (spin probe) / (membrane lipid) ratio of approximately 1:200 was used [6]. The order parameter was calculated from spectral parameters as described in Fig. 1.

EPR spectra were recorded at room temperature using a Varian E104-A EPR spectrometer operating at X-band (9.51GHz) using the following settings: modulation amplitude, 2G; modulation frequency, 100kHz; microwave power, 10mW; time constant, 0.25 s; field centre, 3390G; scan range, 100G; scan time, 8 minutes. Spectra were recorded using EW software (Scientific Software, Bloomington, IL, USA). Samples were drawn into 10cm long gas-permeable Teflon tubes (wall thickness 0.025mm and internal diameter 0.6mm; Zeus industries, Raritan, USA). Measurements were performed using

Table 2: Mean particle size and yield of dry extract of *Castanea sativa* Mill. (2007.)
Tablica 2: Srednji promjer čestica i prinos suhog ekstrakta *Castanea sativa* Mill. (2007.)

Sample	Mean particle size (mm)	Yield of dry extract % (w/w)
Sweet chestnut		
Leaf	0.25 ± 0.006	14.19 ± 0.063
Catkin	0.32 ± 0.005	9.33 ± 0.045
Lovran's Marrone cultivar		
Leaf	0.26 ± 0.006	5.01 ± 0.073
Catkin	0.25 ± 0.008	7.67 ± 0.039
Grafted Italian ″Marrone″ cultivar		
Leaf	0.25 ± 0.010	13 ± 0.036

Table 3: Total phenolics and flavonoids contents (2006.)
Tablica 3: Sadržaj ukupnih fenola i flavonoida (2006.)

Sample	Total phenolics content (%; w/w, i.e. % GAE)	Total flavonoids content (%; w/w, i.e. % CE)
Sweet chestnut		
Seed	0.42 ± 0.067	0.17 ± 0.008
Brown seed shell	1.19 ± 0.126	0.65 ± 0.021
Red seed coat	2.82 ± 0.045	1.44 ± 0.012
Peeled chestnut	0.58 ± 0.029	0.09 ± 0.003
Leaf	1.40 ± 0.011	0.32 ± 0.021
Catkin	3.28 ± 0.154	0.60 ± 0.031
Old chestnut bark	1.70 ± 0.097	0.69 ± 0.056
Young chesnut bark	3.00 ± 0.076	0.75 ± 0.027
Spiny burs	0.49 ± 0.023	0.13 ± 0.017
Lovran's Marrone cultivar		
Seed	0.66 ± 0.045	0.16 ± 0.010
Brown seed shell	3.24 ± 0.023	1.06 ± 0.056
Red seed coat	6.16 ± 0.085	1.62 ± 0.018
Peeled chestnut	0.11 ± 0.004	0.03 ± 0.002
Leaf	2.42 ± 0.056	0.61 ± 0.024
Grafted Italian ″Marrone″cultivar		
Leaf	1.71 ± 0.065	0.42 ± 0.031
Catkin	3.96 ± 0.012	0.83 ± 0.092

quartz capillaries in which Teflon tubes were placed. From the spectra of 7-DS incorporated in liposomes, evaluation was made of the order parameter (S) that is reciprocally proportional to the membrane fluidity.

Statistical analysis

All determinations have been made in the triplicate and the values have been averaged and reported along with the standard deviation (± Standard Deviation). Statistical analysis has been carried out by using Microsoft Excel 2000 software (CORREL statistical function). Pearson correlation test has conducted to determine the correlation among variables. Significant levels were defined using $P \leq 0.05$. All experiments have been performed at least in triplicate. The results have been presented as mean values ± S.D.

RESULTS AND DISCUSSION

The plant material gained in 2006. was milled to the mean particle size from (0.18 ± 0.015mm) to (0.40 ± 0.021mm). The yield of dry extract of sweet chestnut (Table 1), expressed as in % (w/w), was from 1.82 ± 0.066% for

Table 4: Total phenolics and flavonoids contents (2007.)
Tablica 4: Sadržaj ukupnih fenola i flavonoida (2007.)

Sample	Total phenolics content (%; w/w, i.e. % GAE)	Total flavonoids content (%; w/w, i.e. % CE)
Sweet chestnut		
Leaf	4.03 ± 0.035	1.51 ± 0.075
Catkin	3.99 ± 0.011	0.96 ± 0.013
Lovran's Marrone cultivar		
Leaf	2.55 ± 0.063	0.72 ± 0.033
Catkin	3.43 ± 0.079	0.62 ± 0.016
Grafted Italian ″Marrone″cultivar		
Leaf	4.42 ± 0.059	1.10 ± 0.046

spiny burs of sweet chestnut to 13.32 ± 0.074% for red seed coat of Lovran's Marrone.

In 2007. selected plant material was milled to the mean particle size from (0.25 ± 0.010mm) to (0.32 ± 0.005mm) (Table 2), while the yield of dry extract ranged from (5.01 ± 0.073%) for leaf Lovran's Marrone cultivar to (14.19 ± 0.063%) for leaf of sweet chestnut.

The highest content of total phenolics compounds (6.16 ± 0.085 %GAE) was determined in dry extract of red seed coat, while the lowest content (0.11 ± 0.004 %GAE) was obtained for the dry extract of peeled chestnut Lovran's Marrone cultivar, in 1996, Table 3. The total flavonoids content was in the interval from 0.03 ± 0.002 %CE for peeled chestnut of Lovran's Marrone cultivar, to 1.62 ± 0.018%CE for red seed coat of Lovran Marrone.

In the second year of investigations we focused on extracts of C. sativa leaves (CSL) and catkin. CSL used in folk medicine as a tea in France to treat hacking cough and diarrhea and has already been demonstrated that CSL contain phenolic compounds [20]. It was demonstrated [4] that the aqueous, methanol, and ethyl acetate extracts of CSL had good antioxidant potential as compared to Vitis Vinifera.

Total phenolics contents (Table 4) are the lowest (2.55 ± 0.063 %GAE) in dry extract of leaf of Lovran's Marrone and the highest (4.42 ± 0.059 % GAE) in leaf of grafted Italian ″Marrone″ cultivar. The total flavonoid contet ranged from (0.62 ± 0.016 % CE) for catkin of Lovran's Marrone to (1.51 ± 0.075 % CE) obtained for leaf of sweet chestnut.

Lipid peroxidation can be induced by reactive oxygen species, which can break down membrane integrity. The capacity of extracts of chestnut to prevent and remove lipid peroxidation of liposomes was tested using the Fenton reaction as producing system of ·OH, which efficiently provokes peroxidation. EPR spin probing technique and the membrane spin probe 7-Doxyl Stearate were used to assess membrane fluidity decrement which is known to correlate with the increased lipid peroxidation [22]. From the spectra of 7-DS incorporated in liposomes, evaluation of the order parameter (S) was made that is reciprocally proportional to the membrane fluidity.

Table 5 shows order parameters (S) of: i) pure liposomes, ii) liposomes exposed to the Fenton system, iii) liposomes mixed with chestnut extracts, and iv) liposomes mixed with chestnut extracts exposed to the Fenton system. Measurements of S parameter for pure liposomes and liposomes exposed to the Fenton system were performed in order to evaluate the level of decrease of the membrane fluidity (which is reciprocal to S) provoked by the ·OH radical generated in the Fenton system. S of untreated liposome/extract mixtures was acquired in order to determine whether components of the extracts provoke changes in the membrane fluidity, which could imply mechanisms of protection from peroxidation and some other possible applications of extracts.

Extracts of catkin of sweet chestnut, peeled chestnut and seed Lovran's Marrone cultivar did not show the ability to protect liposomes from peroxidation. All the other extracts showed activity. Extracts of seed and leaf of sweet chestnut also induced decrease of the membrane fluidity in untreated samples (not exposed to ·OH radicals from the Fenton reaction). Changes in the fluidity of untreated liposomes predict that some compound(s) in the extracted seed, leaf and spiny burs intercalated into the membrane. It seems that they contain some lipophilic component(s) changing fluidity of liposomes, and preferentially remove lipid radicals inside the membrane. Other extracts most likely prevent lipid peroxidation by removing ·OH radicals in the solution. Hydroxyl radical is known to induce peroxidation of lipids, H_2O_2 passes cell membrane, and can directly affect intracellular metabolism, provoking DNA damage, mitochondria

Table 5: Order parameter (S) of liposomes and liposomes mixed with chestnut extracts, measured using EPR and spin-probe 7-DS. In treated samples Fenton reaction is applied as a radical-generating system (2006.)
Tablica 5: Parametar (S) liposoma i liposoma pomiješanih s ekstraktom kestena, određen upotrebom EPR-a i spin probe 7-DS. U tretiranim uzorcima tijekom Fentonove reakcije aplicira se kao radikal-generirajući sistem (2006.)

	Samples	S
Controls	Untreated liposomes	0.590 ± 0.002
	Treated liposomes	0.609 ± 0.004
Extracts		
	Sweet chestnut	
Seed	Untreated liposomes/extract	0.621 ± 0.002
	Treated liposomes/extract	0.619 ± 0.010
Brown seed shell	Untreated	0.613 ± 0.003
	Treated	0.611 ± 0.003
Red seed coat	Untreated	0.612 ± 0.003
	Treated	0.617 ± 0.003
Peeled chestnut	Untreated	0.595 ± 0.004
	Treated	0.587 ± 0.005
Leaf	Untreated	0.610 ± 0.004
	Treated	0.604 ± 0.003
Catkin	Untreated	0.590 ± 0.004
	Treated	0.601 ± 0.002
Old chestnut bark	Untreated	0.597 ± 0.002
	Treated	0.597 ± 0.004
Young chestnut bark	Untreated	0.620 ± 0.005
	Treated	0.619 ± 0.003
Spiny burs	Untreated	0.579 ± 0.002
	Treated	0.587 ± 0.010
	Lovran's Marrone cultivar	
Seed	Untreated	0.612 ± 0.004
	Treated	0.621 ± 0.002
Brown seed shell	Untreated	0.604 ± 0.007
	Treated	0.603 ± 0.009
Red seed coat	Untreated	0.606 ± 0.007
	Treated	0.603 ± 0.009
Peeled chestnut	Untreated	0.606 ± 0.002
	Treated	0.620 ± 0.005
Leaf	Untreated	0.592 ± 0.003
	Treated	0.588 ± 0.003
	Grafted Italian ″Marrone″ cultivar	
Leaf	Untreated	0.593 ± 0.002
	Treated	0.597 ± 0.002
Catkin	Untreated	0.598 ± 0.005
	Treated	0.606 ± 0.003

disorder, and cell death [5].

In the other hand, extracts of young chestnut bark induced significant decease of membrane fluidity in liposomes untreated by the Fenton system, while extracts of seed of Lovran's Marrone cultivar, red seed coat and brown seed shell of sweet chestnut provoked slight decrease. Extract of red seed coat of sweet chestnut is the richest source of total phenolics (Table 3). It has been demonstrated by [1] that flavonoids and isoflavonoids partition preferentially into the hydrophobic core of membranes. They stabilize the membrane through decrease in lipid fluidity, and this may be a contributory mechanism toward their known ability to inhibit membrane peroxidation. The obtained results shown that chestnut extracts have some active

compounds which are hydrophilic as well as some which are hydrophobic, protection of liposomes from lipid peroxidation most likely goes through two mechanisms – prevention, by removing •OH in the solution, and removing lipid radicals inside the membrane.

Increase of the membrane fluidity provoked by the extract of spiny burs should be taken into account for their further application in diet and medicine, since changes in the cell membrane fluidity are known to affect properties and functions of the cell [26]. Previous studies concerning EPR spin probing method have shown that hypertension has been correlated to decreased fluidity of erythrocytes membrane. Some compounds that have hypotensive effects increased significantly membrane fluidity of erythrocytes in hypertensive patients [24]. Hypertension is associated with an increased risk of atherosclerosis. This indicates that extract of spiny burs could potentially have beneficial effects in diet of hypertensive patients.

Results obtained for extracts produced in 1997 were shown in Table 6.

Extract of catkin of sweet chestnut slightly increased the fluidity of untreated liposomes and prevents lipid peroxidation. All other samples showed activity in removing/preventing lipid peroxidation. Extract of leaf of Grafted Italian ″Marrone″cultivar induced significant decease of membrane fluidity in liposomes untreated by the Fenton system. That particular extracts also have the highest content of total phenolics. Increase of membrane fluidity observed for extract of catkin of sweet chestnut should be also taken into account for its further use.

In correlation analyses, very significant ($r = 0.63$) ($P < 0.01$) linear correlations were observed to exist between yield of dry extract and total phenolics content ($r = 0.59$), and total phenolics and total flavonoids content ($r = 0.87$) ($P < 0.01$). Significant ($r = 0.49$) ($P < 0.05$) correlations existed between yield of dry extract and total flavonoids ($P < 0.01$). Extracts, rich in phenolic compounds also possess sufficiently high yield of dry extract, what is important parameter in their application.

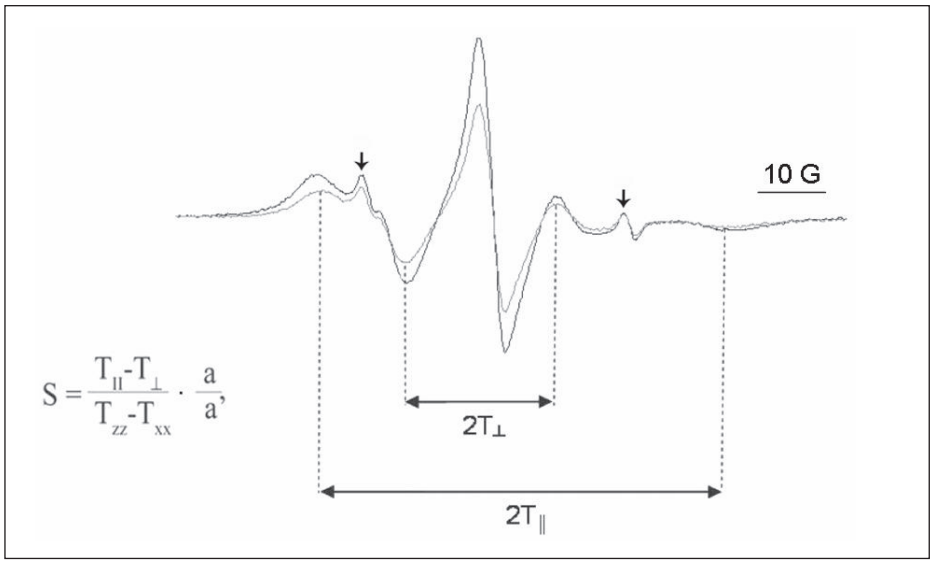

Fig 1: EPR spectra of liposomes labeled with 7-DS (dark: liposomes treated with •OH radicals; pale: untreated liposomes). S: order parameter. 2TII: outer hyperfine splitting. 2T□: inner hyperfine splitting. a: isotropic hyperfine coupling constant in crystal [a = 1/3(Txx + Tyy + Tzz)]. a': isotropic hyperfine coupling constant in membrane [a' = 1/3(TII + 2T_)]. Txx, Tyy, Tzz: hyperfine constants (for 7-DS, they were taken to be Txx = Tyy = 6.1 G, Tzz = 32.4 G). Figure shows how 2TII and 2T□ for untreated liposomes were measured. Two narrow lines (vertical arrows) originate from the 7-DS in the solution.

Slika 1: EPR spektar liposoma obilježenih s 7-DS-om (tamno: liposomi tretirani s •OH radikalima; svijetlo: netretirani liposomi) S: parameter. 2TII: vanjsko hiperfino cijepanje. 2T□: unutrašnje hiperfino sprezanje. a: izotropna hiperfina konstanta sprezanja kristala [a = 1/3(Txx + Tyy + Tzz)]. a': izotropna hiperfina konstanta sprezanje membrane [a' = 1/3(TII + 2T_)]. Txx, Tyy, Tzz: hyperfine konstante (za 7-DS, uzima se da je Txx = Tyy = 6,1 G, Tzz = 32,4 G). Slika pokazuje kako se 2TII i 2T□: određuju za netretirane liposome. Dvije bliske linije (vertikalne linije) potječu od 7-DS u rastvoru.

Table 6: Order parameter (S) of liposomes and liposomes mixed with chestnut extracts, measured using EPR and spin-probe 7-DS. In treated samples Fenton reaction is applied as a radical-generating system (2007.)
Tablica 6: Parametar (S) liposoma i liposoma pomiješanih s ekstraktom kestena, određen upotrebom EPR-a i spin probe 7-DS. U tretiranim uzorcima tijekom Fentonove reakcije aplicira se kao radikal-generirajući sistem (2007.)

	Samples	S
Controls	Untreated liposomes	0.600 ± 0.005
	Treated liposomes	0.616 ± 0.002
	Extracts	
	Sweet chestnut	
Leaf	Untreated	0.599 ± 0.004
	Treated	0.599 ± 0.003
Catkin	Untreated	0.593 ± 0.002
	Treated	0.605 ± 0.010
	Lovran's Marrone cultivar	
Leaf	Untreated	0.605 ± 0.004
	Treated	0.605 ± 0.005
Catkin	Untreated	0.603 ± 0.002
	Treated	0.606 ± 0.002
	Grafted Italian ″Marrone″ cultivar	
Leaf	Untreated	0.621 ± 0.002
	Treated	0.616 ± 0.004

CONCLUSIONS

The results are promising and indicate that phenolics and flavonoids of ethanol extracts of different tissues and cultivar of Castanea sativa Mill. may be useful food compounds. It proved that purified phenolic compounds are difficult to get. There is a growing interest for the use of plant extracts because sometimes they have antioxidant activities higher than those of pure molecules [25].

Summarizing, all extracts, excluding extracts of peeled chestnut, seed of Lovran's Marrone cultivar and catkin of sweet chestnut, protected liposomes from lipid peroxidation, and could be used as protective reagents for obstruction of oxidative stress provoked by $^{\bullet}OH$ and H_2O_2. Catkin of Lovran's Marrone cultivar slightly increased the fluidity of untreated liposomes. Extract of spiny burs provoked decrease of fluidity of liposomes untreated with radical-generating system. Extracts of young chestnut bark, seed of sweet chestnut, seed of Lovran's Marrone cultivar and brown seed shell of sweet chestnut and leaf, as well as leaf of Grafted Italian Marrone cultivar gained in 1997 induced decease of membrane fluidity in untreated liposomes.

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