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Full Length Research Paper

Investigation of antiradical activity of *Salvia officinalis* L., *Urtica dioica* L., and *Thymus vulgaris* L. extracts as potential candidates for a complex therapeutic preparation

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The aim of this study was to investigate free radical-scavenging activity of *Salvia officinalis* L., *Thymus vulgaris* L., *Urtica dioica* L. extracts and mixture of extracts, and to evaluate the effect of these extracts on the ability of mitochondria to generate free radicals. The evaluation of the activity of extracts using 2,2-diphenyl-1-picrylhydrazyl (DDPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radicals scavenging assays and tyrosine nitration inhibition (TNI) techniques showed that thyme extract had the strongest free-radical-scavenging activity. This extract was also found to contain the highest amount of phenols. Mixture of extracts showed slow and identical DPPH and ABTS radical-scavenging activity, with the strongest activity demonstrated during the first minute. Investigation of the ability of extracts to neutralize hydrogen peroxide showed that the lowest extract amount used (0.01 mg) neutralized 24 to 50% and the highest amount (0.1 mg) – 72 to 88% of H_2O_2. In all cases, thyme extract, had the strongest antioxidant effect. Rat liver mitochondria incubated with 0.1 mg of extracts generated by 45 to 55% less radicals than control mitochondria did. Mitochondria incubated with 1 mg of extracts generated smaller amounts of ROS than incubated with 0.1 mg of extract – in case of thyme extract or even more – in case of stinging nettle or sage extracts.

Key words: *Salvia officinalis* L., *Thymus vulgaris* L., *Urtica dioica* L., antioxidant activity.

INTRODUCTION

Antioxidant effect of crude medicines has been widely investigated, and it has been evaluated using spectroscopy, chemiluminescence, enzymes, and other techniques (Medić-Sarić et al., 2009). Cation scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH·) or 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radicals and peroxynitrite induced inhibition of tyrosine nitration (TNI) are the simplest but informative, and therefore most extensively used for plant samples tests (Mavi et al., 2004; Krishnaiah et al., 2010). According to some authors, antioxidant activity of plant extract is directly proportional to the concentration of phenolic compounds and flavonoids (Komes et al., 2010). *Salvia officinalis* L. (*g*arden sage), *Thymus vulgaris* L. (common thyme), and *Urtica dioica* L. (stinging nettle) extracts were selected for experimental testing due to their great antioxidant

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potential (Krishnaiah et al., 2010; Gülçin et al., 2004; Tepe et al., 2007). The selection of these plants was based on the data from literature. It is known that stinging nettle extract exhibits stronger free radical scavenging properties compared with commonly used antioxidant α-tocopherol (Gülçin et al., 2004). Common sage is a well known for its antioxidant properties that are due to its phenolic-rich composition.

Extract from common sage is rich in phenolic acids such as rosmarinic acid, caffeic acid, ferulic acid, and these compounds possess high anti-radical properties (Krishnaiah et al., 2010). Antioxidant activity of common thyme and its extracts is dependent mainly on essential oil content and composition (Chizzola et al., 2008). However, there are no experimental data on the antioxidant activity of a mixture consisting of *Salvia officinalis* L., *Thymus vulgaris* L. and *Urtica dioica* L. In the scientific literature it is indicated that multi-drug strategy is based on a long awareness that many diseases have a multi-causal etiology and a complex pathophysiology. Diseases can obviously be treated more effectively with well-chosen pharmaceutical combinations than with a single drug (Gajdziok et al., 2010; Wagner et al., 2009).

Investigation of the antioxidant activity of plants or extracts using several techniques enables one to evaluate their ability to neutralize various reactive forms of compounds that are characteristic of the normal condition of an aerobic cell. Mitochondria are known to be a very important source of intracellular reactive oxygen species (ROS). Mitochondrial respiration chain is composed of large enzymatic complexes located within the phospholipid bilayer of the internal membrane. Two of these complexes (I and III) are responsible for the major part of the generated superoxide radicals. Transformation of the latter into hydrogen peroxide and hydroxyl radicals is an important process that may result in cellular structure damage (Duchen, 2004). Heat, trauma, infections, other negative environmental factors, and also transition of the cell from the aerobic into the anaerobic state increase ROS concentration in the cells to the level that results in toxicity of these compounds: free radicals may react with membrane lipids, proteins, and nucleic acids, initiating auto-catalytic reactions, and severe oxidative stress causes cell death (apoptosis or necrosis). ROS play an important role in the pathogennesis in a number of diseases such as, inflammatory or degenerative diseases, ischemia/reperfusion, atherosclerosis, cataract, cancer and aging of the organism (Drummond et al., 2011). For this reason, the protection of the organism from oxidative stress is a highly relevant issue.

Thus, the aim of this study was to investigate the ability of garden sage, common thyme and stinging nettle extracts, and a mixture of extracts to scavenge free radicals, and to evaluate the effect of these extracts on the ability of mitochondria, the main source of ROS in cells, to generate free radicals.

MATERIALS AND METHODS

Dry extract from *T. vulgaris* L. leaves (Naturex, France); dry extract from *S. officinalis* L. (Shaanxi Jiahe Phytochem, China); dry extract from *U. dioica* L. leaves (Naturex, France).

Preparation of plant extracts mixture and Trolox solution

A mixture of plant extracts consisted from 6 parts of thyme extracts, 2 parts of sage extract and 2 parts of stinging nettle extract. A certain amount of nettle, sage, thyme extracts and the mixture of extracts were dissolved in water (10 mg/ml). Solutions were filtered through the membrane filter with pore size 0.45 µm.

The standard solutions of Trolox were made for evaluation of Trolox equvivalent antioxidant activity (TEAC) by the DPPH, ABTS and peroxynitrite scavenging tests. A certain amount of Trolox was diluted in ethanol and the concentration was from 0 to 500 µmol/L.

Determination of the content of phenolic compounds

All spectrophotometric measurements were performed using the Unicam Heλios α 9423 spectrophotometer. The appropriately diluted plant extract was oxidized with 0.2 N Folin reagent (Folin-Ciocalteu reagent) and subsequently neutralized with sodium carbonate solution (75 g/L). After 2 h, the suspension was centrifuged (5000x g, 10 min), and absorption level was measured at 760 nm wavelength. The content of phenolic compounds was determined according to the calibration curve of gallic acid. The results were expressed as gallic acid equivalent (GAE) per 1 g of the extract. The number of experiments was n = 5.

Isolation of rat liver mitochondria

The liver was washed with 0.9% KCl solution (0°C). The tissue was comminuted and homogenized using a mechanical homogenizer (glass/Teflon), using 10 mL/g of tissue homogenization medium (70 mM sucrose, 210 mM mannitol, 10 mM HEPES, 1 mM EGTA, and 5 mg/mL bovine serum albumin, pH 7.4; 2°C). The homogenate was centrifuged at 750x g for 5 min., the supernatant was filtered through a double gauze filter, and then repeatedly centrifuged at 10,000x g for 10 min. Mitochondrial sediments were suspended in the medium (70 mM sucrose, 210 mM mannitol, and 10 mM HEPES, pH 7.4; 2°C), and centrifuged again at 10,000xg for 10 min. Mitochondrial sediments were suspended again, and the resulting mitochondrial suspension was stored in ice.

Evaluation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

One of methods to evaluate antioxidant activity was DPPH assay (Molyneux, 2004). The solution of 75 µM DPPH in ethanol was prepared daily before the measurement. 2.0 mL of DPPH solution were mixed with 50 µl of plant extract solution (0.8 mg/mL) in a 1 cm^3-thick disposable cuvette. The absorption of remaining DPPH was determined after 30 min. at 517 nm. Antioxidant activity of extracts was expressed as Trolox equivalent antioxidant capacity (TEAC) (mmol/100 g of extract). TEAC of extracts was determined according to the calibration curve of Trolox. Antioxidant activity of mixture was evaluated by measuring the percentage of DPPH

Table 1. The content of phenolic compounds determined in the studied extracts.

Studied extract	Total polyphenol content (GAE mg/g)[a]
Salvia officinalis L.	24.6±0.4*[#]
Thymus vulgaris L.	96.0±0.9
Urtica dioica L.	59.6±0.7*
Mixture of extracts	55.64±0.4

[a] data expressed as gallic acid equivalent (GAE) mg per 1 g of extract. * p<0.05 vs. *Thymus vulgaris* L. extract; [#] p<0.05 vs. *Urtica dioica* L. extract; values are means (n=5) ± SE (standard error).

radical neutralized by antioxidant compounds in the tested sample. The same procedure was applied to measure it, except the absorption was measured at certain times (1, 5, 15 min etc. till 6 h). Simultaneously, the absorption level of the control sample was measured. The DPPH radical scavenging activity was calculated by the formula:

$$Q = \left(1 - \frac{A_s}{A_b}\right) \cdot 100 \qquad (1)$$

Where Q is inhibition of DPPH, A_s is absorbance of mixture and A_b is absorbance of control. The number of experiments was n = 5.

Evaluation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging

Other method to evaluate antioxidant activity was ABTS radical cation decolorization assay (Re et al, 1999). ABTS was dissolved in water to the concentration 15 mmol/l and a certain amount of potassium persulfate was dissolved in water to the concentration 5 mmol/L. These two solutions were mixed and kept in a dark at room temperature for 16 h in order to produce ABTS radical. This solution was diluted with water to reach the absorbance of 0.800 (±0.03) at 734 nm. 2.0 ml of ABTS solution was mixed with 20 µl of extract solution, which concentration was 0.8 mg/mL in 1 cm^3-thick cuvette. The absorption of sample was measured after 6 min at 734 nm. Results were expressed as TEAC according to the Trolox calibration curve.

Antioxidant activity of mixture from extracts was evaluated by measuring the percentage of ABTS radical bleaching. The same procedure was applied as described therein; just the absorption was measured at time intervals (1, 5, 15 min etc. till 6 h). Simultaneously, the absorption level of the control sample was measured. The ABTS radical scavenging activity was calculated by the formula in Equation (1). The number of experiments was n = 5.

Evaluation of the inhibition of tyrosine nitration (TNI)

For the investigation, high-performance liquid chromatography (HPLC) system HP 1100 (Agilent Technologies) with diode array detector (HP 8453, Agilent Technologies) was used. 250 µl of 2 mM tyrosine solution in 0.15 M phosphate buffer (pH 7.4) was prepared, and mixed with 250 µl of a mixture. 42 µl of this solution in the injector of the autosampler was mixed with 8 µl of 2.5 mM peroxynitrite solution in 0.05 M sodium hydroxide, and then was injected into HPLC column *Supelcosil ABZ Plus* 250 × 4.6 mm, 5 µm. The mobile phase consisted of 90% 40 mM formic acid and 10% methylcyanide, flow velocity – 1 ml/min. Chromatograms were recorded at 356 nm wavelength. The activity of a mixture was calculated on the basis of 3-nitrotyrosine peak, compared to the control. The result was expressed as percents of inhibited radical. The number of experiments was n = 3.

Fluorimetric evaluation of antioxidant activity of extracts

For investigation of antioxidant activity by fluorimetric analysis different amounts of plants extracts (10 mg of extracts were dissolved in 1 ml of water) were added. The incubation of mitochondria was performed using the following procedure: mitochondria (1 mg of protein), a certain amount of extracts solutions (10, 40, 70 or 100 µl), and mitochondrial media up to 200 µL were placed in Eppendorf test-tubes. The content of the test-tubes was shaken and stored in ice for 10 min. Following that, the test tubes were centrifuged, the medium was decanted, and the mitochondria were suspended in another portion of the medium and stored in ice until the testing.

Each fluorimetric well was filled in with the following: (1) a set amount of hydrogen peroxide and the studied amount of the extract, or (2) isolated rat liver mitochondria (0.25 mg/ml protein) incubated with extracts, pyruvate + malate (6 mM both), and antimycin A (1 µM). Subsequently, the following substances were added: horseradish peroxidase (1 U/ml), Amplex Red (10 µM), and measurement medium (up to 200 µl). The measurements were performed for 10 min at the temperature of 37°C. The samples were continuously stirred during measurements. The intensity of fluorescence in the wells was measured (at 544 nm excitation and 590 nm emission wavelengths) immediately after adding the ingredients and at 5 and 10 min thereafter. The number of experiments was n = 3.

Statistical data analysis

Data are presented as Means ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Dunnett's post test using the software package Statistica 1999, v. 5.5 (StatSoft Inc.). A value of p <0.05 was taken as the level of significance.

RESULTS

Phenolic compound content in the tested extracts, calculated as equivalent to gallic acid, ranged from 24.6 ± 0.4 to 96.0 ± 0.9 mg/g (Table 1). The highest amount of phenolic compounds was detected in thyme extract that is, by 1.67-fold more than in stinging nettle extract, and by 4.04-fold more, compared to sage extract. Mixture of

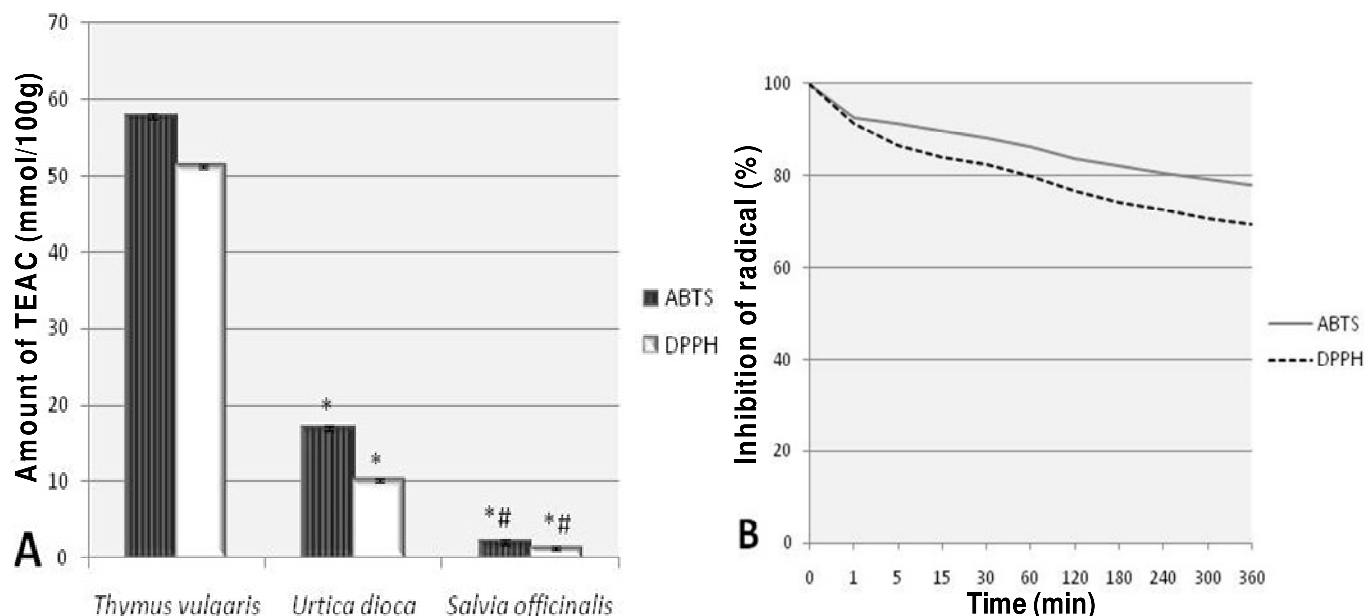

Figure 1. Free radical-scavenging activity of *Thymus vulgaris* L., *Salvia officinalis* L., *Urtica dioica* L. extracts (A) and of mixture of extracts (B). * $p<0.05$ vs. *Thymus vulgaris* L. extract, # $p<0.05$ vs. *Urtica dioica* L. extract.

extracts contained 55.64 ± 0.4 mg/g of phenolic compounds. The evaluation of the antioxidant activity of extracts was first performed by applying DPPH and ABTS radical-scavenging assays. Free radical-scavenging activity of extracts was expressed as TEAC.

Figure 1A indicates that thyme extract had the strongest free radical-scavenging effect ($TEAC_{DPPH}$ was 51.16 mmol/100 g, $TEAC_{ABTS}$ was 57.74 mmol/100 g), stinging nettle was fivefold weaker antioxidant comparing results of DPPH assay and 3.4-fold weaker free radical-scavenger comparing TEAC of ABTS assay than thyme. Common sage exhibited weak free radical scavenging properties ($TEAC_{DPPH}$ was 1.24 mmol/100 g, $TEAC_{ABTS}$ was 2.08 mmol/100 g).

In order to determine antioxidant activity of mixture from extracts the DPPH and ABTS assays were used, just results were expressed as percents of radical inhibition. We were interested in kinetic behavior of mixture of extracts, for this reason bleaching of radicals was measured for six hours (Figure 1B). During first five minutes, mixture demonstrated fast antioxidant activity; DPPH was bleached almost 14% compared with control (water). Later mixture of extracts ensures slow but tantamount antioxidant activity, and during five hours a mixture inhibited more than one third of DPPH radical compared with control. Results of ABTS assay were similar, but not the same. Mixture exhibited slow and tantamount radical-scavenging activity but inhibition was weaker compared with DPPH method. During first five minutes mixture inhibited 5% weaker effect than in the DPPH case, during all experiment decolorization of ABTS cation radical was 22%. The third technique that was applied to determine the free radical-scavenging activity of the tested extracts was inhibition of peroxynitrite induced tyrosine nitration. The inhibition of tyrosine nitration of mixture (0.2 mg/mL) was 65.3±1.1%. Trolox equivalent antioxidant activity of this sample was 72.6±1.9%.

The last technique applied to determine the antioxidant activity of the studied extracts was the evaluation of the neutralization of hydrogen peroxide in the presence of horseradish peroxidase and Amplex Red. In this series of experiments the ability of various amounts (0.01 to 0.1 mg) of extracts to neutralize hydrogen peroxide was investigated. Testing results showed that the lowest used amount of extracts (0.01 mg) neutralized 24 to 50% of H_2O_2, while the highest amount (0.1 mg) neutralized 72 to 88% of the compound (Figure 2).

Subsequently, the effect of various amounts of the studied extracts (0.1 to 1 mg) on the ability of rat liver mitochondria to generate ROS was investigated. Evaluation of ROS generation by mitochondria incubated with 0.1 mg of extracts (Figure 3) showed that in all cases mitochondria affected by extracts generated by ~48 to 57% less radicals, compared to control mitochondria. When mitochondria were incubated with highest amounts of thyme extract, ROS generation decreased with increasing amount of the extract, and dropped by 72%, when 1 mg of the extract was used (Figure 3). In case of stinging nettle extract, the highest effect was observed when 0.4 mg of the extract was used, and further increase of the concentration resulted in reduction of its effect. The highest inhibitory effect on ROS generation exhibited by garden sage extract was observed at

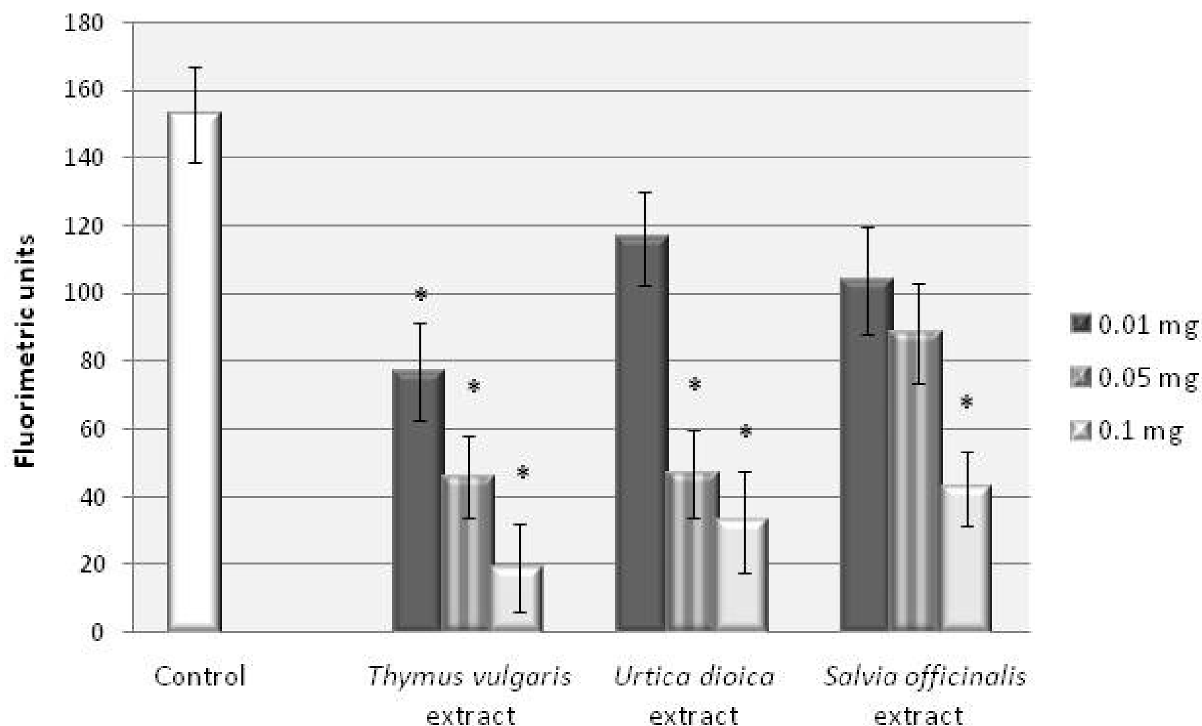

Figure 2. The ability of *Thymus vulgaris* L., *Salvia officinalis* L., *Urtica dioica* L. extracts at various concentrations to neutralize H_2O_2. * $p<0.05$ vs. control.

the lowest studied amount (0.1 mg) of this extract, higher concentrations resulted in reduced effect, and the highest studied concentration demonstrated strong ROS generation-stimulating effect.

DISCUSSION

The DPPH technique was applied in order to determine how effectively the tested extracts inactivated free radicals that subsequently transformed into DPPH-H type compounds. When applying the DPPH and ABTS technique, common thyme extract was found to have the strongest free radical-scavenging effect (Figure 1A). This is probably due to the high amount of phenolic compounds in the thyme extract, compared to garden sage or stinging nettle extracts (Table 1). Literature indicates that phenolic compounds have not only antioxidant, but also antiseptic and antimicrobial activity; information that is relevant in designing a new pharmaceutical composition of three medicinal herbs (Mavi et al., 2004).

U. dioica L. demonstrated weaker antioxidant properties than *T. vulgaris* L. Dadé et al. (2009) investigated antioxidant activity of *U. dioica* L. and determined that $TEAC_{DPPH}$ is 3 mmol/100 g of stinging nettle herb and $TEAC_{ABTS}$ is 19 mmol/100 g. These findings differ from our results, which may be due to different preparation of extract solution. Dadé et al. (2009) used infusions or decoctions prepared by common method in which teas are consumed, whereas in our experiment water solution of ethanolic dry stinging nettle extract was used.

Surprisingly, and in contrast with some sources of literature (Brewer, 2011; Suhaj, 2006), garden sage extract exhibited weak DPPH and ABTS radical-scavenging properties (Figure 1A). Likewise, it was found to contain statistically significant lower amount of phenolic compounds (Table 1). Tepe et al. (2007) investigated six species of sage (*S. caespitosa, S. hypargeia, S. euphratica, S. sclarea, S. candidissima,* and *S. aethiopis*) and found that the free radical-scavenging effect depended not on the amount of phenolic compounds, but on the ortho- and para- position of the hydroxyl group, which increases the polarity of the compound and increases its radical-scavenging capacity. Aruoma et al. (1992) found that in sage, orthodihydroxyl groups can be detected in the aromatic ring of carnosol and carnosic acid, which are characterized by strong antioxidant properties. Thus, weak antioxidant effect of sage extract suggests that the extract had few phenolic compounds with hydroxyl groups in ortho- and para-positions. Low amount of phenolic compounds in sage extract as well as weak radical-scavenging properties may induce the loss of some compounds in extract e.g. rosmarinic acid, which has low long-term stability (Kim et

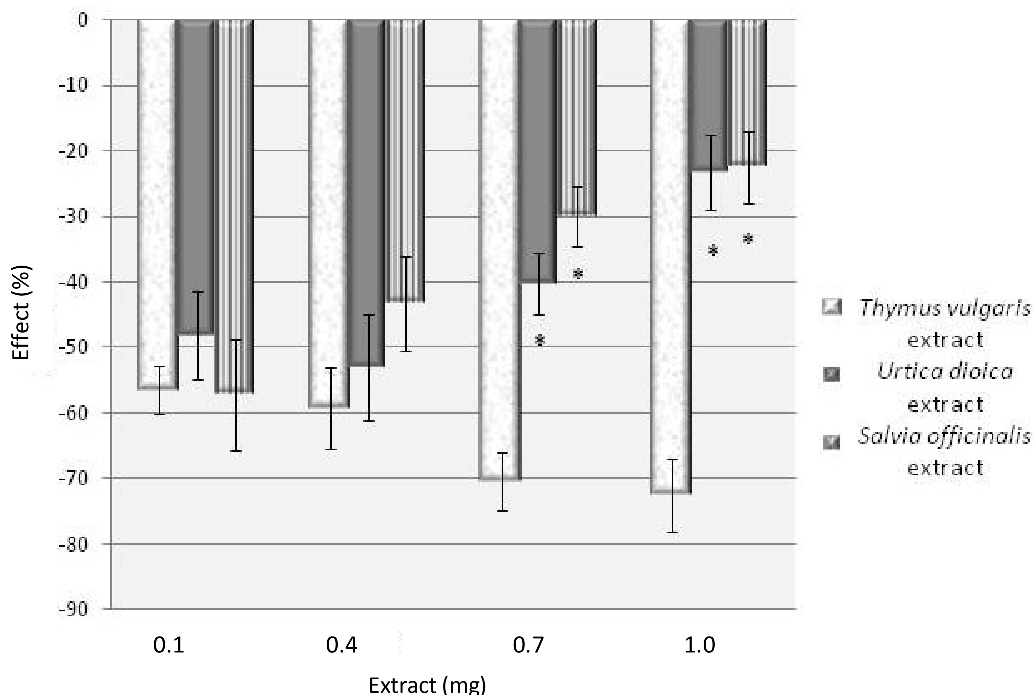

Figure 3. The effect of incubation with 0.1-1.0 mg of different extracts on the ability of isolated rat liver mitochondria to generate ROS (%). * $p < 0.05$ vs. *Thymus vulgaris* L. extract.

al., 2010), during manufacturing or storage too. Mixture of these extracts showed initial faster antioxidant activity following by slow kinetic behavior. Antioxidants can be divided in two groups: “fast-acting” antioxidants (ascorbic acid) or “slow-acting” antioxidants to which complex antioxidants as phenolic compounds can be assigned (Schauss et al., 2006). In our case, a mixture of extract was rich in phenolic compounds therefore its action was characteristic rather as the “slow” antioxidant.

TNI was used to determine how effectively studied preparations scavenged radicals forming during nitration of tyrosine by peroxynitrite ($ONOO^-$). Peroxynitrite is a potent oxidant that forms during a reaction of superoxide with nitrogen monoxide (Beckman et al., 1993; Squadrito et al., 1995; Beckman, 1996). In the presence of physiological disorders of the organism, peroxynitrite rapidly reacts with carbon dioxide, resulting in the formation of nitrogen dioxide and carbon oxide radical anions (Uppu et al., 1996; Lymar et al., 1995), which during nitration reactions may bind to tyrosine (Ramezanian et al., 1996; Van der Vliet, 1995). Mixture of extracts was effective scavenger of peroxynitrite.

All these tests showed that sage, nettle and thyme extracts also their mixture can be scavengers of different free radicals. The last technique was fluorimetric analysis of extracts. In all cases of neutralization of hydrogen peroxide, the strongest effect was observed in thyme extract – which had the highest content of phenolic compounds. Even though the content of phenolic compounds in stinging nettle extract was a half of that in thyme extract, and sage extract contained by fourfold less phenolic compounds, compared to thyme extract, they also effectively neutralized hydrogen peroxide.

We did not find any studies in literature that would analyze the effect of these extracts to the ability of mitochondria to generate ROS, and thus our study is new in this field. However, it has been proven that the majority of natural biologically active substances at low concentrations act as antioxidants (Medić-Sarić et al., 2009), while at higher concentrations they may have a pro-oxidant effect. In our study, low concentrations of stinging nettle and sage extracts resulted in stronger inhibition of ROS generation in mitochondria, compared to 1 mg, and thus we concluded that active substances in small amounts of these extracts acted as antioxidants, while at higher concentrations their effect was pro-oxidant. Active substances in thyme extract effectively inhibited ROS generation in mitochondria at all studied concentrations.

Conclusions

The tested plant extracts and mixture demonstrated effective antioxidant characteristics. For this reason, preparations produced from these extracts may be used as effective prevention and valuable additional treatment for diseases caused by oxidative stress. The strongest

effectiveness in scavenging DPPH and ABTS radicals was observed in thyme extract. Probably there is direct correlation between the amount of phenolic compounds in the preparation and its antioxidant activity. Mixture from sage, stinging nettle, and thyme extracts can provide slow but constant free radical-scavenging activity.

Low concentrations of all studied extracts (0.1 mg) in rat liver mitochondria had an antioxidant effect, while 10-fold higher concentrations of garden sage and stinging nettle extracts may have a pro-oxidant effect on the mitochondria. In case of thyme extract, the increase of its concentration led to higher antioxidant effect. Further studies would be required to investigate the effect of extracts and their active compounds on the mitochondrial respiratory chain components and their functions.

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REFERENCES

Aruoma OI, Halliwell B, Aeschbach R, Löligers J (1992). Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. Xenobiotica, 22(2): 257-268.

Beckman JS, Crow JP (1993). Pathological implications of nitric oxide, superoxide and peroxynitrite formation. Biochem. Soc. T., 21(2): 330-334.

Beckman JS (1996). Oxidative damage and tyrosine nitration from peroxinitrite. Chem. Res. Toxicol., 9(5): 836-844.

Brewer MS (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Compr. Rev. Food Sci. Food Safety, 10: 221-247.

Chizzola R, Michitsch H, Franz C (2008). Antioxidative properties of *Thymus vulgaris* leaves: Comparison of different extracts and essential oil chemotypes. J. Agric. Food Chem., 56: 6897-6904.

Dadé MM, Fioravanti DE, Schinella GR, Tournier HA (2009). Total antioxidant capacity and polyphenol content of 21 aqueous extracts obtained from native of Traslasierra valley (Argentina). BLACPMA, 8(6): 529-539.

Drummond GR, Selemidis S, Griendling KK, Sobey CG (2011). Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. Nat. Rev. Drug Discov., 10(6): 453-471.

Duchen MR (2004). Roles of mitochondria in health and diseases. Diabetes, 53: 98-102.

Gajdziok J, Bernatoniene J, Muselik J, Masteikova R, Dvoračkova K, Petkeviciute Z, Lazauskas R, Kalveniene Z, Bernatoniene R (2011). The evaluation of formulations for the preparation of new formula pellets. Pharm. Dev. Technol., 16(5): 520-528.

Gülçin I, Küfrevioğlu I, Oktay M, Büyükokuroğlu ME (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol., 90: 205-215.

Kim H-J, Kim T-H, Kang K-C, Pyo H-B, Jeong H-H (2010). Microencapsulation of rosmarinic acid using polycaprolactone and various surfactants. Int. J. Cosmet. Sci., 32: 185-191.

Komes D, Belščak-Cvitanović A, Horžić D, Rusak G, Likić S, Berendika M (2011). Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. Phytochem. Anal., 22: 172-180.

Krishnaiah D, Sarbatly R, Nithyanandam R (2011). A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process, 89: 217-233.

Lin CW, Yu CW, Wu SC, Yin KH (2009). Free-Radical scavenging activity, total phenolic contents and chemical composition analysis of forty-two kinds of essential oils. J. Food Drug Anal., 17(5): 386-395.

Lymar SV, Hurst JK (1995). Rapid reaction between peroxonitrite ion and carbon-dioxide: Implications for biological-activity. J. Am. Chem. Soc., 117(34): 8867-8868.

Mavi A, Terzi Z, Özgen U, Yildirim A, Coşkun M (2004). Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* L. (Urticaceae). Biol. Pharm. Bull., 27: 702-705.

Medić-Sarić M, Rastija V, Bojić M, Males Z (2009). From functional food to medicinal product: systematic approach in analysis of polyphenolics from propolis and wine. Nutri., 8: 33-51.

Molyneux P (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sc. Technol., 26(2): 211-219.

Ramezanian MS, Padmaja S, Koppenol WH (1996). Nitration and hydroxylation of phenolic compounds by peroxynitrite. Chem. Res. Toxicol., 9(1): 232-240.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice- Evans CA (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med., 26: 1231-1237.

Schauss AG, Wu X, Prior RL, Ou B, Huang D, Owens J, Agarwal A, Jensen GS, Hart AN, Shanbrom E (2006). Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, *Euterpe oleraceae* Mart. (Acai). J. Agric. Food Chem., 54: 8604-8610.

Silva MM, Santos MR, Caroco GA, Rocha R, Justino G, Mira L (2002). Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radical Res., 36(11): 1219-1227.

Squadrito GL, Pryor WA (1995). The formation of peroxynitrite in vivo from nitric oxide and superoxide. Chem.-Biol. Interact., 96(2): 203-206.

Suhaj M (2006). Spice antioxidants isolation and their antiradical activity: a review. J. Food Comp. Anal., 19: 531-537.

Tepe B, Eminagaoglu O, Akpulat HA, Aydin E (2007). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *amasiaca* (Freyn and Bornm.) *Bornm.* Food Chem., 100(3): 985-989.

Uppu RM, Squadrito GL, Pryor WA (1996). Acceleration of peroxynitrite oxidations by carbon dioxide. Arch. Biochem. Biophys., 327(2): 335-343.

Van der Vliet A, Eiserich JP, O'Neill CA, Halliwell B, Cross CE (1995). Tyrosine modification by reactive nitrogen species: a closer look. Arch. Biochem. Biophys., 319(2): 341-349.

Weng XC (1993). Antioxidant and their antioxidation mechanism. J. Zhengzhou Grain Coll., 3: 20-29.