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In vitro antioxidant activity evaluation of rosmaric acid and honey based supplement

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Abstract

Disrupted balance between free radicals and antioxidants leads to a pathophysiological state of oxidative stress, which is the underlying cause of many diseases. Since some synthetic antioxidants show some potentially adverse effects, many studies aim to find natural antioxidants. Secondary plant metabolites, such as polyphenols, phenolic acids, which include rosmarinic acid, terpenes and terpenoids have antioxidative properties and can be found in different hydrosols and honey. The aim of this study is to evaluate the in vitro antioxidative activity of the supplement based on hydrosol mixture, rosmarinic acid and honey. A series of diluted (100%, 50%, and 25%) supplements were added to the serum pool, collected from healthy donors. A second series of samples was prepared with the same amounts of supplement and addition of tert-Butyl hydroperoxide (TBH) as a prooxidant. Alongside these samples, sera samples with Trolox (hydrophilic vitamin E analog) and Trolox with TBH were prepared. All these samples were tested for the following oxidative stress parameters: total antioxidative status (TAS), paraoxonase-1 (PON-1), total sulfhydryl groups content (SHG), total oxidative status (TOS), prooxidative-antioxidative balance (PAB) and advanced oxidation protein products (AOPP). TAS/TOS ratio was calculated as a quantitative measurement of antioxidative and oxidative substances ratio in the serum. Antioxidative parameters TAS, SHG, TAS/TOS ratio and prooxidant/antioxidant balance parameter PAB, were not significantly different for the supplemened samples with and without the addition of TBH, which indicates that the supplement keeps its antioxidative properties, despite the addition of TBH. The supplement showed significant antioxidative properties as a result of the synergistic effect of rosmarinic acid, terpenes, and terpenoids from hydrosols and polyphenols and other antioxidant substances from honey.

Key words: *Oxidative stress; Antioxidants; Hydrosol; Terpenes; Polyphenols; Rosmarinic acid; Honey.*

INTRODUCTION

Free radicals represent atoms, ions or molecules which have one or more unpaired electrons in their outer shell, making them very reactive towards the other molecules. Antioxidants are molecules which have the ability to neutralize free radicals action. In the order for the normal physiological processes to take place, the balance between free radicals and antioxidants is necessary. Disrupting that balance in any way, could lead to pathophysiological state called oxidative stress. Due to the free radicals ability to damage lipids, proteins and DNA, it is understandable that oxidative stress may be a cause of many diseases. For that reason, the additional intake of antioxidants may be helpful in order to keep balance between free radicals and antioxidants. Since some of the synthetic antioxidants showed some potentially adverse effects, there are more studies aiming to find natural antioxidants, which should have less adverse effects and more efficiency [1,2].

Medical plants of the family *Lamiaceae* produce wide range of secondary metabolites, including antioxidants, such as ascorbic acid, tocopherol, carotenoids, terpenes and polyphenols. Polyphenols are large group of phytochemical compounds and their antioxidant activity depends on their structure and aromatic rings substituents. Flavonoids, tannins and phenolic acids, such as rosmarinic acid, are polyphenols with high antioxidative activity [3]. Some studies suggested that diets rich in plant polyphenols can offer some protection against development of cancer, cardiovascular diseases, diabetes, osteoporosis and

neurodegenerative diseases [4]. Over 8000 polyphenolic compounds have been found in different plant species, of which the largest group with the most bioactive potential are flavonoids [5]. The mechanisms involved in the antioxidant capacity of polyphenols include suppression of reactive oxygen species (ROS) formation by either inhibition of enzymes involved in their production, scavenging of ROS, metal ion chelation ability, or upregulation or protection of antioxidant defenses. Some polyphenols may react in plasma membrane with nonpolar compounds present in the hydrophobic inner membrane layer. In this way, they can affect oxidation rate of lipids or proteins and may prevent access of oxidants and protect the structure and function of the membrane [6].

Rosmarinic acid (RA) was, for the first time, isolated from rosemary leaves (*Rosmarinus Officinalis*), and it has been later isolated from other *Lamiaceae* and *Boraginaceae* family plants. Despite the fact that RA has been isolated from various plants, rosemary is still the biggest RA source. Rosmarinic acid is caffeic acid and 3,4-dihydroxyphenylacetic acid ester. Caffeic acid part of RA originates from phenylalanine, while 3,4-dihydroxyphenylacetic acid part of RA originates from tyrosine [7–9]. Antioxidative effect of phenolic acids, including rosmarinic acid, depends on their structure, substituent and its position on aromatic ring and side chain structure. Greater number of hydroxyl and methoxy groups, especially presence of o-hydroxyl groups on phenolic group, increases antioxidative activity [10]. RA works as a ROS "catcher" by hydrogen donating. More precisely, o-hydroxyl groups in A and B rings are electron donors. This leads to forming of semiquinone and quinone structures, which stabilizes newly formed free radical structure [11]. Furthermore, RA inhibits enzyme xanthine-oxidase (it catalyzes hypoxanthine to xanthine reaction, followed by xanthine to uric acid reaction, while creating H_2O_2), which is one of endogenic sources of free radicals in some pathophysiological states like ischemia or tissue damage. RA also reduces Mo(VI) to Mo(V), reducing metal induced free radical forming [11,12]. RA can infiltrate into the inner, nonpolar layer of plasma membrane, preventing lipid peroxidation by intercepting intramembrane radicals and increasing membrane flow, reorganizing lipid chains and making free radical propagation harder [13]. In addition, RA increases production of enzymes that participate in primary antioxidative protection, such as superoxide-dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx) [14]. RA prevents DNA damage with its antioxidative properties, while it also induces damaged DNA reparation by mediating intracellular mechanism responsible for DNA reparation [15].

Beside polyphenols, terpenes also belong to a significant group of plants' secondary metabolites. Their antioxidative activity is based on ROS "catching" and effect on endogenous antioxidants. Terpenes also show anti-inflammatory, antiallergic, anticoagulative, antitumor, as well as, sedative and analgesic effect [3,16]. Terpenes activity depends on their concentration. Low concentrations of terpenes show antioxidative effect, but high concentrations show prooxidative effect [17].

Alongside plants, polyphenolic compounds can be found in honey, which gives honey a significant antioxidative properties, with its' primary antibacterial activity. Antioxidative properties of honey are based on flavonoids, phenolic acids, vitamins C and E, enzymes (catalase and peroxidase) and trace elements. Antibacterial and antioxidative properties of honey are connected, they effect on each other and together they give special therapeutic characteristics [18].

Hydrolats (hydrosols, flower waters) represent a water phase that forms during steam or water distillation of aromatic plants in essential oil production process. Hydrolat composition is very different from the essential oil of the same plant, because of the water solubility of components, which gives different activity to oils and hydrolats [19]. Main components of hydrolats are, mostly, terpenes and terpenoids, which give hydrolats their antioxidative, as well as antibacterial, antiviral, and other properties of different hydrolats [20].

The aim of this study was to evaluate the *in vitro* antioxidative activity of the supplement based on hydrosol mixture, rosmarinic acid and honey in biological material. The supplement is defined as functional beverage, intended for human diet. This model enables us to predict supplement's activity in blood after the predicted resorption.

MATERIAL AND METHODS

Study design

Med(i)ra supplement (ROSA VITA d.o.o, Pula, Croatia) is a herbal complex based on hydrosol mixture, rosmarinic acid (75 mg RA per 10 mL of drink) and honey. Rosemary (*Rosmarinus officinalis*) hydrosol, lavender (*Lavandula angustifolia*) hydrosol, fennel (*Foeniculum vulgare*) hydrosol and cade juniper (*Juniperus oxycedrus*) hydrosol were used for the mixture. Honey, used in this supplement, originates from aromatic plant fields, which are also used for hydrosol production.

A series of diluted (100%, 50%, and 25%) supplement (25 µL) was added to the serum pool (450 µL), collected from healthy donors. Since there wasn't any significant difference in results between different dilutions of a supplement, we pooled all results from series of dilutions into one group. A second series of samples was prepared with the same supplement amounts and tert-Butyl hydroperoxide (TBH) added as a prooxidant. Alongside these samples, sera samples with Trolox (hydrophilic vitamin E analog) and Trolox with TBH were prepared. All these samples were tested for the fol-

lowing oxidative stress parameters: total antioxidative status (TAS), paraoxonase-1 (PON-1), total sulfhydryl groups content (SHG), total oxidative status (TOS), prooxidative-antioxidative balance (PAB) and advanced oxidation protein products (AOPP). TAS/TOS ratio was calculated as a quantitative measurement of antioxidative and oxidative substances ratio in the serum.

Laboratory analysis

Each of the hydrosols used in herbal mixture was analyzed with gas chromatography – mass spectrometry (GC-MS) on HP-5MS capilary column. *(Faculty of Chemistry & Technology, Split, Croatia)*.

Total antioxidative status (TAS) was determined by a colorimetric test using stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation as a chromogen. The reaction was performed on the *Ilab 300+ Instrumentation Laboratory, (Milan, Italy)*. Paraoxonase-1 (PON-1) activity was measured in a kinetic reaction based on interaction of PON1 enzyme, from the sample, with the paraoxone substrate. The Ellmann method was performed in order to determine total sulfhydryl groups content (SHG). Total oxidative status (TOS) was measured spectrophotometrically after colorimetric reaction in which the oxidants presented in the sample oxidize the ferro-ortho-dianiside complex to the ferric ion. Advanced oxidation protein products (AOPP) was measured after the addition of glacial acetic acid to a diluted sample with phosphate buffer (pH 7.4) and a potassium iodide solution. All PON-1, SHG, TOS and AOPP analyses were performed on *Ilab 300+ Instrumentation Laboratory, (Milan, Italy)*. Prooxidative-antioxidative balance (PAB) test is based on colorimetric reaction of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with H_2O_2 and antioxidants (uric acid) at the same time, since they are in the same serum sample. The calibration curve is formed and used for the results calculation. [21]

Statistical analysis

All data are presented as median and interquartile range (25th and 75th percentiles). Mann-Whitney U test was used to calculate p-values between sample groups for each of the parameters. A p-value of less than 0.05 was considered statistically significant. All data were analyzed using IBM® SPSS® ver. 26.0 (IBM, Armonk, USA) software.

RESULTS

Table 1 represents some of the most significant compounds of hydrosols used in the supplement.

Table 1. The most significant compounds of hydrosols used in the Med(i)ra supplement (represented as peak area %).

	Juniperus oxycedrus	*Lavandula angustifolia*	*Rosmarinus officinalis*	*Foeniculum vulgare*
Compound	Peak area %			
1. 1,8-Cineole	0.3	0.2	6.4	5.3
2. Fenchone	0.4	–	–	40.0
3. Linalool	1.4	19.6	2.4	4.6
4. *cis*-Linalool oxide	–	3.8	0.1	–
5. *trans*-Linalool oxide	–	3.5	–	–
6. Borneol	1.9	5.8	10.9	–
7. Terpinene-4-ol	2.5	17.0	3.4	3.2
8. *p*-Cymen-8-ol	3.4	2.2	0.3	0.3
9. α-Terpineol	7.6	13.6	8.3	2.9
10. Verbenone	3.4	0.4	41.8	–
11. *trans*-Carveol	3.6	0.2	0.2	0.2
12. *cis*-Carveol	1.4	–	–	–
13. Carvacrol	2.1	–	–	–
14. Methyleugenol	4.3	0.6	1.2	2.3
15. 4-Methyl-2,6-bis(1,1-dimethylethyl)- phenol	14.8	1.9	2.0	8.8
16. (*Z*)-9-octadecen-1-ol	2.8	0.3	0.3	7.2
17. Camphor	–	0.7	7.6	1.7
18. Methyl Chavicol (Estragole)	–	–	–	4.4
19. Geraniol	–	–	0.3	0.3
20. Eugenol	1.3	0.2	0.6	0.8
21. 3,7-Dimethyloct-1-en-3,7-diol	–	4.0	0.2	–
21. Coumarin	–	3.1	–	0.2
Total identified	76.5 %	90.2 %	91.3 %	89.7 %

The main compounds of cade juniper (*Juniperus oxycedrus*) hydrosol are 4-methyl-2,6-bis(1,1-dimethylethyl)- phenol (14.8%) (used in BHT production) and α-terpineol (7.6%). Linalool (19.6%), terpenine-4-ol (17.0%) and α-terpineol (13.6%) dominate in lavender (*Lavandula angustifolia*) hydrosol, while the main compounds of rosemary (*Rosmarinus officinalis*) hydrosol are verbenone (41.8%), borneol (10.9%) and α-terpineol (8.3%). Fenchone (40%) is the main compound in fennel (*Foeniculum vulgare)* hydrosol. 1,8-cineol is one of the terpenes with significant antioxidant activity and can be found in all hydrosols in varies amounts (0.2-6.4%).

The concentrations of oxidative stress parameters in samples without TBH addition are shown in **Table 2**.

Table 2. The concentrations of oxidative stress parameters in samples without added TBH.

	Medi(r)a + serum pool (*c*=25%,50%, 100%)	Serum	Trolox + serum pool
TAS (μmol/L)	1638 (1598-1657)	662 [a] (628-696)	810 [a] (752-868)
TOS (μmol/L)	125 (123-130)	123 (111-136)	113 [a] (110-116)
PAB (U/L)	3.23 (2.77-4.23)	97.8 [a] (94.6-101)	84.6 [b] (81.6-87.5)
AOPP (μmol/L)	90.6 (90.4-90.6)	118 [a] (112-125)	118 [a] (114-121)
SHG (mmol/l)	1.17 (0.84-1.73)	0.55 [a] (0.53-0.58)	0.51 [a] (0.50-0.51)
PON1 (U/L)	208 (198-216)	170 [a] (164-176)	184 (182-185)

a - Med(i)ra vs serum; Med(i)ra vs Trolox; b – serum vs Trolox; a, b, p<0,05 (compared using non-parametric Mann-Whitney U test)

Antioxidative parameters TAS and SHG were significantly higher ($p<0.05$), while PAB and oxidative paremeter AOPP were significantly lower ($p<0.05$) in all Med(i)ra samples without added TBH compared to serum and Trolox samples without TBH addition. PON1 parameter was significantly higher in Med(i)ra samples than in serum samples, while TOS was the only parameter that showed significantly lower value in Trolox samples compared to Med(i)ra samples ($p<0.05$).

The concentrations of oxidative stress parameters in samples with TBH addition are shown in **Table 3**.

After TBH has been added to all samples, antioxidative parameters TAS and SHG were higher ($p<0.05$), while PAB was significantly lower ($p<0.05$) in Med(i)ra samples compared to serum and Trolox samples. The other oxidative stress parameters, TOS, AOPP and PON1, didn't show any significant difference in samples after TBH addition. Also, it has been noticed that there wasn't significant difference between oxidative stress parameters measured in Trolox and serum samples.

Table 3. The concentrations of oxidative stress parameters in samples with TBH addition.

	Medi(r)a (*c*=25%,50%,100%) with TBH	Serum with TBH	Trolox with TBH
TAS (μmol/L)	1605 (1584-1642)	734 [a] (712-756)	752 [a] (733-771)
TOS (μmol/L)	127 (125-128)	129 (127-130)	126 (124-128)
PAB (U/L)	2.30 (1.97-3.10)	113.10 [a] (112-114)	96.0 [a] (95.4-96.6)
AOPP (μmol/L)	109 (91.0-129)	122 (121-123)	120 (115-124)
SHG (mmol/l)	0.77 (0.64-0.98)	0.47 [a] (0.46-0.48)	0.48 [a] (0.44-0.52)
PON1 (U/L)	195 (169-213)	217 (188-247)	196 (194-199)

a - Med(i)ra with TBH vs serum with TBH; Med(i)ra with TBH vs Trolox with TBH; b – serum with TBH vs Trolox with TBH; a, b - p<0,05 (compared using non-parametric Mann-Whitney U test)

Table 4. Med(i)ra with added TBH vs Trolox without added TBH.

	Medi(r)a (*c*=25%,50%,100%) with TBH	Trolox	***p***
TAS (μmol/L)	1605 (1584-1642)	810 (752-868)	***<0,05***
TOS (μmol/L)	127 (125-128)	113 (110-116)	***<0,05***
PAB (U/L)	2.30 (1.97-3.10)	84.6 (81.6-87.5)	***<0,05***
AOPP (μmol/L)	109 (90.8-129)	118 (114-121)	*1,00*
SHG (mmol/l)	0.77 (0.64-0.98)	0.51 (0.50-0.51)	***<0,05***
PON1 (U/L)	195 (169-213)	184 (182-185)	*0,505*

When the concentration values of parameters measured in Med(i)ra samples with added TBH were com-

pared to the concentration values measured in Trolox samples without added TBH, it has been noticed that despite TBH addition, Med(i)ra showed a more favorable redox status comparing to serum sample. Data are showed in **Table 4**.

All oxidative stress parameters, except PON1 and AOPP, measured in Med(i)ra samples with added TBH and Trolox samples without added TBH showed significant difference with $p<0.05$. TAS and SHG were significantly higher and PAB was significantly lower in Med(i)ra samples despite TBH addition, while only TOS was significantly lower in Trolox samples without added TBH.

In order to show more precise relation between antioxidative capacity and oxidative stress in all samples, we calculated TAS/TOS ratio which represents quantitative measure of antioxidative substance and oxidative substance ratio in samples. **Figure 1** shows TAS/TOS ratio for all sample series.

TAS/TOS ratio does not show any significant difference in the same samples before and after TBH addition, but data from the figure obviously show that TAS/TOS ratio is significantly higher ($p<0.05$) in all Med(i)ra samples with and without added TBH, when it is compared with those in serum and Trolox samples with and without TBH.

The only parameter that provides determination of oxidants and antioxidants simultaneously in one single test and shows their balance in sample is PAB. PAB data in all samples are shown on **Figure 2**.

The data from the **Figure 2**. clearly shows that PAB values are significantly lower in all Med(i)ra samples compared to serum or Trolox samples, with or without added TBH ($p<0.05$). Despite that **Figure 2**. shows difference between serum (serum with added TBH) and Trolox (Trolox with added TBH) samples, that difference isn't significant. Also, there is not significant difference in same samples before and after TBH addition.

DISCUSSION

The aim of this study was to estimate antioxidative effects of the supplement based on herbal complex (hydrosol mixture), rosmarinic acid (RA) and honey. The main compounds of this supplement are terpenes and terpenoids, phenolic acid (rosmarinic acid) and other polyphenolic compounds with antioxidative activity.

TAS and SHG showed antioxidative protection capacity, while PAB shows balance between prooxidants and antioxidants in organism. Data collected in this study showed that TAS and SHG levels were significantly higher in all Med(i)ra samples (with and without added TBH), while PAB was significantly lower in these samples compared to serum (with and without added TBH) and Trolox samples (with and without added TBH), despite Trolox antioxidative activity. This suggest that Med(i)ra, despite addition of TBH, has high antioxidative capacity. Preliminary *in vitro* study preformed in our laboratory, which tested antioxidative activity of eight aromatic plants hydrosols (which include rosemary, cade juniper, fennel and lavender hydrosols) with and without added TBH, shows that hydrosols didn't significantly changed TAS values in samples without TBH addition, but TOS was significantly lower in samples with rosemary and cade juniper hydrosols

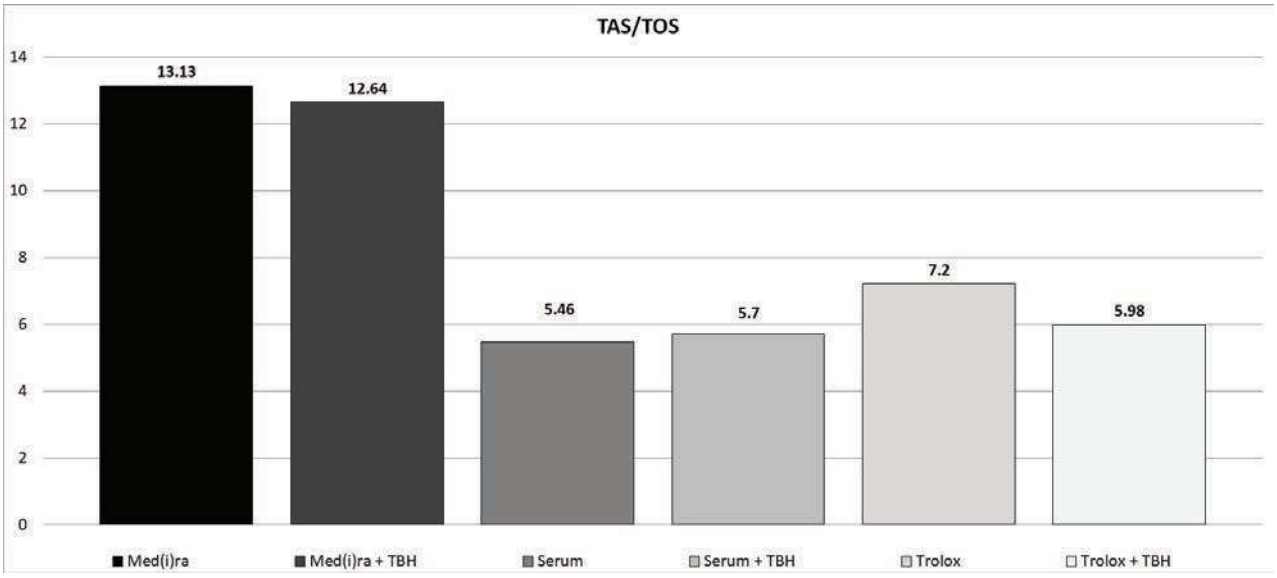

Figure 1. TAS/TOS ratio in all sample series

*p values between same groups with and without added TBH are showed on graph; a – Med(i)ra vs serum; Med(i)ra vs Trolox; b-Med(i)ra with added TBH vs serum with added TBH; Med(i)ra with added TBH vs Trolox with added TBH; a,b - $p<0,05$ (compared using non-parametric Mann-Whitney U test).

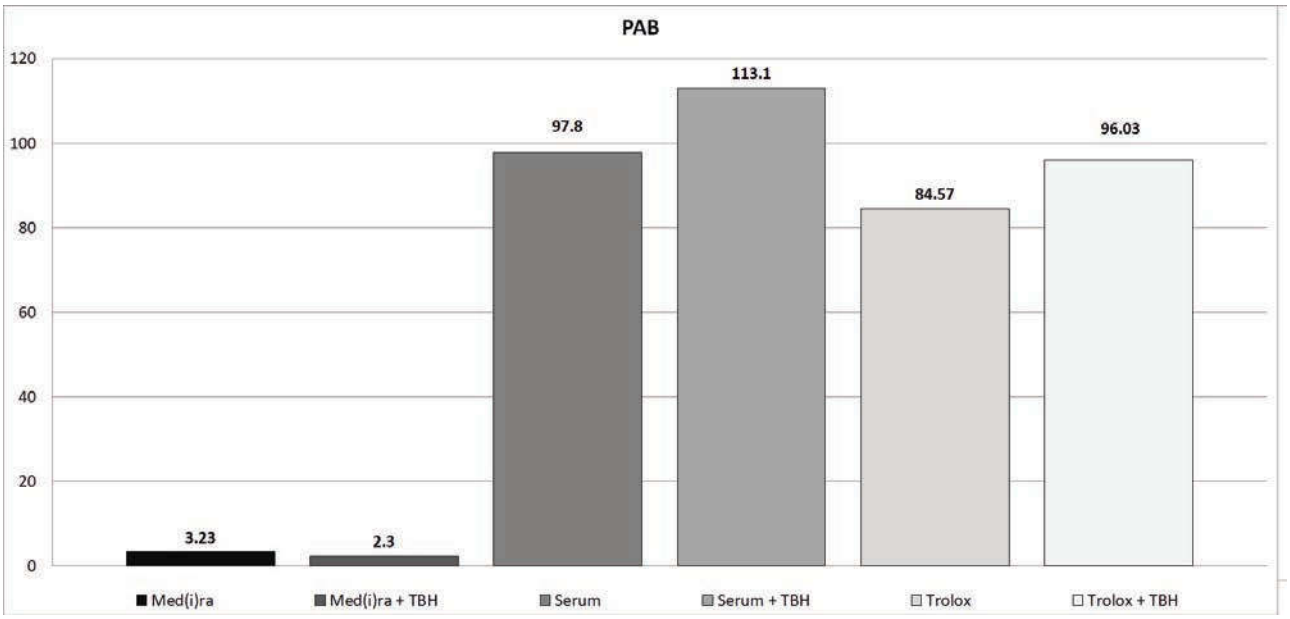

Figure 2. PAB values in all sample series

*p values between same groups with and without added TBH are showed on graph; a - Med(i)ra vs serum; Med(i)ra vs Trolox; b – Med(i)ra with added TBH vs serum with added TBH; Med(i)ra with added TBH vs Trolox with added TBH; a,b – $p<0,05$ (compared using non-parametric Mann-Whitney U test).

(unpublished results). After TBH addition in all hydrosol's samples, TAS value of lavender hydrosol sample was significantly higher compared to fennel and cade juniper hydrosol samples, while TOS was significantly lower compared to serum sample, which lead to higher TAS/TOS ratio values of lavender hydrosol samples. Also, TAS/TOS ratio of lavender hydrosol sample was significantly higher compared to cade juniper hydrosol sample. Rosemary hydrosol significantly decreased TOS and AOPP values compared to their values in serum and showed the best antioxidative capacity compared with other hydrosols used in this study. All data in this study suggests that rosemary, cade juniper, lavender and fennel hydrosols have antioxidative potential [22]. The other study showed that rosemary hydrosol has the ability to act like scavenger of hydroxyl radical, while lavender hydrosol has great potential to act like superoxide anion scavenger [20]. All of these indicate that RA has a great role in Med(i)ra's antioxidative activity. Many studies, including study that investigates antiapoptotic and antioxidative effects of RA on astrocytes [23] and also study that researched protective effect of RA on hydrogen peroxide-induced apoptosis of neural cells [24], showed that RA has a great antioxidative capacity. In study, that compared antioxidative activity of hydrosols to antioxidative activity of hydrosols with added RA, has been shown that TAS/TOS ratio was significantly higher in samples after the RA addition, which suggests that RA significantly improves antioxydative activity of hydrosols [25]. In this case RA probably works as a ROS "catcher" by hydrogen donating and, in that way stabilizes newly formed free radical structure [11].

In addition to significantly higher TAS and SHG levels, and significantly lower PAB in Med(i)ra samples without added TBH, Med(i)ra provides significantly higher PON1 levels and significant decrease of AOPP levles compared to those in serum or Trolox samples. *In vivo* study in estrogen-deficient rats shows that RA has a capability to decrease AOPP values in rat's blood. It is assumed that RA leads to decreasing of AOPP value by inhibition of myeloperoxidase, which catalyzes reaction between chloride ion and hydrogen-peroxide. This reaction leads to hypochlorous acid production, which may induce the AOPP formation [26]. However, after the TBH addition in all samples, AOPP and PON1 values remained lower in Med(i)ra's samples compared to serum and Trolox samples, but that difference wasn't significant (**Table 3**). All of these indicate that Med(i)ra can increase antioxidative activity of PON1 enzyme and decrease the protein oxidation to some extent. In study, which examined the protective effects of RA on the memory impairment in a mouse model induced by amiloid beta protein (Aβ), it has been shown that RA decrease protein damage, induced by protein nitration, by acting like peroxynitrites scavenger [27].

In this study, TAS and SHG levels were significantly higher and PAB was significantly lower in Med(i)ra samples compared to Trolox samples, while TOS levels was significantly lower in Trolox samples (**Table 2**). Other studies also showed that RA provide more efficient antioxidative effect compared to Trolox and other forms

of vitamin E, like γ-tocopherol. However, the most efficient antioxidative effect was gained by synergistic action of RA and vitamin E. RA probably achieves synergistic effect with vitamin E by decreasing vitamin E oxidation, rather than regeneration, like other antioxidants that act synergistically with vitamin E [30, 31]. When TAS, TOS and PAB values and TAS/TOS ratio of Med(i)ra samples were compared before and after TBH addition, no significant difference was observed. That indicates that Med(i)ra can keep its antioxidative potential despite TBH addition. After TBH addition only SHG value was significantly lower, while AOPP value was significantly higher. Beside significant decrease of SHG value in Medi(i)ra samples after TBH addition, that value was significantly higher compared to those values in serum and Trolox samples with added TBH. In addition to studies that showed antioxidative effects of hydrosols and rosmarinic acid [11,12,14,15,20], study performed on endothelial cells showed significant antioxidative protection of honey in presence of various prooxidants. Honey protects GSH as well as regenerates GSH from GSSG, which is, most probably, effect of honeys lipophilic components [30].

CONCLUSION

Results of this study showed that Med(i)ra significantly increased concentrations of antioxidative parameters TAS and SHG, decreased concentration of prooxidative/antioxidative balance parameter (PAB), while significantly increased TAS/TOS ratio, even with added TBH. In this *in vitro* study Med(i)ra also affected PON1 and AOPP parameters, by which it proved itself to be a very efficient antioxidative agent. From all of the above, it is clear that antioxidative activity originates from synergistic effect of this supplements antioxidative components (hydrosols, rosmarinic acid and honey).

Because of the supplement's complex composition, as well as metabolic changes of the supplement's components due to its *in vivo* admission, further *in vivo* studies are necessary in order to test this supplement's antioxidative potency within organism.

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In vitro ispitivanje antioksidativne aktivnosti preparata ruzmarinske kiseline i meda

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Kratak sadržaj

Usled narušavanja ravnoteže između slobodnih radikala i antioksidanasa, dolazi do oksidativnog stresa, patofiziološkog stanja, koje je povezano sa nastankom i razvojem mnogih oboljenja. Kako su se određeni sintetski antioksidansi pokazali kao potencijalno štetni po zdravlje ljudi, sve se više pažnje usmerava ka pronalaženju prirodnih antioksidanasa. Polifenoli, fenolne kiseline, u koje spada i ruzmarinska kiselina, terpeni i terpenoidi su sekundarnii metaboliti biljaka koji poseduju antioksidativne osobine, a mogu se naći u različitim biljnim kompleksima (hidrolatima) i medu. Cilj ovog rada je utvrđivanje in vitro antioksidativne aktivnosti preparata koji predstavlja mešavinu biljnih kompleksa (hidrolata), ruzmarinske kiseline i meda. U „pool" seruma, koji je dobijen mešanjem seruma zdravih pojedinaca, dodavana su serijska razblaženja (100%, 50%, 25%) ispitivanog preparata, kao i serijska razblaženja ispitivanog preparata (100%, 50%, 25%) u prisustvu terc-butil hidroperokisda (TBH), kao prooksidansa. Pored ovih uzoraka, pripremljeni su i uzorci u koje je dodavan troloks (hidrofilni analog vitamina E), kao i troloks u prisustvu TBH. Iz svih uzoraka vršeno je određivanje parametara antioksidativnog stresa: totalnog antioksidativnog statusa (TAS), paraoksonaze-1 (PON1), ukupnog sadržaja sulfhidrilnih grupa (SHG), totalnog oksidativnog statusa (TOS), prooksidativnog-antioksidativnog balansa (PAB) i uznapredovalih produkata oksidacije proteina (AOPP). Takođe, izračunat je i odnos TAS/TOS koji predstavlja kvantitativnu meru odnosa antioksidativnih redukujućih supstanci i oksidujućih supstanci u serumu. Poređenjem vrednosti antioksidativnih parametara TAS, SHG, TAS/TOS odnosa i parametara ravnoteže prooksidanasa i antioksidanasa PAB za uzorke preparata bez i sa dodatkom TBH, nije uočena značajna razlika, što ukazuje da je i pored dodatka TBH ispitivani preparat održavao svoje antioksidativno delovanje. Ispitivani preparat pokazao je značajno antioksidativno delovanje, što se može pripisati sinergističkom delovanju terpena i terpenoida iz hidrolata, ruzmarinske kiseline, polifenola i ostalih antioksidativnih supstanci iz meda.

Ključne reči: *Oksidativni stres; Antioksidans; Hidrolati; Terpeni; Polifenoli; Ruzmarinska kiselina; Med.*