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Influence of immortelle (*Helichrysum italicum* (Roth) G. Don) and lavender (*Lavandula angustifolia* Mill.) hydrolates on oxidative stress and antioxidative parameters in human serum

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Abstract

A number of studies suggest that different antioxidants play important roles in the body's antioxidant defense system. Hydrolates, by-products of essential oils, commonly obtained by steam distillation, are recognized to possess remarkable pharmacological activities, in particular an antioxidant activity. This study aimed to investigate and compare the antioxidant properties of immortelle hydrolate (IH) and lavender hydrolate (LH) using an *ex vivo* platform, i.e. human serum. To assess their impact on oxidative stress / antioxidative parameters, spectrophotometric biochemical assays were applied. The GC/MS analysis revealed that the main constituents were italidione I (17.2%), linalool (15.6%), α-terpineol (13.7%), terpinen-4-ol (6.3%) and nerol (5.2%) in IH, and linalool (19.6%), terpinen-4-ol (17.0%), α-terpineol (13.6%) and borneol (5.8%) in LH. The results showed that serum samples with LH, along with *tert*-butyl hydroperoxide as a

pro-oxidant, had lower concentrations of the TOP (total oxidant potency) parameter, and slightly higher concentrations of the TAC (total antioxidant capacity) and SHG (total sulfhydryl groups) parameters compared to IH samples. Moreover, a significant difference in the OSI (oxidative stress index) parameter was observed (IH vs. LH – 49 (41–58) vs. 70 (61–75), $p<0.05$). The current *ex vivo* platform demonstrated IH and LH distinct antioxidant potency, highlighting LH as a potentially stronger antioxidant than IH.

Key words: hydrolates, human serum, oxidative stress, antioxidant activity

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Introduction

The term “oxidative stress” may be described as the state of cells or tissues induced by the increased production and accumulation of pro-oxidant molecules – reactive oxygen species (ROS) and reactive nitrogen species (RNS), accompanied concurrently with the decreased capacity of protective molecules – antioxidants (1, 2). It is recognized that oxidative stress has been implicated in the pathogenesis of many diseases, including cardiovascular, neurological, respiratory, kidney and chronic inflammatory diseases, along with other age-related disorders and cancer (3). In order to minimize oxidative damage, aerobic organisms synthesize several antioxidant enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (1, 4). Moreover, non-enzymatic proteins (ferritin and ceruloplasmin) also play important roles in antioxidant defense (4). So far, our cells utilize dietary antioxidants, particularly vitamins (tocopherol and ascorbic acid) and minerals (selenium, manganese, zinc and copper), to combat oxidative stress (3, 4). In line with this evidence, great scientific interest focuses on the possible protective potential of plant-based antioxidant preparation.

It is well documented that plants synthesize a range of secondary metabolites with antioxidant properties (5), including essential oils. Essential oils represent a complex mixture of aromatic components, belonging to two main structural families – terpenoids (monoterpene and sesquiterpene) and phenylpropanoids, the most commonly obtained by steam distillation (6, 7). In the same isolation process, by-products called hydrolates, hydrosols or aromatic waters, are also produced (8). Hydrolates primarily contain hydrophilic, oxygenated compounds, namely monoterpene alcohols, aldehydes and ketones, along with sesquiterpene alcohols, originated from essential oils (9). Several studies have shown that hydrolates possess pharmacological activities similar to corresponding essential oils, such as antimicrobial, antioxidant and anti-inflammatory activity (8–10). Due to these attributes, hydrolates are used in aromatherapy, as functional drinks and preservative agents in food industry, active ingredients of cosmetic products (9, 10), and traditional remedies in the treatment of mental and gynecological disorders (11, 12).

Since scientific literature suggests the health potential of hydrolates, the evaluation of insufficiently examined hydrolates as sustainable and effective natural antioxidants seems reasonable. In this direction, immortelle and lavender are two representatives of medicinal plants that are worth investigating for these purposes.

Immortelle (*Helichrysum italicum* (Roth) G. Don) is a perennial thermophilus plant belonging to the Asteraceae family, predominantly distributed in the Mediterranean region (13). The flowers of *H. italicum* are valuable and frequently used in traditional medicine for the treatment of digestive complaints, hepatobiliary disorders and skin impairments (14, 15). The health benefits of immortelle flowers are accredited mainly to flavonoids, phenolic acids and essential oil (15). The essential oil of *H. italicum* has gained the attention of many cosmetic and perfume industries on account of its antioxidant (16), antimicrobial (17), anti-collagenase and anti-elastase activities (18).

Components of *H. italicum* essential oil present in a notable degree are mostly α-pinene, limonene, α-terpineol, curcumenes, nerol and selinenes (13, 17, 19).

Lavender (*Lavandula angustifolia* Mill., Lamiaceae) is a perennial shrub native to the Mediterranean area (20). According to the 10th European Pharmacopoeia, dried flowers (*Lavandulae flos*) represent herbal substance rich in pharmacologically active ingredients – essential oil, flavonoids, phenolcarboxylic acids, triterpenic acids and tannins (21, 22). The most valuable ingredient is essential oil, containing linalool and linalyl acetate as the main compounds, along with borneol, α-terpineol and terpinene-4-ol (20). Lavender is a plant known for its activity on the central nervous system (20, 23), used for the relief of mild symptoms of mental stress and exhaustion and to aid sleep (24), as well as on the gastrointestinal tract as carminative and spasmolytic (25). Furthermore, lavender essential oil possesses antioxidant, antifungal and antibacterial effects (20), representing an important raw material for the cosmetic industry.

Taking into consideration the importance and potential of natural products, the aim of this study was to investigate and compare the antioxidant properties of immortelle and lavender hydrolates using an *ex vivo* platform, i.e. human serum, to assess their impact on oxidative stress-related parameters. Moreover, this study addresses the gap in the current scientific literature concerning the pharmacological effects of hydrolates.

Material and Methods

Hydrolates

In this study, two hydrolates obtained from *Lavandula angustifolia* Mill. (Lamiaceae) and *Helichrysum italicum* (Roth) G. Don (Asteraceae) were used. Both were obtained as commercially available products: Lavender Hydrolate and Immortelle Hydrolate (World of Plants, Vodnjan, Croatia). The composition of investigated hydrolates was provided by the company.

Sample collection

Human serum, used for the evaluation of the antioxidant potential of the hydrolates, was collected from healthy volunteers who had planned check-ups at the Military Medical Academy in Belgrade, and had given agreement that the serum could be used for this study. Only the samples whose basic biochemical parameters were within the reference ranges were selected for preparing the serum pool. After the serum pool was aliquoted, the portions (450 µL) were frozen and kept at –80°C until analysis.

Sample preparation

The same volumes (25 µL) of the investigated hydrolates (concentration range 25–100%) and 0.5 mmol/L *tert*-butyl hydroperoxide solution (TBH), as a pro-oxidant substance, were added to serum (450 µL). The mixture was stirred and incubated at 37°C for 2 hours. The samples were prepared in duplicate. The controls contained either serum and hydrolates or serum and TBH.

Total oxidant potency (TOP)

The total oxidant potency was determined using Erel's advanced method (26, 27). The assay is based on the ability of all oxidants in the sample (H_2O_2, lipid hydroperoxides, etc.) to oxidize the ferrous ion-ortho-dianisidine complex to ferric ion. In the acidic medium and in the presence of glycerol, the ferric ion forms a colored complex with xylenol orange wherein the colour intensity is proportional to the total oxidant content. Immediately before use, two reagents were prepared. Reagent 1 contained 150 µM xylenol orange, 140 mM NaCl and 1.35 M glycerol in 25 mM H_2SO_4 solution, pH 1.75. Reagent 2 contained 5 mM ferrous ammonium sulphate and 10 mM o-dianisidine in 25 mM H_2SO_4 solution. Briefly, reagent 1 (225 µL), reagent 2 (11 µL) and the sample (35 µL) were mixed, and after 3–4 minutes, the absorbance was measured at 560 nm against deionized water as a blank. A standard solution of H_2O_2 was used to obtain the calibration curve in a 10–200 µmol/L range. The results were expressed in µmol H_2O_2 equivalents per liter (µmol/L).

Prooxidant-antioxidant balance (PAB)

The prooxidant-antioxidant balance was measured as described in previously published papers (26, 28). The assay determines the concentration of hydrogen peroxide in the presence of antioxidants. The 3,3′,5,5′-tetramethylbenzidine (TMB) represents a chromogen which simultaneously reacts with hydrogen peroxide (the reaction is catalyzed by the enzyme peroxidase) and antioxidants (the reaction is non-enzymatic), undergoing changes in its oxidation state and color. The intensity of the generated color is proportional to the ratio of pro-oxidants (cause the formation of blue TMB cations) and antioxidants (cause cation reduction and discoloration). Prior to the assay, TMB cation and TMB reagent II solutions were prepared. The 6 g/L solution of TMB in DMSO (TMB reagent I; 1 mL) was added to 0.05 M acetate buffer (pH 4.5; 50 mL) along with 100 mmol/L chloramine T (175 µL) for the preparation of the TMB cation solution. To prepare the TMB reagent II solution, TMB reagent I (200 µL) was dissolved in 0.05 M acetate buffer (pH 5.6; 10 mL). The working solution was prepared by mixing TMB cation (1 mL) and TMB reagent II (10 mL) solutions. The tested hydrolates (10 µL) were mixed with the working solution (180 µL) and incubated at 37°C in the dark. After 10 minutes, the reaction was stopped by the addition of 2M HCl (40 µL), and the absorbance was measured at 450 nm. Standard solutions, used for calibration curve construction, were prepared by mixing an oxidant (hydrogen peroxide) and antioxidant (uric acid) in different proportions (0–100%). The results were expressed in arbitrary HK (Hamidi-Koliakos) units (HKU), representing the hydrogen peroxide concentration (%) evaluated in the standard solution.

Total antioxidant capacity (TAC)

The total antioxidant capacity was evaluated by colorimetric assay using the stable $ABTS^{\bullet+}$ radical cation as a chromogen (26, 29). The colorless 2,2-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) solution is oxidized by hydrogen peroxide

(2 mmol/L) in the acetate buffer (30 mmol/L, pH 3.6) to the deep-green colored $ABTS^{\bullet+}$ radical cation. When diluted with the acetate buffer solution (0.4 mol/L, pH 5.8), the $ABTS^{\bullet+}$ solution slowly bleaches. Antioxidants present in the samples accelerate the bleaching of the $ABTS^{\bullet+}$ solution in varying grades proportional to their concentrations. Briefly, the acetate buffer (0.4 mol/L, pH 5.8; 200 µL), the sample (12.5 µL) and ABTS solution (37.5 µL) were mixed and incubated for 10 minutes at room temperature. The absorbance was measured at 660 nm against deionized water as a blank. The calibration curve was constructed using Trolox, and the results were expressed in µmol Trolox equivalents per liter (µmol/L).

Total sulphydryl groups content (SHG)

The total sulphydryl groups content in serum was determined by a slightly modified Ellman's method (26, 30), based on the formation of a yellow-coloured p-nitrophenol anion between 2,2'-dinitro-5,5'-dithio-benzoic acid (DTNB) and aliphatic thiol compounds in a basic medium. Briefly, samples (15 µL) were mixed with phosphate buffer (0.2 mol/L, pH 9.0; 270 µL) and the DTNB solution (10 mmol/L in 50 mmol/L phosphate buffer, pH 7.0; 10 µL). After 25 minutes of incubation at room temperature, the absorbance was measured at 412 nm. A standard solution of reduced glutathione was used to obtain the calibration curve with a 0.1–1.0 mmol/L range. The results were expressed in mmol glutathione equivalents per liter (mmol/L).

Oxidative stress index (OSI)

The oxidative stress index was calculated as the ratio of TAC and TOP parameters. The higher OSI values indicate better antioxidant activity.

Statistical analysis

Statistical analysis was done with the Statistics Package for the Social Sciences for Windows (SPSS; Chicago, Illinois, USA), version 18.0, whereby the non-parametric tests – the Mann-Whitney U test and the Kruskall-Wallis test – were employed to calculate p-values between the sample groups. A p-value of less than 0.05 ($p<0.05$) was considered a statistically significant difference. The results of all parameters were represented by median and interquartile range, i.e. 25th–75th percentile, since the parameters did not follow a normal distribution. Regarding insignificant differences for all parameters between 3 different hydrolate dilutions, we collected all the data and considered all 3 different dilutions as the same sample.

Results

The antioxidant activity of hydrolates was evaluated using biochemical assays, i.e. by determination of biochemical parameters in human serum. The results of oxidative stress (TOP and PAB) and antioxidative (TAC, SHG and OSI) parameter levels in human serum pool samples with immortelle and lavender hydrolates as antioxidants, TBH as pro-oxidant, as well as with their combinations, are shown in Table I.

Table I Redox status parameters in samples with immortelle and lavender hydrolates

Tabela I Parametri redoks statusa u uzorcima sa hidrolatom smilja i hidrolatom lavande

Parameter	Serum blank (a)	IH (b)	LH (c)	Serum-TBH (d)	IH-TBH (e)	LH-TBH	p
TOP (μmol/L)	6.7 (6.5–6.9)	5.7 [a] (5.4–5.8)	7.6 (5.8–7.8)	14.8 [aa] (6.6–22.9)	26.0 [a, bb] (22.4–30.1)	20.6 [a, c, d, e] (18.6–22.4)	<0.001
PAB (HKU)	217 (215–219)	206 [a] (201–210)	193 [aa, b] (192–197)	219 [c] (214–223)	198 [a, d] (193–199)	205 [c] (199–215)	<0.01
TAC (μmol/L)	1391 (1386–1396)	1290[aa] (1290–1335)	1321 (1290–1335)	1344 [aa] (1312–1376)	1293 [a] (1281–1313)	1341 [d] (1326–1357)	0.422
SHG (mmol/L)	0.446 (0.432–0460)	0.384 [aaa] (0.325–0.403)	0.272 [aa] (0.262–0.368)	0.261 [aaa] (0.174–0.349)	0.200 [a, bb] (0.130–0.225)	0.248 [a, e] (0.217–0.281)	<0.001
OSI	211 (207–220)	235 [aaa] (220–243)	177 [a, b] (171–226)	52 [aaa, bbb, ccc] (44–60)	49 [aaa, bbb] (41–58)	70 [a, c, d, e] (61–75)	<0.001

p – Kruskall-Wallis test; a, aa, aaa – p<0.05, 0.01, 0.001, respectively vs. serum sample (blank); b, bb – p<0.05, 0.01, respectively vs. immortelle sample; c – p<0.05 vs. lavender sample; d – p<0.05 vs. serum + TBH sample; e – p<0.05 vs. immortelle + TBH sample by Mann-Whitney U test.

In the case of the TOP parameter, representing the cumulative action of pro-oxidants and their interactions, the results show that serum samples with IH and serum samples with LH have similar concentrations of this parameter, while an increasing trend was observed after the TBH addition in both samples. A significant difference in TOP values was observed in the comparison subgroup IH-TBH vs. LH-TBH (26.0 (22.4–30.1) vs. 20.6 (18.6–22.4), p<0.05). A higher level of the TOP parameter in the IH-TBH sample indicates better antioxidant activity of lavender hydrolate, i.e. better resistance to exogenous oxidative stress.

For the PAB parameter, representing the pro-oxidant/antioxidant balance, a decrease in the value of this parameter was observed in LH and IH samples, compared to serum blank. However, the differences between the examined subgroups were not significant.

Between the tested antioxidants, IH and LH, no statistically significant difference was observed in the concentrations of the TAC parameter, which considers the cumulative action of all antioxidants present in the serum. Higher concentrations of TAC were observed in samples with a combination of LH-TBH compared to those with IH-TBH at the same concentrations.

By comparing the concentrations of SH groups between LH and IH samples, we observed that LH provided better antioxidant protection (higher concentration of SH groups) than IH in the presence of TBH. This difference in the comparison subgroup IH-TBH vs. LH-TBH (0.200 (0.130–0.225) vs. 0.248 (0.217–0.281)) was statistically significant (p<0.05).

When it comes to the OSI parameter, determined by the ratio of TAC and TOP parameters, its values decreased after the addition of TBH in IH and LH samples.

The undesirable change in oxidative stress status was less pronounced in LH samples since higher levels of the OSI parameter indicate better antioxidant activity. A significant difference in OSI values was observed in the comparison subgroup IH-TBH vs. LH-TBH (49 (41–58) vs. 70 (61–75), $p<0.05$). Additionally, a significant difference was observed between Serum-TBH and LH-TBH samples (52 (44–60) vs. 70 (61–75), $p<0.05$).

In order to get more precise insight into the redox changes upon hydrolate effects on serum biomolecules redox scores, Pro-oxidant, Antioxidant and summary Oxy scores were calculated (Figure 1).

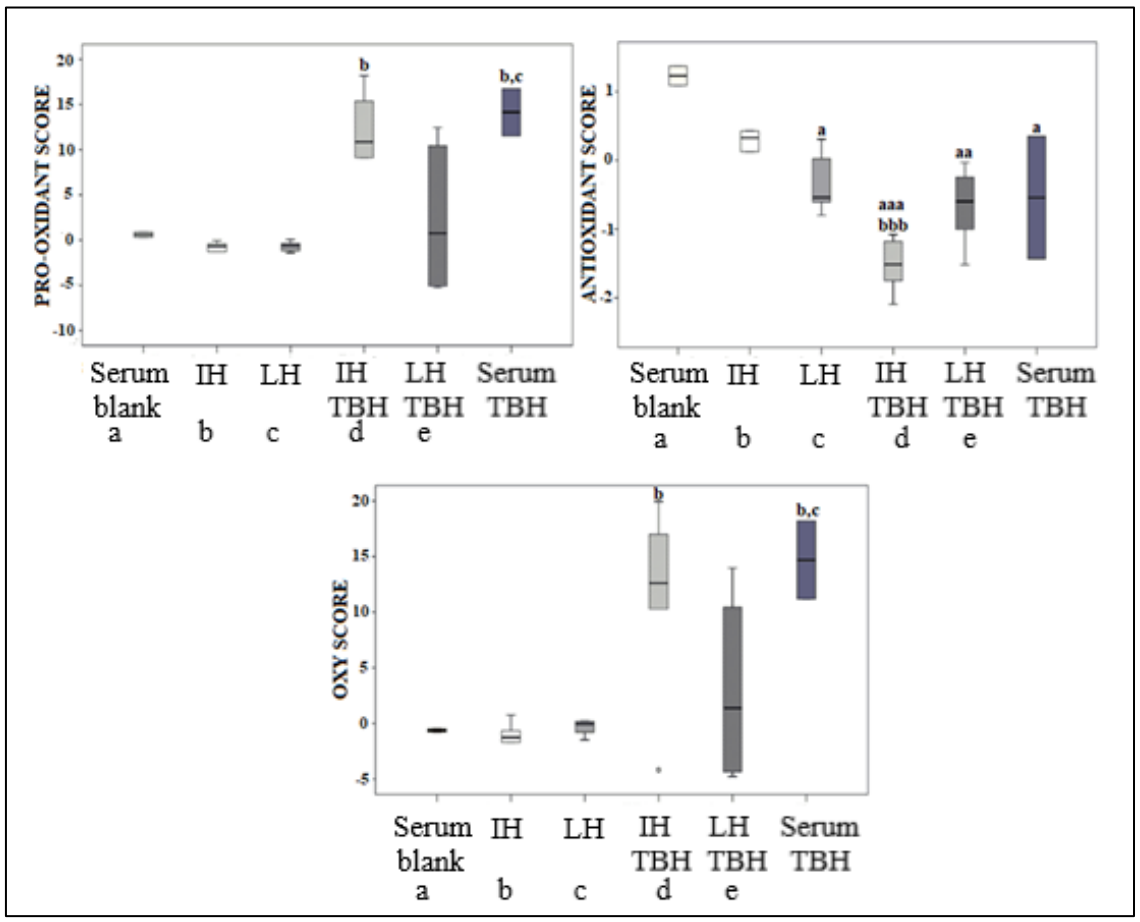

Figure 1. Redox scores for immortelle and lavender hydrolates' effects in healthy people's serum pool

Slika 1. Redoks skorovi u zbirnom serumu zdravih ljudi kao rezultat uticaja hidrolata smilja i lavande

a, aa, aaa – $p<0.05$, 0.01, 0.001 vs. serum, respectively; b, bbb – $p<0.05$, 0.001 vs. immortelle sample, respectively; c – $p<0.05$ vs. lavender sample, by Mann-Whitney U test

The both hydrolates' combination with serum pool produced a low Oxy score, which confirmed their distinct antioxidant potency. However, TBH addition caused an

increase in the IH Oxy score, while LH's Oxy score remained low even in the presence of TBH, and significantly lower compared to the Oxy score of the TBH sample. This finding revealed the strong potency of LH components to neutralize pro-oxidative species generated upon TBH reaction in serum.

Discussion

In a healthy organism, the development of oxidative stress, induced by pro-oxidants, is under the control of antioxidant protection. That is why, in the experiment we conducted, in order to mimic the conditions existing in a live organism, an antioxidant (IH or LH) was added to the samples before the pro-oxidant (TBH). This order of substance addition presents a sequence of events according to which pathological processes developed in a cellular compartment were restricted, at least in part, by a powerful antioxidant system, already existing in the same compartment, or in close proximity.

Hydrolates contain, mainly, essential oil components with oxygen functional groups, such as alcohols, aldehydes, ketones and carboxylic acids, while lipophilic components are significantly less represented (9). The composition of essential oils, and therefore hydrolates, is influenced by many factors (environmental, growth stage, the method of preparation of plant material and the method of essential oil isolation) (31). It is assumed that certain effects possessed by essential oils could also be partially exhibited by the corresponding hydrolates (8, 9). Therefore, the aim of our research was to investigate the antioxidant activity of hydrolates.

The TAC parameter is a summary indicator of the non-enzymatic antioxidant protection of the organism. Here, the omnibus Kruskal-Wallis analysis didn't reveal any significant difference regarding TAC, but post-hoc analysis showed significantly higher TAC content in the LH-TBH sample compared to the IH-TBH sample. This stressed LH's greater ability compared to IH in the pro-oxidative effects of TBH counteraction, i.e. a stronger antioxidant potency of the former.

Hydrogen peroxide and lipid peroxides, oxidants, as well as TBH, contribute to increasing the value of the TOP parameter. Even though the TOP concentration was initially higher in the LH sample compared to the IH sample (without significance), the results show that LH had better antioxidant activity upon TBH addition, and effectively coped with oxidative stress, unlike IH ($p<0.05$).

Observed differences between samples with IH and LH can be explained by the differences in dominant constituents in the investigated hydrolate samples. Main constituents in hydrolates used in the present research were italidione I (17.2%), linalool (15.6%), α-terpineol (13.7%), terpinen-4-ol (6.3%) and nerol (5.2%) in IH, and linalool (19.6%), terpinen-4-ol (17.0%), α-terpineol (13.6%) and borneol (5.8%) in LH. It is evident that both investigated hydrolates share main constituents, namely linalool, terpinen-4-ol and α-terpineol (Figure 2).

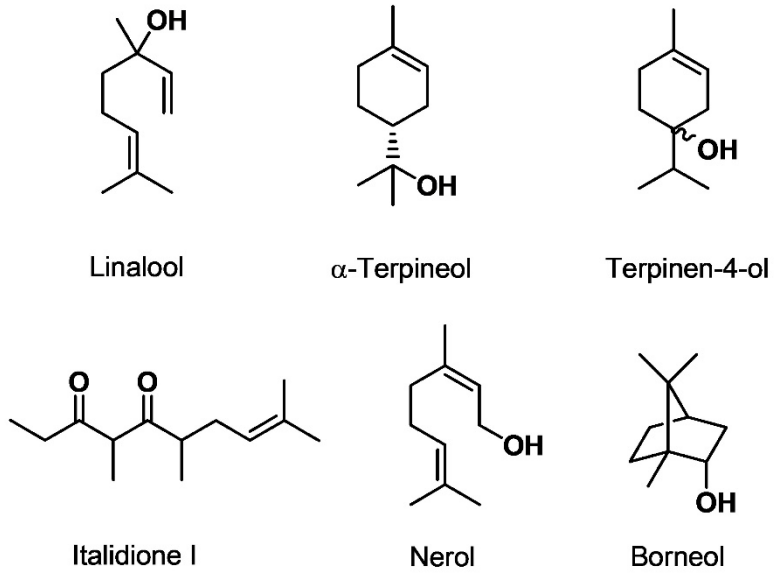

Figure 2. Structures of main constituents of IH and LH
Slika 2. Strukture glavnih komponenti hidrolata smilja i hidrolata lavande

As for the LH composition, it is in line with some previous studies. In the research whose objectives were the qualitative and quantitative analysis of LH obtained from samples from Poland, and their antioxidant activity, the main components were linalool, linalool oxide, borneol, α-terpineol and terpinen-4-ol (32). As for the chemical composition of IH, literature data are scarce (14, 33, 34), but there is still good compliance with the results of the current study. Besides the aforementioned oxygenated monoterpenes, this sample is characterized by high amounts of aliphatic di- and triketones (26.9%). Figure 3 shows the structures of some other compounds present in IH and LH at a slightly lower percentage (3–5%).

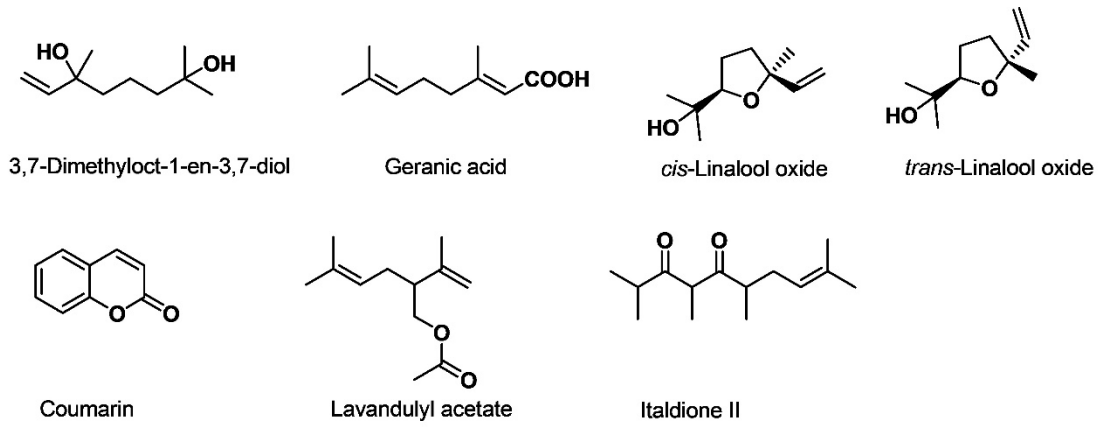

Figure 3. Structures of minor constituents of IH and LH
Slika 3. Strukture sporednih komponenti hidrolata smilja i hidrolata lavande

The antioxidant potential of essential oils has been proven in a number of previous research projects, which demonstrated their ability to neutralize free radicals, to prevent lipid oxidation, as well as to reduce oxidative stress in biological systems (6, 35). Moreover, it was shown that the phenol-rich essential oils (like cinnamon and clove oils rich in eugenol, thyme oil rich in thymol) possess stronger antioxidant activity than non-phenolic essential oils (like lavender oil with linalool and peppermint oil with menthol as main components) (35). Apart from essential oils, hydrolates also appear to have a high antioxidant potential (8).

Studies dedicated to the evaluation of the *ex vivo* antioxidant activity of immortelle and lavender hydrolates are scarce. However, the antioxidant potential of lavender essential oil has been demonstrated in *in vitro* (human hepatoma cell line HepG2), as well as in *ex vivo* (freshly isolated rat hepatocytes) experiments in terms of its protective effect against the oxidant-induced DNA damage, alongside the elevation of glutathione peroxidase activity (36). In addition, the antioxidant activities of immortelle and lavender essential oils / hydrolates were demonstrated in numerous *in vitro* (like DPPH, ABTS and FRAP assays) and in a limited number of *in vivo* experiments (32, 35–38).

Earlier studies have shown that some of the main constituents in IH and LH are responsible for the observed antioxidant effect (39). An *in vivo* study on rats showed that linalool reduces the formation of malondialdehyde, a product of lipid peroxidation, and increases the amount of glutathione (40), thereby achieving an antioxidant effect. The same was shown for terpinen-4-ol, along with catalase activity increase (41). It is known that the radical scavenger properties of some essential oils are based on a high concentration of terpinen-4-ol (42), though this activity was mostly described as weak if compared to some other monoterpenes, such as α-terpineol (43). α-Terpineol is capable to inhibit lipid peroxidation (44) and along with α- and γ-terpinene was identified as the main antioxidant component in tea tree (*Melaleuca alternifolia*) oil (43).

Borneol also exerts antioxidant and free radical scavenging effects, and protects primary rat hepatocytes against exogenous oxidative DNA damage (45).

Aliphatic di-and-triketones, known as italidiones, are specific ingredients of immortelle (19, 46, 47). A specific part of the structure of these compounds is the β-dicarbonyl fragment (Figures 2 and 3), which is responsible for the antioxidant properties of these compounds. An active methylene group between two keto functionalities can form a resonance-stable radical that acts as a scavenger of various reactive oxygen species (48–50).

Though the biological effects of investigated samples could be explained by the presence of their main components, the overall activity is due to their specific composition. The presence and relative abundance of other minor components of hydrolates and effects such as synergism, additive effects and antagonism, should also be considered (51).

In order to determine the influence of antioxidants, with and without the added exogenous oxidant, we calculated the ratio of concentrations of TAC and TOP, embodied

as OSI, as a marker of antioxidant potency. This index showed the extent of antioxidant capacity, at the expense of the pro-oxidant capacity. The conclusion of the Juneja et al. (52) study was that OSI is a real parameter for oxidative stress assessment in cancer patients. As in the case of TOP, which was initially higher in the LH sample, after the addition of TBH, it became a stronger antioxidant compared to the IH-TBH sample; OSI was slightly lower in the LH sample, but became significantly higher after TBH addition in LH compared to the IH sample ($p<0.05$).

The total concentration of sulfhydryl groups serves as an indicator of the ability of antioxidant protection of the tested compound. Linalool, according to research results (40), can increase the amount of glutathione, the most abundant sulfhydryl-containing molecule in human organisms. In this sense, linalool probably had the greatest influence on the SHG parameter due to the higher value of the total content of SH groups detected in the LH-TBH sample compared to the IH-TBH sample.

Redox scores calculation enables a better comparison between different samples, as previously published (53, 54). Both samples, LH and IH, showed a convincingly low Oxy score at the level of serum sample blank (Figure 1). The addition of exogenous pro-oxidants caused an Oxy score level switch, and the LH-TBH sample succeeded in preserving this general oxidant sum at the low level, while IH-TBH failed to control pro-oxidant activity.

Conclusion

Nowadays, hydrolates are no longer considered as by-products in essential oil production. They are important components of natural origin, widely used in the manufacture of preparations for external use, such as cosmetic preparations for use on the skin, hair, teeth or nails. They could also be used as a substitute for synthetic antioxidants and as antimicrobial agents in food products, and as dietary supplements.

Our current *ex vivo* platform showed that LH is potentially a stronger antioxidant than IH, which is evident from higher values of the OSI and SHG parameters, as well as a smaller value of the TOP parameter obtained for LH. The observed antioxidant effects can be attributed to the presence of different components with proven antioxidant potential. Nevertheless, the observed differences in activity between LH and IH are due to the difference in their qualitative and quantitative composition. A higher percentage of terpinen-4-ol in lavender hydrolate may be the explanation for its better antioxidant properties compared to immortelle hydrolate.

Additional research is needed to determine the clear antioxidant potential of IH and LH.

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Declaration of Competing Interest

None.

Author contributions

NK: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. GMV: Resources, Writing – review & editing. JKM: Data curation, Software, Writing – original draft. Milena Simić: Data curation, Software, Writing – original draft. MM: Resources, Writing – review & editing. JKS: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Software, Writing – original draft.

Data Availability Statement

Data will be made available on request.

References

1. Chatterjee S. Oxidative Stress, Inflammation, and Disease. In: Dziubla T, Butterfield DA, editors. Oxidative Stress and Biomaterials. Cambridge (MA): Academic Press; 2016; pp. 35–58.
2. Dobros N, Zawada KD, Paradowska K. Phytochemical Profiling, Antioxidant and Anti-Inflammatory Activity of Plants Belonging to the *Lavandula* Genus. Molecules. 2023;28(1). doi: 10.3390/molecules28010256.
3. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757–72. doi: 10.2147/cia.s158513.
4. Davies KJA. Oxidative stress: the paradox of aerobic life. Biochem Soc Symp. 1995;61:1–31. doi: 10.1042/bss0610001.
5. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. Int J Biol Sci. 2015;11(8):982–91. doi: 10.7150/ijbs.12096.
6. Amorati R, Foti MC, Valgimigli L. Antioxidant Activity of Essential Oils. J Agric Food Chem. 2013;61(46):10835–47. doi: 10.1021/jf403496k.
7. Kar S, Gupta P, Gupta J. Essential Oils: Biological Activity Beyond Aromatherapy. Nat Prod Sci. 2018;24(3). doi: 10.20307/nps.2018.24.3.139.
8. Jakubczyk K, Tuchowska A, Janda-Milczarek K. Plant hydrolates – Antioxidant properties, chemical composition and potential applications. Biomed Pharmacother. 2021;142:112033. doi: 10.1016/j.biopha.2021.112033.
9. Aćimović MG, Tešević VV, Smiljanić KT, Cvetković MT, Stanković JM, Kiprovski BM, et al. Hydrolates: By-products of essential oil distillation: Chemical composition, biological activity and potential uses. Adv Technol. 2020;9:54–70. doi: 10.5937/savteh2002054A.

10. Shafie MH, Kamal ML, Abdul Razak NA, Hasan S, Uyup NH, Abdul Rashid NF, Zafarina Z. Antioxidant and Antimicrobial Activity of Plant Hydrosol and Its Potential Application in Cosmeceutical Products. Jundishapur J Nat Pharm Prod. 2022;17(4):e124018. doi: 10.5812/jjnpp-124018.
11. Hamedi A, Afifi M, Etemadfard H. Investigating chemical composition and indications of hydrosol soft drinks (aromatic waters) used in persian folk medicine for women's hormonal and reproductive health conditions. J Evid Based Complement Altern Med. 2017;22:824–39.
12. Hamedi A, Pasdaran A, Zebarjad Z, Moein M. A survey on chemical constituents and indications of aromatic waters soft drinks (hydrosols) used in persian nutrition culture and folk medicine for neurological disorders and mental health. J Evid Based Complement Altern Med. 2017;22:744–52.
13. Ninčević T, Grdiša M, Šatović Z, Jug-Dujaković M. *Helichrysum italicum* (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity. Ind Crops Prod. 2019;138:111487. doi: 10.1016/j.indcrop.2019.111487.
14. Kokalj Ladan M, Kočevar Glavač N. GC–MS Analysis of A *Helichrysum italicum* Hydrosol: Sensitivity, Repeatability and Reliability of Solvent Extraction versus Direct Hydrosol Analysis. Appl Sci. 2022;12(19):10040. doi: 10.3390/app121910040.
15. Furlan V, Bren U. *Helichrysum italicum*: From Extraction, Distillation, and Encapsulation Techniques to Beneficial Health Effects. Foods. 2023;12(4). doi: 10.3390/foods12040802.
16. Kladar NV, Anačkov GT, Rat MM, Srđenović BU, Grujić NN, Šefer EI, Božin BN. Biochemical characterization of *Helichrysum italicum* (Roth) G. Don subsp. *italicum* (Asteraceae) from Montenegro: phytochemical screening, chemotaxonomy, and antioxidant properties. Chem Biodivers. 2015;12(3):419–31. doi: 10.1002/cbdv.201400174.
17. Oliva A, Garzoli S, Sabatino M, Tadić V, Costantini S, Ragno R, Božović M. Chemical composition and antimicrobial activity of essential oil of *Helichrysum Italicum* (Roth) G. Don Fil. (Asteraceae) from Montenegro. Nat Prod Res. 2020;34:445–8.
18. Fraternale D, Flamini G, Ascrizzi R. In Vitro Anticollagenase and Antielastase Activities of Essential Oil of *Helichrysum italicum* subsp. *italicum* (Roth) G. Don. J Med Food. 2019;22(10):1041–6.
19. Glumac M, Jažo Z, Paštar V, Golemac A, Čikeš Čulić V, Bektić S, et al. Chemical Profiling and Bioactivity Assessment of *Helichrysum italicum* (Roth) G. Don. Essential Oil: Exploring Pure Compounds and Synergistic Combinations. Molecules. 2023;28(14). doi: 10.3390/molecules28145299.
20. Prusinowska R, Śmigielski KB. Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L). A review. Herba Pol. 2014;60(2):56–66. doi: 10.2478/hepo-2014-0010.
21. European Pharmacopoeia. 10th ed, Strasbourg: Council of Europe, 2019.
22. Assessment report on *Lavandula angustifolia* Miller, aetheroleum and *Lavandula angustifolia* Miller, flos [Internet]. European Medicines Agency; 2012 [cited 2024 May 21]. Available from: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-lavandula-angustifolia-miller-aetheroleum-and-lavandula-angustifolia-miller-flos_en.pdf.
23. Garzoli S, Laghezza Masci V, Franceschi S, Tiezzi A, Giacomello P, Ovidi E. Headspace/GC–MS Analysis and Investigation of Antibacterial, Antioxidant and Cytotoxic Activity of Essential Oils and Hydrolates from *Rosmarinus officinalis* L. and *Lavandula angustifolia* Miller. Foods. 2021;10(8). doi: 10.3390/foods10081768.

24. Community herbal monograph on *Lavandula angustifolia* Miller, flos [Internet]. European Medicines Agency; 2012 [cited 2024 May 21]. Available from: https://www.e-lactancia.org/media/papers/LavandaFlor-EMA2012.pdf.
25. Lis-Balchin M, Hart S. Studies on the mode of action of the essential oil of Lavender (*Lavandula angustifolia* P. Miller). Phytother Res. 1999;13(6):540–2.
26. Kotur-Stevuljevic J, Bogavac-Stanojevic N, Jelic-Ivanovic Z, Stefanovic A, Gojkovic T, Joksic J, et al. Oxidative stress and paraoxonase 1 status in acute ischemic stroke patients. Atherosclerosis. 2015;241(1):192–8. doi: 10.1016/j.atherosclerosis.2015.05.016.
27. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38:1103–11.
28. Alamdari DH, Paletas K, Pegiou T, Sarigianni M, Befani C, Koliakos G. A novel assay for the evaluation of the prooxidant-antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. Clin Biochem. 2007;40:248–54.
29. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37:277–85.
30. Ellman GL. Tissue sulfhydril groups. Arch Biochem Biophys. 1959;82:70–7.
31. Mugao LG. Factors influencing yield, chemical composition and efficacy of essential oils. Int J Multidiscip Res Growth Eval. 2024;5:169–78. doi: 10.54660/.IJMRGE.2024.5.4.169-178.
32. Prusinowska R, Śmigielski K, Stobiecka A, Kunicka-Styczyńska A. Hydrolates from lavender (*Lavandula angustifolia*) – their chemical composition as well as aromatic, antimicrobial and antioxidant properties. Nat Prod Res. 2016;30(4):386–93. doi: 10.1080/14786419.2015.1016939.
33. Kunc N, Frlan A, Baričevič D, Kočevar Glavač N, Kokalj Ladan M. Essential Oil and Hydrosol Composition of Immortelle (*Helichrysum italicum*). Plants. 2022;11(19). doi: 10.3390/ plants11192573.
34. Hydrosol Chemistry [Internet]. Circle H Institute; [cited 2024 May 21]. Available from: https://circlehinstitute.com/hydrosol-chemistry/.
35. Chen X, Shang S, Yan F, Jiang H, Zhao G, Tian S, et al. Antioxidant Activities of Essential Oils and Their Major Components in Scavenging Free Radicals, Inhibiting Lipid Oxidation and Reducing Cellular Oxidative Stress. Molecules. 2023;28(11). doi: 10.3390/molecules28114559.
36. Kozics K, Srancikova A, Sedlackova E, Horvathova E, Melusova M, Melus V, et al. Antioxidant potential of essential oil from *Lavandula angustifolia* in *in vitro* and *ex vivo* cultured liver cells. Neoplasma. 2017;64(4):485–93. doi: 10.4149/neo_2017_401.
37. Stanojević J, Stanojević Lj, Bulatović V, Zvezdanović J, Milenković A, Simonović N, et al. Chemical composition and antioxidant activity of immortelle (*Helichrysum italicum*) (Roth) G. Don) and yarrow (*Achillea millefolium* L.) essential oils. Adv Technol. 2022;11(1):93–103. doi: 10.5937/savteh2201093S.
38. But V, Bulboacă A, Rus V, Ilyés T, Gherman ML, Bolboacă SD. Anti-inflammatory and antioxidant efficacy of lavender oil in experimentally induced thrombosis. Thromb J. 2023;21(1). doi: 10.1186/s12959-023-00516-0.
39. Anthony KP, Deolu-Sobogun SA, Saleh MA. Comprehensive assessment of antioxidant activity of essential oils. J Food Sci. 2012;77(8). doi: 10.1111/j.1750-3841.2012.02795.x.

40. Mehri S, Meshki MA, Hosseinzadeh H. Linalool as a neuroprotective agent against acrylamide-induced neurotoxicity in Wistar rats. Drug Chem Toxicol. 2014;38(2):162–6. doi: 10.3109/01480545.2014.919585.
41. Deen JI, Zawad ANMS, Uddin M, Chowdhury MAH, Al Araby SQ, Rahman MA. Terpinen-4-ol, A volatile terpene molecule, extensively electrifies the biological systems against the oxidative stress-linked pathogenesis. Adv Redox Res. 2023;9:100082. doi: 10.1016/j.arres.2023.100082.
42. Mossa AT, Nawwar GA. Free radical scavenging and antiacetylcholinesterase activities of *Origanum majorana* L. essential oil. Hum Exp Toxicol. 2011;30(10):1501–13. doi: 10.1177/0960327110391686.
43. Kim HJ, Chen F, Wu CQ, Wang X, Chung HY, Jin ZY. Evaluation of antioxidant activity of Australian Tea Tree (*Melaleuca alternifolia*) oil and its components. J Agric Food Chem. 2004;52:2849–54.
44. Ozogul F, Çetinkaya A, El Abed N, Kuley E, Durmus M, Ozogul İ, et al. The effect of carvacrol, thymol, eugenol and α-terpineol in combination with vacuum packaging on quality indicators of anchovy fillets. Food Biosci. 2024;59:104008. doi: 10.1016/j.fbio.2024.104008.
45. Madhuri K, Naik PR. Ameliorative effect of borneol, a natural bicyclic monoterpene against hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic Wistar rats. Biomed Pharmacother. 2017;96:336–47.
46. Andreani S, Uehara A, Blagojević P, Radulović N, Muselli A, Baldovini N. Key odorants of industrially-produced *Helichrysum italicum* subsp. *italicum* essential oil. Ind Crops Prod. 2019;132:275–82. doi: 10.1016/j.indcrop.2019.02.008.
47. Ćavar Zeljković S, Šolić ME, Maksimović M. Volatiles of *Helichrysum italicum* (Roth) G. Don from Croatia. Nat Prod Res. 2015;29(19):1874–7. doi: 10.1080/14786419.2015.1009458.
48. Teixeira D, Lalot T, Brigodiot M, Maréchal E. β-Diketones as Key Compounds in Free-Radical Polymerization by Enzyme-Mediated Initiation. Macromolecules. 1999;32(1):70–2. doi: 10.1021/ma980872+.
49. Barzegar A, Moosavi-Movahedi AA. Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. PLoS One. 2011;6(10):e26012. doi: 10.1371/journal.pone.0026012.
50. Morales NP, Sirijaroonwong S, Yamanont P, Phisalaphong C. Electron Paramagnetic Resonance Study of the Free Radical Scavenging Capacity of Curcumin and Its Demethoxy and Hydrogenated Derivatives. Biol Pharm Bull. 2015;38(10):1478–83. doi: 10.1248/bpb.b15-00209.
51. Koroch AR, Juliani HR, Zygadlo JA. Bioactivity of essential oils and their components. In: Berger RG, editor. Flavours and Fragrances. Berlin: Springer; 2007; pp. 87–115.
52. Juneja S, Rathore AS, Sharma K, Shetty D, Jain A. Antioxidant-Oxidant Index as a Biomarker in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma: A Biochemical Study. J Clin Diagn Res. 2017;11(3). doi: 10.7860/JCDR/2017/22909.9371.
53. Ilić M, Samardžić S, Kotur-Stevuljević J, Ušjak D, Milenković M, Kovačević N, Drobac M. Polyphenol rich extracts of *Geranium* L. species as potential natural antioxidant and antimicrobial agents. Eur Rev Med Pharmacol Sci. 2021;25(20):6283–6294. doi: 10.26355/eurrev_202110_26998.

54. Kotur Stevuljević J, Savić J, Simić M, Ivanišević J. Redox homeostasis, oxidative stress and antioxidant system in health and disease: the possibility of modulation by antioxidants. Arh Farm. 2023;73(4):251–63. doi: 10.5937/arhfarm73-45369.

Uticaj hidrolata smilja (*Helichrysum italicum* (Roth) G. Don) i hidrolata lavande (*Lavandula angustifolia* Mill.) na parametre oksidativnog stresa i antioksidantne zaštite u humanom serumu

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Kratak sadržaj

Brojne studije ukazuju da različiti antioksidansi igraju važnu ulogu u antioksidantnom odbrambenom sistemu organizma. Poznato je da hidrolati, nusproizvodi koji se dobijaju tokom ekstrahovanja etarskih ulja destilacijom vodenom parom, poseduju različite farmakološke aktivnosti, a posebno antioksidantnu. Cilj ovog istraživanja bilo je ispitivanje i upoređivanje antioksidantne aktivnosti hidrolata smilja (*IH*) i hidrolata lavande (*LH*) u humanom serumu kao *ex vivo* platformi. Za evaluaciju njihovog uticaja na parametre oksidativnog stresa i antioksidantne zaštite, korišćeni su spektrofotometrijski biohemijski testovi. GC/MS analizom utvrđeno je da su glavni sastojci italidion I (17,2%), linalol (15,6%), α-terpineol (13,7%), terpinen-4-ol (6,3%) i nerol (5,2%) u *IH*, i linalol (19,6%), terpinen-4-ol (17,0%), α -terpineol (13,6%) i borneol (5,8%) u *LH*. Rezultati su pokazali da su u uzorcima seruma sa *LH*, u prisustvu terc-butil hidroperoksida kao prooksidansa, utvrđene manje koncentracije parametra TOP (*total oxidant potency*), i nešto veće koncentracije parametara TAC (*total antioxidant capacity*) i SHG (*total sulfhydryl groups*) u odnosu na uzorke sa *IH*. Štaviše, primećena je statistički značajna razlika u vrednostima

parametra OSI (*oxidative stress index*) (*IH* u poređenju sa *LH* – 49 (41–58) : 70 (61–75), p<0,05). Korišćenjem ove *ex vivo* platforme, otkriveni su različiti antioksidantni potencijali *IH* i *LH*, pri čemi je *LH* istaknut kao potencijalno snažniji antioksidans od *IH*.

Ključne reči: hidrolati, humani serum, oksidativni stres, antioksidantna aktivnost

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