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ANTIOXIDATIVE ACTIVITY OF SAGE (*Salvia officinalis*) HYDROLATE

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Abstract. *Sage (Salvia officinalisL) hydrolate, a byproduct of sage essential oil distillation, has beneficial antioxidant properties that help protect the body from oxidative stress, which can lead to diseases like cardiovascular conditions, neurodegenerative disorders, and cancer. Rich in bioactive compounds, sage hydrolate neutralizes free radicals, reducing oxidative damage. It is widely used in cosmetics for skin protection against aging and UV radiation, as well as in traditional medicine to reduce inflammation and improve general health. It is typically applied as a spray or dietary supplement to boost the immune system. The study aimed to assess the antioxidant potential of sage hydrosol obtained by Clevenger-type hydrodistillation. Chemical analysis through gas chromatography-mass detector revealed that camphor and eucalyptol were the dominant components. Antioxidant activity was tested using the DPPH method, showing significant antioxidant potential. The results suggest that sage hydrolate offers promising benefits in preventing oxidative damage, supporting immune health, and alleviating inflammation.*

Key words: *sage, hydrolate, distillation, antioxidant*

Introduction

In recent years, there has been an increasing global emphasis on adopting a healthier lifestyle, which includes not only improved dietary habits but also the use of safer, more natural cosmetic products. As part of this movement, attention has turned toward the utilization of natural substances, particularly those derived from medicinal plants. These plants have been valued since ancient times for their therapeutic properties and have played a significant role in traditional medicine and overall human well-being for centuries (1).

In the context of modern scientific and industrial development, there is a growing interest in preserving traditional knowledge and exploring the potential of bioactive compounds from medicinal plants. Their integration into contemporary food, pharmaceutical, and cosmetic industries represents an important step toward sustainable and health-conscious innovation (2).

Equally important is the way in which the potential of medicinal plants is harnessed. One promising and sustainable method is the production of hydrolates—aromatic

waters obtained as a secondary product during the steam distillation of essential oils. Although often considered a by-product, hydrolates contain a variety of bioactive compounds, including water-soluble components of essential oils and plant extracts, which contribute to their therapeutic, antimicrobial, and antioxidant properties (3). Chemically, hydrolates are typically acidic liquids, exhibiting a pH range of 4.5 to 5.5. They contain a small concentration of water-soluble aromatic compounds (generally less than 0.10%) derived from the essential oil, alongside other hydrophilic plant secondary metabolites (4). The volatile compounds present in hydrolates are predominantly highly polar (hydrophilic) molecules, such as monoterpene alcohols, aldehydes, and ketones (5). Many medicinal plants hydrolates contain phytochemicals and thus serve as valuable sources of natural antioxidants that are highly effective in combating oxidative cellular stress (6). Oxidative stress is characterized by the excessive production of reactive oxygen species (ROS) within cells and tissues, leading to damage of critical biomolecules such as DNA, lipids, and proteins. This molecular damage increases the risk of mutations and contributes to the development of various pathological conditions, including cancer, neurodegenerative diseases, and cardiovascular disorders (7, 8).

In this study, we produced sage (*Salvia officinalis*) hydrolate as a by-product of essential oil extraction through hydrodistillation and further investigated its antioxidant activity. Building upon these results, we then explored a combination of two medicinal plants—sage and bay laurel (*Laurus nobilis*)—to produce a mixed hydrolate, with the aim of evaluating its antioxidant potential. This approach was based on the well-documented synergistic interactions between plant-derived compounds, which can enhance overall bioactivity. The study emphasizes not only the value of hydrolates as functional by-products but also the potential of combining botanicals to develop more effective, natural antioxidant formulations (9, 10).

Materials and methods

Plant material

As plant material for hydrolate production, we used non-cultivated sage, cultivated sage purchased from a local pharmacy and commonly used for tea preparation, and domestically sourced bay laurel leaves. Non-cultivated sage (*Salvia officinalis*), including leaves and flowers and domestically cultivated laurel (*Laurus nobilis*), including leaves, were used as fresh plant material. The non-cultivated, naturally occurring sage and laurel was collected from the area of Mostar, located in the southern region of Bosnia and Herzegovina. The plant material was collected in May 2025, carefully packed in paper bags to preserve its integrity, and subsequently transported to the laboratory for further analysis.

Hydrodistillation

The hydrolates were obtained as a by-product of essential oil extraction through the method of hydrodistillation, using a Clevenger-type apparatus. For every distillation, 100 grams of plant material were placed into a laboratory glass flask and immersed in 500 milliliters of distilled water. The flask was then positioned on a heating element and connected to the distillation apparatus. The hydrodistillation process was carried

out for two hours, starting from the point of boiling. During distillation, the hydrolate appeared in the graduated capillary of the Clevenger apparatus, clearly separated from the essential oil. The collected hydrolate was stored in dark glass bottles to prevent light-induced degradation and kept refrigerated until further analysis. Table 1 shows hydrolates we produced: non-cultivated sage hydrolate (NCSH), cultivated sage hydrolate (CSH), cultivated bay laurel hydrolate (CLH), combined non-cultivated sage and lurel hydrolate (NCSLH) and combined cultivated sage and laurel hydrolate (CSLH). Combined hydrolates of sage and bay laurel was prepared by mixing 50 grams of each plant material and then adding 500 mililiters of distilled water. The hydrodistillation was carried out under the same conditions as those used for obtaining the sage hydrolate.

Table 1. Hydrolates produced by hydrodistillation

Plant material	NCSH	CSH	CLH	NCSLH	CSLH
non-cultivated sage	·			·	
cultivated sage		·			·
cultivated laurel			·	·	·

GC-MS analysis of hydrolates

The quantitative chemical characterization of hydrolates was performed by instrumental technique of gas chromatography (GC; Agilent 6890B GC-FID instrument, Waldbronn, Germany) coupled to mass spectrometry (MS; Agilent 5977 MSD instrument, Waldbronn, Germany). The samples (500 mg) were extracted by 1mL of internal standard solution (n-octanol in hexane, c=1μL/mL) and injected in split mode (1 : 50) at inlet temperature of 220 °C. The compounds were separated using HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm; Agilent,Waldbronn, Germany) by application of the following temperature programme: initial oven temperature (set at 60 °C)was increased at a rate of 3 °C/min until reaching 246 °C. The carrier gas was helium, with the flow rate of 1 mL/min. The quantification of compounds was based on the calibration curves obtained for chemical standard substances (α-pinene, β-pinene, eucalyptol, linalool, menthol, pulegone, thymol, carvacrol, and α-bisabolol) analysed under the same experimental conditions.

Antioxidant activity

The antioxidant capacity of non-cultivated sage hydrolate (NCSH), cultivated sage hydrolate (CSH), cultivated laurel hydrolate (CLH), combined non-cultivated sage and bay laurel hydrolate (NCSLH) and combined cultivated sage and lurel hydrolate (CSLH) was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, as described by Mensor et al. and Stanojević et al. (11, 12). For the preparation of the reaction mixture, DPPH was dissolved in ethanol to obtain a final concentration of 3×10^{-4} mol/dm³ and stored in a dark glass vial to protect it from light. Complete dissolution of the DPPH radical was achieved through ultrasonic treatment. This ethanolic DPPH solution was used as the control (Ac).

All hydrolates were treated with the DPPH radical and assumed as samples (As), while separate ethanolic solutions of the hydrolates were used as blanks to eliminate

background absorbance. The antioxidant activity was determined by measuring the decrease in absorbance at 517 nm, which corresponds to the characteristic absorption peak of the DPPH radical. The final concentration of hydrolates in the assay was 571 µg/L. Solutions were incubated at room temperature and measurements were carried out until 20, 40 and 60 min. The free radical scavenging activity was determined using the following formula:

$$DPPH\ radical\ scavening\ capacity\ (\%) = 100 - \left[(A_s - A_B) \times \frac{100}{A_c}\right] \quad \text{Eq. 1(12)}$$

Results and discussion

The results of the GC-MS analysis showed that non-cultivated sage hydrolate contains eucalyptol, *β*-thujone, and camphor in individual concentrations of 0.46, 0.27, and 0.83 mg/g, respectively while results for bay laurel indicated presence of eucalyptol, camphor, verbenone and linalool in individual concentration 0.37, 0.39, 0.32 and 0.13 mg/g. The results revealed the presence of compounds belonging to the group of oxygenated monoterpenes, as also demonstrated in previous studies by other authors (13-15).

Hydrolates, also known as hydrosols, are co-products of plant hydrodistillation and are often considered by-products or even waste. However, hydrolates have found application in cosmetics as ingredients in various products, and in the food industry as natural preservatives that inhibit the growth of microorganisms. They can also be used in diluted form as ingredients in refreshing beverages (16). Their usefulness stems from their chemical composition, as well as from the fact that they are safe for use (4, 17).

Despite these beneficial properties, hydrolates still lack widespread application. Their chemical composition and biological activity have not been sufficiently investigated, as scientific research is usually focused on essential oils. Consequently, it is often challenging to find adequate literature data.

The composition and activity of hydrolates depend on various factors, such as the geographical origin and climatic conditions where the plant grew, the stage of vegetation at the time of collection, the parts of the plant used for hydrodistillation, whether the plant material was dried (and how) or fresh. In this context, more studies are needed to enable comparative analyses (15).

The antioxidant activity of the obtained hydrolates was evaluated using the DPPH scavenging assay. The capacity of the hydrolate to inhibit the DPPH radical was assessed, as well as the influence of incubation time on their antioxidant potential. The results are presented in Figure 1.

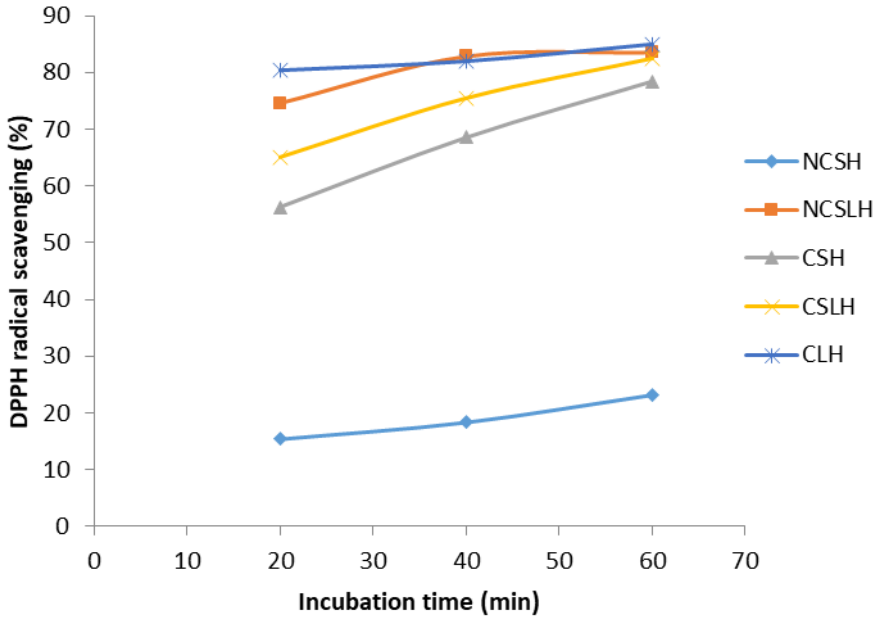

Figure 2. Antioxidant activity of cultivated bay lurel (CLH), non-cultivated sage hydrolate (NCSH), cultivated sage hydrolate (CSH), combined non-cultivated sage and lurel hydrolate (NCSLH) and combined cultivated sage and lurel hydrolate (CSLH)

The antioxidant activity assessment demonstrated that all tested hydrolates exhibit a clear antioxidant effect.

Previous studies on bay laurel and sage hydrolates, as well as plants from the Lamiaceae family originating from other geographical regions, support the findings of this study (18, 19). *Laurus nobilis* exhibited the highest antioxidant potential, with inhibition values ranging from 80.4% to 85%. As shown, the DPPH radical scavenging activity increased slightly over time, following a linear trend.

Earlier studies investigating the antioxidant potential of bay laurel hydrolate also reported notable results when compared to hydrolates derived from other medicinal plants (15, 18). The analysis of all tested hydrolates in this study revealed that antioxidant activity increased with incubation time. After 60 minutes of incubation, three hydrolates displayed comparable antioxidant values on the diagram.

Among them, the hydrolate of wild, non-cultivated sage showed the lowest antioxidant activity, especially when compared to the hydrolate of cultivated sage grown under controlled conditions. Jakubczyk et al (17). evaluated DPPH radical inhibition of hydrolates obtained from 16 different plant species and found that hydrolates derived from organically cultivated plants and specifically from floral parts demonstrated superior radical scavenging ability. In contrast, hydrolates produced from non-organically grown plants or obtained from roots and stems exhibited reduced antioxidant potential.

In this study, we also investigated the antioxidant activity of hydrolates obtained by mixing the plant material of *Laurus nobilis* (bay laurel) and *Salvia officinalis* (sage), with the aim of exploring potential interactions and synergistic effects. The results proved to be particularly interesting and revealed a complex relationship between the two species.

Bay laurel hydrolate, when analyzed independently, exhibited the highest antioxidant capacity among all tested samples, whereas the hydrolate derived from non-cultivated sage showed the lowest antioxidant activity. However, when the plant materials were combined prior to distillation, a notable increase in the antioxidant activity of the non-cultivated sage hydrolate was observed, suggesting a beneficial and possibly synergistic effect induced by the presence of bay laurel.

In contrast, the hydrolate obtained from the mixture of cultivated sage and bay laurel showed only a slight improvement compared to the individual samples. Despite these combinations, bay laurel hydrolate still retained the highest antioxidant potential overall.

Interestingly, these findings suggest a **directional or unidirectional interaction**, where bay laurel enhances the antioxidant profile of sage, particularly the non-cultivated type, but does not benefit reciprocally. In fact, in terms of its own antioxidant capacity, bay laurel may experience a slight reduction when combined with sage, indicating a **mild antagonistic effect** on the part of the bay laurel hydrolate within the mixture.

The synergistic effect is of great importance, as it can be utilized to enhance the biological activity of hydrolates that exhibit lower intrinsic efficacy. In this context, the observed interactions likely arise from the combined action of both major and minor constituents present in the hydrolate (20). Previous studies have demonstrated that **monoterpenes and phenylpropanoids,** when combined with other phytochemicals, have the potential to enhance biological effects such as antioxidant activity. The effectiveness of these interactions is influenced not only by the chemical nature of the compounds but also by their **concentration and relative proportions** within the hydrolate. Thus, the synergistic enhancement of antioxidant capacity may result from additive or cooperative mechanisms between multiple constituents, which together improve the radical-scavenging efficiency more than each component alone. These findings underscore the potential of strategic blending of plant materials to develop more effective natural antioxidant systems, especially by amplifying the activity of hydrolates that are otherwise less potent (9, 21, 22)

These results emphasize the importance of understanding not only the individual bioactive profiles of plant hydrolates, but also how their interactions can influence functional properties such as antioxidant activity. Further mechanistic studies are needed to elucidate the nature of these interactions and to optimize the use of plant combinations in natural antioxidant formulations.

Conclusions

Our study explored the hydrodistillation of non-cultivated sage, cultivated sage, and laurel, including their mixed hydrolates. Through GC-MS analysis, we identified eucalyptol, β-thujone, and camphor in non-cultivated sage hydrolate, while laurel hydrolate contained eucalyptol, camphor, verbenone, and linalool. Evaluating their antioxidant activity using the DPPH method, we found that laurel hydrolate exhibited the highest activity, with wild sage hydrolate having the lowest. Notably, antioxidant activity increased with incubation time. We observed a significant synergistic effect where laurel considerably boosted the antioxidant activity of wild sage hydrolate and, to a lesser extent, cultivated sage hydrolate. Conversely, sage hydrolate showed an antagonistic effect on laurel hydrolate antioxidant properties, as laurel hydrolate consistently maintained the highest percentage inhibition of DPPH radicals across all mixtures, reinforcing its superior individual antioxidant capacity.

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ANTIOKSIDATIVNA AKTIVNOST HIDROLATA ŽALFIJE (*Salvia officinalis)*

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Sažetak. *Hidrolat žalfije (Salvia officinalis), nusproizvod destilacije eteričnog ulja žalfije, poznat je po svojim korisnim svojstvima, uključujući i antioksidativnu aktivnost. Antioksidansi štite organizam od oksidativnog stresa koji može izazvati oštećenja ćelija i doprinijeti razvoju bolesti, uključujući kardiovaskularne, neurodegenerativne poremećaje i rak. Zahvaljujući svom hemijskom sastavu, hidrolat žalfije neutrališe slobodne radikale i time pomaže smanjenju oksidativnog stresa. Ovaj hidrolat se često koristi u kozmetici za zaštitu kože od starenja i oštećenja izazvanih UV zračenjem, a također se primjenjuje u tradicionalnoj medicini za ublažavanje upala i poboljšanje opšeg zdravlja. Njegova primjena u obliku spreja ili u dodacima ishrani može pomoći u jačanju imunološkog sistema i poboljšanju opšeg zdravlja. Cilj ovog rada je bio ispitati antioksidativni potencijal hidrolata žalfije koji je dobijen hidrodestilacijom u aparaturi po Klevendžeru. Hemijski sastav hidrolata ispitan je gasnom hromatografijom sa masenim detektorom. Ispitivanje je pokazalo da su dominantne komponente kamfor i eukaliptol. Antioksidacijska aktivnost određena je DPPH (2,2,-difenil-1-pikrilhidrazil radikal) metodom. Rezultati pokazuju obećavajuće antioksidativne karakteristike hidrolata žalfije.*

Ključne riječi*: žalfija, hidrolat, destilacija, antioksidans*

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