The potential of hydrolates for use in the production of alfalfa micro sprouts: sanitizers and flavour enhancers

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Research Article

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Abstract

Sprouted seeds and microgreens are emerging as innovative specialty raw salad crops, valued for their health-promoting properties. However, the risk of foodborne illnesses associated with microbial contamination in microgreens underscores the need for effective sanitization in their production. This study explores using hydrolates, by-products of essential oil distillation from six plants, as natural sanitizers in alfalfa microgreen production. The research investigates their impact on seed germination, antimicrobial activity, and sensory attributes. Results revealed that oregano, fennel, lavender, and lemon catmint hydrolates decrease alfalfa seed germination, while peppermint and hop hydrolates have no significant impact. Peppermint and fennel hydrolates demonstrate notable efficacy in antimicrobial testing. Sensory analysis indicates differences in odour and flavour, with peppermint, oregano, and lavender receiving high scores. According to the results, peppermint hydrolate can be considered a favourable option for alfalfa micro sprout production, contributing to sustainable and organic approaches in urban agriculture and underlining the importance of natural sanitizers for food safety.

Statement of Novelty

Essential oils are widely used in the food and pharmaceutical industry, but hydrolates, their accompanying by-products, are usually discarded, or used in a small scale in the cosmetic industry. Research has confirmed that hydrolates possess a pleasant scent, mainly similar to the corresponding oils (rarely unpleasant scent, completely different from essential oil) and certain biological activities (antimicrobial, antioxidant, allelopathic, etc.). They have the potential to be used in organic agriculture, as well as in food processing. Bearing in mind the constant world trend of applying innovative classes of specialty raw salad crops, such as microsprouts and microgreens, the application of hydrolates can be of great importance in order to improve its taste and microbiological safety.

Highlights

- Micro sprouts are prevalentraw salad crops with functional properties
- Alfalfa sprouting was done in water or six selected hydrolates during 10 days
- Peppermint, oregano, fennel, hop, lavender and lemon catmint hydrolates were used
- All hydrolates except peppermint and fennel showed a dose-dependent sprouting inhibition
- Peppermint hydrolate showed the best antimicrobial and sensory potential

Introduction

Sprouted seeds and microgreens are a new innovative class of specialty raw salad crops, especially valued for their health-promoting or disease-preventing properties [1, 2]. Micro sprouts are commonly grown in the dark under high relative humidity, and production time is usually 3–10 days from seed hydration (the cotyledons are still under-developed and true leaves have not begun to emerge) [3, 4]. Production of micro sprouts does not require soil or medium to grow; they grow solely in water or moisture. Some other advantages of the production of micro sprouts are: a large-scale production can be achieved in a very small space, and there is no harvesting, because the obtained product is completely edible [4]. From the other side, the seedlings with first true leaves emerge called microgreens, and are usually harvested 7–28 days after germination [5]. Numerous plant species is used for microsprouts and microgreen production, among them many legumes, crucifers, oilseeds, cereals and pseudocereals [3, 6, 7, 8].

Alfalfa (*Medicago sativa* L.), also known as lucerne, is one of the most common seeds for sprouting [9]. Its sprouts are fluffy, crunchy and sweet, and one of the most nutritionally rich foods [10]. Alfalfa sprouts are highly valuable sources of protein, minerals (including trace elements such as copper, manganese, and selenium), vitamins (B complex, vitamins C and E), phenolic compounds (gallic and caffeic acids), flavonoids (apigenin, kaempferol, myricetin, naringin quercetin, rutin, daidzein, and genistein), glucosinolates, saponins and their derivatives [11, 12]. These compounds have great biological potential, such as antioxidant activity, cholesterol-lowering properties [13], antidiabetic potential [14], as well as anticancer properties [15, 16]. In addition, the phytoestrogenic activity of alfalfa sprouts is also noted [17, 18]. Taking all these in mind, alfalfa micro sprouts are very fashionable functional food for the 21st Century [19].

Unfortunately, numerous food illnesses are caused by the consumption of micro sprouts and microgreens contaminated with enteric bacterial pathogens [20]. Therefore, sanitizing raw material during processing is a critical step in microgreen production [21]. Therefore, scientists worldwide are trying to find solutions to chemical sanitizers suitable for organic food production. Hydrolates, as by-products during the distillation process of essential oils, have the potential for application in the food industry, as natural food sanitizers, for washing fresh-cut fruits and vegetables [22, 23, 24, 25, 26, 27, 28], as well as for disinfection of food and areas in contact with it [29, 30]. In addition, hydrolates are often used in the food industry because of specific organoleptic properties and flavour, in functional (soft) drinks and naturally flavoured herbal ice cream [31, 32]. However, they have a much softer scent and are less biologically active than corresponding essential oils, because they mainly contain less than 0.1% of water-soluble fraction of volatile aromatic compounds, similar to dissimilar in comparison to the essential oils [31].

Due to its growing popularity, it is increasing interest among seed produce companies and scientists to develop and improve growing technology practices under urban agriculture, which can easily be applied in indoor farming systems as well as under home conditions. Bearing in mind the above-mentioned, this paper aimed to examine the effect of hydrolates obtained as a by-product during the processing of six essential oil-bearing plants (peppermint, oregano, fennel, hop, lavender and lemon catmint) on germination and initial growth of alfalfa seedlings. Another goal of the investigation was to determine the antimicrobial activity of investigated hydrolates, and their effect on the taste and odour of alfalfa micro sprouts.

Material and method

Plant material

Selected essential oil-bearing plants were grown at the Institute of Field and Vegetable Crops Novi Sad, at experimental fields localized in Bački Petrovac (45.3378183, 19.6700113). Plants were harvested at the optimal technological period: at the beginning of the flowering (peppermint and oregano), full flowering (hop, lavender and lemon catmint), and in the full ripening stage (fennel). After harvest, plant materials were dried in a solar dryer until constant mass and stored until further processing (less than six months).

Essential oil and hydrolate extraction

Approximately 100 kg of dry plant materials were placed in the stainless steel distillation vessel, covered with a steel lid via a plumbing system and supplied with steam obtained by an external steam boiler. Steam passing through the plant material whereby the steam volatile compounds are volatilized, exited the distillation vessel, then condensed and collected and finally collected in Florentine glass flask. Due to its lower density than water, essential oil floats on the surface of the aqueous phase, and after the end of the distillation process, it is easily separated by decanting. However, the aqueous phase that remains in the Florentine flask is mostly discarded, but we collected it in sterile plastic bottles over filter paper in order to investigate the chemical composition and find additional value of this by-product.

Chemical characterization of hydrolates

Likens-Nickerson apparatus was used for simultaneous distillation-extraction method of hydrolates (400 mL) with dichloromethane (5 mL) as the solvent. Obtained secondary essential oils from hydrolates were analysed by gas chromatography-flame ion detection (GC/FID), and gas chromatography-mass spectrometry (GCMS). All chromatograms were shown in Figure. 1, while the relative mass percentage of the identified compounds were given in Supplementary table.

Alfalfa germination tests

Alfalfa seeds (*Medicago sativa* cv. Mediana) obtained from the Institute of Field and Vegetable Crops Novi Sad, were confirmed and deposited at the BUNS Herbarium, University of Novi Sad, Serbia, under voucher specimens number 2-0681.

A total 100 alfalfa seeds were placed in Petri dishes, in four repetitions, on filter paper soaked with 10 mL of distilled water (control) or different concentrations of hydrolates (10%, 20%, 50% and 100%). The seeds were germinated for 10 days in a climate chamber at 22°C/20°C for 12-hour photoperiod according to ISTA recommendations [33]. Seed germination (%) and seedling length (shoot and root length) were recorded in order to determine the efficiency of hydrolates.

Antimicrobial activity

To observe the microbial growth-suppressing potential of hydrolates, 8 representatives of gram-positive and gram-negative bacteria, as well as fungi and yeasts were involved in the disk diffusion testing method described by Šovljanski et al. [34]. Interpretation of the results can be summarized as: sensitive microorganism (diameter of the inhibition zone above 26 mm), intermediary (diameter between 22 and 26 mm), and resistance (diameter below 22 mm). All tested microorganisms were procured from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) and deposited at the Laboratory of Microbiology in a deep freezer (Snijders Labs, Tilburg, The Netherlands). The following microorganisms are involved in this study: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311, *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404.

Sensory analysis

The sensory study was conducted at the University of Novi Sad, Faculty of Technology Novi Sad to evaluate the sensory qualities of microgreen samples. Six samples of alfalfa microgreens obtained using hydrolates obtained as by-products during the processing of six essential oil-bearing plants (peppermint, oregano, fennel, hop, lavender and lemon catmint) were evaluated in a sensory laboratory by an experienced panel consisting of ten members. The sensory attributes were determined as previously described by Xiao et al. [35], with slight modifications. Seven odour terms (cheesy, herbal, balsamic, floral, mint, mentholic, and spicy) and six flavor terms (cheesy, minty, cooling, citrus, floral and citrus-floral) were chosen for further sensory analysis. Tested alfalfa microgreens were evaluated in triplicate on a 7 Likert scale from very poor (1) to excellent (7). For the detection of the odour and flavour of alfalfa microgreens samples was performed as described by Tan et al. [36].

Statistical analysis

The experimental data underwent principal component analysis (PCA) using StatSoft Statistica 10.0® software.

Results

In this investigation, the aim was to evaluate the influence of hydrolates on (1) germination and seedling length, (2) antimicrobial activity of hydrolates and (3) odour and flavour of hydrolates.

Germination and seedling lenght

Tukey's test, at a $p < 0.05$ level of significance, showed that increasing concentrations (10–100%) of oregano, fennel, lavender and lemon catmint hydrolates resulted in a statistically significant decrease of alfalfa seed germination, in the same peppermint and hop hydrolate had no statistically significant influences on alfalfa seed germination (Table 1, Fig. 2).

Table 1
Alfalfa seed germination (%) under distilled water (control) and different concentrations of hydrolates after 10 days

Sample	Pepermint	Oregano	Fennel	Hop	Lavender	Lemon catmint
control	83 ± 6.00^{a}	83 ± 6.00^{bc}	89 ± 7.57^{e}	86 ± 4.00^{b}	83 ± 6.00^{d}	89 ± 7.57^{d}
10%	85 ± 12.38^{a}	76 ± 8.64^{c}	82 ± 8.33^{c}	78 ± 6.93^{b}	58 ± 21.04^{bc}	90 ± 6.93^{e}
20%	83 ± 10.00^{a}	75 ± 8.87^{bc}	86 ± 8.33^{d}	87 ± 11.02^{b}	57 ± 11.49^{b}	83 ± 8.87^{c}
50%	79 ± 8.87^{a}	46 ± 24.55^{ab}	54 ± 18.04^{b}	69 ± 11.49^{b}	59 ± 30.00^{c}	10 ± 10.07^{b}
100%	77 ± 5.03^{a}	0 ± 0.00^{a}	2 ± 4.00^{a}	3 ± 3.83^{a}	3 ± 2.00^{a}	0 ± 0.00^{a}

Results represent average value ± standard deviation. Different letters in superscript of the same table column indicate the statistically significant difference between values, at a level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test)

As can it be seen from Table 2, the alfalfa root length after ten days was between 3.0 and 40.4 mm. All hydrolates decreases root length except peppermint in lower concentrations (Table 2, Fig. 2).

Table 2
Alfalfa root length (mm) under distilled water (control) and different concentrations of hydrolates after 10 days

Sample	Peppermint	Oregano	Fennel	Hop	Lavender	Lemon catmint
control	34.3 ± 15.928^{c}	34.3 ± 15.928^{e}	52.2 ± 23.422^{e}	33.8 ± 15.949^{c}	34.3 ± 15.928^{b}	52.2 ± 23.422^{b}
10%	40.4 ± 20.017^{e}	19.8 ± 10.738^{c}	26.3 ± 16.138^{d}	28.1 ± 13.221^{bc}	6.2 ± 2.931^{a}	48.1 ± 20.096^{b}
20%	39.6 ± 21.237^{d}	22.5 ± 11.603^{d}	21.1 ± 12.271^{c}	27.5 ± 11.034^{bc}	7.4 ± 2.832^{a}	42.5 ± 16.834^{b}
50%	31.1 ± 15.682^{b}	5.9 ± 3.589^{b}	8.9 ± 7.931^{b}	9.5 ± 7.528^{ab}	5.9 ± 3.652^{a}	5.1 ± 3.247^{a}
100%	20.6 ± 11.484^{a}	0.0 ± 0.000^{a}	1.0 ± 0.000^{a}	3.3 ± 2.082^{a}	3.0 ± 2.000^{a}	0.0 ± 0.000^{a}

Results represent average value ± standard deviation. Different letters in superscript of the same table column indicate the statistically significant difference between values, at a level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test)

In this study, after ten days shoot length was between 2.3 and 14.8 mm. All hydrolates in all investigated concentration decrease shoot length, except fennel in concentration of 10% and 20% which stimulate shoot length in comparison to control (Table 3, Fig. 2).

Table 3
Alfalfa shoot length (mm) under distilled water (control) and different concentrations of hydrolates after 10 days

Sample	Peppermint	Oregano	Fennel	Hop	Lavender	Lemon catmint
control	14.6 ± 5.943^{ab}	14.6 ± 5.943^{d}	8.1 ± 3.232^{c}	14.4 ± 5.876^{c}	14.6 ± 5.943^{c}	8.1 ± 3.232^{c}
10%	14.8 ± 5.504^{b}	10.3 ± 3.647^{c}	10.2 ± 5.403^{e}	10.3 ± 3.566^{b}	7.8 ± 3.480^{b}	7.9 ± 2.034^{bc}
20%	13.9 ± 5.852^{ab}	10.1 ± 3.342^{c}	9.5 ± 5.568^{d}	11.2 ± 3.422^{b}	7.6 ± 2.735^{b}	7.9 ± 2.505^{bc}
50%	14.8 ± 5.217^{ab}	6.2 ± 3.462^{a}	5.7 ± 5.303^{b}	3.7 ± 2.975^{a}	7.6 ± 3.444^{b}	2.3 ± 2.627^{ab}
100%	8.5 ± 3.401^{a}	0.0 ± 0.000^{b}	0.0 ± 0.000^{a}	2.7 ± 3.786^{a}	3.3 ± 2.887^{a}	0.0 ± 0.000^{a}

Results represent average value ± standard deviation. Different letters in superscript of the same table column indicate the statistically significant difference between values, at a level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test)

Microbial growth-suppressing potential of selected hydrolates

In this study, microbial growth-suppressing potential of hydrolates derived from peppermint, oregano, fennel, hop, lavender, and lemon catmint was investigated against a panel of microorganisms (Table 4). The results revealed varied inhibitory effects, providing insights into the potential applications of these hydrolates in controlling microbial growth during microgreens growth. Namely, the assessment focused on discerning the inhibitory effects of tested hydrolates by measuring the respective inhibition zones (expressed as mean values with standard deviations based on three repetitions).

Table 4
Microbial growth-suppressing potential of investigated hydrolates shows the diameter of the inhibition zone (mm)*

Tested microorganism	Peppermint	Oregano	Fennel	Hop	Lavender	Lemon catmint
B. cereus	nd	22.00 ± 0.00	14.00 ± 1.00	nd	nd	16.00 ± 1.00
S. aureus	24.00 ± 1.00	22.67 ± 1.15	26.33 ± 0.58	nd	nd	19.33 ± 0.58
E. faecalis	nd	18.67 ± 0.58	17.67 ± 0.58	nd	nd	12.00 ± 0.00
E. coli	27.33 ± 0.58	11.00 ± 1.00	27.00 ± 0.00	nd	nd	nd
S. Typhimurium	nd	9.56 ± 0.58	nd	nd	nd	nd
S. cerevisiae	18.67 ± 0.58	nd	13.00 ± 0.00	nd	8.33 ± 0.58	nd
C. albicans	27.00 ± 0.00	nd	23.00 ± 0.00	nd	nd	nd
A. brasiliensis	nd	nd	nd	nd	nd	nd

*Evaluation of the no-growth zone as circle diameter (results are presented as mean and standard deviation based on three repetitions); nd – not detected.

Peppermint hydrolate, characterized by its menthol content, exhibited notable antimicrobial efficacy. This hydrolate demonstrated strong inhibitory effect against bacterium *Escherichia coli* and yeast *Candida albicans*, since the observed substantial inhibition zone was 27.33 and 27.00 mm, respectively. Additionally, intermediary effects against *Staphylococcus aureus* and *Saccharomyces cerevisiae* underscored the broad-spectrum potential of peppermint hydrolate against both bacterial and fungal strains. On the other hand, oregano hydrolate displayed varied efficacy profiles. While demonstrating similar intermediary effects against *S. aureus*, it exhibited lower growth-suppressing potential towards *E. coli* and intermediary effects against *Bacillus cereus*. Fennel hydrolate demonstrated a significant inhibitory level against both *S. aureus* and *E. coli* comparable to the observed effect of peppermint hydrolate. Contrary, its intermediary effects against *Enterococcus faecalis*, *B. cereus* and *C. albicans* highlight its variability across bacterial and fungal targets.

Hop hydrolate generated no specific results for individual microorganisms in this study, necessitating further exploration to ascertain its specific antimicrobial impact. Similarly, lavender hydrolate exhibited minimal growth-suppressing effect against S. cerevisiae emphasizing insufficient amount of antimicrobial components in its composition. On the other hand, medium inhibitory effects of lemon catmint hydrolate are observed *B. cereus*, *E. faecalis*, and *S. aureus*. The absence of specific results for other tested bacteria, as well as yeast and fungi indicated potential resistance of the tested groups of microorganisms as well as absent of targeted plant-derived compounds in the hydrolate. As shown in Table 4, the highest level of resistance was observed for *A. brasiliensis* since any tested hydrolates did not show growth-suppressing effect against this fungi representative. This results highlights the need for further research and comprehensive literature reviews to establish baseline data for hydrolate efficacy against fungal strains related to micro sprouts production.

Unfortunately, literature comparisons for the investigated hydrolates and their growth-suppressing potential against selected microorganisms are limited in scientific literature, making it challenging to draw deeper correlations. The presented results in Table 4 emphasize the dual role of hydrolates in alfalfa micro sprout production. Peppermint and fennel hydrolates stand out as potent sanitizers, with potential flavour-enhancing attributes. On the other hand, oregano hydrolate had the selective efficacy, positioning it as a targeted food safety and sensory attributes in alfalfa micro sprouts. Since the microbial growth-suppressing effect of hop, lavender and lemon catmint hydrolates is minimal, these hydrolates exhibit potential only as flavour contributors.

Odour and tase of hydrolates

The odour and taste of main compounds presented in investigated hydrolates and its percentage are shown in Table 5 and Supplementary table 1. The main compounds of peppermint hydrolate are menthol (30.6%), menthone (14.4%) and 1,8-cineole (12.8%) which gives it minty odour. Oregano hydrolate contain terpinen-4-ol (34.0%) and linalool (13.7%) as dominant compounds, which gives the pleasant floral and spicy odour. The dominant compound in fennel hydrolate was fenchone (90.4%) which gives balsamic odour. Hop possesses very strong cheesy odour which originated from isovaleric acid, main compound from hydrolate. Lavender hydrolate have characteristic floral lavender odour, originates from linalool (39.4%). Lemon catmint contain mixture of nerol and citronellol (54.3%) and geraniol (29.4%), which gives citrus and floral odour.

Table 5
Odor and taste of main compounds from investigated hydrolates (presented with more than 10%)

No	Compound	Odor Type	Flavour type	Peppermint	Oregano	Fennel	Hop	Lavender	Lemon catmint	Odor Strength	Taste Description
1	Isovaleric acid	cheesy	cheesy	0.0	0.0	0.0	**20.6**	0.0	0.0	high	Cheesey, dairy, creamy, fermented, sweet, waxy and berry
2	1,8-Cineole	herbal	minty	**12.8**	3.8	0.6	0.1	3.9	0.0	high	Minty camphoreous cooling eucalyptus medicinal
3	Fenchone	balsamic	cooling	0.4	0.0	**90.4**	0.0	0.0	0.0	medium	Cooling, camphoreous, sweet and minty with a musty, earthy nuance
4	Linalool	floral	citrus	0.2	**13.7**	0.0	**10.5**	**39.4**	1.9	medium	Citrus, orange, lemon, floral, waxy, aldehydic and woody
5	Menthone	minty	minty	**14.4**	0.1	0.0	0.0	0.0	0.0	medium	Cooling, peppermint, fresh green, minty with an herbal nuance
6	Menthol	mentholic	cooling	**30.6**	0.0	0.0	0.0	0.0	0.0	medium	Cooling mentholic minty
7	Terpinen-4-ol	spicy	cooling	7.3	**34.0**	1.2	0.5	**10.2**	0.0	medium	Cooling mentholic woody weedy earthy herbal spicy citrus
8	Nerol + Citronellol	floral	citrus, floral	0.0	0.0	0.0	0.0	0.4	**54.3**	medium	Floral, rose, lemon, green with fruity terpy nuance
9	Geraniol	floral	floral	0.0	0.0	0.0	3.1	1.5	**29.4**	medium	Floral, rosy, waxy and perfumey with a fruity, peach-like nuance

The points in the PCA graph, being near each other, reveal the similarity of the chemical composition of these samples. The vector's orientation representing the variable in factor space indicates an arising tendency of these factors. Meanwhile, the vector size is proportional to the squared correlation among the variables. The angles between related variables indicate the extent of their correlations (sharper angles correspond to higher correlations). Based on the experimental results, PCA analysis was performed, Fig. 3. The PCA biplot of the relationships among characteristic compounds of the obtained alfalfa microgreens samples revealed that the first two principal components explained 62.51% of the total variance in the observed parameters, Fig. 3a. According to the results of the PCA, the content of 1,8-cineole, menthone, and menthol (28.06%, 24.67%, 24.58% of the total variance, based on correlations, respectively) showed a positive influence on the first principal coordinate. In contrast, linalool (19.34%), terpinen-4-ol content (12.63%) showed positive influence on the calculation of the PC2, while mixture of nerol and citronellol (-26.59%), and geraniol (-25.09%) negatively influenced the calculation of the second principal coordinate (Fig. 3a). Furthermore, a positive influence on the third principal component was observed for linalool (17.42%) and terpinen-4-ol content (17.10%), while fenchone content (-44.30%) showed the opposite effect (Fig. 3b).

Sensory Analysis

The sensory analysis results are presented in Fig. 4 and Fig. 5, showing slight differences in the observed microgreen samples. According to the results of the sensory study of odour (Fig. 4), scores for the cheesy, herbal, balsamic, floral, minty, mentholic, and spicy of microgreens were significantly different ($p < 0.05$) between samples. Higher averages of scores for odour were noted for peppermint, oregano, fennel and lavender alfalfa microgreen samples.

Based on Fig. 5, the outcomes of the sensory study focusing on flavour, reveal distinct variations among the analysed microgreen samples, with statistically significant differences ($p < 0.05$) in scores. Notably, the trends observed in flavour scoring align closely with those identified in the odour assessment, further emphasizing the unique and pronounced characteristics of peppermint, oregano, and lavender alfalfa microgreen samples. Furthermore, reinforcing the notion that the choice of hydrolate in the cultivation process significantly influences the sensory attributes of the final microgreen product.

Discussion

Alfalfa is a dicotyledonous plant. Generally, water uptake occurs 2–4 days after planting. The seedling root is the first structure to emerge from the seed during germination, and this process usually lasts next 5–7 days. After that, cotyledons elongate (next several days), and the first true leaf emerges usually 10 to 15 days after planting [37]. Investigation of the effect of thyme hydrolate solutions on the alfalfa seedlings after 10 days, the root length was between 12.5 and 28.5 mm, while the shoot length was between 7.5 and 12.1 mm (by applyng 20% thyme hydrolate and in control, respectively) [38]. In general, there are several studies that indicated allelopathic activities of plant hydrolates on seed germination of cultivated and weed species. Experiments showed that *Brachiaria humidicola* hydrolate has allelopathic effect on four tropical forage legumes and on lettuce [39], while *Haplophyllum tuberculatum* hydrolate inhibited seeds germination of common wheat and radish [40]. Moreover, thyme hydrolate has negative effect on the germination of soybean, sunflower, maize, clover and alfalfa, as well as weeds (*Amaranthus retroflexus*, *Chenopodium album*, *Portulaca oleracea*, *Echinochloa crus-galli*, *Sorghum halepense*, and *Solanum nigrum*) [38]. In addition, hop and angelica hydrolates in lower concentrations (10 and 20%) stimulate germination of big seeds such as corn and in higher concentrations (50 and 100%) as suppressed germination especially in a small seeds such as *A. retroflexus* [41].

Contaminated seeds, as well as the use of contaminated water for sprouting are the most recognized source of microbial hazards associated with sprouts. There is interest in scientists and farmers/growers at home conditions, to resolve this problem by using a strong oxidants as sanitizers, such as chlorine dioxide (ClO_2) or ozone (O_3) to reduce or eliminate human pathogens from sprouts [42]. However, in this study, we evaluated the effect of hydrolates obtained from six aromatic plants widely used in industrial processing of essential oils, as a medium for germinating alfalfa seeds for microsprouts. Hydrolates, as byproducts, are usually discarded, even if they possess significant odour and flavour, as well as antimicrobial and antioxidant biological potential. By using them in the production of micro sprouts, environmental protection is achieved in the first place (flavoured water is no longer waste, but gains added value). Another, much more significant result achieved by the use of hydrolates in the production of micro sprouts is the preservation of human health and the functionality of food.

Peppermint hydrolate possesses a similar odour as essential oil, due to presence menthol, menthone and 1,8-cineole, previously reported [43, 44, 45]. Oregano hydrolate used in this study, possesses pleasant floral and spicy odour, because of presence terpinen-4-ol and linalool as dominant compounds. However, the significantly different chemical composition of oregano hydrolate with dominant thymol or carvacrol are referred depend on chemotype [45, 46]. The dominant compound in fennel hydrolate was fenchone which gives balsamic odour. However, previously reported estragole (33.0-37.4%) and fenchone (22.5–26.5%) as main compounds in fennel hydrolate [47]. Hop possesses very strong cheesy odour which originated from isovaleric acid, main compound from hydrolate, what is previously reported by Lazarević et al. [41]. Lavender hydrolate is the most studied hydrolate, and all authors referred linalool and linalyl acetate as dominant compounds in hydrolate of lavender species [48, 49, 50, 51]. There is no available data about chemical composition of lemon catmint hydrosol of this species. Principal component analysis (PCA) enables the arrangement of patterns in examined data by presenting information on determining variables, that behave likewise to each other [52]. Looking at the PCA figures, we can easily see which hydrolate samples have the highest content of the observed compounds. The closeness of the vectors representing the observed compounds and the points representing the samples makes it clear which hydrolate is the richest in each compound. There are several studies that reported the antimicrobial activity of hydrolates, among them peppermint and oregano [53, 54, 55], while for hop hydrolate previously is reported no antimicrobial activity [41]. On the other side, there are a lack of information about the antimicrobial potential of other investigated hydrolates (fennel, lavender, and lemon catmint), but for their essential oil it is reported [56, 57]. Taking into account that hydrolates mainly contain less than 1% of essential oil, it is understandably less efficiency of hydrolates in comparison to essential oils [58]

The comparative analysis of plant-based by-products against specific microorganisms revealed distinct inhibitory profile which can directed further investigation to define specific concentrations as well as pharmacodynamic potential prior any use in the real conditions [34]. The obtained results in this study are the first step in the analysis of the mentioned potential of hydrolates and requires a deeper and more complex analysis in *in vitro* and *in vivo* conditions for determination of microbial control based on hydrolate utilisation. The various responses observed across different microorganisms also highlight the importance of considering both the hydrolate source and microbial target in formulating effective growth suppressing strategies in micro sprouts and other food-related production [30].

Conclusions

In light of the results from germination tests, evaluations of antimicrobial potential, and sensory analyses, peppermint hydrolate emerges as a promising option for cultivating alfalfa microgreens. The study underlines the considerable potential of peppermint hydrolate as a favourable choice for incorporation into the production of alfalfa microgreens, given its positive impact on germination, antimicrobial efficacy, and sensory qualities. These findings are significant in advancing sustainable and organic practices within urban agriculture, highlighting the importance of employing natural sanitizers to elevate microgreen production's safety and quality standards.

Declarations

Author Contributions

MA: resources, writing (original draft), conceptualisation; NS: formal analysis, investigation; OŠ: formal analysis, investigation; BL: data curation, writing (original draft); JSJ: formal analysis, investigation; LP: data curation, conceptualisation; BK: methodology, supervision; SV: supervision, project administration.

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Data Availability

Data can be made available upon reasonable request.

Declarations Conflict of interest

The authors declare no competing interests.

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Figures

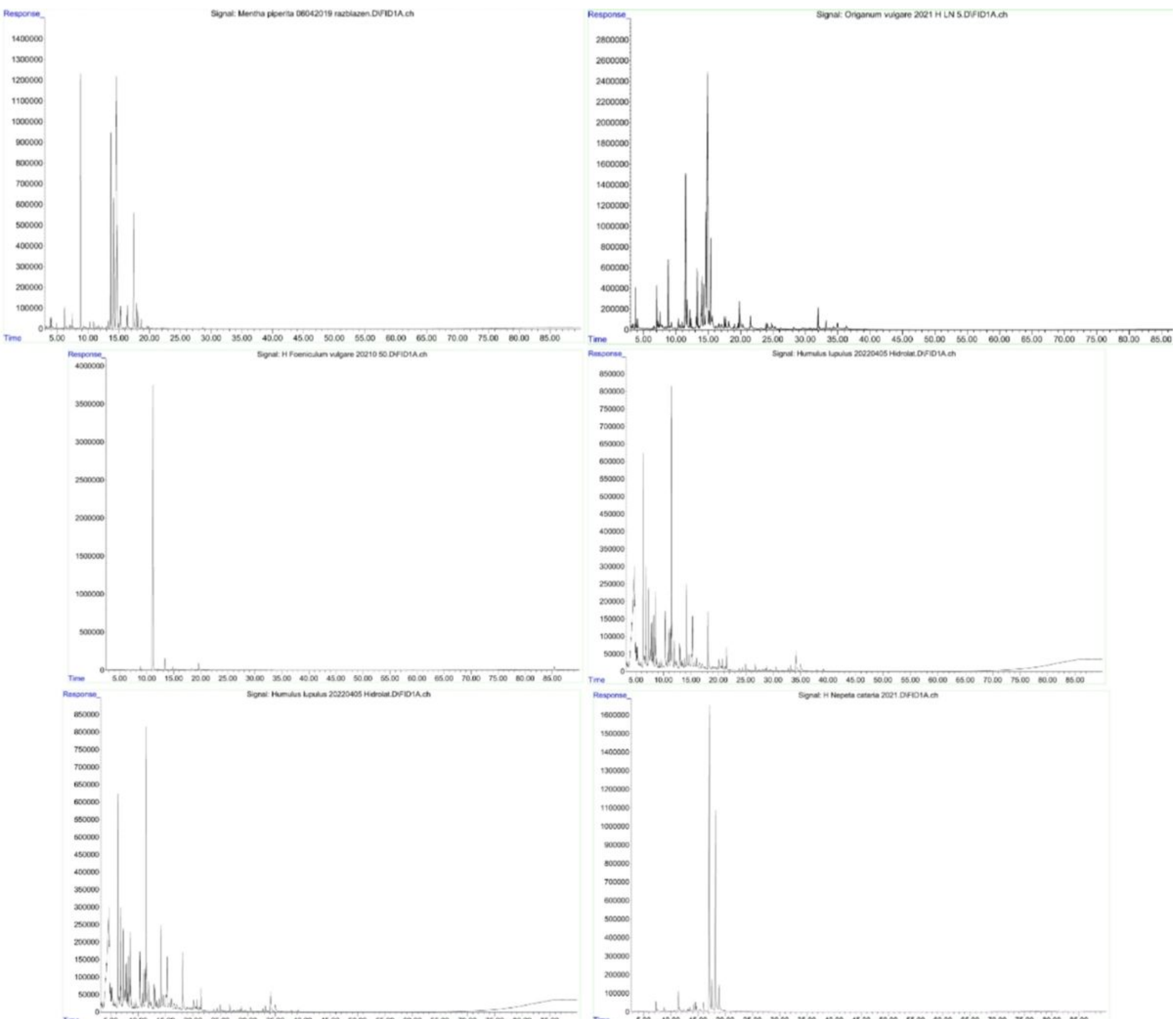

Figure 1

GC-MS chromatographs of investigated hydrolates: (a) peppermint, (b) oregano, (c) fennel, (d) hop, (e) lavender and (f) lemon catmint.

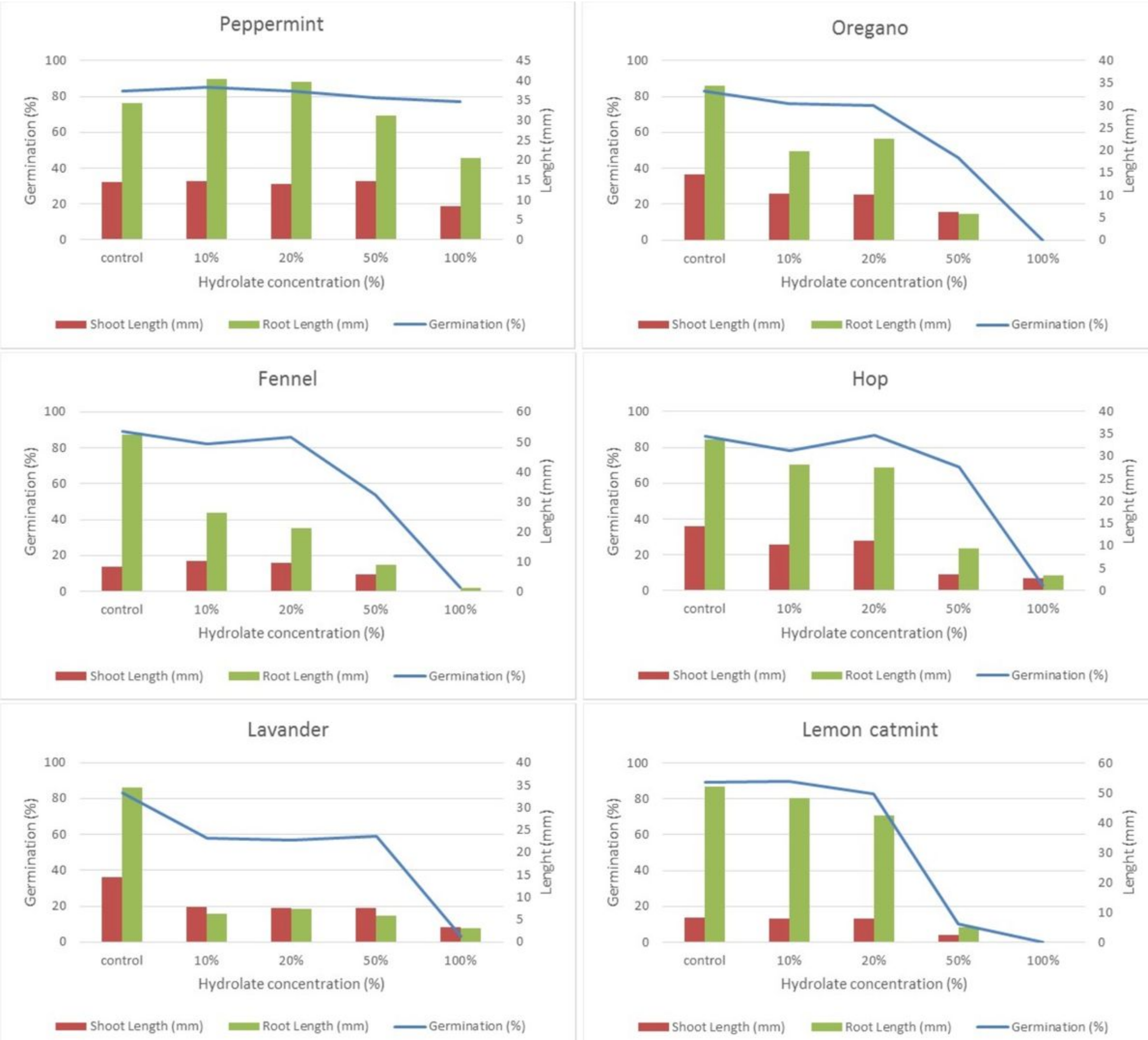

Figure 2

Alfalfa germination, shoot and root length depend on application of selected hydrolates in different concentration

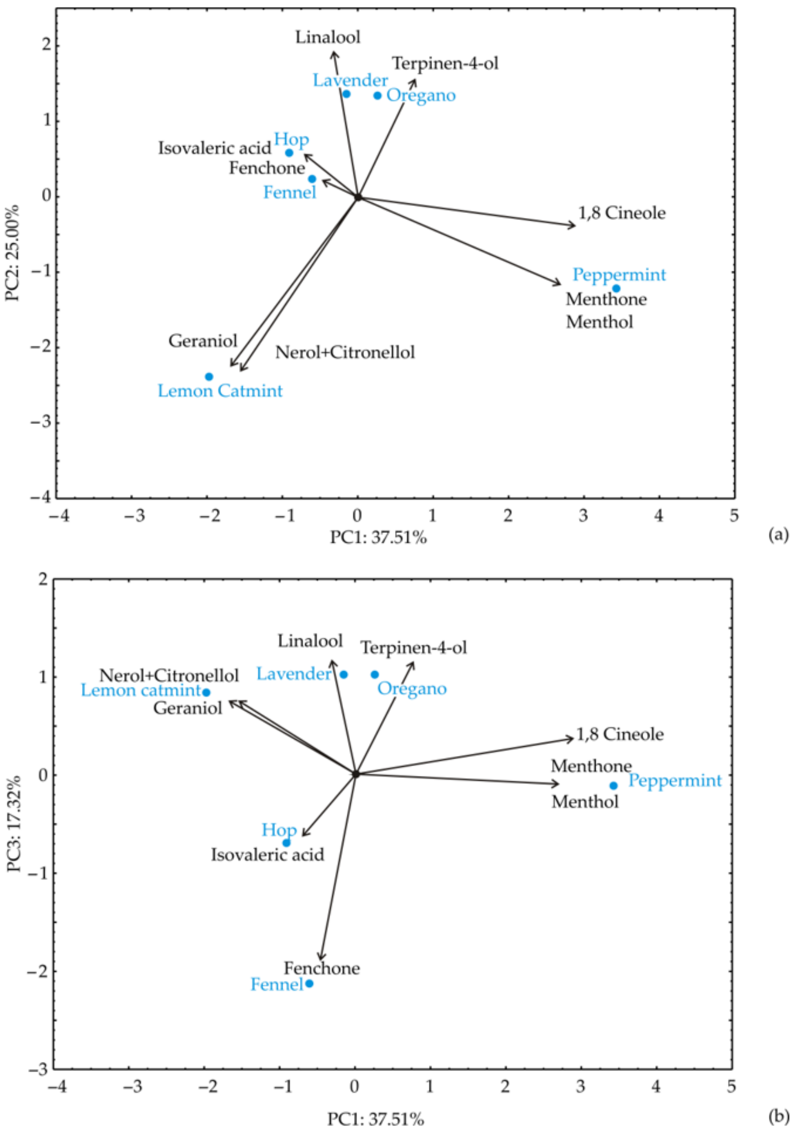

Figure 3

The PCA biplot diagram of the relationships among observed responses the tested alfalfa microgreen samples

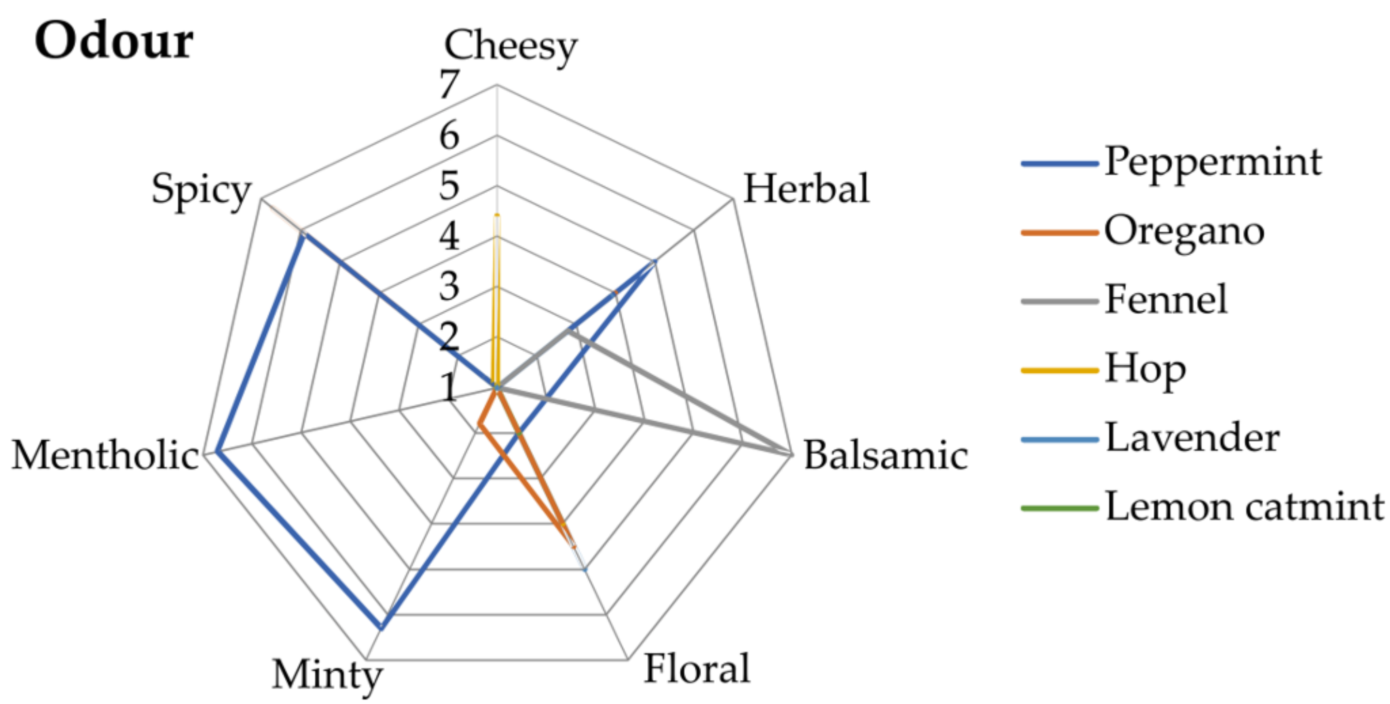

Figure 4

Results of the sensory analysis for odour of the alfalfa microgreens using investigated hydrolates

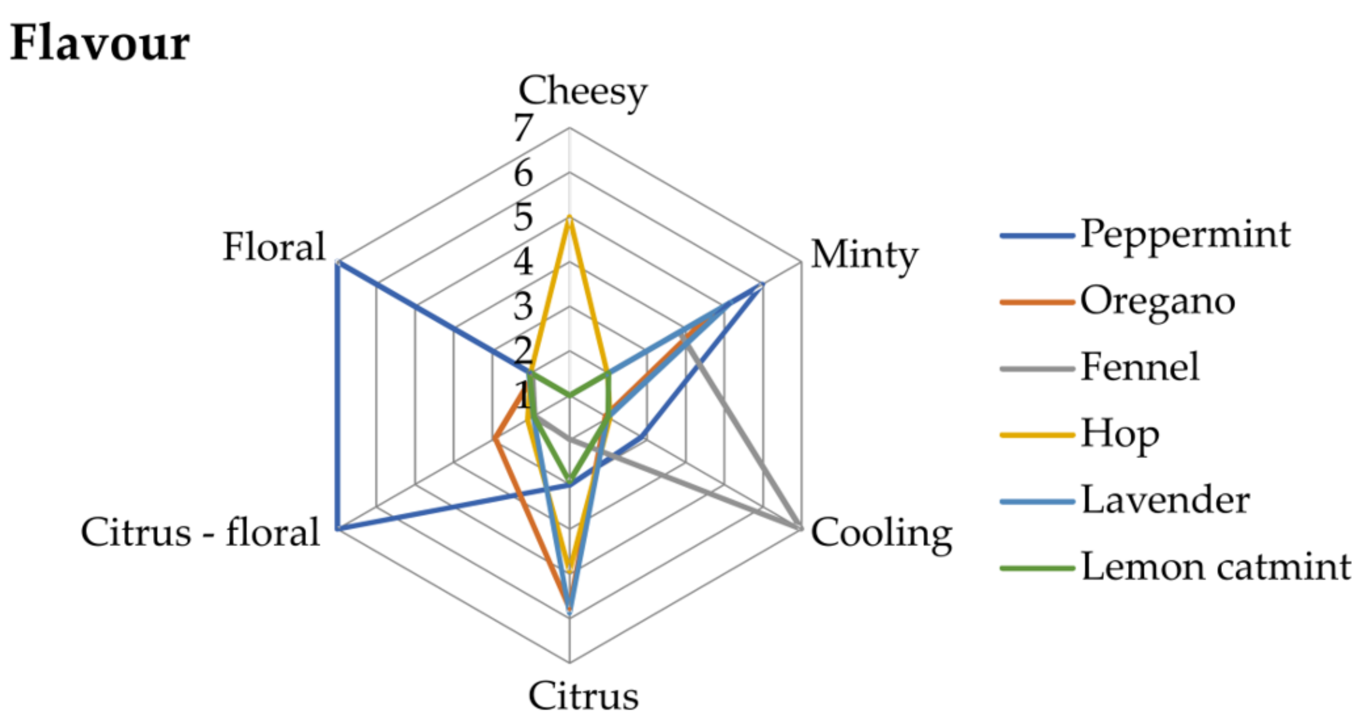

Figure 5

Results of the sensory analysis for flavour of the alfalfa microgreens using investigated hydrolates

Supplementary Files

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