Original research article

THE OCCURRENCE OF UNUSUAL VOLATILE COMPOUNDS IN THE HYDROLATE – A CASE STUDY OF *Cymbopogon citratus* (DC.) Stapf.

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SUMMARY

The hydrolate of *Cymbopogon citratus* (DC.) Stapf., (lemongrass), a by-product of the steam distillation process for essential oil production, has gained interest due to its aroma, biological activity, and potential applications. In this study, the volatile composition of *C. citratus* hydrolate was analyzed and compared to the essential oil and prior research findings. While the essential oil primarily contained citral (72.7%, a mixture of geranial and neral) and myrcene (17.4%), the hydrolate exhibited a significantly altered chemical profile. The dominant compounds in the hydrolate were *trans*-*p*-mentha-1(7)-8-dien-2-ol (27.9%), *p*-mentha-1,5-dien-8-ol (24.7%), and 6-methyl-5-hepten-2-one (12.6%). These results diverged from earlier studies, which showed a hydrolate composition similar to the essential oil. The observed changes may be attributed to citral biodegradation under acidic hydrolate conditions. Sensory analysis further revealed modifications in the hydrolate's aroma, with diminished citrus notes and increased earthy and minty odors. This study highlights the variability and unique characteristics of *C. citratus* hydrolate, emphasizing its potential as a valuable product in green technologies and the need for a qualitative definition of hydrolate.

KEYWORDS: biodegradation, essential oil, green technologies, hydrosol, lemongrass, sensory analysis

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INTRODUCTION

Cymbopogon citratus (DC.) Stapf., commonly known as lemongrass, is a species originally native to tropical and subtropical regions but is now successfully cultivated worldwide, including in Serbia (Aćimović et al., 2024). This perennial plant from the Poaceae family is a rich source of essential oils and polyphenolic compounds (Da Ressurreição et al., 2022; Muala et al., 2021), making it ideal for applications in medicine, cosmetics, food, and as a biopesticide (Aćimović et al., 2019; Aćimović et al., 2020; Gvozdenac et al., 2021). Furthermore, *C. citratus* contains a high concentration of carbohydrates and unsaturated fatty acids, positioning it as a promising feedstock for diesel fuel production (Rodrigues et al., 2022; Vellaiyan, 2023).

The essential oil extracted by steam distillation from the *C. citratus leaves* is a clear, pale yellow to orange-yellow, mobile liquid with a characteristic odor and a strong note of citral (ISO 3217). Due to its distinct aroma, *C. citratus* essential oil is a popular and valuable fragrance ingredient. It is frequently used as a lemon flavoring agent in herbal teas, delicacies such as ice cream and cookies, as well as a spice for soups, curries, poultry, fish, and seafood dishes (Aćimović et al., 2020). *C. citratus* essential oil exhibits a broad range of biological activities, including antimicrobial, antioxidant, anti-

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inflammatory, antihyperlipidemic, and anti-cancer effects (Naik et al., 2010; Onyedikachi et al., 2021; Yen, 2016; Santhosh Kumar et al., 2011). These properties make it a valuable raw material for the pharmaceutical industry and a useful functional food supplement. Furthermore, *C. citratus* has anxiolytic-like properties, making it a popular choice in aromatherapy, cosmetic and household products, as well as perfumery (Alvarado-García et al., 2023). In addition, *C. citratus* essential oil exhibits repellent and insecticidal properties, highlighting its strong potential for the development of natural-based insecticides (Gvozdenac et al., 2021; Johnson et al., 2021).

C. citratus hydrolate (hydrosol) created as a by-product during distillation process shares a similar aroma to the corresponding essential oil (Aćimović et al., 2024). The chemical composition, biological activity, and potential applications of *C. citratus* hydrolate remain relatively understudied, and only several investigations have explored this topic (Benoudjit et al., 2022; Rodrigues et al., 2022; Morales-Aranibar et al., 2023; Erceg et al., 2024; Aćimović et al., 2024). These studies collectively reveal that the hydrolate features a fresh, lemon-like scent dominated by citral and exhibits promising antimicrobial activity, making it suitable for applications in organic agriculture and the food industry.

This study aims to highlight the unusual volatile profile of *C. citratus* hydrolate, a by-product of essential oil production of standard quality, and to compare it with findings from three years of research on the same plantation.

MATERIAL AND METHODS

C. citratus (BUNS voucher specimens No 2-1406) was cultivated in a greenhouse in the village of Banatska Topola (45°40′18.25″ N, 20°27′ 53.91″ E), located in the Vojvodina province of Republic of Serbia. Further details can be found in a previous study (Aćimović et al., 2024). Briefly, the harvested plant material (fresh leaves cut 10 cm above the soil) was dried using a solar dryer and subsequently processed through steam distillation at the Department of Vegetable and Alternative Crops in Bački Petrovac, part of the Institute of Field and Vegetable Crops in Novi Sad. The semi-industrial steam distillation device, handmade in 1984, is made from parts sourced from local manufacturers (ex-Yugoslavia: Ventilator Zagreb, Inox Bački Petrovac, Iskra Poreč). In brief, steam produced by a high-pressure boiler passes through the plant material in a stainless-steel distillation vessel, carrying the volatile compounds in vapor form to a condenser and cooler, where they are collected in a Florentine vessel as essential oil and hydrolate. After 4 hours of steam distillation, the obtained essential oil was decanted and left in a separation funnel overnight, while the hydrolate was filtered and stored in plastic containers. The essential oil was further dehydrated by Na_2SO_4, filtered, and stored in dark glass bottles.

The hydrolate was decanted through filter paper into sterile plastic bottles as a clear, colorless liquid with a pH of 4.4. Before analysis of volatile profile, the hydrolate was processed using the Likens-Nickerson simultaneous distillation and extraction method. The obtained secondary essential oil was stored in vials until further analysis. The identification and quantification of volatile compounds in the essential oil and hydrolate (secondary essential oil) were carried out using gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques (Agilent 7890A GC, HP-5MS, FID, and Agilent 5973 MSD). Identification was conducted using retention indices (RI) relative to C_8-C_{32} *n*-alkanes and compared with the Adams4 and NIST17 databases (Aćimović et al., 2024). The amount of each compound is determined by the area under its peak in the gas chromatogram.

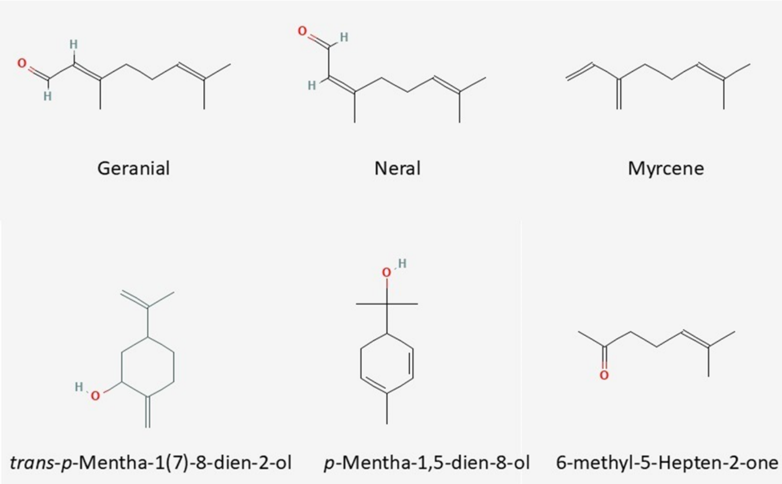
Finally, a sensory evaluation was conducted to assess the aroma of *C. citratus* essential oil and hydrolate samples as previously described by Kiprovski et al. (2023) and Kumar et al. (2023). Briefly, a ten-member expert panel conducted a sensory evaluation of the samples in a dedicated laboratory. Descriptive analysis focused on fifteen aroma attributes: green, herbal, citrus, spicy, minty, terpenic, floral, earthy, phenolic, caramelic, tropical, roasted, cooling, woody, and waxy. Essential oils and hydrolates were assessed in triplicate using a 10-point interval scale (0 = none, 9 = extra strong). Odor detection involved dipping one centimeter of smelling strips into each sample. Panelists took three quick, deep sniffs from the strip, which was then removed. Assessments were conducted with 20-second intervals between samples and with clean air breaks between each.

RESULTS AND DISCUSSION

The main compounds in the *C. citratus* essential oil were citral with 72.7% (i.e. mixture of geranial and neral, 40.8% and 31.9%, respectively), and myrcene (17.4%), Table 1. Similar, in a previous study, we identified geranial (34.7–40.7%), neral (27.1–32.3%), and myrcene (15.6–25.5%) as the dominant compounds in the essential oil. However, in the hydrolate the dominant compounds were *trans*-*p*-mentha-1(7)-8-dien-2-ol (27.9%), *p*-mentha-1,5-dien-8-ol (24.7%), and 6-methyl-5-hepten-2-one (12.6%). Whereas, the hydrolate of our previous study was very similar to essential oil, with the geranial (26.5–32.6%), neral (18.4–26.1%), and 6-methyl-5-hepten-2-one (17.6–23.7%) as predominant compounds (Aćimović et al., 2024). Chemical structures of main volatile compounds from *C. citratus* essential oil and hydrolate are given in Picture 1. Given that the previous study reflects a three-year average, the significantly different composition of the hydrolate in this case study is possible due to the degradation of certain components typically found in the oil or hydrolate distilled species (Park et al., 2015). Therefore, it is very important to consider all the factors that determine the quality of *C. citratus* hydrolate.

This second assumption appears more reasonable, as the main compounds in C. giganteus essential oil from South Africa were trans-p-mentha-1(7)-8-dien-2-ol (23.7%), trans-p-mentha-2,8-dienol (17.9%), cis-p-mentha-1(7)-8-dien-2-ol (16.8%), and limonene (13.8%), Piasecki et al., 2021. Additionally, the primary compounds identified in the fresh leaves essential oil of wild C. giganteus from India were p-mentha-1(7)-8-dien-2-ol (25.89%), trans-p-mentha-2,8-dienol (13.39%), and limonene (9.66%), Majeed et al., 2021. Furthermore, the aroma profile of C. giganteus fresh leaves essential oil from Cameroon is characterized by an intense fresh, green-herbal, spicy, and mildly floral scent (Jirovetz et al., 2007). This is primarily attributed to the high concentration of compounds with a p-menthadiene structure, including cis-p-mentha-1(7),8-dien-2-ol (27.7%), trans-p-mentha-1(7)-8-dien-2-ol (21.6%), and trans-p-mentha-2,8-dien-1-ol (22.1%).

Citral is highly volatile, with an evaporation range of 50°C to 165°C, and is relatively unstable in water (Aytac et al., 2018). Given that hydrolates are acidic liquids with a pH between 4.5 and 5.5 (Aćimović et al., 2020) and that citral rapidly decomposes through cyclization and oxidation reactions during storage under acidic conditions (Park et al., 2015), the presence of compounds with a p-menthadiene structure in *C. citratus* hydrolate may result from biodegradation.



Picture 1. Chemical structures of main volatile compounds from *C. citratus* essential oil and hydrolate

Table 1. A case study on the relative chemical composition (%) of *Cymbopogon citratus* essential oil (EO) and hydrolate (HY), compared to mean value of results from Aćimović et al. (2024)

Rt (min)	Compound	RI$_{lit}$	RI$_{exp}$	This study		Aćimović et al. 2024		Odor*
				EO	HY	EO	HY	
3.785	2,2-dimethyl-3(2H)-Furanone	834	828	nd	0.5±0.3	nd	0.4±0.00	-
3.785	3Z-Hexenol	850	848	nd	0.2±0.00	nd	0.4±0.02	green
6.281	5,5-dimethyl-2(5H)-Furanone	952	948	nd	0.1±0.00	nd	tr	-
6.830	2-ethenyltetrahydro-2,6,6-trimethyl-2H-Pyran	971	967	nd	0.1±0.01	nd	nd	herbal
7.293	6-methyl-5-Hepten-2-one	986	983	2.5±0.04	12.6±2.10	3.0±0.08	20.7±2.23	citrus
7.420	Myrcene	988	988	17.4±0.13	nd	21.2±1.34	nd	spicy
7.434	dehydro-1,8-Cineole	995	989	nd	6.2±0.05	nd	3.9±0.31	minty
8.549	*o*-Cymene	1022	1022	nd	0.6±0.01	nd	nd	-
8.586	*p*-Cymene	1020	1023	0.1±0.00	nd	nd	0.1±0.01	terpenic
8.788	1,8-Cineole	1026	1028	nd	0.1±0.01	nd	0.1±0.01	herbal
9.037	*cis*-*β*-Ocimene	1032	1035	0.1±0.02	nd	0.3±0.01	0.1±0.01	floral
9.238	Benzene acetaldehyde	1036	1040	nd	0.3±0.00	nd	0.2±0.01	green
9.428	*trans*-*β*-Ocimene	1044	1046	0.1±0.01	nd	0.2±0.01	nd	floral
10.306	*cis*-Linalool oxide (furanoid)	1067	1070	nd	1.6±0.03	nd	0.7±0.04	earthy
10.922	*trans*-Linalool oxide (furanoid)	1084	1085	nd	1.4±0.01	nd	0.6±0.04	-
10.972	*p*-Cymenene	1089	1087	nd	0.2±0.00	nd	nd	phenolic
11.100	3-methyl-1,2-Cyclohexanedione	1085	1088	nd	0.1±0.01	nd	nd	caramellic
11.379	Linalool	1095	1098	1.0±0.02	2.9±0.05	0.9±0.06	2.5±0.12	floral
11.458	Perillene	1002	1099	0.3±0.00	nd	0.3±0.03	nd	-
11.578	Hotrienol	1109	1103	nd	0.1±0.00	nd	nd	tropical
11.887	1,3,8-*p*-Menthatriene	1108	1110	nd	0.4±0.01	nd	nd	roasted
12.221	*trans*-*p*-Mentha-2,8-dienol	1118	1118	nd	0.3±0.01	nd	0.2±0.02	minty
12.837	*p*-Mentha-1,5,8-triene	1119	1133	nd	0.7±0.01	nd	nd	minty
13.641	Citronellal	1148	1151	0.2±0.00	nd	0.3±0.03	nd	floral
13.840	*trans*-2-Caren-4-ol	1153	1156	nd	0.7±0.01	nd	nd	-
14.163	*cis*-Isocitral	1165	1163	0.3±0.02	nd	0.8±0.05	nd	green
14.331	*p*-Mentha-1,5-dien-8-ol	1166	1167	nd	24.7±1.65	nd	1.9±0.03	-
14.542	*trans*-Linalool oxide (pyranoid)	1173	1172	nd	0.1±0.00	nd	nd	cooling
14.598	Rosefuran epoxide	1173	1173	0.2±0.00	nd	0.1±0.01	nd	green
14.912	*trans*-Isocitral	1177	1181	0.6±0.01	nd	1.2±0.08	0.1±0.00	-
15.093	*p*-Cymen-8-ol	1179	1184	nd	4.2±0.05	nd	0.3±0.01	-
15.454	*trans*-*p*-Mentha-1(7)-8-dien-2-ol	1187	1193	nd	27.9±1.5	nd	nd	-
16.504	Carveol	1215	1217	nd	0.2±0.01	nd	nd	minty
16.941	Citronellol	1223	1227	0.4±0.02	nd	0.3±0.03	0.2±0.02	floral
17.483	Neral	1227	1241	31.9±2.5	0.6±0.03	29.4±1.11	21.5±1.20	citrus
18.081	Geraniol	1249	1253	nd	2.2±0.00	2.6±0.11	1.8±0.12	floral
18.084	Linalool acetate	1254	1253	2.6±0.01	nd	nd	nd	herbal
18.802	Geranial	1270	1271	40.8±2.13	1.3±0.01	37.0±1.95	30.1±1.60	citrus
20.221	Carvacrol	1298	1301	0.2±0.01	nd	nd	nd	spicy
23.820	Geranyl acetate	1379	1383	0.3±0.01	nd	0.4±0.02	nd	floral
25.336	*trans*-Caryophyllene	1408	1417	0.1±0.00	nd	0.2±0.01	nd	spicy
26.024	*trans*-*α*-Bergamotene	1432	1433	0.1±0.00	nd	0.2±0.01	nd	woody
28.546	2-Tridecanone	1497	1496	0.1±0.00	nd	0.1±0.01	nd	waxy
32.098	Caryophyllene oxide	1582	1580	0.1±0.00	nd	0.1±0.00	nd	woody

Rt – retention time; RI – retention index; nd – not detected;
*according to The Good Scents Company (http://www.thegoodscentscompany.com/index.html assessed 13/12/2024)

The variation in chemical composition had a notable impact on the aroma profile of the hydrolate. The average of the odor description intensities was plotted on a spider diagram in Figure 1. All evaluators identified citrus as the predominant odor in the essential oil and hydrolate of C. citratus. When comparing the scent of the essential oil in this study to a mean value of results from Aćimović et al. (2024), no differences were noted. However, the hydrolate from this study exhibited a significantly weaker citrus intensity. Green and floral scents were moderately significant and consistent across all four samples, while earthy scents showed some variability. Variations in spicy and minty aromas were attributed to differences in the chemical composition of the volatile components. Other aromas, such as phenolic, woody, and caramel-like notes, were minimal but consistently present across the observed samples.

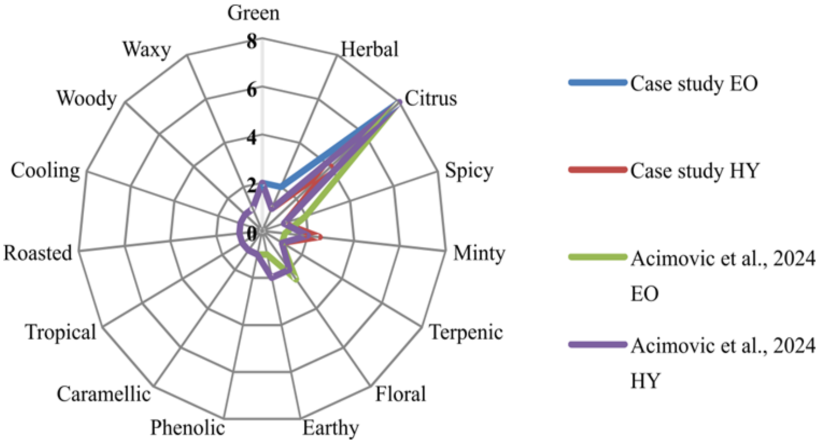


Figure 1. Results of the sensory analysis of the *C. citratus* essential oil and hydrolates for this study and mean value of results from Aćimović et al. (2024)

CONCLUSIONS

Hydrolates, which are often discarded as waste, can actually be valuable by-products of the essential oil distillation process. As a result, they are receiving growing attention in light of the increasing focus on green and zero-waste technologies. These high-value products are now gaining interest from both the scientific community and manufacturers. Given the absence of a well-established system for hydrolate quality, this research is particularly significant, as it presents results that differ from those of a long-term study conducted with plant material from the same plot, while also being similar to other studies. The essential oil produced in this study met standard quality, containing 72.7% citral, while the hydrolate was predominantly made up of compounds with a *p*-menthadiene structure. This unusual volatile composition may be the result of citral biodegradation in the acidic conditions of the hydrolate. Furthermore, these compounds alter the aroma profile of the hydrolate, giving it a more subdued aroma. The results of sensory analysis suggest a strong dominance of citrus odor with notable variations in other key odors (green, floral, spicy, and minty).

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SAŽETAK

POJAVA NEUOBIČAJENIH ISPARLJIVIH JEDINJENJA U HIDROLATU – STUDIJA SLUČAJA *Cymbopogon citratus* (DC.) Stapf.

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Interesovanje za hidrolat *Cymbopogon citratus* (DC.) Stapf. (limunska trava), nusproizvod koji nastaje tokom destilacije etarskog ulja vodenom parom, poraslo je tokom poslednjih godina, pre svega zbog prijatne citrusne arome, ali i biološke aktivnosti, kao i potencijalne primene. U ovoj studiji analizirane su isparljive komponente hidrolata *C. citratus* i upoređene sa etarskim uljem, kao i sa prethodnom studijom. Dok je etarsko ulje prvenstveno sadržalo citral (72,7%, mešavina geranijala i nerala) i mircen (17,4%), hidrolat je pokazao značajno izmenjen hemijski profil. Dominantne komponente u hidrolatu bile su *trans*-*p*-menta-1(7)-8-dien-2-ol (27,9%), *p*-menta-1,5-dien-8-ol (24,7%) i 6-metil-5-hepten-2-on (12,6%). Ovi rezultati odstupali su od prethodne studije, koja je pokazala da je sastav hidrolata sličan etarskom ulju. Uočene promene mogu se pripisati biorazgradnji citrala u uslovima kisele sredine ili rezidualnim isparljivim komponentama iz prethodno destilisanih biljnih vrsta. Senzorska analiza dalje je otkrila modifikacije u aromi hidrolata, sa smanjenim notama citrusa i pojačanim mirisima zemlje i mente. Ova studija naglašava varijabilnost i jedinstvene karakteristike hidrolata *C. citratus*, ističući njegov potencijal kao vrednog proizvoda u zelenim tehnologijama i potrebu za definisanjem kvalitativnog sastava hidrolata ove vrste.

Ključne reči: biodegradacija, etarsko ulje, zelene tehnologije, hidrosol, limunska trava, senzorska analiza

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