Original article UDC: 615.322:582.943.15]:543.51
doi: 10.5633/amm.2024.0304

ORIGANUM VULGARE L.: CHEMICAL PROFILE OF THE EXTRACTED VOLATILE COMPOUNDS AND ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF HYDROLAT

Andjela Dragićević[1], Dušanka Kitić[1], Jelena Matejić[1], Ljiljana Stanojević[2], Jelena Stanojević[2], Dragan Cvetković[2], Dragana Pavlović[1]

Hydrolates or floral waters are the outcomes of the hydrodistillation of aromatic plants. The production of hydrolates is simple and affordable because they are byproducts of the essential oil. The composition and biological activities of hydrolates may differ from those of the corresponding essential oils. The main objective of the study was to assess the chemical profile of the volatiles extracted from the hydrolate obtained from the aerial part of *Origanum vulgare* L., but also to evaluate the anti-inflammatory and antioxidant activity of the hydrolate obtained from the aerial part of *Origanum vulgare*. Qualitative and quantitative analyses of the extracted volatiles, performed using gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization detection (GC/FID), showed that the main components were terpinen-4-ol (36%) and 1-octen-3-ol (33.6%). At all concentrations tested, the hydrolate scavenged 1,1-diphenyl-2 picrylhydrazyl (DPPH) radicals in a way that depended on concentration and showed antioxidant activity in the *β*-carotene/linolenic acid assay. The total antioxidant capacity of oregano hydrolat was calculated using Ferric Reducing Antioxidant Power Assay (FRAP assay), which resulted in a FRAP value of 0.361 ± 0.015 μmol Fe^{2+}/ml. In addition to antioxidant activity, satisfactory anti-inflammatory activity was also observed with the percentage inhibition of BSA denaturation of 71.2 ± 0.006%. Demonstrated antioxidant and anti-inflammatory properties of *O. vulgare* hydrolate may be crucial to its future and use in many industrial fields.

Acta Medica Medianae 2024;63(3):32–41.

Key words: *gas chromatography/mass spectrometry, gas chromatography/flame ionization detection, oregano, hydrosol, terpinen-4-ol, 1-octen-3-ol*

[1]University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
[2]University of Niš, Faculty of Technology, Leskovac, Serbia

Contact: Andjela Dragićević
20/8 Rudnička St., 18000 Niš, Serbia
E-mail: dragicevic.andjela@gmail.com

Introduction

Hydrolates or floral waters are acquired during the essential oil extraction procedure from aromatic plants. According to an international definition, a hydrolate is the distilled aromatic water that is left over after the essential oil has been separated and hydro-distilled or steam-distilled (1). They are made up of volatile oil components that are hydrophilic, polar and oxygenated, and form hydrogen bonds with water and condensed water during the distillation process (2). During the distillation of fragrant plants, it was found that some components of the essential oils were lost to the water (3).

Hydrolates are used as flavorings and refreshing drinks in traditional medicine in Mediterranean countries. Compared to essential oils, hydrolates are simpler and less expensive to make, and they seem to be less harmful to human health (4). Hydrolates produced in the early and late stages of distillation have different chemical compositions and olfactory notes. This can be attributed to the presence of terpenoids with high and low boiling points in them. In addition, the aromatic profile of hydrolates can differ significantly from that of the corresponding essential oils, as they lack hydrophobic, water-insoluble isoprenoid molecules (hydrocarbons). The production of hydrolates is simple and affordable since hydrolates are byproducts of the essential oil industry. The composition and therapeutic capabilities of hydrolates made from the same plant parts in various countries or areas within a country, during various seasons, at various development stages, or under various management approaches, may vary (5).

Origanum vulgare L., oregano, is a perennial herbaceous plant from the family Lamiaceae. It has spikes of white, purple, or pink flowers and dark oval, aromatic leaves. *O. vulgare* is often referred to as the "prince of herbs" and is a well-known aromatic and medicinal plant (6). The name means "joy of the mountains" and is derived from the Greek terms for mountain (oros) and joy (ganos). The ancient Greek goddess Aphrodite treasured oregano, which was once thought to bring good luck. Oregano was used in ancient Egypt as an antidote and as a preservative. The Greeks utilized the aerial part of *O. vulgare* both topically and orally to treat dropsy, convulsions, and skin irritations and infections. It was also a highly effective remedy against poisons (7). The medicinal parts are the aerial part of plant, harvested during the flowering season, dried, the fresh flowering herb, and the essential oil extracted from fresh or dried leaves (8). *O. vulgare* is used in folk medicine to treat a variety of conditions, including rheumatoid arthritis, dyspepsia, painful menstruation, coughing, irritation of the bronchial mucous membranes, urinary tract infections, and diaphoresis (9). The essential oil of *O. vulgare* is also widely used. It is made up of a combination of terpenoid components with antioxidant, antibacterial, antifungal, antiviral, antihyperglycemic, anti-inflammatory and antimutagenic properties (10, 11).

Among the scientific community, hydrolates have long been considered waste materials from hydro- or steam-distillation. Given the sustainability and added value of this by-product of the essential oil industry, there is growing interest in hydrolates, particularly in their composition and biological properties.

Aim

The aim of this study was: (1) determination of the chemical profile of the extracted volatiles from the hydrolate remaining after hydrodistillation of the aerial parts of *O. vulgare*, (2) determination of the antioxidant capacity of the hydrolate of the areal parts of *O. vulgare* using three different assays: DPPH assay, *β*-carotene bleaching assay and FRAP assay, and (3) determination of the anti-inflammatory activity of the hydrolate from the areal parts of *O. vulgare* by *in vitro* protein denaturation assay.

Material and Methods

Plant Material and Chemicals

The hydrolate of the aerial part of *O. vulgare* was obtained through industrial production by the company "PROMONTIS Production", Vilandrica, Gadžin Han. After the isolation of the hydrolate by industrial hydrodistillation, the isolation of volatile compounds from the hydrolate followed the procedure reported by Maciąg and Kalemba by liquid-liquid extraction with diethyl ether (12). The qualitative and quantitative composition of isolated volatiles was examined using GC/MS and GC/FID.

All chemicals used were obtained from Sigma Aldrich (USA), or Zorka Pharma (Šabac, Serbia). All solvents and chemicals were of analytical grade.

Determination of the Chemical Profile of Extracted Volatiles of Oregano Hydrolate

Qualitative and quantitative analyses of the extracted volatiles of oregano hydrolate were performed using GC/MS and GC/FID. An Agilent Technologies 7890B gas chromatograph, fitted with a non-polar silica capillary column for HP-5MS (5% diphenyl and 95% dimethyl polysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA), was used to perform the GC/MS analysis of the extracted volatiles of oregano hydrolate. The column was coupled to an inert, selective 5977 A mass detector manufactured by the same company. The flow rate of the carrier gas, helium, was 1 cm^3/min. A split inlet set to 250 °C in 10:1 split mode was used to introduce one microliter of the prepared diethyl ether solution into the column. The mass spectra were obtained in the 25–550 m/z region in EI mode (70 eV). For the GC/FID analysis, identical analytical parameters were utilized. The corresponding fluxes for the fuel gas (H_2), oxidizing gas (Air), make-up gas (N_2), and carrier gas (He) were 1, 25, 30, and 400 cm^3/min. The flame-ionization detector (FID) had its temperature adjusted to 300 °C.

The MSD ChemStation, AMDIS_32, and MassHunter Qualitative Analysis software (Agilent Technologies, USA) were utilized for data processing. Using a homologous series of n-alkanes from C8–C20 as standards, the retention indices of the constituents from the investigated sample were experimentally calculated. The process of identifying each component involved comparing retention times, their retention indices (RIexp) with literature-available values (13), and their EI mass spectra with authentic standards and mass spectra libraries from RTLPEST 3, NIST 2011, and Willey 6.

Determination of Antioxidant Capacity

DPPH assay

The antioxidant activity of the hydrolate of the aerial part of *O. vulgare* was assessed using 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay. The color changed from violet to yellow when DPPH was reduced to 2,2-diphenyl-1-picrylhydrazine (DPPHH), and an ELISA microplate reader was used to measure it at 540 nm (14). The assay was performed according to Pavlović et al. by incubating different concentrations of hydrolate (20−70% v/v) with DPPH in 96% (v/v) ethanol solution for 30 minutes at room temperature and in the dark (15). The distilled water is present in the blank sample. As a control, 96% (v/v) ethanol containing DPPH was used. Synthetic antioxidants BHT and BHA were used as the reference compounds. The following formula was used to determine the percentage of DPPH free radical inhibition: % DPPH= $(A_c - A_s) / A_c \times 100$ where A_c is the absorbance of the control, and As is the absorbance of the sample.

β-carotene Bleaching Assay

The *β*-carotene bleaching method assesses the capacity of various components to impede the process of lipid peroxidation. Radicals generated by the oxidation of linoleic acid in the assay oxidize *β*-carotene, leading to the loss of the chromophore of the system and distinctive orange color, which is measured spectrophotometrically at 450 nm (16). By Pavlović et al., 200 mg of Tween-20 and 25 µl of linoleic acid were combined with 1 ml of *β*-carotene solution in chloroform (1 mg/5 ml), and that mixture was allowed to evaporate under vacuum at a temperature as high as 40 °C. An emulsion formed as a result of shaking the mixture after 50 ml of distilled water was added. A freshly made *β*-carotene linoleic acid emulsion was added to the sample on a 96-well microtitration plate. Three duplicates of each concentration (1.11–11.1% v/v) were made for testing. The plate was read in a microplate reader immediately (t = 0 min) and after 120 minutes of incubation at 55 °C (t = 120 min) (17). Formula (18) was utilized to determine the percentage (%) inhibition of samples against *β*-carotene bleaching: % inhibition = $100 - (A_{120} / A_0) \times 100$, where A_{120} is the absorbance of the sample at t = 120 min and A_0 is the absorbance of the sample at t = 0 min. Synthetic antioxidants BHT and BHA were used as the reference compounds.

FRAP Assay

The ability of the test sample to reduce iron(III) tripyridyltriazine (Fe^{3+}-TPTZ) at low pH to an intense blue colored iron(II) tripyridyltriazine complex (Fe^{2+}-TPTZ) is the basis for the FRAP method used to estimate the total reduction potential of the hydrolate of the aerial part of *O. vulgare* (19). According to Pellegrini et al., the FRAP reagent was freshly prepared and consisted of the following ingredients: 10 mmol/l TPTZ in 40 mmol/l HCl, sodium acetate buffer (300 mmol/l, pH 3.6) and $FeCl_3 \times 6H_2O$ solution (20 mmol/l), each in a ratio of 10:1:1 (v/v/v). After adding 3000 µl FRAP reagent to 100 µl hydrolate, the absorbance was measured at 593 nm and compared after 5 minutes with the blank sample which consisted of 100 µl distilled water and 3000 µl FRAP reagent (20). For the construction of the calibration curve, six concentrations of $FeSO_4 \times 7H_2O$ (100, 200, 400, 600, 800 and 1000 mmol/l) were used. The resulting FRAP value is presented as µmol ferric iron reduced per ml of sample.

Anti-Inflammatory Activity

The protein denaturation assay was performed with a 5% w/v aqueous solution of BSA (bovine serum albumin) according to Lavanya et al. (21). The test solution is an aqueous solution of hydrolate and bovine serum albumin with a weight percentage of 5% w/v. An aqueous solution of distilled water and bovine serum albumin at a concentration of 5% w/v served as the control. The 5% w/v aqueous solution of diclofenac sodium and bovine serum albumin served as the standard solution against which the findings were evaluated. Using 1N HCl, the pH of each of the aforementioned solutions was brought to 6.3. The samples underwent a 20-minute incubation period at 37 °C, after which they were heated to 57 °C for three minutes. Phosphate buffer was added to the aforementioned solutions after chilling. An ELISA microplate reader was used to measure the absorbance at 340 nm. The following formula was used to determine the inhibition percentage of protein denaturation:

Protein denaturation (%) = 100 - ((optical density of test solution—optical density of product)/optical density of test control) x 100). The control represents 100% protein denaturation. The results were compared with diclofenac (100 µg/ml).

Results

Determination of the Chemical Profile of Extracted Volatiles of Oregano Hydrolate

The percentage composition of the extracted volatiles obtained as well as the main classes of the identified constituents is shown in Figure 1 and Table 1. Sixteen compounds were identified in the extracted volatiles of oregano hydrolate. Terpenes represented the most abundant compound class (59.4%): terpinen-4-ol, 1,8-cineole, *α*-terpinene, γ-terpinene, terpinolene, *trans*-linalool oxide (furanoid), linalool, cis-linalool oxide (furanoid) and *α*-terpineol. According to the analysis, alcohols (3-methyl-1-butanol, 2-methyl-1-butanol, 3-(*cis*)-hexenol, 1-octen-3-ol, and 3-octanol) make up 40.6% of the total. Traces of aromatic components were detected.

In the extracted volatiles of oregano hydrolate isolated from aerial parts, the main components were terpinen-4-ol (36%) and 1-octen-3-ol (33.6%).

Determination of the Ability to Neutralize Free Radicals by the DPPH Test

According to DPPH test, although the tested sample possesses anti-radical activity, none of the tested concentrations of oregano hydrolate failed to reach the IC_{50}, Table 2. The range of free radical neutralizing ability was from 27.81 ± 0.002% (at lowest concentration) to 35.82 ± 0.002% (at high concentration). To compare antiradical activity, the ability of commercial synthetic antioxidants, BHT and BHA, to remove free radicals was also studied. Under the same conditions under which the different concentrations of hydrolate were tested, IC_{50} values for BHT and BHA were: 22.82 ± 2.07 μg/ml and 2.44 ± 0.09 μg/ml, respectively.

Table 1. Chemical composition of the extracted volatiles of oregano hydrolate

No.	t_{ret}, min	Compound	RI^{exp}	RI^{lit}	Method of identification	Relatieve amount, %
1.	4.88	3-Methyl-1-butanol	732	731	RI, MS	tr
2.	4.95	2-Methyl-1-butanol	734	724	RI, MS	tr
3.	7.53	3-(*cis*)-Hexenol	849	850	RI, MS	4.5
4.	**10.45**	**1-Octen-3-ol**	**976**	**974**	**RI, MS**	**33.6**
5.	10.78	3-Octanol	990	988	RI, MS	2.5
6.	11.13	*α*-Terpinene	1006	1014	RI, MS	tr
7.	11.37	*p*-Cymene	1016	1020	RI, MS	tr
8.	11.45	1,8-Cineole	1020	1026	RI, MS	7.8
9.	11.92	Phenylacetaldehyde	1041	1036	RI, MS	tr
10.	12.11	*γ*-Terpinene	1049	1054	RI, MS	tr
11.	12.48	*cis*-Linalool oxide (furanoid)	1066	1067	RI, MS	tr
12.	12.76	Terpinolene	1079	1086	RI, MS	tr
13.	12.83	*trans*-Linalool oxide (furanoid)	1082	1084	RI, MS	tr
14.	13.16	Linalool	1096	1095	RI, MS	7.6
15.	**14.91**	**Terpinen-4-ol**	**1180**	**1174**	**RI, MS**	**36.0**
16.	15.26	*α*-Terpineol	1196	1186	RI, MS	8.0
Grouped compounds (%)				Total identified		100
Alcohols (1–5)				40.6		
Terpenes (6, 8, 10–16)				59.4		
Aromatic compounds (7, 9)				tr		

[1] t^{ret}: Retention time; RIlit: Retention indices from literature (Adams, 2007); RI^{exp}: Experimentally determined retention indices using a homologous series of n-alkanes (C8-C20) on the HP-5MS column; MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching; tr: trace amount (< 0.05%).

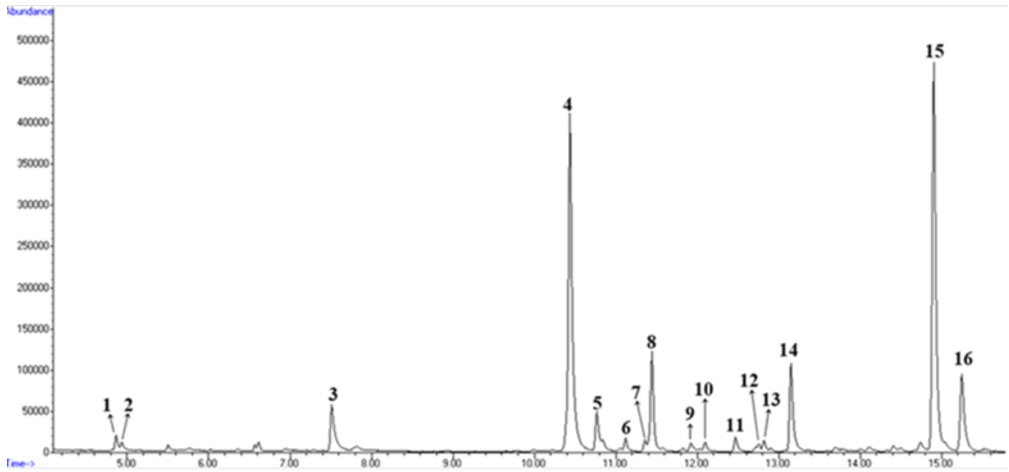

Figure 1. GC/FID chromatogram of the extracted volatiles of oregano hydrolate

Table 2. *In vitro* antioxidant and anti-inflammatory activity of *O. vulgare* hydrolate

Concentration of hydrolate in DPPH assay (% v/v)	Results of DPPH assay (%)	Concentration of hydrolate in β-carotene bleaching assay (% v/v)	Results of β-carotene bleaching assay (%)	Concentration of hydrolate in FRAP assay (% v/v)	Result of FRAP assay (μmol Fe^{2+}/ml)	Concentration of hydrolate in BSA assay (% v/v)	Result of BSA assay (%)
70	35.82 ± 0.002	11.1	46.62 ± 0.023	100	361 ± 0.015	100	71.2±0.006
60	32.2 ± 0.008	8.3	38.67 ± 0.026				
50	31.37 ± 0.015	5.56	36.72 ± 0.045				
30	30.12 ± 0.001	2.78	17.06 ± 0.03				
20	27.81 ± 0.002	1.11	6.46 ± 0.07				
BHT (IC_{50})	22.82 ± 2.07 μg/ml	BHT (IC_{50})	0.03 ± 0.00 μg/ml	/	/	Diclofenac	95.6±0.001
BHA (IC_{50})	2.44 ± 0.09 μg/ml	BHA (IC_{50})	0.04 ± 0.01 μg/ml	/	/		

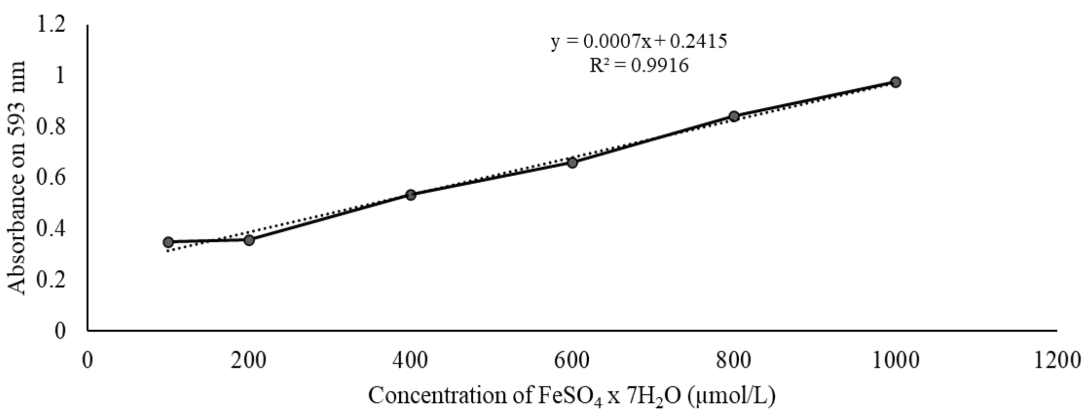

Figure 2. The standard curve obtained using ferrous sulfate solutions (100−1000 μmol/l)

Determination of the Ability to Inhibit β-carotene Bleaching

The results of the determination of β-carotene discoloration in the β-carotene/linoleic acid system as a function of hydrolate concentration expressed in percentage inhibition are presented in Table 2. In the current study hydrolate of *O. vulgare* showed mean inhibition of protein denaturation of 46.62 ± 0.023, 38.67 ± 0.026, 36.72 ± 0.045, 17.06 ± 0.03 and 6.46 ± 0.07% for doses of 11.1, 8.3, 5.56, 2.78 and 1.11% (v/v), respectively. Under the same conditions under which the different concentrations of hydrolate were tested, synthetic antioxidants showed activity in disrupting the chain reaction of lipid peroxidation: IC_{50} values were 0.03 ± 0.00 µg/ml (BHT) and 0.04 ± 0.01 µg/ml (BHA).

Determination of the Total Antioxidant Potential by the FRAP Method

The standard curve constructed by using ferrous sulfate solutions of known concentrations (Fe^{2+} of 100−1000 mmol/l) (Figure 2) was used to calculate the antioxidant potential: $y = 0.0007x + 0.2415$; $R^2 = 0.9916$.

The results determining the total antioxidant potential of oregano hydrolate showed that the FRAP value was 0.361 ± 0.015 µmol Fe^{2+}/ml (Table 2).

Anti-Inflammatory Activity

The percentage of BSA denaturation inhibition of 71.2 ± 0.006% (Table 2), which was lower than the standard value for diclofenac (95.6 ± 0.001%), indicated a considerable anti-inflammatory effect from *O. vulgare* hydrolate.

Discussion

Based on GC/MS and GC/FID, the main compounds of the volatiles extracted from the hydrolates obtained from the aerial part of *O. vulgare* are terpenes (59.4%) and alcohols (40.6%). The most abundant terpenes among the extracted volatile compounds from the hydrolate were terpinen-4-ol (36%), 1,8-cineole (7.8%) and linalool (7.6%); while the most abundant alcohols were 1-octen-3-ol (33.6%), 3-(*cis*)-hexenol (4.5%) and 3-octanol (2.5%). In addition, terpinen-4-ol and 1-octen-3-ol were the most abundant volatiles extracted from the oregano hydrolate. The primary bioactive ingredient in a range of aromatic plants is terpinen-4-ol, a naturally occurring monoterpene (22). Khan et al. found similar results after analyzing the volatile components of hydrolate *O. vulgare* from Saudi Arabia and determining that the primary chemicals were terpinen-4-ol and carvacrol (23). According to the results of the current study, 1-octen-3-ol was also one of the primary components of the volatiles extracted from the hydrolates of *O. vulgare*, accounting for 33.6%. Known as mushroom alcohol, 1-octen-3-ol was isolated from a variety of plants and fungi (24).

As far as we know, there has not been much information on antioxidant properties of *O. vulgare* hydrolate published in the literature. Three complimentary test systems—DPPH free radical scavenging, lipid peroxidation inhibition, and total antioxidant capacity (FRAP) were used to measure antioxidant activity. In the concentration range we tested, *O. vulgare* hydrolate was able to scavenge DPPH radicals and showed concentration-dependent antioxidant activity in the β-carotene/linolenic acid assay. Additionally, a reducing effect on iron(III) ions was observed. It is assumed that terpinen-4-ol is responsible for the observed antioxidant effect, which, at 36%, is the most abundant among the extracted volatile compounds of oregano hydrolates isolated from aerial parts. In the study conducted by Aslam et al., terpinen-4–ol exhibited a DPPH radical scavenging potential of 48.7 ± 0.87% in comparison to BHA which was 44.2 ± 0.08%, and also demonstrated a reducing power at the highest dose of 60 mg/kg in the FRAP assay 72.68% (25).

Protein denaturation occurs via an unexpected mechanism involving changes in hydrophobic, disulfide, and electrostatic hydrogen bonds (26). Protein denaturation results in the creation of autoantigens in inflammatory illnesses such as cancer, diabetes, and rheumatoid arthritis. (27). Therefore, it is possible to reduce inflammatory activity by inhibiting protein denaturation. A nonsteroidal anti-inflammatory drug (NSAID), diclofenac, was used as the reference drug in this study. By inhibiting the activity of the enzyme cyclooxygenase, NSAIDs have an anti-inflammatory effect. On the other hand, ulceration, bleeding, perforation, and constipation are negative effects of these drugs (28). In comparison to the denaturation process with bovine serum albumin, the hydrolate of *O. vulgare* showed an anti-inflammatory effect (Table 2). The functional groups which might influence the anti-inflammatory activity observed herein, are terpene and alcohol compounds (29). The anti-inflammatory activity of terpenes is determined by the presence of methylene groups and phenolic O-H. Studies have shown that these substances block the signaling pathways for mitogen-activated protein kinase and nuclear transcription factor-β (29). Of the volatiles extracted from oregano hydrolate, terpinen-4-ol is the main constituent whose analysis and anti-inflammatory activities have been demonstrated in previous studies (30, 31). Increased intracellular inflammatory factors are the result of activation of nuclear factor kappa β (NF-κβ) in response to LPS lipopolysaccharide-triggered cells. By significantly preventing NF-κβ activation, terpinen-4-ol can reduce the inflammatory response (30). Hydrolate of the aerial part of *O. vulgare* has attracted considerable interest due to its biological activity, including antioxidant and anti-inflammatory effects (32). In the analysis of the volatile compounds, we were only able to detect a part of the compounds present in the hydrolate; a part remained in the aqueous phase after extraction with ether and could be responsible for the activities we investigated, which is why further investigations are required.

Conclusion

Terpinen-4-ol (36%) and 1-octen-3-ol (33.6%) were found to be the main volatiles extracted from the hydrolate obtained after distillation of the essential oil isolated from the aerial part of the plant *O. vulgare*. GC/MS and GC/FID analyses were performed to determine the chemical composition of the volatile compounds extracted from the hydrolate. The hydrolate sample affected the neutralization of DPPH radicals and β-caroten bleaching to some extent at all concentrations tested. The total reducing power in the FRAP assay was 0.361 ± 0.015 µmol Fe^{2+}/ml hydrolate. Additionally, the anti-inflammatory activity was shown in the BSA denaturation inhibition test. Based on the promising results we have presented, hydrolate obtained from the aerial part of *O. vulgare* might have potential application as a natural additive as the result of antioxidant and anti-inflammatory activity.

Acknowlegments

This research was supported by the Ministry of Education and Science of the Republic of Serbia (Grant No. 451-03-65/2024-03/200113 and 451-03-66/2024-03/200113) and the Faculty of Medicine University of Niš Internal Scientific Project No. 15.

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Originalni rad

UDC: 615.322:582.943.15]:543.51
doi: 10.5633/amm.2024.0304

ORIGANUM VULGARE L.: HEMIJSKI PROFIL EKSTRAHOVANIH ISPARLJIVIH KOMPONENATA I ANTIOKSIDATIVNA I ANTIINFLAMATORNA AKTIVNOST HIDROLATA

Anđela Dragićević[1], Dušanka Kitić[1], Jelena Matejić[1], Ljiljana Stanojević[2], Jelena Stanojević[2], Dragan Cvetković[2], Dragana Pavlović[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za farmaciju, Niš, Srbija
[2]Univerzitet u Nišu, Tehnološki fakultet, Leskovac, Srbija

Kontakt: Anđela Dragićević
Rudnička 20/8, 18000 Niš, Srbija
E-mail: dragicevic.andjela@gmail.com

Hidrolati ili cvetne vode proizvodi su hidrodestilacije aromatičnih biljaka. Predstavljaju nusproizvode etarskog ulja, pa je njihova proizvodnja jednostavna i pristupačna. Hemijski sastav i biološka aktivnost hidrolata i odgovarajućih etarskih ulja mogu se razlikovati. Osnovni cilj ove studije bio je da se ispita hemijski profil ekstrahovanih isparljivih komponenata hidrolata dobijenih iz nadzemnog dela biljne vrste *Origanum vulgare* L., ali i da se ispita antiinflamatorna i antioksidativna aktivnost hidrolata. Kvalitativna i kvantitativna analiza ekstahovanih isparljivih komponenata hidrolata izvršena pomoću gasne hematografije i masene spektomerije (engl. *gas chromatography/mass spectrometry* – GC/MS), kao i pomoću gasne hematografije / detekcije plamene jonizacije (engl. *gas chromatography-flame ionization detection* – GC/FID) pokazala je da su glavne komponente terpinen-4-ol (36%) i 1-okten-3-ol (33,6%). Sve ispitivane koncentracije hidrolata pokazale su sposobnost uklanjanja slobodnih DPPH radikala na način zavisan od koncentracije, kao i aktivnost u *β*-karoten / linolna kiselina testu. Ukupni antioksidativni kapacitet origana procenjen je korišćenjem testa kojim se ispituje antioksidativna moć redukcijom gvožđa (engl. *ferric reducing ability of plasma* – FRAP test), čija je vrednost iznosila 0,361 µmol Fe^{2+}/ml ± 0,015 µmol Fe^{2+}/ml. Pored antioksidativne aktivnosti, zabeležena je i zadovoljavajuća antiinflamatorna aktivnost sa procentom inhibicije denaturacije BSA od 71,2% ± 0,006%. Pokazana antioksidativna i antiinflamatorna aktivnost hidrolata *O. vulgare* mogu biti važne za njegovu buduću upotrebu u mnogim industrijskim oblastima.

Acta Medica Medianae 2024; 63(3):32−41.

Ključne reči: *gasna hematografija / masena spektomerija, gasna hematografija / detekcija plamene jonizacije, divlji origano, hidrosol, terpinen-4-ol, 1-okten-3-ol*