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Broj: 517/8-1
Podgorica, 11.04.2022. godine

Univerzitet Crne Gore
Odbor za doktorske studije
n/r predsjednici – prof. dr Biljani Šćepanović

Poštovana,

U skladu sa članom 41 i 55 Pravila doktorskih studija, i tačkom 3.8. Vodiča za doktorske studije, u prilogu akta dostavljamo obrazac D2 uz Prijedlog Odluke Vijeća o imenovanju Komisije za ocjenu doktorske disertacije dr pharm Tijane Mićović, pod nazivom „Farmakognozijska ispitivanja herbe izopa, *Hyssopus officinalis* L. (Lamiaceae) iz Crne Gore i Srbije" sa pratećom dokumentacijom.

S poštovanjem.

MEDICINSKI FAKULTET
D E K A N,
Prof. dr Miodrag Radunović

ISPUNJENOST USLOVA DOKTORANDA

OPŠTI PODACI O DOKTORANDU			
Titula, ime, ime roditelja, prezime	dr pharm Tijana (Zoran i Olivera) Mićović		
Fakultet	Medicinski fakultet u Podgorici – Univerzitet Crne Gore		
Studijski program	Farmacija		
Broj indeksa	10/17		
NAZIV DOKTORSKE DISERTACIJE			
Na službenom jeziku	Farmakognozijska ispitivanja herbe izopa, *Hyssopus officinalis* L. (Lamiaceae) iz Crne Gore i Srbije		
Na engleskom jeziku	Pharmacognostic investigation of aerial parts of *Hyssopus officinalis* L. (Lamiaceae) from Montenegro and Serbia		
Naučna oblast	Farmakognozija		
MENTOR/MENTORI			
Prvi mentor	Prof. dr Zoran Maksimović	Farmaceutski fakultet Univerziteta u Beogradu; Srbija	Farmakognozija
Drugi mentor	(Titula, ime i prezime)	(Ustanova i država)	(Naučna oblast)
KOMISIJA ZA PREGLED I OCJENU DOKTORSKE DISERTACIJE			
Prof. dr Zorica Potpara, vanredni profesor - predsjednik komisije		Medicinski fakultet Univerziteta Crne Gore	Farmaceutska tehnologija
Prof. dr Zoran Maksimović, redovni profesor - član komisije		Farmaceutski fakultet Univerziteta u Beogradu	Farmakognozija
Prof. dr Danijela Stešević, redovni profesor - član komisije		Prirodno-matematički fakultet Univerziteta Crne Gore	Botanika

Datum značajni za ocjenu doktorske disertacije	
Sjednica Senata na kojoj je data saglasnost na ocjenu teme i kandidata	12.03.2020.
Dostavljanja doktorske disertacije organizacionoj jedinici i saglasanost mentora	10.03.2022.
Sjednica Vijeća organizacione jedinice na kojoj je dat prijedlog za imenovanje komisija za pregled i ocjenu doktorske disertacije	07.04.2022.

ISPUNJENOST USLOVA DOKTORANDA

U skladu sa članom 38 pravila doktorskih studija kandidat **je** dio sopstvenih istraživanja vezanih za doktorsku disertaciju publikovao u časopisu sa **SCI/SCIE** liste kao prvi autor.

Spisak radova doktoranda iz oblasti doktorskih studija koje je publikovao u časopisima sa (upisati odgovarajuću listu)

1. **Mićović T,** Topalović D, Živković L, Spremo-Potparević B, Jakovljević V, Matić S, Popović S, Baskić D, Stešević D, Samardžić S, Stojanović D, Maksimović Z. Antioxidant, Antigenotoxic and Cytotoxic Activity of Essential Oils and Methanol Extracts of *Hyssopus officinalis* L. Subsp. *aristatus* (Godr.) Nyman (Lamiaceae). Plants. 2021; 10(4):711. https://doi.org/10.3390/plants10040711

Link ka radu: https://www.mdpi.com/2223-7747/10/4/711

Obrazloženje mentora o korišćenju doktorske disertacije u publikovanim radovima

Navedeni rad predstavlja jedan deo doktorske disertacije kandidata dr pharm. Tijane Mićović i odnosi se na delove disertacije koji su posvećeni ispitivanju hemijskog sastava i karakterizaciji ekstrakata i etarskih ulja izopa, nekih njihovih farmakoloških aktivnosti (antioksidantna, antigenotoksična, citotoksična) i uspostavljanju veze izmedu uočenih dejstava i identifikovanih hemijskih sastojaka. Rad je originalno naučno ispitivanje i objavljen je u vodećem međunarodnom časopisu (Plants, izdavača MDPI Švajcarska; Impact Factor 3.935; JCR category rank Q1: Plant Sciences).

U međuvremenu, u martu tekuće (2022.) godine, prihvaćen je i objavljen online još jedan rad kandidata dr pharm. Tijane Mićović koji predstavlja originalno naučno ispitivanje, proisteklo iz doktorske disertacije, a odnosi se na ispitivanje antiinflamatorne aktivnosti preparata herbe izopa. U pitanju je takođe vodeći međunarodni časopis (Journal of Ethnopharmacology, izdavača Elsevier Ireland Ltd; Impact Factor 4.360; JCR category rank Q2: Drug Discovery; Pharmacology):

Mićović T, Katanić Stanković JS, Bauer R, Nöst X, Marković Z, Milenković D, Jakovljević V, Tomović M, Bradić J, Stešević D, Stojanović D, Maksimović Z. *In vitro*, *in vivo* and *in silico* evaluation of the anti-inflammatory potential of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (Lamiaceae). J Ethnopharmacol. 2022 Mar 28:115201. doi: https://doi.org/10.1016/j.jep.2022.115201. Epub ahead of print. PMID: 35358622.

Link ka radu: https://www.sciencedirect.com/science/article/abs/pii/S0378874122002409

Datum i ovjera (pečat i potpis odgovorne osobe)

U Podgorici,
(07.04.2022.)

MP

DEKAN

Prilog dokumenta sadrži:

1. Potvrdu o predaji doktorske disertacije organizacionoj jedinici
2. Odluku o imenovanju komisije za pregled i ocjenu doktorske disertacije
3. Kopiju rada publikovanog u časopisu sa odgovarajuće liste
4. Biografiju i bibliografiju kandidata
5. Biografiju i bibliografiju članova komisije za pregled i ocjenu doktorske disertacije sa potvrdom o izboru u odgovarajuće akademsko zvanje i potvrdom da barem jedan član komisije nije u radnom odnosu na Univerzitetu Crne Gore

UNIVERZITET CRNE GORE

MEDICINSKI FAKLTET

Broj: 412/1

Podgorica 10.03.2022 godine

POTVRDA

Potvrđuje se da je dr pharm Tijana Mićović predala 7 primjeraka doktorske disertacije, pod nazivom „ **Farmakognozijska ispitivanja herbe izopa, Hyssopus officinalis L.(Lamiaceae) iz Crne Gore i Srbije** " dana 10.03.2022.godine .

Potvrda se izdaje u svrhu pregleda i ocjene doktorske disrtacije.

ŠEF STUDENTSKE SLUŽBE

Sonja Vukićević, diplomirani pravnik

UNIVERZITET CRNE GORE
MEDICINSKI FAKULTET
Broj: 517/8
Podgorica, 07.04.2022. godine

Na osnovu člana 64 stav 1 tačka 9 Statuta Univerziteta Crne Gore, (Bilten UCG br.337/2015 i br 447/2018), člana 41 i 55 Pravila doktorskih studija, inicijalnog predloga Komisije za doktorske studije Medicinskog fakulteta broj: 412/2 od 18.03.2022 godine i tačke 3.8 Vodiča za doktorske studije Univerziteta Crne Gore, Vijeće Medicinskog fakulteta na sjednici održanoj 07.04.2022. godine, donijelo je

O D L U K U

I

Kandidat dr pharm Tijana Mićović, ispunjava formalne uslove za ocjenu doktorske disertacije: „**Farmakognozijska ispitivanja herbe izopa, Hyssopus officinalis L. (Lamiaceae) iz Crne Gore i Srbije**".

II

Predlaže se Komisija za ocjenu doktorske disertacije dr pharm Tijane Mićović, pod navedenim nazivom: „ **Farmakognozijska ispitivanja herbe izopa, Hyssopus officinalis L. (Lamiaceae) iz Crne Gore i Srbije** " u sastavu:

1. **Prof. dr Zorica Potpara**, vanredni profesor Medicinskog fakulteta Univerziteta Crne Gore, naučna oblast: farmaceutska tehnologija;
2. **Prof. dr Zoran Maksimović**, redovni profesor Farmaceutskog fakulteta Univerziteta u Beogradu, naučna oblast: farmakognozija;
3. **Prof. dr Danijela Stešević**, redovni profesor Prirodno-matematičkog fakulteta Univerziteta Crne Gore, naučna oblast: botanika;

III

Komisija za ocjenu doktorske disertacije je dužna da Vijeću Medicinskog fakulteta, podnese izvještaj koji sadrži ocjenu doktorske disertacije.

Obrazloženje

Dr pharm Tijana Mićović je predala doktorsku disertaciju pod nazivom: „ **Farmakognozijska ispitivanja herbe izopa, Hyssopus officinalis L. (Lamiaceae) iz Crne Gore i Srbije** " dana 10.03.2022. godine.
Vijeće Medicinskog fakulteta je utvrdilo da kandidat ispunjava uslove iz člana 38 Pravila doktorskih studija, da kandidat dr pharm Tijana Mićović ima, kao prvi autor dva rada sa rezultatima iz teze objavljene u časopisu sa SCI/**SCIE** liste, od kojih je jedan obljavljen online. Samim tim su se stekli uslovi da se imenuje Komisija za ocjenu pomenute doktorske disertacije. Na osnovu svega navedenog, odlučeno je kao u dispozitivu ove Odluke.

VIJEĆE MEDICINSKOG FAKULTETA
PREDSJEDAVAJUĆI,

Prof. dr Miodrag Radunović, dekan

plants

MDPI

Article

Antioxidant, Antigenotoxic and Cytotoxic Activity of Essential Oils and Methanol Extracts of *Hyssopus officinalis* L. Subsp. *aristatus* (Godr.) Nyman (Lamiaceae)

Tijana Mićović [1], Dijana Topalović [2], Lada Živković [2], Biljana Spremo-Potparević [2], Vladimir Jakovljević [3,4], Sanja Matić [5], Suzana Popović [6], Dejan Baskić [6,7], Danijela Stešević [8], Stevan Samardžić [9], Danilo Stojanović [10] and Zoran Maksimović [9,*]

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Citation: Mićović, T.; Topalović, D.; Živković, L.; Spremo-Potparević, B.; Jakovljević, V.; Matić, S.; Popović, S.; Baskić, D.; Stešević, D.; Samardžić, S.; et al. Antioxidant, Antigenotoxic and Cytotoxic Activity of Essential Oils and Methanol Extracts of *Hyssopus officinalis* L. Subsp. *aristatus* (Godr.) Nyman (Lamiaceae). *Plants* **2021**, *10*, 711. https://doi.org/10.3390/plants10040711

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Abstract: *Hyssopus officinalis* L. is a well-known aromatic plant used in traditional medicine and the food and cosmetics industry. The aim of this study is to assess the antioxidant, genotoxic, antigenotoxic and cytotoxic properties of characterized hyssop essential oils and methanol extracts. Chemical composition was analyzed by gas chromatography - mass spectrometry (GC-MS) and liquid chromatography with diode array detection and mass spectrometry (LC-DAD-MS), respectively. Antioxidant activity was examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) tests; genotoxic and antigenotoxic activity were examined by the comet assay, while cytotoxicity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye (MTT) test against tumor cell lines (SW480, MDA-MB 231, HeLa) and non-transformed human lung fibroblast cell lines (MRC-5). The essential oils were rich in monoterpene hydrocarbons (e.g., limonene; 7.99–23.81%), oxygenated monoterpenes (1,8-cineole; 38.19–67.1%) and phenylpropanoids (methyl eugenol; 0.00–28.33%). In methanol extracts, the most abundant phenolics were chlorogenic and rosmarinic acid (23.35–33.46 and 3.53–17.98 mg/g, respectively). Methanol extracts expressed moderate to weak antioxidant activity (DPPH IC_{50} = 56.04–199.89 µg/mL, FRAP = 0.667–0.959 mmol Fe^{2+}/g). Hyssop preparations significantly reduced DNA damage in human whole blood cells, induced by pretreatment with hydrogen peroxide. Methanol extracts exhibited selective and potent dose- and time-dependent activity against the HeLa cell line. Results of the current study demonstrated notable *H. officinalis* medicinal potential, which calls for further investigation.

Keywords: *Hyssopus officinalis*; antioxidant activity; antigenotoxic activity; comet assay; cytotoxic activity; HeLa cell line; essential oil; methanol extract; GC-MS; LC-DAD-MS

1. Introduction

Hyssop, *Hyssopus officinalis* L. (Lamiaceae), is a shrubby perennial herbaceous plant, distributed mostly in the Mediterranean area [1–3]. In Montenegro and Serbia, *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (syn. *H. officinalis* L. subsp. *pilifer* (Gris. ex Pant.) Murb.) can be found in plant communities of rocky pastures [2].

Hyssop herb (*Hyssopi herba*) and its pharmaceutical preparations (infusions, syrups, tinctures, extracts) have been used in traditional medicine since ancient times as antiseptic, carminative, diaphoretic, emenagogue, expectorant, muscle relaxant, stomachic and tonic agents. As an aromatic plant, it is also used in the food and cosmetics industry [4–6].

Essential oil is the most important and the most frequently investigated product of hyssop. Available literature data on wild and cultivated plants indicate that its herb yields 0.3–1% of essential oil with isopinocamphone as the dominant compound, along with pinocamphone, β-pinene, 1,8-cineole, pinocarvone, linalool, sabinene and methyl eugenol [7–9]. Beside the essential oil, hyssop herb contains flavonoids and phenolic acids, tannins, diterpene lactones (marrubiin) and triterpenoid compounds such as ursolic and oleanolic acid [5,7,10].

Antimicrobial activity is one of the most commonly examined pharmacological effects of various hyssop preparations. Extensive experimental evidence also speak in favor of antioxidant [10], antiviral [11], sedative and anxiolytic [12,13], spasmolytic [14], anti-inflammatory [15], antiulcer [16], anti-asthmatic [17] and antidiabetic activities of the hyssop herb [18]. However, despite the diversity in scientific information on pharmacological activities of *Hyssopi herba* and its preparations, genotoxicity, antigenotoxicity and cytotoxic activity of the essential oils and polar extracts of this herbal drug are still insufficiently investigated.

In attempt to better understand medicinal potentials of *H. officinalis* herb in this field, we designed and performed a set of chemical and physiological investigations on the plant material collected from five wild-growing populations in Montenegro and on one commercial sample available in herbal apothecaries in Serbia, and manufactured by a local enterprise from wild-growing sources.

Hyssopi herba, as an herbal medicinal substance and a raw material for pharmaceutical and related industries, is neither official in pharmacopoeias, nor listed in the other well-established documents. Therefore, the question of its pharmaceutical quality still remains open. Having in mind that growing (for plants harvested from the wild) and/or cultivation conditions can significantly affect the composition and activity of a given plant, it would explain the need for sampling at different sites, as well as the use of a commercial sample-that is something that a person can find, if needed.

Consequently, the main objectives of the present work were to quantify the levels of in vitro antioxidant activity by commonly used 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) tests, to assess potential genotoxicity/antigenotoxicity by comet assay, and to determine the overall cytotoxic, cytostatic and cytocidal effects against human tumor and non-transformed human lung fibroblast cell lines of investigated hyssop essential oils and/or extracts, with respect to their chemical composition.

2. Results and Discussion

2.1. Essential Oils Compositions

Produced oils were pale yellowish-green liquids with characteristic pleasant odors. The yields ranged from 0.4% to 0.79% (*v/w*) for samples collected from the wild in Montenegro. However, the highest yield was obtained from the commercial sample (Serbia), amounting to 1% (*v/w*). The results of the gas chromatography coupled with mass spectrometry (GC-MS) analysis of the essential oils (1EO–6EO) obtained from tested samples of *H. officinalis* subsp. *aristatus* (1–6, Table 1) are presented in Table 2. Overall, 12 to 16 compounds were identified depending on the sample, which is more than 98% of the total oil on average, with one exception (sample 2EO), where the percentage of identified compounds was lower (86.84%).

Table 1. Plant material: origin, collection data and yields of essential oils and extracts. [a] Values are the means of five consecutive determinations.

Sample	Plant Material Origin	Site of Collection	Geographic Coordinates	Altitude (m)	Habitat	Collection Date (dd/mm/yyyy)	Voucher Specimen	Essential Oil (EO) Yield (mL/100 g) [a]	MeOH Extr. (E) Yield (% w/w)
1	Commercial sample (Serbia)	Southeastern Serbia	N/A	N/A	N/A	N/A	N/A	1.00	12.02
2	Wild-growing (Montenegro)	Kuči	N 42°31′55″ E 19°24′07″	870	rocky	13/09/2018	1420263	0.40	9.48
3	Wild-growing (Montenegro)	Šavnik	N 42°57′16″ E 19°05′59″	880	rocky pasture	19/09/2018	1420261	0.54	10.24
4	Wild-growing (Montenegro)	Piva	N 43°9′25″ E 18°50′46″	750	rocky	14/09/2018	1420162	0.65	9.05
5	Wild-growing (Montenegro)	Piperi	N 42°34′23″ E 19°16′0.8″	800	rocky pasture	07/09/2018	1420259	0.79	10.21
6	Wild-growing (Montenegro)	Cuce	N 42°35′19″ E 18°47′40″	820	rocky	12/09/2018	1420260	0.48	9.64

Table 2. Essential oil composition of *Hyssopus officinalis* subsp. *aristatus*. * Arithmetic retention index.

			Amount (%)					
t_R [min]	AI *	Compound	1EO	2EO	3EO	4EO	5EO	6EO
5.517	925	α-Thujene	0.00	0.51	0.00	1.05	1.44	0.00
5.706	932	α-Pinene	2.08	4.13	1.12	0.53	0.79	1.03
6.762	972	Sabinene	1.86	1.24	0.57	0.47	0.54	0.56
6.872	976	β-Pinene	6.73	9.13	16.33	15.79	9.69	5.48
7.238	990	β-Myrcene	0.93	0.46	0.46	0.00	0.43	0.36
8.343	1024	*p*-Cymene	0.27	1.92	0.00	0.00	0.00	0.28
8.482	1028	Limonene	7.99	7.99	16.11	23.81	21.77	15.43
8.569	1030	1,8-Cineole	67.10	42.07	9.77	1.42	38.19	56.08
8.765	1036	*Z*-β-Ocimene	3.57	2.94	2.06	1.88	3.11	3.06
9.142	1046	*E*-β-Ocimene	0.27	0.00	0.00	0.00	0.00	0.00
9.531	1057	γ-Terpinene	0.31	0.58	0.00	0.00	0.00	0.00
12.592	1138	*trans*-Pinocarveol	0.23	2.26	0.83	0.54	0.00	0.61
13.463	1159	*trans*-Pinocamphone	0.00	1.84	3.34	8.34	4.72	0.00
13.556	1162	Pinocarvone	0.00	1.20	3.99	1.67	0.00	0.41
14.027	1173	*cis*-Pinocamphone	1.15	5.61	22.75	14.72	14.54	0.00
14.961	1196	Myrtenal	0.32	3.71	1.02	0.66	0.69	0.80
20.403	1325	Myrtenyl acetate	0.00	1.25	0.00	0.00	0.00	0.00
22.856	1384	β-Bourbonene	0.00	0.00	0.00	0.00	0.00	0.31
23.758	1406	Methyl eugenol	5.43	0.00	19.24	28.33	3.52	13.70
24.265	1418	*E*-β-Caryophyllene	0.47	0.00	0.00	0.00	0.00	0.00
26.771	1480	Germacrene D	0.40	0.00	0.00	0.00	0.00	0.36
Monoterpene hydrocarbons			24.01	28.9	36.65	43.53	37.77	26.2
Oxygenated monoterpenes			68.8	57.94	41.7	27.35	58.14	57.9
Sesquiterpene hydrocarbons			0.87	0.00	0.00	0.00	0.00	0.67
Phenylpropanoids			5.43	0.00	19.24	28.33	3.52	13.70
Total identified			99.11	86.84	97.59	99.21	99.43	98.47

The dominant group of identified volatiles was monoterpenes (70.88–95.91%). Their oxygenated derivatives were the most abundant (41.7–68.8%), among which 1,8-cineole and *cis*-pinocamphone were found in the highest contents. Only in the sample 4EO, the content of monoterpene hydrocarbons (43.53%) was higher than the content of oxygenated monoterpene derivatives. With regard to the monoterpene hydrocarbons, the dominant constituents were β-pinene and limonene. Methyl eugenol (up to 28.33%), the only compound belonging to the phenylpropanoid group, and sesquiterpene hydrocarbons (up to 0.87%) were present in a lower percentage. Monoterpene hydrocarbons, such as β-pinene, limonene, Z-β-ocimene, α-pinene, sabinene and the oxygenated monoterpene derivative myrtenal, were present in all the investigated samples.

The principal component analysis (PCA) confirmed the existence of significant chemical variation in the investigated essential oils. Performed on the entire dataset, PCA detected five principal components (PCs), with the first three accounting together for more than 86% of total variance (Figure 1). The constituents of the oils which contribute the most to the corresponding PCs are listed in Table S1 (Supplementary Material), along with their loadings and scores. Along the first PC axis, the highest number of significant characters of separation (factor loadings higher than ±0.7) was detected. The second and third PCs further underscored the chemical variations between samples.

The cluster analysis of entire dataset revealed the similarity in the composition of essential oils from the commercial sample of *H. officinalis* and plants collected from the locality Cuce in Montenegro (Cluster 1), plants collected from localities Kuči and Piperi (Cluster 2) and plants collected from localities Šavnik and Piva (Cluster 3), as shown in Figure 2. The results indicated that the classification proposed by the PCA and hierarchical cluster analysis (HCA) are in good agreement.

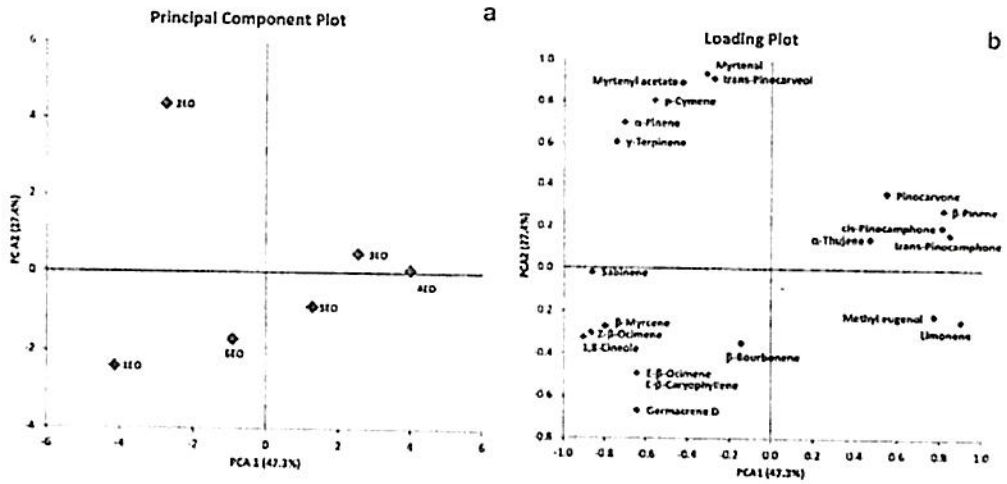

Figure 1. Plots of principal component analysis (PCA) scores (a) and loadings (b) along the first two principal components (PCs) extracted from the dataset of *Hyssopus officinalis* essential oils from six mutually independent sources, as listed in Table 2.

Considering the previously reported literature data, numerous compounds hav formerly been identified in essential oils of hyssop and several chromatographic profile have been described.

Differences in oil composition (deriving from climatic conditions, the origin of plan material, subspecies or variety, developmental stages, soil type, cultivation technologie extraction methods, etc.) determine its organoleptic and physiological properties, an hence, its possibilities of application [1,5,7,19–23].

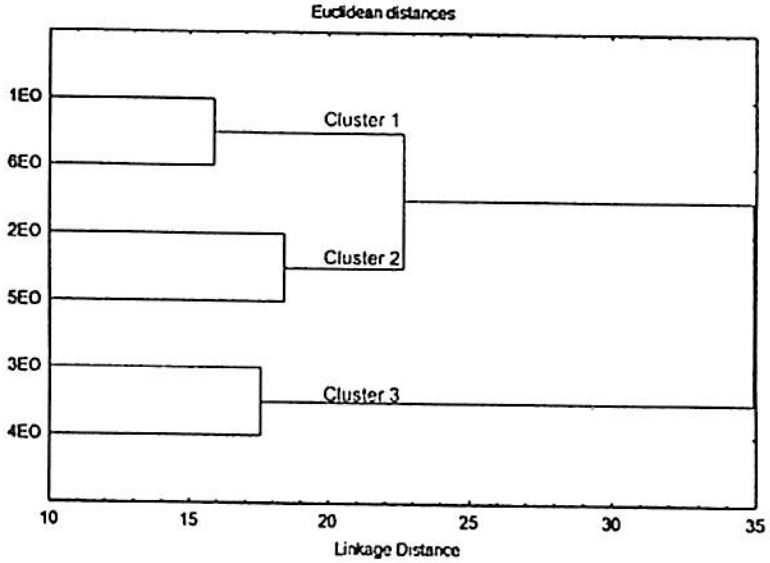

Figure 2. Dendrogram representing the similarity relations of the chemical composition of *Hyssopu officinalis* essential oils. For this analysis, an entire chromatographic dataset was taken into consider tion. Amalgamation rule: single-linkage. Distance metric is Euclidean distances (non-standardized)

The most characteristic and important components of so far investigated *H. officinali* essential oils are 1,8-cineole [1,21,22], *cis*-pinocamphone, *trans*-pinocamphone and thei precursor β-pinene [1,19]. Among the other principal constituents, pinocarvone, sabinene germacrene D, germacren D-4-ol, α-, β-phellandrene, 4-carvomenthenol, thymol, carvacro

elemol, limonene, linalool, α-terpinene, myrtenol, myrtenyl acetate and methyl eugenol were also reported [7].

With regard to the hyssop growing in Serbia, Mitić et al. (2000) identified *cis*-pinocamphone (44.7%) as the most abundant constituent of its essential oil, followed by *trans*-pinocamphone (14.1%), germacren-D-11-ol (5.7%) and elemol (5.6%) [19]. Gorunović et al. (1995) examined hyssop from the territory of Montenegro. The main constituents were methyl eugenol (38.30%), limonene (37.40%) and β-pinene (9.6%) [20].

Hajdari A. et al. (2018) investigated the composition of the essential oil of wild-growing *H. officinalis* subsp. *aristatus* (aerial parts) from five different localities in Kosovo, and found that in four out of five samples, the dominant compound was *cis*-pinocamphone, with the content ranging between 30.44% and 57.73%. In a sample from one of the localities, the dominant compound was 1,8-cineole (45.27%). The same authors found that the content of *trans*-pinocamphone (14.76%) was significant in one of the samples, as well as that of β-pinene (23.31%) and caryophyllene oxide (12.66%) [21].

The essential oils obtained from wild-growing *H. officinalis* L. subsp. *aristatus* in Bulgaria in two stages of development (during the flower bud formation and in the full bloom) were similar in composition, with 1,8-cineole (48.2% and 39.6%), isopinocamphone (16.3% and 29.2%) and β-pinene (11.4% and 39.6%) as the major constituents. The essential oil obtained from cultivated *H. officinalis* contained larger amounts of isopinocamphone (40.2%), pinocamphone (10.3%) and β-pinene (14.2%), but no traces of 1,8-cineole [22].

In the essential oil of wild-growing *H. officinalis* subsp. *aristatus* (aerial parts) native to Italy, the main compound was linalool (35.3–51.2%), whereas methyl eugenol (7.3–22.7%), limonene (3.7–4.4%), germacrene D (1.9–4.1%), (Z)-β-ocimene (5.1–5.8%) and (E)-β-ocimene (2.1–5.3%) were reported as well [5].

Our results revealed three chromatographic profiles in investigated essential oils of wild-growing plants from Montenegro. The essential oil obtained from plants collected from the locality Cuce in Montenegro (sample 6EO) was similar with the essential oil of the commercial sample from southeastern Serbia (sample 1EO), being high in 1,8-cineole and relatively rich in β-pinene, but low in *cis*-pinocamphone. On the other hand, the essential oils of plants collected from the localities Šavnik and Piva in Montenegro (samples 3EO and 4EO, respectively) stood out for being high in β-pinene, limonene, *cis*-pinocamphone and methyl eugenol, but relatively low in 1,8-cineol at the same time. Finally, the essential oils obtained from the plants collected from the localities Kuči and Piperi in Montenegro (samples 2EO and 5EO, respectively) appeared to be relatively rich in 1,8-cineole, limonene, β-pinene and *cis*-pinocamphone.

2.2. Methanol Extract Compositions and Contents of Total Polyphenols

The results of the liquid chromatography with diode array and mass spectrometry (LC-DAD-MS) analysis of methanol extracts (1E–6E) obtained from the tested samples of *H. officinalis* subsp. *aristatus* (1–6, Table 1) are presented in Tables 3 and 4. LC-DAD-MS analysis of methanol extracts of hyssop flowering aerial parts revealed the presence of phenolic compounds, specifically benzoic acid derivative (syringic acid), hydroxycinnamic acid derivatives (chlorogenic, feruloylquinic and rosmarinic acids, as well as caffeoyl pentoside) and flavonoids (derivatives of quercetin and diosmetin). The identified compounds, their spectral characteristics and their retention times are given in Table 3. The comparative view of chromatograms of 1E–6E recorded at 320 nm is given in Supplementary Material Figure S1. It showed that chlorogenic and rosmarinic acids can be considered quantitatively dominant compounds based on their relative peak areas (%).

Table 3. Assignment, retention times, UV and MS spectral data of phenolic compounds in methanol extracts of *Hyssopus officinalis* subsp. *aristatus*. [a] Identification by comparing with commercial reference compounds. [b] Tentative identification by comparing acquired UV and MS spectral data with literature data.

Peak No.	t_r (min)	UV λ_{max} (nm)	ESI-MS Data (*m/z*)	Assignment
1	4.255	280	395.1 $[2M-H]^-$, 197 $[M-H]^-$, 153.1	Syringic acid [b]
2	9.107	218, 240, 298 sh, 326	707.1 $[2M-H]^-$, 353.1 $[M-H]^-$, 191	Chlorogenic acid (5-*O*-caffeoylquinic acid) [a]
3	10.954	218, 238, 298 sh, 328	623.1 $[2M-H]^-$, 311.1 $[M-H]^-$, 134.1	Caffeoyl pentoside [b]
4	12.087	218, 238, 296 sh, 326	735.2 $[2M-H]^-$, 367.1 $[M-H]^-$, 173.1	Feruloylquinic acid [b]
5	15.339	256, 266 sh, 356	463.1 $[M-H]^-$, 300.1	Quercetin *O*-hexoside [b]
6	17.215	252, 266, 348	607.2 $[M-H]^-$, 299.1, 284	Diosmetin *O*-deoxyhexosyl-hexoside [b]
7	18.461	286 sh, 328	719.1 $[2M-H]^-$, 359 $[M-H]^-$, 197, 161.1	Rosmarinic acid [a]

Table 4. Content of total phenols, chlorogenic acid and rosmarinic acid in methanolic extracts of *Hyssopus officinalis* subsp. *aristatus*. Different letters in the superscript indicate statistically different values at $p < 0.05$.

Sample	Total Phenols (mg GAE/g)	Chlorogenic Acid (mg/g)	Rosmarinic Acid (mg/g)
1E	74.7 ± 0.8^{c}	23.35 ± 0.2^{a}	13.71 ± 0.19^{d}
2E	68.2 ± 0.8^{b}	30.94 ± 0.11^{d}	5.35 ± 0.02^{b}
3E	64.1 ± 1.3^{a}	24.12 ± 0.11^{b}	3.53 ± 0.03^{a}
4E	112.0 ± 1.6^{e}	33.46 ± 0.08^{e}	17.98 ± 0.25^{e}
5E	81.8 ± 0.8^{d}	33.17 ± 0.1^{e}	4.97 ± 0.12^{b}
6E	69.0 ± 0.3^{b}	30.19 ± 0.1^{c}	8.13 ± 0.04^{c}

Identified phenolics were present in all extracts, regardless of the site of the plan material collection. Variability was reflected through relatively small differences in th concentrations of individual constituents. The content of chlorogenic acid was in the rang between 23.35 and 33.46 mg/g, whereas rosmarinic acid was present in lower amount (3.53–17.98 mg/g) (Table 4). Among the analyzed preparations, sample 4E was the riches in chlorogenic and rosmarinic acids.

The results are in good agreement with the literature data. Previous studies of ethano and deodorized aqueous extracts of the aerial parts of wild-growing *H. officinalis* subsp *aristatus* (originating from central Italy and eastern Serbia) showed the presence of chlorc genic acid, rosmarinic acid, 4-*O*-feruloylquinic acid and syringic acid [1,5]. Flavonoids isoquercitrin (quercetin 3-*O*-glucoside) and diosmin (diosmetin 7-*O*-rutinoside), were alsc previously detected in extracts of hyssop herb [10,24]. In a study conducted by Borrell et al. (2019), ethanol macerate of the hyssop aerial parts was chemically analyzed anc the occurrence of caffeoyl pentoside, a hydroxycinnamate derivative, was confirmed [25 In addition, a phenylethanoid glycoside martynoside was reported as a constituent of *H. seravshanicus* [26]. The findings of other authors regarding the quantitative composition of different extracts of hyssop herb are consistent with the presented results. Namely Venditti et al. (2015), as well as Hatipoğlu et al. (2013), demonstrated that the content o chlorogenic acid is the highest among the quantities of phenolics [5,27]. Detailed analysis indicated that the contents of chlorogenic acid in the examined samples were 4–5 times higher than the corresponding values formerly reported, whereas the rosmarinic acic contents were closer to the literature values. However, certain variations can be expected and explained by a number of factors, e.g., differences in the extraction solvent used, the extraction methodology, the origin of the plant material and/or the developmental stage o the plant during collection.

The contents of total polyphenols (TPC) in tested samples ranged between 64.1 anc 112.0 mg GAE/g (Table 4). The highest TPC was determined in sample 4E (112 mg GAE/g)

whereas the lowest one was obtained in sample 3E (64.1 mg GAE/g). The sample richest in chlorogenic and rosmarinic acids was also the richest in total polyphenols. The order of the remaining extracts, by the decreasing TPCs, was: 5E > 1E > 6E > 2E.

Previous studies have yielded variable results, which is expected given that TPC can be affected by numerous factors. Namely, reported values for TPC in several different preparations of *H. officinalis* aerial parts were in a wide range, between 2.69 and 497.6 mg GAE/g [1,10,21,25,28].

2.3. *Antioxidant Activity*

Dry methanol extracts (1E–6E) of hyssop herb exhibited notable antioxidant activity in DPPH and FRAP assays (Table 5).

Table 5. Total antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in methanolic extracts of *Hyssopus officinalis* subsp. *aristatus*. Different letters in the superscript indicate statistically different values at $p < 0.05$.

Sample	DPPH-IC_{50} (μg/mL)	FRAP mmol Fe^{2+}/g
1E	88.42 ± 3.48 ^{d}	0.815 ± 0.012 ^{b}
2E	175.41 ± 2.92 ^{e}	0.781 ± 0.012 ^{a,b}
3E	199.89 ± 0.60 ^{f}	0.667 ± 0.004 ^{a}
4E	56.04 ± 0.19 ^{b}	0.959 ± 0.003 ^{c}
5E	79.37 ± 1.51 ^{c}	0.877 ± 0.007 ^{b,c}
6E	87.90 ± 0.67 ^{d}	0.736 ± 0.023 ^{a,b}
Rutin	4.67 ± 1.41 ^{a}	4.111 ± 0.0253 ^{d}
Ascorbic acid	-	8.181 ± 0.136 ^{e}

The lowest IC_{50} value, i.e., the best ability to neutralize DPPH radicals, was shown for the extract 4E (56.04 μg/mL), followed by 5E (79.37 μg/mL), whereas the lowest activity was observed in the case of 3E (199.89 μg/mL).

These results correlate well with the values of total antioxidant activity estimated by the FRAP assay. Namely, the highest FRAP value was obtained for the 4E extract (0.959 mmol Fe^{2+}/g), followed by the 5E extract (0.877 mmol Fe^{2+}/g), whereas the lowest value was demonstrated for the 3E extract (0.667 mmol Fe^{2+}/g).

The data obtained in antioxidant assays correlate well with the contents of total polyphenols, which are known as constituents that contribute to the antioxidant activity of the plants. With regard to extracts 1E, 2E and 6E, there was no such strong link between the antioxidant activity and total polyphenol contents as there was with the aforementioned extracts. Compared to standard substances (rutin and ascorbic acid), tested hyssop preparations were less effective in DPPH radical scavenging and in the reduction of ferrous ion-2,4,6-tri(2-pyridyl)-*s*-triazine complex (Table 5).

Taking into account the presented results, it can be concluded that moderate antioxidant efficacy (IC_{50} < 100 μg/mL) was demonstrated for four of the six analyzed samples, with the best activity shown for sample 4E.

Literature reports on hyssop aerial parts preparations indicate considerable variability in IC_{50} values (25–2970 μg/mL) obtained in the DPPH test [1,10,25,28], which could be expected as the geographical origin of plant material, extraction procedures and antioxidant activity test protocols differ. With regard to the total antioxidant activity, Stanković et al. (2016) examined methanol extract of vegetative parts of *H. officinalis* from southeastern Serbia and found its FRAP value to be 0.73 mmol Fe^{2+}/g [29]. The current study FRAP value is in good agreement with this reported value, as it ranged from 0.667 mmol Fe^{2+}/g to 0.959 mmol Fe^{2+}/g. The chemical composition may help explain the documented antioxidant activity. Namely, earlier published papers provide evidence that the dominant compounds of the tested extracts (chlorogenic and rosmarinic acids) exhibit significant efficacy in neutralizing DPPH radicals and reducing the ferrous ion complex [30–34].

2.4. Genotoxic and Antigenotoxic Activity

Potential genotoxicity and antigenotoxicity of methanol extracts and essential oils of hyssop herb were assessed using the Comet assay.

2.4.1. Genotoxic Activity

Hyssop herb extracts did not exhibit a genotoxic effect at concentrations 100, 200 and 400 µg/mL (data not shown). With regard to the essential oils, the genotoxic effect did not manifest at the lowest tested concentration (2.5 µg/mL). These results were used to select concentrations for antigenotoxic activity testing.

2.4.2. Antigenotoxic Activity

At 400 µg/mL, all tested *H. officinalis* extracts significantly ($p < 0.0001$) reduced DNA damage in human peripheral blood leukocytes, induced by the pretreatment with hydrogen peroxide (Figure 3).

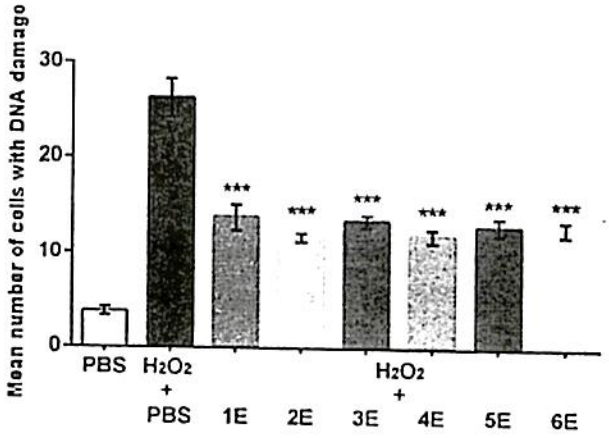

Figure 3. Antigenotoxic properties of methanol extracts of *H. officinalis* subsp. *aristatus* (1E–6E) against DNA damage in human peripheral blood leukocytes, induced by hydrogen peroxide (H_2O_2) in post-treatment protocol. Bars represent the mean value of cells with DNA damage ± standard error of the mean (SEM) versus the control treated with H_2O_2 ($n = 3$). PBS: phosphate-buffered saline solution. *** $p < 0.0001$.

A decrease in the mean number of cells with DNA damage was the most pronounced for extracts 2E and 4E; however, there were no major differences in the antigenotoxic activity among the tested extracts. Similarly, Borrelli et al. (2019) indicated that the ethanol extracts of aerial parts of wild-growing *H. officinalis* subsp. *aristatus*, native to southern Italy, did not display genotoxicity, but counteracted DNA damage in Caco-2 cells caused by hydrogen peroxide [25]. The notable antigenotoxic activity of the tested extracts can be attributed at least in part to the significant content of polyphenols and their ability to neutralize free radicals. Chlorogenic and rosmarinic acids, as dominant compounds in the examined preparations, could be important for the observed activity, considering that earlier published data have shown that these compounds are effective in the Comet test [35,36].

The statistically significant antigenotoxic activity of the essential oils of hyssop aerial parts, applied at the concentration of 2.5 µg/mL, was revealed in the post-treatment. The best activity was shown for the essential oil 4EO ($p < 0.0001$), followed by commercial sample 1EO ($p < 0.001$), whereas other samples (2EO, 3EO, 5EO and 6EO) exhibited weaker, but statistically significant activity ($p < 0.01$) (Figure 4).

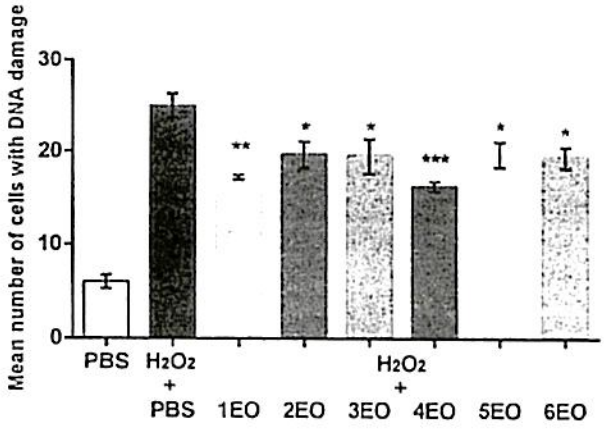

Figure 4. Antigenotoxic properties of essential oils of *H. officinalis* subsp. *aristatus* (1EO–6EO) against DNA damage in human peripheral blood leukocytes, induced by H_2O_2 in post-treatment protocol. Bars represent the mean value of cells with DNA damage ± standard error of the mean (SEM) versus control treated with H_2O_2 (n = 3). PBS: phosphate-buffered saline solution. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$.

The dominant compound in the 4EO was phenylpropanoid methyl eugenol. With regard to the 1EO, 2EO, 5EO and 6EO samples, the dominant constituent was an oxygenated monoterpene 1,8-cineole, whereas *cis*-pinocamphone was present in the highest amount among components of 3EO. For methyl eugenol [37] and 1,8-cineole [38], there are literature data that confirm their ability to neutralize free radicals, which could contribute to the antigenotoxic effect. The beneficial action of essential oils could be based on their participation in the direct neutralization of free radicals, but also their contribution to DNA molecule repair.

These results show that hyssop extracts and essential oils exhibit statistically significant antigenotoxic activity. Therefore, conduction of in vivo tests is needed to estimate the potential of *H. officinalis* preparations more reliably.

2.5. Cytotoxic Activity

A cytotoxic agent can induce cell death when it leads to cell demise or, on the other hand, it can cause reproductive cell death, inhibiting cell growth and proliferation, while the cell remains alive. This study aimed to determine the overall cytotoxic potential, cytostatic and cytocidal effects of extracts (1E–6E) against human tumor cell lines (SW480, MDA-MB 231 and HeLa). Additionally, these effects were examined on non-transformed human lung fibroblast cell line (MRC-5).

Cytotoxic effects of these extracts were examined in a range of seven concentrations after 24, 48 and 72 h of treatment by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye (MTT) colorimetric assay. MTT assay is a method that indirectly determines cell viability. MTT is a water-soluble yellow-colored crystal that easily passes through the cell membrane because of its positive charge. In metabolically active cells, MTT is reduced to non-soluble purple formazan crystals. Mitochondrial reductase (succinate dehydrogenase), active only in viable cells, catalyzes this reaction, so the reduction of the original compound to formazan is directly proportional to the number of viable cells. The obtained results are represented as dose-response curves (Supplementary Figure S2) and IC_{50}, SI (Table 6) parameters.

Table 6. Concentrations of extracts (μg/mL) that induce inhibition of biological activity in 50% cells (IC_{50}), 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and 50% lethality (LC_{50}) in the HeLa cell line, expressed as X ± SD. SI: selectivity index.

HeLa		1E	2E	3E	4E	5E	6E
IC_{50}	24 h	>100	>100	>100	>100	>100	>100
	48 h	22.72 ± 3.53	16.97 ± 2.10	44.38 ± 1.96	16.74 ± 1.43	25.90 ± 4.60	25.32 ± 7.80
	72 h	19.53 ± 1.03	15.15 ± 1.72	33.43 ± 1.36	14.97 ± 0.78	18.73 ± 0.53	20.04 ± 5.10
SI	24 h	0.97	1.52	1.71	1.31	1.10	3.61
	48 h	14.19	20.14	12.08	19.61	8.34	11.87
	72 h	12.17	13.87	8.30	15.04	11.31	7.82
GI_{50}	24 h	6.95 ±0.95	6.00 ± 0.34	98.09 ± 11.08	5.56 ± 0.18	7.67 ± 1.45	7.46 ± 0.99
	48 h	4.91 ± 0.84	3.49 ± 0.46	65.64 ± 3.66	2.54 ± 0.45	5.61 ± 1.22	5.75 ± 0.68
	72 h	0.86 ± 0.51	<0.3	59.38 ± 1.85	<0.3	4.97 ± 0.54	4.76 ± 0.52
TGI	24 h	16.90 ± 4.96	14.67 ± 0.58	>100	12.91 ± 0.44	47.25 ± 5.66	27.55 ± 1.89
	48 h	13.60 ± 1.75	11.00 ± 0.56	>100	9.18 ± 0.59	19.90 ± 4.38	16.77 ± 1.52
	72 h	13.27 ± 1.33	1.69 ± 0.36	>100	<0.3	18.42 ± 3.57	13.63 ± 1.26
LC_{50}	24 h	>100	>100	>100	>100	>100	>100
	48 h	61.09 ± 16.15	35.65 ± 1.16	>100	27.07 ± 1.56	63.66 ± 2.30	41.15 ± 6.75
	72 h	43.19 ± 10.03	26.02 ± 2.88	>100	20.28 ± 1.10	60.65 ± 1.59	31.20 ± 5.93

The data revealed that extracts 1E–6E displayed a statistically significant percentage of growth inhibition in a dose-dependent manner on all designated cell lines after 48 h and 72 h ($p < 0.05$); however, that trend was not noticed after 24 h of treatment ($p > 0.05$). Time-dependent growth inhibition was present only on the HeLa cell line with high statistical significance ($p < 0.0001$), while a significant time-dependent effect was revealed only at the highest examined concentration on MRC-5 and MDA-MB 231 cells ($p < 0.05$). On the other hand, the increase in growth inhibition of the SW480 cell line was independent of the exposure period.

To evaluate the overall inhibitory potential of the examined extracts, we calculated IC_{50} as a parameter of growth inhibition in relation to the control, which did not take into account the initial cell number at time zero. Examined extracts showed very low overall inhibitory activity against healthy cell line MRC-5, but also against the SW480 and MDA-MB 231 tumor cell lines, because their IC_{50} values exceeded the highest examined concentration (data not shown). On the other hand, the HeLa cell line was susceptible to their effect with high overall inhibition indicated by low IC_{50} values. Extracts 2E and 4E exhibited the strongest overall inhibitory activity after 48 h and 72 h of treatment, followed by extracts 1E, 5E and 6E, while extract 3E had the highest IC_{50}. Regardless, there was no statically significant difference among the tested extracts. Importantly, the extracts displayed activity highly selective for HeLa cells with selectivity index (SI) values that ranged between 8 and 20. Antitumor activity of most clinically applied agents is restricted because of their large spectrum of side effects and general toxicity, including to some normal cells. Although scientists continue to develop compounds with a targeted mechanism of action, many of those compounds still lack selectivity for tumor cells [39]. In that term, natural products are considered as less toxic for normal cells and as a biologically friendly approach, as evidenced by the large number of extracts and secondary metabolites in clinical trials [40].

Further, according to National Cancer Institute (NCI) recommendations [41], we calculated three parameters to disclose whether the examined extracts had cytostatic (GI_{50}, TGI) or cytocidal (LC_{50}) effects on designated cell lines (Table 6). The calculated parameters showed low to absent cytostatic or cytocidal activity of tested extracts 1E–6E against the SW480 and MDA-MB 231 cancer cell lines and, importantly, against the non-transformed cell line MRC-5 (data not shown). Contrarily, on the HeLa cell line, all examined extracts acted as very potent inhibitors of net cell growth with very low

GI_{50} values, especially extracts 2E and 4E, which exhibited a net cell growth inhibition for 50% of cells at concentrations lower than the minimum concentration examined after 72 h of treatment (GI_{50} < 0.3 μg/mL). Extracts 1E, 5E and 6E exhibited a net cell growth inhibition of 50%, with similar potency as the previous extracts. Compared to the extract of commercial hyssop herb (1E), only extract 3E had lower cell growth inhibition activity with a high statistical significance ($p < 0.0001$). The same trend was present in the perspective of total growth inhibition and cytocidal activity. Namely, extracts 2E and 4E provoked strong cytostatic effect after 72 h of treatment with TGI values of 1.69 μg/mL and <0.3 μg/mL, respectively. Also, LC_{50} values of these extracts were significantly lower than for other extracts ($p < 0.05$), indicating their potent cytocidal nature. Extracts 1E, 5E and 6E followed the same trend. The tumor grows when the total rate of division of its cells exceeds the total mortality rate. The ability to grow uncontrollably is gained through the accumulation of mutations of genes that manage cell proliferation and cell death. Therefore, the agents that can override these defects, stop uncontrolled cell division and kill cancer cells are beneficial in cancer treatment. Tested extracts 1E, 2E and 4E–6E showed, along with high selectivity, a potent ability both to inhibit cell proliferation and to induce cell death in a human cervical cancer cell line. Therefore, those extracts and their compounds should be further examined for their possible application in the therapy of this type of cancer.

The dominant compounds in the extracts are, as mentioned above, chlorogenic and rosmarinic acids, whose cytotoxic potential has been reported earlier [42,43]. The extract 4E had the highest content of chlorogenic and rosmarinic acids, as well as total phenolic compounds, while the extract 3E, which exhibited the weakest cytotoxic activity compared to other tested extracts, had the lowest contents of total phenols and rosmarinic acid. On the other hand, the extract 2E, which also gave very good results in this study, together with the extract 4E, was distinguished neither by the content of total phenols nor by chlorogenic or rosmarinic acids. The extracts 2E and 4E also showed antigenotoxic activity in the comet assay (post-treatment protocol). Therefore, we can conclude that chlorogenic and rosmarinic acid probably contributed to the overall cytotoxic potential of the methanol extract of hyssop herb. The contribution of individual components of the extract and/or their synergistic/additive action to selective cytotoxicity against HeLa cells is of particular interest and should be further investigated in the future.

3. Material and Methods

3.1. Chemicals and Reagents

The acetonitrile and methanol for the chemical analysis and antioxidant activity testing were from J.T. Baker Chemicals Co. (Phillipsburg, NJ, USA); formic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous sulphate, Folin–Ciocalteu (FC) reagent, ferric chloride and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA); L-ascorbic acid was procured from Acros (Geel, Belgium), whereas rosmarinic acid (≥99%), chlorogenic acid (>97%) and rutin were supplied by Carl Roth (Karlsruhe, Germany). Solvents used for the LC-DAD-MS analysis were of LC-MS grade, whereas the other solvents and reagents were of analytical purity.

For determination of genotoxic and antigenotoxic activity, phosphate-buffered saline solution (PBS) was purchased from Fisher Scientific (Pittsburgh, PA, USA); hydrogen peroxide was purchased from Zorka Pharma (Šabac, Serbia), low-melting-point agarose (LMPA) and normal-melting-point agarose (NMPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

For the investigation of cytotoxic activity, Dulbecco's modified Eagle medium (DMEM), heat-inactivated fetal bovine serum (FBS), L-glutamine, non-essential amino acids, dimethyl sulfoxide (DMSO), penicillin and streptomycin, as well as a trypsin and ethylenediaminetetraacetic acid (EDTA) combination dissolved in phosphate-buffered saline (PBS) were purchased from Sigma Aldrich (St. Louis, MO, USA). Finally, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye (MTT) was purchased from Sigma Aldrich (St. Louis, MO, USA).

3.2. Plant Material

The flowering aerial parts of *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman were collected from five localities in the territory of Montenegro (Table 1).

Identification of the plant material was carried out according to the *Flora Europaea* [44]. Voucher specimens (Table 1) were deposited in the herbarium of the Faculty of Natural Sciences and Mathematics in Podgorica (Montenegro), Department of Biology (TGU). The collected plant material was air-dried at room temperature.

In addition, a commercial sample of hyssop herb was purchased from a local production enterprise in Serbia. Commercial material was produced from the wild-growing plants collected from the sites located in southeastern Serbia (Pirot and Nišava Districts).

Prior to hydrodistillation or extraction, dried plant material was ground to a coarse powder.

3.3. Essential Oil Isolation

Essential oils were isolated from the plant material by hydrodistillation in a clevenger-type apparatus, according to Procedure I of the *Pharmacopoea Jugoslavica IV* (1984), suitable for isolation of essential oils lighter than water [45].

For calculating the yield of essential oils (%, based on the dry weight of the plant material), five consecutive volumetric determinations were performed.

The essential oils were kept at 4 °C in amber glass vials that were tightly closed and protected from light.

3.4. Extraction Procedure

Methanol extracts were prepared by bimaceration, according to the *Pharmacopoea Jugoslavica IV* (1984) [45]. Obtained methanol extracts were brought to dryness using a rotary evaporator under reduced pressure and a temperature below 50 °C, and subsequently, by a stream of nitrogen. Prior to analysis, the extracts were stored at 4 °C in tightly closed glass jars. The extracts were reconstituted just before the analysis by the addition of methanol up to the concentration of 5 mg/mL and filtered through a 0.45 µm membrane filter (Captive Syringe Filters, Agilent, Germany).

3.5. GC-MS Analysis of Essential Oils

Qualitative and semi-quantitative chemical analysis of essential oils was performed by gas chromatography coupled with mass spectrometry (GC-MS) on an Agilent Technologies 6890 Series gas chromatograph.

A 0.2 µL aliquot of each essential oil solution (10 µL/mL in hexane) was injected in split mode, with a split ratio of 1:20, at the temperature 220 °C. The components were separated on a nonpolar poly (tetramethyl-1,4-sulfenylenesiloxane) HP-5ms column (Agilent Technologies; 30 m × 0.25 mm, layer thickness 0.25 µm). The column was eluted in the temperature-programmed mode: initial temperature 60 °C, increase 3 °C/min to 246 °C (total analysis time 62 min). High-purity helium (5.0) was used as the carrier gas with a constant flow of 0.9 mL/min. The effluent was transferred to the Agilent Technologies 5975 series electron ionization mass spectrometer via a transfer line maintained at 280 °C The parameters of the mass spectrometer were as follows: electron energy 70 eV, ion source temperature 230 °C, quadrupole temperature 150 °C. Acquisition scan mode was applied in the range *m/z* 35–400 with a solvent delay of 2.30 min. To achieve a better agreement between the experimental and library spectra, the standard spectra tune was used.

Acquired data were processed using Agilent Technologies MSD ChemStation software (revision E01.01.335) in combination with NIST MS Search software (ver. 2.0d). The spectral libraries Wiley Registry of Mass Spectral Data [46], NIST/EPA/NIH Mass Spectral Library [47] and Adams' mass spectral library of essential oils (3rd Edition) [48] were used to identify mass spectra. The identity of the compounds was demonstrated by comparing the MS spectra and linear retention indices with the literature data. The relative proportion of compounds was determined using the area standardization method and expressed as area %.

3.6. LC-DAD-MS Analysis of Methanol Extracts

Liquid chromatography with diode array and mass spectrometry (LC-DAD-MS) was carried out on Agilent LC/MS System 1260/6130 (Agilent Technologies, Waldbronn, Germany), equipped with ChemStation software Rev. B.04.03-SP1, a degasser (model G1311B), a quaternary pump (G1311B/1260), an autosampler (G1329B), a diode array detector (DAD) (G4212B), a single quadrupole atmospheric pressure ionization - electrospray ionization (API-ESI) mass selective detector (MSD) (6130) and a reverse-phase column Zorbax SB-Aq (150 × 3.0 mm; particle diameter 3.5 µm, Agilent Technologies), maintained at an operating temperature of 25 °C.

The mobile phase consisted of 0.1% aqueous formic acid (phase A) and acetonitrile (phase B). The following gradient elution program was used: 10% B to 35% B (0–20 min), 35% B to 90% B (20–24 min), 90% B (24–25 min) and 90% B to 10% B (25–30 min), at a total operating time interval of 30 min, a mobile phase flow rate of 0.35 mL/min and an injection volume of 3.00 µL. UV spectral data of all peaks were collected in the range 190 to 640 nm, and chromatograms were recorded at 210, 270, 320 and 350 nm. API-ESI in the negative polarity and the range *m/z* 100–1000 was used for analyte ionization. The parameters of the ion source were as follows: fragmentation voltages 100 and 250 V, drying gas flow (nitrogen) 10.0 L/min, drying gas temperature 350 °C, nebulization pressure 40 psi and capillary voltage 3500 V.

The compounds were identified by comparing their UV and MS spectral data, and the retention times (Rt) with the corresponding data obtained for the standard compounds under the same chromatographic conditions, as well as by comparing the data with previously published literature data.

The contents of chlorogenic and rosmarinic acids were determined using the external standard method at 320 nm.

Stock solutions of chlorogenic acid (0.4 mg/mL) and rosmarinic acid (0.5 mg/mL) were prepared by dissolving the reference substances in methanol and their subsequent filtration through a syringe filter (0.45 µM, Captiva, Agilent). By further dilution with the same solvent, calibration standards with a wide range of concentrations were prepared. This procedure (including the preparation of stock solutions) was repeated three times so that three measurements were made for each calibration point. Calibration curves and coefficients of determination were obtained by linear regression analysis. Limit of detection (LoD) and limit of quantification (LoQ) were calculated according to the International Conference on Harmonization guidelines [49].

Chlorogenic acid calibration curve ($y = 26733x + 70.594$, $R^2 = 0.9998$; linearity range 0.02–0.4 mg/mL; LoD = 0.005 mg/mL; LoQ = 0.015 mg/mL) and rosmarinic acid ($y = 18000x + 39.94$, $R^2 = 0.9998$; 0.00625–0.5 mg/mL; LoD = 0.003 mg/mL; LoQ = 0.010 mg/mL) were used for contents determination.

3.7. Total Phenols

The contents of total phenolic compounds in dry methanol extracts were determined using Folin–Ciocalteu (FC) reagent according to the method described by Velioglu et al. [50]. The results were expressed as mg of gallic acid equivalents (GAE) per g of dry extract (mg GAE/g) and represent the mean value of the three consecutive measurements.

3.8. Antioxidant Activity

The antioxidant activity of investigated extracts was assessed by a set of two commonly used tests: DPPH radical scavenging assay and FRAP assay.

3.8.1. DPPH Assay

The DPPH radical scavenging assay was carried out according to the methodology described by Kukić et al. [51] with slight adaptations that were necessary for conducting the assay on microtiter plates.

The methanol solutions of the tested hyssop extracts were prepared in different concentrations, along with the standard methanol solutions of rutin. The test solution consisted of a mixture of 0.1 mL of the methanol solution of tested extract, 0.1 mL of methanol and 0.05 mL of 0.5 mM methanol solution of DPPH. The mixtures were shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbances were measured at 492 nm against methanol as a blank test on Biochrom EZ Read 400 microtiter plate reader. The negative control consisted of 0.2 mL of methanol and 0.05 mL of 0.5 mmol/L DPPH solution. DPPH inhibition was calculated according to the following formula:

$$I\,(\%) = (Ac - At)/Ac \times 100 \quad (1)$$

where Ac is the absorbance of the control and At is the absorbance of test solutions.

The results are expressed as half-maximum inhibitory concentration (IC_{50} values; µg/mL) values, which denote the concentrations that neutralize 50% of DPPH radicals and are the mean values of the three consecutive determinations.

3.8.2. FRAP Assay

The total antioxidant activity of dry methanol extracts of hyssop herb and standard solutions was determined using the ferric reducing/antioxidant power (FRAP) assay, essentially as described by Pellegrini et al. (2003) [52].

In brief, the test, standard (rutin, 0.1 mg/mL; ascorbic acid, 0.05 mg/mL) or control solutions were transferred (0.1 mL) into test tubes and 3.0 mL of ex tempore prepared FRAP reagent (25 mL acetate buffer, 300 mmol/L, pH 3.6 + 2.5 mL 10 mmol/L TPTZ in 40 mmol/L HCl + 2.5 mL 20 mmol/L $FeCl_3 \times 6H_2O$) was added. The absorbances were recorded at 593 nm against a blank containing 0.1 mL of solvent after 30 min incubation at 37 °C. FRAP values were calculated from the calibration curve of $FeSO_4 \times 7H_2O$ solutions, covering the concentration range between 100 and 1000 µmol/L, and expressed as mmol Fe^{2+}/g dry extract (mmol Fe^{2+}/g). All measurements were performed in triplicate.

3.9. Genotoxic and Antigenotoxic Activity

Peripheral blood samples from three volunteer subjects (21–35 years of age), were collected using a method of finger extraction into heparinized containers and immediately subjected to the experiment. The subjects were non-smokers and they had taken neither medications nor alcohol and dietary supplements. They signed their written consent, in accordance with the regulations of the ethical standards of the Ethics Committee for Biomedical Investigations at the Faculty of Pharmacy in Belgrade.

Dry methanol extracts of the hyssop herb were dissolved in the phosphate-buffered saline solution (PBS); the essential oils were dissolved in absolute ethanol and then diluted with PBS.

3.9.1. Genotoxic Activity Assay

Human whole blood cells (WBC) were incubated with different concentrations of essential oils (12.5, 5 and 2.5 µg/mL) or methanol extracts of hyssop herb (100, 200 and 400 µg/mL) for 30 min at 37 °C. Tested concentrations were selected on the basis of the literature data [53,54]. The samples were treated in parallel with a negative control of PBS for 30 min at 37 °C, as well as with a positive control of hydrogen peroxide (H_2O_2, 50 µM) for 20 min at 4 °C and PBS for 30 min at 37 °C. A concentration of 50 µM H_2O_2 was the lowest one that induced a statistically significant increase of DNA damage in the tested cells.

3.9.2. Antigenotoxic Activity Assay

The antigenotoxic activity of extracts or essential oils (which did not show activity in the genotoxicity assay) was analyzed in the post-treatment [55,56]. WBC were treated with H_2O_2 for 20 min at 4 °C and washed with PBS; afterward, they were incubated for 30 min at 37 °C with tested essential oils/methanol extracts. The concentration of

400 µg/mL was chosen for further testing, because it was the most effective concentration in the antigenotoxic assessment of commercial extract. The attenuation of H_2O_2-induced DNA damage in human peripheral blood leukocytes in the post-treatment with EO was assessed using the concentration that did not induce a statistically significant increase of DNA damage in the tested cells in the genotoxicity assessment (2.5 µg/mL).

3.9.3. Comet Assay

The comet assay (single-cell gel electrophoresis), which is normally used to detect DNA damage, was performed according to the protocol described by Singh et al. (1988), called the alkaline method, according to which DNA breaks and alkaline labile sites are detected, while the degree of DNA damage is indicated by the extent of DNA molecule migration [57].

A quantity of 100 µL of the test suspension, containing 6 µL of peripheral blood suspended in 0.67% of LMPA, was evenly applied to the prepared microscopic plates coated with a layer of 1% NMPA. Cover glasses were placed on the plates and then left at 4 °C for 5 min. The cover glasses were slowly removed and the cells were exposed to the appropriate treatment (as previously described for genotoxic or antigenotoxic activities). After treatment, the third layer of 100 µL of 0.5% LMPA was applied, cover glasses were placed and the plates were again left at 4 °C for 5 min. The cover glasses were again carefully removed, the microscope plates were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X100 and 10% DMSO; pH 10 adjusted with NaOH) and kept at 4 °C overnight. After cell lysis, DNA denaturation was performed in the same solution that was later used for electrophoresis for 30 min (10 M NaOH, 200 mM EDTA, pH $\geq$ 13).

Electrophoresis was performed at 25 V and 300 mA for 30 min. After electrophoresis, alkali neutralization in gels was performed by rinsing twice with a neutralizing buffer (0.4 M Tris, pH 7.5) for 10 min, and then with distilled water. After rinsing, the comet was stained and visualized with fluorescent dye-ethidium bromide solution (20 µg/L). After staining (15 min), the plates were ready for analysis.

All experiments were done three times, in duplicate.

To determine the degree of DNA damage for each donor and each concentration, 200 nucleoids (comets) were selected and analyzed by random selection, or 100 from each duplicate microscope plate, using an Olympus BX 50 fluorescent microscope (Olympus Optical Co., GmbH, Hamburg, Germany) with a mercury short arc lamp (HBO) (50 W, 516–560 nm Carl Zeiss Microscopy, Jena, Germany) at 100 × magnification.

The analysis was performed by determining the length and density of DNA in the "tail" based on which of the nucleoids (comets) were classified into five groups, as described by Anderson et al. (1994) [58]: A—without the "tail" (<5% DNA damage), B—low degree of damage (5%–20%), C—medium degree of damage (20%–40%), D—high degree of damage (40%–95%) and E—complete DNA damage (>95%). The degree of total DNA damage was expressed as the sum of all DNA damage/migrations over 5% (B + C + D + E).

3.10. Investigation of Cytotoxic Activity of Hyssop Herb

3.10.1. Cell Lines and Cultures

The potential cytotoxic effect of methanolic extracts was examined on the human cervix (HeLa), breast (MDA-MB 231) and colon (SW480) cancer cell lines, while their selectivity was tested on non-transformed human lung fibroblasts (MRC-5). Cell lines were obtained from American Type Culture Collection (ATCC).

Cells were cultured in DMEM at pH = 7.4, enriched with 10% heat-inactivated FBS, L-glutamine, non-essential amino acids (0.1 mM), penicillin (100 IU/mL) and streptomycin (100 µg/mL). Cultivation was performed in T-25 flasks (ThermoFisher Scientific, Waltham, MA, USA) in an aseptic environment under standard culture conditions (37 °C, absolute humidified air and 5% CO_2). The media were changed when necessary and cells were subcultured every fifth day. Just before in vitro experiments, subconfluent cell monolayers

(~80%) in the logarithmic growth phase were detached from the bottom of the flask b short-term treatment with 0.25% trypsin and 0.53 mM EDTA combination dissolved i phosphate-buffered saline.

3.10.2. Extract Solutions

The stock solutions were prepared by dissolving methanol extracts in DMSO 50 mg/mL and stored at 4 °C. Preceding the treatment, fresh working solutions of the e tracts at different concentrations were prepared by diluting stock solution in supplemente DMEM. The final DMSO concentration in working solutions was lower than 0.5% (*v/v*).

3.10.3. MTT Assay

The cytotoxic potential of the extracts against HeLa, MDA-MB 231, SW480 and MR 5 was evaluated in vitro by MTT assay as a common colorimetric technique for cell viabili determination [59].

The cells were seeded in 96-well flat-bottom microtiter plates (ThermoFisher Scientifi Waltham, MA, USA) at a density of 5×10^3 cells per well and incubated overnight t adhere. After 24 h, the supernatant was replaced with extract solutions at seven differer concentrations (0.3, 1, 3, 10, 30, 100 and 300 µg/mL). In the control wells, cells grew in th presence of supplemented nutrient media only. The cells were incubated for 24, 48 and 72 The MTT solution, at a final concentration 0.5 mg/mL in the unsupplemented mediur was added to each well at time zero (after overnight incubation) and the end of differer incubation periods. Following 2 h of incubation, the MTT solution was discarded an formazan crystals were solubilized with 150 µL of DMSO. The plates were shaken for 5 mi and the absorbance was measured at 550 nm with a multiplate reader (Zenyth 3100, Anthc Labtec Instruments GmbH, Wals-Salzburg, Austria).

All experiments were repeated at least three times in triplicate.

3.10.4. Cytotoxicity Parameters

The results of the MTT assay are presented as the percentage of the values for contr cells that was arbitrarily set to 100%. Cell growth inhibition was calculated according t the expression:

$$(A_0 - A) \times 100/A_0 \quad (3$$

where A_0 is absorbance from control wells and A is absorbance from wells exposed to th tested extracts.

The measure of the overall inhibitory activity of the agent was evaluated through IC_5 which is defined as the concentration of the agent that inhibits the biological activity of th target cells by 50%.

The selectivity index (SI) was calculated as the quotient of IC_{50} values for the treate non-transformed cell line and the IC_{50} values for the tested extracts on malignant cell $SI < 2$ indicates general toxicity of a compound, $SI \geq 2$ indicates selective toxicity an $SI \geq 3$ indicates highly selective toxicity [60].

Following the NCI recommendations [41], GI_{50}, TGI and LC_{50} parameters were calc lated for each extract:

- The GI_{50} value is the concentration where $100 \times (T - T0)/(C - T0)$ equals 50 an measures the growth inhibitory power of the examined extracts;
- The TGI value is the concentration of the tested extract where $100 \times (T - T_0)/(C - T$ equals 0 and measures the cytostatic effect;
- The LC_{50} value is the concentration of the drug where $100 \times (T - T_0)/T_0$ equal 50 and measures the cytotoxic effect of extracts.

In these formulas, T_0 is the absorbance at time zero (when the compound is added Tis the absorbance of the test well after 24, 48 or 72 h of exposure to the test compound an C is the optical density of the control wells (cells incubated for 48 h with no additives).

the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

3.11. Statistics

The results of the experiments are represented as mean ± standard deviation.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) using Statistica® v.8.0 (www.statsoft.com, accessed on 1 April 2021) and StatistiXL® Version 2.0 add-in for MS Excel® (www.statistixl.com, accessed on 1 April 2021) were applied to examine the interrelationships between the chemical compositions of the essential oils.

The results of LC-MS quantitative analysis and assays on antioxidant activity were analyzed by SPSS software (version 20.0) using one-way ANOVA and post hoc Tukey's test. Differences between the mean values were considered statistically significant if $p < 0.05$.

The IC_{50}, GI_{50}, TGI and LC_{50} parameters were calculated using MS Office Excel® free add-in ED50 plus v1.0 software (www.sciencegateway.org/protocols/cellbio/drug/data/, accessed on 1 April 2021). SPSS software version 20 was used for statistical data analysis. The Shapiro–Wilk test was used to test the normality of data distribution. Depending on the results normality test, for the comparison of groups, one-way analysis of variance (ANOVA) or its non-parametric equivalent Kruskal–Wallis test was used.

For the genotoxic and antigenotoxic activity assays, the results are expressed as the mean value ($n = 3$) ± standard error of the mean (SEM). Statistical analysis of the comet assay results was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test for comparisons of different treatments vs. the respective controls. GraphPad Prism 6.0 software was used. A regression was used to determine the effect of the bioactive substance concentration on the outcome. The values of the obtained data were considered statistically significant if $p < 0.05$, and statistically highly significant if $p < 0.001$.

4. Conclusions

This study evaluated the antioxidant activity and genotoxicity/antigenotoxicity, as well as cytotoxic, cytostatic and cytocidal effects against human tumor and non-transformed human lung fibroblast cell lines of the investigated *Hyssopus officinalis* essential oils and/or extracts, with respect to their chemical composition. Our results revealed high variability in the composition of essential oils, as three chromatographic profiles of the investigated essential oils of wild-growing plants from Montenegro could be distinguished: oils rich in 1,8-cineole and relatively rich in β-pinene, but low in *cis*-pinocamphone; oils rich in β-pinene, limonene, *cis*-pinocamphone and methyl eugenol, but relatively low in 1,8-cineol; and oils relatively rich in 1,8-cineole, limonene, β-pinene and *cis*-pinocamphone. The essential oil from the commercial plant material from Serbia, being rich in 1,8-cineole and β-pinene, but low in *cis*-pinocamphone, appeared similar to only one of the samples obtained from wild-growing plants from Montenegro. Both the extracts and the essential oils significantly reduced in vitro DNA damage. In addition, potent and selective cytotoxic action of the hyssop methanol extracts on the HeLa cell line was observed. These findings deserve closer attention and our further investigations, which will be performed in the prospective future, and should be directed to the panel of cancer cell lines derived from the most sensitive tissue (cervix), along with a detailed mechanism of antitumor effect and the isolation/chemical characterization of the constituents that are presumably responsible for observed activity.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10040711/s1, Figure S1: A comparative view of LC-DAD chromatograms of methanol extracts (1E–6E) of *H. officinalis* recorded at 320 nm, Figure S2: Dose-response curves in MTT assay after 24, 48 and 72 h treatment of MRC-5 (a–f), SW480 (g–l), MDA-MB 231 (m–r) and HeLa (s–x) cell lines with extracts 1E–6E, Table S1: Component loadings and score coefficients for constituents of *Hyssopus officinalis* essential oils.

Author Contributions: T.M. participated in all the experiments (as a part of her PhD work) and wrote the manuscript draft. D.T. performed investigation on genotoxicity and antigenotoxicity, the visualization of the data and contributed to writing the original draft. L.Ž. performed investigation and provided resources for the tests on genotoxicity and antigenotoxicity. B.S.-P. performed investigation provided methodology for the tests of genotoxicity and antigenotoxicity, and conducted supervision. V.J. provided resources, methodology and supervision for investigations on cytotoxic activity S.M., S.P. and D.B. performed investigations on cytotoxic activity, their validation, visualization and writing of the original draft. D.S. (Danijela Stešević) and D.S. (Danilo Stojanović) performed field investigations, identified plant material and performed the writing, review and editing process. S.S performed chromatographic analyses and the interpretation of collected data, and also contributed to writing of the original draft and the review and editing processes. Z.M. was responsible for the conceptualization, resources, supervision, and the writing, review and editing process. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study on genotoxic and antigenotoxic activity was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committee for biomedical investigations at the Faculty of Pharmacy in Belgrade (protocol code 1121/2; date of approval: 31 August 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study on genotoxic and antigenotoxic activity.

Data Availability Statement: Data available upon request.

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In vitro, *in vivo* and *in silico* evaluation of the anti-inflammatory potential of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (Lamiaceae)

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ABSTRACT

Ethnopharmacological relevance: Medicinal properties of hyssop have been used in traditional medicine since ancient times, inter alia, in diseases/conditions with an inherent inflammatory process.
Aim of the study: Accordingly, the aim of this study was to investigate the anti-inflammatory properties of hyssop herb preparations (essential oil and methanol extracts) *in vivo*, *in vitro* and *in silico*.
Materials and methods: For *in vitro* testing of essential oils and extracts of hyssop herb, the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme assays were used. *In vivo* anti-inflammatory potential of the extracts (at doses of 50, 100 and 200 mg/kg) was assessed using the carrageenan-induced rat paw edema test. Molecular docking and dynamics were used for *in silico* testing of the inhibitory activity of chlorogenic (CA) and rosmarinic (RA) acids, as the dominant compounds in the tested methanol extracts against COX-1 and COX-2 enzymes.
Results: Significant inhibitory activity was shown in the COX-2 test regarding extracts (essential oils did not exhibit any significant activity). Namely, all analyzed extracts, at a concentration of 20 μg/mL, showed a percentage of inhibition of COX-2 enzyme (54.04–63.04%), which did not indicate a statistically significant difference from the positive control of celecoxib (61.60%) at a concentration of 8.8 μM. *In vivo* testing showed that all methanol extracts of hyssop herb, at the highest test dose of 200 mg/kg in the third and fourth hours, after carrageenan administration, exhibited a statistically significant ($p < 0.05$) inhibitory effect on the increase in rat paw edema in relation to control. This activity is comparable or higher in relation to the reference substance, indomethacin, at a concentration of 8 mg/kg. The preliminary *in silico* results suggest that investigated compounds (RA and CA) showed better inhibitory activity against COX-1 and COX-2 than standard non-steroidal anti-inflammatory drug (NSAID), ibuprofen, as evident from the free binding energy (ΔG_{bind} in kJ mol^{-1}). The binding energies of the docked compounds to COX-1 and -2 were found to be in the range between −47.4 and −49.2 kJ mol^{-1}. Ibuprofen, as the one NSAID, for the same receptors targets, showed remarkably higher binding energy (ΔG_{bind} = −31.3 kJ mol^{-1} to COX-1, and ΔG_{bind} = −30.9 kJ mol^{-1} to COX-2).

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Conclusion: The results obtained not only support the traditional use of hyssop herb in inflammatory conditions in folk medicine, but also open the door to and the need for further *in vivo* testing of extracts in order to examine the molecular mechanism of anti-inflammatory activity in living systems and possibly develop a new anti-inflammatory drug or supplement.

1. Introduction

Inflammation is a protective response of the microcirculation triggered after infection/injury, which aims to eliminate the effects of harmful stimuli (Ballerini and Gilroy, 2016). Inflammatory processes are associated with various acute and chronic diseases (Yang et al., 2017), many of which pose a current problem today such as rheumatoid arthritis, multiple sclerosis, chronic asthma, inflammatory bowel disease, psoriasis, cardiovascular and malignant diseases (Coussens and Lachab, 2000). The prevalence of these diseases, especially obesity, cardiovascular, neurodegenerative diseases, and cancer, has increased rapidly over the past decades (Okin and Medzhitov, 2012).

The main anti-inflammatory drugs are glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs (aspirin, ibuprofen, naproxen) manifest their activity by inhibiting enzymatic activities of cyclooxygenases-1 and -2 (COX-1 and COX-2), key enzymes in the conversion of arachidonic acid to prostaglandins, most often via non-selective inhibition (Furst and DuBois, 2002). However, due to numerous adverse reactions, which can be serious, especially with long-term use, associated with the use of existing anti-inflammatory drugs, e.g. NSAID: cardiotoxicity and gastrointestinal toxicity (Singh et al., 2021); glucocorticoids: osteoporosis, diabetes, hypertension, skin hypertrophy, adrenal suppression, and glaucoma (Oray et al., 2011), alternative compounds, which would exhibit a satisfactory anti-inflammatory effect with fewer or no side effects, have been sought. This primarily refers to natural compounds such as plant polyphenols (Pushpa and Mahesh, 2017). Particular problem, in terms of side effects, represents a group of selective COX-2 inhibitors called COXIBs, thus commercially available were celecoxib (Celebrex) and rofecoxib (Vioxx). Many compounds that are selective COX-2 inhibitors have been developed for therapeutic use because they were thought to selectively block inflammation caused by the action of COX-2, without the danger of causing any gastrointestinal problems and side effects by inhibition of the COX-1 enzyme. However, rofecoxib was withdrawn from the market due to a significant increase in cardiac stroke risk (Vonkeman and Van de Laar, 2010). This is just one of the reasons for directing the attention of the scientific community towards discovery of new sources of COX-2 inhibitors, with a special focus on plant sources (Rosano et al., 2016; Samadder et al., 2018; Wang et al., 2013). Many medicinal plants are known for their anti-inflammatory action and are used not only in traditional medicine, but also in conventional therapy. Based on their action, they are also classified in numerous world pharmacopoeias, e.g. *Calendula officinalis* L., *Matricaria chamomilla* L., *Salvia officinalis* L., *Achillea millefolium* L., etc., have widespread use in treatment of various inflammation-based ailments (Shikov et al., 2014). Of natural products from plant origin, there are several groups of compounds that are carriers of anti-inflammatory effects, e.g., phenolic compounds (phenolic acids, flavonoids, anthocyanins), terpenes, lignans, etc. (Calixto et al., 2003, 2004; Fürst and Zündorf, 2014) detected in medicinal plants of diverse genera. For these purposes, the plant species, which have been found to be effective in inflammatory processes over the years of use in traditional medicine, are first examined. One such plant is hyssop, *Hyssopus officinalis* L. (Lamiaceae).

Hyssop is a medicinal and aromatic plant, whose medicinal properties have been used in traditional medicine since ancient times. It is also used in the food and cosmetics industry and as a decorative plant. In traditional medicine, it is, inter alia, used for digestive and intestinal disorders, as well as to treat respiratory diseases such as tuberculosis, asthma, chronic catarrh, and bronchitis; it is also valued in the treatment of rheumatic pains, bruises, wounds, burns, frostbite, skin irritation (Dulgerova, 2010; Teodor, 2010). This herb has been used traditionally as an expectorant, carminative, anti-inflammatory, anti-catarrhal, and antispasmodic medicine in many parts of the world (Srivastava et al., 2018; Vlase et al., 2014). Since ancient times hyssop has been used to treat rheumatic pains, bruises, wounds, etc. (Hajdari et al., 2018). The herb was used to alleviate digestive disorders, cure laryngitis and to accelerate wound healing in folk medicine of different nations. It is used in tea blends for cough relief, antispasmodic effects and relieving catarrh (Srivastava et al., 2018). In Southern Europe, it was used in the treatment of nose, throat, and lung afflictions due to its anti-inflammatory properties. Generally, these therapeutic uses and health benefits of hyssop are largely based on folklore rather than on scientific substantiation (Vlase et al., 2014). There are also literature data speaking in favour of the anti-inflammatory activity of this species; for example, Wang et al. (2011) indicated that the aqueous extract of hyssop herb had a potential to regulate T helper cell differentiation, thus contributing to anti-inflammatory activity. Ma et al. (2014) showed that *in vivo* application of *H. officinalis* aqueous extract regulated the levels of eosinophil in bronchoalveolar fluid and immunoglobulin IgG and IgE in serum. *H. officinalis* was reported not only to exhibit anti-inflammatory activity but also to affect immune regulation (Ma et al., 2014).

Traditional use and previous literature data provide a good basis for testing the anti-inflammatory activity of hyssop herb preparations. There is little scientific evidence on *H. officinalis* anti-inflammatory activity regarding its impact on cyclooxygenase enzymatic activities and *in vivo* assessment of anti-inflammatory potential. This study aims to reveal the anti-inflammatory activity of *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman essential oils and methanolic extracts from the aerial parts of the plant. Namely, the anti-inflammatory activity of methanol extracts and essential oils of hyssop herb was tested *in vitro*, and then the testing of extracts was continued *in vivo*; the ability of the dominant compounds of the methanol extracts (chlorogenic and rosmarinic acids) to inhibit the activity of cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes, was assessed *in silico*. The results of basic phytochemical analyses of essential oils (gas chromatography-mass spectrometry, GC-MS) and methanol extracts (liquid chromatography with diode array detection and mass spectrometry, LC-DAD-MS), samples used in this study, were reported recently by our research group (Mićović et al., 2021).

2. Material and methods

2.1. Chemicals and reagents

For determination of *in vivo* anti-inflammatory activity, carrageenan and indomethacin were purchased from Sigma (Sigma, St. Louis, MO, USA); purified COX-1 from ram seminal vesicles, human recombinant COX-2 and arachidonic acid from Cayman Chemical (Ann Arbor, MI, USA); porcine hematin and indomethacin from MP biomedicals LLC (Solon, OH, USA), L-epinephrine bitartrate from Fluka (Buchs, Switzerland), Na_2EDTA from Merck (Darmstadt, Germany), TRIS/HCl-buffer from Roth (Karlsruhe, Germany). Celecoxib was purchased from Sigma-Aldrich Handels Gmbh (Vienna, Austria) and PGE_2 ELISA kit from Enzo Life Sciences (Farmingdale, NY, USA).

2.2. Plant material and samples preparation

The aerial parts of wild-growing *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman were collected during blooming in September 2018,

from five different locations in the territory of Montenegro. The taxonomical evaluation was done by Prof. Dr. D. Stešević and voucher specimens are kept at the Herbarium of the Department of Biology of the Faculty of Natural Sciences and Mathematics in Podgorica (University of Montenegro). Additionally, a commercial sample of hyssop herb, which was produced from the wild-growing plants collected at localities in southeastern Serbia (Pirot and Nisava Districts), was purchased from a local production enterprise in Serbia and identified by Prof. Dr. D. Stojanović.

Depending on the origin of the plant material, the following designations from 1 to 6 were used in the paper: 1 (Comercial sample, Serbia); 2 (Kuci, Montenegro, Voucher No. 1420263), 3 (Savnik, Montenegro, Voucher No. 1420261); 4 (Piva, Montenegro, Voucher No. 1420162); 5 (Piperi, Montenegro, Voucher No. 1420259); 6 (Cuce, Montenegro, Voucher No. 1420260). The procedures of essential oils distillation and extracts preparation were already described in details as published by Mićović et al. (2021). Disstillation of essential oils was done according to Procedure I of the Pharmacopoea Jugoslavica IV (1984) while the extracts preparation was done with methanol by bimaceration according to the same reference. Prior to further analysis, the obtained essential oils (EO) were kept at 4 °C in sealed glass vials, protected from light. The extracts were stored at 4 °C in glass, well-sealed jars, protected from light, prior to further analysis.

2.3. In vitro prostaglandin synthase inhibitory activity (cyclooxygenase-1 and -2 assays)

The method for testing the ability of a sample to inhibit the activity of COX-1 and COX-2 enzymes is based on the enzyme-linked immunosorbent assay (ELISA) quantification of the formed product, prostaglandin E_2 (PGE_2). The tests were performed in 96-well flat-bottomed microtiter plates using COX-1 from ram seminal vesicles, or human recombinant COX-2, according to the method described by Dobrić et al. (2005). The incubation mixture contained 180 μL 0.1 M TRIS/HCl buffer (pH 8.0), 5 μM hematin, 18 mM epinephrine hydrogen tartrate, 0.2 U enzyme and 50 μM sodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) (only for COX-2 test). Samples (solutions of tested essential oils or methanol extracts of *H. officinalis* herb in dimethyl sulfoxide (DMSO)) were added in a volume of 10 μL and the mixture was preincubated for 5 min at room temperature. Indomethacin (purity ≥99%) and celecoxib (purity ≥98%), previously dissolved in ethanol p.a. (*pro analysi*), were used for the positive control. The following concentrations were used in the experiment: essential oils and extracts: 20 μg/mL; indomethacin 1.2 μM; celecoxib 8.8 μM. To start the reaction, 10 mL of 5 mM arachidonic acid in ethanol p.a. was added and the mixture was incubated for 20 min at 37 °C. Thereafter, 10 mL of 10% formic acid was added to stop the reaction. The concentration of PGE_2, the major arachidonic acid metabolite in this reaction, was determined using a competitive PGE_2 EIA kit, which was assessed using a microtiter plate reader (Tecan Rainbow, Tecan Group Ltd., Maennedorf, Switzerland), according to the method described by Fiebich et al. (2000).

The inhibition of COX enzyme activity refers to a decrease in PGE_2 production compared to a blank test without inhibitor. All results were expressed as percentage of inhibition of COX-1 and COX-2 enzyme activity, respectively. Each analysis was performed in two replicates on two consecutive days, and finally the mean value of 4 measurements ± standard deviation was taken for both tests (COX-1 and COX-2).

2.4. In vivo anti-inflammatory activity

2.4.1. Animals

Male *Wistar albino* rats (200–250 g), eight weeks old, were kept under strictly controlled conditions (at a temperature of 22 ± 2 °C, light: darkness cycle 12:12 h). Water and food were available *ad libitum* (Nosov et al., 2016).

This investigation was performed at the Faculty of Medical Sciences, University of Kragujevac, Serbia. The study protocol (code 01–6121) was performed in accordance with the regulations of the Faculty's Ethical committee for the welfare of laboratory animals and principles of the Good laboratory practice and European Council Directive (86/609/EEC).

2.4.2. Carrageenan-induced paw edema and determination of the anti-inflammatory activity of extracts

Testing of the anti-edematous activity was performed in the carrageenan-induced rat paw edema model (Nosov et al., 2016; Ganga et al., 2012). Inflammation in all rats was induced by intraplantar injection of 1 mL of 0.5% carrageenan saline in the left hind paw. The tested extracts were administered by intraperitoneal (IP) injection in three doses: 50, 100 and 200 mg/kg. Each of the three experimental groups consisted of 10 rats. Animals from the indomethacin group (10 rats) were treated with IP injection of indomethacin suspended in saline at a dose of 8 mg/kg. Animals from the control group (10 rats) were treated with IP injection of saline. All tested agents (extracts, indomethacin, saline) were administered for 60 min before inducing inflammation (Nosov et al., 2016; Ganga et al., 2012).

In order to quantify the anti-inflammatory effect, the thickness of the left paw tissue of each rat was measured at the following time intervals: immediately before inducing inflammation (moment 0) and 1, 2, 3, 4 h (moments 1, 2, 3 and 4) after inflammation. Tissue thickness was measured in the middle of rat paw using a Digital vernier caliper (Aerospace, China). The percentage of inhibition of paw edema was calculated according to the formula, Eq (1):

$$\% \text{ Inhibition} = 100 \times [1 - (Yt / Yc)] \quad (1)$$

where Yt = average increase in paw thickness in the treated group of rats between two measurement moments, and Yc = average increase in paw thickness in the untreated group of rats between two measurement moments (Ganga et al., 2012).

2.5. In silico studies

2.5.1. Molecular docking

The binding affinity of rosmarinic acid (RA) and chlorogenic acid (CA) towards COX-1 and COX-2 receptors was estimated using molecular docking simulations using the AutoDock 4.2 software (Morris et al., 2009). The pockets and binding sites of COX-1 and COX-2 were determined by the AutoGridFR (AGFR) program. The crystal structures of COX-1 (PDB ID: 1EQG (Sidhu et al., 2001)) and COX-2 (PDB ID: 4PH9 (Orlando et al., 2015)) were extracted from RCSB Protein Data Bank in PDB format. The target receptors were prepared for docking by removing the co-crystallized ligand, water molecules, and cofactors. For this purpose, the Discovery Studio 4.0 (BIOVIA, 2020) was employed. The AutoDockTools (ADT) (Morris et al., 2009) graphical user interface was used to calculate the Kollman partial charges and add the polar hydrogens. The flexibility of the ligands was analyzed, while the protein kept on as the rigid structure in the ADT. The bonds of ligands were set to be rotatable to express their flexibility. The Lamarckian Genetic Algorithm (LGA) method was used for protein-ligand flexible docking. The parameters for the LGA method were determined as follows: a maximum number of energy evaluations was 250,000, a maximum number of generations was 27,000, and mutation and crossover rates were 0.02 and 0.8, respectively. The algorithms in the AutoDock 4.2 software were set up to predict the position of compounds within the protein target and to assess them by scoring functions by setting the grid box. The grid boxes with dimensions 48 × 44 × 44 $Å^3$ and 44 × 38 × 32 $Å^3$ in -x, -y, and -z directions of COX-1 and COX-2 receptors were used to cover the protein binding site and accommodate ligand to move freely. For Auto Grid runs, a grid point spacing of 0.375 Å was used. The interactions between the target protein and investigated compounds as the three-dimensional (3D) results were analyzed and illustrated in

Discovery Studio 4.0 and AutoDockTools.

The AutoDock program calculates these values according to the following equation, Eq (2):

$$\Delta G_{bind} = \Delta G_{vdw + hbond + desolv} + \Delta G_{elec} + \Delta G_{total} + \Delta G_{tor} - \Delta G_{unb} \quad (2)$$

where ΔG_{bind} is the estimated free energy of binding, the $\Delta G_{vdw + hbond + desolv}$ denotes the sum of the energies of dispersion and repulsion (ΔG_{vdw}), hydrogen bond (ΔG_{hbond}), and desolvation (ΔG_{desolv}). The ΔG_{total} represents the final total internal energy, the ΔG_{tor} is torsional free energy, ΔG_{unb} is unbound system's energy, and ΔG_{elec} is electrostatic energy. Ligand efficiency (LE) denotes the binding energy of ligand to protein per atom. LE (Eq (3)) has a unit of kJ mol^{-1}/heavy atom.

$$LE = \frac{\Delta G_{bind}}{N} \quad (3)$$

where N is the number of non-hydrogen atoms.

2.5.2. Molecular dynamics

The best docked complex RA-COX-1 was used as starting model for MD simulations. The RA was parameterized by the CHARMM36 force (Best et al., 2012). The CHARMM-GUI web server (Lee et al., 2016) was employed for the preparation of complex protein-ligand input for equilibration and the production. The solvation of investigated system was performed by the 0TIP3P solvation model. The sodium chloride ions were added to neutralize the systems to a salt concentration of 0.15 M in KCl. The energetically minimization of the neutralized system was done by steepest descent and conjugate gradient algorithms with up to a tolerance of 1000 kJ mol^{-1} nm^{-1} during 5000 steps. After that, system was equilibrated at 310.15 K using the Berendsen weak coupling method in NVT (constant volume) ensemble condition with a 2 ns time scale. The LINCS algorithm was use for the production MD phase in the NPT ensemble for 20 ns time scale including a modified Berendsen thermostat ($\tau T = 1$ ps) and a Parrinello-Rahman barostat ($\tau P = 2$ ps) (Berendsen et al., 1984). The GROMACS 5.1.5 package (Abraham et al., 2015) was employed for the simulation trajectories propagation to 20 ns.

2.6. Statistical analysis

In the COX-1 and COX-2 tests, the methods of descriptive statistics (arithmetic mean, standard deviation, and standard error of the arithmetic mean) were employed. Student's t-test for two independent samples was used to compare the arithmetic means, assuming a significance level of 0.05.

For *in vivo* anti-inflammatory activity, average value ± standard deviation (SD) and minimal and maximal values were used as parameters of descriptive statistics. The normality of the parameter distribution was evaluated with the Shapiro–Wilk and Kolmogorov-Smirnov tests. Additionally, data were analyzed using a one-way analysis of variance (ANOVA) and the post hoc Bonferroni test for multiple comparisons. The statistical significance was based on $p < 0.05$. Complete statistical evaluation was performed with SPSS Statistics 22 (SPSS, Chicago, IL).

3. Results and discussion

3.1. Effects of methanol extracts and essential oils of hyssop herb on COX-1 and COX-2 enzyme activity

The anti-inflammatory activity of the essential oils and methanol extracts of hyssop herb was tested *in vitro* by measuring their ability to inhibit the activity of COX-1 and COX-2 enzymes responsible for prostaglandin synthesis. The tested essential oils and extracts of the *H. officinalis* herb were found to have an inhibitory effect on the activity of COX-1 and COX-2 enzymes at a concentration of 20 μg/mL, as outlined in Table 1.

Table 1
The results of the ability of methanol extracts (1–6 E, 20 μg/mL) and essential oils (1–6 EO, 20 μg/mL) of the *H. officinalis* herb to inhibit the activity of COX-1 and COX-2 enzymes (% inhibition).

Sample	COX-1 Inhibition (%)	COX-2 Inhibition (%)
1E	16.15 ± 4.70	63.04 ± 21.44
2E	4.34 ± 8.50 *	54.04 ± 16.28
3E	2.15 ± 3.28 *	59.42 ± 15.75
4E	2.31 ± 2.68 *	61.19 ± 11.47
5E	2.14 ± 3.70 *	61.79 ± 14.44
6E	3.36 ± 1.73 *	55.51 ± 8.98
1EO	15.16 ± 3.56 *	52.37 ± 7.53
2EO	7.90 ± 7.14 *	49.03 ± 10.00 *
3EO	3.40 ± 6.72 *	38.52 ± 9.71 *
4EO	6.35 ± 6.62 *	36.24 ± 8.72 *
5EO	10.12 ± 4.47 *	43.77 ± 10.24 *
6EO	11.04 ± 8.47	43.65 ± 12.26 *
Indomethacin	22.11 ± 3.76	/
Celecoxib	/	61.60 ± 1.77

Indomethacin (1.2 μM) was used as a positive control in the COX-1 test, while celecoxib (8.8 μM) was used in the COX-2 test. The percent of inhibition of COX enzyme activity, which was obtained in two independent experiments (the mean value ± SD); * A statistically significant difference compared to the positive control ($p < 0.05$).

With respect to the extracts, significant inhibitory activity was shown in the COX-2 assay. At a concentration of 20 μg/mL all analyzed extracts exhibited inhibition in the range of 54.04–63.04%, which was not statistically significant compared to the positive control celecoxib at 8.8 μM concentration (61.60%). With respect to the essential oils, significant activity was found for the 1EO essential oil (52.37%) at a concentration of 20 μg/mL, i.e. its inhibitory activity on COX-2 enzyme did not show a statistically significant difference from the positive control (celecoxib at a concentration of 8.8 μM).

In the COX-1 assay, weaker activity was found. Namely, 1E extract (16.15%) and 6EO essential oil (11.04%) stood out for their activity, which at a concentration of 20 μg/mL did not show a statistically significant difference in the activity in relation to the positive control, indomethacin (22.11% inhibition at a concentration of 1.2 μM).

The dominant ingredient in the tested essential oils 1-6EO, that exhibited significant activity in the COX-1 and COX-2 assays, is monoterpenoid 1,8-cineole (eucalyptol), as reported in our previous study (Mićović et al., 2021). Generally, the activity of tested essential oils toward the inhibition of COX-1/2 enzymes can be correlated with the content of 1,8-cineole in each of them. The sample 1EO had the highest content of 1,8-cineole (67.10%) followed by 6EO, 2EO, 5EO, 3EO and, finally, 4EO. The content of total oxygenated monoterpenes followed similar trend, the highest concentration was found in 1EO (68.8%) while the lowest concentration was in 4EO (27.35%). This trend of COX-1/2 inhibitory potential of *Hyssopus* essential oils is certainly not surprising when it is known that 1,8-cineole has been proven to be an excellent antioxidant, analgesic and anti-inflammatory compound (Juergens et al., 2020). It exhibited anti-inflammatory activity which is mainly achieved through the regulation of nuclear factor-kappa B (NF-kB) pathway and its ability to remove reactive oxygen species (ROS) (Cai et al., 2021). In various *in vivo* tests, 1,8-cineole showed significant anti-inflammatory and analgesic activities that are reflected in amelioration of inflammation and pain responses during gout arthritis induced in mice (Cai et al., 2020), neuroprotective activity against early brain injury via anti-inflammatory and antioxidant modes of action in rats (Xu et al., 2021), as well as the inhibition of platelet activation, thrombus development and haemostasis in mice (Matawi et al., 2021). Those health benefits are used in clinically approved drug with 1,8-cineole as the active ingredient (Soledum™) for the in bronchial asthma and other respiratory tract diseases (Galan et al., 2020; Juergens et al., 2003). Based on all the above, it can be concluded that the activity of tested essential oils is closely related to the content of 1,8-cineole, so 6EO has

the greatest potential, while 4EO showed the weakest inhibitory activity against COX-1/2 enzymes.

In our previous study was demonstrated that all tested methanol extracts of the aerial parts of hyssop contain various phenolic compounds, namely benzoic acid derivatives (syringic acid), hydroxycinnamic acid derivatives (rosmarinic, chlorogenic, and feruloylquinic acids, as well as caffeoyl pentoside) and flavonoids (quercetin-*O*-hexoside and diosmetin-*O*-deoxyhexosyl-hexoside). The dominat compounds in all samples were shown to be chlorogenic and rosmarinic acids. The chlorogenic acid content ranged from 23.35 to 33.46 mg/g, while the rosmarinic acid content was slightly lower (03.53–19.98 mg/g) (Mićović et al., 2021). All phenolic constituents detected are likely to contribute to the anti-inflammatory activity of the *Hyssopus* extracts. Various mechanisms through which polyphenolic compounds achieve the anti-inflammatory effect have been described. One of them is the inhibition of regulatory enzymes and transcription factors that play an important role in the control of mediators involved in the inflammatory process (e.g. inhibition of protein kinases involved in signal transduction during cellular activation in inflammation; inhibition of the transcription factor NF-kB which regulates certain cytokines, chemokines and cell adhesion molecules involved in the inflammatory process; flavonoids, for example, may lead to an increased level of cAMP by inhibiting cyclic adenosine monophosphate (cAMP) phosphodiesterase, which contributes to anti-inflammatory activity). Polyphenols also contribute to anti-inflammatory activity due to their antioxidant activity (inhibition of free radical production and neutralization of reactive oxygen species (ROS), reactive nitrogen species (RNS) and other reactive species). Polyphenolic compounds can also inhibit enzymes like phospholipase A2 (PLA2), cyclooxygenase (COX) and lipoxygenase (LOX) leading to decreased production of prostaglandins (PG) and leukotrienes (LT), and consequently to inhibition of inflammation. Polyphenols also affect some cells of the immune system and its mechanisms, which are important in inflammatory processes (Maleki et al., 2019; Yahfoufi et al., 2018).

With respect to the quantitatively dominant compounds, chlorogenic and rosmarinic acids, there are also literature data supporting their anti-inflammatory activity. Shin et al. (2017) explained the mechanism of the anti-inflammatory activity of chlorogenic acid in human intestinal epithelial cells by inhibition of protein kinase D-NF-κB signaling pathway and hydrogen peroxide-induced production of interleukin-8 (IL-8), due to the presence of catechol groups capturing intracellular reactive oxygen species. Besides, some other *in vitro* studies have reported the anti-inflammatory effect of chlorogenic acid, which is mainly based on the removal of ROS and RNS (Han et al., 2017; Paloez et al., 2016). Hwang et al. (2014) investigated the effects of chlorogenic acid on lipopolysaccharide (LPS) stimulated murine macrophages (RAW 264.7) and BV2 microglial cells. The inhibition of nitric oxide (NO) production and inhibition of COX-2 expression and inducible NO synthase (iNOS) were found to occur without inducing cytotoxicity. Chlorogenic acid also led to an attenuated effect of pro-inflammatory cytokines (including interleukin 1 beta (IL-1b) and tumour necrosis factor-alpha (TNF-α)), as well as other markers associated with inflammation such as interleukin 6 (IL- 6), in a dose-dependent manner. Moreover, endotoxin-induced macrophage adhesion was reduced by chlorogenic acid, as well as expression level of ninjurin 1 (Ninj 1); nuclear translocation of NF-kB was also inhibited (Hwang et al., 2014). Shen et al. (2009) also conducted a study with murine macrophages RAW 264.7, in which chlorogenic acid was found to suppress LPS-induced expression of COX-2 by attenuating the activation of NF-kB and c-Jun N-terminal kinase/activator protein-1 (JNK/AP-1) signaling pathways.

With respect to rosmarinic acid, various possible mechanisms of anti-inflammatory activity have been described in the literature; however, the interaction between rosmarinic acid and the complement pathway is most often mentioned. Namely, rosmarinic acid has been found to inhibit the activation of the complement system *in vivo* and *in vitro*, binding covalently to C3b, the activated complement component at the site of inflammation (where the complement system is activated) (Colica et al., 2018). Scheckel et al. (2008) indicated that rosmarinic acid could be an effective inhibitor of the expression of the proinflammatory gene COX-2 (which is considered a risk factor for tumour development). Some literature data suggest that rosmarinic acid protects brain cells from ischemia/reperfusion injury in diabetes, which could include a high mobility group box 1 (HMGB1) and NF-kB signaling pathway; the results suggesting the antiseptic effect of rosmarinic acid indicate that it is mediated by a decrease in local and systemic levels of inflammatory mediators (Colica et al., 2018). Ghasemzadeh Rahbardar et al. (2017) demonstrated that the use of ethanol extracts of the *Rosmarinus officinalis* L. herb (400 mg/kg), as well as rosmarinic acid (40 mg/kg), in a rat model of sciatic nerve chronic constriction injury (CCI)-induced neuropathic pain, led to a decrease in inflammatory markers in the spine, metallopeptidase 2 (MMP2), prostaglandin E_2 (PGE_2), interleukin-1 (IL-1) and COX-2. Administration of rosmarinic acid (10 mg/kg) in Wistar rats with spinal cord injury (SCI) led to an improved antioxidant status and reduced oxidative stress and inflammatory activity by decreasing pro-inflammatory cytokines and regulating NF-κB (Shang et al., 2017).

In addition to these phenolic acids, other identified polyphenols can contribute to the overall anti-inflammatory potential of tested hyssop extracts. For example, diosmetin derivate hydrolysis may led to the display of significant anti-inflammatory activity of diosmetin. It was recently reported that this *O*-methylated flavone showed a great level of inhibition of inflammation in co-cultured adipocytes and macrophages via the MAPKs/NF-κB pathway inactivation (H. Lee et al., 2020). Moreover, diosmetin anti-inflammatory potential manifested in atopic dermatitis model via, inter alia, inhibition of the expression of inducible nitric oxide synthase (iNOS) and lowering the levels of inflammatory cytokines (D. Lee et al., 2020). Diosmetin applied *in vivo* displayed decreased inflammatory response during ulcerative colitis in rats via suppression of NF-κB, TNF-α and IL-6 signaling pathways (Yu and Liu, 2021). Quercetin and its numerous derivatives are also well known for their anti-inflammatory action by inhibiting the activity or expression of enzymes that participate in inflammatory response or via modulation of various signaling pathways (Boots et al., 2008; Garcia-Lafuente et al., 2009). Syringic acid, found in tested hyssop extracts, also exerted significant role in ameliorating the inflammatory processes, either via reducing the inflammatory cells and inflammatory markers like interleukins and TNF-α or down-regulating inflammatory genes (Ham et al., 2016; Li et al., 2019).

The inhibitory activity of the *H. officinalis* herb extracts on COX-2 enzyme was found to be higher compared to the same samples tested for the activity of COX-1 enzyme, which could have practical significance and open possibilities for further studies, considering that numerous side effects of drugs, which have an inhibitory effect on the activity of COX enzymes, originate from simultaneous strong inhibition of COX-1 enzyme (which is a constitutive enzyme in many healthy tissues), in addition to COX-2. According to our knowledge, this is the first study of the inhibitory activity of the *H. officinalis* herb extracts on COX-1 and COX-2 enzymes.

3.2. Carrageenan-induced paw edema and determination of the anti-inflammatory activity of extracts

Since the extracts exhibited much better activity, especially when inhibiting COX-2 enzyme activity, *in vivo* testing of the extracts was continued. The results of *in vivo* testing of the anti-inflammatory activity of the extracts (1–6 E), depending both on the time elapsed from the carrageenan-induced rat paw inflammation (1, 2, 3 and 4 h) and the applied concentration of the extracts (50, 100 and 200 mg/kg), are shown in Table 2.

According to the results obtained, all methanol extracts of hyssop herb (1–6 E), which consist of several compounds, in the highest tested

Table 2
Anti-inflammatory activity of methanol extracts of hyssop herb (1–6 E) in the carrageenan-induced rat paw edema model. The results are presented as the mean value ± standard deviation (SD); *A statistically significant difference at the level of $p < 0.05$ in relation to the control group.

Rat paw thickness (mm)(% inhibition)					
Experimental groups	*0 h*	*1 h*	*2 h*	*3 h*	*4 h*
1E 50 mg/kg	4.1 ± 0.3	4.92 ± 0.1 (18)	5.5 ± 0.2 (0)	5.31 ± 0.25 (28.82)	5.15 ± 0.15 (25)
1E 100 mg/kg	4.3 ± 0.2	4.95 ± 0.3 (35)	5.7 ± 0.4 (0)	5.75 ± 0.2 (14.71)	5.45 ± 0.1 (17.86)
1E 200 mg/kg	4.18 ± 0.1	4.62 ± 0.3 (56)	5.2 ± 0.3 (27.14)	5.12 ± 0.2 (44.71)*	4.95 ± 0.4 (45)*
2E 50 mg/kg	4.05 ± 0.2	4.83 ± 0.1 (22)	5.55 ± 0.3 (-7.14)	5.16 ± 0.2 (34.71)	4.95 ± 0.13 (35.71)
2E 100 mg/kg	4.13 ± 0.1	4.55 ± 0.2 (58)	5.62 ± 0.2 (-6.43)	5.1 ± 0.2 (42.94)	4.65 ± 0.1 (62.86)*
2E 200 mg/kg	3.98 ± 0.1	4.65 ± 0.1 (33)	5.45 ± 0.2 (-5)	4.72 ± 0.3 (56.47)*	4.6 ± 0.3 (55.71)*
3E 50 mg/kg	4.2 ± 0.1	4.87 ± 0.2 (33)	5.75 ± 0.4 (-10.71)	5.28 ± 0.2 (36.47)	5.1 ± 0.1 (35.71)
3E 100 mg/kg	4.25 ± 0.2	5.07 ± 0.1 (18)	5.47 ± 0.1 (12.86)	5.2 ± 0.1 (44.12)	4.97 ± 0.2 (48.57)
3E 200 mg/kg	4.2 ± 0.1	5 ± 0.2 (20)	5.44 ± 0.1 (11.43)	5.18 ± 0.2 (42.35)*	4.87 ± 0.1 (52.14)*
4E 50 mg/kg	4.22 ± 0.2	4.9 ± 0.3 (32)	5.35 ± 0.2 (19.29)	5.33 ± 0.1 (34.71)	5.05 ± 0.2 (40.71)*
4E 100 mg/kg	4.2 ± 0.1	5.32 ± 0.2 (-12)	5.37 ± 0.2 (16.43)	5.32 ± 0.1 (34.12)	5.15 ± 0.3 (32.14)*
4E 200 mg/kg	4.25 ± 0.4	5.45 ± 0.3 (-20)	5.52 ± 0.2 (9.29)	5.26 ± 0.3 (40.59)*	5.02 ± 0.4 (45)*
5E 50 mg/kg	4.15 ± 0.1	5.1 ± 0.1 (5)	5.22 ± 0.3 (23.57)	5.45 ± 0.2 (23.53)	5.18 ± 0.2 (26.43)
5E 100 mg/kg	4.08 ± 0.3	5.3 ± 0.1 (-22)	5.5 ± 0.2 (-1.43)	5.37 ± 0.3 (24.12)	5.12 ± 0.1 (25.71)
5E 200 mg/kg	4.5 ± 0.2	5.37 ± 0.1 (13)	5.6 ± 0.4 (24.43)	5.12 ± 0.5 (63.53)*	5.01 ± 0.1 (63.57)*
6E 50 mg/kg	4.22 ± 0.1	5.52 ± 0.2 (-30)	5.21 ± 0.2 (29.29)	5.25 ± 0.3 (39.41)*	4.87 ± 0.3 (53.57)*
6E 100 mg/kg	4.12 ± 0.2	5.45 ± 0.1 (-33)	5.65 ± 0.1 (-9.29)	5.25 ± 0.1 (33.53)*	5.3 ± 0.1 (15.71)
6E 200 mg/kg	4.47 ± 0.1	5.37 ± 0.2 (10)	5.32 ± 0.3 (39.29)	5.29 ± 0.2 (51.76)*	5.07 ± 0.2 (57.14)*
Indomethacin 8 mg/kg	4.1 ± 0.2	4.9 ± 0.2 (20)	5.2 ± 0.3 (21.43)	5.02 ± 0.1 (45.88)	4.7 ± 0.1 (57.14)
Control	4.2 ± 0.4	5.2 ± 0.2	5.6 ± 0.3	5.9 ± 0.4	5.6 ± 0.4

dose of 200 mg/kg in the third and fourth hours after carrageenan administration, showed a statistically significant ($p < 0.05$) inhibitory effect on the increase of rat paw edema compared to control. This activity is similar or higher as the activity of the reference substance indomethacin at a concentration of 8 mg/kg:

- In the third hour (200 mg/kg), the extracts 2E (56.47%), 5E (63.53%) and 6E (51.76%) showed a higher percentage of inhibition than the indomethacin-treated group (8 mg/kg) in the same hour (45.88%). Besides, the percentage of inhibition exhibited by the 5E extract (63.53%) was higher than indomethacin (8 mg/kg) in the last (the fourth) hour (57.14%).

- In the fourth hour, the percentage of inhibition of rat paw edema for the indomethacin-treated group (8 mg/kg) was 57.14%. The same percentage of inhibition was shown by the 6E extract at a dose of 200 mg/kg, while the extracts 2E (100 mg/kg) - 62.86% and 5E (200 mg/kg) - 63.57% in the last (the fourth) hour exhibited a higher percentage of inhibition in relation to the indomethacin group. The highest percentage of inhibition was exhibited by the 5E extract (200 mg/kg) in the last hour - 63.57%. However, it should be noted that the results obtained cannot be directly compared with the positive control (indomethacin), since a much lower dose of indomethacin was used; that is, the dose-wise anti-inflammatory response of indomethacin is better. On the other hand, it should be noted that indomethacin is a pure, laboratory-synthesized substance with proven anti-inflammatory action, while extracts are multicomponent systems.

Experimentally induced carrageenan inflammation is a frequently used method for measuring the anti-inflammatory activity of an agent *in vivo*. In the induced inflammatory response, two phases are distinguished, the initial one (about 1h after carrageenan administration) which is mainly characterized by the production of histamine and serotonin by mast cells, the consequent increase in vascular permeability, and the later phase (after 1h) which brings about neutrophil infiltration, prostaglandin production and edema development (William Carey et al., 2016).

NSAIDs, such as indomethacin, exhibit anti-inflammatory activity during the second phase of carrageenan-induced inflammation by inhibiting prostaglandin synthesis through the inhibition of COX enzymes (Singh et al., 2014), as shown in this study (indomethacin had the highest percentage of inhibition in the third and fourth hours after carrageenan administration). According to the results obtained, all tested extracts (at the highest dose of 200 mg/kg) exhibited a statistically significant degree of inhibition of rat paw edema compared to the control in the second phase of carrageenan-induced inflammation (in the third and fourth hours), which is inter alia characterized by prostaglandin synthesis; and considering that *in vitro* testing showed the inhibition of COX enzyme activity (especially COX-2), it can be preliminary concluded that the tested methanol extracts of hyssop herb act by the same or similar mechanism of action as the reference substance, indomethacin or other NSAIDs.

Saleh and Setorki (2018) have also demonstrated that the ethanol extract of hyssop herb at doses of 25, 50 and 75 mg/kg exhibited high anti-inflammatory activity on the rat ear edema (13.33 ± 3.1, 20 ± 3.1 and 19.83 ± 2.8, respectively) induced by xylene ($p < 0.05$). There is also a recent study by Abdel-Megeed et al. (2020) that showed significant anti-inflammatory effects of *H. officinalis* extract on streptozotocin-induced diabetic rats. It exerted its activity via down-regulation of TNF-α and NF-κB gene expression. As stated above, polyphenolic compounds, which are contained in the extracts, are very much responsible for this behavior of the extract *in vivo* conditions. The effects of phenolic compounds (phenolic, acids, flavonoids, flavonols, tannins, etc.) can be focused on various inflammation pathways so their use in

various health conditions (diabetes mellitus, cancer, cardiovascular diseases, neurological disorders, etc.), as well in the prevention is highly recommended. It should definitely be considered the fact that polyphenolics can be variously metabolized in the organism, in intestine and liver, then reabsorbed, and thus obtained metabolites can also contribute to overall bioactivity (Maleki et al., 2019).

3.3. Active site confirmation and molecular docking analysis

Given that the tested extracts exhibited significant anti-inflammatory activity in *in vitro* and *in vivo* studies, the effect of the dominant phenolic compounds in the extracts, chlorogenic (CA) and rosmarinic (RA) acids, on the activity of COX-1 and COX-2 enzymes was examined *in silico*.

In this study, the molecular interactions between active binding sites of COX-1 and COX-2 receptors and analyzed compounds (RA and CA) were investigated by molecular docking simulations. Before molecular docking, the pockets and binding sites of targeted receptors were determined. For this purpose, the AGFR software was applied to configure and computing affinity maps for a receptor molecule to be used for AutoDock4. The native bound ligand (ibuprofen) was extracted from COX 1, and binding pocket analysis was performed. After that, re-docking was performed with the investigated compounds to generate the same docking pose as found in its co-crystallized form. The same protocol was done for the co-crystallized form of COX-2 where the ibuprofen ligand was used. This step was performed to compare the theoretical binding affinity of RA and CA with the reference drug, ibuprofen (Khan et al., 2018), and correlate it with the experimental inhibition constant.

The most stable docking conformations of the investigated compound are presented in Fig. 1 and Table 3. The lower value of the inhibition constant (Ki) and more negative value of free energy of binding (ΔG_{bind}) indicate better inhibition. The inhibitory activities of the compounds RA and CA towards COXs were ranked based on their lowest binding energy involved in the complex formation at the active sites. The binding energies of the docked compounds to COX-1 and -2 were found to be in the range between −47.4 and −49.2 kJ mol^{-1} (Table 3).

As can be seen from Table 3, the ligands strongly bind to receptors COX-1 and 2. The docking analyses of investigated molecules revealed that several non-covalent interactions existed between investigated molecules and target receptors. The most prominent interactions are hydrogen bonds, alkyl-π, and π-π interactions (Fig. 1). ARG, MET, TYR, VAL, SER, and GLY in positions 120, 522, 355, 349, 530, and 526 in the primary structure of the COX-1 chain have a predominant role as the

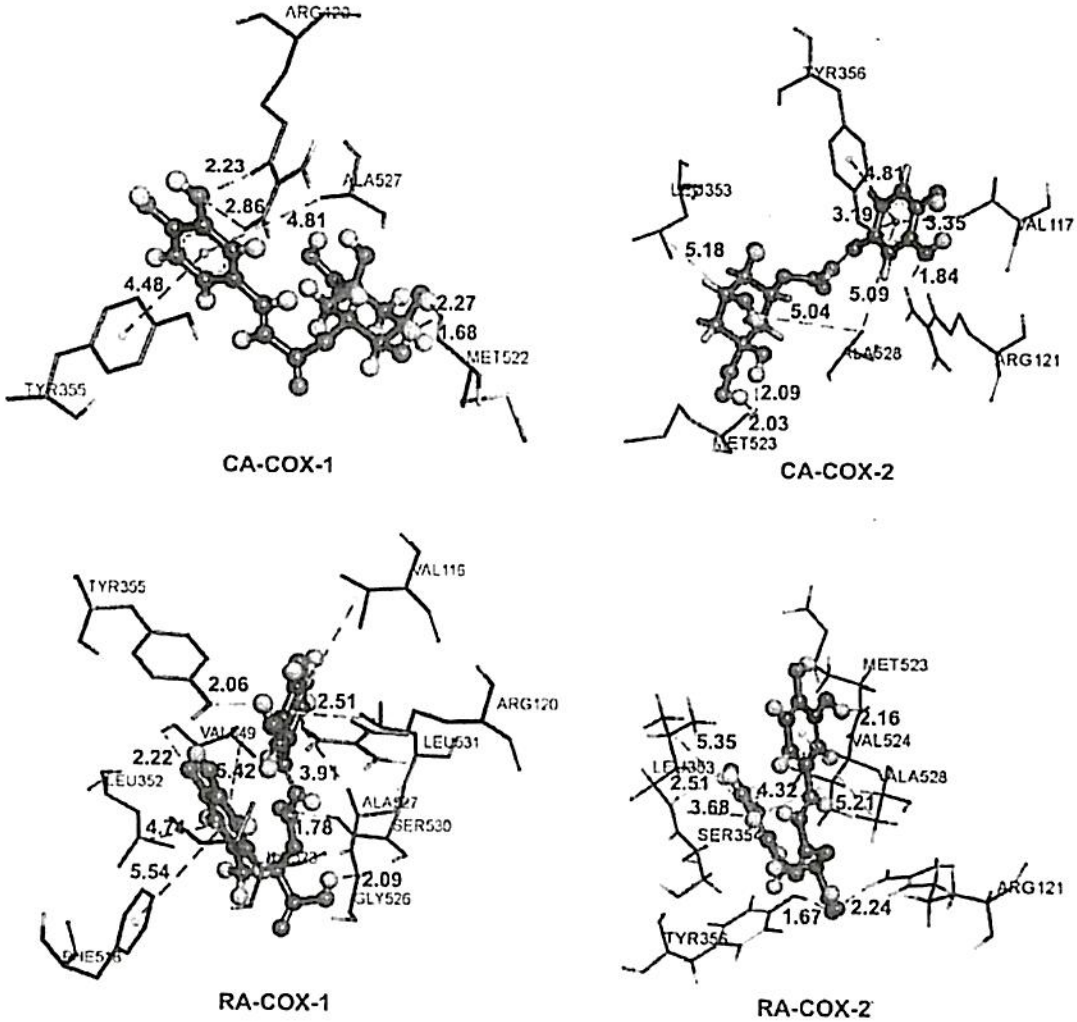

Fig. 1. The hydrogen bond (green dotted lines) and hydrophobic (rose pink dotted lines) docking interactions of the most stable conformations of RA and CA with COX-1 and COX-2.

Table 3
The important thermodynamic parameters for best docking conformations of investigated molecules with COX-1 and COX-2 (PDB IDs: 1EQG and 4PH9).

Conformations	ΔG_{bind} (kJ mol^{-1})	K, (nM)	$\Delta G_{Intermol.\ Energy}$ (vdw + hbond + desolv) (kJ mol^{-1})	ΔG_{elec} (kJ mol^{-1})	$\Delta G_{Final\ Intermol.\ Energy}$ (kJ mol^{-1})	ΔG_{Total} (kJ mol^{-1})	ΔG_{tor} (kJ mol^{-1})	ΔG_{unb} (kJ mol^{-1})	LE
Ibuprofen-COX-1	−31.3	3180	−33.4	0.1	−33.3	−1.84	3.8	0.0	−2.1
Ibuprofen-COX-2	−30.9	3840	−32.3	0.4	−32.7	−2.05	3.8	0.0	−2.1
RA-COX-1	−49.2	2.4	−47.8	0.8	−48.6	−12.0	11.4	0.0	−1.9
RA-COX-2	−48.1	3.8	−47.6	−1.1	−47.6	−13.5	11.4	0.0	−2.0
CA-COX-1	−48.2	3.6	−44.8	−1.6	−46.4	−12.3	10.5	0.0	−1.9
CA-COX-2	−47.4	5.1	−46.1	−0.9	−46.9	−14.1	10.5	0.0	−2.0

active site of these receptors regarding ligands, RA and CA. These amino acids form strong hydrogen bonds (bond lengths range from 1.68 to 2.86 Å), while TYR355, ALA527, VAL116, LEU531, VAL349, ILE523, LEU532, and PHE518 form weak alkyl-π, and π-π interactions with the benzene ring of investigated ligands (Fig. 1). On the other hand, ARG121, TYR356, MET523, and SER354 in the primary structure of COX-2 form hydrogen bonds with OH and C=O groups of RA and CA. In addition, TYR356, ALA528, VAL117, VAL524, VAL528, and LEU533 form weak alkyl-π, and π-π interactions with the benzene ring of investigated ligands. The obtained results for the binding energy of ligands are in good accordance with the experimental biological data (Khan et al., 2018).

Ibuprofen, as the one NSAID, for the same receptors targets, showed remarkably higher binding energy (ΔG_{bind} = −31.3 kJ mol^{-1} to COX-1, and ΔG_{bind} = −30.9 kJ mol^{-1} to COX-2) indicating that investigated ligands show better inhibitory activity to the COX-1 and 2 receptors (Table 3), in comparison to Ibuprofen (Khan et al., 2018).

Based on the results presented in Table 3, it is clear that ligand efficiency is not determining factor for the value of the binding energy. On the other hand, the main contribution to the binding energy comes from the sum of the dispersion, repulsion, and hydrogen bond energies. A higher number of OH groups leads to a higher probability of hydrogen bond formation, which directly leads to significantly lower values of binding energy. It should be noted that electrostatic interactions also significantly contribute to the stabilization of the complex with rosmarinic and chlorogenic acids compared to Ibuprofen. The torsional energies are lower for ibuprofen due to the smaller size and lower flexibility of this molecule in comparison to RA and CA.

The obtained results of molecular docking studies revealed that COX-1 had active sites (TYR355 and ARG120) consistent with COX-2 (TYR356 and ARG121) (Fig. 1). These results indicated that the COX-1 receptor-binding pocket conformation resembles that of the COX-2 binding pocket and raises the possibility that inhibitors intended for COX-1 may also inhibit the activity of COX-2 (Dvorakova et al., 2021; Jan et al., 2020; Khan et al., 2018; Oniga et al., 2019).

Additional *in vivo* studies with RA and CA should be performed to see how these two compounds act individually in a living system and to confirm their action, as metabolic transformations must also be considered.

3.4. Molecular dynamics simulation and analysis

The structure obtained after the molecular docking simulation was used as initial for the molecular dynamics simulation. To examine the system properties, including the overall stability, local residue and general structure fluctuations through, the molecular dynamic analyses were performed using Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of gyration (Rg) (Fig. 2).

The direct changes in the protein from the initial coordinates can be measured by the RMSD. These values of the protein COX-1 backbone (C–Cα–N) with and without ligand in the active site of protein were computed for the initial structure as a frame reference (0–20 ns) (Fig. 2A). The RMSD value for RA-COX-1 steadily increased from 0 to 10 ns and reached an equilibration that remained throughout the simulation period. The average RMSD value for the RA-COX-1 complex (0.34 ± 0.08) is higher than the unbound COX-1 receptor (0.25 ± 0.04) indicating that there are conformational changes and a slight decrease in receptor rigidity after binding of RA to active site. The difference in the average RMSD values is due to the larger fluctuation of the values indicating that RA in the active site has a more significant effect on the conformational changes and accommodation of investigated compound.

The average of RMSF values of the 8867 amino acids of COX-1 protein in the presence RA and without RA, over the simulation period, was calculated to explore the local protein flexibility (Fig. 2B). The average RMSF value for RA-COX-1 is 0.11 ± 0.06 nm and slightly higher than unbound COX-1 receptor (0.10 ± 0.06 nm). Changes in local flexibility are a consequence of ligand accommodation in the active site of the receptor. Amino acids ARG120, TYR355, VAL349, SER354, ALA527, ILE523, LEU532, and MET523 (amino acid residues as discussed in molecular docking analysis) in active sites of RA-COX-1 complex show little change flexibility (intense oscillations) than the unbound COX-1 receptor. The fluctuations of amino acids in complex is lower due to the new interactions formed with RA.

The radius of gyration (Rg) of the protein is associated with its size and compactness. The Rg values of the complex between a COX-1 and RA and unbound COX-1 were calculated and presented in Fig. 2C. The average Rg value of RA-COX-1 complex (2.43 ± 0.01 nm) is higher than unbound COX-1 (2.40 ± 0.008 nm). Slightly higher fluctuation Rg value indicate larger conformational changes in the secondary structure of COX-1 when the investigation compound bound to the active site. The MD simulation results confirmed the stability of investigated compound at the active site of the target receptor.

4. Conclusion

This study examined the anti-inflammatory activity of essential oils (*in vitro*) and methanol extracts (*in vitro* and *in vivo*) of hyssop herb, as well as the inhibitory effect of the dominant ingredients of the tested extracts (chlorogenic and rosmarinic acids) on the activity of COX-1 and COX-2 enzymes (*in silico*).

Significant inhibitory activity was shown in the COX-2 test regarding extracts (essential oils did not show any significant activity). Namely, all analyzed extracts (at a concentration of 20 μg/ml) showed a percentage of inhibition of COX-2 enzyme activity, which did not indicate a statistically significant difference from the positive control of celecoxib (at a concentration of 8.8 μM). All extracts tested *in vivo* (at a dose of 200 mg/kg) exhibited a statistically significant ($p < 0.05$) degree of inhibition of rat paw edema compared to the control in the second phase of carrageenan-induced inflammation, which is inter alia characterized by prostaglandin synthesis. The inhibitory nature of investigated ligands toward the COX-1 and COX-2 receptors was examined *in silico* by the means of molecular docking studies. According to the results of the molecular docking analysis, the RA and CA achieve a more effective interaction with target receptors. The most important interactions are H-

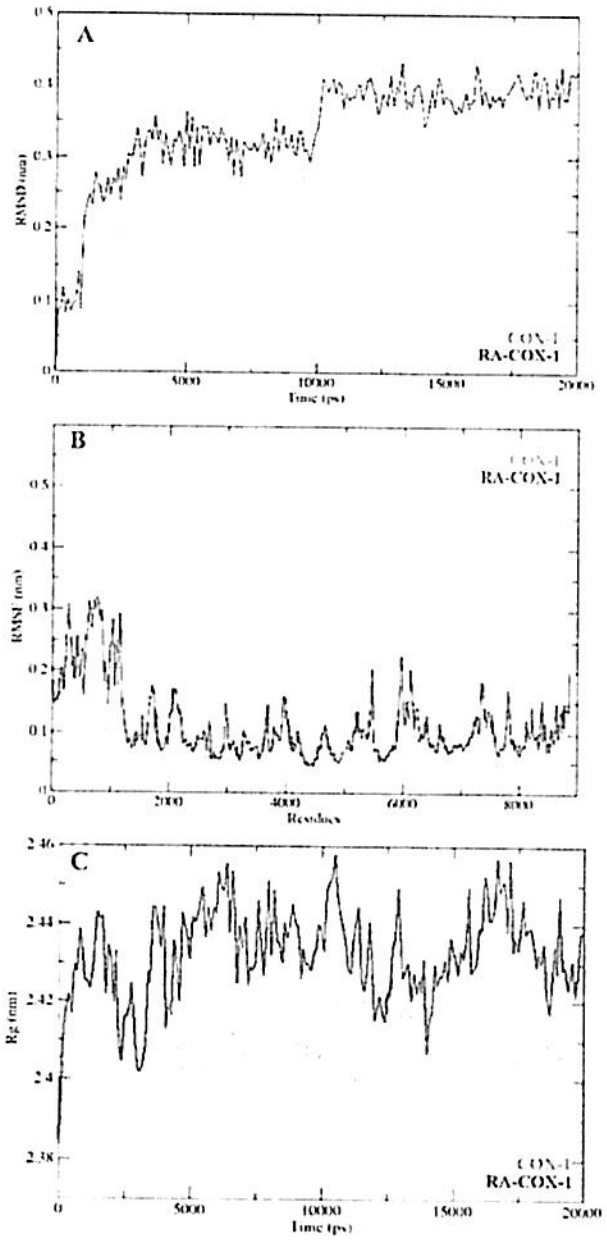

Fig. 2. The results of molecular dynamics simulation and analysis: A – The plot of root mean square deviation (RMSD) of C–Cα–N backbone vs. simulation time for COX-1 in complex with RA and without RA during 20 ns molecular dynamics simulations; B – The root mean square fluctuation (RMSF) values of RA-COX-1 and COX-1 was plotted against residue numbers; C – Rg plots of COX-1 receptor with and without RA in active sites during 20 ns MD simulation.

bonds, π-π, and π-alkyl. The preliminary results suggest that investigated compounds show better inhibitory activity against COX-1 and COX-2 than standard NSAID, ibuprofen, as evident from the free binding energy (ΔG_{bind} in kJ mol^{-1}).

The results obtained indicate a good anti-inflammatory potential of hyssop herb and support the traditional use of the herb in some inflammatory processes in folk medicine. They also lead to the preliminary conclusion that the mechanism of action of the tested methanol extracts of hyssop herb, inhibition of prostaglandin synthesis, is most likely through the inhibition of COX enzyme activity (probably, among others, contributed by the dominant ingredients, chlorogenic and rosmarinic acids), especially COX-2, as shown in *in vitro* testing, which could have practical significance, given that numerous side effects of drugs, which have an inhibitory effect on COX enzyme activity, originate from simultaneous strong inhibition of COX-1 enzyme (which is a constitutive enzyme in many healthy tissues), in addition to COX-2. However, further studies are required to determine the exact mechanism of action, optimal doses, and method of use.

Data availability

The data used to support the findings of this study are available from the corresponding authors upon request.

CRediT authorship contribution statement

Tijana Mićović: Investigation, Writing – original draft, Visualization, Writing – review & editing, participated in all the experiments (as a part of her PhD work), Investigation, Writing – Original draft and Visualization. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Jelena S. Katanić Stanković:** Resources, Methodology, Investigation, Writing – review & editing, performed Investigation and provided Resources and Methodology for in vitro anti-inflammatory assays on COX-1 and -2 inhibitory properties. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Rudolf Bauer:** Resources, Methodology, Writing – review & editing, performed Investigation and provided Resources and Methodology for in vitro anti-inflammatory assays on COX-1 and -2 inhibitory properties. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Xuehong Nöst:** Investigation, Methodology, Writing – review & editing, performed Investigation and provided Resources and Methodology for in vitro anti-inflammatory assays on COX-1 and -2 inhibitory properties. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Zoran Marković:** Investigation, Writing – review & editing, conducted in silico Investigation of the inhibitory activity of examined compounds against COX-1 and COX-2 receptors using the molecular docking simulations; also performed Visualization. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Dejan Milenković:** Investigation, Writing – review & editing, conducted in silico Investigation of the inhibitory activity of examined compounds against COX-1 and COX-2 receptors using the molecular docking simulations; also performed Visualization. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Vladimir Jakovljević:** Investigation, Writing – review & editing, performed Investigation on carrageenan-induced paw edema in rats and evaluated in vivo anti-inflammatory potential of extracts. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Marina Tomović:** Investigation, Writing – review & editing, performed Investigation on carrageenan-induced paw edema in rats and evaluated in vivo anti-inflammatory potential of extracts. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Jovana Bradić:** Investigation, Writing – review & editing, performed Investigation on carrageenan-induced paw edema in rats and evaluated in vivo anti-inflammatory potential of extracts. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Danijela Stesević:** Investigation, Writing – review & editing, performed field investigations and identified plant material. **Danilo Stojanović:** Investigation, Writing – review & editing, performed field investigations and identified plant material. All authors participated in Writing – Review & Editing. All authors read and approved the final manuscript. **Zoran Maksimović:** Investigation, Writing – review & editing, was responsible for the Conceptualization, Resources and Supervision, All

authors participated in Writing – Review & Editing. All authors read and approved the final manuscript.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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BIOGRAFIJA - Tijana Mićović

Tijana Mićović je rođena 15.09.1990. godine u Nikšiću, gdje je završila osnovnu i srednju školu, kao đak generacije i dobitnik diplome "Luča". Kao učenicu Gimnazije "Stojan Cerović", na prirodno-matematičkom smjeru, posebno je zanimala oblast hemije, iz koje je nadograđivala znanja i učestvovala na brojnim takmičenjima. Tako je na Državnom takmičenju učenika srednjih škola, 2006. godine osvojila prvo mjesto iz oblasti opšte i neorganske hemije, a 2008. godine, prvo mjesto iz oblasti organske hemije.

Farmaceutski fakultet u Podgorici UCG (petogodišnje studije) je završila u roku, 2014. godine, sa prosječnom ocjenom **"A" (9.99)**, kao student generacije i dobitnica **Plakete Univerziteta Crne Gore za najboljeg svršenog studenta u Crnoj Gori iz oblasti tehničkih, prirodno-matematičkih i medicinskih nauka** za studijsku 2014/15. godinu. Tokom perioda studija, dobila je brojne nagrade i priznanja za postignute rezultate (nagrada Univerziteta Crne Gore za studenta generacije Farmaceutskog fakulteta sa prosječnom ocjenom 10.00, 2011. godine; Atlas stipendija za najboljeg studenta Farmaceutskog fakulteta, 2014. godine; Studentska nagrada "18. septembar" Opštine Nikšić, za najboljeg studenta Farmaceutskog fakulteta, 2014. godine; stipendije Ministarstva prosvjete i nauke).

Od 2015. godine, zaposlena je u Institutu za ljekove i medicinska sredstva Crne Gore.

Od završetka studija (2014. godine), angažovana je i kao saradnik u nastavi na Medicinskom fakultetu u Podgorici (studijski program Farmacija); od 2017. godine, praktičnu nastavu izvodi na predmetima Farmakognozija I, II i Fitoterapija.

Jezici: engleski i ruski.

Hobi: Pisanje poezije i kratkih priča (2007. godine izdala je knjigu refleksivne poezije „Isprekidani glasovi").

BIBLIOGRAFIJA

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- **Mićović, T.;** Katanić-Stanković, J.; Bauer, R.; Nöst, X.; Marković, Z.; Milenković, D.; Maksimović, Z. Promising anti-inflammatory potential of methanol extracts of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Nyman (Lamiaceae). Poster presentation at the 10th Conference of the Serbian Biochemical Society in Kragujevac, September, 2021.
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- **Mićović T.**, Marković D., Goločorbin-Kon S. Cosmetics vs Dermocosmetics vs Cosmeceutics. Poster presentation at the 8th International Medical Students' Congress in Novi Sad, Serbia, July, 2013.

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Broj / Ref 03 - 1332
Datum / Date 19.04.2019

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br. 44/14, 47/15,40/16,42/17,71/17 55/18 i 3/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore, na sjednici održanoj 19. aprila 2019.godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr ZORICA POTPARA bira se u akademsko zvanje **vanredni profesor Univerziteta Crne Gore za oblasti: Socijalna farmacija i Farmaceutska tehnologija i kozmetologija** (Osnovi industrijske farmacije, Osnovi farmaceutskog menadžmenta, Farmaceutska etika i zakonodavstvo i Uvod u farmaciju, na studijskom programu Farmacija) **na Medicinskom fakultetu Univerziteta Crne Gore,** na period od pet godina.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK

Prof.dr Danilo Nikolić, rektor

BIOGRAFIJA ZORICA POTPARA

Rođena sam 08.01.1963.god. u Nikšiću, Crna Gora. Osnovnu školu završila sam u Nikšiću kao nosilac diplome Luča I. Gimnaziju, smjer biohemija i molekularna biologija, završila sam 1981.god. u Beogradu kao nosilac Vukove diplome.
Školske 1981/82.godine upisala sam Farmaceutski fakultet Univerziteta u Beogradu, a diplomirala na istom u novembru 1985.god.
Školske 1988/89.godine upisala sam zdravstvenu specijalizaciju iz farmaceutske tehnologije na Farmaceutskom fakultetu u Beogradu, u trajanju od tri godine. Specijalizacija je bila raspisana za potrebe proizvodnje Galenske laboratorije pri AU "Montefarm". Specijalistički ispit sa temom "Tablete Paracetamola á 500mg" (mentor prof.dr Milica Jovanović, prof.dr Zorica Đurić), odbranila sam u novembru 1991.godine, stekavši naziv specijalista farmaceutske tehnologije. Rezultati istraživanja su iskorišteni za izradu formulacije tableta Paracetamola, koje su se izrađivale kao galenski preparat.
2007.god. upisala sam doktorske studije na Medicinskom fakultetu u Kragujevcu, smjer Eksperimentalna i klinička farmakologija. Doktorsku disertaciju „ Ispitivanje biološke aktivnosti preparata peloida sa lokaliteta ulcinjske obale Jadranskog mora", pod mentorstvom prof.dr Slobodana Jankovića, odbranila sam u decembru 2011.god. na Medicinskom fakultetu u Kragujevcu i stekla akademsko zvanje doktora medicinskih nauka. Eksperimentalni dio teze je rađen u PZU"Fontis", koji je obuhvatio ispitivanja dejstva dermokozmetičkog preparata za akne i njegu kože, sa prirodnim resursom-morskim peloidom sa područja ulcinjske Solane. Rezultati ispitivanja su potvrđeni kroz dugogodišnju primjenu preparata, sa odličnim ishodima.

Obavezni pripravnički staž za farmaceute obavila sam u apoteci „Zemun" u Zemunu, opština Beograd, 1986.god. a nakon toga položila državni ispit.
1987.god. počinjem da radim u apoteci „Podgorica" u Podgorici u okviru Apotekarske Ustanove Crne Gore.
1988.godine prelazim u novootvorenu galensku laboratoriju i iste godine zbog potreba proizvodnje upisujem specijalizaciju iz farmaceutske tehnologije u Beogradu gdje provodim naredne tri godine.
Po povratku u Podgoricu, nastavila sam sa radom u galenskoj laboratoriji gdje se proizvodilo više od 40 galenskih pripravaka. Za potrebe proizvodnje sirupa, boravila sam u fabrici ljekova "Galenika" u Beogradu, gdje sam radila transfer iz laboratorijske u serijsku proizvodnju, zbog potreba tržišta Crne Gore.
Kao specijalista farmaceutske tehnologije, učestvovala sam u izradi mnogih formulacija galenskih preparata, koji su distribuirani na teritoriji Crne Gore.
Od 1997. do 2001.god. radila sam u ICN-u Crna Gora, gdje sam bila rukovodilac pogona za izradu Flonivina BS.
2001.god. sam na mjestu direktora proizvodnje u novootvorenoj fabrici čvrstih oblika „Habitpharm", u Podgorici.
2002. godine fabriku preuzima „Hemomont" i kao rukovodilac čvrstih formi radim na transferu tehnologije čvrstih farmaceutskih oblika iz fabrike „Hemofarm" Vršac.
2003.godine prelazim u privatnu dermatovenerološku ordinaciju „Fontis", koja u svom sastavu ima proizvodnju dermokozmetičkih preparata za njegu zdrave i liječenje problematične kože . Radim na kreiranju novih preparata kao rukovodilac proizvodnje.

Aktivno sam učestvovala u pripremi elaborata za otvaranje Samostalnog studijskog programa Farmacije u Podgorici, koji je otvoren 2007.god. a 2010.god transformisan u Farmaceutski fakultet. Tokom ovog perioda bila sam saradnik na predmetima:
Farmaceutska tehnologija I, Farmaceutska tehnologija II,
Farmaceutska tehnologija III, Industrijska farmacija i koordinator za stručnu praksu.
Odlukom Senata br.08-2713 od 19.12. 2013. izabrana sam u zvanje docenta na Univerzitetu Crne Gore, a potom zasnovala radni odnos na Farmaceutskom fakultetu, danas studijskom programu Farmacija na Medicinskom fakultetu.
Bila sam član Strukovnog vijeća za prirodne i tehničke nauke, član Senata Univerziteta Crne Gore, dekan Farmaceutskog fakulteta (jun-septembar 2015.godine), do integracije sa Medicinskim fakultetom.
17.03.2016.godine imenovana sam za rukovodioca studijskog programa Farmacija na Medicinskom fakultetu u Podgorici. Prvi sam farmaceut doktor nauka u Crnoj Gori i prvi farmaceut-nastavnik na Farmaceutskom fakultetu u Podgorici, danas studijskom programu Farmacija.

PREGLED RADOVA

I -Radovi objavljeni u časopisima koji se nalaze u međunarodnim bazama podataka

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II-Poglavlja u knjizi

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III- Međunarodni kongresi, simpozijumi i seminari

- Dragana Marković, Ana Todorović, **Zorica Potpara**. *Modern Pharmaceutical forms whith pulsatile drug release- Pulsincap*. 37th International medical scientific congress in Ohrid. May 2014. ISBN 978-608-4596-59-2
- Jovanović-Djurašković M, Karadzic J, **Potpara Z**. *Development of Pharmacy in the History of Montenegro*. 40th International Congress for the History of Pharmacy, Berlin 2011; Pharmacy and Books, p. 92-3.
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- Miomir Šoškić, Vanja Tadić, Dragica Bojović, **Zorica Potpara**, Zinaida Kalač, Andela Drašković. Uporedna hemijska analiza etarskih ulja izolovanih iz cvijeta lavande (Lavandulae flos, Lamiaceae) različitog geografskog porijekla. II Kongres farmaceuta sa medunarodnim učešćem, Bečići, 2015. Zbornik sažetaka ISBN 978-9940-9314-3-8; p.183-5
- Šabotić Sabina, Anđelić Kristina, Koprivica Tijana, **Potpara Zorica**. Use of dietary supplements for weight loss. II Kongres farmaceuta sa medunarodnim učešćem Bečići, 2015. Zbornik radova
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V- Uvodno, objavljeno plenarno predavanje

- **Zorica Potpara**. *Farmaceutski fakultet u Podgorici-sadašnjost i perspektive.* II Kongres farmaceuta sa međunarodnim učešćem, Bečići, 2015. Zbornik sažetaka, ISBN 978-9940-9314-3-8.p.68-9
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- **Zorica Potpara**. *Primjena peloida u dermokozmetičkim preparatima u tretmanu akni i njezi kože.* I Konferencija "Značaj i uloga balneologije u rehabilitaciji inflamatornih stanja", Igalo, septembar 2018.

VI - Recenziranje

- *Serbian Journal of Experimental and Clinical Research.* ISSN 1820 – 8665 1 rad .(M. Sovrlić and N Manojlović. Plants from the genus daphne: a review of its traditional uses, phytochemistry, biological and pharmacological activity)
- *Int J Pharm.* ISSN: 0378-5173 . 1 rad (J.Đuriš. Z.Đurić Modeling in the Quality by Design environment: regulatory requirements and recommendations for design space and control strategy applications)
- *Saudi Pharmaceutical Journal (SPJ).* ISSN: 1319-0164. 1 rad . Telmisartan-meglumine solid dispersions: influence of physico-chemical characteristics on telmisartan intrinsic dissolution

УНИВЕРЗИТЕТ У БЕОГРАДУ

16.3.2020.

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Београд, 11. март 2020. године
02 Број: 61202-707/3-20
СЋ

На основу чл. 75 Закона о високом образовању („Службени гласник РС", бр. 88/17, 73/18 и 67/19), чл. 43 ст. 1 тач. 22 и чл. 44 ст. 4 Статута Универзитета у Београду („Гласник Универзитета у Београду", бр. 201/18, 207/19 и 213/20), чл. 26 ст. 1 и ст. 2 тач. 1 Правилника о начину и поступку стицања звања и заснивања радног односа наставника Универзитета у Београду („Гласник Универзитета у Београду", бр. 200/17 и 210/19) и Правилника о минималним условима за стицање звања наставника на Универзитету у Београду („Гласник Универзитета у Београду", бр. 192/16, 195/16, 197/17, 199/17 и 203/18), а на предлог Изборног већа Фармацеутског факултета, бр. 225/1 од 30. јануара 2020. године и мишљења Већа научних области медицинских наука, 02-01 бр. 61202-707/2-20 од 25. фебруара 2020. године, Сенат Универзитета, на седници одржаној 11. марта 2020. године, донео је

О Д Л У К У

БИРА СЕ др Зоран Максимовић, у звање редовног професора на Универзитету у Београду – Фармацеутски факултет, за ужу научну област Фармакогнозија.

О Б Р А З Л О Ж Е Њ Е

Фармацеутски факултет („Факултет") је дана 30. октобра 2019. године, у публикацији „Послови", објавио конкурс за избор у звање редовног професора, за ужу научну област Фармакогнозија, због истека изборног периода.

Реферат Комисије за припрему реферата о пријављеним кандидатима стављен је на увид јавности дана 24. децембра 2019. године на сајту Факултета.

На основу предлога Комисије за припрему реферата о пријављеним кандидатима, Изборно веће Факултета, на седници одржаној дана 31. јануара 2020. године, донело је одлуку о утврђивању предлога да се кандидат др Зоран Максимовић изабере у звање редовног професора.

Факултет је дана 5. фебруара 2020. године доставио Универзитету комплетан захтев за избор у звање на прописаним обрасцима.

Универзитет је комплетну документацију коју је доставио Факултет ставио на web страницу Универзитета дана 18. фебруара 2020. године.

УНИВЕРЗИТЕТ У БЕОГРАДУ

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Веће научних области медицинских наука, на седници одржаној дана 25. фебруара 2020. године дало је мишљење да се др Зоран Максимовић може изабрати у звање редовног професора.

Сенат Универзитета, на седници одржаној дана 11. марта 2020. године разматрао је захтев Факултета и утврдио да кандидат испуњава услове прописане чл. 74 и 75 Закона о високом образовању, чланом 135 Статута Универзитета у Београду, као и услове прописане Правилником о минималним условима за стицање звања наставника на Универзитету у Београду, па је донета одлука као у изреци.

ПОУКА О ПРАВНОМ ЛЕКУ:

Против ове одлуке кандидат пријављен на конкурс може изјавити жалбу Сенату Универзитета, преко Факултета. Жалба се доставља Факултету у року од 8 дана од дана достављања одлуке.

ПРЕДСЕДНИЦА СЕНАТА
РЕКТОРКА
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Bibliografija, sa referencama grupisanim prema indikatorima naučne kompetentnosti

1. Monografije, monografske studije, tematski zbornici, leksikografske i kartografske publikacije međunarodnog značaja

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2. Radovi objavljeni u naučnim časopisima međunarodnog značaja

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4. Nacionalne monografije, tematski zbornici, leksikografske i kartografske publikacije nacionalnog značaja; naučni prevodi i kritička izdanja građe, bibliografske publikacije

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5. Radovi u časopisima nacionalnog značaja

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8. Davidović V, Lazarević M, Joksimović-Todorović M, Maksimović Z, Jovanović M, Miljas N (2010). Uticaj ekstrakata podzemnih organa *Helleborus odorus* W. et K. na crvenu krvnu sliku Wistar pacova. Veterinarski glasnik 64(3-4): 219-229
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32. Maksimović Z. 6. Kongres medicine sporta i 3. Kongres prevencije dopinga u sportu „Promena igre". Beograd, 10-11.05.2018, Knjiga sažetaka, 39-41.
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7. Tehnička rešenja

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Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 28.05.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Milena Tadić bira se u akademsko zvanje vanredni profesor Univerziteta Crne Gore za **oblasti Hemijsko inženjerstvo i Inženjerstvo zaštite životne sredine,** na Metalurško-tehnološkom fakultetu Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2297
Podgorica, 28.05.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 28.05.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Anđelka Šćepanović bira se u akademsko zvanje vanredni profesor Univerziteta Crne Gore za **oblasti Opšta grupa bioloških predmeta i Ekologija,** na Prirodno-matematičkom fakultetu i nematičnim fakultetima Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2295
Podgorica, 28.05.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 28.05.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Mehmed Dečević bira se u akademsko zvanje docent Univerziteta Crne Gore za **oblast Socijalni rad,** na Fakultetu političkih nauka Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2299
Podgorica, 28.05.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 28.05.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dušan Kasalica bira se u akademsko zvanje docent Univerziteta Crne Gore za **oblast Film i mediji,** na Fakultetu dramskih umjetnosti Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2297
Podgorica, 28.05.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 28.05.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Sreten Tomović bira se u akademsko zvanje vanredni profesor Univerziteta Crne Gore za **oblast Hidrotehnika,** na Građevinskom fakultetu i nematičnim fakultetima Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2296
Podgorica, 28.05.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 04.06.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Miloje Šundić bira se u akademsko zvanje docent Univerziteta Crne Gore za **oblasti Opšta grupa bioloških predmeta i Ekologija,** na Prirodno-matematičkom fakultetu Univerziteta Crne Gore, na period od pet godina.

Broj: 03-2398
Podgorica 04.06.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

Na osnovu člana 72 stav 2 Zakona o visokom obrazovanju („Službeni list Crne Gore" br 44/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19) i člana 32 stav 1 tačka 9 Statuta Univerziteta Crne Gore, Senat Univerziteta Crne Gore na sjednici održanoj 04.06.2020. godine, donio je

O D L U K U
O IZBORU U ZVANJE

Dr Danijela Stešević bira se u akademsko zvanje redovni profesor Univerziteta Crne Gore za **oblasti Opšta botanika i Opšta grupa bioloških predmeta,** na Prirodno-matematičkom fakultetu Univerziteta Crne Gore, na neodređeno vrijeme.

Broj: 03-2399
Podgorica 04.06.2020.g.

SENAT UNIVERZITETA CRNE GORE
PREDSJEDNIK
Prof. dr Danilo Nikolić, rektor

KRATKA BIOGRAFIJA PROF. DR DANIJELE STEŠEVIĆ

LIČNI PODACI

Rođena sam 16.07.1976. godine u Titogradu, gdje sam završila osnovnu školu »Savo Pejanović« i srednju školu gimnaziju »Slobodan Škerović«.

PODACI O VISOKOM OBRAZOVANJU

Školske 1994/95 godine upisala sam studije Biologije na Prirodno-matematičkom fakultetu u Podgorici, gdje sam diplomirala 6. 10. 1998. godine sa prosječnom ocjenom 9,45 i stekla zvanje diplomirani biolog.

Poslijediplomske studije upisala sam školske 1998/99. godine na Biološkom fakultetu Univerziteta u Beogradu (smjer: Ekologija i geografija biljaka) i završila ih sa prosječnom ocjenom 10. Magistarsku tezu pod nazivom: "Flora kraških polja u Piperskom kraju Crne Gore" odbranila sam 15. 05. 2001. godine i stekla zvanje magistra bioloških nauka.

Doktorsku disertaciju pod nazivom: "Ekološka-fitogeografska analiza flore šireg urbanog područja Podgorice", odbranila sam 24. 06. 2009. godine, na Biološkom fakultetu Univerziteta u Beogradu i stekla zvanje doktora bioloških nauka. Rješenje o priznavanju Uvjerenja o stečenom naučnom stepenu Doktora bioloških nauka izdato mi je od strane Ministarstvo Nauke i Prosvjete 26. 10. 2009. godine.

PODACI O RADNIM MJESTIMA I IZBORIMA U AKADEMSKA ZVANJA

Od 1999. godine zasnovala sam radni odnos na Prirodno-matematičkom fakultetu u Podgorici (Studijski program Biologija), gdje sam januara 1999. godine izabrana u zvanje asistenta. U toku svog desetogodišnjeg staža asistirala sam u laboratorijskim vježbama na predmetima: *Anatomija biljaka, Ekologija biljaka, Ekologija životinja, Sistematika i filogenija viših biljaka, Limnologija, Sistematika i filogenija nižih biljaka, Biologija mora, Botanika* na akademskim studijskim programima Biljna proizvodnja i Farmacija.

Zvanje docenta na Prirodno-matematičkom fakultetu u Podgorici (predmeti *Sistematika i filogenija viših biljaka I i II*, na studijskom programu Biologija i *Botanika,* na studijskom programu Biljna proizvodnja) stekla sam 27.05.2010. godine. Školskih 2010/2011 i 2011/2012. godine bila sam angažovana kao predavač Botanike na Farmaceutskom fakultetu. Od školske 2012/2013 držim dio predavanja iz "Bioloških zbirki" koje se slušaju na specijalističkim studijama, na studisjkom programu Biologija. Od izbora u zvanje docenta, nastavila sam da držim vježbe na predmetima *Sistematika i filogenija viših biljaka I* i *II*, na studijskom programu Biologija.

Zvanje vanrednog profesora na Prirodno-matematičkom fakultetu u Podgorici (predmeti *Sistematika i filogenija viših biljaka I i II*, na studijskom programu Biologija i *Botanika,* na studijskom programu Biljna proizvodnja) stekla sam 24.06.2015. godine.

Zvanje redovnog profesora na Univerzitetu Crne Gore za oblast Opšta botanika i Opšta grupa bioloških predmeta na Prirodno-matematičkom fakultetu u Podgorici stekla sam 04.06.2020. godine.

ODABRANE PUBLIKACIJE

Godina	Kategorija	Autori	Naslov	Izvor
2021	SCI, SCIE, SSCI, A&HCI	Danijela Stešević, Đorđije Milanović Milica Stanišić-Vujačić, Urban Šilc	Aristida oligantha – a new alien species on the eastern Adriatic coast"	Acta Botanica Croatica
2021	SCI, SCIE, SSCI, A&HCI	Kremer D, Stabentheiner E, Bogunić F, Ballian D, Eleftheriadou E, Stešević D, Matevski V, Randelović V, Ivanova D, Ruščić M, Dunkić V.	Micromorphological Traits of Balcanic Micromeria and Closely Related Clinopodium Species (Lamiaceae)	Plants
2021	SCI, SCIE, SSCI, A&HCI	Mićović T, Topalović D, Živković L, Spremo-Potparević B, Jakovljević V, Matić S, Popović S, Baskić D, Stešević D, Samardžić S, Stojanović D, Maksimović Z.	Antioxidant, Antigenotoxic and Cytotoxic Activity of Essential Oils and Methanol Extracts of Hyssopus officinalis L. Subsp. aristatus (Godr.) Nyman (Lamiaceae).	Plants
2021	SCI, SCIE, SSCI, A&HCI	Pedja JANAĆKOVIĆ, Milan GAVRILOVIĆ, Dragana RANČIĆ, Danijela STEŠEVIĆ, Zora DAJIĆ-STEVANOVIĆ and Petar D. MARIN	Anatomical traits of Artemisia umbelliformis subsp. eriantha (Asteraceae) alpine glacial relict from Mt. Durmitor (Montenegro)	Botanica Serbica
2021	SCI, SCIE, SSCI, A&HCI	Danka Caković Leonardo Cresti Danijela Stešević Peter Schönswetter Božo Frajman	High genetic and morphological diversification of the Euphorbia verrucosa alliance (Euphorbiaceae) in the Balkan and Iberian peninsulas	Taxon
2021	SCI, SCIE, SSCI, A&HCI	Nenad Jasprica, Željko Škvorc, Marija Pandža, Milenko Milović, Dragica Purger, Daniel Krstonošić, Sanja Kovačić, Dubravka Sandev, Anđelka Lasić, Danka Caković, Danijela Stešević, Branko Anđić & Milica Stanišić-Vujačić	Phytogeographic and syntaxonomic diversity of wall vegetation (Cymbalario-Parietarietea diffusae) in southeastern Europe	Plant Biosystems
2020	SCI, SCIE, SSCI, A&HCI	Radisav Dubljević,Božidarka Markovic, Dušica Radonjić, Danijela Stešević and Milan Marković	Influence of Changes in Botanical Diversity and Quality of Wet Grasslands through Phenological Phases on Cow Milk Fatty Acid Composition	Sustainability
2020	SCI, SCIE, SSCI, A&HCI	Danijela Stešević, Branko Anđić, Milica Stanišić-Vijačić	Physcomitrium eurystomum Sendtn., a new moss species in the bryophyte flora of Montenegro	Acta Botanica Croatica
2020	SCI, SCIE, SSCI, A&HCI	"JELICA NOVAKOVIĆ, PATRIK MRÁZ, DANIJELA STEŠEVIĆ, PETAR D. MARIN, BOJAN ZLATKOVIĆ, DMITAR LAKUŠIĆ, PEDJA JANAĆKOVIĆ "	Typification and taxonomic re-evaluation of Centaurea crnogorica and C. gjurasinii, two neglected yellow-flowered endemics of the Balkan Peninsula (Centaurea sect. Acrocentron, Asteraceae)	Phytotaxa
2020	SCI, SCIE, SSCI, A&HCI	Urban Šilc, Danijela Stešević, Milica Luković, Danka Caković	Changes of a sand dune system and vegetation between 1950 and 2015 on Velika plaža (Montenegro, E Mediterranean)	Regional Studies in Marine Science
2020	SCI, SCIE, SSCI, A&HCI	Danijela Stešević, Filip Küzmič , Milica Stanišić-Vijačić, Urban Šilc	Coastal sand dune vegetation of Velika plaža (Montenegro)	Acta Botanica Croatica
2020	SCI, SCIE, SSCI, A&HCI	Radisav Dubljević, Božidarka Marković, Dušica Radonjić, Danijela Stešević, Milan Marković	Influence of Changes in Botanical Diversity and Quality of Wet Grasslands through Phenological Phases on Cow Milk Fatty Acid Composition	Sustainability - MDPI Journal
2019	SCI, SCIE, SSCI, A&HCI	Radonjić, D., Đorđević, N., Marković, B., Marković, M., Stešević, D. and Dajić-Stevanović,	Effect of phenological phase of dry grazing pasture on fatty acid composition of cows' milk	Chilean journal of agricultural research

Godina	Kategorija	Autori	Naslov	Izvor
		Z.		
2019	SCI, SCIE, SSCI, A&HCI	Andjić Branko, Cvijetićanin Stanko, Hayhoe Simon, Grujičić Rade, Stešević Danijela	DICHOTOMOUS KEYS IN THE BOTANICAL LEARNING OF NON-VISUAL (BLIND) PEOPLE	Journal of Baltic Science Education
2018	SCI, SCIE, SSCI, A&HCI	Branko Andić, Christian Berg & Danijela Stešević	New and interesting bryophytes of Albania and Montenegro	Herzogia
2018	SCI, SCIE, SSCI, A&HCI	Urban Šilc, Filip Küzmič, Danka Caković, Danijela Stešević	Beach litter along various sand dune habitats in the southern Adriatic (E Mediterranean)	Marine Pollution Bulletin
2018	SCI, SCIE, SSCI, A&HCI	Stešević, D., Jaćimović, Ž., Šatović, Z., Šapčanin, A., Jančan, G., Kosović, M., Damjanović-Vratnica, B.	Chemical characterization of wild growing Origanum vulgare populations in Montenegro	Natural Product Communications
2018	SCI, SCIE, SSCI, A&HCI	Branko Andić, Stanko Cvijetićanin, Mirjana Maričić and Danijela Stešević	The contribution of dichotomous keys to the quality of biological-botanical knowledge of eighth grade students	Journal of Biological Education
2017	SCI, SCIE, SSCI, A&HCI	Marcin Nobis, Andrey Erst, Arkadiusz Nowak, Dmitry Shaulo, Marina Olonova, Yuriy Kotukhov, Asli Doğru-Koca, Ali A. Dönmez, Gergely Király, Aleksandr L. Ebel, Maria Kushunina, Renata Piwowarczyk, Alexander P. Sukhorukov, Agnieszka Nobis, Filip Verloove, Joanna Zalewska-Gałosz, Golshan Zare, Jean-François Burri, Danka Caković, Elżbieta Jędrzejczak, Nejc Jogan, Ewelina Klichowska, Artur Pliszko, Anton V. Popovich, Danijela Stešević, Urban Šilc, Natalia Tupitsyna, Vladimir M. Vasjukov, Wei Wang, Philippe Werner, Magdalena N. Wolanin, Mateusz M. Wolanin & Kun-Li Xiang	Contribution to the flora of Asian and European countries: new national and regional vascular plant records, 6,	Botany Letters
2017	SCI, SCIE, SSCI, A&HCI	Šilc, U., Caković, D., Kuzmič, F., Stešević, D.	Trampling impact of vegetation of embryonic and stabilised sand dunes in Montenegro.	Journal of Coastal Conservation
2017	SCI, SCIE, SSCI, A&HCI	Danijela Stešević, Milica Luković, Danka Caković, Nemanja Ružić, Nada Bubanja , Urban Sulc	Distribution of alien species along sand dune plant communities zonation	Periodicum biologorum
2017	SCI, SCIE, SSCI, A&HCI	Danka Cakovic, Danijela Stesevic, Peter Schonswetter, Bozo Frajman	Long neglected diversity in the Accursed Mountains of northern Albania: Cerastium hekuravense is genetically and morphologically divergent from C. dinaricum	Plant Systematics and Evolution
2016	SCI, SCIE, SSCI, A&HCI	Stešević Danijela, Božović Mijat, Tadić Vanja, Rančić Dragana, Dajić Stevanović Zora	Plant-part anatomy related composition of essential oils and phenolic compounds in Chaerophyllum coloratum,a Balkan endemic species	Flora
2015	SCI, SCIE, SSCI, A&HCI	Caković Danka, Stešević Danijela, Schonswetter Peter, Frajman Božo	How many taxa? Spatiotemporal evolution and taxonomy of Amphoricarpos (Asteraceae, Carduoideae) on the Balkan Peninsula	Organisms Diversity & Evolution
2015	SCI, SCIE, SSCI, A&HCI	Kremer D., Bolarić S., Ballian, D., Bogunić, F., Stešević, D., Karlović, K., Kosalec, I., Vokurka, A., Vuković Rodríguez, J., Randić, M., Bezić, N., Dunkić, V.	Morphological, genetic and phytochemical variation of the endemic Teucrium arduini L. (Lamiaceae),	Phytochemistry
2015	SCI, SCIE,	Stešević, D., Berg, C.	Botrychium matricariifolium, a new fern species	Acta Botanica

Godina	Kategorija	Autori	Naslov	Izvor
	SSCI, A&HCI		for the flora of Montenegro	Croatica
2015	SCI, SCIE, SSCI, A&HCI	Caković, D, Stešević, D., Schönswetter, P., Frajman, B.	How many taxa? Spatiotemporal evolution and taxonomy of Amphoricarpos (Asteraceae, Carduoideae) on the Balkan Peninsula	Organisms Diversity & Evolution
2014	SCI, SCIE, SSCI, A&HCI	Stešević, D., Ristić, M., Nikolić, V., Nedović, M, Caković, D., Zlatko Šatović	Chemotype Diversity of Indigenous Dalmatian Sage (Salvia officinalis L.) Populations in Montenegro	Chemistry & Biodiversity
2014	SCI, SCIE, SSCI, A&HCI	Kremer, D., Dunkić, V., Stešević, D., Kosalec, I., Ballian, D., Bogunić, F., Bezić, N., Stabentheiner, E.	Micromorphological traits and essential oil of Micromeria longipedunculata Bräuchler (Lamiaceae)	Cental European Journal of Botany
2014	SCI, SCIE, SSCI, A&HCI	Vučković, I., Vujisić, Lj., Todosijević, M., Stešević D., Milosavljević, S., Trifunović, S.	Volatile Constituents of Different Plant Parts and Populations of Malabaila aurea Boiss. from Montenegro	Records of Natural Products
2014	SCI, SCIE, SSCI, A&HCI	Caković, D., Stešević, D., Vuksanović, S., Tan, K	Colchicum cupanii Guss. subsp. glossophyllum (Heldr.) Rouy, Datura innoxia Mill. and Eclipta prostrata (L.) L., new floristic records in Montenegro and western Balkan	Acta Botanica Croatica
2013	SCI, SCIE, SSCI, A&HCI	Mayrhofer, H., Drescher, A., Stešević, D., Bilovitz, P.	Lichenized fungi of a chestnut grove in Livari (Rumija, Montenegro)	Acta Botanica Croatica
2013	SCI, SCIE, SSCI, A&HCI	Kremer, D., Dunkić, V., Ruščić, M., Matevski, V., Ballian, D., Bogunić, F., Eleftheriadou, E., Stešević, D., Kosalec, I.., Bezić, N., Stabentheiner E.	Micromorphological traits and essential oil contents of Micromeria kerneri Murb. and M. juliana (L.) Benth. (Lamiaceae)	Phytochemistry
2012	SCI, SCIE, SSCI, A&HCI	Janković, T., Zdunić, G., Beara, I., Balog, K., Pljevljakušić, D., Stešević, D. Šavikin, K.	Comparative study of some polyphenols in Plantago species	Biochemical Systematics and Ecology
2011	SCI, SCIE, SSCI, A&HCI	Milić Čurović, Danijela Stešević, Milan Medarević, Rade Cvjetićanin, Damjan Pantić, Velibor Spalević	Ecological and structural characteristics of monodominant montane beech forests in the National Park Biogradska Gora	Arhiv bioloških nauka - Archives of biological sciences
2011	SCI, SCIE, SSCI, A&HCI	Menković, N., Šavikin, K., TasiĆ, S., Zdunić, G., Stešević, D., Milosavljević, S., Vincek, D.	Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro)	Journal of Ethnopharmacology
2011	SCI, SCIE, SSCI, A&HCI	Šiljegović, J., Glamočlija, J., Soković, M., Vučković, I., Tešević, V., Milosavljević, S., Stešević, D.	Composition and atimicorbial activity of Seseli montanum subsp. tommasinii Essential oil	Natural Product Communication
2011	SCI, SCIE, SSCI, A&HCI	Čurović, M., Stešević, D., Medarević, M., Cvjetićanin, R, Pantić, D., Spalević, V.	Ecological and structural characteristics of monodominant montane beech forest in the National Park Biogradska Gora, Montenegro	Archives of Biological Sciences
2011	SCI, SCIE, SSCI, A&HCI	Petrović D., Stešević D.	Shift of the western boundary of the distribution area of Micromeria cristata (Hampe) Griseb. and Steptorhamphus tuberosus (Jacq.) Grossh.	Acta Botanica Croatica
2010	SCI, SCIE, SSCI, A&HCI	Vučković, I., Vujisić, Lj., Stešević, D., Radulović, S., Lazić, M., Milosavljević, S.	Cytotoxic guaianolide from Anthemis segetalis (Asteraceae)	Phytotherapy Research
2009	SCI, SCIE, SSCI, A&HCI	Stešević, D., Jovanović, S. & Šćepanović S.	Flora of the city of Podgorica- a chorological structure, and comparative analysis with floras of Roma, Patra, and Thessaloniki	Archives of Biological Sciences
2008	SCI, SCIE, SSCI, A&HCI	Godevac, D., Pejin, B, Zdunjić, G., Šavikin, K., Stešević, D., Vajs, V., Milosavljević, S.	Flavonoids from the arial part of Onobrychis montana subsp. scardica,	Journal of Serbian Chemical Society
2008	SCI, SCIE, SSCI, A&HCI	Stešević, D. & Jovanović, S.	Flora of the city of Podgorica, Montenegro (Taxonomic analysis)	Archives of Biological Sciences

Godina	Kategorija	Autori	Naslov	Izvor
2007	SCI, SCIE, SSCI, A&HCI	Danijela Stešević, Ute Feiler, Danijela Šundić, Slavoljub Mijović, Lothar Erdinger, Thomas-Benjamin Seiler, Peter Henninger, Henner Hollert,	Application of a New Sediment Contact Test with Myriophyllum aquaticum and of the Aquatic Lemna Test to Assess the Sediment Quality of Lake Skadar	Journal of Soils and Sediments

Vijeću Medicinskog fakulteta

Na osnovu Odluke Vijeća Medicinskog fakulteta o formiranju Komisije za doktorske studije,broj: 392/7 od 21.02.2019. godine a u skladu sa članom 41 Pravila doktorskihs tudija i tačkom 3.8 Vodiča za doktorske studije UCG-Centar za doktorske studije, nakon nakon razmatranja ispunjavanja formalnih uslova za ocjenu doktorske disertacije i poštujući princip kompetentnosti, Komisija za doktorske studije dostavlja Vijeću Medicinskog fakulteta

INICIJALNI PRIJEDLOG

Sastava Komisije za ocjenu doktorske disertacije

I. DOKTORAND: **Dr pharm Tijana Mićović**

Naziv doktorske disertacije: "**Farmakognozijska ispitivanja herbe izopa, *Hyssopus officinalis* L. (Lamiaceae) iz Crne Gore i Srbije"**

II. U skladu sa članom 38 Pravila doktorskih studija, doktorand dr pharm Tijana Mićović ispunjava uslove za ocjenu doktorske disertacije.

III. Komisija za ocjenu doktorske disertacije:

- **Prof. dr Zorica Potpara**, vanredni profesor Medicinskog fakulteta Univerziteta Crne Gore - predsjednik

- **Prof. dr Zoran Maksimović**, redovni profesor Farmaceutskog fakulteta Univerziteta u Beogradu – mentor

- **Prof. dr Danijela Stešević**, redovni profesor Prirodno-matematičkog fakulteta Univerzieta Crne Gore - član

KOMISIJA ZA DOKTORSKE STUDIJE

Prof. dr Filip Vukmirović

UNIVERZITET CRNE GORE

Vijeću Medicinskog fakulteta

Komisiji za doktorske studije

UNIVERZITET CRNE GORE
MEDICINSKI FAKULTET

Primljeno: 10.03.2022			
Org. jed.	Broj	Prilog	Vrijednost
med	412		

PREDMET: Zahtjev za ocjenu doktorske disertacije

Poštovani,

U skladu sa Pravilima studiranja na doktorskim studijama Univerziteta Crne Gore, ovim putem podnosim zahtjev za ocjenu doktorske disertacije pod nazivom:

„Farmakognozijska ispitivanja herbe izopa, *Hyssopus officinalis* L. (Lamiaceae) iz Crne Gore i Srbije“

Završetkom doktorske disertacije i objavom rada u časopisu sa SCI/SCIE liste koji sadrži djelove sopstvenih istraživanja sprovedenih u okviru izrade doktorske disertacije, ispunila sam uslove za predaju disertacije na pregled i ocjenu, predviđene Pravilima doktorskih studija Univerziteta Crne Gore.

Ovim putem se obraćam Komisiji za doktorske studije Medicinskog fakulteta, sa molbom da inicira predlog Komisije za ocjenu gorenevedene doktorske disertacije.

Uz Zahtjev, u prilogu dostavljam sljedeće:

- Pismenu saglasnost mentora

- Sedam primjeraka doktorske disertacije (u štampanoj formi)

- Fotokopiju rada objavljenog u časopisu sa SCI/SCIE liste koji sadrži dio rezultata iz doktorske disertacije

- Biografiju i bibliografiju

- CD sa cjelokupnim sadržajem doktorske disertacije u PDF formatu i objavljenim radom

- Potpisanu izjavu o autorstvu (Prilog 1 iz Uputstva o oblikovanju doktorske disertacije)

S poštovanjem,

Dr pharm. Tijana Mićović

Tijana Mićović

U Podgorici, 10.03.2022. godine

UNIVERZITET CRNE GORE

MEDICINSKI FAKULTET

Na osnovu Odluke Senata Univerziteta Crne Gore br. 03-1727/2 od 03.07.2018. imenovan sam za mentora za izradu doktorske disertacije kandidata dr pharm. Tijane Mićović. U fazi predaje doktorske disertacije na pregled i ocjenu, u skladu sa Pravilima doktorskih studija Univerziteta Crne Gore, dajem:

SAGLASNOST

Saglasan sam da kandidat dr pharm. Tijana Mićović može predati doktorsku disertaciju pod nazivom „Farmakognozijska ispitivanja herbe izopa, *Hyssopus officinalis* L. (Lamiaceae) iz Crne Gore i Srbije" na pregled i ocjenu.

U Beogradu, 03.03.2022. godine

Mentor

Prof. dr Zoran Maksimović

Prilog 1.

Izjava o autorstvu

Potpisani-a Tijana Mičović
Broj indeksa/upisa 10117

Izjavljujem

da je doktorska disertacija pod naslovom

" Farmakognazijska ispitivanja lerbe vrpa, Hyssopus officinalis L. (Lamiaceae) iz Crne Gore i Sr

- rezultat sopstvenog istraživačkog rada,
- da predložena disertacija ni u cjelini ni u djelovima nije bila predložena za dobijanje bilo koje diplome prema studijskim programima drugih ustanova visokog obrazovanja,
- da su rezultati korektno navedeni, i
- da nijesam povrijedio/la autorska i druga prava intelektualne svojine koja pripadaju trećim licima.

U Podgorici, 10.03.2022. godine

Potpis doktoranda
Tijana Mičović

UNIVERZITET CRNE GORE
MEDICINSKI FAKULTET
Broj: 412/4
Podgorica, 06.04.2022. godine

Uvidom u službenu evidenciju, izdaje se

P O T V R D A

Prof. dr Zoran Maksimović, redovni profesor Farmaceutskog fakulteta Univerziteta u Beogradu, nije u radnom odnosu na Medicinskom fakultetu Univerziteta Crne Gore.

Potvrda se izdaje kao prilog obrascu D2 za kandidata dr pharm Tijanu Mićović , i u druge svrhe se ne može koristiti.

RUKOVODILAC STUDENTSKE SLUUŽBE
Sonja Vukićević
Sonja Vukićević,diplomirani pravnik

UNIVERZITET CRNE GORE
MEDICINSKI FAKULTET
FARMACIJA
Broj dosijea: **10/2017**

Na osnovu člana 33 Zakona o upravnom postupku ("Službeni list CG", br. 56/14, 20/15, 40/16 i 37/17), člana 115 Zakona o visokom obrazovanju("Službeni list CG", br. 44/14, 52/14, 47/15, 40/16, 42/17, 71/17, 55/18, 3/19, 17/19, 47/19, 72/19, 74/20 104/21) i službene evidencije, a po zahtjevu studenta Mićović Zoran Tijana, izdaje se

UVJERENJE O POLOŽENIM ISPITIMA

Student **Mićović Zoran Tijana**, rođena **15-09-1990** godine u mjestu **Nikšić**, opština **Nikšić**, Republika **Crna Gora**, upisana je studijske **2017/2018** godine, u **I** godinu studija, kao student koji se **samofinansira** na **doktorske akademske studije**, studijski program **FARMACIJA**, koji realizuje **MEDICINSKI FAKULTET** - Podgorica Univerziteta Crne Gore u trajanju od **3 (tri)** godine sa obimom **180** ECTS kredita.

Student je položio ispite iz sljedećih predmeta:

Redni broj	Semestar	Naziv predmeta	Ocjena	Uspjeh	Broj ECTS kredita
1.	1	BIOSTATISTIKA	"A"	(odličan)	10.00
2.	1	MEDICINSKA INFORMATIKA	"A"	(odličan)	10.00
3.	1	METODOLOGIJA NAUČNOG ISTRAŽIVANJA	"A"	(odličan)	10.00
4.	2	IZRADA / ODBRANA POLAZNIH ISTRAŽIVANJA	"A"	(odličan)	15.00
5.	2	OSNOVI ĆELIJSKE BIOLOGIJE	"A"	(odličan)	5.00
6.	2	OSNOVI MOLEKULARNE GENETIKE	"A"	(odličan)	5.00
7.	2	PRIJAVA TEME DOKTORSKE DISERTACIJE	"A"	(odličan)	5.00

Zaključno sa rednim brojem **7.**

Ostvareni uspjeh u toku dosadašnjih studija je:
- srednja ocjena položenih ispita **"A" (10.00)**
- ukupan broj osvojenih ECTS kredita **60.00** ili **100.00%**
- indeks uspjeha **10.00.**

Uvjerenje se izdaje na osnovu službene evidencije, a u svrhu ostvarivanja prava na: (dječji dodatak, porodičnu penziju, invalidski dodatak, zdravstvenu legitimaciju, povlašćenu vožnju za gradski saobraćaj, studentski dom, studentski kredit, stipendiju, regulisanje vojne obaveze i slično).

Broj:
Podgorica, 11.04.2022 godine

M. P.

SEKRETAR: