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VARIATION OF ESSENTIAL OIL COMPOSITION OF *EUCALYPTUS CAMALDULENSIS* (MYRTACEAE) FROM THE MONTENGERO COASTLINE

Slavenko Grbović, Dejan Orčić, Maria Couladis, Emilija Jovin, Dušan Bugarin, Kristina Balog and Neda Mimica-Dukić

In the current study the essential oil obtained from the leaves of Eucalyptus camaldulensis plants collected from five localities of the Montenegro coastline was analyzed. The oil yield varied from 0.63 % (Kotor) up to 1.59% (Tivat). The chemical composition of the leaf essential oil was analyzed using GC-MS technique. Monoterpene hydrocarbons were a major class of compounds. Among them, dominant compounds were p-cymene (17.38-28.60%), β-phellandrene (12.35-14.47%) and β-pinene (0.94-11.48%). The second largest group was oxygenated monoterpenes with cryptone (4.97-7.25) and terpinene-4-ol (2.75-4.21%) as predominant. Besides high content of sesquiterpene alcohol spathulenol (7.83-14.15%) was found. According to the results obtained E. camaldulensis from Montenegro can be classified in the chemotype with low 1,8-cineole and high p-cymene and cryptone ratio.

KEYWORDS: 1,8-cineole, cryptone, GC-MS, *Eucalyptus camaldulensis*, p-cymene

INTRODUCTION

In recent years, natural extracts have been in high demand by the food manufacturers, cosmetics, and pharmaceuticals due to the growing interest of consumers in the ingredients from the natural sources. Spices and essential oils are also well known for their various beneficial effects on human health. The use of aromatic plants and spices in phytotherapy is mostly due to the essential oils and their various biological activities, such as antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, and anticarcinogenic (1,2). However, besides having a wide spectrum of well-known biological and pharmacological activities, essential oil composition is frequently used in distinguishing particular chemoraces among the species and genera.

Eucalyptus (Myrtaceae) is one of the most important and most widely planted genera. Although being Australia's native, more than 700 species wildly grow in many parts of the world. In fact, Eucalyptus species are one of the most-extensively planted pulpwood

Slavenko Grbović, PhD student, Emilija Jovin, PhD student , Dejan Orčić, PhD student, Kristina Balog, PhD student, Dušan Bugarin PhD student and Dr Neda Mimica-Dukić, Prof., Department of Chemistry Faculty of Scineces, University of Novi Sad, Trg Dositeja Obradovića 3, 21 000 Novi Sad, Serbia. Dr Maria Couladis, Department of Pharmacognosy and Natural Product Chemistry, Faculty of Pharmacy, University of Athens, Greece

species. The trees are planted largely for their leaves that are rich in essential oils and are exploited commercially for their use in food, flavors, pharmaceutical and perfumery industries (3). Used for centuries as a traditional Aboriginal herbal remedy, eucalyptus leaves and their essential oils have found various applications in everyday life due to their antiseptic, anti-inflammatory and antipyretic properties (4, 5).

Around 15 eucalyptus species grow in the Mediterranean region, out of which less than 10 species were introduced in the coastal area of Montenegro at the beginning of the 20th century. Among them, *Eucalyptus camaldulensis* Dehn. (syn. *Eucalyptus rostrata* Schl.) is the most commonly found in Montenegro. This species is used in the indigenous system of medicine to cure various human ailments such as diarrhea, chronic dysentery, malaria, infection of upper respiratory tract, and certain skin diseases (6). Essential oils obtained from the leaves are of particular commercial interest. However, no information about the chemical composition of *Eucalyptus camaldulensis* grown in Montenegro had been published before we initiated the corresponding studies. Here, for the first time, we report the composition of essential oil from the leaves of *Eucalyptus camaldulensis* plants collected from the different locations of the Montenegro coastline.

EXPERIMENTAL

Plant material and chemicals

Plant material: For essential oil analysis leaves were collected from the trees of *Eucalyptus camaldulensis* Dehn. growing at five locations at Montenegro coastline: Bar (No 2-1812), Sutomore (No 2-1813), Tivat (No2-1814), Kotor (No2-1815), and Herceg Novi (No2-18-16), in August 2006. Voucher specimens were prepared and identified by Goran Anačkov, PhD, and deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad.

Essential oil isolation and analysis

***Essential oils isolation*:** Air-dried plant materials were submitted to hydrodistillation according to Eur. Pharm. 4 (7), using *n*-hexane as a collecting solvent. The solvent was removed under vacuum, and the quantities of the essential oils were determined gravimetrically.

***GC-MS analysis of essential oil*:** Qualitative analysis of essential oils was performed by gas chromatography-mass spectrometry (GC-MS). Agilent Technologies 6890N-5975B system was used, with data acquisition parameters as follows: carrier gas - He, flow rate 1.0 mL/min, constant flow mode; injection volume 0.2 μL (split 50:1), inlet temperature 250°C; Agilent Technologies HP-5MS 30 m × 0.25 mm × 0.25 μm column, temperature program: 50°C for 1 min, 5°C/min to 100°C, 9°C /min to 200°C, hold 7.89 min; transfer line temperature 280°C; electron ionization, electron energy 70 eV, scan mode, mass range 35-400 Da, quadrupole temperature 150°C, source temperature 230°C. Acquired data were analyzed by Agilent Technologies MSD ChemStation software in conjunction with AMDIS (Automated Mass Spectral Deconvolution and Identification

System) and NIST MS Search software. Two different mass spectra libraries were used for mass spectra identification: Wiley Registry of Mass Spectral Data 7th Edition (338000 spectra, 289000 unique compounds) (8), NIST/EPA/NIH Mass Spectral Library 05 with 190825 spectra, 163198 unique compounds (9). Identity is confirmed by comparison of Kovat`s retention indices.

RESULTS AND DISCUSSION

The amount of essential oil obtained by hydrodistillation from the dried leaves is presented in Table 1. The oil quantity ranged from 0.63 % (Kotor) up to 1.59% (Tivat). Comparison with available literature data (10, 11, 12, 13) shows that only plants collected from the Tivat location, have a satisfactory oil yield, whereas the other plants are rather poor in oil content.

Table 1. Essential oil content (%) in dried leaves of *Eucalyptus camaldulensis* Dehn. collected from five locations from Montenegro coastline.

Sample	Bar	Sutomore	Tivat	Kotor	Herceg Novi
Oil Content %	0.67	0.70	1.59	0.63	0.68

In all the investigated samples monoterpene hydrocarbons were identified as the major class of compounds (Table 2). Among them dominant were: *p*-cymene (17.38-28.60%) and β-phellandrene (12.35-14.47%). The next largest group was the one of oxygenated monoterpenes that ranged from 20.63% (Kotor) up to 25.62% (Tivat). Interestingly, the major volatile compound in all oils was irregular monoterpene cryptone (Figure 1) whose content ranged from 4.97% (Kotor) to 7.25% (Tivat).

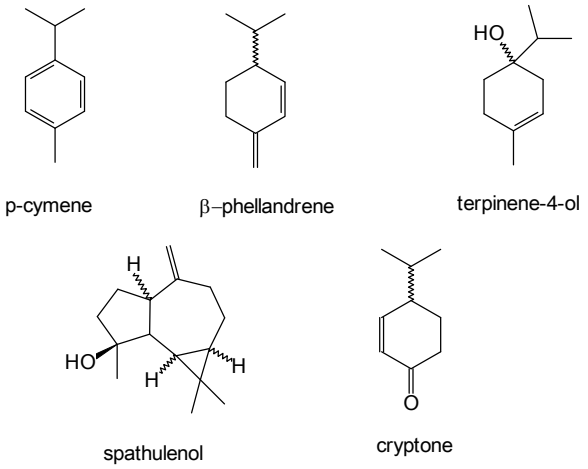

Figure 1. Molecular structures of major compounds in *Eucalyptus camaldulensis* essential oil

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Table 2. Percentage content of volatile compounds in essential oil of the leaves from examined *Eucalyptus camaldulensis* Dehn. from Montenegro

Compound	KI[a]	Tivat	Sutomore	Kotor	Herceg Novi	Bar
α-Thujene	927	2.68	3.01	3.04	2.28	2.19
α-Pinene	934	1.66	5.01	3.88	3.75	3.59
Sabinene	974	1.26	1.47	1.05	1.28	1.02
β-Pinene	978	0.94	11.48	6.34	9.94	9.54
β-Myrcene	992	0.77	0.93	0.84	0.77	0.87
α-Phellandrene	1006	4.26	3.81	2.36	3.43	4.00
α-Terpinene	1018	0.87	0.98	0.73	0.68	1.04
p-Cymene	1026	28.60	20.02	24.63	17.38	18.15
β-Phellandrene	1030	13.99	14.46	12.35	13.79	14.47
Eucalyptol	1033	2.89	2.03	1.70	1.65	1.78
γ-Terpinene	1060	1.12	1.31	0.95	1.02	1.36
α-Terpinolene	1090	0.56	0.57	0.42	0.44	0.65
Linalool L	1101	0.81	0.96	0.85	0.76	0.99
cis-p-menth-2-en-1-ol	1126	0.85	0.69	0.83	0.70	0.68
menthenol isomer + trans-Pinocarveol	1144	0.66	0.98	0.97	0.89	1.11
n.i.	1155	0.38	0.38	0.48	0.25	0.51
Pinocarvone	1168	tr	0.33	0.24	0.30	0.41
Terpinene-4-ol	1182	4.21	3.11	3.99	2.75	3.50
Cuminyl alcohol	1188	0.47	0.28	0.29	0.31	0.32
Cryptone	1190	7.25	5.27	4.97	6.12	5.78
α-Terpineol	1194	0.58	0.66	0.48	0.62	0.71
Myrtenal	1199	0.25	0.73	0.61	0.71	0.86
Cuminaldehyde	1246	2.57	1.69	1.81	1.59	1.87
Phellandral	1281	1.62	1.54	1.59	1.67	1.88
Cumyl alcohol	1294	0.41	0.31	0.28	0.42	0.37
Thymol	1302	0.33	0.32	0.34	0.53	0.43
α-Terpineyl acetate	1355	tr	tr	tr	tr	tr
Aromadendrene	1451	0.30	tr	tr	tr	tr
Allo-aromadendrene	1474	1.79	1.63	2.18	1.47	1.99
n.i.	1501	0.44	0.38	0.57	0.38	0.44
Bicyclogermacrene	1509	3.73	3.88	3.77	3.64	3.60
Spathulenol	1593	7.83	8.52	11.63	14.15	9.77
sesquiterpene	1599	0.90	0.83	1.12	1.34	0.94
Viridiflorol	1606	0.14	tr	0.14	0.18	0.16
Sesquiterpene	1639	0.30	0.38	0.51	0.93	0.58
sesquiterpene	1642	0.38	0.51	0.52	0.77	0.69
Lepidozenal	1648	0.12	0.28	0.42	0.49	0.42
Total identified		**95.92**	**98.73**	**96.89**	**97.38**	**96.65**
Monoterpene hydrocarbons		**57.37**	**61.95**	**56.09**	**54.73**	**56.77**
Oxygenized monoterpenes		**25.62**	**21.04**	**20.63**	**21.07**	**23.48**
Sesquiterpene hydrocarbons		**6.06**	**5.58**	**6.04**	**5.19**	**5.78**
Oxygenized sesquiterpene		**8.30**	**8.62**	**11.95**	**14.56**	**10.37**

[a] *Retention indices relative to C_9-C_{24} n-alkanes on the HP 5MS column. GC, identification based on retention times of authentic compounds on HP 5MS column; MS, tentatively identified on the basis of computer matching of the mass spectra of peaks with the NIST/NBS and Wiley libraries; tr - ratio in essential oil below 0.1%; n.i. non identified*

Oxygenated sesquiterpenes were also found in a considerable amount. The highest ratio was found in the essential oil obtained from the plants collected from Herceg Novi (14.15%) and the lowest from plants from the vicinity of Tivat (7.83%). Sesquiterpene alcohol spathulenol was the dominant compound in this class. Sesquiterpene hydrocarbons did not significantly vary between the samples. Allo-aromadendrene was one of the most abundant compounds. Evidently, no significant qualitative and quantitative difference in volatile composition between the samples examined was found. Only the plants from the Tivat region can be distinguished as containing the highest portion of *p*-cymene and the lowest amount of β-pinene.

The composition of the essential oil from *E. camaldulensis*, especially from the leaves, has been widely studied. By surveying the data reported we found a great diversity of oil composition, which was effected by many factors such as: geographical origin, tissue explored, date of harvest, genetic factors etc. (14, 15). Two groups of *E. camaldulensis* essential oils can be distinguished: those that contain 1,8-cineole as the main compound, which include *E. camaldulensis* from Mali, Mozambique, Nigeria, Egypt and Iran (16-21) and those that contain spathulenol, *p*-cymene and cryptone as main compounds, and small quantities of 1,8-cineol, like the oil examined here, and which are similar to *E. camaldulensis* from the south of Florida, Jerusalem and Greece (22, 13, 14).

Our results certainly suggest that only one chemotype of *E. camadulensis* exists on the Montenegro coastline. The main characteristic of this chemotype is a high amount of aromatic monoterpene hydrocarbon *p*-cymene, followed by monoterpenes: β-phellandrene, β-pinene (except for the plants from Kotor), cryptone, β-phellandrene, terpinene-4-ol and sesquiterpene, spathulenol. According to the high *p*-cymene, cryptone and terpinene-4-ol ratio, a great similarity was found with the plants cultivated in Spain-Valencia (23) and the south of Florida (22).

As already mentioned, the essential oil of eucalyptus species is of great commercial value. In the south-eastern countries such as Thailand, *E. camaldulensis* is mainly planted for the use as a pulpwood. Evidently, during the process of papermaking, a large amount of waste such as leaves is disposed. Therefore, the possibility of exploiting the leaves as a source of oil production is being extensively investigated (24).

Some recent studies show that *E. camaldulensis* oil exhibits a great antimicrobial and repellent activity (25, 26). Eucamalol and epi-eucamalol were identified as potent repellents. Interestingly enough, these compounds can be synthesized from irregular monoterpene cryptone (25). Cheng et al., 2009. (27) found that the volatile oil obtained from *E. camaldulensis* had an excellent mosquito larvicidal activity. The oil composition was similar to the one examined here, in respect to high *p*-cymene and phellandrene portion. Having these facts in mind, one can conclude that leaves of *E. camaldulensis* from Montenegro should be exploited as a source of a valuable essential oil with considerable commercial value.

CONCLUSION

The GC-MS analysis of essential oil yield and composition in *Eucalyptus camaldulensis* Dehn., samples collected from five different locations of the Montenegro coast-

line shows that all plants belong to one chemotype which is characterized by the high portion of *p*-cymene, β-phellandrene, β-pinene, cryptone, spathulenol and a low ratio of 1,8-cineol. This is the first report on chemical study of the eucalyptus species from Montenegro. Investigations of the biological activity of oil and extracts of *Eucalyptus* species in Montenegro are in progress.

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ЕТАРСКО УЉЕ *EUCALYPTUS CAMALDULENSIS* (MYRTACEAE) СА ЦРНОГОРСКОГ ПРИМОРЈА

Славенко Грбовић, Дејан Орчић, Maria Couladis, Емилија Јовин, Кристина Балог и Неда Мимица-Дукић

Иако аутохтоне у Аустралије и Тасманији, врсте еукалиптуса гаје се широм света као украсне биљке али и као значајна индустријска сировина. Етарско уље врста еукалиптуса је од посебног комерцијалног значаја и широко се примењује у козметичкој, фармацеутској и прехрамбеној индустрији. Хемијски састав и биолошка активност етарског уља условљени су бројним факторима од генетских, климатских, времена брања, до старости биљака и др. Од преко 700 врста еукалиптуса на Медитерану има око 15, а у Црној Гори мање од 10 врста. У овом раду по први пут су представљени резултати о хемијском саставу етарског уља еукалиптуса са црногорског приморја, и то врсте *Eucalyptus camaldulensis* Dehn. која је најраспрострањенија у Црној Гори. Испитивани су количина и хемијски састав етарског уља добијеног хидродестилацијом из осушених листова биљака сакупљених са пет различитих локалитета: Котор, Тиват, Сутоморе, Херцег Нови и Бар. Садржај уља кретао се од 0,63% (Котор) до 1,59% (Бар). У етарском уљу доминирала су једињења из класе монотерпенских угљоводоника од којих су доминантни били: *p*-цимен (17,38 – 28,60%), β-феландрен (12,35-14,47%), β-пинен (0,94-11,48%). Од оксидованих монотерпена доминантни су били иregуларни монотерпен криптон (4,97-7,25%) и терпинен-4-ол (2,75-4,21%), док је од сесквитерпена доминантна компонента био сесквитерпенски алкохол спатуленол (7,83-14,15%). Добијени резултати показују да на црногорском приморју доминира специфичан хемотип врсте *Eucalyptus camaldulensis* Dehn, сиромашан у 1,8-цинеолу (најчешћа доминантна компонента еукалиптуса), а богат у *p*-цимену и криптону.

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