Nat. Volatiles & Essent. Oils, 2021; 8(1): 29-38
DOI: 10.37929/nveo.772848

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Monarda essential oils as natural cosmetic preservative systems

Łukasz Gontar[1,2], Anna Herman[1,3,*], Ewa Osińska[2]

[1]Faculty of Cosmetology, The Academy of Cosmetics and Health Care, Warsaw, POLAND
[2]Department of Vegetable and Medicinal Plants, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences, Warsaw, POLAND
[3]Faculty of Health Sciences, Warsaw School of Engineering and Health, Bitwy Warszawskiej 1920 18 street, 02-366 Warsaw, POLAND

*Corresponding author. Email: anna.herman@onet.pl

Submitted: 30.07.2020; Accepted: 26.01.2021

Abstract

The aim of the study was to compare the antimicrobial activity of essential oils (EOs) obtained from leaves and inflorescences of *Monarda media, M. didyma,* and *M. citriodora* found in different phenological stages of development, to examine the inhibition of microbial growth by *Monarda* EOs in O/W emulsions. Thymol, *p*-cymene, and carvacrol were the main constituents characterized in *Monarda* EOs. The strongest antimicrobial activity was obtained from the leaves of *Monarda* EOs in the flowering phase of the development. *M. media* EO fulfilled the criterion of the preservative effectiveness test for all evaluated bacteria and fungi, while *M. didyma* and *M. citriodora* EOs were not sufficient for Gram-negative bacteria. As an overall result, *M. media* EO may be an effective candidate as natural cosmetic preservative, and finally create self-preserving system in O/W emulsion, while *M. didyma* and *M. citriodora* EOs may only reduce the amount of synthetic preservatives used in O/W emulsions.

Keywords: *Monarda citriodora*, *Monarda didyma*, *Monarda media*, essential oils, preservative system

Introduction

The preservatives are added to cosmetic formulations to prevent microbial growth during production process, packing, storage and entire period of use by consumers to ensure their safety. The cosmetic industry recommends the use of combination of various preservatives in the smallest concentrations for the protection of cosmetic products from potential microorganism contaminations. Unfortunately, synthetic preservatives are one of the main factors causing allergies and irritant contact dermatitis to users (Andersen, 1993). Therefore, many cosmetic manufacturers, also increasingly aware consumers draw their attention to cosmetics marked as preservative-free, which are referred as formulations without the well known preservatives listed in Annex VI of the Commission Directive 76/768/EEC, but other cosmetics ingredients with antimicrobial activity. Among the cosmetic ingredients used in self-preserving preparations are essential oils (EOs) and plant extracts (Herman, 2019). Therefore, EOs with antimicrobial activity can reduce the amounts as well as the concentration of synthetic preservatives in cosmetic products, or even completely eliminate the use and finally create a preservative free or self-preserving system.

The aim of the present study was to compare the antimicrobial activity of EOs obtained from leaves and inflorescences of *M. media* L., *M. didyma* L., and *Monarda citriodora* L. found in different phenological stages of development, to examine the inhibition of microbial growth by *Monarda* EOs in O/W emulsions.

Materials and Methods

Microorganisms

Pseudomonas aeruginosa ATCC 9027*, Escherichia coli* ATCC*8739, Staphylococcus aureus* ATCC 6538*, Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 were used. The microorganisms were

activated through double passaging: bacteria on TSA medium (Trypticase Soy Agar; BioMerieux, France) (37°C, 48h), yeast on SDA medium (Sabouraud Dextrose Agar; BioMerieux, France) (25°C, 72h) and mould on SDA medium (BioMerieux, France) (25°C, 7days).

Plant collection

The fresh herbs of *M. citriodora*, *M. didyma* and *M. media* were harvested from June to September 2016 from an experimental field at the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences (Poland) at 3 different stages of development: I – budding stage (shoots with only leaves), II – flowering stage (full bloom stage), III - maturity stage (seed forming stage). The raw materials, leaves and inflorescences (if available), were air-dried at 35°C in the dark.

Essential oils extraction

The *Monarda* EOs were isolated according to the European Pharmacopeia 9th edition. 30 g of each air-dried raw materials were distilled with 1 L of distilled water in a 2-liter round bottom glass flask using a Clevenger-type apparatus. The distillation time took about 180 minutes. Obtained *Monarda* EOs were stored in amber vials at 4°C until chemical and microbiological investigations.

Analysis of essential oils

The EOs from leaves of *Monarda* in the flowering phase of the development were analysed by Agilent 7890A/5975C GC-MS system inert XL MSD with Triple-Axis Detector (Agilent-Technologies, Little Falls, CA, USA), equipped with a non-polar capillary column (HP-5MS 5% phenylmethylsiloxane; 30.00 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 50°C for 5 min initially, and then raised at the rate of 3°C min^{-1} to 240°C, and then was kept for 1 min at 240°C. Injector temperature was set at 290 °C. Helium was used as carrier gas at a flow rate of 0.8 mL min^{-1}, and 1 µL samples (5% of EO in hexane) were injected automatically by the (7683B Injector) in the split mode (1:20). For MS detection, an electron ionization mode was used with ionization energy of 70 eV and scan range of 35 to 350 *m/z*. Acquiring and analyses of data were carried out using a built-in data-handling program provided by the manufacturer of the GC/MS (Agilent ChemStation). Quantification by % peak area calculations was performed using the non-polar HP-5MS column.

The identification of the EOs constituents were based on a comparison of their MS spectra with Mass spectra library of NIST 08 and Wiley 8th ed. Mass spectra were confirmed by comparing linear retention indices (LRI) calculated relative to (C_8-C_{20}) *n*-alkanes with LRI database, included in NIST 08 mass spectra library.

Determination of antimicrobial activity of *Monarda* essential oils

Several colonies of overnight cultures of individual organisms were suspended in saline to obtain density equal to 0.5 McFarland turbidity standard (approximate cell density of 1.5 × 10^8 CFU/mL). The antibacterial and antifungal activity was evaluated by using the disc diffusion method. Suspensions of microorganisms were spread over the TSA and SDA agar plates (BioMerieux, France), respectively by using sterile cotton swabs. The sterile paper discs of 6 mm in diameter (BTL, Poland) were impregnated with 10 µL mixture of *Monarda* EO / 96 % ethanol (1:1) and placed on the agar surface. Tetracycline (30 µg), Erythromycin (15 µg), Miconazole (10 µg) and Nystatin (100 U) (BTL, Poland) were used as controls. All bacterial plates were incubated at 37°C for 24 h and fungal plates at 25°C for 72h. The diameter of the zone of inhibition was measured in mm. For each test, three replicates were performed.

DOI: 10.37929/nveo.772848

Preparation of O/W emulsions

The composition of formulations is showed in Table 1. Emulsifier Ceteareth-20 (Eumulgin ® B2,CognisPolska Sp. z.o.o.), Isopropyl Myristate (Cognis Polska Sp. z.o.o.), Ethylhexyl stearate (Cetiol® 868, Cognis Polska Sp. z.o.o.), Octyldecanol (Eutanol G®, Cognis Polska Sp. z.o.o.), Cyclopentasiloxane and Dimethicone (Dow Corning®1411 Fluid, Dow Corning Europe S.A.), water, glycerine (POCH S.A, Poland) and preservative - Germaben II (Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben; Ashland, USA) /essential oil were homogenized for 3 min using Heidolph SilentCrusher M homogenizer (Heidolph Instruments GmbH & Co. KG, Germany) at approximately 15 000 rpm. Then emulsion was gently stirred using blender RW 16 (IKA® Werke GmbH & Co. KG, Germany) and essential oils and the Sodium Polyacrylate (Cosmedia SP, Cognis Polska Sp. z.o.o.) was added stepwise. Stirring continued for an additional 30 min. *Monarda* EOs from leaves in flowering phase of the development were added at 1.5% concentration. Preservative was added at 1.0 % concentration as recommended use level for emulsion specified by producer (Ashland, USA). Emulsion without preservative and EOs was a references sample.

Table 1. Composition of the emulsions: E1 - emulsion without preservative / essential oils; E2 - emulsion with preservative; E3 – emulsion with essential oils

Ingredients	Percentage by weight		
	E1	**E2**	**E3**
SodiumPolyacrylate	0.8	0.8	0.8
Ceteareth-20	3	3	3
IsopropylcMyristate	4	4	4
EthylhexylSearate	3	3	3
CetearylIsononanoate	3	3	3
CyclopentasiloxaneandDimethicone	3	3	3
Glycerine	3	3	3
PropyleneGlycol (and) DiazolidinylUrea (and) Methylparaben (and) Propylparaben	0	1	0
Essential oil	0	0	1.5
Aqua	80.2	79.2	78.7

[a]INCI Name, [b]preservative system (Germaben II)

Determination of the preservation efficacy of the O/W emulsions

Evaluation of the antimicrobial protection of a cosmetic product was performed according to the EN ISO 11930:2012 (Cosmetics - Microbiology - Evaluation of the antimicrobial protection of a cosmetic product). Inoculation of test microorganism suspension was prepared by addition of 0.02 mL calibrated inoculum (each strain separately) to 20 g emulsion sample to obtained final concentration of bacteria between 1 x 10^5 - 1 x 10^6 CFU/ml and fungi between 1 x 10^4 - 1 x 10^5 CFU/ml in the formulation. The inoculated containers were mixed thoroughly and incubated in the dark at 20°C to 25°C. The number of viable microorganism in formulations was determined by the plate count method at the proper times 0, 7, 14 and 28 days after inoculation. A sample of 1 mL emulsion was transferred to 9 mL Eugon LT 100 broth (Graso Biotech, Poland) and pre-incubated for 30 minutes at room temperature, then 10-fold dilutions method was done. Triplicate plating of each dilution was performed with TSA agar for bacteria, SDA agar for yeast and PDA agar for mould. The plates were incubated at 37°C for 72h (bacteria and *C. albicans*) and 25°C for 7 days (*A. brasiliensis*), respectively. The number of surviving microorganisms per gram of tested cosmetic product was determined by count the CFU per plate (30-300 colonies for bacteria and *C. albicans*, 15-150

DOI: 10.37929/nveo.772848

colonies for *A. brasiliensis*). The results were expressed as log reduction value (log CFU/g). For each time and each strain, the log reduction value is calculated and compared to the minimum values required for evaluation criteria A or B of preservation efficacy test (Annex B in EN ISO 11930:2012). Formulation meets Criterion A when the cosmetic product is protected against microbial proliferation (recommended efficacy of antimicrobial preservation for topical preparations). Formulation meets Criterion B when the microbiological risk analysis shall demonstrate the existence of control factors not related to the formulation (e.g. a protective package such as pomp provides a higher level of protection than a jar) indicating that the microbiological risk for topical preparations is tolerable. All tests were conducted in triplicate and data from experiments were calculated as mean ±SD. Standard deviation for the test of microorganism population viability not exceeding 0.3 logarithmic unit.

Statistical analysis

All tests were conducted in triplicate and data from experiments were calculated as mean ±SD. Standard deviation for the test of microorganism population viability not exceeding 0.3 logarithmic unit.

Results and Discussion

Chemical composition and antimicrobial activity of *Monarda sp.* EOs

In the recent years, many publications have notified that antimicrobial activity of EOs is closely related to their chemical compositions, which depend on growth stage and the part from which the raw material is obtained (Mohammadi and Saharkhiz, 2011; Saharkhiz et al., 2009). The main constituents of *Monarda sp*. EOs determined by the GC-MS method were: *M. citriodora* EO: thymol (58.0%), *p*-cymene (13.4 %) and carvacrol (11.7 %); *M. media* EO: thymol (41.9 %) and *p*-cymene (18.0 %); *M. didyma* EO: carvacrol (26.2 %) and p-cymene (11.6 %) (Table 2), what corresponds with the results obtained by other researchers (Collins et al., 1994; Fraternale et al., 2006; Mattarelli et al., 2017). Moreover, it is well known that thymol and carvacrol are bioactive compounds with strong antimicrobial activity against bacteria and fungi, even resistant isolates (Memar et al., 2017; Naghdi Badi et al., 2017).

Table 2. Chemical composition of *Monarda sp*. essential oils obtained from leaves in flowering phase of the development

Essential oil constituents	Retention time	LRI calculated	LRI database (Nist 08)	Relative content [%]		
				M. citriodora	*M. didyma*	*M. media*
α-Thujene	8.421	925	928	1.37	1.31	1.96
α-Pinene	8.675	930	932	0.57	0.91	0.79
Camphene	9.309	944	946	0.10	0.69	0.13
Sabinene	10.610	970	975	0.02	7.77	0.17
1-Octen-3-ol	10.951	980	980	1.54	2.58	3.66
3-Octanone	11.300	987	988	0.05	0.23	0.06
Myrcene	11.461	991	993	0.34	1.09	0.40
3-Octanol	11.746	997	997	0.06	1.38	0.14
α-Phellandrene	11.978	1002	1006	0.04	0.17	0.18
α-Terpinene	12.600	1015	1018	1.19	2.00	3.14
p-Cymene	13.091	1026	1027	13.43	11.55	17.99
Limonene	13.211	1028	1030	0.72	1.18	4.09
1,8-Cineole	13.335	1031	1036	0.10	2.82	0

γ-Terpinene	14.703	1057	1058	0.76	5.79	0.94
cis-Sabinene hydrate	15.108	1065	1069	0.70	0.58	0.87
α-Terpinolene	16.147	1087	1090	0.11	0.33	0.12
trans-Sabinene hydrate	16.660	1095	1098	0.04	0.24	0.12
Linalool	16.833	1101	1101	0.16	6.46	0.16
Nonanal	17.057	1104	1106	0.03	0.22	0.14
Borneol	19.880	1164	1169	0.20	0.88	0.15
Terpinen-4-ol	20.472	1176	1180	0.94	1.07	0.72
α-Terpineol	21.155	1188	1193	0.08	2.55	0.12
Thymol methyl ether	23.269	1236	1234	0.02	0.67	0.15
Carvacrol methyl ether	23.693	1245	1243	2.14	10.99	6.69
Thymoquinone	23.929	1254	1252	0.66	0.21	1.81
Thymol	26.399	1295	1293	57.96	0.99	41.92
Carvacrol	26.654	1306	1303	11.70	26.15	4.44
β-Bourbonene	29.848	1382	1382	0.08	0.39	0.10
Caryophyllene	31.280	1417	1423	0.42	1.03	0.92
Germacrene D	33.821	1478	1479	0.06	2.67	0.90
δ-Cadinene	35.564	1511	1514	0.14	0.15	0.24
Thymohydroquinone	36.888	1556	1558	0.82	0.50	1.04
Caryophyllene oxide	37.806	1580	1580	0.34	0.14	0.22
			Total identified (%)	96.9	95.68	94.48

The antimicrobial activity of *Monarda sp.* EOs were determined using disc-diffusion methods and presented in Table 3. The EOs from the leaves of *M. media* collected in the budding phase, and the EOs from inflorescences of *M. media* and *M. didyma* in the maturity phase showed the strongest antimicrobial activity against *S. aureus* growth. EOs from the leaves of *M. media* and *M. citriodora* inflorescences, both collected during the full flowering phase have the strongest antibacterial activity against *P. aeruginosa* and *E. coli* growth, respectively. The EOs from the leaves of *M. citriodora* harvested in the budding phase and full flowering phase as well EO from *M. citriodora* inflorescences collected in the maturity phase showed the stronger antifungal activity against *C. albicans* growth. The EOs from the inflorescences of *M. citriodora* harvested in the full flowering phase showed the strongest antifungal activity against *A. brasiliensis*, while EOs from the inflorescences of *M. didyma* collected in the maturity phase showed no activity against mould. Summarizing, the results showed that *Monarda* EOs obtained from the leaves in the flowering phase strongly inhibited the growth of Gram-positive bacteria and fungi but was less effective against Gram-negative bacteria. Literature data showed that *M. citriodora* EO has a strong antibacterial activity against *E. coli, B. subtilis* and *S. albus* (Lu et al., 2011) as well antifungal activity against 15 fungal species (Bishop and Thornton, 1997). *M. didyma* EO strongly inhibited the growth of *S. aureus*, *E. coli, B. subtilis, B. cereus, P. fluorescens, S. typhimurium* and *C. albicans,* while weak antibacterial activity was observed against *L. monocytogenes* and *P. aeruginosa* (Ghabraie et al., 2016; Wróblewska et al., 2019). Antifungal activity of *M. didyma* EO was completely inhibited mycelial growth and spore germination of *Botrytis cinerea* (Adebayo et al., 2013). The EOs from leaves and flower *M. didyma* collected during the vegetative phase inhibited the growth of fungi *Rhizoctonia solani* and *B. cinerea* (Fraternale et al., 2006). Moreover, it was shown that the main active ingredient of *M. didyma* EO - thymol and carvacrol showed inhibitory activity against *R. solani* (Gwinn et al., 2010). Unfortunately, the strong antimicrobial activity of *Monarda* EOs confirmed by disc-diffusion test are not enough to confirm their potential use as preservatives in cosmetics. Only a challenge test is able to confirm whether EOs can replace synthetic preservatives in

cosmetics / reduce the concentration of preservatives added to cosmetics / or do not have sufficiently strong antimicrobial activities that would protect the cosmetics against microbial contamination. However, some literature data showed that the use of EOs in formulation significantly reduce or even eliminate the use of synthetic preservatives (Herman, 2019).

Table 3. Antibacterial and antifungal activity of *Monarda* essential oils (10 µl) in agar disc diffusion method

Species	Plant growth stage	Raw material	Microorganisms				
			S. aureus	***P. aeruginosa***	***E. coli***	***C. albicans***	***A. brasiliensis***
M. citrodora	Budding	Leaf	57±2.0	11±0.5	41±6.0	66±0.0	62±2.0
	Flowering	Leaf	65±1.5	16±1.0	44±1.0	66±1.0	67±2.0
		Inflorenscence	56±1.5	15±4.0	46±0.5	65±0.0	68±2.5
	Maturity	Leaf	74±3.5	14±0.0	43±2.5	61±0.5	63±1.0
		Inflorenscence	53±0.5	17±2.0	43±3.0	66±2.0	60±5.0
M. didyma	Budding	Leaf	73±6.5	17±0.5	36±0.5	52±1.0	52±1.5
	Flowering	Leaf	24±0.5	11±0.5	24±1.5	47±3.0	26±6.0
		Inflorenscence	84±0.0	15±0.5	30±2.0	49±4.0	33±4.0
	Maturity	Leaf	53±3.0	20±5.5	29±0.0	23±1.0	12±0.0
		Inflorenscence	89±0.0	9±0.5	14±0.5	17±0.0	0 ± 0.0
M. media	Budding	Leaf	89±0.0	19±2.0	34±1.5	59±2.0	55±1.5
	Flowering	Leaf	87±1.0	22±2.0	34±0.5	56±2.0	58±2.0
		Inflorenscence	80±2.0	21±1.0	31±0.5	58±2.0	55±5.0
	Maturity	Leaf	82±2.0	21±0.5	37±1.5	60±0.5	54±1.0
		Inflorenscence	89±0.0	12±0.5	19±1.0	24±1.0	9±0.5
Ethanol			0±0.0	0±0.0	0±0.0	10±0.5	0±0.0
Tetracycline (30 µg)			40±0.0	16±1.5	25±2.5	-	-
Erythromycin (15 µg)			38±0.5	26±6.0	13±0.5	-	-
Miconazole (10 µg)			-	-	-	14±0.5	14±0.5
Nystatin (100 U)			-	-	-	20±0.5	12±0.5

Diameter of inhibition zones (mm) including the diameter of disc (6 mm), values are given as mean ± SD of triplicate experiment.

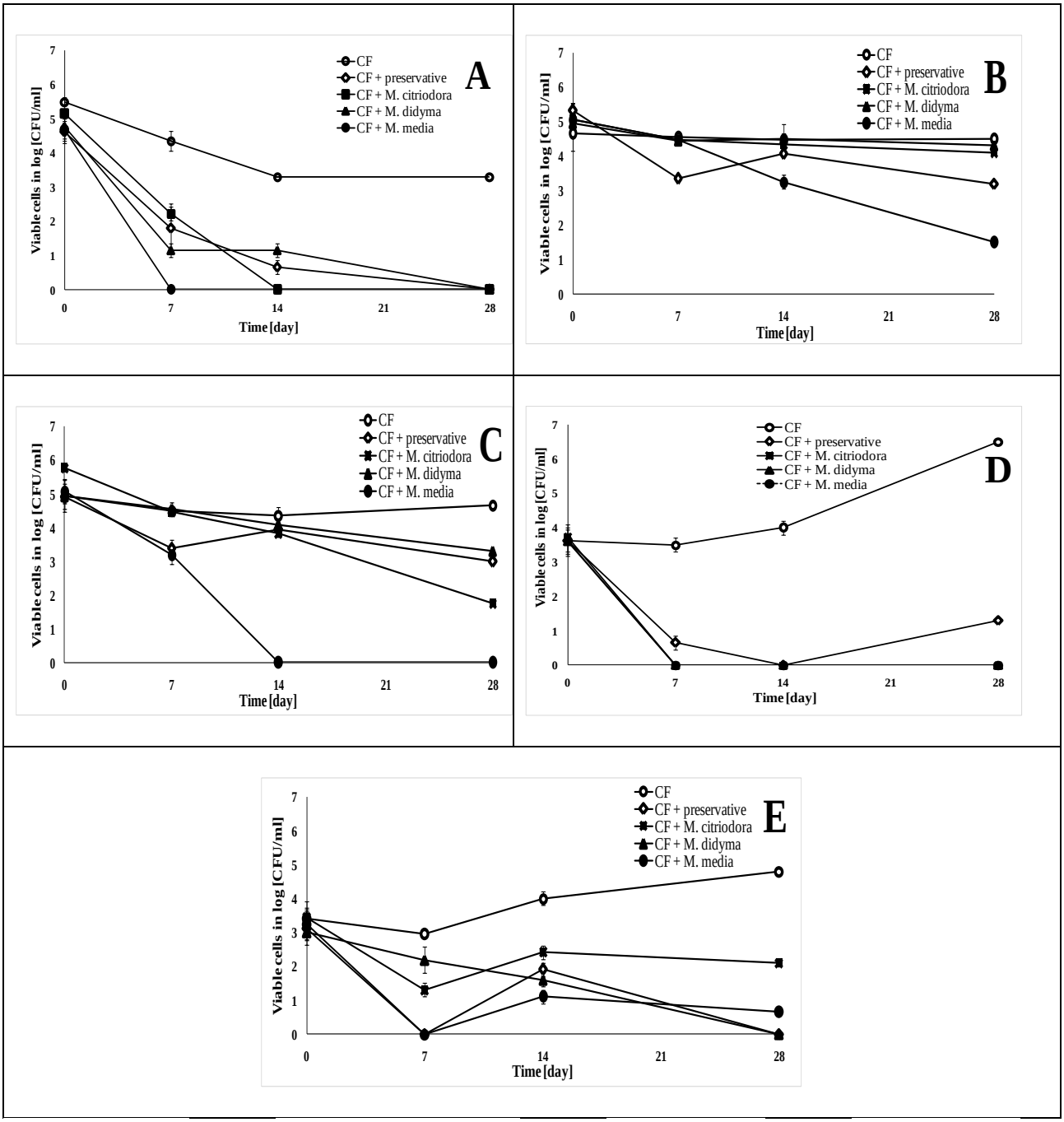
Antimicrobial effectiveness

EOs from leaves of *M. citriodora, M. didyma* and *M. media* added to the O/W emulsion at a concentration of 1.5% effectively reduced the growth of all tested references microorganisms (Figure 1). Among all tested essential oils, *M. media* EO completely inhibited growth of *S. aureus, E. coli* and *C. albicans* in emulsion after 28 days of incubation and significant inhibited the growth of *P. aeruginosa* and *A. brasiliensis* compared to emulsions with *M. didyma*, *M. citriodora* EOs and synthetic preservative. Antimicrobial activity of *M. didyma* and *M. citriodora* EOs in emulsions were comparable with activity of synthetic preservative against all tested references strains. The weakest antimicrobial activity against *P. aeruginosa* was observed in all tested emulsion with *Monarda* EOs and preservative. According to the PN-EN ISO 11930: 2012 standard, cosmetic formulations must meet the criterion A or B of the preservative effectiveness test for all references microorganisms, what is equivalent that the cosmetic product is protected against microbial proliferation (Table 4). Therefore, the EO from the leaves of *M. media* (1.5%) can acts a substitutes for synthetic preservatives in O/W emulsions and create self-preserving system. In turn, EOs from the leaves of

DOI: 10.37929/nveo.772848

M. citriodora and *M. didyma* can be valuable raw materials supporting the preservatives system, which can significantly reduce the concentration of preservatives used in cosmetic formulation.

Figure 1. Inhibition of growth of the microorganisms



Staphylococcus aureus ATCC 6538(**A**), *Pseudomonas aeruginosa* ATCC 9027(**B**), *Escherichia coli* ATCC 8739(**C**), *Candida albicans*ATCC 10231(**D**), *Aspergillus brasiliensis* ATCC 16404(E) in o/w formulation(CF) with *Monarda citriodora*, *Monarda didyma*, *Monarda media* essential oils and preservative Germaben II (Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparabe).

Table 4. Criteria of preservation efficacy test for o/w emulsion with preservatives and *Monarda* EOs according to PN-EN ISO 11930: 2012 standard

o/w emulsion	***S. aureus***	***P. aeruginosa***	***E. coli***	***C. albicans***	***A. brasiliensis***
Base	-[a]	-	-	-	-
Preservative	B[c]	-	-	-	A[b]
M. citriodora	B	-	-	A	A
M. didyma	A	-	-	A	A
M. media	A	B	B	A	A

[a]Beyond the criterion. [b]Recommended efficacy of antimicrobial preservation for topical preparations. [c]Microbiological risk for topical preparations is tolerable.

Literature data showed that EOs from *Calaminta officinalis*(2%) in O/W emulsion and shampoo (Nostro et al., 2004), *Cinnamomum zeylanicum* (2.5%) in O/W emulsion (Herman et al., 2013; Herman, 2014), *Artemisia afra* and *Pteronia incana* in aqueous cream formulation (Muyima et al., 2002), *Lavandula angustifolia* (Turgut et al., 2017),*Lavandulla officinallis* (1.5%), *Rosmarinus officinalis* (1.5%) or their mixture (0.5% *L. officinallis* oil and 0.5% *R. officinalis* oil) in an aqueous cream (Muyima et al., 2002), and *C. officinalis* (0.5% and 1% v/v) in combination with 2.0 mM EDTA in cetomacrogol cream (Nostro et al., 2002) can create a self-preservative system. In turn, *Thymus vulgaris* EO (3%) in O/W and W/O emulsions (Manou et al., 1998), *L. officinalis* and *Melaleuca alternifolia* essential oils (2,5%) in O/W emulsion (Herman et al., 2013), *C. officinalis* essential oil (1%) in O/W emulsion (Nostro et al., 2004), lavender, lemon and tea tree oils alone (0.5%) or as mixtures (1%) of EOs in O/W body milks formulation (Kunicka-Styczynska et al., 2009), lemon oil (1%) / lavender oil (1%) / tea tree oil (1%) as well their mixtures (0.5% each oil) in washing liquids (Kunicka-Styczyńska et al., 2011) can reduce the addition of preservatives used to cosmetic formulations. Moreover, using the lowest possible concentration of EOs with preservative activities will exclude from cosmetics too intense fragrance and formation allergies and skin irritation to users. Additionally, EOs as multifunctional ingredients can not only effect on cosmetics preservation system but also offers cosmetic industries many valuable properties, i.e. EOs can independently penetrate through the skin or increase the penetration of other active compounds from topically applied formulation into the lower layers of the skin (Herman and Herman, 2015), EOs with antimicrobial, antioxidant and anti-inflammatory properties are used in topical formulation for the treatment of many skin diseases as acnes, alopecia, psoriasis and wound healing (Reuter et al., 2010).

Conclusions

According to the experimental results observed it can be concluded that, *M. media* EO may be suggested as an effective candidate as natural cosmetic preservative in O/W emulsion, and create an artificial preservative free or self-preserving system. In addition, the *M. didyma* and *M. citriodora* EOs can reduce concentration of preservatives in O/W emulsions, at the same time contributing for the microbiological safety of the cosmetic formulation for its use and storage.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Adebayo, O., Bélanger, A., Khanizadeh, S. (2013). Variable inhibitory activities of essential oils of three *Monarda* species on the growth of *Botrytis cinerea*. *Canadian Journal of Plant Science, 93*, 987-995.

Andersen, K.E. (1993). Contact allergy and irritation from preservatives. *Journal of Applied Cosmetology, 11,*65-68.

Bishop, C.D., Thornton, I.B. (1997). Evaluation of the antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on post-harvest pathogens. *Journal of Essential Oil Research, 9*, 77-82.

Collins, J.E., Bishop, C.D., Deans, S.G., Svoboda, K.P. (1994). Composition of the essential oil from the leaves and flowers of *Monarda citriodora* var. *citriodora* grown in the United Kingdom. *Journal of Essential Oil Research, 6,* 27-29.

Fraternale, D., Giamperi, L., Bucchini, A., Ricci, D., Epifano, F., Burini, G., Curini, M. (2006). Chemical composition, antifungal and in vitro antioxidant properties of *Monarda didyma* L. essential oil. *Journal of Essential Oil Research, 18,* 581-585.

Ghabraie, M., Vu, K.D., Tata, L., Salmieri, S., Lacroix, M. (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT-Food Science Technology, 66,* 332-339.

Gwinn, K.D., Ownley, B.H., Greene, S.E., Clark, M.M., Taylor, C.L., Springfield, T.N., Trently, D.J., Green, J.F., Reed, A., Hamilton, S.L. (2010). Role of essential oils in control of *Rhizoctonia* damping-off in tomato with bioactive *Monarda* herbage. *Phytopathology, 100,* 493-501.

Herman, A. (2014). Comparison of antimicrobial activity of essential oils, plant extracts and methyl paraben in cosmetic emulsions: 2 months study. *Indian Journal of Microbiology, 54,* 361-364.

Herman, A. (2019). Antimicrobial ingredients as preservative booster and components of self-preserving cosmetic products. *Current Microbiology, 76,* 744-754.

Herman, A., Herman, A.P. (2015). Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. *Journal of Pharmacy and Pharmacology, 67,* 473-485.

Herman, A., Herman, A.P., Domagalska, B.W., Młynarczyk, A. (2013). Essential oils and herbal extracts as antimicrobial agents in cosmetic emulsion. *Indian Journal of Microbiology, 53,* 232–237.

Kunicka-Styczynska, A., Sikora, M., Kalemba, D. (2009). Antimicrobial activity of lavender, tea tree and lemon oils in cosmetic preservative systems. *Journal of Applied Microbiology, 107,* 1903-1911.

Kunicka-Styczyńska, A., Sikora, M., Kalemba, D. (2011). Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. *International Journal of Cosmetic Science, 33,* 53-61.

Lu, Z.G., Li, X.H., Li, W. (2011). Chemical composition of antibacterial activity of essential oil from *Monardacitriodora* flowers. *Advanced Material Research, 183,* 920-923.

Manou, I., Bouillard, L., Devleeschouwer, M.J., Barel, A.O. (1998). Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. *Journal of Applied Microbiology, 84,* 368-376.

Mattarelli, P., Epifano, F., Minardi, P., Di Vito, M., Modesto, M., Barbanti, L., Bellardi, M.G. (2017). Chemical composition and antimicrobial activity of essential oils from aerial parts of *Monardadidyma* and *Monardafistulosa* cultivated in Italy. *Journal of Essential Oil Bearing Plants, 20,* 76-86.

Memar, M.Y., Raei, P., Alizadeh, N., Aghdam, M.A., Kafil, H.S. (2017). Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Review Medical Microbiology, 28,* 63-68.

Mohammadi, S., Saharkhiz, M.J. (2011). Changes in essential oil content and composition of catnip (*Nepetacataria* L.) during different developmental stages. *Journal of Essential Oil Bearing Plants, 14,* 396–400.

Muyima, N.Y.O., Zulu, G., Bhengu, T., Popplewell, D. (2002). The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. *Flavour and Fragrance Journal, 17,* 258-266.

NaghdiBadi, H., Abdollahi, M., Mehrafarin, A., Ghorbanpour, M., Tolyat, M., Qaderi, A., Ghiaci Yekta, M. (2017). An overview on two valuable natural and bioactive compounds, thymol and carvacrol, in medicinal plants. *Journal of Medicinal Plants, 3,* 1-32.

Nostro, A., Cannatelli, M.A., Morelli, I., Cioni, P.L., Bader, A., Marino, A., Alonzo, V. (2002). Preservative properties of *Calaminthaofficinalis* essential oil with and without EDTA. *Letters of Applied Microbiology, 35,* 385-389.

Nostro, A., Cannatell, M.A., Morelli, I., Musolino, A.D., Scuderi, F., Pizzimenti, F., Alonzo, V. (2004). Efficiency of *Calaminthaofficinalis* essential oil as preservative in two topical product types. *Journal of Applied Microbiology, 97,* 395-401.

Reuter, J., Merfort, I., Schempp, C.M. (2010). Botanicals in dermatology: an evidence-based review. *American Journal of Clinical Dermatology, 11,* 247-267.

Saharkhiz, M.J., Ghani, A., HassanzadehKhayyat, M. (2009). Changes in essential oil content and composition of clary sage (*Salvia sclarea*) aerial parts during different phenological stages. *Medicinal and Aromatic Plant Science and Biotechnology, 3,* 90–93.

Turgut, A.C., Emen, F.M., Canbay, H.S., Demirdöğen, R.E., Çam, N., Kılıç, D., Yeşilkaynak, T. (2017). Chemical characterization of *Lavandula angustifolia* Mill. which is a phytocosmetic species and investigation of its antimicrobial effect in cosmetic products. *Journal of the Turkish Chemical Society Section A: Chemistry, 4,* 283-298.

Wróblewska, K., Szumny, A., Żarowska, B., Kromer, K., Dębicz, R., &Fabian, S. (2019). Impact of mulching on growth essential oil composition and its biological activity in *Monarda didyma* L. *Industrial Crops and Products*, *129*, 299-308.