Antiphytoviral Activity of Essential Oils of Some Lamiaceae Species and There Most Important Compounds on CMV and TMV

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1. Essential oils of family Lamiaseae

The aromatic plants produce a large, diverse array of organic compounds, which are known as secondary metabolites. These substances are typically found in only one plant species or a taxonomically related group of species. Secondary metabolites can be divided into three groups according to their mode of biosynthesis: terpenes, phenolic and nitrogen-containing compounds. Terpenes are lipids synthesized from acetyl-CoA via the mevalonic acid pathway. There are classified by the number of 5-carbon units which they contain: 10-carbon terpens (two C_5 units) are called monoterpenes, 15-carbon terpens (three C_5 units) are sesquiterpenes, 20-carbon (four C_5 units) are diterpenes … and polyterpenoids $(C_5)_n$ carbons [1].

Lamiaceae family includes about 3500 species spread all over the world but mostly in the Mediterranean area. The species of the investigated family are well known as aromatic species, as they contain substantial quantities of the essential oils. In these studies we have used species of three genuses (*Satureja, Teucrium* and *Micromeria*) which grow in unpolluted areas of the coastal part of Croatia. In all cases there are aromatic plants that produce essential oils as normal metabolic activity. Producing essential oil is one of the morphological and anatomical adaptations of xerophyte plant which was formed in response to the specific conditions of plant life, as well as a hypersensitivity reaction to the temperature cross flow and water stress [2]. In these plants essential oils mainly producing glands that are part of the epidermis [3]. Essential oils are variable mixtures, principally of terpenoids, and specifically of monoterpenes and sesquiterpenes, and represent secondary metabolites that occur as intracellular cytoplasmic products and they are isolated from dried aerial plant parts by distillation or extraction. Monoterpenes, are divided into two groups of compounds regular and irregular, of which is precursor geranylpyrophosphate. Regular monoterpenes divided into acyclic, monocyclic and bicyclic groups. Sesquiterpenes are acyclic and cyclic C15 components, which is precursor farnesylpyrophosphate [1].

A significant number of plants of this family have been used in traditional medicine and their posses a wide spectrum of biological activities, which may be of great importance in several fields such as antimicrobial [4, 5] and antiphytoviral activity [6, 7]. A novel way to reduce the proliferation of plant viruses is the use of essential oils or there compounds. The ability of plants to defend themselves from infection is related to the activation of the immune response. Antiviral substances are not only protection for a species that produces them, but also for species that breed in its immediate neighbourhood. This is the reason that we have to isolate the essential oils used wild plants that grow on uncontaminated and isolated habitat.

Teucrium is a genus of Lamiaceae family within which we can find 49 species, of which 13 are widespread in the Croatian flora, including the five species studied in this chapter: *T. polium* L., *T. flavum* L., *T. montanum* L., *T. chamaedrys* L. and endemic one *T. arduini* L. [6, 7, 8]. All species showed with potential for preservation from plant viruses, primarily due to their essential oils, which are a mixture of a great number of volatile constituents [9]. The oils of other *Teucrium* species are characterized by the presence of sesquiterpenes such as caryophyllene, caryophyllene oxide, germacrene D, α-humulene, α-muurolene, *(E)-β*-farnesene and the monoterpene carvacrol [10, 11]. In *T. arduini* species the major compound are *β*-caryophyllene (19.9%) as the sesquiterpene hydrocarbons and the caryophyllene oxide (14.6%) as the oxygenated sesquiterpenes (Table 1). The other four investigated *Teucrium* species are characterized by similar essential oil compositions and high proportion of the sesquiterpene hydrocarbons E-caryophyllene (7.1–52.0%) and germacrene D (8.7-17.0%). The monoterpene hydrocarbons such as α- pinene is in 10.5% in *T. flavum* and β-pinene 12.3% in *T. montanum* (Table 1).

The genus *Satureja* is comprised of some 200 species of often aromatic herbs and shrubs widely distributed in the Mediterranean Area, Asia, boreal America and in the Croatian flora 9 of them been reported. In our previous research for four species from the Mediterranean region (*Satureja montana* L., *S. cuneifolia* Ten., *S. subspicata* Vis. and an endemic one *S. visianii Šilić*) affiliation relationships are established based on the analysis of its DNA sequences for an internal transcribed spacer (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA [12]. All of them are annual or perennial semi-bushy plants that inhabit arid, sunny, stony and rocky habitats. The main constituents of *Satureja* species oils are monoterpenes such as carvacrol and thymol. In investgated *S. montana* the phenolic compound cavacrol comes in

19.4% and thymol in 16.6% (Table 1). For antiphytoviral research was used essential oil from S. montana most widely along the Adriatic coast [6]. The antiviral activity of savory's essential oils against HIV has been documented [13].

The genus *Micromeria* in addition belongs to the family Lamiaceae and includes 70–90 herbs, sub-shrub and shrubs distribute in temperate area [14]. According to earlier perception these species were placed in genus *Satureja* L. [15]. The essential oil composition of genera *Micromeria* are very variable. The composition of the essential oils of *M. cristata*, *M. juliana*, *M. dalmatica*, *M. albanica*, *M. thymifolia*, *M. graeca*, *M. libanotica* and *M. parviflora* has been reported [16-20]. Several of the species have considerable antimicrobial [21] and antioxidant [22] activity, and some are used in folk medicine [31]. The main constituent of investigated *M. graeca* is α-bisabolol (13.9%) which belonging in the oxigenated sesquiterpenes (Table 1).

Table1 Phytochemical composition, identification [%], and major groups of chemical composition of essential oil of *Satureja, Teucrium* and *Micromeria* species

Component	R	*S. montana*	*T. arduini*	*T. polium*	*T. flavum*	*T. montanum*	*T. chamaedrys*	*M. graeca*	Identification
Monoterpene hydrocarbons		**21**	**14**	**6.3**	**28.4**	**24.4**	**3.9**	**7.7**	
α-Thujene	924	0.8	0.6	-	-	-	-	0.1	RI, MS
α-Pinene	938	0.2	0.4	tr	10.5	1.9	1	3.8	RI, MS, Co-GC
Camphene	962	0.2	0.2	-	0.1	-	-	-	RI, MS
β-Pinene	982	-	-	0.3	8.4	12.3	1.9	2.1	RI, MS, Co-GC
Myrcene	992	4	0.3	0.1	0.7	4.2	0.2	-	RI, MS
δ-3-Carene	1008	-	-	-	-	-	-	0.7	RI, MS
α-Terpinene	1016	4.9	0.9	-	-	-	-	-	RI, MS
p-Cymene	1021	0.3	1.7	-	-	-	-	0.3	RI, MS
Limonene	1032	0.8	5.7	5.9	7.9	4.6	0.6	0.5	RI, MS, Co-GC
(*Z*)-*β*-Ocimene	1052	1.6	0.6	tr	0.6	0.8	0.2	0.2	RI, MS
γ-Terpinene	1057	6.9	0.9	-	-	-	-	-	RI, MS
cis-Sabinene hydrate	1065	0.8	1.8	-	-	-	-	-	RI, MS
Terpinolene	1089	-	-	-	0.2	0.6	-	-	RI, MS
allo-Ocimene	1128	0.5	0.9	-	-	-	-	-	RI, MS
Oxygenated monoterpenes		**33.7**	**15.6**	**14.4**	**4.9**	**16**	**0.2**	**30**	
trans -Linalool oxide (furanoid)	1088	0.9	-	1.9	1.5	3.6	-	6.8	RI, MS, Co-GC
Linalool	1099	5.9	1.9	1.9	1.5	3.6	-	3.5	RI, MS, Co-GC
β-Thujone	1121	-	-	5.7	-	0.3	-	-	RI, MS
trans-Pinocarveol	1147	-	.-	-	0.4	1.2	-	4.1	RI, MS
Camphor	1151	0.6	1.5	1.4	-	1.3	-	8.1	RI, MS, Co-GC
Borneol	1176	3	1.9	1.4	-	1.6	-	0.2	RI, MS
Terpinen-4-ol	1184	2.1	1.6	0.2	0.2	1.5	-	0.6	RI, MS
α-Terpineol	1186	1.9	1.7	-	-	-	-	-	RI, MS
Myrtenol	1197	0.8	1.3	-	0.6	1.2	0.2	2.2	RI, MS
Verbenone	1204	-	-	-	-	-	-	0.5	RI, MS
endo-Fenchyl acetate	1218	-	-	-	-	-	-	1.7	RI, MS
β-Cyclocitral	1223	-	-	-	0.2	-	tr	-	RI, MS
Nerol	1227	3	0.7	-	-	-	-	-	RI, MS
Thymol methyl ether	1230	4.1	1.4	-	-	-	-	-	RI, MS
Carvacrol methyl ether	1241	5.5	1.7	-	-	-	-	-	RI, MS
Geraniol	1249	1.7	1.2	-	-	-	-	-	RI, MS
Linalyl acetate	1252	-	-	0.8	0.3	0.5	-	0.3	RI, MS
Bornyl acetate	1285	-	-	1.1	0.2	0.2	-	1.2	RI, MS
α-Terpenyl acetate	1349	-	-	-	-	1	-	0.8	RI, MS
Neryl acetate	1358	4.2	0.7	-	-	-	-	-	

Sesquiterpene hydrocarbons		**0.9**	**44.9**	**76**	**50.8**	**35.1**	**86.9**	**17.3**	
α-Copaene	1377	-	-	0.2	0.7	-	0.7	0.6	RI, MS
β-Bourbonene	1383	-	-	0.7	2.6	3.4	3.7	0.3	RI, MS
α-Gurjunene	1407	-	-	-	0.3	-	0.2	0.8	RI, MS
β -Caryophyllene	1424	-	19.9	52	23.1	7.1	47.6	0.4	RI, MS, Co-GC
β -Copaene	1429	-	-	1.4	2.7	-	5.7	2.4	RI, MS
trans-*α*-Bergamotene	1433	-	-	4.1	-	-	-	-	RI, MS
(*Z*)-*β*-Farnesene	1454	-	5.6	4.3	2.1	2.9	-	1.9	RI, MS
α-Humulene	1456	-	4.8	4.6	-	-	-	0.6	RI, MS
allo-Aromadendrene	1465	0.4	4.9	-	1.2	-	-	5.2	RI, MS
β-Chamigrene	1477	-	-	-	-	-	-	0.4	RI, MS
Germacrene D	1481	-	-	8.7	15.3	17.2	29	3.2	RI, MS
Viridiflorene	1496	-	4.4	-	-	-	-	-	RI, MS
Bicyclogermacrene	1500	-	-	-	-	-	-	0.8	RI, MS
β-Bisabolene	1494	-	-	tr	1.8	1.8	-	0.7	RI, MS
δ-Cadinene	1517	0.5	5.3	tr	1	2.7	-	-	RI, MS
Oxygenated sesquiterpenes		**1.5**	**19.6**	**tr**	**5**	**5.1**	**5.9**	**23.2**	
Spathulenol	1577	0.8	5	tr	1.6	1.9	tr	3.2	RI, MS
Caryophyllene oxide	1581	0.7	14.6	tr	2.6	1	4.5	1.9	RI, MS, Co-GC
γ-Eudesmol	1632	-	-	-	-	-	-	2.6	RI, MS
α-Cadinol	1655	-	-	tr	0.8	2.2	1.4	-	RI, MS
α-Bisabolol	1688	-	-	-	-	-	-	13.9	RI, MS
α-Bisabolol oxide	1748	-	-	-	-	-	-	1.6	RI, MS
Phenolic compounds		**36**	**1.6**	**0.1**	**1.1**	**-**	**0.6**	**1**	
p-Vinylanisole	1159	-	-	-	0.5	-	-	-	RI, MS
Methyl salicylate	1194	-	-	-	-	-	0.3	-	RI, MS
Thymol	1290	16.6	1.6	-	-	-	-	0.3	RI, MS, Co-GC
Carvacrol	1298	19.4	-	-	-	-	-	0.4	RI, MS, Co-GC
p-Vinyl-guaiacol	1312	-	-	-	0.2	-	0.1	-	RI, MS
Eugenol	1370	-	-	0.1	0.4	-	0.2	0.3	RI, MS, Co-GC
Carbonylic compounds		**0.8**	**0.8**	**tr**	**7.2**	**0.9**	**0.4**	**3.8**	
1-Octen-3-ol	974	0.8	0.8	-	-	-	-	-	
n-Amyl isovalerate	1113	-	-	-	3.7	0.5	-	-	RI, MS
3-Octanol acetate	1125	-	-	-	0.4	-	-	0.3	RI, MS
Isobutyl hexanoate	1155	-	-	-	0.4	-	-	-	RI, MS
Butylhexanoate	1193	-	-	-	0.5	-	-	-	RI, MS
Hexyl isovalerate	1245	-	-	-	0.1	-	-	-	RI, MS
Isoamyl hexanoate	1256	-	-	-	1.6	-	-	-	RI, MS
β-Ionone	1487	-	-	-	-	-	-	3.5	RI, MS
6,10,14-Trimethyl-2-pentadecanone	1839	-	-	tr	0.5	0.4	0.4	-	RI, MS
Hydrocarbons		**0.2**	**0.3**	**0.4**	**1.4**	**16.6**	**0.4**	**6.9**	
Eicosane	2000	-	-	-	-	0.2	-	0.3	RI, MS, Co-GC

Heneicosane	2100	-	-	tr	-	1	0.4	0.1	RI, MS, Co-GC
Docosane	2200	-	0.2	-	0.1	1.9	-	1.5	RI, MS, Co-GC
Tricosane	2300	-	-	tr	0.2	2.8	-	0.9	RI, MS, Co-GC
Tetracosane	2400	0.1	0.1	-	0.1	0.1	-	1.6	RI, MS, Co-GC
Pentacosane	2500	0.1	-	0.2	0.3	1.3	-	1.4	RI, MS, Co-GC
Hexacosane	2600	-	-	-	0.1	3.4	-	0.6	RI, MS, Co-GC
Heptacosane	2700	-	-	0.1	0.3	2.7	-	0.3	RI, MS, Co-GC
Octacosane	2800	-	-	0.1	0.1	2	-	0.2	RI, MS, Co-GC
Nonacosane	2900	-	-	tr	0.2	1.2	-	-	RI, MS, Co-GC
Total identified (%)		**94.1**	**96.8**	**97.2**	**98.8**	**98.1**	**98.3**	**88.9**	
Yield (%)		**2.5**	**0.5**	**0.5**	**0.4**	**0.4**	**0.3**	**0.5**	

R, retention time on capillary column VF5-ms; RI, identification using literature data (Adams 2007); MS, identification using database NIST02 and Wiley 7; Co-GC, identification by reference components; –, component is not identified; tr, traces (mean value below 0.1%)

2. Antiyphitoviral activity of essential oils and the major components of oil

Wide range of biological activities displayed by the essential oils makes these compounds subject to different researches. Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries [23]. Onwards, essential oils are of great importance in several fields such as physiological function of growth, ecological function, development and resistance against diseases and insects [24, 25]. Increasingly, essential oils are becoming the subject of different researches because of their numerous biological effects, which are attributed mostly to prooxidant effects on the cellular level [23]. A recent area of research that is of particular interest is their antiviral activity. Regarding phytopathogenic viruses, various substances of natural and synthetic origin have been assessed for their antiphytoviral activity [26-28]. Still limited number of reports provides data about antiphytoviral activities of essential oils [6, 7, 9, 29-31]. Therefore, this area of research is still insufficiently explored and requires further gathering of information that would enable a more complete understanding of the ways and mechanisms of antiviral actions of these natural substances.

Tobacco mosaic virus (TMV) is a positive-sense single stranded RNA virus belonging to genus Tobamovirus that causes mottling and discoloration of leaves of an infected plant, especially tobacco and other members of the Solanaceae. It was the first infectious agent to be recognised as being distinct from bacteria and fungi and thus the first to bear the designation "virus". TMV is named for one of the first plants in which it was found in the 1800s. However, it can infect well over 350 different species of plants. TMV can multiply only inside a living cell but it can survive in a dormant state in dead tissue, retaining its ability to infect growing plants for years after the infected plant part died. Most other viruses die when the plant tissue dies. TMV is known as one of the most stable viruses. Infection by tobacco mosaic virus causes serious losses on several crops including tomatoes, peppers, and many ornamentals. The virus almost never kills plants but lowers the quality and quantity of the crop, particularly when the plants are infected while young. Unlike fungicidal chemicals used to control fungal diseases, to date there are no efficient chemical treatments that protect plant parts from virus infection. Additionally, there are no known chemical treatments used under field conditions that eliminate viral infections from plant tissues once they do occur. Practically speaking, plants infected by viruses remain so. Thus, control of tobacco mosaic virus is primarily focused on reducing and eliminating sources of the virus and limiting the spread by insects. Therefore, sanitation is the single most important practice in controlling tobacco mosaic virus.

Cucumber mosaic virus (CMV) is a plant pathogenic virus in the family *Bromoviridae*. It is the type member of the plant virus genus, *Cucumovirus*. This virus has a worldwide distribution and the widest host range for any plant virus comprising about one thousand plant species, including some of the most important crops. As a worldwide occuring disease agent, it has been reported in tomatoes, peppers, cucurbits and cucumbers inducing various types of symptoms [32]. No chemicals can cure a plant of this virus infection or of any other. CMV is a small icosahedral virus with a tripartite genome and two subgenomic RNAs [33-35]. Some strains of CMV encapsidate evolutionary unrelated satellite RNA (satRNA) that is completely dependet on the helper virus for its replication and spread. Accompained by their helper, several variants of satRNA can induce severe symptoms, particulary in tomato plants [36].

Since chemicals cannot cure a plant of any viruses, we tried to find natural substances that can help in a control of plant virus diseases. Current knowledge about the antiviral effects of essential oils, although limited and insufficiently explored, indicates the potential of these secondary metabolites to control or at least reduce the spread of viral infections. Our research was directed in a parallel investigation of the composition and antiviral potential of essential oils of various plant species. In this sense, we have proved that essential oils from several plant species may inhibit development of local lesions in tobacco mosaic virus and cucumber mosaic virus infected plants [6, 7, 30, 31]. Essential oil of *Satureja montana* L. ssp. *variegata* (Host) P. W. Ball (Lamiaceae) applied on local hosts *Chenopodium amaranticolor* Coste & Reyn. and *Chenopodium quinoa* Willd. simultaneously with the infecting virus, reduced the number of local lesions on both TMV and CMV infected plants for 29.2% and 24.1%, respectively (Table 2). Furthermore, the main components of these oil, oxygenated monoterpenes thymol and carvacrol, reduced local lesions number when applied on the leaves of local hosts simultaneously with the infecting virus. Thymol was more effective in reducing CMV infection (33.2%), while carvacrol was more effective in reducing the TMV infection (34.3%) (Table 3). Synergistic effect of both monoterpenes was not observed in the antiviral activity of the oil. Onwards, sesquiterpenes-rich essential oils of *Teucrium polium*, *T. flavum*, *T. montanum*, *T. chamaedrys* and *T. arduini* inhibited local lesions number in CMV infected plants and *Micromeria graeca* (L.) Rchb essential oil in CMVsat infected plants (Table 2) [6, 7, 30, 31]. Essential oils of above listed species were rich in sesquiterpenes including *β*-caryophyllene and caryophyllene oxide, germacrene D, and α-bisabolol (Table 1) [7, 30, 31]. Antiviral activity of essential oils isolated from *T. montanum, T. polium, T. chamaedrys and T. flavum* showed that percentage of *β*-caryophyllene (Table 3) in the oil correlates with antiviral activity of the oil [30]. Exception among these is *T. montanum* essential oil which showed the strongest antiviral activity among *Teucrium* species although the most abundant component in essential oil of *T. montanum* was germacrene D, followed by *β*-pinene, *β*-caryophyllene and limonene (Table 1) [30]. Sesquiterpene oil component *β*-caryophyllene and oxygenated sesquiterpene caryophyllene oxide, both applied individually as spray solution before virus inoculation, showed significant antiviral activity by reducing the number of local symptoms in CMV infected plants (Table 3) [7]. Therefore, this study confirmed that monoterpenes and sesquiterpenes-rich essential oils can inhibit TMV, CMV or CMVsat infection. All of the above listed essential oil components, some of which are present as major constituents of the oil and some are present in a relatively low percentage in the oil composition, can have a synergistic effect and contribute to the antiviral efficacy of the oil. Antiviral testing of many oils could help us to gain insight into the relationship between oil composition and antiviral effectiveness. This results support further research of antiphytoviral activity of essential oils and individual components of the oils. Understanding of mode of antiviral activity of essential oils may help to find and adjust these natural substances for possible use in the control of plant virus diseases.

Table 2 Effect of essential oils (EO) of different plant species on tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) and CMV associated with satellite RNA (CMVsat) infectivity. Local host plants (*Chenopodium amaranticolor* for TMV and *C. quinoa* for CMV and CMVsat) were sprayed with EO of *Teucrium polium*, *T. flavum*, *T. montanum*, T. *chamaedrys*, *T. arduini* and *Micromeria graeca* (500 ppm) for two succesive days prior to virus inoculation; essential oil of *Satureja montana* was added directly in viral inoculum. Percentage of local lesions inhibition is calculated according to local lesions number on the treated and untreated (control) leaves of the host plants.

	% of inhibition of local lesions number		
	TMV	CMV	CMVsat
Satureja montana EO	29.2	24.1	/
Teucrium polium EO	/	41.4	/
T. flavum EO	/	22.9	/
T. montanum EO	/	44.3	/
T. chamaedrys EO	/	25.7	/
T. arduini EO	25.7	21.9	/
Micromeria graeca EO	/	/	59.3

/ - not tested

Table 3 Effect of essential oil components on cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) infectivity. Local host plants (*Chenopodium amaranticolor* for TMV and *C. quinoa* for CMV) were sprayed with *β*-caryophyllene or caryophyllene-oxide for two succesive days prior to virus inoculation; thymol and carvacrol were added directly in viral inoculum. Percentage of local lesions inhibition is calculated according to local lesions number on the treated and untreated (control) leaves of the host plants.

Components of essential oil	Percentage of inhibition of local lesions number		Conc.
	TMV	CMV	
Thymol	26.1	33.2	1 mmolL^{-1}
Carvacrol	34.3	28.3	4.2 mmolL^{-1}
β-caryophyllene	7.6	30.8	500 ppm
Caryophyllene-oxide	5.9	36.9	500 ppm

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References

[1] Breitmair E, *Terpenes*. WILEY-VCH Verlage GmbH & Co. KgaA, Weinheim; 2008.

[2] Kremer D, Dragojević MI, Stabentheiner E, Vitali D, Kopričanec M, Ruščić M, Kosalec I, Bezić N, Dunkić V, Phytochemical and micromorphological traits of endemic *Micromeria pseudocroatica* Šilić (Lamiaceae). *Natural Product Comunication*. 2012; 7 (12):1667-1670.

[3] Dunkić V, Bezić N, Ljubešić N, Bočina I. Glandular Hair ultrastructure and Essential Oils in *Satureja subspicata* Vis. ssp. S*ubspicata* and ssp. *Liburnica* Šilić. *Acta Biologica Cracoviensis. Series Botanuca*. 2007;49/2:45-52.

[4] Skočibušić M, Bezić N, Dunkić V. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chemistry*. 2006;96:20-28.

[5] Skočibušić M, Bezić N. Phytochemical Analysis and *In vitro* Antimicrobial Activity of Two *Satureja* Species Essential Oils. *Phytotherapy Research*. 2004;18:967-970.

[6] Dunkić V, Bezić N, Vuko E, Cukrov D. Antiphytoviral Activity of *Satureja montana* L. ssp. *Variegata* (Host) P. W. Ball Essential Oil and Phenol Compounds on CMV and TMV. *Molecules*. 2010;15:6713-6721.

[7] Dunkić V, Bezić N, Vuko E. Antiphytoviral Activity of Essential Oil from Endemic Species *Teucrium arduini* L. *Natural Product Comunication*. 2011; 6: 1-3.

[8] Stojanović G, Palić I, Ursić-Janković J. Composition and antimicrobial activity of the essential oil of *Micromeria cristata* and *Micromeria juliana*. *Flavour and Fragrance Journal*. 2006;21:77–79.

[9] Bishop CD. Antiviral activity of the essential oil of *Melaluca alternifolia* (Maiden & Betche) cheel (tea tree) against tobacco mosaic virus. *J. Essent.Oil Res*. 1995;7:641-644.

[10] Kazemizadeh Z, Basiri A, Habibi Z. Chemical Composition of the Essential Oil of *Teucrium hyrcanicum* and *T. chamaedrys* L. subsp. *chamaedrys* from Iran. *Chem. Nat. Comp.* 2008;44:651-653.

[11] De Martino L, Formisano C, Mancini E, De Feo V, Piozzi F, Rigano D, Senatore F. Chemical Composition and Phytotoxic Effects of Essential Oils from Four *Teucrium* Species*. Nat. Prod. Comm.* 2010;5:1969-1976.

[12] Bezić N, Šamanić I, Dunkić V, Besendorfer V, Puizina J. Essential Oil Composition and Internal Transcribed Spacer (ITS) Sequence Variability of Four South-Croatian *Satureja* Species (Lamiaceae). *Molecules*. 2009;14:925-938.

[13] Yamasaki K, Nakano M, Kawahata T, Mori H, Otake T, Ueba N, Oishi I, Inami R, Yamane M, Nakamura M, Murata H, Nakanishi T. Anti-HIV-1 activity of herbs in Labiatae. *Biol. Pharm. Bull*. 1998;21:829-833.

[14] Chater AO, Guinea E. *Micromeria* Benth. In: *Flora Europaea*. Vol. ***3.*** Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters S, Webb DA (Ed). Cambridge University Press, Cambridge; 1972:167–170.

[15] Wielgorskaya T. *Dictionary of generic names of seed plants.* Columbia University, New York. 1995:369.

[16] Slavkovska V, Couladis M, Bojovic S, Tzakou O, Pavlovic M, Lakušić B, Jančić R. Essential oil and its systematic significance in species of *Micromeria* Bentham from Serbia & Montenegro. *Plant Systematics and Evolution*. 2005;255:1–15.

[17] Konstandinova E, Aplieva K, Stefova M, Stafilov T, Antonova D, Evstatieva LJ, Matevski V, Kulevanova S, Stefkov G, Bankova V. Chemical composition of the essential oils of three *Micromeria* species growing in Macedonia and Bulgaria. *Macedonian Journal of Chemistry and Chemical Engineering* 2007;26:3-7.

[18] Mastelić J, Jerković I, Kuštrak D. Aromatic compounds of *Micromeria juliana* (L) Bentham ex Reicheneb. from Croatia*. Journal of Essential Oil Research* 2005;17:516–518.

[19] Chalchat JC, Diab Y, Auezova L, Chebib H. Chemical composition of the essential oil of *Micromeria libanotica* Boiss., an endemic species of Lebanon. *Journal of Essential Oil Research*. 2005;17:449–450.

[20] Tzakou O, Couladis M. The essential oil of *Micromeria graeca* (L.) Bentham et Reichenb. growing in Greece. *Flavour and FragranceJournal*. 2001;16:107–109.

[21] Stojanović G, Palić I, Ursić-Janković J. Composition and antimicrobial activity of the essential oil of *Micromeria cristata* and *Micromeriajuliana*. *Flavour and Fragrance Journal*. 2006;21:77–79.

[22] Öztürk M, Kolak U, Duru ME, Harmandar M. GC-MS analysis of the antioxidant active fractions of *Micromeria juliana* with anticholinesterase activity. *Natural Product Communications*. 2009;4:1271-1276.

[23] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. *Food Chem Toxicol*. 2008;46: 446–475.

[24] Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*. 2003;64: 3–19.

[25] Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. *Nat Chem Biol*. 2007;3:408–414.

[26] Krcatović E, Rusak G, Bezić N, Krajačić M. Inhibition of tobacco mosaic virus infection by quercetin and vitexin. *Acta Virolog*. 2008;52:119–124.

[27] Rusak G, Krajačić M, Pleše N. Inhibition of tomato bushy stunt virus infection using a quercetagetin flavonoid isolated from *Centaurea rupestris* L. *Antiviral Res.* 1997;36:125–129.

[28] Yordanova A, Korparov N, Stomenova E, Starcheva M. Antiphytoviral activity of 1-morpholinomethyl tetrahydro 2-Pyrimidinone (DDB). *Plant Pathol*. 1996; 45:547–551.

[29] Othman BA, Shoman SA. Antiphytoviral activity of the *Plectranthus tenuiflorus* on some important viruses. *Int J Agri Biol.* 2004; 6:844–849.

[30] Bezić N, Vuko E, Dunkić V, Ruščić M, Blažević I, Burčul F. Antiphytoviral activity of sesquiterpene-rich essential oils from four Croatian *Teucrium* species. *Molecules*. 2011;16:8119–8129.

[31] Vuko E, Dunkić V, Bezić N, Ruščić M, Kremer D. Chemical composition and antiphytoviral activity of essential oil of *Micromeria graeca*. *Nat Prod Comm.* 2012;7:1227–1230.

[32] Gallitelli D. The ecology of cucumber mosaic virus and sustainble agriculture. *Virus Res*. 2000;71:9–21.

[33] Palukaitis P**,** Roosinck MJ**,** Dietzgen RG, Francki RIB. Cucumber mosaic virus**.** *Adv Virus Res.* 1992;41:281–348.

[34] Shi BJ, Ding SW, Symons RH. In vivo expression of an overlapping gene encoded by the cucumoviruses. *J Gen Virol* 1997;78:237-241.

[35] Kwon CS, Chung WI. Differential roles of the 5' untranslated regions of cucumber mosaic virus RNAs 1, 2, 3 and 4 in translational competition. *Virus Res.* 2000;66:175–185.

[36] Garciá-Arenal F, Palukaitis P. Satellites and defective viral RNAs. In: Vogt: PK, Jackson AO, eds. *Current Topics in Microbiology and Immunology*. Berlin, Heidelberg, New York: Springer-Verlag;1999:37-63.