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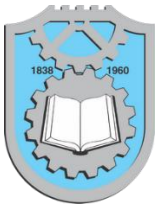

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DETAILED GC-MS ANALYSES OF THE *MYRTUS COMMUNIS* L., ESSENTIAL OIL AND THE ANTIFUNGAL ACTIVITY ASSESSMENT

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Abstract

Myrtus communis L., Myrtaceae, known as true myrtle, is a widely distributed evergreen shrub native to Mediterranean regions. This medicinal plant, used worldwide, possess a broad spectrum of phytochemical, pharmacological and therapeutic effects, mostly due to a large number of up to now detected, or isolated, essential oil components, considered the main biologically active ones. Multifarious secondary metabolite content, dependent on a geographic region, season of harvest and the length of distillation, depicts the variability of the essential oil composition obtained in general from its leaves, branches, fruits and flowers. Herein, we present the results of meticulous analyses of the essential oil (2.2%, w/w) constituents obtained from *M. communis* leaves, collected during the summer period from the coastal regions of peninsula Luštica, Montenegro. Detailed GC-MS analyses enabled the identification of 66 constituents, among which 1,8-cineole (28.4%), linalool (18.4%), α-pinene (16.6%), geranyl acetate (6.6%), α-terpineol (6.3%) and linalyl acetate (4.2%) were the major ones. Together with the secondary metabolite profile, determination of the Montenegro true myrtle essential oil agricultural plant protection potential is also estimated, by assessing mycelia growth of *Colletotrichum acutatum* J.H. Simmonds C.A.2 isolates, causative of strawberry antrachnose, *in vitro.*

Keywords: *Myrtus communis* L., essential oil, antifungal activity

1. Introduction

Myrtle (*Myrtus communis* L., Myrtaceae) is a Mediterranean essential oil bearing shrub used worldwide in traditional medicine for the treatment of various disorders. Traditional claim on the *M. communis* antifungal activity was supported by the existence of the numerous reports against many fungal species, such as *C. albicans*, *A. flavus*, *A. fumigatus*, *P. variotii*, *A. niger*, *T. mentagrophytes*, *E. floccosume*, *Rhizoctoniasolani Kuhn*. In this work we present the composition of the essential oil of *M. communis*, from the coastal regions of peninsula Luštica, and the evaluation of it's antifungal activity against *Colletotrichum acutatum* J.H. Simmonds, a plant pathogen infecting both the root and crown of strawberries (*Fragaria* × *ananassa* Duchesne, Rosaceae) causing necrosis and significant production losses worldwide, with the aim of finding new natural bactericides and fungicides.

2. Material and methods

Aerial parts (300 g) of *Myrtus communis* L. originating from peninsula Luštica, Montenegro were collected in the blooming stage, air dried and further subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus yielding ca 2.2%, (w/w, based on dry weight) of pale yellow highly fragrant oils. Mass spectra were recorded on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% diphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Lexington, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 °C and 300 °C, respectively. Oven temperature was raised from 70 to 290 °C at a heating rate of 5 °C/min; the heating program ended with an isothermal period of 10 min. As carrier gas helium, He, at 1.0 mL/min was used. The samples, 1 μl of the appropriate solutions in diethyl ether (1 mg/mL) were injected in a splitless mode. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-650, scan time 0.32 s. The constituents of the essential oil were identified by using their retention indices (using the generalized equation by Van Del Dool) and comparing their mass spectra with those obtained from authentic samples and/or the MS library (Wiley 7N and NIST/NBS libraries).
In this experiment, the used isolate of *C. acutatum* J.H. Simmonds (C.A.2) was obtained from *Fragaria × ananassa* (Weston) Duchesne ex Rozier, Rosaceae grown in Serbia. The isolate was determined based on morphological, pathogenic and molecular characteristic, and maintained on a potato-dextrose agar (PDA) at the temperature of 25 °C. The antifungal activity of essential oil was tested on PDA in Petri dishes 90 mm in diameter. The oil was applied in the form of drops placed on the inside of the lid of the Petri dish at concentrations of 0.02, 0.04, 0.08 and 0.16μL/mL of air. Exposure of the isolate to the vapors of the studied oil lasted seven days at 25 °C. The assay was performed in four replicates for oil concentration. The isolates grown on the medium with added fungicide Method 480 SC (a.m. captan, Galenika phytopharmacy) were used as the positive control variant. The negative control variant, fungi isolates grown on PDA were used under identical conditions without the described treatment.

3. Results and discussion

3.1 Chemical composition of the essential oil

Detailed GC-MS analyses of the *M. communis* essential oil enabled the identification of 70 constituents, which made up 98.7% of analyzed e.oil, where monoterpenes were the dominant ones (93.2%). Among the 74.1% of identified oxygenated monoterpenes, 1,8-cineol (28.4%), linalool (18.4%), geranyl acetate (6.6%) and α-terpineol (6.3%) prevailed indicating, according to previous reports which regarded them as ones underlying antimicrobic activity, that e.oil in our hands can possess antifungal activity. *α*-pinene, another major constituent represented by 16.6% was also previously reported as antifungal. Sesquiterpenes occupied a minor portion (3.4%) of the analysed e.oil, among which *α*-humulene (1.1%) and humulene epoxide II (1.0%) were the dominant ones, Table 1.

Table 1. Percentage composition of *M. communis* essential oil

RI[a]	Compound	Content[b] [%]	Compound class[c]	Identification method[d]
832	Furfural	tr	O	MS, RI, CoI
846	(*E*)-2-Hexenal	0.2	O	MS, RI
901	Heptanal	tr	O	MS, RI, CoI
902	Isobutyl isobutyrate	0.6	O	MS, RI, CoI
922	*α*-Thujene	0.2	MT	MS, RI
935	*α*-Pinene	16.6	MT	MS, RI, CoI
949	Camphene	0.1	MT	MS, RI, CoI
953	Thuja-2,4(10)-diene	tr	MT	MS, RI
980	*β*-Pinene	0.3	MT	MS, RI, CoI
999	Myrcene	0.5	MT	MS, RI, CoI

1002	Isobutyl 2-methylbutanoate	tr	O	MS, RI, CoI
1014	*δ*-3-Carene	0.2	MT	MS, RI, CoI
1017	*α*-Terpinene	0.1	MT	MS, RI, CoI
1026	*p*-Cymene	0.3	MT	MS, RI, CoI
1038	1,8-Cineole	28.4	MT*	MS, RI, CoI
1043	Camphene hydrate	tr	MT*	MS, RI
1046	(*E*)-*β*-Ocimene	0.4	MT	MS, RI
1059	*γ*-Terpinene	0.4	MT	MS, RI, CoI
1072	*cis*-Linalool oxide (furanoid)	0.4	MT*	MS, RI, CoI
1090	*trans*-Linalool oxide (furanoid)	0.7	MT*	MS, RI, CoI
1106	Linalool	18.4	MT*	MS, RI, CoI
1107	*trans*-Hotrienol	0.2	MT*	MS, RI
1116	*endo*-Fenchol	0.1	MT*	MS, RI, CoI
1118	*trans*-Sabinene hydrate	tr	MT*	MS, RI
1125	*α*-Campholenal	0.1	MT*	MS, RI
1130	*cis*-*p*-Mentha-2,8-dien-1-ol	tr	MT*	MS, RI
1140	*trans*-Pinocarveol	0.1	MT*	MS, RI, CoI
1148	Camphene hydrate	0.1	MT*	MS, RI
1152	Nerol oxide	tr	MT*	MS, RI
1161	Pinocarvone	tr	MT*	MS, RI, CoI
1166	*δ*-Terpineol	0.2	MT*	MS, RI, CoI
1177	Terpinen-4-ol	0.5	MT*	MS, RI, CoI
1185	*p*-Cymen-8-ol	0.2	MT*	MS, RI, CoI
1194	*α*-Terpineol	6.3	MT*	MS, RI, CoI
1198	Estragol	0.7	MT*	MS, RI, CoI
1208	Verbenone	0.1	MT*	MS, RI, CoI
1219	*trans*-Carveol	0.1	MT*	MS, RI, CoI
1227	Nerol	0.3	MT*	MS, RI, CoI
1241	Carvone	tr	MT*	MS, RI, CoI
1254	Linalyl acetate	4.2	MT*	MS, RI, CoI
1284	Isobornyl acetate	tr	MT*	MS, RI, CoI
1298	*trans*-Pinocarvyl acetate	0.1	MT*	MS, RI
1321	Methyl geranate	tr	MT*	MS, RI, CoI
1323	Myrtenyl acetate	tr	MT*	MS, RI, CoI
1339	*exo*-2-Hydroxycineole acetate	0.3	MT*	MS, RI
1348	*α*-Terpinyl acetate	1.8	MT*	MS, RI
1351	Dehydro-*ar*-ionene	0.2	O	MS, RI
1356	Eugenol	0.1	MT*	MS, RI, CoI
1361	Neryl acetate	0.9	MT*	MS, RI, CoI
1382	Geranyl acetate	6.6	MT*	MS, RI, CoI
1392	Phenethylisobutyrate	tr	O	MS, RI, CoI
1403	Methyl eugenol	3.2	MT*	MS, RI, CoI
1420	(*E*)-Caryophyllene	0.4	ST	MS, RI, CoI
1440	Aromadendrene	tr	ST	MS, RI
1449	Geranyl acetone	0.1	O	MS, RI, CoI
1454	*α*-Humulene	1.1	ST	MS, RI, CoI
1461	*allo*-Aromadendrene	tr	ST	MS, RI, CoI
1483	*β*-Ionone	tr	O	MS, RI, CoI
1492	2-Tridecanone	0.1	O	MS, RI, CoI
1516	5-Hydroxy-2,2,6,6-tetramethyl-4- (2-methylprop-1-en -1-yl)cyclohex-4-ene-1,3-dione	0.8	O	MS, RI
1578	Spathulenol	0.1	ST*	MS, RI

1581	Caryophyllene oxide	0.5	ST*	MS, RI, CoI
1600	Humulene epoxide I	0.2	ST*	MS, RI
1608	Humulene epoxide II	1.0	ST*	MS, RI
1623	Dill apiole	0.1	O	MS, RI
1628	Humulene epoxide III	0.1	ST*	MS, RI
Total identified (%)			**98.7**	
Number of components			70	
	MT – Monoterpenes		19.1	
	MT* – Oxygenated monoterpenes		74.1	
	ST – Sesquiterpenes		1.5	
	ST* – Oxygenated sesquiterpenes		1.9	
	O – Others		2.1	

[a]) Linear retention indices (RI) determined experimentally on the DB-5MS column relative to a series of C7 – C32 n-alkanes. [b]) Values are means of the individual analysis; tr, trace amounts (< 0.05%). [c])Compound identification: RI, retention indices matching with literature data; MS, mass spectra matching.

3.2 Antifungal activity; Effect of volatile phase of essential oil on the growth of Colletotrichum acutatum isolates

M. communis essential oil inhibited the growth of *C. acutatum* mycelium and the percentage of the inhibition was concentration dependent. To be exact, inhibition of mycelial growth was observed at concentrations of 0.02μL/mL of air, and higher. The minimum inhibitory concentration (MIC) of the tested EO, Figure 2, was at the concentration of 0.02μL/mL of air, while the minimal lethal concentration (MLC) was at the concentration of 0.08 μL/mL of air. Inhibition of mycelial growth by *M. communis* essential oil varied from 24%, (0.02μL/mL of air) up to 60% (0.16μL/mL of air).

3. Conclusions

In this work, we present the secondary metabolite composition of the essential oil of *M. communis* and the evaluation of its agricultural plant protection potential in the *in vitro* antifungal activity assessment against plant pathogenic fungus *Colletotrichum acutatum* J.H. Simmonds C.A.2 isolates. *M. communis* essential oil had observable antifungal activity at concentrations of 0.02 μL/mL of air, and lower, and the mechanism of fungicidal and fungistatic action should be yet clarified.