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COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE FRUIT ESSENTIAL OILS OF TWO *Athamanta turbith* SUBSPECIES

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Athamanta turbith (L.) Brot. (Umbelliferae) inhabits limestone rock crevices in the mountains and gorges of southeastern Europe (Italy, the Balkans, and the Carpathians). It is currently considered to contain three subspecies: *A. turbith* ssp. *turbith*, *A. turbith* ssp. *hungarica* (Borbas) Tutin, and *A. turbith* ssp. *haynaldii* (Borbas & Uechtr.) Tutin [1]. In the Flora of FR Serbia, *A. turbith* ssp. *hungarica* is registered as the species *A. hungarica* Borbas [2], and *A. turbith* ssp. *haynaldii* as *A. haynaldii* Borbas & Uechtr. [3].

Previously, we reported on the fatty acid composition of the fatty oils isolated from mature fruits of *A. turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii* [4]. In this study, the composition and antimicrobial properties of the fruit essential oils of these plants were analysed.

The essential oils were obtained from powdered mature fruits by hydrodistillation, according to the procedure of the European Pharmacopoeia 4 [5]. The fruits of *A. turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii* yielded 7.1 and 7.7% (v/w) of essential oil, respectively.

The chemical composition of the isolated essential oils was determined using GC/FID and GC/MS analysis. Results are reported in the Table 1. Thirty-seven compounds (99.7% of the total oil) were identified in the essential oil of *A. turbith* ssp. *hungarica* (sample 1) and thirteen compounds (98.9% of the total oil) in the essential oil of *A. turbith* ssp. *haynaldii* (sample 2). The major component in both essential oils was myristicin (58.6 and 75.9%, in sample 1 and sample 2, respectively), representing the only phenylpropane in the oils. The content of sesquiterpene hydrocarbones was 36.4% in sample 1 and 16.3% in sample 2, while monoterpene hydrocarbones were present in smaller quantities 3.0 and 6.7%, respectively. In the *A. turbith* ssp. *hungarica* essential oil oxygenated mono- and sesquiterpenes were present in small quantities (0.5–1.2%), while in the *A. turbith* ssp. *haynaldii* essential oil they were absent.

In previous studies on the essential oil composition of various *Athamanta* species a diversity has been noticed. Zivanovic et al. (1994) reported myristicin and β-pinene as the major compounds in the fruit essential oil of *A. haynaldii* [6]. Myristicin was the main compound in the fruit and leaf essential oil of *A. sicula* [7]. The most abundant components in the essential oil obtained from aerial parts of *A. macrophylla* were *p*-cymene, thymol, and carvacrol [8]. In the essential oil of *A. macedonica* ssp. *macedonica*, sabinene was found to be the major constituent [9].

Antimicrobial activity of the isolated essential oils was assayed using the agar diffusion and broth microdilution method, against Gram (+) bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, Gram (–) bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, and a yeast *Candida albicans* (Tables 2 and 3) [10, 11]. The best inhibitory effect was detected against *S. aureus* and *K. pneumoniae,* followed by the effects against *E. faecalis, C. albicans*, and *E. coli*. Neither of the oils had activity against *P. aeruginosa*. The antimicrobial activity of *A. turbith* ssp. *haynaldii* essential oil was about two times higher than that of *A. turbith* ssp. *hungarica* essential oil, except for *E. faecalis*, against which the examined essential oils exerted the same activity.

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TABLE 1. Composition of *A. turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii* Fruit Essential Oils

Compound	RI_{exp} [a]	RI_{lit} [b]	Percentage		Compound	RI_{exp} [a]	RI_{lit} [b]	Percentage	
			Sample 1[c]	Sample 2[d]				Sample 1[c]	Sample 2[d]
α-Pinene	937	939	Tr.[g]	0.5	β-Bourbonene	1387	1388	0.6	Tr.
Camphene	950	954	Tr.	-	β-Elemene	1390	1391	1.0	0.4
Sabinene	971	975	Tr.	-	(*E*)-Caryophyllene	1418	1419	2.0	2.1
β-Pinene	980	979	Tr.	6.2	β-Gurjunene	1430	1434	0.2	-
α-Terpinene	1015	1017	Tr.	-	γ-Elemene	1435	1437	7.4	1.1
p-Cymene	1022	1025	0.9	-	Aromadendrene	1438	1441	Tr.	-
Limonene	1027	1029	0.2	-	α-Humulene	1450	1455	5.5	1.7
γ-Terpinene	1058	1060	1.9	-	*allo*-Aromadendrene	1455	1460	Tr.	-
Terpinolene	1085	1089	1.1	-	γ-Muurolene	1475	1480	7.0	6.5
Linalool	1095	1097	Tr.	-	Bicyclogermacrene	1497	1500	3.0	0.3
n-Nonanal	1098	1101	Tr.	-	α-Muurolene	1498	1500	Tr.	-
1,3,8-*p*-Menthatriene	1105	1110	Tr.	-	δ-Amorphene	1509	1512	0.2	-
p-Cymen-8-ol	1180	1183	Tr.	-	Myristicin	1515	1519	58.6	75.9
Dihydrocarveol	1190	1194	0.1	-	Germacrene B	1555	1561	8.9	4.2
n-Decanal	1199	1202	Tr.	-	Spathulenol	1574	1578	0.5	-
Citronellol	1222	1226	Tr.	-	Grouped components				
Bornyl acetate	1285	1289	Tr.	-	Phenylpropanes			58.6	75.9
δ-Elemene	1335	1338	0.4	-	Monoterpene hydrocarbons			3.0	6.7
α-Cubebene	1346	1351	Tr.	-	Oxygenated monoterpenes			1.2	-
Citronellyl acetate	1348	1353	Tr.	-	Sesquiterpene hydrocarbons			36.4	16.3
α-Ylangene	1372	1375	Tr.	Tr.	Oxygenated sesquiterpenes			0.5	-
α-Copaene	1375	1377	0.2	Tr.	Total			99.7	98.9

[a]RI_{exp}: retention indices relative to C_9-C_{23} *n*-alkanes on the HP 5MS; [b]RI_{lit}: RI values from literature data; [c]sample 1, essential oil from fruit of *A. turbith* ssp. *hungarica*; [d]sample 2, essential oil from fruit of *A. turbith* ssp. *haynaldii*.
[g]Tr.: trace (<0.1%).

TABLE 2. Antimicrobial Properties of *A. turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii* Fruit Essential Oils

Microorganism	*A. turbith* ssp. *hungarica*		*A. turbith* ssp. *haynaldii*		Ampicillin	Amikacin	Nystatin
	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	10 μg/disk	30 μg/disk	100 IU/disk
Staphylococcus aureus ATCC 25923	8.0±0.7	10.0±0.0	7.3±0.4	13.0±0.0	27.0±0.5	26.0±0.0	Nt[a]
Enterococcus faecalis ATCC 29212	6.0±0.8	12.0±1.9	6.0±0.0	12.8±1.2	26.0±0.0	Nt	Nt
Escherichia coli ATCC 25922	12.2±0.9	15.0±0.7	12.0±0.0	13.0±2.1	18.0±0.0	24.0±0.0	Nt
Klebsiella pneumoniae ATCC 25922	8.0±0.7	14.0±0.0	12.5±1.5	12.5±1.5	17.0±0.0	Nt	Nt
Pseudomonas aeruginosa ATCC 27853	Na[b]	Na	Na	Na	Nt	26.0±0.5	Nt
Candida albicans ATCC 24433	8.0±0.7	8.5±0.5	15.8±1.3	15.5±0.5	Nt	Nt	20.0±0.8

Results are presented in diameters of zones of inhibition (mm) and values are the average of four determinations (±S.D.).
[a]Nt: not tested.
[b]Na: not active.

TABLE 3. Minimal Inhibitory Concentrations (MIC) of *A. turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii* Fruit Essential Oils

Microorganism	*A. turbith* ssp. *hungarica*	*A. turbith* ssp. *haynaldii*	Ampicillin	Amikacin	Nystatin
	mg/mL		μg/mL		
Staphylococcus aureus ATCC 25923	10.8	5.4	1	2	Nt[a]
Enterococcus faecalis ATCC 29212	21.6	21.7	2	Nt	Nt
Escherichia coli ATCC 25922	>86.6	43.4	8	4	Nt
Klebsiella pneumoniae ATCC 25922	10.8	5.4	Nt	Nt	Nt
Pseudomonas aeruginosa ATCC 27853	Na[b]	Na	Nt	2	Nt
Candida albicans ATCC 24433	43.3	21.7	Nt	Nt	3

[a]Nt: not tested.
[b]Na: not active.

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REFERENCES

1. T. G. Tutin, *Athamanta* L. In: T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb (Eds.), *Flora Europaea 2*, Cambridge University Press, Cambridge, 1968, pp. 340-341.
2. M. Obradovic, *Athamanta hungarica* Borb. In: M. R. Saric and N. Diklic, (Eds.), *The Flora of FR Serbia*, vol. **10**, SANU, Belgrade, 1986, pp. 150-152.
3. V. Nikolic, *Athamanta* L. In: M. Josifovic (Ed.), *The Flora of FR Serbia*, vol. **5**, SANU, Belgrade, 1973, pp. 258-260.
4. A. Tomic, S. Petrovic, M. Pucarevic, S. Sobajic, M. Ristic, and M. Niketic, *Chem. Nat. Comp.*, **42**, 391 (2006).
5. *European Pharmacopoeia*, 4th Edition, Council of Europe, Strasbourg, 2001, pp. 183-184.
6. P. Zivanovic, D. Dokovic, V. Vajs, V. Slavkovska, B. Todorovic, and S. Milosavljevic*, Pharmazie,* **49**, 463 (1994).
7. L. Camarda and V. Di Stefano, *J. Essent. Oil Res.*, **15**, 133 (2003).
8. K. H. C. Baser, T. Ozek, Kh. R. Nuriddinov, A. M. Nigmatullaev, K. Kh. Khadzimatov, and Kh. N. Aripov, *J. Essent. Oil Res.*, **9**, 249 (1997).
9. E. Verykokidou, O. Tzakou, A. Loukis, and V. Roussis, *J. Essent. Oil Res.*, **7**, 335 (1995).
10. J. F. Acar and F. W. Goldstein, *Disc susceptibility test*. In: V. Lorian (Ed.), *Antibiotics in Laboratory Medicine*, 4th Edition, Williams & Wilkins, Baltimore, 1996, pp. 1-51.
11. National Committee for Clinical Laboratory Standards (NCCLS), *Performance Standards for Antimicrobial Susceptibility Testing*: Elevent informational supplement. Document M100-S11, 2001, Wayne*, Pennsylvania*.