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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Hypericum hircinum* L. Subsp. *majus* ESSENTIAL OIL

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Hypericum hircinum L. (Guttiferae) is a semi-evergreen shrub, called "goat St John′s wort" due to the strong goat-like smell of the leaves. It is distributed in the Mediterranean region, on damp and shady places [1]; often it is transplanted from its wild environment to home gardens where it is cultivated and easily naturalized. In particular, it occurs in Marche (central Italy) with the subspecies *majus* (Aiton) N. Robson [2]. In folk medicine this plant is used for healing sore throats, colds, and as an antitussive [3]. In this study we report the chemical composition of the essential oils hydrodistilled from different parts during the phenological cycle and analyzed by GC and GC-MS, and the antimicrobial activity of the oils against a panel of human opportunistic pathogenic bacteria and fungi by using the agar diffusion and dilution method [4, 5].

Ninety-two volatile components were identified in the oils of *H. hircinum* subsp. *majus* (61 in leaves, 53 in flowers, and 64 in fruits), representing 81.41–94.36% of the total essential oils (Table 1). The oils from leaves and flowers were dominated by sesquiterpene hydrocarbons, while the fruits oils were rich in monoterpenes. The major compounds were *cis*-β-guaiene (23.25–41.23%) and δ-selinene (8.48–25.20%) in leaves, δ-selinene (18.29%) and limonene (15.23%) in flowers, and limonene (14.01–38.72%) and β-pinene (9.88–16.31%) in fruits. The development stages of the plant during the annual phenological cycle influence the composition of the fruits oils much more than the leaves oils.

The results of antimicrobial activity (Tables 2 and 3) indicated that the essential oils were active against the gram-positive bacteria *S. aureus*, *S. mutans,* and *B. subtilis*, the gram-negative *E. coli*, and the yeast *C. albicans*; the latter was the most sensitive microorganism tested, with the lowest MIC value (155 μg/mL). The ANOVA test ($P \leq 0.05$) showed significantly different activities for different harvesting times. In particular, the most active oils were those obtained during the fruiting stage, confirming the harvesting time of the plant in the traditional uses [3]. The maximum inhibition zones and MIC values of the bacteria and yeast species sensitive to the essential oils were in the range of 9.6–20.5 mm and 155–625 μg/mL. In particular, *S. mutans*, an oral pathogen responsible for caries, showed good sensitivity to the oils (MIC values in the range of 625–1250). Recently, it has been shown that oral bacteria are associated with many systemic diseases, such as pneumonia and cardiovascular diseases [6].

The results support the traditional use of this plant in Southern Italy, and suggest its use in the treatment of sore throats and colds.

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TABLE 1. Chemical Composition (Area Percent) of *H. hircinum* subsp. *majus* Essential Oil during the Phenological Cycle

Compound[b]	RI[c]	Phenological cycle[a]									
		Vegetative stage			Floral budding stage	Flowering stage		Fruiting stage			
		12.06	02.07	04.07	06.07	June 2007		August 2007		October 2007	
		leaves				leaves	flowers	leaves	fruit	leaves	fruit
Nonane*	900	0.49	Tr.	1.10	0.41	1.30	5.29	1.05	1.59	1.78	0.62
α-Thujene	927								Tr.		Tr.
α-Pinene*	933			0.27	Tr.	Tr.	0.84	0.10	1.26	Tr.	1.30
Camphene	948								Tr.		0.11
Benzaldehyde	962								Tr.		
3-Methylnonane	970			0.23	0.29	0.33	0.25	0.39		0.65	
β-Pinene*	976			Tr.	Tr.	0.21	5.86	0.47	9.88	0.16	16.31
Myrcene*	993						0.48		0.13		1.04
Decane	1000							Tr.		Tr.	
p-Cymene*	1028			Tr.			0.12		0.75		0.48
Limonene*	1031			Tr.	0.33	0.43	15.23	0.63	14.01	0.79	38.72
(*Z*)-β-Ocimene	1044							Tr.		0.18	
Benzene acetaldehyde	1049			Tr.			0.15				
(*E*)-β-Ocimene	1054			0.13		0.13		0.10		0.37	
γ-Terpinene*	1057						0.15		Tr.		0.12
Acetophenone	1063								Tr.		0.58
Terpinolene*	1089						0.12		0.23		0.59
p-Cymenene	1092								Tr.		Tr.
Undecane*	1100	0.80	0.31	0.91	1.11	0.93	4.13	0.85	2.13	1.33	5.57
Nonanal	1110			0.10			0.42	Tr.	0.24	0.11	0.32
endo-Fenchol*	1116						0.11		Tr.		
trans-*p*-Mentha-2,8-dien-1-ol	1123								0.86		0.11
α-Campholenal	1129								0.52		0.14
trans-Pinocarveol	1141						1.42	Tr.	12.40		1.51
trans-Limonene oxide	1143						Tr.				
cis-Verbenol	1146								0.52		0.14
trans-Verbenol	1149								2.01		0.49
Pinocarvone	1164						0.15		2.17		0.41
Borneol*	1168								0.50		0.46
p-Mentha-1,5-dien-8-ol	1172								0.61		0.10
Terpinen-4-ol	1179						0.18		0.80		0.60
ρ-Cymen-8-ol	1190								0.60		0.19
α-Terpineol*	1193						2.31	Tr.	3.57	Tr.	5.02
Myrtenal*	1196							Tr.	6.38		0.75
Myrtenol*	1197						1.11		10.33		1.37
Dodecane*	1199	0.73	0.14	0.28							
trans-Dihydrocarvone	1207								0.24		Tr.
Decanal	1209	0.49	0.11	0.31	0.19	0.18	0.39	0.25		0.36	
Verbenone*	1210								0.98		0.28
trans-Carveol*	1223						1.00	Tr.	3.05	Tr.	0.43
cis-Carveol*	1236						0.49		1.38		0.23
Cumin aldehyde	1243								0.11		
Carvone	1248						0.42		2.54		0.38
Piperitone*	1259								0.13		Tr.
Perilla aldehyde	1276								0.59		Tr.
Perilla alcohol	1301								1.51		0.21
Cyclosativene	1364							Tr.		Tr.	
α-Copaene*	1373	0.63	0.59	0.44	0.82	0.73	0.65	0.91	0.14	0.87	0.45
Isoledene	1378		0.40	0.42	0.51	0.43	0.24	0.43		0.46	
β-Maaliene	1391		0.29	0.42	0.50	0.43	0.10	0.39		0.39	
α-Gurjunene*	1405	0.84	0.65	0.17	0.67	0.71	0.31	1.07	0.14	0.86	0.38
(*E*)-Caryophyllene*	1415	9.88	8.45	7.15	9.28	8.49	5.12	9.09	0.46	11.10	1.30
Aromadendrene*	1435		Tr.			0.11		0.18	0.13	0.21	0.20
α-Himachalene	1445								0.10		0.23

TABLE 1. (continued)

Compound[b]	RI[c]	Phenological cycle[a]									
		Vegetative stage			Floral budding stage	Flowering stage		Fruiting stage			
		12.06	02.07	04.07	06.07	June 2007		August 2007		October 2007	
		leaves				leaves	flowers	leaves	fruit	leaves	fruit
α-Humulene*	1446		0.18	0.23	Tr.	Tr.	0.45	0.19		0.29	
allo-Aromadendrene*	1452	1.77	1.73	1.38	1.87	1.78	0.69	1.77		1.85	
9-epi-(*E*)-Caryophyllene	1454		Tr.					0.19			
(*E*)-β-Farnesene	1461	2.05	1.98	2.22	2.34	1.99		1.78		1.71	
trans-Cadina-1(6),4-diene	1475	3.58	3.56	2.75	3.79	3.52	1.53	3.52	0.22	3.77	0.56
β-Selinene	1482	0.87	1.11	0.80	1.23	0.84	0.53	0.91	Tr.	1.10	0.13
γ-Gurjunene	1485		Tr.	Tr.	Tr.	0.25	0.52	0.38		0.26	
γ-Muurolene	1488	0.31	2.41	0.50	0.69	1.98	0.85	2.00		2.22	
α-Selinene	1491	2.21	0.78	0.61	0.88	0.81	0.47	0.93	Tr.	0.98	0.26
δ-Selinene	1500	8.48	19.05	11.14	10.95	15.90	18.29	25.20	1.27	22.77	4.58
cis-β-Guaiene	1503	39.35	33.07	28.92	38.29	41.23		26.29		23.25	
(*E,E*)-α-Farnesene	1511	0.82	0.86	0.42	0.74	0.97	0.22	0.94		1.32	
γ-Cadinene	1511								Tr.		Tr.
δ-Cadinene	1523	0.29	0.23	0.15	0.26	0.29	0.22	0.52	0.10	0.48	0.16
Methyl dodecanoate	1529					0.17	0.31	0.17	Tr.	0.14	
β-Calacorene	1564		0.23	0.24	0.19	0.19		0.41		0.45	
Spathulenol	1577								0.27		0.31
Caryophyllene oxide*	1580	2.23	1.23	1.30	1.25	0.76	2.45	1.63	1.53	1.28	0.99
Viridiflorol	1589						1.32		0.21		Tr.
Hexadecane	1600		0.62								
Isolongifolan-7-α-ol	1626	10.19	6.45	8.92	7.98	6.08	6.48	6.02	1.40	5.84	2.58
epi-α-Cadinol	1642				0.11	0.14		0.23	0.30	0.24	0.31
β-Eudesmol	1649			0.17				0.23		0.30	
Selin-11-en-4-α-ol	1659							Tr.		Tr.	
Benzyl benzoate	1767		0.14	0.14	0.25	0.16	1.10	0.15	0.63	0.20	1.27
Guaiazulene*	1773		0.55	0.26	0.17	0.12	0.16	0.10	Tr.	Tr.	Tr.
Tetradecanoic acid	1774	0.25	Tr.								
Hexadecanoic acid	1969	0.41	1.41	1.89			1.43				
Phyllocladene	2030						0.30				
Phytol	2107		0.20	0.48			0.39				
Linoleic acid	2133		0.12	0.16							
Docosane*	2200		Tr.	0.13	Tr.	Tr.		Tr.		Tr.	
Tricosane*	2300		0.33	1.38	Tr.	0.14	0.60	Tr.		Tr.	Tr.
Tetracosane*	2400	Tr.	0.23	0.31	Tr.		0.17	Tr.		Tr.	Tr.
Pentacosane*	2500	0.39	1.04	3.04	0.13	0.19	0.95	0.17	Tr.	0.15	0.24
Hexacosane*	2600		0.20	0.17		Tr.	Tr.	Tr.		Tr.	Tr.
Heptacosane*	2700	0.33	0.85	1.18	Tr.	Tr.	1.07	Tr.	0.23	Tr.	0.54
Nonacosane*	2900	0.28	0.51	0.37	Tr.	Tr.	0.58	Tr.	0.43	Tr.	0.83
Monoterpene hydrocarbons				0.58	0.44	0.80	22.80	1.36	26.42	1.53	58.71
Oxygenated monoterpenes							7.19	0.24	54.46	Tr.	13.44
Sesquiterpene hydrocarbons		71.06	76.80	58.20	73.16	80.73	30.34	77.23	2.84	74.47	8.41
Oxygenated sesquiterpenes		12.42	7.68	10.40	9.35	6.98	10.25	8.18	3.70	7.74	4.28
Alkanes		3.08	4.36	9.10	2.11	3.01	13.05	2.79	4.46	4.24	7.94
Others		1.16	2.07	3.14	0.43	0.51	4.48	0.64	0.97	0.80	1.59
Identified compounds		25	40	45	36	39	53	51	60	48	59
Total identified		87.72	90.31	81.41	85.49	92.03	88.11	90.45	90.30	88.83	94.36
Yield (%, w/w)[a]		0.22	0.35	0.31	0.29	0.22	0.05	0.21	0.10	0.21	0.04

[a]12.06 – December 2006, 02.07 – February 2007, 04.07 – April 2007, 06.07 – June 2007; [b]compounds are listed in order of their elution from a HP-5 column; [c]RI: retention indices as determined on HP-5 column using homologous series of C8-C26 alkanes; [d]Essential oil yields are given on moisture free basis (w/w).

Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those reported from Adams [7]; *MS, RI and std; std, by injection of an authentic sample. Tr.: trace (<0.1%).

TABLE 2. Antimicrobial Activity of *Hypericum hircinum* subsp. *majus* Essential Oils Given as Inhibition Zone (measured in mm)[a]

Organism	Doses, μg/disk	Essential oil samples						Positive control[b]
		Vegetative	Floral budding	Flowering	Fruiting			
		April 2007	June 2007		August 2007		October 2007	
		leaves			leaves	fruits	leaves	
S. aureus ATCC 25923	100	N.t.	9.2	10.0	8.8	17.4	12.2	25.5
	50	N.t.	9.2	9.8	9.8	14.0	13.9	
	25	N.t.	9.1	9.4	10.4	13.1	13.5	
S. mutans DSM 20523	100	18.6	N.t.	N.t.	20.2	20.5	17.4	30.3
	50	16.8	N.t.	N.t.	16.9	19.2	15.2	
	25	13.8	N.t.	N.t.	14.7	18.1	13.6	
B. subtilis ATCC 6633	100	14.5	9.2	12.2	16.8	19.1	15.1	25.8
	50	13.6	9.5	11.2	12.7	17.3	12.7	
	25	13.5	10.2	10.7	11.2	13.5	13.1	
E. faecalis ATCC 29212	100	–	9.1	–	–	–	–	27.7
	50	–	9.8	–	–	–	–	
	25	–	9.6	–	–	–	–	
E. coli ATCC 13706	100	9.6	9.2	9.2	9.6	12.5	9.4	16.4
	50	11.7	11.3	11.2	10.0	12.3	10.0	
	25	12.5	12.6	11.7	11.1	11.7	11.2	
C. albicans ATCC 14053	100	13.4	15.0	12.8	16.3	17.0	16.5	18.4
	50	14.0	14.9	13.6	16.4	15.7	17.9	
	25	15.2	14.6	15.4	15.6	14.8	17.5	

[a]Mean value, n = 3 (as the diameter of the disk was 5.5 mm). [b]Ampicillin (10 μg) for gram-positive bacteria and gram-negative bacteria, Amphotericin B (10 μg) for yeast; solvent control (acetone) was negative for all tested strains. –: not active. N.t.: not tested owing to reduced amount of the oils.

TABLE 3. Antimicrobial Activity of *Hypericum hircinum* subsp. *majus* Essential Oils Given as Minimum Inhibitory Concentration (MIC, μg/mL)

Organism	Essential oil samples					Positive control[b]
	Vegetative	Floral budding	Fruiting			
	April 2007	June 2007	August 2007		October 2007	
	leaves			fruits	leaves	
S. aureus ATCC 25923	N.t.[a]	625	625	310	625	5
S. mutans DSM 20523	N.t.	1250	625	625	625	10
B. subtilis ATCC 6633	625	N.t.	625	625	1250	10
E. faecalis ATCC 29212	N.t.	1250	2500	2500	5000	10
E. coli ATCC 13706	310	N.t.	310	310	625	5
C. albicans ATCC 14053	625	625	310	155	625	1

[a]N.t.: not tested owing to reduced amount of the oils. [b]Chloramphenicol for gram-positive and gram-negative bacteria, Amphotericin B for yeast; solvent control (acetone) was negative for all tested strains.

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