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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OIL OF *Satureja montana* FROM CENTRAL ITALY

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Satureja montana L. is a perennial semi-shrubbery plant growing in the submediterranean area and is also known as "Winter savory". This plant is one of the best honey plants and its honey is well known as folk remedy to cure bronchitis [1]. It contains various biologically active constituents such as essential oil, tannins, flavonoids, and rosmarinic acid and is important for pharmacy and medicine [2].

The plant and its essential oil and extracts are used in folk remedies for many diseases, exerting bactericidal, carminative, digestive, expectorant, fungicide, laxative, antidiuretic, sedative, and antioxidant activities. The essential oil is also an ingredient of flavoring condiments, relishes, soups, sausages, canned meats, and in spicy table sauces [1].

The aim of this study was to determine the composition of the essential oil extracted from *S. montana* grown in central Italy and to evaluate its antifungal activity against nine phytopathogenic fungi: *Fusarium poae, F. equiseti, F. graminearum, F. sporotrichoides, F. culmorum, Alternaria solani, Rhizoctonia solani, Phytophthora cryptogea,* and *Botrytis cinerea*. The above-mentioned activity was evaluated by two methods: the agar diffusion method and the microatmosphere method, which has been used herein for the first time for the essential oil of *S. montana*.

Flowering tops of *Satureja montana* L. were collected near Urbino (central Italy) at 500 m altitude on sea level in July 2004 and authenticated by prof. Donata Ricci, Botanical Institute, University of Urbino. Voucher specimens of the plants have been deposited at the Herbarium of the Botanical garden of the University of Urbino (15-07). Air-dried aerial parts of plants collected were submitted for 3 h to steam distillation using a Clevenger apparatus to produce the essential oil (yield 0.59% v/w). Oil was dried over anhydrous sodium sulphate and, after filtration, stored at + 4°C until tested and analyzed. The GC and GC/MS analysis were carried out as already described [3]. Fungal strains were kindly supplied by DI.PRO.VAL (Faculty of Agriculture) of the University of Bologna, Italy. Activity against phytopathogenic fungi was assayed by the agar dilution method [4] and by a modification of the micro-atmosphere method already reported by Delespaul and coworkers [5]. The fungicidal activity of the oils was determined using the technique of Thompson [6] (1989) and Carta and Arras [7].

The chemical composition of the essential oil is reported in Table 1. Thirty-five compounds were identified by GC-MS, and carvacrol (18.00%), *p*-cymene (14.30%), thymol (9.92%), β-phellandrene (5.60%), β-caryophyllene (4.97%), carvacrol methyl ether (4.86%), and linalool (4.81%) were the most abundant ones.

The *in vitro* antimicrobial activity of the essential oil of *S. montana* is reported in Table 2 , and values are expressed as percentage of inhibition and minimal inhibitory concentration (MIC) (μg/mL) respectively.

A concentration of 600 ppm was fungicidal for all the tested fungi.

The results obtained in the micro-atmosphere method and are expressed as minimal inhibitory volumes of the essential oil in μL.

For all the tested fungi, 1 μL of the oil inhibited the growth of mycelium for 2 days; the inhibitory effect of 2 μL of oil lasted 10 days for all fungi. After exposure for 10 days, 4 μL of oil was fungicidal for *F. poae, P. cryptogea,* and *B. cinerea,* while for all the other tested fungi this concentration of oil was fungistatic. The fungicidal effect for these last fungi was achieved with 8 μL of oil after10 days.

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TABLE 1. Chemical Composition of the Oil of the Aerial Parts of *S. montana*

Compound	%	RI	Compound	%	RI
α-Pinene	3.34	942	β-Cubebene	1.01	1400
Camphene	2.20	952	α-Caryophyllene	0.91	1425
β-Pinene	2.18	980	β-Caryophyllene	4.97	1434
Myrcene	2.95	985	Germacrene D	0.34	1468
(*Z*)-β-Ocimene	0.04	1013	Aromadendrene	1.39	1472
(*E*)-β-Ocimene	0.21	1023	γ-Muurolene	0.91	1486
Terpinolene	0.34	1062	δ-Cadinene	0.58	1504
Linalool	4.81	1092	β-Bisabolene	1.43	1505
Tujone	0.11	1101	γ-Cadinene	1.13	1507
Terpinen-4-ol	2.66	1129	Spatulenol	2.41	1560
β-Terpineol	1.79	1137	Bourbonene	1.12	1586
Camphor	2.03	1138	Borneol	2.69	1698
Δ^3-Carene	0.03	1142	Thymol methyl ether	0.37	1735
α-Terpineol	0.13	1169	Carvacrol methyl ether	4.86	1736
β-Phellandrene	5.60	1213	Thymol	9.92	1739
Pulegone	2.14	1229	Carvacrol	18.00	1741
Geraniol	1.41	1230	Ledol	1.69	2098
p-Cymene	14.30	1272			

TABLE 2. Effect of *Satureja montana* Essential Oil on *in vitro* Growth of Phytopathogen Fungi; Minimal Inhibitory (MIC) and Fungicidal (MFC) Concentration of Essential Oil

Fungus	Inhibition, %[a]				MIC (μg/mL)[a]	MFC (μg/mL)[a]
	100 ppm	200 ppm	400 ppm	6400 ppm		
F. poae	70.2	100[c]	100[b]	100	150	250
F. equiseti	62.3	100[c]	100[b]	100	150	300
F. graminearum	57.1	100[c]	100[b]	100	100	250
F. sporotrichoides	66.4	100[c]	100[c]	100[b]	100	450
F. culmorum	30.4	70.0	100[c]	-	250	450
A. solani	26.6	46.6	100[c]	-	300	450
R. solani	17.5	77.5	100[c]	-	250	500
P. cryptogea	25.0	65.0	100[b]	-	250	300
B. cinerea	18.8	58.8	100[b]	-	250	300

[a]Calculated as mean of 3 tests; [b]fungicidal; [c]fungistatic.
Inhibition, %[a]: 600 ppm, 800 ppm = 100[b]; Nystatine (50 ppm) = 100[b].

A very high antifungal activity of the oil of *S. montana* has been observed using both the agar dilution method and the micro-atmosphere method. This is noteworthy as we show that the essential oil of *S. montana* can be effective not only by direct contact with fungi but also in the vapor phase, and in this state it can inhibit the growth of post-harvest pathogens of a range of crops.

In conclusion, in this study we reported that the essential oil of *S. montana* grown in central Italy showed very good antifungal activity using two different methods. Our study represents, to the best of our knowledge, the first application of the micro-atmosphere method to evaluate the antifungal activity of the essential oil of *S. montana*.

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