Acta Bot. Croat. 58, 99–104, 1999

CODEN: ABCRA 25
ISSN 0365–0588

Dedicated to Prof. dr. MERCEDES WRISCHER
on the occasion of her 70th birthday.

UDC 547.913

ANTIMICROBIAL EFFECT OF *Satureja cuneifolia* Ten. ESSENTIAL OIL

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The essential oil composition of *Satureja cuneifolia* Ten., was analyzed with respect to samples picked in different vegetative stages in the region of Rotimlje (Stolac, Herzegovina). We did not find oil of a composition characteristic of the *Satureja* genus. The researched species mostly did not contain either Thymol or Carvacrol. The antimicrobial effect of the oil was highly marked during the post – flowering period, especially for the *Staphylococcus aureus* bacterium. Essential oil fungicidal activity was best marked on *Candida albicans* yeast during the flowering period. Picking this plant at the proper time can considerably increase its therapeutic effect.

Key words: *Satureja cuneifolia*, oil, antimicrobial effect, thymol, carvacrol, *Staphylococcus aureus, Candida albicans*

Introduction

Excessive use of antibiotics has led to the selection of microorganisms resistant to many of the antimicrobic preparations used, which has resulted in turn in the appearance of complex bacterial infections. The complexity of this problem is best proved by the World Health Organization (WHO) statement that in 1996 alone, 17,3 million people died of infective illnesses, accounting for 33 % of the total mortality rate (JONES and PFALLER 1998).

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The return to nature and its resources, along with the growth of material well-being, has contributed to ever greater attention being paid to the use of medicinal herbs in nutrition and health care. A prominent position in this respect is occupied by plants growing wild in non-polluted areas. Such plants represent a source of natural organic compounds that have a protective function for the plants themselves and also for the organisms surrounding them. Modern phytotherapy aims at solving the resistance problem, thus developing better protection for human beings.

The *Satureja* genus consists of multi-annual semi-shrubs of a more or less aromatic nature, concentrated in Mediterranean region. The *Satureja cuneifolia* Ten. species grows mostly on rocky meadows sheltered from strong winds. Due to its arid habitat this plant developed xeromorphic properties, the production of essential oil being among them. Different species have different essential oil compositions, and the composition also changes during the various vegetative stages of a single species. This study deals with research into the composition and antimicrobial effect of the essential oil (*S. cuneifolia* species) of a plant from the same habitat but from different vegetative – generative stages of the plant's annual growth.

Materials and methods

The plant material used in the elaboration of this work was picked in the region of Rotimlje (Stolac, Herzegovina). The leaves from the younger parts of the stem and the flowers of *Satureja cuneifolia* species, gathered from June to October 1998 (before, during and after flowering) were analyzed. Hundred grams of dried leaves was extracted using water distillation. Analysis of the oil was made on a gas chromatograph, Hewlet Packard, GC 5890 series II; MSD 5971A. The column was HP-20M (polyethylene glycol 50 m × 0.2 mm) directly connected to MS. Helium flow rate was 1 mL/min^{-1}. Temperature was programmed at 700 °C for 4 min, then 70–180 °C, increase 4 °C, maintaining temperature for 10 minutes.

The antimicrobial effect tests of the essential oil of *S. cuneifolia* were carried out in vitro on different kinds of microorganisms representing the most frequent infective agents isolated from biological samples. *Escherichia coli* and *Pseudomonas aeruginosa* were used as Gram negative bacteria in these tests, *Staphylococcus aureus* as Gram positive *and Candida albicans* as yeast. The standard procedure of antibiogram elaboration was performed by the diffusion method in agar, as often applied in similar research (KONEMAN et al. 1997; MILES and AMYES 1996).

24 hour bacteria culture was used as inoculum. The inoculum size and the number of bacteria in a suspension were calculated in relation to the final volume and presented as colony forming units in one millilitre (cfu/mL). Antibiograms were done on solid M - H agar. Inhibition zone diameter was given in millimetres.

Antibiograms were also made on other culture media such as: MacConkey's agar (MC) and Saburaud-dextrose agar for yeast cultivation.

Filter paper discs were placed over adequate solid culture media, onto which pure and fresh bacteria and yeast cultures had previously been inoculated.

Three discs containing a dose of 10 μL of the tested essential oil were placed over each culture medium. The growth inhibition zone was read separately after 24 hours of incubation at 37 and 22 °C.

Results

By the process of water distillation of *Satureja cuneifolia* Ten. 0.25 mL of essential oil was isolated in the pre-flowering period, 0.30 mL during the flowering and 0.20 mL in post-flowering period.

Depending on picking time, this species shows different essential oil contents.

Expressed in percentages, *S. cuneifolia* contains most Linallol, beta-Cubebene, trans-Caryophylene, 1-alpha-Terpineol and 1- Borneol in the pre-flowering period, and considerably less Thymol and Carvacrol (Tab. 1). No Thymol or Carvacrol are to be found either during or after the flowering period.

Tab. 1. Composition (%) of essential oils from *Satureja cuneifolia* Ten.

Oil composition	Flowering period		
	before	during	after
Beta-Myrcene	0.85	1.17	0.26
1-Limonene	3.22	5.17	1.15
Ocimen	–	1.22	0.20
1-octen-3-ol	0.96	0.73	0.18
Alloocimen	–	–	0.14
Trans-Sabinene hydrate	0.59	1.25	0.11
Alpha-Copaene	1.67	2.33	3.61
Beta-Bourbonene	1.83	2.10	5.27
Linalool	9.25	3.37	–
Terpinen-4-ol	5.48	5.05	–
Trans-Caryophyllene	6.00	2.00	6.12
Z-Citral	1.66	2.34	–
1-alpha-Terpineol	5.99	3.15	1.47
1-Borneol	4.51	5.02	4.84
Beta-Cubebene	6.88	14.79	–
Neryl acetate	1.56	–	–
Geranyl acetate	0.59	–	–
Delta-Cadinene	2.06	1.89	2.61
Mytrenol	0.74	0.30	0.31
Nerol	1.03	0.70	0.83
Geraniol	0.37	–	–
Beta-Ionone	0.13	–	–
- (-)Carryo-phyllen oxide	2.00	0.83	–
Thymol	0.24	–	–
T-Muurolol	0.39	0.27	0.85
Carvacrol	1.75	–	–

It was noted however that in the post-flowering period the *S. cuneifolia* species generally contains a smaller number of detected compounds and in a smaller quantity, trans-Caryophyllen, Beta-Bourbonene, 1-Borneol and Alpha--Copaene prevailing (Tab. 1).

Illustration 1 shows the spectrum of essential oil *S. cuneifolia* antimicrobial activity in different vegetative stages. The biggest growth inhibition of the tested microorganisms was noted in the oil isolated from the plant in the post-flowering period.

Growth inhibition zones in all microorganisms show lower antimicrobial oil activity during the flowering period. The essential oil isolated from the plant in the pre-flowering period has considerably reduced antibiotic effects.

Growth inhibition zones in *Staphylococcus aureus*, the representative of Gram positive bacteria, ranged from 32 mm in the pre-flowering to 41 mm in the post-flowering period with 10 μL doses used. The representatives of Gram negative bacteria are less sensitive to the effects of *S. cuneifolia* essential oil in all the vegetative stages examined. In *Escherichia coli* the growth inhibition zones ranged from 0 to 6 mm, while the *Pseudomonas aeruginosa* species showed comparatively high level of resistance to the essential oil tested.

A high level of essential oil activity was observed in *Candida albicans*, especially in the flowering period.

Discussion

Among thirty known species of the *Satureja* genus (ŠILIĆ 1979), *Satureja montana* (CAVE 1995) is mostly used in phytoformation. The representatives of the *Satureja* genus have fairly similar essential oil composition. The best represented compounds are Thymol and Carvacrol. This is the case with *S. montana* (PALIĆ et al.1983, PICCAGLIA et al. 1991, KUŠTRAK et al.1996), and also with the *Satureja subspicata*. and *Satureja liburnica* species (LOKAR et al.1983), as well as with *Satureja obovata* (ARREBOLA et al. 1994), so that most authors believe that with above species it is a matter of Carvacrol chemotype.

The fact that the existing data on *Satureja cuneifolia* species essential oil composition are similar to those on other *Satureja* genus representatives and are rich in Thymol and Carvacrol (TUMEN et al. 1998) is particularly important for our study. The evidence that the species used in this research shows almost no presence of Thymol or Carvacrol indicates that it cannot be classified under a separate chemotype. These compounds appear only in the pre-flowering period (Tab. 1) and are not formed in later vegetative stages. We believe that this composition of the tested species' essential oil is the result of the specific environmental conditions of its habitat.

Essential oils are known for their antibacterial, antimicotic and antiviral effects (KUŠTRAK et al.1996, WAGNER 1993) and we therefore tested the essential oil's antimicrobial effect at different vegetative stages of the *S. cuneifolia* species. The test results of antimicrobial activity and the manner of action of *S. montana* essential oil (BEZIĆ et al. 1998) are similar to the results obtained under this study with respect to the sensitivity spectrum of the tested pathogen

microorganisms. The high level of growth inhibition of *Candida albicans* yeast indicates the exceptional fungicidal effect of the oil, especially in the flowering period of the tested plant. The level of antimicrobial effect on Gram positive bacteria was generally lower than that on yeast, but definitely higher than that on Gram negative microorganisms. *S. cuneifolia* essential oil shows different levels of antimicrobial activity on all the tested bacteria, the highest being in the post--flowering period, while the lowest antimicrobial activity was observed in plants picked in pre-flowering period. (Fig. 1). We believe that the maturing of a plant brings forth changes in the composition and the percentage of some oil components as to enhance the effect on microorganisms.

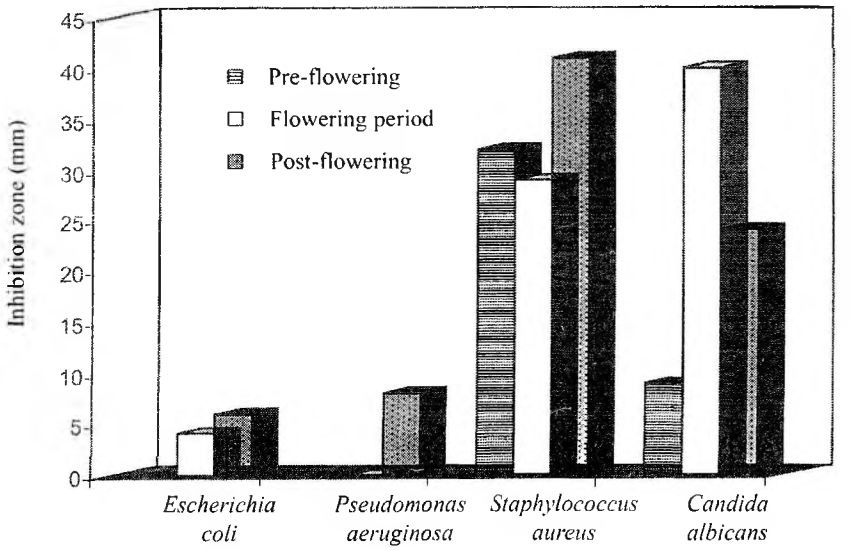

Fig. 1. Antimicrobial activity of essential oils from *Satureja cuneifolia* Ten. in different vegetative stages: before, during and after flowering.

Acknowledgements

My special thanks goes to Dr. Mercedes Wrischer, for her cooperation and generous support throughout the period of my scientific education.

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