# Satureja horvatii essential oil: *In vitro* antimicrobial and antiradical properties and *in situ* control of *Listeria monocytogenes* in pork meat

Danka Bukvički [a,c], Dejan Stojković [b,*], Marina Soković [b], Lucia Vannini [c], Chiara Montanari [c], Boris Pejin [d], Aleksandar Savić [d], Milan Veljić [a], Slavica Grujić [a], Petar D. Marin [a]

[a] University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", 11000 Belgrade, Serbia
[b] University of Belgrade, Institute for Biological Research 'Siniša Stankovic', Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
[c] University of Bologna, Department of Agricultural and Food Sciences, Via Fanin 46, 40127 Bologna, Italy
[d] University of Belgrade, Institute for Multidisciplinary Research, Department of Life Science, Kneza Višeslava 1, 11030, Belgrade, Serbia

## A B S T R A C T

The dominant compounds in *Satureja horvatii* oil were *p*-cymene (33.14%), thymol (26.11%) and thymol methyl ether (15.08%). The minimum inhibitory concentration (MIC) varied from 0.03 to 0.57 mg/mL for bacteria, and from 0.56 to 2.23 mg/mL for yeast strains, while minimum bactericidal/yeast-cidal concentration (MBC/MYC) varied from 0.07 to 1.15 mg/mL and 1.11 to 5.57 mg/mL for bacteria and yeasts, respectively. The antiradical potential of the essential oil was evaluated using hydroxyl radical (•OH) generated in Fenton reaction. The meat preserving potential of essential oil from *Satureja horvatii* was investigated against *L. monocytogenes*. Essential oil successfully inhibited development of *L. monocytogenes* in pork meat. Sensorial evaluation on flavor and color of meat was performed. The color and flavor of meat treated with essential oil improved after 4 days of storage. *S. horvatii* essential oil can act as a potent inhibitor of food spoiling microorganisms, in meat products and also can be a useful source of natural antioxidants.

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## 1. Introduction

The genus *Satureja* L. includes about 200 species of herbs and shrubs, often aromatic, with a centre of distribution in the Mediterranean Basin. In the area of the central and western Balkans, nine species of this genus have been registered (Lakusic, Ristic, Slavkovska, Stankovic, & Milenkovic, 2008). Many of these species from genus *Satureja* are endemic from the Orjen–Lovćen mountain massive (Montenegro), with a high content of essential oil, up to 4% and are widely applied in ethnomedicine and ethnobotany. Significant proportions of *Satureja* species are plants that have an important role in the pharmaceutical industry. The essential oils isolated from various *Satureja* species have shown antibacterial, fungicidal, antiviral and antioxidant activities (Lakusic et al., 2008).

Due to antimicrobial effects attributed to the composition of these essential oils, they are widely used in various types of phytotherapies and cosmetics (Momtaz & Abdollai, 2010; Redžić, Tuka, & Pajević, 2006).

A wide range of preservatives or antimicrobial treatments is used to extend the shelf-life of a product by inhibiting microbial growth. However, an increasingly negative consumer consideration of food additives,

perceived as non-natural, has spurred an interest in finding natural alternatives to the traditional solutions (Smith-Palmer, Stewart, & Fyfe, 2001). Although essential oils were originally added to change or improve taste, their antimicrobial activity makes them an attractive choice for substituting chemical preservatives (Burt, 2004; Hyldgaard, Mygind, & Meyer, 2012; Lanciotti et al., 2004; Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009). Many food products are perishable and require protection from microbial spoilage during preparation, storage and distribution to give them an acceptable shelf-life and organoleptic characteristics. Because of recent trends in food marketing, the need for an extended shelf-life for these products has increased together with the application of less severe technological treatments (Rasooli, 2007). In addition, the risks due the presence and the growth of pathogenic microrganisms or bacteria producing toxins can be increased by these trends. As an example, contamination of some foods with *Listeria monocytogenes* is almost inevitable due to its ubiquitous nature in the environment.

Oxidative reactions can also affect the shelf-life and overall quality of a food. In particular, oxidative damages by free radicals are implicated in the etiology of many diseases, cancer and cardiovascular diseases being the most common. Antioxidants have been widely used as food additives to prevent oxidative degradation by free radicals (Razali, Mat-Junit, Abdul-Muthalib, Subramaniam, & Abdul-Aziz, 2012). Chemical antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are widely used as inhibitors of lipid peroxidation.

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Several side effects of these additives have been reported, including a carcinogenic effect (Ames, 1983; Baardseth, 1989).

In the present study, the chemical composition of S. horvatii oil was analyzed by GC-MS. The effect of S. horvatii oil against different food spoiling yeasts and bacteria was studied through in vitro antimicrobial assays. Moreover, the potential use of the plant essential oil as a natural preservative in pork against L. monocytogenes and as an antioxidant (more specifically, antihydroxyl radical agent) was assessed.

## 2. Materials and methods

### 2.1. Plant material

Plant material of S. horvatii Šilic was collected from Orjen Mt. (Montenegro) in July 2010. A specimen has been deposited in the Herbarium at the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade (BEOU). The material was dried at room temperature (25 °C) with constant aeration for ten days.

### 2.2. Distillation of essential oil

The aerial parts of the plant were dried at room temperature and hydrodistilled (100 g) for 2 h, using a Clevenger-type apparatus. The oil yield was 3.2%. After hydrodistillation, water was removed by decantation and the essential oil obtained was stored at 4 °C and protected against light.

### 2.3. Gas chromatographic mass spectrometry (GC-MS) analysis of Satureja horvatii oil

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5975 mass selective detector operating in electron impact mode (ionization voltage, 70 eV). A CP-Wax 52 CB capillary column (50 m length, 0.32 mm inner diameter, 1.2 μm film diameter) was used. The temperature program started from 50 °C, then was programmed at 3 °C/min to 240 °C, which was maintained for 1 min. Injector, interface, and ion source temperatures were 250 °C, 250 °C, and 230 °C, respectively. Injections were performed in split mode and helium (1 mL/min) was used as the carrier gas. The mass selective detector was operated in the scan mode between 20 and 400 m/z. Data acquisition started 4 min after injection. One microliter of the sample was injected directly into the column with a split ratio of 1:100. Component separation was achieved as described above. The identification of the molecules was based on comparison of mass spectra of compounds, both with those contained in available databases (NIST version 2005) and with those of pure standards (Sigma–Aldrich, Milan, Italy) analyzed under the same conditions.

### 2.4. Yeast, bacteria strains and culture conditions

Yeasts (Saccharomyces cerevisiae 635, Zygosacharomyces bailii 45, Aureobasidium pullulans L6F, Pichia membranaefaciens OC 71, Pichia membranaefaciens OC 70, Pichia anomala CBS 5759 and Pichia anomala DBVPG 3003) obtained from the strain collection of the Department of Agricultural and Food Sciences of the University of Bologna (Italy) were used to evaluate the effect of essential oil. The bacterial strains (Listeria monocytogenes NCTC 7973, Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 35210 and Salmonella typhimurium ATCC 13311) were obtained from the strain collection of the Department of Plant Physiology, Laboratory of Mycology, Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia. Yeast strains were grown in Yeast extract Peptone Dextrose (YPD) broth at 27 °C for 48 h, while bacterial strains were grown in Tryptic Soy Broth (TSB) at 37 °C for 24 h. Microorganisms were used immediately to make appropriate cell dilutions in media for further experiments.

### 2.5. In vitro antimicrobial assay

In order to investigate the antimicrobial activity of the essential oil, the modified micro-dilution technique was used (Daouk, Dagher, & Sattout, 1995; NCCLS, 1999).

Minimum Inhibitory Concentration (MIC) determination was performed by serial dilution using 96-well microtitre plates (Sarstedt, Milan, Italy). The tested oil was added to the TSB medium for bacteria and YPD medium for yeasts and then filled into 96-wells microplates (100 μL/well) with inoculum of the target microbial species previously adjusted to a concentration of approximately $1.0 \times 10^6$ CFU/mL. The microplates were incubated for 24 h at 37 °C for bacteria and 48 h at 27 °C for yeasts. A sterile medium incubated under the same condition was used as a blank, while the medium inoculated with the target microorganisms (without the oil) was used as a positive control of growth. The lowest concentrations of the oil showing complete inhibition of visible growth were defined as MICs. The absence of visible growth was determined under a binocular microscope. All determinations were performed in triplicate. Also the Minimum Bactericidal Concentration (MBC) and Minimum Yeast-cidal Concentration (MYC) were determined. Generally, MBC/MYC values are defined as the minimum concentrations of the tested molecule not allowing any microbial growth when 10 μL of the cultures taken from the wells with no visible growth after incubation is plated into solid medium (YPD and TSB for yeasts and bacteria, respectively). Streptomycin and cyclohexamide were used as positive controls.

### 2.6. Fluorescence measurements

Fluorescence spectra in time domain (kinetics mode) were collected using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. Measurements were performed in a well stirred and tempered quartz cuvette (1 cm optical path length, 1.5 mL volume, 25 °C). The slits on the excitation and emission beams were fixed at 2 nm. Wider slits can be used if white noise is present. Total time of measurement was 600 s, while the integration time was set to 0.1 s. Excitation/emission wavelengths were 500/520 nm for hydroxyl radical measurements (Gomes, Frenandes, & Lima, 2005).

APF (2-[6-(4'-amino)phenoxy-3H-xanten-3-on-9-yl] benzoic acid) was dissolved directly in a cuvette during the Fenton reaction (0.5 μL, undiluted). Standard Fenton reaction was used for generation of hydroxyl radicals (•OH) (0.5 mM FeSO$_4$ and 1 mM H$_2$O$_2$, and 0.5 μL APF in a cuvette of 1.5 mL). This was used as the blank. Anti hydroxyl radical was determined in the same reaction system, with addition of 0.1 μL of essential oil.

All chemicals (analytical grade or higher) were used as received from Sigma–Aldrich without further purification. All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm.

#### 2.6.1. Signal processing

Kinetic profile of APF probe represents linear function of time:

$$A(t) = at + A_0 \tag{1}$$

$A(t)$ — amplitude of fluorescence emission in given moment of time, a — parameter which determines the slope of line, $A_0$ — starting intensity of fluorescence emission.

In the presence of (•OH) radical, fluorescence emission intensity increases with time due to –O–degradation of the fluorescence probe (Setsukinai, Urano, Kakinuma, Majima, & Nagano, 2003).