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Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil

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Abstract

The essential oils of *Salvia officinalis* and *Salvia triloba* cultivated in South Brazil were analyzed by GC–MS. The major constituents of the oil of *S. officinalis* were α-thujone, 1,8-cineole, camphor, borneol and β-pinene, whereas those of *S. triloba* were α-thujone, 1,8-cineole, camphor, and β-caryophyllene. The essential oils of both species exhibited remarkable bacteriostatic and bactericidal activities against *Bacillus cereus*, *Bacillus megatherium, Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria*, and *Klebsiella oxytoca*. Moreover, the essential oil of *S. triloba* efficiently inhibited the growth of *Staphylococcus aureus. S. aureus* and *A. hydrophila* growth were drastically reduced even in the presence of 0.05 mg/ml of the essential oil of *S. triloba*.

Keywords: *Salvia officinalis*; *Salvia fruticosa*; Essential oil; Antimicrobial activity; GC–MS

1. Introduction

Since ancient times the crude herbal extracts of aromatic plants have been in use for different purposes, such as food, drugs and perfumery (Heath, 1981). The essential oils are considered among the most important antimicrobial agents present in these plants, and may also have antioxidant and antiinflammatory activities. Volatile oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Other volatile compounds include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, the oil composition is a balance of various compounds, although in many species one constituent may prevail over all others (Cowan, 1999).

In the recent decades, antimicrobial plant products have gained special interest because of the resistance to antibiotics that some microorganisms have acquired (Essawi & Srour, 2000), the increasing popular concern about the safety of food and the potential impact of synthetic additives on health (Reische, Lillard, & Eintenmiller, 1998).

Salvia, the largest genus of the Lamiaceae family, includes about 900 species, spread throughout the world, some of which are economically important since they have use as spices and flavouring agents in perfumery and cosmetics. The analysis of the essential oil composition of several *Salvia* species indicates that 1,8-cineole (eucalyptol), and borneol are its main constituents. However, several authors have documented significant species specific variations in the concentration of these compounds and/or presence of others in high concentrations (Ahmadi & Mirza, 1999; Baser, Ozek, & Kirimer, 1993; Baser, Duman, Vural, Adiguzel, & Aytac, 1997; Haznedaroglu, Karabay, & Zeybek, 2001; Holeman, Berrada, Bellakhdar, Hidrissi, & Pinel, 1984; Perry et al., 1999; Putievsky, Ravid, Diwan-Rinzler, & Zohary, 1990; Sivropoulou et al., 1997; Torres, Velasco-Negueruela, Perez-Alonso, & Pinilla, 1997). Moreover, the essential oil composition of *Salvia* species, as

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occurs with other medicinal and aromatic plants, is highly influenced by genetic and environmental factors (Dean & Ritchie, 1987; Piccaglia & Marottu, 1993).

The antimicrobial activity of *Salvia officinalis* was recognized decades ago (Jalsenjak, Peljnajk, & Kustrak, 1987), and was attributed to the presence of 1,8-cineole, thujone and camphor (Jalsenjak et al., 1987; Sur, Tuljupa, & Sur, 1991). More recently, Sivropoulou et al. (1997) reported the antimicrobial activity of *Salvia triloba* (syn. *Salvia fruticosa*) collected in Greece. As with *S. officinalis*, its oil was also characterized by high concentrations of 1,8-cineole, thujone, and camphor.

The aim of this study was to compare the antimicrobial activities of the essential oils of *S. officinalis* and *S. triloba* cultivated in South Brazil, against a range of foodborne pathogenic and spoilage bacteria, evaluating minimal inhibitory concentrations, and kinetic parameters, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

2. Materials and methods

2.1. Plant material

Two commercial cultivars were handled in this study, one of *S. officinalis* L., obtained from Isla S.A., and the other of *S. triloba* L., obtained from the North of Italy and cultivated for long time by the Italian immigrants of the northeast region of Rio Grande do Sul State, Brazil. The plants were cultivated in the experimental field of the Institute of Biotechnology at Caxias do Sul, Rio Grande do Sul, Brazil, under standard conditions. Two year-old flowering plants were collected for oil extraction and analysis in November, 2001.

2.2. Essential oil extraction and analysis

Aerial parts of 10 plants were collected and dried in a chamber at 40 °C. The dried samples were subjected to hydrodistillation for 1 h using a Clevenger-type apparatus. The oil obtained was separated from water and dried over anhydrous Na_2SO_4. The identification of the chemical components of the oil samples was done in a complete HP 6890 gas chromatograph using a mass selective detector HP 5973, equipped with Chemstation software and Wiley 275 spectra data. A HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The chromatographic conditions were: column temperature 60 °C (8 min), 60–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), interface 180 °C, split ratio 1:100, carrier gas, He (55.4 kPa), flow rate 1.0 ml/min, ionization energy 70 eV, mass range 40–350, volume injected 0.5 μl, solvent cut, 3.5 min.

GC analysis was performed on a HP 5973 gas chromatograph with FID detector using a HP-Innowax fused silica capillary column (30 m × 0.25 mm, 0.50 μm film thickness). The chromatographic conditions were: column temperature 40 °C (8 min), 40–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min), injector temperature 250 °C, split ratio 1:50, detector temperature 250 °C, carrier gas hydrogen (34 kPa), flow rate 1.0 ml/min, volume injected 0.2 μl.

2.3. Bacterial strains

The following bacterial strains and isolates were used in the antimicrobial tests: *Escherichia coli* IBEc-01, *Proteus mirabilis* IBPm-101, *Salmonella typhimurium* IBSt-101, *Aeromonas hydrophila* ATCC 7966, *A. hydrophila* CECT 389, *Aeromonas sobria* ATCC 43979, *Klebsiella oxytoca* IBKle-101, *Citrobacter sp.* IBCs-101, *Serratia marcescens* IBSm-101, *Bacillus megatherium* IBBac-103, *Bacillus cereus* IBBac-102, *Bacillus subtilis* IBBac-101, *Pseudomonas aeruginosa* IBPa-101, *Pseudomonas fluorescens* IBPf-101, *Staphylococcus aureus* ATCC 6538, *S. aureus* IBAs-102, and *Staphylococcus epidermidis* IBSe-101 (IB strains, Institute of Biotechnology, University of Caxias do Sul, CECT, Spanish Type Culture Collection, and ATCC, American Type Culture Collection).

2.4. Antimicrobial activity assay

A broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of MIC (NCCLS, 1999). Briefly, bacterial strains were cultured overnight at 37 °C on Mueller Hinton broth (MHB, BBL) and adjusted to a final density of 10^6 cfu/ml, and used to inoculate (1/10) 96-well microtitre plates containing serial twofold dilutions of the essential oils (10–0.01 mg/ml) on MHB supplemented with 0.5% (v/v) Tween 80. Plates were incubated under normal atmospheric conditions at 37 °C for 24 h. Bacterial growth was monitored by absorbance at 560 nm in a microtitre plate reader (Metrolab, Argentina).

Bacterial viability was monitored, transferring 10 μl of the cultures of the above cultures to 3 ml of LB broth and incubating them at 37 °C for 18 h. Minimal cidal concentration (MCC) was determined visually by the evaluation of the presence of bacterial white pellet on the well bottom.

To evaluate the effect of *S. officinalis* and *S. triloba*'s essential oils on *S. aureus* and *A. hydrophila*, cultures were grown on LB medium at 37 °C for 18 h, from which 0.5 ml was used to inoculate 50 ml of LB medium supplemented with 0.5% Tween 80 and dilutions of each essential oil (0, 0.05, 0.1, 0.2 or 0.5 mg/l). Cultures were incubated at 37 °C on a rotatory shaker (150 rpm) and samples were collected at regular time intervals. Bacterial growth was evaluated by absorbance at 560 nm in a Pharmacia spectrophotometer.

All the experiments were conducted in triplicate and average values were plotted. For growth kinetic experiments, maximum growth rates and yields were calculated and submitted to ANOVA analysis of variance.

3. Results and discussion

The main constituents (>1%) of the essential oils of *S. officinalis* and *S. triloba* cultivated in South Brazil and used in the experiments are presented in Table 1. The main components of both oils were α-thujone, 1,8-cineole and camphor. *S. officinalis* also presented substantial amounts of β-pinene, borneol and δ-gurjunene, whereas *S. triloba* was characterized by high concentrations of β-caryophyllene, α-humulene and the presence of viridiflorol.

For the main constituents in the essential oil of *S. officinalis*, the concentration observed was similar to that obtained in Italy (Grella & Picci, 1988; Marino, Bersani, & Comi, 2001), Yugoslavia (Tucker, Maciarello, & Howell, 1980), and Israel (Putievsky et al., 1990). However, the concentrations of β-caryophyllene and α-humulene were significantly lower than those previously reported. Conversely, the essential oil of *S. triloba* (syn, *S. fruticosa*) was different from that reported in Greece by Sivropoulou et al. (1997) in that it presents significantly lower concentrations of 1,8-cineole, and higher concentrations of α-thujone and β-caryophyllene. These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences (Perry et al., 1999).

The antimicrobial activity of the essential oils was examined by broth microdilution susceptibility assay against a panel of 17 bacterial strains selected on the basis of their relevance as food contaminants. The results, presented in Table 2, reveal that the oil of *S. officinalis* inhibited the growth of *B. cereus, B. megatherium, B. subtilis, A. hydrophila, A. sobria*, and *K. oxytoca*. This antimicrobial spectrum obtained with the essential oil of *S. officinalis*, is comparable to that reported by Hammer, Carson, and Riley (1999) and Marino et al. (2001). However, we observed very small effects on *E. coli* and *S. aureus*. This discrepancy can be attributed to the bacterial strains and the composition of the essential oils used in each particular study.

The essential oil of *S. triloba* not only inhibited the growth of the above bacteria but also that of *S. aureus* (Table 2). The MIC and MCC values indicate that the oil of *S. triloba* was more efficient than that of *S. officinalis*. The effectiveness of the essential oil of *S. triloba* (MIC and MCC) against susceptible bacteria was higher than that previously reported for this species (Sivropoulou et al., 1997), and for *Salvia*, *Salvia pratensis*, *Salvia glutinosa*, and *Salvia aethiopis* (Velickovic, Randjelovic, Ristic, Smelocerovic, & Velickovic, 2002), *Salvia tomentosa* (Tepe, Daferera, Sokmen, Sokmen, & Polissiou, 2005), and *Salvia cryptantha* and *Salvia multicaulis* (Tepe et al., 2004).

In low concentrations, both oils exhibited bacteriostatic activity. However, in high concentrations, above MIC values,

Table 1
Essential oil composition (% of major components) of *S. officinalis* and *S. triloba* cultivated in South Brazil

Compound	RT	*S. officinalis*	*S. triloba*
α-Pinene	6.20	3.07	1.38
Camphene	6.86	4.40	1.85
β-Pinene	8.49	9.87	3.95
Myrcene	9.13	–	1.17
1,8-Cineole	12.5	14.8	15.7
α-Thujone	18.1	24.8	20.1
β-Thujone	18.8	3.97	4.76
Camphor	19.3	10.9	12.6
Borneol	20.5	11.1	1.64
β-Caryophyllene	33.2	2.89	11.8
α-Humulene	34.6	1.47	7.52
Viridiflorol	41.6	–	6.34
δ-Gurjunene	48.2	8.20	–

RT, retention time.

Table 2
Minimal inhibitory concentration (MIC) and minimal cidal concentration (MCC) of the essential oils of *S. officinalis* and *S. triloba* against some foodborne and spoiling bacteria

Test organism	*S. officinalis*		*S. triloba*	
	MIC (mg/ml)	MCC (mg/ml)	MIC (mg/ml)	MCC (mg/ml)
Escherichia coli IBEc-101	5.0 – 10.0	>10.0	5.0	7.0
Proteus mirabilis IBPm-101	5.0 – 10.0	>10.0	5.0 – 10.0	>10.0
Salmonella typhimurium IBSal-101	5.0 – 10.0	>10.0	1.0	2.0
Aeromonas hydrophila ATCC 7966	0.5	0.5	0.3	0.4
Aeromonas hydrophila CECT 389	0.5	0.5	0.4	0.4
Aeromonas sobria ATCC 43979	0.5	0.5	0.1	0.1
Klebsiella oxytoca IBKle-101	0.1	0.1	0.1	0.1
Citrobacter sp. IBCs-101	5.0–10.0	>10.0	5.0–10.0	>10.0
Serratia marcescens IBSm-101	5.0–10.0	>10.0	5.0–10.0	>10.0
Bacillus megatherium IBBac-103	0.5	0.5	0.05	0.1
Bacillus cereus IBBac-102	0.3	0.4	0.05	0.1
Bacillus subtilis IBBac- 101	0.4	1.0	0.1	0.1
Pseudomonas aeruginosa IBPa-101	5.0 – 10.0	>10.0	5.0 – 10.0	>10.0
Pseudomonas fluorescens IBPf-101	5.0	5.0	3.0	4.0
Staphylococcus aureus IBSa-102	5.0 – 10.0	>10.0	0.3	0.4
Staphylococcus aureus ATCC 6538	5.0 – 10.0	>10.0	0.2	0.3
Staphylococcus epidermidis IBSe-01	5.0 – 10.0	>10.0	1.0	3.0

bactericidal activity was pronounced, especially in the case of *S. triloba* oil.

The antimicrobial activities of *S. officinalis* and *S. triloba* can be attributed to the presence of high concentrations of thujone, 1,8-cineole and camphor, three monoterpenes with well documented antibacterial and antifungic potential (Jalsenjak et al., 1987; Sivropoulou et al., 1997; Sur et al., 1991). The stronger activity of the essential oil of *S. triloba* against almost all the susceptible bacteria may be due to the presence of a high concentration of β-caryophyllene, since the antimicrobial properties of caryophyllene and caryophyllene oxide were observed by Azaz, Demirci, Satil, Kurkçuoglu, and Baser (2002) and Ulubelen et al. (1994), studying *Salvia sclarea* and *Satuleja coerulea*, respectively.

Other than the major compounds, α-pinene (a monoterpene hydrocarbon) and borneol (an oxygenated monoterpene), as well as other minor constituents of the essential oils of *S. officinalis* and *S. triloba* have antimicrobial activity (Dorman & Deans, 2000). In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity.

In general, the antimicrobial activity of the essential oils tested was more pronounced against Gram-positive than against Gram-negative bacteria, a general observation derived from studies with essential oils from many other plant species (Nostro, Germano, D'Angelo, Marino, & Cannatelli, 2000; Ouattara, Simard, Holley, Piette, & Bégin, 1997). This generally higher resistance among Gram-negative bacteria could be ascribed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds (Nikaido & Vaara, 1985). The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipid bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Cowan, 1999; Wendakoon & Sakaguchi, 1995).

The high efficiency of the essential oils, particularly that of *S. triloba*, against *S. aureus* and *A. hydrophyla*, two important foodborne pathogens, prompted us to examine the effect of sublethal concentrations on bacterial growth and growth kinetics. As can be observed in Fig. 1, concentrations as low as 0.15 mg/ml of the essential oil of *S. triloba* drastically inhibit (>1 log) the growth *S. aureus* and *A. hydrophila*. This oil also reduced the growth of *E. coli*, and other Gram-negative bacteria (data not shown).

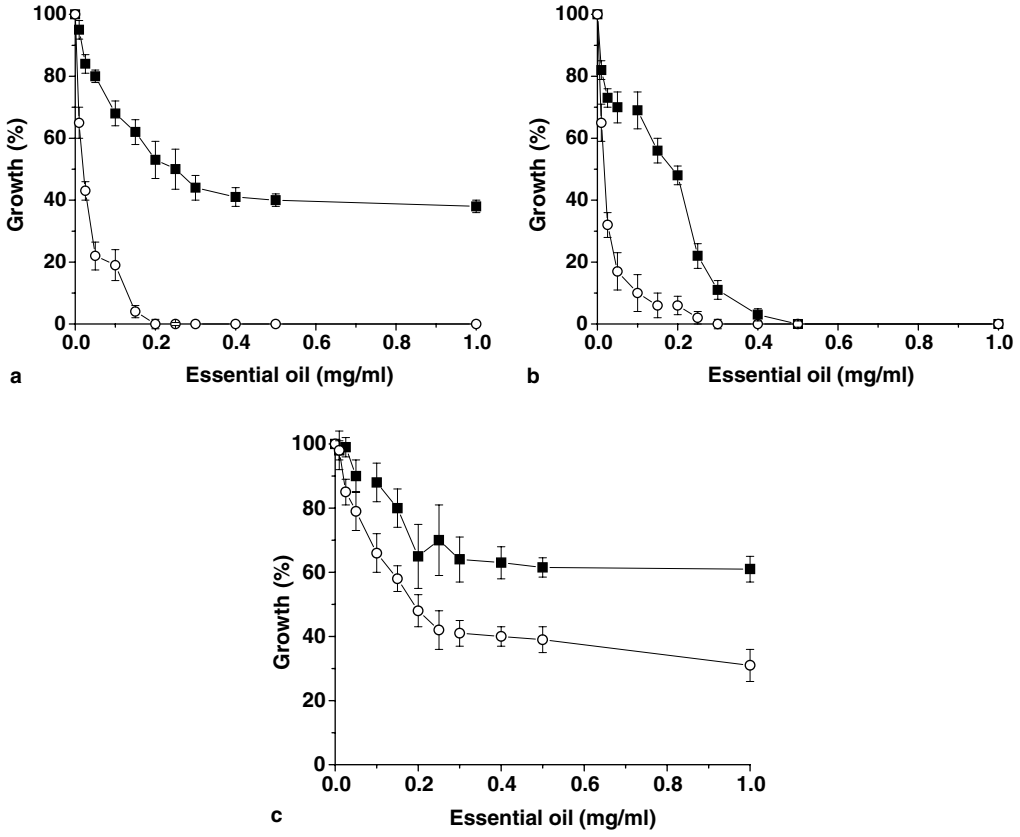

Fig. 1. Antibacterial activity of the oils of *Salvia officinalis* (■) and *Salvia triloba* (○) against *Staphylococcus aureus* ATCC 6538 (a), *Aeromonas hydrophila* ATCC 7966 (b), and *Escherichia coli* IBEc-101 (c). Growth on LB medium at 37 °C for 48 h. Data represent the means ± standard errors for three replicates.

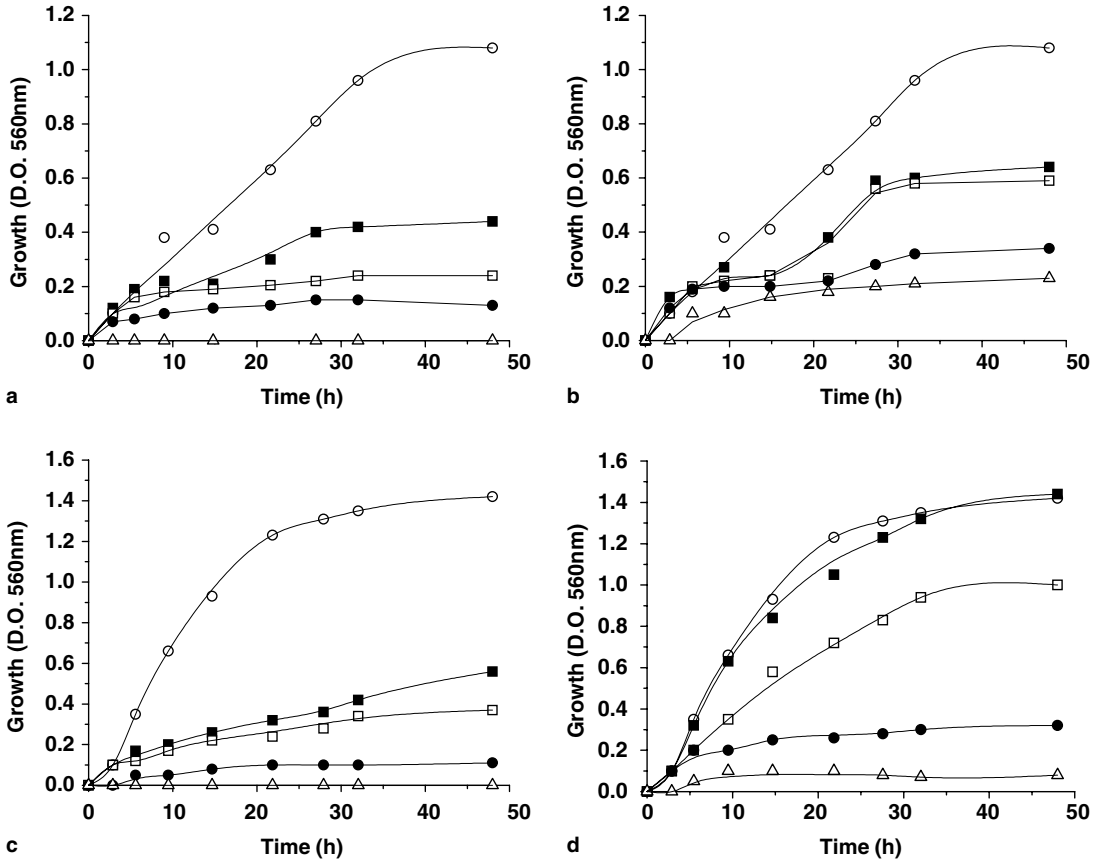

Fig. 2. Growth kinetics of *S. aureus* ATCC 6538 (a–b) and *A. hydrophila* ATCC 7966 (c–d) on LB medium in the absence (○) and in the presence of 0.05 (■), 0.1 (□), 0.2 (●) and 0.5 (△) mg/ml of the essential oils of *S. triloba* (a–c) and *S. officinalis* (b–d). Data represent the average values of three replications (variation coefficient <5.7%).

Growth kinetics of *S. aureus* and *A. hydrophila* in the presence of increasing concentrations of *S. officinalis* and *S. triloba* essential oils (Fig. 2) showed that, even at low concentrations, these oils drastically impair the maximum yield and growth rate of both bacteria. The reduction of growth parameters increased with the concentration of the oils. In this sense, *S. triloba* essential oil, at 0.05 mg/ml, leads to a reduction of 64% and 57%, and 63% and 93%, of the maximum yield and growth rate of *S. aureus*, and *A. hydrophila*, respectively. A comparison of the curves obtained with different concentrations of *S. triloba* and *S. officinalis* oils confirms the highest efficiency of the first plant extract on these bacteria.

Although tests on food are necessary, the present study indicates that *S. triloba* oil extracts can be considered as an alternative to "traditional food preservatives", eliminating or reducing the growth of important foodborne pathogens and spoilage bacteria, and contributing to enhance food safety and shelf life.

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