Comparative study of chemical and antimicrobial characteristics of essential oils from in-house farmed Salvia species (*Salvia officinalis* & *Salvia triloba*)

E. Wogiatzi[1], S. Dalakoura[2], K. Petrotos[3], I. Giavasis*[2]

[1] Department of Crop Production, TEI of Larissa, 41110 Larissa, Greece; [2*]Department of Food Technology, Food Microbiology & Biotechnology Lab, TEI of Larissa, End of Temponera Str., 43100 Karditsa, Greece; [3] Department of Biosystems Engineering, TEI of Larissa, 41110 Larissa, Greece
igiavasis@teilar.gr

Abstract

Salvia (sage) blooms and leaves contain essential oils characterized by health-promoting properties.*Salvia officinalis and Salvia triloba* (two of the most scientifically and industrially interesting species) were comparatively tested for their antimicrobial properties in view of their potential use as natural antimicrobials. *S.triloba* and S.*officinalis* originating from mount Olympus were cultivated in institutional farms,and two harvests of 2-year-old plants were carried out.Essential oils were isolated by steam-distillation using naturally-dried leaves.Essential oil concentration between two harvests of the same plant,and between the two types of essential oil was compared.The bioactive chemical compounds of each essential oil were identified by gas chromatography and their antimicrobial effect against food-borne pathogens was comparatively studied. Cultures of *Staphylococcus aureus, Listeria monocytogenes and Escherichia coli* were inoculated on solid media containing Salvia essential oils at concentrations of 0% (blind), 0.1%, 0.2% and 0.5%.

Chemical analysis showed that essential oil content between the two harvest periods was similar. S.officinalis contained less essential oil(0.7%) than S. triloba(1.8%),but much higher content of thujone (37.17%),which is a bioactive compound. The essential oil of both Salvia species exhibits significant antimicrobial activity against *Staphylococcus aureus*[strongly inhibited by 3.7 log(cfu/gr) at high essential oil concentrations (0.5%) of *S. officinalis* essential oil],while *Listeria monocytogenes* is inhibited by 3.9 log(cfu/gr) at 0.5% *S. triloba* essential oil,and by more than 2 log(cfu/gr) at moderate(0.2%) concentration of both essential oils.*E. coli* was less affected by both essential oils. Overall, Salvia species exhibited significant antimicrobial effects even at moderate concentrations (0,2%),but no specific link with the thujone content could be established.

Introduction

Salvia (sage) belongs to the family of *Labiatae,* in which *Salvia officinalis* and *Salvia triloba* represent some of the most scientifically and industrially interesting species. All upper parts of Salvia, and especially flowers (blooms) and leaves, contain essential oils characterized by a distinct odor and a yellow-greenish colour. This is used in food (canned food), pharmaceutical and cosmetics applications [1].

Common sage was used since the copper age, to induce female fertility and fight diarrhea. Ancient Greek philosophers-doctors Galinos and Hippocrates used it as a medicine, while Aristophanes refers to it in one of his ancient plays. Romans also considered it life-boosting medicine. It was thought to be a nerve-stimulating and stomach-soothing drug, [1]. Sage has antibacterial, constringent, anti-tussive, anti-spasmic, soothing, diuretic and styptic properties [1], 4]. It is also used in soothing throat and pharynx inflammations or against ulcer [1], [4]. However, overdose and long-lasting use may cause fever and tachycardia [4].

In order for the plant to be commercialized it must have a minimum concentration of 1,5% essential oil , a maximum concentration of 3% stems and 2% in foreign matter, a maximum concentration of 10% water, a maximum concentration of 10% in pulverized matter, [2], [3], [4].

In terms of bioactive substances, *Salvia officinalis* contains a total concentration of 1-2,5% essential oil [2], [3], [4], [6], [7], a 35-60% thujone [2], [3], [4], [6], [7], a iso thujone [4], 6-16% of 1,8 sineol [2], [3], [4], [6], [7], [8], 14-32% of camphor [2], [4], [6], [8], borneol [2], [4], [6], [7], [8], a 7% depsides [2], [4], bitter compounds [2], [3], [4], [7], flavonoides [3], [4], glycosites [4].

Similarly, *Salvia triloba* contains bioactive substances as a total concentration of 2-3% essential oil (Greek sage) [4], [6], about 5% thygione in the essential oil [6], 40-67% of 1,8-sineol [2], [6], up to 15% of camphor [6], depsides [4], triterpenes [4], flavonoids [4].

The aim of the present work is to compare the essential oil of the two farmed species of Salvia under realistic farming conditions, with the scope of proposing one or both of the species as an attractive option for alternative farming. Also, to study the antimicrobial effect of the essential oil of Salvia species against food pathogens, in order to investigate the potential use of this oil as a natural antimicrobial agent.

Materials and Methods

Cultivation of Salvia species was done in a soil of medium texture, with a moderate content in organic matter (1,2%), slightly acid (pH 6,3), containing sufficient available amounts of available phosphorous (P-Olsen 33 ppm) and potassium (242 ppm), free of limestone, at an altitude of 88,5 m above sea level.

The plant seeds were initially isolated from naturally growing plants on mount Olympus, which were identified and characterized by the Greek National Research Foundation. The seeds were kept refrigerated for three weeks before sowing, in order to facilitate vegetation. Before sowing, a mixture of black and blond turf (ratio 1:1) was prepared in an outdoor facility at the TEI of Larissa to support seeding. Seeding started in the first two weeks of August, while vegetation began by the end of the same month and was completed after 75-80 days. The young plants were then transferred to farmland (at the end of October) having a mutual distance of 1X1m .

Two of the most interesting species of Salvia were cultivated, namely, *Salvia triloba* and S*alvia officinalis,* and two harvests of two-year old plants were carried out.

The isolation of the essential oil was done by the steam-distillation method. Forty five samples were used for the first harvest of *S. officinalis* and 28 samples from the second harvest. Similarly, 40 samples and 33 samples were used for the first and second harvest of *S. triloba,* respectively. All samples were naturally-dried leaves.

The essential oil concentration between two harvests of the same plant, and between the two types of essential oil was compared. The bioactive compound of each essential oil were identified and their antimicrobial effect against food-borne pathogens was comparatively studied. Gas-chromatography was used for the analysis of essential oil samples in an Agilent 7890 analyzer with a Mass Spectrometer (MS) - detector type 5975 using a capillary column type J&W 112-88A7 100m X 250μm X 0.25μm. Ultra pure Helion was used as an eluent at 3ml/min elution rate. The elution volume was 0,5 μL and the elution type was split ratio 100:1. The temperature of the incoming current was regulated at 300 °C and the heating program was at 3 steps (Step 1: Stay at 50 °C for 5 min, Step 2: Heating from 50 °C to 130 °C at a rate of 5°C /min and 0°C min stay, Step 3: Heating from 130 °C to 250 °C at 3 °C /min rate and 0°C min stay).

The characteristic temperatures of the MS detector were 230 °C for the source and 150 °C for the dipole, while the lag for detecting the solvent was regulated at 3,50 °C min.

Finally, the *in vitro* antimicrobial effects of the two essential oils was studied in solid culture media. Salvia essential oil (from each of the two species) was added by swirling into the nutrient media at a concentration of 0% (blind), 0.1%, 0.2% and 0.5% (as a percentage of the solid agar media). Pure cultures of pathogens *Staphylococcus aureus* (cultivated onto Baird Parker agar with egg yolk tellurite, for up to 48h at 37°C), *Listeria monocytogenes* (cultivated onto Oxford agar with Oxford selective supplement, for up to 48h at 37°C), *and Escherichia coli* (Cultivated into TBX agar, for up to 24h at 44°C),

Results and Discussion

Essential oil concentration of *Salvia officinalis* varied between 0.60 to 0.70 % in the first and second harvest, respectively, while the respective concentrations for *Salvia triloba* were higher, namely, 1.48 – 2.00 % (Figure. 1). The relatively low yield of essential oil compared to other studies [2,3,4,6,7] is probably due to the fact that only dried leaves and no inflorescences (no flowers), which have the highest essential oil content, were used for isolating the essential oil. In addition, the leaves were not used fresh, but only after drying, which allowed some of the volatile compounds to evaporate [1].

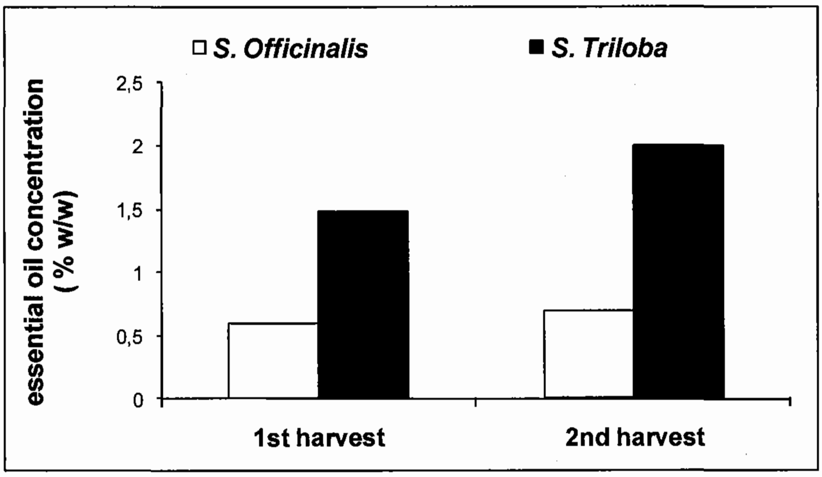

Figure.1. Essential oil concentration in *Salvia officinalis* and *Salvia triloba* from two different harvest periods.

The concentration of separated and characterized active compounds from *Salvia triloba* essential oil were: 1,4-cineol 70%, thujone 3.35%, camphor 25.55% και borneol 1%, while *Salvia officinalis* contained: 0,4-cineol 44.2%, thujone 37.17%, camphor 16,3% και borneol 2.31%. Thujone, which is an active pharmaceutical compound, was obtained in much higher concentration in *S. officinalis* essential oil, which is in agreement with other relative studies [9]. This is the only bioactive compound in which *S. officinalis* appears to outmatch *S. triloba* essential oil (Figure. 2).

The antimicrobial effect of each of the two essential oils depended on essential oil concentration and type of pathogen (Figure. 3). *Salvia triloba* essential oil reduced *Staphylococcus aureus* population by 2.2 log (cfu/gr) when added at a 0.5% concentration (on solid culture media), but S.officinalis essential oil was even more effective at the same concentration, leading to a 3.7 log (cfu/gr) reduction. Conversely, *S. triloba* essential oil at 0.5% concentration produces a maximum 3.9 log (cfu/gr) decrease in *Listeria monocytogenes* population and is very effective even at 0.2% concentration causing a 3.2 log (cfu/gr) reduction, while *S.officinalis* essential oil causes a somewhat milder 2.5 log (cfu/gr) decrease at 0.5% concentration. This inhibitive/bacteriocidal activity of Salvia essential oils is comparable to or higher than that of other established antimicrobial essential oils (e.g. oregano, dill, coriander essential oils) [11,12, 13].

On the other hand, *Escherichia coli* was only slightly affected by the essential oil of the two Salvia species, showing a total decrease of approximately 0.9 log (cfu/gr) with *S. triloba* and 1.6 log with *S.*

officinalis when added at 0.5% concentration into the solid growth medium. Similarly, other Salvia species, such as *Salvia albicaulis* and *Salvia dolomitica* show limited antimicrobial activity against *E. coli*, despite being more active against other microbial pathogens (Figure.3), [14].

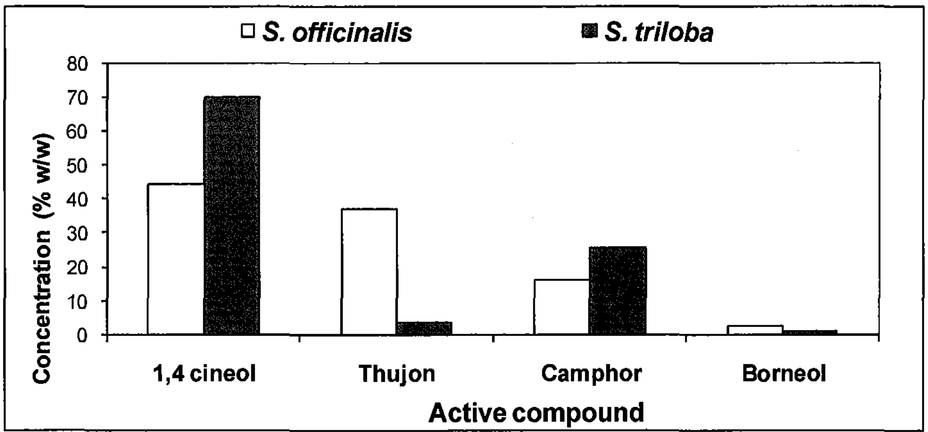

Figure.2. Concentration of active compounds in essential oils from *S. triloba and S. officinalis.*

In general, the essential oils from the two species of Salvia appear to have distinct antimicrobial properties which need to be investigated in more detail and against a larger range of microbial pathogens or spoilage organisms. Also, "*in vivo*" experiments with these essential oils added into real food/pharmaceutical products or formulations would allow us to evaluate their antimicrobial/pharmaceutical properties in a real commercial environment and assess their potential as natural antimicrobial compounds, especially in matrices where a fruity/flowery flavor would be expected, which might allow the use of Salvia essential oil in relatively high (i.e. 0.5%) concentrations.

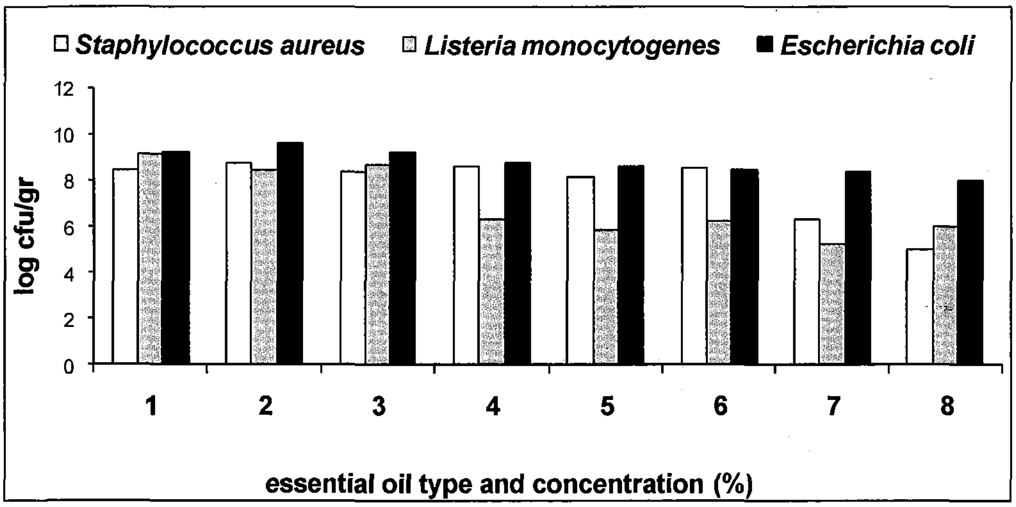

Figure.3. Antimicrobial effects of *S. triloba* (samples 1,3,5,7) and *S. officinalis* (samples 2,4,6,8) essential oil in *in vitro* studies with food pathogens. (1): Blind sample - *S. triloba* 0.0%, (2): Blind sample - *S. officinalis* 0.0%, (3): S. *triloba* 0.1%, (4): *S. Officinalis* 0.1%, (5) *S. triloba* 0.2%, (6) *S. officinalis* 0.2%, (7) *S. triloba* 0.5%, (8): *S.officinalis* 0.5%.

Conclusions

The content of essential oil between the two harvest periods was not significantly different for the two Salvia species. *S. officinalis* contains less essential oil (0.7%) than *S. triloba* (1.8%), but higher content of thujone (37.17%), which is a pharmaceutical active compound.

The essential oil of both Salvia species exhibits significant antimicrobial activity against food pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. *Staphylococcus aureus* is strongly inhibited by 3.7 log (cfu/gr) at high essential oil concentrations (0.5%) of *S. officinalis* essential oil, while *Listeria monocytogenes* is inhibited by 3.9 log (cfu/gr) at 0.5% *S. triloba* essential oil, and by more than 2 log (cfu/gr) at moderate concentration of both essential oils (0.2%), which is important in exerting an antimicrobial effect without adversely affecting the sensory attributes of a food or pharmaceutical product.

References

Wogiatzi E., 2004. A selection of Aromatic and Pharmaceutical Plants, Edited by Modern Education, Thessaloniki, ISBN: 960357-065-6.

Dachler M. & Pelzman H., 1999. Arznei-und Gewurzpflanzen, Agrarverlag Klosterneuburg, ISBN:3-7040-1360-9 s.

Linder, U, 1987. Salbei (Salvia officinalis). Unsere Küchenkräuter. Gemüse, 6: 302-303.

Marquard R., Kroth E, 2001.Anbau und Qualitätsanforderungen ausgewählter Arzneipflanzen,Agrimedia GmbH s.126-134.Jan-Öjvin Swahn, 1991. Kräuter und Gewürze der Welt, Gothenburg, Sweden, ORBIS VERLAG, s.126-134.

Teuscher, E., 1997. Biogene Arzneimittel:5. Auflage. Wissenschaftliche, Verlagsgesellschaft mbH, Stuttgart.

Rimpler, H., 1999. Biogene Arzneistoffe. 2. Auflage. Deutscher Apotheker Verlag, Stuttgart.

Franke, W.,1992. Salvia officinalis Nutzpflanzenkunde. 5. Auflage. Georg Thieme Verlag, Stuttgart.

Daunderer-Kormann R.,1984. GIFTPFLANZEN PFLANZENGIFTE, Vorkommen-Wirkung-Therarie- Allergische und phototoxische Reaktionen, Nikol Verlangsgesellschaft mbH & Co.KG, Hamburg, ISBN13 978-3-933203-31-1, ISBN 10 933203-31-7.

Sarac, N., Ugur, A., 2009. The in Vitro antimicrobial activities of the essential oils of some lamiaceae species from Turkey. Journal of Medicinal Food 12 (4), pp. 902-907.

Barbosa, L.N., Rall, V.L.M., Fernandes, A.A.H., Ushimaru, P.I., Da Silva Probst, I., Fernandes Jr., A. 2009. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease 6 (6), pp. 725-728 .

Gutierrez, J., Barry-Ryan, C., Bourke, P. , 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components, Food Microbiology 26 (2), pp. 142-150 .

Petrović, S., Pavlović, M., Tzakou, O., Couladis, M., Milenković, M., Vučićević, D., Niketić, M. 2009. Composition and antimicrobial activity of Salvia amplexicaulis Lam. essential oil. Journal of Essential Oil Research 21 (6), pp. 563-566.

Kamatou, G. P. P., Viljoen, A. M., Figueiredo, A. C., Tilney, P. M., Van Zyl, R. L.,Barroso, J. G., et al., 2007. Trichomes, essential oil composition and biological activities of Salvia albicaulis benth. and S. dolomitica codd, two species from the cape region of South Africa.South African, Journal of Botany, 73, 102–108.