Antimicrobial Activity of Greek Oregano and Winter Savory Extracts (Essential Oil and SCFE) Investigated by Impedimetry

Cs. Mohácsi-Farkas
Department of Microbiology and Biotechnology, SZIE University, Somlói út 14-16. H-1118 Budapest, Hungary

M. Tulok and B. Balogh
Department of Medicinal and Aromatic Plants, SZIE University, P.O. Box 53
H-1502 Budapest, Hungary

Keywords: *Satureja montana, Oreganum vulgare* ssp *hirtum*, essential oil, supercritical fluid extract, inhibition, rapid method, conductometry

Abstract

The inhibitory activity of the essential oil and supercritical fluid extract (SCFE) of *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart and *Satureja montana* L. were tested by impedimetry on a number of food-borne bacteria and fungi. Both essential oil and SCFE of oregano had a significant growth-inhibitory effect against *Escherichia coli, Pseudomonas fluorescens, Bacillus cereus* and *Aspergillus niger* strains in 0.05 and 0.1 % (v/v) concentrations. SCFE of oregano in 0.1% concentration proved to be able to inhibit growth of *Saccharomyces cerevisiae*. Winter savory extracts (essential oil and SCFE) had antimicrobial activity against *Ps. fluorescens, S. aureus, B. cereus* and *A. niger* at both concentrations. However, there was no significant difference in conductimetric detection times between control and winter savory-treated samples in case of *E. coli* and *Sacch. cerevisiae*. In general, the microorganism strains were more sensitive to oregano extracts than to winter savory extracts, and essential oils were more effective than SCFEs added in the same concentrations.

INTRODUCTION

In food processing there is an increasing consumer demand on food products with the quality characteristics of fresh products, and foods containing only natural compounds. The extracts of medicinal or aromatic plants are one of the potential biopreservatives (Smid and Gorris, 1999). Both oregano and winter savory are perennial shrubs, members of the Lamiaceae family of which dried shoots with leaves are used in the food industry as a flavouring agent. Greek oregano is a popular culinary herb and it is often used as a flavourant in tomato-based products. However, these herbs are also used in therapy and they have provable pharmacological effects (Halászné Zelnik and Szabó, 2000). Oregano has antiseptic, expectorant and antispasmodic effects. Winter savory has a mild vasopressor effect and, in replacing of black pepper, it can be used as a dietetic spice (Halászné Zelnik, 2000).

There are various methods that are used to investigate antimicrobial activity of essential oils. One of the most successful of the rapid and automated microbiological techniques is based on electrical measurements. Microbial metabolism usually results in an increase in both conductance and capacitance causing a decrease in impedance of the culture media.

Supercritical fluid extraction with carbon dioxide is a milder extraction method than the conventional steam distillation and solvent extraction. The most natural, true-tasting extracts of botanical feed-stocks are obtained by this technique, which utilizes low temperatures and avoids degradative heat processes and reactive solvents (Moyler, 1993).

Inhibitory activity of essential oils and supercritical fluid extracts of *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart and *Satureja montana* L. was tested by impedimetry on a number of food-borne bacteria and fungi.

Proc. Int. Conf. on MAP
Eds. J. Bernáth et al.
Acta Hort. 597, ISHS 2003

MATERIALS AND METHODS

Plant Extracts

Greek oregano (*Origanum vulgare* ssp. *hirtum*, epithet of Department of Medicinal and Aromatic Plants) and winter savory (*Satureja montana* „Bokroska”, variety of Department of Medicinal and Aromatic Plants) grown at the Research Station of the Department of Medicinal and Aromatic Plants of SZIE, Soroksár were used.

Essential oils were produced by hydrodistillation in a Clevenger apparatus according to VIIth Ph.Hg.

Supercritical fluid extracts (SCFE) were obtained using an Isco SFX 2-10 laboratory-scale apparatus, where a pressure of 100 Bar and temperature of 40 °C were used.

Samples were stored in a refridgerator at 5 °C.

Test Organisms

Pseudomonas fluorescens (NCAIM B 8436)
Escherichia coli (NCAIM B 200)
Staphylococcus aureus (ATCC 6538)
Bacillus cereus (ATO/DLO, NL)
Saccharomyces cerevisiae (CBS 1782)
Aspergillus niger (SZIE, Dpt. of Microbiology and Biotechnology)
Stationary phase cultures were diluted in sterile saline to give suspensions containing about 10^5 cfu/mL.

Assessment of Antimicrobial Activity by the Impedance Method

Inhibitory activity was investigated by using a RABIT type automated impedimeter from DonWhitley Scientific (Shipley, U.K.).

Direct impedimetric technique: In this technique the test organisms are in direct contact with the system electrodes, the changes in conductance of the growth medium are directly resultant of changes taking place in the bulk electrolyte.

500 µL of cultures of bacteria were inoculated in triplicate into 4.5 mL of DonWhitley impedimetric broth (produced by Don Whitley Sci., Ltd).

Indirect technique: Indirect conductance measurements utilized changes of conductance in CO_2 traps produced by the microorganisms. In this case, CO_2 evolving from the culture is absorbed by potassium hydroxide solution which is in contact with the electrodes. The method of indirect assay was adopted from Bolton (1990). 4.5 mL volumes of 0.5 % yeast-extract 0.5 % peptone and 1 % glucose broth were inoculated with 500 µL of the diluted yeast and mould cultures.

Conductance of the cultures was recorded automatically at 6 min intervals for 48 hours at 30 °C either without or after addition of essential oils or SCFEs in concentrations of 0, 0.05%, and 0.1% (v/v). The impedimetric detection times (TTD) were automatically determined by the instrument, as indicated by three consecutive changes in conductivity greater than 5 µS 6 min^{-1} for direct, and -10 µS 6 min^{-1} for indirect measurements.

RESULTS AND DISCUSSION

Impedimetric evaluation of antimicrobial effects of oregano and winter savory essential oils and SCFE are shown in Fig. 1 (direct method) and Fig. 2 (indirect method). Both essential oil and SCFE of *Origanum vulgare* ssp. *hirtum* had significant growth-inhibitory effects (Table 1) against *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus cereus* and *Aspergillus niger* strains at both concentrations tested. SCFE of oregano in 0.1% concentration proved to be able to inhibit growth of *Saccharomyces cerevisiae*.

Satureja montana „Bokroska” extracts (essential oil and SCFE) had antimicrobial activity against *Ps. fluorescens, S. aureus, B. cereus* and *A. niger* at both concentrations. However, there was no significant difference in conductimetric detection times between control and winter savory-treated samples in the case of *E. coli* and *Sacch. cerevisiae*.

There are several microbiological methods to investigate the antimicrobial effect of essential oils of which the most widely used is the agar diffusion method. The traditional microbiological methods are very time-, labour- and cost consuming. The results obtained demonstrated that the kinetic method based on automated conductimetry provides a tool for characterization of the antimicrobial activity.

Both oregano and winter savory extracts had considerable inhibitory effects. In agreement with the studies of Dorman and Deans (2000) on the antimicrobial activities of plant volatile oils, *O. vulgare* ssp. *hirtum* appeared to be equally effective against both Gram-positive and Gram-negative bacteria. Data presented by Ciani et al. (2000) showed that essential oil of *Satureja montana* can be used to control potential pathogenic and spoilage yeasts at a MIC of 0.1 to 0.25 µL/mL. In general, microbial strains were more sensitive to oregano extracts than to winter savory extracts.

The main components of the essential oil of *O. vulgare* ssp. *hirtum* are thymol, carvacrol and p-cymene (Kokkini et al., 1997, Russo et al., 1998). Ultee (2000) showed that with addition of carvacrol to food products at doses below the MIC-value (0.11 mg/mL) the risk of toxin production by *B. cereus* can be reduced and the safety of the product can be increased.

These in vitro tests showed that, in general, essential oils were more effective than SCFEs added in the same concentrations. Oszagyán et al (1996) compared the SCFE products of lavandin and thyme with essential oils obtained by conventional steam distillation. SCFE products were markedly different from the corresponding steam distilled oil. Further studies are in progress to identify the main components and the antimicrobial activity of the essential oils and supercritical extracts used in our experiments.

Literature Cited

Bolton, F.J. 1990. An investigation of indirect conductimetry for detection of some food-borne bacteria, J. Appl. Bacteriol., 69:655-661.

Ciani, M., Menghini, L., Mariani, F., Pagiotti, R., Menghini, A. and Fatichenti, F. 2000. Biotechnology Letters, 22:1007-1010.

Dorman, H.J.D. and Deans, S.G. 2000. Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. J. Appl. Microbiol., 88:308-316.

Halászné Zelnik, K. 2000. *Satureja hortensis*. p. 525-528. In: J. Bernáth (ed.), Gyógy- és Aromanövények, Mezőgazda Kiadó, Budapest.

Halászné Zelnik, K. and Szabó, K. 2000. *Origanum vulgare*. p. 444-447. In: J. Bernáth (ed.), Gyógy- és Aromanövények, Mezőgazda Kiadó, Budapest.

Kokkini, S., Karousou, R., Dardioti, A., Krigas, N. and Lanaras, T. 1997. Autumn essential oils of Greek oregano. Phytochemistry, 44:883-886.

Lackowicz, K.J., Jones, G.P.,Brigop, D.R., Bienvenu, F.E., Wan, J., Wilcock, A. and Coventry, M.J. 1998. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acid-tolerant food microflora, Letters in Applied Microbiology, 26:209-214.

Moyler, D.A. 1993. Extraction of essential oils with carbon dioxide. Flavour and Fragrance Journal, 8:235-247.

Oszagyán, M., Simándi, B., Sawinsky, J., Kéry, Á., Lemberkovics, E. and Fekete, J. 1996. Supercritical fluid extraction of volatile compounds from lavandin and thyme. Flavour and Fragrance Journal, 11:157-165.

Pharmacopoea Hungarica VII. 1986. Medicina Könyvkiadó, Budapest.

Russo, M., Galletti, G.C., Bocchini, P. and Carnacini, A. 1998. Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): a preliminary evaluation of their use in chemotaxonomy by cluster analysis. I. Inflorescences. J. Agricultural and Food Chemistry, 46:3741-3746.

Smid, E.J. and Gorris, L.G.M. 1999. Natural Antimicrobials for Food Preservation, In: Handbook of Food Preservation, Marcel Dekker Inc., New York, pp. 285-308

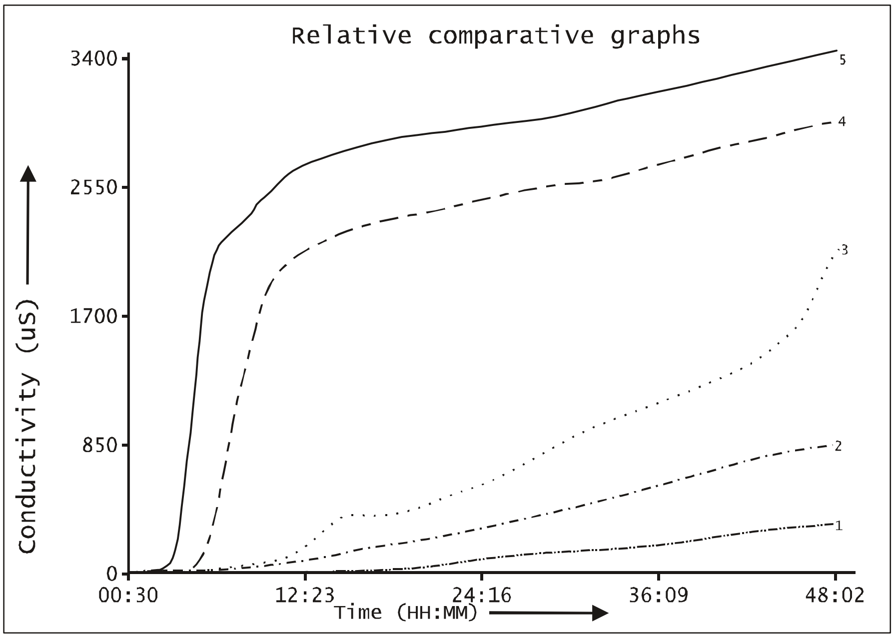
Ultee, A. 2000. Bactericidal action of carvacrol towards the food pathogen *Bacillus* *cereus*. A case study of a novel approach to mild food preservation. Thesis Wageningen University, The Netherlands, 73-82.

Tables

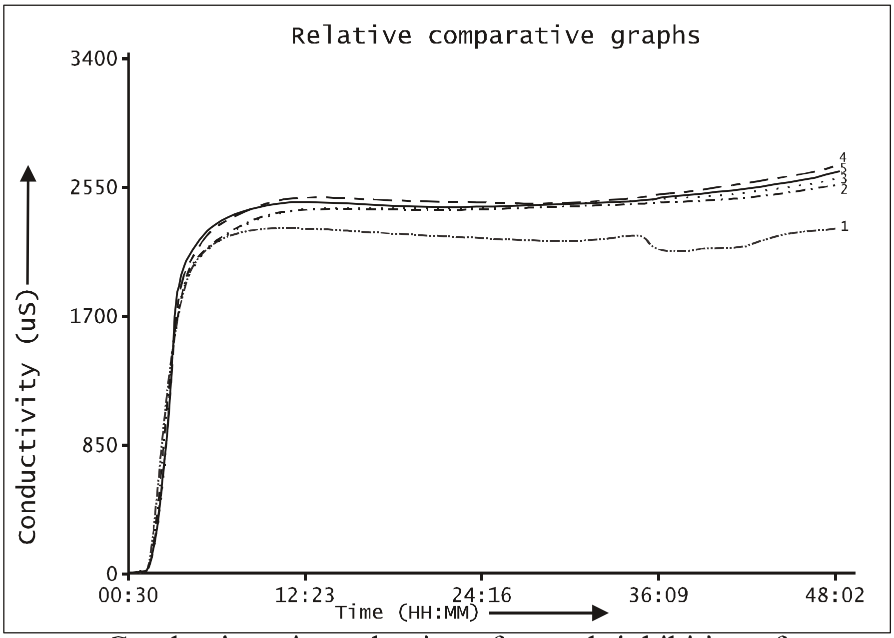
Table 1. Effect of oregano and winter savory extracts on growth of microorganisms investigated by automated conductimetry at 30 °C

Test organism	**Plant**	**TTD (h) Concentration (%) of**				
		Essential oil			**SCFE**	
		0	0.05	0.1	0.05	0.1
Escherichia coli	Oregano	3.2	31.3	>48	5.1	10.9
	Winter savory	2.5	2.4	2.6	2.4	2.5
Pseudomonas fluorescens	Oregano	4.2	25.6	35.1	24.3	31.8
	Winter savory	3.4	6.3	6.2	5.8	6.2
Staphylococcus aureus	Oregano	6.8	>48	>48	>48	>48
	Winter savory	3.6	5.7	5.8	4.3	4.8
Bacillus cereus	Oregano	8.35	>48	>48	>48	>48
	Winter savory	7.85	>48	>48	9.0	>48
Aspergillus niger	Oregano	17.95	nd*	>48	nd	>48
	Winter savory	18.6	nd	>48	nd	>48
Saccharomyces cerevisiae	Oregano	9.7	nd	11.4	nd	11.9
	Winter savory	9.1	nd	9.2	nd	10.2

*: not determined

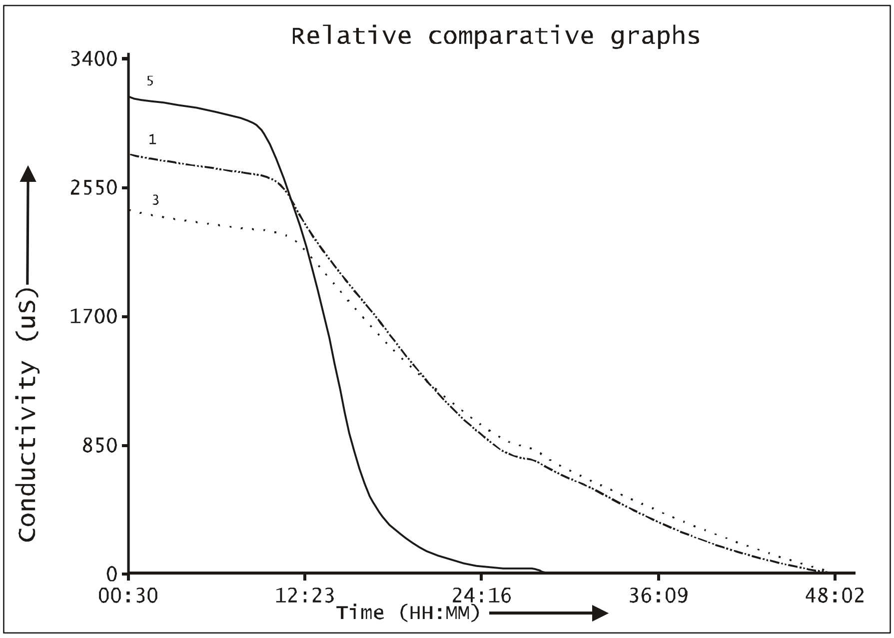


Conductimetric evaluation of growth-inhibition of
Escherichia coli by oregano extracts

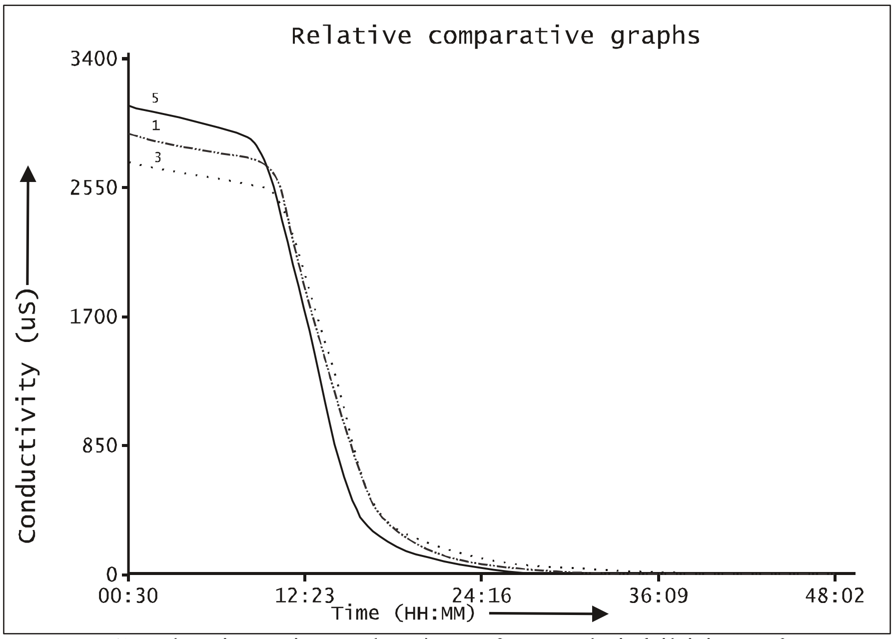


Conductimetric evaluation of growth-inhibition of
Escherichia coli by summerwinter extracts

Fig. 1. **1**. 0.1 % concentration of essential oil, **2**. 0.05 % concentration of essential oil, **3**. 0.1 % concentration of SCFE, **4**. 0.05 % concentration of SCFE, **5**. Control



Conductimetric evaluation of growth-inhibition of
Saccharomyces cerevisiae by oregano extracts



Conductimetric evaluation of growth-inhibition of
Saccharomyes cerevisiae by summerwinter extracts

Fig. 2. **1**.0.1 % concentration of essential oil, **2**. 0.05 % concentration of essential oil, **3.** 0.1 % concentration of SCFE, **4**. 0.05 % concentration of SCFE, **5**. Control