

Correlation between Major Constituents and Antibacterial Activities of Some Plant Essential Oils against Some Pathogenic Bacteria

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(Received: 19.11.2008; Accepted: 31.03.2009)

Abstract

Five different plant essential oils (*Satureja hortensis*, *Thymus sipyleus* ssp. *rosulans*, *Thymus haussknechtii*, *Origanum rotundifolium* (cultured form) and *Origanum acutidens* (wild and cultured form)) and their two major constituents carvacrol and thymol were evaluated for antibacterial activity against food-borne Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis*) and Gram positive bacteria (*Bacillus subtilis*, *Streptococcus pyogenes* and *Enterococcus faecalis*). Our results showed that the tested pathogen bacteria were more or less inhibited by whole essential oils and pure compounds. Thymol, carvacrol, the essential oils of *Satureja hortensis* and *Origanum* species showed a strong antibacterial activity against both Gram positive and negative bacteria with an inhibition zone ranging from 21.0 to 42.33 mm, and a MIC (Minimal Inhibition Concentration) ranging from 6.25 to 600 µl/ml. The main compounds of effective essential oils were carvacrol and/or thymol. This study indicated that especially carvacrol and thymol, *Satureja hortensis*, selected *Thymus* and *Origanum* species are beneficial to human health, having the potential to be used for medical and food preservation purposes. Furthermore, we think that there is a strong correlation between major constituents and antibacterial activities of plant essential oils.

Keywords: Antibacterial activity, Carvacrol, Thymol, *Origanum* sp., *Satureja* sp., *Thymus* sp.

Bazı Patojenik Bakterilere Karşı Bitkisel Yağların Ana Bileşenleri ile Antibakteriyel Aktiviteleri Arasındaki İlişki

Özet

Beş farklı bitkisel yağ (*Satureja hortensis*, *Thymus sipyleus* ssp. *rosulans*, *Thymus haussknechtii*, *Origanum rotundifolium* (kültür formu), *Origanum acutidens* (yabani ve kültür formu)) ve bunların ana bileşenleri olan thymol ile carvacrolün gıda orijinli gram negatif (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* ve *Salmonella enteritidis*) ve gram pozitif (*Bacillus subtilis*, *Streptococcus pyogenes* ve *Enterococcus faecalis*) bakterilere karşı antibakteriyel etkinlikleri belirlenmiştir. Elde edilen sonuçlar; test edilen bitkisel yağlar ve bunların saf haldeki bileşenlerinin patojen bakterilerin gelişimlerini az ya da çok engellediğini göstermiştir. *Satureja hortensis* ve *Origanum* türlerinin bitkisel yağlarında ana bileşen olan thymol ve carvacrol, hem gram negatif hem de gram pozitif bakterilere karşı 21,0-42,33 mm arasında değişen inhibisyon zonları ile güçlü bir antibakteriyel etki göstermiş ve MIC (Minimal İnhibisyon Konsantrasyonu) değerlerindeki değişimin 6,25 ila 600 µl/ml arasında olduğu saptanmıştır. Bu çalışmada; özellikle thymol ve carvacrol, *Satureja hortensis* ve bazı *Thymus* ve *Origanum* türlerinin tıp ve gıda muhafazasında sahip oldukları kullanım potansiyelleri dolayısı ile insan sağlığı için faydalı olduklarını göstermektedir. Ayrıca, bitkisel yağların antibakteriyel aktiviteleri ile ana bileşenleri arasında güçlü bir ilişkinin olduğu da düşünülmektedir.

Anahtar Kelimeler: Antibakteriyel aktivite, Carvacrol, Thymol, *Origanum* sp., *Satureja* sp., *Thymus* sp.

1. Introduction

Food borne illness resulting from consumption of food contaminated with Gram

positive and Gram negative bacteria has been of vital concern to public health. In developed countries, good hygienic practices, adequate preservative technique for processed foods and

use of antimicrobial substances such as antibiotics are the various ways of control and treatment [1]. But, the development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents [2].

Plant materials play a major role in primary health care as therapeutic remedies in many developing countries. Researchers are increasingly turning their attention to folk medicine. Therefore, there are many reports on that many plant extracts and essential oils from medicinal plants, herbs and spices have been shown to possess antimicrobial activity and could serve as a source for antimicrobial agents against food spoilage and pathogens [3-9].

In the previous studies were demonstrated that the extract or essential oils of tested plant species in this study had antagonistic activity against many food borne pathogenic bacteria [10-16]. But, it is also known that antimicrobial effects or biological activities of essential oils and extracts of medicinal plants may be subject to change, based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material [10].

The objective of this study was to evaluate the inhibition zone and minimum inhibitory concentration (MIC) of essential oils obtained from five different plant species (*Satureja hortensis*, *Thymus sipyleus* ssp. *rosulans*, *Thymus haussknechtii*, *Origanum rotundifolium* (cultured form) and *Origanum acutidens* (wild and cultured form)) against six important food borne pathogenic Gram negative and positive bacteria, and to determine the carvacrol and thymol rate in main compounds of plant essential oils.

2. Materials And Methods

2.1. Pathogenic bacteria

The following six food borne Gram negative (*Escherichia coli* RK-58, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29213 and *Salmonella enteritidis* ATCC 13076) and Gram positive bacteria (*Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes*

ATCC 176 and *Enterococcus faecalis* ATCC 29122) were used in this study. The used bacterial strains were provided by Biotechnology Research and Application Centre at Atatürk University, Erzurum, Turkey. Identity of them was confirmed by Microbial Identification System. The bacterial cultures preserved in Loria Broth and 15% glycerol solution at -80 °C to for using further studies.

2.2. Plant Materials and Pure Compounds

The aerial parts of used plant samples (*Satureja hortensis* L., *Thymus sipyleus* ssp. *rosulans* Boiss, *Thymus haussknechtii* Velen (endemic in Turkey), *Origanum rotundifolium* Boiss and *Origanum acutidens* (Hand.-Mazz.) Letswaart (endemic in Turkey) were collected from Erzurum province in eastern Anatolia region of Turkey in July 2006 at the flowering stages and were dried in shade. The plant samples were identified by Dr. Saban Kordali, and now they have been deposited in the Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum (Turkey). Pure compounds carvacrol and thymol were purchased from Sigma (Lot Number: 28, 2197; T 0501, respectively).

2.3. The Isolation of The Essential Oils

The dried plant samples (500 g) were subjected to hydro distillation using a Clevenger-type apparatus for 4 hours. The oils were extracted with CHCl_3 and then were dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20°C in a sealed vial until use.

2.4. Determination of Antibacterial Activities

Antibacterial activity assays were carried out by disc diffusion method [17] with a minor modification. The original essential oils and pure compounds delusions sterilized by filtration by 0.45 μm Millipore filters. Bacterial suspension (100 μl) containing 10^8 CFU/ml of bacteria spread by a sterile swab on Tryptic Soy Agar (TSA) medium. The discs (6 mm in diameter) were impregnated with 12.5 μl of the essential oil and pure compounds carvacrol (1 g/ml) and thymol (1 ml/ml) solutions and put in the middle of the inoculated plates. Bacterial cultures were incubated at 35 ± 2 °C for 48 h, and then inhibition zones were measured in diametr

(mm) around of the discs. Negative controls were prepared using the dimethylsulfoxide (10% DMSO) employed to dissolve the pure compounds. Commonly used antibiotic Unacefin[®] was used as a positive control and dissolved sdH₂O as 0.5 g/ml. The assays were performed three times with three triplicates.

2.5. Determination of Minimum Inhibitory Concentration (MIC):

The minimal inhibition concentration (MIC) values were determined for the bacteria to the essential oils and pure compounds by using the modified agar well diffusion method [18]. In the agar-well diffusion technique, a two-fold serial dilution of the oils and pure compounds were prepared by diluting 10% DMSO to achieve a decreasing concentration range from 600 µl/ml to 6.25 µl/ml. Using 100 µl of suspension containing 10⁸ CFU/ml of each bacteria spread on TSA plates. The discs were impregnated with 12.5 µl of essential oils, and then were put in the middle of inoculated agar plates, containing TSA agar. All test plates were incubated at 35±2 °C for 48 h. At the end of this period, inhibition zones were measured as mm. The least concentration of each the essential oils and the pure compounds showing a clear zone of inhibition were taken as the MIC. The assays were performed three times with three triplicates.

2.6. GC Analysis Conditions

The analysis of the essential oil was performed using a Thermofinnigan Trace GC/A1300, (E.I) equipped with a SGE-BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Helium was the carrier gas, at a flow rate of 1 mL/min. Injection temperature was set at 220 °C. The programme was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected in the splitless mode. Quantitative data were obtained from FID area percentage data.

2.7. GC-MS Analysis Conditions

The analysis of essential oil was performed using a Thermofinning Trace GC/Trace DSQ/A1300, (E.I Quadropole) equipped with a SGE-BPX5 MS capillary column (30 m x 0.25

mm i.d., 0.25 µm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The programme used was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually and in the splitless mode. The main components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N library data of the GC-MS system and literature data [19]. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature [19].

2.8. Statistical Analysis

In order to determine whether there is a statistically significant difference among the results of obtained from antibacterial effect of tested plant essential oils and sensitivity of tested bacteria; variance analyses were carried out using SPSS 10.0 software package. Values of $p < 0.05$ were considered as significantly different.

3. Results

The main inhibition zone and minimum inhibitory concentration values were given in Table 1 and 2, respectively. According to these results, the tested essential oils, carvacrol, thymol and Unacefin[®] antibiotic showed more or less antagonistic activity against all tested bacteria on Petri plates assays, based on the zone of inhibition. Unacefin[®] used as a positive control showed the strongest mean inhibition zone (41.23 mm). Negative control DMSO didn't show any inhibition zone against the pathogens. Minimal inhibitory concentration of the tested essential oils and pure compounds were changed from 6.25 and 600 µl/ml. *O. acutidens* (both wild and cultured form) essential oils showed the lower minimal inhibition concentration (MIC, equal to 6.25 to 12.5 µl/ml). Thymol and carvacrol rate of tested essential oils were given in Table 3. According to these results obtained, effective essential oils were generally

characterized by high amounts of thymol and carvacrol, with small variations. As can be seen in Table 1, essential oils obtained from the wild forms of *O. acutidens* that had the strongest antibacterial activity are mainly consisted of carvacrol (87.0%). In addition, *S. hortensis* oil had a high content of thymol (67.5%).

4. Discussion

In this study, the mean inhibition zones of the whole essential oils were less than that of positive control antibiotic Unacefin®. But, pure compound thymol and/or carvacrol generally had stronger antibacterial activity than Unacefin® for Gram positive bacteria. The most successful results were obtained from pure thymol and carvacrol compounds, and the essential oils of *O. acutidens*, *S. hortensis* and *O. rotundifolium*. Mean inhibition zones and minimal inhibitory concentration of them, respectively were changed from 21 and 42.33 mm and 6.25 and 400 µl/ml. Some of the essential oils used in this study previously had been screened for antimicrobial activity of them against pathogenic bacteria and fungi by other investigator. It was reported that the extract or essential oils of *S. hortensis* [10,16] and *O. acutidens* [14] had antagonistic activity against a lot of bacterial species. Our data generally confirmed that the findings of these studies. But, according to our knowledge on food borne pathogenic bacteria of the essential oils obtained from *T. s. ssp. rosulans*, *T. haussknechtii* and *O. rotundifolium* have never been studied before against Gram negative and positive bacteria together. Therefore, this is the first study showed that essential oils of these species have inhibitory activity against food borne bacteria.

In the previous studies have been demonstrated that *Origanum* and *Thymus* species have shown a stronger antibacterial activity against tested bacteria. Carvacrol and thymol showed the highest inhibition zone when compared with that of essential oils. Up to now, there are a lot of studies that previously cited *Origanum* and *Thymus* species have shown that are rich in carvacrol and thymol [14]. Our results generally confirmed that the findings of these studies. The essential oils of *Thymus* and *Origanum* species tested in this study were also

characterized by high amounts of thymol and carvacrol, with small variations. Most studies agree that, generally, the essential oils are slightly more active against Gram positive than Gram negative bacteria [20]. It was indicated that *Cinnamomum verum*, *O. vulgare* and *Thymus vulgaris* essential oils showed the greatest inhibition zones against Gram positive bacteria than Gram negative bacteria [15]. Our results were in not agreement with these reports. The tested bacterial species exhibited different susceptibility to essential oils, thymol, carvacrol and Unicefin antibiotic. In addition, we observed that Gram negative bacterial strain *Salmonella enteritidis* ATCC 13076, and Gram positive bacterial strains *Bacillus subtilis* ATCC 6633 and *Streptococcus pyogenes* ATCC 176 had approximately equal sensitivity to the oils, pure compounds and antibiotic based on the general means of inhibition zones. Similar results were also obtained for Gram negative bacterial strains *Escherichia coli* RK-58 and *Staphylococcus aureus* ATCC 29213, and Gram positive bacterial strain *Enterococcus faecalis* ATCC 29122.

Many plant extracts and essential oils from medicinal plants, herbs and spices have been shown to possess antimicrobial activity and could serve as a source for antimicrobial agents against food spoilage and pathogens. The findings of the present investigations also indicate that plant essential oils might be useful as control agents of food borne diseases. Furthermore, we think that there is a strong correlation between major constituents and antibacterial activities of plant essential oils. They might provide alternative technologies to conventional antimicrobial additives in foods. The results of this study suggest the possibility of using the selected essential oils or their main compounds such as carvacrol and thymol as natural food preservatives, because some of these posses strong antibacterial activities.

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Table 1. Zone of inhibitory activity (in millimeter) of different essential oils of some plants, pure carvacrol and tymol, average inhibitory activity of them against Gram negative and Gram positive bacteria, and general means of inhibition zone of bacteria

| Applications | Gram negative bacteria (zone) | | | | Gram positive bacteria (zone) | | | GMB* |
|---|-------------------------------|-------|-------|-------|-------------------------------|-------|-------|--------------------|
| | Ec | Pa | Sa | Se | Bs | Sp | Ef | |
| Positive control (Unacefin [®]) | 42.66 | 44.33 | 55.66 | 41.66 | 20.00 | 33.33 | 51.00 | 41.23 ^a |
| Tymol | 34.00 | 33.66 | 42.33 | 32.66 | 25.33 | 35.33 | 38.00 | 34.57 ^b |
| Carvacrol | 32.00 | 28.00 | 31.33 | 26.33 | 30.66 | 25.33 | 40.66 | 30.61 ^c |
| <i>Origanum acutidens</i> (wild) | 31.33 | 28.66 | 29.66 | 24.00 | 18.00 | 27.66 | 36.33 | 27.95 ^d |
| <i>Satureja hortensis</i> | 36.00 | 23.66 | 34.00 | 26.66 | 25.33 | 21.00 | 26.33 | 27.57 ^d |
| <i>Origanum acutidens</i> (cultured) | 28.33 | 22.33 | 24.00 | 21.00 | 29.66 | 27.00 | 27.00 | 25.61 ^e |
| <i>Origanum rotundifolium</i> (cultured) | 29.66 | 20.33 | 32.00 | 21.00 | 28.66 | 21.66 | 24.66 | 25.42 ^e |
| <i>Thymus sipyleus</i> ssp. <i>rosulans</i> | 18.66 | 18.66 | 20.33 | 19.00 | 16.33 | 17.66 | 21.00 | 18.80 ^f |
| <i>Thymus haussknechtii</i> | 17.33 | 18.00 | 13.00 | 11.33 | 12.00 | 11.00 | 20.33 | 14.71 ^g |
| Negative control (DMSO) | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 ^h |

Ec: *Escherichia coli* RK-58, Pa: *Pseudomonas aeruginosa* ATCC 9027, Sa: *Staphylococcus aureus* ATCC 29213, Se: *Salmonella enteritidis* ATCC 13076, Bs: *Bacillus subtilis* ATCC 6633, Sp: *Streptococcus pyogenes* ATCC 176 and Ef: *Enterococcus faecalis* ATCC 29122

GMB: General means of inhibition zone of bacteria

*Data given are mean of three replicates and data in column and row with different letters are statistically different according to Duncan's multiple range test at p<0.05

Table 2. Minimal inhibitory concentration (MIC) of different essential oils of some plants, pure carvacrol and tymol against Gram negative bacteria and Gram positive bacteria

| Applications | Gram negative bacteria (MIC) | | | | Gram positive bacteria (MIC) | | |
|---|------------------------------|-------|-------|-------|------------------------------|-------|-------|
| | Ec | Pa | Sa | Se | Bs | Sp | Ef |
| Positive control (Unacefin®) | ≤6.25 | ≤6.25 | ≤6.25 | ≤6.25 | 100 | ≤6.25 | ≤6.25 |
| Tymol | 50 | 100 | 200 | 100 | 50 | 100 | 600 |
| Carvacrol | 50 | 100 | 100 | 50 | 50 | 50 | 100 |
| <i>Origanum acutidens</i> (wild) | 6.25 | 6.25 | 6.25 | 12.5 | 12.5 | 6.25 | 6.25 |
| <i>Satureja hortensis</i> | 100 | 100 | 100 | 25 | 50 | 50 | 100 |
| <i>Origanum acutidens</i> (cultured) | 25 | 12.5 | 6.25 | 6.25 | 12.5 | 12.5 | 50 |
| <i>Origanum rotundifolium</i> (cultured) | 25 | 25 | 25 | 12.5 | 25 | 25 | 50 |
| <i>Thymus sipyleus</i> ssp. <i>rosulans</i> | 100 | 200 | 200 | 100 | 200 | 100 | 200 |
| <i>Thymus haussknechtii</i> | 200 | 600 | 600 | 100 | 600 | 200 | 200 |
| Negative control (DMSO) | - | - | - | - | - | - | - |

MIC: Minimal Inhibitory Consantration

Ec: *Escherichia coli* RK-58, Pa: *Pseudomonas aeruginosa* ATCC 9027, Sa: *Staphylococcus aureus* ATCC 29213, Se: *Salmonella enteritidis* ATCC 13076, Bs: *Bacillus subtilis* ATCC 6633, Sp: *Streptococcus pyogenes* ATCC 176 and Ef: *Enterococcus faecalis* ATCC 291

Table 3. Tymol and carvacrol rate of essential oils from plant species tested in this study

| Plant species | Rate (%) | | Average Inhibition zone (mm) |
|---|----------|-----------|------------------------------|
| | Tymol | Carvacrol | |
| <i>Origanum acutidens</i> (wild) | <0.5 | 87.0 | 27.95 |
| <i>Origanum rotundifolium</i> (cultured) | <0.5 | 64.6 | 25.42 |
| <i>Origanum acutidens</i> (cultured) | <0.5 | 47.4 | 25.61 |
| <i>Satureja hortensis</i> | 67.5 | 17.9 | 27.57 |
| <i>Thymus sipyleus</i> ssp. <i>rosulans</i> | 29.9 | 14.4 | 18.80 |
| <i>Thymus haussknechtii</i> | 21.6 | 15.3 | 14.71 |

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