Antibacterial Activity of Several Concentrations of Sater (*Satureja hortensis* L.) Essential Oil on Spoilage and Pathogenic Food - Related Microorganisms

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Abstract: The present study was designed to establish the antibacterial activities of several concentrations of sater (Satureja hortensis L.) essential oil which is widely used as a condiment and herbal tea and collected from Mersin (Büyükeceli-Gülnar) in Turkey. Sater commonly consumed and grown naturally in Turkey was tested against eleven bacterial strains to compare their antibacterial effects with seven antibiotics. The results showed that the essential oil tested varied in their antimicrobial activity. All concentrations of oil were found to posses an activity against tested microorganisms depending on increasing doses. While inhibition zones of 10, 20, 30 and 40 µg concentrations of oil against Streptococcus salivarius, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus mutans were found smaller than on Bacillus cereus, Salmonella enteritidis, Escherichia coli and Bacillus anthracis strains. Generally, oil at 70, 80, 90 and 100 µg, were more effective on Salmonella enteritidis, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus mutans When compared with penicillin, amikacine, cefepime, gentamicin, ceftriaxone, cefaperazon sulbaktam. As a result, the use of sater essential oil as antiseptics and disinfectants may be useful in the field of food preservation and food processing plants.

Key words: Sater • S. hortensis • Essential oil • Antimicrobial effect • Antibiotics

INTRODUCTION

The antibacterial activities of spices and essential oils have been known for a long time and a number of researches on the antibacterial effect of spices, essential oils and their derivatives have been reported. Plant essential oils are a potentially useful source of antimicrobial compounds. Although numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including foodborne pathogens [1-17] Most of the synthetic chemicals used to control microbial deterioration of food commodities are either hazardous to or responsible for altering the palatability of the treated commodity [18-20]. Some investigations showed that the usage of spices in place chemicals and synthetics have become indispensable because of their antimicrobial effects and also because of the increasing risk from the side effects on health of the chemical and synthetic prefers the use of natural compounds as chemical and

synthetic preservatives. The chemical composition, quality and quantity of plant oils are known to vary due to several factors such as geographical origin of the plant, variants of the same species, part of the plant used to extract oil, time of harvest, crushing intensity, distillation method and time and duration of storage[21-23]. Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Common culinary herbs, spices and aromatic plants that exhibit antimicrobial activity could provide sources of acceptable, natural alternatives. Bioactive phytochemicals from these plants are often recovered as "essential oils" by hydrodistillation of whole tissues or seed. The chemical composition and antimicrobial properties of essential oils extracted from diverse plant species have been demonstrated using a variety of experimental methods [24-27].

In recent years, there has been a rising interest in the discovery of new antimicrobial compounds, due to an alarming increase in the rate of infections with antibiotic

resistant microorganisms [28]. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [22].

Spices and their derivatives such as essential oil and oleoresin are used with the primary purpose of flavoring foods and beverages although it has long been known some spice have an antimicrobial activity [29]. The aim of this work was to indicate antimicrobial effects of sater (*Satureja hortensis* L.) essential oil on bacteria in *in vitro* conditions at concentrations from 0.1% to 1.0% (v/v).

MATERIALS AND METHODS

Plant Sample: The plant (*Satureja hortensis* L.) used in this work was collected from Mersin (Büyükeceli-Gülnar) in Turkey in 2004 year. The species were identified by Dr Y. Bağcı, staff of the herbarium Section, Konya. Herbarium specimen was deposited at the Department of Food Engineering, Faculty of Agriculture, University of Selcuk, Konya in Turkey.

Antibiotics: Seven antibiotics were used to compare their antibacterial effects with those of spice extracts. Antibiotics used were: Ciprofloxacin, Cefaperazon Sulbaktam, Cefriaxone, Gentamicine, Penicillin, Cefepime and Amikacine. All the antibiotics (each 4 mm diameter disc) were obtained from Oxoid (Hampshire, England).

Extraction of Essential Oil: Essential oil of sater plant was obtained by hydrodistillation method by using Clevenger Apparatus. The plant material (about 100 g), cutted into small pieces, were placed in a flask (2 L.) together with double distilled water (1.5 L.). The mixture was boiled for 3 h. The extract was condensed in cooling vapor to collect the essential oil of plant. The extracted oil was dried over anhydrous sodium sulfate. Oil was hold at freezing temperature (-18 °C) by using.

Antimicrobial Activity Assay: 7 gram (+) Streptococcus salivarius RSHE 605, Listeria monocytogenes NCTC 5348, Bacillus cereus ATCC 11778, Staphylococcus aureus ATCC 25923, Streptococcus pneumoniae ATCC 10015, Streptococcus mutans RSHE 676, Bacillus anthracis (S.Ü. Vet Fak.) and 4 gram (-) Salmonella enteritidis ATCC 13076, Escherichia coli ATCC 25922, Klebsiella pneumoniae NCTC 5049, Pseudomonas aeruginosa ATCC 27853 of bacteria strains were used to determine the antimicrobial activities of the volatile oil synthesized

from thyme. All bacteria strains were the pathogens for human and animal. The determination of the thyme oil effectiveness on these strains was thought to be an indicative regarding how its effect on the other strains is. The disc diffusion method was used to determine the antimicrobial activity [30]. After raised on Brain Heart Infusion broth, the lyophilized strains were inoculated on %5 of blood agar and then incubated for 24 h at 37°C. The pre-cultures of microorganisms were prepared for the susceptibility tests. For this purpose, the bacteria strains were taken by sterile inoculating loop, touching to 4-5 colonies raised from pure microorganism culture and these strains were inoculated at the concentration of 1x10⁸ cfu/ml (in order to achieve the Mc. Farland No: 0.5 density) and then incubated at 37°C. The 500 µg, 1000 µg, $1500 \, \mu g$, $2000 \, \mu g$, $2500 \, \mu g$, $3000 \, \mu g$, $3500 \, \mu g$, $4000 \, \mu g$, $4500 \, \mu g$ ug and 5000 ug concentrations of sater oil were dissolved in 1 ml of dimethyl sulfoxide (DMSO) solution having no any antimicrobial activity. All these solutions prepared were provided to be absorbed by 6 mm diameter of blank (Oxoid) disks, absorbing capacity of which is two fold of weight itself, in order that the final concentration of each disc could be 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 80 μg, 90 μg and 100 μg, respectively. For the negative control, a second party of the blank disks was provided to absorb DMSO. For each bacterium strain, Ciprofloxacin (CPR), Cefaperazon Sulbaktam (CES), Ceftriaxone (CRO), Gentamicine (GM), Penicillin (P), Cefepime (FEP), Amikacine (AK) antibiotic discs were used as the positive control. The prepared discs were placed in inoculated petri dishes. These petri dishes were incubated for 24 h at 37°C. The diameter of formed inhibition zones were measured as millimeter. The results were evaluated according to NCCLS [31] criteria. The study was conducted in 3 replicates. The obtained results were the mean of three measurements.

RESULTS AND DISCUSSION

Antimicrobial properties of *Satureja hortensis* oil and some discs contained antibiotic are summarized in Table 1. Also, variance analyses of microorganism and dosage on diameter are given in (Table 2).

According to the table of variance, difference of the microorganisms and doses were statistically significant (p<0.01).

The results showed that all ten concentrations had antimicrobial activity against tested bacteria. The growth characteristic of *Str. salivarius* RSHE 605, *Bacillus cereus* ATCC 11778, *E. coli* ATCC 25922, *K. pneumoniae* NCTC

Table 1: Means and standards deviations of the inhibition zones of the microorganism (mm)

	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	P	AK	FEP	GM	CRO	CES	CPR
Str. salivarius	8.00	8.75	9.25	11.00	13.00	14.75	18.00	22.75	26.25	27.75	23.75	8.75		9.75		13.75	29.75
RSHE 605	± 0.82	± 0.50	± 0.50	±1.41	± 0.82	±0.96	± 0.82	± 0.96	±1.26	± 0.82	± 0.96	± 0.82	R	± 0.86	R	± 0.58	± 0.88
L. monocytogenes	8.00	8.25	7.25	8.75	9.00	11.00	12.75	15.00	15.75	17.50		8.75		7.75	11.75	14.75	24.75
NCTC 5348	±1.63	± 0.96	± 0.50	± 0.50	± 0.82	± 0.82	± 0.50	± 0.82	± 0.82	± 0.26	R	± 0.60	R	± 0.49	±0.28	± 0.67	±1.05
Bacillus cereus	7.75	9.25	12.75	19.25	24.25	25.00	28.75	30.00	34.75	39.75	24.75	21.75	14.75	17.75	16.75	25.75	33.75
ATCC 11778	±1.26	± 0.96	± 0.96	± 0.96	± 2.87	±2.45	±1.89	± 1.63	± 0.96	± 1.63	± 0.96	± 0.96	± 0.82	± 0.26	± 0.73	±1.26	±1.06
S. aureus	6.00	8.00	10.00	12.25	14.00	15.75	15.75	19.00	20.75	24.75	9.75	19.75	17.75	19.75	24.75	17.75	19.75
ATCC 25923	± 0.00	±1.63	± 1.63	± 0.50	± 0.00	± 2.06	±1.26	± 0.82	± 0.82	± 0.82	± 0.82	± 0.80	± 0.86	± 0.28	±1.24	±0.29	±0.92
Salm. enteritidis	7.75	11.00	14.00	16.00	19.25	20.75	22.25	22.00	25.00	27.25	13.00	17.00	15.00	14.00	15.00	28.00	30.00
ATCC 13076	± 0.50	± 0.82	± 0.82	± 1.63	± 0.96	±1.89	± 0.50	± 2.16	± 0.82	± 2.50	± 0.82	± 0.64	± 0.57	± 0.28	±0.28	± 0.82	± 0.84
Str. pneumoniae	6.00	7.00	13.00	14.00	16.75	17.00	20.25	22.75	24.75	26.00	15.00	21.00	22.00	14.00	28.00	18.00	30.00
ATCC 10015	± 0.00	± 0.00	± 0.82	± 0.00	± 0.50	± 0.82	± 0.50	± 0.50	± 0.50	± 0.82	± 0.82	±0.59	± 1.01	± 0.76	±0.49	± 0.72	±0.43
E. coli ATCC 25922	10.75	13.00	15.75	19.25	19.00	21.00	25.00	29.00	33.25	39.25		15.00	26.00	14.00	28.00	30.00	30.00
	± 2.06	± 2.16	± 3.30	± 0.96	± 0.82	± 0.82	± 0.82	± 0.82	± 0.50	± 4.11	R	± 0.89	± 0.98	± 0.26	± 0.67	± 0.69	±0.22
K. pneumoniae	7.00	8.00	8.25	12.00	12.25	14.25	20.00	25.00	27.75	32.00	9.00	21.00	35.00	20.00	36.00	35.00	40.00
NCTC 5049	± 0.82	± 0.00	± 1.26	± 0.82	± 0.50	± 0.50	± 1.63	± 0.82	± 0.50	± 1.00	± 0.82	±1.21	± 0.76	± 0.59	± 0.58	± 0.79	± 0.83
P. aeruginosa	6.00	8.00	8.75	9.25	10.25	11.00	13.25	15.00	18.25	21.00		20.00	25.00	17.00	12.00	20.00	30.00
ATCC 27853	± 0.00	± 0.00	± 0.50	± 0.50	± 0.50	± 0.82	± 0.50	± 0.82	± 0.50	± 0.82	R	± 0.84	± 0.26	± 0.83	± 0.76	± 0.66	± 0.47
Str. mutans	7.25	8.00	9.00	10.25	13.25	15.75	19.75	21.00	24.00	27.25	15.00	21.00	22.00	14.00	28.00	18.00	30.00
RSHE 676	± 0.50	± 0.82	± 0.00	± 0.50	± 0.96	± 0.50	± 0.50	± 0.00	± 0.82	± 0.96	± 0.82	±0.25	± 0.37	± 0.47	±0.94	± 0.81	±0.65
Bacillus anthracis	12.00	13	14.75	16.00	20.75	23.00	24.00	29.75	33.00	37.25	35.00	25.00	15.00	23.00	24.00	30.00	32.00
(S.Ü. Vet Fac.)	±1.63	±0.82	±0.50	±0.82	±0.96	±0.82	±1.63	±0.50	±0.82	±0.96	±0.82	±0.82	±0.67	±0.53	±0.82	±0.99	±0.42

Table 2. Table of variance of microorganism and dosage on diameter.

		Diameter (mm)				
Source of variance	Degree of Freedom	Mean Square	F			
Difference of the microorganisms (A)	10	11069.45	1262.829**			
Difference of dosages (B)	16	29073.23	2072.964**			
AXB	160	15664.78	111.692**			
Fault	561					

^{**}p<0.01

5049, Bacillus anthracis in the presence of sater essential oil are shown in Fig. 1. The essential oil exerted varying levels of antimicrobial effect against microorganisms. The essential oil of sater at low concentrations (0.1%) showed antibacterial activity against bacterial species. The high concentrations of oil processed greater antimicrobial effects against all microbial species than other low concentrations. 10, 20, 30 doses of oil were partly ineffective on Str. salivarius, L. monocytogenes, S. pneumoniae, K. pneumoniae, aureus, Str. P. aeruginosa and Str. mutans. Generally, sater oil at 70, 80, 90 and 100 were more effective on Salm. enteritidis, Str. pneumoniae, E. coli, K. pneumoniae, P. aeruginosa and Str. mutans when compared with penicillin, amikacine, cefepime, gentamicine, ceftriaxone, cefaperazon sulbaktam.

The active fraction is probably responsible for the antimicrobial activity of the essential oil. It has been shown that the phenolic components of essential oils showed the strongest antimicrobial activity, followed by aldehydes, ketones and alcohols [19,32,33]. Different antimicrobial effects were obtained with basil, cumin, fennel, rosemary, sage, sater, savory, oregano and thyme oils on *S. aureus*, *E. coli, Pseudomonas aeruginosa*, *E. faecalis*, *B. subtilis*, *S. cerevisiae*, *C. albicans*, *S. typhimurium*, *L. monocytogenes*, *Y. enterocolitica*, Gram-negative bacteria, gram-positive bacteria, yeasts

[6,11,12,14-17,34]. Antibacterial effects varied as a function of a microbial species considered. With a few exceptions, the effects of the highest concentrations of oil were found to be quite similar. Therefore, the antimicrobial effect of our samples should be closely related to the high percentage of this compound. The variation in the antimicrobial activities of essential oils between results and the previous reports [11,13,17,19,29,33] may be attributed to the different environmental growth conditions of plant and microbial species. Generally, the inhibition of thyme oil increased by the increase of the dosage for all the microorganisms tested (Fig 2.). For Str. salivarius RSHE 605 the inhibition effect of CPR and FEP was lower than 0.1 dose of the thyme oil. The effect of CES and GM was similar with 0.5 and 0.3 dosages, respectively. Inhibition effect of CPR was very high but the effect of 0.1 dosage was very close to its. L. monocytogenes NCTC 5348 had the smallest diameters generally for all dosages. Except for CPR, the effect of all thyme oil dosages was generally higher than the effect of other antimicrobials. According to the figure generally the effect of all doses of the thyme oil was high for Bacillus cereus ATCC 11778. While, CPR was the most effective antibiotic, the dosages of thyme oil for 90 and 100 were higher than that. The effect of CRO and 1.0 dosage was the same and those was the most effective inhibitors for the S. aureus ATCC 25923. Also, 90 dosage was higher

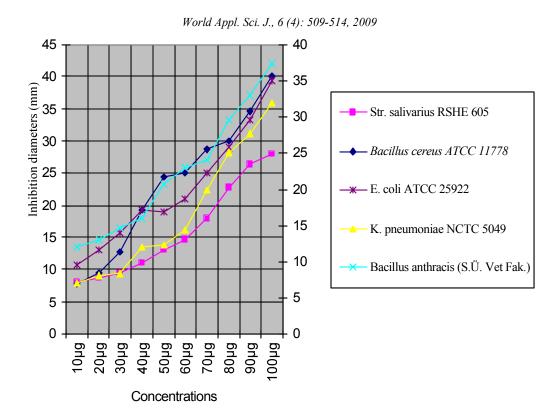


Fig. 1: Inhibition curves of various concentrations of savory essential oil against some tested microorganisms

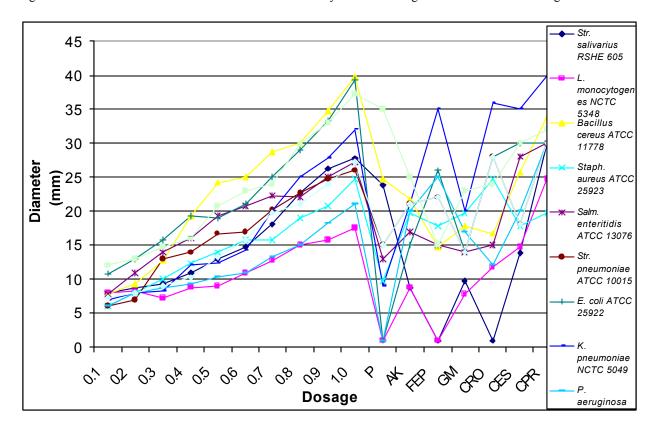


Fig. 2: Interaction of differences of microorganism and dosage on diameter

than all other antimicrobials. The diameter for CRO and CPR was 28 and 30. *Salm. enteritidis* ATCC 13076. The diameters obtained for *P. aeruginosa* ATCC 27853 was as low as *L. monocytogenes* NCTC 5348.

As a result, the inhibitory effect of both oils on the growth of certain bacteria is an interesting finding in view of their eventual application as natural antimicrobial compounds taking into account the increasing alarm on the use of traditional antibiotics. Several aspects need to be studied before adding plant essential oils into food, such as their toxicity, their allergenicity, the effect of processing conditions on their efficiency and the concentration of oil required in foods. Organoleptic effects on foods and safety studies may also be necessary before use as food additives. The literature revealed that the antimicrobial activity of essential oils against bacteria may vary [35,36]. Three main factors can influence the results of a test of the antimicrobial activity of a plant oil: the composition and solubility of the oil, the microorganism and the method of growing and enumerating the surviving bacteria [1,29]. Partial studies are recommended on the use of essential oils of selected spices during production of foods. A food product requires a very low initial microbial load and inhibition during the production period for an adequate shelf-life. The results suggest the potential use of some essential oils as antimicrobial preservatives in food. Further studies on the combined effects of many local plant essential oils and components in food products are in progress in our model systems.

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