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CHEMICAL COMPOSITION AND ANTISTAPHYLOCOCCAL ACTIVITY OF AN ENDEMIC *SALVIA CHRYSOPHYLLA* STAPF. NATURALLY DISRIBUTED DENIZLI PROVINCE (TURKEY) AND ITS VICINITY

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Abstract

In present study, antistaphylococcal activity of endemic *Salvia chrysophylla* Stapf. naturally distributed in Denizli (Turkey) and its vicinity was investigated. The antistaphylococcal activity of the crude extracts was evaluated against two strong microorganisms *Staphylococcus aureus* and *Cowan liyofili.* The activity was detected by using broth microdilution methods. When compared with other studies, mic value of our study is further low. The essential oils of endemic *Salvia chrysophyla* in Denizli was analyzed by GC-MS. The major constituents of the oil of *S. chrysophyla* were 3-oktanol, α-phellandren-8-ol, camphor and limonene.

Introduction

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes, such as food, drugs and perfumery (Heath, 1981). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Essawi & Srour, 2000). Plant products are also known to possess potential for food preservation (Baratta *et al*. 1998 a; 1998b; Deans& Ritchie, 1987). They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants (Barlow, 1990).

Salvia, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. This genus is represented, in Turkish flora, by 88 species and 93 taxa, 45 of which are endemic (Guner *et al*., 2000). Some members of this genus are of economic importance since they have been used as flavouring agents in perfumery and cosmetics. Sage (*S. officinalis*) has been credited with a long list of medicinal uses: e.g. spasmolytic, antiseptic, astringent (Newall *et al*., 1996). Despite themedicinal potential of plants in Turkey being considerable, knowledge of this area and studies on these plants are scarce (Digrak *et al*., 2001).

The aim of this study was to avaluate the antimicrobial activities of the crude extracts isolated from *S. chrysophyla* in Denizli (Turkey), against a range of foodborne pathogenic and spoilage bacteria, evaluating minimal inhibitory concentrations in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

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Materials and Methods

Plant material: *S. chrysophyla* was collected from Geyran Plateau (1450m), Denizli, when flowering (June, 2005). The voucher speciemens were identified by Dr. Ali Celik at the Department of Biology, Pamukkale University, Denizli-Turkey and deposited at the Herbarium.

Isolation of essential oil: A portion (100g) of parts of *S.chrysophyla* was submitted for 3 h to water-distillation, using a Clevenger-type apparatus (yield 0.51 v/w). The obtained essential oil was dried over anhydrous sodium sulphate and, after filtration, stored at + 4 C until tested and analysed.

Preparation of the hexane, dichloromethane and methanol extracts: A portion (100 g) of dried plant material was extracted with hexane (HE) (5.36%, w/w), followed by dichloromethane (DCM) (1.67%, w/w) and ethanol (EtOH) (7.83%, w/w) in a Soxhlet apparatus (6 h for each solvent) (Sokmen *et al*., 1999). All extracts obtained were kept in the dark at +4 C until used.

Gas chromatography/mass spectrometry analysis conditions

Gas chromatography analysis: The essential oil was analysed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5 MS capillary column (30 m0.25 mm, film thickness 0.25 lm). Injector and detector temperatures were set at 220 and 290 C, respectively. Oven temperature was kept at 50 C for 3 min, then gradually raised to 160 C at 3 C/min, held for 10 min and finally raised to 240 C at 3 C/min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples (1/100 in acetone, v/v) of 1.0 ll were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

Gas chromatography/mass spectrometry analysis: Analysis of the oils was performed using a Hewlett Packard 5890 II GC, equipped with a HP 5972 mass selective detector and a HP-5 MS capillary column (30 m0.25 mm, film thickness 0.25 lm). 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 and 290 C, respectively. Oven programme temperature was the same with GC analysis. Diluted samples (1/100 in acetone, v/v) of 1.0 ll were injected manually and in the splitless mode. The components were identified by comparison of their relative retention times and mass spectra with those of standards (for the main components), NBS75K library data of the GC/MS system and literature data, as described by Adams (2001). The results were also confirmed by comparison of the compounds elution order with their relativeretention indices on non-polar phases as reported by Adams (2001).

Antistaphylococcal activity

Microbial strains: The extracts were individually tested against two microorganisms. Test microorganisms were *Staphylococcus aureus* ATCC 25923 and *Cowan liyofili* ATCC 12598. Bacterial strains were cultured overnight at 37 °C.

Antimicrobial screening: 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 106 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. The MICs of vancomisin (VA) and erythromisin (E) were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

Results and Discussion

It is the first report about biologycal tests with *S. chrysophylla,* therefore our report is an original report. The main constituents of the essential oils of an endemic *S.chrysphylla* used in the experiments are presented in Table 2. The essential oil of *S. chrysophyla* were 3-oktanol, α-phellandren-8-ol, camphor and limonene as the major constituents. The results showed that oils had significant antistaphylococcal activity on of test microorganisms. The antistaphylococcal activity of the extracts was examined by broth microdilution susceptibility assay against 2 bacterial strains selected on the basis of their relevance as food contaminants. The results, presented in Table 1. This

Table 1:

Test Microorganisms	**MIC Value of Crude Extracts (µg/ml)**			**ABT(µg/ml)**	
	HE	DCM	EtOH	VA	E
***S.aureus* ATCC25923**	80	80	120	50	80
***C.liyofili* ATCC12598**	320	200	250	200	250

ATB: Antibiotics, VA; Vancomisin, E; Erythromisin; **MIC**; Minimal Inhibitory Concentration, **HE**; Hekzan, **DCM**; Dichloromethane, **EtOH**;Ethanol

Table 2. Chemical composition of the essential oils of *S .chrysophylla*

	Compounds	**RT**	**%**
1	α-Tujen	9,37	1,38
2	α–Pinene	9,63	1,85
3	Camphene	10,29	3,95
4	Pinene	11,55	1,17
5	α–Phellandren-8-ol	11,85	15,7
6	3-Oktanol	18,1	20,1
7	α–Terpinene	18,8	4,76
8	Camphor	19,3	12,6
9	Borneol	20,5	1,64
10	Limonene	33,2	11,8
11	α–Humulene	34,6	7,52
12	*p*-Simene	13,97	6,34
13	Bornile asetate	26,87	8,2
14	Cadinene isomer	36,83	0,5

RT : Retention time; % : Percantage of the content of each constituent in total essential oil.

antistaphylococcal activity obtained with the extracts of *S. chrysophyla* , is comparable to that reported by Delamare (2005). The effectiveness of the extracts of *S. chrysophyla* (MIC) against susceptible bacteria was higher than that previously reported for this species (Sivropoulou *et al*., 1997), and for Salvia , *Salvia glutinosa* , and *Salvia aethiopis* (Velickovic *et al*., 2002), *Salvia tomentosa* (Daferera *et al.*, 2005), and *Salvia cryptantha* and *Salvia multicaulis* (Tepe *et al*., 2004), *Salvia triloba* and *Salvia officinalis* (Delamera *et al*. 2005).

In low concentrations, extracts exhibited bacteriostatic activity. 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. Although tests on food are necessary, the present study indicates that *S. chrysophyla* 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.

Finally, the antimicrobial activity of the crude extract from *S.chrysophyla* may be due to the presence of both antifungal and antibacterial compounds. The present study provides an important basis for the use of extracts from these plants for the treatment of infections associated to the studied microorganisms. The crude extract as well as the isolated compounds found active could be useful for the development of new antimicrobial drug. However, pharmacological and toxicity studies currently going on in our laboratory will be necessary to confirm this hypothesis.

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