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Antimicrobial Effect of Plant Oils against Some Bacterias Izolated from Patients Samples

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Abstract

Aim: The aim of present study was to compare the antibacterial potential of *Thymus sipyleus* boiss. subsp. *sipyleus* boiss. var. *sipyleus* L. (Thymol and camphor both tested), *Satureja thymbra* L. and *Origanum onites* L. by Kirby Bauer disc diffusion method.

Methods: We isolated *Enterococcus* spp., *E. coli, Morgonella morganii, Pseudomonas aeruginosa*, MSSA, *Klebsiella pneumonia* and *Proteus mirabilis* from different patient samples. The antibacterial activity of each oils was evaluated by the Kirby Bauer disc diffusion method.

Results: *Proteus mirabilis* were the most susceptible bacteria against essential oils, followed by *Klebsiella pneumonia* and *Morganella morganii*. *Pseudomonas aeruginosa* did not show susceptibility to any oil.

Conclusion: The essential oils of the *T. sipyleus, O. onites and S. thymbra* were especially very effective against the resistant strains such as *Enterococcus spp*. and *Klebsiella pneumonia*. In this group the maximum antimicrobial activity was observed with the essential oils of *Origanum onites and Satureja thymbra*. We think that these oils can be proposed in treatment process as an alternative application structures in the future.

Keywords: Antimicrobial; Disc diffusion; Essential oils

Introduction

This study was undertaken to determine the *in vitro* antimicrobial activities of three commercial essential oils and their main components in order to pre-select candidates for potential infections. The antibacterial effects against pathogenic bacteria which are isolated from patient's samples (*Enterococcus spp., E. coli, Morgonella morganii, Pseudomonas aeruginosa,* MSSA, *Klebsiella pneumonia* and *Proteus mirabilis*) were tested using paper disk diffusion method. These materials could be served as an important natural alternative to prevent bacterial infections in the future (Table 1).

One of the most popular spices used around the world is *Origanum onites* L. It is used also as a medicinal therapy, arousing interest not only in the use of its leaves in folk medicine, but also in relation to its essential oil for therapeutic purposes and treatment [1].

Species of the genus *Satureja* (family Lamiaceae) are widely distributed in the Mediterranean area, Asia, regularly found in warm, dry, rocky habitats also in east part of Turkey [2]. *Satureja* consists of about 200 species, usually aromatic herbs and used for treatment. *S. thymbra* oil has been found to have a good antimicrobial activity against various bacteria [3-5].

The genus Thymus, member of the Lamiaceae family, contains about 400 species of perennial aromatic, evergreen or semi-evergreen various plants with many subspecies, varieties, sub-varieties and forms are mostly distributed in the Mediterranean area and also in Turkey [6].

Materials and Methods

Plant material and isolation of essential oil

Origanum onites L. (Lamiaceae), L. (Labiatae), *Satureja thymbra* L. (Labiatae), *Thymus sipyleus boiss*. subsp. *sipyleus* boiss. var. *sipyleus* L. (Lamiaceae) were collected at the flowering stage from different regions of Turkey between June and August. Voucher specimens have been deposited in the herbarium of Ataturk University, Faculty of Agriculture, the Department of Plant Protection, Erzurum, Turkey. Aerial parts of the plants were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydro distillation for 4 h using a Clevenger-type apparatus. The oil yields of *O. onites*, *S. thymbra* and *T. sipyleus* were 4.5%, 2.3%, 1.17% and 0.98% (w/w, dry weight basis), respectively. The yield was based on dry materials of plant samples. The oils were dried over a hydrous Na_2SO_4 and stored under N_2 in a sealed vial until required, and then stored at 4°C until used for toxicity bioassays. The pure compounds were purchased commercially from Fluka and Sigma. [Camphor (Fluka, purity 97%), Thymol (Sigma, purity 95%)]. The compounds tested for toxicity against *Enterococcus spp., E. coli, Morgonella morganii, Pseudomonas aeruginosa,* MSSA, *Klebsiella pneumonia* and *Proteus mirabilis.*

Collection of bacteria

Enterococcus spp., E. coli, Morgonella morganii, Pseudomonas aeruginosa, MSSA, Klebsiella pneumonia and Proteus mirabilis isolated from different patient samples in the laboratory of clinical microbiology with VITEC microbial identification system.

Kirby-Bauer disc diffusion method

The experiment was performed with a bacterial inoculum of 0.5 McFarland; Mueller-Hinton (MH) was inoculated with each bacterial

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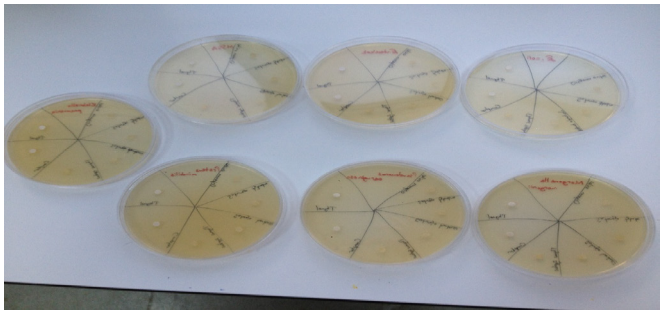

Figure 1: Muller-Hinton agar base with each bacteria and oils in a sterile filter paper disc. After 24 hours period inhibition zones were measured.

Thymus sypleus	*Entero-coccus Spp.*	*E. coli*	*Morgan-ella mor-ganii*	*Pseudomo-nas aerugi-nosa*	MSSA	*Klebsiella pneumo-nia*	*Proteus mirabilis*
Kirby-Bauer	17 mm	14 mm	20 mm	0 mm	13 mm	22 mm	23 mm
Satura-ja thym-bra	*Entero-coccus Spp*	*E. coli*	*Morgan-ella mor-ganii*	*Pseudomo-nas aerugi-nosa*	MSSA	*Klebsiella pneumo-nia*	*Proteus mirabilis*
Kirby-Bauer	21 mm	18 mm	30 mm	0 mm	14 mm	18 mm	30 mm
Origa-num onites	*Entero-coccus Spp*	*E. coli*	*Morgan-ella mor-ganii*	*Pseudomo-nas aerugi-nosa*	MSSA	*Klebsiella pneumo-nia*	*Proteus mirabilis*
Kirby-Bauer	18 mm	18 mm	16 mm	*0 mm*	*9 mm*	13 mm	18 mm
Cam-phor	*Entero-coccus Spp*	*E. coli*	*Morgan-ella mor-ganii*	*Pseudomo-nas aerugi-nosa*	MSSA	*Klebsiella pneumo-nia*	*Proteus mirabilis*
Kirby-Bauer	0 mm	0 mm	0 mm	0 mm	0 mm	12 mm	0 mm
*Mean values in Kirby-Bauer disc diffusion method expressed as mm							

Table 1: The results of the antibacterial assays in terms of Kirby Bauer (Inhibition ranged from 0 mm to 30 mm).

strain. Each oil was applied to a sterile filter paper disc (6-mm diameter) 2 µl placed on the surface of inoculated plates; duplicate plates for each oil were used. After overnight incubation at 37°C, the inhibition zones were measured. Control plates were prepared by placing sterile water for negative controls (Figure 1 and Table 1).

Results

Proteus mirabilis were the most susceptible, followed by *Klebsiella pneumonia* and *Morganella morganii*, *Pseudomonas aeruginosa* did not show susceptibility to any oil. *Klebsiella pneumonia* susceptible, other oils did not show susceptibility to the camphor oil.

Discussion

In the literature, the results of experiments showed that the oil from *Thymus* exhibited extremely strong activity against all of the clinical strains. In traditional popular medicine, *Thymus spp*. essential oil has been traditionally used to treat respiratory tract disorders and wound infections; using of *Thymus* derivatives has been used to treat wound infections, cough, and sputum treatment. Over the past few years, the interest in natural medicine has been increasing in industrialized societies and in medical area also. It is evident that there is a relationship between the high activity of thymol oil and the presence of phenol components such as thymol and carvacrol to the bacterias [7-11].

According to literatures, the mode of action of essential oils against pathogen or non-pathogen bacteria cannot be attributed to one specific mechanism [12].

Enterococcus spp., E. coli, Morgonella morganii, Pseudomonas aeruginosa, MSSA, *Klebsiella pneumonia* and *Proteus mirabilis* are the most important causes of some infections, and the emergence of resistance to antibiotics is a serious public health problem.

Conclusion

In our hands, comparison of their results of this study with previously published data is difficult because the composition of plant oil products is known to vary according to local air conditions, soil composition and to extraction techniques. Moreover, the results obtained may differ because of the method used to assess antimicrobial activity and quantity of oil.

The essential oils of the *T. sipyleus, O. onites and S. thymbra* were especially very effective against the resistant strains such as *Enterococcus spp.* and *Klebsiella pneumonia.* The maximum antimicrobial activity was observed with the essential oils of *Origanum onites* and *Satureja thymbra.*

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