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Antibacterial Effect Of Essential Oils From Medicinal Plants

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The present study aimed to evaluate the antimicrobial activity of essential oils of the 2 thymus (*Thymus vulgaris* L. and *Th. kotschyanus* Boiss. et Hohen.) species with antimicrobial potential of four antibiotics (gentamicin, penicilin, nalidixic acid and amoxicillin). The selected essential oils were screened against Gram (-) bacteria (*Escherichia coli*) and Gram (+) bacteria (*Staphylococcus aureus)* using disc diffusion method. According to results of our study of the bacterial inhibition zone diameter these oils lack any antibacterial effect.

Key words: *Antibiotics, antimicrobial activity, thymus, essential oil, gram-negative bacteria, gram-positive bacteria*

INTRODUCTION

In the present time, drug resistance in microbes is a very serious problem. Hence, herbal medicines are considered as safe alternatives of synthetic drugs. There are varied methods of medicines like Aurveda, Homeopathy and Unani, which utilize plant materials for drug production. Currently, Aurveda considered as a vital system of medicine and governed the worldwide recognition and having non-toxic substances. However, newly discovered non-antibiotic substances such as certain essential oils (Sonboli et al., 2006) and their constituent chemicals (Chavan et al., 2006) have shown good fighting potential against drug resistant pathogens (Cowan, 1999; Ahmad and Beg, 2001).

Plant essential oils and extracts have been used for many thousands of years (Jones, 1996), in food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis, 1997). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds (Mitscher, 1987) especially against bacterial pathogens. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Knobloch, 1986; Sikkema, 1994). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Denyer, 1991). Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria (Zaika, 1998).

Essential oils are aromatic oily liquids, which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation. Scientifically these oils have been proved highly potent antimicrobial agents in comparison to antibiotics. These plant essential oils are rich source of scents and used in food preservation and aromatherapy.

Plant essential oils possess multiple antimicrobial, i.e., antibacterial (Ozcan et al., 2006), antifungal (Cafarchia et al., 2002), anticancer, antiviral and antioxidant properties (Salehi et al., 2005; Vardar-Unlu et al., 2003), against viruses, bacteria and fungi (Kalemba and Kunicka, 2003). Some essential oils such as aniseed, calms, thyme, camphor, cedar-wood, cinnamon, eucalyptus, geranium, lavender, lemon, lemongrass, lime, peppermint, nutmeg, rosemary, basil, vetiver and winter green are traditionally used by people in different parts of the world. Cinnamon (Prabuseenivasn et al., 2006), clove, rosemary and lavender oils have shown both antibacterial and antifungal properties (Quale et al., 1996; Chang et al., 2001; Wilkinson and Cavanagh, 2005). Besides this, Cinnamon oil possesses anti-diabetic and anti-inflammatory activity (Mitra et al., 2000), while lemon, rosemary and peppermint exhibit anticancer activities (Imai et al., 2001). Peppermint oil (*Menthae piperitae aetheroleum*) is a naturally occurring carminative that is typically obtained from the fresh leaves of peppermint (*Mentha piperita* L.) by steam distillation (Grigoleitet al., 2005; Beesley et al., 1996). It has been used to treat recurrent abdominal pain, irritable bowel syndrome (IBS), nausea, and coughs and colds (Charrois et al., 2006). Recent studies have also indicated that peppermint oil possesses antibacterial and antifungal activities (Edriset al., 2003; Imai et al., 2001).

Giordani et al. (2004) carried out a study on the antifungal potential of essential oils of various chemotypes of *Th. vulgaris* against *Candida albicans*. According to Klaric et al. (2007), both thymol and the essential oil of *T. vulgaris*, whose main components are *p*-cymene (36.5%) and thymol (33.0%) showed strong fungicidal and/or fungistatic activities against *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Rhizopus*. Thymol exhibited three times greater inhibition compared with the essential oil of *Th. vulgaris*. Many investigators have demonstrated the antifungal potential of thymol against species of yeasts and filamentous fungi. Thymol inhibits the growth of *Candida* species sensitive and resistant (clinical isolates) to azoles and amphotericin B (Ahmad et al., 2010), and interferes with the formation and viability of hyphae of *C. albicans* (Braga et al., 2007). Similar results were reported for *Aspergillus fumigates* and *Trichophyton rubrum* resistant to azoles and amphotericin B (Hammer et al., 2003). However, there are few studies on the antifungal activity of *p*-cymene. *p*-Cymene and 1,8-cineol have been found to be much less effective against *Aspergillus spp*. and *Penicillium spp*. (MIC ≥4 or 8%, v/v), when compared with thymol (Hammer et al., 2003). However, with regard to opportunistic yeasts, thymol and *p*-cymene, alone or in combination, exhibit strong antifungal activity against *Candida spp*. (Pina-Vazetal., 2004).

Other essential oils such as thyme are well known aromatic plant and its essential oil and aromatic water are used in the mountain regions of the Mediterranean parts of Turkey. Thyme was used by the Greeks as incense in their temples and by the Romans in cooking and as a source of honey. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics (Simon et al., 1999 and Senatore, 1996). Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction (Simon et al., 1999). Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene et al, 2003). The main essential oil in thyme, thymol is active against *Salmonella* and *Ataphylococcus* bacteria. The main constituents of thyme include thymol, carvacrol and flavonoids. The known primary constituents of thyme include essential oil (borneol, carvacrol, linalool, thymol), bitter principle, tannin, saponins and triterpenic acids. It is used to suppress coughing ease chest congestion and stimulate production of saliva (Jellin et al., 2000; Barnes et al., 2002). Thyme also known as creeping thyme, mountain thyme and wild thyme a small shrubby plant with a strong spicy taste and order is extensively cultivated in Europe and US for culinary use. Thymol shows spontaneous contractile activity (SCA) of smooth muscle strips (SCA) from the stomach and vena portae of guinea pigs (Beer et al., 2007).

In present study antimicrobial potential of four antibiotics (gentamicin, penicilin, nalidixic acid and amoxicilin) and essential oils of two thymus species was screened against two pathogenic bacterial strains, i.e., *Staphylococcus aureus* (PTCC 1112) and *Eschericia coli* (PTCC 1399) in various antibacterial bioassays. For antimicrobial susceptibility of each essential oil, growth inhibition zone diameters were determined.

MATERIALS AND METHODS

In order to examine *Thymus vulgaris* L. and *Th. kotschayus* Boiss. et Hohen. oils, 10 µl of each were placed on standard papers using a sterilized sampler under aseptic conditions. Then they were kept in incubator for 6 h at 37°C to dry completely. *E.coli* and *S.aureus* bacteria strains were separately cultured in Mueller Hinton agar medium under aseptic conditions. Then nalidixic acid, gentamicin, penicillin and amoxicillin antibiotic disks and the disks prepared from oils at specific intervals were placed in the medium. Aforesaid mediums were kept in incubator for 24 h at 37°C to inhibit bacterial growth. In this study we measured the minimal inhibition concentration (MIC) and minimal bacteriostatic concentration (MBC) for index of antibiotic and were measured chemical compound with GC-MS.

Essential oils were extracted from 4 samples of 2 species of the *Thymus* spp. and were measuredtheir yield (Table 1). The result showed that the thymus simple of No 10 had significant highest oil yields. In order to evaluation of antibacterial characteristic of essential oil of thymus species and compared with synthetic antibiotics we designed experiment with 6 treatments in complete randomized design (CRD).The inhibition zone diameter around each disk was measured by a ruler and it is shown below (Table 2).

Table 1. Used plant material and their oil yields

Simples No	Plant name	Oil yield (%)
1	*Thymus vulgaris*	0.577
2	“---”	0.367
3	*Thymus kotschyanus*	0.413
4	“---”	1.252

Table 2. Measuring bacterial growth inhibition zone diameter in relation to different antibiotics (mm).

Bacteria	Antibiotics				Essential oils	
	Pen.	**Gent.**	**Amp.**	**Nalidixic acid**	***Th. kotschyanus***	***Th. vulgaris***
S. aureus	10	19	25	0	27	24
E.coli	20	0	22	5	25	20

Table 3. Means of MIC and MBC on the antibiogram test of *Thymus kotschyanus* and *Th. vulgaris*

Bacteria sample	*Th. kotschyanus*		*Th. vulgaris*	
	MIC	**MBC**	**MIC**	**MBC**
S.aureus	0.001	0.010	0.001	0.01
E.coli	0.010	0.100	0.001	0.01

RESULTS AND DISCUSSION

In the present study, we have investigated the antimicrobial activity of *Thymus vulgaris* and *Th. kotschyanus* oils against *Staphylococcus aureus* and *Escherichia coli*.

Measuring bacterial growth inhibition zone diameter in relation to different antibiotics showed that the essential oil of *Th.kotschyanus* and *Th. vulgaris* inhibited of *S.aureus* and *E.coli* growth, in this result same the effect of ampicillin and highest control of these bacteria with thymus essential oil.Considering the results were sample of *Th. kotschyanus* and *Th. vulgaris* to test antibiogarm for determination of MIC and MBC, and the results were showed on the Table 3.

The results of antibiograms of 2 species of *Thymus* were accepted of results in exercise of measuring bacterial growth inhibition zone diameter.

In this project we resulted that essential oil of *Th.kotschyanus* and *Th. vulgaris* inhibited of *S.aureus* and *E.coli* bacterial growth (Table 2 and Table 3). It can be considering for their chemical compositions (for example see:Table 4).

Table 4. Chemical composition of *T. vulgaris* essential oil

Constituent	%
α-Pinene	3.3
Camphene	1.0
β-Pinene	0.6
Myrcene	1.7
p-Cymene	38.9
Limonene	0.8
1,8-Cineole	1.2
γ-Terpinene	0.3
Linalool	3.8
Thymol	46.6

***Peaks less than 0.1% were excluded.**

In our study accords to results, bacterial inhibition zone diameter indicates these oils lack any antibacterial effect. In the other hand, the essential oils of peppermint and Thymus vulgaris had not took apart in antibacterial effect, but the combination of these essential oils with antibiotics showed different results i.e the combination of three antibiotics (except penicillin) with *Thymus* oil showed different bacterial growth inhibition zone diameter against *S.aureus*, the combination of peppermint and antibiotics (except amoxicillin and nalidixic acid) showed different bacterial growth inhibition zone diameter against *S.aureus.* Other studies showed different results and the importance of these essential oils.

Correlating structure with activity, it can be speculated that the fungicidal and/or fungistatic activity of the essential oil of *Th. vulgaris* can be attributed to thymol, its principal constituent, especially the hydroxyl group of this compound, since *p*-cymene (benzene), the second major component, does not possess substantial antifungal activity. This would explain the lower potency of the oil when compared to thymol, supporting the idea that the efficacy of essential oils depends on its chemical composition, mainly phenolic components. These results are of great importance, because they facilitate the utilization of individual components, instead of a mixture, giving more predictability and probably less collateral effects.

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Dərman Bitkilərinin Efir Yağlarının Antibakterial Təsiri

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Təqdim olunan işin məqsədi potensial olaraq dörd antibiotikin (gentamisin, penisilin, nalidiks turşusu və amoksisilin) antimikrob fəallığına malik iki növ kəklikotu (*Thymus vulgaris* L.və *Th. kotschyanus* Boiss.et Hohen.) efir yağlarının antimikrob fəallığının qiymətləndirilməsi olmuşdur. Seçilən efir yağları disk diffuziya metodundan istifadə etməklə qram-mənfi *Escherichia coli* və qram-müsbət *Staphylococcus aureus* bakteriyalarına qarşı skrininq olunmuşdur. Bakterial inhibirləşmə zonasının tədqiqindən aldığımız nəticələrə görə bu yağların heç bir antibakterial təsiri yoxdur.

Açar sözlər: *Antibiotiklər, antimikrob fəallıq, kəklikotu, efir yağı, qram-mənfi bakteriya, qram-müsbət bakteriya*

Антибактериальное Действие Эфирных Масел Лекарственных Растений

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Целью настоящего исследования была оценка антимикробной активности эфирных масел двух видов рода Тимьян (тимьян обыкновенный – *Thymus vulgaris* L. и тимьян Кочи – *Th. kotschyanus* Boiss. et Hohen.) с потенциальной антимикробной активностью четырех антибиотиков (гентамицина, пенициллина, налидиксовой кислоты и амоксициллина). С помощью диск-диффузионного метода, выбранные эфирные масла были подвержены скринингу против грамотрицательных (*Escherichia coli*) и грамположительных (*Staphylococci saureus*) бактерий. По результатам наших исследований, согласно диаметрам зон бактериального ингибирования, эти масла не обладают каким-либо антибактериальным эффектом.

Ключевые слова: *Антибиотики, антимикробная активность, тимьян, эфирное масло, грамотрицательная бактерия, грамположительная бактерия*