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Comparing of Plant Antibacterial Essential Oils With Unique Synthetic Antibiotics on Different Bacteria

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In the present investigation antimicrobial activity of three different plant essential oils i.e., *Thymus vulgaris, Th. serphyllum* and *Mentha longifolia* oils have been evaluated. After bioassays, gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria i.e., *Escherichia coli* and *Pseudomonas aeruginosa* were found susceptible to most of the essential oils. For screening of antimicrobial susceptibility in each essential oil, both positive and negative controls were set to determine MIC (minimum inhibitory concentration), MBC values (minimum bactericidal concentration) and growth inhibition zone diameters. Among all essential oils *Th. vulgaris* oil was found to be highly bactericidal, as it has shown lowest MIC and MBC values and high growth inhibition zone diameter in comparison to antibiotics. Present study reveals significantly higher broad-spectrum antibacterial activity in essential oils than antibiotics i.e., penicillin, gentamicin, amoxicillin and nalidix acid.

Key words: *Essential oil, Thymus, antibacterial activity*

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe et al., 2004). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary health care. Medicinal and aromatic plants which are widely used as medicine constitute are a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt et al., 2004). Some oils have been used in cancer treatment (Sylvestre et al., 2006). Some other oils have been used in food preservation (Faid et al., 1995), aromatherapy (Buttner et al., 1996) and fragrance industries (Van de Braak et al., 1999). Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau et al., 2004). Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential (Darokar et al., 2006). Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is the most common used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market (Van de Braak et al., 1999). Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. Essential oils such as aniseed, thyme, calamus, camphor, cedar wood, cinnamon, citronella, clove, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver and wintergreen have been traditionally used by people for various purposes in different parts of the world. Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity (Ouattara et al., 2006); cinnamon oil also possesses antidiabetic property (Tepe et al., 2004). Peppermint and orange oils have shown anticancer activity (Kumar et al., 2004). In case of thymemany pharmacological *in vitro*experiments carried out during the last decade’s revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma (Inouye et al., 2001). Thyme is employed to season and suppress offensive odors, such as trimethylamine odor, in foods. The essential oil is well recognized for its medicinal properties in the treatment of bronchitis, whooping cough and toothache. The herb or its infusion is also given for several disorders. It is possible that the flavonoids present maybe important, such as in the spasmolytic activity of the smooth muscles guinea pig ileum and trachea. It was found that the main component of the essential oil were thymol and carvacrol and it had antimicrobial activity against fungi (some aflatoxins producers), viruses, helminthes, gram-positive bacteria (included *C.botulinum*) and gram-negative bacteria. In the present study antimicrobial potential of four antibiotics (Gentamicin, Penicilin, Nalidix acid and Amoxicilin) and different plant essential oils (thyme and peppermint) was screened against three pathogenic bacterial strains i.e., *Staphylococcusaureus* (PTCC 1112), *E. coli* (PTCC 1399) and *Pseudomonas aeruginosa* (PTCC 10)in various antibacterial bioassays. For antimicrobial susceptibility of each essential oil, MIC value, MBC value and growth inhibition zone diameters were determined.

MATERIAL AND METHODS

In this research the studied Microbiological Methods are provided from Industrial Scientific Research Center of Iran. First, Mueller Hinton broth medium was used to prepare appropriate concentration of suspension of the bacteria strain. Then the strains were incubated for 24 h at 37°C, and Mueller Hinton agar medium was used to study anti-bacterial activity. First, 10 µg of each essence were placed on sterile standard papers under aseptic conditions. Thence, it was kept in Incubator for 6 h to dry completely. Then antibiogram was applied by using standard antibiotic discs and negative control discs. In the next stage, tube dilution test was performed to determine MIC and MBC levels only for essences with growth inhibition zone and anti-bacterial properties, and 0.00000001 µg/ml… 0.0001 µg/ml, 0.001 µg/ml, 0.1 µg/ml, 0.1 µg/ml, 1.0 µg/ml concentrations of each were prepared. Then 10 ml of prepared bacterial suspension (equivalent to 5 McFarland) was added to each tube. Tubes were kept for 24 h at 37°C. After 24 h turbidity of tubes was examined. The tube with appearance of turbidity was considered MIC, and for further examination all tubes were cultured in solid medium (MHA) under aseptic conditions, and after placing in Incubator the culture results were studied.

RESULTS AND DISCUSSION

The results of the bacterial inhibition zone diameter with unique codes using paper disk method are shown in table (1) and the results of it using tube dilution method are shown in table (2).

Considering the results, n-4, n-2, and 1 specimen were selected for antibiogram testing using dilution method to determine MBC and MIC levels.

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-Balchin et al., 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 et al., 2006) especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied.

Table 1. Mean bacterial inhibition zone diameter ratio to antibiotics and essence with unique code (in mm).

Bacteria sample	Antibiotics				The essence of the plants					
	penicillin	gentamicin	amoxicilin	nalidix	20	1	n-4	n-5	n-2	10
S.aureus	10	19	25	0	5	27	24	6	15	10
E.coli	20	0	22	5	0	25	20	5	15	5
P.aeruginosa	12	0	18	0	0	18	15	5	11	5

**Thymus vulgaris*=(1), *Th. vulgaris*=(n-2), *Th. vulgaris*=(n-4), *Th. vulgaris*=(10), *Th.serphyllum*=(20) and *Mentha longifolia*=(n-5).

Table 2. Mean MBC and MIC of each essence (µg/ml)

Bacteria sample	1		n-2		n-4	
	MIC	MBC	MIC	MBC	MIC	MBC
S.aureus	0.001	0.010	0.0001	0.001	0.001	0.010
E.coli	0.010	0.100	0.001	0.010	0.001	0.010
P.aeruginosa	0.001	0.010	0.010	0.100	0.001	0.010

**Thymus vulgaris*=(1), *Th. vulgaris*=(n-2), *Th. vulgaris*=(n-4)

The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak (Zaika, 2000). 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 et al., 2005; Sikkema et al., 2007). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Denyer and Hugo, 2008). Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria (Kordali et al., 2005).

Present study reveals the antimicrobial susceptibility of essential oils against three bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.* It is proved by low MIC and MBC values obtained in essential oils whenused against each bacterial culture. Among all essential oils, *Thymus vulgaris* (n-2) essence has shown lowest MIC values (0.0001:µg/ml) against *Staphylococcus aureus* while *Thymus vulgaris* (n-4) has shown 0.001: µg/ml MIC value against all three bacteria strains (Table 2). *Th.vulgaris* (1) oil has shown anti-microbial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* at a 0.001: µg/ml concentration. Besides this, *Th.vulgaris* oil (n-2) was found highly bactericidal to *Staphylococcus aureus*, which was proved by low MBC values obtained i.e. in a range of 0.001: µg/ml (Table 2). More specifically all essential oils tested have shown lowest MBC values than MIC values against each bacterial strain. Finally, essential oils have shown significantly very low MIC and MBC values in comparison to broad-spectrum antibiotic drugs. This suggests that the essential oils are highly bactericidal. Similar MIC values are also reported in juniper oil (70% v/v) (Pepeljnjak et al., 2005) against Gram +ve and Gram -ve bacteria. It has also shown stronger fungicidal activity against *Candida* sp. (MIC from 0.78 to 2% v/v). Similarly garlic oil, garlic powder and diallyl sulfur components have shown MIC and MBC values in a range of 8 to 32: l/ml and 16 to 32: l/ml against *Helicobacter pylori* (Gara et al., 2000). Few essential oils obtained from different *Mentha* species, i.e. *Mentha longifolia* L., *Mentha aquatic* and *Mentha piperita.* L. (Mimica-Dukic et al., 2003), *Hypericum scabrum, Hypericum scabroides* and *Hypericum triquetrifolium* exhibited broad-spectrum antibacterial activity against disease pathogens (Kizil et al., 2004). Similarly *Dracocepha lumfoetidum* essential oil also exhibited strong antibacterial activity against menthicilin resistant *Staphylococcus aureus* (MRSA) at a very low MIC value i.e., 26-2592: l/ml (Lee et al., 2007). Besides essential oils, oil constituents such as allicin and diallyl sulfur (Gara et al., 2000), luteolin (Brenes et al., 1999), thymus (Shin and Kim, 2005), phenolics (Eduardo-Medina et al., 2006) and carvacrol (Burt et al., 2005) isolated from various essential oils have also shown stronger antimicrobial activity against few bacteria. Olive oil and its phenolic constituents showed antimicrobial activity against food borne pathogens such as *Helicobacter pylori* (Romero et al., 2007). In the present study, essential oils have show n nearly equal antimicrobial effects on both gram positive and gram-negative bacteria in suspension culture. *Thymus vulgaris*, *Thymus serphyllum* and *Mentha longifolia* oils was found to be the most effective. However, inhibition zone diameters obtained in filterpaper disc diffusion assays have shown better effectiveness of essential oils against Gram-positivebacteria.It may be due to volatile action of essential oils and due to absence of lipopolysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming biomolecule (Inouye et al., 2001; Delaquis et al., 2002). There might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive ion leakage (Lambert et al., 2001; Walsh et al., 2003). It may also happen due to hydrophilic nature of bacterial cell wall (Knobloch et al., 1986). In the present study, almost all essential oils tested have shown strong antibacterial potential against pathogenic bacteria.

CONCLUSION

This study emphasizes antimicrobial properties of plant essential oils against human pathogenic bacteria. It has been observed that all the essential oils possess both bacteriostatic and bactericidal activity much higher than that of synthetic antibiotics when tested *in vitro*. These essential oils may be effective on other Gram-ve and Gram+ve bacteria. More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Hence, essential oils and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

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Antibakterial Bitki Efir YağlarınınVə Unikal Sintetik Antibiotiklərin Müxtəlif Bakteriyalara Qarşı Təsirinin Müqayisəli Tədqiqi

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Tədqiqat işində üç müxtəlif bitkinin (*Thymus vulgaris, Th. serphyllum* və *Mentha longifolia*) efir yağlarının antimikrob fəallığı tədqiq edilərək qiymətləndirilmişdir. Bioloji analizlərdən sonra məlum olmuşdur ki, *Staphylococcus aureus* kimi qram-pozitiv və *Escherichia coli* və *Pseudomonas aeruginosa* kimi qram-neqativ bakteriayalar efir yağlarının əksəriyyətinə qarşı həssasdırlar. Hər iki tip bakteriyaların hər bir efir yağına qarşı həssaslığının skrininqi üçün MİQ (minimal inhibirləşdirmə qatılığı (MİC)), MBQ (minimum bakterisid qatılığı (MBC)) və inkişafın inhibirləşmə zonasının diametri kimi parametrlər təyin olunmuşdur. Tədqiq olunan efir yağları içəricində *Th. vulgaris*-in efir yağı sintettik antibiotiklərlə müqayisədə ən aşağı MİQ, MBQ-yə və ən böyük inkişafın inhibirləşmə zonasının diametrinə malik olduğu üçün ən yüksək bakterisidlik nümayiş etdirmişdir. Hazırkı tədqiqatın nəticələri göstərir ki, bitki mənşəli efir yağları penisillin, gentamisin, amoksisillin və nalidiks turşusu kimi sintetik antibiotiklərlə müqayisədə əhəmiyyətli dərəcədə daha geniş antibakterial fəallıq spektrinə malikdirlər.

Açar sözlər: *Efir yağı, Thymus, antibakterial fəallıq*

Сравнение Антибактериальной Активности Эфирных Масел Растений И Уникальных Синтетических Антибиотиков Против Различных Бактерий

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Данное исследование посвящено оценке антимикробной активности эфирных масел трех различных растений (*Thymus vulgaris, Th. serphyllum* и *Mentha longifolia*). После биоанализов выявлено, что такие грам-положительные бактерии, как *Staphylococcus aureus* и грамотрицательные бактерии, как *Escherichia coli* и *Pseudomonas aeruginosa* чувствительны к большинству тестированных эфирных масел. Для скрининга антимикробной активности каждого эфирного масла против обоих типов бактерий были определены параметры МИК (минимальная ингибирующая концентрация (МИС)), значения МБК (минимальная бактерицидная концентрация (МВС)) и диаметры зон задержки роста. Среди исследованных эфирных масел, масло *Th.vulgaris*, проявляя наиболее низкие значения МИК и МБК и наибольший диаметр зоны задержки роста, по сравнению с синтетическими антибиотиками, характеризовалось наивысшей бактерицидностью. Результаты данного исследования показывают, что растительные эфирные масла обладают значительно более широким спектром антибактериальной активности, чем такие синтетические антибиотики, как пенициллин, гентамицин, амоксициллин и налидиксовая кислота.

Ключевые слова: *Эфирное масло, Thymus, антибактериальная активность*