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The Determination of the Antibacterial Activities of Rose, Thyme, Centaury and Ozone Oils Against Some Pathogenic Microorganisms

Ozon, Kantaron, Kekik ve Gül Yağlarının Bazı Patojenik Mikroorganizmalara Karşı Antimikrobiyal Aktivitelerinin Belirlenmesi

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ABSTRACT

AIM: *The increase in antibiotic-resistant bacteria has dramatically revived the interest in plant products as alternative antimicrobial agents to prevent the efficiency of pathogenic microorganisms. Our aim in this study is to show the antimicrobial activities of commercially obtained thyme, rose, centaury and ozone oils against the clinically important bacteria and yeasts.*

METHODS: *The antimicrobial activity of the thyme, rose, ozone and centaury oils were tested against Escherichia coli, Proteus vulgaris, Proteus mirabilis, Stenotrophomonas maltophilia, Enterococcus spp., Acinetobacter baumannii, Streptococcus spp., Citrobacter freundii, Staphylococcus aureus and Candida albicans strains. Disc diffusion method (Kirby-Bauer) was used to show the antimicrobial activity by measuring the zone diameters.*

RESULTS: *Most bacteria including Stenotrophomonas maltophilia (which is only sensitive to a few antibiotics) are found sensitive to the thyme oil. Gram positive bacteria and yeasts found more resistant than the Gram negative bacteria to the thyme oil. Escherichia coli and Staphylococcus aureus found sensitive to the rose oil. The anti-microbial activities of some herbal oils and ozone oil and rose oil were tried to be shown.*

CONCLUSION: *The thyme oil has a stronger antimicrobial activity than the rose, ozone and centaury oils. Herbal essential oils, especially thyme oil, are candidates to be alternatives in medical applications due to their anti-microbial effects.*

Key words: *bacteria; antimicrobial activity; disc diffusion method; herbal oils*

ÖZET

AMAÇ: *Antibiyotiklere dirençli bakterilerin sayısındaki artış, tedavi basamağında alternatif bitkisel ürünlerin kullanılmasına olan ilginin dramatik bir şekilde artışına da sebep olmuştur. Bu çalışmadaki amacımız, ticari olarak elde ettiğimiz ozon, gül, kantaron ve kekik yağlarının klinik olarak önemli bakteri ve mantarlara karşı olan antimikrobiyal etkilerinin gösterilmesidir.*

YÖNTEM: *Ozon, gül, kantaron ve kekik yağlarının Escherichia coli, Proteusvulgaris, Proteusmirabilis, Stenotrophomonas maltophilia, Enterococcus spp., Acinetobacter baumannii, Streptococcus spp., Citrobacter freundii, Staphylococcus aureus ve Candida albicans isimli mikroorganizmalara karşı antimikrobiyal aktiviteleri test edilmiştir; Disk difüzyon metodu (Kirby-Bauer) kullanılmış ve oluşan zon çapları ölçülerek anti-mikrobiyal etki ortaya konulmuştur.*

BULGULAR: *Sadece birkaç antibiyotiğe karşı duyarlı olan Stenotrophomonas maltophilia başta olmak üzere birçok bakteri, kekik yağına karşı duyarlı olarak tespit edilmiştir. Gram pozitif bakterilerin ve Kandida suşlarının kekik yağına, Gram negatif bakterilerden daha dirençli oldukları görülmüştür. Bunun yanı sıra Escherichia coli ve Staphylococcus aureus suşlarının ise gül yağına karşı duyarlı oldukları tespit edilmiştir. Bazı herbal yağların ve ozon yağının muhtemel antimikrobiyal etkileri bu çalışma ile ortaya koyulmaya çalışılmıştır.*

SONUÇ: *Kekik yağının antimikrobiyal etkinliği ozon, gül ve kantaron yağından oldukça yüksek düzeyde tespit edilmiştir. Sonuç olarak başta kekik yağı olmak üzere bitkisel uçucu yağlar, antimikrobiyal aktivitelerinden dolayı tıbbi ilaç uygulamalarına iyi bir alternatif olabilme potansiyeli taşımaktadır.*

Anahtar kelimeler: *bakteriler; anti-mikrobiyal aktivite; disk difüzyon testi; bitkisel yağlar*

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Introduction

A big percentage of the population uses herbal products for preventative and therapeutic purposes. The increase in antibiotic-resistant bacteria has dramatically revived the interest in plant products as alternative antimicrobial agents to prevent the efficiency of pathogenic microorganisms. A major group of plant antimicrobial compounds is represented by essential oils, which are complex mixtures of volatile secondary metabolites. They are mostly used in the food industry because of their preservative activity against food-borne pathogens, thanks to their antimicrobial, antibacterial, and antifungal properties[1,2].

Plant essential oils are generally isolated from non-woody plant material by distillation methods, usually by evaporation or hydro-distillation. These oils contains variable mixtures such as terpenoids, specifically monoterpenes [C10] and sesquiterpenes [C15] although diterpenes [C20] is also present, and a variety of low molecular weight aliphatic hydrocarbons acids (linear, ramified, saturated and unsaturated), alcohols, aldehydes, acyclic esters or lactones, nitrogen- and sulphur-containing compounds and homologues of phenyl-propanoids in their ingredients. Terpenes are one of the important chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants[3].

Hypericumperforatum (centaury oil) is one of the best-studied medicinal plants all over the world and its chemical ingredients are well-characterized. The phytopharmaceuticals based on standardized extracts have been approved against mild to moderate depression and for the short-term treatment of symptoms in mild depressive disorders. Moreover, *Hypericumperforatum* can be effective in the treatment of somatoform disorders, anxiety disorder, sleep disorders, obsessive compulsive disorder and seasonal affective disorder[4–7].

In recent years, interest in natural products has increased, and medicinal plants have been investigated for various biological activities and therapeutic potentials[8]. Oil of thyme is derived from thyme, also known as *Thymus vulgaris*. Thyme also has a number of medicinal properties, which is due to the herb's essential oils. The health benefits of thyme essential oil can be attributed to its properties as an antispasmodic, antirheumatic, antiseptic, bactericidal, cardiac, carminative, cicatrisant, diuretic, expectorant, hypertensive, insecticide, stimulant and tonic substance. Oil of *Thymus vulgaris* has been shown to exhibit antimicrobial activities against pathogenic microorganisms[9,10].

Rosa damascena is popular in the world for its perfume[11]. This plant has several therapeutic effects such as treatment of menstrual bleeding, digestive problems, anti-inflammatory, the analgesic, anticonvulsant, antitussive, and bronchodilatory effects[12,13]. Addition to these activities, rose oil (*Rosa damascena*), has an antimicrobial performance against some microorganisms[14].

And the final oil which is used in this study is ozone oil. The biocidal activity of ozone reveals by a combination of its high oxidation potential, reacting with organic-material up to 3,000 times faster than chlorine, and its ability to diffuse through biological cell membranes[15].

Our aim in this study is to show the antimicrobial activities of these herbal oils and compare the possible antimicrobial effects.

Method

The essential oils of thymus, rose, centaury and ozone were screened for antimicrobial activity using an agar diffusion technique (Kirby-Bauer Disc Diffusion Method) against the following pathogenic microorganisms; *Escherichiacoli, Proteus vulgaris, Proteus mirabilis, Stenotrophomonasmaltophilia, Enterococcus faecalis, Acinetobacter baumannii, Streptococcus spp, Citrobacterfreundii, Staphylococcusaureus and Candidaalbicans.*

At first, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) were standard bacterial strains which provided from American Type Culture Collection (ATCC). Then, other microorganisms were isolated from the samples by using Automatic Bacteria Identification Machine (VITEK-2 Compact System, BioMerieux, France). Then identified bacteria were cultured with stock medium and stored at -80°C till the experiment day.

The fresh passages were performed before the study and for the inoculum, colonies were selected from 18-24 h old plates. Turbidity was visually adjusted to that of a 0.5 McFarland turbidity standard (1.5×10 ~ CFU/ml) using sterile Mueller-Hinton broth. Sterile filter paper disks were prepared to a diameter of 6.35 mm and sterilized in a Pasteur-oven, (at 170 ~ for 2 hour). On the other hand, essential oils and extracts were sterilized by passing through 0.22 mm pore-size membrane filters and then 20 HI (0.02 ml) of the solution of essential oils was pipetted (0.1 ml) into the center of each disk to achieve the desired potency. By the way, the herbal oils were commercially provided. The manufacturer extraction protocol is seen in the following sentence: "It is extracted from the fresh or partly dried flowering tops and leaves of the plant by water or steam distillation and the yield is 0.7–1.0%". Also, the concentration of herbal oils were 100%.

To compare the antimicrobial activity of these oils, we used some standard commercial antibiotics onto these microorganisms such as Ampicillin 25µg (Oxoid, USA), Imipenem 10µg (Oxoid, USA), Gentamycin30µg (Oxoid, USA), Cefoxitine 30µg (Oxoid, USA) and Flucanozole 25µg (Oxoid, USA).

Disks were air-dried in a contamination free environment. *E.coli, P.vulgaris, P.mirabilis, S.maltophilia, E. faecalis, A.baumannii, Streptococcus spp, C.freundii, S.aureus and C.albicans* were swabbed onto the surface of Mueller-Hinton agar plates by rotating the plates approximately with a sterile cotton swab. Inoculated plates were allowed to stand for at least 3 minutes before applying antimicrobial disks.Disks were not placed closer to each other than 24 mm, measured from center to center. Plates were incubated at 37°C for 18–24 h. After overnight incubation, the diameter of the zone of inhibition around each disk was measured in mm. The measurement was performed by a scale and evaluated by 2 different microbiologists. The obtained data were compared with the standard antibiotics zone diameters which were evaluated according to The Clinical and Laboratory Standards Institute (CLSI) criteria.

Results

Antimicrobial activities of rose, thyme, centaury and ozone oils were determined by agar diffusion against nine pathogenic bacteria and yeasts. On the other hand, standard commercial antibiotics were also applied to the same pathogens. Rose oil was only effective on *E.coli* and *S.aureus* while centaury oil and ozonized oil have no antimicrobial effects of all microorganisms. However, rose oil zone diameters were quite low when compared with standard commercial antibiotics. Additionally, the thyme oil has a stronger antimicrobial activity than other oils, when the data were evaluated. It has no antimicrobial effects only on *A.baumannii* and *Candida albicans.* The biggest effect of thyme oil was detected on *Citrobacter* and *Streptococcus* strains. The zone diameters were 24 and 21 mm respectively. On the other hand, thyme oil had higher zone diameters from Gentamycin and nearly same diameters for cefoxitin (Table 1).

Table 1. *The data about the antimicrobial activity of oils*

	Bacteria									
Oils	E.coli	Proteus vulgaris	Proteus mirabilis	S.maltophilia	E.faecalis	Acinetobacter baumannii	Streptococcus spp.	Citrobacter freundii	S.aureus	Candida albicans
Rose	Zone ≥5 mm	No zone	No zone	No zone	No zone	No zone	No zone	No zone	Zone ≥3 mm	No zone
Thyme	Zone ≥19 mm	Zone ≥17 mm	Zone ≥17 mm	Zone ≥20 mm	Zone ≥19 mm	No zone	Zone ≥21 mm	Zone ≥24 mm	Zone ≥17 mm	No zone
Centaury	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
Ozone	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
Ampicilin	≥28 mm	≥21 mm	≥20 mm	Not applied	≥18 mm	Not applied	≥24 mm	≥26 mm	≥33 mm	Not applied
Imipenem	≥23 mm	≥21 mm	≥19 mm	Not applied	Not applied	≥16 mm	≥22 mm	≥20 mm	≥18 mm	Not applied
Gentamycin	≥18 mm	≥17 mm	≥15 mm	Not applied	Not applied	≥14 mm	Not applied	≥19 mm	≥15 mm	Not applied
Cefoxitin	≥19 mm	≥20 mm	≥22 mm	Not applied	Not applied	Not applied	Not applied	≥23 mm	≥25 mm	Not applied
Flucanozole	Not applied	Not applied	Not applied	Not applied	Not applied	Not applied	Not applied	Not applied	Not applied	≥18 mm

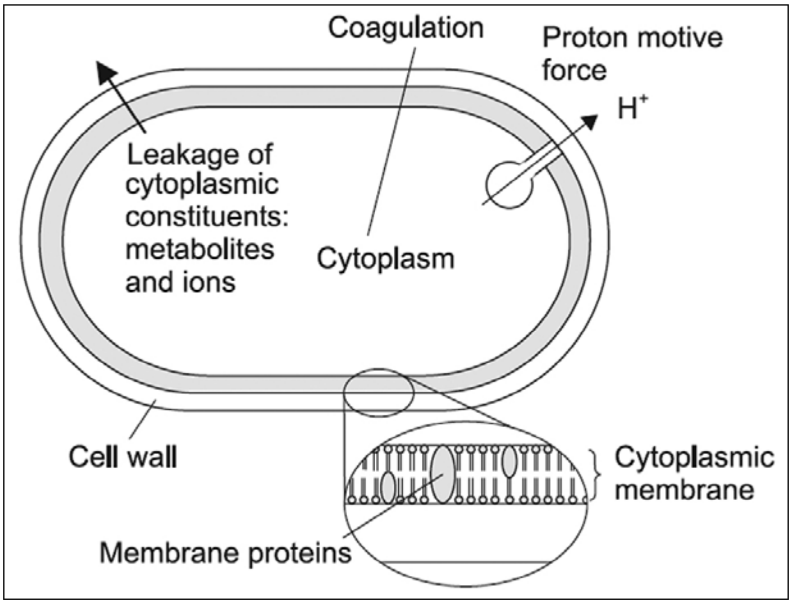

Figure 1. *Essential oils components effects: degradation of the cell wall, damage tocytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm,and depletion of the proton motive force*[25].

Some antimicrobial agents which are routinely used in Microbiology Laboratories were used for this study to compare the antimicrobial activities. Ampicillin was the antimicrobial agent which had the largest diameter zones. Also imipenem had larger zones after ampicillin. At that point, it was seen that thyme oil had close diameter zones with these two important antibiotics. In addition, thyme oil was effective to all bacteria except *Acinetobacter baumannii*.

Discussion

One of the alternative strategies to eliminate antibiotic-resistant bacteria is the use of natural antimicrobial substances such as plant essential oils and their components[16]. On the other hand comparing the antibacterial effect of these plants is important for choosing the most appropriate ones. In this study, the effects of rose, thyme, centaury and ozone oils were determined and compared against 9 different pathogenic and resistant microorganisms.

The results of the present study indicate that the thyme oil has important antimicrobial effects on some pathogenic microorganisms. In our country, thyme was traditionally used to treat medical symptoms such as coughing, upper respiratory congestion, gastritis, bronchitis, spasm, sprains, stomach cramps, dysmenorrhea, dyspepsia, and urinary tract infection (Figure 1)[17]. Because of the ingredient of *Thymus vulgaris* (Thymol 10–64%, Carvacrol 2–11%, g-Terpinene 2–31%, p-Cymene 10–56%), it may has a strong antimicrobial activity as seen in this study[18,19]. It seems reasonable that mechanism of action of thyme oil would therefore be similar to other phenolic; this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents[20].

Other herbal oils were also evaluated and it was seen that rose oil has an antimicrobial activity. However, this activity was limited with 2 microorganisms. In the current literature, antibacterial effect of major components of rose oil (citronellal, geraniol and nerol) was reported previously[21–24]. Rose oil is a volatile oil obtained by distillation of the fresh flowers of *R.damascena*. The chief producing countries are Bulgaria, Turkey and Morocco[11]. The identified compounds from rose oils were; β-citronellal (14.5–47.5%), nonadecane (10.5–40.5%), geraniol (5.5–18%), and nerol and kaempferol were the major components of the oil[25]. The in vitro antibacterial activities of essential oil from R.damascene were also shown by disk diffusion testing against *E.coli, S.aureus and P.aeruginosa*. R.damascene showed antimicrobial activity against S.aureus in some studies[21]. Our results suggest that essential oils have potential use as antimicrobials especially thyme and rose oils. Essential oils and main components of some of these oils, such as carvacrol, citronellal, geraniol, and nerol have been previously reported to have antibacterial effects[26].

On the other hand, there were no antimicrobial effects for centaury and ozone oils .n this study. No zone diameters were detected for these 2 oils. However, there must be 2 reasons to explain this issue. First one; the bacteria used in this study were selected from the most resistant to the most antibiotics and Extended spectrum beta-lactamase (ESBL) positives. And the second reason is that the oil dose might be inadequate. So, new doses experiments should be performed to put out the real data about the antimicrobial activities of ozone and centaury oils.

In conclusion, our study showed that because of strong antibacterial effects of the thymus and rose oil (Table 1). These oils can be used in treatment process as an alternative application structures. The intensive use of antibiotics has often resulted in the development of resistant strains.As found in this study,plant essential oils may be an alternative treatment options and may be used for elimination of some bacteria.

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