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The antibacterial and antifungal activities of commonly used herbal oils

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ABSTRACT

The antibacterial and antifungal activities of herbal oils and their derivatives has been studied for several years; however, more studies are needed to develop alternative strategies to destroy pathogenic microorganisms due to increasing concerns about the development of antimicrobial resistance amongst them. In this study, our aim was to investigate the minimal inhibitory concentrations (MIC) of 23 different commercially available herbal oils on both yeasts and bacteria strains. Twenty three commercially available herbal oils including centaury, ginger, curcumin, eucalyptus, black cumin, cinnamon, sesame, rosemary, safflower, cardamom, argan, thyme, etc. were used to determine the antibacterial and antifungal activities on both yeasts and bacteria (standard ATCC strains). *Candida albicans, Candida parapsilosis, Candida glabrata* from yeasts, *Escherichia coli* from gram-negative bacteria, *Acinetobacter baumannii* from non-fermentative bacteria, and *Staphylococcus aureus* from gram-positive bacteria were selected. The effective MIC values of herbal oils were detected by using resazurin microtiter assay plate (REMA) technique. All herbal oils were effective on standard bacteria and yeast strains in different concentrations. The effective concentration ranges of herbal oils on each bacteria and yeast were as following; 15.625-31.25 µg/ml for *Candida parapsilosis* (ATCC 22019), 15.625-125 µg/ml for *Acinetobacter baumannii* (ATCC 49139), 31.25-62.5 µg/ml for *Candida albicans* (ATCC 14053), *Candida glabrata* (ATCC 15126), and *Staphylococcus aureus* (ATCC 29213), 62.5-125 µg/ml for *Escherichia coli* (ATCC 25923). In conclusion, antimicrobial capacities of some herbal oils that provide alternative solutions to pathogen microorganisms inhibition, which are made more difficult due to widespread resistance to antimicrobial agents, were evaluated in this study. We believe that this study will contribute to other related studies on the identification of herbal oil antimicrobial mechanisms of action.

1. Introduction

The percentage of infectious diseases causing human deaths is quite high. On the other hand, the widespread resistance to antimicrobials among pathogenic microorganisms poses a serious threat in the treatment of microbial diseases (Maurice et al., 1990; Faydalıoğlu and Sürücüoğlu, 2011). This resistance has led to a necessity for new strategies in the treatment or prevention of infectious diseases (Ozmen et al., 2015; Karamese et al., 2016). At that point, the use of plant extracts and herbal oils as a natural product source for combating resistant and/or non-resistant microorganisms offers alternative solutions (Prabuseenivasan et al., 2006). The World Health Organization (WHO) reported that the traditional medicine for primary healthcare has been preferred by the majority of the world's

population. Medicinal and aromatic plants are a major source of natural organic compounds widely used as medicine (Solmaz and Ata, 2009). Herbal oils which are natural, concentrated, volatile aromatic compounds isolated from plants have some preventive/therapeutic effects including antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. The number of plants used to provide an alternative solution against antibiotic resistance is quite high. In current literature, the most known and used commercially available herbal oils were obtained from *Pinus terebenthinae, Copaifera officinalis, Salvia officinalis, Cedrus libani, Aesculus hippocastanum, Hypericum perforatum, Santalum album, Foeniculum vulgare, Lavandula stoechas, Urtica dioica,* and *Citrus bergamia* plants (Burt, 2004; Kordali et al., 2005; Mickiene, 2011; Bilenler and Gökbulut, 2019). The bioactive compounds of these plants may involve multiple modes of antimicrobial action including changes in the synthesis of DNA and RNA, degradation of bacterial cell-wall, disruption the structure of cytoplasmic membrane, changes in the level of fatty acid and phospholipid constituents, and destruction of protein translocation. Hence, it is possible to use the herbal oils for antimicrobial effects against pathogenic microorganisms (Lambert et al., 2001; Shan et al., 2007; Witkowska et al., 2013).

In the present study, antimicrobial activity of 23 most known and used commercially available herbal oils was investigated against gram-negative, gram-positive, non-fermentative and yeast strains for minimal inhibitory activity.

2. Materials and methods

Oils, microorganisms, and culture conditions

The herbal oils were purchased from Biotama Natural Products (Biotama, Ankara, Turkey). The names of plants and oils used in this study are seen in Table 1.

The initial concentration of herbal oils with different concentrations obtained to 1mg/ml by the dissolving in Dimethyl Sulfoxide (DMSO) and filtered through 0.22 µM membrane filters. Reference microbial strains of American Type Culture Collection (ATCC, USA) were used in this study. The antimicrobial activity of Gram-positive bacterial strain [*Staphylococcus aureus* (ATCC 29213)], Gram-negative bacterial strains [*Escherichia coli* (ATCC 25923), *Acinetobacter baumannii* (ATCC 49139)] and yeast strains [Candida albicans (ATCC 14053), *Candida glabrata* (ATCC 15126) and *Candida parapsilosis* (ATCC 22019)] were investigated. The bacterial strains were stored at -80ºC until the experiment day. Blood Agar and Sabouraud Dextrose Agar (SDA) supplemented with 8% glucose were used for production bacteria and yeast, respectively. Mueller Hinton Broth (MHB) for bacteria and Tryptic Soy Broth (TSB) for yeast were used to determine the minimum inhibitory concentrations (MIC). The mediums were sterilized with autoclave at 121°C for 15-20 minutes and prepared according to the manufacturer's instructions.

Table 1. The names of plants and oils used in this study.

No	Plants	Oils
1	*Hypericum perforatum*	Centaury oil
2	*Cinnamomum verum*	Cinnamon oil
3	*Simmondsia chinensis*	Jojoba oil
4	*Carthamus tinctorius*	Safflower oil
5	*Eucalyptus globulus*	Eucalyptus oil
6	*Ocimum basilicum*	Basil oil
7	*Nigella sativa*	Black cumin oil
8	*Argania spinosa*	Argan oil
9	*Jasminum nudiflorum*	Jasmine oil
10	*Thymus vulgaris*	Thyme oil
11	*Sesamum indicum*	Sesame oil
12	*Rosa canina*	Rosehip oil
13	*Urtica dioica*	Nettle oil
14	*Ricinus communis*	Indian oil
15	*Cananga odorata*	Ylang ylang oil
16	*Rosmarinus officinalis*	Rosemary oil
17	*Curcuma longa*	Turmeric oil
18	*Lilium candidum*	Lily oil
19	*Elettaria cardamomum*	Cardamom oil
20	*Zingiber officinale*	Ginger oil
21	*Foeniculum vulgare*	Fennel oil
22	*Syzygium aromaticum*	Clove oil
23	*Cuminum cyminum*	Cumin oil

Inoculum and resazurin preparation

The stock bacterial and yeast suspensions used for inoculation were prepared at 105 CFU/ml by diluting fresh cultures at McFarland 0.5 density in sterile tubes. Suspensions of bacteria at McFarland density was diluted 1:20. Suspensions of the yeast at McFarland density was diluted 1:50 and 1:20 respectively. Resazurin sodium salt powder was used. Resazurin is a non-fluorescent blue dye used to test samples for bacterial and yeast contamination. It is also useful in sperm viability and semen quality test. Resazurin is applicable in cytotoxicity determination. A working solution was prepared at a 0.01% (w/v) concentration in distilled water and a 0.22 µM membrane filter was used for filtration and sterilization procedures.

Resazurin microtiter assay (REMA)

A sterile 96-well microplates were used for determination MIC. A volume of 100 µl of test medium (TSB for yeast and MHB for bacteria) was pipetted into the each well of the microplate. The stock concentration of oils (1 mg/ml) were added into the first well of microplates and two-fold dilutions were performed.

Serial dilutions were performed using multichannel pipette. Finally, 10 µl of bacterial suspension and 100 µl of yeast suspension was added to each well. Microplates including bacteria and yeast were covered with lids and incubated at 37°C for 24-48 hours. After incubation, 10 ml of freshly prepared 0.01% resazurin solution was added to each well and the plates were re-incubated at 37°C at 24 hours. A growth control containing any oils and a sterile control without bacteria and yeast were also used. Any color changes from purple to pink were considered as positive (Fig. 1). REMA was carried out in triplicate (Nateche et al., 2006).

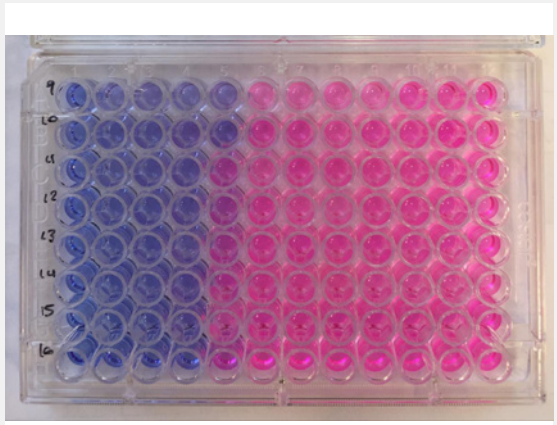

Fig. 1. Resazurin Microtiter Assay (REMA) performed in our study.

3. Results

The antibacterial and antifungal activities of 23 different herbal oils on six microorganisms are seen in Table 2. All herbal oils were effective on reference bacteria and yeast strains in different concentrations. The effective

Table 2. The MIC values of herbal oils on tested microorganisms.

	Minimal Inhibitory Concentrations (µg/ml) Microorganisms					
Herbal oils	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*
Hypericum perforatum (centaury oil)	62.5	31.25	62.5	31.25	62.5	15.625
Zingiber officinale (ginger oil)	62.5	62.5	62.5	31.25	31.25	15.625
Sesamum indicum (sesame oil)	125	31.25	62.5	31.25	31.25	15.625
Jasminum nudiflorum (jasmine oil)	125	62.5	62.5	31.25	31.25	15.625
Cuminum cyminum (cumin oil)	125	62.5	62.5	31.25	31.25	15.625
Ocimum basilicum (basil oil)	125	62.5	62.5	31.25	31.25	15.625
Nigella sativa (black cumin oil)	62.5	62.5	31.25	31.25	31.25	15.625
Cinnamomum verum (cinnamon oil)	62.5	62.5	31.25	31.25	31.25	15.625
Eucalyptus globulus (eucalyptus oil)	62.5	62.5	62.5	31.25	31.25	15.625
Rosmarinus officinalis (rosemary oil)	62.5	62.5	31.25	31.25	31.25	15.625
Argania spinosa (argan oil)	62.5	62.5	31.25	31.25	31.25	15.625
Foeniculum vulgare (fennel oil)	62.5	62.5	62.5	31.25	31.25	31.25
Rosa canina (rosehip oil)	62.5	62.5	62.5	31.25	31.25	31.25
Ricinus communis (indian oil)	62.5	62.5	62.5	31.25	31.25	31.25
Cananga odorata (ylang ylang oil)	62.5	31.25	62.5	31.25	31.25	31.25
Simmondsia chinensis (jojoba oil)	62.5	31.25	62.5	31.25	31.25	31.25
Carthamus tinctorius (safflower oil)	62.5	31.25	62.5	31.25	31.25	31.25
Curcuma longa (turmeric oil)	62.5	31.25	31.25	31.25	31.25	31.25
Elettaria cardamomum (cardamom oil)	62.5	31.25	31.25	31.25	31.25	31.25
Lilium candidum (lily oil)	62.5	31.25	31.25	31.25	31.25	31.25
Urtica dioica (nettle oil)	62.5	31.25	31.25	62.5	31.25	31.25
Syzygium aromaticum (clove oil)	62.5	15.625	62.5	62.5	31.25	31.25
Thymus vulgaris (thyme oil)	62.5	125	62.5	62.5	31.25	31.25

concentration ranges of oils on each bacteria and yeast were as following; 15.625-31.25 μg/ml for *Candida parapsilosis* (ATCC 22019), 15.625-125 μg/ml for *Acinetobacter baumannii* (ATCC 49139), 31.25-62.5 μg/ml for *Candida albicans* (ATCC 14053), *Candida glabrata* (ATCC 15126), and *Staphylococcus aureus* (ATCC 29213), 62.5-125 μg/ml for *Escherichia coli* (ATCC 25923). The most effective oils were centaury, ginger, sesame, jasmine, cumin, basil, black cumin, cinnamon, eucalyptus, rosemary, and argan oils for *Candida parapsilosis* (15.625 μg/ml); and black cumin, cinnamon, rosemary, argan, turmeric, cardamom, lily and nettle oils for *Staphylococcus aureus* (31.5 μg/ml). All tested herbal oils had antibacterial effects in different ranges on *Escheriehia coli* and *Acinetobacter baumannii*; however, this efficiency was less than the effect on other microorganisms. On the other hand, black cumin, cinnamon, rosemary, and argan oils were the most effective on all tested microorganisms (both bacteria and yeast strains).

4. Discussion

For centuries, herbal oils have been used extensively in different fields for the protection of foods, pharmaceuticals, medicine and natural therapeutic. In order to increase the quality in the field of health, it is essential to scientifically examine the herbal oils used in traditional medicine. Herbal oils have a high potential for the development of new antimicrobial agents. In our study, 20 herbal oils showed different rates of antimicrobial activity against six microorganisms (three bacteria and three yeasts).

In current literature, it has been reported that these herbal oils exhibit antibacterial activity on a variable scale against different microorganisms. Nostro et al., determined that plant extracts have inhibitory effects against some Gram (+), Gram (-) bacteria and yeast strains (Nostro et al., 2000). Another study reported that herbal oils obtained from eight different aromatic plants showed inhibitory effects on 11 different microorganisms (Sartoratta et al., 2004). Similarly, Witkowska et al. showed the efficiency of 30 different herbs and spices on *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas fluorescens* (Witkowska et al., 2013). They detected the MIC values of basil, cinnamon, cumin, fennel, ginger, rosemary, clove, thyme, and turmeric oils on *Escherichia coli* (40, 20-40, >40, >40, 20-40, 20-40, 5-10, 20-40, and 20-40 mg/ml-1, respectively) and *Staphylococcus aureus* (40, 20-40, >40, >40, >40, 20-40, 5-10, 20-40, and >40 mg/ml-1, respectively) standard bacteria strains. Our study shows similarity regarding current data about the antibacterial activities of herbal oils. In our study, the MIC value ranges were detected >40 μg/ml for *Escherichia coli*, and 31.25-62.5 μg/ml for *Staphylococcus aureus*.

Furthermore, two studies also reported the antimicrobial activities of rosemary, clove, cinnamon, cumin, eucalyptus, thyme, basil, fennel oils on 4 Gram (+) and 2 Gram (-) bacteria including *Escherichia coli* O157:H7 strain and expressed that the most effective ones were cloves, cinnamon, and rosemary oils (Ouattara et al., 1997; Roura et al., 2005). When compared to our study, the results were close to each other. Black cumin, cinnamon, rosemary, argan, turmeric, cardamom, lily and nettle oils were the most effective oils for Gram (+) bacteria.

On the other hand, the antifungal activity of herbal oils was also investigated in some researches. Çenet and Toroğlu showed antibacterial and antifungal activities of fennel, thyme, and ginger oils (Çenet and Toroğlu, 2006; Balkan et al., 2016). Another study performed in 2003 reported that some herbal oils had significant inhibitory effects on *Candida albicans* and *Candida vaginalis* yeast strains (Al-Howiriny, 2003). Furthermore, a group of researchers applied Kirby-Bauer disk diffusion test with herbal oils. They showed both antibacterial and antifungal effects of those; however, they observed that these oils were more effective in Gram (+) bacteria and yeast strains than Gram (-) bacteria (Dağcı et al., 2002). The centaury, ginger, sesame, jasmine, cumin, basil, black cumin, cinnamon, eucalyptus, rosemary, and argan oils were the most effective oils for *Candida* strains in our study. Similarly, Rabe and Staden studied the antimicrobial effects of 21 different herbal oils and reported that those were more effective on Gram (+) bacteria while no efficiency was detected on *Klebsiella pneumonia* (Rabe and Van Staden, 1997). Shan et al. performed a study about the same issue with 46 herbal oils on five food pathogen bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella anatum*) and detected the most of oils were more effective on Gram (+) bacteria (Shan et al., 2007).

Despite the widespread use of medical herbal oils in the fight against microorganisms, the mechanisms of antimicrobial action have not been fully defined. In literature, different approaches regarding the mechanism of action have been proposed; bacterial inhibition due to deterioration of membrane integrity, loss of cell content (molecules and ions) due to damage of selective permeable structure of membrane, secondary metabolites (phenolic compounds) in volatile oil composition causing damage to cell membrane, cell vital activities (energy production, protein synthesis) (Beyaz, 2014; Şengün and Öztürk, 2018; Bilenler and Gökbulut, 2019).

In conclusion, antimicrobial capacities of some herbal oils that provide alternative solutions to pathogen microorganism inhibition, which are made more difficult due to widespread resistance to antimicrobial agents, were evaluated in this study. We believe that

this study will contribute to other related studies on the identification of herbal oil antimicrobial mechanisms of action. For this reason, further detailed molecular studies should be performed.

Conflict of interest

The author declares that there is no conflict of interest.

REFERENCES

Al-Howiriny, T.A., 2003. Composition and antimicrobial activity of essential oil of Salvia lanigera. Pak. J. Biol. Sci. 6, 133-135.

Balkan, Ç.E., Karameşe, M., Çelebi, D., Aydoğdu, S., Dicle, Y., Çalık, Z., 2016. The determination of the antibacterial activities of rose, thyme, centaury and ozone oils against some pathogenic microorganisms. Kafkas. J. Med. Sci. 6, 18-22.

Beyaz, M., 2014. Essential Oils: Antimicrobial, antioxidant and antimutagenic activities. Academic Food Journal. 12, 45-53.

Bilenler, T., Gökbulut, İ., 2019. Determination of sensitivity of hospital pathogens against commercial essential oil. GUSTIJ . 9, 716-723.

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods. Int. J. Food. Microbiol. 94, 223-253.

Çenet, M., Toroğlu, S., 2006. Medical and aromatic plants' antimicrobial, antioxidant activities and use opportunities. KSU J. Eng. Sci. 9, 12-20.

Dağcı, E., İzmirli, K., Dığrak, M., 2002. A research of the antimicrobial activities of some tree species grown in Kahramanmaras City. KSU J. Eng. Sci. 5, 38-46.

Faydalıoğlu, E., Sürücüoğlu, M.S., 2011. History of the use of medical and aromatic plants and their economic importance. J. Kas. Forf. 11, 52-67.

Karamese, M., Guvendi, B., Aksak Karamese, S., Cinar, I., Can, S., Erol, H.S., Aydin, H., Gelen, V., Karakus, E., 2016. The protective effects of epigallocatechin gallate on lipopolysa ccharide-induced hepatotoxicity: An in vitro study on Hep3B cells. Iran. J. Basic. Med. Sci. 19, 483-489.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., Yildirim, A., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils. J. Agric. Food. Chem. 53, 9452-9458.

Lambert, R.J., Skandamis, P.N., Coote, P.J., Nychas, G.J., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91, 453-462.

Maurice, M.I., Angela, R.D., Chiris, O.O., 1990. New antimicrobials of plant origin. Perspectives on new crops and new uses. 8, 457-460.

Mickiene, R., Bakutis, B., Baliukoniene, V., 2011. Antimicrobial activity of two essential oils. Ann. Agric. Environ. Med. 18, 139-144.

Nateche, F., Martin, A., Baraka, S., Palomino, J.C., Khaled, S., Portaels, F., 2006. Application of the resazurin microtitre assay for detection of multidrug resistance in Mycobacterium tuberculosis in Algiers. J. Med. Microbiol. 55, 857-860.

Nostro, A., Germano, M.P., D'Angelo, V., Marino, A., Cannatelli, M.A., 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30, 379-384.

Ouattara, B., Simard, R.E., Holley, R.A., Piette, G.J., Begin, A., 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food. Microbiol. 37, 155-162.

Ozmen, E., Yazgi, H., Aktas, O., Uyanik, M.H., Aydin, H., 2015. The comparison of in-vitro activity of doripenem and other carbapenems on gram negative bacteria isolated from clinical samples. Türk. Mikrobiyol. Cem. Derg. 45, 83-87.

Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S., 2006. In vitro antibacterial activity of some plant Essential oils. Biomed. Cent. 6, 39-45.

Rabe, T., Van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81-87.

Roura, S.I., Valle, C.E., Ponce, A.G., Moreira, M.R., 2005. Inhibitory parameters of essential oils to reduce a food borne pathogen. -Lebensm. Wiss. Technol. 38, 565-570.

Sartoratta, A., Machado, A.L., Delarmelina, C., Figueria, G.M., Duarte, M.C.T., 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian J. Microbiol. 35, 275-280.

Şengün, İ.Y., Öztürk, B., 2018. Some natural antimicrobials of plant origin. Anadolu Univ. J.Sci. 7, 256-276.

Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H., 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food. Microbiol. 117, 112-119.

Solmaz, E.S., Ata, E.P., 2009. Adverse effects of herbal medicines and products. Türk. Hij. Den. Biyol. Derg. 66, 133-141.

Witkowska, A.M., Hickey, D.K., Alonso-Gomez, M., Wilkinson, M., 2013. Evaluation of antimicrobial activities of commercial herb and spice extracts against selected food-borne bacteria. J. Food. Res. 2, 37-54.