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Antibacterial effect of myrtle (*Myrtus communis* L.) leaves extract on microorganisms

Antibakterielle Wirkung von Myrtenblätterextrakten (Myrtus communis L.) auf Mikroorganismen

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Summary

The antibacterial activity of the extracts of myrtle black and white leaves was determined. By the increase of the concentration of the extracts, antibacterial activity also increased. The most effective extract was the methanol extract of the leaves of the white myrtle against *S. aureus*. While, effect of ethyl acetate extracts of white and black myrtle leaves were very low to *S. aureus* and *P. vulgaris,* methanol extracts of the leaves of the black myrtle inhibited the growth of it. Acetone extracts of white and black myrtle leaves were very effective against *P. mirabilis*. *B. cereus* was most resistant to ethyl acetate extract of myrtle leaves, but the reduction effect of methanol extract was very high.

Keywords: *Myrtus communis,* extract, composition, antibacterial

Zusammenfassung

Die antibakterielle Wirkung von Extrakten aus Blättern von schwarz- und weißbeerigen Myrtepflanzen wurde bestimmt. Durch die Erhöhung der Extraktkonzentrationen, wurde die antibakterielle Wirkung ebenfalls erhöht. Das wirksamste Extrakt war das Methanol-Extrakt aus den Blättern der weißen Myrte gegenüber *S. aureus*. Während die Wirkung der Ethylacetat-Extrakte beider Myrtenarten sich als sehr gering gegenüber *S. aureus* und *P. vulgaris* erwies. Die Methanol-Extrakte aus den Blättern der schwarzen Myrte hemmte hingegen deren Wachstum. Die Aceton-Extrakte der weißen und schwarzen Myrte waren sehr wirksam gegen *P. mirabilis*. *B. cereus* zeigte sich am widerstandsfähigsten gegenüber dem Ethylacetat-Extrakt, während die Hemmwirkung des Methanol-Extraktes gegen *B. cereus* sehr ausgeprägt war.

Schlüsselwörter: Myrte, *Myrtus communis* L., Extrakt, Zusammensetzung, antibakteriell

1. Introduction

Myrtle tree (*Myrtus communis* L.) belongs to the Myrtaceae family, is a typical representative of the Mediterranean flora. It is distributed in Asia, Africa, America and Europe (Özek et al. 2000). In Turkey, myrtle tree is found growing in pine forest and river sides, particularly in the Taurus mountains. Several uses of myrtle leave oil are known for culinary purposes. Myrtle leaves have some important constituents with aromatic and medicinal properties. Various constituents of *M. communis* leaves were found to be pharmacologically active. The essential oil of *M. commumis* leaves has been the subject of many chemical and pharmacological studies. At the folk medicine, leaf decoction or infusion are used as stomachic, hypoglycemic, cough and oral antiseptic (Garg and Dengre, 1988). Recently, the chemical composition of the essential oil of *M. communis* leaves has been reported (Chalchat et al. 1998; Asllani, 2000; Jamoussi et al. 2005; Kaukos et al. 2011; Messaoud et al. 2005).

Investigations have been conducted into the antimicrobial effects of various spices and derivatives (Özcan and Erkmen, 2001; Hsieh et al. 20001; Sağdıç et al. 2002). Spices are rich in essential oils that can be used to delay or inhibit the growth of microorganisms. The consumer prefers the use of natural compounds as chemical preservatives. A number of spice and herb oils have already been described to have antimicrobial activity against various bacteria such as *Staphylococcus aureus, Vibria parahaemolyticus, Salmonella* Typhimurium, *Enterococcus faecalis, Escherichia coli* and *Listeria monocytogenes* (Sağdıç et al. 2002; Aureli et al. 1992). Studies have been reported on the antimicrobial activity of leave oils of savory, basil, laurel, cumin sea fennel, myrtle , pickling herb and mint (Aureli et al. 1992). The aim of this study was to establish the antimicrobial activity of leave extracts towards microorganisms which have an important role in the agriculture, food and pharmaceutical fields of essential oil and extracts of both myrtle plants.

2. Material and Methods

2.1. Plant Material

The leave of myrtle plants (black and white berry color) were collected from Antalya (Serik) province in Turkey. The samples were transported in polypropylene bags, and were dried to constant weight. A specimen has been deposited in the Food Engineer Museum of the University of the Selçuk in Konya in Turkey.

2.2. Extraction of myrtle leaves

Ethyl acetate, methanol, ethanol, acetone, ethyl acetate-methanol (1:1, v/v) or ethanol-water (7:3, v/v) were used for extraction of leaves of black and white myrtle. About 10 g ground sample was weighed into a flask and 200 ml solvent was added to each one. Residue was kept in the dark and sterile bottle at 4 °C until use.

2.3. Bacterial cultures

Nine bacterial specie were used as test organisms: *S. aureus, P. vulgaris, P. mirabilis, B. cereus, A. hydrophila, E. faecalis, K. pneumoniae, S.* Typhimurium, *E. aerogenes* and *E. coli.*

2.4. Determination of antibacterial effects by paper disc diffusion method

Several concentrations (0.1, 0.5, 2.5 and 5.0 %) of myrtle leave extracts were used in the experiments. Also, extracts were obtained by using ethyl acetate, acetone, alcohol and methanol solutions. Stock cultures of microorganisms were grown in nutrient broth (Acumedia Manufacturers, Inc., Maryland) at 25 °C for 22 h. Final cell concentrations were 10^6–10^7 cfu/ml. 250 µl of bacterial suspansion was inoculated into flask containing 20 ml sterile nutrient agar (Acumedia Manufacturers, Inc., Maryland) at 43–45 °C. These bacterial cultures were poured into petri dish (9 cm diameter) and the agar was allowed to solidify at 4 °C for 1 h. The well method was used to detect the antibacterial activity of myrtl extracts. (Kelmanson et al. 2000; Sağdıç et al. 2002). Microorganisms were incubated at 37 °C for 18–24 h. Diameter of inhibition zones were measured as three times (mm).

2.5. Statistical analysis

The data were subjected to Anova using randomized complete block design with statistical analyses system Anova procedure. This research was performed by three duplicates with a replicate (Düzgüneş et al., 1987).

TABLE 1: *Effect of extracts of myrtle leaves against microorganisms (n:3).*

Microorganism	Ethyl Ascetate-Methanol (White myrtle)	Ethyl Acetate (White myrtle)	Alcohol-Water (White myrtle)	Aceton (White myrtle)	Ethyl Acetate-Methanol (Black myrtle)	Metha-nol (Black myrtle)	Ethyl Acetate (Black myrtle)	Alcohol-Water (Black myrtle)	Aceton (Black myrtle)
S. aureus	14.625a**	14.125bc	14.875bcd	14.792b	13.083a	15.875ab	14.750c	15.958cd	17.833e
P. vulgaris	14.792a	1.000E-03a	14.958	14.208a	16.167de	16.250bc	22.667a	15.125ab	15.500b
Proteus mirabilis	16.292c	12.167b	16.292e	17.500f	16.792de	16.500bc	17.125c	15.458abc	17.958e
B. cereus	15.417abc	13.250b	15.250cde	16.875e	16.083d	18.083cd	16.417c	15.250ab	16.542cd
A. hydrophila	14.667a	15.125bc	14.708bcd	16.083d	15.167c	14.208a	18.875b	16.292d	14.458a
E. feacalis	15.083ab	18.333bc	15.875de	15.333c	16.958e	17.375bcd	16.375c	15.667bcd	14.917ab
K. pneumoniae	14.708a	15.500bc	13.333a	16.375de	14.417b	16.292bc	14.708c	15.542abc	15.750bc
S. Typhimurium	15.708bc	16.292bc	13.917ab	15.500c	16.458de	15.708ab	15.375c	15.583bc	19.000f
E. aerogenes	15.083ab	14.958bc	14.333abc	14.625ab	16.708de	17.667bcd	14.875c	16.333d	15.458b
E. coli	16.167c	20.250c	15.833de	18.208g	16.958e	18.958d	16.833c	14.875a	16.708d

*Results are the diameter of the the inhibition zone (mm); **Differences between means indicated by the same letters are not statistically significant (Duncan's multiple range test, $P < 0.05$).

Journal of Food Safety and Food Quality 66, Heft 1 (2015), Seiten 1–24

3. Results and Discussion

S. aureus, A. hydrophila and *K. pneumoniae* were the most resistant microorganisms to ethyl acetate-methanol, methanol and alcohol-water extracts, respectively and *P. vulgaris* to acetone and ethyl acetate extracts of the leaves of the white myrtle (Tab. 1). *S. aureus, P. vulgaris, E. coli* were the most resistant microorganisms to ethyl acetate-methanol, ethyl acetate and alcohol extracts, respectively and *A. hydrophila* to water and acetone extracts of the leaves of the black myrtle. The most effective extract was the methanol extract of the leaves of the white myrtle against *S. aureus.* While effect of ethyl acetate extracts of white and black myrtle leaves were very low to *P. vulgaris,* methanol extracts of the leaves of the black myrtle inhibited the growth of it (Tab. 1). Acetone extracts of white and black myrtle leaves were very effective against *Proteus mirabilis. B. cereus* was most resistant to ethyl acetate extract of myrtle leaves, but the effect of methanol extract was very high. Ethyl acetate extracts of white myrtle leaves were the most effective one to stop the growth of *E. feacalis.* The most effective extracts on *S.* Typhimurium was the ethyl acetate extracts of white myrtle leaves and the aceton extracts of black myrtle leaves. While *E. aerogenes* was too much effected by application of ethyl acetate-methanol extract and the methanol extracts of the black myrtle leaves, alcohol-water extract of white myrtle and the ethyl acetate extracts of black myrtle leaves were vice versa. Resistance of *E. coli* against ethyl acetate extracts of black myrtle and alcohol-water extracts of white myrtle were very high, while ethyl acetate extracts of white myrtle and methanol extracts of black myrtle leaves inhibited the growth (Tab. 1).

Sağdıç et al. (2002) tested the antimicrobial activity of many spice extracts on *Escherichia coli* 0157:H7 at concentrations 0.5 %, 1.0 %, 1.5 % and 2.0 %. They reported that by the increase of the concentration, effect of the extracts were increased. 2.0 % concentration was the most effective. Özcan (1998) tested the antifungal activity of some spice extracts on *Aspergillus parasiticus* NRRL 2999 at 1 % and 2 % concentrations for ten days time period. According to their results, myrtle extracts were effective after 5 days at 2 % concentration. Akgül and Kıvanç (1989), tested antimicrobial activity of some spices on 30 microorganisms at concentrations of 0.1 %, 0.5 %, 1.0 % and 2.0 %. They have reported that myrtle, sage and sumach was partly effective on growth of yeast and fungi. Sağdıç et al. (2003) reported that the antimicrobial effect of myrtle extract were the best in comparison with the tested 11 antibiotics, except for carbenicillin on *L. plantarum* C 27, *L. plantarum* C 32 and *L. plantarum* P 33. Ilçim et al. (1998), reported that, while, clorophorm extracts of myrtle leaves were effective on *S. aureus,* they not effective on *E. coli.* Researchers were expressed that the differences of the antimicrobial activity of the extracts may be due to growing conditions, soil properties and the variation of the species of the microorganisms (Nostro et al. 1989; Özcan and Erkmen, 2001). Özcan ve Erkmen (2001) reported that the antibacterial activity of the essential oil of myrtle at 1 %, 10 % and 15 % concentrations and determined no activity on *S.* Typhimurium, *B. cereus, S. aureus, E. faecalis, E. coli, C. ruposa, R. oryzae* and *A. niger.* Generally, by the increase of concentration, the antibacterial activity had also increased. It is apparent that 5 % concentration is the most effective (Tab. 2). Friedman et. al (2002) tested the antimicrobial activity of essential oils of some spices on *Campylobacter jejumi, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica.* They reported that the essential oil of the leaves of myrtle had very low effect. Akgül and Kıvanç (1989) determined antimicrobial activity of some spices on 30 microorganisms at concentrations of 0.1 %, 0.5 %, 1.0 % and 2.0 %. They have reported that myrtle, sage and sumach were partly effective on growth of yeast and fungi. Researchers expressed that the differences of the antimicrobial activity of the extracts may be due to growing conditions, soil properties and the variation of the species of the microorganisms (Deans and Svoboda, 1990; Nostro et al., 2000; Özcan and Erkmen, 2001).

As a result, except for the methanol extract of the white myrtle leaves, all extracts were very effective on inhibition of the tested microorganisms. Inhibition increases by the increase of the concentration.

TABLE 2: *Duncan test of extracts of myrtle leaves against concentration*.*

Concentration (%)	Methanol	Ethyl acetate	Alcohol-Water Aceton		Ethyl acetate-Methanol
Black myrtle extracts					
0.1	14.133a**	10.634a	12.283a	13.467a	13.233a
0.5	16.167b	12.967b	14.617b	15.417b	15.000b
2.5	17.450c	14.717b	16.433c	17.183c	16.633c
5	19.017d	16.883c	19.100d	19.583d	18.650d
White myrtle extracts					
0.1	11.550a**	12.950a	11.533a	13.017a	12.967a
0.5	14.567b	14.350a	13.667b	15.033b	14.750b
2.5	16.600c	15.400a	16.100c	16.883c	16.000c
5	18.283c	13.300a	18.450d	18.867d	17.300d

*Results are the diameter of the inhibited area (mm); **Differences between means indicated by the same letters are not statistically significant (Duncan's multiple range test, $P < 0.05$).

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