Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi

Afyon Kocatepe University Journal of Science and Engineering

AKÜ FEMÜBİD **18** (2018) **011002** (757-762) AKU J. Sci. Eng. **18** (2018) **011002** (757-762)

DOİ: 10.5578/fmbd.67554

Araştırma Makalesi / Research Article

Melissa officinalis Ekstraktlarının Antimikrobiyal Aktivitesinin ve DNA Koruyucu Kapasitesinin Araştırılması

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Geliş Tarihi: 10.01.2018 ; Kabul Tarihi: 18.10.2018

Anahtar kelimeler
Antimikrobiyal aktivite; Antioksidan; Comet testi; DNA koruyucu etki; Melissa officinalis

Özet

Bu çalışmada, *Melissa officinalis* yaprak ekstraktlarının antimikrobiyal, antioksidan kapasiteleri ve H_2O_2-indüklenmiş oksidatif hasara karşı DNA koruyucu etkisi incelendi. *Melissa officinalis* yaprak ekstraktlarının oksidatif stresten DNA'yı koruyucu etkisi maya comet testi kullanılarak belirlendi. Comet testi *Saccharomyces cerevisiae* BY4741 üzerinde uygulandı. Oksidatif strese karşı, ekstrenin İnkübasyon öncesi ve inkubasyon sırasında uygulanmasında doza bağlı olarak DNA hasarında azalma gözlendi. Sonuçlar; *Melissa officinalis* methanolik yaprak ekstraktlarının hidrojen peroksitin zararlı etkisine karşı DNA'yı koruduğunu gösterdi. Ayrıca *Melissa officinalis* ekstraktlarının farklı mikroorganizmalar üzerindeki antimikrobiyal etkisi incelendi. *Melissa officinalis* ekstraktları tüm test mikroorganizmaları üzerinde antimikrobiyal etki göstermiştir.

Investigation of Antimicrobial Activity and DNA Protective Capacity of *Melissa officinalis* Extracts

Keywords
Antimicrobial activity; Antioxidant; Comet assay; DNA protective effect; Melissa officinalis

Abstract

In this study, antimicrobial activity, antioxidant capacity of *Melissa officinalis* leaf extracts and DNA protective effect aginst H_2O_2-induced oxidative damage were investigated. DNA protective effect of *Melissa officinalis's* leaf extract from oxidative stress was determined by using yeast comet assay. The comet assay, applied on *Saccharomyces cerevisiae* BY4741. We observed that DNA damage decreased in a dose dependent manner in experiments of preincubation and simultaneous incubation with the extract upon oxidative shock. Results indicated that *Melissa officinalis*'s methanolic leaf extract protect the DNA against the damaging effect of hydrogen peroxide. Also we indicated that the antimicrobial effects of *Melissa officinalis*'s extract on the different microorganisms. Extract of *Melissa officinalis* showed antimicrobial effect on all test test microorganisms.

1. Introduction

Many plant extracts contain various phytochemicals and traditionally used for medicinal applications. They have antioxidant activity and protect our cells against oxidative damage (Manach *et al*. 2009). A lot of studies have demonstrated that plant extracts exhibit antimicrobial activity and protective activity against genotoxicity caused by oxidative stress (Akyıl *et al*. 2013, Behrevan *et al*. 2011, Glei and Pool-Zobel 2006, Karadağlı *et al*. 2014).

Melissa officinalis L. (fam. Lamiaceae) is a perennial, aromatic herb (Canadanovic Brunet *et al*. 2008). Lamiaceae family members have been widely used in traditional and medical purpose. They used as a mild sedative, anxiolytic, digestive, carminative, spasmolytic, antimicrobial, antitumoral (Birdane *et al*. 2007, Lopez *et al*. 2009) and antiviral agent (Mazzanti *et al*. 2008). Lemon

balm is an aromatic herb and has a lot of natural antioxidants antioxidants (Cortes-Cabrera and Prieto 2010, Topal *et al*. 2008). Antioxidant compounds can deactivate and scavenge the free radicals. Some studies suggested that methanolic extract of *M. officinalis* caused a considerable concentration dependent inhibition of lipid peroxidation. Phenolic components of *M. officinalis* demonstrated antioxidant activity. Researchers suggested that extracts of *M. officinalis* have antioxidant activity due to the high portion of phenolic acids (Hohmann *et al*. 1999, Zandi and Ahmadi 2000). Genotoxicity assays are designed to detect compounds that induce directly or indirectly damage the genetic material by different mechanisms. One of the widely used test is comet assay which is a reliable and can be carried out very rapid and cheap assay for detecting DNA damage (Azevedo *et al*. 2011, Karadağlı *et al*. 2014, Korcan *et al*. 2013, Marques *et al*. 2011, Oliveira and Johansson 2012, Serpeloni *et al*. 2008, Yılmaz *et al*. 2016).

In this study we were evaluating the antimicrobial effect and potential DNA protective and repair effects of *M. officinalis* leaf extract against oxidative stress on *S. cerevisiae* BY4741 in vitro. The present study is the first research on antioxidant capacity of *M. officinalis* leaf extract by *S. cerevisiae.*

2. Materials and Methods

2. 1 Plant extract

M. officinallis were purchased from a herbal market in Afyonkarahisar, Turkey. 25 g of *M. officinallis* was subjected to extraction with 100 mL of dH_2O (MOWE) and methanol (MOME) by using soxhlet and rotary evaporator in vacuom. All the experiments were carried out for 4 hours.

2.2. Comet assay in S. cerevisiae strain BY4741 In the procedure

S. cerevisiae strain BY4741 (*MATa his3D1 leu2D0 met15D0 ura3D0*) used as a test microorganisms for comet assay. Test microorganism *S. cerevisiae strain BY4741* cells at stock culture were taken and suspended in 10 ml of liquid YPD medium. YPD medium contains 1% yeast extract, 2% peptone, 2% glucose and 2% agar. This strain incubated at 24 h, 30 °C. Cells were harvested by centrifuging at 5000 rpm, 4 °C for 2 min and then the pellet was suspended in the same volume of S buffer. S buffer contains; 1 M sorbitol, 25 mM KH_2PO_4 (pH 6.5). Cells were harvested by centrifugation at 15000 rpm, 4 °C for 2 min and resuspended in lyticase buffer (2 mg\ ml^{-1} lyticase, 500 µl S buffer , 300 µl deionized H_2O and 50 mM *β*-mercaptoethanol) and incubated at 200 rpm and 30 °C for 30 min in order to obtain spheroplasts. Comet test was used for evaluation of DNA damage (Korcan *et al*. 2013, Marques *et al*. 2011). Application of MOWE concentration was performed on spheroplasts both before and after 5 Mm H_2O_2 treatment for evaluating the antioxidant capacity and DNA protective capacity. Comets/slide were scored visually as belonging to one of five classes (0-undamaged, 1-mild damage, 2-moderate damage, 3-severe damage, 4-complete damage) by using a fluorescence microscope.

2. 3. Determination of antimicrobial activity

The antibacterial screening was carried out using the disc diffusion method as described by Bauer *et al*. (1996). *Proteus vulgaris* (ATCC 7644)*, Klebsiella sp., Escherichia coli* **(**ATCC 25922)*, Salmonella typhimurium* (NRRLB-4420) *Micrococcus luteus* (ATCC 7644)*, Bacillus subtilis, Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 7644), *Candida albicans* were used as test microorganisms. The test bacteria were inoculated into tubes of Nutrient broth separately and incubated at 37 ^oC for 18 h. The yeast was inoculated into tubes of Malt Extract Broth (DIFCO) and incubated at 30 ^oC for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standard and inoculated (0.2 ml each) onto Mueller Hinton Agar (MHA, Oxoid) plates. Filter paper discs (6 mm diameter), impregnated with MOME (50 and 100 µl). Positive control [oxacillin (OX), penicillin (P)] negative control (its solvents) were used. The plates were incubated at 37 °C, 18 h. All experiments were performed in triplicate. Inhibition zones (mm) was investigated for antimicrobial activity of the plant extracts.

3. Results

In present study antioxidant activity of *M. officinallis* extract and protective effect aginst H_2O_2 induced oxidative damage were investigated. Yeast comet assay were used to evaluate oxidative DNA damage. Four different concentrations of MOWE were tested. The protective effects of MOWE against H_2O_2 induced genotoxic effects on *S. cerevisiae* BY4741 were shown in Table 1. DNA

damage decreased in a dose-dependent manner in experiments. Reduction of DNA damage of %50 (0,2 µl) MOWE +5 mM H_2O_2 and %50 (4µl) MOWE +5 mM H_2O_2 dose applications were statistically significant compared with positive control (**p** < 0.05).

Table 1. The protective effects of MOWE extract

Application Dose	N	The percent change in DNA damage scores	
		According to NC	According to PC
NK	2	-	47.27(-)
PK	2	89.65(+)	-
%25 (0,2 µl) MOWE+5MmH_2O_2	2	74.13(+)	8.18(-)
%25 (4 µl) MOWE +5Mm H_2 O_2	2	81.03(+)	4.54(-)
%50 (0,2 µl) MOWE +5Mm H_2 O_2	2	51.72(+)	20.00(-)
%50 (4 µl) MOWE +5Mm H_2O_2	2	51.72(+)	27.27(-)

NC: Negative control, PC:Positive control

The repair potential effects of MOWE extract against H_2O_2-induced genotoxic effects were showed in Table 2.

Table 2. The repair potential effects of MOWE extract

Application Dose	N	The percent change in DNA damage scores	
		According to NC	According to PC
NK	2	-	47.27(-)
PK	2	89.65(+)	-
%25 (0,2 µl) MOWE+5Mm H_2O_2	2	89.65(+)	8.18(-)
%25 (4 µl) MOWE +5Mm H_2O_2	2	84.48(+)	2.72(-)
%50 (0,2Mm) MOWE +5Mm H_2O_2	2	84.48(+)	2.72(-)
%50 (4 µl) MOWE +5Mm H_2O_2	2	77.58(+)	6.36(+)

NC: Negative control, PC:Positive control

Repair potential effects of different MOWE concentrations against H_2O_2 induced genotoxic effects on *S. cerevisiae* BY4741 statistically significant compared with control groups (**p** < 0.05). DNA damages increased were increased with all of application dose of MOWE compared with negative control. Evalation of protective and repair potential effects of MOWE extract against H_2O_2-induced genotoxic effects on *S. cerevisiae* BY4741 were shown in Table 3.

Table 3. Evalation of protective and repair potential effects of MOWE extract

H_2O_2	N	Score of DNA Damage (Mean±SD)	
		Protective Potential	Repair İnduction Potential
NC	3	58.00 ±5.29a	58.00 ±5.29a
PC	3	110.33±6.42c	110.33±6.42b
%25 (0,2 µl) MOWE +5Mm H_2O_2	3	101.00±6.08c	101.00±4.35b
%25 (4 µl) MOWE +5Mm H_2O_2	3	105.33±7.50c	106.66±8.50b
%50 (0,2 µl) MOWE +5Mm H_2O_2	3	87.66±12.50bc	107.00±2.64b
%50 (4 µl) MOWE +5Mm H_2O_2	3	80.00±6.00b	103.33±11.54b

NC: Negative control, PC:Positive control, SD: Standart deviation

The highest value of protective potential value (105.33±7.50) was investigated on %25 (4 µl) MOWE +5Mm H_2O_2. The highest value of repair induction potential (107.00±2.64) was obtained on %50 (0,2 µl) MOWE +5Mm H_2O_2. The antimicrobial activity of MOME is shown in Table 4. The antimicrobial activities of MOME was compared whith standard antibiotics such as penicillin and oxacillin being employed as positive controls. All bacteria strains were susceptible to the plant extracts and the gram positive and negative strains displayed a variable degree of susceptibility against MOME. Generally the antimicrobial activity of 50 µl extract was found to be weaker than 100 µl extract. Especially considerable is that the highest sensitivity to MOME was observed by *M. luteus* (ATCC 7644) (18 and 24mm) and the strain of *S.typhimurium* (NRRLB-4420) (18 and 20 mm).

Table 4. Disk diffusion test results of MOME

Microorganisms	MOME(50µl)	MOME(100µl)	MET	P	OX
P. vulgaris (ATCC 7644)	16	19	13	-	-
Klebsiella sp	7	17	12	16	14
E. coli (ATCC 25922)	10	14	12	-	-
S.typhimurium (NRRLB-4420)	18	20	11	10	-
M. luteus (ATCC 7644)	18	24	11	28	16
B. subtilis	9	13	7	10	-
B. cereus	16	19	12	21	17
L. monocytogenes	9	11	10	8	-
C. albicans	0.9	16	17	-	-

MET: Methanol , OX:Oxacillin, P:Penicillin

4. Discussion

Living cells have antioxidant defense potential to protect themselves from damages caused by reactive oxygen species (ROS) and there is a balance between production and scavenging of ROS. When free radicals exceed the cellular

antioxidant defense, oxidative stress occurs and consequently may cause the oxidative damage to lipids, proteins, and DNA, leading development of chronic diseases (Atanassova *et al*. 2011). DNA is probably the most biologically significant target of oxidative attack and these cause strand breaks. The DNA damage can be evaluated by using comet assay. This method is sensitive, reliable and rapid for evaluation DNA damage (Azevedo *et al*. 2011).

In this study, antimicrobial activity, antioxidant capacity and DNA protective effect of *M. officinalis* leaf extract were investigated. DNA damage decreased in a dose-dependent manner in experiments of preincubation and simultaneous incubation with the extracts. Reduction of DNA damage of %50 (0,2 µl) MOWE +5 mM H_2O_2 and %50 (4µl) MOWE +5 mM H_2O_2 dose applications were statistically significant compared with positive control ($p < 0.05$). Smilarly in our study Marques *et al*. (2011), used *S. cerevisiae* as experimental model to determine DNA protective effect of extracts of *Ginkgo biloba* from oxidative stress. They suggested that the extract of *Ginkgo biloba* protect DNA from oxidation. In many studies, antioxidant and antigenotoxic properties have been reported for plant extracts and phytochemicals (Anter *et al*. 2010, Babota *et al*. 2018, Behrevan *et al*. 2011, Karuna *et al*. 2009, Kaur *et al*. 2010, Marques *et al*. 2011, Phalanisong *et al*. 2018, Quincozes-Santos *et al*. 2010, Sinha *et al*. 2010, Vasconcellos *et al*. 2010, Zandi and Ahmadi 2000). Ribeiro *et al*. (2001) investigated that extract of *M. officinalis* has got high value phenol compounds. Zeraatpishe *et al*. (2011) demonstrated that oral administration of Lemon balm infusion may be helpful for the protection of the radiology staff against radiation-induced oxidative stress.

Also, we have evaluated the antimicrobial effects of MOME on 9 different test microorganisms. In this study, MOME showed antimicrobial effect on all test microorganisms. The strongest activity was recorded on *M. luteus* (ATCC 7644) with 18 and 20 mm zone of inhibition at 50 and 100 µl concentration, respectively. The lowest antimicrobial activity was observed in *C. albicans* (0.9 mm) and *L. monocytogenes* (16 mm) in 50 µl and 100 µl. Dukic *et al*. (2014) reported that *M. officinalis* extract shows antimicrobial activity at different levels. In another study Abdellatif *et al*. (2014) essential oil obtained from leaves of *M. officinalis* L. was evaluated for its chemical composition and antimicrobial activity. The chemical composition was determined by GC/MS and GC-FID. According to their results, the essential oil presented high antimicrobial activity against all test microorganisms. Romeo *et al*. (2008) and Hussain *et al*. (2011) reported that *M. officinalis* oil has got antimicrobial activity. The antibacterial effect was investigated against ***v***arious microorganisms. Ehsani *et al*. (2017), investigated that chemical composition, antimicrobial activity and antioxidant properties of *M. officinalis* and *Deracocephalum moldavica* essential oils (EOs). Their results indicated strong antimicrobial effects of the oils against tested bacteria (*Salmonella typhimorium, Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus*). *Staphylococcus aureus* with the lowest MIC value (0.12 mg mL-1) for both EOs was the most sensitive bacterium, although, antibacterial effect of *M. officinalis* EO was stronger than *D. moldavica*. In another study, Okmen (2017), evaluated that antibacterial effects of *M. officinalis* extracts against bacteria isolated from football player's shoes and its antioxidant effects. Antibacterial activities of the extracts were tested against eight bacterial strains. Smilarly in our study, his study results suggested that *M. officinalis* has significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents of plant origin.

5. Conclusion

Antibiotic resistance is one of the world's most important problems. The discovery of new antimicrobials is very important. Plants has been used to improve human health and quality of life for many years. Turkey is one of the world's richest countries in terms of plant diversity. In recent years, researchers have done a lot of research on new antimicrobials of plant origin. Plants also contain phenolic compounds, and antioxidant effects of these compounds on organisms. Living cells have antioxidant defense potential to protect themselves from damages caused by reactive oxygen species The yeast comet assay is useful method to evaluate antioxidant potantial of plant extracts and DNA protective effect aginst H_2O_2-induced oxidative damage. In present study, we evaluated that DNA damage decreased in a dose dependent manner in experiments. *Melissa officinalis*'s methanolic leaf extract protect the DNA against the damaging effect of hydrogen peroxide. Also, *M. officinalis*'s extract has got antimicrobial activity on the different microorganisms. It was

conclueded that *M. officinalis* has potential antibacterial and antioxidant activities. *M. officinalis* extracts can be used as natural sources in the pharmaceutical industry due to their strong antimicrobial and antioxidant activities.

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