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# Antimicrobial, antioxidant and DNA protective effects and phenolic content of *Lallementia canescens* (L.) Fisch. & C.A.Mey. and *Lallementia peltata* (L.) Fisch. & C.A.Mey

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**Abstract**

The healing effects of plants are known to stem from the components in their contents. In today's science, the studies on plant contents and biological activities as well as the discovery or development of new therapeutic agents are very popular. In this study, the biological activities of *Lallementia canescens* (*L. canescens*) and *Lallementia peltata* (*L. peltata*) were investigated to provide valuable data for the literature in the treatment of various diseases and new active substance design. The corresponding plants, which benefits and contents have not been completely elucidated yet, were collected from Bitlis province in Turkey and converted into herbarium material. Ethanol (EtOH) extracts were prepared from the aerial parts (leafs) of the plants using the soxhlet extraction method. The concentrations of 17 phenolic compounds (antioxidants) were determined in these extracts by HPLC. Moreover, the *in vitro* antioxidant activities of such extracts were compared with the standard antioxidants: Butylated hydroxyanisol (BHA), Butylated hydroxytoluene (BHT) and Alpha tocopherol (α-Toc). Hollow agar and agarose gel electrophoresis methods were used to investigate the antimicrobial effects on various microorganisms and protective effects on pBR322 plasmid DNA, respectively. The results showed that some phenolics such as kaempferol, gallic acid, and salicylic acid could not be identified in EtOH extracts, while the extracts showed strong antioxidant and antimicrobial activity, particularly on the yeasts. On the other hand, DNA protective activities of the extracts were found to be very limited. It can be concluded that the plants are generally rich resources of phenolic compounds. The plants have potential to exhibit strong antioxidant antifungal activities as similar to the standards.

**Key words:** Antimicrobial; antioxidant; DNA protection; HPLC; *Lallemantia*

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## *Lallementia canescens* (L.) Fisch. & C.A.Mey. ve *Lallementia peltata* (L.) Fisch. & C.A.Mey. fenolik içerikleri ve antimikrobiyal, antioksidan ve DNA koruyucu etkileri

**Özet**

Bitkilerin iyileştirici etkilerinin içerdikleri bileşenlerden kaynaklandığı bilinmektedir. Bugünün bilim dünyasında, yeni terapötik ajanların geliştirilmesi ve keşfinde olduğu gibi bitki içerikleri ve biyolojik aktiviteleri üzerine çalışmalar çok popülerdir. Bu çalışmada, çeşitli hastalıkların tedavisi ve yeni aktif madde dizaynında literatür için değerli data elde etmek amacıyla, *Lallementia canescens* (*L. canescens*) ve *Lallementia peltata* (*L. peltata*)'nın biyolojik aktiviteleri araştırıldı. Henüz yararları ve içerikleri tam aydınlatılmamış olan bu bitkiler Bitlis ilinden toplandı ve herbaryum materyaline çevrildi. Bitkilerin toprak üstü kısımları kullanılarak soxalet ekstraksiyon metodu ile etanol

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(EtOH) özleri hazırlandı ve HPLC kullanılarak bu özlerde 17 fenoliğin konsantrasyonları araştırıldı. Daha sonra standart antioksidan olarak bilinen bütillenmiş hidroksianisol (BHA), bütillenmiş hidroksitoluen (BHT) ve alfa tokoferol (α-Toc)'e karşı özlerin *in vitro* antioksidan aktiviteleri, oyuk agar metodu ile çeşitli mikroorganizmalar üzerindeki antimikrobiyal etkileri ve agaroz jel elektroforezi ile pBR322 plazmid DNA'sı üzerindeki koruyucu etkileri araştırıldı. Kamferol (kaempferol), gallik asit (gallic acid) ve salisilik asit (salycilic acid) gibi bazı fenolikler saptanamasa da genel olarak özlerin zengin antioksidan içeriğe sahip olduğu belirlendi. Bu etkiye paralel olarak bitki özlerinin standartlara yakın *in vitro* antioksidan aktivite sergilediği belirlendi. Ayrıca özütlerin özellikle mantarlar üzerinde daha güçlü antimikrobiyal etki gösterdikleri ancak sınırlı derecede DNA koruyucu aktiviteye sahip oldukları saptandı.

**Anahtar kelimeler:** Antimikrobiyal; Antioksidan; DNA koruma; HPLC; *Lallemantia*

## 1. Introduction

The world health organization (WHO) has reported that the total amounts of medicinal plants used for treatment are approximately 20,000 based on the compilation of publications on pharmacy and medical plants from 91 different countries [18]. The plants, mainly used as a source of food and medicine in the past, have been used as tea and spice in the present day [3]. The studies on this topic have revealed that the healing properties of plants stem from various components such as essential oils [36], phenolic substances and various antioxidants [1; 16]. It is known that these components are important in the regulation of cellular ROS, antimicrobial protection and in the fight against several diseases such as cancer [20]. Increased ROS concentration in a cell by the effects of endogenous and exogenous agents can cause various neurodegenerative diseases by interacting with the many macromolecules such as DNA, lipid and protein [7; 8; 21]. Although excessive concentration of ROS is harmful to the cell, many plants can voluntarily produce the ROS in cellular metabolic pathways, which are essential both for pathogen defense and for progression of many mechanisms carrying with the signal transduction [25] and most scientists have stated that plants have antioxidants and antimicrobial properties [23].

The cosmopolitan family of *Lamiaceae*, which is a member of the flowering plants, includes mostly fragrant single or perennial herbaceous plants and several shrubs. The endemism rate in Turkey is 44% and it has got 250 genera and 6900-7200 species in the world [15; 34]. Furthermore, Turkey has got 45 genera, 546 species and 731 taxon including the other subspecies [2]. *Lallementia* genera, which can be used as food and medicine, is represented by three species [26]. These are *L. peltata*, *L. iberica* and *L. canescens* [9]. The length of the *Lallementia canescens* can be 20-45 cm and has an important distribution in Turkey. It blooms between May and July, and grows usually on the slopes, roadsides, volcanic and limestone ridges. The length of the *L. peltata* can be 10-40 cm and shows an important distribution in Turkey and grows on empty fields, worn slopes, roadsides, and on the creek beds in the inner parts of the north and south of Turkey.

This study focused on the biological activities of *Lallemantia canescens* and *Lallemantia peltata* and thus, it was aimed to expand the plant literature by providing important data for advance pharmacological studies. For this purpose the EtOH extracts were prepared from aerial parts of *L. canescens* and *L. peltata*, and then the concentrations of 17 phenolic and *in vitro* biological activities in this extracts were investigated.

## 2. Materials and methods

### *2.1. Plant collection and extract preparation*

The plants collected from Bitlis province during vegetation period and stored in Science and Technology Application and Research Center Laboratory in Bitlis Eren University with herbarium codes (*L. canescens*: M.KURŞAT 6041; *L. peltata*: M.KURŞAT 6042). The aerial parts of the plants which were dried in the shade and pulverized by a mixer. Ethanol extracts were then prepared from 50 g of plants using the soxhlet extraction method and stored at -18 $^{o}$C in dark glass bottles [1]. Soxhlet extraction method is one of the methods used to obtain extracts from plants in the simplest and cheapest way.

### *2.2. Quantitative analysis of phenolic compounds by HPLC*

The phenolic contents of EtOH extracts were determined by HPLC. For this purpose, standard phenolics were prepared at 10 mg/mL concentrations and placed into 50 ml flasks and then 1% acetic acid and acetonitrile were then mixed (9:1 respectively) and a homogeneous solution was obtained. Stock solution was prepared by mixing the previous prepared homogeneous solution and methanol (1:1). Previous prepared 10 mg/mL phenolic standards were dissolved in stock solution and standard samples (100 mM, 75 mM, 50 mM, 25 mM, and 10 mM) were prepared to form the HPLC standard chromatograms [28]. EtOH extract samples were then prepared at 20 mg/mL concentration and filtered using the 0.45 μm membrane filter. The experimental conditions followed in HPLC were shown in Table 1.

Table 1. HPLC operating conditions and gradient elution program

| | **HPLC operating conditions** | **Gradient elution** | | |
|---|---|---|---|---|
| **Model** | Agilent Technologies 1260 Infinity II | Time (min) | A (%) | B (%) |
| **Colon** | ACE 5 C18 (250x4.6 mm id) | 0 | 90 | 10 |
| **Colon Oven** | G7130A | 25 | 60 | 40 |
| **Detection** | 1260 DAD WR | 39 | 40 | 60 |
| **Pump** | 1260 Quat Pump VL | 50 | 10 | 90 |
| **Mobile phase** | A: 1% Acetic Acid / B: Acetonitrile | 55 | 90 | 10 |
| **Wavelength** | 272, 280 and 310 nm | | | |
| **Auto sampler** | 1260 Vialsampler | | | |
| **Flow Rate** | 1 mL/min | | | |
| **Colon Temperature** | 28 ºC | | | |
| **Injection** | 20 µl | | | |

*2.3. Sample preparation and antimicrobial activity assay*

Nutrient Broth and Sabourand 2% Glucose Broth were used respectively in the production of bacteria and yeast. 1% of each microorganism developed in liquid media was again planted in the same media and incubated for 18 hours. The microorganisms were inoculated into the previous prepared Nutrient Broth and Sabourand 2% Glucose Broth media and the wells with a diameter of 10 mm were formed on the media. The 0.1 g of previous prepared extracts was dissolved in 1 mL of DMSO to prepare the extract solutions. The 5 µl, 10 µl, 20 µl, 40 µl and 80 µl of the extract solutions were inoculated into the wells and incubated at 4 °C for 1 hour. Bacteria and yeasts were inoculated into culture media and then incubated at 37 °C and 27 °C (bacteria and yeasts respectively) for 18-24 hours. The blurs ($10^6$ CFU s/mL) of bacteria and yeasts are adjusted according to the 0.5 standard of Mc Farland [17]. Antimicrobial activity assays were performed on 10 different microorganisms according to the method of [27]. According to this, EtOH extracts were prepared as 100 mg/mL and inoculated into hollow agar wells. The diameters of inhibition zones were measured and compared with the results of antibiotics used as control.

The microorganisms were obtained from the Central Research and Application Center in Muş Alparslan University and these are as follows; the seven of microorganisms are pathogenic bacteria; $Gr^+$; *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus ATCC 25923* and *Bacillus megaterium* DSM 32 and $Gr^-$; *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* 9027 and *Klebsiella pneumonia* 13883 and three of organisms are fungi; pathogenic: *Yarrowia lipolytica* and *Candida albicans* ATCC 10231 and non-pathogenic: *Saccharomyces cerevisiae*. Antibiotics were purchased from OXOID and these are Erythromycin (E-15), Ampicillin/Sulbactam (SAM-20), Rifampicin (RD-5), Amikacin (AK-30) and Fluconazole (FCA-25).

2.4. *Determination of in Vitro Antioxidant Activities*

*2.4.1 Total Antioxidant Activity assay*

Total antioxidant activity assay was performed by ferric thiocyanate method of [24] using Shimadzu 1800 spectrophotometer. According to this, 1 mg/mL stock solutions were prepared and, by diluting the stock solutions, 100 µL samples were prepared at 25 µM, 50 µM and 100 µM concentrations. The volume of each sample was then completed to 2.5 mL by adding previous prepared buffer solution and 2.5 mL of linoleic acid was added to each tube and thus total volume was elevated to 5 mL. In this assay, control tube contains 2.5 mL of buffer solution, 2.5 mL of linoleic acid and 100 µL of distilled water, and blank tube contains 4.8 mL of ethanol and 100 µL of $Fe^{2+}$ and $SCN^-$. The samples, control, and blank tubes were incubated at 37 °C and then 4.7 mL of ethanol and 100 µL of $Fe^{2+}$ and $SCN^-$ were added on, respectively. Absorbance values were read at 500 nm and the inhibition percentages of linoleic acid emulsions were calculated at 40$^{th}$ hour when the control absorbance reached its maximum value.

*2.4.2. Ferric iron ($Fe^{3+}$) reduction assay*

*2.4.3. Cupric ion ($Cu^{+2}$) reduction assay*

The cupric cation ($Cu^{+2}$) reduction capacity was carried out according to the CUPRAC method used by [37]. For this purpose, 0.1 mL of samples having the different concentrations (25 µg/mL, 50 µg/mL and 75 µg/mL) were prepared and their volumes were completed to 1 mL with the distilled water and then 0.25 mL of $CuCl_2$ solution (0.01 M), ethanolic neocuprin solution and 1M ammonium acetate buffer were added in the empty test tubes and briefly vortexed. Previous prepared samples at different concentrations were transferred to test tubes and incubated for 30 min. The increase in absorbance values at 450 nm shows the reduction capacity of cupric cation.

*2.4.4. ABTS radical scavenging*

$ABTS^{\bullet+}$ (2.2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) scavenging activity was performed according to the method of [35]. According to this, the same proportions of 2.45 mM potassium persulphate ($K_2S_2O_8$) and 7 mM ABTS solution were reacted and the samples were incubated for 16 hours to generate the $ABTS^{\bullet+}$. The radical solution was

diluted with methanol until the absorbance value of them reach to control absorbance (1.660±0.02) at 734 nm. 4 mL of $ABTS^{\bullet+}$ solution and 2 mL of extracts having different concentrations were transferred into test tubes and the values of sample absorbance were measured against the blank (Phosphate Buffer pH: 7.4) at 734 nm.

*2.4.5. DPPH free radical scavenging*

The DPPH (1,1-Diphenyl 2-picrylhydrazyl) free radical scavenging activity was performed according to the method of [5]. The different concentrations of extracts and standards (25 µg/µL, 50 µg/µL and 100 µg/µL) were prepared in test tubes and total volumes were completed to 3 mL by adding pure ethanol. 1 mM DPPH radical solution was prepared and transferred into test tubes. After the incubation for 30 min at room temperature and dark, absorbance values were measured against the blank (ethanol) at 517 nm. The following equation was used in the calculation of DPPH radical scavenging percentages.

$$ScavengingCapacityofDPPHRadical(\%) = \left(1 - \frac{Absorbanceofsample}{Absorbanceofcontrol}\right)x10 \quad (1)$$

*2.5. DNA protective activity study*

Protective effects of the extracts on pBR322 plasmid DNA were imaged by agarose gel electrophoresis according to the [29] method. Stock extract solutions at 200 mg/mL concentration were prepared with the dissolution of extracts in DMSO. By the re-dilution of them in DMSO at different concentrations, fresh extracts were prepared and mixed with the different chemicals and materials (pBR322 DNA (200 ng), $H_2O_2$ and DMSO) according to the amounts given in Table 2 and loading samples at different concentrations (25 mg/mL, 50 mg/mL and 100 mg/mL) were obtained. These samples were incubated at 37 ºC in dark for 3, 6, 12 and 24 hours and 5 µL of them were mixed with loading buffer. After loading the samples onto the agarose gel, Electrophoresis was performed in TBE buffer at 40 volts for 2 hours and agarose gel was viewed by Londershausen (1996) imaging system (Figure 2).

Table 2. The amounts and components of samples for electrophoresis

| DNA (µL) | $H_2O_2$ (µL) | DMSO (µL) | Extracts (10 µL) and Concentrations | PW (µL) | Total Volume (µL) |
|---|---|---|---|---|---|
| **10** | - | - | - | 15 | 25 |
| **10** | 5 | - | - | 10 | 25 |
| **10** | 5 | 10 | - | - | 25 |
| **10** | - | 10 | - | 5 | 25 |
| **10** | 5 | - | Leaf/ EtOH (100 mg/mL) | - | 25 |
| **10** | 5 | - | Leaf/ EtOH (50 mg/mL) | - | 25 |
| **10** | 5 | - | leaf/ EtOH (25 mg/mL) | - | 25 |
| **10** | - | - | Leaf/ EtOH (100 mg/mL) | 5 | 25 |
| **10** | - | - | Leaf/ EtOH (50 mg/mL) | 5 | 25 |
| **10** | - | - | Leaf/ EtOH (25 mg/mL) | 5 | 25 |

*2.6. Statistical Analysis*

All experimental assays were repeated as three measurements. Statistical comparisons of both antioxidant activity results and antimicrobial activity results were performed by One-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA. All data compared statistically were presented as Mean ± standard deviation and P values below 0.05 were considered significant. $Fe^{3+}$cation reduction study was performed according to the FRAP method proposed by [33], and BHA, BHT, and α- Toc were used as standard antioxidants. Extracts and standards at different concentrations were added in test tubes and total volume was completed to 1 mL with the distilled water. 2.5 mL of phosphate buffer (0.2 M, pH:6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were added and incubated at 50 °C for 20 minutes. After the incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added in the reaction mixture and centrifuged at 3000 RPM for 10 min. 2.5 mL from top phases of sample tubes was taken and transferred in empty tubes, and then 2.5 mL distilled water and 0.5 mL $FeCl_3$ were added on, respectively. The absorbance values of control and samples were read against the blank (pure water) at 700 nm..

## 3. Results

*3.1. Quantitative Phenolic contents*

Concentrations (µg/mL) of 17 phenolics were determined by HPLC (Table 3) and according to this, salycilic acid and gallic acid could not be detected in both extracts. Apigenin as the highest phenolic (504.710 µg/mL) and 3,4-Dihydroxybenzoic acid as the lowest phenolic (0.016 µg/mL) were found in the *L. canescens* extract. Besides catechol as the highest phenolic (112.701 µg/mL) and abscisic acid as the lowest phenolic (1.164 µg/mL) were detected in the *L. peltata* extract (Table 3). The phenolic chromatograms of plant extracts are given in supplemental data Figure 2 and Figure 3.

Table 3. The phenolic concentration (µg/mL) in the EtOH extracts of *L. canescens* ve *L. peltata*. The lowest and highest concentrations of phenolics in the both of extracts were written in bold type

| **Phenolics** | ***L. canescens*** | ***L. peltata*** | **Phenolics** | ***L. canescens*** | ***L. peltata*** |
|---|---|---|---|---|---|
| **Ascorbic acid** | 71.176 | 92.862 | Curcumin | 7.848 | 5.939 |
| **Gallic acid** | N/A | N/A | Catechol | 20.182 | **112.701** |
| **Myricetin** | 2.229 | 9.941 | Vanillin | 0.522 | 12.801 |
| **Abscisic acid** | 64.687 | **1.164** | Caffeic acid | 9.663 | 69.673 |
| **Quercetin** | 13.789 | 15.958 | Cinnamic acid | 3.661 | 2.837 |
| **Apigenin** | **504.710** | 72.485 | Rosmarinic acid | 21.012 | 31.435 |
| **Kaempferol** | 20.182 | 5.295 | Salicylic acid | N/A | N/A |
| **3,4-Dihydroxybenzoic acid** | **0.016** | N/A | Trans-p-coumaric acid | 3.99 | 1.589 |
| **4-Hydroxybenzoic acid** | 2.003 | 2.605 | | | |

*3.2. Antimicrobial activities*

Antimicrobial activity studies were performed by hollow agar method. The antimicrobial effects of the extracts were tested on 10 microorganisms at different volumes (50 µL, 100 µL, and 150 µL) and the most significant effect was observed at 150 µL (Table 4). The diameters of hallow agar wells treated with antibiotic and EtOH extracts were measured as mm and 150 µL results were compared statistically by One-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA. Statistical evaluation results were expressed as Mean ± Standard Deviation in Table 4. According to this, the highest activity of *L. canescens* was observed on *Y. lipolytica* (30 mm) and the highest activity of *L. peltata* extract was observed on *C. albicans* (26±0.57 mm). The antimicrobial effects for 50 µL and 100 µL are given in supplemental data Table 1.

Table 4. The effects of 150 mg/mL EtOH extract and antibiotics on the microorganisms and statistical evaluation of results

| Organism | | Antibiotics □ | | Erythromycin | Ampicillin/ Sulbactam | Amikacin | Rifampicin | Fluconazole |
|---|---|---|---|---|---|---|---|---|
| Gram positive | *B. subtilis* | Antibiotic results | | 20±0.00 | 14±1.15 | 11±1.00 | 21±0.00 | ─ |
| | | *L. canescens* | 15±1.00 | **** | ns | +*** | **** | |
| | | *L. peltata* | ─ | N/A | N/A | N/A | N/A | N/A |
| | *S. aureus* | Antibiotic results | | 21±1.00 | 10±0.00 | 9±0.00 | 18±1.15 | ─ |
| | | *L. canescens* | ─ | N/A | N/A | N/A | N/A | N/A |
| | | *L. peltata* | 17±1.00 | ** | +**** | +**** | ns | N/A |
| | *B. megaterium* | Antibiotic results | | 25±0.00 | ─ | 10±1.00 | 16±0.00 | ─ |
| | | *L. canescens* | 15±0.00 | **** | N/A | +**** | ns | N/A |
| | | *L. peltata* | 16±1.00 | **** | N/A | +**** | ns | N/A |
| Gram Negative | *E. aerogenes* | Antibiotic results | | 27±1.00 | 10±1.00 | 9±0.00 | 16±1.00 | ─ |
| | | *L. canescens* | 15±0.57 | **** | +**** | +**** | ns | N/A |
| | | *L. peltata* | 18±1.15 | **** | +**** | +**** | ns | N/A |
| | *E. coli* | Antibiotic results | | 19±1.52 | 13±0.00 | 13±0.00 | 18±0.00 | ─ |
| | | *L. canescens* | 16±1.52 | * | +* | +* | ns | N/A |
| | | *L. peltata* | 17±0.57 | ns | +** | +** | ns | N/A |
| | *P. aeroginosa* | Antibiotic results | | 19±0.00 | ─ | 14±1.15 | 8±0.00 | ─ |
| | | *L. canescens* | 17±1.15 | * | N/A | +** | +**** | N/A |
| | | *L. peltata* | 15±1.00 | -*** | N/A | ns | +**** | N/A |
| | *K. pneumonia* | Antibiotic results | | 19±1.73 | 16±0.57 | 10±0.00 | 19±1.73 | ─ |
| | | *L. canescens* | ─ | N/A | N/A | N/A | N/A | N/A |
| | | *L. peltata* | 18±1.00 | ns | ns | +**** | ns | N/A |
| Fungus | *Y. lipolytica* | Antibiotic results | | ─ | ─ | ─ | ─ | 21±0.00 |
| | | *L. canescens* | 30±1.00 | N/A | N/A | N/A | N/A | +**** |
| | | *L. peltata* | 23±1.00 | N/A | N/A | N/A | N/A | +* |
| | *C. albicans* | Antibiotic results | | ─ | ─ | ─ | ─ | 23±1.52 |
| | | *L. canescens* | 26±1.73 | N/A | N/A | N/A | N/A | ns |
| | | *L. peltata* | 26±0.57 | N/A | N/A | N/A | N/A | ns |
| | *S. cerevisiae* | Antibiotic results | | ─ | ─ | ─ | ─ | ─ |
| | | *L. canescens* | 19±0.57 | N/A | N/A | N/A | N/A | N/A |
| | | *L. peltata* | 20±0.57 | N/A | N/A | N/A | N/A | N/A |

N/A; Not Available, ─; Inhibition zone not formed, +; shows a higher antimicrobial effect than antibiotics. *; was used as a symbol of statistical significance in the comparison of antimicrobial effects, **P<0.05 (significant); **P<0.01 (very significant); ***P<0.001 and ****P<0.0001 (extremely significant); $^{ns}$P>0.05 (not significant), All data are shown as Mean±SD (n=3); All p values are derived from Dunnett's multiple comparisons test, One Way ANOVA

**3.3.***In vitro antioxidant studies*

Total antioxidant activities of the extract and standards were performed by measuring one time per 10 hours at 500 nm. The measurements were terminated with the maximum absorbance of control. The extracts showed the most important activity at a concentration of 100 µg/mL. The change in total antioxidant activity for 40 hours is shown in Figure 1a. The line graph of total antioxidant activity was given in supplemental data Figure 1a. The calculations were determined according to the following equation (2) and the definitions of the parameters used in the equation are as follows; Asample: the value of sample absorbance when the control absorbance reached to maximum and Acontrol: the value of control absorbance when control absorbance reached the maximum.

$$\text{Lipid Peroxidation Inhibition (\%)} = 100 - \left(\frac{\text{Asample}}{\text{Acontrol}} \text{x} 100\right) \qquad (2)$$

The peroxidation percentages of linoleic acid emulsion for 100 µg/mL were as follows; *L. canescens* and *L. peltata*: 73.25% and 75.32%, respectively and standard antioxidants (BHT, BHA, and α-Toc) 72.76%, 72.34%, 57.87%, respectively (Figure 1a).

Iron reduction activity for different concentrations of extracts were tested and statistical analysis for 100 µg/mL having the most effect was performed (Figure 1b). Accordingly, iron reduction results were listed as follows; BHA > *L. peltata* > BHT > α- Toc > *L. canescens*. The other results were present in supplemental data Figure 1b.

Cupric reduction capacities for different concentrations of extracts and standards were determined at 505 nm. Consequently, cupric ion reduction capacity increased depending on the extract concentration and the statistical comparison was performed for 75 µg/mL concentration results having the most important effect. The results and statistical evaluation are present in Figure 1c. Eventually, the cupric reduction capacity of *L. peltata* EtOH extract was found to be particularly similar to α-Toc. However, the cupric ion reduction capacity of *L. canescens* EtOH extract was lower than all standards. The other concentration results were given in supplemental data Figure 1c.

ABTS radical scavenging activities for two concentration (50 µg/mL and 100 µg/mL) were measured. Although the results of both concentrations were close to the standards, statistical evaluation was performed for 100 µg/mL results due to the optimum dose in such studies. Accordingly, ABTS radical scavenging percentages and rankings for 100 µg/mL were as follows; BHT (96.16%) ≥ α-Toc (96.15%) ≥ BHA (96.04%) ≥ *L. peltata* (95.86%) ≥ *L. canescens* (95.50%) (Figure 1d). 50 µg/mL results are present in the supplemental data Figure 1d.

DPPH radical scavenging activities were tested for different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) and the best effects were observed for 100 µg/mL concentration known as optimum dose (Figure 1e). Statistical comparison was performed for 100 µg/mL concentration and especially the DPPH radical scavenging activity of *L. peltata* EtOH extract was found to be significantly close to the standards. However, DPPH radical scavenging activity of *L. canescens* EtOH extract was significantly lower than standards (Figure 1e). DPPH radical scavenging percentages were as follows; BHA (91.74%) ≥ α-Toc (91.23%) ≥ *L. peltata* (90.67%) ≥BHT (90.50%) > *L. canescens* (75.56%). The other concentration results were present in supplemental data Figure 1e.

*3.4. DNA protection activity results*

Protective effects of different concentrations of extracts on pBR322 plasmid DNA were tested using agarose gel electrophoresis (Figure 2). Contrary to the destruction of DNA in the samples with $H_2O_2$ alone, it was observed that form I and II structures were stabilized in the samples with only DMSO as pure DNA sample. As a result, *L. canescens* extract did not remove the harmful effect of $H_2O_2$ even when applied alone (Line 5, 6, 7) and did not have an important positive effect on DNA stabilization (Line 8, 9, 10). As seen in the results of the study, when $H_2O_2$ was applied alone, form I was erased (Figure 2 Line 2), but preserved when *L. peltata* extract was added on the same sample (Figure 2 Line 11).

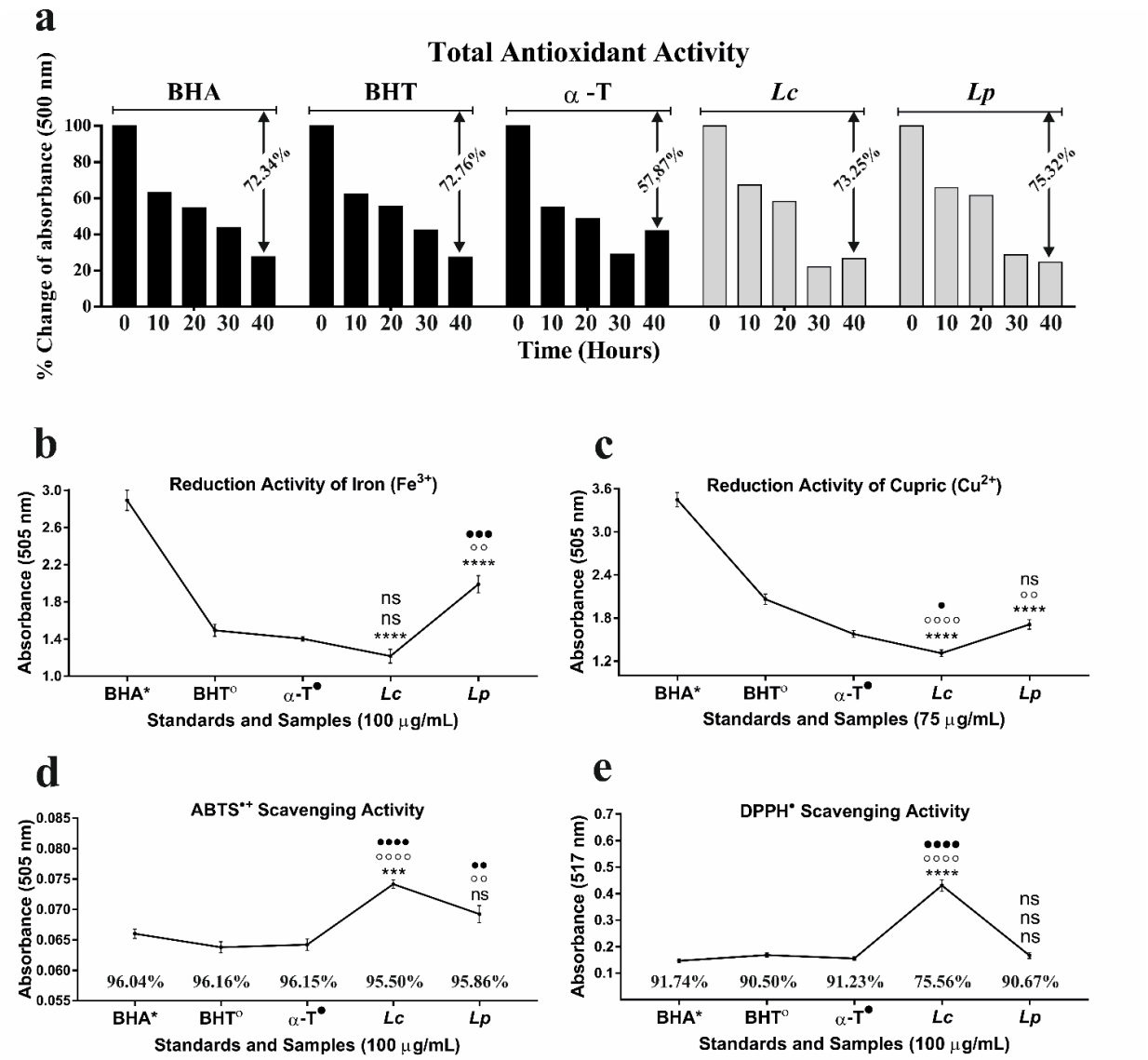


Figure 1. Graphs of *in vitro* antioxidant studies **a)** Total antioxidant activity by thiocyanate method, **b)** $Fe^{3+}$ reducing power by FRAP**, c)** $Cu^{2+}$ reducing power by CUPRAC, **d)** ABTS radical scavenging activity and percentages, **e)** DPPH radical scavenging activity and percentages. Standard antioxidants; BHA (Butylated hydroxytoluene), BHT (Butylated hydroxytoluene) and α-Toc (Alpha tocopherol) Extracts; *Lc* **(***L. canescens*), *Lp* (*L. peltata*). All data are shown as Mean±SEM (n=3); All p values are derived from Dunnet's multiple comparisons test, One Way ANOVA *; Symbol of statistical significance in comparison of BHA and extracts; º; symbol of statistical significance in comparison of BHT and extracts; •; Symbol of statistical significance in comparison of α-T and extracts; */º/•P<0.05 (significant); **/ºº/••P<0.01 (very significant); ***/ººº/•••P<0.001 and ****/ºººº/••••P<0.0001 (extremely significant). The symbols are also shown as the exponent of standard antioxidants

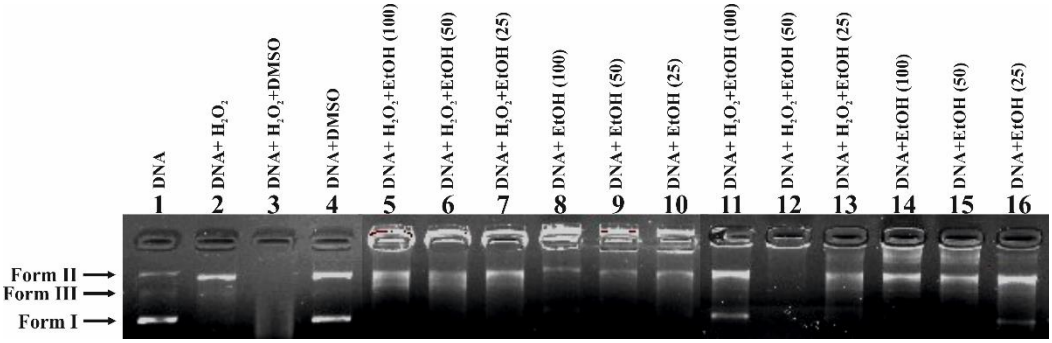


Figure 2. Visualization of the effects of *L. canescens* (Line 5-10) and *L. peltata* (Line 11-16) on DNA by Agarose Gel Electrophoresis, The numbers in parentheses are the concentrations of extracts used in the preparation phase of the electrophoresis solution, EtOH: Ethanol extract

## 4. Conclusions and discussion

### *4.1. Quantitative analysis of Phenolic contents*

In total phenolic concentration, it is clear that *L. canescens* extract is richer than *L. peltata* extract. However, this result was not observed in several *in vitro* antioxidant activities and DNA protective activity results. Biological activity studies are of great importance in the scientific world as they are often a starting point for advance studies. A study performed emphasized that high concentration of rosmarinic acid (4.23 mg/g) and the presence of caffeic acid (0.19-0.62 mg/g) in the *Lamiaceae* family [6] and another study again emphasized the high concentration of rosmarinic acid and the absence of caffeic acid [10]. A study reported that phenolic contents and their concentration may vary according to the extract type [30]. The reason for the differences in phenolic and their concentrations in this study may be the differences in plant species and solvents used and this difference may allow the usage of the plants in different scientific fields, the production of new active substances, and the treatment of new diseases.

### *4.2. Antimicrobial activities of extracts*

Both plant extracts were found to be more effective on fungal media than bacterial media. The antibiotics except for fluconazole were shown to be partially effective on fungal media. In addition, higher inhibition diameters were observed on fungal media treated with 150 µL volume of the plant extracts. This data may be proof that plants can be used in the treatment of some fungal diseases or a new beginning point for pharmaceutical industry studying on the treatment of microbial disease. A study on a genus of *Lallementia* reported that the extracts other than water extract have antibacterial activity [22] and showed high antibacterial activity on *B. subtilis*, *B. sphaericus* and *P. aeruginosa* [19]. Similarly, a study on *Lamiaceae* family reported that extracts prepared by using solvents other than water showed antibacterial activity [37]. It is clear that both extracts are also power antimicrobial agents and this study provides important data for advance studies performed on this field when compared with the previous studies.

### *4.3.In vitro antioxidant studies*

Similarity of total antioxidant activity with the standards or higher than them at $40^{th}$ hours is remarkable data and supports previous studies (Figure 1a). As a matter of fact, in a previous study, it was stated that the extracts prepared from a member of *Lamiaceae* family showed higher activity than BHA and BHT [12]. Similarly, *L. peltata* extract showed higher iron ($Fe^{3+}$) reduction activity other than BHA. However, the iron reduction activity of *L. canescens* extract was lower than all standards (Figure 1b). It is well known that heavy metals such as iron and copper may increase $H_2O_2$ production through the fenton reaction [7; 8] or by causing cellular toxicity in the cell [13]. According to this data, the high activity of the extracts in cupric and iron reduction activities may be a proof to limit cellular $H_2O_2$ production and this shows that the extracts are antioxidants. *L. peltata* extract showed similar positive results in the other *in vitro* studies such as total antioxidant activity, DPPH and ABTS radical reduction activities (Figure 1), and especially antifungal activity (Table 4). Although *L. canescens* extract was generally weaker than *L. peltata* extract in antimicrobial and *in vitro* antioxidant activities, it is clear that the phenolic content of *L. canescens* was quite high (Figure 3). *L. peltata* may have undetermined rich phenolic content in this study, which causes this reverse effect. Although total antioxidant activity, ABTS radical scavenging activity, and phenolic content of *L. canescens* extract were quite good, it generally showed a weaker effect compared to *L. peltata* extract (Figure 1). As a result, both plants are good antioxidants, but *L. peltata* is better. Essential oils of *S. hortensis*, a member of the Lamiaceae family, have been reported to exhibit lower iron reduction activity than the standards used [37]. This result is on the line of the *L. canescens* results. A similar effect was observed in cupric ion reduction activity results. When the results of ABTS radical scavenging activity are examined, it can be said that both extracts are good radical scavengers. In the previous study, it was stated that methanol extract obtained from *Ajuga postii* Briq and *Ajuga relicta* had 70% power in the ABTS radical scavenging capacity [31]. The reason for incompatibility in our results may be due to the different solvents and plant species used. Similarly, the DPPH radical scavenging activity of *L. peltata* extract was highly similar to the standards, but *L. canescens* extract was found to be weaker. A study performed on *Salvia steminea* extract emphasized that the plant extract have lower DPPH radical scavenging activity than standards [11] and the another study emphasizes that *Origanum marorana* extract exhibited lower DPPH radical scavenging activity than standards such as BHA, BHT and ascorbic acid [12]. It can be said that the results of *L. canescens* are similar to the literature, but the *L. peltata* results showed better activity than literature. According to the *in vitro* antioxidant results, we can say that both plants are a good source of antioxidants and good antimicrobial agents. Thus, we believe that our study can provide important basic data for advance studies focusing on active substance design and drug production.

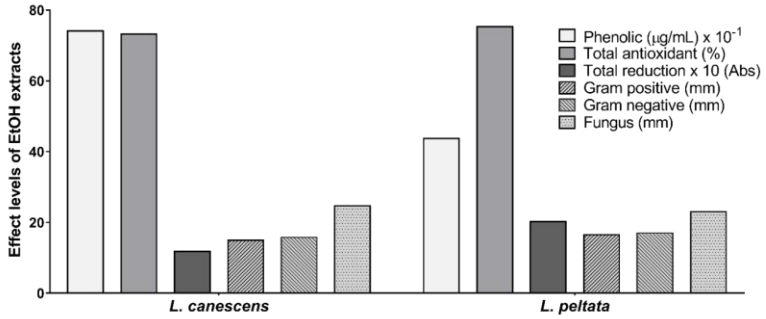


Figure 3. Various biological activity levels of EtOH extracts. The actual phenolic concentrations (µg/mL) were multiplied with $10^{-1}$ and actual total reduction results were multiplied with 10 to compare easly by displaying all graph columns clearly on graph. Total antioxidant results are shown as percent (%) and antibacterial effects are shown as diameter values of inhibition zones (mm).

*4.4. DNA protection activity results*

From the simplest compounds to the most complex molecules, many molecules can interact with DNA and disrupt its natural structure. As a result, form I structure by breaking the first strand of DNA and form II by breaking the second strand of the DNA can be occurred [38]. These construction forms were shown in Figure 2. Although these plants are expected to have power DNA protective activity due to their antioxidant properties, this effect was minimal. The reason for this may be due to insufficient antioxidant content in plants. As a data supporting this result, we have already mentioned that *L. peltata* extract was better than *L. canescens* extract in the DNA protective activity results. It may also be another molecule produced by the plant, which may inhibit the protective effect of the plants on DNA. As stated in many previous studies, plants can voluntarily produce reactive oxygen species to defend themselves against pathogens or other adverse conditions [25]. Despite the positive effect of *L. peltata* extract on the form I structure in the line 11, the absence of this effect in the line 14, 15 and 16$^{th}$ is quite ambiguous. Both *L. peltata* extract and $H_2O_2$ alone were observed to have deleterious effect on plasmid DNA, however, this effect was partially disappeared when they were added together. The minimal view of form I in Figure 2 line 16 supports to this. The phenolic content of *L. peltata* extract was observed to be dramatically lower than *L. canescens* extract (Figure 3). The reason for this may be due to the excessive height of undetermined phenolic content in *L. peltata* extract. As a matter of fact, the efficacy of *L. peltata* on form II stabilization supports this idea and both of the plants extracts did not show generally a significant protective effect on plasmid DNA damaged by $H_2O_2$. This can be also attributed to an inhibitory molecule produced by plant or insufficient antioxidant content [32] reported that *Lamiaceae* family water extracts, depending on concentration, showed protective effect on the pBR322 plasmid DNA damaged by $H_2O_2$ and UV and essential oils of the same family were reported to have the stabilization of plasmid DNA [4]. In addition, *Leucas aspera* water extract was reported to have a protective effect on DNA [14]. In addition to the partial effect of our EtOH extracts on the stabilization of plasmid DNA, the form I was transformed into form II by an unknown effect of the high concentration of the extracts.

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