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# Some biological properties of ethanol extract prepared from the aerial parts of *Scutellaria albida* L. subsp. *condensata* (Rech.f.) J.R. Edm.

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ABSTRACT

Moderate effects of some plants with proven biological properties in the treatment of various diseases have increased the importance of them and the interest in alternative medicine. The members of the Lamiaceae family are one of the widely used in alternative medicine and agriculture due to their metabolite content. In order to gain valuable biological data for alternative medicine and new studies, *Scutellaria albida* subsp. *condensata* is a member of the Lamiaceae family and this plant was collected from a height of 1500 meters in Bitlis province in Turkey. Ethanol (EtOH) extract was prepared by using the aerial parts of the plant and used in all stages of the study. Firstly, the phenolic content of the extract was determined by HPLC. Myricetin and 4-Hydroxybenzoic acid at the highest concentrations were detected, but ascorbic acid, gallic acid, quercetin, and 3,4-Dihydroxybenzoic acid could not be determined in the extract. In order to test the antioxidant properties based on phenolic content, several *in vitro* antioxidant tests were performed and DNA protective properties were investigated. In the biological activity results, the extract was determined to have a similar antioxidant effect to standards or lower than them and exhibited relatively DNA protective activity at high concentration. Finally, the effects of the extract on some types of bacteria and fungi were investigated by the hollow agar method and 150 µL volume of the extract was shown to have better activity than ampicillin and Amikacin. Due to the limited studies on *Scutellaria albida* subsp. *condensata*, it is thought that this study will contribute to the literature.



## 1. Introduction

Plants are important medical factors for our health because of their healing effects or positive effects on the some of diseases. Secondary metabolites (SMs), also known as phytochemicals, natural products, or plant constituents are important molecules that play a role in the cell's interaction with its environment **[1]**. The main classification system for SMs includes three major groups: terpenoids, alkaloids, and phenolics and each of them contain subclasses with complexity in structure **[2]**. Phenolics, one of the most important, have high antioxidant value and are known to be protective against neurodegenerative diseases, diabetes, and cancer associated with reactive oxygen species such as hydrogen peroxide ($H_2O_2$), superoxide ($O^{2-}$), and hydroxyl ($OH^{\bullet}$) **[3, 4]**.

The members of the Lamiaceae family are widely used in traditional medicine and agriculture and they include plant species with economic value **[5]**. The use of this family in pharmaceutical production and agricultural fields increased the importance of family members and attracted the attention of scientific world. They are spread almost all over the world and have 46 different genera and 758 taxa in our country, and the rate of endemism in our country is 45% **[6]**. The members of *Scutellaria* L. genus generally grow on stony and rocky slopes and have medical importance due to their SMs and other contents **[7]**. Previous studies have reported that the members of this family has many biological effects such as antimicrobial, antifungal, anti-inflammatory, anticancer, and antioxidant effects **[8-12]**.

Based on the data suggested about the Lamiaceae genus in previous studies, it is aimed to gain a new plant species and its valuable biological properties for the literature and to provide a starting point for advanced studies carried out on similar studies and especially pharmacological research. For this purpose, ethanol (EtOH) extract was prepared from the aerial parts of *Scutellaria albida* subsp. *condensata* collected in Bitlis province in Turkey and various biological properties of the extracts were investigated. Within the scope of the study, the concentrations of 17 phenolics were tried to determine by HPLC. Moreover, Various *in vitro* antioxidant properties and DNA protective properties on pBR322 plasmid

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DNA were studied using different concentrations of the extract to validate HPLC results. Finally, by investigating the antimicrobial effects of the extract, their compatibility with the effects of the antioxidant and DNA protective properties were tested.

## 2. Material and Method

### *2.1. Collection of the plants and extract preparation*

*Scutellaria albida* subsp. *condensata* was collected during the vegetation period between 2014-2015 years in Bitlis province in Turkey. EtOH extract was prepared with the 50 g of aerial parts of the using the soxhlet extraction apparatus and stored at -18 $^{o}$C, in dark bottles. The Plant was also converted into herbarium material and stored in Bitlis Eren University Science and Technology Application and Research Center with the code M.KURŞAT: 6049.

### *2.2. Determination of phenolic concentrations by HPLC*

HPLC chromatograms using the 10mg/mL standard concentrations and calibration curves using their different concentrations were created. The chromatogram of phenolics is shown in **Figure 1**. Five different concentrations of standards were used to generate the calibration curve and prepared standard solutions were filtered through 0.45µm membrane filter. HPLC conditions were performed according to the method used in our previous article **[13]**.

### *2.3. Microorganisms and antimicrobial activity*

The microorganisms used in this study are as follows; *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM 32, *Enterobacter aerogenes* ATCC 13048, *Eshericha coli* ATCC 11229, *Pseudomonas aeroginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 13883, *Yarrowia lipolytica*, *Candida albicans* ATCC 10231 and *Saccharomyces cereviciae* ATCC 834. Standard antibiotics known to be effective on microorganisms were purchased from OXOID company and their antimicrobial effects were determined using the hollow agar method and the results were compared with the effects of the extract. The antibiotics used in this study are Erythromycin; E-15, Ampicillin/Sulbactam; SAM-20, Rifampicin; RD-5, Amikacin; AK-30 and Fluconazole; FCA-25. Test microorganisms were obtained from Muş Alparslan University Central research and application laboratory, and bacterial production was performed using the method of Hindler [14]. The blur of microorganisms was adjusted according to the 0.5 standard of Mc Farland. The different concentrations (75 µL, 100 µL, and 150 µL) of extract were prepared and antimicrobial effects of the extract and antibiotics were determined using the method of Sagdic, Karahan [15].

### *2.4. Antioxidant properties using in vitro studies*

#### *2.4.1. Total antioxidant activity by ferric thiocyanate method*

The effect of the extract on lipid peroxidation was determined using the ferric thiocyanate method proposed by Mitsuda [16]. This method is based on the spectrophotometric measurement of the peroxide formed as a result of linoleic acid oxidation at 500 nm at 10 hours intervals. The test was terminated when the control absorbance reached the highest value. Absorbance percentages in the inhibition of linoleic acid emulsion were calculated according to the following equation 1. In the equation, A sample; the value of the sample absorbance when the control absorbance is the highest and A control; the value of control absorbance when the control absorbance is the highest.

$$\text{Lipid Peroxidation Inhibition (\%)} = 100 - \left(\frac{\text{A sample}}{\text{A control}} \text{x} 100\right) \qquad (1)$$

#### *2.4.2. Determination of total reduction*

Total reduction power was performed according to the method proposed by Oyaizu [17]. According to this, the extract was prepared at different concentrations (25µg/mL, 50µg/mL, and 100µg/mL) and absorbance values were spectrophotometrically recorded at 700 nm.

#### *2.4.3. DPPH radical scavenging activity*

The effect of DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging was performed by Blois [18] method. This method is based on the reduction of DPPH radicals dissolved in alcohol in the presence of antioxidants having hydrogen donating groups and absorbance values are measured at 517 nm.

#### *2.4.4. Cupric ($Cu^{2+}$) reduction by the CUPRAC method*

Cupric ions ($Cu^{2+}$) reduction activities were performed using the method proposed by Apak, Güçlü [19]. According to the method, the absorbance values of different concentrations of the samples were recorded at 450 nm. The increase in the absorbance of the reaction mixture indicates the cupric ion reduction capacity.

### *2.5. Determination of DNA protective effect*

In this study, pBR3222 plasmid DNA was used as model nucleic acid and DNA damage was created using $H_2O_2$. The protective effect of the extract on pBR322 plasmid DNA was demonstrated by agarose gel electrophoresis **[20]**. According to this, sample mixtures were prepared as shown in **Table 1** and 5µl of prepared extract and 5µl of loading buffer were mixed with each other and loaded to electrophoresis with the other electrophoresis components (**Figure 3**). Electrophoresis was performed at 40V for 2 hours and stained with ethidium bromide and agarose gel was then visualized using Londershausen 1996 imaging system.

## 3.Results

### *3.1. Phenolic Concentrations of the Extract*

The phenolic concentration results are shown in **Table 1**. The study showed the myricetin concentration as the highest (28.795µg/mL) and the vanillin concentration as the lowest (0.728µg/mL). However, ascorbic acid, gallic acid, 3,4-dihydroxybenzoic acid, and quercetin could not be determined

in the extract.

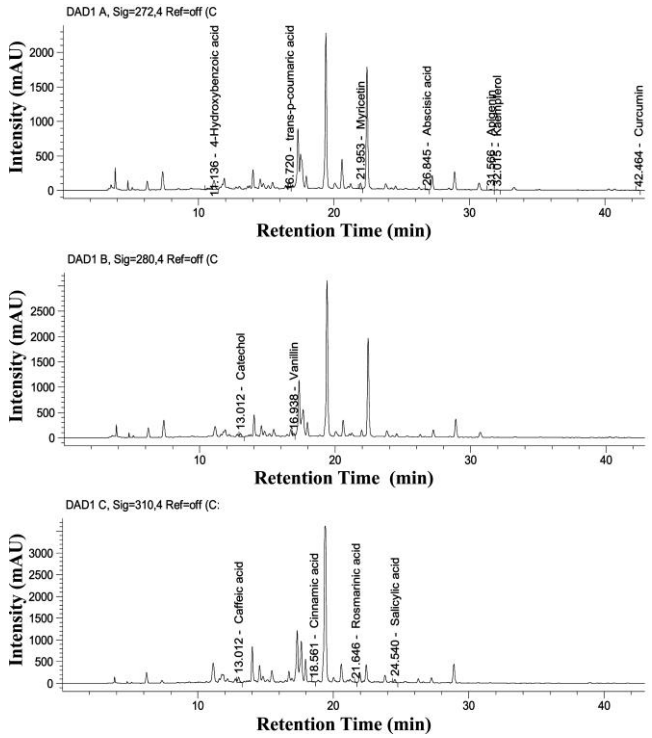


**Figure 1.** HPLC chromatogram showing retention times of phenolic content in the extract.

**Table 1.** Phenolic concentrations of EtOH extract

| Phenolic content | Concentration (µg/mL) |
|---|---|
| Ascorbic acid | N/A |
| Gallic acid | N/A |
| 3,4-Dihydroxybenzoic acid | N/A |
| 4-Hydroxybenzoic acid | 24.823 |
| Trans-p-coumaric acid | 10.808 |
| Myricetin | **28.795** |
| Abscisic acid | 6.773 |
| Quercetin | N/A |
| Apigenin | 18.462 |
| Kaempferol | 2.799 |
| Curcumin | 4.111 |
| Catechol | 17.951 |
| Vanillin | **0.728** |
| Caffeic acid | 8.360 |
| Cinnamic acid | 1.428 |
| Rosmarinic acid | 0.780 |
| Salicylic acid | 14.913 |

### *3.2. Antimicrobial effects of the extract*

*Scutellaria albida* subsp. *condensata* extract was tested on 3 Gram positive (*B. subtilis*, *S. aureus* ve *B. megaterium*), 4 gram negative (*E. aerogenes*, *E. coli*, *P. aeroginosa* ve *K. pneumonia*), and 3 Fungus (*Y. lipolytica*, *C. albicans* ve *S. cereviciae*) and the results of the antimicrobial effects were calculated as mm (**Table 2**). When the antimicrobial effect results are evaluated, it is clear that primarily the antimicrobial effect increases depending on the extract concentration and it can be said that although the extract has both antibacterial and antifungal effects, fluconazole has only antifungal effect and other antibiotics have only antibacterial effect. In addition to this, extract showed the lowest antimicrobial effect on *B. subtilis*, *E. aerogenes* and *C. albicans* (14±0,00 mm) and the highest antimicrobial effect on *B. megaterium* (20±1,00 mm).

**Table 2.** **A**: Diameters of the antimicrobial effect depending on the EtOH extract concentration (mm), **B:** Diameters of the antimicrobial effect depending on the type of antibiotics (mm)

**A**

| | Microorganisms | EtOH extract 75µl | 100µl | 150µl |
|---|---|---|---|---|
| Gram (+) | ***B. subtilis*** | n/a | n/a | 14±0,00 |
| | ***S. aureus*** | n/a | n/a | n/a |
| | ***B. megaterium*** | 15±0,00 | 16±0,00 | 20±1,00 |
| Gram (-) | ***E. aerogenes*** | n/a | n/a | 14±0,00 |
| | ***E. coli*** | 12±0,00 | 14±0,57 | 16±1,15 |
| | ***P. aeroginosa*** | 12±0,00 | 14±0,00 | 17±1,00 |
| | ***K. pneumonia*** | n/a | n/a | n/a |
| Fungus | ***Y. lipolytica*** | 14±0,00 | 15±0,57 | 15±0,57 |
| | ***C. albicans*** | n/a | n/a | 14±0,00 |
| | ***S. cereviciae*** | n/a | n/a | 17±0,57 |

**B**

| Antibiotics | | | | |
|---|---|---|---|---|
| Erythromycin | Ampicillin / Sulbactam | Amikacin | Rifampicin | Fluconazole |
| 20±0,00 | 14±1,15 | 11±1,00 | 21±0,00 | n/a |
| 21±1,00 | 10±0,00 | 9±0,00 | 18±1,15 | n/a |
| 25±0,00 | n/a | 10±1,00 | 16±0,00 | n/a |
| 27±1,00 | 10±1,00 | 9±0,00 | 16±1,00 | n/a |
| 19±1,52 | 13±0,00 | 13±0,00 | 18±0,00 | n/a |
| 19±0,00 | - | 14±1,15 | 8±0,00 | n/a |
| 19±1,73 | 16±0,57 | 10±0,00 | 19±1,73 | n/a |
| n/a | n/a | n/a | n/a | 21±0,00 |
| n/a | n/a | n/a | n/a | 23±1,52 |
| n/a | n/a | n/a | n/a | n/a |

n/a: not available

### *3.3. Antioxidant study results*

#### *3.3.1. Total antioxidant activity results*

Total antioxidant activity was investigated at different concentrations (25µg/mL, 50µg/mL, and 100µg/mL) and generally increased depending on extract concentration. Inhibition effects of the extract and standards on linoleic acid emulsion were calculated based on the 30$^{th}$ hour when the control absorbance value reached the maximum. When the result of total antioxidant activity was evaluated, the highest

effect was observed at 100µg/mL concentration (**Figure 2A**) and total antioxidant activity percentages were calculated for this test. According to this, the order of total antioxidant activities for the highest concentration is as follows; EtOH extract (73.19%) > BHT (72.76%) > BHA 72.34(%) > α-Tocopherol (57.87%)

### *3.3.2. Total reduction activity results*

The reduction capacities of the samples were determined by measuring spectrophotometrically the absorbance values at 700 nm and their reduction capacities increased in parallel with the concentration and the order of the results obtained is as follows; BHA > BHT > α-Tocopherol > EtOH extract. (**Figure 2B**)

### *3.3.3. DPPH radical scavenging results*

DPPH radical scavenging activities were evaluated for 25 µg/mL, 50 µg/mL and 100 µg/mL extract concentrations at 517 nm and the strongest effect was seen at 100 µg/mL (**Figure 2C**). Then, DPPH radical scavenging activity percentages for 100 µg/mL were calculated. When looking at 100 µg / mL concentration results, besides the strong effect in the extract, it is clear that the extract has a lower radical scavenging effect than the standards and DPPH radical scavenging sequence is as follows; BHA(91,74%) ≥ α-Tocopherol (91,23%) ≥ BHT (90,50%) > EtOH extract (69,94%).

### *3.3.4. Cupric ion ($Cu^{2+}$) reduction results*

Cupric ions ($Cu^{2+}$) reduction activities were performed using the method proposed by Apak, Güçlü [19]. The absorbances in different concentrations of the extract and standards were recorded at 450 nm. The cupric ion reduction capacity of the extract increased in parallel with extract concentration (**Figure 2D**). The order of cupric ion reduction capacities is as follows; BHA > BHT > α-Tocopherol > EtOH extract.

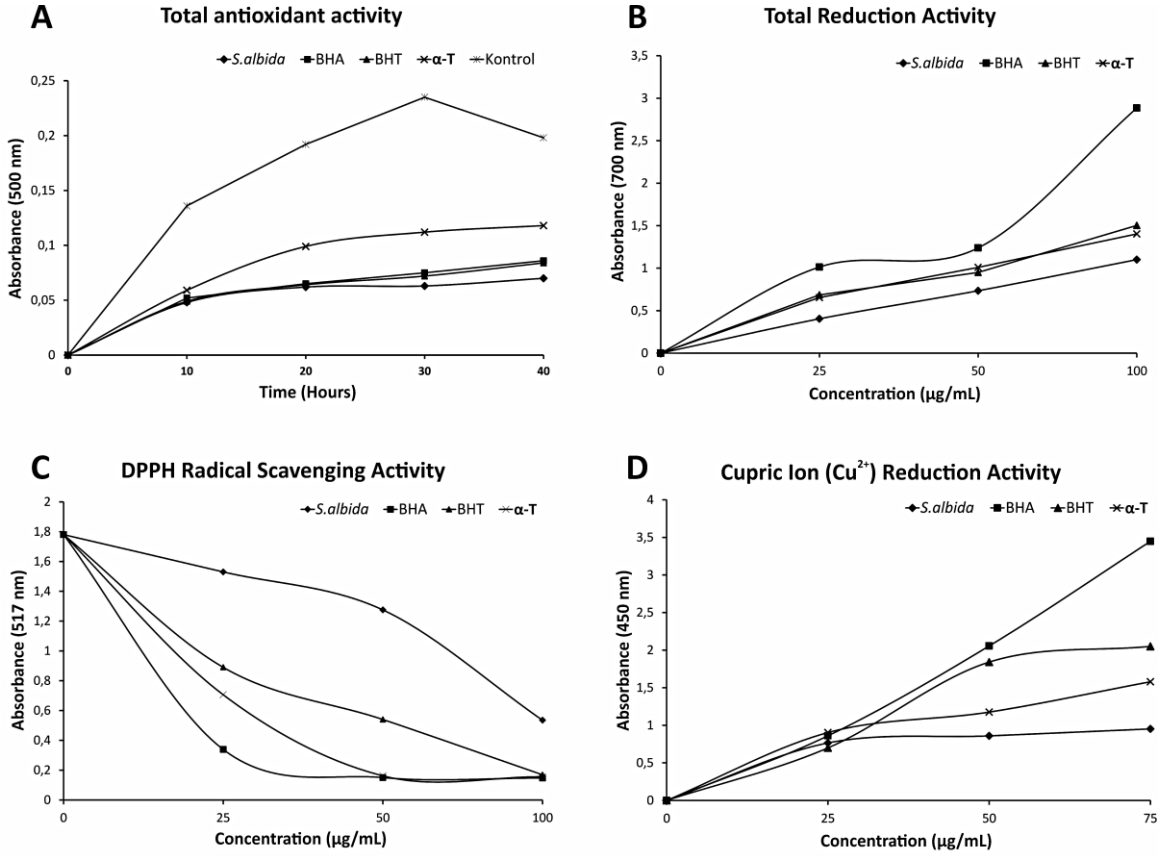


**Figure 2.** *In vitro* antioxidant activity results of EtOH extract prepared from S. albida subsp. condensata (*S. albida*) and standards. **A;** Total antioxidant activity depending on hours, **B;** Total reduction activity on concentrations **C;** DPPH radical scavenging activity on concentrations and, **D;** Cupric ion ($Cu^{2+}$) reduction activity on concentrations.

### *3.4. DNA protective activity results*

Whether the extract has a protective effect on plasmid DNA was determined by observing the form I, form II, and form III structures in pBR322 plasmid DNA (**Figure 3**). In the results of agarose gel electrophoresis, it is understood that $H_2O_2$ disrupted the form I structure (**Line 2 in Figure 3)** and the mixture of $H_2O_2$ and DMSO destroyed completely the plasmid DNA (**Line 3 in Figure 3**). It was also observed that the high extract of *Scutellaria albida* subsp. *condensata* relatively

decreased the scavenging effects of the mixture of $H_2O_2$ and DMSO (**Line 5 in Figure 3**). In addition, the extract did not have a serious adverse effect on plasmid DNA when applied alone (**Line 8, 9, 10 in Figure 3**) however, when applied together with $H_2O_2$, it had a seriously positive effect on the stability of the form II structure, especially at 100 µg/mL concentration (**Line 5 in Figure 3**).

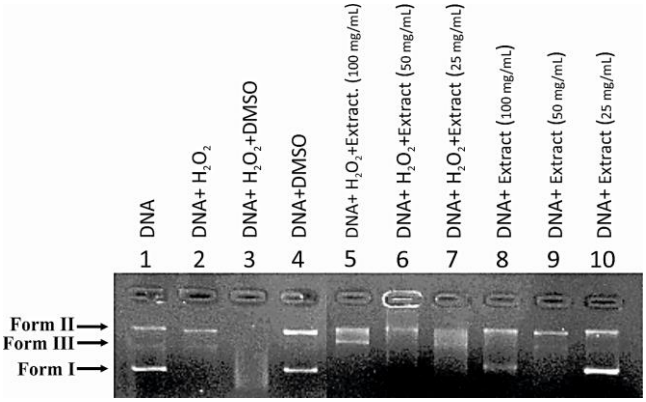


**Figure 3.** Agarose gel image showing the protective effect of EtOH extract on pBR322 plasmid DNA.

## 4. Discussion

It is known that plants have a protective effect against some diseases by supporting the immune system. In particular, plants whose healing effects have become the focus of researchers and new plant and biological property discoveries have gained momentum. As a result, it was understood that some of them have direct healing effects and the others have an indirect healing effect, and the reason for this is attributed to the types and concentrations of molecules in plant content. Lamiaceae family is widely researched and known to have strong biological properties. Previous studies have mentioned the high levels of rosmarinic acid in this family **[21-23]**. Gallic acid, myricetin, quercetin, apigenin, kaempferol, caffeic acid, cinnamic acid, and rosmarinic acid were reported to find in different species of this family **[24]**. Another study reports that this family has high phenolic concentrations known as antioxidants such as rosmarinic acid, coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, luteolin, apigenin, quercetin, rutin, epicatechin, and catechin **[25-27]**. In the HPLC results of our study, it was detected that the extract contains the highest level of myricetin (28.795µg/mL) and was found to have very low curcumin concentration which is used extensively as food additive (4.111µg/mL).

Extracts prepared from plants known to have antimicrobial effects were used in the treatment of bacterial infections in ancient times. Studies have reported that ethanol and methanol extracts prepared from the members of the Lamiaceae family have a strong effect on *S. aureus* and *B. subtilis*. The essential oils of the *Saturja hortensis* which is a member of the Lamiaceae family have been reported to show high antimicrobial activity **[28]** and acetone extract of the *Scutellaria genus* show the highest antibacterial activity against *Streptococcus mutans* **[29]**. 150 µL volume of extract in our study exhibited the highest antimicrobial activity on *B. megaterium* and generally showed a higher effect than antibiotics other than erythromycin and rifampicin. A study conducted with the Lamiaceae family emphasized that the extracts exhibited lower activity than ampicillin and kanamycin **[30]**. It is clear that in this study, the antimicrobial effect at 150 µL extract volume was similar to ampicillin/sulbactam and amikacin or higher than them.

Some factors such as adverse environmental conditions and the misuse of medications cause oxidative stress and various metabolic diseases by increasing cellular radical concentration **[31, 32]**. Moreover, the radicals can transform the form I structure of DNA into form II and form III by disrupting the double helix of DNA **[33]**. Contrary to this, antioxidant molecules inhibit the radicals that are the product of adverse conditions and hereby prevent DNA structure against oxidative stress and DNA damage **[34]**. In the results of antioxidant studies, it was clear that the extract has high antioxidant properties, but still exhibited lower activity than standard antioxidants. It was also determined that total antioxidant activity was higher than standards but, the effects of the extract were weaker in the other *in vitro* tests. This result is evidence that *Scutellaria albida* subsp. *condensata* is a potential antioxidant but not kinetically active. A study emphasizes that the Lamiaceae family have a protective role on plasmid DNA damaged by $H_2O_2$ and UV **[35]** and reported that *Leucas aspera* is a member of the Lamiaceae family and it has a positive effect on DNA stability **[36]**. Similar to previous studies, it was shown in this study that EtOH extract reduced the DNA damage caused by only $H_2O_2$ or DMSO and $H_2O_2$ together by allowing the regeneration of the form II structure depending on extract concentration. It can also be said that the lowest volume of extract does not have a significant effect on pBR322 plasmid DNA.

## 5. Conclusions

In conclusion, *Scutellaria albida* subsp. *condensata* extract was found to contain optimum concentrations of phenolic antioxidants. In parallel with this result, they were also found to have quite power antioxidant properties despite being weak by standards and exhibited the valuable antibiotic effect by providing the desired effect at high concentrations. The extract did not show a serious negative effect on pBR322 plasmid DNA when applied alone at low concentration, but when applied together with $H_2O_2$, the 100 µg/mL concentration of the extract reduced relatively the scavenging effect of $H_2O_2$.

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