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Determination of Antibiofilm Activity Species of *Sideritis trojana*

Yağmur TUNCA PINARLI[1] and Kerem CANLI[2]

[1]Department of Biology, the Graduate School of Natural and Applied Sciences, Dokuz Eylul University
Izmir, Turkey
yagmur.tunca.pinarli@gmail.com
[2]Department of Biology, Faculty of Science, Dokuz Eylul University, Dokuz Eylul University Fauna and Flora Research Center
Izmir, Turkey
biyoloji@gmail.com

Abstract*: The discovery of antibiotics is an important turning point in humanity's fight against diseases throughout history. However, the rapidly increasing world population and the increase in the use of antibiotics cause the emergence of antimicrobial resistance among bacterial agents and more biofilm structure in bacteria. Humanity is facing an antibiotic crisis due to the increasing number of people with bacterial biofilm-related infectious diseases. The formation of a biofilm structure that inhibits the effect of antibiotics is associated with the ability of pathogenic bacteria to make them sick. Bacteria with biofilm structures are 1000 times more resistant to antibiotics and disinfectants than bacteria with planktonic structures. In this situation that humanity is facing today, it is of great importance to discover agents with antibiofilm effects. In this study, antibiofilm activities of Sideritis trojona against Bacillus subtilis DSMZ 1971, Escherichia coli ATCC 25922, Listeria monocytogenes ATCC 7644, Listeria innocua, Escherichia coli 1214816 strains were determined. The test study determined that the plant extract had a positive antibiofilm relationship to Listeria monocytogenes strain and a negative antibiofilm relationship to Escherichia coli 1214329 strain.*

Keywords; Antibiofilm, Antibiotic, Sideritis trojona

1. INTRODUCTION

From past to present, all civilizations have struggled against diseases, and they are trying various treatment methods in this ongoing struggle. Humanity has used the plants found in the geographies where they live to heal against diseases as the first treatment method. Today, 25% of the drugs available in our prescriptions are of herbal origin, for example, drugs such as aspirin, reserpine, and quinine have herbal-based active ingredients [1-2].

Today, interest in studies in the field of phytotherapy is increasing due to the high side effects of synthetic substances used in the pharmaceutical industry and the inability to provide the desired treatment. According to the results of the research conducted by the World Health Organization based on phytotherapy studies, around 20,000 plants are used for treatment in the world. It is estimated that around 500 herbal drugs are used for medical treatment in Turkey [3].

The increasing world population and increasing consumption of antibiotics out of control in societies have revealed the problem of antibiotic-resistant strains. Antibiotic-resistant strains have caused an increase in patients with impaired immune systems, an increase in patient burden in intensive care units, and an increase in deaths from infectious diseases. One of the mechanisms that provide resistance of microorganisms to antibiotics is the biofilm structure [4-5]

Biofilm has been explained as the structure formed by microorganisms that are immobile embedded in the exopolysaccharide matrix produced by microorganisms on living or inanimate surfaces, adhered to the surface irreversibly, and are completely different from each other in terms of genetic and protein structure [6-7].

When the structure of the biofilm is examined microscopically, it is seen that it consists of an exopolysaccharide layer in which the water channels that provide the transport of nutrients and oxygen are embedded. Microorganisms in the biofilm layer show different characteristics from their planktonic forms and do not lose their acquired properties even if they leave the biofilm layer. Microorganisms that make up the biofilm structure may be the same species or may be formed by the combination of different species. Microorganisms with a biofilm layer are more resistant to antibiotics, humidity, heat, environmental changes, and the immune system of the host they are in compared to microorganisms in planktonic form [8-9-10].

Sideritis trojona is an endemic species that spreads in Kaz Mountains, known as yellow maiden tea. The most distinctive feature of *Sideritis trojona*, which is in the form of a bush and herbaceous, is the white wooly hair structure of its leaves. There is a flower structure consisting of 3-9 dark yellow flower clusters [11]. It is used as herbal tea among people. It is known to affect the nervous and digestive systems [12-13].

2. MATERIAL AND METHOD

2.1 PLANT SAMPLE

Sideritis trojona used in this study, Kaz Mountains were collected and identified from Çanakkale by Dr. Mustafa Eray BOZYEL. Witness samples are in Dokuz Eylul University, Faculty of Science, Department of Biology.

2.2 Microorganisms

In this study, 3 standard strains, gram-positive *Bacillus subtilis* DSMZ 1971, *Listeria monocytogenes* ATCC 7644 and gram-negative *Escherichia coli* ATCC 25922, 1 food isolate strain gram-positive *Listeria innocua*, and 1 gram-negative *Escherichia coli* working with Efflux pump system were used to determine the antibiofilm activity of *Sideritis trojona* endemic plant.

2.3 Antibiofilm Test

In this study, the Antibiofilm test was modified from Karaca (2020) [14]. The test consists of two stages: first step determination of biofilm formation conditions and the second step determination of antibiofilm activity.

Determination of biofilm formation conditions:

Bacillus subtilis DSMZ 1971, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Listeria innocua*, and *Escherichia coli* 1214816 strains used in the study was adjusted to 0.5 McFarland in saline. Then, it was transferred to microplates with six different glucose monophosphate concentrations. These are 0%, 0.5%, 1%, 1.5%, 2%, and 2.5%, and incubated at 37 degrees for 24 and 48 hours.

After the incubation process, the microplates removed from the incubator were poured and washed with distilled water, and 200 µL of crystal violet was placed in all the wells of the microplate and left for 15 minutes. After the waiting period was completed, the crystal violet was emptied, and the microplates were washed again with distilled water. At the last stage, 200 µL of ethyl alcohol-acetone solution was placed in the wells and after waiting for 15 minutes, the ethyl alcohol-acetone solution was transferred to clean microplates with the help of a micropipette, and measurements were made at 550 nm in the microplate reader device.

As a result of the study, ideal biofilm formation conditions for *Bacillus subtilis* DSMZ 1971, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Listeria innocua*, and *Escherichia coli* 1214816 strains were determined as 48 hours and 1.5% glucose monophosphate concentration.

Determination of antibiofilm activity:

Bacteria were adjusted to 0.5 McFarland in physiological saline and transferred to microplates with 1.5% glucose monophosphate concentration for 48 hours determined during the determination of biofilm formation conditions and incubated at 37 degrees.

After the incubation process, the procedures described in the stage of determining the biofilm formation conditions were applied to the microplates removed from the incubator, and their antibiofilm activities were determined by making measurements at 550 nm in the microplate reader device.

3. RESULT AND DISCUSSION

The correlation graphs obtained from the antibiofilm activity test performed on 5 strains of the plant extract obtained from the *Sideritis trojona* endemic plant are available in Table 1-5. Correlation results obtained from *Sideritis trojona* plant against strains in the test study: *Escherichia coli* 1214329 strongly negative, *Listeria innocua* weakly negative, *Bacillus subtilis* DSMZ 1971 medium positive, *Escherichia coli* ATCC 25922 negative, *Listeria monocytogenes* ATCC 7644.

Table 1: Antibiofilm activity correlation graph of *Sideritis trojona* endemic plant to *Escherichia coli* 1214329 strain

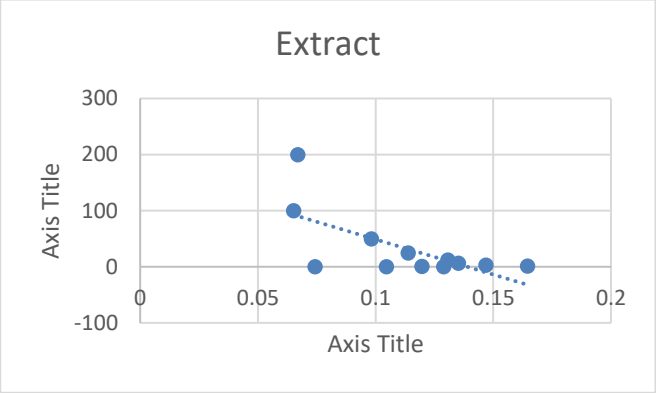

Table 2: Antibiofilm activity correlation graph of *Sideritis trojona* endemic plant to *Listeria innocua* strain

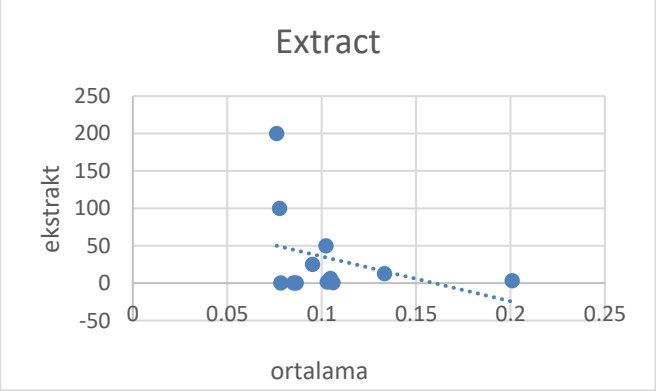

Table 3: Antibiofilm activity correlation graph of *Sideritis trojona* endemic plant to *Bacillus subtilis* DSMZ 1971 strain

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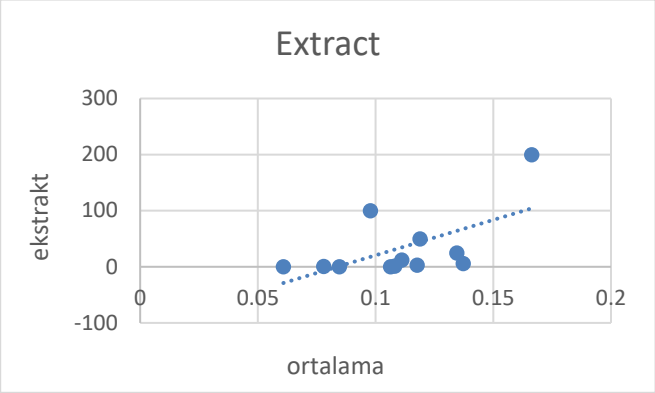


Table 4: Antibiofilm activity correlation graph of *Sideritis trojona* endemic plant to *Escherichia coli* ATCC 25922 strain

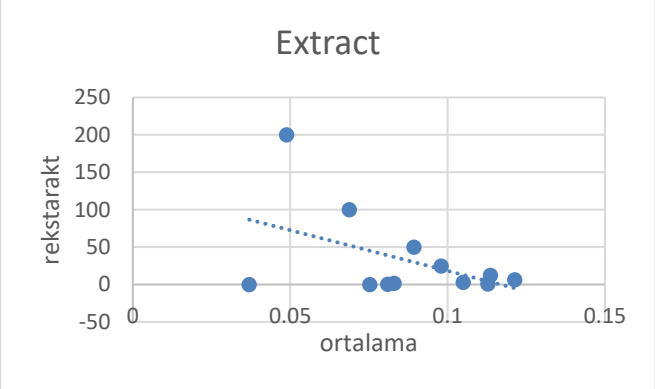
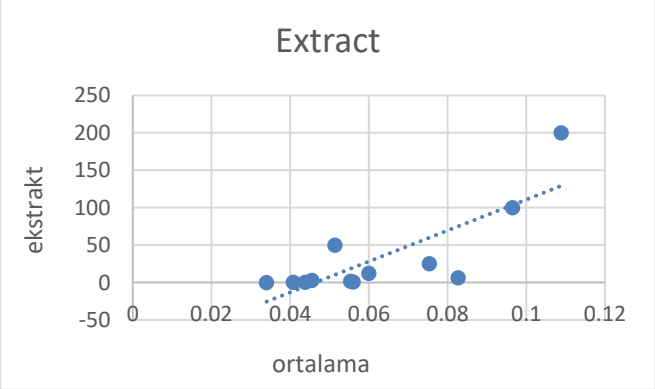


Table 5: Antibiofilm activity correlation graph of *Sideritis trojona* endemic plant to *Listeria monocytogenes* ATCC 7644 strain



According to the statistical data of the test study, the positive correlation relationship of the endemic plant *Sideritis trojona* against the strain of *Listeria monocytogenes* ATCC 7644 reveals that it has antibiofilm properties. *Escherichia coli* 1214329 strain, on the other hand, shows that it can be used as an alternative in the field of antibiofilm because of the negative correlation with the plant extract.

In the study conducted by Koç and Erginkaya [15], which investigated the antibiofilm effect of *Bacillus coagulans*, which is an important probiotic, on the *Listeria innocua* strain, it showed an antibiofilm effect, while the *Sideritis trojona* plant extract used in this study showed a weak negative correlation.

Pelemir seed oil extract concentrations by Atalan [16] did not show any effect against the strain of *Listeria monocytogenes* in the antibiofilm activity test. In the antibiofilm test performed in this study, a very strong positive correlation was obtained against the plant extract concentrations of *Listeria monocytogenes*, *Sideritis trojona.*

Üstün et al. [17] showed that the bioactive compounds obtained from the fungus *Aspergillus sclerotiorum* influenced the *Bacillus subtilis* strain in the antibiofilm activity test.

In the antibiofilm study conducted with the extract concentrations of the *Ginkgo biloba* plant species by Karakaya et al. [18], it was found that it showed no effect against *Escherichia coli* ATCC 25922 strain. In this study, it was shown that *Sideritis trojona* plant extract concentrations had a negative correlation in inhibiting the biofilm of the *E. coli* strain.

4. CONCLUSION

There is no study in the literature of the medicinally important *Sideritis trojana* plant in the field of antibiofilm. The study makes an important contribution to the literature. Since the purification process of the *Sideritis trojona* plant, whose antibiofilm activity was determined in this study, was not carried out, the active substance and effective dosage in the plant extract are not known.

After the purification and dosage amount studies to be done, it can be revealed whether it will be used in the medical field and food industry. More comprehensive studies are needed to determine the antibiofilm effect mechanism and active ingredient of *Sideritis trojana*.

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