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RESEARCH ARTICLE

ANTIBACTERIAL, ANTIFUNGAL, ANTIBIOFILM, ANTIOXIDANT, AND ANTICANCER PROPERTIES OF METHANOL EXTRACTS OF SOME *ACANTHOLIMON BOISS* (PLUMBAGINACEAE) SPECIES

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ABSTRACT

Therapeutic properties of plants have been used since the existence of mankind. To benefit from this feature, the discovery of the active components of plants has gained momentum today. One of these plant species is *Acantholimon Boiss*. This plant extract has antifungal, antimicrobial, and anticancer properties. In this article, antimicrobial, antifungal and antioxidant capacities and antibiofilm properties of *Acantholimon Boiss* plant extract on 15 bacterial and 2 fungal species were evaluated. Antimicrobial properties were evaluated by Agar Well Diffusion method. It was observed that the plants formed a zone diameter of 12-18 cm. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were used to determine the minimum active property. Biofilms of almost all bacteria were reduced by 10-50% by Acantholimon species at doses of 8 mg/ml-16 mg/ml. For MCF-7 cells, the IC50 values for *A. acerosum subsp. brachystachyum (Boiss.)*, *A. acenum var. balansae*, *A. armenum var. balansae and A. kotschyi* were 0.143 mg/ml, 0.63 mg/ml, 0.63 mg/ml, 0.63 mg/ml, 0.63 mg/ml, 0.63 mg/ml and 0.107 mg/ml, respectively. The same doses did not show a cytotoxic effect on HUVEC cell lines. With this article, it has given important results in terms of having a wide range of bacterial studies and examining its activities in fungi and cell lines. These findings suggest that extracts of *Acantholimon Boiss* species may be a potential source of natural medicines and antibacterial, antifungal, antibiofilm, antioxidant, and anticancer activities may support their therapeutic use.

Keyword: *Acantholimon species; Anticancer activity; Antimicrobial activity; Antioxidant activity.*

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1. INTRODUCTION

Plants are highly valued in various businesses due to their fragrant, medicinal, aromatic, or therapeutic capabilities. Medical plants supply fundamental raw ingredients for a variety of businesses, including medicine, cosmetics, fragrance, and food [1]. The ancient scriptures and customary wisdom of numerous cultures demonstrate that people's search for plant benefits dates back to ancient times [2,3]. Most of the medications used today are derived from plants. Aspirin and quinine are two examples of common medications derived from plants. These medications are created by extracting active compounds in plants or by enhancing these components [3–5]. Almost every compound contains compounds with medical content. One of the plant species to be used for this purpose is *Plumbaginaceae* family. This family is a family of perennial (rarely annual) plants that prefer cold, dry, and saline habitats, usually in mountainous and coastal areas. Plants in the family may be herbs, shrubs, or semi-shrubs. The diversity and endemism of the plants in the family are highest in the cold and dry mountainous regions of Central Asia [6,7]. There are six breeds representative of the family in Türkiye and the East Aegean islands [8]. *Acantholimon* species is a plant of the genus *Plumbaginaceae*, which forms spiny cushions, flowers are simple or branched, flowers are arranged in two rows and leaves are almost always spiny [9]. The arid mountainous regions of southwest and northeast Asia are the most dominant regions of this genus. The genus contains many plants as endemic species [6,10]. Extracts of this plant species are known to exhibit very good inhibitory effects on a wide variety of bacteria, fungi, and different cell lines [11–14].

When the literature studies are examined, *Acantholimon austroiranicum*, *Acantholimon bracteatum (Girard) Boiss.*, *Acantholimon gilliati*, *Acantholimon longiscapum*, *Acantholimon serotinum*, *Acantholimon scorpius*, and *Acantholimon chlorostegium* plants were tested for antimicrobial, antitumor, insecticide, cytotoxicity, liver protective, and antioxidant activity. Studies have shown that Acantholimon species have therapeutic properties [15–17].

The activity of these plants on various fungi is also frequently observed in the literature. One of the fungal species used in the study was *Candida albicans*. *Candida* species cause the majority of fungal-associated systemic bloodstream infections in intensive care units worldwide. Although Candida albicans is the most common and invasive species, non-albicans *Candida* species have become more prevalent in the last 20 years, leading to a decrease in the dominance of *Candida albicans*. However, there is still a significant risk of pathogens [18,19]. Another fungus, *Candida parapsilosis*, can build biofilms on central venous catheters and other surgically implanted devices. Additionally, when critical care patients get complete parenteral nutrition, *C. parapsilosis* grows quickly. Particularly in underweight newborns and malnourished youngsters, this poses a danger of mortality. Although infections with *C. parapsilosis* typically have lower rates of morbidity and mortality than infections with *C. albicans*, some clinical isolates of this species are known to be less susceptible to echinocandins, and in some regions, resistance to azole therapy has also been recorded [19–21].

Although some studies with Acantholimon species are included in the literature, they have generally covered a limited area. Accordingly, Karimzadeh et al. (2020) reported that the *Acantholimon serotinum* plant showed very strong cytotoxic activity on Breast cancer (MDA-MB231). It is reported to have a medically traditional use among people in Asia [22,23]. The biological activities of extracts and some fractions of the species *Acantholimon lycopodioides* were studied by Parvez et al. (2020)[24]. In the study, they reported that *Acantholimon* species have important biological activities in terms of antimicrobial, antioxidant, anticancer, and hepatoprotective properties. Soltanian et al.

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(2020) evaluated the cytotoxicity, free radical scavenging activity, and antimicrobial activity against some bacterial species of three species belonging to the genus *Acantholimon* on various cell lines and found quite ambitious results [14]. Parvez et al. (2020) emphasized that *Acantholimon lycopodioides* have important biological activities in terms of antimicrobial, antioxidant, and anticancer properties [13].

Within the framework of these aims, in this study, the activities of *Plumbaginaceae* family members on various bioactive and anticancer properties on a wide variety of bacterial groups, fungal groups, and cell lines were monitored. The fact that a wide range of bacterial groups was tested in the study, as well as the experiments with fungi and cell lines, were carried out in the same study, the results of the study will have a wide range of effects. In addition, since biological activity and cell culture studies on *Acantholimon* species are rare, the study is intended to fill a very important gap in the scientific literature.

2.MATERIAL AND METHOD

2.1. Plant Samples

Acantholimon species which are used in this study were collected by Dr. Cihangir Uygun from different locations on Ahir Mountain in Kahramanmaras **(Table:1).**

Table 1. Features, Locations, and Photos of Plants.

Plants	Morphological Features	Location
Acantholimon acerosum subsp. *brachystachyum*	Monomorphic leaf, 7-8 mm in the outer brakte and the inner brakte are equal. Calix is in the form of a 12-14 mm long pillow [9].	Kahramanmaras Ahir Mountain, Transmitter Antenna Zone.1800m Altitude, Habitat properties Alpin region, step, intense windy hill.
Acantholimon armenum var. *balansae*	In the outer bracte, it is 8–9 mm long. Leaf width 1.5 mm, exterior brakte is equal to the internal brakte or slightly shorter [25].	Kahramanmaras Ahir Mountain, Sulutarla around the region. 1600m altitude. Habitat properties Step, sub- alpin- alpin.
Acantholimon kotschyi	Linear 1.5-4 cm x 1-2 mm leaf, brakte equal 5-6 mm, calix 1-1.4 mm [26].	Kahramanmaras Ahir Mountain Summit, Karagol around the region,1600m altitude. Habitat properties, Step.
Acantholimon libanoticum	Monomorphic, 1.5-2.5 cm leaf, calix lop, and funnel-like form of 12-13 mm [9,26].	Kahramanmaras Ahir Mountain, Kucukgol around the region, 1650m altitude. Habitat Properties Step.

2.2. Extract Preparation

The technology developed by Comlekcioglu and Aygan in 2020 was modified to create plant extracts. [27]. The above-ground parts of the plants were crushed into a powder with a magnetic grinder. The powder was added to the sample at 1g/10 ml of methanol. After this period, the mixture was applied in an ultrasonic water bath for 30 minutes. After half an hour, the extracts were filtered into another

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container, the filtered portion was returned to the bottle, and 1g/10ml methanol was added. There have been three iterations of these actions. After the extraction process, the liquid extracts were centrifuged and allowed to dry.

2.3. Antimicrobial Activity

Revitalized bacteria in the range of 0.5-1 were utilized. Bacteria were seeded into Muller Hilton Agar using sterile exchanges. 50 µl from each plant sample, *A.armenum* var. *balansae, A.libanoticum* together, *A.kotschyi*, and *A.acerosum* subsp. *brachystachyum* together were tested. Bacteria inoculated on the Petri plate were incubated at 25 °C for two days and fungi at 37 °C for 18 to 24 hours. After incubation, the zone diameters on the Petri plates were measured with a ruler and recorded with an acetate pen.

2.4. Minimum Inhibition Concentration (MIC)

MIC was used to determine the lowest concentration of a material that would inhibit bacterial growth. 96 Well plates were used for this test. Nutrient Broth 100µl is put on each well of Well plates. Then 100 µL extracts were placed in the first well and mixed homogeneously. By taking 100 µl, this technique was repeated up to 6 wells. Wells 7 and 8 served as controls. OD values (600 nm) were obtained after 18 to 24 hours of incubation at 37 °C.

2.5. Minimum Bactericidal Concentration (MBC)

The MIC test was used to determine minimum inhibitory concentrations (MBC). After incubation, samples from plate wells without bacterial growth for MBC were zigzag injected into Muller Hilton. After injection, the plates were incubated at 37 °C for 18 to 24 hours. As a result of the experiment, minimal bactericidal doses that killed 99.9% of the microorganisms were obtained.

2.6. Antibiofilm Activity

Literature was taken into consideration for the determination of antibiofilm activity [28]. Plate wells were empty after incubation. The distilled water was then cleaned and allowed to air dry. The dried wells received 130 µl of 95 percent methanol, which was then poured into them and allowed to fix for 15 minutes before the methanol was drained and the plates were allowed to dry once again. The dry microplate wells were filled with 125 µl of 0.1 percent crystal violet solution and left at room temperature for 10 minutes. The plate was given a second distilled water washing after drying for ten minutes. Gram-positive bacteria were present in the drying wells, therefore, to dissolve the adhering bacteria, 200 µl of a 33 percent glacial acetic acid solution was applied. 200 µl of a 33 percent glacial acetic acid solution and 200 µl of a 95 percent ethanol solution, correspondingly, were poured into the wells containing gram-positive bacteria and gram-negative bacteria, respectively, and allowed for 15 minutes to dissolve the adhering bacteria (eq. 1).

$$\% \text{ Decrease: } ((C-Co)/C)x100 \quad (1)$$

C: Positive control sample. Co: Test sample.

2.7. Antioxidant Activity

Using the Blois (1958) [29] approach, a 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazil) solution was made and added to the antioxidant test apparatus. We prepared and vortexed plant extracts with concentrations of 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml. 150 µl of DPPH and 50 µl of extract were applied to the wells of a 96 cm plate. The wells received 200 µl of DPPH as a blank, 50

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µl of methanol, and 150 µl of DPPH. An ELISA reader was used to read the sample at 517 nm after it had been incubated for 30 minutes at room temperature and in the dark. The percentage of radical scavenging was estimated from the absorbance values obtained after the spectrophotometric tests using the following formula. The GraphPad Prism 8 software and IC_{50} values were used to determine the percentage findings of the three repeats (eq. 2).

Radical sweep % = (Ao – A) / Ao x 100 (2)

A: *AbsSample517nm* ***Ao:*** *AbsBlank517nm*

2.8. Anticancer Activity (MTT Method)
The study was also tested in comparison with the MCF-7 Breast cancer cell line and Human Umbilical Vein Endothelial Cells (HUVEC).

2.8.1. Preparing the medium
The completed medium was prepared by adding the components in the following ratio to the medium required to culture the cells.

1- 445 ml DMEM (Dulbecco's Modified Eagle's Medium),
2- 50 ml FBS (Fatal Bovine Serum),
3- 5 ml PS(Penicillin-Streptomycin).

The media content prepared for MCF-7 cells was added to the content of 0.05 mg of insulin. The prepared completed medium was filtered through a 0.20 µm filter before use and stored in the refrigerator at +4 °C.

2.8.2. Cell passage and freezing
Cells stored at 80°C were thawed under laboratory conditions for use. The media and cells were transferred to falcon tubes of 15 cm3 volume and centrifuged at 1000 rpm for 5 minutes. After centrifugation, the supernatant was aspirated to remove dead cells and wastes and 2 ml of the completed medium suitable for each cell was carefully pipetted onto the pellet. Cells were transferred to 75 cm2 flasks and 10 ml of complete medium was added. The flask was placed in an incubator containing 5% CO2 at 37°C. The morphological structure and density of the cells were monitored and the cell media was changed 2-3 times during the week. The passage was performed to cover the cell density of the flask surface. In the incubation step of this procedure, the medium was first aspirated from the cell flask. After PBS was added, the flask surface was washed and aspirated, the flask was filled. Following this step, 2/3 ml trypsin was injected and the live cells adhering to the flask surface were incubated for 5 minutes and separated. After incubation, a microscope was used to examine the detachment of the cells. The mixture was poured into a 3 ml flask and centrifuged at 1000 rpm for 5 minutes to separate the cells. From the absorbance values obtained after spectrophotometric tests, the percentage of radical scavenging was estimated using the following formula. A further 10 ml of prepared medium was poured into a new flask containing the cells and then placed in an incubator containing 5% CO2 at 37 °C. After this procedure, the cell density for transplantation was determined.

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2.8.3. Cell counting and transplantation

The completed medium was added after the cells had been incubated, and they were then withdrawn, centrifuged, and treated with PBS and Trypsin at rates appropriate for counting the cells during the passage process. To count the cells in the Thoma slide, 50 µl of Trypan Blue dye was added to 50 µl of Eppendorf and 50 µl of cell suspension. We counted the divisions on the slide and computed the average number of cells. Each well of the Well Plate would hold 5,000 cells for the duration of the experiment. From the prepared suspension, the necessary cell was obtained, and it was then added to the working volume with the prepared medium. A 100 µl plate's wells are occupied by the cells. After being placed on the plate, the cells were incubated for 24 hours at 37°C with 5 percent CO_2.

2.8.4. Preparation of plant concentrations and application to cells

By diluting with the finished medium, concentrations of 10, 10-1, 10-2, and 10-3 from the plant extract were created. The respective wells were filled with vortexed plant concentrations, 100 µl was added and then the plate was incubated once more. This technique was repeated three times with three different controlled analyses of the experiments.

2.8.5. Control of cell vitality by MTT method

Before staining, MTT stain was made up at a rate of 5 mg/ml. By limiting direct light in the cabin, the prepared paint is filtered. The remaining Well plate cabinet from the incubator was stolen, and the functional supplies within were sucked. Then, 10 µl of the ready-made MTT paint was added to each well, and the mixture was allowed to incubate for 3–4 hours. 100 µl of DMSO was added to the wells after the paint had been aspirated during incubation. An ELISA reader operating at a wavelength of 570 nm read the plate after it had been incubated for 5 minutes. Using the GraphPad Prism 8 application, the study results were assessed.

2.8.6. Statistical analysis

Statistical analyzes were performed using the software application GraphPad Prism version 8. Statistical differences between cell proliferation and apoptosis studies were calculated using the two-way ANOVA test. IC_{50} values were determined using the GraphPad tool. P values below 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Activity, MIC-MBC Results

Plant extracts in terms of antibacterial properties *Enterobacter aerogenes* ATCC 13048, *Salmonella infantis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* DSMZ 50071, *Salmonella kentucky*, *Enterococcus faecalis* ATCC 29212, *Listeria innocua*, *Salmonella enteritidis* ATCC 13075, *Enterococcus durans*, *Salmonella typhimurium*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Bacillus subtilis* DSMZ 1971, *Escherichia coli* CFAI ATCC 25922 and *Serratia marcescens* ATCC 13048 bacteria tested against strains. It has also been tested on antifungal properties *Candida parapsilosis* and *Candida albicans.* In line with the findings; *Acantholimon kotschyi* and *Acantholimon acerosum* subsp. *brachystachyum* methanol extracts showed a zone diameter effect of 12-17 mm on *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Escherichia coli* species. *Acantholimon libanoticum* and *Acantholimon armenum var. balansae* methanol extracts showed a zone diameter of 14-18 mm on *Klebsiella pneumoniae*, *Listeria innocua*, *Enterococcus durans*, and *Salmonella typhimurium* species in addition to *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Escherichia coli* species (Table:2).

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Table 2. Antibacterial activity zone diameters of *Acantholimon* plant extracts (mm).

Bacteria	***A. kotschyi***	***A.libanoticum***	***A.acerosum subsp. brachystachyum***	***A. armenum* var. *balansae***	**AZM 10**
***Enterobacter aerogenes* ATCC 13048**	-	-	-	-	17
Salmonella infantis	-	-	-	-	19
Klebsiella pneumoniae	-	17±1	-	18±1,73	14
***Pseudomonas aeruginosa* DSMZ 50071**	15,33±0,57	16,66±0,57	14,66±0,57	15,66±2,08	20
Salmonella kentucky	-	-	-	-	18
***Enterococcus faecalis* ATCC 29212**	-	-	-	-	17
Listeria innocua	-	16±0	-	15,33±0,57	20
***Salmonella enteritidis* ATCC 13075**	12,66±0,57	15,33±0,57	14,33±0,57	16±0	15
Enterococcus durans	-	15±1	-	14,33±0,57	18
Salmonella typhimurium	-	15,33±0,57	-	15,33±1,15	20
***Staphylococcus aureus* ATCC 25923**	-	-	-	-	19
***Staphylococcus epidermidis* DSMZ 20044**	-	-	-	-	18
***Bacillus subtilis* DSMZ 1971**	-	-	-	-	13
***Escherichia coli* CFAI ATCC 25922**	17±1	18,33±1,52	17±1	18±1	13

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***Serratia marcescens* ATCC 13048**	-	-	-	-	17

(-): No inhibition zone was formed. AZM: Azithromycin 10 mg/ml

Plant extracts have been tested on *Candida albicans* and *Candida parapsilosis*. The tests were studied 3 times and the arithmetic means of the findings and the standard deviation value were shown in the tables. All the extracts yielded near-control findings on both species. Among the species, the most effective antifungal effects were *A.acerosum* subsp. *brachystachyum* and *A.armenum* var. *balansae* plants (Table: 3).

Table 3. Antifungal activity zone diameters (mm) in fungal species of plant extracts.

Fungus	***A. kotschyi***	***A.libanoticum***	***A. acerosum* subsp. *brachystachyum***	***A.armenum* var. *balansae***	**K**
Candida albicans	17,66±0,57	18,66±1,15	19,33±0,57	19,33±0,57	25
C*andida parapsilosis*	19,66±1,15	20,33±1,15	21,33±0,57	21,33±0,57	18

(K): Control Fungus Syrup

Dilution laws were used in conjunction with the MIC technique to determine the lowest inhibitory concentrations. Decreasing concentrations of the same amount of bacteria were tested. The MIC was calculated as the first concentration to inhibit colony formation. Bacteria can be inhibited by some *Acantholimon species*, usually at doses of 2 mg/ml to 4 mg/ml. In the inhibitory concentration test results on fungi, two *Candida species* were inhibited by *Acantholimon species* at concentrations between 2 and 4 mg/ml. At doses of 4 mg/ml to 8 mg/ml, *Candida* fungal species have demonstrated a fatal impact on *Acantholimon* plants.

3.2. Antibiofilm Activity Results

Antibiofilm; Biofilm creation is characterized as an activity blocking feature. The biofilm surface reduction percentages of the plants were tested by the crystal violet method.

Acantholimon kotschyi showed biofilm reduction activity most on *Enterococcus durans* at a concentration of 16 mg/ml. While *Acantholimon kotschyi* extract did not exert any effect on the bacteria *Salmonella kentucky*, *Listeria innocua*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Staphylococcus aureus*, it did act on other microorganisms in proportion to concentrations. The highest concentration used, 16 mg/ml plant concentration, was capable of blocking more than 50% biofilm areas on *Enterobacter aerogenes*, *Salmonella infantis*, *Kleibsiella pneumoniae*, *Enterococcus durans* and *Bacillus subtilis* bacteria.

Acantholimon libanoticum showed a directly proportional reduction effect on biofilm field formation at concentrations of 16 mg/ml and 8 mg/ml. *Salmonella enteridis*, *Enterococcus durans*, *Salmonella typhimurium*, and *Staphylococcus epidermidis* species have shown approximately 50% biofilm surface reduction efficacy. *Salmonella infantis*, *Listeria innocua*, *Escherichia coli*, and *Serratia marcescens*

did not show any reduction effect on bacterial species, and no effect was observed in all bacterial species at a concentration of 4 mg/ml.

All concentrations on *Acantholimon armenum var. balansae* , *Escherichia coli*, and *Seratia marrescens* bacteria also showed no biofilm surface reduction effect. It had a reduction effect of close to 40% on *Salmonella kentucky*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Staphylococcus epidermidis* bacteria, and about 30% on *Enterobacter aerogenes*, *Salmonella infantis*, *Kleibsiella pneumoniae*, *Listeria innocua*, and *Enterococcus durans* bacteria.

Acantholimon acerosum subsp. brachystachyum showed an effect on biofilm field formation in microorganisms tested other than *Salmonella infantis* and *Listeria innocua* bacteria at 16 mg/ml, 8 mg/ml, 4 mg/ml, and 2 mg/ml concentrations. The tested concentration of 16 mg/ml showed the highest reduction effect with 63.45% on *Escherichia coli*, followed by 57.26% on *Salmonella kentucky*. The concentration of 16 mg/ml shows effects between 40-10% in other bacteria. It tended to reduce biofilm on the bacterium *Bacillus subtilis* by over 30% at all concentrations. All the findings were directly proportional to the concentrations tested, and *Acantholimon acerosum subsp. brachystachyum* was the plant that gave the most reduction tendency to microorganisms tested among the *Acantholimon* species.

3.3. Antioxidant Activity Results

4 different concentrations (1 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml) were tested 3 times by DPPH (2,2-diphenyl-1-picryhydrarizyl) Radical Sweep Method. The plants whose antioxidant capacities were tested by the DPPH method created graphs by calculating the average values of the three studies studied and IC_{50} values were calculated using the GraphPad program. Ascorbic Acid data from the antioxidant activity findings of Tozyilmaz et al. were taken as a reference as the standard substance [30]. Figure 1 shows the graph of IC_{50} values of antioxidant activity of *Acantholimon species*.

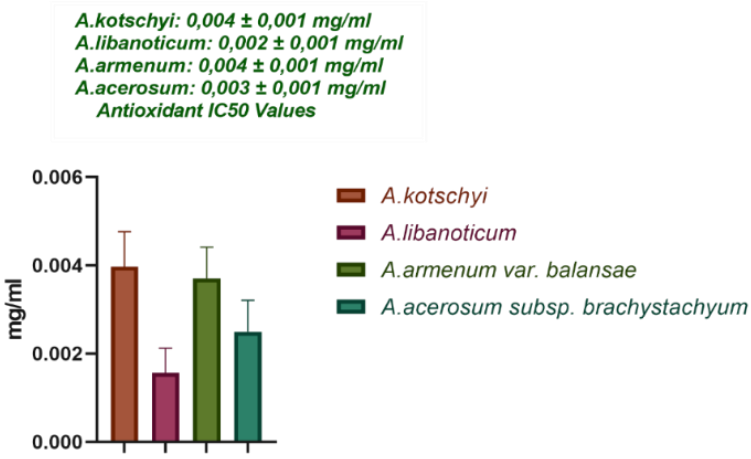

Figure 1. Graphic of *IC_{50}* values of antioxidant activity of *Acantholimon species.*

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When the DPPH˙ radical scavenging percentage values of *Acantholimon kotschyi* concentrations were examined, the concentrations of 1 mg/ml and 0.1 mg/ml used showed 73% and 75% effects. Compared to these two concentrations, which had an effect of over 70%, the other two concentrations tested (0.01 mg/ml and 0.001 mg/ml) also showed similar values despite the decrease in the plant extract. When the antioxidant capacity of *Acantholimon kotschyi* is examined by considering the effect values of 41% and 32%.. When the DPPH Radical sweep percentage values of *Acantholimon libanoticum* methanol extract were examined, the plant tested at a concentration of 1 mg/ml showed a 100% effect. The concentration of 0.1, 0.01 mg/ml, and 0.001 mg/ml showed a radical sweeping effect in direct proportion to the decrease in plant concentration. These findings obtained as a result of repetitions show that the antioxidant capacity of the *Acantholimon libanoticum* plant is high.

When the DPPH radical sweeping percentage values of *Acantholimon armenum* var. *balansae* methanol extracts are examined, it is seen that the first two high concentrations of the plant used (1 mg/ml and 0.1 mg/ml) show an average radical sweeping effect of 75%. At concentrations of 0.01 and 0.001, it gave a radical sweep percentage directly proportional to plant concentration. An examination of the *Acantholimon armenum* var. *balansae* DPPH radical sweep chart showed that the plant had a good antioxidant capacity.
When the DPPH radical sweep percentage values of *Acantholimon acerosum subsp. brachystachyum* methanol extracts were examined, 1 mg/ml concentration showed 76% radical sweeping effect, while 0.001 mg/ml concentration showed 34% sweeping effect. In line with this proportion, *Acantholimon acerosum* subsp. *brachystachyum* extract shows that the antioxidant capacity is good.

3.4. Anticancer Activity Results

Acantholimon kotschyi, *Acantholimon libanoticum*, *Acantholimon armenum* var. *balansae*, and *Acantholimon acerosum* subsp. *brachystachyum* methanol extracts on HUVEC and MCF-7 cell lines were tested by the MTT method in the concentration range of 0.01-10 mg/ml in a cell culture medium. The test results were calculated statistically using the GraphPad Prism 8 program ($p< 0.05$). IC_{50} values of cell viability rates of plant extracts of Acantholimon species at different concentrations to HUVEC cells in Figure 2a and Figure 2b.

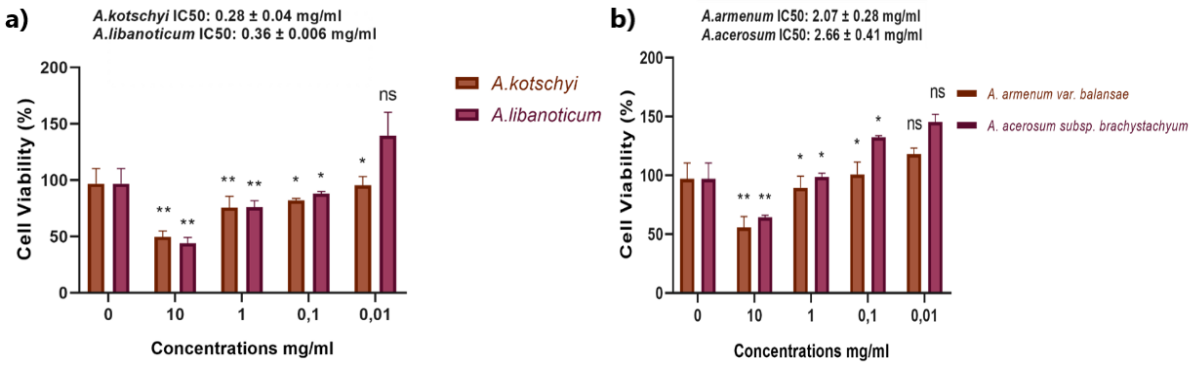

Figure 2. a) *Acantholimon kotschyi* and *Acantholimon libanoticum* HUVEC Cell Line Vitality Graph and IC_{50} Values. **b)** *Acantholimon armenum* var. *balansae* and *Acantholimon acerosum subsp. brachystachyum* HUVEC Cell Line Vitality Graph and IC_{50} Values.

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Figure 2a. and Figure 2b. show the cell viability rates of plant extracts of *Acantholimon* species applied to HUVEC cells at different concentrations (0.01-10 mg/ml). The decrease in cell viability appears to be associated with concentrations. HUVEC cell line IC50 values were calculated as 0.28 mg/ml for *A.kotschyi*, 0.36 mg/ml for *A.libanoticum*, 2.07 mg/ml for *A.armenum* var. *balansae* and 2.66 mg/ml for *A.acerosum* subsp. *brachystachyum.* IC_{50} values of cell viability rates of plant extracts of Acantholimon species at different concentrations to HUVEC cells in Figure 3a and Figure 3b.

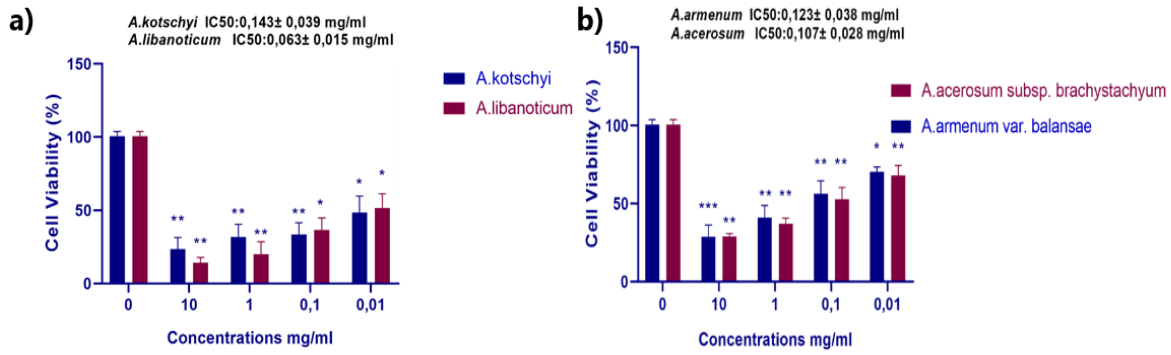

Figure 3. a) *Acantholimon kotschyi* and *Acantholimon libanoticum* MCF-7 Cell Line Vitality Graph and IC_{50} Values. **b)** *Acantholimon armenum var. balansae* and *Acantholimon acerosum subsp. brachystachyum* MCF-7 Cell Line Vitality Graph and IC_{50}Values.

Figure 3a. and Figure 3b. show the cell viability rates of plant extracts of *Acantholimon* species applied to MCF-7 cells at different concentrations (0.01-10 mg/ml). Analysis of the IC_{50} values on the HUVEC and MCF-7 cell lines revealed that the tested Acantholimon species exhibited cytotoxicity on the MCF-7 cell line but not on the HUVEC cell line.

4. CONCLUSION

Plants are increasingly being used as therapeutic agents, and their significance in this area is rising. Very significant research has been conducted on the use of plants as microbiological agents and as one of the active ingredients in anti-cancer medications. In this study, the use of *Acantholimon Boiss*. species as biologically active agents were investigated. According to the results, according to the findings of the antibiofilm activity test by crystal violet method; *Acantholimon kotschyi* showed a biofilm surface reduction effect on *Enterococcus durans* at a concentration of 16 mg/ml. It blocked more than 50% biofilm area on *Enterobacter aerogenes, Salmonella infantis, Kleibsiella pneumoniae*, *Enterococcus durans*, and *Bacillus subtilis bacteria*. *Acantholimon libanoticum* also reduced the biofilm surface by approximately 50% on *Salmonella enteridis*, *Enterococcus durans*, *Salmonella typhimurium*, and *Staphylococcus epidermidis species*. In addition, all concentrations reduced the biofilm surface on *Acantholimon armenum var. balansae, Escherichia coli and Serratia marrescens bacteria*. However, on other bacteria, it decreased by 30-40% in proportion to the concentrations. *Acantholimon acerosum subsp. brachystachyum* formed biofilms on microorganisms other than *Salmonella infantis* and *Listeria innocua* in the tested concentration range of 2 to 16 mg/ml. In

addition to this effect obtained in bacterial species, its activity in cell lines was also observed quite well. It was observed that the decrease in cell viability was related to the concentrations of plant extracts of *Acantholimon species* in the range of 0.01-10 mg/ml applied to HUVEC cells. Analysis of the IC50 values on the HUVEC and MCF-7 cell lines revealed that the tested Acantholimon species exhibited cytotoxicity on the MCF-7 cell line but not on the HUVEC cell line.

In line with these data, it was determined that the study plants within the *Acantholimon* species have important biological activities and show similar properties to other species. In this study, antibacterial, antifungal, and cell line assays of local plant sources were carried out together and biological agent activities were emphasized. The results provided important contributions to the literature to emphasize the biological activity of *Acantholimon* plant species.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

CONSENT FOR PUBLICATION

Authors accept the publication policy of the journal.

AVAILABILITY OF DATA AND MATERIAL

None

COMPETING INTERESTS

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M.T.B, A.S.B., C.U.,M.B., R.B., and F. S. organized all experiments and wrote the manuscript. M.T.B., and A.S.B. performed all experiments and characterizations.

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