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RESEARCH ARTICLE

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Silver nanoparticle synthesis by biogenic reduction method and investigation of antimicrobial, antibiofilm, anticancer activities

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Abstract

In this study, R. aculeatus plant extract and biogenically formed AgNPs were investigated for their potential antibacterial, antibiofilm and anticancer abilities. AgNPs were characterised using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and UV-vis spectroscopy (UV-VIS). According to the Debye Scherrer formula, the particle size was found to be 32.56 nm. Disc diffusion and microdilution methods were used to investigate the antibacterial activity. In the disc diffusion study, the best results were obtained from the extract and AgNP. In the tests using plant extracts, Staphylococcus aureus ATCC 25923 showed the lowest antibiofilm activity, while Bacillus subtilis and Enterobacter aerogenes ATCC 13048 showed the highest activity. Salmonella infantis was most affected by AgNP, while Escherichia coli CFAI ATCC 25922 was least affected. Biogenically synthesised AgNPs were also investigated in cytotoxic activity studies. It was found to have the lowest concentration value on MCF-7 and HUVEC cell lines at the determined concentrations. The extract did not have any cytotoxic effect on MCF-7 cell line. HUVEC cell line showed the lowest cytotoxic activity of 10^{-3} g/mL. The antibacterial, antibiofilm and anticancer properties of R. aculeatus plant extract and biogenically produced AgNPs have been the subject of an important study. Furthermore, the comparison of the effects of plant extract and AgNPs on breast cancer cell lines and healthy cell lines provides a rich scientific material.

Keywords: Antimicrobial activity, Antibiofilm activity, Anticancer activity, Biogenic agent.

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1. Introduction

Treatment of diseases is a major economic concern for both individuals and countries. At the same time, finding appropriate treatment methods for the disease is also very important and is another important cause of individual concern. To eliminate these concerns, both plant sources and nanoparticles (NP), in which plants are used as biological reducing agents, are used in the treatment of many diseases [1].

Since ancient times, people have used plants both as nutrients and for therapeutic purposes to eliminate health problems. Today, plants have begun to be used for medicinal purposes all over the world and in our country [2]. Many of the drugs that are used extensively in the field of medicine are also obtained from plants [3]. Herbal medicines are prepared by obtaining active substances from plants that are generally included in the group of medicinal plants. Herbal products are often used in the treatment of diseases with chronic medical conditions, including breast cancer (2%), liver diseases (21%), HIV (22%), asthma (24%), and rheumatological disorders (26%) [4].

Ruscus aculeatus L. plant is notable among the medicinally important species. This species, which belongs to the Ruscaceae family, is found in maquis forests and its dimensions are around 20-50 cm. [5]. The active components of the plant are steroid saponins. These active ingredients have beneficial and anti-inflammatory effects on the veins, such as increasing blood circulation [6]. In addition, the main phenolics in the leaves and stems of the plant have been identified as quercetin and p-coumaric acid [7]. These identified phenolic compounds can be used as reducing agents [8]. The method, which includes these and similar biological compounds and is also known as green synthesis, allows the synthesis of nanoparticles with economical and relatively easy methods without the use of heavy chemicals [9–11].

The produced new age drugs are polymer, metal, or ceramic nanoparticles that can fight diseases such as cancer and human pathogens such as bacteria [12]. With nanotechnology applications, significant acceleration has been gained in drug release systems, sensor systems, innovative energy production systems, and antibacterial research [13–16]. Cancer is undoubtedly one of the most important of these diseases.

In addition, cancer, a complex and deadly disease, continues to pose major challenges to world health. Despite advances in treatment alternatives, the creation of effective anticancer drugs remains an important subject of study. Recently, nanotechnology has become a suitable field for the creation of new and focused methods in cancer treatment [17]. One of the nanotechnological applications is AgNPs. The anticancer effects of Ag NPs are due to their capacity to precisely target and destroy cancer cells while leaving healthy cells unharmed. The different size-dependent properties of AgNPs are responsible for this selectivity, making them more effective than conventional therapeutic agents in penetrating malignant tissues. AgNPs can also be functionalized with ligands or antibodies to directly target cancer cells, increasing their selectivity, and minimizing side effects [18–20]. AgNPs are important in the field of nanotechnology due to their unique properties such as chemical stability, good conductivity, catalytic, and most importantly antibacterial, antiviral, and antifungal properties [21,22]. Therefore, silver shows the best activity against bacteria, viruses, and other eukaryotic microorganisms [23,24]. Silver nanoparticles (AgNP), which draw attention with their antimicrobial properties, have many advantages such as the fact that silver is a very broad-spectrum antibiotic, almost no bacterial resistance in silver, and non-toxicity at low concentrations [25]. There are also different studies in the literature on obtaining AgNPs by green synthesis and their applications. One of these studies was performed with jujube seed extract. It was reported that the plant and silver structure used in the synthesis showed good antibacterial properties [26]. In a similar study, AgNPs were obtained with Diospyros malabarica Fruit Extract. According to the results, it was found to inhibit bacterial growth against *Staphylococcus aureus,* and *Escherichia coli* pathogenic microorganism strains at concentrations of 500 µg/ml as 8.4 ± 0.3 mm and 6.1 ± 0.7 mm, respectively [27]. In the study conducted with Moringa Oleifera biological agent, it was reported that an active NP was obtained against carcinoma cells and this NP was also used in different applications [28].

According to the research, it is important to select the right herbal agent and to put forward a suitable antibacterial-anticancer molecule by considering economic concerns.

For this reason, silver, which has remarkable biological activities compared to other metals, and the green synthesis method, which has been used frequently in recent years, were preferred in this study. In this study, AgNPs were synthesized for the first time using the R. aculeatus plant, and their antibacterial, antibiofilm, and anticancer activities were investigated.

2. MATERIAL AND METHOD

2.1. Preparation of Biogenic Agent

The plant sample used in the study was collected from the Balamba location in the center of Bartin province, located between 41° 53' north latitude and 32° 45' east longitude on the northwest coast of the Black Sea. After the identification of the species, the plant part to be used was washed, dried in a cool and non-humid environment, and made ready for grinding. Then, the leaf part of the plant was crushed in a mortar with the help of liquid nitrogen. This mixture was extracted for 4 hours in a magnetic heater set at 80 ºC. At the end of the period, the mixture was filtered through filter paper, and the extract obtained was stored at +4ºC to be used in the analyses [29,30].

2.2. Synthesis of Silver Nanoparticles

1 mM silver nitrate ($AgNO_3$) solution was added to 50 ml of the obtained biological agent and diluted. The liquid was stirred at 100 ^oC in a magnetic heater for 1 hour. The mixture was washed 3 times in a centrifuge with H_2O. Characterization processes were started by drying in an oven at 35 ^oC for 24 hours [31]. Figure 1 briefly summarizes the synthesis phase and the implementation step.

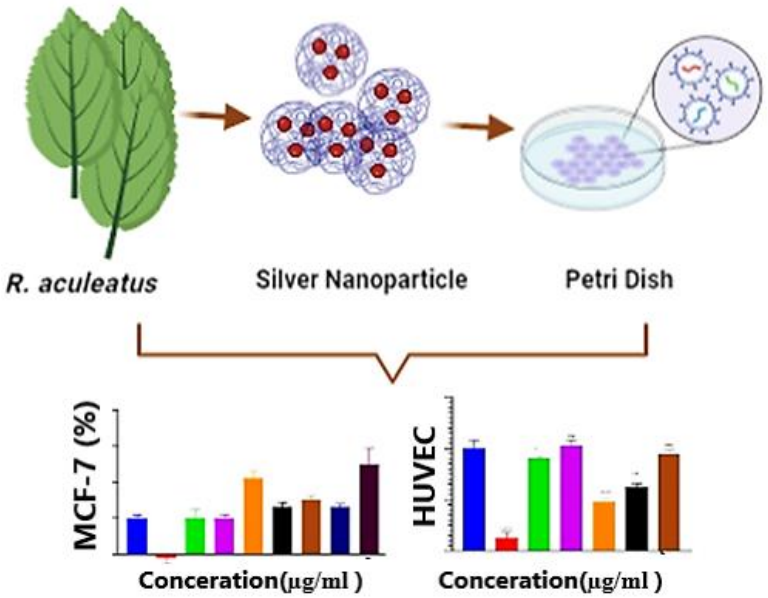

Fig. 1. Graphical representation of synthesis step and application method.

2.3. Characterization of AgNPs

Thermo Scientific Multiskan GO was used to take spectroscopic measurements in UV-vis analysis and measurements were made in the 300-800 nm range. Nanoparticles obtained by green synthesis were analyzed in 1, 24, and 48-hour working samples. X-ray diffraction (XRD) was used for the analysis of the crystal structure. A scanning electron microscope (SEM) was used for morphological analysis. In the FT-IR analysis, the presence of various functional groups in the molecules responsible for the biological reduction and stabilization of AgNPs, whether the structure is aromatic or aliphatic, the state of the bonds in the structure, and their binding sites were determined.

2.4. Concentration Preparation

0.2 g of dried AgNP was weighed and dissolved in 10 ml dH_2O in an ultrasonicated bath. The plant extract was taken into a 50 ml falcon tube and used as the initial concentration. Working concentrations were prepared by making 1/10 dilutions.

2.5. Disk Diffusion Method

Strains from stock microorganisms were suspended in Luria Bertoni (LB) broth and kept in a shaking incubator for 16-18 hours. After this period was completed, a 0.5 cell/ml McFarland turbidity test was performed on the microorganism strains and a dilution containing 1.5x106 cells/ml microorganism was prepared. Mueller Hinton Agar (MHA) solid medium was prepared for bacterial dilutions. Then, bacteria were planted on the surface of the petri dishes with the help of disposable sterile swabs.

Antimicrobial activity trials of AgNPs and plant extract were first analyzed by the disk diffusion method [32]. Fifteen microorganisms were used in the study. 6 were gram-positive and 9 were gram-negative. The solutions produced were impregnated on sterilized discs. Antibiotic discs containing the active substance Tetracycline (TE: 10 mg/ml) prepared from antibiotic drugs were used for positive control. Discs containing AgNP and plant extract were placed in Petri dishes appropriately. Bacteria were left in the incubator at 37^{o}C for 16-18 hours and the inhibition zone diameters around the discs were measured at the end of the period. The study was performed in triplicate for AgNP and plant extract, and the arithmetic mean and standard deviation of the results were calculated.

2.6. Minimum Inhibition Concentration (MIC)

The MIC of the plant extract and AgNP were determined using sterile 96-well microplates. After mixing 100 µL of LB Broth and 100 µL of AgNP and plant extract into the first wells of the microplates, 100 µL was taken and pipetted into the next well, and these dilutions were continued until the last well, and the concentration in each well was adjusted to be half of the previous one. Finally, 10 µL of microorganisms was added to all wells except for the negative control well. After these steps were completed, the microplates were left to incubate at 37ºC for 16-18 hours. At the end of the period, measurements were made in the spectrophotometer device at 600 nm.

2.7. Minimum Bactericidal Concentration (MBC)

After determining the MIC of the plant extracts and AgNPs used in the study against bacteria, the MBC were investigated. After determining the MIC, samples were taken from the wells where bacteria did not grow and inoculated into MHA medium using sterile rings. Then they were incubated at 37ºC for 16-18 hours. After this period was completed, 99.9% of the bacteria inoculated into the media The minimum antimicrobial agent concentration that kills was accepted as the MBC value.

2.8. Determination of Antibiofilm Activities

The MBC were investigated after the MIC of the plant extract and AgNPs used in the study against bacteria had been determined. After determining the MIC values, samples were taken from the wells where bacteria did not grow and injected onto an MHA medium using sterile loops. The impact of the plant extract and AgNP on the antibiofilm was compared to the data from the positive control, and the percentage decrease value of biofilm inhibition was computed using the following formula (Eq. 1):

$$\%\text{Reduction} = ((A_0 - A_1)/A_0) \times 100 \quad (1)$$

A_0: positive control well
A_1: test wells

2.9. Determination of Anticancer Activities

Cytotoxic effect of plant extract and green synthesized AgNP on breast cancer (MCF-7) and human umbilical vein endothelial cells (HUVEC) cell lines MTT (3-(4,5-dimethyltriazol-2-yl)-2,5-diphenyltetrazolium bromide) was determined by the method [33,34]. The mitochondrial dehydrogenase enzyme reduces MTT salt, which is a yellow, water-soluble salt, to water-insoluble formazan, resulting in purple-colored crystals. Formazan crystals dissolved because of treatment with dimethyl sulfoxide (DMSO) can be calculated quantitatively as a measure of viability by the calorimetric method in an ELISA reader [35].

Enough MCF-7 and HUVEC cells obtained by the passage in this study were each treated separately. First, the cells were suspended with trypan blue at a ratio of 1:1, and cell counts were made under the microscope with the help of a Thoma slide. Then, 105 cells/mL were added to each well of 96-well microplates and incubated for 24 hours at 37 °C in an incubator containing 5% CO_2. After incubation, plant extract and green synthesized AgNP concentrations of 10, 20, 30, 40, and 50 µg/mL were suspended and added to the wells. They were left to incubate again for 24 hours and the changes that occurred afterward were examined under the microscope and compared with the control groups. After this step, 10 µL of MTT solution was added to each well and incubated for 3 hours. At the end of the time, the microplates were read by ELISA at 630 nm and the results were calculated.

3. Results and Discussion

3.1. Analyzing the synthesized AgNPs

After the addition of silver nitrate, a color change was observed over time. The reason for the color change here is the stimulation of the surface plasmon resonance with silver nanoparticles [36]. The UV-VIS spectra recorded at 1 hour, 24 hours, and 48 hours after the start of the reaction are shown in Figure 2. As soon as the plant leaf extract and silver nitrate solution were mixed, the reduction of pure Ag^+ ions to Ag^0 was monitored by measuring the UV-vis spectrum at certain time intervals [37].

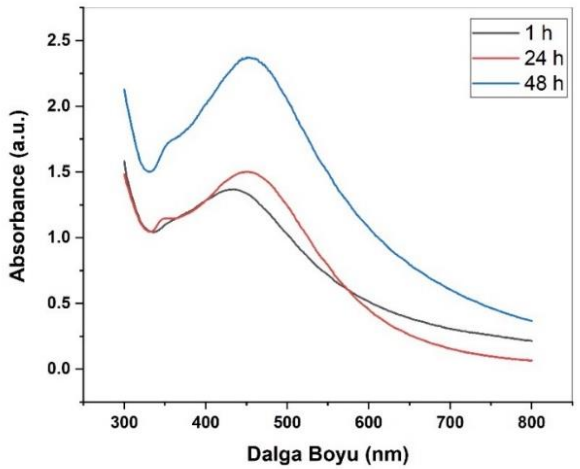

Fig. 2. UV-vis spectra of *R. aculeatus L.* recorded as a function of leaf extract and aqueous silver nitrate solution over time [38].

In the production of silver nanoparticles, it was observed that the plasmon resonance occurred at 426nm after 1 hour and the peak length increased steadily as the reaction time was extended to 24 (444nm) and 48 (444nm) hours.

The crystal structure of the particles was confirmed by analyzing them by X-ray diffraction (XRD). The analysis revealed a significant number of Braggs reflections that can be indexed. The peaks with a value of 2θ in our XRD spectrum were compared with the literature and showed that the AgNPs produced were nanocrystals. The XRD graph is given in Figure 3a. The XRD spectrum of the prepared AgNPs showed four diffraction bands at 2θ= 38.35, 46.46, 64.75, and 77.62, which are the characteristic Bragg diffraction plans of the face-centered cube (111), (200), (220) and (311). Through the biological agent, the reduction of Ag+ ions resulted in the formation of AgNPs with a crystalline structure by XRD. According to this result, it was determined that there is metallic silver in the face-centered cubic (FMC) structure. The Debye-Scherrer equation can be used to determine the typical particle size of AgNPs produced using the present green technique. The average nanoparticle size of AgNPs was calculated as 32.56 nm according to the Debye-Scherrer formula (Eq. 2):

$$d(\text{Å}) = k\lambda / \beta\cos\theta \quad (2)$$

d=Average size of NPs,
k= Factor (0,9),
λ= X-Wavelenght (1,54056Å),
β=Half-maximum point of the full width of the corresponding refractive peak (rad),
θ= Represents the maximum height angle (rad) of the peak.

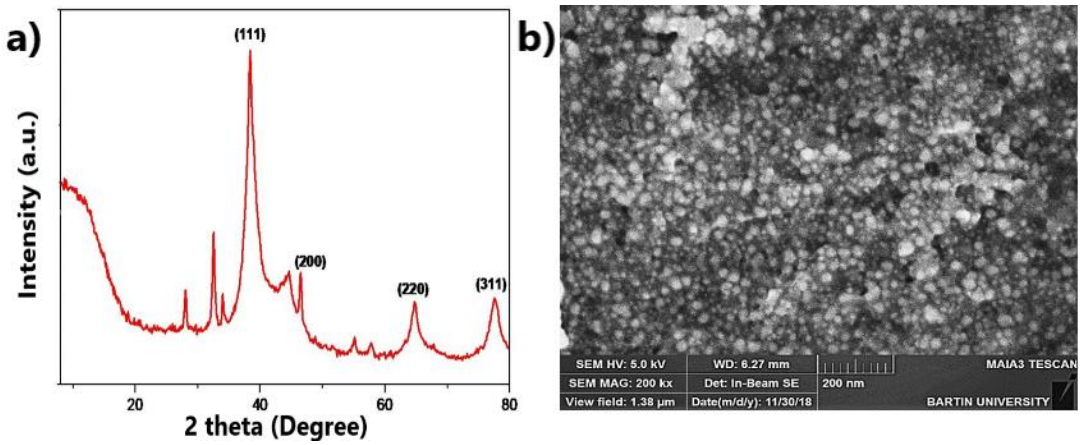

Fig. 3. a) AgNP XRD image b) AgNP SEM image at 200 nm [38].

Surface morphologies and dimensions of AgNPs obtained by the green synthesis method were analyzed by SEM. As a result of the imaging, it was determined that the biological AgNP was spherical in size and approximately 32.56 nm in size. The SEM image of AgNP is shown in Figure 3b. According to the FTIR spectrum results of the plant, the peak at wave number 3302 cm^{-1} is the O-H vibration from hydroxyl groups and similar peaks are hydroxyl groups at wave number 1616 cm^{-1}. C-C stretching vibration was observed due to the high phenyl levels in the polyphenolic components (Figure 4) [39]. The peak, which corresponds to a wave number of approximately 1443 cm^{-1}, corresponds to the -C-O bond vibration, which is composed of carbohydrates, glycogen, and oligosaccharides [40]. By comparing both spectra, it was determined that the peaks corresponding to 2917 cm^{-1} wave number were caused by saturated alkane (-C-H) stretching [41]. It was observed that the peaks observed at approximately 1022 cm^{-1} and 1029 cm^{-1} wave numbers are the maximum band absorption of glucose [42,43]. The presence of small shift-like peaks in the spectra reveals that the synthesized AgNPs contain natural compounds originating from the plant extract [44].

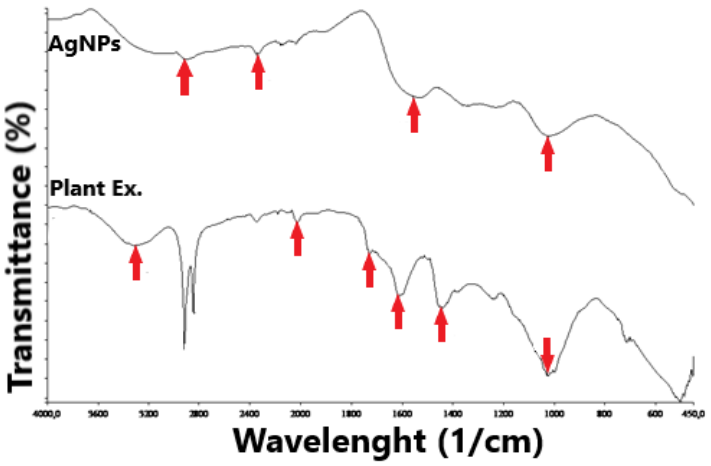

Fig. 4. a) Green synthesis AgNP b) FT-IR spectra of *Ruscus aculeatus* leaf extract [38].

3.2. Disk Diffusion Results

Table 1 presents the antimicrobial activity results of the plant extract and synthesis AgNP by disk diffusion method. When the data obtained were examined, it was observed that the plant extract showed inhibition zones on other species except for *S. marcescent* ATCC 13048 bacterial strain. AgNPs obtained as a result of synthesis showed an inhibition zone against *S. kebtucky*, *S. enteriditis* ATCC 13075, *E.durs*, and *S. typhimurium* microorganisms, but did not show an inhibition zone against other microorganisms.

Table 1. Measurements of zones of inhibition of plant extract and green synthesis AgNP against test microorganisms (mm) [38].

Microorganisms	**Extract**	**AgNP**	**TE 10**
Pseudomonas aeruginosa DSMZ 50071	8±0.00	-	17.5
Klebs iella pneumoniae	7±0.00	-	19.5
Salmonella infantis	8.3±1.52	-	15
Enterobacter aerogenes ATCC 13048	7±0.00	-	14
Salmonella enteritidis ATCC 13075	9.6±0.57	7±0.00	17.5
Salmonella typhimurium	7.3±0.57	7±0.00	18.5
Enterococcusfaecalis ATCC 29212	7±0.00	-	16.5
Bacillus subtilis DSMZ 1971	7.3±0.57	-	14
Listeria innocua	7.3±0.57	-	15.5
Staphylococcus aureus ATCC 25923	-	-	14.5
Saratia marrescens ATCC 13048	6.6±0.57	-	9
Salmonella kentucky	7.6±0.57	7.6±1.15	17.5
Staphylococcus epidermidis DSMZ 20044	7±0.00	-	11
Escherichia coli CFAI ATCC 25922	6±0.00	-	16

(-): There is no zone of inhibition. **(TE 10)**: Tetrasiklin (10 mg/ml) [38].

3.3. Minimum Inhibition Concentration (MIC) Results

MIC values of the plant extract and synthesized AgNP are shown in Table 2. Accordingly, it has been shown that the prepared 100% plant extract has no inhibitory effect against all microorganisms. On the other hand, the synthesized AgNP showed a minimum inhibitory effect against *Salmonella infantis*, one of the microorganisms tested, at a concentration of 0.12 mg/ml. While AgNP showed a minimum inhibitory effect at a concentration of 0.06 mg/ml against the tested microorganisms, *Pseudomonas* aeruginosa DSMZ 50071 and *Listeria innocua* strains, it showed a minimum inhibitory effect at a concentration of 0.03 mg/ml against the other 12 bacterial strains.

Table 2. MIC results of green synthesis AgNP against test microorganisms [38].

Microorganism Name	**AgNP- mg/mL**
Staphylococcus epidermidis DSMZ 20044	0.03
Salmonella kentucky	0.03
Enterococcus faecais ATCC 29212	0.03
Salmonella enteritidis ATCC 13075	0.03
Salmonella infantis	0.12
Bacillus subtilis DSMZ 1971	0.03
Enterobacter aerogenes ATCC 13048	0.03
Klebsiella pneumoniae	0.03
Enterococcus durans	0.03
Salmonella Typhimurium	0.03
Listeria innocua	0.06
Escherichia coli CFAI ATCC 25922	0.03
Pseudomonas aeruginosa DSMZ 50071	0.06
Staphylococcus aureus ATCC 25923	0.03
Saratia marrescens ATCC 13048	0.03

3.4. Minimum Bactericidal Concentration (MBC) Results

Table 3 shows the lowest bactericidal concentrations (MBC) that inhibit the growth of the plant extract and biosynthesized AgNP up to 99.9%. Accordingly, it was determined that the prepared 100% plant extract did not have an inhibitory effect of 99.9% against all microorganisms (Figure 5). The lowest bactericidal concentration of biologically synthesized AgNP, which inhibited 99.9% against *S. infantis*, was determined as 0.25 mg/ml. The lowest bactericidal concentration of biologically synthesized AgNP, which inhibited 99.9% of S. enteritidis ATCC 13075 bacteria, was determined as 0.06 mg/ml, while 0.12 mg against *P. aeruginosa* DSMZ 50071 and *Listeria innocua* bacterial strains. It was determined as /ml. In addition, the lowest bactericidal concentration, which inhibited 99.9% against 11 other bacterial strains, was determined as 0.03 mg/ml. Compared to our study, Kaviya et al. (2011) found that because of the silver nanoparticle synthesis and the use of fewer types of bacteria, the antimicrobial effect on *E. coli* and *P. aeruginosa* bacteria showed higher antibacterial activity against *S. aureus* bacteria, as in our study. Concentration (A1=A2=A3: %100, B1=B2=B3: %50 concentration - MBC) AgNP concentrations (A1: 1 mg/ml, B1: 0.5 mg/ml, C1: 0.25 mg/ml, D1: 0.12 mg/ml, E1: 0.06 mg/ml, F1: 0.03 mg/ml).

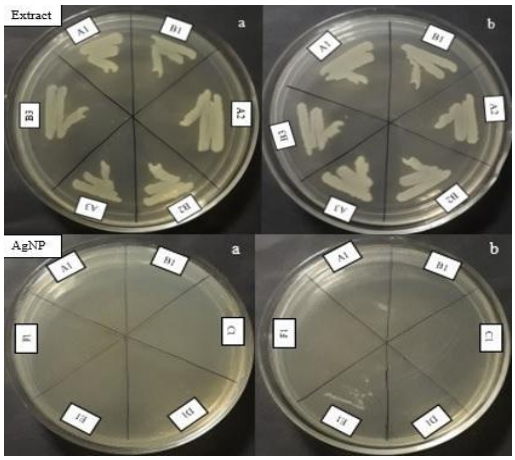

Fig. 5. Plant extract a: *P. aeruginosa* (A1,B1), *E. faecalis* (A3,B3), *S. kentucky* (A2,B2). b: *L. innocua* (A1,B1), *E. durans* (A3,B3), *S. enteritidis* (A2,B2), and MBC Results of AgNP [38].

Table 3. Minimum bactericidal concentrations of the green synthesis AgNP studied [38].

Microorganism Name	**AgNP- mg/mL**
Enterobacter aerogenes ATCC 13048	0.03
Klebsiella pneumoniae	0.03
PPseudomonas aeruginosa DSMZ 50071	0.12
Salmonella infantis	0.25
Listeria innocua	0.12
Salmonella enteritidis ATCC 13075	0.06
Salmonella Typhimurium	0.03
Staphylococcus aureus ATCC 25923	0.03
Escherichia coli CFAI ATCC 25922	0.03
Saratia marrescens ATCC 13048	0.03
Salmonella Kentucky	0.03
Enterococcusfaecalis ATCC 29212	0.03
Bacillus subtilis DSMZ 1971	0.03
Enterococcus durans	0.03
Staphylococcus epidermidis DSMZ 20044	0.03

3.5. Antibiofilm Results

When the antibiofilm effect results of the plant extract and AgNP against the test microorganisms at different concentrations in Table 4 and Table 5 are examined, it is seen that the extract did not inhibit biofilm formation at the lowest concentration (3.13%) and that the *S. infantis* strain did not inhibit the biofilm formation at the lowest concentration of AgNP (0.03 mg/ml) did not inhibit biofilm formation of *E. coli* CFAI ATCC 25922 strain. In addition, it was determined that all the test microorganisms at different concentrations inhibited biofilm formation. Based on the antibiofilm study, Mohanty et al. (2012) showed that they reduced the nanoparticles they synthesized by using starch material at 1-2 µM concentrations on *P. aeruginosa* and *S. aureus* bacterial species by 65% and 88%, respectively.

Table 4. Biofilm inhibition values of the plant extract at different concentrations (%) [38].

Microorganism Name	100	50	25	12.5	6.25	3.13
Enterobacter aerogenes ATCC 13048	43.8	39.4	48.9	48.3	43.2	9
Escherichia coli CFAI ATCC 25922	30.9	44.5	32	44.2	44.8	40.7
Salmonella infantis	45.8	41.4	37.5	30.5	12.4	-
Staphylococcus epidermidis DSMZ 20044	25	19.6	24.7	21.9	9.4	15
Staphylococcus aureus ATCC 25923	6	12.2	10.1	2.7	1.3	6.8
Bacillus subtilis DSMZ 1971	46.5	44.2	41.1	38	24	13.3

Table 5. Biofilm inhibition values of AgNP at different concentrations (mg/ml) [38].

Microorganism Name	1	0.5	0.25	0.12	0.06	0.03
Enterobacter aerogenes ATCC 13048	15	29.7	31.1	31.9	26.8	10.7
Staphylococcus aureus ATCC 25923	25.2	36.3	34.3	33.8	17.2	8.6
Salmonella infantis	60	65.5	62.6	63.4	55.8	53.2
Escherichia coli CFAI ATCC 25922	1 1.1	10.7	1 1 .1	11.7	7.9	-
Staphylococcus epidermidis DSMZ 20044	17.2	18	24.6	22.8	8.4	1.1
Bacillus subtilis DSMZ 1971	38	36.9	38.4	34.7	25	28.4

3.6. Anticancer Activity Results

Figure 6a shows the cell viability rates of AgNP and plant extract applied to the MCF-7 cell line at different concentrations (10^{-3}, 10^{-5}, $10^{-7,}$ and 10^{-9} µg/ml). Compared to the control group, the lowest value determined in the applied AgNP concentrations is 10^{-3} µg/ml. Other concentrations applied were found to have less cytotoxic effects. This result indicates that the cytotoxic effect of biosynthesized AgNPs against MCF-7 is stronger. The IC50 value of the green synthesized AgNP is 0.06 µg/ml for the MCF-7 cell line, while the IC50 value of the plant extract is 6.91 µg/ml.

Figure 6b shows the cell viability rates at different concentrations (10^{-3}, $10^{-4,}$ and 10^{-5} µg/ml) of AgNP and plant extract applied to the HUVEC cell line. In comparison to the control group, the lowest value determined in the applied AgNP concentrations is 10^{-3} g/ml. It was observed that the cytotoxic effect was less at other applied concentrations. In plant extract, the lowest value determined when compared to the control group is 10^{-3} µg/ml, and concentration values of 10^{-4} and 10^{-5} µg/ml have less cytotoxic effect compared to this value. The IC50 value of the green synthesized AgNP is 0.04 µg/ml for the HUVEC cell line, while the IC50 value of the plant extract is 18.4 µg/ml. In this context, Suman et al. (2013) reported that they exhibited a significant cytotoxic effect compared to other chemical-based synthetic drugs in their study on the HeLa cell line with the AgNPs they synthesized [45]. In this context, our study contributed by examining the effect of AgNP not only on a cancer cell line but also on a healthy cell line. A wider spectrum can be contributed to the literature with studies that can be performed on various cancer cell lines and on healthy cell lines that can be obtained from other tissues.

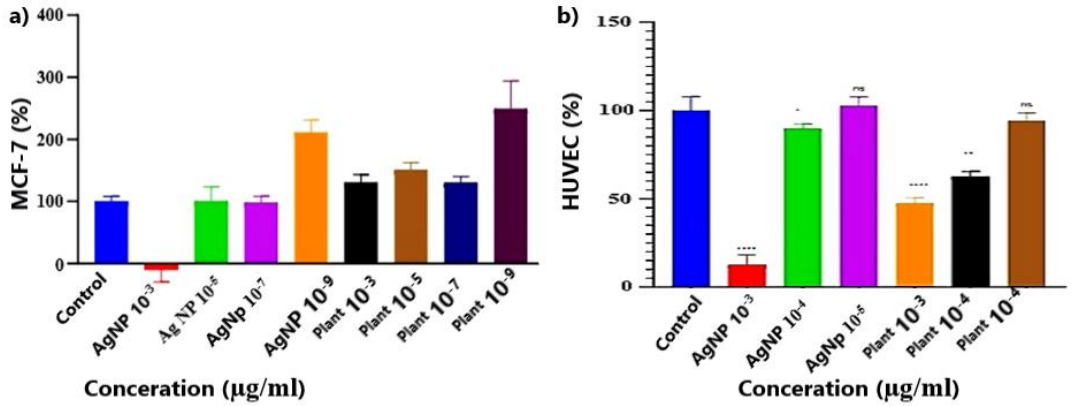

Fig. 6. a: Cytotoxic effects of. *Biogenic agent* extract and green synthesized AgNP on MCF-7 cell line b: Cytotoxic effects of *biogenic agent* extract and green synthesized AgNP on HUVEC cell line [38].

4. Conclusion

In this study, AgNP synthesis was carried out from grass wastes of the R. aculeatus plant by the green synthesis method. The synthesis result was calculated according to the Debye Scherer formula, in which 32.56 nm structures were seen. Antimicrobial and anticancer studies of the obtained AgNPs were investigated. The results calculated that the AgNPs had a MIC value of approximately 3 µg/ml *against Pseudomonas aeruginosa* and *Acinetobacter baumannii*. This value was calculated as the highest inhibitory effect against *Fusarium solani* 20 µg/ml. They determined that it is close to 90% in ml AgNP concentration. They showed that an inhibitory effect on cancer cell growth was achieved by increasing the AgNP concentration to 5 µg/ml, thus reducing the survival of cancer cells by about 30%. According to our study, the MIC values of AgNP synthesized against 15 different test microorganisms were observed between 0.03 and 0.12 mg/ml, while MBK values differed between 0.03-0.25 mg/ml. It has been observed that 100% plant extract has no inhibitory effect against all microorganisms. In the plant extract, it was observed that the cytotoxic effect of the applied concentrations was low. This result shows that the cytotoxic effect of biosynthesized AgNPs against MCF-7 is stronger. The IC50 value of the green synthesized AgNP is 0.06 µg/ml for the MCF-7 cell line, while the IC50 value of the plant extract is 6.91 µg/ ml. At the same time, tests were made against different bacterial species in our study. It was determined that all microorganisms except the E. coli bacterial strain inhibited biofilm formation at the lowest concentration of AgNP (0.03 mg/ml).

It was observed that the antimicrobial, anti-biophilic, and anticancer activity of the *R. aculeatus* plant was quite high with AgNP synthesis. With the study we have done, a source is presented to the literature for possible future studies. However, it may enable the use of new therapeutic agents and show promise for effective antibiofilm, and antimicrobial agent designs in the biomedical field.

Author Contribution

F.K., A.S.B., M.B., R.B., E.K., and F. S. organized all experiments and wrote the manuscript. F.K., and F.S. performed all experiments and characterizations.

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