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Research Article

Investigation of antimicrobial and anticancer activity of extracts obtained following UV application to *Althaea officinalis* L. callus cultures

Fethi Ahmet Özdemir[1,*], **Mesut Turan**[2]

[1]Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Bingol, 1200, Türkiye.
[2]Department of Molecular Biology and Genetics, Institute of Science, Bingol University, Bingol, 1200, Türkiye.

Abstract: *Althaea officinalis* L., a plant of the Malvaceae family, is widely used in alternative medicine. The aim of this study is to cultivate the *Althaea officinalis* plant under *in vitro* conditions to create an appropriate callus regeneration protocol and investigate the antimicrobial and anticancer activities of methanol and ethyl acetate extracts of calli after UV-C application. Leaf, petiole, and root parts of *A. officinalis* plants germinated in a sterile environment were used as explant sources. Explants were cultured on MS medium containing different concentrations of 2,4-D (1, 2 mg/l) and BAP (0.25, 0.50, 0.75 mg/l). The most effective (100%) callus growth and callus weight (516.24±0.48 mg) was observed on petiole explants using MS medium containing 1 mg/l 2,4-D + 0.25 mg/l BAP. Calli obtained from leaf and petiole explants were exposed to UV-C treatment. Extractions of calli were carried out using methanol and ethyl acetate solutions. 1 mg/ml, 5 mg/ml, and 10 mg/ml solutions of methanol and ethyl acetate extracts were prepared and their antimicrobial activity on bacteria was investigated using the disc diffusion method for 7 different gram-positive and 9 different gram-negative bacteria. None of the three extract concentrations used had any antimicrobial activities. The anticancer activities of the extracts on SH-SY5Y human neuroblastoma cells were studied using the WST-1 viability kit. 1000, 500, 250, 125, and 62.5 µg/ml concentrations of ethyl acetate extracts of leaf and petiole calli had anticancer activity.

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1. INTRODUCTION

Althaea officinalis L. or Marshmallow is a perennial and herbaceous plant. *A. officinalis* is widely distributed in the Mediterranean, North Africa, France, the United Kingdom, the Balkans, Central Asia, and Russia. It can grow on seasides, salt marshes, and damp meadows (Altan, 2001). Since ancient times, medicinal and aromatic plants have been used for therapeutic purposes in folk medicine, but there was no extensive knowledge about the content of the plants. Plants are of great importance and use in the fields of chemistry, biology, pharmacy, medicine, and biotechnology (Bodeker *et al*., 2002, Al-Snafi, 2013). Marshmallow is used as a pain reliever against infections, a diuretic effect, a chest softener and protector, an immune system booster for persistent coughs, weight loss, softening of skin wounds, protective

*CONTACT: Fethi Ahmet ÖZDEMİR ozdemirfethiahmet23@yahoo.com Bingöl University, Faculty of Science and Art, Department of Molecular Biology and Genetics, Bingöl, Türkiye

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and wound healing, and relieving respiratory tract damage due to cough. The roots, leaves, and flowers of the plant are used for different medicinal purposes in many countries worldwide (Elmastas *et al*., 2004). It is used in the treating irritation of the mouth and throat mucosa, in the treatments of dry cough, mild gastritis, skin burns and insect bites, ulcers, abscesses, burns, and constipation In addition to these medicinal purposes, it has also been used in the food industry (Changizi *et al*., 2015). *A. officinalis*, also provides a natural and beautiful appearance to the hair and strengthens vision. It helps to regulate body weight by melting fats, increases performance during exercise, and relieves thirst (Diplock, 1998; Baytop, 1984).

It increases sexual performance and heals wounds on the skin. It removes wrinkles, freckles, and brown spots on the skin. It moisturises the skin. It is used in the treatment of haemorrhoids. While it has a calming effect, it eliminates the feeling of tiredness and exhaustion. It is effective in normalising blood pressure. It treats cough and bronchitis. It relieves muscle and headaches. It prevents insomnia by regulating the nervous system (Diplock, 1998). It allows the hardened liver to soften and renew itself and helps to stabilise blood glucose levels by regulating it. It helps in the treatment of digestive system and stomach ailments. It is effective in lowering high blood pressure and relieves restlessness.

It improves kidney activities. It is used to prevent and treat cancer, and it ensures the regeneration of the cell wall. It helps to remove uric acid and cholesterol from the body by dissolving them in water. It stimulates the secretory glands, encourages the release of hormones, helps the waste and toxic substances in the body become water-soluble and expelled, and accelerates blood circulation. It has a stimulating effect on metabolism. Moreover, it regulates the heartbeat and it cleans the blood (Baytop, 1984).

This study aims to investigate the antimicrobial and anticancer activities of methanol and ethyl acetate extracts of calli by using different types of explants from *in vitro* germinated *A. officinalis* as a source and creating a suitable callus regeneration protocol after UV-C application.

2. MATERIAL and METHODS

2. 1. Plant Material

The seeds of the *A. officinalis* used in this study were collected from its natural growing environment. The species was identified according to “The Flora of Turkey and East Aegean Islands” (Davis 1984).

2.2. Sterilization

The surface sterilisation of *A. officinalis* was carried out by using commercial bleach (ACE – Turkey, 5% NaOCl). The most suitable sterilisation protocol was developed by keeping the seeds in bleach for 10, 15, 20, 25, and 30 minutes. Following the sterilisation, the seeds were rinsed with sterile distilled water three times to remove the sodium hypochlorite traces on their surface.

2.3. Nutrient Environment and Culture Conditions

In this study, MS medium containing 3% sucrose and solidified with 0.35% gelrite was used to ensure the germination of seeds. The pH of the media was adjusted to 5.8 ± 0.2 using 1N NaOH and HCl. Leaf, petiole, and root parts of sterile *A. officinalis* plantlets grown by germination of sterile seeds were used as explant sources. Explants were cultured on MS nutrient medium containing 3% sucrose, solidified with 0.35% gelrite, pH adjusted to 5.8 ± 0.2 using 1N NaOH and HCl, amended with 2,4-D (1.2 mg/l) and BAP (0.25, 0.50, 0.75 mg/l). To ensure optimal conditions for calli development, the culture dishes were placed in an environmental cabinet and maintained at a constant temperature of 25±1 °C. The photoperiod consisted of 16 hours of light followed by 8 hours of darkness, with a light intensity of 500 μmol $m^{-2}s^{-1}$.

2.4. UV-C Application to Callus Cultures

Short-wavelength UV-C light was used in the study. UV-C light was applied for 15 minutes from a distance of 15 cm by opening the covers of the magenta vessels where calli were developed.

2.5. Obtaining Methanol and Ethyl Acetate Extracts from Calli

After the calli developed in the callus development medium, they were dried and powdered, 100 g of callus was taken, and 2000 ml of methanol or ethyl acetate was added to it and kept in a shaker incubator for 24 hours under normal conditions. At the end of the 24 hours, the macerated solution was passed through the filter paper, and the large particles were removed. Then the solutions were removed with the help of a rotary evaporator.

2.6. Antimicrobial Activity

The antimicrobial activities of the extracts were determined using the disk diffusion method. For this, the microorganisms used in the study were multiplied in a liquid nutrient medium (LB Broth, Germany) with a final concentration of 10^6. The final concentrations of the extracts were adjusted to be 1 mg/ml, 5 mg/ml, and 10 mg/ml.

In the antimicrobial activity studies, seven different gram-positive (*Bacillus subtilis* ATCC 6337, *Brevibacillus brevis*, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IM 622, *Bacillus cereus* EMC 19, *Staphylococcus aureus* 6538 P, *Listeria monocytogenes* NCTC 5348), nine different gram-negative (*Salmonella typhimurium* NRRLE 4413, *Pseudomonas fluorescens*, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* EMCS, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC II, *Pseudomonas aeruginosa* DSM 50070, *Proteus vulgaris*, *Salmonella enterica* ATCC 13311) bacteria were used.

2.7. Anticancer Activity

Human neuroblastoma cancer cells were cultured in an incubator at 37 °C in DMEM F12 nutrient medium containing 12% fetal bovine serum and 0.5% antibiotic (penicillin-streptomycin) in a humidified medium with 5% CO_2. After 24 hours of incubation, anticancer activity results of 7.8125, 15.625, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL doses of methanol and ethyl acetate extracts of leaf and petiole calli treated with UV-C rays in 96 well plates were examined. The anti-cancer activities of plant extracts were determined by adding 4 µl of WST-1 viability kit to each well in 96 well plates, waiting for 2 hours, and measuring at 450 nm wavelength in the Elisa Reader device.

2.8. Statistical analysis

The descriptive statistics were given as “mean ± standard deviation” in combinations of MS nutrient medium and added plant growth regulators to ensure callus development for the characteristics discussed in the study. No comparison was made in these combinations. Ten leaf, petiole, and root explants were placed in each culture container, using three replications. The students’ test was used to compare cell viability (%) with the control group for each concentration discussed in the study. The statistical significance level was taken as 5%, and the GraphPad package statistics program was used for analysis.

3. RESULTS

3.1. Results on Sterilization and Seed Germination

In this study, it was concluded that the most suitable seed sterilisation for *A. officinalis* seeds is to soak them in commercially available bleach for 30 minutes. After sterilisation, the seeds were cultured on MS nutrient medium which contains 3% sucrose, and solidified with 0.35% gelrite and pH adjusted to 5.8±0.2 using 1N NaOH and HCl. It was determined that the most effective germination was on MS medium + gelrite at a rate of 28%.

3.2. The Results on Callus Growth Rate and Callus Weight

Leaves, petiole, and root parts of *A. officinalis* plants germinated in the sterile environment were used as explant sources. Explants properly separated from the plant were cultured on MS nutrient medium containing different doses of 2,4-D (1, 2 mg/l) and BAP (0.25, 0.50, 0.75 mg/l), 5 combinations each.

The cultures were kept in the climate cabinet for 35 days under suitable conditions. Following a period of 35 days, the callus growth rate and callus weights were determined. The studies were planned as three replications, and the mean and standard deviation values of the data obtained from these replications were calculated and are shown in Tables 1, 2, and 3.

Table 1. The callus growth rate and callus weights in petiole explants.

Plant Growth Regulators		The callus growth rate (%)	The callus weights (mg)
2,4-D (mg/l)	BAP (mg/l)		
1	-	64	324.65±1.43
1	0.25	100	516.24±0.48
1	0.50	51	294.31±0.87
1	0.75	43	254.86±1.73
2	-	78	347.82±0.58
2	0.25	62	320.15±0.47
2	0.50	58	298.37±0.13
2	0.75	47	263.58±1.02

±: refers to the standard deviation values obtained with at least three repetitions.

Table 1 illustrates the callus development rates in petiole explants under different medium compositions. The most effective callus development, reaching 100%, was observed on MS medium supplemented with 1 mg/l 2,4-D and 0.25 mg/l BAP. Following this, the medium containing 2 mg/l 2,4-D exhibited a callus development rate of 78%. The medium with 1 mg/l 2,4-D showed a slightly lower rate of 64%. Similarly, the medium consisting of 2 mg/l 2,4-D combined with 0.25 mg/l BAP displayed a callus development rate of 58%. Further, the medium with 1 mg/l 2,4-D and 0.50 mg/l BAP yielded a rate of 51%. The combination of 2 mg/l 2,4-D and 0.75 mg/l BAP resulted in a callus development rate of 47%. Lastly, the medium containing 1 mg/l 2,4-D and 0.75 mg/l BAP showed the lowest callus development rate at 43%.Although the presence of 2,4-D, was effective on callus development, its use with BAP, a cytokinin, improved callus development. However, the increase in the amount of BAP had a negative effect on callus development. Increasing the amount of 2,4-D from 1 mg/l to 2 mg/l increased the percentage of callus development.

Table 1 presents the weights of calli developed under different nutrient medium compositions. The highest callus weight of 516.24±0.48 mg was observed in the medium containing 1 mg/l 2,4-D and 0.25 mg/l BAP. This was followed by the medium with 2 mg/l 2,4-D, which resulted in a callus weight of 347.82±0.58 mg. The medium containing 1 mg/l 2,4-D yielded a callus weight of 324.65±1.43 mg. Similarly, the medium comprising of 2 mg/l 2,4-D combined with 0.25 mg/l BAP exhibited a callus weight of 320.15±0.47 mg. The combination of 2 mg/l 2,4-D and 0.50 mg/l BAP resulted in a callus weight of 298.37±0.13 mg. Furthermore, the medium containing 1 mg/l 2,4-D and 0.50 mg/l BAP produced a callus weight of 294.31±0.87 mg. The medium with 2 mg/l 2,4-D and 0.75 mg/l BAP showed a callus weight of 263.58±1.02 mg. Lastly, the medium containing 1 mg/l 2,4-D and 0.75 mg/l BAP exhibited the lowest callus weight at 254.86±1.73 mg.

Table 2. Callus growth rate and callus weights in leaf explants

Plant Growth Regulators		The callus growth rate	The callus weights
2,4-D (mg/l)	BAP (mg/l)	(%)	(mg)
1	-	59	257.71±1.39
1	0.25	71	332.54 ±0.78
1	0.50	39	224.62±0.98
1	0.75	29	196.38±1.73
2	-	63	260.48 ±0.46
2	0.25	54	245.93±0.21
2	0.50	41	238.06 ±0.81
2	0.75	34	211.37±0.21

±: refers to the standard deviation value obtained with at least three repetitions.

In leaf explants, the most effective callus development was detected on MS nutrient medium containing 1 mg/l 2,4-D + 0.25 mg/l BAP at a rate of 71%. This is followed by the medium containing 2 mg/l 2,4-D at the rate of 63%, the medium containing 59% of 1 mg/l 2,4-D, the medium containing 2 mg/l 2,4-D + 0.25 mg/l BAP at the rate of 54%, the medium containing 2 mg/l 2,4-D + 0. 50 mg/l BAP at the rate of 41%, the medium containing 1 mg/l 2,4-D + 0. 50 mg/l BAP at the rate of 39%, the medium containing 2 mg/l 2,4-D + 0. 75 mg/l BAP at the rate of 34%, and lastly, the medium containing 1 mg/l 2,4-D + 0. 75 mg/l BAP at the rate of 29% respectively (Table 2).

Although the presence of only one auxin, 2,4-D, was effective on the development of calli from leaf explants, its use with BAP, a cytokinin, was more effective on the rate of callus growth. However, the increase in the amount of BAP used negatively affected the callus growth rate from leaf explants. Increasing the amount of 2,4-D from 1 mg/l to 2 mg/l increased the percentage of callus development in leaf explants (Table 2).

Table 2 presents the weights of calli developed under various nutrient medium compositions. The highest callus weight of 332.54±0.78 mg was observed in the medium containing 1 mg/l 2,4-D and 0.25 mg/l BAP. Following this, the medium with 2 mg/l 2,4-D resulted in a callus weight of 260.48±0.46 mg. The medium containing 1 mg/l 2,4-D yielded a callus weight of 257.71±1.39 mg. Similarly, the medium comprising of 2 mg/l 2,4-D combined with 0.25 mg/l BAP exhibited a callus weight of 245.93±0.21 mg. The combination of 2 mg/l 2,4-D and 0.50 mg/l BAP resulted in a callus weight of 238.06±0.81 mg. Furthermore, the medium containing 1 mg/l 2,4-D and 0.50 mg/l BAP produced a callus weight of 224.62±0.98 mg. The medium with 2 mg/l 2,4-D and 0.75 mg/l BAP showed a callus weight of 211.37±0.21 mg. Lastly, the medium containing 1 mg/l 2,4-D and 0.75 mg/l BAP exhibited the lowest callus weight at 196.38±1.73 mg.

Table 3 presents the results of callus development under different hormone combinations. Out of the various environments tested, callus development was observed in only three hormone combinations, while no callus development was detected in other conditions.

The medium containing 1 mg/l 2,4-D and 0.25 mg/l BAP exhibited the highest callus growth rate of 18%. Following this, the medium with 2 mg/l 2,4-D had a callus growth rate of 8%. Lastly, in the medium containing 1 mg/l 2,4-D, the callus growth rate was observed to be 3%.

Table 3. Callus growth rate and callus weights in root explants.

Plant Growth Regulators		The callus growth rate	The callus weights
2,4-D (mg/l)	BAP (mg/l)	(%)	(mg)
1	-	3	25.74±1.68
1	0.25	18	78.26 ±2.34
1	0.50	-	-
1	0.75	-	-
2	-	8	48.06±0.97
2	0.25	-	-
2	0.50	-	-
2	0.75	-	-

±: refers to the standard deviation value obtained with at least three repetitions.
Root explants were observed to be the most inefficient explants in terms of callus development.

Regarding callus weights, they ranged between 78.26±2.34 mg and 25.74±1.68 mg. The medium with the highest callus weight was determined to be the one containing 1 mg/l 2,4-D and 0.25 mg/l BAP. On the other hand, the lightest calli were developed in the medium containing 1 mg/l 2,4-D (Table 3).

When the callus development rates and callus weights of the petiole, leaf, and root explants were compared with each other, it was observed that the petiole explant was more efficient than the other two explants in terms of callus development rate and callus weight. The best results regarding both callus growth rate and callus weight were obtained from petiole explants. After the development of the calli was observed, UV-C was applied for 15 minutes from a distance of 15 cm in the airflow cabinet while opening the lids of the culture vessels in which the calli developed. After drying the calli growing from the UV-C treated leaf and petiole explants, their extraction was carried out using methanol and ethyl acetate solutions. Extraction from root explants was not performed as there was not enough callus development from root explants.

3.3. Antimicrobial Activity Results

While methanol extracts of leaf and petiole calli were prepared with sterile distilled water, ethyl acetate extractions were prepared using DMSO with final concentrations of 1 mg/ml, 5 mg/ml, and 10 mg/ml. The antimicrobial activities of the solutions of all three concentrations were determined using the disk diffusion method. It was determined that the methanol and ethyl acetate extracts of the calli of the leaf and petiole, solutions of all three concentrations (1, 5, 10 mg/ml) did not have antimicrobial activities against the bacteria used in the study.

3.4. Anticancer Activity Results

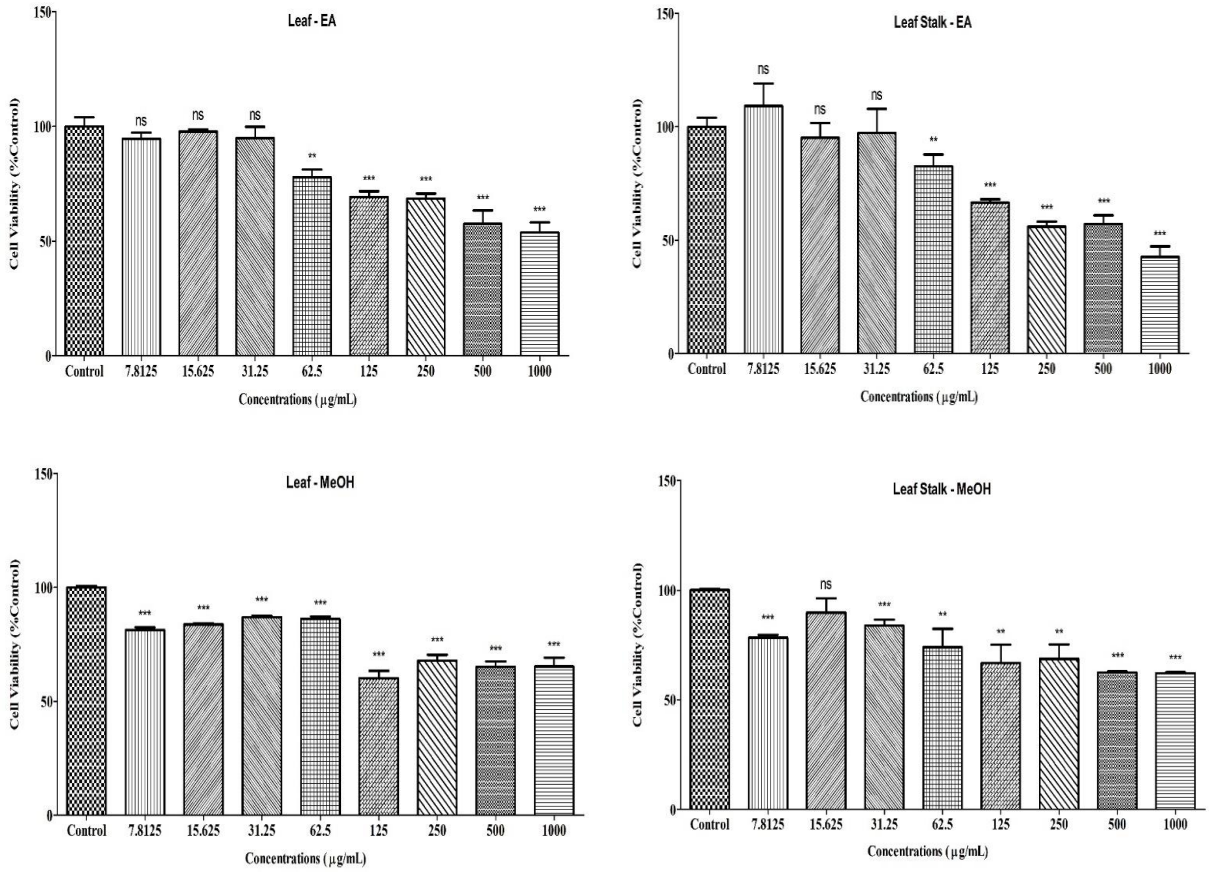
The anticancer activities of methanol and ethyl acetate extracts of calli, of which leaves and petioles were exposed to UV-C treatment, on SH-SY5Y human neuroblastoma cells were determined using the WST-1 viability kit. The anticancer activity results of 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL doses of the extracts are shown in Figure 1.

It was observed that 1000, 500, 250, and 125 µg/mL doses of ethyl acetate extracts obtained from leaf calluses showed a statistically (p<0.001) highly significant level of anticancer activity on SH-SY5Y human neuroblastoma cells compared to the control. It was determined that the dose of 62.5 µg/ml had a statistically significant (p<0.01) anticancer activity compared to the control. Still, the doses of 31.25, 15.625, and 7.8125 µg/ml did not have any anticancer activity

statistically compared to the control (Figure 1). It was observed that the anticancer activity decreased due to the decrease in the doses of the extracts obtained from the leaf calluses. In other words, as the doses increased, there was an increase in the activity.

It was observed that 1000, 500, 250, and 125 µg/ml doses of ethyl acetate extract of petiole calli showed highly significant anticancer activity on SH-SY5Y human neuroblastoma cells compared to control ($p< 0.001$). It was determined that the 62.5 µg/ml dose had a statistically significant ($p<0.01$) anticancer activity compared to the control, but the 31.25, 15.625, 7.8125 µg/mL doses did not have any anticancer activity statistically (Figure 1). It was concluded that ethyl acetate extracts obtained from the calli grown from leaf and petiole explants showed similar anticancer activities.

Figure 1. Show the effect of the extracts of *Althaea officinalis* L. calli on cell viability in SH-SY5Y human neuroblastoma cells.



The data are represented by the mean ± S.D. from 3 independent experiments and are statistically significant at $p< 0.05$.^{ns} $p>0.05$,$^{**}p <0.01$, $^{***}p <0.001$ vs control treatment, as indicated by the brackets. EA: ethyl acetate; MeOH: methanol.

All doses of methanol extract of leaf calli (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 µg/mL) showed anticancer activity on SH-SY5Y human neuroblastoma cells. This activity was statistically significant compared to the control group ($p <0.001$). The most effective anticancer activity was obtained using 125 µg/ml of methanol extract (Figure 1).

It was determined that the methanol extract of petiole calli showed anti-cancer activity on SH-SY5Y human neuroblastoma cells at all doses (1000, 500, 250, 125, 62.5, 31.25, 7.8125 µg/mL) except for the dose of 15.625 µg/ml. Compared with the control group, this activity was found statistically significant ($p <0.01$) (Figure 1). It was determined that methanol extracts

of leaf and petiole calluses showed almost similar results in terms of anti-cancer activity. When the anti-cancer activities of methanol and ethyl acetate extracts were compared, it was observed that methanol extracts showed a more effective anti-cancer activity compared to ethyl acetate extracts. The reason for this situation can be explained by the fact that methanol is a more effective organic solvent.

4. DISCUSSION and CONCLUSION

The reason why plants are used in alternative medicine is the valuable secondary metabolites they contain. The different parts of *A. officinalis* contain many components. Pectins, mono and disaccharides, mucilage, flavonoids, isoquercitrin, camphor, caffeic, p-coumaric acid, coumarins, scopoletin, phytosterol, tannins, asparagines are the main components (Al-Snafi, 2013). Previous studies have shown that *A. officinalis* has many pharmacological effects, including antimicrobial, anti-inflammatory, cardiovascular, antiestrogenic, cytotoxic, immunological and immunomodulatory effects (Ahmad *et al*., 1998; Lin *et al*., 1999; Bonjar *et al*., 2004; Guatem *et al*., 2015; Rezai *et al*., 2015; Zhang *et al*., 2016; Twaij *et al*., 2018; Qaralleh *et al*., 2020). Besides, this study, it was determined that methanol and ethyl acetate extracts obtained after UV-C application on calli which were developed using leaves and petiole of *A. officinalis* showed anti-cancer activity.

Our findings are consistent with previous literature on the subject. In a study conducted by Naz and Anis (2012) on the interaction of exogenous hormone concentration and adenine sulfate in *A. officinalis*, three distinct phases of callus formation were investigated. The study presented results obtained from various leaf segments.

As a result of the study, an effective callus development protocol was created by adding 2,4-D at 15 µM concentration in a liquid MS nutrient medium. Our study used 1 and 2 mg/l doses of 2,4-D to ensure callus development. A similar result was obtained using the same plant growth regulator. However, in our study, 2,4-D was used in combination with a cytokinin, BAP, and the amount of use of 2,4-D differs from the aforementioned study. Another similar aspect of both studies is the use of the leaf as an explant source. However, while we used the leaf as an explant source by dividing it in half in our study, different leaf segments were used in the aforementioned study. This makes the two studies different from each other.

In another study on developing an effective shoot regeneration protocol in *A. officinalis* (Naz et al., 2015), the node parts of the plant were used as an explant source. Effective shoot regeneration was obtained by adding BA, Kn, and 2-iP together with combinations of IBA, IAA, and NAA to the MS nutrient medium. In the study, different types of cytokinin and auxin were used to ensure shoot formation and an effective shoot regeneration protocol was obtained. Our study received an effective callus development protocol using BAP, a cytokinin, with 2,4-D, an auxin, at different concentrations. However, the fact that the study is a shoot regeneration and our study is a callus development study separates the two studies from each other. In addition, the explants used in both studies are different from each other.

In another study on the effect of plant growth regulators on *in vitro* production of *A. officinalis* (Mujib et al., 2017), the root, node, and leaf parts of the plant were used as explant source and when 2,4-D was added to the medium, the callus was obtained from explants which were used. The most effective callus growth was obtained from the node explants with a rate of 62%, the leaf explants with a rate of 39%, and the root explants with a rate of 27%. However, not only 2,4-D but also 0.5, 1, and 2 mg/l BAP were added to the medium. Similarly, in our study, an effective callus development protocol was obtained as a result of using 2,4-D and BAP together. Although the plant hormones used in the mentioned study and our study are the same, the concentrations which were used differ from each other. In addition, while the most effective callus growth rate was obtained from the node explant in that study, petiole was

determined as the most efficient explant in terms of callus development in our study. In both studies, root explant was found to be an inefficient explant source in terms of callus development. In the root explants we used in our study, the callus development was observed at 18% as the highest rate while this rate was 27% in the mentioned study.

In a study conducted to determine the antibacterial activities of *A. officinalis* and *A. hirsute* extracts (Lin et al., 1999), it was determined that both plant extracts did not show antibacterial effects. In our study, methanol and ethyl acetate extracts of the calli were made after UV-C application to the calli obtained from the leaf, and petiole. In this sense, the findings of our study and the literature findings support each other.

In another study investigating the antibacterial effects of the methanol extracts of *A. officinalis* and *A. cannabina* (Ahmad et al., 1998), it was observed that both plant extracts produced inhibition. In this study, which was conducted with 52 different bacterial species, very good results were obtained in 17 bacteria. However, the lack of antimicrobial activity of the methanol and ethyl acetate extracts of the calli developed in our study caused the two studies to be different from each other. The disparity in results may be attributed to the contrasting methodologies employed. In the previous study, the plant itself was utilized, whereas in our study, we specifically developed callus from the leaf and petiole of the plant for experimentation. This discrepancy in experimental approaches likely accounts for the observed differences between the two studies. In obtaining such a result, the fact that the biologically active compounds of the plant itself and the calli are different may be effective. For this reason, more detailed studies should be carried out, and the biologically active compounds of the plant and calli collected from the natural growing environment should be compared.

In another study investigating the antimicrobial activities of extracts obtained from *A. officinalis* flowers (Bonjar, 2004), it was emphasized that while an effective antimicrobial activity was observed on some of the used pathogenic microorganisms, there was no antimicrobial activity on others. Likewise, in our study, the determination of the absence of antimicrobial activity of the methanol and ethyl acetate extracts of calli is supported by the literature findings. However, it is important to note that our study differs from the previous one in terms of the methodology employed. While the previous study utilized extracts obtained from the flowers of the plant, our study focused on utilizing callus extracts developed from leaves and petiole, which were then subjected to UV-C treatment. As a result, these differing approaches between the two studies contribute to their variations in findings.In the study in which the antimicrobial activities of ethanol, hexane, ethyl acetate, and water extracts of *A. officinalis* on *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* bacteria were investigated using the methods of disc diffusion and microdilution (Qaralleh et al., 2020). It was determined that the extracts had weak antimicrobial activity. Above all, it was emphasized that the microdilution method is a more effective method for the determination of antimicrobial activity. In our study, the disc diffusion method was correspondingly used in the determination of antimicrobial activity, and a similar result was obtained. The use of a different antimicrobial activity measurement technique such as microdilution in our study might have led to more positive outcomes as a result of the study.

In a study investigating the antimicrobial activities and wound healing potential of hydroalcoholic extracts of *A. officinalis* on clinical strains as well as pathogenic bacteria such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes* in comparison with ciprofloxacin, gentamicin, and penicillin antibiotics (Rezai et al., 2015), disc diffusion and MIC methods were used in antimicrobial activity studies. Consequently, it was emphasized that although the extract was not effective on gram-negative bacteria, it was effective on gram-positive bacteria. The absence of antimicrobial activities of methanol and ethyl acetate extracts obtained from leaf and petiole calluses on both gram-

negative and gram-positive bacteria, which we used in our study, enabled both studies to partially support each other. No antimicrobial activity was detected on gram-negative bacteria in either study. Another difference is that while methanol and ethyl acetate extracts were used in our study, hydroalcoholic extract was used in the current study.

A study that examined the anticancer activity on A549 cells using root extracts of *A. officinalis* (Zhang et al., 2016) stated that the root extract at a dose of 25 mg/ml showed a highly effective anticancer activity. Moreover, in our study, it was determined that both methanol and inert acetate extracts showed very effective anticancer activity, and this suggests that the same biologically active substance or substances may be present in the extracts used. This should be demonstrated using detailed chromatographic techniques. However, we used SH-SY5Y neuroblastoma cancer cells in our study while A549 cells were used in the aforementioned study.

In a study using water extracts of the flower, leaf, and root parts of the *A. officinalis* plant (May & Willuhn, 1985), it was determined that 10% concentrations of the extracts had an inactivation effect on HeLa cells. Our findings also showed that the methanol and ethyl acetate extracts we used had a highly effective inactivation on SH-SY5Y neuroblastoma cancer cells. Both studies differ from each other: they use different cancer cells, use different solvents for extraction, and make use of the different extracted plant parts.

In a study investigating the anti-cancer activities of *A. officinalis* and *A. esculentus* flower and root extracts on HeLa and SK-Hep1 cells (Park et al., 2010), it was observed that both plants have anticancer activities. It was determined that *A. officinalis* showed a stronger anticancer activity compared to *Abelomoschus esculentus*. It was stated that the anti-cancer activity of the extracts obtained by using the whole plant was more effective than the flower and root parts. In our study, it was determined that the callus that was developed by using the leaf and petiole of *A. officinalis* and treated with UV-C showed a very effective anti-cancer activity. In the aforementioned study, the effect of flower and root extracts of *A. officinalis* on HeLa and SK-Hep1 cancer cells was investigated. On the other hand, in our study, the anti-cancer activity of methanol and ethyl acetate extracts of UV-C-treated calli developed using the leaf and petiole of *A. officinalis* on SH-SY5Y neuroblastoma cancer cells was investigated. This situation caused these studies to be different from each other.

In the study in which the methanol extract was obtained by using the aerial parts of *A.ludwigii* L. and its anti-cancer activity against MCF-7 cells was investigated, it was noted that the used plant extract has a highly effective anti-cancer activity against MCF-7 cancer cells. It was pointed out that the plant extract is rich in rutin in terms of its bioactive components, and that rutin, which has a strong anti-cancer activity, may have played a role in the formation of such an effect (Alshaya et al., 2019). It raises such questions: Could this be the reason why the extracts we used in our study have strong anti-cancer activity because they are rich in rutin content? In addition, how does the routine amount change in calli before and after UV-C application? In order to clarify these questions, routine content analyses of calli should be performed before and after UV-C application.

Although the plant in the aforementioned study is in the same genus as the plant we used in our study, the fact that they are different species and the use of different cancer cells in both studies caused these studies to differ from each other.

It was shown that scopoletin (7-hydroxy-6-methoxy coumarin), which is abundant in *A. officinalis*, has anti-cancer activity by destroying the tumoral lymphocyte effect (Ding *et al*., 2008). Is the reason why the extracts we used in our study showed effective anti-cancer activity because of the high content of scopoletin? This situation can be demonstrated by

comprehensively studying the biologically active ingredient contents of the extracts used in the study.

To conclude, with this study, an effective sterilization protocol was developed to culture the seeds of the *A. officinalis* plant *in vitro*, and then an effective germination environment was tried to be created because germination difficulties were encountered. Leaf, petiole, and root parts of plants grown *in vitro* were used as explant sources and an effective callus regeneration protocol was developed. It was determined that the most effective medium for callus development was MS nutrient medium containing 1 mg/l 2,4-D and 0.25 mg/l BAP, and the most efficient explant for callus development was petiole. Following UV-C application to the obtained calli, methanol, and ethyl acetate extracts were obtained from these calli. The antimicrobial and anti-cancer activities of the extracts were investigated, and it was observed that the used extracts did not have an effective antimicrobial activity but showed strong anticancer activity. Whereas the microorganisms used in the study had a prokaryotic cell structure, the fact that the cancer cells we used in the study had a eukaryotic cell structure may have caused such a result. The fact that we do not know the biologically active component content of the extracts we used in the study creates a deficiency, but it provides a research area for new studies to be made on this subject in the future.

After the routine and scopoletin content analyses were made, the calli used with the normal plant, these two components, even different component contents, were compared with each other before and after the UV-C application. The question of how these components can affect prokaryotic and eukaryotic cells was put forward by this study.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Fethi Ahmet Özdemir: Investigation, Methodology, Resources, Supervision, Visualization, Software, Formal Analysis, and Writing-original draft. **Mesut Turan:** Investigation, Methodology, Resources, Visualization, Software, Formal Analysis

Orcid

Fethi Ahmet Özdemir https://orcid.org/0000-0001-7215-9692
Mesut Turan https://orcid.org/0000-0002-4980-7337

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