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Research Article

Salvia ceratophylla L. and *Ricotia aucheri* (BOISS.) B.L. INVESTIGATION OF BIOLOGICAL AND CYTOTOXIC ACTIVITIES OF BURTT PLANTS IN VITRO CONDITIONS

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ABSTRACT. In this study, was aimed to investigate the antimicrobial activities, antioxidant capacity assays and cytotoxic effects of *Salvia ceratophylla* L. and *Ricotia aucheri* (Boiss.) B.L. Burtt on MCF-7 (human breast cancer cell line) and HUVEC (human umbilical vein endothelial cells) cell lines. Disc diffusion method was chosen for antimicrobial activity and capacity and it was determinedon 6 pathogenic microorganisms (*Enterococcus durans*, *Staphylococcus aureus* ATCC 25923, *Enterobacter aerogenes* ATCC 13048, *Bacillus subtilis* DSMZ 1971, *Salmonella typhimurium*). Antioxidant activity capacities were observed by DPPH method. Cytotoxicity tests on MCF-7 (human breast cancer cell line) and HUVEC (human umbilical vein endothelial cells) cell lines were tested by MTS method. While the antimicrobial effect of *Ricotia aucheri* was observed on *Klebsiella pneumoniae* and *Staphylococcus aureus* ATCC 25923 strains, antimicrobial effect of *Salvia ceratophylla* was observed on with other strains except *Enterococcus durans* and *Enterobacter aerogenes* ATCC 13048. It was also observed that *Salvia ceratophylla*, showed a higher antioxidant capacity than BHT when BHT was used as the control group. On the other hand, in the cytotoxicity test, *Ricotia aucheri* plant occurred a significant decrease in MCF-7 cell line. As a result of this study, it has been determined that two plant materials have antimicrobial, antioxidant and cytotoxic properties and it can be assumed that they can be a source for future studies.

Keywords:*Salvia ceratophylla , Ricotia aucheri, MCF-7, HUVEC, DPPH.*

INTRODUCTION

Turkey has a wide variety of flora with 11707 taxa and 3649 of them are endemic.[2]. Salvia genus belong to the Lamiace family which is the third largest family in terms of distribution in the biogeography in Turkey[3]. The plants in this family includes high amount of essential oil and secondary metabolites. Owing to these features, it can be used in medicine, pharmacy, food and cosmetics[4]. According to the data of a study conducted in 2017, the Lamiacea family is represented by 48 genera and 782 taxa in Turkey, and the its endemism rate was determined to be 44%[5]. Salvia L. is the largest genus of the Lamiacea family. In Latin, salvia comes from the word 'salveo' and means healer and savior [6]. As a result of the studies, it has been observed that some bioactive compounds

from this plant reduced DNA synthesis in the cell, thus the diagnosis and treatment of cancer disease can be determined or applied with it [7].

Ricotia aucheri belongs to the Brassicaceae family and 4 main classification systems have been proposed for this family by Hayek, Schulz, Janchen and Avetisian[8,9,10,11]. According to phylogenetic studies, *Ricotia aucheri* differs from all members of the genus with its simple leaves [12].

While we benefit from plants as food, we ensure the intake of foods and antioxidants in our metabolism. By taking antioxidants into metabolism, we can prevent diseases such as cancer, Alzheimer's, obesity and slow down the aging rate. The antioxidant capacities of food-based plants are examined for their effect on these diseases. Antioxidant capacity is divided into two groups, methods based on hydrogen atom transfer (HAT) reactions and methods based on electron transfer[ET]. Methods based on electron transfer test the ability of the antioxidant to reduce the oxidant by creating a color change [13]. The method we used in the study, DPPH (2,2-diphenyl-1-picrylhydrazil) is a practical and fast method based on ET.

Our body reacts as a result of pollution, radiological factors, stress and genetic predisposition occurring in our environment and in our world.[14] Among these reactions, the disease with the most complex structure today is cancer. In general terms, cancer is a disease based on the rapid and irregular proliferation of cells, as well as their invasion of distant healthy tissues by means of metastasis. Lung cancer is the most common type of cancer, followed by breast cancer. 44.5% of women diagnosed with breast cancer in Turkey are between the ages of 50-69[8]. As seen in the data, age and cancer mortality rate are directly proportional[15]. Among the methods used for cytotoxic tests, MTS (3-(4,5-dimethylthiazol-2-yl)-5 (3carboxymethoxyphenyl)-2-(4-sulfophenyl) measurement is used to measure cytotoxicity based on viability of metabolism in in-vitro conditions. is a method[16].

This study was performed on 6 bacteria, and Gram-negative *Enterobacter aerogenes* (ATCC13048), *Klebsiella pneumoniae*, Salmonella typhimurium strains and gram-positive *Staphylacoccus aureus* (ATCC25923), *Bacillus subtilis* (DSMZ1971), Enterococcus durans strains were used. The experimental process was carried out by preparing three different concentrations from the sample plant materials.

MATERIAL AND METHODS

Material

The identification and supply of the plant sample were provided by Dr. Yusuf Ziya Kocabaş from Kahramanmaraş Sütçü Imam University, Department of Herbal and Animal Production. MCF-7 (Human Breast Cancer Line) and HUVEC (Human Umbilical Vein Endothelial Cells) cell lines used in the cytotoxicity stage were obtained from Kahramanmaraş Sütçü Imam University Faculty of Agriculture cell culture laboratory.

Method

Plant Extraction

Plant samples were dried under sterile conditions in the laboratory environment. In order to carry out the extraction process, the plant samples were divided into small pieces with the help of a pestle. Extraction was carried out using methanol as solvent. The Soxhlet method was used as the method.

Disc Diffusion Method

Three concentrations of sample (20 mg/ml, 10 mg/ml and 5 mg/ml) were prepared and cultivated on Müeller Hilton Agar (MHA). Then, the prepared plant concentrations were loaded on sterile discs with a diameter of 6 cm and kept in an incubator at 37ºC for 24 hours. After 24 hours, discs loaded with plant samples were placed on bacteria inoculated with sterile swaps on MHA at regular intervals and kept in the ıncubator at 37ºC for 24 hours. The study was carried out in triplicate. Zone diameters were measured after the completion of the time.

Minimum Inhibition Concentration (MIC)

The activity of plant extracts against microorganisms was tested by disk diffusion. Additionally, the Minimum Inhibition Concentration (MIC) test was applied on 96-well microplates to determine the lowest inhibition concentrations of these plant samples on microorganisms. Plant sample and bacterial strains were added to the relevant wells in the microplates and the microplate was incubated at 37ºC for 18-24 hours in incubator. At the end of the incubation process, the microplate was read in the microplate reader at 550 nm.

Minimum Bactericidal Concentration (MBK)

This test was performed to observe whether there is microorganism growth in three concentrations of the plant sample after the MIC test. For the MBK test, samples were taken from the wells in the microplate with the help of sterile loops and cultivated on MHA, which is a solid medium. For one day, the cultivated petri dishes were kept in incubator at 37ºC for one day. At the end of the incubation process, the concentration that killed all the microorganisms in the petri dishes was measured as the MBK value.

Antibiotic Activity

Biofilms are communities of microorganisms found in the extracellular matrix on surfaces where the necessary moisture is present [17]. After MIC and MBK processes, microplate containing bacteria and plant extract was incubated at 37ºC for 48 hours. After the incubation process was completed, all the wells in the plate were emptied and washed with distilled water (dH_2O) then, and they were left to dry. After these procosses, 130 µl of methanol (95%) was added to all the wells in the drying plate and left for fixation for 15 minutes. The wells were again completely emptied and left at room temperature to dry. 125 µl of 0.1% crystal violet solution was added to the dried plate wells and kept at room temperature for 10 minutes. After this the plates were emptied and washed with distilled water and left to dry. 33% glacial acetic acid was added to the wells containing gram -positive bacteria and 200 µl of 95% ethanol solution was added to the wells containing gram harmful bacteria and left for 15 minutes. At the end of 15 minutes, measurements were done by spectrophotometer device at 550 nm.

Determination of DPPH Free Radical Antioxidant Capacity

To determine the antioxidant capacity, DPPH (2,2-diphenyl-1-picrylhydralysis) method, which is one of the methods based on electron transfer, was applied. DPPH and BHT(2,6-Di-tert-butyl-4-methylphenol) were prepared as 0.1Mm solvent in DMSO (10%). Three concentrations of plant samples (0.01mg/ml, 0.1mg/ml and 1mg/ml) were prepared. The analysis was carried out in 96-well microplates and was repeated in 3 replicates. The absorbance values were read at a wavelength of 517 nm on the microplate, which was kept in the dark for 30 minutes at room temperature and under aseptic conditions.

Cell Culture

MCF-7(Human Breast Cancer Line) and HUVEC(Human Umbilical Vein Endothelial Cells) cell lines were grown in 75cm$_2$ flasks in 15ml medium in DMEM medium containing FBS (fetal bovine serum) at 37°C in 5% CO2 atmosphere.

Cell Count

To calculate the number of cells per milliliter of total cell suspension, a thoma slide with an area of 1mm2, a depth of 0.1mm, divided into 25 small squares, was used so that the total volume could be calculated. Before the cells were treated with the plant sample, they were counted as 5000 cells per well in 96-well culture dishes and waited for 24 hours for the cells to adhere to the surface they were on.

Application of salvia ceratophylla and Ricotiaaucheri

Salvia ceratophylla and Ricotia aucheri dissolved in 10% DMSO were applied on the cells in 96-well culture dishes with 4 different concentrations of 0, 0.01, 0.1 and 1 mg/ml.

MTS Test

10µl of MTS solution was applied to each well and left for 2 hours at 37ºC for cells to metabolize MTS. After 2 hours, the color change was observed and absorbance values were taken at 490 nm wavelength.

Statistical Analysis

The GraphPad Prism 6 program was used for statistical analysis (ANOVA). Statistically, $p \leq 0.05$ was considered significant..

RESULTS

As a result of the antimicrobial activity test, it was observed that Salvia cerattophylla and Ricotiaaucheri samples showed activity at 10 mg/ml and 20 mg/ml concentrations for all strains except for Enterobacter aerogenes ATCC 13048 and Enterococcus durans strains. Table 1 shows the antimicrobial activity results of Salvia ceratophyllawhile Table 2 shows Ricotia aucheri plant.

Table 1. *Salvia ceratophylla* antimicrobial zone diameters (Disc diameters not removed 6 mm) (-): There is no inhibition zone. (IPM10): Rope 10mg/ml. DMSO (10%)

	5 mg/ml	**10 mg/ml**	**20 mg/ml**	**DMSO**	**IPM 10**
Enterobacter aerogenes ATCC 13048	-	-	-	-	**24**
Klebsiella pneumoniae	-	**10**	**12**	-	**20**
Enterococcus durans	-	-	-	-	**22**
Salmonella typhimurium	-	**12**	**14**	-	**25**
Staphylococcus aureus ATCC 25923	-	**15**	**17**	-	**22**
Bacillus subtilis DSMZ 1971	-	**12**	**14**	-	**24**

Table 2. *Ricotia aucheri* antimicrobial zone diameters (Disc diameters not removed 6 mm) (-): There is no inhibition zone. (IPM10): Rope 10mg/ml. DMSO (10%)

	5 mg/ml	**10 mg/ml**	**20 mg/ml**	**DMSO**	**IPM 10**
Enterobacter aerogenes ATCC 13048	-	-	-	-	**25**
Klebsiella pneumoniae	**10**	**13**	**16**	-	**20**
Enterococcus durans	-	-	-	-	**22**
Salmonella typhimurium	-	**13**	**15**	-	**24**
Staphylococcus aureus ATCC 25923	-	**14**	**16**	-	**25**
Bacillus subtilis DSMZ 1971	-	**10**	**12**	-	**20**

MIC and MBK tests also supported the antimicrobial activity. MIC values of two plant samples are shown on Table 3.

Table 3.*Salvia ceratophylla* and *Ricotiaaucheri* MIC values

	Salvia ceratophylla	***Ricotia aucheri***
Enterobacter aeorogenes ATCC 13048	**5 ±0,82352**	**5 ±0,9310775**
Klebsiella pneumoniae	***2.5*** **±0,9437228**	**2.5 ±0,8982658**
Enterococcus durans	***2.5*** **±0,8146098**	**10 ±0,8649213**
Salmonella typhimurium	**5 ±0,9544459**	**5 ±0,8701211**
Staphylococcus aureus ATCC 25923	**5 ±0,7386082**	**10 ±0,9065981**
Bacillus subtilis DSMZ 1971	**10 ±0,8618223**	**10 ±0,9424853**

As a result of the antibiofilm activity, both *Salvia ceratophylla*and *Ricotiaaucheri* showed the highest activity on *Salmonella typhimurium*strain with with 62.98% and 71.98%, rspectively. Table 4 and Table 5 shows antibiofilm reduction capacities of the two plant materials.

Table 4.*Salvia ceratophylla*antibiophile reduction capacities

	20 mg/ml	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**
Enterobacter aerogenes ATCC 13048	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-
Enterococcus durans	**31,06**	**1,94**	-	-	-
Salmonella typhimurium	**62,98**	**58,11**	**56,16**	**43,50**	-
Staphylococcus aureus ATCC 25923	**16,47**	**14,70**	**14,11**	**4,11**	-
Bacillus subtilis DSMZ 1971	**27,22**	**11,51**	**9,94**	**9,42**	**6,28**

Table 5.*Ricotiaaucheri*antibiophile reduction capacities

	20 mg/ml	**10 mg/ml**	**5 mg/ml**	**2.5 mg/ml**	**1.25 mg/ml**
Enterobacter aerogenes ATCC 13048	-	-	-	-	-
Klebsiella pneumoniae	**2,04**	-	-	-	-
Enterococcus durans	**35,62**	**30,47**	**29,61**	**17,16**	**4,29**
Salmonella typhimurium	**71,98**	**65,94**	**53,87**	**22,41**	-
Staphylococcus aureus ATCC 25923	**35,41**	**33,85**	**32,29**	**22,91**	-
Bacillus subtilis DSMZ 1971	**26,16**	**25,08**	**23,65**	**22,22**	**27,22**

The antioxidant capacity determination was applied according to the DPPH method. BHT (Butylated Hydroxytoluene) with known antioxidant capacity was used as control. It has been found that the antioxidant capacity was proportion to concentration. As the concentrations increased from low to high concentrations, the capacity also increased. The antioxidant capacity results are shown on Figure 1 and Figure 2.

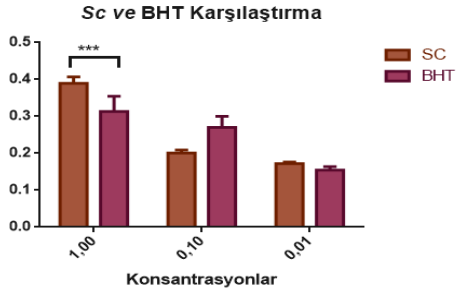

Figure 1. Antioxidant capacity of *salvia ceratophylla* plant material

Salvia ceratophylla showed more activity than the BHT control group, which is known to have the highest concentration (1.00 mg/ml) of antioxidant capacity.

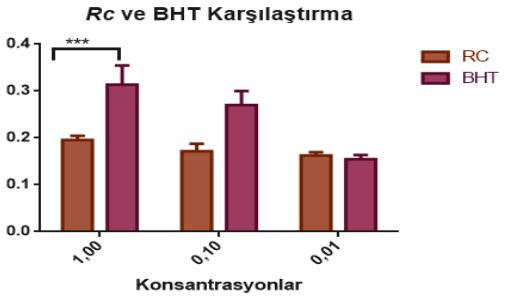

Figure 2. Antioxidant capacity of *Ricotia aucheri* plant material

MTS method was preferred to measure the cytotoxicity effect of the plant samples and the tests were with MCF-7 and HUVEC cell lines at concentrations of 0 , 0.01 , 0.1 , and 1 mg/ml. According to the results of the statistical analysis performed on the MCF-7 cell line, the first significant reductions were observed at 1 mg/ml concentration ($p<0.05$). For both plant samples, it was observed that the cytotoxic effect on the HUVEC cell line was less on the MCF-7 cell line. Furthermore, it was determined that there was a significant decrease in the viability level of the unhealthy cell line as the concentrations of plant samples increased. Figure 3 and Figure 4 show the cytotoxic effects of the two plant materials.

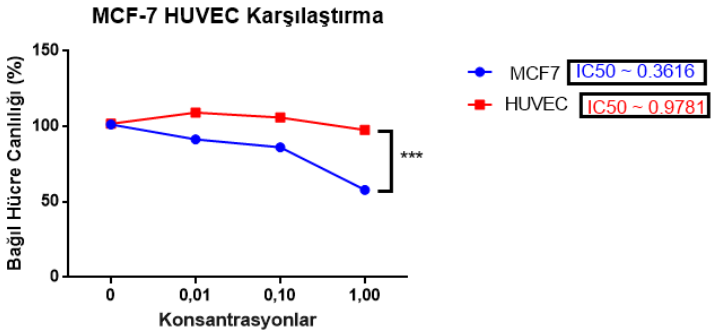

Figure 3. Cytotoxic effect of *Ricotia aucheri* on MCF-7 and HUVEC cell lines

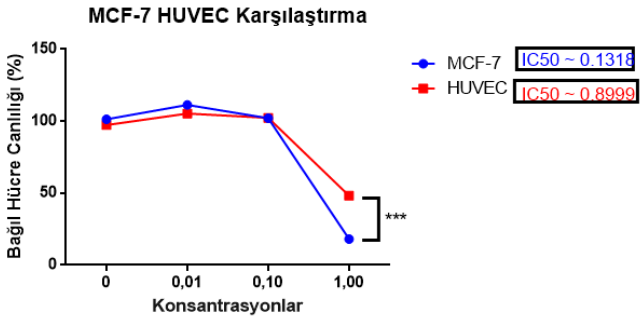

Figure 4. Cytotoxic effect of *Salvia ceratophylla* in MCF-7 and HUVEC cell lines

DISCUSSION AND CONCLUSION

Studies have been carried out on Salvia species in terms of taxonomic, systematic, palynological, biogeographic and their secondary metabolites as well as the essential oils. Özdemir and Şenel(1999) conducted research on Salvia sclarae in terms of morphology, anatomical and karyological features[18].

It was observed that the highest activity in the antibiofilm activity of the Ricotia aucheri plant sample belonged to the Salmonella typhimurium bacteria species. In Salvia ceratophylla plant sample, the highest activity was observed in Salmonella typhimurium bacterial strain.

According to the results of the antioxidant capacity determination, the Salvia ceratophylla sample showed higher antioxidant capacity than the control group BHT. Moreover, antioxidant capacityof Ricotia aucheri has also observed although it is less than Salvia ceratophylla.

When its cytotoxic properties were examined, it was observed that Ricotia aucheri at a concentration of 0.01mg/ml did not cause any death in healthy cells but caused death in cancerous cell lines. In terms of Salvia ceratophylla, it was observed that it also affected the cancerous cell line more than the healthy cell. It is thought that these two plant samples, especially Ricotia aucheri, has a potential on anticancer activity, however, more researches are needed to explore this acitivity.

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