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Antimutagenic and Multi-Biological Activities of *Smilax excelsa* L. Fruit Extract

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Abstract. In this study, antimutagenic, antimicrobial and antioxidant activities of *Smilax excelsa* L. fruit extract were evaluated. The antimicrobial effect was investigated by disk diffusion method and the antimutagenic effect was investigated by Ames/Salmonella/microsom test. The antioxidant properties of S. excelsa fruit samples were determined by investigating the total phenolic, flavonoid contents and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. It was observed that 200 mg plate^{-1} fruit extract was found to have a mutagenicity inhibition as 81% in the absence of S9 mixture and 67% in the presence of S9 mixture against *Salmonella typhimurium* TA1535. *S. excelsa* fruit extract produced an inhibition zone in the range of 11-16 cm against the tested microorganisms. Flavonoid and phenolic contents were found to be 0.7985±0.0124 mgQE 100 mL^{-1} and 11.9847±0.0041 mgGAE 100 mL^{-1} at the 200 mg mL^{-1} extract concentration, respectively. The DPPH removal rate was determined to be 55% at the 200 mg L^{-1} dose. As a result, it was observed that the *S. excelsa* fruit tissues exhibited a highly antimutagenic activity and has been determined as a potential natural antimicrobial and antioxidant source.

***Keywords*:** Ames/Salmonella/microsomal test, antioxidant activity, disc diffusion test, *Smilax excelsa*.

Smilax excelsa L. Meyve Ekstresinin Antimutagenik ve Multi-Biyolojik aktiviteleri

Özet. Bu çalışmada, *Smilax excelsa* L. meyve ektraktının antimikrobiyal, antioksidan ve antimutajenik aktiviteleri araştırılmıştır. Antimikrobiyal aktivite disk difüzyon yöntemi ile antimutajenik aktivite ise Ames/Salmonella/mikrozom testi kullanılarak araştırılmıştır. *S. excelsa* meyve örneklerinin antioksidan özelliği ise toplam fenolik ve flavonoid içeriğinin tespiti ve 1,1-difenil-2-pikrilhidrazil (DPPH) giderme etkisi araştırılarak belirlenmiştir. Meyve ekstraktının 200 mg plak^{-1} dozunda *Salmonella typhimirium* TA1535 suşu ile S9 karışımı yokluğunda %81, S9 karışımı varlığında ise %67 oranında mutajenite inhibisyonu oluşturduğu belirlenmiştir. *S. excelsa* meyve eksraktının test edilen mikroorganizmalara karşı 11-16 cm aralığında inhibisyon zonu oluşturduğu gözlenmiştir. 200 mg mL^{-1} konsantrasyonunda ekstraktta flavonoid içeriği 0.7985±0.0124 mgQE 100 mL^{-1} olarak, fenolik içerik ise 11.9847±0.0041 mgGAE 100 mL^{-1} olarak tespit edilmiştir. 200 mg L^{-1} dozunda DPPH giderme oranı %55 olarak belirlenmiştir. Sonuç olarak, *S. excelsa* meyve dokularının yüksek antimutajenik aktivite sergilediği, potansiyel doğal bir antimutajenik ve antioksidan kaynak olduğu olduğu belirlenmiştir.

***Anahtar Kelimeler*:** Ames/Salmonella/mikrozomal test, antioksidan aktivite, disk difüzyon testi, *Smilax excelsa*.

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1. INTRODUCTION

The accelerated industrialization has increased the use of chemicals in various sectors, and this increase has brought the need for natural components that can eliminate the negative effects of the chemicals. Natural components that can reduce the toxicity of chemicals on living organisms can be consumed as a drug or as a diet in daily consumption. Different tissues of the plants play a protective role against many diseases such as acute/chronic diseases and degenerative defects resulting from the adverse effects of chemicals. This feature of plants is related to the active ingredients found in different tissues with different ratios. It is suggested that regular use of these active compounds in daily life may be effective in preventing cancer and genetic diseases [1, 2]. And also by this way, the secondary diseases caused by synthetic drugs, drug side effects and over-loading of the drug will be prevented [3, 4]. In this study, antimicrobial, antioxidant and antimutagenic activities of *S. excelsa* fruit extract were investigated.

Smilax excelsa L. belongs to the family of Smilacaceae, and these family members are woody, spiny, perennial plants that can be sized up to 15 m, with an average length greater than 3 m. In the Smilacaceae family *S. medica*, *S. ornata*, *S. officinalis*, *S. syphilitica*, *S. papyracea* species are grown in Central America, *S. aspera* and *S. excelsa* species are grown in Anatolia. *S. excelsa*, which is widespread throughout Northern Anatolia, has narrow, cylindrical roots and bakka type fruit [5, 6]. The shoots of *S. excelsa*, which is a spiny plant and started to give young shoots in spring, are consumed as vegetable. The rhizomes have various pharmacological properties such as immunomodulator, antibacterial, antifungal and antioxidant. *S. excelsa* has a variety of active ingredients. These active ingredients are responsible for the antitumor, anti-mutagenic, antibacterial, antifungal, antioxidant, anti-inflammatory properties of *Smilax* species. It is reported that *S. excelsa*, which is known for its blood cleaning and perspiration, is used for therapeutic purposes in the syphilis [7-9].

Because of its importance and frequently consumption in the Black Sea Region, antimicrobial, antioxidant and antimutagenic activities of *S. excelsa* fruit extract have been investigated. Antimicrobial activity was determined by disc diffusion method, antimutagenic activity was determined by Ames/Salmonella test, antioxidant activity was determined by total phenolic content, flavonoid content analysis and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity test.

2. MATERIALS AND METHODS

2.1. Sample Preparation and Extraction

S. excelsa fruit samples were dried under sterile conditions in an oven at 30°C. After grinding samples, 0.2 g of ground sample was extracted in 10 ml of methanol at room temperature for 24 hours in a shaking incubator. At the end of the incubation period, the extract was filtered to remove solid particles and the filtrate was centrifuged at 10000 rpm for 10 minutes. After centrifugation, the supernatant was evaporated by using liquid phase evaporator. Extracts were stored at -18°C and used for determine the antimicrobial, antioxidant and antimutagenic activity [10].

2.2. Antimicrobial Activity

The antimicrobial activity of *S. excelsa* fruit extract was determined by using disc diffusion method with the strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus mutans*, *Staphylococcus epidermidis*, *Candida albicans* and *Candida krusei*. Fresh inoculum of strains (10^7-10^8 pcs L^{-1}) was spread over the surface of Mueller Hinton Agar plates. The sterile filter paper and standart antibiotic discs (6 mm) were placed over the medium surface and 20 mg mL^{-1} extract were impregnated to free sterile discs (20 µl $disc^{-1}$). Plates were incubated for 1 hour at 4 °C and then for 18-24 hours at 37 °C [11]. At the end of the period, the inhibition zones formed around the disc were evaluated as mm.

2.3. Antioxidant Activity

2.3.1. Total Phenol and Flavonoid Determination

The phenolic content of *S. excelsa* fruit extract was determined by Folin-Ciocaltaeu method. Gallic acid was used as standard material with five different concentrations. For experimental procedures, 0.5 mL of the sample in 2.5 mL of Folin-Ciocaltaeu reagent (10%) and 7.5 mL of Na_2CO_3 (20%) were mixed in a test tube. The mixture was incubated at room temperature in the dark for 2 hours and then the absorbance was determined spectrophotometrically at 750 nm [12]. The total phenolic concentration was estimated as equivalent gallic acid (mg GAE 100 mL^{-1}).

For flavonoid determination, quercetin as a standard substance was prepared in methanol at concentrations of 50-200 mg L^{-1}. 10 mL of the sample was mixed with 1 mL of sodium nitrite (5%) and allowed to stand for 6 minutes, after 1 mL of aluminum nitrate (10%) was added. The mixture was left to stand for 6 min, 10 mL NaOH (4.3%) was added and then the volume was completed to 25 mL with dH2O. After incubation for 15 min at room temperature, the absorbance of the solution was determined spectrophotometrically at 510 nm [13]. Total flavonoid content was expressed in mg QE 100 mL^{-1}.

2.3.2. DPPH Assay

The DPPH method was used to determine the radical scavenging activity of the *S. excelsa* fruit extract. For this aim, fruit extracts, BHA and BHT solutions were prepared at a concentration of 50-200 mg L^{-1}. BHA and BHT were used as the standard substance. 80 µl of samples were mixed with 1185 µl DPPH ($6x10^{-5}$ M) solution. The mixture was allowed to stand in the dark for 60 minutes and at the end of time the absorbance of the solution was determined spectrophotometrically at 517 nm. DPPH radical scavenging activity as % Inhibition was calculated from the following equation [14].

$$\% \text{ Inhibition} = Abs_{control} - Abs_{sample} / Abs_{control} \times 100$$

2.4. Antimutagenic Activity

The antimutagenic activities of *S. excelsa* fruit extracts were determined by Ames/Salmonella test with the strains of mutant *S. typhimurium* TA1535 [15, 16]. For this aim, the mutagenity inhibition rates of extract against mutagenic substances have been investigated. 100 µl bacterial strain (1-2 x 10^9 bacteria mL^{-1}), 100 µl extract, 100 µl sodium azide as positive mutagen solution and 500 µl S9 mixture or phosphate buffer (for S9 (-) assay) were added to 2.5 ml top agar. The mixture was shaken with vortex and poured onto the surface of minimal glucose agar plates and the plates were allowed to incubate at 37 ° C for 48-72 hours. At the end of the incubation period the revertant colonies were counted by Stuart Colony meter. The rate of mutagenity in plates without extract, only contain bacterial strain and mutagenic material, was accepted as 100% (ie 0% antimutagenicity). The antimutagenic activities of the extracts were evaluated by using the following equation.

$$\text{Antimutagenic activity (\%)} = [(A-B)/(A-C)] \times 100$$

(A: Numbers of revertant colonies in bacteria and mutagen containing plate; B: Numbers of revertant colonies in bacteria, mutagen and extract containing plate; C: Numbers of self-returning revertant colonies).

3. RESULTS

In this study, antimicrobial, antioxidant and antimutagenic activities of *S. excelsa* fruit extract were investigated. The antimicrobial activity of *S. excelsa* fruit extract is given in Figure 1. To determine the change of antimicrobial activity according to microorganism species, disc diffusion method was tested against fungi, gram positive and gram negative bacteria. It was determined that *S. excelsa* fruit extract showed different antimicrobial activity against all tested microorganisms. The maximum antimicrobial effect of extract was obtained with a 16 mm inhibition zone against *E. coli*. The lowest antimicrobial effect was obtained with 11 mm inhibition zone against *S. aureus*. In general, it has been determined that fruit extracts are more effective against gram-negative bacteria compared to gram-positives and fungi. It is also an essential

point that the extracts have an antimicrobial activity against *Klebsiella pneumonia* and *Candida albicans* strains in which the test antibiotics are ineffective.

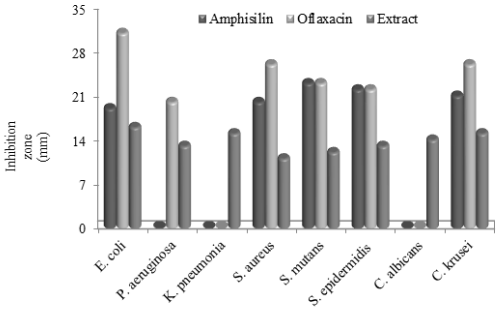

Figure 1. The antimicrobial activity of *S. excelsa* fruit extract

The phenolic content of *S. excelsa* fruit extract was determined by Folin-Ciocaltaeu. Phenolic and flavonoid contents were investigated at a concentration of 200 mg mL^{-1}. The flavonoid content of extract was determined as 0.7985±0.0124 mgQE 100 mL^{-1} and the phenolic content was determined as 11.9847±0.0041 mgGAE 100 mL^{-1}.

One of the methods used for evaluating antioxidant activity is the DPPH radical scavenging activity assay. DPPH removal activity of *S. excelsa* fruit extract investigated in this study is given in Figure 2. Standard substances and extract were tested at concentrations of 50-200 mg L^{-1} and it was determined that the DPPH removal efficiency increased with increasing the concentration of each sample. The highest radical scavenging activity for BHA, BHT and fruit extracts at the concentration of 200 mg L^{-1} was found as 85%, 80% and 55%, respectively. The DPPH scavenging activity obtained at the 200 mg L^{-1} dose of the fruit extract was 1.7 times higher than the effect obtained at the concentration of 50 mg L^{-1}. The DPPH removal effect of the extract may be associated with the active ingredients, especially the antioxidant phenolic compounds.

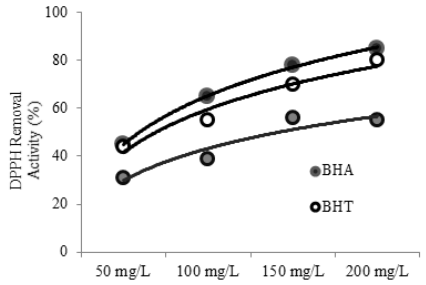

Figure 2. DPPH removal activity of *S.excelsa* fruit extract

The antimutagenicity test results of *S. excelsa* fruit extract against TA 1535 strain are given in Figure 3 and 4. It was determined that 200 mg $plate^{-1}$ fruit extract caused an inhibition rate of 81% in the absence of S9 mixture. The inhibition rate increased with the extract dose increased and the inhibition rate obtained with 200 mg $plate^{-1}$ extract was found to be 1.37 times higher than that obtained with 50 mg $plate^{-1}$ extract. When the percentages of inhibition were evaluated, it was determined that the 50-200 mg $plate^{-1}$ extracts exhibited strong antimutagenic activity in the absence of S9 mixture. In the presence of S9 mixture, the fruit extract was resulted an inhibition rate of 67% against TA 1535 strain at 200 mg $plate^{-1}$ extract. It was determined that the inhibition rate obtained with the 200 mg $plate^{-1}$ extract increased by 1.48 times as much as that obtained with the 50 mg $plate^{-1}$. It was also determined that the inhibition results obtained in the presence of the S9 mixture are lower than those obtained in the absence of the S9 mixture.

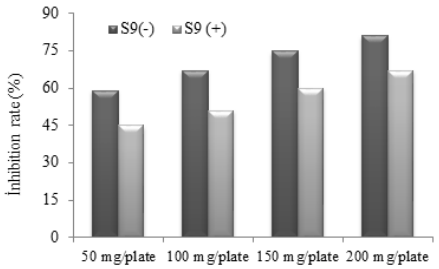

Figure 3. Antimutagenic activities of *S. excelsa* fruit extracts against TA 1535

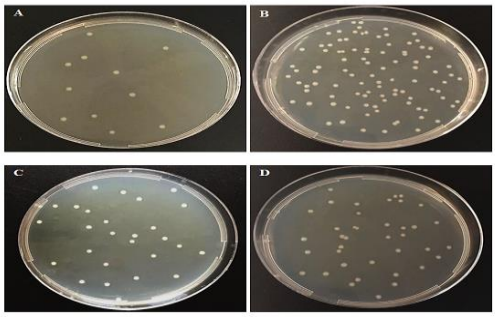

Figure 4. A: self-returning revertant colonies, B: revertant colonies in bacteria and mutagen containing plate, C: revertant colonies in bacteria, mutagen and 200 mg plate^{-1} extract containing plate in the absence of S9 mixture, D: revertant colonies in bacteria, mutagen and 200 mg plate^{-1} extract containing plate in the presence of S9 mixture

4. DISCUSSION

With the increase of mutagenic, carcinogenic and contaminant chemical compounds, the importance of antimicrobial, antioxidant and antimutagenic compounds in the natural structure is increasing day by day. There are many studies on the antimicrobial, antiviral and antioxidant effects of naturally active compounds. However, the increasing frequency of cancer diseases increases the importance of natural products with anticarcinogenic or antimutagenic activity. Although there are many studies on antimutagenic effects but the diversity of natural sources makes these studies insufficient. In this study, antimicrobial, antioxidant and antimutagenic activities of *S. excelsa* fruit extract were investigated. It was determined that the fruit extract provided 81% protection against mutagenity in the absence of S9 mixture at 200 mg plate^{-1} dose. In the presence of S9 mixture, it was determined that it provides 67% protection. Based on the inhibition percentages, it was determined that *S. excelsa* fruit extracts exhibited strong antimutagenic activity. In literature, catechin [8], trans-resveratrol, 5-O-caffeoylbicimic acid, 6-O-caffeoyl-β-D-fructofuranosyl-(2→1)-α-D glucopyranozide [17] and phytochemical compounds [18] are reported in *Smilax* tissues. And also in this study the presence of phenolic and flavonoid compounds in *S. excelsa* fruit extracts was determined. These compounds have antioxidant effect and provide protection against oxidative stress and thus show anti-mutagenic properties.

S. excelsa fruit extract showed different antimicrobial effect with the 11-16 cm inhibition zone range against tested microorganisms. This antimicrobial activity was comparable to the effect of the ampicillin and oflaxacin antibiotics tested in the study. It was determined that the oflaxacin did not show activity against *K. pneomoniae* and *C. albicans* and ampicillin did not show activity against *P. aureginosa*, *K. pneomoniae* and *C. albicans*. However, the fruit extract was also effective on these bacteria and showed a broad-spectrum antimicrobial activity. The antimicrobial activity exhibited by *Smilax* species is due to phenolic acids and the structure of the phenol group is very important in antimicrobial action. Oxygen-free phenolic compounds having a C3 side chain are classified as essential oils and exhibit high antimicrobial activity [19]. Ozsoy et al. [8] reported the presence of catechin in *S. excelsa* tissues. The antimicrobial effect observed in this study can be related to catechin which is the most reduced form of the C_3 unit from the flavonoid compounds. Another important detail of this study is that fruit extract exhibit higher antibacterial properties against gram negative bacteria than gram positive bacteria. This result can be explained by the structural differences of gram-positive and gram-negative cells. Gram-positive bacteria have thick peptidoglycan layer, while gram-positive bacteria have a thin layer. This layer acts as a barrier to reduce the transport of external molecules to the gram positive bacteria and reduces the toxic effects of extract. This structural differentiation makes the gram positives more resistant to antimicrobial agents and the gram negatives to be more sensitive to these agents [20].

The antioxidant property of *S. excelsa* fruit extract was evaluated by the DPPH removal activity, total phenolic and flavonoid content of extract. In 200 mg mL^{-1} *S. excelsa* fruit extract concentration, flavonoid and phenolic content were determined

as 0.7985±0.0124 mgQE 100mL^{-1} and 11.9847±0.0041 mgGAE 100 mL^{-1}, respectively. The DPPH removal rate of BHA, BHT and S. *excelsa* fruit extract was found to be 85%, 80% and 55%, respectively. In the literature, antioxidant activity activities of *S.excelsa* and different *Smilax* species have been reported. Ozsoy et al. [8] reported that *S. excelsa* leaf extracts have a total phenol content of 8.8–35.7 GAE mg g^{-1} and a flavonoid content of 0.61–28.7 mg g^{-1} catechin equivalent. Khaligh et al. [17] reported the isolation of trans-resveratrol, 5-O-caffeoylshikimic acid, 6-O-caffeoyl-β-D-fructofuranosyl- (2→1) -α-D glucopyranozide structures from *S. excelsa* tissues. And the antioxidant activity exhibited by the tissues was associated with these structures. The flavonoid and phenolic structures presence in the *Smilax* tissues have an active role in the prevention of diseases such as cardiovascular diseases, cancer and chronic inflammation by preventing free oxygen radicals and lipid peroxidation [21]. It is also known that flavonoids and phenolic compounds inhibit the enzymatic system involved in the radical formation of flavonoids, and decrease lipid oxidation by binding metal ions [9]. And also many studies have shown that these structures have antimutagenic and anticarcinogenic effects [18, 22].

5. CONCLUSION

Plant tissues have been used for many years in the treatment of various diseases. Increased industrial pollution and the access of contaminants to people through food chain has increased the risk of many diseases, especially cancer. In parallel with this increase, the use of synthetic drug substances increased, the side effects of drugs and the formation of various resistance mechanisms of microorganisms have brought the usage of plants for therapeutic purposes. For this aim, in literature many effects of plant tissues such as antifungal, antioxidative, antibacterial, antimutagenic, antiviral and anticarcinogenic have been studied. In this study, antimicrobial, antioxidant and antimutagenic activities of *S. excelsa* fruit exract, which is consumed as food in the Black Sea Region, were investigated. It was determined that the fruit extracts exhibited antimicrobial activity against *E.coli, P. aureginosa, S. aureus, K. pneumoniae, S. mutans, S. epidermidis, C. albicans* and *C. krusei*. And also, fruit extracts have a high antioxidant phenolic and flavonoid content and have a significant DPPH removal effect as 85%. In the antimutagenic activity assay, it was determined that the fruit extract inhibited mutagenicity in the 67-81% range and could be classified as a strong antimutagenic compound. As a result, it was determined that *S. excelsa* fruit tissues are a potential natural antimicrobial and antimutagenic source and exhibit strong antioxidant activity.

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