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Investigation of *In Vitro* Antimicrobial Activity of Ethanol Extracts obtained from *Asparagus acutifolius*

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Abstract: *In this study, the antimicrobial activity of Asparagus acutifolius, called "Tilkişen" and used in the treatment of diseases, was investigated in and around Aydın. The ethanol extracts obtained from fresh shoot tips and stems of A. acutifolius were tested against 45 bacteria and 3 fungi strains by the disk diffusion method. As a result of the study, it was determined that the ethanol extracts had antimicrobial activity against various strains tested, especially against S. mutans (Clinic isolated), S. aureus MRSA, S. aureus MRSA+MDR, S. epidermidis DSMZ 20044, S. aureus ATCC 25923, and L. monocytogenes ATCC 7644 strains.*

Keywords: *Asparagus acutifolius*; Fresh shoot tips; Fresh shoot stems; Disk diffusion method; Antimicrobial activity

1. INTRODUCTION

Humanity, especially in recent history, has made significant progress in medicine [1]. One of the main reasons for this progress is due to the evolution of the microorganisms and their newfound resistance to antibiotics [2]. Therefore, scientists continue their work to find new antimicrobial agents. In this case, plants come to the fore. Because plants have been used as medicines against infectious viruses, fungi, and parasites for hundreds of years. At present, new effects of plants against viruses are still being discovered [3-5]. Thanks to the diversity of plants in Anatolia, many important studies are carried out. Anatolian plants have been used to treat various diseases until today. The traditional uses of plants have been modernized by the modern technology of the 21st century and have begun to be used as medicines on humans. Accordingly, as in the world, the use of biotechnological drugs in Turkey has rapidly gained importance [6].

Asparagus (Asparagaceae), which is a valuable source of significant compounds, essential nutrients, oligosaccharides, vitamins, and minerals, has more than 250 species, making it a very important plant genus [7-9]. One of the species belonging to the *Asparagus* genus is *Asparagus acutifolius* L. (Wild asparagus), which is a native plant species commonly found throughout the Mediterranean [10]. It is also known as Tilkişen [11]. *A. acutifolius* is an herbaceous, perennial, evergreen dioecious species with a tetraploid set of chromosomes [12, 13]. *A. acutifolius* is rich in flavonoids and vitamin C, showing high antioxidant activity [14]. In western Anatolia, the young shoots of this plant are consumed as vegetables and are added to dishes such as omelets and soups. Furthermore, *A. acutifolius* is used in traditional medicinal treatments as an antiatherosclerotic, anticancer, anticoagulant, antimicrobial, antineuralgic, antirheumatic, antiurolithiatic, antiviral, blood depurative, diuretic, hepatoprotective, laxative, cardiotonic, and vasodilator [15-23].

In this study, the antimicrobial activity of ethanol extracts obtained from fresh shoot tips and stems of *A. acutifolius* were investigated.

2. MATERIALS AND METHODS

Plant Sample: *A. acutifolius* was collected from the Aydın mountains and identified by Dr. Mustafa Eray BOZYEL.

Extraction: Plant samples were dried after collection and powdered with a grinder. Powdered samples of fresh shoot tips and stems of *A. acutifolius* were shaken in ethanol (Sigma-Aldrich) at 160 rpm for 2 days at room temperature. The whole mixture was then filtered into flasks with Whatman No.1 filter paper. Filtrates were evaporated at 42 °C with a rotary evaporator (Buchi R3) [24, 25]. Finally, the residual material in the flask was collected and weighed as 0.400 and 0.570 mg, and used to prepare the extracts.

Disk Diffusion Test: The activity of the extracts was tested against 45 bacteria and 3 fungi strains, of which are standard, food isolated (FI), clinic isolated (CI), and multidrug resistant (MDR). All bacterial strains were incubated at 37 °C for 24 hours; however, *C. albicans*, *C. tropicalis*, and *C. glabrata* were incubated at 27 °C for 48 hours [26]. Each bacteria and fungi were inoculated in saline water and adjusted to the 0.5 McFarland standard to standardize the inoculum to contain approximately 10^8 cfu • mL^{-1} for bacteria and 10^7 cfu • mL^{-1} for fungi. First, Mueller - Hinton agar (BD Difco, USA) was poured into a 90 mm sterile petri dish to achieve an average thickness of 4.0 mm ± 0.5 mm. The extracts were loaded on 6 mm Oxoid Antimicrobial Susceptibility Test Disks in three doses of 50,

100, and 200 µl. The disks were allowed to dry in sterile conditions at 30 °C during the day to avoid any solvent residue that could affect the results. The prepared microorganisms, which were then inoculated in saline water, were passaged onto the surface of petri dishes. These plates were left to freeze under aseptic conditions at room temperature [27]. The discs were then firmly applied to the surface of the petri dishes. Finally, these petri dishes were incubated and the diameters of the inhibition zone were recorded.

3. RESULTS AND DISCUSSION

The antimicrobial activities of ethanol extracts obtained from fresh shoot tips and stems of *A. acutifolius* were analyzed. To load extracts, empty sterile disks were used. These disks were applied on a Mueller-Hinton Agar after they were inoculated with microorganisms. An inhibition zone was observed when the extracts had activity against these microorganisms. The diameters of inhibition zones were measured in millimeters as Table 1.

Table 1. Disk diffusion test results for *A. acutifolius* (Inhibition zones in mm)

	Fresh Shoot Tips			Fresh Shoot Stems		
Microorganisms	**50µL**	**100µL**	**200µL**	**50µL**	**100µL**	**200µL**
Bacillus subtilis DSMZ 1971	7	9	10	-	8	10
Candida albicans DSMZ 1386	-	-	8	-	-	-
Enterobacter aerogenes ATCC 2912	-	-	-	-	-	-
Escherichia coli ATCC 25922	-	-	-	-	-	-
Listeria monocytogenes ATCC 7644	-	8	11	-	7	9
Pseudomonas aeruginosa DSMZ 50071	-	7	10	7	8	10
Pseudomonas fluorescens P1	-	-	10	-	-	-
Salmonella enteritidis ATCC 13076	-	-	-	-	-	-
Salmonella typhimurium SL1344	-	-	-	-	-	-
Staphylococcus aureus ATCC 25923	-	10	11	7	8	9
Staphylococcus epidermidis DSMZ 20044	10	11	12	-	-	8
Staphylococcus hominis ATCC 27844	-	7	10	-	-	9
Staphylococcus warneri ATCC 27836	-	7	9	-	7	9
Bacillus cereus RSKK 863	7	9	10	-	7	10
Shigella flexneri RSKK 184	-	-	-	-	-	-
Acinetobacter baumanni CECT 9111	-	-	-	-	-	9
Enterococcus durans (FI)	-	-	-	-	-	-
Enterococcus faecium (FI)	-	-	9	-	-	8
Klebsiella pneumoniae (FI)	-	-	-	-	-	-
Listeria innocua (FI)	-	-	-	-	-	-
Salmonella infantis (FI)	-	-	-	-	-	-
Salmonella kentucky (FI)	-	-	-	-	-	-
Escherichia coli (FI)	-	-	-	-	-	-
Staphylococcus aureus (CI)	-	-	-	-	-	-
Staphylococcus mutans (CI)	12	13	14	-	8	10
Staphylococcus hominis (CI)	-	-	-	-	-	-
Staphylococcus haemolyticus (CI)	-	-	-	-	-	-
Staphylococcus lugdunensis (CI)	-	-	-	-	-	-
Shigella boydi (CI)	-	-	-	-	-	-
Acinetobacter baumannii (CI)	-	-	-	-	-	-

Shigella flexneri (CI)	-	-	-	-	-	-
Staphylococcus aureus (CI-2)	-	-	8	-	-	8
Enterococcus faecalis (CI)	-	-	-	-	-	-
Klebsiella pneumoniae (CI)	-	-	-	-	-	-
Candida tropicalis (CI)	-	-	-	-	-	-
Candida glabrata (CI)	-	-	-	-	-	-
Escherichia coli (MDR)	-	-	-	-	-	-
Klebsiella pneumonia (MDR)	-	-	-	-	-	-
Acinetobacter baumannii (MDR)	-	-	-	-	-	-
Enterobacter aerogenes (MDR)	-	-	-	-	-	-
Serratia odorifera (MDR)	-	-	-	-	-	-
Proteus vulgaris (MDR)	-	-	-	-	-	-
Streptococcus pneumonia (MDR)	-	-	-	-	-	-
Staphylococcus aureus MRSA	10	12	14	7	8	9
Staphylococcus aureus MRSA+MDR	-	-	12	-	7	10
Providencia rustigianii (MDR)	-	-	-	-	-	-
Achromobacter sp. (MDR)	-	-	-	-	-	-

"-": No inhibition, CI: Clinic isolated, FI: Food isolated, MDR: Multidrug resistant

In our study, the antimicrobial activity of ethanol extracts obtained from fresh shoot tips and stems of *A. acutifolius* was determined against 48 microorganisms with disk diffusion method at 50µL, 100 µL, and 200 µL. According to our results, *A. acutifolius* has antimicrobial activity against 15 of them. The extract that is effective in all of these is the fresh shoot tips extract. 12 of them are gram-positive strains, except *P. aeroginosa*, *P. fluorescens*, and *A. baumanni*. The most effective extract is the ethanol extracts obtained from fresh shoot tips at 200 µL. This extract showed high antimicrobial activity against 8 *Staphylococcus* strains. These are *S. aureus* ATCC 25923, *S. epidermidis* DSMZ 20044, *S. hominis* ATCC 27844, *S. warneri* ATCC 27836, *S. mutans* (CI), *S. aureus* (CI-2), *S. aureus* MRSA, and *S. aureus* MRSA+MDR.

S. aureus is known as one of the common nosocomial infections in medical intensive care units [28]. Several researchers study the antimicrobial activity of some plant extracts on *S. aureus* strains. In our study, we observed a 14 mm zone against *S. aureus* MRSA, a 12 mm zone against *S. aureus* MRSA+MDR, an 11 mm zone against *S. aureus* ATCC 25923, and an 8 mm zone against *S. aureus* (CI-2) strains. *A. acutifolius* is active against *S. aureus* when compared to some other higher plants [29].

In another study, the antimicrobial activity of ethanol extract of *A. acutifolius* was analyzed against 15 microorganisms. The authors found that the extract they used showed low antimicrobial activity against *E. faecalis*, *S. typhimurium*, *M. luteus*, and high antimicrobial activity against *P. fluorescens*, *B. cereus*, *S. vestibularis*, and *C. glabata*. Also, the extract they used was not affected *Staphylococcus* strains. [30]. According to our results, ethanol extract obtained from the fresh shoot tips of *A. acutifolius* is most effective against *Staphylococcus* strains.

4. CONCLUSION

Our study makes it clear that *A. acutifolius* has the potential for a possible medical drug candidate. Especially it has antimicrobial activity against *Staphylococcus* strains. However, further researches are needed to analyze the active substances and their activity mechanisms in detail. Data obtained because of studies using biotechnological drug technologies can reveal the true potential of this plant.

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