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Investigation of Antimicrobial Activity of Some Natural Plants which are Not-Cultivated and are Sold at Bazaars in Aydın Vicinity

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Abstract:

In this study, the antimicrobial activity of *Malva vulgaris*, *Salicornia europaea* L., *Asparagus officinalis* L. and *Asparagus acutifolius* L., which are edible plants and amply sold at bazaars and in Aydin vicinity were tested against some microorganisms. Plants were dried and extracted with ether, acetone, ethanol and water. Antimicrobial activity of the extracts were determined by the disc diffusion method. Test microorganisms were 10 bacteria including, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* CCM 99, *Micrococcus luteus* ATCC 9341, *Salmonella typhimirium* ATCC 14028, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas fluorescens* DSMZ 50090, *Streptococcus vestibularis* DSMZ 5636, *Serratia marcescens* and 5 yeast including, *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 90028, *Candida glabata*, *Candida utilis*, *Candida trophicalis*. Also different antibiotic discs were used for comparison of inhibition zones.

On the contrary, *Asparagus officinalis* L. and *Asparagus acutifolius* L. showed antimicrobial effect against microorganisms more than *Malva vulgaris* and *Salicornia europaea* L. Moreover, antimicrobial activity of ether extract was higher than those of acetone, ethanol and water extracts.

Keywords: Antibiotic resistant bacteria, antimicrobial effect, folkloric medicine

INTRODUCTION

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments [1]. From ancient time, plants are rich source of effective and safe medicines. In recent years there has been focus on plants with antimicrobial activity. There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram negative microorganisms, and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases [2]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [3-5].

Some foods contain naturally occuring substances showing antimicrobial activity. Some spices are known to contain sinnamic aldehyde, allicin in garlic and alliin in onion. This substances can be use for protection against microorganisms [6].

There are some studies concerning with antimicrobial and biological activity of various organic and inorganic substances in the publications [7, 8]. According to the some researches, it was shown that the use of herbal drugs increased instead of synthetic drugs [9, 10]. Although the antimicrobial activity of various plant extracts has been studied on the growth of many microorganisms in Turkey, ethnobotanical and pharmaceutical studies on these plants are inadequate. Hence this in vitro study aimed to investigate antimicrobial activity of some selected natural plants sold at bazaars in Aydın vicinity.

MATERIALS AND METHODS

Plant materials

The following plant materials *Malva vulgaris*, *Salicornia europaea* L., *Asparagus officinalis* L. and *Asparagus acutifolius* L. were bought from bazaars in Aydin vicinity.

Preparation of extracts

The plant samples were freeze-dried and powdered. 10g of these materials were subjected to Soxhlet extraction for 6h each using 200ml of the following solvents ether, acetone, ethanol and water. The extracts were concentrated and then kept at 4°C.

Microorganisms and condition for cultivation

In this study; 10 bacteria including, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* CCM 99, *Micrococcus luteus* ATCC 9341, *Salmonella typhimirium* ATCC 14028, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas fluorescens* DSMZ 50090, *Streptococcus vestibularis* DSMZ 5636, *Serratia marcescens* and 5 yeast including, *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 90028, *Candida glabata*, *Candida utilis*, and *Candida trophicalis* were used.

E. coli ATCC 35218, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *B. cereus* CCM 99, *M. luteus* ATCC 9341, *S. typhimirium* ATCC 14028, *P. fluorescens* DSMZ 50090, *S. marcescens* were cultured in Nutrient Broth (NB) (Merck) at 30-37°C; *S. vestibularis* DSMZ 5636 and *E. faecalis* ATCC 29212 were cultured in Brain Heart Infusion Broth (BHIB) (Merck) at 37°C for 24 h.; *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 90028, *C. glabata*, *C. utilis* and *C. trophicalis* were cultured in Malt Extract Broth (MEB) (Merck) at 27-30°C for 24 h.

Antimicrobial assays

The antimicrobial activities of the ether, acetone, ethanol and water plants were analyzed by the disk diffusion

method [11, 12]. The inoculum size of each group of bacteria and yeast were prepared by using a no. 0.5 McFarland tube to give a concentration of $1x10^8$ bacteria and $1x10^6$ yeast per milliliter. In order to test the antimicrobial activity of plants, 15 ml of Mueller Hinton Agar were poured in petri dishes which were then inoculated with strains of bacteria by taking 0.1 ml from cell culture media. It was kept to solidify at room temperature for a while and then holes were made on top with a sterile stick. These holes were filled with 10µl of plant extracts. Then, bacterial cultures were incubated at 30-37°C and yeast cultures were incubated at 27-30°C for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA was evaluated in millimetres. Discs of Chloramphenicol (C30), Ampicillin (AM10), Gentamycine (CN10), Eritromycine (E15), Bacitracin (B), Nystatine (NS30) were used as positive controls. Studies were performed in triplicate, and the developing inhibition zones were compared with those of the reference discs.

RESULTS AND DISCUSSION

The antimicrobial activity of ether, acetone, ethanol and water extracts of *Malva vulgaris*, *Salicornia europaea* L., *Asparagus officinalis* L. and *Asparagus acutifolius* L. plants were investigated and the results were given in Tables. The inhibition zone diameter of the reference antibiotics to the test microorganisims are shown in Table 3.

Table 1. Antimicrobial activities of the extracts of *Malva vulgaris* and *Salicornia europaea* L. on some bacteria and yeasts.

Test Microorganisms	Inhibition zone (mm)							
	Malva vulgaris				*Salicornia europaea* L			
	E	A	EtOH	D	E	A	EtOH	D
Escherichia coli ATCC 35218	-	-	-	-	14	-	-	-
Staphylococcus aureus ATCC 25923	-	-	-	-	-	-	-	-
Enterococcus faecalis ATCC 29212	12	15	11	-	13	-	-	-
Staphylococcus epidermidis ATCC 12228	-	-	10	-	11	-	-	-
Salmonella thphimirium ATCC	-	-	8	-	14	-	-	-
Pseudomonas fluorescens DSMZ 50090	-	-	7	-	14	-	-	-
Bacillus cereus CCM 99	-	-	15	-	11	-	-	-
Micrococcus luteus ATCC 9341	-	-	-	-	13	-	-	-
Streptococcus vestibularis DSMZ 5636	16	-	15	-	15	-	-	-
**Serratia marcescens*	-	-	-	-	16	-	-	-
Saccharomyces cerevisiae ATCC 9763	-	-	-	-	12	-	10	-
Candida albicans	-	-	-	-	10	-	-	-
Candida utilis	-	-	-	-	19	-	-	-
Candida glabata	-	-	-	-	15	-	-	-
Candida trophicalis	-	-	10	-	9	-	-	-

E: Diethyl ether extract, A: Acetone extract, EtOH: Ethanol extract, D: Water extract
(-): No zone, NT: Not tested
*Special gift from Adnan Menderes University Faculty of Medicine

Table 2. Antimicrobial activities of the extracts of *Asparagus officinalis* L. and *Asparagus acutifolius* L. on some bacteria and yeasts.

Test Microorganisms	Inhibition zone (mm)							
	Malva vulgaris				*Salicornia europaea* L			
	E	A	EtOH	D	E	A	EtOH	D
Escherichia coli ATCC 35218	17	-	-	-	15	-	-	-
Staphylococcus aureus ATCC 25923	33	-	-	-	17	-	-	-
Enterococcus faecalis ATCC 29212	18	-	-	-	11	-	9	-
Staphylococcus epidermidis ATCC 12228	29	-	-	-	25	-	-	-
Salmonella thphimirium ATCC	18	-	-	-	18	-	8	-
Pseudomonas fluorescens DSMZ 50090	18	-	8	-	18	-	16	-
Bacillus cereus CCM 99	16	-	-	-	12	8	17	-
Micrococcus luteus ATCC 9341	15	-	-	-	-	-	6	-
Streptococcus vestibularis DSMZ 5636	27	9	-	-	24	12	21	-
**Serratia marcescens*	20	-	-	-	23	-	-	-
Saccharomyces cerevisiae ATCC 9763	17	-	-	-	15	-	-	10
Candida albicans	18	-	-	-	11	-	-	-
Candida utilis	19	-	-	-	20	-	-	-
Candida glabata	20	-	-	-	-	-	20	9
Candida trophicalis	16	-	-	-	15	-	-	-

E: Diethyl ether extract, A: Acetone extract, EtOH: Ethanol extract, D: Water extract
(-): No zone, NT: Not tested
*Special gift from Adnan Menderes University Faculty of Medicine

Table 3. Inhibition zone diameter of the reference antibiotics to the test microorganisms.

Test Microorganisms	Inhibition zone (mm)					
	Reference antibiotics					
	C30	AM10	CN10	E15	B	N_s
Escherichia coli ATCC 35218	22	17	12	-	-	NT
Staphylococcus aureus ATCC 25923	21	16	7	10	-	NT
Enterococcus faecalis ATCC 29212	25	14	25	7	-	NT
Staphylococcus epidermidis ATCC 12228	20	17	9	11	-	NT
Salmonella thphimirium ATCC	22	7	18	-	-	NT
Pseudomonas fluorescens DSMZ 50090	22	-	19	21	-	NT
Bacillus cereus CCM 99	20	-	15	26	-	NT
Micrococcus luteus ATCC 9341	30	17	18	12	15	NT
Streptococcus vestibularis DSMZ 5636	25	22	-	27	9	NT
**Serratia marcescens*	20	15	10	7	-	NT
Saccharomyces cerevisiae ATCC 9763	NT	NT	NT	NT	NT	22
Candida albicans	NT	NT	NT	NT	NT	17
Candida utilis	NT	NT	NT	NT	NT	20
Candida glabata	NT	NT	NT	NT	NT	15
Candida trophicalis	NT	NT	NT	NT	NT	16

C30: Cloramphenicol (30µg\ml), AM10: Ampicillin (10µg\ml), CN10: Gentamycin (10µg\ml), E15: Eritromycin (15µg\ml), B: Bacitracin (0.04 U), NS: Nystatin (30 U)
(-): No zone, NT: Not tested
*Special gift from Adnan Menderes University Faculty of Medicine

Ether and acetone extracts of *Malva vulgaris* had antimicrobial effect against *E. faecalis* ATCC 29212 and *Streptococcus vestibularis* DSMZ 5636 bacteria. In addition, ethanol extract of *Malva vulgaris* showed antimicrobial effects against five pathogen bacteria and one yeast. Water extract of *Malva vulgaris* was active against *E. faecalis* ATCC 29212. None of the extract showed antimicrobial effect against yeast cells. Ether extract of *Salicornia europaea* L. had antimicrobial effect against nine pathogen bacteria and five yeasts. Ethanol extract of *Salicornia europaea* L. was only active against *Saccharomyces cerevisiae* ATCC 9763 yeast. Acetone and water extracts did not show any antimicrobial effects against used microorganisms.

Ether extract of *Asparagus officinalis* L., displayed test antimicrobial effect against ten pathogen bacteria and five yeasts. Acetone extract of *Asparagus officinalis* L. had antimicrobial activity against *Streptococcus vestibularis* DSMZ 5636. In addition, ethanol extract of *Asparagus officinalis* L. was active against *Pseudomonas fluorescens* DSMZ 50090. Water extract of *Asparagus officinalis* L. showed antimicrobial effect against none of the microorganisms.

Ether extract of *Asparagus acutifolius* L., displayed test antimicrobial effect against nine pathogen bacteria and four yeasts. Acetone extract of *Asparagus acutifolius* L. had antimicrobial activity against *Bacillus cereus* CCM 99 and *Streptococcus vestibularis* DSMZ 5636. In addition, ethanol extract of *Asparagus acutifolius* L. was active against six pathogen bacteria and one yeast. Although water extract of *Asparagus acutifolius* L. showed antimicrobial effect against none of bacteria, showed antimicrobial effect against *Saccharomyces cerevisiae* ATCC 9763 and *Candida glabata* yeasts.

According to our findings; Ether extract of *Salicornia europaea* L., *Asparagus officinalis* L and *Asparagus acutifolius* L. showed antimicrobial activity against microorganisms higher that other extracts.

As a result of this study, *Asparagus officinalis* L. and *Asparagus acutifolius* L. showed antimicrobial effect against microorganisms more than *Malva vulgaris* and *Salicornia europaea* L. Moreover, antimicrobial activities of ether extracts were higher those of acetone, ethanol and water extracts. We that active constituents in the plant have higher solubility in ether.

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