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Preliminary Study on the Antifungal and Antimicrobial Activities of Some Medicinal Plants of Turkey

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Abstract: In this study, acetone, alchol and hexane extracts *Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis* and *Rumex acetosa* used for medicinal purpose in Turkey were tested for antimicrobial activity by the diffusion method. These extracts were tested on *Staphylococcus aureus* NRRL-B 767, *Micrococcus luteus, Bacillus subtilis* NRS-744, *B.cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Listeria monocytoponus* ATCC 7644, *Klebsiella pneumoniae, Yersinia enterocolitica, Proteus vulgaris, Enterococcus fecolis* ATCC 29212, *Xanthomonas phaseoli, Pseudomonas fluorescens, Fusarium oxysporum, F. culmorum, F.aveneceum, F.moniliforme, Gaeumannomycetes graminis* var. *tritici, Alternaria citri* and *Trichoderma harzianum*. As conclusion, extracts of *Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis* and *Rumex acetosa* inhibited the growth of microorganisms used in these tests at different ratios. We have found that acetone extracts of tested plants relealed antimicrobial activity against bacteria but it had no antifungal activity against fungi used in this study.

Keywords: Antimicrobial activity; Medicinal plants; Disc diffusion method.

1. Introduction

The use of plants in treatment has begun in ancient times. Medical plants in turkey are used among the public. The use of natural products has increased because of the inadequate efficacy of the drugs against the diseases and side effects. For this reason, many plants are being investigated in terms of microbiological, pharmacological and plant defense mechanisms [1]. Important properties of microorganisms kill and human health of plants have been investigated in laboratory studies [2, 3]. Studies conducted with antimicrobial activities of plants are quite extensive.

Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis used and *Rumex acetosa* used in this study traditionally used by Turkish people for treating gastro-enteritics, poor digestion. We chose six plant species used in folk medicine to determine their antimicrobial and antifungal activity. This study describe the *in vitro* antifungal and antimicrobial activity of three different extracts from *Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis* and *Rumex acetosa* leaves.

2. Material and Methods

2.1. Plants Materials

Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis and Rumex acetosa. Plants were obtained Soil and Water Research Institute, Şanlıurfa, Turkey.

2.2. Preparation of Extracts

The aerial parts of plants were ground with a mill. The samples (10 g) were prepared with 100 ml of alcohol, acetone and hexan solvent (MERCK) in a shaker at room temperature for 24 h. Then, the samples extracted were filtered. All extracts were concentrated in vacuo at 55 ^{o}C. The yields from the different extracts of different plants were weighed, recorded and dissolved in the same solvents (alcohol, acetone and hexan) at a concentration of 100 mg ml^{-1} [4, 5]. The extracts were stored in a refrigerator.

2.3. Test Microorganisms

Ten species of medical bacteria (*Staphylococcus aureus* NRRL-B 767, *Micrococcus luteus, Bacillus subtilis* NRS-744, *B.cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Listeria monocytoponus* ATCC 7644, *Klebsiella pneumoniae, Yersinia enterocolitica, Proteus vulgaris, Enterococcus faecalis* ATCC 29212) and nine species of

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agricultural microorganims (*Xanthomonas phaseoli*, *Pseudomonas fluorescens*, *Fusarium oxysporum*, *F. culmorum*, *F.avenaceum*, *F. moniliforme*, *Gaeumannomycetes graminis* var. *tritici*, *Alternaria citri* and *Trichoderma harzianum*) were used in this study. The bacterial isolates were grown in Mueller Hinton Agar (MERCK) while fungal isolates were grown on Sabouraud Dextrose Agar (Difco).

2.4. Antimicrobial and Antifungal Activity

Antimicrobial activity was measured using the Standard method of diffusion disc plates on agar [6]. Each of bacterial or fungal cultures (0.1 ml) was spread on agar surface. Bacterial cultures were grown in Mueller Hinton Broth for 24 h at 37 ^{o}C. The concentration of bacterial cultures were adjusted to 10^8 cfu ml^{-1} using McFarland No. 0.5 standard solution. Paper discs (6 mm in diameter) were placed on the Mueller Hinton Agar to load 10 µl of each sample.

For antifungal assays, 10^5 spore ml^{-1} of fungi were prepared and spread onto the surface of the Sabouraud Dextrose Agar plates. Petri plates were incubated at 37 ^{o}C for 24 h for bacterial cultures and 72 h for fungi at 25 ^{o}C. Vancomycine (30 mg ml^{-1}) for bacteria and Chloramphenicol (10 mg ml^{-1}) for fungi were used as positive controls. At the end of the period, the antimicrobial and antifungal activities of the plant extracts were evaluated by measuring the inhibition zones formed on the agar. All experiments were repeated three times.

3. Results and Discussion

In search for antimicrobial agents among natural products many plants and their components have been screened. In many studies have strongly suggested that Polyphenols are natural chemicals found in plants. Polyphenols have antioxidant properties [7]. Poliphenols are phytoalexins produced by plants in response to an infection or another physiological stimulation, which suggested that alcoholic extracts from plants may display antimicrobial properties [8-10].

In this study, the antimicrobial influence of extracts (acetone, alcohol and hexane) from *Aloysia triphylla, Mentha aquatica, Foeniculum vulgare, Calendula officinalis, Salvia officinalis and Rumex acetosa* against bacteria and fungi was determined. As shown in Table 1, the extracts from the *Aloysia triphylla, Mentha aquatica, Foeniculum vulgare* and *Rumex acetosa* plants displayed antibacterial activity against and/or some of the tested gram positive and gram negative bacterial strains with the diameters of zone inhibition ranging between 4 and 22 mm but acetone and hexan extracts of *Rumex acetosa* and *Foeniculum vulgare* did not inhibit the growth of *L. monocytoponus*. Hexan extracts of *Mentha aquatica* and *Aloysia triphylla* did not inhibit the growth of *S.aureus, M.luteus, E.coli* and *L. monocytoponus*, respectively (Table 1).

The most active extract was obtained from acetone extracts of tested plants and *Aloysia triphylla* acetone extract inhibited the growth of all the bacteria tested, specifically *E.coli* (22 mm), *L. monocytoponus* (18 mm) and acetone extract of *Mentha aquatica* inhibited the growth of *M.lutes* (20 mm), *S.aureus* (19 mm) and *L. monocytoponus* (18 mm). However, acetone extracts of *Salvia officinalis* exhibited significant antimicrobial activities against all the tested bacteria, but, to *E.faecalis* and *P.fluorescens* with a diameter greater than vancomycin. The inhibition zone diameter of *E.faecalis* and *P.fluorescens* was 28 mm and 25 mm, respectively with acetone extract, although vancomycin gave only 20 mm and 28 mm inhibition zones, respectively (Table 2).

Fungal cultures were not inhibited by any of the extracts of tested plants (Table 1). Many researchers have reported that some bacteria and fungi may be inhibited by *Rumex acetosa*, *Foeniculum vulgare*, *Salvia officinalis* extracts [4, 11-13].

Table-1. Inhibitor effects of *Aloysia triphylla, Mentha aquatica, Rumex acetosa* and *Foeniculum vulgare* extracts against tested microorganisms (inhibitory zone diameter, mm)

Test microorganism	*Aloysia triphylla*			*Mentha aquatica*			*Rumex acetosa*			*Foeniculum vulgare*		
	A	B	C	A	B	C	A	B	C	A	B	C
Staphylococcus aureus NRRL-B 767	12	19	-	10	19	-	6	9	-	7	12	-
Micrococcus luteus	15	15	-	13	20	-	14	8	-	6	14	-
Bacillus subtilis NRS-744	13	12	10	10	12	6	6	10	8	9	14	8
B.cereus ATCC 11778	12	10	8	12	12	6	5	9	8	12	9	11
Escherichia coli ATCC 25922	18	22	-	12	16	-	6	11	-	12	-	-
Listeria monocytoponus ATCC 7644	10	18	-	12	18	-	6	-	-	9	-	-
Klebsiella pneumoniae	10	15	7	13	16	4	9	9	9	8	7	12
Yersinia enterocolitica	8	12	8	10	13	5	11	8	8	13	9	11
Proteus vulgaris	12	10	9	10	10	8	10	8	6	15	8	10
Enterococcus faecalis	14	13	9	10	12	9	8	9	4	14	8	9

ATCC 29212												
Xanthomonas phaseoli	16	12	8	14	12	9	7	12	4	14	8	12
Pseudomonas fluorescens	12	9	7	12	12	8	5	10	6	10	8	12
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-	-	-
F. culmorum	-	-	-	-	-	-	-	-	-	-	-	-
F.avenaceum	-	-	-	-	-	-	-	-	-	-	-	-
F. moniliforme	-	-	-	-	-	-	-	-	-	-	-	-
Gaeumannomycetes graminis var. *tritici*	-	-	-	-	-	-	-	-	-	-	-	-
Alternaria citri	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-	-	-	-	-	-	-	-

A:Alcohol extract; B: Acetone; C: Hexane

Table-2. Effects of *Calendula officinalis*, *Salvia officinalis* extracts and antibiotics against tested microorganisms (inhibitory zone diameter, mm)

Test microorganism	***Calendula officinalis***			***Salvia officinalis***			**Antibiotic**	
	A	**B**	**C**	**A**	**B**	**C**	**Vancomycin**	**Chloramphenicol**
Staphylococcus aureus NRRL-B 767	-	10	-	19	14	-	23	-
Micrococcus luteus	5	6	4	22	21	8	22	-
Bacillus subtilis NRS-744	6	8	-	20	22	-	30	-
B.cereus ATCC 11778	8	8	-	20	24	6	33	-
Escherichia coli ATCC 25922	12	10	-	18	15	-	23	-
Listeria monocytoponus ATCC 7644	14	9	-	14	22	4	25	-
Klebsiella pneumoniae	10	12	4	12	20	4	18	-
Yersinia enterocolitica	8	11	8	10	22	8	24	-
Proteus vulgaris	8	13	-	18	20	6	24	-
Enterococcus faecalis ATCC 29212	6	10	-	15	28	-	20	-
Xanthomonas phaseoli	8	14	8	22	18	8	20	-
Pseudomonas fluorescens	8	12	8	24	25	8	18	-
Fusarium oxysporum	-	-	-	-	-	-	-	22
F. culmorum	-	-	-	-	-	-	-	23
F.avenaceum	-	-	-	-	-	-	-	22
F. moniliforme	-	-	-	-	-	-	-	20
Gaeumannomycetes graminis var. *tritici*	-	-	-	-	-	-	-	21
Alternaria citri	-	-	-	-	-	-	-	18
Trichoderma harzianum	-	-	-	-	-	-	-	15

A:Alcohol extract; B: Acetone; C: Hexane

The *Calendula officinalis* showed some degree of activity against some bacteria (Table 2). Hexane extract of *C.officinalis* did not inhibit the growth of *S.aureus, B.subtilis, E.coli, L. monocytoponus, B.cereus, P.vulgaris* and *Enterococcus fecolis*. The results showed that the extracts of *Salvia officinalis* showed some degree of activity against some bacteria (Table 2). Acetone extract showed significant antimicrobial activity against the bacteria.

Venikar and Jangde [13] reported that the extracts of *Calendula officinalis* were active against *Staphylococcus aureus* and *Escherichia coli*. This result is similar to our studies.

Acetone extract of *S.officinalis* gave an inhibition zone very close to the standard antibiotic vancomycine. No antifungal activity of the extracts of tested any plants was observed in our study.

In previous antimicrobial activity studies with the pure methanol and acetone extracts of *Foeniculum vulgare, Salvia officinalis*, Alzoreky and Nakahara [11] reported that they found antimicrobial activity in *Bacillus cereus, L. monocytoponus.* Simionatto, *et al.* [12] indicated that the essential oils and extracts of *Aloysia triphylla* showed high antimicrobial activity against *S.epidermis, M.luteus, K.pneumoniae, E.coli* and *Helicobacter pylori*.

4. Conclusion

Most of the extracts obtained contain antimicrobial substances against microorganisms. The phytochemical properties of plants vary from species to species. Therefore, the effects of plant extracts used in the study have been different. These findings may provide a basis for further study of larger bacterial and fungal strains in order to clarify the structures of plants and to find new therapeutic principles.

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