Research Article

Antimicrobial Activity of *Echinophora tenuifolia* L. and *Raphanus sativus* L. Extracts

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ABSTRACT

In this study, the antimicrobial activity of *Echinophora tenuifolia* and *Raphanus sativus* extracts were tested against some pathogen microorganisms. Plant leaves were reduced to powder with liquid nitrogen. Three solvents were used for extraction. Disc diffusion method was used to test antimicrobial activity. Ten different microorganisms were used. After incubation zone diameters were measured and evaluated. Both plants extracts discovered effective against four microorganisms with similar zone diameter. Methanol extracts of *Echinophora tenuifolia* L. showed more effect while ethyl acetate extracts of *Raphanus sativus* L. were more effective.

Key words: *Echinophora tenuifolia*, *Raphanus sativus*, Antimicrobial effect, Agar well diffusion method.

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction.[1] Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases.[2] There is still a need to explore prospective antibiotic compounds capable to control pathogens.[3] Plants are the main sources of new treatments and new medicines.[4] They are expected to form new sources of antimicrobial drugs, especially against bacteria.[5] It needs to find cost-effective readily available natural anti-microbial agents with minimum side effects.[6] But the number of new drugs in development is low, raising the question for alternative research.[7] Plants are rich in a wide assortment of auxiliary metabolites, for example, tannins, terpenoids, alkaloids, flavonoids, glycosides, and so on, which have been found *in vitro* to have antimicrobial properties.

The extracts of two plants with three different solvents were used in this study. *Echinophora tenuifolia*, common name tarhana, has been used in Turkish cuisine for a long time and publicly known as a natural healer. *Raphanus sativus* is an aromatic plant which is also a part of both Turkish and Mediterrian cuisine. Besides from methanol and ethyl acetate, boiling water was chosen because these plants we boiled before used for eating. The aim of this study was to found out the antimicrobial effects of these plants with different solvents.

MATERIAL AND METHODS

Plant Materials and Preparation of plant extracts

The sample of leaves of *Echinophora tenuifolia* L. *And Raphanus sativus* L. were collected from Aydın province in Turkey. Leaves of the plant samples were washed with distilled water and reduced to powder with liquid nitrogen. Ten grams of this material was extracted separately in 150 mL of methanol, ethyl acetate, and boiled water for 6 hours at Soxhlet. The extracts were concentrated and then kept at 4°C until use.[8]

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Table 1: Antimicrobial activities of the extracts of *Echinophora tenuifolia* L. and *Raphanus sativus* L. on some bacteria, yeasts and molds.

Test Microorganisms	Inhibition zone (mm)																	
	Echinophora tenuifolia L.						*Raphanus sativus* L.						Reference antibiotics					
	1	2	3	4	5	6	1	2	3	4	5	6	C30	CN 10	TE 30	E15	AMP 10	NS 100
Escherichia coli ATCC 35218	-	-	-	-	-	-	-	-	-	-	-	-	24	21	15	11	-	NT
Stapylococcus aureus ATCC 25923	-	-	-	-	-	-	10	-	-	-	-	-	23	20	22	23	20	NT
Salmonella typhimirium ATCC 14028	-	-	-	-	-	-	-	-	-	-	-	-	17	16	15	8	8	NT
Klebsiella pneumoniae ATCC 13882	-	-	-	-	-	-	-	-	-	-	-	-	21	19	20	14	-	NT
Mycobacterium smegmatis ATCC 607	10	-	-	-	-	-	-	-	-	-	-	-	23	18	26	25	19	NT
Corynebacterium xerosis ATCC 373	11	-	-	-	-	-	-	11	12	-	-	-	20	17	25	26	27	NT
Candida albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	22
Candida utilis ATCC 9950	12	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	21
*Aspergillus niger**	-	-	11	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
*Penicillium expansum**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1: Methanol Extract, 2: Ethyl Acetate Extract, 3: Boiled Water Extract, 4:Pure Methanol, 5:Pure Ethyl Acetate, 6:Pure Distilled Water C_{30}: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erythromycin (15mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid), KET20: Ketaconazole (20 mg Oxoid). (-): No zone (NT): Not tested, (*):Special gift from Adnan Menderes University, Department of Biology.

Microorganisms

The six bacteria, two yeasts and two molds species tested as *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Staphylococcus aureus* ATCC 25923, *Corynebacterium xerosis* ATCC 373, *Mycobacterium smegmatis* ATCC 607, *Candida utilis* ATCC 9950, *Candida albicans* ATCC 10231, *Aspergillus niger*, *Penicillium expansum*. The bacteria, yeasts and molds were cultured in Tryptic Soy Agar (Merck) at 30-37°C, Malt Extract Agar (Merck) at 27-30°C for 24 h and Potato Dextrose Agar (Merck) at 27°C for 5-7 days, respectively.

Antimicrobial assays

Screenings for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms.[9] The inoculum size of each group of bacteria, yeast and mold were prepared by using a no. 0.5 McFarland tube to give a concentration of $1x10^8$ bacteria, $1x10^6$ yeast, and $1x10^4$ molds per milliliter. Mueller Hinton Agar (MHA) was used to test antimicrobial activity. 0.1 ml from cell culture media were inoculated to each plate. 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 30µL of plant extracts. Then, bacterial cultures were incubated at 30-37°C and yeast and mold cultures were incubated at 27-30°C for 18-24 h. After incubation the diameters of the inhibition zones were evaluated in millimeters. Discs of Chloramphenicol (C30), Gentamycin (CN10), Tetracycline (TE30), Erythromycin (E15), Ampicillin (AM10), Nystatin (NS100), and Ketoconazole (KET20) were used as positive controls.

RESULTS AND DISCUSSION

The methanol extract of *E. tenuifolia* was found to be effective against tested bacterial pathogens *M. smegmatis* ATCC 607, *C. xerosis* ATCC 373, *C. utilis* ATCC 9950, while extract in boiled distilled water showed only against *A. niger*. However, ethyl acetate extract of *E. tenuifolia* showed no activity. The extract in boiled distilled water *of R. sativus* demonstrated effect against *C. xerosis* ATCC 373 and *A. niger*. In addition, the methanol

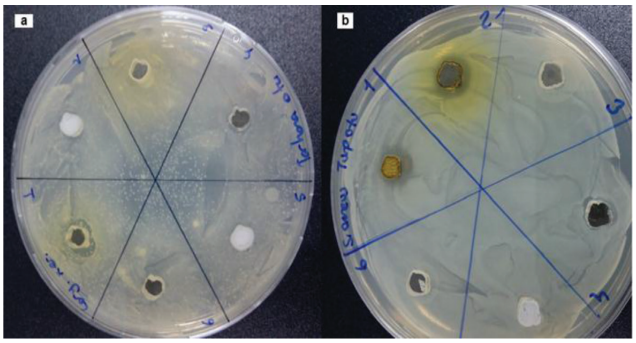

Figure 1: Zones formed by extracts of two plants. a. *Echinophora tenuifolia* L. b. *Raphanus sativus* L.

extract *of R. sativus* showed only against *S. aureus* ATCC 25923, while ethyl acetate extract inferred only against *C. xerosis* ATCC 373 (Figure 1).

The methanol extract of *E. tenuifolia* and boiled water extract of *R. sativus* was more effective than the ethyl acetate extract. The methanol extract of *E. tenuifolia* and the extract in boiled distilled water *of R. sativus* find out phenolic compounds like alkaloids, tannins, and flavonoids and these have an antimicrobial effect.

Gökbulut *et al.* investigated antimicrobial activity of *E. tenuifolia* essential oils and found effective mostly against Gram-positive bacteria.[10] Essential oils might be effective only against Gram-positive bacteria but we found plant extracts are effective both gram-positive and gram-negative bacteria as well as yeast and fungi.

Ahmad *et al.* studied the antibacterial effect of *R. sativus* seed extracts.[11] They found all ethanolic and methanolic extracts has effective against bacterial strains they used. This might be due to the high concentrations of effective components in seeds. Since we used leaves which have lower concentrations we obtained lesser results.

CONCLUSION

Echinophora tenuifolia L. showed more activity against microorganism than *Raphanus sativus* L. while overall antimicrobial activity observed low. This can be due to the extraction method, solvent used in extraction and extract concentration in both extract and plant. Changing these variables can show more antimicrobial activity.

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CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATION USED

MHA: Mueller Hinton Agar; C_{30}: Chloramphenicol; CN10: Gentamycin; TE_{30}: Tetracycline; E15: Erythromycin; AM10: Ampicillin; NS100: Nystatin; KET20: Ketoconazole.

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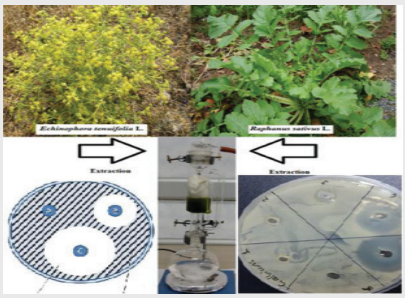
PICTORIAL ABSTRACT



SUMMARY

- Both *Echinophora tenuifolia L.* and *Raphanus sativus L.* are widely used by Turkish people in different areas.
- Different solvents were used to extract active compounds.
- Ten different microorganisms were used.Methanol and ethyl acetate extracts showed antimicrobial activity.

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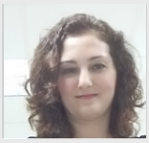
Mr. Bahadır Törün: He complated B.Sc in Biology at Ege University, Turkey. He earned his masters degree in Biology at Anadolu University, Turkey. He is now about to complate his PhD at Adnan Menderes University.



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