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Investigation of Antimicrobial Effects in Different Solvents of P*istacia terebenthus* L. Fruits Collected from Aydın

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Abstract
In this study, the antimicrobial activity of *Pistacia terebenthus* L. fruits extracts were tested against some pathogen microorganisms. Fruits of the plant samples were freeze-dried and powdered. 10 grams of this material was extracted separately in 150mL of methanol, ethyl acetate, and boiled water for 6 h. at Soxhlet. The extracts were concentrated and then kept at 4°C. The agar well diffusion method is used for testing the antimicrobial activities of extracts. The results of methanol extract of *Pistacia terebenthus* L. fruits was found to form larger zones against tested bacterial pathogens *E. coli* ATCC 35218,*S. aureus* ATCC 25923, *S. typhimirium*ATCC 14028, *M. smegmatis* ATCC 607, *C. xerosis* ATCC 373. In addition the ethyl acetate and boiled distilled water extracts of *Pistacia terebenthus* L. fruits demonstrated moderate effect against some bacterial pathogens tested. However, none of the extracts used showed any effect on yeast and molds.The ethyl acetate extract of *Pistacia terebenthus* L. fruits have effects on wider spectrum than methanol and boiled water extracts for revealing secondary metabolites like alkaloids, tannins, and flavonoids.
Keywords: *Pistacia terebenthus*fruits, extracts, antimicrobial effect, agar well diffusion method

INTRODUCTION

Antibiotics are one of our most importantweapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction [1]. Drug resistance has developed due to the high and insensibly use of antimicrobial drugs in the treatment of infectious diseases [2]. There is still a need to explore new antimicrobial compounds that can be used against pathogens [3]. Plants are the main sources of new medicines [4]. They are expected to form new sources of antimicrobial drugs, especially against bacteria and viruses[5]. It is needed to find cheap and easily accessible natural anti-microbial agents with minimum side effects [6]. But pathogens rising resistance to drugs each day the number of new drugs in development is low, and alternative research on natural resources are needed[7]. Plants are rich in a wide assortment of secondary metabolites, for example, tannins, terpenoids, alkaloids, flavonoids, glycosidesthat have been found to have antimicrobial properties.

MATERIALS and METHODS

Plant material

The sample of leaves of *Pistachia terebenthus* were collected from Aydın province in Turkey.

Preparation of extracts

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].

Microorganisms and condition for cultivation

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, 10]. 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 50µL 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

In this study, the antimicrobial activity of *Pistacia terebenthus* L. fruits extracts were tested against some

pathogen microorganisms. Three solvents were used for extraction (Table 1). The findings were given in Tables 2 and Figures 1 and 2.

The bacterial pathogens tested for methanol extracts of *Pistacia terebenthus* fruits showed higher activity on *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *S. typhimirium* ATCC 14028, *M. smegmatis* ATCC 607, and *C. xerosis* ATCC 373 than the other solvents. In addition, ethyl acetate and boiled water extracts of *Pistacia terebenthus* fruits had moderate effects on some pathogens tested. However, none of the extracts had any effect on yeast and molds.

Kavak et al. (2010) investigated various bioactivities, such as antioxidant, antimicrobial and cytotoxic property because of its flavonoid, phenolic and alkaloid contents of Pistacia terebinthus leaf extract. They found that although plant extract showed an antimicrobial effect on gram-positive*S. aureus*, it was ineffective on gram-negative*E. coli* [11].

Durak and Uçak (2015) studied antimicrobial and antioxidant activities of *Pistacia terebinthus* L. (terebinth) fruits and determined that *L. monocytogenes* and *Salmonella typhimurium* were more susceptible to the extracts than *E. coli* O157:H7 and *S. aureus*. [12].

Bleder et al. (2016) researched the antibacterial activity of *Pistacia terebinthus* essential oils against *Staphylococcus aureus*and determinate effect. [13].

Coban et al. (2017)investigated the antimicrobial effects of *Pistacia terebinthus* L. leafextracts against some pathogen microorganisms and found effect against *Staphylococcus aureus*, *Corynebacterium xerosis*, *Mycobacterium smegmatis*, *Klebsiella pneumonia*, *Candida albicans* and *Candida utilis*. [14].

As conclusion, this study supports the findings of previous studies. The different extracts of *Pistacia terebinthus* fruits have strong bioactive and antimicrobial properties.

Defense molecules in plants are known as secondary metabolites. Therefore, the season in which the plant is collected, the temperature of the environment, the rate of heavy metal accumulating in the soil and the rate of pollution change the metabolites of the plant. [15].There may be an antimicrobial agent in our extracts that can be used in the future by carrying out more detailed studies with molecular techniques and other new techniques.

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REFERENCES

[1]Törün B, Çoban EP, Biyik HH, Barisik E. 2017. Antimicrobial Activity of *Echinophora tenuifolia* L. and *Raphanus sativus* L. Extracts. Indian J Pharm. Education Research. 51(1):136-43.

[2] Singh N, Gupta S, Rathore V. 2017. Comparative antimicrobial study of ethanolic extract of leaf and rhizome of Curcuma longa Linn. Pharmacognosy J. 9(2):208-12.

[3]Badakhshan MP, Sasidharan S, Rameshwar NJ, Ramanathan S. 2009. A Comparative study: Antimicrobial activity of methanol extracts of Lantana camara various parts. Phcog. Res. 1(6):348-51.

[4] Tepe B, Daferera D, Sökmen M, Polissiou M, Sökmen A. 2004. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii. J Agric Food Chem. 52(1):132-7.

[5] Bankole MA, Shittu LA, Ahmed TA, Bankole MN, Shittu RK, Kpela T. 2007. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. Afr J Trad Comp Alternative Med. 4(4):427-33.

[6] Nag M, Mukherjee P, Biswas R, Chanda J, Kar A. 2016. Evaluation of antimicrobial potential of some Indian ayurvedic medicinal plants. Pharmacognosy J. 8(6):525-33.

[7] Roumy V, Gutierrez-Choquevilca AL, Lopez Mesia JP, Ruiz L, Ruiz Macedo JC, Abedini A, et al. 2015. In vitro antimicrobial activity of traditional plant used in mestizo shamanism from the Peruvian Amazon in case of infectious diseases. Phcog Mag. 11(4): 625-33.

[8]Çoban EP, Bıyık HH, Uzun C. 2009. Investigation of antimicrobial activity of some natural plants which are not cultivated and are sold at bazaars in Aydın. Int J Nature Eng Sci. 3:59-62.

[9]Collins CH, Lyne PM, Grange JM, Falkinham JO. 2004. Microbiological methods. 8th ed. London: Arnold Publishers.

[10]Clinical and Laboratory Standards Institute. CLSI. 2015. *M02-A12*: performance standards for antimicrobial disk susceptibility tests: approved standard. 12.ed. Wayne: CLSI, p.1-73.

[11]Kavak DD, Altıok E, Bayraktar O, Ülkü S. (2010). *Pistacia terebinthus* extract: As a potential antioxidant, antimicrobial and possible -glucuronidase inhibitor. J Molecular Catalysis B: Enzymatic. 64: 167–171.

[12] Durak MZ, Uçak G. (2015). Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish *Pistacia terebinthus* L. extracts. Turk J Agric For. 39: 10-19.

[13]Bledar P, Behxhet M, Kate N, Cassandra LQ, Avni H. 2016. Chemical composition and in vitro antibacterial activity of *Pistacia terebinthus* essential oils derived from wild populations in Kosovo. BMC Complementary Alternative Med. 16(147): 1-9.

[14]Coban EP, Bıyık H, Törün B, Yaman F. (2017). Evaluation the antimicrobial effects of *Pistacia terebinthus* L. and *Papaver rhoeas* L. extracts against some pathogen microorganisms. Indian J Pharm Education Research. 51(3): 377-380.

[15] Haliki A, Çetingül V, Denizci AA. 1998. An Investigation on Antibacterial Activities of Some Marine Algae (Phaeophyta, Rhodophyta), *Ege UniversityJournal Of The Faculty Of Science,* 21; 1-9.

Table 1: Boiling Point of Solvents

Solvents	Boiling Point
Ethyl acetate	68
Water	100
Methanol	64.7

Table 2: Antimicrobial activities of the extracts of*Pistacia terebenthus* fruits

Test Microorganisms	Inhibition zone (mm)												
	Extracts of*Pistacia terebenthus* L.						Reference Antibiotics						
	1	2	3	4	5	6	C30	CN 10	TE 30	E15	AMP10	NS 100	KET 20
Escherichia coli ATCC 35218	13	-	9	-	-	-	24	21	15	11	-	NT	NT
Stapylococcus aureus ATCC 25923	14	11	10	-	-	-	23	20	22	23	20	NT	NT
Salmonella typhimirium ATCC 14028	12	11	11	-	-	-	17	16	15	8	8	NT	NT
Klebsiella pneumoniae ATCC 13882	-	-	-	-	-	-	21	19	20	14	-	NT	NT
Mycobacterium smegmatis ATCC 607	13	9	8	-	-	-	23	18	26	25	16	NT	NT
Corynebacterium xerosis ATCC 373	14	12	13	-	-	-	20	17	25	26	27	NT	NT
Pseudomonas aeruginosa ATCC 35032	-	11	-	-	-	-	22	22	20	21	-	NT	NT
Serratia marcescens ATCC 13880	-	10	-	-	-	-	23	19	13	-	19	NT	NT
Bacillus subtilis ATCC 6633	-	10	-	-	-	-	22	20	12	25	-	NT	NT
Candida albicans ATCC 10231	-	-	-	-	-	-	NT	NT	NT	NT	NT	22	NT
*Candida glabrata**	-	-	-	-	-	-	NT	NT	NT	NT	NT	21	NT
*Aspergillus niger**	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	18
*Penicillium expansum**	-	-	12	-	-	-	NT	NT	NT	NT	NT	NT	20

1: Methanol Extract2: Ethyl AcetateExtract3: Water Extract4:Pure Methanol5:Pure Ethyl Acetate6:Distilled Water

C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erytromycin (15mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid), KET: Ketaconazole (20 mg Oxoid).

NT: Not Tested

(*):Special gift from Adnan Menderes University Faculty of Medicine

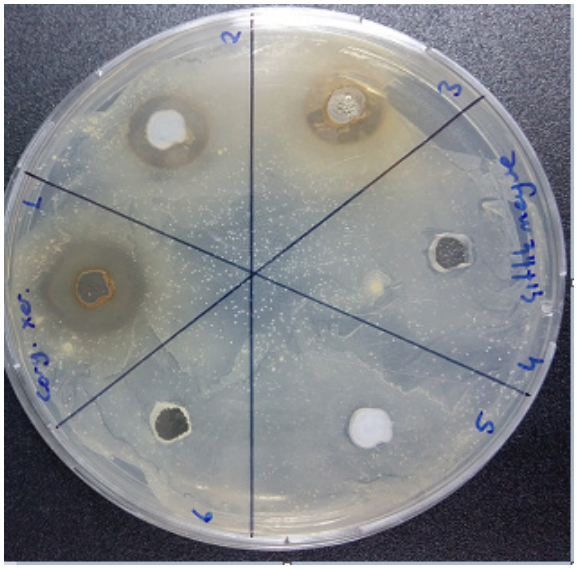

Figure 1. Antibacterial zones formed (1: Methanol Extract, 2: Ethyl Acetate Extract, 3: Water Extract, 4:Pure Methanol, 5:Pure Ethyl Acetate, 6:Distilled Water)

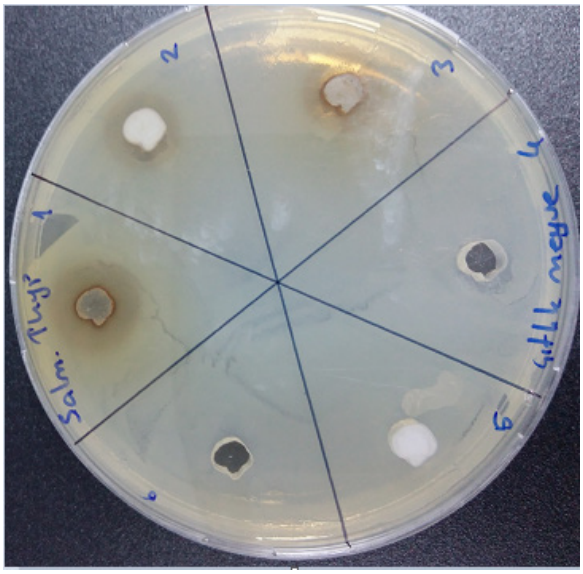

Figure 2. Antibacterial zones formed (1: Methanol Extract, 2: Ethyl Acetate Extract, 3: Water Extract, 4:Pure Methanol, 5:Pure Ethyl Acetate, 6:Distilled Water)