# Evaluation the Antimicrobial Effects of *Pistacia* terebinthus L. and *Papaver rhoeas* I. Extracts Against Some Pathogen Microorganisms

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#### **ABSTRACT**

In this study, the antimicrobial activity of *Pistacia terebinthus L*. and *Papaver rhoeas L*. extracts were tested against some pathogen microorganisms. Leaves of the plant samples were freeze-dried and powdered. Three solvents were used for extraction. The agar well diffusion method is used for the antimicrobial activities of extracts. Six different bacteria, two yeasts, and two molds were used. The extracts of *P. terebinthus L*. was found more effect than *P. rhoeas* L. extracts against tested bacteria and fungi pathogens.

**Key words:** *Pistacia terebinthus L., Papaver rhoeas L.,* Folkloric Medicine, Antimicrobial Effect, Agar well diffusion method.

#### INTRODUCTION

Plants have been used for prevent and treat diseases in pharmacology since ancient times.1 The extracts of plants have been used for the treatments of a wide range of diseases as inflammation, diarrhea, sleep disorders and cough.2 Therefore, the use of phytochemicals for medical purposes has increased in many countries.3 Plants containing secondary metabolites such as phenolic compounds, essential oils have antibacterial, antifungal, insecticidal, acaricidal, and cytotoxic activities.4 Therefore, many researchers have been extremely studied on plants and mechanisms of action in several fields as pharmacology, pharmaceutical botany, medical and clinical microbiology, plant pathology and food preservation.<sup>5</sup>

Pistacia terebinthus L. is native to the Canary Islands and the Mediterranean region from the western regions of Morocco, and Portugal to Greece and western Turkey.<sup>6</sup> P. terebinthus produces a rich mixture of substances, including resin, essential oils, proteins, organic acids, sugars, flavonoids and tannins.<sup>7</sup>

Papaver rhoeas L. (red poppy), which is widely distributed in Turkey, is used to make a cough syrup for children, as a tea for disturbed sleep, for pain relief, and as a sedative in folk medicine. In some regions of Turkey, a decoction prepared from red poppy petals is used for mouth inflammations in children and the infusion of the aerial parts is drunk for lowering the blood sugar level.<sup>8</sup>

#### MATERIAL AND METHODS

#### **Plant Materials**

The sample of leaves of *Pistacia terebinthus* L. and *Papaver rhoeas* L. were collected from Aydın province in Turkey.

# Preparation of plant extracts

Leaves of the plant samples were washed with distilled water and freeze-dried. Dried leaves were powdered and 10 grams of this material was extracted separately in 150 mL of methanol, ethyl acetate, and boiled water for 6 h at Soxhlet. The extracts were concentrated and then kept at 4°C.9

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#### Microorganisms and condition for cultivation

The six bacteria, two yeasts and molds species tested as *Escherichia coli* ATCC 35218, *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 Disc diffusion method

Screenings for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms. <sup>10,11</sup> 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<sup>8</sup> bacteria, 1x10<sup>6</sup> yeast, and 1x10<sup>4</sup> molds per milliliter. In order to test the antimicrobial activity of plants, 20 ml of Mueller Hinton Agar (MHA) were poured in Petri dishes which were then inoculated with strains of bac-

teria 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  $30\mu L$  of plant extracts. Then, bacterial cultures were incubated at  $30\text{-}37^{\circ}\mathrm{C}$  and yeast and mold cultures were incubated at  $27\text{-}30^{\circ}\mathrm{C}$  for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimeters. Discs of Chloramphenicol ( $C_{30}$ ), Gentamycin ( $CN_{10}$ ), Tetracycline ( $TE_{30}$ ), Erythromycin ( $E_{15}$ ), Ampicillin ( $AM_{10}$ ), Nystatin ( $NS_{100}$ ), and Ketoconazole ( $KET_{20}$ ) were used as positive controls.

#### RESULTS AND DISCUSSION

The antimicrobial activity of methanol, ethyl acetate, and boiled distilled water of *Pistacia terebinthus* L. and *Papaver rhoeas* L. plants were investigated and the results were given in Table 1.

According to Table 1 methanol, ethyl acetate and boiled water extracts of *Pistacia terebinthus L.* showed antimicrobial effect against *S. aureus* ATCC 25923 and *M. smegmatis* ATCC 607. In addition, methanol and ethyl acetate extract of *P. terebinthus L.* had antimicrobial effects against

Test Microorganisms	Inhibition zones (mm)																		
	Pistacia terebinthus L.					Papaver rhoeas L.						Reference Antibiotics							
	1	2	3	4	5	6	1	2	3	4	5	6	C 30	CN 10	TE 30	E 15	AMP 10	NS 100	KET 20
Escherichia coli ATCC 35218	-	-	-	-	-	-	-	-	-	-	-	-	24	21	15	11	-	NT	NT
Stapylococcus aureus ATCC 25923	10	12	9	-	-	-	-	-	-	-	-	-	23	20	22	23	20	NT	NT
Salmonella typhimirium ATCC 14028	10	11	-	-	-	-	-	-	-	-	-	-	17	16	15	8	8	NT	NT
Klebsiella pneumoniae ATCC 13882	10	-	-	-	-	-	-	-	-	-	-	-	21	19	20	14	-	NT	NT
Mycobacterium smegmatis ATCC 607	10	12	9	-	-	-	-	-	-	-	-	-	23	18	26	25	19	NT	NT
Corynebacterium xerosis ATCC 373	20	15	-	-	-	-	-	-	-	-	-	-	20	17	25	26	27	NT	NT
Candida albicans ATCC 10231	10	13	-	-	-	-	12	-	12	-	-	-	NT	NT	NT	NT	NT	22	NT
Candida utilis ATCC 9950	10	12	-	-	-	-	-	-	13	-	-	-	NT	NT	NT	NT	NT	21	NT
Aspergillus niger*	-	-	12	-	-	-	-	-	14	-	-	-	NT	NT	NT	NT	NT	NT	20
Penicillium expansum*	-	-	-	-	-	-	-	-	-	-	-	-	NT	NT	NT	NT	NT	NT	19

two pathogen bacteria (*S. typhimirium* ATCC 14028 and *C. xerosis* ATCC 373) and yeasts (*C. albicans* ATCC 10231 and *C. utilis* ATCC 9950). While methanol extract of *P. terebinthus* L. was only active against *K. pneumoniae* ATCC 13882 boiled water extract of *P. terebinthus* L. was active against *S. aureus* ATCC 25923, *M. smegmatis* ATCC 607 and *A. niger.* Methanol and ethyl acetate extract of *P. terebinthus* L. demonstrated only stronger activity against *C. xerosis* ATCC 373.

While methanol extracts of *Papaver rhoeas* L. was only effect against *C. albicans* ATCC 10231 boiled water extracts of *P. rhoeas* L. showed activity against *C. albicans* ATCC 10231, *C. utilis* ATCC 9950 and *A. niger.* However, ethyl acetate extracts of *P. rhoeas* L. did not show any antimicrobial effects against used microorganisms. None of the extracts showed antimicrobial effect against *P. expansum.* 

Kostic *et al.* examined the methanol, ethanol and water extracts of *P. rhoeas* L. and they found that the ethanol extract of *P. rhoeas* L. showed antimicrobial activity against the yeast *Candida albicans*, and all tested bacteria except *Bacillus subtilis*. However, methanol and water extracts of *P. rhoeas* L. showed less activity. Ünsal *et al.* investigated antimicrobial activity the petroleum ether, diethyl ether, chloroform, acetone and ethanol extracts of *Papaver* species growing in Turkey. They showed that diethyl ether, chloroform and acetone extracts of *P. rhoeas* had activity against *S. aureus*. 8

1: Methanol Extract, 2: Ethyl Acetate Extract, 3: Boiled Water Extract, 4: Pure Methanol, 5: Pure Ethyl Acetate, 6:Pure Distilled Water, C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erythromycin (15 mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid), KET20: Ketaconazole (20 mg Oxoid), (\*): Special gift from Adnan Menderes University, Department of Biology, (-): No zone, (NT): Not tested

# CONCLUSION

Pistacia terebinthus L. indicated antimicrobial effect against microorganisms more than Papaver rhoeas L. In addition, antimicrobial activity of methanol extract was higher than those of ethyl acetate and boiled water extracts. The methanol and ethyl acetate extracts of

Pistacia terebinthus reveal secondary metabolites like alkaloids, tannins, and flavonoids and these have an antimicrobial effect.

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### **CONFLICT OF INTEREST**

There is no conflict of interest.

## **ABBREVIATION USED**

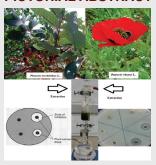
MHA: Mueller Hinton Agar; C30: Chloramphenicol; CN10: Gentamycin; TE30: Tetracycline; E15: Erythromycin; AM10: Ampicillin; NS100: Nystatin; KET20: Ketoconazol.

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#### PICTORIAL ABSTRACT



#### **SUMMARY**

- Antimicrobial effects of Pistachia terebinthis and *Papaver rhoeas* were studied.
- Three differen solvents and ten different microorganisms were used.
- *P. terebinthus* found more effective against tested microorganisms.

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