In Vitro Antimicrobial Activity of *Pistacia lentiscus* L. Extracts: Preliminary Report

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Summary

The *in vitro* antimicrobial activity of *Pistacia lentiscus* L. extracts was determined. *Pistacia lentiscus* L. extracts were tested on bacteria (*Sarcina lutea, Staphylococcus aureus* and *Escherichia coli*) and fungi (*Candida albicans, Candida parapsilosis, Torulopsis glabrata* and *Cryptococcus neoformans*). Of the different plant extractions, decoctions showed the best antibacterial activity, but the activity against fungal cells appears to be much more interesting.

Key words: Antimicrobial activity, *Pistacia lentiscus* L.

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INTRODUCTION

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle Eastern areas. *Pistacia lentiscus* L. is a small evergreen tree or shrub, 1-8 m high, with paripinnate leaves and lanceolate to obovate-lanceolate leaflets. The inflorescence is compact and spike-like, flowers are yellowish or purplish, and the fruit is a drupe [1].

The aerial part of *Pistacia lentiscus* L. has traditionally been used in some regions of Spain as a popular cure for hypertension [2]. Mastic from *Pistacia* has been used by traditional healers for the relief of upper abdominal discomfort, gastralgia, dyspepsia and peptic ulcer [3,4]. It has also been used as a masticatory and by dentists for filling decayed teeth [5]. *Pistacia* has been reported to possess stimulant and diuretic properties [5]. Recently, a double-blind trial of mastic and placebo in the treatment of duodenal ulcer has shown that it produced complete ulcer healing in 70% of the patients compared with 22% of the placebo group [6].

Miniati [7] reported that the phenolic fraction of *Pistacia vera* L. appears to be endowed with antimicrobial activity. Since no further studies have been reported on the antimicrobial activity of *P. lentiscus* L., the object of the present paper was to investigate the antibacterial and antimycotic activity of this plant.

MATERIALS AND METHODS

Plant material

The aerial part of *P. lentiscus* L. was collected near Messina in May 1993, during the flowering season. A voucher specimen of the plant

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was deposited in the herbarium of the Department of Pharmacobiology, University of Messina.

Preparation of extracts

Lyophilized and powdered leaves of *P. lentiscus* L. were used for the preparation of extracts. The extracts which were assayed included:

- 10% decoction
- petroleum ether extract
- ethanol extract
- infusion
- maceration.

Decoctions, infusions, macerations, and extracts were prepared according to the official Pharmacopoeia rules [8] or approved variations thereof. The drug extracts were lyophilized for further use.

Determination of bacterial susceptibility

The antimicrobial activity of the plant extracts was determined on ATCC standard strains of *Sarcina lutea*, *Staphylococcus aureus* and *Escherichia coli*. The minimal inhibitory concentration (MIC) was determined by a broth microdilution assay in microtiter plates, according to the NCCLS reference method [9]. In the first well of each series a quantity of the different extracts was added. Isosensitest broth (Oxoid) (100 µl) was added to the first well and serial twofold dilutions were performed with a multichannel pipette beginning in the second well and discarding the final 100 µl. A total of 11 concentrations (mg/l) of each drug was obtained: 9.75 - 19.5 - 39 - 78 - 156 - 312 - 625 - 1,250 - 2,500 - 5,000 - 10,000. The last well contained broth with no drug: this was used as the control for the growth of the microorganisms assayed. A suspension organism (1 µl) was added to each well containing the drug and to the control well. The final concentration of each microorganism in every well was 1×10^5 CFU/ml. Plates were sealed with transparent acetate and incubated at 37°C under atmospheric conditions for up to 18 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that inhibited visible growth after 18 h. MIC50 and MIC90 were defined as the drug concentrations that inhibited 50% and 90%, respectively, of the bacterial strains assayed.

A drug sterility control, consisting of broth containing the highest concentration of the drug and not inoculated with tested bacteria, was included for each bacterial test. Each assay was repeated twice against each plant extract and six additional times on a different day with all extracts to ensure reproducibility of results.

Successively, the minimal bactericidal concentration (MBC) was determined by carrying out a subculture of the tubes showing no growth on plates of Brain-Heart-Infusion (BHI) agar without drug. The MBC was defined as the lowest concentration of drug that inhibited 99.9% of the growth in subcultures.

Determination of yeast susceptibility

A group of 43 clinical yeast isolates (18 *Candida albicans*, 9 *Candida parapsilosis*, 11 *Torulopsis glabrata* and 5 *Cryptococcus neoformans*) were used for the studies. To prepare fresh starting inocula, the strains were inoculated on Sabouraud dextrose agar plates for overnight growth at 37° C.

Broth macrodilution susceptibility tests were performed according to Galgiani [10].

Briefly, twofold dilutions of the plant extracts were prepared by standard methods, using RPMI - 1640 medium, buffered in morpholinopropanesulfonic acid (MOPS) at a final concentration of 0.165 M, pH 7.0. Final drug concentrations ranged from 9.75 mg/l to 10,000 mg/l, as for the antibacterial tests.

Yeast inocula were prepared by suspending several colonies in RPMI broth for overnight growth at 37°C, and spectrophotometrically adjusted from 1×10^4 to 1×10^5 yeast cells per ml.

Each assay was repeated twice against each plant extract and six additional times on a different day with all extracts to ensure reproducibility of results.

RESULTS AND DISCUSSION

The antibacterial activity of *P. lentiscus* L. against *Sarcina lutea*, *Staphylococcus aureus* and *Escherichia coli*, in the form of different plant extractions, is shown in *Table 1*. The decoctions

TABLE 1 - *Antibacterial activity (MIC and MBC mg/l)*

	S. lutea ATCC 9341		*S. aureus* ATCC 29213		*E. coli* ATCC 35218	
SUBSTANCES	MIC	MBC	MIC	MBC	MIC	MBC
Decoction	312	312	312	312	312	312
Petroleum ether extract	2500	2500	2500	2500	2500	2500
Ethanol extract	2500	2500	2500	2500	2500	2500
Maceration	1250	1250	1250	1250	1250	1250
Infusion	1250	1250	1250	1250	1250	1250

TABLE 2 - *Antimycotic activity (MIC mg/l)*

	C. albicans			*C. parapsilosis*			*T. glabrata*			*C. neoformans*		
SUBSTANCES	MIC50	MIC90	RANGE	MIC50	MIC90	RANGE	MIC50	MIC90	RANGE	MIC50	MIC90	RANGE
Decoction	625	625	156-625	312	312	156-312	78	156	39-156	625	625	312-625
Petroleum ether extract	1250	2500	1250-2500	625	625	625-1250	312	625	312-625	1250	2500	1250-2500
Ethanol extract	1250	2500	1250-2500	625	625	625-1250	312	625	312-625	1250	2500	1250-2500
Maceration	1250	2500	625-2500	312	625	312-625	312	312	156-312	2500	2500	2500
Infusion	1250	2500	625-2500	312	625	312-625	78	156	78-156	1250	1250	1250

showed the best activity (MIC = 312 mg/l for all the three bacterial strains). Since MIC and MBC values are the same, the substances should possess bactericidal activity.

The activity of plant extracts against the yeasts sampled is more differentiated and more interesting. Antimycotic activity is shown in *Table 2*.

The cooked preparation for yeasts also is more active with respect to the others; *Torulopsis glabrata* is the most sensitive species.

The activity of the extracts of *P. lentiscus*, even if of different levels, is similar to those of the other vegetable extracts, such as the ajoene fraction of *Allium sativum* (garlic)[11] active on yeasts and molds but not active on bacteria except for *Staphylococcus aureus*.

Similar to the higher activity of *P. lentiscus* against *T. glabrata* compared to *C. albicans*, (which could be considered a rare event,) is reported in the literature by Nakamoto [12] for the rhizomes of *Coptis japonica* Mak. and the bark of *Phellodendron amurense* Rupr. with values of 0.5 µg/ml and 1.0 µg/ml respectively.

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