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Research Article

Evaluation of the Antimicrobial Properties of the Essential Oil of *Myrtus communis L.* against Clinical Strains of *Mycobacterium spp.*

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Mycobacterium tuberculosis is the etiological agent of tuberculosis. The World Health Organization has estimated that 8 million of people develop active TB every year and the situation is complicated by an increase of *Mycobacterium tuberculosis* strains resistant to drugs used in antitubercular therapy: MDR and XDR-TB. Myrtle leaf extracts, used as an antiseptic in Sardinian traditional medicine, have strong antibacterial activity as several investigations showed. In this study we investigated the antimicrobial properties of the essential oil of *Myrtus communis* against clinical strains of *M. tuberculosis* and *M. paratuberculosis.*

1. Introduction

Myrtle (*Myrtus communis L.*) is an evergreen shrub belonging to the family of Mirtaceae that grows spontaneously throughout the Mediterranean area. In Italy it grows along the coast and in the inner hills, and it is spread especially in the islands, where it is one of the most characteristic species. *Myrtus communis* had history in the popular and traditional medicine: the essential oil obtained from leaves and, sometimes, flowers and berries has been used for its tonic and balsamic properties, and it is used in flavour and fragrance industries. In Sardinia natural formations are still the main source for the production of a traditional liqueur, that every year reaches 3 million bottles. Essential oils are gaining remarkable interest for their potential multipurpose use as antioxidant, antibacterial, and antiseptic agent [1–4]; the essential oil obtained from the leaves was used in the past for the treatment of lung disorders.

The isolation of essential oils from *Myrtus communis* leaves is usually obtained by hydrodistillation method with a Clevenger-type apparatus, according to the Italian Official Pharmacopoeia. The chemical composition of the essential oils, analysed by Gas/Cromatography (G/C), generally exhibits α-pinene, 11%; 1,8-cineole, 16%; linalool, 12%; α-terpineol, 7%; and limonene, 5%. The Sardinian myrtle oil is characterized by the lack of myrtenyl acetate and by a higher content of limonene.

Romani et al. [5] showed the biological activities of tannins, including anticancer and antioxidant. In our previous studies (data not published) the antimicrobial properties of myrtus essential oil against several clinical strains and in particular against *Helicobacter pylori* [6] were studied, and we obtained encouraging results. Considering these results, in this study, we have used this essential oil towards strains of *M. tuberculosis* and strains of *Mycobacterium avium* subsp. *paratuberculosis*.

2. Materials and Methods

In this study we investigated the antimicrobial properties of the essential oil of *Myrtus communis* against two reference strains: *M. tuberculosis* H37*Rv* (virulent strain) and *M. tuberculosis* H37*Ra* (avirulent strain) and 8 clinical isolates of *M. tuberculosis* resistant to one or more drugs, collected in the Department of Biomedical Sciences, Microbiology of the University of Sassari, Italy.

Table 1: Activity of antitubercular drugs and *Myrtus communis* against *Mycobacterium tuberculosis* strains.

Strains	MIC	Antitubercular Drug*						
M. tuberculosis	*Myrtus communis* % (v/v)	SM	INH	RIF	ETH	PZA	CLA	CIP
H37Rv	0.17%	S	S	S	S	—	—	—
H37Ra	0.17%	S	S	S	S	—	—	—
M. tuberculosis	0.17%	S	S	R	S	—	—	—
M. tuberculosis	0.17%	S	S	R	S	—	—	—
M. tuberculosis	0.17%	S	R	R	S	—	—	—
M. tuberculosis	0.17%	R	S	R	S	—	—	—
M. tuberculosis	0.17%	R	R	S	S	—	—	—
M. tuberculosis	0.17%	R	R	S	R	—	—	—
M. tuberculosis	0.17%	R	R	R	S	R	R	R
M. tuberculosis	0.17%	S	S	S	S	—	—	—
M. paratuberculosis (1515)	>2%	—	—	—	—	—	—	—
M. paratuberculosis (1517)	>2%	—	—	—	—	—	—	—

* SM: streptomicin; INH: isoniazid; RIF: rifampicin; ETH: ethambutol; PZA: pyrazinamide; CLA: clarithromycin; CIP: ciprofloxacin.

We also studied the antimicrobial properties of the essential oil towards two human strains of *Mycobacterium avium* subsp. *paratuberculosis* (1515 and 1517).

The antibacterial activity of the oil was assessed by the proportional method used for M*ycobacterium tuberculosis* as described in NCCLS-National Committee for Clinical Laboratory Standards [7].

Briefly, isolate suspensions of *M. tuberculosis* in 7H9 broth were adjusted to an optical density of 1 McFarland, and two dilutions, 10^{-2} and 10^{-4}, were plated onto 7H10 agar with a different concentration of essential oil (16%, 14%, 12%, 10%, 8%, 4%, 2%, 1%, and 0.17% v/v).

We also investigated the antimicrobial properties of some components of *M. communis* and, in particular, limonene, 1-8 cineole and α-pinene, using the proportional method [7].

3. Results and Discussion

In Table 1 we report the data obtained with the essential oil in toto.

Towards all the strains of *M. tuberculosis*, including the extensively drug-resistant (XDR), the oil of *M. communis* tested showed an MIC of 0.17% (v/v), whereas against the two *M. paratuberculosis* strains showed an MIC of 2% (v/v).

Subsequently we tested each different compound (limonene, 1-8 cineole, α-pinene) to see if it had different antimicrobial properties.

As far as limonene concerned, it showed towards all the strains an MIC of 2% (v/v); about 1-8 cineole, for 4 strains, including H37*Rv*, the MIC was of 2% (v/v), while the MIC shown for other 4 strains was of 16% (v/v); α-pinene showed an MIC of 1% (v/v) for 3 strains, and for one strain an MIC of 2% (v/v), for 3 others an MIC of 8% (v/v), and for only one MIC was of 16% (v/v) (Table 2). Limonene and α-pinene are monoterpenic that, according to the literature, are used as expectorant, antalgic, revulsive, antitussive, mucolitic, and decongestant; about 1-8 cineole it is an oxide used as expectorant, mucolitic, and decongestant [8].

We compared the activity of our essential oil with four standard antitubercular drugs: streptomycin, isoniazid, rifampin, and ethambutol, performing according to international protocols [7]. About the mycobacterial strains, one clinical strain was susceptible to all the drugs. Two clinical strains were rifampicin resistant, three strains were resistant to 2 drugs (one streptomicin and rifampicin resistant, one streptomicin and isoniazid resistant, and one isoniazid and rifampicin resistant), one was resistant to 3 drugs (streptomicin, isoniazid, and ethambutol). The last one was a XDR strains. The essential oils screened in toto have a better antimicrobial activity than each single compound against all mycobacteria tested. The results presented here may contribute to the knowledge of the antimicrobial properties of myrtle and our aim is to carry on further studies.

4. Conclusions

The chemical composition of the essential oil of *M. communis* exhibited qualitative differences that depended on different geographical areas and from the season in which the leaves were picked up. The essential oil that we used for antimicrobical *in vitro* assay contained a high quantity of monoterpenic and oxide that, according to literature, do not have antimicrobical activity. From our studies emerged that the essential oil in toto might have a good activity towards *M. tuberculosis*, although the individual compounds (except α-pinene) showed in all the strains a higher MIC. It may be the consequence of a sinergic effect of all the compounds together.

The results from the myrtle oil in toto showed a good activity towards *M. tuberculosis* but not toward *M. paratuberculosis*. The MIC registered against *M. tuberculosis* was 0.17% (v/v) in comparison with an MIC of 2% (v/v) observed toward *M. paratuberculosis*.

A limit of our study is the small amount of the essential oil that did not allow to perform the MIC towards all the strains used. These data are encouraging even if further

Table 2: Activity of *Myrtus communis* and single compound against *Mycobacterium tuberculosis* strains.

Strains	MIC	MIC % (v/v)		
M. tuberculosis	*Myrtus communis* % (v/v)	Extracts of *Myrtus communis* oil		
		Limonene	*1-8 Cineole*	*α Pinene*
H37Rv	0.17%	0.17%	2	1
H37Ra	0.17%	—	—	—
M. tuberculosis	0.17%	2	2	1
M. tuberculosis	0.17%	2	>16	8
M. tuberculosis	0.17%	2	2	1
M. tuberculosis	0.17%	2	2	16
M. tuberculosis	0.17%	2	>16	8
M. tuberculosis	0.17%	2	>16	2
M. tuberculosis	0.17%	2	>16	8
M. tuberculosis	0.17%	—	—	—
M. paratuberculosis (1515)	>2%	—	—	—
M. paratuberculosis (1517)	>2%	—	—	—

additional "*in vitro*" testing and large clinical studies are necessary to verify the potential use of the essential oils of myrtle as antitubercular drug. Given the excellent results that we obtained in this study, we would expand the research with further studies, to value the possible cytotoxic effects, and eventually to perform tests using "*in vivo*" mouse model.

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