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In *vitro* Antibacterial Activity of *Thymus vulgaris* Essential Oil against *Mycobacterium tuberculosis*

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Authors
Pourazar Dizaji Sh.[1] *MSc*,
Soleimani N.* *PhD*,
Afrugh P.[1] *PhD*,
Saedi S.[2] *PhD*

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*Microbiology & Microbial Biotechnology Department, Life Sciences & Biotechnology Faculty, Shahid Beheshti University, Tehran, Iran
[1]Mycobacteriology & Pulmonary Research Department, Pasteur Institute of Iran, Tehran, Iran
[2]Medical Bacteriology Department of Pasteur Institute of Iran, Tehran, Iran

Correspondence
Address: Shahid Beheshti University, Velenjak, Tehran, Iran. Postal Code: 1983969411
Phone: +98 (21) 29905516
Fax: +98 (21) 29901664
n_soleimani@sbu.ac.ir

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ABSTRACT

Aims Nowadays, treatment of bacterial infections is one of the most important challenges in the world. Medicinal plants offer a great hope to overcome these needs because of their chemical diversity and their significant role in the drug development. The aim of this study was to evaluate the in *vitro* antibacterial activity of the thyme (*Thymus vulgaris*) essential oil against *Mycobacterium tuberculosis*.

Materials & Methods In this experimental study, thyme herb plants were collected and thyme essential oil was extracted. The Minimum Inhibitory Concentration (MICs) tests were performed to determine the antimicrobial activity of Thymus plant against the first (Isoniazid, Rifampicin, Ethambutol) and second (Cycloserine, Streptomycin, Kanamycin) drug antibiotics of mycobacterium. Data were analyzed by SPSS 21 software, using one-way ANOVA test.

Findings The MICs for Isoniazid, Ethambutol, Streptomycin and Cycloserine were less than 10µg/ml and the MIC values for Rifampicin and Kanamycin were 40µg/ml. The limits of minimal inhibitory concentration of essential oil was between 0.5-40µg/ml ($p<0.05$).

Conclusion Thyme essential oil has antibacterial activity against *Mycobacterium tuberclusis.*

Keywords Minimum Inhibitory Concentration; *Mycobacterium tuberculosis*; Antibiotic Resistance; *Thymus vulgaris*

CITATION LINKS

[1] In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis strains [2] athogenesis of tuberculosis and other mycobacteriosis [3] Global tuberculosis control 2009, epidemiology, strategy, financing [4] Byrsonima fagifolia niedenzu apolar compounds with antitubercular activity [5] Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant mycobacterium tuberculosis isolates [6] Tuberculosis pathogenesis and immunity [7] Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of Mycobacterium tuberculosis [8] The pathology of Mycobacterium tuberculosis infection [9] Molecular biology of drug resistance in Mycobacterium tuberculosis [10] Detection of mutations in drug resistance genes of Mycobacterium tuberculosis by a dot-blot hybridization strategy [11] Molecular detection and characterization of resistant genes in Mycobacterium tuberculosis complex from DNA isolated from tuberculosis patients in the Eastern Cape province South Africa [12] Gene mutations in Mycobacterium tuberculosis: Multidrug-resistant TB as an emerging global public health crisis [13] Molecular detection of drug-resistant Mycobacterium tuberculosis with a scanning-frame oligonucleotide microarray [14] Chemical composition and antioxidant activity of Shirazi Thymus vulgaris essential oil [15] Aroma profile of Thymus vulgaris L. growing wild in Turkey [16] Volatile components of Thymus vulgaris L. from wild-growing and cultivated plants in Jordan [17] In vitro antimicrobial activity of thymus vulgaris essential oil against major oral pathogens [18] Thymus vulgaris L. extract has antimicrobial and anti-inflammatory effects in the absence of cytotoxicity and genotoxicity [19] Phenotypic and molecular characterization of Mycobacterium tuberculosis isolates resistant to both isoniazid and ethambutol [20] Essential oil of Thymus vulgaris L. and Rosmarinus officinalis L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens [21] Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes [22] Molecular characteristics of rifampin resistance among mycobacterium tuberculosis strains isolated in Northeast of Iran [23] Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts [24] Thymus vulgaris essential oil: Chemical composition and antimicrobial activity [25] Factors that interact with the antibacterial action of thyme essential oil and its active constituents [26] Redox biology of tuberculosis pathogenesis [27] Tuberculosis infection--pathogenesis, diagnosis, treatment and prevention strategies

Introduction

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs. This disease is a major health problem worldwide, particularly in developing countries as well. Currently about one third of the world's population is assumed to be infected with the tubercle bacillus [1-3].

In 90% of cases the infection remains latent and active form of the emergence of the disease in 10%. The global resurgence of TB and the development of drug resistance, multidrug-resistant (MDR) and extensively drug resistant (XDR) and newly Totally Drug-Resistant Tuberculosis (TDR-TB) strains present significant threats to TB control. The emergence of MDR-TB and extensively-drug resistant (XDR) TB to the medicines now in use makes the urgent search for new anti-TB agents worldwide [4-6] and there is a lot of motivation to achieve anti-mycobacterial compounds, particularly in plants. In order medicinal plants offer a great hope to overcome these needs because of their chemical diversity and their significant role in the drug development [7-13].

Thyme is a plant of the mint family native to Iran, Afghanistan and Pakistan. The plants used in traditional medicine have been mentioned as an antiseptic, antispasmodic and anti-inflammatory and they are widely used as a flavoring in foodstuffs. Thyme (*Thymus vulgaris*), belonging to the Lamiaceae family, is a pleasant smelling perennial shrub, which grows in several regions in the world. Thyme and its oil have been used as fumigants, antiseptics and antioxidant. The main constituents of *Thymus vulgaris* include Thymol, carvacrol and flavonoids to have antibacterial properties. The mechanism of the antimicrobial effect of essential oil is in its response to change in the permeability of the cell membrane of microorganisms and compounds such as potassium and hydrogen [14, 15]. Based on research report, the essential oil with high proportions of the phenolic components Thymol and/or carvacrol exhibited the highest antioxidant activity [16-18].

Considering the high incidence of tuberculosis, the growth in rate of reported infections of *Mycobacterium tuberculosis* strains in Iran and spread of antibiotic-resistance, broader and more extensive studies about effective compounds on the bacteria and infection are required.

Since medicinal plants have been widely distributed in our country, studies on the antimicrobial properties of these plants provide fields for replacement natural origin drugs instead of chemical drugs to control and the treatment of bacterial infections and reduction of complications.

The aim of this study was to evaluate the *in vitro* antibacterial activity of the thyme (*Thymus vulgaris*) essential oil, against *M. Tuberculosis*.

Materials and Methods

Plant samples and extraction of oil: In this experimental study, thyme herb plants grown in medicinal plant research center, 25km north of Tehran (Tehran, Iran) were collected. They were dried and powdered and then water distillation (hydro distillation) on Clevenger extraction was performed (The yield was 0.7-1.0%). The seeds were dried at room temperature. Air-dried leaves of thyme and rosemary were submitted to hydro distillation (HD) for 3h with 500ml distilled water using a Clevenger type apparatus according to the European Pharmacopoeia (1975). The extracted oil were collected and dried over anhydrous sodium sulfate, then stored in sealed glass vials in a refrigerator at 4˚C prior to analysis. The quantities of the essential oil were determined gravimetrically [19].

Gas Chromatography/Mass Spectrometry (GC/MS): The volatile compounds isolated by HD were analyzed by GC/MS. The oven temperature was programmed as 50˚C for 1min, then 7˚C/min to 250˚C, and then left at 250˚C for 5min. The injection port temperature was 250˚C and that of the detector was 280˚C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2ml/min. The MS conditions were as follow: ionization voltage, 70eV; ion source temperature, 150˚C; electron ionization mass spectra were acquired over the mass range 50 to 550m/z [20].

Microbial strains: Clinical isolate of *Mycobacterium tuberculosis* resistant to antibiotics of Isoniazid and Rifampin (MDR) obtained from the Tuberculosis and Lung Research Pasteur Institute of Iran. Thyme essential oil was used to assess the effects of anti-tuberculosis and H37RV used for standard strain of *Mycobacterium tuberculosis*.

Drug Susceptibility Testing: The Minimum Inhibitory Concentration (MIC) tests were performed to determine the antimicrobial activity of Thymus plant against the first (Isoniazid, Rifampicin, Ethambutol) and second (Cycloserine, Streptomycin, Kanamycin) drug antibiotics of mycobacterium. Drug susceptibilities against Isoniazid, Rifampin, Ethambutol and Streptomycin were determined by the standard proportional method using Lowenstein-Jensen (L-J) medium. For this purpose several fresh colonies dissolved in sterile deionized water until a bacterial density corresponding to 1 McFarland turbidity standards would be obtained and inoculated to drug containing tubes Rifampin (40µg/ml), Isonaizid

(0.2µg/ml), Ethambutol (2µg/ml) and streptomycin (4µg/ml) then were incubated at 37°C for 28 days. After 28 days visible colonies was interpreted as bacterial growth, if there is no colonies Lowenstein-Jensen medium incubated for two more weeks and results were reported after 42 days and critical ratio were recorded for each drug [21, 22]. MICs were defined as the lowest drug concentration after two-fold serially diluted concentration of the drugs that inhibits growth of more than 99.0% of a bacterial proportion of the tested *M. tuberculosis* within 14 to 21 days of incubation at 37°C.

Data analysis: Data were analyzed by SPSS 21 software, using one-way ANOVA test with 95% confidence interval and a significance level of $p<0.05$.

Findings

The MICs for Isoniazid, Ethambutol, Streptomycin and Cycloserine were less than 10µg/ml and the MIC values for Rifampicin and Kanamycin were 40µg/ml. The limits of minimal inhibitory concentration of essential oil was between 0.5-40µg/ml ($p<0.05$; Diagram 1).

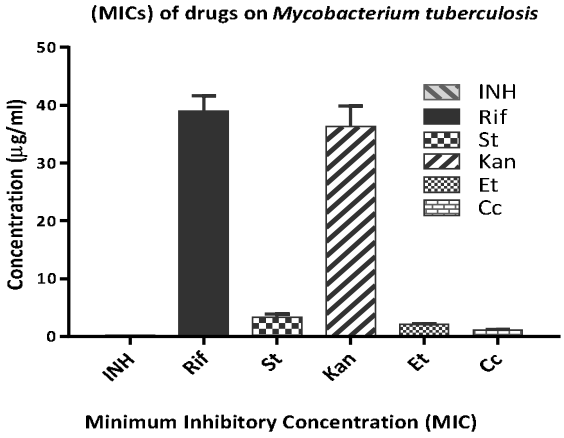

Diagram 1) The MIC of thyme oil (in concentrations of 0.5 to 100µg/ml) against *Mycobacterium tuberculosis* strains

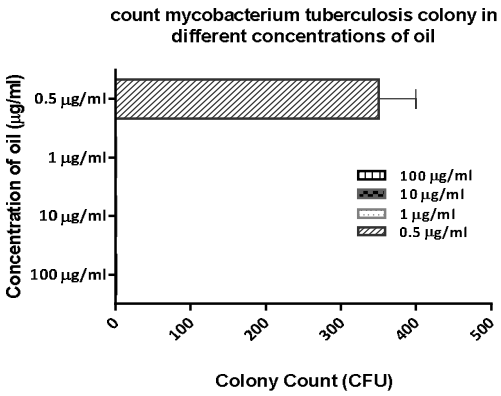

Diagram 2) TB colony count in different concentrations of thyme oil (0.5 to 100µg/ml)

Thyme oil had very strong anti-mycobacterial effect on bacteria. The results showed that essential oil can have bactericidal effect. Any colony of TB was not shown to 1µg/ml (Diagram 2).

Discussion

In many experiments has been proven that thyme essential oil is antibacterial and antifungal when tested on Gram positive and Gram negative bacteria, fungi and yeasts, due to its thymol and carvacrol content [23].

Boruga *et al.* reported that the antimicrobial activity of essential oils depends on their chemical constituents. Apparently, the antimicrobial activity of the essential oil analyzed is related to the presence of phenolic compounds (thymol) and terpene hydrocarbons (γ-terpinene), respectively [24].

Thymol is the main constituents of *Thymus vulgaris* essential oil. It is thought that the phenolic compounds of thyme exert antibacterial activity by complexing with the bacterial membrane proteins [25].

The MDR-TB treatment has global importance. These cases are difficult to treat with second-line drugs and they are more toxic and more expensive than first-line drugs. According who reported nearly 440,000 cases in 2008. The reported frequency of 5% of new cases infected to 48% who have had a history of tuberculosis have been different. Tuberculosis resistance to anti tuberculosis drugs is related to mutations in the genes coding for molecular drug target. Isoniazid (INH) and Rifampin (RIF) are the most important anti-TB drugs. As a result, resistance to INH develops due to point mutations in the *katG* gene. In addition to mutations in *katG*, mutations in several loci, such as the alkylhydroperoxidase AhpC and the enoylreductase *InhA*, may contribute to INH resistance and the development of resistance to Rifampin is due to mutation in 81bp region of *rpoB* (B subunit gene encoding the RNA polymerase) [26, 27].

In many parts of the world, traditional herbal preparations are used to treat some diseases, especially infectious diseases, diarrhea, fever, colds and dental hygiene. These plants have been considered and used without the knowledge of their antimicrobial properties and only through clinical experiences in the treatment of infectious diseases. Traditional natural plant material can be used as antimicrobial agents in the treatment of infections or be used as a food preservative. The food consumption can affect the course of disease and infection.

Carvacrol in thyme oil, increases the penetration, socialization and destruction of bacterial cell membrane and inhibit the activity of the ATPase.

Antimicrobial properties of thymol are about 25 times stronger than phenol in a series of germs.

There are numerous reports about the antimicrobial properties of the plants, but significant studies on the impact of the plant on tuberculosis have not been reported.

The results of MIC determination in mycobacterium indicate that this essential oil at concentration of 1µg/ml inhibits bacterial activity and no growth occurs at subsequent concentrations. The minimum mortality was also determined for this bacterium.

The results showed that the essential oil at the concentration of 1µg/ml causes bacteria to die and the results suggested that *Thymus vulgaris* could be regarded as a source of natural new antimicrobial agents which can affect problematic drug-resistant infection.

These results confirmed the traditional reports. *Thymus vulgaris* is an important source of phenolic compounds. The result of the present study showed that the extract of this plant contain high amount of flavonoids and exhibited a great antioxidant and antibacterial activity. In this study, thyme can be used as an easily accessible source of natural antioxidants and antibiotics in commercial food products and drugs.

Conclusion

Thyme essential oil has antibacterial activity against *Mycobacterium tuberclusis*.

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Ethical Permissions: None declared.

Conflicts of Interests: None declared.

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