Original Article

The antimycobacterium activity of mentha piperita and mentha spicata ethanolic extract against mycobacterium Bovis in comparison with isoniazid

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Abstract

Objectives: The plant materials play a major role in primary health care as therapeutic regimen in many developing countries. In the present study, the ethanol extracts of *mentha spicata* or spearmint (*M. spicata*) and *mentha piperita* or peppermint (*M. piperita*) have been used to inactive *mycobacterium bovis* (*M. bovis*) in comparison to isoniazid.

Patients and Methods: After collecting and identifying the herbs, their ethanolic extract was prepared using percolation method. The extracts of *M. spicata* and *M. piperita* with different dilutions; 0.39,0.78,1.56,3.12,6.25,12.5,25,50,100,200,400 mg/ml were provided. *M. bovis* strain 1173 P2 was used in this study. This microorganism was confirmed by acid-fast staining (Ziehl-Neelsen). The bacteria were incubated at 37 °C for a long time by inoculation into Middle Brook broth (Difco). Biochemical tests such as niacin, nitrate and urease were performed to confirm the organism (e.g. Feingold)(1) Agar diffusion and MIC methods (McFarland standard method and diffusion disk) were used to determine the antimicrobial activity of ethanolic extracts and the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

Results: The *in vitro* antibacterial activities of ethanolic extracts showed 0.39 mg/ ml consistency of *M. spicata* and 100 mg/ml consistency of *M. piperita* as the least concentrations which inhibit growth of *M. bovis* in comparison with isoniazid.

Conclusion: According to our findings, extracts of *M. spicata* and *M. piperita* could be used as raw materials for phytotherapy because of their antibacterial activities against *M. bovis* as TB etiology.

***Keywords**:* mentha spicata, mentha piperita, Mycobacterium bovis, antibacterial activity

Introduction

Various medical plants have been used for years in daily life to treat diseases all over the world. Peppermint *(mentha piperita)* and spearmint (*mentha spicata*) are popular herbs that can be used in numerous forms (i.e. oil, leaf, leaf extract, and leaf water) (1). Peppermint extracts e.g. ones with ethanol components, have various applications in food industries, cosmeceuticals, hygienic and pharmaceutical products for both its flavoring and fragrance properties (2,3).

Apart from that, tuberculosis (TB) is a contagious and chronic disease transmitted from person to person and has not been eradicated completely up to now. It is estimated that one third of world population are suffering from TB caused by Mycobacterium tuberculosis and Mycobacterium bovis that can be treated with antimycobacterial agents such as isoniazid, rifampin and streptomycin. Unfortunately, however, the evolution of drug resistance has led to the recent emergence of TB strains resistance to multiple medications including those of standard first-line therapies (4). Due to long duration of treatment, side effects of drugs, multiple drug resistance (MDR) and high cost of treatments, some researchers have been encouraged to use herbal products to treat TB.

Peppermint leaves and peduncles contain considerable amount of ether oil and essence used in aromatic and cosmetic industries (5,6). Regarding its medicinal use, *in vitro* antibacterial activity of essential oils of this plant was assessed in combination with standard antibiotic therapies and demonstrated that antibacterial activity can change by in vitro interactions and synergistic or antagonistic effects (7,8,9).

The aim of this study is determination of *in vivo* antimycobacterial activity of *mentha spicata (M. spicata)* & *mentha piperita (M. piperita)* ethanol extracts against *Mycobacterium bovis (M. bovis)*.

Patients and Methods

Collecting herbs- *M. piperita and spicata* were respectively collected from Shahid Beheshti pharmaceutical herbs garden and Qhom in the beginning of autumn. Aerial parts of herbs was dried in the shade at 25 c, powdered and conserved in refrigerator.

Preparing the extract- *M. piperita and M. spicata* aerial part powders turned into extract by ethanol 80%, which is equal with menthol by percolation method.

Sterilizing the extract- The extracts were sterilized by filtration method in aseptic situation.

Microorganism- *M. bovis* strain 1173 P2 was used in this study. These bacteria have been provided from BCG vaccine and clinical samples (tissue extracts) of infected

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Guinea pigs by *M. bovis*. This was incubated at 37 °C for a long time by inoculation into Lowenstein –Jensen (L.J) and Middle Brook 7H10 (Difco). Then biochemical tests such as niacin, nitrate and urease were done to confirm the microorganism (10,11).

Disk diffusion method – The turbidity of 0.5 McFarland was prepared from bacterial colony on L.J medium. Different dilutions of herb's extracts were prepared and 800 mg of dried extracts were dissolved in 2 ml distilled water and 0.25 ml DMSO was used as co-dissolvent. Afterwards, the dissolved extracts passed from 0.45 micrometer filter and 1 ml of solution poured into the second tube containing 1 ml sterilized distilled water. Paper disk of containing specific concentration were used and isoniazid disk was considered as control antibiotic. In this way, the whole plate surface of Middle Brook 7H10 was soaked with 1173 p2 *M. bovis* with 0.5 McFarland medium and different concentrations of extract disks were located on the surface of medium and after incubation in 37 °C for more than 10 days, the halo diameter (in millimeter) of non-growth were studied. In all steps isoniazid disks (1 mg/ml) showed no growth of which halo (17mm) was used as positive control (12).

Agar dilution method- In this method, minimum inhibition concentration (MIC) of extracts was studied. The extracts (1 ml) provided from different dilution added to 50 micro litter of microbial suspension. After incubation in 37 °C, growth of bacteria near the different dilution of extracts was shown.

Results

Findings of this research comprise two stages: first stage was the isolation of *M. bovis* 1173 P2 from clinical samples and production of suspension in addition to extraction and preparation of different dilutions of extracts (*M. piperita* and *M. spicata)*. The second stage was antibiogram methods (disk diffusion and MIC method) and *in vitro* antibacterial activities of the ethanol extracts *M. piperita* and *M. spicata.* We observed 0.39 mg/ml consistency of *M. spicata* (Table1) and 100 mg/ml consistency of *M. piperita* (Table2) are the least concentrations which inhibit *M. bovis*'s growth in comparison with isoniazid.

With comparison charts 1 and 2, it can be concluded that the diffusion of extract from disk is low even in high consistency.

Discussion

This study showed that extracts of *M. spicata* and *M. piperita* could be used as raw materials for phytotherapy because of their antibacterial activities against *M. bovis* as one of the etiologies of TB.

Owing to the fact that many studies have been accomplished in order to achieve antimycobacterial combination therapy, ethanol extracts of *M. spicata* and *M. piperita* with various concentrations were provided. In a review by Macky and Blumberg in 2006 on *M. piperita* phenolic leaves, it is demonstrated that the combination of extracts had noticeable anti-tumor and anti-allergic effects as well as its relaxing influences on gastrointestinal tract and anesthetic effects on CNS and PNS in animals. Moreover, another important virtue of *M. piperita* leaves is analgesic effect on pulmonary and digestive system and used for relaxation and treatment of nephrolithiasis as well (7).

Interestingly, Samarth et al in 2006 demonstrated that number of lung tumor cells will decrease from %83 to %31 when increased oral dose of *M. piperita* is considered (13). According to *M. piperita* effectiveness on lung tumor treatment and its high penetration into lung tissue, more studies on Albino mouse is suggested to assess the treatment outcomes of other lung diseases such as infections, especially TB (13, 14). Moreover and in another study by Fit et al in 2007, *M. piperita* effect on Staphylococcus was studied and is observed that 10% consistency of *M. piperita* has antibacterial effect on Staphylococcus bacterial strain (15). Additionally, Marjia and colleagues in 2009 evaluated the *M. piperita* anti-fungal effect with consistency of 0.5%,1%,1.5%,and 2% on pathogenic species of Aspergillus toxin (*A. ochraceus, A. flavus, A. fumigatus*) and proved that *M. piperita* has considerable effects on these species since 0.5% density of *M. piperita* can inhibit about 95% and 1%,1.5%, 2% of *M. piperita* can completely inhibit these species and hinder production of Aflatoxin B. According to these and our findings, *M. piperita* effectiveness by disk diffusion method was effectual in high densities and non-growth halo was observed. Low density of *M. piperita*, however, did not have haltering effect and its MIC was obtained on Mycobacterium Bovis at 100 mg (16). Furthermore, Shkurupii and colleagues in 2002 suggested *M. piperita* essence as fumigation with other TB medicines (17).

Apart from that and in view of the fact that the *M. piperita* essence with density of 300-600 mg has antimicrobial effect *in vitro*, Shkurupiĭ et al used it 20 minutes daily during two months and based on chest x ray results, suggested *M. piperita* as supplementary with other drugs. In 2006, moreover, these researchers have proven that, patients suffering from diffused pulmonary tuberculosis, with the use of *M. piperita* extract inhalation have had less affection by recurrent infection and distinctively decreased lung damage (18).

According to the mentioned two investigations and present study, the *M. piperita* effectiveness on Mycobacterium Bovis derived from tissue sample and standard strain is proven and is equal to 100 mg/ml.

In a research by Molina-Salinas and colleagues in 2005, *M. spicata* methanolic (ethanol 80%) was used to extinguish and halter of Mycobacterium Tuberculosis H37RV and CIBIN:UMF:15:99 is resistance to the drug that mentioned. The conclusion of this research for H37RV MIC is 50 mg/ml and for CIBN:UMF:15:99 strain is 100 mg/ml. (19) and the different findings is because of different strains of bacteria.

We found *M. piperita* and MIC to be, respectively 100 and 0.39 which represents different herb effectual substance in Mycobacterium Bovis inhibition for these two herbs. As main parts of *M. piperita* and *M. spicata* essences are menton and Karon, respectively, the different conclusion goes back to different chemical components in these two herbs.

Since these two herbs do not have any poisonous effect, we can use them for treatment of TB along with other antibiotics with fewer side effects.

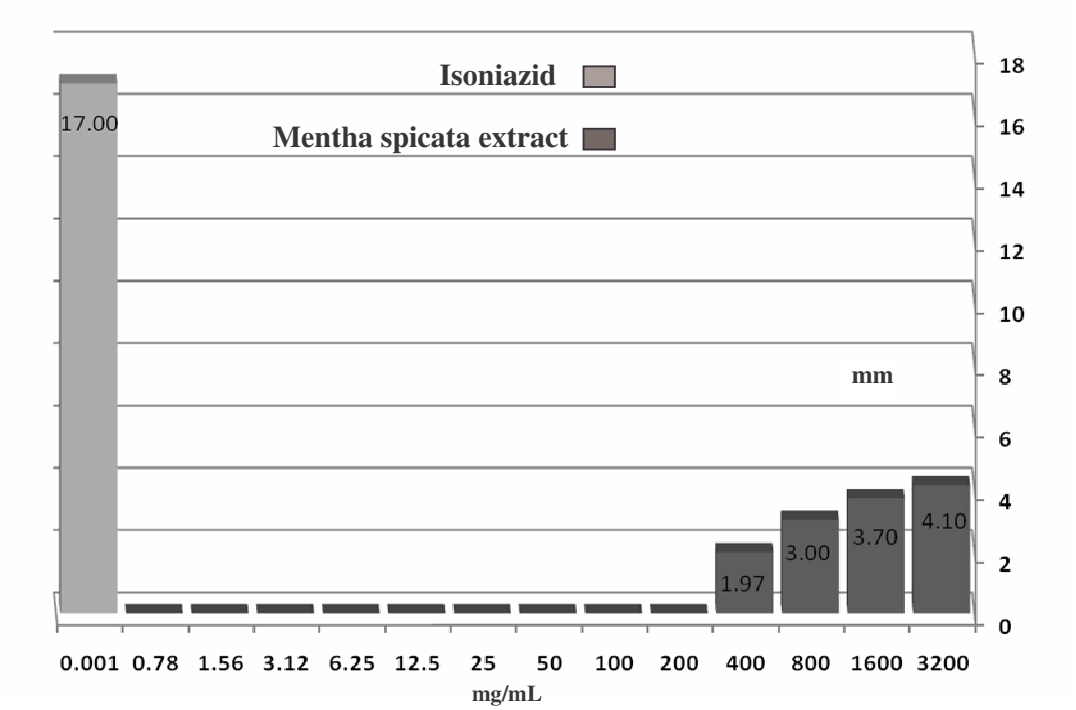

Chart 1: Average distribution of halo growth inhibition (*mm*) in different *Mentha Spicata* extract consistency; mg/ml with isoniazid as positive control.

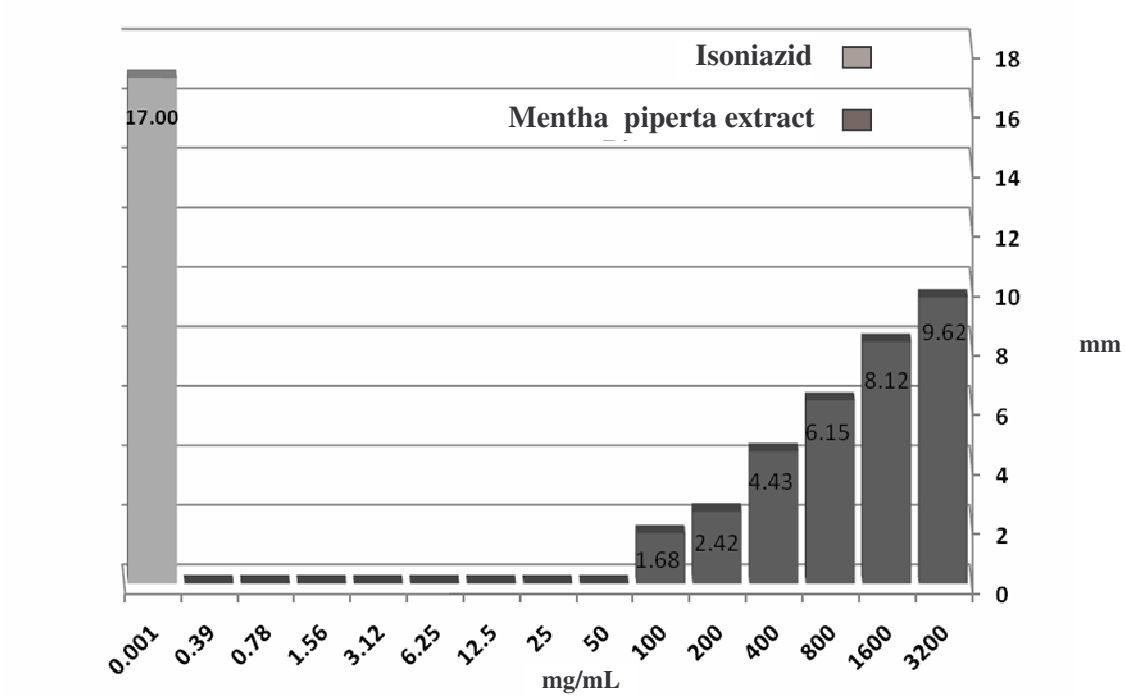

Chart 2: Average distribution of halo growth inhibition (*mm*) in different *Mentha Piperita* consistency; mg/ml with isoniazid as positive control.

Table 1: Distribution of growth rate and growth inhibition of *M. Bovis* derived from Guinea pig tissue extracts and standard strain **ATCC1173P(BCG)**

Isoniazid		*Menta spicata extract*										
0.001	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	200	400	concentration(mg/ml)
-	-	-	-	-	-	-	-	-	-	-	-	resistance
+	+	+	+	+	+	+	+	+	+	+	+	sensitivity

Table 2: Distribution of growth rate and growth inhibition of *M. Bovis* derived from Guinea pig tissue extracts and standard strain **ATCC1173P(BCG)**

Isoniazid		*Mentha piperita extract*										concentration
0.001	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	200	400	(mg/ml)
-	+	+	+	+	+	+	+	+	-	-	-	resistance
+	-	-	-	-	-	-	-	-	+	+	+	sensitivity

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Conflict of interest

None declared.

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