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In vitro activity of garden thyme (*Thymus vulgaris* L.) chloroform extract against non-tuberculous mycobacteria

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Summary

This study was carried out to evaluate the antibacterial activity of chloroform (CHL) extract of *Thyme vulgaris* L. (Lamiaceae). The CHL extract from aerial parts of *T. vulgaris* exhibited antimicrobial activity against slow growth mycobacteria – SGM (n = 49) and rapid growth mycobacteria – RGM (n = 50) strains isolated from diseased ornamental fish. The MIC_{AV}, MIC_{90}, and MIC_{50} values of CHL extract against to RGM strains were found to be at 194.00, 400, and 200 µg/mL, respectively. The MIC_{AV}, MIC_{90}, and MIC_{50} values of CHL extract against to SGM strains were found to be at 54.59, 100, and 50 µg/mL, respectively. In conclusion, the CHL extract exhibits the activity against non tuberculosis mycobacteria isolated from diseased ornamental fish.

Keywords: mycobacteria, chloroform extract, *Thymus vulgaris*

Mycobacteriosis in fish is a serious health problem in ornamental fish farming and aquaculture, caused by various species of mycobacteria, including *Mycobacterium marinum*, *Mycolicibacterium fortuitum* (known previously as *Mycobacterium fortuitum*), *Mycolicibacterium peregrinum* (*Mycobacterium peregrinum*), and *Mycobacteroides chelonae* (*Mycobacterium chelonae*), *Mycobacterium avium* (2, 3, 7, 8, 13, 15, 17, 18). These pathogens can cause chronic infections, leading to significant economic losses and health maintenance issues in fish farms. Mycobacteria are aerobic, acid-fast bacteria characterized by slow growth and a high lipid content in their cell walls. These properties make them exceptionally resistant to many traditional antibiotics and difficult to treat (4). Mycobacterial infections in fish can lead to symptoms such as lethargy, loss of appetite, weight loss, emaciation, ascites, skin ulcers, scale loss, deformities, exophthalmia, and ultimately, the death of the fish (15). Non tuberculous mycobacteria (NTM), which cause diseases in fish, can also cause diseases in humans (17). These bacteria are commonly found in aquatic environments, and humans can become infected through open wounds or abrasions when they come into contact with contaminated water or infected fish. Treating mycobacteriosis presents significant challenges and is frequently ineffective, highlighting the urgency of developing novel therapeutic approaches (4, 5, 14). Currently used medications often fail to completely eliminate the infection, leading to chronic and recurrent health issues.

The growing issue of drug resistance in mycobacteria highlights the urgent need to search for new and effective compounds against these pathogens. Research on finding natural anti-mycobacterial compounds in plant extracts may open new possibilities for combating these difficult-to-treat pathogens. Plant extracts often exhibit multi-targeted actions on various cellular targets, increasing their effectiveness and enabling them to combat drug-resistant strains. One of the plants containing antitubercular compounds is common thyme (*Thymus vulgaris*) (6, 12). Numerous studies have shown that extracts from this plant, especially the essential oil, contain substances such as thymol, carvacrol, and p-cymene (9), which exhibit strong antimicrobial activity. These compounds can effectively inhibit the growth of mycobacteria, including strains resistant to traditional antibiotics. *Thymus vulgaris*, with its multi-targeted properties, represents a promising source of natural antitubercular compounds that

Tab. 1. Sensitivity of the tested NTM strains to CHL extracts of *T. vulgaris*

Strains	Minimal inhibitory concentration (µg/mL)									
	> 800	800	400	200	100	50	25	12.5	6.25	< 6.25
Rapid growth mycobacteria (n = 50)										
M. abscessus (n = 1)				1						
M. chelonae (n = 16)			3	12	1					
M. fortuitum (n = 10)			1	4	3	2				
M. peregrinum (n = 12)			5	4	2	1				
M. neoaurum (n = 2)						2				
M. mucogenicum (n = 1)				1						
M. saopaulense (n = 1)						1				
M. septicum (n = 2)				1	1					
M. salmoniphilum (n = 1)				1						
M. senegalense (n = 4)					2	2				
Slow growth mycobacteria (n = 49)										
M. gordonae (n = 15)					2	9	4			
M. marinum (n = 33)					6	22	5			
M. szulgai (n = 1)					1					

could be utilized in new therapeutic strategies for combating mycobacterial infections.

The aim of this study was to investigate the antimicrobial activity of the chloroform extract from the aerial parts of *T. vulgaris* against mycobacteria isolated from diseased ornamental fish.

Material and methods

Dried *Thymus vulgaris* plants were obtained from the herb wholesaler NANGA (Przemysław Figura, Złotów, Poland). Chloroform (CHL) extract was prepared following the methods previously outlined by Puk et al. (16). In this study, the rapid growth mycobacteria (RGM, n = 50) and slow growth mycobacteria (SGM, n = 49) were investigated. The mycobacterial strains from the collection of the Department of Fish Biology and Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland, were identified based on molecular characteristics as previously described (15). The antimicrobial activity of the extracts was assessed using the method described earlier by Puk et al. (16). The MIC_{AV} (mean inhibitory concentration against the tested strains), MIC_{90} (minimum inhibitory concentration required to inhibit the growth of 90% of bacterial strains), and MIC_{50} (minimum inhibitory concentration required to inhibit the growth of 50% of bacterial strains) were determined.

Results and discussion

The CHL extract from aerial parts of *T. vulgaris* exhibited antimicrobial activity against SGM (n = 49) and RGM (n = 50) strains isolated from diseased ornamental fish (15). The MICs, MIC_{AV}, MIC_{90}, and MIC_{50} values of CHL extract against to SGM and RGM strains were shown in Table 1 and 2.

Al-Hashmi et al. (1) demonstrated that the major chemical constituents analysed by GC-MS in chloroform crude extract were 1-iodo-2-methylundecane (2.7%), 3.7-octadiene-2.6-diol, 2.4-dimethylbenzaldehyde (4.7%), 2.6-dimethyl-(7.6%), thymol (38.8%), ando-thymol (46.0%). The chloroform extract obtained from *T. vulgaris* has been shown to have activity against *Escherichia coli* and *Shigella dysenteriae* (10). However, to the authors' knowledge, its activity against mycobacteria has not been investigated. In the present study, the CHL extract from the aerial parts of *T. vulgaris* demonstrated significant antimicrobial activity against both RGM and SGM strains isolated from diseased ornamental fish. The MIC results indicate that this extract is more effective against SGM strains compared to RGM strains (Tab. 2).

Tab. 2. The MIC values for *T. vulgaris* CHL extract (µg/mL) against isolates of NTMs (n = 99): SGM (n = 49) and RGM (n = 50)

MIC	Non-tuberculosis mycobacteria		
	RGM	SGM	RGM+SGM
MIC_{AV}	194.00	54.59	124.20
MIC_{90}	400	100	200
MIC_{50}	200	50	100

Explanations: RGM – rapid growth mycobacteria; SGM – slow growth mycobacteria; MIC – minimum inhibitory concentration; MIC_{AV} – the mean inhibitory concentration of the tested strains; MIC_{90} – minimum inhibitory concentration required to inhibit the growth of 90% of bacterial strains; MIC_{50} – minimum inhibitory concentration required to inhibit the growth of 50% of bacterial strains

For RGM strains, the MIC_{AV}, MIC_{90}, and MIC_{50} values were 194.00 µg/mL, 400 µg/mL, and 200 µg/mL, respectively. This means that the average inhibitory concentration (MIC_{AV}) and the concentrations necessary to inhibit the growth of 90% and 50% of RGM strains were relatively high. These results suggest that RGM may be less sensitive to the chloroform extract of *T. vulgaris*, which could be due to their faster growth rate and possibly greater ability to rapidly adapt to environmental stresses. In contrast, the MIC values for SGM strains were lower, with an MIC_{AV} of 54.59 µg/mL, an MIC_{90} of 100 µg/mL, and an MIC_{50} of 50 µg/mL. These lower MIC values indicate that SGM are more sensitive to the extract, which may be related to their slower growth rate and, consequently, their lesser ability to adapt to adverse environmental conditions.

The increasing antibiotic resistance necessitates the search for alternative methods to combat mycobacteria (5, 11, 14, 16). In a previous study by Guz and Puk (4), it was demonstrated that all RGM strains tested in that study are highly resistant to rifampicin (100%), isoniazid (100%), and doxycycline (78%), while SGM strains are resistant to rifampicin (93.88%) and isoniazid (97.96%). Moreover, it was shown that RGM strains are most sensitive to kanamycin (92%) and amikacin (90%), and all SGM strains are sensitive to amikacin, kanamycin, and sulfamethoxazole. Although the MICs for the chloroform extract tested in this study are high for the tested SGM and RGM strains, it has been demonstrated that this extract contains a compound or compounds with antimycobacterial activity.

In conclusion, the results of this study represent a significant step towards the use of natural plant compounds in combating bacterial pathogens in aquaculture. They may also open new research opportunities in the context of sustainable management of the health of ornamental fish. Further studies are necessary to more precisely determine the composition, mechanisms of action of this active compounds.

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