Polish Journal of Veterinary Sciences Vol. 29, No. 3 (2026), 371–

DOI

Short communication

Antimicrobial activity of twelve plant-derived compounds against fish-associated non-tuberculous mycobacteria

K. Puk, L. Guz, A. Pastuszka

Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland

Correspondence to: L. Guz, e-mail: leszek.guz@up.lublin.pl

Abstract

Nontuberculous mycobacteria (NTM) are important pathogens of fish, causing chronic infections that are difficult to control in aquaculture. In this study, we evaluated the in vitro antimicrobial activity of twelve plant-derived compounds against six fish-associated NTM strains using a broth microdilution assay. Minimum inhibitory concentrations (MICs) ranged from 0.25 to >32 µg/mL. Our results demonstrated that cinnamaldehyde exhibited the strongest antimycobacterial effect, followed by sesamol and farnesol, while several other phytochemicals showed moderate activity and salicylic acid was inactive. These findings highlight the potential of selected plant-derived compounds, particularly cinnamaldehyde, as promising candidates for alternative or adjunctive strategies against fish mycobacteriosis.

Keywords: non-tuberculous mycobacteria, phytochemicals, fish mycobacteriosis, antimicrobial activity

Introduction

Fish mycobacteriosis is a chronic granulomatous disease affecting both wild and cultured fish species worldwide (Decostere et al. 2004, Gauthier and Rhodes 2009). The disease is caused by non-tuberculous mycobacteria (NTM), primarily *Mycobacterium marinum, M. chelonae, M. peregrinum* and *M. fortuitum* (Puk and Guz 2020). In addition to economic losses in aquaculture and the ornamental fish industry, some NTM species are zoonotic and may pose a health risk to aquarium workers and fish handlers (Szmygin-Milanowska et al. 2016).

Therapeutic management of fish mycobacteriosis is difficult due to subclinical infections, nonspecific clinical signs, and limited treatment options (Strike et al. 2017). Therefore, alternative antimicrobial strategies are needed. Our previous studies have demonstrated that selected plant-derived compounds and plant extracts exhibit antimycobacterial activity against

fish-associated non-tuberculous mycobacteria (Puk and Guz 2022, Puk et al. 2023, Guz et al. 2024, Puk et al. 2024). This study extends this line of research by evaluating an additional panel of plant-derived compounds against fish-associated NTM species. The compounds selected in the present study, including cinnamaldehyde, sesamol and farnesol, were chosen based on their reported antimicrobial activity against various bacterial pathogens, including mycobacteria, as well as their potential mechanisms of action, including membrane disruption and interference with essential bacterial processes (Jabra-Rizk 2006, Zacchino et al. 2017, Álvarez-Martínez et al. 2021, Li et al. 2024). Nevertheless, their activity against fish-associated non-tuberculous mycobacteria remains poorly characterized. The aim of this study was to evaluate the *in vitro* antimycobacterial activity of selected plant-derived compounds against fish-associated NTM strains.

Materials and Methods

Six non-tuberculous mycobacteria (NTM) strains isolated from diseased ornamental fish between 2015 and 2016 were included: *Mycobacterium chelonae* M18, *Mycobacterium marinum* M10, *Mycobacterium peregrinum* M1 and M73, *Mycobacterium senegalense* M41, and *Mycobacterium septicum* M77. The strains were stored at the Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland. Twelve plant-derived compounds (caffeic acid, chlorogenic acid, cinnamaldehyde, farnesol, gallic acid, piperine, reserpine, sabinene, salicylic acid, sesamol, terpinene, thujone) and amikacin (positive control) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

MICs were determined for each strain using the resazurin microtiter assay in Middlebrook 7H9 broth supplemented with 10% OADC, as previously described by Guz and Puk (2022). NTM strains were subcultured and incubated at 25°C until sufficient growth was obtained. Bacterial suspensions were prepared in sterile broth and adjusted to approximately 10^5 CFU/mL before inoculation on microtiter plates. The tested compounds were dissolved in sterile distilled water or dimethyl sulfoxide (DMSO) depending on their solubility, and the final concentration of DMSO in the assay wells did not exceed 1% (v/v). The MIC was defined as the lowest concentration preventing colour change. A growth control containing inoculum without compounds, a sterility control without inoculum, and a positive control with amikacin were included on each plate. All experiments were performed in two independent replicates. MIC values were reported as single values when identical results were obtained in both replicates. Compounds were classified as strongly active (MIC ≤ 8 μg/mL), moderately active (16-32 μg/mL), or inactive (> 32 μg/mL).

Results and Discussion

The in vitro antimicrobial activity of twelve plant-derived compounds was evaluated against six fish-associated NTM strains (Table 1). MIC values ranged from 0.25 μg/mL to >32 μg/mL.

Cinnamaldehyde exhibited the strongest activity, with MICs as low as 0.25 μg/mL against *M. marinum* and 1 μg/mL against the remaining strains. Sesamol and farnesol also showed notable activity (MIC 2-8 μg/mL). Moderate activity (MIC 16-32 μg/mL) was observed for caffeic acid, chlorogenic acid, gallic acid, piperine, reserpine, sabinene, terpinene, and thujone. Salicylic acid was inactive at the tested concentrations (Table 1). The observed activity of cinnamaldehyde, sesamol, and farnesol is consistent with previous reports describing the antimicrobial effects of plant-derived compounds against mycobacteria and other bacterial pathogens (Zacchino et al. 2017, Álvarez Martínez et al. 2021). However, the susceptibility of fish-associated NTM to these compounds has been rarely investigated. The low MIC values obtained in the present study, particularly for cinnamaldehyde, suggest that selected phytochemicals may represent promising candidates for further evaluation against fish mycobacteriosis.

The pronounced activity of cinnamaldehyde may be associated with membrane disruption and interference with essential bacterial enzymes, mechanisms previously described for plant phenolics and terpenoids (Zacchino et al. 2017, Álvarez Martínez et al. 2021). Similarly, sesamol and farnesol are known to affect bacterial membrane integrity and may inhibit quorum sensing, contributing to their antimicrobial effects (Ji et al. 2005, Jabra-Rizk et al. 2006). The relatively consistent MIC ranges across species suggest a broad-spectrum mode of action. These findings indicate that selected phytochemicals, particularly cinnamaldehyde, sesamol, and farnesol, may serve as promising candidates for further investigation as alternative or adjunctive agents in the management of fish mycobacteriosis. Nevertheless, evaluation of cytotoxicity, pharmacokinetics, and in vivo efficacy is required before practical application in aquaculture.

Table 1. Minimum inhibitory concentrations (MICs, µg/mL) of twelve plantderived compounds against six fish-associated nontuberculous mycobacteria (NTM) strains.

Compound /Strain	M18	M10	M1	M73	M41	M77
Caffeic acid	16	16	16	16	16	16
Chlorogenic acid	32	32	32	32	32	32
Cinnamaldehyde	1	0.25	1	1	1	1
Farnesol	8	4	8	8	8	8
Gallic acid	32	16	32	32	32	32
Piperine	32	16	16	16	32	32
Reserpine	>32	32	32	32	32	32
Sabinene	32	32	32	32	32	32
Salicylic acid	>32	>32	>32	>32	>32	>32
Sesamol	8	4	8	8	4	8
Terpinene	32	32	32	32	32	32
Thujone	32	32	16	32	32	32
Amikacin	16	1	1	1	1	1

Author Declarations

Ethics approval

Ethics approval was not required for this study, as no live animals were used. All experiments were conducted in vitro on bacterial strains obtained from previously collected samples, in accordance with institutional biosafety guidelines.

Use of generative artificial intelligence

Generative artificial intelligence (ChatGPT) was used to assist the authors in improving the English language, phrasing and formatting of the manuscript. All scientific content, study design, data analysis, and interpretations were performed solely by the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V (**2021**) Antibacterial plant compounds, extracts and essential oils: an updated review on their effects and putative mechanisms of action. Phytomedicine 90: 153626.

Decostere A, Hermans K, Haesebrouck F (**2004**) Piscine mycobacteriosis: a literature review covering the agent and the disease it causes in fish and humans. Vet Microbiol 99: 159-166.

Gauthier DT, Rhodes MW (**2009**) Mycobacteriosis in fishes: a review. Vet J 180: 33-47.

Guz L, Puk K, Szwajgier D, Pastuszka A (**2024**) Combined antimicrobial action of streptomycin and terpenes against atypical mycobacteria isolated from fish. Pol J Vet Sci 27: 135-138.

Guz L, Puk K (**2022**) Antibiotic susceptibility of mycobacteria isolated from ornamental fish. J Vet Res 66: 69-76.

Jabra-Rizk MA, Meiller TF, James CE, Shirtlif ME (**2006**) Effect of farnesol on Staphylococcus aureus biofilm formation and antimicrobial susceptibility. Antimicrob. Agent. Chemother. 50: 1463-1469.

Ji P, Yin K, Jiang Y, Sun Y, Luo W, Zhou J (**2005**) Sesamol: a novel quorum sensing inhibitor and colistin accelerator against Pseudomonas aeruginosa. Food Sci. Hum. Wellness 14: 9250075.

Li Z, Wu M, Yan H, Meng Z, Gao B, Dong Q (**2024**) Antimicrobial effect and possible mechanism of sesamol against foodborne pathogens. Foods 13: 435.

Puk K, Guz L (**2020**) Occurrence of Mycobacterium spp. in ornamental fish. Ann Agric Environ Med 27: 535-539.

Puk K, Guz L (**2022**) Effect of alkaloid berberine on the susceptibility of nontuberculous mycobacteria to antibiotics. Pol J Vet Sci 25: 479-481.

Puk K, Guz L, Pastuszka A (**2024**) Berberine enhances the antibacterial activity of thymoquinone, carvacrol and thymol against multi-drug resistant nontuberculous mycobacteria. Pol J Vet Sci 27: 469-473.

Puk K, Wawrzykowski J, Guz L (**2023**) Evaluation of the anti-mycobacterial activity and composition of Carlina acaulis L. root extracts. Pol J Vet Sci 26: 57-63.

Strike TB, Feltrer Y, Flach E, Macgregor SK, Guillaume S (**2017**) Investigation and management of an outbreak of multispecies mycobacteriosis in Australian lungfish (Neoceratodus fosteri) including the use of triple antibiotic treatment. J Fish Dis 40: 557-570.

Szmygin-Milanowska K, Grzywa-Celińska A, Zwolska Z, Krawczyk P, Guz L, Milanowski J (**2016**) 'TB or not TB' Problems of differential diagnosis of cutaneous mycobacteriosis and tuberculosis – A case study and interdisciplinary discussion. Ann Agric Environ Med 23: 97-102.

Zacchino SA, Butassi E, Liberto MD, Raimondi M, Postigo A, Sortino M (**2017**) Plant phenolics and terpenoids as adjuvants of antibacterial and antifungal drugs. Phytomedicine 37: 27-48.