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Antibacterial Activity of Extracts Derived from Leaves of *Ficus lyrata* Warb. and its Cultivar Bambino against Some Fish Bacterial Strains

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The bioactive compounds present in various plant extracts offer a sustainable and environmentally friendly approach to the control of bacterial infections in farmed fish, thus contributing to the promotion of animal health, food safety and environmental sustainability in aquaculture. In the present study, we evaluated the antimicrobial activity of the ethanolic extracts of the leaves of *Ficus lyrata* Warb. and its *F. lyrata* cv. Bambino against *Aeromonas sobria*, *Aeromonas hydrophila* and *Aeromonas salmonicida* subsp. *salmonicida*, *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*, to evaluate their possible use of these plants in the prevention of infections caused by these fish pathogens in aquaculture. The isolates used in our studies were *Aeromonas sobria* (K825), *Aeromonas hydrophila* (K886) and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens* (Pt521), *Yersinia ruckeri* (UP 2), *Pseudomonas fluorescens* (Pt 433), *Shewanella putrefaciens* (St15). These strains, derived from freshwater fish species such as common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), were isolated at the Department of Fish Diseases, National *Veterinary Research* Institute in Puławy (Poland). The antimicrobial susceptibility of the tested strains was determined by the Kirby-Bauer disc diffusion method (1966), according to the recommendations of the Clinical and Laboratory Standards Institute (2006, 2014), with our slight modifications. The results of our study showed that a group of *Aeromonas strains* showed resistance to *F. lyrata* and *F. lyrata* cv. Bambino extracts compared to oxytetracycline and enrofloxacin activity. *Serratia liquefaciens*, *Yersinia ruckeri* and *Pseudomonas fluorescens* strains were also resistant to the ethanolic extracts from the leaves of *F. lyrata* and its cv. Bambino. On the other hand, the *Shewanella putrefaciens* strain was sensitive to

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the ethanolic extracts of the leaves of *F. lyrata* and its cv. Bambino. The percentage increase in zone inhibition diameter for the *F. lyrata* cv. Bambino leaf extract was 43.3% ($p < 0.05$) compared to oxytetracycline activity. This finding suggests the presence of bioactive compounds in *F. lyrata* leaves with antimicrobial properties against selected bacterial strains. It highlights the potential of this botanical resource to combat bacterial infections in aquaculture. In conclusion, the antibacterial activity demonstrated by *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts against fish bacterial strains highlights their potential as valuable resources for disease prevention and treatment in aquaculture. Continued research efforts to elucidate their mechanisms of action, optimise their formulation, and validate their efficacy *in vivo* will further enhance their utility and facilitate their integration into sustainable aquaculture production systems.

Keywords: leaves, *Ficus lyrata* antimicrobial efficacy, fish bacterial pathogens, susceptibility, resistance

Introduction

In recent years, there has been a growing interest in investigating the antibacterial potential of plant extracts against fish bacterial pathogens, as bacterial infections pose a significant challenge to aquaculture production worldwide (Balunas and Kinghorn, 2005; Nik Mohamad Nek Rahimi et al., 2022). The use of plant-derived compounds offers a promising avenue for the development of novel antimicrobial agents that are effective, environmentally friendly and potentially less prone to induce bacterial resistance (Vaou et al., 2021; Alaoui Mdarhri et al., 2022).

The search for natural sources of antimicrobial agents has become increasingly important due to the emergence of antibiotic-resistant bacteria and the need for sustainable alternatives in various fields, including aquaculture (Abdallah et al., 2023). *Ficus lyrata* Warb., commonly known as the fiddle leaf fig, is a species of the Moraceae family known for its ornamental value (Fang et al., 2007). However, beyond its aesthetic appeal, *F. lyrata* has also been recognised for its potential medicinal properties, including antimicrobial and antioxidant activities (Rizvi et al., 2009; Pękala-Safińska et al., 2019; Wira et al., 2020). Seraia et al. (2008) showed that *F. lyrata* has gas-absorbing and phytoncide properties that can be used to improve the sanitary properties of indoor air. Elganzoury et al. (2023) investigated the antagonistic efficacy of *F. lyrata* extract (FLE) conjugated with selenium nanoparticles (FLE-SeNPs) against aluminium chloride (AlCl3)-induced hippocampal injury in rats and showed that SeNPs synthesised with FLE exerted a remarkable neuroprotective effect against $AlCl_3^-$ induced neurotoxicity by reversing oxidative damage, neuronal inflammation and apoptosis in exposed rats (Elganzoury et al., 2023).

The present study focuses on evaluating the antibacterial activity of leaf extracts from *F. lyrata* and its cv. Bambino against selected fish bacterial strains. *F. lyrata* cv. Bambino is a dwarf cultivar of *F. lyrata* known for its compact size and distinctive foliage, making it a popular choice for indoor cultivation. By evaluating the antibacterial efficacy of these plant extracts, we aim to explore their potential application in mitigating bacterial infections in aquaculture.

The bacterial strains selected for evaluation represent common pathogens known to affect various fish species in aquaculture, including both freshwater and marine environments (Irshath et al., 2023). These pathogens can cause devastating losses in aquaculture production through diseases such as bacterial septicemia, fin rot and ulcerative disease (Majeed et al., 2023). The genus *Aeromonas* comprises a collection of ubiquitous Gram-negative rods that are widely distributed in aquatic environments (Beaz-Hidalgo et al., 2010; Fernández-Bravo and Figueras, 2020). The presence of several virulent *Aeromonas* spp. in aquatic environments predisposes aquatic animals and humans to infection (Majeed et al., 2023). Several motile species of *Aeromonas* are known to be pathogens of aquatic animals, and interest in this genus has recently increased due to its zoonotic potential (Janda and Abbott, 2010; Gonçalves Pessoa et al., 2019). The most common *Aeromonas* spp. causing infections in aquatic animals and humans are *A. hydrophila*, *A. salmonicida*, *A. caviae* and *A. veronii* biotype *sobria*. The ability of *Aeromonas* spp. to produce a variety of virulence factors enhances their pathogenicity (Majeed et al., 2023).

Serratia liquefaciens is considered a pathogenic bacterium of fish and causes infections that result in high mortality in salmonid populations (McIntosh and Austin, 1990). *Yersinia ruckeri* is the causative agent of enteric redmouth disease in various salmonid species worldwide (Kumar et al., 2015). This microorganism has caused economic losses to the aquaculture industry since it was first described, but the early development of a vaccine has allowed relative control of the disease (Fernández et al., 2007). *Pseudomonas fluorescens* is a gram-negative, rod-shaped organism that is motile with polar flagella and can produce

a fluorescent pigment (fluorescein) (Shabana et al., 2022). *P. fluorescens* is a common opportunistic psychrotrophic microorganism in aquaculture, causing pseudomoniasis in vertebrates such as freshwater and saltwater fish, and invertebrates such as shrimps, is widespread in soil, water, plants and animals, and can grow at 25–30 °C (Wang et al., 2009; Shabana et al., 2022). *Shewanella putrefaciens* is a non-fermentative, motile, gram-negative bacillus with the main phenotypic characteristic of H_2S production, which infects a variety of aquatic animals such as European sea bass (*Dicentrarchus labrax*), loach (*Misgurnus anguillicaudatus*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus auratus*), European eel (*Anguilla anguilla*), American eel (*Anguilla rostrata*), and tilapia (*Oreochromis niloticus*) (Jiang et al., 2022).

In the present study, we investigated the antimicrobial activity of the ethanolic extracts of the leaves of *F. lyrata* and its cultivar *F. lyrata* cv. Bambino against *Aeromonas sobria*, *Aeromonas hydrophila* and *Aeromonas salmonicida* subsp. *salmonicida*, *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*, to evaluate the possible use of these plants in the prevention of infections caused by these fish pathogens in aquaculture.

Material and methodology

Collection of plant materials and preparing plant extracts

The leaves of *F. lyrata* and its cv. Bambino, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Sciences of Ukraine (Kyiv). Specifically, the leaves of *F. lyrata* and its cultivar were sampled for our study. The sampled leaves were taken to the laboratory for antimicrobial testing. Freshly sampled leaves were washed, weighed, crushed, and homogenised in 96% ethanol (at a ratio of 1 : 10) at room temperature and centrifuged at 3,000 g for 5 minutes. The supernatants were stored at -20 °C in vials protected by laminated paper until needed (2–3 months).

The current study was conducted as part of an ongoing project between the Institute of Biology (Pomeranian University in Słupsk, Poland), Faculty of Veterinary Medicine and Animal Sciences, University of Life Sciences (Poznań, Poland), M.M. Gryshko National Botanic Gardens of the National Academy of Sciences of Ukraine (Kyiv, Ukraine) and Ivan Franko National University in Lviv (Lviv, Ukraine), undertaken within the framework of the cooperation programme aimed at the assessment of medicinal properties of tropical and subtropical plants cultivated *in vitro*.

Bacterial strains for antimicrobial activity testing

The isolates used in our studies were *Aeromonas sobria* (K825), *Aeromonas hydrophila* (K886), and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens* (Pt521), *Yersinia ruckeri* (UP 2), *Pseudomonas fluorescens* (Pt 433), *Shewanella putrefaciens* (St15). These strains, derived from freshwater fish species such as common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), were isolated at the Department of Fish Diseases, National *Veterinary Research* Institute in Puławy (Poland). Bacteria were collected from fish showing clinical signs of disease. Each isolate was inoculated on trypticase soy agar (TSA) (bioMériux) and incubated for 24 h at 27 ±2 °C. Pure colonies were used for biochemical identification according to the manufacturer's instructions, except for the incubation temperature, which was 27 ±1 °C. The following identification systems were used in the study: API 20E, API 20NE, API 50CH (bioMériux). Suspected *Aeromonas* isolates were further identified at species level by restriction analysis of 16S rDNA genes amplified by polymerase chain reaction (PCR) (Kozińska, 2007).

Bacterial growth inhibition test of plant extracts by disc diffusion method

The antimicrobial susceptibility of the tested strains was determined by the Kirby-Bauer disc diffusion method (1966), according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2006, 2014), with slight modifications. Each inoculum of specific bacterial species was cultured on Mueller-Hinton agar at a density of 0.5 McFarland. After inoculation of bacteria, a maximum of 5 wells per Petri dish, each 6 mm in diameter, were cut into the medium and plant extracts were added. The plates were incubated for 24 h at 28 ±2 °C and the inhibition zones were measured for each well. Eight replicates (n = 8) were tested for each extract. Plates were observed and photographed. Zone diameters were measured and averaged. Oxytetracycline (OT, 30 µg) and enrofloxacin (ENR, 5 µg) were used as controls for antimicrobial susceptibility testing. After inoculation of media plates and placement of appropriate antimicrobial discs (five discs per plate), the plates were incubated at 28 ±2 °C for 24 hours.

Statistical analysis

Statistical analysis of the data obtained was performed using the mean ± standard error of the mean (S.E.M.). All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$). To find significant differences (significance level, $p < 0.05$) between groups, the Kruskal-Wallis rank test was applied to the data (Zar, 1999). All statistical analyses were performed using STATISTICA software v. 13.3 (TIBCO Software Inc., USA). The following zone diameter criteria were used to classify bacteria as susceptible or resistant to the phytochemicals tested: susceptible (S) ≥15 mm, intermediate (I) = 10–15 mm, and resistant (R) ≤10 mm (Okoth et al., 2013).

Results and discussion

The results of the *in vitro* evaluation of the antimicrobial activity of ethanolic extracts from the leaves of *F. lyrata* and its cv. Bambino against fish bacterial strains, expressed as the mean of the diameters of the inhibition zones, are presented in Figure 1.

Our results showed that the ethanolic extracts from the leaves of *F. lyrata* and its cv. Bambino had low activity against fish pathogenic bacteria compared to the oxytetracycline and enrofloxacin activity of the control samples. A group of *Aeromonas strains* showed resistance to *F. lyrata* and *F. lyrata* cv. Bambino extracts compared to oxytetracycline. The inhibition zone diameters were 9.50 ±0.33 mm and 12.0 ±0.73 mm for *A. sobria*, 9.38 ±0.38 mm and 9.18 ±0.54 mm for *A. hydrophila*, 9.50 ±0.50 mm and 9.13 ±0.44 mm for *A. salmonicida* subsp. *salmonicida* compared to oxytetracycline activity – 29.14 ±1.56 mm, 23.43 ±1.19 mm and 27.0 ±1.0 mm, respectively, and enrofloxacin activity – 35.14 ±1.52 mm, 27.43 ±1.45 mm and 35.14 ±1.83 mm, respectively (Figure 1). The percentage of lesser in zone inhibition diameter for extracts from leaves of *F. lyrata* and its cv. Bambino were 67.4 and 58.8% ($p < 0.05$) for *A. sobria*, 60 and 60.8% ($p < 0.05$) for *A. hydrophila*, 64.8 and 66.2% ($p < 0.05$) for *A. salmonicida* subsp. *salmonicida* compared to oxytetracycline activity. The percentage of smaller zone inhibition diameter for extracts from leaves of *F. lyrata* and its cv. Bambino were 73 and 65.9% ($p < 0.05$) for *A. sobria*, 65.8 and 66.5% ($p < 0.05$) for *A. hydrophila*, 73 and 74% ($p < 0.05$) for *A. salmonicida* subsp. *salmonicida* compared to enrofloxacin activity (Figure 1).

Serratia liquefaciens, *Yersinia ruckeri* and *Pseudomonas fluorescens* strains were also resistant to the ethanolic extracts from the leaves of *F. lyrata* and its cv. Bambino. The inhibition zone diameters were 9.50 ±0.50 mm and 9.25 ±0.49 mm for *S. liquefaciens*, 9.25 ±0.59 mm and 9.75 ±0.65 mm for *Y. ruckeri*, 10.25 ±0.56 mm and 9.38 ±0.73 mm for *P. fluorescens* compared to oxytetracycline activity – 22.43 ±0.72 mm, 25.29 ±0.42 mm and 17.0 ±0.82 mm, respectively, and enrofloxacin activity – 22.14 ±0.59 mm, 28.29 ±0.97 mm and 22.14 ±0.67 mm, respectively (Figure 1). The percentage of less in zone inhibition diameter for extracts from leaves of *F. lyrata* and its cv. Bambino were 57.6 and 58.8% ($p < 0.05$) for *S. liquefaciens*, 63.4 and 61.4% ($p < 0.05$) for *Y. ruckeri*, 39.7 and 44.8% ($p < 0.05$) for *P. fluorescens* compared to oxytetracycline activity. The percentage of less in zone inhibition diameter for extracts from leaves of *F. lyrata* and its cv. Bambino were 57.1 and 58.2% ($p < 0.05$) for *S. liquefaciens*, 67.3 and 65.5% ($p < 0.05$) for *Y. ruckeri*, 53.7 and 57.6% ($p < 0.05$) for *P. fluorescens* compared to enrofloxacin activity (Figure 1).

On the other hand, the *Shewanella putrefaciens* strain was sensitive to the ethanolic extracts of the leaves of *F. lyrata* and its cv. Bambino. The inhibition zone diameters were 20.63 ± 0.71 mm and 30.50 ± 0.57 mm for extracts from leaves of *F. lyrata* and its cv. Bambino compared to 21.29 ± 0.42 mm for oxytetracycline and 30.29 ± 0.52 mm for enrofloxacin activity. The percentage decrease in zone inhibition diameter for the *F. lyrata* leaf extract was 31.9% ($p < 0.05$) compared to enrofloxacin activity. The percentage increase in zone inhibition diameter for the *F. lyrata* cv. Bambino leaf extract was 43.3% ($p < 0.05$) compared to oxytetracycline activity (Figure 1).

The investigation of the antibacterial activity of leaf extracts of *F. lyrata* and its cv. Bambino against selected fish bacterial strains provides promising insights into the potential use of these botanical extracts in aquaculture and fisheries management. The results of this study demonstrate significant antibacterial activity of *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts against the fish bacterial strain *S. putrefaciens*. The observed inhibition zones for other strains indicate a mild efficacy of these extracts in suppressing the growth of pathogenic bacteria commonly associated with fish diseases (Figure 1). This finding suggests the presence of bioactive compounds in *F. lyrata* leaves with antimicrobial properties against selected bacterial strains, highlighting the potential of this botanical resource to combat bacterial infections in aquaculture.

We suggest that the antibacterial activity of *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts can be attributed to various bioactive compounds, including phenolic compounds, flavonoids and terpenoids, which are known for their antimicrobial properties. A total

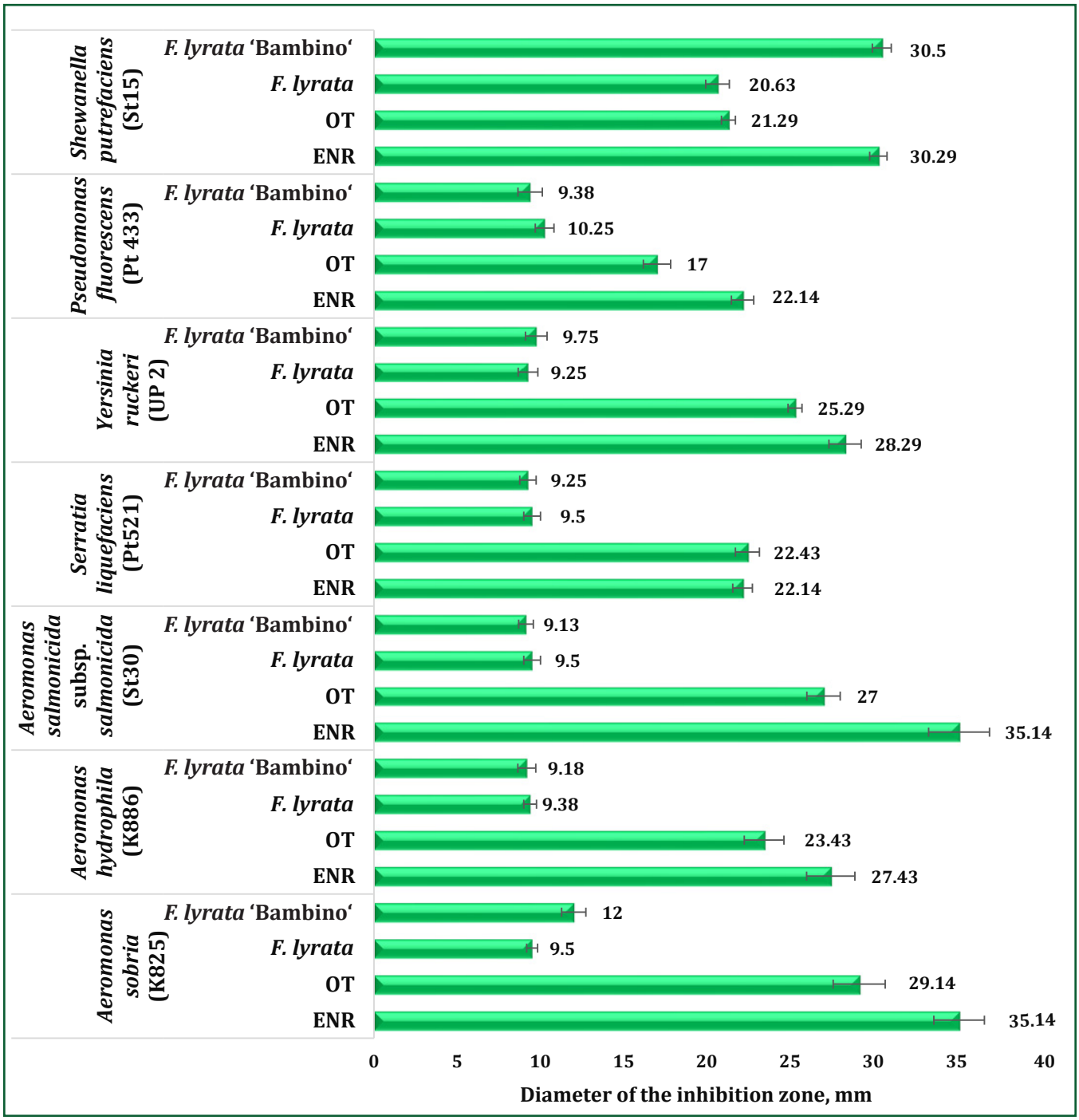

Figure 1 Mean inhibition zone diameters induced by ethanolic extracts of leaves of *F. lyrata* and its cultivar 'Bambino' against fish bacterial strains (1,000 µL inoculum) (M ±m, n = 8)

of 72 metabolites in fruits and leaves of *F. lyrata* were evaluated in a UPLC-qTOF-MS-based metabolomic study by Farag et al. (2014). Seventeen flavonoids were characterised and tentatively identified, with the major components being catechins/procyanidins, O- and C-linked flavonoid glycosides. The major procyanidins were dimers and trimers comprising (epi)catechin and (epi)afzelechin units, whereas the predominant flavones were C-glycosides of luteolin and apigenin. Apart from these major flavonoid classes, a group of benzoic acids, caffeoylquinic acids, fatty acids and sphingolipids were also annotated (Farag et al., 2014).

These compounds can disrupt bacterial cell membranes, inhibit essential enzymatic processes or induce oxidative stress, leading to bacterial growth inhibition or cell death (Tan et al., 2022). Flavonoids are known to be antibacterial agents against a wide

range of pathogenic microorganisms. With the increasing prevalence of untreatable infections caused by antibiotic-resistant bacteria, flavonoids have attracted much interest as a potential substitute for antibiotics (Xie et al., 2015). Flavonoids could exert antibacterial activity via cytoplasmic membrane damage, inhibition of energy metabolism and inhibition of nucleic acid synthesis, thus flavonoids are considered as constitutive antibacterial substances (Tan et al., 2022). The hydroxylation of C5, C7, C3‘ and C4‘ and the geranylation or prenylation at C6 have been extensively studied to enhance the bacterial inhibition of flavonoids. On the other hand, methoxylation at C3‘ and C5 has been reported to decrease the antibacterial activity of flavonoids (Shamsudin et al., 2022). Apigenin induces bacterial apoptosis via activation of cellular oxidative pathways dependent on free radical production and accumulation (Kim et al., 2020), and mitochondrial-mediated apoptotic pathway and mitochondrial calcium signalling in *Candida albicans* (Lee et al., 2019). Further studies to elucidate the specific mechanisms of action of these extracts will improve our understanding of their antimicrobial efficacy and facilitate their targeted use in aquaculture systems.

Many of our studies have confirmed the antioxidant properties of *Ficus* plants against fish pathogens (Tkachenko et al., 2016a-e, 2022; Pękala-Safińska et al., 2021). In our previous study, we evaluated the antimicrobial activity of ethanolic extracts of *Ficus* plant species against *Aeromonas strains* (Pękala-Safińska et al., 2021). As an average of the three *Aeromonas strains*, the highest antimicrobial activity among all the tested ethanolic extracts was observed in *F. binnendijkii* leaves with an inhibition zone diameter (IZD) of 23.75 ±1.64 mm against *A. sobria*, 20.63 ±1.45 mm against *A. hydrophila*, and 15.75 ±0.80 mm against *A. salmonicida*. *F. craterostoma* extract was effective against *A. sobria* with an IZD of 15.25 ±0.90 mm and against *A. salmonicida* with a zone of 15.25 ±1.15 mm, while *F. deltoidea* extract was effective against *A. sobria* over a diameter of 18.81 ±1.25 mm and against *A. salmonicida* over a diameter of 20.13 ±0.79 mm. The extract of *F. hispida* inhibited *A. sobria* best with an IZD of 25.56 ±1.63 mm, followed by the extracts of *F. binnendijkii* with an IZD of 23.75 ±1.64 mm and *F. tinctoria* with an IZD of 22.5 ±1.20 mm. The IZD results also showed that isolates of *A. sobria* had intermediate susceptibility to ethanolic extracts of *F. aspera*, *F. benjamina*, *F. elastica*, *F. formosana*, *F. johannis* subsp. *afghanistanica*, *F. natalensis* subsp. *leprieurii*, *F. religiosa*, *F. villosa*, and *F. virens*, which produced mean IZDs of 10 to 15 mm. The isolates appeared to be resistant to extracts from 18 *Ficus* species (43.9%), which only restricted growth to mean IZDs of less than 10 mm (Pękala-Safińska et al., 2021).

The therapeutic potential of using various plants of the *Ficus* genus in the control of bacterial diseases was evaluated against fish pathogens in an *in vitro* study with promising results (Tkachenko et al., 2016a-e, 2022). In our previous study, the *in vitro* antimicrobial activity of ethanolic leaf extracts of different *Ficus* species against *Citrobacter freundii* was evaluated. The results proved that the extracts of *F. drupacea*, *F. septica*, *F. deltoidea*, as well as *F. hispida*, *F. mucuso*, *F. pumila*, *F. craterostoma* exhibited favourable antibacterial activity against *C. freundii* (200 µL standardised inoculum) (Tkachenko et al., 2016b). Our results also proved that the ethanolic extracts obtained from *F. pumila*, *F. binnendijkii* ‘Amstel Gold‘, *F. carica*, *F. erecta*, *F. hispida*, *F. mucuso*, *F. palmeri*, *F. religiosa* have considerable sufficient antibacterial potential against *C. freundii* (Tkachenko et al., 2016b). Ethanolic extracts of the leaves of ten *Ficus* species, which were screened among different *Ficus* species, i.e. *F. hispida*, *F. binnendijkii*, *F. pumila*, *F. rubiginosa*, *F. erecta*, *F. erecta* var. *sieboldii*, *F. sur*, *F. benjamina*, *F. craterostoma*, *F. lyrata*, *F. palmeri* (the species are listed in the order of effectiveness against the tested pathogen) were the most effective against *P. fluorescens* (200 µL of standardised inoculum) (Tkachenko et al., 2016a). In addition, previous research has shown that the most effective against *P. fluorescens* (400 µL of standardised inoculum) were the ethanolic extracts obtained from the leaves of ten *Ficus* species: *F. craterostoma*, *F. cyathistipula*, *F. drupacea* ‘Black Velvet‘, *F. hispida*, *F. macrophylla*, *F. mucuso*, *F. pumila* and *F. villosa* (Tkachenko et al., 2016e). In our study, most of the ethanolic extracts of *Ficus* spp. proved to be effective against the tested strain of gram-negative *A. hydrophila*, with inhibition zones of 10–12 mm observed. *A. hydrophila* was most sensitive to *F. pumila*. The highest antimicrobial activity against *A. hydrophila* (200 µL of standardised inoculum) was shown by leaf extracts of *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida* and *F. lyrata* (Tkachenko et al., 2016c). Among different species of *Ficus* genus with moderate activity against *A. hydrophila* (400 µL of standardised inoculum), the highest antimicrobial activity was shown by leaf extracts of *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida* and *F. lyrata* (Tkachenko et al., 2016d).

The results of this study have significant implications for aquaculture and fisheries management practices. The use of botanical extracts from *F. lyrata* and its

cultivar 'Bambino' as natural antimicrobial agents offers a sustainable and environmentally friendly alternative to conventional antibiotics for disease prevention and treatment in fish farming. Incorporating these extracts into aquafeed formulations or bath treatments may help reduce the reliance on synthetic antibiotics, reduce the development of antimicrobial resistance in fish pathogens, and improve the health and welfare of farmed fish populations.

The use of other *Ficus* plants has also been shown to have antibacterial activity. The methanolic stem bark extract of *Ficus thonningii* Blume was subjected to preliminary phytochemical screening and *in vitro* antimicrobial testing in the study by Usman et al. (2009). The antimicrobial activity of the plant extract was tested using the agar plate disc diffusion and broth dilution techniques. The microorganisms tested were: *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Salmonella typhi* (Gram-negative strains), *Staphylococcus aureus* and *Streptococcus* spp. (Gram-positive strains). The extracts inhibited the growth of all test organisms at various concentrations, particularly against *Pseudomonas aeruginosa* and *Streptococcus* spp. with a mean inhibition zone of 33.33 ±7.33 mm and 32.33 ±2.51 mm, respectively. The results showed an MIC of 10 mg·ml^{-1} against *P. aeruginosa* and 1.25 against the other organisms tested. The MIC against *S. aureus* was 2.5 mg·ml^{-1} and that against *Streptococcus* spp. was 0.625 mg·ml^{-1}. The extracts showed variable inhibitory activity against the organisms tested (Usman et al., 2009). The enhancement of antibacterial activity by phyto-fabrication of silver nanoparticles with *F. thonningii* aqueous extracts was investigated by Ondigo et al. (2022). Enhanced antimicrobial efficacy was shown by the nanoparticles (inhibition zones of 26.1, 24.1 and 15.2 mm of 11.5, 10.6 and 6.5 mm) for *S. aureus*, *Streptococcus pyrogenes* and *E. coli* respectively compared to flucloxacillin standard which was in the range of 21.5, 23.5 and 25.7 mm (Ondigo et al., 2022).

Methanolic, hexanoic, chloroform and ethyl acetate extracts of *Ficus carica* L. latex were evaluated by Aref et al. (2010) for their *in vitro* antimicrobial properties against five bacterial species and seven fungal strains. The antimicrobial activity of the extracts was evaluated based on the inhibition zone using the disc diffusion assay, the minimum inhibitory concentration (MIC) for bacterial testing and the method of calculating inhibition percentage (I%) for fungal inhibitory activities. The methanolic extract had no effect against bacteria except *Proteus mirabilis*, while the ethyl acetate extract inhibited the multiplication of five bacterial species (*Enterococcus fecalis*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*). For the opportunistic pathogenic yeasts, the ethyl acetate and chlorophormic fractions showed a very strong inhibition (100%); the methanolic fraction showed a total inhibition against *Candida albicans* (100%) at a concentration of 500 µg·ml^{-1} and a negative effect against *Cryptococcus neoformans*. *Microsporum canis* was strongly inhibited by the methanolic extract (75%) and totally inhibited by the ethyl acetate extract at a concentration of 750 µg·ml^{-1}. The hexanoic extract showed moderate results (Aref et al., 2010).

The antimicrobial activity of the methanol extract of *Ficus polita* Vahl. roots (FPR), as well as that of its fractions (FPR1-5) and two of the eight isolated compounds, namely euphol-3-O-cinnamate (1) and (E)-3,5,4'-trihydroxy-stilbene-3,5-O-β-D-diglucopyranoside (8), was evaluated by Kuete et al. (2011). The results of the MIC determination showed that the crude extract, the FPR1, FPR2 fractions and compound 8 were able to inhibit the growth of the eight microorganisms tested. Other samples showed selective activity. The lowest MIC value of 64 µg·ml^{-1} for the crude extract was recorded on 50% of the microbial species tested. The corresponding value for fractions of 32 µg·ml^{-1} was obtained on *Salmonella typhi*, *E. coli* and *C. albicans* ATCC strains. The MIC values obtained with compound 8 on the resistant strain *P. aeruginosa* PA01 were equal to those of chloramphenicol used as reference antibiotic (Kuete et al., 2011).

Kuete et al. (2008) also evaluated the antimicrobial activity of the methanol extracts of *Ficus chlamydocarpa* Mildbr. & Burret (FCR), *Ficus cordata* Hort. Berol. ex Kunth & C.D.Bouché (FCB), a mixture of the two plants (FCM), and the isolated flavonoids alpinumisoflavone (2), genistein (3), laburnetin (4), luteolin (5) (isolated from FCR), catechin (7) and epiafzelechin (8) (isolated from FCB). Mycobacteria, fungi, Gram-positive and Gram-negative bacterial species were tested for their susceptibility to the above samples. All samples except compound 7 were found to be active against Mycobacterium smegmatis and the MIC ranged from 0.61 to 312.50 µg·ml^{-1}. Compound 4 showed the best activity against *Mycobacterium tuberculosis* with an MIC of 4.88 µg·ml^{-1}. The results of the diffusion test indicated that the crude extract of FCB, FCM as well as compounds 5 and 8 were able to inhibit the growth of all tested organisms (fungi, Gram-positive and Gram-negative bacteria). The inhibitory effect of the *F. chlamydocarpa* crude extract was observed against 10 (62.5%) of the 16 microorganisms tested (excluding mycobacteria), whereas that of compounds

4, 2 and 3 was observed against 14 (87.5%), 8 (50.0%) and 7 (39.9%) of the microbial species tested, respectively. FCB was found to be more active than FCR against most of the organisms tested (Kuete et al., 2008).

The antimicrobial activities of hexane extract and decussatin from the stem bark extract of *Ficus congensis* Engl. (syn. *Ficus trichopoda* Baker) were investigated by Alaribe et al. (2011). Decussatin and hexane extract were screened *in vitro* for antibacterial and antifungal activities using broth microdilution (MHB) and disc agar diffusion (DAD) techniques against *E. coli*, *Bacillus substilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *C. albicans*. Hexane extracts showed potent antibacterial activity against *E. coli* and *B. subtilis* with MICs of 8 mg·mL^{-1} and 5 mg·mL^{-1} respectively, while decussatin at the highest concentration (8 mg·mL^{-1}) used in this study showed no appreciable antimicrobial activity. Only hexane extract was active against *C. albicans* with a MIC of 1 mg·mL^{-1} (Alaribe et al., 2011).

Abdsamah et al. (2012) investigated the *in vitro* antimicrobial activity of chloroform, methanol and aqueous extracts of *Ficus deltoidea* Jack at 10 mg·ml^{-1}, 20 mg·ml^{-1} and 50 mg·ml^{-1}, using the disc diffusion method against 2 Gram-positive (*S. aureus* IMR S-277, *B. subtilis* IMR K-1), 2 Gram-negative (*E. coli* IMR E-940, *P. aeruginosa* IMR P-84) and 1 fungal strain, *C. albicans* IMR C-44. All extracts showed inhibitory activity against the fungi, Gram-positive and Gram-negative bacterial strains tested, except the chloroform and aqueous extracts against *B. subtilis*, *E. coli* and *P. aeroginosa*. The methanol extract showed good antibacterial and antifungal activities against the test organisms. The methanol extract significantly inhibited the growth of *S. aureus* forming a wide inhibition zone (15.67 ± 0.58 mm) and lowest MIC value (3.125 mg·ml^{-1}). *B. subtilis* was least sensitive to the chloroform extract (6.33 ± 0.58 mm) and highest MIC value (25 mg·ml^{-1}) (Abdsamah et al., 2012).

Various chemical constituents present in the stem bark of *Ficus racemosa* L. show enhancement of cell migration (which corresponds to cell proliferation), as well as antimicrobial activity against *Staphylococcus* and *Bacillus* species, and antifungal activity against *Saccharomyces* spp. and *Candida albicans*, as described by Bopage et al. (2018). A new antifungal pyranoisoflavone, 5,3‘,4‘-trihydroxy-2″,2″-dimethylpyrano (5″,6″:7,8) isoflavone (1), along with two known isoflavones, wighteone (2) and lupiwighteone (3) (with previously reported antifungal activity), were isolated from ethyl acetate extract by Wei et al. (2012) using bioassay-guided fractionation. The antifungal activities of 1–3 against *Phytophthora infestans* were evaluated by direct spore germination assay and the IC_{50} values were 262.442, 198.153 and 90.365 μg·mL^{-1}, respectively (Wei et al., 2012). A new isoflavone (Z)-5,7,4‘-trihydroxy-3‘-[3-hydroxy-3-methyl-1-butenyl] isoflavone (1) together with seven known isoflavones (2–8) were isolated from the fruits of *Ficus auriculata* Lour. by Shao et al. (2022). All compounds were evaluated for their antibacterial activities against five pathogenic bacteria *in vitro*. Compounds 3 and 4 showed significant antibacterial activities against five pathogenic bacteria with MIC values ranging from 1.25 to 20 μg·mL^{-1} (Shao et al., 2022).

A traditional Ugandan *Ficus natalensis* Hochst. bark cloth exhibits antimicrobial activity against methicillin-resistant *S. aureus* (MRSA) strain, as described by Butler et al. (2021). Antimicrobial contact and disc diffusion assays, coupled with time-kill kinetic assays, demonstrated that bark cloth inhibited the growth of a clinically relevant MRSA strain and acted as a bactericidal agent, causing a seven-log reduction in bacterial viability. Scanning electron microscopy revealed morphological changes in bacterial cell ultrastructure upon exposure to bark cloth, supporting a proposed mechanism of antimicrobial activity. The observed antimicrobial properties, combined with the physical properties induced by bark cloth, suggest that this product is ideally suited for wound and other skin care applications (Butler et al., 2021).

Future research efforts should focus on elucidating the specific bioactive compounds responsible for the antibacterial activity of *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts. The identification and characterisation of these compounds using advanced analytical techniques, such as chromatography and mass spectrometry, will facilitate the development of standardised extracts with improved potency and reproducibility. In addition, *in vivo* studies to assess the safety and efficacy of these extracts in fish models are warranted to validate their therapeutic potential and to evaluate their impact on fish health and performance under real-world aquaculture conditions.

Conclusions

The investigation of the antibacterial activity of *F. lyrata* and its cv. Bambino leaf extracts against fish bacterial strains underlines the potential of these

botanical extracts as natural antimicrobial agents for aquaculture and fisheries management. The significant antibacterial activity observed, as evidenced by the inhibition zones against *Shewanella putrefaciens*, highlights the efficacy of *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts in suppressing the growth of this fish pathogenic bacterium. This finding suggests the presence of bioactive compounds in the extracts with potent antimicrobial properties.

The potential mechanisms of action of these extracts may involve various bioactive compounds, including phenolic compounds, flavonoids and terpenoids, which are known for their antimicrobial activity. Further elucidation of the specific mechanisms underlying the antibacterial activity of *F. lyrata* and *F. lyrata* cv. Bambino leaf extracts will help to optimise their efficacy and facilitate their targeted application in aquaculture. The implications of this study for aquaculture and fisheries management are significant. The use of plant extracts as natural antimicrobials offers a sustainable and environmentally friendly alternative to synthetic antibiotics, helping to reduce the development of antimicrobial resistance in fish pathogens and promoting the health and welfare of farmed fish populations.

Conflicts of interest

The authors have no competing interests to declare.

Ethical statement

This article does not include any studies that would require an ethical statement.

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