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ANTIMICROBIAL POTENTIAL OF EXTRACT DERIVED FROM THE LEAVES OF *FICUS VILLOSA* BLUME AGAINST SOME FISH PATHOGENIC STRAINS

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The use of natural compounds can be found in many species of plants, and this holds much antimicrobial potential against fish pathogens in animal husbandry and fish farmers. In the current study, we studied the antimicrobial activity of the ethanolic extract derived from the leaves of F. villosa against the Aeromonas species: A. sobria, A. hydrophila, and A. salmonicida subsp. salmonicida, as well as Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens to evaluate the possible use of this plant in preventing infections caused by these fish pathogens in aquaculture. The current study was conducted as a part of an ongoing project between five universities undertaken in the frame of a cooperation program aimed at the assessment of medicinal properties of tropical and subtropical plants. The leaves of F. villosa, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. The Aeromonas strains used in our studies were Aeromonas hydrophila (K886), Aeromonas sobria (K825) and Aeromonas salmonicida subsp. salmonicida (St30). These microorganisms originated from the bacterial strain collection of the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, and had been isolated from fish of two farmed freshwater species exhibiting clinical signs of disease: common carp (Cyprinus carpio L.) (K886 and K825) and rainbow trout (Oncorhynchus mykiss Walbaum) (St30). Bacteria Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens were isolated both from apparently healthy rainbow trout (Oncorhynchus mykiss Walbaum), as well as from individuals exhibiting clinical signs of the diseases, according to the procedure developed at the Department of Fish Disease of the National Veterinary Research Institute in Poland. Antimicrobial susceptibility of the tested strains was performed by the Kirby-Bauer disc diffusion method (1966) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014), with our some modifications. Results of the antimicrobial screening revealed, that F. villosa possessed different antibacterial properties against fish pathogens. The ethanolic extract derived from leaves of F. villosa exhibited the maximum antimicrobial activity against Shewanella putrefaciens, Aeromonas sobria, and Pseudomonas fluorescens. The percentage of increase in the diameters of inhibition zones was 122.7% for Shewanella putrefaciens,

54.9% for Aeromonas sobria, and 48.5% for Pseudomonas fluorescens, respectively. F. villosa exhibited mild antibacterial properties against Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida. Thus, the Shewanella putrefaciens strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of F. villosa, while Serratia liquefaciens and Yersinia ruckeri strains were resistant to this extract. These findings demonstrate that plant extracts derived from plants belonging to the Ficus genus are potential sources of botanical drugs for controlling bacterial infection in aquaculture.

Keywords: Ficus villosa Blume, extract, antimicrobial efficacy, Kirby-Bauer disk diffusion technique, fish pathogens, susceptibility, resistance

АНТИМІКРОБНИЙ ПОТЕНЦІАЛ ЕКСТРАКТУ, ОТРИМАНОГО З ЛИСТЯ *FICUS VILLOSA* BLUME, ЩОДО ДЕЯКИХ ПАТОГЕННИХ ШТАМІВ РИБ

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Використання природних сполук, які можна знайти в багатьох видах рослин, має великий антимікробний потенціал щодо збудників хвороб риб у тваринництві та аквакультурі. У цьому дослідженні ми вивчали антимікробну активність етанольного екстракту, отриманого із листя Ficus villosa Blume, щодо 3 видів Aeromonas: A. sobria, A. hydrophila та A. salmonicida subsp. salmonicida, а також Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens, з метою оцінки можливого використання цієї рослини для запобігання інфекціям, викликаними цими збудниками захворювань риб в аквакультурі. Це дослідження було проведено в рамках поточного проекту між п'ятьма університетами, який здійснюється в рамках програми співпраці, спрямованої на оцінку лікувальних властивостей тропічних і субтропічних рослин. Зразки листя F. villosa, культивованого в тепличних умовах, відбирали в Національному ботанічному саду імені М.М. Гришка (НБС) НАН України. Штами Aeromonas, використані в наших дослідженнях, були Aeromonas hydrophila (K886), Aeromonas sobria (K825) і Aeromonas salmonicida subsp. salmonicida (St30). Ці мікроорганізми походять із колекції штамів бактерій Відділу хвороб риб Національного ветеринарного науково-дослідного інституту в Пулавах (Польща), і були виділені з риб двох прісноводних видів, що вирощуються, і мали клінічні ознаки захворювання: короп звичайний (Cyprinus carpio L.) (K886 і K825) і райдужна форель (Oncorhynchus mykiss Walbaum) (St30). Бактерії Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens виділяли як від умовно здорової райдужної форелі (Oncorhynchus mykiss Walbaum), так і від особин із клінічними ознаками захворювань за методикою, розробленою у Відділі хвороб риб Національного ветеринарного науково-дослідного інституту в Пулавах (Польща). Антимікробну чутливість досліджуваних штамів проводили методом дискової дифузії Кірбі-Бауера (1966) згідно з рекомендаціями Інституту

клінічних і лабораторних стандартів (CLSI, 2014) з нашими деякими модифікаціями. Результати антимікробного скринінгу показали, що екстракт з листя F. villosa проявляв різні антибактеріальні властивості щодо патогенів риб. Спиртовий екстракт, отриманий із листя F. villosa, виявляв максимальну антимікробну активність щодо Shewanella putrefaciens, Aeromonas sobria та Pseudomonas fluorescens. Відсоток збільшення діаметрів зон інгібування становив 122,7% для Shewanella putrefaciens, 54,9% – для Aeromonas sobria та 48,5% – для Pseudomonas fluorescens, відповідно. F. villosa виявляв помірні антибактеріальні властивості щодо Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida. Отже, штам Shewanella putrefaciens виявив найбільшу чутливість до спиртового екстракту, отриманого з листя F. villosa, тоді як штами Serratia liquefaciens і Yersinia ruckeri були найбільш стійкими до цього екстракту. Подібні дослідження демонструють, що рослинні екстракти, отримані з рослин роду Ficus, є потенційними джерелами ботанічних препаратів для боротьби з бактеріальними інфекціями в аквакультурі.

Ключові слова: Ficus villosa Blume, екстракти, антимікробна ефективність, методика дискової дифузії Кірбі-Бауера, патогени риб, чутливість, резистентність

Introduction. An intensive farming system puts fish at risk of disease outbreaks, with economic consequences. Antibiotics are commonly used to control the health of aquaculture species, but this has several disadvantages, including increasing antibiotic resistance in pathogenic bacteria and introducing antibiotic residues into the human food chain, which is a public health and environmental problem (Wei, L. S. at al., 2022). Medicinal plants and their use represent a fast, readily available, cost-effective, efficient, and environmentally friendly approach to the socio-economic and sustainable development of modern aquaculture practices (Semwal, A. at al.,2023). The potential of herbal medicines as alternatives to antibiotics for maintaining the health of aquaculture species has been explored in numerous studies (Wei, L. S. at al., 2022). Medicinal plants and their use represent a fast, readily available, cost-effective, efficient, and environmentally friendly approach to the socio-economic and sustainable development of modern aquaculture practices (Semwal, A. at al., 2023).

A wide range of medicinal plants such as herbs, seeds, and spices in various forms such as crude, extracts, blends, and active compounds are used as immunostimulants resulting in a marked enhancement of the fish's immune system to prevent and control microbial diseases (Awad, E., & Awaad, A., 2017). For example, most *in vitro* studies of plant extracts against *Streptococcus iniae* and S. agalactiae have shown antibacterial activity, and essential oils, especially those containing eugenol, carvacrol, or thymol, are more effective. Although the essential oils showed the best anti-streptococcal activity in *in vitro* assays, extracts tested *in vivo* showed moderate efficacy in increasing the survival of infected fish, probably by enhancing immunity (Van Doan, H. at al., 2022). Similar trends were observed in the study with herbal extractives performed against *Lactococcus garvieae* with no strong antibacterial activity (Soltani, M. at al., 2021).

Phytotherapy, either by immersion in water or by incorporation into the diet, is an alternative to synthetic pharmaceuticals to reduce the pathogenicity of pathogens in aquatic environments due to the presence of remarkable phytocomponents such as flavonoids, alkaloids, pigments, terpenoids, steroids, and essential oils, the medicinal plant exhibits antimicrobial, appetite-stimulating, anti-stress, growth-stimulating, and immunostimulating activity (Semwal, A. at al.,2023).

The genus *Ficus* belongs to the family Moraceae (Berg, C. C. 2003) and includes a variety of plants, from trees over 30 m tall in their countries of origin to dwarf, creeping species (Salehi, B. at al., 2021). It is one of the largest genera of angiosperms, numbering more than 800 species of moderately woody plants or trees, epiphytes, and shrubs (Singh, D. at al., 2011). They form an important part of biodiversity in many tropical regions of the world, including Indo-Australasia, Neotropics, and Afrotropics (Mon, A. M. at al., 2020). Figs have been used by humans for nutrition, healthcare, and other functional and cultural purposes throughout their distribution (Mon, A. M. at al., 2020).

Ficus villosa Blume is a (gyno)dioecious evergreen root-climber distributed in continental southern and eastern Asia to Malesia. Leaves are distichous, 9-30 cm long and 4.5-11 cm wide, (sub)coriaceous, puberulous to sub-hirsute or strigillose (entirely on only on the main veins), ovate to oblong or elliptic, symmetric, with subacuminate apex and cordate to obtuse base. Figs are axillary, in pairs or clustered (up to 8 in one cluster), also on up to 1 cm long spurs on the older wood, 0.8-1.8 cm in diameter, pedunculate or sessile, subglobose to ovoid, puberulous to glabrous, at maturity yellow to orange or crimson (Berg C.C., Corner E.J.H. 2005).

In the current study, we studied the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. villosa* against the *Aeromonas* species: *A. sobria, A. hydrophila,* and *A. salmonicida* subsp. *salmonicida,* as well as *Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. The current study was conducted as a part of an ongoing project between the Institute of Biology and Earth Sciences (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 National Academy of Sciences of Ukraine (Kyiv, Ukraine), and Ivan Franko National University in Lviv (Lviv, Ukraine) undertaken in the frame of cooperation program aimed at assessment of medicinal properties of tropical and subtropical plants (Pękala-Safińska, A., 2021; Tkachenko, H. at al., 2021; 2016 (a); 2016 (b); 2016 (c); 2016 (d); 2016 (e); 2023 (a); 2022; 2016 (f); 2023 (b).

Materials and methods.

Collection of plant material and preparing plant extract. The leaves of *F. villosa*, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. Specifically, the leaves of *F. villosa* were sampled for our study.

The sampled leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, crushed, and homogenized in 96% ethanol (in proportion 1:10) at room temperature, and centrifuged at 3,000 g for 5 minutes. Supernatants were stored at -20°C in bottles protected with laminated paper until required.

Bacterial strains for antimicrobial activity assay. The *Aeromonas* strains used in our studies were *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30). These microorganisms originated from the bacterial strain collection of the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, and had been isolated from fish of two farmed freshwater species exhibiting clinical signs of disease: common carp (*Cyprinus carpio* L.) (K886 and K825) and rainbow trout (*Oncorhynchus mykiss* Walbaum) (St30). Each isolate was inoculated onto trypticase soy agar (TSA) (BioMérieux Polska Sp. z o.o.) and incubated at 27°C ± 2°C for 24 h. Pure colonies were used for biochemical

identifications, according to the manufacturer´s instructions, except the temperature of incubation, which was at 27°C ± 1ºC. The following identification systems were used in the study: API 20E, API 20NE, API 50CH (BioMérieux Polska Sp. z o.o.). Presumptive *Aeromonas* isolates were further identified to the species level by restriction analysis of 16S rDNA genes amplified by polymerase chain reactions (PCR) Kozińska A. 2007).

Bacteria *Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens* were isolated both from apparently healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), as well as from individuals exhibiting clinical signs of the diseases, according to the procedure developed at the Department of Fish Disease of the National Veterinary Research Institute in Poland. Collected bacteria were morphologically, physiologically, and biochemically characterized by conventional methods (Austin, B., Austin, D. A., 2016). All isolates were preliminarily identified using the API system (bioMérieux, France) according to the manufacturer's instructions, except for the incubation temperature, which was 27 ± 2°C. The results were interpreted using the "apiweb" system (bioMérieux). In order to confirm the correctness of biochemical identification, sequencing was performed according to the procedures described previously (Pękala-Safińska, A. at al., (2021).

Bacterial growth inhibition test of plant extracts by the disk diffusion method. Antimicrobial susceptibility of the tested strains was performed by the Kirby-Bauer disc diffusion method (1966) (Bauer, A. W.,1966) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (2014; 2006) with some modifications. Each inoculum of particular bacteria species in the density of 0.5 McFarland was cultured on Mueller-Hinton agar. After inoculation of bacteria, a maximum of 5 wells per Petri dish with a diameter of 6 mm each was cut into the medium, and plant extracts were added to them. Plates were incubated for 24 h at 28 ± 2°C and the inhibition zones for each well were measured. For each extract, eight replicates were assayed. The plates were observed and photographs were taken. Zone diameters were determined and averaged. Ethanol (at 96% strength, POCH, Poland) as used to prepare the extracts was also used as the negative control for the microbiological study.

***Statistical analysis*.** Statistical analysis of the data obtained was performed by employing 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 test by ranks was applied to the data (Zar, J.H., 1999). All statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Poland). The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm (Okoth, D. A., at al., 2013).

Results. Results on *in vitro* antimicrobial activity assessment of ethanolic extracts derived from leaves of *F. villosa* against some strains of fish pathogens expressed as a mean of diameters of inhibition zone is presented in Figure 1.

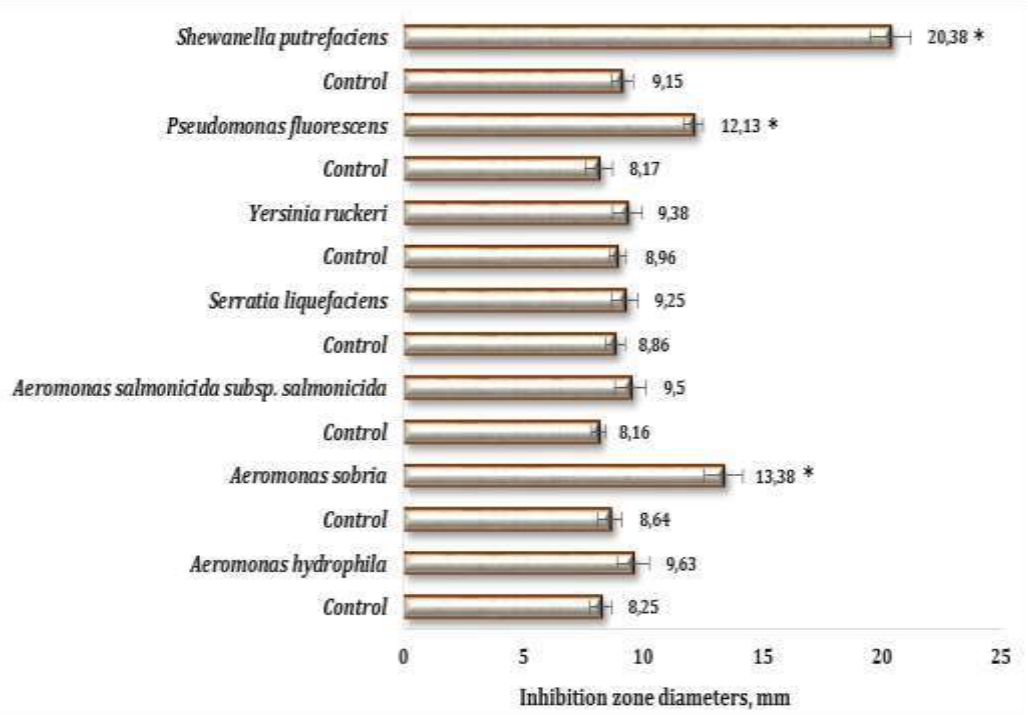

Fig. 1. **The mean inhibition zone diameters induced by ethanolic extract derived from leaves of *F. villosa* against some strains of fish pathogens such as *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens* (1000 µL inoculum) (M ± m, n = 8).**
**– changes are statistically significant compared to the 96% ethanol.*

The ethanolic extract derived from leaves of *F. villosa* exhibited the maximum antimicrobial activity against *Shewanella putrefaciens* (the mean of inhibition zone diameters was 20.38 ± 0.86 mm), *Aeromonas sobria* (13.38 ± 0.82 mm), and *Pseudomonas fluorescens* (12.13 ± 0.40 mm). The percentage of increase in the diameters of inhibition zones was 122.7% for *Shewanella putrefaciens*, 54.9% for *Aeromonas sobria*, and 48.5% for *Pseudomonas fluorescens*, respectively. *F. villosa* exhibited mild antibacterial properties against *Serratia liquefaciens* (the mean of inhibition zone diameters was 9.25 ± 0.56 mm), *Yersinia ruckeri* (9.38 ± 0.63 mm), *Aeromonas hydrophila* (9.63 ± 0.67 mm), *Aeromonas salmonicida* subsp. *salmonicida* (9.50 ± 0. 65 mm). The percentage of increase in the diameters of inhibition zones was 4.41% for *Serratia liquefaciens*, 4.69% for *Yersinia ruckeri*, 16.7% for *Aeromonas hydrophila*, and 16.4% for *Aeromonas salmonicida* subsp. *salmonicida*. Thus, the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and *Yersinia ruckeri* strains were resistant to this extract (Fig. 1).

Discussion. Our results of the antimicrobial screening revealed, that *F. villosa* possessed antibacterial properties against some strains of fish pathogens such as *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30), *Serratia liquefaciens*, *Yersinia ruckeri*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*. Results of our study revealed that the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and *Yersinia ruckeri* strains were resistant to this extract (Fig. 1).

Aeromonas hydrophila is a Gram-negative, rod-shaped, facultatively anaerobic,

non-spore-forming bacteria that is autochthonous and widely distributed in aquatic environments (Yu, H. B.,at al., 2004). Around the globe in warm water fisheries, bacteria belonging to this genus cause the most common diseases (Chowdhury, H., at al., 2023). Plant extracts and essential oils (EO) exhibited different antimicrobial activity against *Aeromonas hydrophila*, and the results of these studies were documented in many works (Al Laham, S. A., & Al Fadel, F. M. 2014) ;Chowdhury, H., at al., 2023; Sutili, F. J., at al., 2015). For example, Sutili and co-workers (2015) investigated the *in vitro* antibacterial activity of the essential oils (EOs) of *Hesperozygis ringens* (Benth.) Epling (HREO), popularly known as 'espanta-pulga' and two different species of basil, *Ocimum gratissimum* L. (OGEO) and *Ocimum americanum* L. (OAEO), as well as, the potential of these products to be used in silver catfish (*Rhamdia quelen*) infected with *Aeromonas hydrophila*. All tested EOs showed *in vitro* antibacterial properties against *A. hydrophila*; HREO and OAEO showed potential to be used in the treatment of infected fish. These products can be used in aquaculture as therapeutic and prophylactic agents against fish pathogens, with antimicrobial and immunostimulant properties (Sutili, F. J., at al., 2015). Essential oil of *Cymbopogon flexuosus* (Nees ex Steud.) Will.Watson (Poaceae family) was studied by Chowdhury and co-workers (2023) *in vitro* for its antibacterial efficacy against two oxytetracycline (OTC) resistant and one sensitive strain of *Aeromonas hydrophila*. This study, based on both *in vitro* and *in silico* methods, indicates the essential oil of *Cymbopogon flexuosus* as a potential antibacterial agent in fish disease control caused by the Aeromonads in aquaculture. The study also illustrates the favorable physicochemical and pharmacokinetic properties and drug-likeness of the essential oil components. However, the toxicological properties of the molecules did not conform with the literature reports necessitating further *in vitro* and *in vivo* toxicological validations (Chowdhury, H. at al., 2023). Al Laham and Al Fadel (2014) investigated the anti-bacterial activity shown by the extracts prepared from different parts of *Olea europea* L., *Myrtus communis* L., *Thymus vulgaris* L., *Rosmarinus officinalis* L., and *Achillea falcata* L. that grow in Syria against *A. hydrophila*. Ethanolic extracts of the studied plants had different antibacterial effects against antibiotic-resistant *A. hydrophila*. *T. vulgaris* had the highest activity, *R. officinalis* had the second, and *M. communis* and *A. falcate* were in the third place, while *O. europea* had the weakest antibacterial activity (Al Laham, S. A., & Al Fadel, F. M., 2014).

Medicinal plant-derived essential oils provide a virtually safer alternative to chemotherapeutics for fish, consumers, and ecosystems. For example, Abd El-Hamid and co-workers (2016) verified the *in vitro* antibacterial activities as well as the *in vivo* potential values of clove oil and ciprofloxacin against *Aeromonas sobria* in African catfish (*Clarias gariepinus*) (Abd El-Hamid, M. I. at al., (2016). Apparent lower mortality rates were correlated well with both decrescent bacterial burden and significant down-regulated transcripts of representative genes in the treated groups with clove oil, followed by ciprofloxacin as a prophylactic use for 15 days; however, the essential oil apart from ciprofloxacin significantly enhanced different hematological parameters (Abd El-Hamid, M. I. at al., (2016).

Non-motile *A. salmonicida* subsp. *salmonicida* is a ubiquitous gram-negative bacterium that is the causative agent of furunculosis in salmonid fish causing hemorrhagic sepsis, ulcerative lesions, pointed bleeding, and death (Dallaire-Dufresne, S., at al., 2014); Lim, J., & Hong, S., 2020). Appiah and co-workers (2022) evaluated the antimicrobial activities of the medicinal plant *Mallotus japonicus* (L.f.) Müll.Arg. against the fish pathogenic bacteria, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardisella tarda*, and *Vibrio anguillarum*, and also describes the antimicrobial

activities of the major and minor active compounds present within the plant extract. The synergistic effects by way of a combination of these compounds were also evaluated and described. Results of the study by Appiah and co-workers (2022) revealed that *Mallotus japonicus* could be used as a prophylaxis to treat bacterial disease infections of fishes and its diethyl ether-extract has the potential of an alternative to antibiotic treatment in aquaculture (Appiah, E. K. at al., 2022). Also, Hayatgheib and co-workers (2020) evaluated *in vitro* efficacy of essential oils and their compounds alone or in combination against *Aeromonas salmonicida* subsp. *salmonicida*, the causative agent of furunculosis in salmonid fish. Cinnamon, oregano, clove, and thyme oils and their major phytochemical compounds showed strong activities against *A. salmonicida* subsp. *salmonicida* strains (Hayatgheib, N. at al.,2020).

Serratia liquefaciens is considered a pathogenic bacterium of fish, and it causes infection leading to heavy mortalities in Atlantic salmon populations (McIntosh, D., & Austin, B.,1990). *Serratia liquefaciens* infections must be taken into consideration in the aquaculture industry due to the fact that the disease can cause economic losses however low the number of infection cases is (Aydin, S., at al., 2001). The antimicrobial activities of lemon oil-based nanoemulsion and two different concentrations of lemon essential oil (100% and 10%) on food-borne pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Salmonella paratyphi* A) and fish spoilage bacteria (*Photobacterium damselae*, *Enterococcus faecalis*, *Vibrio vulnificus*, *Proteus mirabilis*, *Serratia liquefaciens*, and *Pseudomonas luteola*) were compared by Yazgan and co-workers (2019) in terms of disc diffusion, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). It was found that lemon nanoemulsion was more effective on food-borne pathogens except *K. pneumoniae* than 100% lemon essential oil. 10% lemon essential oil showed the highest inhibition effect on *S. paratyphi* A. The conversion of the essential oil into nanoemulsion improved antimicrobial activity. According to the value of MIC, both nanoemulsion and 100% essential oil inhibited bacterial growth of all of the pathogen bacteria tested whereas they were less effective in inhibition of fish spoilage bacteria. However, 10% essential oil was more effective on spoilage bacteria than pathogens (Yazgan, H. at al., 2019).

Enteric redmouth disease (ERM) is a severe septic bacterial disease of salmon species caused by the Gram-negative rod-shaped enterobacterium *Yersinia ruckeri*. It has a wide host range and wide geographic distribution. The disease got its name from subcutaneous hemorrhages; it can occur in the corners of the mouth, on the gums, and on the tongue (Kumar, G. at al., 2015). The disease mainly affects salmonids, and outbreaks have a significant economic impact on fish farms and the fish aquaculture industry around the world (Wrobel, A. at al., 2019). In the 21-day study, Terzi and co-workers (2021) examined the effects of the aqueous methanolic extract of thin-skinned plum (*Prunus domestica*) on growth, immune response, and resistance to a pathogenic bacterium *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). The thin-skinned plum aqueous methanolic extract could improve innate immunity, and survival against *Y. ruckeri* and decrease the feed conversion ratio level (Terzi, E. at al., 2021). The effects of dietary Myrtle (*Myrtus communis* L.) on non-specific immune parameters and bactericidal activity of skin mucus in rainbow trout (*Oncorhynchus mykiss*) fingerlings were conducted by Mansouri Taee and co-workers (2017). No antibacterial activity was detected against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* in all treatments and control group, whereas skin mucus of rainbow trout showed antimicrobial activity against fish pathogens (*Aeromonas hydrophila* and *Yersinia ruckeri*) in 1 and 1.5% Myrtle treatments. These results indicated the beneficial effects

of dietary Myrtle on the mucosal immune parameters of fingerling rainbow trout (Mansouri at al., 2017).

A 56-day research was performed by Hoseinifar and co-workers (2020) to examine the influence of graded levels [0 (control), 0.5, 1, and 2%] of Fern (*Adiantum capillus-veneris*) leaves powder (FLP) in the diet on immune competence and growth of common carp (*Cyprinus carpio*) (Hoseinifar, S. H. at al., 2020). The serum bactericidal activity against human and fish pathogens increased with enhancing the FLP level in diet against *Staphylococcus aureus*, *Escherichia coli* (EHEC ATCC 43895), *Escherichia coli* (CI), *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Aeromonas hydrophila*. The serum antibacterial activity against *Yersinia ruckeri* in the 2% FLP group was higher than the other treatments. Furthermore, the serum bactericidal activity against *P. aeruginosa* (ATCC 27853) was only observed in fish fed on the 1 and 2% FLP-supplemented diets. The skin mucosal bactericidal activity and inhibitory effects increased with enhancing the FLP level in the diet against *E. coli*, *K. pneumonia*, *Y. ruckeri*, and *A. hydrophila* in a dose-response manner. Moreover, the skin mucosal bactericidal activity against *S. aureus* was only observed in fish fed on 1 and 2% FLP-supplemented diets. Thus, by considering serum and mucosal bactericidal activities against different pathogenic bacteria, the supplementation of 2% FLP in the diet is recommended for *C. carpio* during the grow-out phase (Hoseinifar, S. H. at al., 2020). Baba and co-workers (2018) investigated the effects of olive leaf (*Olea europea* L.) extract (OLE) on the control of *Yersinia ruckeri* infection in rainbow (*Oncorhynchus mykiss*) trout and assessed the impact on the expression of immune-related genes in the spleen and serum biochemical parameters of rainbow trout. These researchers revealed that OLE/ especially at 0.1% added feed may effectively enhance the serum biochemical parameters, survival rate, and immune gene expression in rainbow trout (Baba, E. at al., 2018).

Pseudomonas fluorescens is one of the main causes of septicemic diseases among freshwater fish, causing severe economic losses and decreasing farm efficiency (Shabana, B. M., at al., 2022). Moreover, this strain is a specific spoilage microorganism of refrigerated marine fish and is highly adapted to low temperatures (Xu, J. at al.,2021). Hardi and co-workers (2018) described the antibacterial and immunostimulant abilities of *Boesenbergia pandurata* (Roxb.) Schltr. (BP), *Solanum ferox* Mill. ex Dunal (SF), and *Zingiber zerumbet* (L.) Sm. (ZZ) plant extracts to treat and prevent *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection on Tilapia (*Oreochromis niloticus*). Various extract combinations were 60 mg SF extract/kg feed with 40 mg ZZ/kg feed (SF60/ZZ40), SF50/ZZ50, BP90/SF10, and BP50/SF50. The number of pathogenic bacteria in fish-fed combined extracts was lower than the control at week 4. In both trials, the percentage of survival rate and relative percent survival of tilapia fed SF 50/ZZ 50, showed the optimum results compared to the other combinations. Thus, the combined extract in feed, especially SF50/ZZ50 has a positive effect on the tilapia's innate immune system of tilapia to treat and prevent bacterial infections (Hardi, E. H. at al., 2018).

The *Shewanella putrefaciens* group are ubiquitous microorganisms recently isolated from different freshwater fish species and causing serious health disorders, as well as a typical spoilage bacteria organism in seafood (Paździor, E., at al., 2019; Zhang, W., at al., 2022). Wu and co-workers (2018) explored the effect of adding traditional Chinese medicine (TCM) in feed on the intestinal microbiota of gibel carp (*Carassius auratus gibelio*). The results showed that the composition and structure of the bacterial community were significantly altered by the TCM feeding. At the genus level, the addition of TCM tended to reduce the incidence of potential pathogens

(*Aeromonas, Acinetobacter,* and *Shewanella*), while stimulating the emergence of some potential probiotics (*Lactobacillus, Lactococcus, Bacillus,* and *Pseudomonas*). These data suggested that the feed additive could regulate the fish intestinal microbiota by reinforcing the microbial balance (Wu, Z. B. at al., 2018)).

Therapeutic potential for the use of various plants of the *Ficus* genus in the control of bacterial diseases was evaluated against fish pathogens in *in vitro* study with promising results (Pękala-Safińska, A. at al., 2021; Tkachenko, H. at al., 2021; 2016 (a); 2016 (b); 2016 (c); 2016 (d); 2016 (e); 2023 (a); 2022; 2016 (f); 2023 (b). In our previous study, the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* species against *Citrobacter freundii* was evaluated (Tkachenko, H., at al., 2016 (b). The results proved that the extracts from *F. drupacea, F. septica, F. deltoidea*, as well as *F. hispida, F. mucuso, F. pumila, F. craterostoma,* exhibit favorable antibacterial activity against *C. freundii* (200 µL of standardized inoculum) (Tkachenko, H., at al., 2016 (b). 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* possess considerably sufficient antibacterial potential against *C. freundii* (Tkachenko, H. at al., 2016 (b). Among various species of *Ficus* screened ethanolic extracts of the leaves of ten *Ficus* species: *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 pathogen tested) were the most effective against *P. fluorescens* (200 µL of standardized inoculum) (Tkachenko, H. at al., 2016). Moreover, previous investigation has shown that the most effective against *P. fluorescens* (400 µL of standardized inoculum) were the ethanolic extracts obtained from leaves of ten *Ficus* species: *F. craterostoma, F. cyathistipula, F. drupacea* 'Black Velvet', *F. hispida, F. macrophylla, F. mucuso, F. pumila, F. villosa (*Tkachenko, H. at al., 2016 (e). In our study, most ethanolic extracts derived from *Ficus* spp. proved effective against the bacterial strain of Gram-negative *A. hydrophila* tested, with 10-12 mm zones of inhibition being observed. *A. hydrophila* demonstrated the highest susceptibility to *F. pumila*. The highest antibacterial activity against *A. hydrophila* (200 µL of standardized inoculum) was displayed by *F. benghalensis, F. benjamina, F. deltoidea, F. hispida, F. lyrata* leaf extracts (Tkachenko, H. at al., 2016(c). Among various species of *Ficus* genus exhibiting moderate activity against *A. hydrophila* (400 µL of standardized inoculum), the highest antibacterial activity was displayed by *F. benghalensis, F. benjamina, F. deltoidea, F. hispida, F. lyrata* leaf extracts (Tkachenko, H., at al., 2016 (d); Tkachenko, H., at al., 2016 (f).

Conclusions. In the current study, we studied the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. villosa* against the *Aeromonas* species: *A. sobria, A. hydrophila,* and *A. salmonicida* subsp. *salmonicida,* as well as *Serratia liquefaciens, Yersinia ruckeri, Pseudomonas fluorescens, Shewanella putrefaciens* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. Results of the antimicrobial screening revealed, that *F. villosa* possessed different antibacterial properties against fish pathogens. The ethanolic extract derived from leaves of *F. villosa* exhibited the maximum antimicrobial activity against *Shewanella putrefaciens*, *Aeromonas sobria,* and *Pseudomonas fluorescens*. The percentage of increase in the diameters of inhibition zones was 122.7% for *Shewanella putrefaciens*, 54.9% for *Aeromonas sobria,* and 48.5% for *Pseudomonas fluorescens*, respectively. *F. villosa* exhibited mild antibacterial properties against *Serratia liquefaciens, Yersinia ruckeri, Aeromonas hydrophila, Aeromonas salmonicida* subsp. *salmonicida*. Thus, the *Shewanella putrefaciens* strain exhibited the highest sensitivity to the ethanolic extract derived from leaves of *F. villosa*, while *Serratia liquefaciens* and

Yersinia ruckeri strains were resistant to this extract.

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