Research Article

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Antibacterial activity of extracts derived from leaves of *Ficus elastica* Roxb. ex Hornem. (Moraceae) and its cultivars against three *Aeromonas* spp. strains

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Article Details:
Received: 2023-05-04
Accepted: 2023-05-19
Available online: 2023-05-31

DOI: https://doi.org/10.15414/ainhlq.2023.0008

The present study aimed to evaluate the antimicrobial activity of the ethanolic extracts derived from the leaves of *Ficus elastica* Roxb. ex Hornem. and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas salmonicida* subsp. *salmonicida* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. The leaves of *F. elastica* and its cultivars, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine. Specifically, the leaves of *F. elastica* and its cultivars were sampled for our study. Three *Aeromonas* strains: *Aeromonas sobria* (K825) and *Aeromonas hydrophila* (K886), as well as *Aeromonas salmonicida* subsp. *salmonicida* (St30), originated from freshwater fish species such as common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), respectively, were isolated in Department of Fish Diseases, National Veterinary Research Institute in Puławy (Poland). Antimicrobial susceptibility of the tested *Aeromonas* strains was performed by the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014), with some modifications. Our results of the antimicrobial screening revealed, that *F. elastica* and its cultivars possessed mild antibacterial properties against the *A. sobria* and *A. hydrophila* strains. The ethanolic extract derived from leaves of *F. elastica* 'Variegata' exhibited the maximum antimicrobial activity against *A. sobria*, while the ethanolic extract derived from leaves of *F. elastica* exhibited the maximum antimicrobial activity against *A. hydrophila* and *Aeromonas salmonicida* subsp. *salmonicida* strains. The results of this study provide baseline information on the potential validity of extracts derived from leaves of *F. elastica* and its cultivars in the treatment of infections associated with fish pathogen *Aeromonas* spp..

Keywords: *Ficus elastica*, antimicrobial efficacy, Kirby-Bauer disk diffusion technique, fish pathogens, susceptibility, resistance

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Introduction

Bacterial and viral diseases are the most serious type of disease affecting aquatic animals and a serious obstacle to the development of the aquaculture industry (Liao et al., 2022). Antibiotics and chemicals are common means to prevent and control fish diseases, but their use is currently limited or even banned due to serious issues such as drug residues, pathogen resistance, and environmental pollution (Zhang et al., 2022). In aquaculture, medicinal herbs, and extracts are increasingly promising supplements and alternatives due to their effectiveness, safety, environmental friendliness, and less drug resistance (Valladão et al., 2015). Herbal essential oils contain many bioactive components with powerful antibacterial, antioxidant, and immune-boosting properties, suggesting their use in aquatic animals (Dawood et al., 2022). Medicinal herbs and their extracts can affect growth performance and stimulate the immune system when used in a fish diet. In addition, the use of herbal medicines and their extracts can reduce oxidative stress caused by several stressors in fish farming (Ahmadifar et al., 2021). A wide range of medicinal plants such as herbs, seeds, and spices in various forms such as raw, extracts, mixed, and active compounds are used as immunostimulants and result in a marked boost in the immune system of fish to prevent and control microbial diseases (Awad and Awaad, 2017). Some of these herbs are *Ficus* species that have a long history of use as a food source, in medicine, planting, and other industries and fields of human activity, partly owing to their great diversity and wide distribution range. Among popular ethnomedicinal uses of *Ficus* are treatments of skin damage, disorders of the digestive system and related organs, and parasitic infections. Besides these, the range of healing targets for particular *Ficus* species compiled from local medicines can be competitive with that of broad-spectrum traditional remedies (Lansky and Paavilainen, 2011).

Ficus elastica Roxb. ex Hornem. is a large monoecious evergreen (rarely deciduous) tree up to 30 m tall. The species is considered to naturally originate from NE India, Myanmar, Malay Peninsula, Sumatra, and Java, but is also commonly cultivated in that areas and throughout the world. It belongs to those species known as hemi-epiphytes, which start life as an epiphyte in the crown of another tree and then send roots down to the ground enveloping the trunk of the host tree. Although usually occurring in forests, this species can also grow as a terrestrial tree or shrub in dry habitats such as cliffs and limestone hills. Its glabrous coriaceous spirally arranged leaves reach 10–40 cm in length and 5–22 cm in width; they are elliptic to oblong with an acuminate apex and cuneate to obtuse or rounded base. The pedunculate glabrous figs of 1.0–1.5 cm in diameter are born axillary or just below the leaves, in pairs or solitary, and turn yellow at maturity (Berg and Corner, 2005).

Standardized extracts of *F. elastica* could be used in traditional medicine for the treatment of wounds and other topical infections (Mbosso et al., 2012). Also, *F. elastica* extracts revealed significant *Schistosoma mansoni* worm reductions and exhibited antischistosomal activity (Seif el-Din et al., 2014). Mbosso Teinkela et al. (2018) revealed *in vitro* cell-growth inhibition activities by methanolic extract of *F. elastica* against *Plasmodium falciparum* strain 3D7 and *Trypanosoma brucei brucei*, as well as against HeLa human cervical carcinoma cells. At the 25 $\mu g.mL^{-1}$ concentration, the extract of *F. elastica* exhibited plasmodiacidal activity (IC_{50} value of 9.5 $\mu g.mL^{-1}$) and trypanocidal (IC_{50} value of 0.9 $\mu g.mL^{-1}$) activity. Extract presented low cytotoxic effects on the HeLa cancer cell line (Mbosso Teinkela et al., 2018). Leaf extract of *F. elastica* is employed as a diuretic agent besides treating skin infections and allergies (Phan et al., 2012).

In the current study, we studied the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. elastica* and its cultivars (*F. elastica* 'Rubra', 'Robusta', 'Burgundy', 'Variegata') against *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas salmonicida* subsp. *salmonicida* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture.

Material and methodology

Collection of plant materials and preparing plant extract

The leaves of *F. elastica* and its cultivars (Figure 1), cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine (Kyiv). Specifically, the leaves of *F. elastica* and its cultivars, i.e. Rubra, Robusta, Burgundy, Variegata 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 3000 g for 5 minutes. Supernatants were stored at -20 °C in bottles protected with laminated paper until required.

Figure 1 General view of Ficus elastica Roxb. ex Hornem. plant (A) and a leaf of this plant (B)
Photo: Yevhen Sosnovsky

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, cultivated *in vitro*.

Bacterial strains for antimicrobial activity assay

Three *Aeromonas* strains: *Aeromonas sobria* (K825) and *Aeromonas hydrophila* (K886), as well as *Aeromonas salmonicida* subsp. *salmonicida* (St30), originated from freshwater fish species such as common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), respectively, were isolated in the Department of Fish Diseases, National Veterinary Research Institute in Puławy (Poland). Bacteria were collected from fish exhibiting clinical disorders. Each isolate was inoculated onto trypticase soy agar (TSA) (bioMériux) and incubated at 27 ±2 °C for 24 h. Pure colonies were used for biochemical identifications, according to the manufacturer´s instructions, except for the temperature of incubation, which was at 27 ±1 °C. The following identification systems were used in the study: API 20E, API 20NE, and API 50CH (bioMériux). Presumptive *Aeromonas* isolates were further identified to the species level by restriction analysis of 16S rDNA genes amplified by polymerase chain reactions (PCR) (Kozińska, 2007).

Bacterial growth inhibition test of plant extracts by the disk diffusion method

Antimicrobial susceptibility of the tested *Aeromonas* 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 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, 1999). All statistical analyses were performed using Statistica 13.3 software (TIBCO Software Inc.). 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 et al., 2013).

Results and discussion

The genus *Aeromonas* includes a collection of ubiquitous Gram-negative rods widely distributed in the aquatic environment (Colwell et al., 1986). The genus *Aeromonas* can be divided into motile and non-motile species (Janda and Abbott, 2010). Currently, 31 species are described in the genus (Fernández-Bravo and Figueras, 2020). 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; Park et al., 2020). *Aeromonas sobria* is a Gram-negative, uniflagellate, rod-shaped, motile, facultative anaerobic bacterium of the genus *Aeromonas* (Taslimi et al., 2018). It is widely distributed in natural environments, including water, soil, feces, etc., and is an opportunistic bacterium for humans, aquatic animals, livestock, and poultry (Zhang et al., 2021). Results on *in vitro* antimicrobial activity assessment of ethanolic extracts derived from leaves of *F. elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas sobria* strain expressed as a mean of diameters of inhibition zone is presented in Figure 2.

Our results of the antimicrobial screening revealed, that *F. elastica* and its cultivars possessed mild antibacterial properties against the *A. sobria* strain. The ethanolic extract obtained from leaves of *F. elastica* 'Variegata'

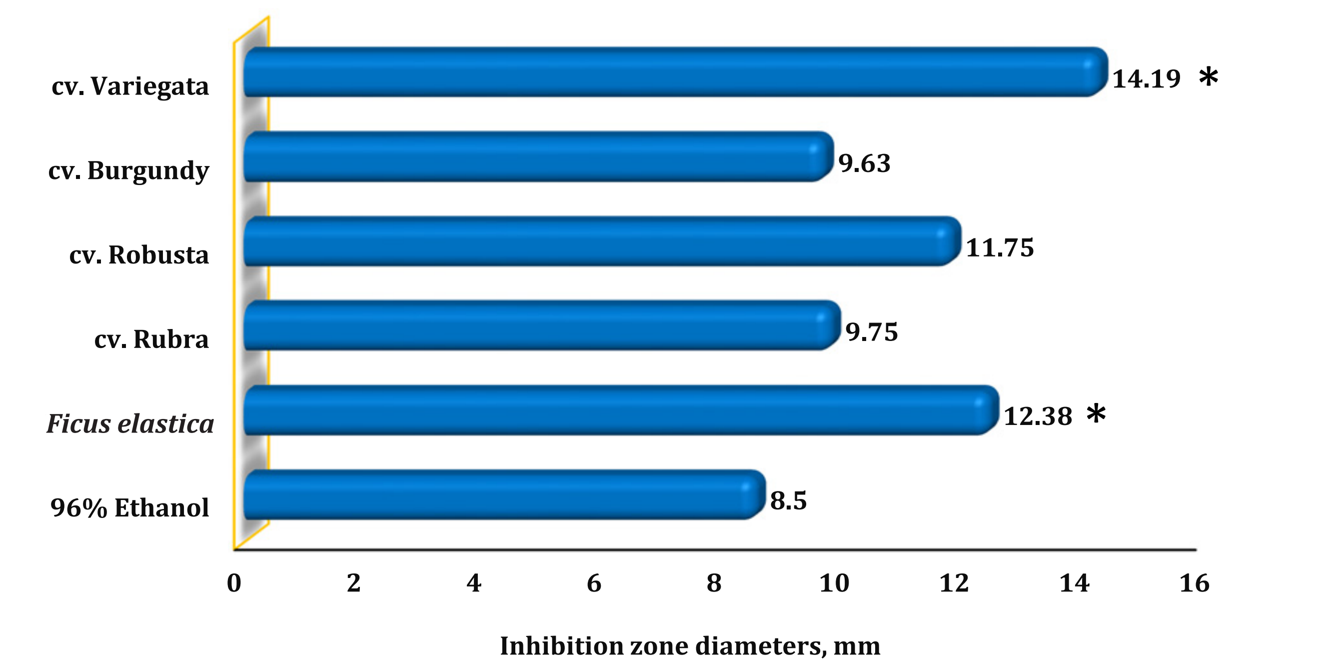

Figure 2 The mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *Ficus elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas sobria* strain (1000 µL inoculum) (M ±m, n = 8)
*– changes are statistically significant compared to the 96% ethanol

exhibited the maximum antimicrobial activity against *A. sobria* (the mean of inhibition zone diameters was 14.19 ±0.73 mm). *A. sobria* strain was susceptible to the *F. elastica* (12.38 ±0.82 mm) and 'Robusta' (11.75 ±0.53 mm). *A. sobria* strain was the most resistant to *F. elastica* 'Rubra' (9.75 ±0.41 mm) and *F. elastica* 'Burgundy' (9.63 ±0.38 mm) leaf extracts. Statistically significant increase in the mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *F. elastica* and its cultivars was demonstrated for *F. elastica* (by 45.6%, p <0.05) and *F. elastica* 'Variegata' (by 66.9%, p <0.05) (Figure 2).

Aeromonas hydrophila is a Gram-negative bacterium that is widely distributed in the aquatic environment and can cause septicemia in both fish and humans (Ji et al., 2015). The disease affects many aquaculture sectors potentially requiring antimicrobial treatments (Gieseker et al., 2022). Results on *in vitro* antimicrobial activity assessment of ethanolic extracts derived from leaves of *F. elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas hydrophila* strain expressed as a mean of diameters of inhibition zone is presented in Figure 3.

Our results of the antimicrobial screening revealed, that *F. elastica* and its cultivars possessed mild antibacterial properties against the *A. hydrophila* strain. The ethanolic extract obtained from leaves of *F. elastica* exhibited the maximum antimicrobial activity against *A. hydrophila* (the mean of inhibition zone diameters was 12.38 ±0.82 mm). *A. hydrophila* strain was susceptible to the *F. elastica* (12.38 ±0.82 mm) and 'Robusta' (10.31 ±0.49 mm). *A. hydrophila* strain was the most resistant to leaf extracts derived from *F. elastica* 'Rubra' (9.25 ±0.59 mm), *F. elastica* 'Variegata' (9.69 ±0.62 mm), and *F. elastica* 'Burgundy' (9.50 ±0.50 mm). A statistically significant increase in the mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *F. elastica* and its cultivars was demonstrated for *F. elastica* (by 43.1%, p <0.05) (Figure 3).

Aeromonas salmonicida, which is known as the only non-motile species in the genus *Aeromonas*, is an important pathogen in salmonid aquaculture and is responsible for typical furunculosis (Vanden Bergh and Frey, 2014). Furunculosis is a ubiquitous disease that affects aquaculture operations worldwide and is characterized by high mortality and morbidity (Dallaire-Dufresne et al., 2014). Results on *in vitro* antimicrobial activity assessment of ethanolic extracts derived from leaves of *F. elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas salmonicida* subsp. *salmonicida* strain expressed as a mean of diameters of the inhibition zone is presented in Figure 4.

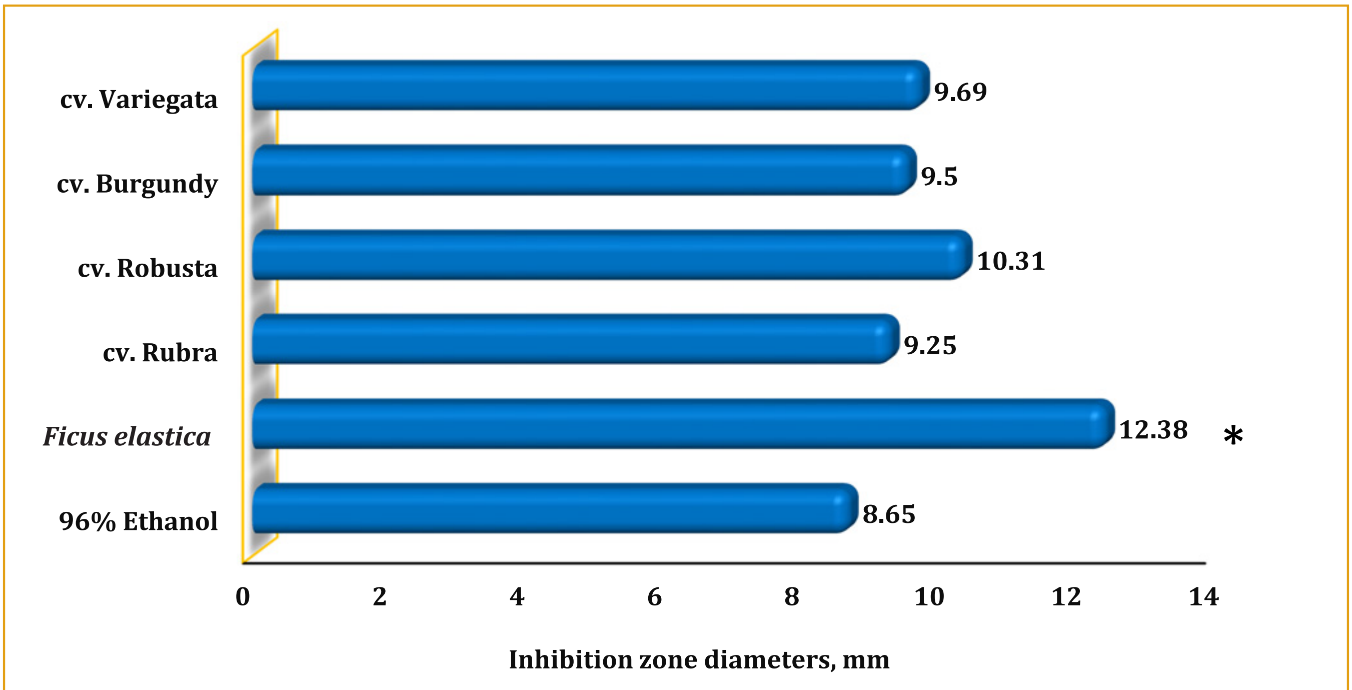

Figure 3 The mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *Ficus elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas hydrophila* strain (1000 μL inoculum) (M ±m, n = 8)

*– changes are statistically significant compared to the 96% ethanol

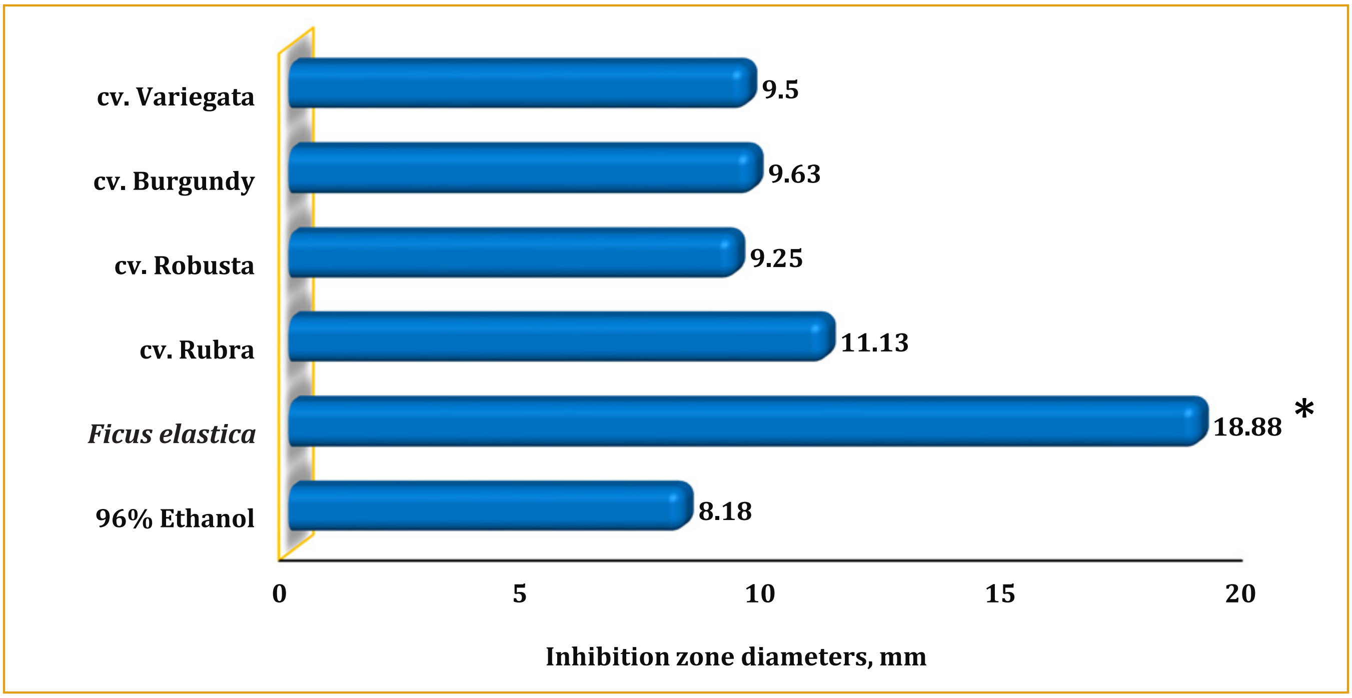

Figure 4 The mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *Ficus elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas salmonicida* subsp. salmonicida strain (1000 μL inoculum) (M ±m, n = 8)
*– changes are statistically significant compared to the 96% ethanol

Our results of the antimicrobial screening revealed, that *F. elastica* and its cultivars possessed mild antibacterial properties against the A. *salmonicida* strain. The ethanolic extract derived from leaves of *F. elastica* exhibited the maximum antimicrobial activity against A. *salmonicida* (the mean of inhibition zone diameters was 18.88 ±0.48 mm). A. *salmonicida* strain was susceptible to *F. elastica* 'Rubra' (11.13 ±0.74 mm). A. *salmonicida* strain was the most resistant to leaf extracts derived from *F. elastica* 'Robusta' (9.25 ±0.56 mm), *F. elastica* 'Variegata' (9.50 ±0.33 mm), and *F. elastica* 'Burgundy' (9.63 ±0.46 mm). A statistically significant increase in the mean inhibition zone diameters induced by ethanolic extracts derived from leaves of *F. elastica* and its cultivars was demonstrated for *F. elastica* (by 130.8%, $p < 0.05$) (Figure 4).

Moreover, in our previous study (Opryshko et al., 2020), we evaluated the *in vitro* possible antioxidant effects of extracts derived from leaves of *F. elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) using oxidative stress biomarker [2-thiobarbituric acid reactive substances (TBARS) as a biomarker of lipid peroxidation] using of human erythrocytes as a cell model after incubation with plant extracts in two doses (5.0 and 0.5 mg.mL^{-1}). Our results revealed that treatment of human erythrocytes by extracts derived from leaves of *F. elastica* and its cultivars 'Rubra' and 'Burgundy' in the dose of 0.5 mg.mL^{-1} caused a statistically significant decrease of TBARS level by 27.3% ($p < 0.05$), 32.4% ($p < 0.05$), and 33.5% ($p < 0.05$), respectively. The increase in TBARS level was observed after the treatment of human erythrocytes by extracts derived from leaves of *F. elastica* 'Robusta' and 'Variegata' (by 12.3 and 9.3%, $p > 0.05$, respectively) compared to untreated controls. After treatment of human erythrocytes by extracts derived from leaves of *F. elastica* and its cultivars (Rubra, Burgundy, and Robusta) in the dose 5 mg.mL^{-1}, the increase of TBARS level (by 5.7%, 39.5%, 82%, and 87.5%, $p < 0.05$) was observed. Only extract derived from leaves of *F. elastica* 'Variegata' (5 mg.mL^{-1}) caused the decrease in TBARS level (by 29.2% $p < 0.05$) compared to untreated controls. Among extracts studied (0.5 mg.mL^{-1}), *F. elastica* 'Burgundy' exhibited the lowest TBARS level (decreased by 33.5%, $p < 0.05$) while in dose 5 mg.mL^{-1}, *F. elastica* 'Variegata' decreased TBARS level by 29.2% ($p < 0.05$) (Opryshko et al., 2020).

We also evaluated the *in vitro* effect of extracts obtained from leaves of *Ficus elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) on the levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of the rainbow trout (*Oncorhynchus mykiss* Walbaum) (Tkachenko et al., 2022). Our results revealed that the incubation of muscle tissue of rainbow trout with extracts derived from the leaves of *F. elastica* and its cultivars resulted in the same levels of aldehydic derivatives of OMP compared to the untreated samples. On the other hand, the levels of ketonic derivatives of OMP were statistically non-significant decreased to the values 12.83 ±1.0 nmol.mg^{-1} protein for *F. elastica* extract, 12.03 ±1.26 nmol.mg^{-1} protein for *F. elastica* 'Rubra' extract, 12.89 ±1.25 nmol.mg^{-1} protein for *F. elastica* 'Robusta' extract, 11.81 ±1.21 nmol.mg^{-1} protein for *F. elastica* 'Burgundy' extract, 12.39 ±1.35 nmol.mg^{-1} protein for *F. elastica* 'Variegata' extract compared to the untreated samples (14.16 ±1.02 nmol.mg^{-1} protein). The percentage of decreased levels of ketonic derivatives of OMP in the muscle tissue of rainbow trout after incubation with extracts derived from leaves of *F. elastica* and its cultivars compared to the values of untreated controls was as follows: 9.4% for *F. elastica* extract, 15% for *F. elastica* 'Rubra' extract, 9% for *F. elastica* 'Robusta' extract, 16.6% for *F. elastica* 'Burgundy' extract, 12.5% for *F. elastica* 'Variegata' extract, respectively. Thus, two extracts derived from leaves of *F. elastica* 'Burgundy' and *F. elastica* 'Rubra' after incubation with muscle tissue of rainbow trout resulted in the maximum decrease in the levels of ketonic derivatives of OMP. The present study ascertained the antioxidant potency of the extracts derived from the leaves of *F. elastica* and its cultivars as a potential source of natural antioxidants (Tkachenko et al., 2022).

Many of our studies confirmed the antioxidant properties of *Ficus* plants against fish pathogens (Pękala-Safińska et al., 2021; Tkachenko et al., 2016a-e, 2022). 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 the average over the three *Aeromonas* species, the highest antimicrobial activity among all the tested ethanolic extracts was observed in *F. binnendijkii* leaves with inhibition zone diameters (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* across 18.81 ±1.25 mm and A. *salmonicida* across 20.13 ±0.79 mm diameters. *F. hispida* extract inhibited *A. sobria* the best and showed an IZD of 25.56 ±1.63 mm followed by the extracts of *F. binnendijkii* presenting an IZD of 23.75 ±1.64 mm and *F. tinctoria giving* one of 22.5 ±1.20 mm. The IZD results also showed that isolates of *A. sobria* revealed 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 created mean IZDs ranging from 10 to 15 mm. The isolates appeared to be resistant to extracts of 18 *Ficus* species (43.9%), which only restricted growth in mean IZDs of less than 10 mm (Pękala-Safińska et al., 2021).

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 (Tkachenko et al., 2016a-e, 2022). In our previous study, the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* species against *Citrobacter freundii* was evaluated. 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 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* possess considerably sufficient antibacterial potential against *C. freundii* (Tkachenko et al., 2016b). 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 et al., 2016a). 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 et al., 2016e). 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 et al., 2016c). 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 et al., 2016d).

It is generally assumed that the antibacterial activity of various *Ficus* species can be explained due to the presence of secondary metabolites that are probably responsible for the test organism's susceptibility to them. The main chemical classes of the phytochemical compounds occurring in the extracts, obtained from the plants belonging to the genus *Ficus*, are alkaloids, anthocyanins, balsams, carbohydrates, flavonoids, free anthraquinones, tannins, glycosides, amino acids, organic acids, fatty acids, terpenes, resins, phytosterols, aliphatic alcohols, volatile components and saponins (Ali and Chaudhary, 2011; Ashraf et al., 2021; Murugesu et al., 2021). The presence of alkaloids and flavonoids both reveals their activity against pathogenic bacteria and suggests a role in the limitation of fungal infection, given that many flavonoids exhibit antifungal activity (Wan et al., 2017). Among polyphenols, flavan-3-ols, flavonols, and tannins received the most attention due to their wide spectrum and higher antimicrobial activity in comparison with other polyphenols, and to the fact that most of them are able to suppress a number of microbial virulence factors (such as inhibition of biofilm formation, reduction of host ligands adhesion, and neutralization of bacterial toxins) and show synergism with antibiotics (Coppo and Marchese, 2014).

Conclusions

In the current study, we investigated the antimicrobial activity of the ethanolic extracts derived from the leaves of *F. elastica* and its cultivars (Rubra, Robusta, Burgundy, Variegata) against *Aeromonas sobria*, *Aeromonas hydrophila*, and *Aeromonas salmonicida* subsp. *salmonicida* to evaluate the possible use of this plant in preventing infections caused by this fish pathogen in aquaculture. Our results of the antimicrobial screening revealed, that *F. elastica* and its cultivars possessed mild antibacterial properties against the *A. sobria* and *A. hydrophila* strains. The ethanolic extract derived from leaves of *F. elastica* 'Variegata' exhibited the maximum antimicrobial activity against *A. sobria*, while the ethanolic extract derived from leaves of *F. elastica* exhibited the maximum antimicrobial activity against *A. hydrophila* and *Aeromonas salmonicida* subsp. *salmonicida* strains. The results of this study provide baseline information on the potential validity of extracts derived from leaves of *F. elastica* and its cultivars in the treatment of infections associated with fish pathogen *Aeromonas* spp.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

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