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# ANTIBACTERIAL EFFICACY OF LEAF EXTRACT OBTAINED FROM *FICUS LINGUA* WARB. EX DE WILD. & T. DURAND (MORACEAE) AGAINST *AEROMONAS* SPP. STRAINS

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Although many species within the genus *Ficus* L. have been encompassed by pharmacological investigations in previous years, there are many species that have not been studied and whose ethnobotanical relevance is yet to be investigated. With this background, an attempt was made to study the *in vitro* antimicrobial activity of the ethanolic extracts of various plants belonging to the genus *Ficus* against fish pathogens. This is especially important in view of the increasing resistance of bacteria observed during the last years and is an alternative to antibiotic therapy. Therefore, the aim of this study was to screen the antimicrobial activity of the ethanolic extract of *Ficus lingua* Warb. ex De Wild. & T. Durand leaf against fish pathogen – three *Aeromonas* strains (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*). The focus of this paper is on the analytical methodologies, which include the extraction of active compounds and antimicrobial activity assay by the disk diffusion technique. It was revealed that the leaf extract of *F. lingua* used in this study has bactericidal properties which make it very attractive for use in fish aquaculture. Its uses will reduce the side effects of applying synthetic compounds. The ethanolic extract obtained from leaves of *F. lingua* exhibited the maximum antimicrobial activity against *Aeromonas sobria* strain (inhibition zone diameter was 19.38 ±1.27 mm), *Aeromonas hydrophila* (16.06 ±1.05 mm), and *Aeromonas salmonicida* subsp. *salmonicida* (11.25 ±1.16 mm). The most susceptible strain to the antimicrobial activity of *F. lingua* was *Aeromonas sobria*. However, further study is needed to determine the effects of the active compounds presented in the leaf extract of *F. lingua* on fish metabolism in *in vitro* and *in vivo* study. Present results suggest the possibility of using such extracts in *in vivo* studies in order to corroborate if it could be possible using those extracts in aquaculture in order to achieve protection against pathogenic infections.

**Keywords:** *Ficus lingua* Warb. ex De Wild. & T. Durand, *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*, antimicrobial activity, disc diffusion technique, ethanolic extract

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## Introduction

The aquaculture industry is one of the fastest-growing sectors in recent decades. The global aquaculture production of food fish reached 62.7 million tonnes in 2011 and is continuously increasing with an estimated production of food fish of 66.5 million tonnes in 2012 (a 9.4% increase in 1 year (FAO, www.fao.org/fishery/topic/16140; Clarke et al., 2013). High intensity of aquacultural practices led to negative situations, i.e. environmental harm (e.g. poor quality of water), increased number of various opportunistic microorganisms and stress conditions for fish growth and health (Beltrán et al., 2018). Disease prevention is the key issue to maintain the sustainable development of the aquaculture sector, both environmentally and economically. Widespread use of antibiotics in aquaculture has led to the development of antibiotic-resistant bacteria and the accumulation of antibiotics in the environment, resulting in water and soil pollution (Clarke et al., 2013). It has been reported that antimicrobials used in aquaculture are administered to fish mostly in food and only rarely by injection or bath (Armstrong et al., 2005; Sørum, 2006). Consequently, this method of administration leads to their affecting both diseased and healthy fish (metaphylaxis) in the population (Sørum, 2006; Okocha et al., 2018). Moreover, it was highlighted that the application of antibiotics or other chemical treatments might have an effect on non-target organisms (e.g. other non-target culture fish and shellfish), whereas, the residues from plant-derived compounds tend to be biodegradable in the water (Kumar and Bossier, 2018).

In recent years the use of antibiotics to treat and control fish diseases has been banned in the EU (Beltrán et al., 2018). Prophylactic methods based on stimulation of the fish immune system (i.e., vaccination, probiotics, and immunostimulation) have been successfully used for this purpose and have become an integrated part of the management of modern aquaculture processes (Magnadottir, 2010).

Due to the increasing demand for chemical diversity in screening programs, an extensive search for therapeutic drugs from natural products to be used in aquaculture has grown throughout the world (Defoirdt et al., 2011; Cobello et al., 2013; Kumar and Bossier, 2018).

Plant-based products, such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectins, and polypeptides have been shown to be very effective alternatives to antibiotics and other synthetic compounds for an efficient treatment against microbial infection in aquaculture (Citarasu, 2010; Wunderlich et al., 2017; Kumar and Bossier, 2018).

The pantropical genus *Ficus* L., with its approximately 750 species, is the largest within the family and one of the most speciose genera of flowering plants and is a well-known genus used worldwide since ancient times in traditional and folk medicine (Berg and Wiebes, 1992; Cook and Rasplus, 2003; Berg and Corner, 2005). *Ficus* trees have a number of uses in various industries and fields of human activity. Virtually all parts of their body are utilized in ethnomedicine to cure disorders of digestive and respiratory systems, skin diseases, parasitic infections, etc. Some species have been cited to have analgesic, tonic, and ecbolic effects (Lansky and Paavilainen, 2011). Numerous studies have demonstrated that the plants belonging to the genus *Ficus* present a wide variety of secondary metabolites, most of the phenolic compounds such as flavonoids, terpenoids, phenolic acids, alkaloids, tannins and active proteins and fatty acids among others, which are the principal components responsible for its activities and allows its use in traditional medicine (Ahmed and Urooj, 2010; Singh et al., 2011; Dangarembizi et al., 2012; Badgujar et al., 2014; Bunawan et al., 2014). Among the pharmacological properties demonstrated for the compounds present in genus *Ficus* are anticonvulsant, anti-inflammatory, analgesic, antimicrobial, antiviral, hypolipidemic, antioxidant, immunomodulatory, antiasthmatic, parasympathetic modulatory, estrogenic, antitumor, antiulcer, antianxiety, antihelmintic, analgesic, tonic, anti-diabetic, antipyretic, anti-inflammatory, antitussive, hepatoprotective activities etc. (Ahmed and Urooj, 2010; Lansky and Paavilainen, 2011; Singh et al., 2011; Dangarembizi et al., 2012; Badgujar et al., 2014; Bunawan et al., 2014; Yadav et al., 2015). For all these reasons, plants belonging to the genus *Ficus* could be considered a priori as a good source of new natural compounds to treat, prevent and control fish diseases in aquaculture.

At the current time, there are intense and active investigations into natural products with biocidal activities for fish (Galina et al., 2009). It is well known that many active compounds of plants are responsible for potential bio-activities. For that reason, there has been considerable interest in the use of medicinal plants in aquaculture with a view to providing safe and eco-friendly compounds for replacing antibiotics and chemical compounds as well as to enhance immune status and control fish diseases (Awad and Awaad, 2017). In addition to the immunostimulant properties, it has also been demonstrated that many medicinal plants are also able to have other positive effects on fish, such as the stimulation of fish growth, weight gain and early maturation of cultured species (Galina et al., 2009; Biller-Takahashi and Urbinati, 2014; Newaj-Fyzul and Austin, 2015; Vallejos-Vidal et al., 2016).

*Ficus lingua* Warb. ex De Wild. & T. Durand is an evergreen shrub, sometimes with a climbing habit, or can become a tree with a spreading, much-branched crown; it can grow up to 30 meters tall (African Flowering Plants Database, Conservatoire et Jardin Botaniques; Tropical Plants Database). It often starts life as an epiphyte in the branch of a tree and can eventually send down aerial roots that, once they reach the ground, provide extra nutrients that help the plant grow more vigorously. These aerial roots can completely encircle the trunk of the host tree, constricting its growth - this, coupled with the more vigorous top growth, can lead to the fig outcompeting and killing the tree in which it is growing (Protabase – Plant Resources of Tropical Africa). The tree is traditionally used as a source of fiber from which cloth can be made. The cloth is traditionally made by removing pieces of bark from the bole and large branches, then soaking it in water for several days, drying it in the shade and then beating it with a mallet to make it supple enough for use It is sometimes grown to provide shade and as an ornamental and bonsai tree (Protabase – Plant Resources of Tropical Africa; Tropical Plants Database).

The range of *F. lingua* is tropical Africa – Liberia, through the moister parts of Africa to Uganda and Kenya, south to DR Congo, Malawi, Mozambique, and Swaziland. The habitat – evergreen humid forest; coastal bushland; coral outcrops; Hemi-epiphytic, strangler or secondarily terrestrial; sometimes growing on rocks. At elevations up to 1,200 meters (African Flowering Plants Database, Conservatoire et Jardin Botaniques; Protabase – Plant Resources of Tropical Africa).In this study, we evaluated the antimicrobial activity of the ethanolic extract of *Ficus* *lingua* against fish pathogen – three *Aeromonas* strains (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*) in order to evaluate the possible use of this plant in preventing infections caused by these bacteria in aquaculture.

**Figure 1** Twig of *Ficus lingua* Warb. ex De Wild. & T. Durand

The role of *Aeromonas* spp. as a causative agent of fish diseases has been known for decades, longer than their comparable role in causing systemic illnesses in humans (Janda and Abbott, 2010).

The current investigation was conducted as a part of an ongoing project between the Institute of Biology and Environmental Protection (Pomeranian University in Slupsk, Poland), National Veterinary Research Institute (Puławy, 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 plants, cultivated *in vitro*.

## Materials and methodology

### Collection of plant material and preparing plant extract

The leaves of *Ficus lingua* were sampled at National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine) and Botanic Garden of Ivan Franko Lviv National University (Lviv, Ukraine). The sampled leaves were brought into the laboratory for antimicrobial studies. Freshly collected leaves were washed, weighed, 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 the laminated paper until required.

### 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 (*Cyprinys carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), respectively, were isolated in Department of Fish Diseases, The National Veterinary Research Institute in Pulawy (Poland). Bacteria were collected from fish exhibiting clinical disorders. Each isolate was inoculated onto trypticase soy agar (TSA) (BioMérieux) and incubated at 27 ±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 ±1 °C. The following identification systems were used in the study: API 20E, API 20NE, API 50CH (BioMérieux). 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* isolates was performed by the Kirby-Bauer disc diffusion method (1966), according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (2014). Each inoculum of bacteria in the density of 0.5 Mc McFarland was cultured on Mueller–Hinton agar for 24 h at 28 ±2 °C prior to the determination of results. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria provided by the Clinical and Laboratory Standards Institute (2014). The results of the disk diffusion test are "qualitative," in that a category of susceptibility (i.e., susceptible, intermediate, or resistant) is derived from the test rather than a MIC (Jorgensen and Ferraro, 2009). Seven drugs representing different antimicrobial classes as quinolones, tetracyclines, sulphonamides, and phenicols were used (Table 1).

**Table 1** Antimicrobial agents used in the study

| Group of antimicrobial agents | Symbol (Oxoid®) | Active substance(s) | Concentration (µg) |
|---|---|---|---|
| **Sulphonamides** | S3 | sulphonamides | 300 |
| | SXT | sulphonamides and trimethoprim | 25 |
| **Quinolones** | OA | oxolinic acid | 2 |
| | UB | flumequine | 30 |
| | ENR | enrofloxacin | 5 |
| **Tetracycline** | OT | oxytetracycline | 30 |
| **Phenicols** | FFC | florfenicol | 30 |

After incubation, the inhibition zones were measured. Interpretation criteria have been adopted from that available for *Aeromonas salmonicida* (CLSI, 2006)

### 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). In order 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 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*) = 11–14 mm, and Resistant (*R*) ≤10 mm (Okoth et al., 2013).

## Results and discussion

Results on *in vitro* antimicrobial activity assessment of ethanolic extract obtained from *F. lingua* leaves against three *Aeromonas* strains (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*) expressed as a mean of diameters of inhibition zone is presented in Figure 2.

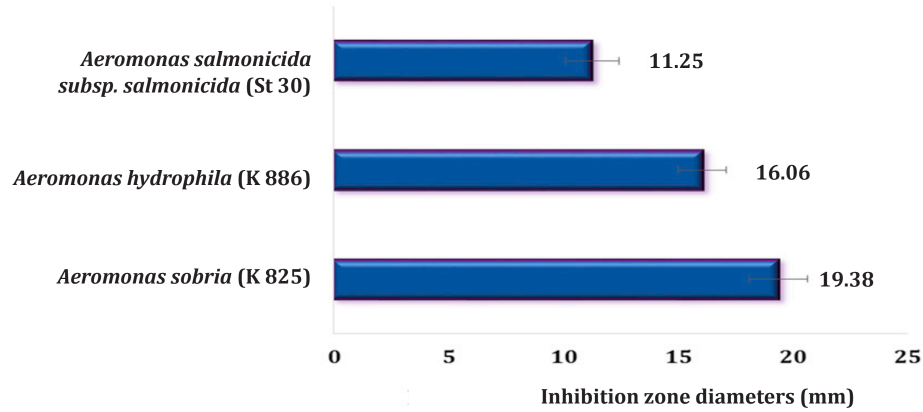


**Figure 2** The mean inhibition zone diameters induced by ethanolic extract obtained from leaves of *Ficus lingua* against *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida* strains (1,000 µL inoculum) (M ±m, $n$ = 8)

Our results of the disc diffusion screening revealed, that *F. lingua* possessed antibacterial properties against *Aeromonas* strains. The ethanolic extract obtained from leaves of *F. lingua* exhibited the maximum antimicrobial activity against *Aeromonas sobria* strain (inhibition zone diameter was 19.38 ±1.27 mm), *Aeromonas hydrophila* (16.06±1.05 mm), and *Aeromonas salmonicida* subsp. *salmonicida* (11.25 ±1.16 mm) (Figure 2). The most susceptible strain to the antimicrobial activity of *F. lingua* was *Aeromonas sobria*.

In response to the development of drug-resistant pathogens in aquaculture remarkable progress in the field of antibacterial herbal therapy has been made in recent years based on *ex situ* cultivated plants, *Ficus* genus, in particular (Tkachenko et al., 2016a-d; 2017a-c; 2018; 2019).

In our previous studies, the 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., 2016, 2017, 2018, 2019). Most ethanolic extracts obtained from *Ficus* spp. according to previous studies proved effective against the bacterial strain of Gram-negative *A. hydrophila* tested, with 10–12 mm zones of inhibition were 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., 2016). 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., 2016). Antibacterial properties of plant extracts have been by far the most studied bioactivity with potential applications in aquaculture systems (Reverter et al., 2014). Castro et al. (2008) have revealed by agar diffusion assay that 31 methanolic extracts of Brazilian plants presented antibacterial activity against the fish pathogenic bacteria, i.e. *Streptococcus agalactiae*, *Flavobacterium columnare*, and *A. hydrophila*. *F. columnare* being the most susceptible microorganism to most of the tested extracts. Similarly, Wei and Musa (2008) also studied the susceptibility by assay of minimum inhibitory concentration (MIC) of two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*), four Gram-negative bacteria (*C. freundii*, *Escherichia coli*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*) and 18 isolates of *Edwardsiella tarda* to aqueous extract of garlic (500, 250, 125, 62.5 mg/mL), and found that all garlic extracts were effective against the tested pathogenic bacteria.

The antimicrobial activity of *F. religiosa* L. bark, leaf, stem, and fruit aqueous extracts against a number of major pathogens (*Aeromonas hydrophila*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger*, and *Candida albicans*) and conducted their phytochemical analysis was screened by Rajiv and Rajeshwari (2012). All tested extracts appeared active against the pathogens at concentrations 25–100 mg/ml, the widest inhibition zone (15–16 mm) resulting from the highest concentration. Fruit extract showed generally the weakest activity and only the leaf extract affected the whole set of tested organisms at maximal concentration. Antibacterial properties of the extracts were generally better pronounced than antifungal ones. *E. coli* was most strongly inhibited by the maximal concentration extracts from the stem (inhibition zone diameter 16 mm) and leaves (15 mm) and it was unaffected by bark extract. The reference antibiotic tetracycline showed a very similar activity (16 mm) for *E. coli* at concentration 5 μg/ml. Qualitative phytochemical analysis showed the bark extract to have the richest chemical composition (sugar, alkaloids, phenols, and tannins present), being poorer in fruits (phenols and flavonoids), stem (sugar and tannins), and leaves (only tannins). Glycosides and terpenoids featured all extracts tested. Hence the most specific chemicals appeared to be alkaloids (found only in bark) and flavonoids (only in fruits), while tannins were common for the plant parts with the highest antimicrobial activity in general (i.e., bark, leaves, and stem). Although the authors present the results of the phytochemical analysis, they do not make any inferences concerning the possible contribution of particular chemical classes to the antimicrobial activity of plant extracts.

Several works have studied the immune parameters of various fish species after the dietary administration of plant extracts as well as *in vitro* and *in vivo* antibacterial and immunostimulant activity against the fish pathogens. In general, treated fish showed increased humoral (e.g. plasma protein, lysozyme, and complement activity) and cellular (phagocytic and respiratory burst activities) immune activities. For example, Sheikhlar et al. (2017) have tested *in vitro* antimicrobial activities of aqueous and methanol extracts of lemon *Citrus limon* peel, *Euphorbia hirta* (aerial parts), and fenugreek *Trigonella foenum-graecum* seeds against the bacterium *Aeromonas hydrophila*. A swab paper disk method showed that the methanol extract of *E. hirta* (EHE) had the largest inhibition zone and the lowest minimum inhibitory concentration compared to all other herbal extracts. Based on these results, EHE was included in the diets of Sharptooth Catfish *Clarias gariepinus* at 0 (control), 2, 5, or 7 g/kg of diet (experiment 1). After 30 d, the growth, feed intake, hepatosomatic index (HSI), and plasma biochemical parameters were measured. With a separate batch of Sharptooth Catfish, the efficacy of the EHE diets in conferring fish resistance to *A. hydrophila* over 30 d was compared to that of a diet containing oxytetracycline (OTC; experiment 2). The un-treated fish (Control group) were fed the control diet and were not injected with *A. hydrophila*, while the Control-AH and OTC-AH groups were infected with *A. hydrophila* and were fed either the control diet or the diet containing OTC at 1 g/199 g. The other three treatments included fish that were injected with *A. hydrophila* but fed diets with increasing EHE at 2, 5, or 7 g/kg. Experiment 1 showed no change to growth, feeding efficiency, HSI, or plasma biochemical parameters. In experiment 2, however, fish that were fed dietary EHE at 5 g/kg had significantly lower mortality than the Control-AH group, with further resistance observed for fish fed EHE at 7 g/kg. Dietary OTC was more effective than EHE as a prophylactic to *A. hydrophila* infection in Sharptooth Catfish. Nevertheless, EHE can potentially be a valuable dietary supplement to improve the resistance of Sharptooth Catfish to *A. hydrophila* infection.

Herbals such as *Ixora coccinea*, *Daemia extensa*, and *Tridax procumbens* were selected by Anusha et al. (2014) to screen *in vitro* antibacterial and immunostimulant activity against the freshwater fish pathogen *Aeromonas hydrophila* using different organic polar and non-polar solvents. Initial screening results revealed that ethyl acetate extracts and its purified fraction of *I. coccinea* were able to suppress the *A. hydrophila* strains at more than 15 mm of the zone of inhibition and positive immunostimulant activity. In order to study the *in vivo* immunostimulant influence of the compounds, the crude extracts (ICE) and purified fractions (ICF) were incorporated into the artificial diets at the concentration of 400 mg/kg and fed to the ornamental goldfish *Carassius auratus* for 30 days. After termination of the feeding experiment, they were challenged with highly virulent *A. hydrophila* AHV-1 which was isolated from infected goldfish and studied the survival, specific bacterial load reduction, serum biochemistry, hematology, immunology, and histological parameters. The control diet-fed fishes succumbed to death within five days at 100% mortality whereas ICE and ICF fed groups survived 60 and 80% respectively after 10 days. The diets also helped to decrease the *Aeromonas* load after challenge and significantly improved the serum albumin, globulin, and protein. The diets also helped to increase the RBC and hemoglobin levels significantly from the control group. The immunological parameters like phagocytic activity, serum bactericidal activity, and lysozyme activity were significantly increased in the experimental diets.

Macrophages and erythrocytes were abundantly expressed in the treated groups. Anusha et al. (2014) revealed that the Phthalate derivatives from *I. coccinea* help to stimulate the immune system against *A. hydrophila* challenge in *C. auratus*.

The effects of dietary inclusion of peels and cloves of garlic (*Allium sativum*) on the growth performance and disease resistance of African catfish (*Clarias gariepinus*) fingerlings against *Aeromonas hydrophila* infection were studied by Eirna-liza et al. (2016). Seven isonitrogenous (36% protein) experimental diets were formulated to contain graded levels of garlic (peels and cloves) at 0, 10, 20 and 30 g/kg. Fish were fed twice a day for 12 weeks. The results demonstrated that no significant differences were observed with respect to growth performance or feed utilization efficiency, specific growth rate (SGR), and feed conversion ratio (FCR) of fish fed with different inclusion levels of garlic peels and cloves as compared to control group. The plasma biochemical results showed higher total protein, albumin and globulin content in the control group as compared to the experimental groups, but these results were not significant. After the fish were challenged with *A. hydrophila*, low survival (13%) was found in the control group which was significantly lower as compared to all the treatment groups (>35%). Meanwhile, the highest survival (64%) was observed for fish fed with garlic cloves at 20 g/kg (T3). The results showed that the inclusion of garlic cloves at 20 g/kg could enhance the resistance of African catfish fingerlings to *A. hydrophila* infection (Eirna-liza et al., 2016).

Harikrishnan and Balasundaram (2008) have evaluated the antimicrobial potency of aqueous and ethanolic decoction (individual extract) and concoction (mixed extract) of three common medicinal herbs, turmeric *Curcuma longa*, Tulsi plant *Ocimum sanctum*, and neem *Azadirachta indica*, against the *in vitro* growth of *A. hydrophila*. Among the decoctions, *A. indica* exhibited the most potent antibacterial property against *A. hydrophila*. Among the concoctions, both the aqueous and ethanolic triherbal extracts mixed in the ratio of 1 : 1 : 1 had higher antibacterial activity than the other concoctions and decoctions. Goldfish *Carassius auratus* were challenged with *A. hydrophila* intramuscularly in the caudal region with two separate doses (days 1 and 3) of 50 μL/fish ($1.8 \times 10^3$ colony-forming units per milliliter). On days 9 (early) and 15 (late) of infection, fish were held in a net and dip treated for 5 min daily in a 1-L solution of 1% aqueous triherbal concoction. Red blood cell (RBC) count, hemoglobin, and hematocrit levels of the infected group were significantly higher than those of the control group. In the early treated group, all of the affected profile values returned to near normal, while the late-treated group registered a partial recovery, such as improved RBC count. The derived hematological values, such as mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, of the early and late-treated groups also significantly declined but were restored to near normal only in the early treated group. The dip treatment of *A. hydrophila*-infected goldfish in an aqueous triherbal concoction had a synergistic restorative effect on the hematological variables (Harikrishnan and Balasundaram, 2008).

Ngugi et al. (2015) results demonstrate that using stinging nettle (*Urtica dioica*) can stimulate fish immunity and make adult Victoria Labeo (*Labeo victorianus*) more resistant to bacterial infection (*A. hydrophila*). They investigated the effects of dietary administration of *U. dioica* on growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo against *A. hydrophila*. Fish were divided into 4 groups and fed for 4 and 16 weeks with 0%, 1%, 2% and 5% of *U. dioica* incorporated into the diet. The use of *U. dioica* in the diet resulted in improved biochemical, hematological and immunological parameters. Among the biochemical parameters; plasma cortisol, glucose, triglyceride, and cholesterol decreased while total protein and albumin in fish increased with increasing dietary inclusion of *U. dioica*. Among the hematology parameters: red blood cell (RBC), white blood cell (WBC) counts, hematocrit (Htc), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and neutrophils increased with increasing dietary inclusion levels of *U. dioica*, some depending on the fish age. Serum immunoglobulins, lysozyme activity, and respiratory burst were the main immunological parameters in the adult and juvenile *L. victorianus* measured and they all increased with increasing herbal inclusion of *U. dioica* in the diet. Dietary incorporation of *U. dioica* at 5% showed significantly higher relative percentage survival (up to 95%) against *A. hydrophila* (Ngugi et al., 2015).

Mansouri Taee et al. (2017) examined 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. The treatments were feeding trout with experimental diets containing different levels (0, 0.5, 1 and 1.5%) of Myrtle powder. The fingerlings were fed on the experimental diet for sixty days and then skin mucus non-specific immune parameters, as well as bactericidal activity, were measured. At the end of the trial, the highest skin mucus soluble protein level was observed in the group fed with 1.5% Myrtle. The alkaline phosphatase (ALP) activity was significantly increased in fish groups fed 1 and 1.5% Myrtle compared with the control group. However, the evaluation of skin mucus lysozyme activity showed no significant difference between the treatments and the control group. Also, no antibacterial activity was detected against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* in all treatments and control groups. Whereas skin mucus of rainbow trout showed antimicrobial activity against fish pathogens (*Aeromonas hydrophila* and *Yersinia ruckeri*) in 1 and 1.5% Myrtle treatments. The results of Mansouri Taee et al. (2017) indicated beneficial effects of dietary Myrtle on mucosal immune parameters of fingerling rainbow trout.

The ability of some herbs and seaweeds to inhibit the activity of bacterial fish pathogens is of great interest. The antibacterial screening of extracts of the leaves of *Finlaysonia obovata* with hexane, chloroform, and alcohol was carried out by Mishra and Sree (2008) against freshwater fish pathogenic bacteria viz., *Micrococcus* sp. (multidrug-resistant strain), *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Edwardsiella tarda* by the disc-assay method. The hexane and chloroform extracts were found active against four and five pathogens, respectively.

The different bactericidal activity observed in this study may be due to the different components extracted. Among the most abundant components present in leaves of various *Ficus* plants, flavonoids, phenolic acids and especially terpenoids present bactericidal activity. Guimarães et al. (2019) have investigated the antibacterial activity of 33 free terpenes commonly found in essential oils and evaluate the cellular ultrastructure to verify possible damage to the cellular membrane. The higher antimicrobial activity was related to the presence of hydroxyl groups (phenolic and alcohol compounds), whereas hydrocarbons resulted in less activity.

The first group, such as carvacrol, l-carveol, eugenol, trans-geraniol, and thymol, showed higher activity when compared to sulfanilamide. The mechanism causing the cell death of the evaluated bacteria is based on the loss of cellular membrane integrity of function (Guimarães et al., 2019). Moreover, the natural phenols and their semisynthetic derivatives were tested by Pinheiro et al. (2018) for their antimicrobial activity against the bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Listeria innocua*, *Pseudomonas aeruginosa*, *Salmonella enterica typhimurium*, *Salmonella enterica* ssp. *enterica*, and *Bacillus cereus*. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined using concentrations from 220 to 3.44 μg/mL. Most of the tested compounds presented MIC values ≤220 μg/mL for all the bacteria used in the assays (Pinheiro et al., 2018).

The relationship between the structure and antibacterial activity of 22 polyphenols was analyzed by Taguri et al. (2006) using minimum inhibitory concentration (MIC) as a criterion against 26 species of bacteria that can grow in Mueller-Hinton medium. There was no clear correlation between Gram-staining and bacterial susceptibility to polyphenols, and the extent of the susceptibility was approximately dependent on the species of bacteria. In the same Gram-negative bacteria, the antibacterial activity of the polyphenols against *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* was comparatively strong. On the other hand, the activity against 11 species of the Enterobacteriaceae was comparatively weak, and the activity against six species of aerobic bacteria causing plant disease was moderate. Polyphenols having pyrogallol groups showed strong antibacterial activity, and those with catechol and resorcinol rings showed lower activity. The structure-activity relationship was extended to 26 polyphenol-rich plant extracts which could have potent antibacterial activity (Taguri et al., 2006).

It was assumed that the important function of plant-based compounds as antimicrobial includes binding to substrate or metal ions and making unavailable for microbial pathogens, microbial cell membrane disruption, binding to bacterial cell adhesins or other proteins and inhibiting the binding of bacteria to cell membranes, inactivating the microbial enzymes, blocking the viral cell fusion or adsorption in host cell (Kumar and Bossier, 2018). In addition, it was evidenced that phytochemicals are able to inhibit peptidoglycan synthesis, damage microbial membrane structures, modify bacterial membrane surface hydrophobicity and also modulate quorum-sensing (*QS*) (Rasooli et al., 2008).

## Conclusions

Based on the above investigation it can be concluded that the leaf extract of *F. lingua* has bactericidal properties which make it very attractive for use in fish aquaculture. Based on the above investigation it can be concluded that Its uses will reduce the side effects of applying synthetic compounds. The ethanolic extract obtained from leaves of *F. lingua* exhibited the maximum antimicrobial activity against *Aeromonas sobria* strain (inhibition zone diameter was 19.38 ±1.27 mm), *Aeromonas hydrophila* (16.06 ±1.05 mm), and *Aeromonas salmonicida* subsp. *salmonicida* (11.25 ±1.16 mm). The most susceptible strain to the antimicrobial activity of *F. lingua* was *Aeromonas sobria*. However, further study is needed to determine the effects of the active compounds presented in the leaf extract of *F. lingua* on fish metabolism in *in vitro* and *in vivo* study. Based on the above investigation it can be concluded that Present results suggest the possibility of using such extracts in *in vivo* studies in order to corroborate if it could be possible using those extracts in aquaculture in order to achieve protection against pathogenic infections.

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## References

AFRICAN FLOWERING PLANTS DATABASE, Conservatoire et Jardin Botaniques. 2019-07-20. Website http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php

AHMED, F., UROOJ, A. 2010. Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: a review. In *Pharm. Biol.*, vol. 48(6), p. 672–681. https://doi.org/10.3109/13880200903241861

ANUSHA, P., THANGAVIJI, V., VELMURUGAN, S., MICHAELBABU, M., CITARASU, T. 2014. Protection of ornamental gold fish *Carassius auratus* against *Aeromonas hydrophila* by treating *Ixora coccinea* active principles. In *Fish Shellfish Immunol.*, vol. 36(2), p. 485–493. https://doi.org/10.1016/j.fsi.2013.12.006

ARMSTRONG, S.M., HARGRAVE, B.T., HAYA, K. 2005. Antibiotic use in finfish aquaculture: modes of action, environ-mental fate, and microbial resistance. In *Environmental Effects of Marine Finfish Aquaculture*, pp. 341–357. https://doi.org/10.1007/b136017

AWAD, E., AWAAD, A. 2017. Role of medicinal plants on growth performance and immune status in fish. In *Fish Shellfish Immunol.*, vol. 67, p. 40–54. https://doi.org/10.1016/j.fsi.2017.05.034

BADGUJAR, S.B., PATEL, V.V., BANDIVDEKAR, A.H., MAHAJAN, R.T. 2014. Traditional uses, phytochemistry and pharmacology of *Ficus carica*: a review. In *Pharm. Biol.*, vol. 52(11), p. 1487–1503. https://doi.org/10.3109/13880209.2014.892515

BAUER, A.W., KIRBY, W.M., SHERRIS, J.C., TURCK, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. In *Am. J. Clin. Pathol.*, vol. 45(4), p. 493–496.

BELTRÁN, J.M.G., ESPINOSA, C., GUARDIOLA, F.A., ESTEBAN, M.Á. 2018. *In vitro* effects of *Origanum vulgare* leaf extracts on gilthead seabream (*Sparus aurata* L.) leucocytes, cytotoxic, bactericidal and antioxidant activities. In *Fish Shellfish Immunol.*, vol. 79, p. 1–10. https://doi.org/10.1016/j.fsi.2018.05.005

BERG, C.C., CORNER, E.J.H. 2005. Moraceae (*Ficus*). In *Noteboom H.P. (ed.) Flora Malesiana*, Ser. 1, vol. 17, Part 2. National Herbarium Nederland, Leiden, p. 1–730.

BERG, C.C., WIEBES, J.T. 1992. *African fig trees and fig wasps*. Koninklijke Nederlandse Akademie van Wetenschappen, Verhandelingen Afdeling Natuurkunde, 2nd reeks, deel 89. North-Holland, Amsterdam, p. 298.

BILLER-TAKAHASHI, J.D., URBINATI, E.C. 2014. Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. In *An. Acad. Bras. Cienc.*, vol. 86(3), p. 1484–1506.

BUNAWAN, H., AMIN, N.M., BUNAWAN, S.N., BAHARUM, S.N., MOHD NOOR, N. 2014. *Ficus* deltoidea Jack: A Review on Its Phytochemical and Pharmacological Importance. In *Evid. Based Complement. Alternat. Med.*, vol. 2014, p. 902734. *https://doi.org/10.1155/2014/902734*

CASTRO, S.B., LEAL, C.A., FREIRE, F.R. CARVALHO, D.A., OLIVEIRA, D.F., FIGUEIREDO, H.C. 2008. Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. In *Braz. J. Microbiol.*, vol. 39(4), p. 756-760. https://doi.org/10.1590/S1517-838220080004000030

CITARASU, T. 2010. Herbal biomedicines: A new opportunity for aquaculture industry. In *Aquaculture International*, vol. 18(3), p. 403–414. https://doi.org/10.1007/s10499-009-9253-7

CLARKE, J.L., WAHEED, M.T., LÖSSL, A.G., MARTINUSSEN, I., DANIELL, H. 2013. How can plant genetic engineering contribute to cost-effective fish vaccine development for promoting sustainable aquaculture? In *Plant Mol. Biol.*, vol. 83(1-2), p. 33–40. https://doi.org/10.1007/s11103-013-0081-9

COBELLO, F.C., GODFREY, H.P., TOMOVA, A., IVANOVA, L., DÖLZ, H., MILLANAO, A., BUSCHMANN, A. 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. In *Environmental Microbiology*, vol. 15(7), p. 1917–1942.

COOK, J.M., RASPLUS, J.-Y. 2003. Mutualists with attitude: coevolving fig wasps and figs. In *Trends in Ecology & Evolution*, vol. 18(5), p. 241–248.

DANGAREMBIZI, R., ERLWANGER, K.H., MOYO, D., CHIVANDI, E. 2012. Phytochemistry, pharmacology and ethnomedicinal uses of *Ficus thonningii* (Blume Moraceae): a review. In *Afr. J. Tradit. Complement. Altern. Med.*, vol. 10(2), p. 203–212. https://doi.org/10.4314/ajtcam.v10i2.4

DEFOIRDT, T., SORGELOOS, P., BOSSIER, P. 2011. Alternatives to antibiotics for the control of bacterial disease in aquaculture. In *Current Opinion in Microbiology*, vol. 14, p. 251–258. https://doi.org/10.1016/j.mib.2011.03.004

EIRNA-LIZA, N., SAAD, C.R., HASSIM, H.A., KARIM, M. 2016. The effects of dietary inclusion of garlic on growth performance and disease resistance of African catfish (*Clarias gariepinus*) fingerlings against *Aeromonas hydrophila* infection. In *J. Environ. Biol.*, vol. 37(4 Spec No), p. 817-824.

GALINA, J., YIN, G., ARDÓ, L., JENEY, Z. 2009. The use of immunostimulating herbs in fish. An overview of research. In *Fish Physiol. Biochem*., vol. 35(4), p. 669–676. https://doi.org/10.1007/s10695-009-9304-z

GUIMARÃES, A.C., MEIRELES, L.M., LEMOS, M.F., GUIMARÃES, M.C.C., ENDRINGER, D.C., FRONZA, M., SCHERER, R. 2019. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. In *Molecules*, vol. 24(13). pii: E2471. https://doi.org/10.3390/molecules24132471

HARIKRISHNAN, R., BALASUNDARAM, C. 2008. *In vitro* and *in vivo* studies of the use of some medicinal herbals against the pathogen *Aeromonas hydrophila* in goldfish. In *J. Aquat. Anim. Health*, vol. 20(3), p. 165–176. https://doi.org/10.1577/H05-035.1

JANDA, J.M., ABBOTT, S.L. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. In *Clin Microbiol Rev.*, vol. 23(1), p. 35–73. https://doi.org/10.1128/CMR.00039-09

JORGENSEN, J.H., FERRARO, M.J. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. In *Clin. Infect. Dis.*, vol. 49(11), p. 1749–1755. https://doi.org/10.1086/647952

KOZIŃSKA, A. 2007. Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland. In *J. Fish Dis*., vol. 30(5), p. 293–301. https://doi.org/10.1111/j.1365-2761.2007.00813.x

KUMAR, V., BOSSIER, P. 2018. Importance of plant-derived compounds and/or natural products in aquaculture. In *Aqua feed: Advances in Processing & Formulation*, vol. 10(3), p. 43–46.

LANSKY, E.P., PAAVILAINEN, H.M. 2011. *Figs: the genus Ficus.* In Hardman R. (ed.) *Traditional herbal medicines for modern times*, vol. 9. CRC Press, Boca Raton, p. 1–357.

MAGNADOTTIR, B. 2010. Immunological control of fish diseases. In *Mar. Biotechnol*. (NY), vol. 12(4), p. 361–379. https://doi.org/10.1007/s10126-010-9279-x

MANSOURI TAEE, H., HAJIMORADLOO, A., HOSEINIFAR, S.H., AHMADVAND, H. 2017. Dietary Myrtle (*Myrtus communis* L.) improved non-specific immune parameters and bactericidal activity of skin mucus in rainbow trout (*Oncorhynchus mykiss*) fingerlings. In *Fish Shellfish Immunol*., vol. 64, p. 320–324. https://doi.org/10.1016/j.fsi.2017.03.034

MISHRA, P.M., SREE, A. 2008. Chemical investigation of *Finlaysonia obovata*: part I – a rare triterpene acid showing antibacterial activity against fish pathogens. In *Nat. Prod. Res.*, vol. 22(9), p. 801–807. https://doi.org/10.1080/14786410701640403

NEWAJ-FYZUL, A., AUSTIN, B. 2015. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. In *J. Fish Dis.*, vol. 38(11), p. 937–955. https://doi.org/10.1111/jfd.12313

NGUGI, C.C., OYOO-OKOTH, E., MUGO-BUNDI, J., ORINA, P.S., CHEMOIWA, E.J., ALOO, P.A. 2015. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. In *Fish Shellfish Immunol.*, vol. 44(2), p. 533–541. https://doi.org/10.1016/j.fsi.2015.03.025

OKOCHA, R.C., OLATOYE, I.O., ADEDEJI, O.B. 2018. Food safety impacts of antimicrobial use and their residues in aquaculture. In *Public Health Rev.*, vol. 39, p. 21. https://doi.org/10.1186/s40985-018-0099-2

OKOTH, D.A., CHENIA, H.Y., KOORBANALLY, N.A. 2013. Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). In *Phytochem. Lett.*, vol. 6, p. 476–481. http://dx.doi.org/10.1016/j.phytol.2013.06.003

PINHEIRO, P.F., MENINI, L.A.P., BERNARDES, P.C., SARAIVA, S.H., CARNEIRO, J.W.M., COSTA, A.V., ARRUDA, T.R., LAGE, M.R., GONÇALVES, P.M., BERNARDES, C.O., ALVARENGA, E.S., MENINI, L. 2018. Semisynthetic Phenol Derivatives Obtained from Natural Phenols: Antimicrobial Activity and Molecular Properties. In *J. Agric. Food Chem.*, vol. 66(1), p. 323–330. https://doi.org/10.1021/acs.jafc.7b04418

PROTABASE – PLANT RESOURCES OF TROPICAL AFRICA. 2019-07-20. Website http://www.prota.org

RAJIV, P., RAJESHWARI, S. 2012. Screening for phytochemicals and antimicrobial activity of aqueous extract of *Ficus* religiosa Linn. In *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 4, p. 207–209.

RASOOLI, I., SHAYEGH, S., TAGHIZADEH, M., ASTANEH, S.D.A. 2008. Phytotherapeutic prevention of dental biofilm formation. In *Phytother. Res.*, vol. 22, p. 1162–1167. https://doi.org/10.1002/ptr.2387

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B., SASAL, P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. In *Aquaculture*, vol. 433, p. 50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

SHEIKHLAR, A., MENG, G.Y., ALIMON, R., ROMANO, N., EBRAHIMI, M. 2017. Dietary *Euphorbia hirta* Extract Improved the Resistance of Sharptooth Catfish *Clarias gariepinus* to *Aeromonas hydrophila*. In *J. Aquat. Anim. Health*, vol. 29(4), p. 225–235. https://doi.org/10.1080/08997659.2017.1374310

SINGH, D., SINGH, B., GOEL, R.K. 2011. Traditional uses, phytochemistry and pharmacology of *Ficus* religiosa: a review. In *J. Ethnopharmacol.*, vol. 134(3), p. 565–583. https://doi.org/10.1016/j.jep.2011.01.046

SØRUM, H. 2006. Antimicrobial drug resistance in fish pathogens. In *Antimicrobial Resistance in Bacteria of Animal Origin*. Ed. Aarestrup, F.M. Washington, DC, USA: ASM Press, p. 213–238.

TAGURI, T., TANAKA, T., KOUNO, I. 2006. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. In *Biol. Pharm. Bull.*, vol. 29(11), p. 2226–2235. https://doi.org/10.1248/bpb.29.2226

TKACHENKO, H., BUYUN, L., KASIYAN, O., TERECH-MAJEWSKA, E., HONCHARENKO, V., PROKOPIV, A., OSADOWSKI, Z. 2018. Preliminary *in vitro* screening of antibacterial activity of leaf extract from *Ficus natalensis* subsp. *natalensis* Hochst. (Moraceae) against fish pathogens. In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, vol. 2, p. 170–183. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.170-183

TKACHENKO, H., BUYUN, L., OSADOWSKI, Z., HONCHARENKO, V., PROKOPIV, A. 2017a. The antimicrobial efficacy of ethanolic extract obtained from *Ficus benghalensis* L. (Moraceae) leaves. In *Agrobiodiversity for improving nutrition, health, and life quality*, vol. 1, p. 438–445. http://dx.doi.org/10.15414/agrobiodiversity.2017.2585-8246.438-445

TKACHENKO, H., BUYUN, L., OSADOWSKI, Z., TERECH-MAJEWSKA, E., HONCHARENKO, V., PROKOPIV, A. 2017b. Comparative study of the antimicrobial efficacy of the ethanolic leaf extract of *Ficus benghalensis* L. (Moraceae) against bacterial fish pathogens. In *Słupskie Prace Biologiczne*, vol. 14, p. 209–228.

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA E., OSADOWSKI, Z., SOSNOVSKYI, Y., HONCHARENKO, V., PROKOPIV, A. 2016d. *In vitro* antibacterial efficacy of various ethanolic extracts obtained from *Ficus* spp. leaves against the fish pathogen, *Pseudomonas fluorescens*. In *Globalisation and regional environment protection. Technique, technology, ecology.* Sci. eds T. Noch, W. Mikołajczewska, A. Wesołowska. Gdańsk, Gdańsk High School Publ., 2016, p. 265–286.

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA, E., HONCHARENKO, V., PROKOPIV, A., OSADOWSKI, Z. 2019. Preliminary *in vitro* screening of the antibacterial activity of leaf extracts from various *Ficus* species (Moraceae) against *Yersinia ruckeri*. In *Fish. Aquat. Life*, vol. 27, p. 15–26. https://doi.org/10.1248/bpb.29.2226. 10.2478/aopf-2019-0002

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA, E., OSADOWSKI, Z. 2016a. Antibacterial activity of ethanolic leaf extracts obtained from various *Ficus* species (Moraceae) against the fish pathogen, *Citrobacter freundii*. In *Baltic Coastal Zone – Journal of Ecology and Protection of the Coastline*, vol. 20, p. 117–136.

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA, E., OSADOWSKI, Z. 2016b. *In vitro* antimicrobial activity of ethanolic extracts obtained from *Ficus* spp. leaves against the fish pathogen *Aeromonas hydrophila*. In *Arch. Pol. Fish.*, vol. 24, p. 219–230. https://doi.org/10.1515/aopf-2016-0019

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA, E., OSADOWSKI, Z. 2017c. Antibacterial screening of ethanolic extracts obtained from leaves of various *Ficus* species (Moraceae) against *Citrobacter freundii*. In *Trudy VNIRO*, vol. 167, p. 138–149.

TKACHENKO, H., BUYUN, L., TERECH-MAJEWSKA, E., OSADOWSKI, Z., SOSNOVSKYI, Y., HONCHARENKO, V., PROKOPIV, A. 2016c. The antimicrobial activity of some ethanolic extracts obtained from *Ficus* spp. leaves against *Aeromonas hydrophila*. In *Trudy VNIRO*, vol. 162, p. 172–183.

TROPICAL PLANTS DATABASE, Ken Fern. tropical.theferns.info. 2019-07-20. Website <tropical.theferns.info/viewtropical.php?id=*Ficus*+lingua>

VALLEJOS-VIDAL, E., REYES-LÓPEZ, F., TELES, M., MACKENZIE, S. 2016. The response of fish to immunostimulant diets. In *Fish Shellfish Immunol.*, vol. 56, p. 34-69. https://doi.org/10.1016/j.fsi.2016.06.028

WEI, L.S., MUSA, N. 2008. Inhibition of *Edwardsiella tarda* and Other Fish *Pathogens* by *Allium sativum* L. (Alliaceae) Extract. In *American-Eurasian J. Agric. & Environ. Sci.*, vol. 3(5), p. 692–696.

WUNDERLICH, A.C., ZICA, É.D.O.P., DOS SANTOS AYRES, V.F., GUIMARÃES, A.C., TAKEARA, R., 2017. Plant-Derived Compounds as an Alternative Treatment Against Parasites in Fish Farming: A Review. In *Natural Remedies in the Fight Against Parasites*, p. 115–135. http://dx.doi.org/10.5772/67668

YADAV, R.K., NANDY, B.C., MAITY, S., SARKAR, S., SAHA S. 2015. Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. In *Pharmacogn. Rev.*, vol. 9(17), p. 73–80. https://doi.org/10.4103/0973-7847.156356

ZAR, J.H. 1999. *Biostatistical Analysis*. 4$^{th}$ ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.