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The antimicrobial activity of some ethanolic extracts obtained from *Ficus* spp. leaves against *Aeromonas hydrophila*

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Nowadays numerous medicinal plants possessing antiviral, antibacterial, antifungal and antiparasitic activities have a significant role in aquaculture as prophylactic and therapeutic agents against fish pathogens. This prompted us to determine the *in vitro* antimicrobial activity of ethanolic extracts from leaves of various *Ficus* species (Moraceae) against the bacterial strain of *Aeromonas hydrophila* isolated locally from infected rainbow trout (*Oncorhynchus mykiss*) with the aim of providing a scientific proof for the use of the plants in the treatment of bacterial infections induced by *Aeromonas* spp. in fish. The antimicrobial susceptibility testing was done on Muller-Hinton agar by disc diffusion method. Our results indicated that extracts offer a promising alternative to the use of antibiotics in controlling *A. hydrophila* growth. In our study, most ethanolic extracts obtained 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 against *F. pumila*. Among various species of *Ficus* with moderate activity against *A. hydrophila*, the highest antibacterial activity for *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida*, *F. lyrata* was displayed. Thus, *Ficus* spp. leaves possess great medicinal potential for the therapy of bacterial and fungal infections and may be used as a natural antiseptic and antimicrobial agent in veterinary. It is concluded from the present study that these products can be used in aquaculture as therapeutic and prophylactic agents against fish pathogens, with antimicrobial and immunostimulant properties. Further investigation is necessary to identify those bioactive compounds, which will be a platform for further pharmacological studies and clinical applications.

Key words: antibacterial activity, *Aeromonas hydrophila*, infected rainbow trout *Oncorhynchus mykiss*, *Ficus* spp., ethanolic extracts, zone of growth inhibition

Introduction.

The genus *Aeromonas* of the family *Aeromonadaceae* includes Gram-negative facultative anaerobes that have worldwide distribution in aquatic environments and are pathogenic to fish and other animals [Cipriano, Austin, 2011]. *A. hydrophila* is an autochthonous species in freshwater environments and a member of the normal microflora in the fish intestinal tract [Cipriano, 2001]. On the other hand, *A. hydrophila* causes diverse pathologic conditions that include acute, chronic, and latent infections. *Aeromonas* bacteria are also the etiologic agents responsible for a variety of infections in both immunocompetent and immunocompromised humans. Severity of disease is influenced by a number of interrelated factors, including bacterial virulence, the kind and degree of stress exerted on a population of fish, as well as the resistance and physiological condition of the host. Pathologic conditions attributed to members of the *A. hydrophila* complex include dermal ulceration, hemorrhagic septicemia, red sore disease, red rot disease, and scale protrusion disease [Cipriano, 2001]. In salmonids, *A. salmonicida* causes furunculosis, a disease characterized by skin ulcers and septicemia. Moreover, other *Aeromonas* species are involved in similar pathological diseases [Austin, Austin, 2007]. Findings of Zepeda-Velázquez and co-workers [2015] confirmed that the *Aeromonas* species, *A. hydrophila*, *A. salmonicida*, and *A. veronii*, are associated with septicemia and dermal lesions in rainbow trout. Our previous study revealed that cytoplasmic vacuolation of hepatocytes, loss of normal basophilia by cytoplasm, presence of pigment deposition in the parenchyma are a signs of liver deterioration in trout affected by *A. hydrophila*. Impaired circulation in the liver manifested in oedema and an occurrence of a large number of erythrocytes in the sinusoids and the intercellular space [Tkachenko et al., 2014].

One of the overriding considerations is how disease may be controlled. The application of vaccine technology to fish diseases started with *A. salmonicida* in the 1940s and now commercial products based on iron-regulated outer membrane proteins (IROMPs) of typical strains are available. Motile aeromonads have attracted less interest in vaccine development. However, these pathogens have been the focus of probiotics, immunostimulants and medicinal plants, some of which stimulate immune memory. Finally, the use of genetically disease-resistant stock has already been considered for *A. salmonicida* [Cipriano, Austin, 2011].

The benefits of medicinal plants have been the focus in many studies originating from Asia in controlling infections induced by *Aeromonas* [Cipriano, Austin, 2011]. The active compounds of herbs possess characteristics that could be useful in fish and shrimp culture; various herbs can stimulate growth and appetite, enhance immune system responses, and have broad spectrum of antimicrobial activity [Friedman et al., 2002].

Resistance against antibiotics of many bacteria is accumulating. Therefore, searches for new substances with antimicrobial activity have become an urgent necessity. Medicinal plants involved in traditional medicine are potential sources of antimicrobial compounds. Recently, there has been increasing interest in *Ficus* spp. (Moraceae) due to its chemical composition and the potential health benefits. *Ficus* spp. have been extensively used in traditional medicine for a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system and infectious disorders [Kumar, Augusti, 1989; Cherian, Augusti, 1993; Kirana et al., 2009, 2011; Usman et al., 2009; Ahmad et al., 2011; Ilyanie et al., 2011; Dangarembizi et al., 2012; Arunachalam, Parimelazhagan, 2013; Gul-e-Rana et al., 2013; Farsi et al., 2014]. However, although many species within the genus *Ficus* have been encompassed by phytochemical and pharmacological investigations in previous years, there are many species that have not been studied and whose ethnobotanical relevance is yet to be investigated. This prompted us to determine the *in vitro* antimicrobial activity of ethanolic extracts from leaves of various *Ficus* species against the bacterial strain of *A. hydrophila* isolated locally from infected rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) with the aim of providing a scientific rationale for the use of the plant in the treatment of bacterial infections induced by *Aeromonas* spp. in fish.

Materials and methods.

Collection of Plant Material. The leaves of *F. aspera* G.Forst, *F. benghalensis* L., *F. benjamina* L., *F. benjamina* 'Reginald', *F. binnendijkii* (Miq.) Miq., *F. binnendijkii* 'Amstel Gold', *F. binnendijkii* 'Amstel King', *F. binnendijkii* 'Amstel Gold', *F. carica* L., *F. craterostoma* Mildbr. & Burret, *F. cyathistipula* Warb., *F. deltoidea* Jack, *F. drupacea* Thunb., *F. drupacea* 'Black Velvet', *F. elastica* Roxb., *F. elastica* 'Variegata', *F. erecta* Thunb., *F. erecta* var. *sieboldii* (Miq.)

King, *F. hispida* L.f., *F. luschanthiana* (Miq.) Miq., *F. lyrata* Warb., *F. macrophylla* Desf. ex Pers., *F. mucuso* Welw. ex Ficalho, *F. natalensis* Hochst. subsp. *natalensis*, *F. natalensis* Hochst. subsp. *leprieurii* (Miq.) C.C.Berg, *F. palmeri* S.Watson, *F. platypoda* (Miq.) A.Cunn. ex Miq., *F. pumila* L., *F. religiosa* L., *F. rubiginosa* Desf. ex Vent., *F. sagittata* J.Koenig ex Vahl, *F. septica* Burm. f., *F. sur* Forssk., *F. sycomorus* L., *F. vasta* Forssk., *F. villosa* Blume were collected in M.Gryshko National Botanical Garden (Kyiv, Ukraine) and Botanical Garden of Ivan Franko Lviv National University (Lviv, Ukraine) during March and September, 2015. The whole collection of tropical and subtropical plants both at NBG and Botanical Garden of Ivan Franko Lviv National University (including *Ficus* spp. plants) has the status of a National Heritage Collection of Ukraine. The species author abbreviations were followed by Brummitt and Powell [1992].

Preparation of Plant Extracts. The sampled leaves of *Ficus* spp. were brought into the laboratory for antimicrobial studies. Freshly crushed leaves were washed, weighted, and homogenized in 96% ethanol (in proportion 1:10) at room temperature.

Method of Culturing Pathological Sample. *Aeromonas hydrophila* (strain E2/7/15) isolated locally from gill of rainbow trout (*Oncorhynchus mykiss* Walbaum) with clinical features of furunculosis (kidneys were gray, liver was pale and fragile, enlarged spleen with exudate in the body cavity) was used as test organism. Fish infection had a mixed character (*Pseudomonas fluorescens*, *A. hydrophila* complex, *Shewanella putrefaciens*). The increased mortality among trout has been occurred. Fish with clinical signs of disease were euthanized with Propiscin (Inland Fisheries Institute, Poland), anesthetic designed for fish, in immersion using 2 mL per liter of water. Samples of internal organs (kidneys, spleen, liver) weighting 2 g were taken and homogenized before preincubation in TSB broth (Tripticase Soya Broth, Oxoid) for 24 hrs.

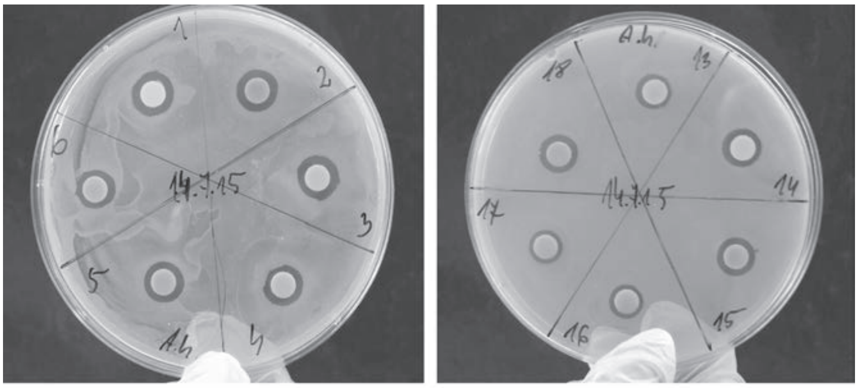
Identification Method of the Bacteria. After preincubation, bacterial culture was transferred to two different cultivation media: TSA (Tripticase Soya Agar, Oxoid) and BHIA (Brain Heart Infusion Agar, Oxoid) supplemented with 5% of sheep blood [OIE Fish Diseases Commission 2000, Fredrichs, 1993]. After 48 hrs of incubation at 27 °C, characteristic pink colonies were selected for further examination. Bacterial species were identified with the oxidase test and API E test kit (Biomerieux, France). The results of the test were interpreted in accordance with the manufacturer's protocol, after 24 hrs of incubation at 27 °C. Codes ++V–V––+V+++––+–VV in API E test were identified as *A. hydrophila*.

Bacterial Growth Inhibition Test of Plant Extracts by the Disk Diffusion Method. Strain tested was plated on TSA medium (Tryptone Soya Agar) and incubated for 24 hrs at 25 °C. Then the suspensions of microorganisms were suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. Antimicrobial activity of extracts was evaluated by using agar well diffusion method [Bauer et al., 1966]. Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (10^8 CFU/mL) of bacterium and spread with sterile swabs.

Sterile filter paper discs impregnated by extract were applied over each of the culture plates, 15 min after bacteria suspension was placed. The antimicrobial susceptibility testing was done on Muller-Hinton agar by disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol). The *A. hydrophila* isolates were individually tested against 4 antibiotics. The tested antibiotics were as follows: oxytetracycline (30 µg), enrofloksacin (5 µg), gentamicin (10 µg); sulphamethoxazole/trimethoprim (25 µg). A negative control disc impregnated by sterile ethanol was used in each experiment. The sensitivity of strain was also studied to the commercial preparation with extracts of garlic (in dilution 1:10, 1:100 and 1:1000). After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 hrs at 25 °C. The diameters of the inhibition zones were measured in millimeters, and compared with those of the control and standard susceptibility disks. Activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated six times. 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].

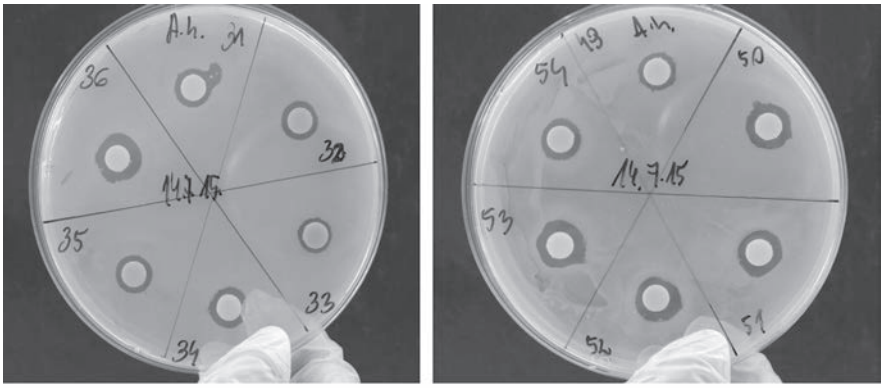
Results.

The results of screening study of antimicrobial activity of ethanolic extracts obtained from *Ficus* spp. leaves are presented in Table 1, Figs 1—5. A comparison of susceptibility categories, i. e. susceptible, intermediate, and resistant, for the disk diffusion method is shown in Table 1.



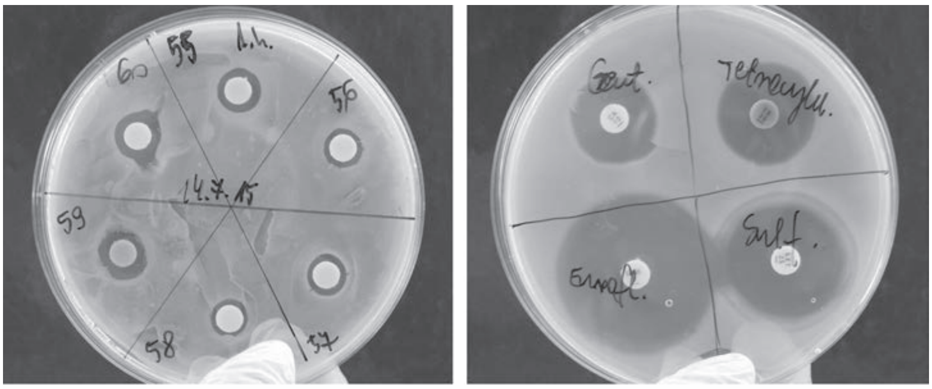
A B

Fig. 1. Antimicrobial activity of ethanolic extracts obtained from *F. benghalensis* (4), *F. benjamina* (5) (A), *F. cyathistipula* (14), and *F. lyrata* (15) against *A. hydrophila* (B) measured as inhibition zone diameter.



A B

Fig. 2. Antimicrobial activity of ethanolic extracts obtained from *F. natalensis* subsp. *leprieurii* (34) and *F. deltoidea* (36) (A), as well as *F. palmeri* (50), *F. hispida* (51), and *F. natalensis* subsp. *natalensis* (52) against *A. hydrophila* (B) measured as inhibition zone diameter.



A B

Fig. 3. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* (55, 60) against *A. hydrophila* (A), and tested antibiotics measured as inhibition zone diameter.

Table 1. Susceptibility or resistance of *A. hydrophila* against ethanolic extracts obtained from *Ficus* spp. leaves

Ficus spp.	Susceptibility or resistance of *A. hydrophila*	
	Intermediate (I) = 11–14 mm	Resistant (R) ≤ 10 mm
F. aspera		+
F. benghalensis	+	
F. benjamina	+	
F. benjamina 'Reginald'		+
F. binnendijkii	+	
F. binnendijkii 'Amstel Gold'		+
F. binnendijkii 'Amstel King'		+
F. carica		+
F. craterostoma		+
F. cyathistipula	+	
F. deltoidea	+	
F. drupacea		+
F. drupacea 'Black Velvet'		+
F. elastica		+
F. elastica 'Variegata'		+
F. erecta	+	
F. erecta var. *sieboldii*	+	
F. hispida	+	
F. luschanthiana	+	
F. lyrata	+	
F. macrophylla	+	
F. mucuso	+	
F. natalensis subsp. *leprieurii*	+	
F. natalensis subsp. *natalensis*	+	
F. palmeri	+	
F. platypoda	+	
F. pumila	+	
F. religiosa		+
F. rubiginosa	+	
F. sagittata		+
F. septica		+
F. sur	+	
F. sycomorus	+	
F. vasta		+
F. villosa	+	

Our results showed that the *A. hydrophila* revealed intermediate susceptibility (diameter of inhibition zone) concerning to ethanolic extracts obtained from *F. benghalensis*, *F. benjamina*, *F. binnendijkii*, *F. cyathistipula*, *F. deltoidea*, *F. erecta*, *F. erecta* var. *sieboldii*, *F. hispida*, *F. luschanthiana*, *F. lyrata*, *F. macrophylla*, *F. mucuso*, *F. natalensis* subsp. *leprieurii*, *F. natalensis* subsp. *natalensis*, *F. palmeri*, *F. platypoda*, *F. pumila*, *F. rubiginosa*, *F. sur*, *F. sycomorus*, and *F. villosa*. *A. hydrophila* was resistant against ethanolic exstracts from *F. aspera*, *F. benjamina* 'Reginald', *F. binnendijkii* 'Amstel Gold', *F. binnendijkii* 'Amstel King', *F. carica*, *F. craterostoma*, *F. drupacea*, *F. drupacea* 'Black Velvet', *F. elastica*, *F. elastica* 'Variegata', *F. religiosa*, *F. sagittata*, *F. septica*, and *F. vasta* (Table 1).

Discussion.

Our results indicated that extracts offer a promising alternative to the use of antibiotics in controlling *A. hydrophila*. In our study, most ethanolic extracts obtained 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 against *F. pumila* (Table 1, Fig. 3). Among various species of *Ficus* with moderate activity against *A. hydrophila*, the highest antibacterial activity for *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida*, *F. lyrata* was noted.

It is well documented that various *Ficus* spp. have been used against Gram-positive and Gram-negative bacteria [Salem et al., 2013]. The scientific research on *Ficus* spp. indicated that these plants have received increasing interest in recent years. For instance, *F. deltoidea* has been reported to have beneficial pharmaceutical uses as an antidiabetic, anti-inflammatory, antinociceptive, antimelanogenic, antiphotoaging, antioxidant, antiulcerogenic, and antibacterial agent with antioxidant, antihyperglycemic, anti-inflammatory, antiulcerogenic and antinociceptive activity [Bunawan et al., 2014; Dzolin et al., 2015]. Adam and co-workers (2007) reported that almost all of the parts of *F. deltoidea* plant including the root, bark, leaf and fig have their own medicinal properties. Abdsamah and co-workers (2012) studied the *in vitro* antimicrobial activity of the chloroform, methanol and aqueous extracts of *F. deltoidea* against 2 Gram-positive [*Staphylococcus aureus* (IMR S-277), *Bacillus subtilis* (IMR K-1)], 2 Gram-negative [*Escherichia coli* (IMR E-940), *Pseudomonas aeruginosa* (IMR P-84)] and 1 fungal strain, *Candida albicans* (IMR C-44). All the extracts showed inhibitory activity on the fungus, Gram-positive and Gram-negative bacteria strains tested except for the chloroform and aqueous extracts on *B. subtilis*, *E. coli*, and *P. aeruginosa* [Abdsamah et al., 2012]. In line with these studies, in our investigation, ethanolic extract obtained from *F. deltoidea* showed intermediate antibacterial efficiency against *A. hydrophila* with 11—13 mm zone of growth inhibition (Fig. 2A).

Similar antibacterial activity was shown for ethanolic exstract from *F. benjamina* (Fig. 1). *F. benjamina* also possesses high medicinal potential. The plant is also used as antimicrobial, antinociceptive, antipyretic, hypotensive and anti-dysentery remedy [Imran et al., 2014]. Sirisha and co-workers (2010) suggested that the leaves, bark and fruits of *F. benjamina* contain various bioactive constituents like cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol. The fruit extract of *F. benjamina* possessed both antitumor and significant antibacterial activities. Medicinal importance of this plant encouraged us to carry out the antimicrobial study of the ethanolic extract from the leaves of *F. benjamina* plants against *A. hydrophila*. Parveen and co-workers [2009] reported about the isolation and characterization of a new triterpene, (9,11), (18,19)-disecoolean-12-en-28-oic acid (1) along with β-amyrin (2). The compound 1 exhibited significant antimicrobial activity against *Salmonella typhimurium* (MTCC-98), *C. albicans* (IAO-109), *S. aureus* (IAO-SA-22), *E. coli* (K-12) and low activity against *A. niger* (lab isolate ICAR) and A. *brassicola*.

Our results demonstrate that *F. benghalensis* has intermediate *in vitro* activity against *A. hydrophila*. Our study is in agreement with studies of other authors. The bark of *F. benghalensis* exhibited significant antibacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [Gayathri et al., 2009].

Verma and co-workers [2012, 2013] confirmed immunostimulatory role of *F. benghalensis* (prop-roots) and *L. leucocephala* (pod seed) in

Clarias gariepinus when supplemented in artificial feed. They evaluated the antibacterial activity of methanolic extracts of *F. benghalensis* (prop-root) by measurement of zone of inhibition against pathogenic bacteria, *E. coli* and *A. hydrophila*. Moreover, juvenile *C. gariepinus* were fed with 5% powder of *F. benghalensis* with respective feeds for 20 days prior to the experiment. Immunomodulatory response of supplementary feed was studied by challenging the fish intraperitoneally at weekly intervals, with *A. hydrophila*. One set of fish, not challenged with *A. hydrophila* was used as a negative control, to analyze any detrimental effect of supplementary feed, while positive control, comprised of challenged fish fed with non-supplemented feed. Other two groups of fish were challenged with *A. hydrophila* and fed with respective supplementary feeds. Fish fed with supplementary feed showed increased lysozyme activity and phagocytic index indicating an increase in non-specific immune response. Moreover, serum lysozyme, tissue superoxide dismutase, percentage phagocytosis, phagocytotic index, nitric oxide (NO), total serum protein and immunoglobulin increased significantly in the treated fish compared to control fish.

In our study, ethanolic extracts of *F. lyrata* and *F. hispida* leaves inhibited growth of *A. hydrophila* (Fig. 1B, 2B). In similar studies considerable antimicrobial activity of *F. lyrata* also has been found. Ethyl acetate extract of *F. lyrata* latex comprises compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases. The methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*E. faecalis*, *Citobacter freundii*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis*) [Bidarigh et al., 2011]. *F. lyrata* has been reported to have numerous bioactive compounds such as arabinose, β-amyrins, β-carotenes, glycosides, β-setosterols and xanthotoxol [Jeong and Lachance, 2001; Vaya and Mahmood, 2006]. Findings of Rizvi and co-workers [2010] suggest that Ursolic acid from *F. lyrata* has excellent antibacterial activity against several problematic bacteria like MRSA and ESBL producing bacteria, *Pseudomonas*, *Salmonella*, *Shigella* and *Vibrio cholerae* and other known pathogens with drug resistance. Ursolic acid and Acacetin-7-O-neohesperidoside contribute significantly to the antimicrobial activities of the crude extract of *F. lyrata* [Rizvi et al., 2010]. Ahmad and Beg [2001] revealed that glycosides and saponins extracted from leaves using alcohol had biological effects but they had no effects on *C. albicans*, *S. aureus* and *E. coli*.

F. hispida was chosen for its abundance of alkaloids, carbohydrates, proteins and amino acids, sterols, phenols, flavonoids, gums and mucilage, glycosides, saponins, and terpenes [Ghosh et al., 2004]. The broad antibacterial activities of this extract, apparently, could be explained as a result of the plant secondary metabolites. Previously have been reported [Salem et al., 2013], that the therapeutic properties of *Ficus* species may be attributed to the presence of a wide range of phytochemical compounds. In general, *Ficus* species are rich sources of polyphenolic compounds. In particular, flavonoids and isoflavonoids are responsible for the extract's strong antioxidant activity that may be useful in preventing diseases involving oxidative stress [Sirisha et al., 2010]. Ali and Chaudhary [2011] have reported that *F. hispida* contains wide varieties of bioactive compounds from different phytochemical groups like alkaloids, carbohydrates, proteins and amino acids, sterols, phenols, flavonoids, gums and mucilage, glycosides, saponins, and terpenes. Two substantial phenanthroindolizidine alkaloids, 6-O-methyltylophorinidine and 2-demethoxytylophorine, and a novel biphenylhexahydroindolizine hispidine from stem and leaves of *F. hispida* were isolated by Venkatachalam and Mulchandani [1982]. Recently, hispidin has been reported to have anticancer activity [Ali and Chaudhary, 2011]. All the detected phenolic acids are known to have antimicrobial and antioxidant properties [Jaafar et al., 2012]. The antimicrobial property of *F. hispida* extract may be due to its constituents.

A wide range of chemical compounds have been also isolated and characterized from *F. deltoidea*, particularly from the leaves and figs [Bunawan et al., 2014]. An initial comprehensive study on volatile compounds produced by the fruits was conducted by Grison-Pigé and co-workers [2002]. The volatile compounds isolated and identified are mainly products of the shikimic acid pathway, terpenoids, and aliphatic groups, generally present as floral fragrances in plants. Mohd Lip and co-workers [2009] have isolated and identified moretenol from *F. deltoidea* leaves. Later on, an antibacterial compound known as lupeol ($C_{30}H_{50}O$) was also isolated from the leaves of *F. deltoidea* and exhibited toxicity against *S. aureus*, *B. subtilis*, and *E. coli* [Suryati et al., 2011]. Ong and co-workers [2011] demonstrated enhancement of flavonoid compounds (rutin, quercetin, and naringenin) in cell cultures of *F. deltoidea* influenced by different carbon sources as well as plant growth regulators. A comprehensive study on flavonoid compounds of aqueous extract of *F. deltoidea* was conducted by Omar and co-workers [2011], and more than 25 compounds were identified. Currently, two bioactive constituents known as vitexin and isovitexin have been isolated, identified, and evaluated to show α-glucosidase inhibition [Choo et al., 2012]. The antibacterial activity of this extract is possibly linked to the presence of flavonoid compounds. Antibacterial activities of flavonoid compounds isolated from plant species are well documented [Hendrich, 2006; Ferrazzano et al., 2011; Farzaei et al., 2013]. A high amount of epicatechin found in *F. deltoidea* may be responsible for the strong radical scavenging activities found in the extract. A positive correlation was observed between flavonoid constituents present and radical scavenging activities of the aqueous extracts of *F. deltoidea* [Dzolin et al., 2015]. Chemical analysis found four phenolic compounds (chlorogenic, p-coumaric, ferulic and syringic acids) in roots, three (chlorogenic, p-coumaric and ferulic acids) in stem and only one (caffeic acid) in leaves of *F. benjamina* [Imran et al., 2014]. A glucoside, bengalenoside, was isolated from *F. benghalensis* and evaluated for hypoglycemic activity [Garg, Paliwal, 2011]. The phytochemical screening of *F. benghalensis* revealed the presence of saponins, tannins and flavonoids in aqueous and methanolic extract [Aswar et al., 2008]. Levels of total phenolic, total flavonol and total flavonoid compounds in aerial roots in 70 mg/g of extract, 3 mg/g quercetin equivalent and 5 mg quercetin equivalent/g extract have also been reported [Sharma et al., 2009]. Some natural compounds, i. e. glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentariacontan-5-one, β-sitosterol-α-D-glucose and meso-inositol have been isolated from the bark [Subramanian and Misra, 1978].

Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes [Kumar, Pandey, 2013].

It is desirable that antibiotic use in fish cultures will be reduced and replaced by natural medicines to prevent the emergence of bacterial resistance in aquatic animals and their environment. Antibiotics are widely used in fish farms to prophylactically treat bacterial infections and as a growth promoter. Despite its widespread use, there is no regulation on this drug class in fish [Rigos, Troisi, 2005]. Therefore, use of plant extracts can be more effective for preventive and therapeutically aims in aquaculture. In our study, the ethanolic extracts obtained from various species of *Ficus* leaves showed varying inhibitory activities against *A. hydrophila*.

Conclusions.

Present study aimed to investigate the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* spp., respectively using the disc diffusion method, against *A. hydrophila*. Our results demonstrate that various species of *Ficus* had intermediate antibacterial *in vitro* activity against *A. hydrophila*. The highest antibacterial activity against *A. hydrophila* was noted for *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida*, *F. lyrata*. Further studies should be conducted to verify this activity against other pathogenic bacteria of interest in aquaculture and to confirm immune response involvement or its potential as a virulence factors inhibitor. Thus, *Ficus* spp. leaves possess great medicinal potential for the therapy of bacterial and fungal infections and may be used as a natural antiseptic and antimicrobial agent in veterinary. These products can be used in aquaculture as therapeutic and prophylactic agents against fish pathogens, with antimicrobial and/or immunostimulant properties. Further investigation is necessary to identify those bioactive compounds, which will be a platform for further pharmacological studies and clinical applications.

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Антимикробная активность некоторых спиртовых экстрактов, полученных из листьев видов *Ficus*, в отношении *Aeromonas hydrophila*

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В настоящее время многочисленные лекарственные растения, обладающие противовирусными, антибактериальными, противогрибковыми и противопаразитарными свойствами, играют важную роль в аквакультуре в качестве профилактических и терапевтических агентов в лечении паразитозов рыб. Это послужило основанием для сравнения антимикробной активности спиртовых экстрактов из листьев различных видов фикусов (*Moraceae*) в отношении бактериального штамма *Aeromonas hydrophila*, изолированного локально из инфицированной радужной форели (*Oncorhynchus mykiss*) с целью выявления научных доказательств для использования этих растений в лечении бактериальных инфекций рыб, вызванных *Aeromonas*. Наши результаты показали, что использование исследованных экстрактов является перспективной альтернативой применению антибиотиков с целью контроля роста *A. hydrophila*. Полученные результаты свидетельствуют о том, что большинство спиртовых экстрактов, полученных из фикусов, являются эффективными в отношении роста бактериального штамма *A. hydrophila*, с 10—12 мм зонами ингибирования роста. *A. hydrophila* оказался наиболее восприимчивым в отношении экстрактов листьев *F. pumila*. Среди различных видов фикуса с умеренной активностью в отношении *A. hydrophila*, высокая антибактериальная активность отмечена у *F. benghalensis*, *F. benjamina*, *F. deltoidea*, *F. hispida*, *F. lyrata*. Таким образом, экстракты из листьев фикусов обладают большим терапевтическим потенциалом в лечении бактериальных и грибковых инфекций и могут быть использованы в качестве природных антисептиков и противомикробных агентов в ветеринарии. Из данного исследования сделан вывод, что эти продукты могут быть использованы в лечебно-профилактических мероприятиях в аквакультуре в качестве антимикробных средств. Дальнейшие исследования необходимы для идентификации биологически активных соединений, которые послужат платформой для дальнейших фармакологических исследований и клинического применения.

Ключевые слова: антибактериальная активность, *Aeromonas hydrophila*, радужная форель *Oncorhynchus mykiss*, *Ficus* spp., спиртовые экстракты, зона ингибирования роста.