

macrophages and fibroblasts. The liver exhibited focal necrosis of the hepatocytes with tubular degeneration of the intestinal microvilli and hepatocellular necrosis. The liver tissues appeared oedematous and were congested with necrotic foci showing fibrin deposition or slight haemorrhage in the pulp, inflammation, free pre-granulomatous tissue, and mature granuloma.

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IN VITRO INHIBITION OF AEROMONAS HYDROPHILA GROWTH BY ETHANOLIC EXTRACTS OBTAINED FROM LEAVES OF VARIOUS FICUS SPECIES (MORACEAE)

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Introduction. *Aeromonas hydrophila* is an autochthonous species in freshwater environments and a member of the normal microflora in the fish intestinal tract [4]. On the other hand, *A. hydrophila* causes diverse pathologic conditions that include acute, chronic, and latent infections. 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, and 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 [4]. In salmonids, *A. salmonicida* causes furunculosis, a disease characterized by skin ulcers and septicemia. Other *Aeromonas*

species are involved in similar pathological conditions [1].

One of the overriding considerations is how disease may be controlled. 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. The use of genetically disease-resistant stock has already been considered for *A. salmonicida* [3].

The benefits of medicinal plants have been the focus in many studies originating from Asia in controlling infections induced by *Aeromonas* [3]. 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. However, although there are many phytochemical and pharmacological reports on species within the genus *Ficus*, 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) 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 of the research. 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. elastic* Roxb., *F. elastica* 'Variegata', *F. erecta* Thunb., *F. erectavar. sieboldii* (Miq.) King, *F. hispida* L.f., *F. luschanthiana* (Miq.) Miq., *F. lyrata* Warb., *F. macrophylla* Desf. ex Pers., *F. mucoso* 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 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 ratio 1:10) at room temperature.

A. hydrophila (strain E 2/7/15) isolated locally from gill of rainbow trout with clinical features of furunculosis (kidney were gray, liver was pale and fragile, enlarged spleen with exudate in the body cavity) was used as a test organism. Fish infection had a mixed character (*Pseudomonas fluorescens*, *A. hydrophila* complex, *Shewanella putrefaciens*). Bacterial species were identified with the use of the oxidase test and API E test kit (Biomérieux, France). The results of the test were interpreted in accordance with the manufacturer's protocol, after 24 hrs of incubation at 27°C. Code ++V-V---+V+++---+VV+ in API E test were identified as *A. hydrophila*.

Strain tested was plated on TSA medium (Tryptone Soya Agar) and

incubated 24 hrs at 25°C. Then the suspension 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 [2]. Muller-Hinton agar plates were inoculated with 200 μ L of standardized inoculum (10^8 CFU/mL) of bacterium and spread with sterile swabs. Activity was evidenced by the presence of a zone of inhibition surrounding the well.

The results of the research. Our results showed that the *A. hydrophila* (200 μ L of standardized inoculum) showed 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. mucoso*, *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 extracts 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*.

The scientific research on *Ficus* spp. indicated that these plants have received increasing interest in recent years. The antibacterial activity of *Ficus* spp. is possibly linked to the presence of flavonoid compounds. Antibacterial activity of flavonoid compounds isolated from plant species are well documented [5, 6]. Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action [7]. 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 [7].

It is desirable that antibiotic use in fish cultures be reduced and replaced by natural medicines to prevent the emergence of bacterial resistance in aquatic animals and its 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 [8]. 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*.

Conclusion. Our results indicated that extracts offers 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*. 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. 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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SARCOCYSTOSIS OF CATTLE IN UKRAINE

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Introduction. In recent years, parasitic diseases have a tendency for spreading and cause significant economic losses to the national economy. Such a disease as sarcocystosis is dangerous to human health, so the problem of the spread of this infestation also has a social nature [2]. Unfortunately, proper attention is not always given to controlling of parasitic infestations, primarily due to the fact that the disease is often asymptomatic. Nowadays more than 150 species of sarcocystis, which affect domestic, wild animals, reptiles, birds, marine mammals and humans, are known. In productive animals (intermediate hosts) the parasites are localized in different muscles, forming cysts under the sarcolemma of muscle fibers. The intensity of sarcocystis infestation of different muscles in cattle is not the same [1]. Based on the results of studies carried out by Fukuyo M. et al. among beef cattle in Mongolia in 1998-1999, the heart muscle had the highest intensity of sarcocystis infestation compared to other muscles [3]. W. Hirschman (1974) reported that the sarcocystis cysts were often found in the muscular tissue of the esophagus, and M. Saito, T. Nakajima, A. Wa-