DOI 10.32900/2312-8402-2019-122-18-30

УДК 639.3:615.32:615.38

AN IN VITRO ASSESSMENT OF THE ANTI-AEROMONAS PROPERTIES OF LEAF EXTRACT OBTAINED FROM *FICUS* *LYRATA* WARB. (MORACEAE)

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The aim of the present study was to assess the antibacterial efficacy of ethanolic extracts derived from F. lyrata and its cultivar F. lyrata 'Bambino' against three Aeromonas strains (Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida). The leaves of plants were collected in M. M. Gryshko National Botanic Garden (NBG, Kyiv, Ukraine) and Botanic Garden of Ivan Franko Lviv National University (Lviv, Ukraine). Freshly collected leaves were weighed and homogenized in 96 % ethanol (in proportion 1:10) at room temperature. Three Aeromonas strains: Aeromonas sobria (K825) and Aeromonas hydrophila (K886), as well as Aeromonas salmonicida subsp. salmonicida (St30), originated from freshwater fish species such as common carp (Cyprinus carpio L.) and rainbow trout (Oncorhynchus mykiss Walbaum), respectively, were isolated in Department of Fish Diseases, The National Veterinary Research Institute in Pulawy (Poland). Bacteria were collected from fish exhibiting clinical disorders. Our results demonstrated that three Aeromonas strains (Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida subsp. salmonicida) were resistant to ethanolic extract derived from F. lyrata. The inhibition zone diameters were (9.50±0.33 mm), (9.38±0.38 mm), and (9.5±0.5 mm) for Aeromonas sobria, Aeromonas hydrophila and Aeromonas salmonicida subsp. salmonicida (St30), respectively. F. lyrata 'Bambino' extract exhibited the intermediate activity against Aeromonas sobria (inhibition zone diameter was 12±0.73 mm), while Aeromonas hydrophila and Aeromonas salmonicida subsp. salmonicida (St30) were resistant (inhibition zone diameters were 9.18±0.54 mm and 9.13±0.44 mm). These results pave the way for the possible development of natural additives to replace synthetic ones. Therefore, further investigations for the isolation of active constituents and their pharmacological evaluation as well as in vitro and in vivo study are required.

Keywords: *Ficus lyrata*, *Ficus lyrata* 'Bambino', *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*, antimicrobial activity, disc diffusion technique, ethanolic extracts.

The genus *Aeromonas*, currently with 32 recognized species [13], is constituted by facultative anaerobic, Gram-negative, rod-shaped and non-spore-forming bacteria of

approximately 1–3 μm in length [14, 26, 27]. Moreover, they are oxidase-positive, capable of fermenting glucose and characterized by tolerating increasing concentrations of NaCl varying from 0.3 to 5 % [14, 26]. *Aeromonas* are emerging pathogens capable of colonizing and infecting several hosts [1]. They are inhabitants of marine environments, so fish and other seafood are the most common sources for isolating these microorganisms. Therefore, they are widely known in aquaculture as potentially infectious organisms and can cause diseases such as septicemia and furunculosis [14, 46].

The role of *Aeromonas* spp. as a causative agent of fish diseases have been known for decades, longer than their comparable role in causing systemic illnesses in humans [15]. Nevertheless, there are contradicting suggestions on whether the microbe is a primary agent of diseases or an opportunistic one causing diseases to hosts that are immune-compromised and stressed [23].

The fish disease is one of the major threats to the sustainable development of aquaculture causing loss of millions of dollars annually [47]. 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 [8]. In recent years the use of antibiotics to treat and control fish diseases has been banned in the EU [4]. 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 [20].

Plant extracts have been reported to favor various activities like anti-stress, growth promotion, appetite stimulation, enhancement of tonicity and immunostimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish and shrimp aquaculture due to active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils [7, 30]. Plants belonging to *Ficus* L. genus and their constituents are used widely to prevent and treat human and animal diseases. The possible role and mode of action of these natural products are discussed with regard to the prevention and treatment of cancer, cardiovascular diseases including atherosclerosis and thrombosis, as well as their bioactivity as antibacterial, antiviral, antioxidants and antidiabetic agents [3, 6, 11, 19, 32, 49].

In our previous studies, the therapeutic potential for the use of various plants of *Ficus* genus in the control of bacterial diseases was evaluated against fish pathogens in *in vitro* study with promising results [33–44]. Most ethanolic extracts obtained from *Ficus* spp. in our 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 [39]. 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 [41].

We have reported already data on the antioxidant and antibacterial effects of extracts from various plant belonged to the *Ficus* genus [33–44]. Our current scientific project undertaken in the frame of cooperation programme between Institute of Biology and Environmental Protection (Pomeranian University in Slupsk, Poland), Department of Fish Diseases, National Veterinary Research Institute (Pulawy, Poland), M. M. Gryshko National Botanic Gardens of National Academy of Sciences of

Ukraine (Kyiv, Ukraine), and Ivan Franko Lviv National University (Lviv, Ukraine) directed to assessment of medicinal properties of tropical plants.

Therefore, the aim of the present study was to evaluate the antibacterial efficacy of ethanolic extracts derived from *F. lyrata* and its cultivar *F. lyrata* 'Bambino' against three *Aeromonas* strains (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*).

Materials and methods. Collection of plant material and preparing plant extract. The leaves of *F. Lyrata* and its cultivar *F. lyrata* 'Bambino' were collected in M. M. Gryshko National Botanic Garden (NBG, Kyiv, Ukraine) and Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine). The whole collections of tropical and subtropical plants both at NBG and Botanical Garden of Ivan Franko National University in Lviv (including *Ficus* spp. plants) have the status of a National Heritage Collection of Ukraine. The species author abbreviations were followed by Brummitt and Powell (1992).

Ficus lyrata Warb., commonly known as the fiddle-leaf fig, is a monoecious evergreen tree up to 15 m tall, native to western Africa. It belongs to those species known as hemi-epiphytes, which start life as an epiphyte in the crown of another tree and then send roots down to the ground enveloping the trunk of the host tree (Fig. 1). It can also grow as a terrestrial tree or shrub, especially in cultivation. Its leaves are broadly pandurate, 20-40 cm long and 10-30 cm wide, coriaceous and glabrous, with shortly acuminate apex and cordate base. Figs are axillary, sessile or occasionally pedunculate, globose, 4-6.5 cm in diameter, wrinkled, puberulous, at maturity greenish, often with paler spots [5, 28].

Fig. 1. Twigs of *Ficus lyrata* (A) and *Ficus lyrata* 'Bambino' (B), cultivated at M. M. Gryshko National Botanic Garden, National Academy of Science of Ukraine

The sampled leaves were brought into the laboratory for antimicrobial studies. Then, freshly collected leaves were 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.

Method of culturing pathological sample and identification method of the *Aeromonas* strains. Three *Aeromonas* strains: *Aeromonas sobria* (K825) and *Aeromonas hydrophila* (K886), as well as *Aeromonas salmonicida* subsp. *salmonicida* (St30), originated from freshwater fish species such as common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum), respectively, were isolated in Department of Fish Diseases, 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) [17].

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 according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014) [9, 10]. 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. Seven drugs representing different antimicrobial classes as quinolones, tetracyclines, sulphonamides, and phenicols were used. After incubation, the inhibition zones were measured. Interpretation criteria have been adopted from that available for *Aeromonas salmonicida* [17].

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 randomized according to the phytochemical activity of strains tested. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm [25].

Results and discussion. The results of antimicrobial activity of an ethanolic extract derived from *F. lyrata* and *F. lyrata* 'Bambino' leaves against three *Aeromonas* strains are presented in Fig. 2.

Fig. 2. The inhibition zone diameters of *Aeromonas* strains' growth (1000 µL inoculum) exhibited by the ethanolic extract derived from *Ficus lyrata* and *F. lyrata* 'Bambino' leaves (M ± m, n = 8).

Our results demonstrated that three *Aeromonas* strains (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*) were resistant to ethanolic extract derived from *F. lyrata*. The inhibition zone diameters were (9.50±0.33 mm), (9.38±0.38 mm), and (9.5±0.5 mm) for *Aeromonas sobria*, *Aeromonas hydrophila* and *Aeromonas salmonicida* subsp. *salmonicida* (St30), respectively. *F. lyrata* 'Bambino' extract exhibited the intermediate activity against *Aeromonas sobria* (inhibition zone diameter was 12±0.73 mm), while *Aeromonas hydrophila* and *Aer-*

omonas salmonicida subsp. *salmonicida* (St30) were resistant (inhibition zone diameters were 9.18 ± 0.54 mm and 9.13 ± 0.44 mm) (Fig. 2).

The same results on *F. lyrata* were obtained in case of other bacterial strains in our previous study. Our results showed that the ethanolic extract of *F. lyrata* leaves exhibited moderate activity against the Gram-positive bacteria (11.3 mm of inhibition zone diameter for *Staphylococcus aureus*), and the Gram-negative bacteria (10.3 mm for *Escherichia coli*). *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and *Streptococcus pneumoniae* appeared to be less sensitive to the extracts, the inhibition zone was 8.9 mm, 8.5 mm, 8.9 mm, and 8.4 mm, respectively [33].

F. lyrata showed potent antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Shigella boydii*, *Citrobacter freundii*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella* in the study of Rizvi and co-workers (2009). The aqueous extract appeared more potent than alcoholic extract. Furthermore, the isolated compounds were more potent and showed an improved spectrum of activity as compared to the crude extract. The minimum inhibitory concentration (MIC) of aqueous extract of *F. lyrata* and the isolated compounds were found to be significantly low for all the tested bacterial strains. The study Rizvi and co-workers (2009) suggests that the extracts obtained from the leaves of *F. lyrata* possess excellent antibacterial activity [31].

Wira and co-workers (2018) have compared the potential utilization of *F. lyrata* fruit and leaf extract as antimicrobial agents. The predicted result of their research is a difference in the concentration of compound bioactive and antimicrobial activity between fruit and leaf extract. This research showed that extracts of *F. lyrata* fruit and leaf could use as antimicrobial resources. The phytochemical screening resulted in the water fruit leaf extract the presence of secondary metabolites like flavonoids, phenolic, and tannin. Antimicrobial activity was observed by the disc diffusion method against bacterial pathogens including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. The result showed that the leaf extract has a higher of bioactive compounds than fruit extract, on the other side, antimicrobial activity is not a significant difference [48].

Other researchers also demonstrated the antimicrobial effects of plants belonging to the *Ficus* genus. For example, Valsaraj and co-workers (1997) evaluated activity of ethanolic extracts from *F. benghalensis* aerial roots and *F. religiosa* leaves, among a large number of plants, against four bacterial strains (*Bacillus subtilis* ATCC 6633, *E. coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538) and two fungi (*Aspergillus niger* IMI 076837 and *Candida albicans* IMI 349010), using the agar dilution method for the former and agar-well diffusion method for the latter. Of these, only *F. religiosa* extracts were active against *P. aeruginosa*, showing weak inhibition at a concentration of 25 mg/ml [45].

Nimri and co-workers (1999) evaluated activity of 15 medicinal plant species ethanolic extracts against 14 bacterial strains, including Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus* ATCC 8095, *S. epidermidis*, *Streptococcus pyogenes* ATCC 12351, and *Enterococcus faecalis*) and Gram-negative bacteria (*Shigella dysenteriae* ATCC 49345, *Yersinia enterocolitica* ATCC 9610, *E. coli* ATCC 25922, clinical isolates *E. coli* B, 0111 and 2759, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*). *P. aeruginosa* was inhibited by most of the plant extracts, being among the most susceptible microorganisms tested. However, all bacteria appeared resistant to the fruit extract of *F. carica*, the only *Ficus* species studied [24].

Farrukh and Ahmad (2003) investigated antimicrobial activity of ethanolic extracts of 22 Indian medicinal plant species against seven bacteria (*Escherichia coli*,

Pseudomonas aeruginosa, *Salmonella typhimurium*, *S. paratyphi*, *S. typhi*, *Shigella dysenteriae*, and *Staphylococcus aureus*) and five filamentous fungi species (*Alternaria alternata*, *Aspergillus niger*, *Fusarium chlamydosporum*, *Rhizoctonia bataticola*, and *Trichoderma viride*). Of these, extracts from *F. carica* fruits and *F. religiosa* leaves were generally weakly effective (mostly 10 to 15 mm of inhibition zone diameter or no effect) with higher expression of antibacterial action. However, only *F. carica* extract was tested against *P. aeruginosa*, showing an inhibition zone of 11-15 mm. No inhibition of any organism tested caused the extracts from *F. rumphii* and *F. benghalensis* leaves [12].

Rajiv and Rajeshwari (2012) screened antimicrobial activity of *F. religiosa* bark, leaf, stem, and fruit aqueous extracts against a number of major pathogens (*Aeromonas hydrophila*, *Enterobacter aerogenes*, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger*, and *Candida albicans*) and conducted their phytochemical analysis. 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. All extracts at all concentrations tested affected *P. aeruginosa*, although the strongest inhibition showed the maximal concentration extracts from leaves and stems (inhibition zone diameter 14 mm) and the slighter effect was produced by bark (13 mm) and fruit (12 mm) extracts. 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 phytochemical analysis, they do not make any inferences concerning possible contribution of particular chemical classes to the antimicrobial activity of plant extracts [29].

Nair and Chanda (2006) screened aqueous and ethanol extracts from 20 plant species, among which were four species of *Ficus* (*F. benghalensis*, *F. racemosa*, *F. religiosa*, and *F. tiselae*), against seven Gram-negative (*Pseudomonas aeruginosa* ATCC27853, *Pseudomonas testosteroni* NCIM5098, *Proteus mirabilis* NCIM2241, *Proteus vulgaris* NCTC8313, *Enterobacter aerogenes* ATCC10240, *Escherichia coli* ATCC25922, and *Citrobacter freundii* ATCC10787) and five Gram-positive (*Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* ATCC11778, *Streptococcus fecalis* ATCC29212, *Streptococcus cremoris* NCIM2179, and *Streptococcus agalactiae* NCIM2401) bacterial strains. Aqueous extracts generally showed less activity than ethanol extracts and Gram-positive bacteria were generally more affected than Gram-negative ones. The examined *Ficus* species, of which bark extracts were used, showed low inhibition activity in general. Only their methanolic extracts affected *P. aeruginosa* with small inhibition zone diameter, namely 3 mm for *F. tiselae*, 2,5 mm for *F. racemosa*, and 2 mm for *F. benghalensis*. Neither *F. religiosa* extract showed activity against *P. aeruginosa* [21].

Further studies (Nair and Chanda, 2007) tested aqueous and ethanol extracts from ten Indian plant species, including the same species of *Ficus* (*F. benghalensis*, *F. racemosa*, *F. religiosa*, and *F. tiselae*), against several medically important bacterial strains (*Alcaligenes faecalis* ATCC 8750, *Bacillus cereus* ATCC 11778, *Pseudomonas*

aeruginosa ATCC 27853, *Proteus mirabilis* NCIM 2241, *Salmonella typhimurium* ATCC 23564, and *Staphylococcus aureus* ATCC 25923). The ethanol extracts were more potent than aqueous extracts of all the plants studied. Almost all *Ficus* bark extracts showed activity against each of the tested bacteria, although the strength of inhibition varied. *P. aeruginosa* was among the most resistant bacteria tested. *Ficus* species demonstrated low inhibition of *P. aeruginosa*, with inhibition zone diameter values of 3 mm (*F. benghalensis* and *F. tiselae* ethanolic extracts) and 2 mm (*F. racemosa* ethanol extract). Aqueous extracts of all *Ficus* species and both extracts of *F. religiosa* appeared inactive against *P. aeruginosa* [22].

Atindehou and co-workers (2002) tested crude ethanol extracts from 115 plant species against Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Cladosporium cucumerinum*). Among the examined plants, there were three *Ficus* species, namely *F. exasperata*, *F. mucuso*, and *F. sur*. The Gram-negative bacteria appeared unaffected by any plant extract tested, whereas the Gram-positive bacteria and fungi were inhibited by at least several plant species. Among *Ficus* species tested, *F. exasperata* and *F. mucuso* had no significant effect on any microorganism, while *F. sur* appeared among the most active plant species against Gram-positive bacteria [2].

Koné and co-workers (2004) screened crude ethanol extracts from 50 plant species of 31 families, among which were *F. thonningii* and *F. vallis-choudae*, for *in vitro* activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus subtilis*) bacteria. Only Gram-positive bacteria appeared inhibited by the tested extracts. Of two *Ficus* species examined, only *F. thonningii* leaf extracts showed activity against strains of *E. faecalis* and *S. pyogenes*. Furthermore, it was found one of the most active plant species against these two bacteria, showing inhibitory concentrations (IC₁₀₀) of 94 µg/ml on some resistant strains of *E. faecalis* and of 23-47 µg/ml on hospital strains of *S. pyogenes*. The inhibitory concentrations (IC₁₀₀) was defined as the lowest concentration of crude plant extract at which the visible growth of a strain was completely inhibited (no turbidity in wells) [16].

Kubmarawa and co-workers (2007) carried out an antimicrobial and phytochemical screening of 50 Nigerian plant species ethanolic extracts, among which were five species of *Ficus* (i.e., *F. abutilifolia* (Miq.) Miq., *F. platyphylla* Del., *F. polita* Vahl, *F. sycomorus* L., and *F. thonningii* Blume). Microbial strains used in the study were *Bacillus subtilis* NCTC 8236, *E. coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 13709, and *Candida albicans* ATCC 10231. *Ficus* stem bark extracts demonstrated comparatively low antimicrobial activity, with the broadest activity spectrum being of *F. thonningii* extract (active against all microorganisms except *P. aeruginosa* and *S. aureus*). Extracts from *F. polita* and *F. sycomorus* showed no activity at all. *P. aeruginosa* was in general moderately susceptible compared to other bacteria tested, although no *Ficus* extract was active against it. Phytochemical analysis revealed the presence of only saponins and volatile oil in *F. thonningii* extract and saponins and flavonoids in *F. polita* extract, while richer chemical content was found in *F. abutilifolia* (tannins, alkaloids, and volatile oil), *F. platyphylla* (saponins, flavonoids, alkaloids, and volatile oil), and *F. sycomorus* (glycosides, tannins, flavonoids, and volatile oil) extracts. However, the authors do not make any speculations regarding the contribution of particular chemical classes to the antimicrobial activity of plant extracts tested. Authors also suggest the presence of some compound

classes (such as alkaloids) in plants to be affected by climatic and environmental factors [18].

Conclusions. The ethanolic extracts of *Ficus lyrata* and *Ficus lyrata* ‘Bambino’ were found to exhibit a mild antibacterial growth inhibitory effect against three *Aeromonas* strains. These results pave the way for the possible development of natural additives to replace synthetic ones. Therefore, further investigations for the isolation of active constituents and their pharmacological evaluation as well as *in vitro* and *in vivo* study are required.

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ИССЛЕДОВАНИЕ IN VITRO АНТИМИКРОБНЫХ СВОЙСТВ ЭКСТРАКТА ЛИСТЬЕВ *FICUS LYRATA* WARB. (MORACEAE) В ОТНОШЕНИИ ШТАММОВ *AEROMONAS*

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Цель данного исследования состояла в определении антимикробной активности этанольного экстракта листьев *F. lyrata* и сорта *F. lyrata* 'Bambino' в отношении трех штаммов *Aeromonas* (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*). Образцы листьев были собраны в Национальном ботаническом саду имени Н.Н. Гришко (НБС, Киев, Украина) и Ботаническом саду Львовского национального университета имени Ивана Франко (Львов, Украина). Свежесобранные листья взвешивали и гомогенизировали в 96 %-ном этаноле (в пропорции 1:10) при комнатной температуре. Три штамма *Aeromonas*: *Aeromonas sobria* (K825), *Aeromonas hydrophila* (K886), а также *Aeromonas salmonicida* subsp. *salmonicida* (St30), выделенные из видов пресноводных рыб, таких как карп обыкновенный (*Cyprinus carpio* L.) и радужная форель (*Oncorhynchus mykiss* Walbaum), соответственно, были получены в Отделе заболеваний рыб Национального научно-исследовательского института ветеринарии в Пулавах (Польша). Материал для бактериологических исследований был выделен из рыб с видимыми клиническими проявлениями заболевания. Наши результаты свидетельствуют о том, что три штамма *Aeromonas* (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*) оказались резистентными к этанольным экстрактам листьев *F. lyrata*. Диаметр зоны ингибирования составил $(9,50 \pm 0,33)$ мм, $(9,38 \pm 0,38)$ мм и $(9,5 \pm 0,50)$ мм для *Aeromonas sobria*, *Aeromonas hydrophila* и *Aeromonas salmonicida* subsp. *salmonicida* (St30), соответственно. Экстракт листьев *F. lyrata* 'Bambino' проявил промежуточную активность в отношении *Aeromonas sobria* (диаметр зоны ингибирования составил $12 \pm 0,73$ мм), в то время как штаммы *Aeromonas hydrophila* и *Aeromonas salmonicida* subsp. *salmonicida* (St30) выявили резистентность (диаметр зоны ингибирования составил $9,18 \pm 0,54$ мм и $9,13 \pm 0,44$ мм). Полученные результаты являются предпосылкой для создания природных добавок, способных заменить синтетические. Следовательно, дальнейшие исследования, направленные на выделение активных веществ и их фармакологическое исследование как *in vitro*, так и *in vivo*, представляются нам крайне необходимыми.

Ключевые слова: *Ficus lyrata*, *Ficus lyrata* 'Bambino', *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*, антимикробная активность, диско-диффузионный метод, этанольные экстракты.

ДОСЛІДЖЕННЯ IN VITRO ВЛАСТИВОСТЕЙ ЕКСТРАКТУ ЛИСТКІВ FICUS LYRATA WARB. (MORACEAE) ПО ВІДНОШЕННЮ ДО ШТАМІВ AEROMONAS

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Мета даного дослідження полягала у визначенні антимікробної активності етанольного екстракту листків *F. lyrata* та сорту *F. lyrata* 'Bambino' по відношенню до трьох штамів *Aeromonas* (*Aeromonas sobria*, *Aeromonas*

hydrophila, *Aeromonas salmonicida* subsp. *salmonicida*). Зразки листків було зібрано в Національному ботанічному саду імені М. М. Гришка (НБС, Київ, Україна) та Ботанічному саду Львівського національного університету імені Івана Франка (Львів, Україна). Свіжозібрані листки зважували та гомогенізували в 96%-ному етанолі (у співвідношенні 1:10) при кімнатній температурі. Три штами *Aeromonas*: *Aeromonas sobria* (K825), *Aeromonas hydrophila* (K886), а також *Aeromonas salmonicida* subsp. *salmonicida* (St30), виділені з видів прісноводних риб, таких як короп звичайний (*Cyprinus carpio* L.) та райдужная форель (*Oncorhynchus mykiss* Walbaum), відповідно, були отримані у Відділі захворювань риб Національного науково-дослідного ветеринарного інституту в Пулавах (Польща). Матеріал для бактеріологічних досліджень було виділено з риб із вираженими клінічними проявами захворювання. Наші результати свідчать про те, що три штами *Aeromonas* (*Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*) виявили резистентність до етанольних екстрактів листків *F. lyrata*. Діаметр зони інгібування становив ($9,50 \pm 0,33$ мм), ($9,38 \pm 0,38$ мм) і ($9,5 \pm 0,50$ мм) для *Aeromonas sobria*, *Aeromonas hydrophila* та *Aeromonas salmonicida* subsp. *salmonicida* (St30), відповідно. Екстракт листків *F. lyrata* 'Bambino' виявив проміжну активність стосовно *Aeromonas sobria* (діаметр зони інгібування становив $12 \pm 0,73$ мм); натомість штами *Aeromonas hydrophila* і *Aeromonas salmonicida* subsp. *salmonicida* (St30) виявили резистентність до дії екстракту (діаметр зони інгібування становив $9,18 \pm 0,54$ мм і $9,13 \pm 0,44$ мм). Отримані результати є передумовою для створення природних добавок, здатних замінити синтетичні. Відповідно, подальші дослідження, спрямовані на виділення активних сполук та їх фармакологічне дослідження як *in vitro*, так і *in vivo*, є, на наш погляд, вкрай необхідними.

Ключові слова: *Ficus lyrata*, *Ficus lyrata* 'Bambino', *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*, антимікробна активність, диско-дифузійний метод, етанольні екстракти.

DOI 10.32900/2312-8402-2019-122-30-42

УДК 639.3:615.32:615.38

EVALUATION OF OXIDATIVE STRESS BIOMARKERS LEVELS IN THE EQUINE BLOOD AFTER *IN VITRO* TREATMENT WITH *SANSEVIERIA CAULESCENS* N.E.BR. EXTRACT

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The main goal of present study was to evaluate the level of the 2-thiobarbituric acid reactive substances (TBARS) as lipid peroxidation biomarker, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes' suspension induced by treatment of leaf extracts obtained from