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THE ANTIBACTERIAL ACTIVITY OF THE ETHANOLIC LEAF EXTRACT OF *FICUS PUMILA* L. (MORACEAE) AGAINST FISH BACTERIAL PATHOGENS

The aim of this study was to test the antibacterial properties of ethanolic extract prepared from Ficus pumila L. leaves against fish pathogens, Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri to evaluate the possible use of this plant in preventing infections caused by these bacteria in aquaculture. The antimicrobial susceptibility testing was done on Muller-Hinton agar by disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol). Muller-Hinton agar plates were inoculated with 200 and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium and spread with sterile swabs. Aeromonas hydrophila (strain E 2/7/15) isolated locally from gills of rainbow trout (Oncorhynchus mykiss Walbaum) and Pseudomonas fluorescens (strain E 1/7/15) isolated locally from internal organs of rainbow trout with clinical features of furunculosis, Citrobacter freundii isolated locally from gills of eel (Anguilla anguilla L.) with clinical features of disease, as well as Yersinia ruckeri collected from clinically healthy fish and fish with clinical symptoms of yersiniosis were used as test organisms. In our study, the A. hydrophila and C. freundii strain (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility to ethanolic extract obtained from leaves of F. pumila (inhibition zone diameters ranged from 10 to 11 mm). Ethanolic extract derived from F. pumila leaves exhibited the highest antibacterial activity against Pseudomonas fluorescens causing a zone of inhibition, comprising at least 13-14 mm for 200 µL and 9-10 mm for 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium strain. Y. ruckeri isolate (200 and 400 µl of standardized inoculum) revealed high susceptibility to ethanolic extract obtained from leaves of F. pumila (inhibition zone diameters ranged between 12 and 14 mm). Further studies aimed at the isolation and identification of active substances from the ethanolic extracts obtained from leaves of F. pumila could also disclose compounds with better therapeutic value. It is believed that screening of plants from Ficus genus for other biological activities including antimicrobial activity is essential. Finally, it seems important not only to determine the substances involved in the activity against pathogens but also to establish the induced response in the fish physiology.

Key words: *Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri, antimicrobial activity, disc diffusion technique, ethanolic extrac.t*

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АНТИМИКРОБНАЯ ЭФФЕКТИВНОСТЬ ЭТАНОЛЬНОГО ЭКСТРАКТА, ПОЛУЧЕННОГО ИЗ ЛИСТЬЕВ *FICUS* PUMILA L. (MORACEAE) ОТНОСИТЕЛЬНО БАКТЕРИАЛЬНЫХ ПАТОГЕНОВ РЫБ

Целью этого исследования была оценка антимикробной эффективности этанольного экстракта, полученного из листьев Ficus pumila L. относительно бактериальных патогенов рыб (Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri), и возможность применения этого растения для предотвращения инфекций, вызванных этими бактериями в аквакультуре. Листья F. pumila были собраны в Национальном ботаническом саду им. Н.Н. Гришко НАН Украины (Киев, Украина). Свежие листья промывали, измельчали, взвешивали и гомогенизировали

в 96%-м этаноле для получения 10 % экстрактов. Aeromonas hydrophila (штамм Е 2/7/15), Pseudomonas fluorescens (штамм Е 1/7/15), а также Yersinia ruckeri, выделенные из внутренних органов радужной форели (Oncorhynchus mykiss Walbaum) с клиническими признаками фурункулеза и иерсиниоза, а также Citrobacter freundii, изолированный из внутренних органов угря (Anguilla anguilla L.) с клиническими признаками заболевания, мы использовали в этом исследовании как тестовые штаммы в дискодиффузионном методе Кирби-Бауэра (1966). В чашки с агаром Muller-Hinton инокулировали 200 и 400 мкл стандартизованного инокулята (10^8 КОЕ/мл) бактерий. В нашем исследовании штамм A. hydrophila и C. freundii (200 и 400 мкл стандартизированного инокулята) продемонстрировал среднюю восприимчивость к этанольному экстракту, полученному из листьев F. pumila (диаметры зон ингибирования роста штамма варьировались от 10 до 11 мм). Самая высокая антибактериальная активность экстракта продемонстрирована относительно штамма Pseudomonas fluorescens (диаметр зоны ингибирования роста штамма составлял 13–14 мм для 200 мкл стандартизированного инокулята и 9–10 мм – для 400 мкл инокулята бактериального штамма). Изолят Y. ruckeri (200 и 400 мкл посевного материала) проявил высокую восприимчивость к этанольному экстракту, полученному из листьев F. pumila (диаметры зон ингибирования составляли от 12 до 14 мм). Дальнейшие исследования, направленные на выделение и идентификацию активных веществ из этанольных экстрактов, полученных из листьев F. pumila, помогут целенаправленно улучшить терапевтическую ценность экстрактов. Важно не только определить вещества, участвующие в активности относительно патогенов, но и установить индуцированный физиологический ответ рыб в исследованиях in vivo.

Ключевые слова: *Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri, антимикробная активность, дискодиффузионный метод Байера-Кирби, этанольный экстракт.*

Introduction

Infectious diseases in aquaculture are of major concern to the industry and are typically controlled by eradication of the pathogen, treatment with antibiotic or chemotherapeutics [6]. Many plants were shown to have potential for being effective herbal drugs against the fish and other aquaculture pathogens [17]. Therefore, use of natural products has been considered as an alternative to antibiotics in fish health management to control bacterial infections in aquaculture. Additionally, it is an attractive method for increasing the protective capabilities of fish [15]. One of the potential plants that can be used as antimicrobial to enhance survival and immune competence is *Ficus* genus plants.

Among 37 genera of *Moraceae* comprising 1050-1100 species in total, *Ficus* L. is the largest one with ca 750 species of tropical and subtropical distribution worldwide [4]. *Ficus* trees have widely been used by humans over their history in a variety of industries and fields of activity. Virtually all parts of their body are utilized by local people in various medicinal practices to cure wounds, sores, stomach and eye problems, headaches and toothaches, and even tumours and cancer, etc. A number of species are known helpful in healing disorders of digestive and respiratory systems, parasitic infections, and also as painkillers, tonics, and ecbolics [12].

Ficus L. is one of the largest genera of angiosperms, with about 750 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics of the world [4]. *Ficus* trees have widely been used by humans over their history in a variety of industries and fields of activity. Virtually all parts of their body are utilized by local people in various medicinal practices to cure wounds, sores, stomach and eye problems, headaches and toothaches, and even tumours and cancer, etc. A number of species are known helpful in healing disorders of digestive and respiratory systems, parasitic infections, and also as painkillers, tonics, and ecbolics [12].

Ficus pumila L. is a (gyno)dioecious evergreen root-climber naturally occurring in continental southern and eastern Asia. Leaves are distichous, 1-10 cm long and 0.7-6 cm wide, elliptic to

oblong or ovate. The species represents dimorphy of leaves and stems: sterile climbing stems possess short adventitious roots and small asymmetric leaves, while the fertile non-climbing stems without roots bear large symmetric coriaceous leaves. A separate plant may possess either two or one stem (and leaf) type, with the fertile branches tenting to develop in mature plants reaching the forest canopy. Figs are axillary or below the leaves, solitary, 3.5-6.5 cm in diameter, pedunculate and obovoid, pubescent, purple to blackish at maturity [4].

Ficus (Moraceae) species are reported to have antimicrobial activity against several pathogenic bacteria and have been used as traditional medicines for the treatment of human diseases [2, 19, 20]. Moreover, in line with the growing interest in the antibacterial properties of different plants, in our previous researches, we have used ethanolic extracts derived from leaves of various *Ficus* species to assess antibacterial activity against harmful fish pathogens, *Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens* [21-26].

Therefore, the aim of this study was to test the efficacy of ethanolic extract prepared from *F. pumila* leaves against fish pathogens, *Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens,* and *Yersinia ruckeri* to evaluate the possible use of this plant in preventing infections caused by these bacteria in aquaculture.

Materials and methods

Collection of Plant Material and Preparing of Plant Extract. The leaves of *F. mucuso* were sampled in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The whole collection of tropical and subtropical plants at M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) (including *Ficus* spp. plants) has the status of a National Heritage Collection of Ukraine. 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, and centrifuged at 3,000 g for 5 minutes. Supernatants were stored at -20°C in bottles protected with laminated paper until required.

Method of Culturing Pathological Sample and identification Method of the Bacteria. *Aeromonas hydrophila* (strain E 2/7/15) isolated locally from gill of rainbow trout (*Oncorhynchus mykiss* Walbaum) and *Pseudomonas fluorescens* (strain E 1/7/15) isolated locally from internal organs 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), as well as *Citrobacter freundii* isolated locally from gill of eel (*Anguilla anguilla* L.) with clinical features of disease were used as test organisms. 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. 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). After 48 hrs of incubation at 27˚C, characteristic pink colonies were selected for further examination.

The isolates of *Y. ruckeri* were collected from clinically healthy fish and fish with clinical symptoms of yersiniosis. Internal organs (predominantly pronephros and gills) as well as intestinal swabs were sampled. Tissue samples were homogenized and inoculated on nutritional agar with 5% blood (Columbia Blood Agar, Oxoid). Following a 24 h incubation period at 25˚C ± 2˚C, distinctive colonies were transferred onto TSA. Round, elevated, shining and whitish colonies without haemolytic properties were considered typical of *Y. ruckeri.* After 24h-incubation at 25˚C ± 2˚C, an oxidase test and Gram-staining were performed. Gram-negative and oxidase-negative isolates were cultured on TSA medium and incubated for 24 h at 25˚C ± 2˚C.

Preliminary characterization of isolates. Bacterial species were identified with the use of 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*. The strain was obtained from Diagnostics Laboratory of Fish and Crayfish Diseases, Department of Veterinary Hygiene, Provincial Veterinary Inspectorate in Olsztyn (Poland).

For characterization of *Y. ruckeri* isolates, bacteria were Gram-stained and then morphologically evaluated. 24h bacterial culture was wet-mounted and a microscopic smear on the slide was prepared. Following fixation over the flame, the slide was Gram-stained with a Gram colour set (Merck) according to the manufacturer's instructions. The shape of the bacteria was determined by observing the microorganisms under a light microscope at 1000^x with immersion oil [9, 28]. Motility was examined on a wet mount. A drop of distilled water was put on a cover slip and bacteria were mounted on it with drops of distilled water put on the corners of a slip. The slip was then covered with a special microscopic slide with an indentation and the whole set was vigorously turned. The motility of the bacteria was evaluated under a light microscope at 400x [9].

Oxidase test was performed according to manufacturer's instruction (Merck). Biochemical properties of individual *Y. ruckeri* isolates were investigated with the API 20E system (BioMerieux). Tests were performed according to the manufacturer's instructions. The results, namely, the presence or a lack of reaction, were read based on the key featured in the operating procedure provided by the manufacturer of the assay. The results were analysed with the Apiweb software (BioMerieux) to identify the investigated bacterium.

Bacterial Growth Inhibition Test of Plant Extracts by the Disk Diffusion Method. Strains tested were plated on TSA medium (Tryptone Soya Agar) and incubated for 24 hrs at 25°C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibacterial activity [3]. Muller-Hinton agar plates were inoculated with 200 and 400 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). 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.

Statistical analysis. Each test was repeated six times and the average values of antimicrobial activity were calculated. All statistical calculation was performed on separate data from each species with STATISTICA 8.0 software (StatSoft, Poland) [29]. 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 [21-26].

Results

Antimicrobial activity of ethanolic extracts obtained from *F. pumila* against *Aeromonas hydrophila*, *Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri* expressed as mean of diameters of inhibition zone is presented in Figs 1-5.

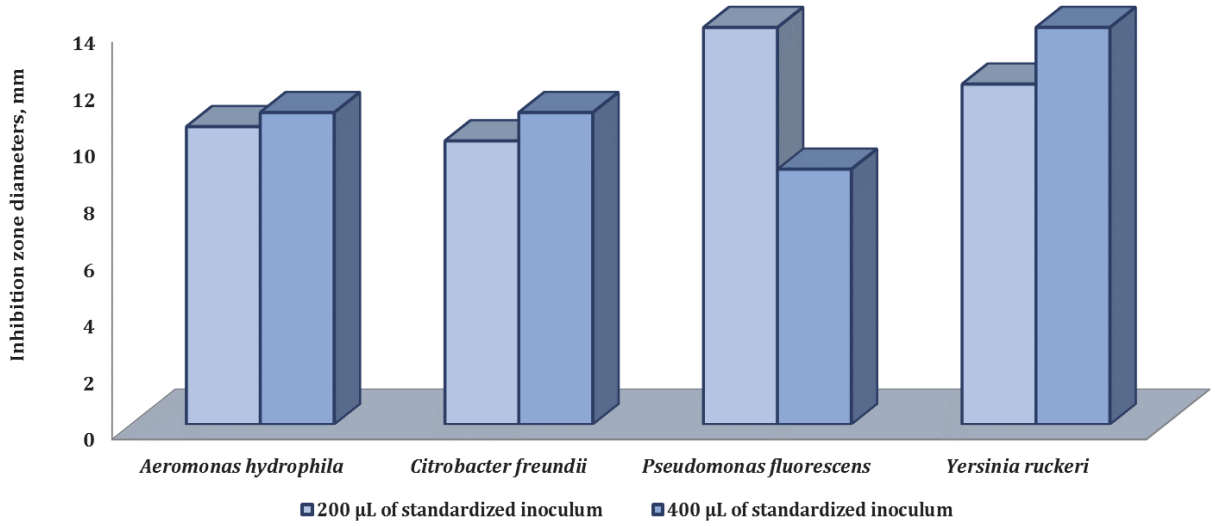
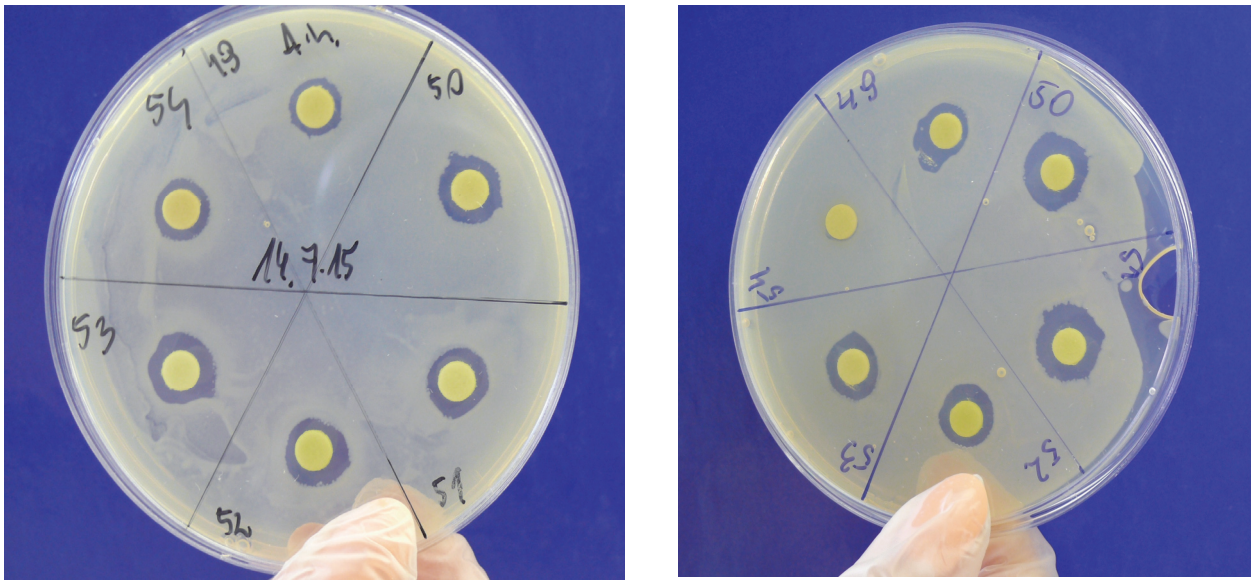


Fig. 1. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* against *Aeromonas hydrophila*, *Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri.* Muller-Hinton agar plates inoculated with 200 and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium

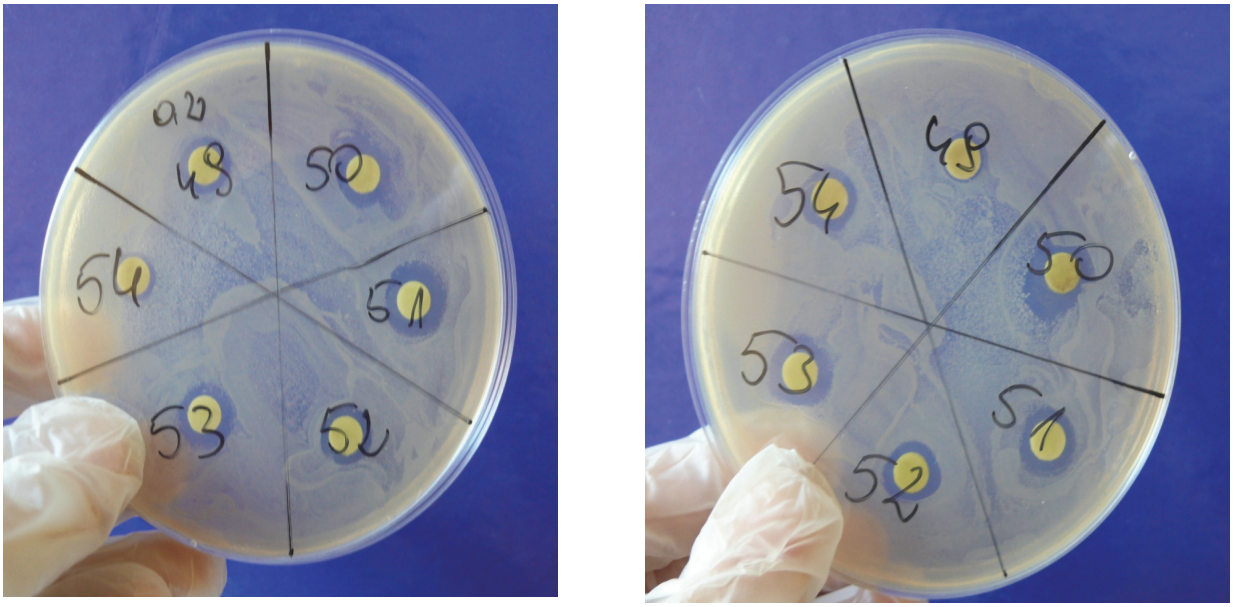
In our study, the *A. hydrophila* and *C. freundii* strain (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility to ethanolic extract obtained from leaves of *F. pumila* (inhibition zone diameters ranged from 10 to 11 mm) (Figs 1-3).



A B

Fig. 2. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* (53) against *Aeromonas hydrophila*. Muller-Hinton agar plates inoculated with 200 (A) and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium (B)

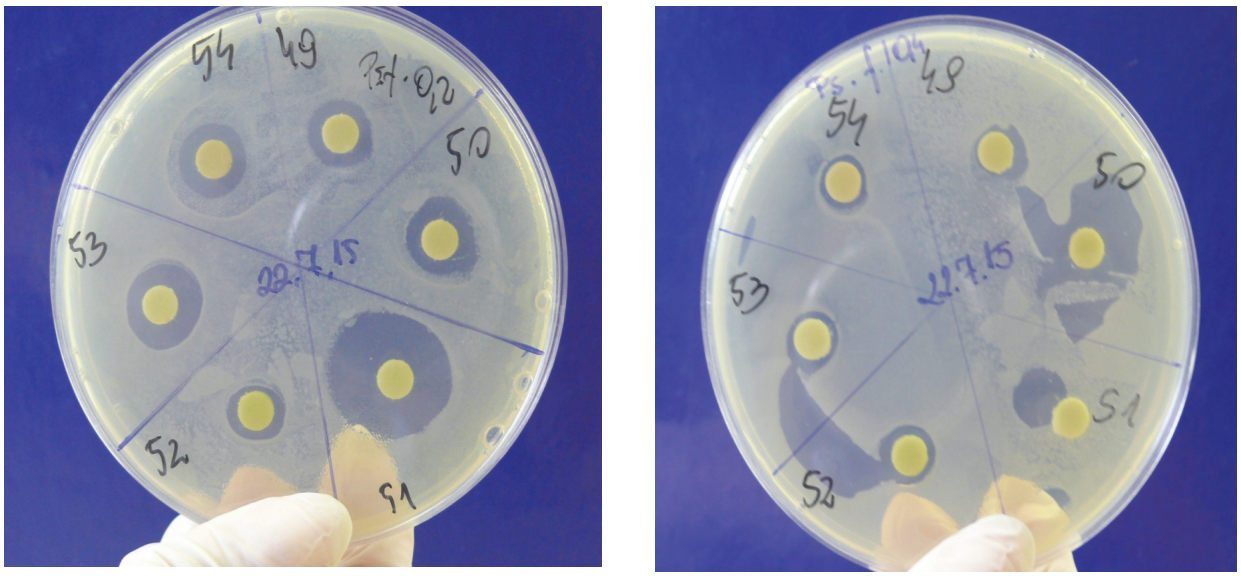
Our results demonstrated that the *C. freundii* (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility to ethanolic extract obtained from leaves of *F. pumila* (inhibition zone diameters were ranged between 10 and 12 mm) (Fig. 3).



A B

Fig. 3. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* (53) against *Citrobacter freundii*. Muller-Hinton agar plates inoculated with 200 (A) and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium (B)

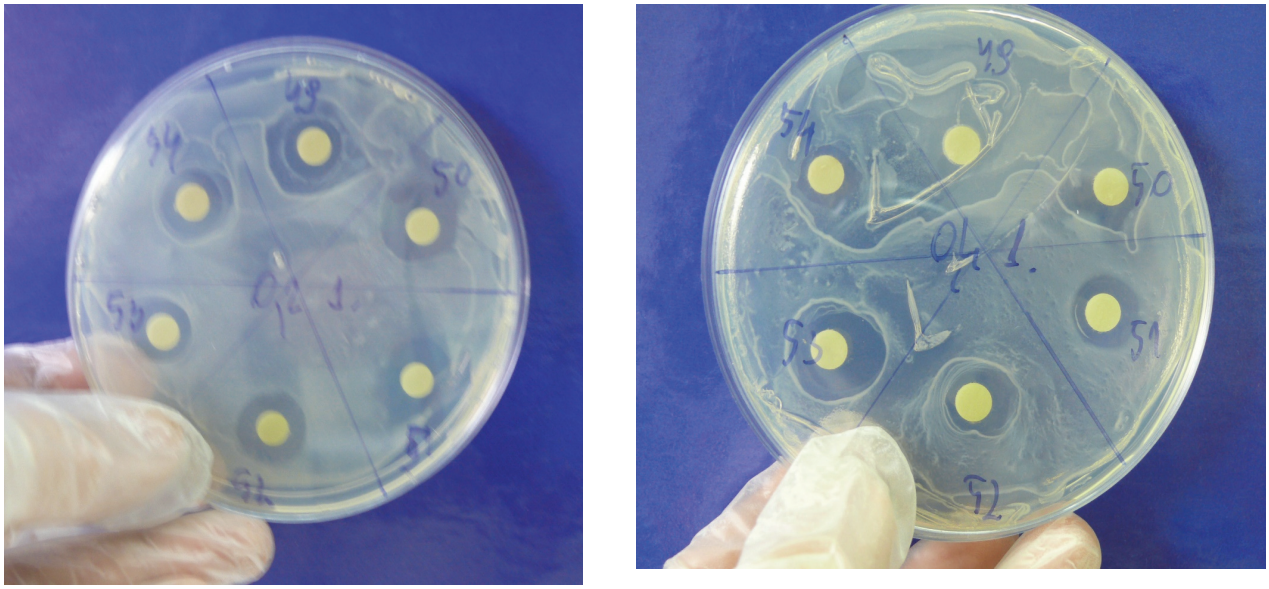
Ethanolic extract derived from *F. pumila* leaves exhibited the highest antibacterial activity against *Pseudomonas fluorescens* causing a zone of inhibition, comprising at least 13-14 mm for 200 µL and 9-10 mm for 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium strain. *Y. ruckeri* isolate (200 and 400 µl of standardized inoculum) revealed high susceptibility to ethanolic extract obtained from leaves of *F. pumila* (inhibition zone diameters ranged between 12 and 14 mm) (Figs 1, 4, 5).



A B

Fig. 4. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* (53) against *Pseudomonas fluorescens*. Muller-Hinton agar plates inoculated with 200 (A) and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium (B)

Y. ruckeri (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility to ethanolic extract obtained from leaves of *F. pumila* (inhibition zone diameters were ranged between 12 and 14 mm) (Fig. 5).



A B

Fig. 5. Antimicrobial activity of ethanolic extracts obtained from *F. pumila* (53) against *Yersinia ruckeri*. Muller-Hinton agar plates inoculated with 200 (A) and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium (B)

Discussion

The antimicrobial activity profile of ethanolic extract obtained from leaves of *F. pumila* against the tested pathogen strains indicated that *Yersinia ruckeri* was the most susceptible bacterium (200 and 400 µl of standardized inoculum) among all the bacterial test strains (Figs 1, 5). Similarly, *P. fluorescens* was found to be sensitive strain (13-14 mm for 200 µL and 9-10 mm for 400 µL of standardized inoculum of bacterium strain) although *A. hydrophila* and *C. freundii* was found to be least susceptible to ethanolic extract obtained from leaves of *F. pumila* (Figs 1-4). Of all the bacterial strains included in the test, *Y. ruckeri* (200 and 400 µl of standardized inoculum dilution) and *P. fluorescens* (200 µl) were found to be the most susceptible and *C. freundii*, which is an isolate from gills of eel, was found to be the least inhibited bacterium (Figs 1, 3-5).

The comprehensive review of usefulness of some medicinal plants (herbal drugs) against fish diseases has been presented by many researchers [5, 7, 8, 13, 19, 20]. In our previous studies, therapeutic potential for the use of various plants of *Ficus* genus in the control of bacterial diseases were evaluated against fish pathogens in *in vitro* study with promising results [21-26]. Antibacterial properties of plant extracts have been by far the most studied bioactivity with potential application in aquaculture systems [18]. Castro and co-workers (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 [5]. 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 [27].

This investigation is in line with our previous works which have revealed a great potential of *Ficus* species as plants with potent antimicrobial properties. In our previous study, the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* species against *Citrobacter freundii* was evaluated. The results proved that the extracts from *F. drupacea, F. septica, F. deltoidea* as well as *F. hispida, F. mucuso, F. pumila, F. craterostoma* exhibit a favorable antibacterial activity against *C. freundii* (200 μL of standardized inoculum) [21]. Our results also proved that the ethanolic extracts obtained from *F. pumila, F. binnendijkii* 'Amstel Gold', *F. carica, F. erecta, F. hispida, F. mucuso, F. palmeri, F. religiosa* possess considerably sufficient antibacterial potential against *C. freundii*. Among various species of *Ficus* screened ethanolic extracts of the leaves of ten *Ficus* species: *F. hispida*, *F. binnendijkii, F. pumila, F. rubiginosa, F. erecta, F. erecta* var. *sieboldii, F. sur, F. benjamina, F. craterostoma, F. lyrata, F. palmeri* (the species are listed in the order of effectiveness against pathogen tested) were the most effective against *P. fluorescens* (200 μL of standardized inoculum) [22]. Moreover, previous investigation has shown that the most effective against *P. fluorescens* (400 μL of standardized inoculum) were the ethanolic extracts obtained from leaves of ten *Ficus* species: *F. craterostoma, F. cyathistipula, F. drupacea* 'Black Velvet', *F. hispida, F. macrophylla, F. mucuso, F. pumila, F. villosa.* 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 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. 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 [23, 26].

In line with these general findings, there are copious evidences that various species of genus *Ficus* exhibit antimicrobial properties against broad spectrum of microorganisms. The scientific research on *Ficus* spp. indicated that these plants have received increasing interest in recent years. It is well documented that various *Ficus* spp. have been used against Gram-positive and Gram-negative bacteria [19]. For instance, 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 untifungal 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 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) [16].

Al Askari et al. (2013) screened aqueous and ethanolic leaf extracts of *F. carica* from different regions of Morocco against 16 bacterial strains (*Acinetobacter baumannii*, *Escherichia coli*, *Hafnia alveie*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Salmonella arizonae*, *S. entericatyphimurium*, *S. enteritidis*, *Staphylococcus aureus*, *S. aureus* meticillin-resistant, *S. epidermidis*, *Streptococcus pyogenes*, *S. sanguins*, and *Yersinia*

enterocolitica) and 8 yeasts (*Candida famata*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, two strains for each species). In general, aqueous extract was found more active against Gram-positive bateria than Gram-negative ones and it was not active against yeasts. Ethanolic extract demonstrated stronger inhibitory activity compared to aqueous extract and inhibited growth of both bacteria and fungi. *E. coli* showed comparatively high susceptibility among all bacteria tested. Aqueous extracts from plants collected in different regions affected *E. coli* with the inhibition zone diameter values of 0-11 mm (MIC 100 μg/ml), while ethanolic extracts showed zone diameters of 13.3-18.7 mm (MICs 25-50 μg/ml) [1].

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. tisela*), 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. Among bacteria tested, *E. coli* appeared the most resistant. Neither *Ficus* extract acted against *E. coli* [14].

Further studies focused on antimicrobial evaluation of methanolic extracts, hexane-ethyl acetate and ethyl acetate-methanol extract fractions, and isolated compounds from stem bark of *F. ovata* Vahl., testing a range of microbe clinical isolates, including Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* LMP805, *Streptococcus faecalis* LMP806, and *Bacillus İcereus* LMP716), Gram-negative bacteria (β-lactamase positive *Escherichia coli* LMP701, ampicillin-resistant *Klebsiella pneumoniae* LMP803, carbenicillin-resistant *Pseudomonas aeruginosa* LMP804, chloramphenicol-resistant *Salmonella typhi* LMP706, and chloramphenicol-resistant *Citrobacter freundii* LMP802), and fungi (*Candida albicans* LMP709U and *Microsporum audouinii* LMP725D) [11]. The crude extracts and certain fractions and compounds were found active against all organisms tested. *E. coli* was most strongly inhibited (MIC 78 μg/ml) by Hex–EtOAc 25% fraction and two compounds, namely taraxeryl acetate (terpenoid) and protocatechuic (phenolic) acid. Other compounds showed MIC values of 156 μg/ml, fractions 156 to 312 μg/ml, and crude extract 312 μg/ml against *E. coli*. Among all organisms tested, *E. coli* demonstrated relatively high susceptibility to the evaluated antimicrobial agents. In general, the most inhibitory-active compounds appeared to be 2′-hydroxyisoprunetin (isoflavonoid) and protocatechuic acid, affecting (nearly) all pathogens tested with relatively low MIC values. Antimicrobial activity of flavonoids and isoflavonoids was suggested to come from their ability to complex with bacterial cell wall, while that of terpenoids to cause membrane disruption, both resulting in the microbial growth inhibition [10, 11].

Conclusions

In conclusion, ethanolic extract obtained from leaves of *F. benghalensis* investigated possessed activity against at least two strains of bacteria (*Y. ruckeri* and *P. fluorescens*). The data on susceptibility patterns of pathogens responsible for fish diseases are very important for aquaculture. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Therefore, it seems a promising strategy to apply plant-derived products to gain control of infections in fish used for aquaculture. Further studies aimed at the isolation and identification of active substances from the ethanolic extract obtained from leaves of *F. benghalensis* could also disclose compounds with better therapeutic value.

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