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SCREENING FOR ANTIMICROBIAL ACTIVITIES OF THE ETHANOLIC EXTRACT DERIVED FROM *FICUS MUCUSO* WELW. EX FICALHO LEAVES (MORACEAE) AGAINST BACTERIAL FISH PATHOGENS

Use of natural products has been considered as an alternative to antibiotics in fish health management to control bacterial infections in aquaculture. Many plants were shown to have potential for being effective herbal drugs against the fish and other aquaculture pathogens. Therefore, the aim of this study was to test the efficacy of ethanolic extract prepared from Ficus mucuso Welw. ex Ficalho 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, ethanolic extracts obtained from F. mucuso proved effective against bacteria tested, with 10-15 mm zones of inhibition being observed. F. mucuso demonstrated the highest antibacterial activity against Citrobacter freundii and Pseudomonas fluorescens. Among various bacteria tested, the highest susceptibility for 400 µL of standardized inoculum (10^8 CFU/mL) of Citrobacter freundii and Pseudomonas fluorescens was noted. Thus, the preliminary screening assay indicated that F. mucuso leaves extract possess a great potential for the therapy of bacterial infections and may be used as a natural antiseptic and antimicrobial agent in aquaculture. Further investigation needs to be focused on isolation and identification of bioactive compounds from F. mucuso, which would be a platform for further pharmacological studies, in vivo tests and practical applications in fish health management.

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

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СКРИНИНГ АНТИМИКРОБНОЙ АКТИВНОСТИ ЭТАНОЛЬНОГО ЭКСТРАКТА, ПОЛУЧЕННОГО ИЗ ЛИСТЬЕВ *FICUS MUCUSO* WELW. EX FICALHO (MORACEAE), ОТНОСИТЕЛЬНО БАКТЕРИАЛЬНЫХ ПАТОГЕНОВ РЫБ

Использование натуральных продуктов в аквакультуре рассматривается как альтернатива антибиотикам для борьбы с микробными инфекциями. Многие растения потенциально могут быть эффективным средством в лечении заболеваний, вызванных бактериальными, грибковыми и вирусными возбудителями. Кроме того, фитотерапия – это эффективный метод для повышения защитных свойств у рыб. Преимущество фитопрепаратов перед синтетическими лекарственными средствами заключается в их малой токсичности, большей биодоступности, отсутствии выраженных побочных эффектов. Целью этого исследования было проверить антимикробную

эффективность этанольного экстракта, полученного из листьев Ficus mucuso Welw. ex Ficalho, относительно бактериальных возбудителей у рыб (Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri), чтобы оценить возможное использование этого растения для предотвращения инфекций, вызванных этими бактериями в аквакультуре. Листья F. mucuso были собраны в Национальном ботаническом саду им. Н.Н. Гришко НАН Украины (Киев, Украина). Свежие листья промывали, измельчали, взвешивали и гомогенизировали в 96 % этаноле для получения 10 % экстрактов. Aeromonas hydrophila (штамм E 2/7/15), Pseudomonas fluorescens (штамм E 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 диско-диффузионном 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 этанольный экстракт, полученный из листьев F. mucuso, проявил антимикробную активность относительно тестируемых бактерий (диаметр зон ингибирования роста составлял 10–15 мм). Экстракт из листьев F. mucuso продемонстрировал самую высокую антибактериальную активность относительно Citrobacter freundii и Pseudomonas fluorescens (400 мкл стандартизованного инокулята). Таким образом, предварительный скрининговый анализ показал, что экстракт из листьев F. mucuso обладает большим антимикробным потенциалом для лечения бактериальных инфекций рыб и может использоваться в качестве природного антисептика и антимикробного агента в аквакультуре. Дальнейшие исследования будут сосредоточены на изоляции и идентификации биологически активных соединений из F. mucuso, что станет платформой для фармакологических исследований in vivo с целью практического применения в аквакультуре.

Ключевые слова: *Ficus mucuso Welw. ex Ficalho, Moraceae, антимикробная активность, Aeromonas hydrophila, Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri, диско-диффузионный метод Байера-Кирби.*

Introduction

Recently, application of herbals as a immunostimulants have potential to alternative for drugs, antibiotics or chemicals being used to control fish diseases in fish culture [9]. The alternative herbal bio-medicinal products in the aquacultural have the characteristics of growth promoting ability and tonic to improve the immune system and appetite stimulators. They increase consumption, induce maturation, and have antimicrobial capability without any environmental and hazardous problems. Herbal compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines and polypeptides have been shown to be very effective alternatives to antibiotics and other synthetic compounds [7]. Moreover, herbal plants may provide a low-cost agents in aquaculture and greater accuracy than chemotherapeutic agents in this field [1].

Use of natural products has been considered as an alternative to antibiotics in fish health management to control bacterial infections in aquaculture. Many plants were shown to have potential for being effective herbal drugs against the fish and other aquaculture pathogens [20]. Additionally, it is an attractive method for increasing the protective capabilities of fish [18].

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 [6]. 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 [16].

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, 10, 21, 23]. 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* [26-30].

Ficus mucuso Welw. ex Ficalho is an African monoecious evergreen terrestrial tree, growing to a height of 30-40 m, with tall trunk, white puberulous leafy twigs, and often forming buttress roots. The leaves are 6-17 cm long and 4-15 cm broad, chartaceous to subcoriaceous, nearly orbicular to elliptic or obovate, with acuminate apex and mostly cordate base, puberulous to hirsute mostly on the veins. The pedunculate syconia are born on up to 30-40 cm long branched leafless branchlets on the main branches or also on the trunk; they are depressed-globose to obovoid, 2.5-4 cm in diameter, puberulous, at maturity red to dark orange [5].

Therefore, the aim of this study was to test the efficacy of ethanolic extract prepared from *F. mucuso* 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 1000x with immersion oil (Kocwowa 1981, Whitman and MacNair 2004). 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 [12, 32].

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 [4]. 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) [33]. 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 [17].

Results

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

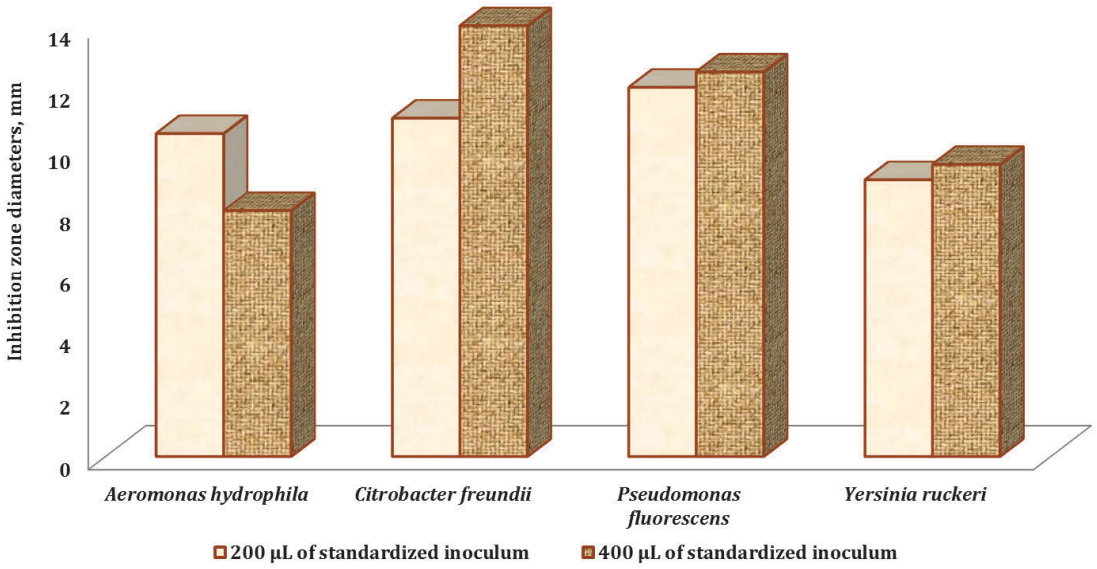
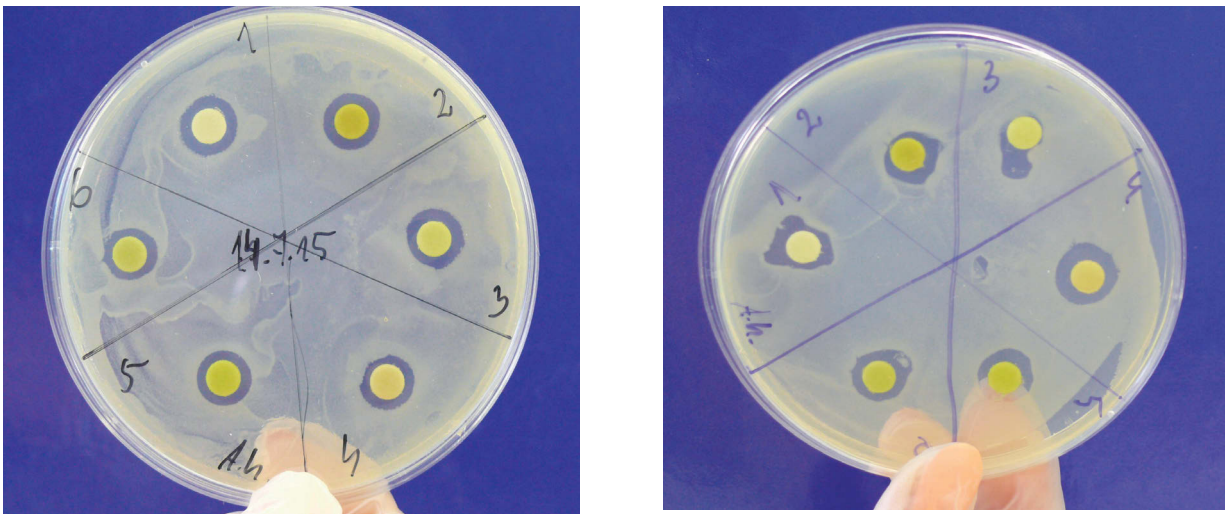


Fig. 1. Antimicrobial activity of ethanolic extracts obtained from *F. mucuso* against *Aeromonas hydrophila*, *Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri.* Muller-Hinton agar plates inoculated with 200 and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium

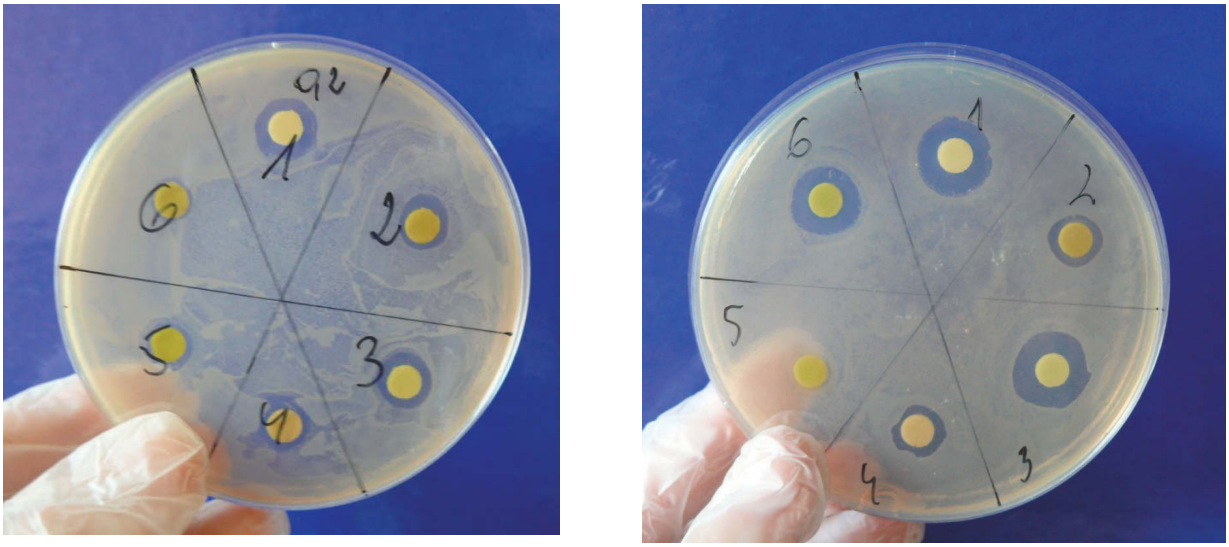
Our results from the disc diffusion assay indicated that the *A. hydrophila* (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility concerning to ethanolic extract obtained from leaves of *F. mucuso* (inhibition zone diameters were ranged from 8 to 11.5 mm) (Figs 1 and 2).



A B

Fig. 2. Antimicrobial activity of ethanolic extracts obtained from *F. mucuso* (3) against *Aeromonas hydrophila*. Muller-Hinton agar plates inoculated with 200 (A) and 400 µL of standardized inoculum (10^8 CFU/mL) of bacterium (B)

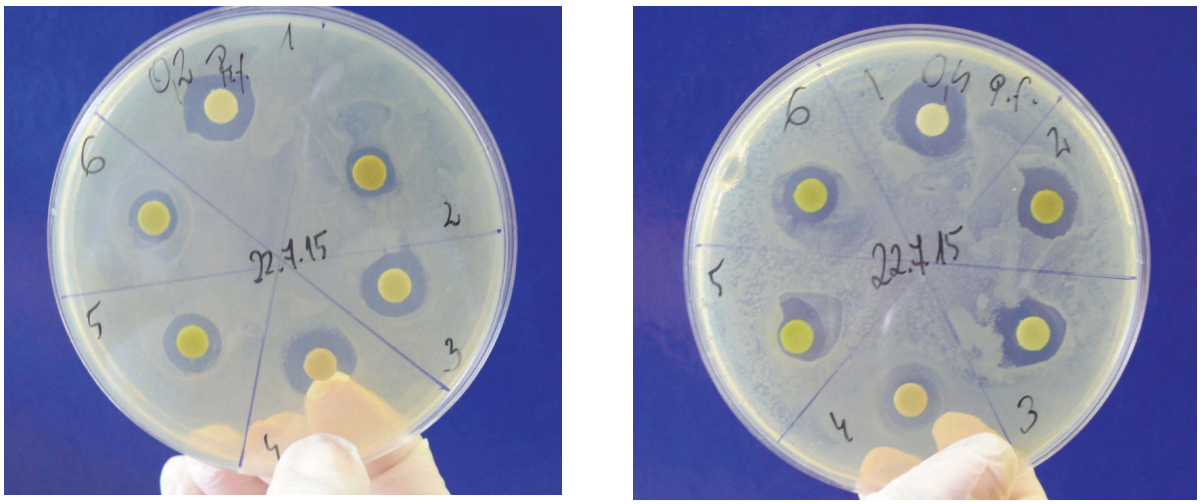
Our results demonstrated that the *C. freundii* (200 and 400 µl of standardized inoculum) revealed intermediate susceptibility to ethanolic extract obtained from leaves of *F. mucuso* (inhibition zone diameters were ranged between 10 and 15 mm) (Fig. 3).



A B

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

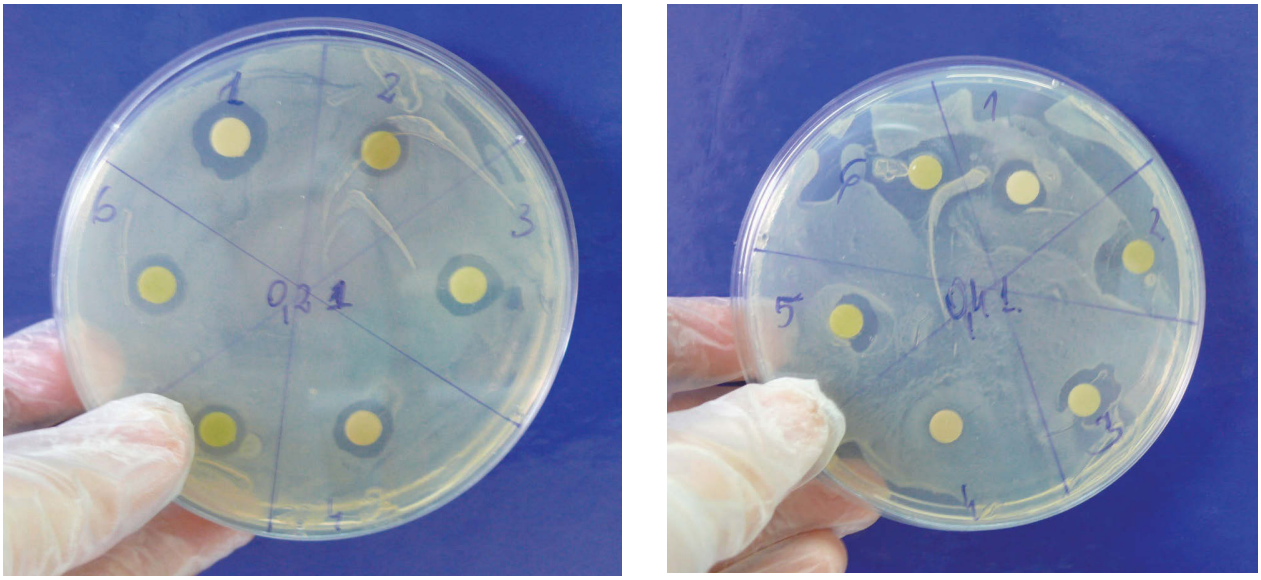
The most effective at least causing a zone of inhibition 14-16 mm was ethanolic extract from *F. mucuso* against *Pseudomonas fluorescens* both in 200 µL of standardized inoculum (10^8 CFU/mL) of bacterium (inhibition zone diameters were ranged from 11 to 12.5 mm) and 400 µL (11-15 mm) (Fig. 4).



A B

Fig. 4. Antimicrobial activity of ethanolic extracts obtained from *F. mucuso* (3) 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. mucuso* (inhibition zone diameters were ranged between 8 and 10 mm) (Fig. 5).



A B

Fig. 5. Antimicrobial activity of ethanolic extracts obtained from *F. mucuso* (3) 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

Our results indicated that extract obtained from *F. mucuso* offer a promising alternative to the use of antibiotics in controlling of infection caused by *Aeromonas hydrophila*, *Citrobacter freundii, Pseudomonas fluorescens, Yersinia ruckeri*. In our study, ethanolic extracts obtained from *F. mucuso* proved effective against bacteria tested, with 10-15 mm zones of inhibition being observed. *F. mucuso* demonstrated the highest antibacterial activity against *Citrobacter freundii* and *Pseudomonas fluorescens* (Figs 1, 3 and 4). Among various bacteria tested, the highest susceptibility for 400 µL of standardized inoculum (10^8 CFU/mL) of *Citrobacter freundii* and *Pseudomonas fluorescens* was noted (Figs 1, 3 and 4).

The comprehensive review of usefulness of some medicinal plants (herbal drugs) against fish diseases has been presented by many researchers. For example, 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 [3].

Essien and colleagues (2016) obtained essential oils from leaves of *F. mucuso* (misspelled as "*Ficus mucoso*") and *Casuarina equisetifolia* by hydrodistillation technique and screened them for chemical content as well as cytotoxic (against human cancer cells) and antimicrobial activities. Microorganisms tested included Gram-positive (*Bacillus cereus* ATCC 14579 and *Staphylococcus aureus* ATCC 29213) and Gram-negative (*Escherichia coli* ATCC 254922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria and fungi (*Aspergillus niger* ATCC 16401 and *Candida albicans* ATCC 10231). In *F. mucuso*, identified were 35 constituents representing 100% of the leaf essential oil content. The leaf oil was found to be rich in terpenoids (89.6% of its con-

tent), similar to that in *C. equisetifolia* (94.7% of terpenoids). Antimicrobial activity of *F. mucuso* essential oil was generally lower compared to that of *C. equisetifolia*. Gram-positive bacteria appeared more sensitive to the treatments than Gram-negative ones. *P. aeruginosa* showed generally low susceptibility compared to other organisms tested. *F. mucuso* oil was active against *P. aeruginosa* with MIC of 625 μg/ml (the same value for *C. equisetifolia* oil). However, the authors did not discuss regarding which of the identified constituents could most significantly contribute to antimicrobial activity of the analysed essential oils [8].

The crude ethanolic extracts from 50 plant species of 31 family, among which were *F. thonningii* and *F. vallis-choudae*, for *in vitro* activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphyloccocus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus subtilis*) bacteria were screened by Koné and co-workers (2004). 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 (IC_{100}) 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 (IC_{100}) 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) [13].

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. abutifolia* (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. abutifolia* (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 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 [14].

Phytochemical analysis of the extracts obtained from various parts of *Ficus* spp. showed the presence of flavonoids, tannins, steroids, glycosides, and saponins, as well as absence of alkaloids and amino acids. Total phenolic content of the extract was almost twice as high as total flavonoid content [21, 23].

The broad antibacterial activities of this extract, apparently, could be explained as a result of the plant secondary metabolites. Previously it has been reported [21, 23], that the therapeutic properties of *Ficus species* may be attributed to the presence of a wide range of phytochemical compounds. In general, *Ficus* species were reported to have the rich array 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 [23]. For example, 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) [31]. Recently, hispidin has been reported to have anticancer activity [2]. All the detected phenolic acids are known to have antimicrobial and antioxidant properties [11].

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 [21]. 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) [19].

Solomon-Wisdom and co-workers (2011) tested leaf and stem bark methanolic extracts from *F. sur* against several bacterial and fungal pathogens (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimorium*, *Staphylococcus aureus*, and *Candida pseudotropicalis*) and carried out their phytochemical screening. Leaf extract showed inhibition of all tested organisms except *P. aeruginosa* and *S. typhimorium* at a range of concentrations from 1.0 to 2.5 mg/ml, while stem bark extract was active against the same pathogens at concentrations from 0.5 to 2.5 mg/ml. *P. aeruginosa* was not affected by any of the extracts tested. Phytochemical analysis showed leaf extract to have richer content compared to stem bark extract. Saponins, steroids, and tannins were present in both extracts. Leaf extract additionally contained volatile oils and phenols, while stem bark extract was distinct in containing saponin glycosides [24].

Thagriki and co-workers (2015) studied chemical composition, radical scavenging activity, hydrogen peroxide scavenging radical activity, ferric reducing power, hemolytic inhibition, hemagglutination inhibition, and antibacterial activity of the leaf methanol extracts from *Ficus sycomorus* and *Piliostigma thonningii*. Bacteria tested included *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. Antibacterial essay through a broth dilution method resulted in *F. sycomorus* extract to have generally higher inhibitory activity compared to *P. thonningii* extract. In general, no significant difference among tested bacteria was observed as to their susceptibility, but the difference was between the extract concentrations tested (ranging from 2 to 10 mg/ml), the higher concentration resulting in stronger inhibition. The lowest concentration used showed growth inhibition percentage value of 7.55% for *P. aeruginosa*, whereas the highest concentration showed 54.66%. Phytochemical screening of the extracts showed their similar qualitative composition in *F. sycomorus* and *P. thonningii* (alkaloids, tannins, saponins, flavonoids, glycosides, and phenols present, while terpenoids and

steroids absent in both species) with the only difference in the presence of proteins in *F. sycomorus* extract and their absence in *P. thonningii*. The authors suggest that the antibacterial activity of the plant extracts may be due to the strong extraction capacity of methanol producing great numbers of active constituents responsible for this activity [25].

Consequently, the antimicrobial property of *F. mucuso* extract may be due to its constituents. Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action [15]. 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 [15]. It is reported that flavones have been used as Efflux pump inhibitors (EPIs). Flavones also exhibit bactericidal activity by interference with iDNA synthesis. A series of flavones was studied for their DNA-gyrase inhibitory activities. It was proposed that the ring-B of flavones is involved in intercalation or hydrogen bonding with the stacking of nucleic acid bases, thus imparting inhibitory action on DNA and RNA synthesis [22].

Conclusions

The ethanolic extract obtained from *F. mucuso* leaves showed varying inhibitory activities against all the test organisms. Thus, the preliminary screening assay indicated that *F. mucuso* leaves extract possess great potential for the therapy of bacterial infections and may be used as a natural antiseptic and antimicrobial agent. Further investigation needs to be focused on isolation and identification of those bioactive compounds, which would be a platform for further pharmacological studies, *in vivo* tests and practical applications in fish health management.

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