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Antibacterial screening of ethanolic extracts obtained from leaves of various *Ficus* species (Moraceae) against *Citrobacter freundii*

Halyna Tkachenko[1], Lyudmyla Buyun[2], Elżbieta Terech-Majewska[3], Zbigniew Osadowski[1]

[1] Dep. of Zoology and Animal Physiology, Institute of Biology and Environmental Protection, Pomeranian University in Slupsk, Poland

[2] M. Gryshko National Botanical Garden, NASU, Kyiv, Ukraine

[3] Dep. of Epizootiology, University of Warmia and Mazury, Olsztyn, Poland

Medicinal herbs play an alternative role to antibiotic therapy in aquaculture. *Ficus* species (Moraceae) leaves possess great medicinal potential for the therapy of bacterial and fungal infections and may be used as a natural antiseptic and antimicrobial agent in veterinary. Present study aimed to investigate the *in vitro* antimicrobial activity of the ethanolic leaf extracts of various *Ficus* species against fish pathogen, *Citrobacter freundii*. The leaves of *Ficus* species were collected in M. Gryshko National Botanical Garden (Kyiv, Ukraine). Freshly crushed leaves were washed, weighted, and homogenized in 96% ethanol (in proportion 1:10) at room temperature. *Citrobacter freundii* was isolated locally from gill of eel (*Anguilla anguilla*) with clinical features of disease. 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 400 µl of standardized inoculum (10^8 CFU/mL) of bacterium and spread with sterile swabs. Our results demonstrate that various species of *Ficus* had mild antibacterial *in vitro* activity against *C. freundii*. The results 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*.

Key words: *Ficus* species, ethanolic extracts, antibacterial *in vitro* activity, zone of growth inhibition, *Citrobacter freundii*, eel *Anguilla anguilla*.

Introduction

The parasitic, bacterial, and fungal diseases act as major limiting factors for fish farming, meaning that massive amounts of antibiotics, disinfectants, and pesticides in order to control mortality and avoid huge economic losses have to make use [Valladão et al., 2015]. However, most chemical products are toxic towards fish and compromise their tissues. On the other hand, such chemotherapeutics are potential xenobiotics in the nature and may give rise to serious risks to human health and cause environmental pollution [Malheiros et al., 2016].

The herbal extracts can be used in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents [Galina et al., 2009]. Herbal medicines appear as sustainable and effective alternatives for conventional drugs for disease control in aquaculture. Plant-based products have shown promise, in that it is natural and biodegradable and has antimicrobial activity against various pathogens relating to fish [Valladão et al., 2015].

Many medicinal plants and their extracts act as immunostimulants. The use of immunostimulants has been considered to be the most environmentally friendly method of preventing piscine disease [Carbone and Faggio, 2016]. One group of immunostimulants that has shown numerous beneficial effects in aquatic animals, is tropical herbs. Indeed, Chinese herbs can stimulate the development of organs directly associated with the immune response, such as the thymus and spleen as well as increase antibody production [Galina et al., 2009]. Moreover, one of the therapeutic strategies in ayurvedic medicine (plant-derived therapeutics) is to increase the body's natural resistance to the disease-causing agent rather than to directly neutralize the agent itself [Devasagayam and Sainis, 2002]. Successful results on controlling diseases in shrimp and fish by herbs have been reported in Mexico, Thailand, Japan and Turkey [Galina et al., 2009].

One of the potential plants that can be used as antimicrobial to enhance survival and immune competence is *Ficus* genus. Recently, researchers have reported promising effects from many species from *Ficus* genus for treating parasitic diseases and broad activity against bacteria and fungi [Salem et al., 2013]. *Ficus* trees have a number of uses in various industries and fields of human activity. Virtually all parts of their body are utilized in ethnomedicine to cure disorders of digestive and respiratory systems, skin diseases, parasitic infections, etc. Some species have been cited to have analgesic, tonic, and ecbolic effects [Lansky and Paavilainen, 2011].

The pantropical genus *Ficus* L., with its approximately 750 species, is the largest within the family *Moraceae* and one of the most speciose genera of flowering plants. Among all *Moraceae*, it is characterized by the presence of waxy glands on vegetative organs, heterostyly, and prolonged protogyny, that is the anthesis of staminate flowers in already mature fruits. These features are functionally linked to the unique pollination mode in *Ficus* involving mutualistic relationships with agaonid wasps (order *Hymenoptera*). The closed urceolate inflorescences provide a shelter for the development of wasps, which, in turn, are the only pollinators of these plants ensuring their reproductive propagation [Cook, Rasplus, 2003; Berg, Corner, 2005].

Ficus spp. has been extensively used in traditional medicine for a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system and infectious disorders [Usman et al., 2009; Ahmad et al., 2011; Dangarembizi et al., 2012; Arunachalam and Parimelazhagan, 2013]. However, although many species within the genus *Ficus* have been encompassed by phytochemical and pharmacological investigations in previous years, there are many species that have not been studied and whose ethnobotanical relevance is yet to be investigated. Therefore, this study assessed the antibacterial activity of ethanolic extracts of various *Ficus* species against *Citrobacter freundii*, a fish pathogen isolated locally from gill of eel (*Anguilla anguilla* L.) with clinical features of disease.

MATERIALS AND METHODS

Plant Materials. The leaves of *F. aspera* G. Forst, *F. benghalensis* L., *F. benjamina* L., *F. benjamina* 'Reginald', *F. binnendijkii* Miq., *F. binnendijkii* 'Amstel Gold', *F. binnendijkii* 'Amstel King', *F. carica* L., *F. craterostoma* Warb. ex Mildbr. & Burret, *F. cyathistipula* Warb., *F. deltoidea* Jack, *F. drupacea* Thunb., *F. drupacea* 'Black Velvet', *F. elastica* Roxb., *F. elastica* 'Variegata', *F. erecta* Thunb., *F. erecta* var. *sieboldii* (Miq.) King, *F. hispida* L.f., *F. luschnathiana* (Miq.) Miq., *F. lyrata* Warb., *F. macrophylla* Desf. ex Pers., *F. mucuso* Welw. ex Ficalho, *F. natalensis* Hochst. subsp. *natalensis*, *F. natalensis* Hochst. subsp. *leprieurii* (Miq.) C.C. Berg, *F. palmeri* S. Watson, *F. platypoda* (Miq.) A. Cunn. ex Miq., *F. pumila* L., *F. religiosa* L., *F. rubiginosa* Desf. ex Vent., *F. sagittata* J. Koenig ex Vahl, *F. septica* Burm. f., *F. sur* Forssk., *F. sycomorus* L., *F. vasta* Forssk., *F. villosa* Blume were collected in M. Gryshko National Botanical Garden (NBG, Kyiv, Ukraine). The whole collections of tropical and subtropical plants at NBG (including *Ficus* spp. plants) have the status of a National Heritage Collections of Ukraine. The species author abbreviations were followed by Brummitt and Powell (1992).

Preparing Plant Extracts. The sampled leaves of *Ficus* spp. were brought into the laboratory for antimicrobial studies. Freshly crushed leaves were washed, weighted, and homogenized in 96% ethanol (in proportion 1:10) at room temperature. The extracts were then filtered and investigated for their antimicrobial activity. All extracts were stored at 4 °C until use.

Method of Culturing Pathological Sample and identification Method of the Bacteria. *Citrobacter freundii* isolated locally from gill of eel (*Anguilla anguilla* L.) with clinical features of disease. Samples of internal organs (kidney, spleen, liver) 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 mediums: 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 round, smooth colonies were selected for further examination. They were not pigment and did not induce haemolysis on blood agar. Bacterial species was identified on the basis of key phenotypic characters and with the use of the oxidase test and API E test kit (Biomerieux, France). The results of test were interpreted in accordance with the manufacturer's protocol, after 24 hrs of incubation at 37 °C.

Bacterial Growth Inhibition Test of Plant Extracts by the Disk Diffusion Method. Strain tested was plated on TSA medium (Tryptone Soya Agar) and incubated for 24 hrs at 25 °C. Then the suspension of microorganisms were suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. Muller-Hinton agar plates were inoculated with 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) [Bauer et al., 1966]. The *C. freundii* isolates were individually tested against 4 antibiotics. The results were determined using the disk diffusion method. The tested antibiotics were as follows: gentamicin, tetracycline, enrofloxacin, and sulfonamide. 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 assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the disks.

The diameters of the inhibition zones were measured in millimeters, and compared with those of the control and standard susceptibility disks. Activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated six times. The following zone diameter criteria were used to assign susceptibility or resistance of bacterium to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 11–14 mm, and Resistant (R) ≤ 10 mm [Okoth et al., 2013].

Results

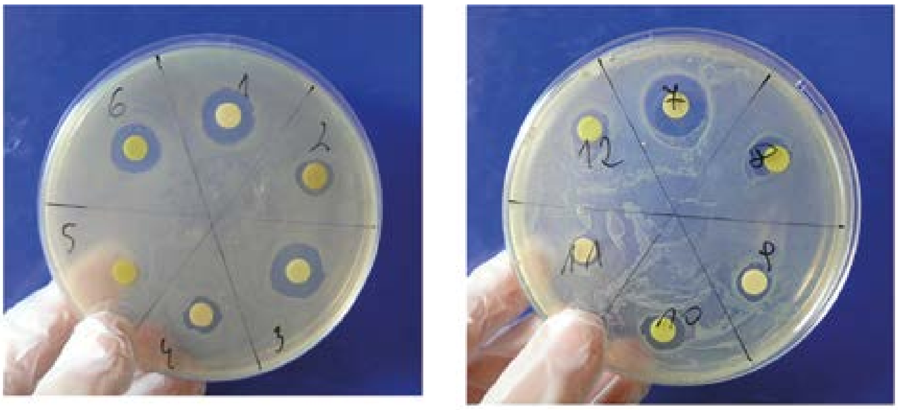
The results of antimicrobial activity of ethanolic extracts obtained from leaves of various *Ficus* species are presented in Table 1 and Figs 1–5. A comparison of susceptibility categories, i. e. intermediate and resistant, for the disk diffusion technique is demonstrated in Table 1.

Our results revealed that *C. freundii* (400 µl of standardized inoculum) had intermediate susceptibility (diameter of inhibition zone) concerning to ethanolic extracts obtained from leaves of *F. binnendijkii* 'Amstel Gold' (mean of inhibition zone diameter was 11 mm), *F. carica* (13 mm), *F. erecta* (12 mm), *F. hispida* (14 mm), *F. mucuso* (14 mm), *F. palmeri* (13 mm), *F. religiosa* (12 mm). The highest anti-*Citrobacter* activity was demonstrated for *F. pumila* (15 mm). *C. freundii* was resistant against ethanolic extracts from *F. aspera*, *F. benghalensis*, *F. benjamina*, *F. benjamina* 'Reginald', *F. binnendijkii*, *F. binnendijkii* 'Amstel King', *F. craterostoma*, *F. cyathistipula*, *F. deltoidea*, *F. drupacea*, *F. drupacea* 'Black Velvet', *F. elastica*, *F. elastica* 'Variegata', *F. erecta* var. *sieboldii*, *F. luschnathiana*, *F. lyrata*, *F. macrophylla*, *F. natalensis* subsp. *natalensis*, *F. natalensis* subsp. *leprieurii*, *F. platypoda*, *F. rubiginosa*, *F. sagittata*, *F. septica*, *F. sur*, *F. sycomorus*, *F. vasta*, *F. villosa* (inhibition zone diameters were ranged from 6 to 10 mm) (Table 1).

Antimicrobial activity of ethanolic extracts obtained from leaves of various *Ficus* species are shown in Figs 1–5.

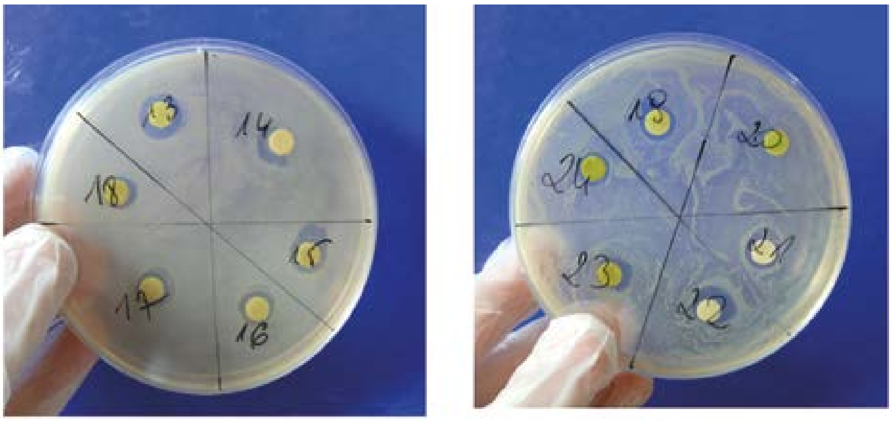
Table 1. Susceptibility or resistance of *Citrobacter freundii* against ethanolic extracts obtained from leaves of various *Ficus* species

Ficus species	Susceptibility or resistance of *Citrobacter freundii*		
	Susceptible (S) ≥ 15 mm	Intermediate (I) = 11–14 mm	Resistant (R) ≤ 10 mm
F. aspera			+
F. benghalensis			+
F. benjamina			+
F. benjamina 'Reginald'			+
F. binnendijkii			+
F. binnendijkii 'Amstel Gold'		+	
F. binnendijkii 'Amstel King'			+
F. carica		+	
F. craterostoma			+
F. cyathistipula			+
F. deltoidea			+
F. drupacea			+
F. drupacea 'Black Velvet'			+
F. elastica			+
F. elastica 'Variegata'			+
F. erecta		+	
F. erecta var. *sieboldii*			+
F. hispida		+	
F. luschnathiana			+
F. lyrata			+
F. macrophylla			+
F. mucuso		+	
F. natalensis subsp. *leprieurii*			+
F. natalensis subsp. *natalensis*			+
F. palmeri		+	
F. platypoda			+
F. pumila	+		
F. religiosa		+	
F. rubiginosa			+
F. sagittata			+
F. septica			+
F. sur			+
F. sycomorus			+
F. vasta			+
F. villosa			+



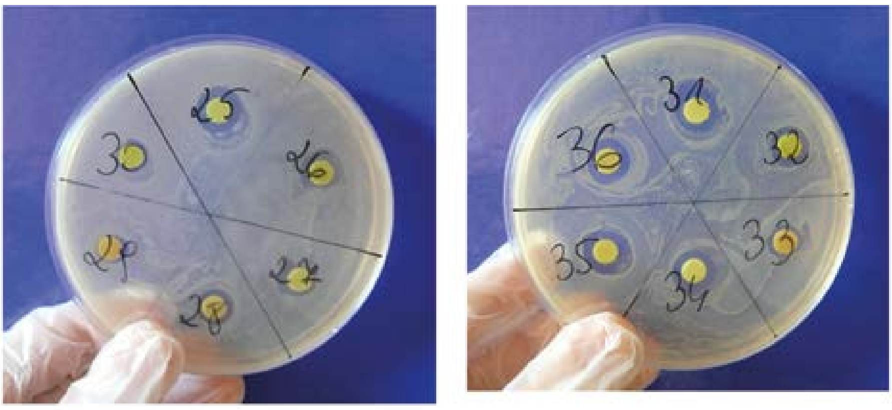
A B

Fig. 1. Antimicrobial activity of ethanolic extracts obtained from leaves of *F. hispida* (1), *F. villosa* (2), *F. mucuso* (3), *F. benghalensis* (4), *F. carica* (6) (A), *F. pumila* (7), *F. macrophylla* (8), *F. sycomorus* (9), *F. luschnathiana* (10) *F. elastica* 'Variegata' (11), *F. aspera* (12) (B) against *C. freundii*.



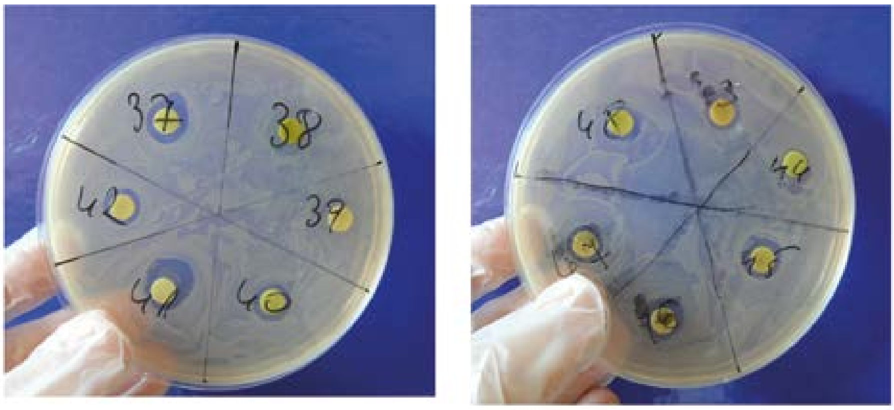
A B

Fig. 2. Antimicrobial activity of ethanolic extracts obtained from leaves of *F. religiosa* (13), *F. cyathistipula* (14), *F. lyrata* (15), *F. binnendijkii* (18) (A), *F. elastica* (19), *F. benjamina* 'Reginald' (20), *F. binnendijkii* 'Amstel King' (23), *F. binnendijkii* (24) (B) against *C. freundii*.



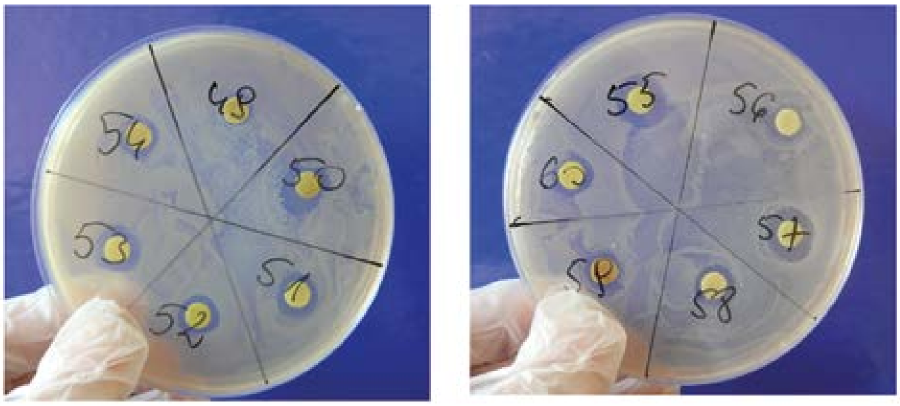
A B

Fig. 3. Antimicrobial activity of ethanolic extracts obtained from leaves of *F. luschnathiana* (26), *F. craterostoma* (27), *F. drupacea* 'Black Velvet' (28), *F. elastica* (29), *F. drupacea* (30) (A), *F. septica* (31), *F. natalensis* subsp. *leprieurii* (34), *F. binnendijkii* 'Amstel Gold' (35), *F. deltoidea* (36) (B) against *C. freundii*.



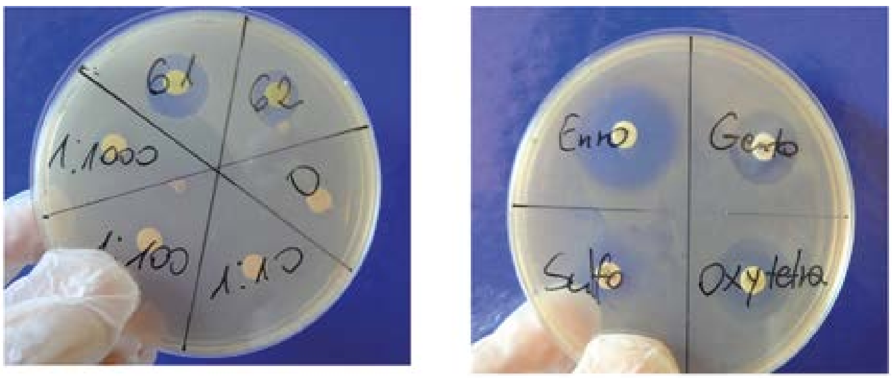
A B

Fig. 4. Antimicrobial activity of ethanolic extracts obtained from leaves of *F. erecta* var. *sieboldii* (37), *F. rubiginosa* (40), *F. erecta* (41), *F. sagittata* (42) (A), *F. lyrata* (45), *F. vasta* (48) (B) against *C. freundii*.



A B

Fig. 5. Antimicrobial activity of ethanolic extracts obtained from leaves of *F. palmeri* (50), *F. natalensis* subsp. *natalensis* (52), *F. platypoda* (54), *F. sagittata* (42) (A), *F. pumila* (55) (B) against *C. freundii*.



A B

Fig. 6. Antimicrobial activity of different concentrations of garlic (*Allium sativum*) extract (A), and tested antibiotics (B) against *C. freundii*.

Discussion

Researchers are in continuous search for the exploration of novel sources of more effective compounds for the treatment of infectious diseases in aquaculture [Das et al., 2008; Manivasagan et al., 2013]. As previously reported, plants possess antibacterial secondary metabolites which are getting more and more importance due to their negligible toxicity and adverse effects for aquatic organisms and environment [Galina et al., 2009; Hai, 2015]. The aim of the current study is to take a step towards the achievement of novel natural antimicrobial agents against fish pathogen, *Citrobacter freundii*. Results of the current study revealed that *F. pumila* possess high antibacterial results against *C. freundii*. This significance is in the terms of therapeutic applications against various disorders, i. e. skin diseases, enlargement of liver and spleen, dysentery, diarrhea, diabetes, leprosy, lung complaints, leucorrhoea, heart diseases, cough, asthma, piles, ulcers, gonorrhea and rheumatism [Kaur, 2012]. Similarly, the antimicrobial activity of ethanolic extracts from leaves of *F. binnendijkii* 'Amstel Gold', *F. carica*, *F. erecta*, *F. hispida*, *F. mucuso*, *F. palmeri*, *F. religiosa* is also dominant. It was also observed that the *F. hispida* and *F. mucuso* were the most active one against *C. freundii*.

The results of our previous reports are in line with the findings of our current study which indicate that like other species of *Ficus* genus, *F. pumila*, *F. binnendijkii* 'Amstel Gold', *F. carica*, *F. erecta*, *F. hispida*, *F. mucuso*, *F. palmeri*, *F. religiosa* are also prominent candidates of this genus as alternative antimicrobial agents in aquaculture. In our previous study, we determined the *in vitro* antimicrobial activity of ethanolic extracts from the leaves of various *Ficus* species against the bacterial strain of *A. hydrophila* isolated locally from infected rainbow trout (*Oncorhynchus mykiss* Walbaum) with the aim of providing scientific rationale for using these plants in the treatment of bacterial infections induced by *Aeromonas* spp. in fish (Tkachenko et al. 2016a, b). Interestingly, ethanolic extracts obtained from *Ficus* species demonstrated different antibacterial activity against *A. hydrophila* inoculated with 200 µl and 400 µl of standardized inoculum (10^8 CFU ml^{-1}) of bacterium strain. Previous study using 200 µl of *A. hydrophila* inoculum revealed that *F. benghalensis*, *F. benjamina*, *F. binnendijkii*, *F. cyathistipula*, *F. deltoidea*, *F. erecta*, *F. erecta* var. *sieboldii*, *F. hispida*, *F. luschnathiana*, *F. lyrata*, *F. macrophylla*, *F. mucuso*, *F. natalensis* subsp. *leprieurii*, *F. natalensis* subsp. *natalensis*, *F. palmeri*, *F. platypoda*, *F. pumila*, *F. rubiginosa*, *F. sur*, *F. sycomorus*, and *F. villosa* possessed good antibacterial activity (diameters of inhibition zones ranged from 10 to 14 mm), while *A. hydrophila* was resistant against ethanolic exstracts from *F. aspera*, *F. benjamina* 'Reginald', *F. binnendijkii* 'Amstel Gold', *F. binnendijkii* 'Amstel King', *F. carica*, *F. craterostoma*, *F. drupacea*, *F. drupacea* 'Black Velvet', *F. elastica*, *F. elastica* 'Variegata', *F. religiosa*, *F. sagittata*, *F. septica*, and *F. vasta* [Tkachenko et al., 2016a]. On the other hand, in study with 400 µl of *A. hydrophila* inoculum, bacterium demonstrated the highest susceptibility to *F. pumila* leaves extract (diameters of inhibition zones ranged from 16 to 18 mm), while intermediate susceptibility was demonstrated to ethanolic extracts obtained from *F. benghalensis*, *F. benjamina*, *F. benjamina* 'Reginald', *F. binnendijkii*, *F. carica*, *F. cyathistipula*, *F. deltoidea*, *F. drupacea*, *F. drupacea* 'Black Velvet', *F. elastica*, *F. erecta*, *F. erecta* var. *sieboldii*, *F. hispida*, *F. lyrata*, *F. macrophylla*, *F. natalensis* subsp. *leprieurii*, *F. natalensis* subsp. *natalensis*, *F. palmeri*, *F. religiosa*, *F. rubiginosa*, *F. septica*, and *F. sycomorus* leaves [Tkachenko et al., 2016b]. In other our study, most ethanolic extracts obtained from *Ficus* spp., proved effective against the bacterial strain of Gram-negative *P. fluorescens* (strain E1/7/15) isolated locally from internal organs of rainbow trout (*Oncorhynchus mykiss* Walbaum) with clinical features of furunculosis, with 8–15 mm zones of inhibition being observed. Among various species of *Ficus* the most effective against *P. fluorescens* (400 µl of standardized inoculum) were the ethanolic extracts of the leaves of ten *Ficus* species: *F. craterostoma*, *F. cyathistipula*, *F. drupacea* '*Black Velvet*', *F. hispida*, *F. macrophylla*, *F. mucuso*, *F. pumila*, *F. villosa* (Tkachenko et al. 2016c). Various species of *Ficus* had mild antibacterial in vitro activity against *C. freundii* isolated locally from infected eel (*Anguilla anguilla* L.) in dissolved to 200 µl of standardized inoculum (Kirby-Bauer disk diffusion susceptibility test protocol). Our 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* [Tkachenko et al., 2016d].

The results obtained in the current study can be correlated with the previous publications. Other species of *Ficus* genus also possess effective potential against microbes i. e., *F. religiosa* have been reported to possess strong antibacterial and antifungal activities [Preethi et al., 2010; Gayathri, Kannabiran, 2009; Solomon-Wisdom et al., 2011; Rajiv, Rajeshwari, 2012; Al Askari et al., 2013; Essien et al., 2016]. In a study of Preethi and co-workers (2010), aqueous extract of *F. religiosa* leaves compared to the ethanol and methanol extracts showed the strongest inhibitory activity against bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) as evaluated by the agar diffusion method. 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 [Rajiv, Rajeshwari, 2012]. Gayathri and Kannabiran (2009) reported quite high antibacterial activity of *F. benghalensis* bark aqueous extract against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Phytochemical screening of the extracts showed tannins and saponins to significantly prevail over other chemical classes, suggesting their substantial contribution to the antimicrobial properties of the species [Gayathri, Kannabiran, 2009]. Solomon-Wisdom and co-workers (2011) tested leaf and stem bark methanolic extracts from *F. sur* against several bacterial and fungal pathogens (*Bacillus subtilis*, *E. coli*, *P. aeruginosa*, *Salmonella typhimorium*, *S. aureus*, and *Candida pseudotropicalis*) and carried out their phytochemical screening. Leaf and bark extracts showed inhibition of all tested organisms except *P. aeruginosa* and *S. typhimorium*. 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 [Solomon-Wisdom et al., 2011].

In the same way, essential oils from leaves of *Ficus* species have been reported to possess antimicrobial potential. Essien and co-workers (2016) obtained essential oils from leaves of *F. mucuso* (misspelled as "*Ficus mucoso*") and *Casuarina equisetifolia* and screened them for chemical content as well as cytotoxic (against human cancer cells) and antimicrobial activities. Microorganisms tested included Gram-positive (*Bacillus cereus* ATCC14579 and *S. aureus* ATCC29213) and Gram-negative (*E. coli* ATCC254922 and *P. aeruginosa* ATCC27853) bacteria and fungi (*Aspergillus niger* ATCC16401 and *Candida albicans* ATCC10231). 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 content). Gram-positive bacteria appeared more sensitive to the treatments than Gram-negative ones (Essien et al. 2016). 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* NCTC8236, *E. coli* ATCC9637, *P. aeruginosa* ATCC27853, *S. aureus* ATCC13709, and *C. albicans* ATCC10231. *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 (Kubmarawa et al. 2007). Al Askari and co-workers (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 pneumoniae*, *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 bacteria 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 [Al Askari et al., 2013].

Several classes of secondary metabolites such as alkaloids, triterpenes, sterols, flavonoids, polyphenols and saponins have been reported to have antibacterial properties [Cowan, 1999; Kuete et al., 2008; Mbaveng et al., 2015; Voukeng et al., 2016]. Their presence in the studied plant extracts could explain the antibacterial effects of the tested samples. Antibacterial activity of tannins and saponins isolated from plant species are well documented [Gayathri, Kannabiran, 2009; Usman et al., 2009]. The presence of flavonoids and polyphenols is the basis for the analgesic and anti-inflammatory activities of various parts of *Ficus* including the fruit, latex, bark, roots, and leaves [Modi et al., 2012; Eteraf-Oskouei et al., 2015]. The antimicrobial activity of purified flavonoids may result in susceptibility differences against species with different origins and background [Taguri et al., 2004]. This could explain the difference in sensitivity to *Ficus* extracts used in this work and a previously tested other species of *Ficus* [Tkachenko et al., 2016a-d]. The highest antibacterial potential of *Ficus* could be explained by the amount of flavonoids present. However, the activity showed by ethanolic extracts of *Ficus* species may result from the interactions of different polyphenols. Most studies on the antimicrobial potential of polyphenols have focused on the inhibitory activity of individual components. The inhibitory effect of phenolics could be explained by absorption across cell membranes, interactions with enzymes, substrate and metal ion deprivation [Scalbert, 1991]. Direct interaction between the two compounds may result in changes of the structural conformation thus reducing the inhibitory activity [Mandalari et al., 2010]. Antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action [Kumar, Pandey, 2013]. 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 [Cowan, 1999; Kumar, Pandey, 2013]. Moreover, many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities [Kumar, Pandey, 2013].

Conclusions

The study reveals that among the plants of *Ficus* species screened, *F. pumila*, *F. binnendijkii* 'Amstel Gold', *F. carica*, *F. erecta*, *F. hispida*, *F. mucuso*, *F. palmeri*, *F. religiosa* were rich in antimicrobial agents. Secondly it may also be implied that the ethanolic fraction was rich in such secondary metabolites which confer antimicrobial potential to these plants. Based on our current investigation it can be concluded that *F. pumila*, *F. binnendijkii* 'Amstel Gold', *F. carica*, *F. erecta*, *F. hispida*, *F. mucuso*, *F. palmeri*, *F. religiosa* possess considerably antibacterial potential against *C. freundii*. It may also be concluded that antimicrobial potential of ethanolic extracts obtained from leaves of various *Ficus* species might be due to wide variety of compounds present in these plants. Moreover, we observed that specifically the ethanolic fraction of *F. pumila* possesses considerably high activity against *C. freundii*. Therefore, these species are potent targets to be subjected to bio-guided isolation and exploration of novel natural antimicrobials for application in aquaculture.

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Антимикробный скрининг спиртовых экстрактов, полученных из листьев разных видов *Ficus* в отношении *Citrobacter freundii*

Г.М. Ткаченко[1], Л.И. Буюн[2], Э. Терех-Маевская[3], З. Осадовский[1]

[1] Институт биологии и охраны среды, Поморская Академия, Слупск, Польша

[2] Национальный ботанический сад им. Н.Н. Гришко НАНУ, Киев, Украина

[3] Варминско-Мазурский Университет, Ольштын, Польша

Лекарственные растения играют альтернативную роль в антибиотикотерапии в аквакультуре. Виды *Ficus* (Moraceae) обладают большим лечебным потенциалом в терапии бактериальных и грибковых инфекций, и могут быть использованы в качестве антисептиков и антимикробных агентов в ветеринарии. Настоящее исследование предназначено для изучения *in vitro* антимикробной активности этанольных листовых экстрактов различных видов *Ficus* против возбудителя *Citrobacter freundii*. Листья были собраны в Национальном ботаническом саду им. Н.Н. Гришко (Киев, Украина). Свежесрезанные листья промывали, взвешивали и гомогенизировали в 96% этаноле (в пропорции 1:10) при комнатной температуре. *Citrobacter freundii* выделяли локально из жабр угря (*Anguilla anguilla*) с клиническими признаками заболевания. Испытание на антимикробную чувствительность проводили методом Кирби-Бауэра. В чашки с агаром Muller-Hinton инокулировали 400 мкл стандартизированного инокулята (10^8 КОЕ/мл) бактерии и распределяли равномерно стерильными тампонами. Наши результаты показывают, что различные виды фикусов имели слабую антибактериальную активность *in vitro* против *C. freundii*. Результаты также показали, что этанольные экстракты, полученные от *F. pumila*, *F. binnendijkii* 'Amstel Gold', *F. carica*, *F. erecta*, *F. hispida*, *F.mucuso*, *F. palmeri*, *F. religiosa* обладают значительным антибактериальным потенциалом против *C. freundii*.

Ключевые слова: *Ficus* spp., листья, спиртовые экстракты, антибактериальная активность, зона ингибирования роста, *Citrobacter freundii*, угорь *Anguilla anguilla*.