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Studies on the inhibitory properties of leaf ethanolic extracts obtained from *Ficus* (*Moraceae*) species against *Aeromonas* spp. strains

Agnieszka Pękala-Safińska[1✉], Halyna Tkachenko[2], Natalia Kurhaluk[2], Lyudmyla Buyun[3], Zbigniew Osadowski[2], Vitaliy Honcharenko[4], Andriy Prokopiv[4,5]

[1]Department of Fish Diseases, National Veterinary Research Institute, 24-100 Puławy, Poland
[2]Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, 76-200 Słupsk, Poland
[3]Department of Tropical and Subtropical Plants, M.M. Gryshko National Botanic Garden, National Academy of Sciences of Ukraine, 01-014 Kyiv, Ukraine
[4]Department of Botany, Biology Faculty, [5]Botanic Garden, Ivan Franko National University in Lviv, 79-005 Lviv, Ukraine
a.pekala@piwet.pulawy.pl

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Abstract

Introduction: The most frequently isolated bacteria in Polish aquaculture are of the *Aeromonas* genus; also pathogenic to human fish consumers, they cause substantial economic losses, and require antibiotic therapy to treat. Antibiotic residues in animal-derived food provoke concern. The aim of the study was to appraise the antimicrobial activity of ethanolic extracts of *Ficus* plant species against *Aeromonas* strains. **Material and Methods:** Leaves of 41 *Ficus* species were collected from two Ukrainian botanic gardens. They were crushed, washed, homogenized in ethanol and centrifuged, and the supernatants were applied in the Kirby–Bauer disc-diffusion method to assess the susceptibility to them of *Aeromonas hydrophila*, *A. sobria*, and *A. salmonicia* subsp. *salmonicida* isolates confirmed as K886, K825, and St30 strains. Analogous assessment was also made of these bacteria's susceptibility to sulfonamides, quinolones, tetracyclines, and one amphenicol. Data were analysed statistically. **Results:** The majority of the extracts considerably inhibited bacterial growth, *A. sobria* being susceptible to 14 *Ficus* species, *A. salmonicida* subsp. *salmonicida* to 13, and *A. hydrophila* to 10. **Conclusion:** Treatment with plant extracts has promise as an alternative to antibiotic therapy. Botanic gardens may offer new sources of plant-derived agents with a broad spectrum of biological and antimicrobial action. Further research will be useful to broaden knowledge of *Ficus'* therapeutic potential.

Keywords: inhibitory properties, antibacterial activity, *Aeromonas* spp., ethanolic leaf extracts, *Ficus* (*Moraceae*).

Introduction

In recent years, the demand for substitutes for antibiotics and other therapeutic chemical preparations has increased, setting in motion research focusing on plant-derived products as alternatives. Screening assays have been performed on some plant species whose antibacterial properties have proved useful as another choice besides classical antimicrobial therapy against bacterial infections in fish farming (14).

Various plants and their extracts have been used experimentally as antibacterial agents in many diverse studies. Some of the plants, which can be considered for use as antimicrobials and immune competence enhancers in animals, belong to the *Ficus* L. (*Moraceae*) genus. Ethanolic leaf extracts were the material form selected for the assessment of the antibacterial activity of this ecologically important multiple-species group of plants. The *Ficus* genus has long been of particular interest to researchers, especially in the context of its use by humans as a food source, in medicine, and in other industries and areas of human activity, partly due to its wide variety and range of distribution. Among the most popular ethnomedicinal uses of *Ficus* species are as treatments of skin infections and damage, parasitic invasions, and disorders of the digestive system and its related organs (2, 20). The therapeutic range of particular *Ficus* species may even equal that of the traditional broad-spectrum agents. An example of the use of various *Ficus* species is Ayurvedic and traditional Chinese medicine, where people apply many of these plants as a dietary supplement and treat various diseases and disorders with them (3, 13, 25).

Recently, many species of the *Ficus* genus have shown promise in the treatment of parasitic diseases and appeared to have a broad spectrum of activity, also against bacteria and fungi (25). Although numerous plants of the *Ficus* genus have already been characterised phytochemically and pharmacologically, there are still some whose ethnobotanic significance has not yet been studied and requires further research. Considering this need for elucidation, we have attempted to perform *in vitro* analysis of the antimicrobial activity of ethanolic extracts derived from various *Ficus* species.

Various crucial *Ficus* species were chosen to be evaluated for their antimicrobial efficacy against the *Aeromonas* species: *A. sobria*, *A. hydrophila*, and *A. salmonicida* subsp. *salmonicida*. This group of microorganisms is very important due to their pathogenicity to fish and humans and their impact in food spoilage processes (17). In the context of the increasing resistance of these bacteria to antimicrobials observed in recent years, the proposition of an alternative antibacterial therapy is especially important (16). Therefore, the aim of our study was to assess the *in vitro* effectiveness of the antibacterial activity of ethanolic extracts obtained from various *Ficus* species against *Aeromonas* strains, as the most frequently isolated bacteria in Polish aquaculture. Verification of the inhibitory effect of these plants on *Aeromonas* is the basis for proposing a new, alternative source of antimicrobials to prevent and treat the infections caused by these microorganisms in aquaculture.

Material and Methods

Plant collection and extract preparation. Leaves of the following *Ficus* species were collected in Gryshko National Botanic Garden (Kiev, Ukraine) and the Botanic Garden of Ivan Franko National University in Lviv (Ukraine): *F. aspera* G. Forst, *F. barteri* Sprague, *F. benghalensis* L., *F. benjamina* L., *F. binnendijkii* Miq., *F. carica* L., *F. craterostoma* Warb. ex Mildbr. & Burret, *F. cyathistipula* Warb., *F. deltoidea* Jack, *F. drupacea* Thunb., *F. elastica* Roxb. ex Hornem, *F. erecta* Thunb., *F. formosana* Maxim., *F. hederacea* Roxb., *F. hispida* L. f., *F. johannis* subsp. *afghanistanica* (Warb.) Browicz, *F. lingua* Warb. ex De Wild. & T. Durand, *F. luschnathiana* (Miq.) Miq., *F. lyrata* Warb., *F. macrophylla* Desf. ex Pers., *F. malayana* C. C. Berg & Chantaras., *F. microcarpa* L. f., *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. retusa* L., *F. rubiginosa* Desf. ex Vent., *F. sagittata* J. König ex Vahl, *F. sarmentosa* var. *henryi* (King ex D. Oliv.) Corner, *F. septica* Burm. f., *F. sur* Forssk., *F. sycomorus* L., *F. taiwaniana* Hayata, *F. tinctoria* G. Forst., *F. vasta* Forssk., *F. villosa* Blume, and *F. virens* Aiton. The plant collections located in the National Botanic Garden in Kiev and the Botanic Garden of Ivan Franko National University in Lviv are parts of the National Heritage Collection of Ukraine. Standardised names of plant species and appropriate botanical nomenclature were cited follow Brummitt and Powell (4). An authoritative digitised global source of plant taxonomy was used by the authors, namely The Plant List (http://www.theplantlist.org).

The freshly crushed sampled leaves were washed and weighed. After homogenisation in 96% ethanol (in the ratio 1:10, v/v) at room temperature, the samples were centrifuged at 3,000 g for 5 min. The supernatants were kept frozen at −20°C for further studies.

Bacteria isolation and identification. The *Aeromonas* strains used in our studies were *Aeromonas hydrophila* (K886), *Aeromonas sobria* (K825) and *Aeromonas salmonicida* subsp. *salmonicida* (St30). These microorganisms originated from the bacterial strain collection of the Department of Fish Diseases at the National Veterinary Research Institute in Puławy, Poland, and had been isolated from fish of two farmed freshwater species exhibiting clinical signs of disease: common carp (*Cyprinus carpio* L.) (K886 and K825) and rainbow trout (*Oncorhynchus mykiss* Walbaum) (St30). In order to identify the strains, Gram staining, assessment of the morphological characteristics, and biochemical characterisation using the API system (bioMérieux, France) according to the manufacturer's instructions were carried out. The initial identification of *Aeromonas* isolates was confirmed by restriction analysis of 16S rDNA genes (PCR-RFLP) (19). Pure cultures were kept frozen for further studies at −80°C in tryptic soy broth (TSB) (bioMérieux) supplemented with 15% glycerol.

Susceptibility to antimicrobial agents. The antimicrobial sensitivity of each selected *Aeromonas* isolate was investigated with the Kirby–Bauer technique. The disc-diffusion method was carried out on Mueller–Hinton agar (Oxoid, UK) according to the recommendations of the Clinical and Laboratory Standards Institute (8). The following chemotherapeutics (Oxoid, UK) from different groups of drugs were used: the sulfonamides consisted of compound sulfonamides (S3) and sulfamethoxazole with trimethroprim (SXT); the quinolones were oxolinic acid (OA), flumequine (UB), and enrofloxacin (ENR); the tetracyclines comprised only oxytetracycline (OT); and florfenicol (FFC) was the single selection from the amphenicols (Table 1). After media plate inoculation and the placing of appropriate antimicrobial discs (five discs per plate) on them, the plates were incubated at 28 ± 2°C for 24 h.

Table 1. Antimicrobials used in the study

Group of antimicrobial agents	Symbol (Oxoid)	Antimicrobial	Concentration (μg)
Sulfonamides	S3	compound sulfonamides	300
	SXT	sulphamethoxazole/trimethoprim	23.75/1.25
Quinolones	OA	oxolinic acid	2
	UB	flumequine	30
	ENR	enrofloxacin	5
Tetracycline	OT	oxytetracycline	30
Phenicols	FFC	florfenicol	30

Table 2. Results of antimicrobial susceptibility of *Aeromonas* strains

Strains	Inhibition zone diameter (IZD), mm						
	S3	SXT	OA	UB	ENR	OT	FFC
Aeromonas sobria	6.37 ± 0.28	30 ± 1.8	32.86 ± 1.44	35.29 ± 1.61	35.14 ± 1.52	29.14 ± 1.56	35 ± 0.98
Aeromonas hydrophila	14.86 ± 0.91	24.14 ± 2.53	30.14 ± 1.24	30.57 ± 0.37	27.43 ± 1.45	23.43 ± 1.19	24.14 ± 0.88
Aeromonas salmonicida subsp. *salmonicida*	6.43 ± 0.30	25.14 ± 1.28	31.0 ± 0.9	32.86 ± 1.08	35.14 ± 1.83	27.0 ± 1.0	35.29 ± 1.11

After that, the diameters of the growth inhibition zones were measured to estimate the zone diameter breakpoints (mm) of tested isolates. Because very few internationally harmonised interpretive criteria were available for bacteria isolated from aquatic animals, we generated our own to establish the meaning of the obtained results, adapting those available for *Aeromonas salmonicida* (9).

Bacterial susceptibility to extracts of different *Ficus* species. The sensitivity of *Aeromonas* strains to selected *Ficus* extracts was determined by the Kirby–Bauer technique, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (8), with our modifications. A suspension of each bacterial species was inoculated on Mueller–Hinton agar. Five wells per Petri dish with a diameter of 6 mm were made in the medium, and plant extracts were added into them. Plates were incubated at 28 ± 2°C for 24 h and the growth inhibition zones for each well were measured. The interpretation criteria for the phytochemicals tested were that a zone ≥15 mm was termed susceptible (S), one of 10–15 mm intermediate (I), and a ≤10 mm zone was indicative of a resistant microorganism (R) (21).

Negative control. Ethanol at 96% strength (POCH, Poland) as used to prepare the extracts was used as the negative control.

Statistical analysis. Obtained data were analysed statistically by employing the mean ± standard error of the mean (S.E.M.). All variables were randomised according to the phytochemical activity of the tested ethanolic extracts.

Results

The presented study demonstrates the antibacterial activity *in vitro* of 41 ethanolic extracts of different species of *Ficus* plant against selected bacteria belonging to the *Aeromonas* genus. This potential activity was indicated qualitatively and quantitatively assessed by the diameter of the bacterial growth inhibition zone visible around the particular plant extract. The results described in Tables 2−5 show that ethanolic extracts obtained from the various *Ficus* species exhibited *in vitro* antibacterial activity against one or more tested *Aeromonas* strains. This efficacy was compared with selected antibiotics commonly used in aquaculture (Table 1).

The results of antimicrobial disc susceptibility testing of *A. sobria*, *A. hydrophila*, and *A. salmonicida* subsp. *salmonicida* are presented in Table 2. An inhibition zone of 6 mm, indicating possible resistance to S3, was noted for *A. sobria* and *A. salmonicida.* Sulfamethoxazole with trimethoprim, quinolones, tetracyclines, and phenicols yielded inhibition zones >24 mm for all tested isolates (Table 2).

The results of the research on the antimicrobial activity of the ethanolic extracts of various *Ficus* species against *A. sobria* bacteria using the disc-diffusion method are summarised in Table 3.

Variable antimicrobial activity of the 41 tested ethanolic extracts against the selected *Aeromonas* strains was observed. The *A. sobria* strain was susceptible to 14 (34.2%) extracts out of 41 tested (Table 3), while *A. hydrophila* and *A. salmonicida* subsp. *salmonicida* were susceptible to 10 (24.4%) and 13 (31.7%), respectively (Tables 4 and 5). No bacterial growth inhibition zone was observed around the wells containing ethanol, which were the negative control of the assay.

As the average over the three *Aeromonas* species, the highest antimicrobial activity among all the tested ethanolic extracts was observed in *F. binnendijkii* leaves with inhibition zone diameters (IZD) of 23.75 ± 1.64 mm against *A. sobria*, 20.63 ± 1.45 mm against *A. hydrophila*, and 15.75 ± 0.80 mm against *A. salmonicida*. *F. craterostoma* extract was effective against *A. sobria* with an IZD of 15.25 ± 0.90 mm and against *A. salmonicida* with a zone of 15.25 ± 1.15 mm, while *F. deltoidea* extract was effective against *A. sobria* across 18.81 ± 1.25 mm and *A. salmonicida* across 20.13 ± 0.79 mm diameters. *F. hispida* extract inhibited *A. sobria* the best and showed an IZD of 25.56 ± 1.63 mm followed by the extracts of *F. binnendijkii* presenting an IZD of 23.75 ± 1.64 mm and *F. tinctoria* giving one of 22.5 ± 1.20 mm.

The IZD results also showed that isolates of *A. sobria* revealed intermediate susceptibility to ethanolic extracts of *F. aspera*, *F. benjamina*, *F. elastica*, *F. formosana*, *F. johannis* subsp. *afghanistanica*, *F. natalensis* subsp. *leprieurii*, *F. religiosa*, *F. villosa*, and *F. virens*, which created mean IZDs ranging from 10 to 15 mm (Table 3). The isolates appeared to be resistant to extracts of 18 *Ficus* species (43.9%), which only restricted growth in mean IZDs of less than 10 mm (Table 3).

In the case of *A. hydrophila* isolates, high susceptibility of this bacteria was observed to ethanol extracts obtained from leaves of *F. virens*, *F. sagittata*, and *F. religiosa*, indicated by mean IZDs of 25.44 ± 1.35, 22.56 ± 1.66, and 21.25 ± 1.33 mm, respectively (Table 4). Moreover, in the group of *Ficus* species with significant inhibitive properties against *A. hydrophila*, high IZD values were observed for *F. binnendijkii*, *F. luschnathiana*, *F. hispida*, *F. lingua*, *F. mucuso*, *F. retusa*, and *F. tinctoria*. In addition, 11 extracts (26.8%) showed intermediate susceptibility against *A. hydrophila* with IZDs between 10 and 15 mm. Among the group of extracts obtained from *Ficus* species with intermediate inhibitiveness to *A. hydrophila*, the largest IZDs were observed for *F. formosana*, which prevented growth over 14.13 ± 0.69 mm, *F. craterostoma*, which did so over 13.75 ± 0.86 mm, and *F. aspera*, which inhibited in a 13.38 ± 0.68 mm zone. The IZD for 20 species (48.8%) was in a range of less than 10 mm at its maximum and *A. hydrophila* was resistant to these (Table 4).

Table 3. Diameters of the growth inhibition zones of *Aeromonas sobria* bacteria made by ethanolic extracts obtained from the leaves of various *Ficus* species

Ficus species	Inhibition zone diameter (IZD), mm
	High susceptibility, IZD ≥ 15 mm
F. binnendijkii	23.75 ± 1.64
F. craterostoma	15.25 ± 0.90
F. cyathistipula	15.31 ± 1.01
F. deltoidea	18.81 ± 1.25
F. drupacea	18.31 ± 1.13
F. erecta	17.63 ± 0.92
F. hispida	25.56 ± 1.63
F. lingua	19.38 ± 1.27
F. luschnathiana	18.56 ± 1.29
F. malayana	20.25 ± 1.06
F. sur	17.63 ± 1.07
F. taiwaniana	15.19 ± 0.84
F. tinctoria	22.5 ± 1.20
F. vasta	20.63 ± 1.44
	Intermediate susceptibility, IZD = 10–15 mm
F. aspera	14.5 ± 0.94
F. benjamina	12.5 ± 0.80
F. elastica	12.38 ± 0.82
F. formosana	14.19 ± 0.82
F. johannis subsp. *afghanistanica*	12.38 ± 0.83
F. natalensis subsp. *leprieurii*	13.5 ± 0.76
F. religiosa	14.44 ± 0.85
F. villosa	13.38 ± 0.82
F. virens	14.25 ± 0.80
	Resistance, IZD ≤ 10 mm
F. barteri	9.25 ± 0.53
F. benghalensis	9.5 ± 0.54
F. carica	9.75 ± 0.60
F. hederacea	9.19 ± 0.55
F. lyrata	9.5 ± 0.33
F. macrophylla	9.5 ± 0.62
F. microcarpa	9.63 ± 0.50
F. mucuso	9.62 ± 0.67
F. natalensis subsp. *natalensis*	9.38 ± 0.62
F. palmeri	9.5 ± 0.62
F. platypoda	9.75 ± 0.64
F. pumila	9.25 ± 0.45
F. retusa	9.88 ± 0.52
F. rubiginosa	9.56 ± 0.65
F. sagittata	9.5 ± 0.77
F. sarmentosa var. *henryi*	9.75 ± 0.53
F. septica	9.56 ± 0.59
F. sycomorus	9.63 ± 0.65

Table 4. Diameter values of the growth inhibition zone of bacteria *Aeromonas hydrophila* caused by ethanolic extracts obtained from the leaves of various *Ficus* species

Ficus species	Inhibition zone diameter (IZD), mm
	High susceptibility, IZD ≥ 15 mm
F. binnendijkii	20.63 ± 1.45
F. hispida	17.25 ± 1.10
F. lingua	16.06 ± 1.05
F. luschnathiana	17.5 ± 1.27
F. mucuso	15.25 ± 1.05
F. religiosa	21.25 ± 1.33
F. retusa	15.19 ± 0.80
F. sagittata	22.56 ± 1.66
F. tinctoria	15.06 ± 0.83
F. virens	25.44 ± 1.35
	Intermediate susceptibility, IZD = 10–15 mm
F. aspera	13.38 ± 0.68
F. barteri	11.5 ± 0.76
F. benghalensis	11.25 ± 0.37
F. craterostoma	13.75 ± 0.86
F. elastica	12.38 ± 0.82
F. formosana	14.13 ± 0.69
F. malayana	12.25 ± 0.65
F. natalensis subsp. *leprieurii*	10.88 ± 0.58
F. palmeri	13.13 ± 0.91
F. sur	11.38 ± 0.60
F. vasta	13.0 ± 0.94
	Resistance, IZD ≤ 10 mm
F. benjamina	9.31 ± 0.73
F. carica	9.63 ± 0.63
F. cyathistipula	9.13 ± 0.69
F. deltoidea	9.5 ± 0.77
F. drupacea	9.5 ± 0.62
F. erecta	9.38 ± 0.6
F. hederacea	9.5 ± 0.6
F. johannis subsp. *afghanistanica*	9.06 ± 0.71
F. lyrata	9.38 ± 0.38
F. macrophylla	9.13 ± 0.6
F. microcarpa	9.38 ± 0.42
F. natalensis subsp. *natalensis*	9.5 ± 0.67
F. platypoda	9.31 ± 0.52
F. pumila	9.44 ± 0.76
F. rubiginosa	9.45 ± 0.8
F. sarmentosa var. *henryi*	9.48 ± 0.47
F. septica	9.5 ± 0.47
F. sycomorus	9.69 ± 0.62
F. taiwaniana	9.75 ± 0.37
F. villosa	9.63 ± 0.67

Table 5. Diameter values of the growth inhibition zone of bacteria *Aeromonas salmonicida* subsp. *salmonicida* caused by ethanolic extracts obtained from the leaves of various *Ficus* species

Ficus species	Inhibition zone diameter (IZD), mm
	High susceptibility, IZD ≥ 15 mm
F. aspera	20.0 ± 0.53
F. binnendijkii	15.75 ± 0.80
F. craterostoma	15.25 ± 1.15
F. deltoidea	20.13 ± 0.79
F. elastica	18.88 ± 0.48
F. formosana	17.75 ± 0.53
F. natalensis subsp. *leprieurii*	20.63 ± 0.71
F. pumila	20.64 ± 1.16
F. sarmentosa var. *henryi*	17.88 ± 0.74
F. septica	15.25 ± 0.82
F. sycomorus	17.38 ± 0.68
F. taiwaniana	20.5 ± 0.77
F. virens	20.63 ± 0.53
	Intermediate susceptibility, IZD = 10–15 mm
F. benghalensis	12.25 ± 0.73
F. carica	12.5 ± 0.57
F. drupacea	12.38 ± 0.53
F. erecta	13.63 ± 0.89
F. hederacea	13.75 ± 0.62
F. hispida	12.63 ± 0.50
F. lingua	11.25 ± 1.16
F. luschnathiana	12.13 ± 0.44
F. malayana	10.38 ± 0.32
F. natalensis subsp. *natalensis*	10.19 ± 0.52
F. palmeri	14.75 ± 0.92
F. religiosa	14.25 ± 1.05
F. sur	14.13 ± 1.11
F. tinctoria	12.13 ± 0.88
F. vasta	13.38 ± 0.42
	Resistance, IZD ≤ 10 mm
F. barteri	9.25 ± 0.41
F. benjamina	9.13 ± 0.35
F. cyathistipula	9.63 ± 0.91
F. johannis subsp. *afghanistanica*	9.38 ± 0.53
F. lyrata	9.5 ± 0.5
F. macrophylla	9.38 ± 0.65
F. microcarpa	9.25 ± 0.31
F. mucuso	9.25 ± 0.65
F. platypoda	9.38 ± 0.56
F. retusa	9.25 ± 0.59
F. rubiginosa	9.38 ± 0.42
F. sagittata	8.88 ± 0.48
F. villosa	9.5 ± 0.65

The results of the antibacterial activity testing of ethanolic extracts obtained from the leaves of different *Ficus* species against *A. salmonicida* subsp. *salmonicida* are illustrated in Table 5. In the group of extracts to which the bacterium was highly susceptible, the ethanolic *F. pumila* extract inhibited it in the largest zone, one of 20.64 ± 1.16 mm, followed by *F. natalensis* subsp. *leprieurii* with a 20.63 ± 0.71 zone, *F. virens* with a 20.63 ± 0.53 mm zone, and *F. taiwaniana* with a 20.5 ± 0.77 mm zone. Out of 15 extracts in the group to which *A. salmonicida* subsp. *salmonicida* was intermediate susceptible, the 3 extracts derived from *F. palmeri*, *F. religiosa*, and *F. sur* exhibited the highest antibacterial activity with IZDs of 14.75 ± 0.92, 14.25 ± 1.05, and 14.13 ± 1.11 mm, respectively. *Aeromonas salmonicida* subsp. *salmonicida* isolates were resistant to 13 extracts (31.7%) (Table 5).

Discussion

In the present study we investigated the antimicrobial activity of ethanolic extracts of various *Ficus* species against *Aeromonas* strains. We proved that the majority of those substances considerably inhibited bacterial growth: 14 extracts restricted *A. sobria*, 13 inhibited *A. salmonicida* subsp. *salmonicida*, and 10 impeded *A. hydrophila*. Moreover, our studies indicated that among all *Aeromonas* strains, the psychrophilic strain *A. salmonicida* subsp. *salmonicida* associated with the pathogenesis of furunculosis in salmonids (5) showed the highest sensitivity to the susbstances contained in the extracts.

The long-noted overuse of antibiotics both in human and in veterinary medicine has increased bacterial resistance to the antimicrobials used, causing side effects to the therapy which are often life-threatening. Therefore prescription of chemotherapeutics should be significantly reduced and they should be replaced with newly developed substances and technologies, including alternative methods of deriving antibacterial activity for medical purposes. One of the possible methods is plant-derived products or phytobiotics with antibacterial and antifungal properties, which are widely studied for their potential application in aquaculture systems (32). Nevertheless, although the properties of medicinal plants are well documented and exploited in human herbal medicine around the world, currently very few plant-derived antibacterial agents are available commercially for use in large-scale aquaculture (31). Plant extracts containing natural substances such as flavonoids, phenolic compounds, polysaccharides, and proteoglycans have been shown to stimulate the fish immune system, however, making them potentially valuable in preventing bacterial infections (23).

The presence of alkaloids, balsams, carbohydrates, flavonoids, free anthraquinones, glycosides, resins, saponins, sterols, tannins, and terpenes, which are known to be helpful in inactivating Gram-positive and Gram-negative bacteria, has been described in various plants belonging to the *Ficus* species (25). That account, our preliminary examinations, and the results of other researchers (26−30) are all in accord in showing that a number of ethanolic extracts obtained from the leaves of various *Ficus* species are antimicrobial against pathogenic and antibiotic-resistant bacteria. These substances could be used as an alternative therapy to treat infections caused by *Aeromonas* strains.

In our study, *F. hispida* extract exhibited the highest antibacterial activity against *A. sobria* and the extracts of *F. binnendijkii* and *F. tinctoria* were next. The significant antimicrobial effect of the *F. hispida* extract can be explained by the presence and role of the plant's secondary metabolites. The therapeutic features of this plant may be attributed to the occurrence of a wide range of phytochemical compounds, *i.e.* alkaloids, sterols, phenols, flavonoids, glycosides, saponins, and terpenes (15). One of them, a biphenylhexahydroindolizine hispidine isolated from the stem and leaves, has been found to act anti-oncologically (2). The other described compounds such as phenolic acids show antibacterial and antioxidant properties (18). Therefore, the considerable antimicrobial efficacy of *F. hispida* extract against the assayed *A. sobria* strain may be due to the sum effect of its constituents.

Chatterjee *et al.* (6) screened a methanolic leaf extract of *F. hispida* for chemical content and antioxidant and antibacterial activity. The bacteria tested included five strains of Gram-negative *Salmonella typhi* (NCTC-74, B-111, C-145, E-3404, and A-2467) and five strains of Gram-positive *Staphylococcus aureus* (ML-357, ML-15, ML-366, ML-276, and ML-145). The results showed that *S. aureus* strains generally had lower susceptibility to the extracts than *S. typhi*. Phytochemical analysis of the extract displayed the presence of flavonoids, glycosides, saponins, steroids, and tannins but the absence of alkaloids and amino acids. The total phenolic content of the extract was almost twice as high as the total flavonoid content (6).

Potent antimicrobial activity has been demonstrated among several flavonoids: apigenin, chalcones, flavone and flavonol glycosides, galangin, and isoflavones (11). This antibacterial function of flavonoids is associated with the capability to construct multiple cellular targets. An example of forming such a molecular mechanism is building a non-specific protein complex from covalent bonds and hygrogenic and hydrophobic bonding (10). Moreover, the B ring flavonoids can form a hydrogen bond with bases of nucleic acid, which can consequently lead to inhibition of bacterial DNA and RNA synthesis. Due to this ability, flavonoids' mode of antibacterial action is to inactivate microbial adhesins, cell envelope transport proteins and enzymes. Lipophilic flavonoid activity can also cause destruction of bacterial cell membranes (10). Among the group of polyphenols, three substances merit the most attention: flavan-3-ols, flavonols, and tannins. Compared to others, they show greater synergism with antibiotics and are characterised by higher and wider spectra of antimicrobial activity and a consequent capacity to suppress many microbial virulence factors including adhesion properties, biofilm formation, and bacterial toxins (in neutralising them) (12). Moreover, recent research has shown that the crude extracts obtained from plants possess more pharmacologically active properties than particular isolated active principles. This is due to the synergistic effects of different components present in the whole extract (22).

Thus, both literature data and our own results indicate that the control of bacterial diseases in fish by means other than traditional antibiotic administration is an actively developing field of research permitting well-founded hope of success. Considering the numerous threats to public health associated with the use of antibiotics in aquaculture, *i.e.* the spread of drug-resistant bacteria, the presence of resistance genes, and residues of antibacterial substances in aquaculture products and the environment (24), the search for environmentally friendly antimicrobial agents as alternatives to antibiotics is particularly urgent. Therefore, the introduction of plant extracts into treatment regimes can be considered a promising and very desirable alternative to antibiotic therapy.

For these reasons, future studies should focus on the chemical characterisation of plant extracts in order to identify and quantify the active compounds contained in them and determine the appropriate doses (1). The basis for this research could be the botanic gardens that play an important role in *ex situ* conservation and exploration of plant biodiversity (7) but are an untapped resource of practical applications. Our findings highlight how valuable collections of tropical plants accumulated at botanic gardens can be the searching ground for new plant-derived agents with a broad spectrum of biological and in particular antimicrobial action.

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