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ANTIBACTERIAL ACTIVITY OF AN ETHANOLIC EXTRACT DERIVED FROM LEAVES OF *FICUS LINGUA* WARB. EX DE WILD. & T.DURAND (MORACEAE) AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE STRAINS

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Many species belonging to the Ficus genus (Moraceae) contain several active compounds such as flavonoids, tannins, sesquiterpenes, alkaloids, and saponins which possess biological activities such as antioxidant, anticancer, anti-inflammation, antiviral, antibacterial, and others. In this study, we evaluated the antimicrobial activity of the ethanolic extract derived from the leaves of Ficus lingua Warb. ex De Wild. & T.Durand against some Gram-positive and Gram-negative strains in order to evaluate the possible use of this plant in preventing infections caused by these bacteria both in veterinary and medicine. The leaves of Ficus lingua, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. The testing of the antibacterial activity of the plant extracts was carried out in vitro by the Kirby-Bauer disc diffusion technique. In the current study, Gram-negative strains such as Escherichia coli (Migula) Castellani and Chalmers (ATCC® 25922™), Escherichia coli (Migula) Castellani and Chalmers (ATCC® 35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213™), Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 25923™), methicillin-resistant (MRSA), mecA positive Staphylococcus aureus (NCTC® 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used. Results of the current study revealed that both Gram-positive and Gram-negative strains were sensitive to the F. lingua extract. Gram-positive strains such as S. aureus subsp. aureus Rosenbach (ATCC® 29213™), S. aureus subsp. aureus Rosenbach (ATCC® 25923™), E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to the F. lingua extract. The highest diameters of inhibition zones after the application of the F. lingua extract were observed for S. aureus subsp. aureus strains. This study demonstrates the antibacterial potential of ethanolic extract derived from the leaves of F. lingua and for use in the treatment of bacterial infection. The bioactive compounds of F. lingua extract, as well as its main biological activities, make it a promising candidate for communicable disease management.

Keywords: *Ficus lingua* Warb. ex De Wild. & T.Durand, Kirby-Bauer disc diffusion technique, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis.*

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АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ ЕТАНОЛОВОГО ЕКСТРАКТУ, ОТРИМАНОГО З ЛИСТЯ *FICUS LINGUA* WARB. EX DE WILD. & T.DURAND (MORACEAE) ПРОТИ ДЕЯКИХ ГРАМПОЗИТИВНИХ І ГРАМНЕГАТИВНИХ ШТАМІВ

Багато видів, що належать до роду Ficus (Moraceae), містять кілька активних сполук, таких як флавоноїди, дубильні речовини, сесквітерпени, алкалоїди та сапоніни, які мають антиоксидантну, протипухлинну, протизапальну, противірусну, антибактеріальну та інші властивості. У цьому дослідженні ми вивчали антимікробну активність спиртового екстракту, отриманого з листя Ficus lingua Warb. ex De Wild. & T.Durand проти деяких грампозитивних і грамнегативних штамів, щоб оцінити можливе використання цієї рослини для запобігання інфекціям, викликаним цими бактеріями, як у ветеринарії, так і в медицині. Листя Ficus lingua, культивованих в тепличних умовах, відбирали у Національному ботанічному саду імені М.М. Гришка (НБС) НАН України.

Випробування антибактеріальної активності рослинних екстрактів проводили in vitro методом дискової дифузії Кірбі-Бауера. У поточному дослідженні грамнегативні штами, такі як Escherichia coli (Migula) Castellani і Chalmers (ATCC® 25922™), Escherichia coli (Migula) Castellani та Chalmers (ATCC® 35218™), Pseudomonas aeruginosa (Schroeter) Migula (ATCC® 27853™) та грампозитивні штами, такі як Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213™), Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 25923™), methicillin-resistant (MRSA), mecA positive Staphylococcus aureus (NCTC® 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (стійкий проти ванкоміцину; чутливий до тейкопланіну) і Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) було використано. Результати поточного дослідження показали, що як грампозитивні, так і грамнегативні штами були чутливі до екстракту F. lingua. Грам-позитивні штами, такі як S. aureus subsp. aureus Rosenbach (ATCC® 29213™), S. aureus subsp. aureus Rosenbach (ATCC® 25923™), E. faecalis (Andrewes and Horder) Schleifer та Kilpper-Balz (ATCC® 51299™) і E. faecalis (Andrewes and Horder) Schleifer та Kilpper-Balz (ATCC® 29212™) був чутливий до екстракту F. lingua. Найбільший діаметр зон інгібування після застосування екстракту F. lingua спостерігався для S. aureus subsp. aureus strains. Це дослідження демонструє антибактеріальний потенціал етанольного екстракту, отриманого з листя F. lingua, для використання при лікуванні бактеріальних інфекцій. Біоактивні сполуки екстракту F. lingua, а також його основна біологічна активність роблять його перспективним кандидатом для лікування інфекційних захворювань.

Ключові слова: *Ficus lingua* Warb. ex De Wild. & T.Durand, дискодифузійний метод Кірбі-Бауера, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis.*

INTRODUCTION

The mulberry family (Moraceae) is represented by mainly woody tropical or (more rarely) temperate species with specialized canals within their body containing milky latex, the feature most obviously distinguishing the family from other members of the order Urticales in which it is currently placed. The family comprises 37 genera and 1050-1100 species with a diversity of growth forms, including terrestrial trees, shrubs, climbers, hemi-epiphytes, subshrubs, and herbs [9, 10, 14]. *Ficus* L. is the largest within the family, containing ca 750 species distributed in the tropics and subtropics worldwide. Despite the exceptionally large species diversity of *Ficus* unproportional to that of other moraceous taxa, its consideration as a single entity is well-grounded on a number of

specific features, among which are the presence of waxy glands on vegetative plant parts, heterostyly, and anthesis of staminate flowers when the fruits are mature. The last two characteristics are functionally linked to the unique pollination mode in the genus involving mutualistic relationships with insects from the family Agaonidae (*Hymenoptera*) commonly referred to as «fig wasps» [8, 9, 11, 25].

Among the pharmacological properties demonstrated for the species belonging to the *Ficus* genus are antioxidants [4], antidiabetic [15], anti-inflammatory [24], anticancer [3], antitumor [35] and antiproliferative [29], antimutagenic [28], antimicrobial [23], anti-helminthic [6], hepatoprotective [17], wound healing [19], anticoagulant [21], immunomodulatory activities [16], antistress [38], toxicity studies [26], and larvicidal effects [28, 36]. Many reports have revealed that *Ficus* species contained a wide range of phytoconstituents, including phenols, flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides, sugar, protein, essential and volatile oils, and steroids [28]. Cruz and co-workers [12] have compiled the main reports over the last 5 years concerning the Ficus spp. fruits based on chemistry, properties, and applications as products. About 30 Ficus spp. fruits were reported focusing on their chemical composition rich in phenolic acids such as gallic, caffeic, and chlorogenic acids, as well as quercetin and cyanidin derivatives. The fruits from the Moraceae family presented mainly antioxidant and antimicrobial properties in addition to other functional properties to consumer's health [12]. Thus, plants belonging to the *Ficus* genus could be considered a priori as a good source of natural compounds to treat, prevent and control many diseases and disorders [15].

Many plants of the family Moraceae are used in the treatment of infectious diseases [22]. In our previous studies, we also assessed the antibacterial activity of ethanolic extracts derived from leaves of *Ficus* species against many bacterial and fungus strains [39-42]. In this study, we evaluated the antimicrobial activity of the ethanolic extract derived from the leaves of *Ficus lingua* Warb. ex De Wild. & T.Durand against some Gram-positive and Gram-negative strains in order to evaluate the possible use of this plant in preventing infections caused by these bacteria both in veterinary and medicine.

Ficus lingua is an evergreen shrub, sometimes with a climbing habit, or can become a tree with a spreading, much-branched crown; it can grow up to 30 meters tall (*African Flowering Plants Database, Conservatoire et Jardin Botaniques; Tropical Plants Database*) [46]. It often starts life as an epiphyte in the branch of a tree and can eventually send down aerial roots that, once they reach the ground, provide extra nutrients that help the plant grow more vigorously. These aerial roots can completely encircle the trunk of the host tree, constricting its growth – this, coupled with the more vigorous top growth, can lead to the fig outcompeting and killing the tree in which it is growing (*Protabase – Plant Resources of Tropical Africa*) [33]. The tree is traditionally used as a source of fiber from which cloth can be made. The cloth is traditionally made by removing pieces of bark from the bole and large branches, then soaking it in water for several days, drying it in the shade and then beating it with a mallet to make it supple enough for use It is sometimes grown to provide shade and as an ornamental and bonsai tree (*Protabase – Plant Resources of Tropical Africa; Tropical Plants Database*). The range of *F. lingua* in tropical Africa is Liberia, through the moister parts of Africa to Uganda and Kenya, south to DR Congo, Malawi, Mozambique, and Swaziland. The habitat - evergreen humid forest; coastal bushland; coral outcrops; Hemi-epiphytic, strangler or secondarily terrestrial; sometimes growing on rocks. At elevations up to 1,200 meters (*African Flowering Plants Database, Conservatoire et Jardin Botaniques; Protabase – Plant Resources of Tropical Africa*) [2].

The current study was conducted as a part of an ongoing project between the Institute of Biology and Earth Sciences (Pomeranian University in Słupsk, Poland), M.M. Gryshko National Botanic Gardens of National Academy of Sciences of Ukraine (Kyiv, Ukraine), and Ivan Franko Lviv National University (Lviv, Ukraine) undertaken in the frame of cooperation program aimed at assessment of medicinal properties of tropical plants, cultivated *ex-situ*.

MATERIALS AND METHODOLOGY

Collection of Plant Materials and Preparation of Plant Extracts. The leaves of *F. lingua*, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. The leaves (fig. 1) were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, and homogenized in 96% ethanol (in the ratio of 1:19, w/w) at room temperature. The extracts were then filtered and investigated for their antimicrobial activity.

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Fig. 1. Twig of *Ficus lingua* Warb. ex De Wild. & T. Durand.
Photo: Lyudmyla I. Buyun

Determination of the antibacterial activity of plant extracts by the disk diffusion method. The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique [7]. In the current study, Gram-negative strains such as *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), methicillin-resistant (MRSA), mecA positive *Staphylococcus aureus* (NCTC® 12493), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with extract were applied over each of the culture dishes. Isolates of bacteria with extract were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of

inhibition produced by the antibacterial activity of F. lingua extract. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24-h period, the inhibition zones formed were measured in millimeters using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the *F. lingua* extract was indicated by a clear zone of inhibition around the discs containing the extract and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤ 10 mm [30, 43, 44].

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the extract tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) [50].

RESULTS AND DISCUSSION

The antibacterial activity induced by the ethanolic extract derived from the leaves of F. lingua estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains was presented in Figures 2 and 3.

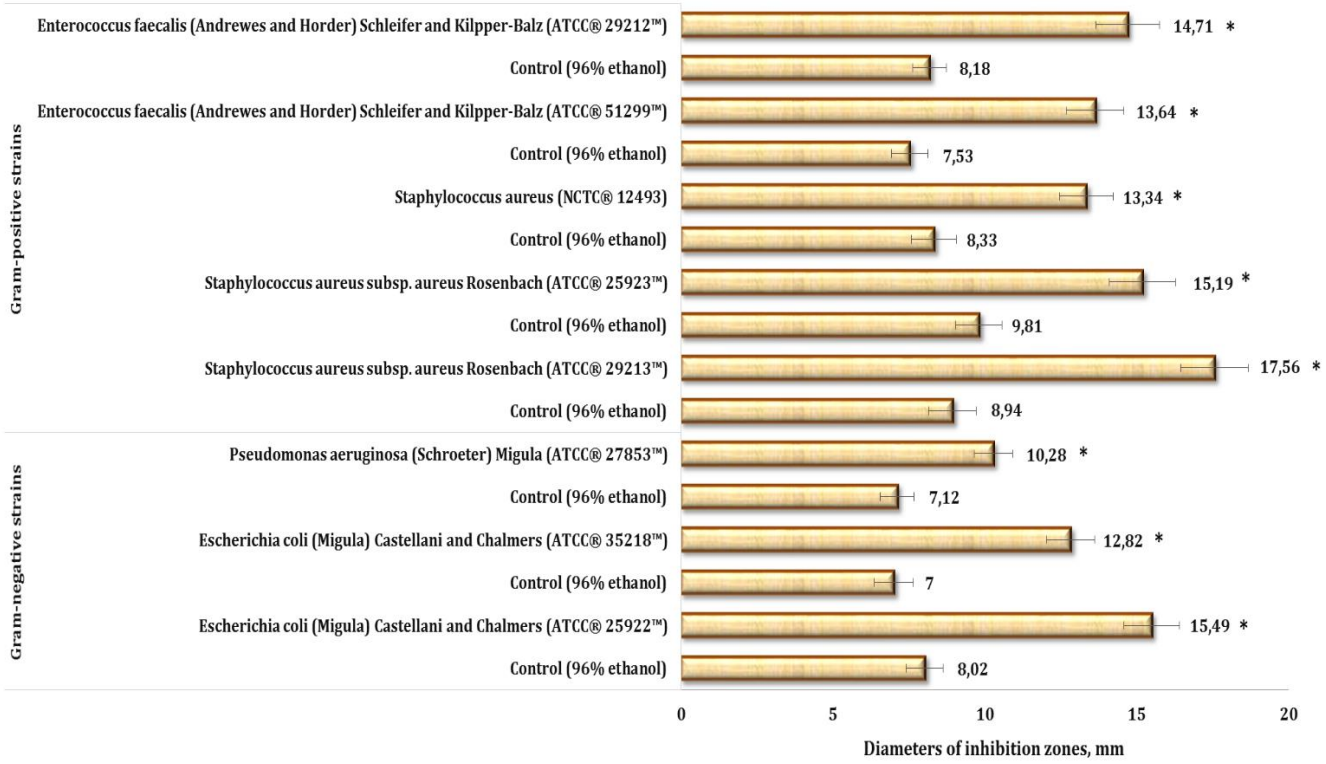
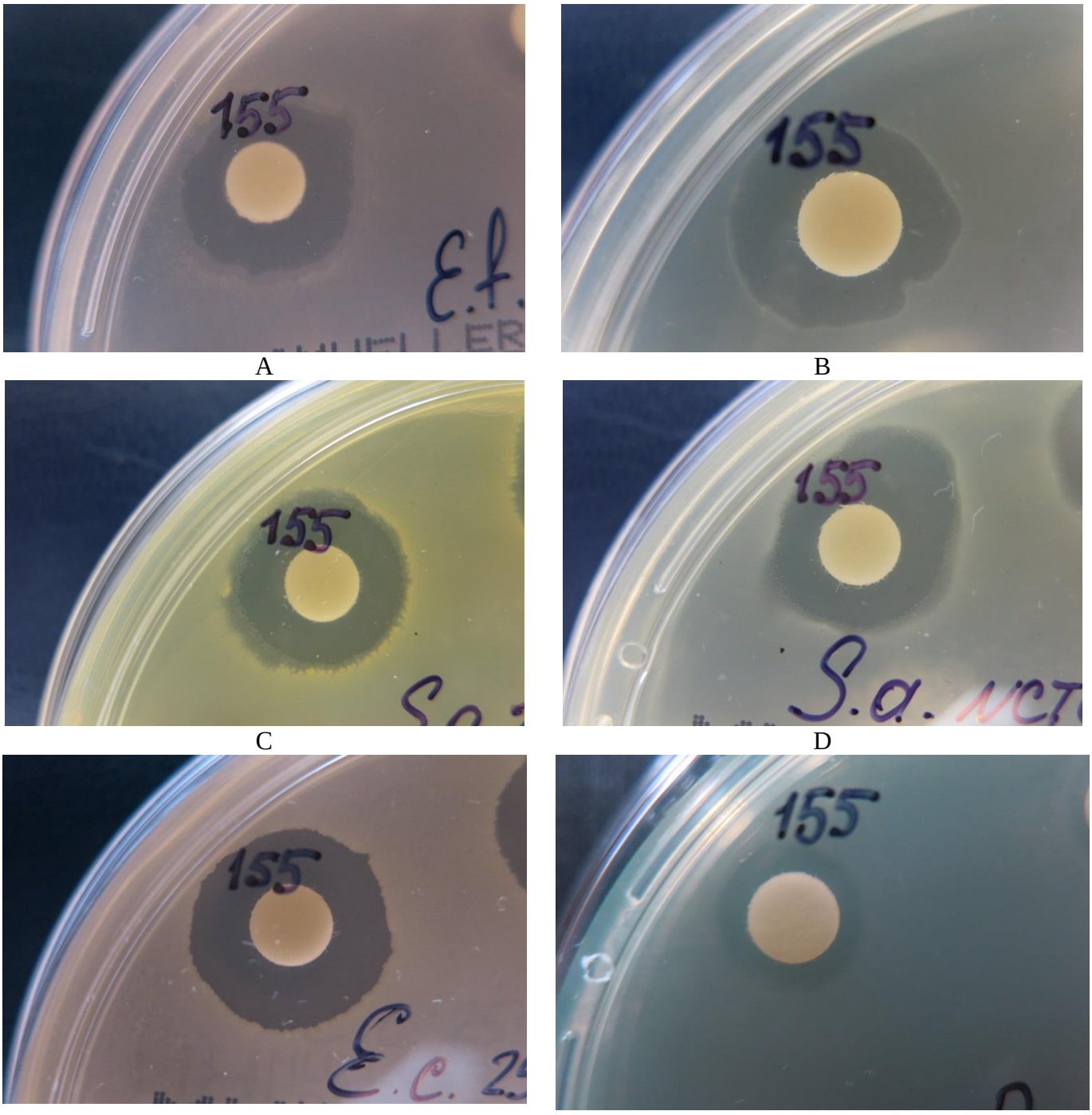


Fig. 2. The antibacterial activity of the ethanolic extract derived from the leaves of *Ficus lingua* estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains

The data were presented as the mean ± the standard error of the mean (S.E.M.).

* denote significant differences between the control (96% ethanol) and *F. lingua* extract ($p < 0.05$).



A B C D E F

Fig. 3. Inhibition growth zones induced by the *F. lingua* extract against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (B), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) (C), *Staphylococcus aureus* (NCTC® 12493) (D), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) (E), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) (F)

Results of the current study revealed that both Gram-positive and Gram-negative strains were sensitive to the ethanolic extract derived from the leaves of *F. lingua*. The diameters of inhibition zones for *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain after the application of *F. lingua* extract were increased to (15.49 ± 0.91 mm) compared to the 96% ethanol as control samples (8.02 ± 0.61 mm). Similar results were obtained for the *E. coli* (Migula) Castellani and

Chalmers (ATCC® 35218™) strain. The diameters of inhibition zones after the application of *F. lingua* extract were increase to (12.82 ± 0.79 mm) compared to the 96% ethanol as control samples (7.0 ± 0.64 mm). The percentage of increase in the inhibition zone diameters was 93% and 83% for *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) and *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains, respectively. *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain was resistant to the *F. lingua* extract. The diameters of inhibition zones after the application of *F. lingua* extract were (10.28 ± 0.63 mm) compared to the 96% ethanol as control samples (7.12 ± 0.56 mm) (Fig. 2).

Gram-positive strains were also sensitive to the *F. lingua* extract. *S. aureus* strains exhibited intermediate activity to the *F. lingua* extract. *S. aureus* (NCTC® 12493) strain was less sensitive then *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) strains. Diameters of inhibition zones after application of the *F. lingua* extract were (17.56 ±1.11 mm) compared to the 96% ethanol as control samples (8.94 ± 0.79 mm) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain, (15.19 ± 1.08 mm) compared to the 96% ethanol as control samples (9.81 ± 0.77 mm) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) strain, and (13.34 ± 0.88 mm) compared to the 96% ethanol as control samples (8.33 ±0.74 mm) for *S. aureus* (NCTC® 12493) strain. The increase of diameters of inhibition zones after the application of the *F. lingua* extract was 96% ($p < 0.05$), 55% ($p < 0.05$), and 60% ($p < 0.05$) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), and *S. aureus* (NCTC® 12493) strains, respectively, compared to the control samples (96% ethanol) (Fig. 2).

E. faecalis strains were also sensitive to the *F. lingua* extract (Fig. 2). Diameters of inhibition zones after application of the *F. lingua* extract were (13.64 ± 0.95 mm) compared to the 96% ethanol as control samples (7.53 ± 0.60 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain and (14.71 ± 1.05 mm) compared to the 96% ethanol as control samples (8.18 ± 0.55 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strain. The increase of diameters of inhibition zones after the application of the *F. lingua* extract was 81% ($p < 0.05$) and 80% ($p < 0.05$) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strains, respectively (Fig. 2).

Detailed photos regarding the zones of inhibition induced by the *F. lingua* extract against Gram-positive and Gram-negative bacterial strains were recorded and presented in Figure 3.

In line with our previous studies according to the antibacterial potential of different plant extracts, in the current study, we examined the antibacterial potential of an ethanolic extract derived from the leaves of *F. lingua* against Gram-positive and Gram-negative bacterial strains. Sensitivity to the *F. lingua* extract were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strains. The diameters of inhibition zones after the application of the *F. lingua* extract ranged from 10.1 to 16.8 mm. On the other hand, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), methicillin-resistant *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to the *F. lingua* extract. The highest diameters of inhibition zones after the application of the *F. lingua* extract were observed for *S. aureus* subsp. *aureus* strains (Figures 2 and 3).

In our previous study, we screened the antimicrobial activity of the ethanolic extract of *F. lingua* against fish pathogen – three *Aeromonas* strains (*Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida* subsp. *salmonicida*). The leaf extract of *F. lingua* used in this study has bactericidal properties which make it very attractive for use in fish aquaculture. Its uses will reduce the side effects of applying synthetic compounds. The ethanolic extract obtained from leaves of *F. lingua* exhibited the maximum antimicrobial activity against *Aeromonas sobria* strain (inhibition

zone diameter was 19.38 ± 1.27 mm), *Aeromonas hydrophila* (16.06 ± 1.05 mm), and *Aeromonas salmonicida* subsp. *salmonicida* (11.25 ± 1.16 mm). The most susceptible strain to the antimicrobial activity of *F. lingua* was *Aeromonas sobria.* However, further study is needed to determine the effects of the active compounds present in the leaf extract of *F. lingua* on fish metabolism in *in vitro* and *in vivo* studies. Present results suggest the possibility of using such extracts in *in vivo* studies in order to corroborate if could be possible to use those extracts in aquaculture in order to achieve protection against pathogenic infections [31].

Many researchers have revealed the antioxidant properties of many species belonging to the *Ficus* genus. For example, Aref and co-workers [5] have investigated methanolic, hexanoic, chloroformic, and ethyl acetate extracts of *Ficus carica* L. latex collected from Chott Mariam Souse, Middle East coast of Tunisia against five bacteria species and seven strains of fungi. The antimicrobial activity of the extracts was evaluated and based respectively on the inhibition zone using the disc-diffusion assay, minimal inhibition concentration (MIC) for bacterial testing, and the method by calculating inhibition percentage (I%) for fungi-inhibiting activities. The methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had an inhibition effect on the multiplication of five bacteria species (*Enterococcus faecalis*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus mirabilis*). For the opportunist pathogenic yeasts, ethyl acetate and chloroformic fractions showed a very strong inhibition (100%); methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 0.500 mg/ml and a negative effect against *Cryptococcus neoformans*. *Microsporum canis* was strongly inhibited with methanolic extract (75%) and totally with ethyl acetate extract at a concentration of 0.750 mg/ml. The hexanoic extract showed medium results [5].

In our previous study [45], an ethanolic extract derived from leaves of *F. carica* was tested for its antibacterial activity against Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922), Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* (ATCC 49619), as well as fungus *Candida albicans.* The ethanolic extract of *F. carica* exhibited mild antimicrobial activity against the Gram-positive bacteria (10.4 mm of inhibition zone diameter for methicillin-resistant *S. aureus* and 14.28 mm for *S. aureus*), and the Gram-negative bacteria (13.25 mm for *E. coli*). *K. pneumonia, P. aeruginosa,* and *S. pneumoniae* appeared to be less sensitive to the extract, the inhibition zones were 9.75 mm, 8.69 mm, and 8.56 mm, respectively. The antibacterial activity of leaf extract is possible could be explained by the presence of flavonoids, steroids, saponins, and/or tannins. The high antimicrobial activity may perhaps be due to leaves content of rutin, quercetin, luteolin, phenolic acids, and phytosterols [34].

Antimicrobial activities of some Thai traditional medical longevity formulations from plants and antibacterial compounds from *Ficus foveolata* (Miq.) Wall. ex Miq. (synonym of *Ficus sarmentosa* Buch.-Ham. ex Sm.) were studied by Meerungrueang and Panichayupakaranant [27]. The ethyl acetate extract of *F. foveolata* showed the strongest antibacterial activity with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 19.5-39.0 and 39.0-156.2 μg/mL, respectively. On the basis of antibacterial assay-guided isolation, seven antibacterial compounds, including 2,6-dimethoxy-1,4-benzoquinone (1), syringaldehyde (2), sinapaldehyde (3), coniferaldehyde (4), 3β-hydroxystigmast-5-en-7-one (5), umbelliferone (6), and scopoletin (7), were purified. Among these isolated compounds, 2,6-dimethoxy-1,4-benzoquinone (1) exhibited the strongest antibacterial activities against *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus mutans* with MIC values of 7.8, 7.8, and 15.6 μg/mL, and MBC values of 7.8, 7.8, and 31.2 μg/mL, respectively [27].

Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* Blume were done by Usman and co-workers [47]. The phytochemical tests revealed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, and tannins. The antimicrobial activity of the plant extract was assayed using the agar plate disc

diffusion and nutrient broth dilution techniques. Test microorganisms were *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Salmonella typhi* (Gram-negative), *Staphylococcus aureus*, and *Streptococcus* spp. (Gram-positive). The extracts inhibited the growth of all the test organisms at different concentrations, especially against *Pseudomonas aeruginosa* and *Streptococcus* spp. The results showed a MIC of 10 $mg \cdot ml^{-1}$ against *Pseudomonas* and 1.25 against the remaining organisms tested. The MBC against *Staphylococcus aureus* was 2.5 $mg \cdot ml^{-1}$ and that of *Streptococcus* spp. was found to be 0.625 $mg \cdot ml^{-1}$. The extracts showed varied inhibitory activity against the organisms studied [47].

Abdsamah and co-workers [1] have investigated the *in vitro* antimicrobial activity of the chloroformic, methanolic and aqueous extracts of *Ficus deltoidea* Jack at 10 mg/ml, 20 mg/ml and 50 mg/ml, respectively using the disc diffusion method against 2 Gram-positive [*Staphylococcus aureus* (IMR S-277), *Bacillus subtilis* (IMR K-1)], 2 Gram-negative [*Escherichia coli* (IMR E-940), *Pseudomonas aeruginosa* (IMR P-84)] and 1 fungal strain, *Candida albicans* (IMR C-44). All the extracts showed inhibitory activity on the fungus, Gram-positive and Gram-negative bacteria strains tested except for the chloroformic and aqueous extracts on *B. subtilis*, *E. coli*, and *P. aeruginosa*. The methanolic extract exhibited good antibacterial and antifungal activities against the test organisms. The methanolic extract significantly inhibited the growth of *S. aureus* forming a wide inhibition zone (15.67 ± 0.58 mm) and the lowest minimum inhibitory concentration (MIC) value (3.125 mg/ml). *B. subtilis* was the least sensitive to the chloroform extract (6.33 ± 0.58 mm) and highest minimum inhibitory concentration (MIC) value (25 mg/ml) [1].

Also, Kuete and co-workers [23] have assessed the antimicrobial activity of the methanol extract from the roots of *Ficus polita* Vahl. (FPR), as well as that of its fractions (FPR1-5) and two of the eight isolated compounds, namely euphol-3-O-cinnamate (1) and (E)-3,5,4'-trihydroxy-stilbene-3,5-O-β-D-diglucopyranoside (8). The results of the MIC determination showed that the crude extract, fractions FPR1, FPR2, and compound 8 were able to prevent the growth of the eight tested microorganisms. Other samples showed selective activity. The lowest MIC value of 64 μg/ml for the crude extract was recorded on 50% of the studied microbial species. The corresponding value for fractions of 32 μg/ml was obtained on *Salmonella typhi*, *Escherichia coli*, and *Candida albicans* ATCC strains. The MIC values recorded with compound 8 on the resistant *Pseudomonas aeruginosa* PA01 strain were equal to that of chloramphenicol used as a reference antibiotic. The obtained results highlighted the interesting antimicrobial potency of *F. polita* as well as that of compound 8 and provided the scientific basis for the traditional use of this taxon in the treatment of microbial infections [23].

The different bactericidal activity observed in this study may be due to the different components extracted. Among the most abundant components present in leaves of various *Ficus* plants, flavonoids, phenolic acids and especially terpenoids present bactericidal activity. Terpenoids are natural compounds originating from five-carbon isoprene units and are responsible for the protection of various plants against herbivores and pathogens [49]. The perturbation of ion homeostasis upon an increase in cell wall permeability is key for the action of terpenoids against bacteria cells [18]. In the study of Ergüden [18], the antibacterial activity of 12 different terpenoids or related structures in a comparative way and revealed that the phenolic terpenoids are superior to other substituted derivatives against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacteria. An immediate loss of cell membrane integrity and ion leakage upon treatment of both Gram-negative and Gram-positive cells with phenolic terpenoids carvacrol and thymol were observed [18]. The combination of carvacrol and thymol has bacteriostatic and bactericidal activities. Four terpenoids (carvacrol, thymol, eugenol, and nootkatone) exhibited bacteriostatic and bactericidal activities when used at low concentrations for 5–10 min. The most effective bactericidal activity was observed for Gram-negative bacteria [49]. After prolonged treatment, leakage of genetic material and an increase in membrane permeability for molecules were also observed [18]. Guimarães and co-workers [20] have investigated the antibacterial activity of 33 free terpenes commonly found in essential oils and evaluated the cellular ultrastructure to

verify possible damage to the cellular membrane. The higher antimicrobial activity was related to the presence of hydroxyl groups (phenolic and alcohol compounds), whereas hydrocarbons resulted in less activity. The first group, such as carvacrol, l-carveol, eugenol, trans-geraniol, and thymol, showed higher activity when compared to sulfanilamide. The mechanism causing the cell death of the evaluated bacteria is based on the loss of cellular membrane integrity of function [20].

Flavonoids, a wide variety of phenolic secondary metabolites, are also well-known as antibacterial agents against a wide range of pathogenic microorganisms [37, 48]. Several high-quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement [13]. The proposed antibacterial mechanisms of flavonoids are as follows: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane permeability, and attenuation of the pathogenicity [13, 48]. The natural phenols and their semisynthetic derivatives were tested by Pinheiro and co-workers [32] for their antimicrobial activity against the bacteria: *Staphylococcus aureus, Escherichia coli, Listeria innocua, Pseudomonas aeruginosa, Salmonella enterica typhimurium, Salmonella enterica* ssp. *enterica*, and *Bacillus cereus* [32].

CONCLUSIONS

In summary, this study provides insight into the *in vitro* antibacterial activity of an ethanolic extract derived from the leaves of *Ficus lingua* against Gram-negative strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), methicillin-resistant (MRSA) *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™). Results of the current study revealed that both Gram-positive and Gram-negative strains were sensitive to the *F. lingua* extract. Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to the *F. lingua* extract. The highest diameters of inhibition zones after the application of the *F. lingua* extract were observed for *S. aureus* subsp. *aureus* strains. This study demonstrates the antibacterial potential of ethanolic extract derived from the leaves of *F. lingua* and for use in the treatment of bacterial infection. The bioactive compounds of *F. lingua* extract, as well as its main biological activities, make it a promising candidate for communicable disease management.

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