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Оценка антимикробной активности экстрактов
из листьев различных видов фикусов
относительно синегнойной палочки**

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Evaluation of antimicrobial activity of *Ficus* species
leaves extracts against *Pseudomonas aeruginosa***

В последнее время растет интерес к видам рода *Ficus* (Moraceae) из-за их химического состава и потенциальной терапевтической пользы. Род *Ficus* широко используется в традиционной медицине для лечения широкого круга заболеваний центральной нервной системы, желудочно-кишечного тракта, эндокринной, репродуктивной, дыхательной систем, а также для лечения инфекционных заболеваний. Целью данного исследования была оценка влияния экстрактов выделенных с растений рода *Ficus* на рост синегнойной палочки как наиболее характерного представителя условно-патогенной микрофлоры. Экстракты видов фикуса обладают антимикробной активностью в отношении синегнойной палочки. Такие виды как *F. natalensis* subsp. *leprieurii*, *F. binnendijkii* 'Amstel Gold', *F. septica*, *F. rubiginosa* и *F. sagittata* оказались наиболее эффективными в качестве антибактериальных агентов. Виды рода *Ficus* являются перспективными кандидатами для разработки новых лекарственных средств в будущих клинических применениях

Many *Ficus* species have been used in ayurvedic and traditional medicine in the treatment or management of a variety of ailments and diseases and to have antioxidant, antidiabetic, antibacterial, antifungal, antiviral, antiprotozoal, anticancer, cytotoxic, antiulcer, antiinflammatory, antihyperglycemic, antidiarrhoeal, hepatoprotective, mucoprotective and gastroprotective activities. This study was undertaken to evaluate the effect of various ethanolic extracts of *Ficus* leaves on the growth of *Pseudomonas aeruginosa*. *Ficus* species were collected in M. Gryshko National Botanical Garden (Kyiv, Ukraine) and Botanical Garden of Ivan Franko Lviv National University (Lviv, Ukraine). Freshly crushed leaves were washed, weighted, and homogenized in 96% ethanol (in proportion 1:10) at room temperature. Antimicrobial activity was determined using the agar diffusion method. Our results show that extracts of *Ficus* species have antimicrobial activity against *Pseudomonas aeruginosa*, and *F. natalensis* subsp. *leprieurii*, *F. binnendijkii* 'Amstel Gold', *F. septica*, *F. rubiginosa* and *F. sagittata* proved to be most effective as antibacterial agents. Thus, *Ficus* species are a promising candidates in pharmaceutical biology for the development and formulation of new drugs and future clinical uses

Ключевые слова: *Ficus*, *Pseudomonas aeruginosa*, антимикробная активность, экстракты

Key words: *Ficus* species, *Pseudomonas aeruginosa*, antimicrobial activity, ethanolic extracts

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Introduction

Furuncle, also known as boil represents the commonest example of an abscess, and pyogenic organisms such as Staphylococcus aureus, and Pseudomonas aeruginosa have been implicated as causal agents [22,32]. Infection by these organisms evokes the acute inflammatory response, causing tissue swelling and the associated pain and leading to pyogenic or suppurative inflammation [38]. Pseudomonas aeruginosa is a common Gram-negative, rod-shaped bacterium, which is optimally adapted to various environmental conditions. P. aeruginosa is ubiquitously present in soil, water or sewage as well as in human, animal or plant hosts and why it is widespread around the world due to use aerobic respiration as its optimal metabolism; however, it can also respire anaerobically on nitrate or other alternative electron acceptors [28,39]. The infections range from endophthalmitis, endocarditis, meningitis, and septicemia to chronic lung infections [11]. P. aeruginosa is one of the most important bacteria with documented resistance to multiple

antimicrobial agents including β -lactams, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins [4]. Due to its intrinsic and acquired antimicrobial resistance, only limited classes of antibiotics are effective for the treatment of *P. aeruginosa* infections [13]. From the pathophysiology, it is evident that the medicinal potency of an agent with such a popular use and acclaimed effectiveness in the treatment of furuncles may derive from direct inhibition of the growth of the causal organisms, suppression of acute inflammatory response and or enhancement of the host's ability to combat the infection through stimulation of immune responses [22].

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay [34]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [21,27,29]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings [20].

Most of the information currently available on *Ficus* spp. reveals that they are rich source of compounds, which have multidimensional curative properties. Many *Ficus* species have been used in ayurvedic and traditional medicine in the treatment or management of a variety of ailments and diseases and to have antioxidant, antidiabetic, antibacterial, antifungal, antiviral, antiprotozoal, anticancer, cytotoxic, antiulcer, anti-inflammatory, antihyperglycemic, antidiarrhoeal, hepatoprotective, mucoprotective and gastroprotective activities [3,16].

Our recent study [37] revealed that ethanolic extract from leaves of *F. carica* showed potent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. *S. aureus* demonstrated susceptible test for five of the leaf ethanolic extracts: *F. erecta* var. *sieboldii*, *F. rubiginosa*, *F. benamina*, *F. septica*, and *F. erecta* [35]. *E. coli* ATCC 25922 demonstrated susceptible test for nine of the ethanolic leaf extracts: *F. villosa*, *F. benamina*, *F. religiosa*, *F. elastica*, *F. sur*, *F. aspera*, *F. vasta*, *F. hispida*, and *F. craterostoma*. Intermediate susceptibility was noted for three species: *F. benghalensis*, *F. sycomorus*, and *F. binnendijkii*. The high anti-*E.coli* activity demonstrated by the *F. sur* (inhibition zone diameter is 19 mm), *F. villosa* (17.5 mm), *F. aspera* (17 mm), *F. elastica* (16 mm), *F. vasta* (16 mm). *F. sur* proved to be most effective as an antibacterial agent against Gram-negative *E. coli* [36]. This study was undertaken to evaluate the effect of various ethanolic extracts of *Ficus* leaves on the growth of *Pseudomonas aeruginosa*.

Materials and Methods

The leaves of *F. aspera* G. Forst, *F. benghalensis* L., *F. benamina* L., *F. binnendijkii* (Miq.) Miq., *F. binnendijkii* 'Amstel Gold', *F. binnendijkii* 'Amstel King', *F. carica* L., *F. craterostoma* Mildbr. & Burret, *F. cyathistipula* Warb., *F. deltoidea* Jack, *F. drupacea* Thunb., *F. elastica* Roxb., *F. elastica* 'Variegata', *F. erecta* Thunb., *F. erecta* var. *sieboldii*, *F. hispida* L. f., *F. hispida* 'Parcelii', *F. luschanthiana* (Miq.) Miq., *F. lyrata* Warb., *F. macrophylla* Desf. Ex Pers., *F. mucoso* Ficalho, *F. natalensis* Hochst. subsp. *natalensis*, *F. natalensis* Hochst. subsp. *leprieurii* (Miq) C.C. Berg, *F. platypoda* (Miq.) A. Cunn. ex Miq., *F. petiolaris* Kunth, *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 (Kyiv, Ukraine) and Botanical Garden of Ivan Franko Lviv National University (Lviv, Ukraine) during March, 2015. The whole collection of tropical and subtropical plants at NBG (including *Ficus* spp. plants) has the status of a National Heritage Collection of Ukraine. The sampled leaves of *Ficus* 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. Antimicrobial activity was determined using the agar diffusion method. Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 27853) was used as test organism. Mueller Hinton Agar solid medium was used for culturing of bacteria. Then the sterile filter paper discs containing sample were immersed in plant extracts and were placed over the solidified agar in such a way that there was no overlapping of zone of inhibition. The plates were incubated at 37° for 24 h. Activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated six times and the antibacterial activity was expressed as the mean of diameter of inhibition zones (mm) produced by the extracts. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 11-14 mm, and Resistant (R) ≤ 10 mm [23].

Results

The ethanolic leaves extracts of *Ficus* species were used to determine the antimicrobial activity against *P. aeruginosa*. The highest antimicrobial activities against *P. aeruginosa* (zone diameters were above 15 mm) were noted for *F. natalensis* subsp. *leprieurii*, *F. deltoidea*, *F. binnendijkii* 'Amstel Gold', *F. septica*, *F. rubiginosa*, *F. sagittata*, and *F. benghalensis* (Fig. 1). Less pronounced antibacterial activity was observed for the extracts of *F. drupacea*, *F. platypoda*, *F. lyrata*, and *F. pumila* (zones of growth inhibition were varied between 10.5 and 14.6 mm) (Figs 1 and 2).

Fig. 1. Inhibition zone diameters produced by the leaf ethanolic extract of *Ficus* species against *Pseudomonas aeruginosa*

Fig. 2. Antimicrobial activity of leaf ethanolic extract of *F. natalensis* subsp. *leprieurii* (A), *F. deltoidea* (B), *F. binnendijkii* 'Amstel Gold' (C), *F. septica* (D) against *Pseudomonas aeruginosa*

Discussion and Conclusion

Abdsamah et al. (2012) studied the *in vitro* antimicrobial activity of the chloroform, methanol and aqueous extracts of *F. deltoidea* 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) [1]. All the extracts showed inhibitory activity on the fungus, Gram-positive and Gram-negative bacteria strains tested except for the chloroform and aqueous extracts on *B. subtilis*, *E. coli*, and *P. aeruginosa*. The methanol extract exhibited good antibacterial and antifungal activities against the test organisms. Methanol extracts of whole *F. deltoidea* exhibited antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* and antifungal activity towards *C. albicans*. Antimicrobial activity of *F. deltoidea* *in vitro* further justifies its utility in folkloric medicines for the treatment of infections of microbial origin [1].

A wide range of chemical compounds have been isolated and characterized from *F. deltoidea*, particularly from the leaves and figs [5]. An initial comprehensive study

on volatile compounds produced by the fruits was conducted by Grison-Pigé et al. (2002) [12]. The volatile compounds isolated and identified are mainly products of the shikimic acid pathway, terpenoids, and aliphatic groups, generally present as floral fragrances in plants. In 2009, Mohd Lip et al. (2009) isolated and identified moretenol from *F. deltoidea* leaves using nuclear magnetic resonance (NMR) and mass spectrometers [18]. Later on, an antibacterial compound known as lupeol (C₃₀H₅₀O) was also isolated from the leaves of *F. deltoidea* and exhibited toxicity against *S. aureus*, *B. subtilis*, and *E. coli* [33]. Ong et al. (2011) demonstrated enhancement of flavonoid compounds (rutin, quercetin, and naringenin) in cell cultures of *F. deltoidea* influenced by different carbon sources as well as plant growth regulators [25]. A comprehensive study on flavonoid compounds of aqueous extract of *F. deltoidea* was conducted by Omar et al. (2011) using HPLC-MS-based methodology and more than 25 compounds were identified [24]. Currently, two bioactive constituents known as vitexin and isovitexin have been isolated, identified, and evaluated to show α -glucosidase inhibition [6]. The antibacterial activity of this extract is possibly linked to the presence of flavonoid compounds. Antibacterial activity of flavonoid compounds isolated from plant species are well documented [9]. A high amount of epicatechin found in *F. deltoidea* may be responsible for the strong radical scavenging activities found in the extract. A positive correlation was observed between flavonoid constituents present and radical scavenging activities of the aqueous extracts of *F. deltoidea* [7].

The bark of *F. benghalensis* exhibited significant antibacterial activity against *S. aureus*, *P. aeruginosa* and *Klebsiella pneumoniae* [10]. Aquatic and hexane aerial root extracts of *F. benghalensis* showed sustained activity against all bacterial strains and the highest activity was observed against *S. aureus* [31]. The methanolic extract showed good antimicrobial activity and was more potent towards *B. subtilis* [14]. Mousa et al. (1994) reported the fruit extracts had significant antibacterial activity but no antifungal activity [19]. The aquatic or alcoholic extracts of various parts of this plant were found to have antibacterial activity [2].

Murti and Kumar (2011) reported that the ethanolic extract at different concentrations of roots showed moderate antibacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumonia* [20]. Study of Koona and Rao (2012) revealed that hexane leaves extract was found to be resistance against *K. pneumoniae*, *P. aeruginosa* and *Micrococcus luteus* for low concentration, while *K. pneumoniae* showed intermediate activity to its high concentration [15]. *E. coli*, *P. vulgaris*, *B. subtilis* and *Enterococcus faecalis* did not show any inhibition zones. CHCl₃ extract was found effective against *K. pneumoniae* and *M. luteus* showed intermediate activity against *P. aeruginosa* irrespective of its concentrations whereas *E. coli*, *P. vulgaris* and *Enterococcus faecalis* were resistance to low concentration and evinced intermediate activity to high concentration. Methanolic extract exhibited promising activity against all tested bacteria for both concentrations [15]. The acetonic, methanolic and ethanolic bark extracts showed good antibacterial activity against *P. aeruginosa*, *E. coli*, *P. vulgaris*, *B. subtilis*, and *S. aureus* [17].

Moreover, other *Ficus* species possess antimicrobial activity. Among the different leaf extracts of *F. tsiela*, diethyl ether extract exhibited better inhibitory effect against *K. pneumoniae* (20 mm) followed by *E. coli* (12 mm), *P. aeruginosa* (12 mm)

and least activity was noted against *S. aureus* (10 mm) [30]. The acetone extract showed maximum inhibitory activity (11 mm) against *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. Moderate activity (9 mm) has been recorded against *E. coli* [30]. The fruit extracts of *F. sycomorus* L., *F. benjamina* L. had significant antibacterial activity [19]. El-Fishawy et al. (2011) reported that the alcoholic extracts of the leaves and fruits were effective against *S. aureus*, *B. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* [8]. All the extracts of *F. deltoidea* showed inhibitory activity on the fungus, gram-positive and gram-negative bacteria strains tested [1]. The methanolic extract exhibited good antibacterial and anti-fungal activities against the test organisms [1]. The leaf extracts of *F. thonningii*, *F. saussureana*, *F. exasperata* and *F. sur* were screened for antimicrobial properties on eight fungal species and two bacterial species. The presence of alkaloids, flavonoids and cardiac glycosides in the leaves of these species may have conferred the antimicrobial properties on these species. The extracts from all the four *Ficus* species exerted significant antimicrobial effect on all the test organisms at 75 and 100 mg/ml [26].

Thus, these plants have great medicinal potential for the therapy of infection. Further investigation is necessary to identify those bioactive compounds, which will be a platform for clinical applications. These may help to strengthen our understanding of this highly therapeutic plant for commercial exploitation.

Our results show that extracts of *Ficus* species have antimicrobial activity against *Pseudomonas aeruginosa*, and *F. natalensis* subsp. *leprieurii*, *F. binnendijkii* 'Amstel Gold', *F. septica*, *F. rubiginosa* and *F. sagittata* proved to be most effective as an antibacterial agents. Thus, *Ficus* species are a promising candidates in pharmaceutical biology for the development and formulation of new drugs and future clinical uses.

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Evaluation of antimicrobial activity of *Ficus* species
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