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IN VITRO SCREENING OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OBTAINED FROM *FICUS LYRATA* WARB. (MORACEAE) LEAVES

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Проведено скринінгове дослідження антимікробної активності етанольного екстракту, отриманого з листків *Ficus lyrata*, щодо патогенних мікроорганізмів з метою виявлення нових джерел антимікробних засобів. Антимікробну активність екстракту оцінювали за допомогою диско-дифузійного методу з використанням стандартних дисків. Антимікробну активність екстракту *F. lyrata* визначали відносно патогенних мікроорганізмів людини — як грампозитивних (*Staphylococcus aureus*, метицилін-резистентного *S. aureus* та *Streptococcus pneumoniae*), так і грамнегативних (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* та *Escherichia coli*) штамів. Результати дослідження свідчать, що етанольний екстракт листків *F. lyrata* виявляє помірну антимікробну активність, очевидно, обумовлену присутністю різних вторинних метаболітів, що підтверджує традиційне використання цієї рослини для лікування захворювань, викликаних патогенними мікроорганізмами. Отримані дані дають змогу висловити припущення, що екстракт *F. lyrata* може бути використаний для виявлення нових антимікробних сполук та розробки на їх основі нових фармацевтичних препаратів для профілактики та лікування бактеріальних інфекцій.

Ключові слова: *Ficus lyrata*, етанольний екстракт листків, патогенні бактерії, in vitro антимікробна активність, вторинні метаболіти.

Medicinal plant products exhibiting antimicrobial activity continue to be the subject of extensive research aimed at the development of new therapeutic agents for treatment of various infectious diseases. Antimicrobial properties of plants have been investigated by a number of researchers worldwide. In particular, up to now medicinal values of many *Ficus* species as a source of natural antimicrobial agents have been well documented [1].

Ficus L. is one of the largest genera of angiosperms, with about 750 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers distributed in the tropics and subtropics of the world [2].

A number of *Ficus* species have been used as food source and due to their medicinal properties in both Ayurvedic and traditional Chinese medicine, especially among the inhabitants of the areas where these species are distributed. It is widely used for treatment of numerous diseases such as various disorders of the central nervous system, endocrine sys-

tem (diabetes, etc.), gastrointestinal tract, reproductive and respiratory systems and infectious diseases [3].

However, although many species within the *Ficus* genus 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.

Ficus lyrata Warb., the fiddle-leaf or banjo fig ([syn. *Ficus sycomorus*] family *Moraceae*) is a small or average size (up to 12 m), slow growing deciduous tree with broad ovate or nearly orbicular leaves, more or less deeply 3–5 lobed, rough above and pubescent below; fruits axillary, usually pear shaped, variable in size and color [4]. Its fruit, root and leaves are used in the native system of medicine in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders. Findings of Bidarigh and co-workers [4] justified the use of *F. lyrata* latex in traditional medicine for the treatment of various disease conditions whose symptoms might involve fungal infections and pointed to the importance of ethnobotanical approach as useful measure for the discovery of new bioactive compounds. The ethnomedicinal and traditional uses of the various part of *F. lyrata* plants in the treatment of aforementioned unhealthy symptoms suggest that these plants should possess antibacterial efficacy.

It has been shown that *in vitro* screening methods could provide the needed preliminary data necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. Therefore, the main aim of this research was to conduct preliminary antimicrobial screening of ethanolic extract obtained from *Ficus lyrata* leaves against standard and locally isolated strains of Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

The leaves of *F. lyrata* were collected in M.M. Gryshko National Botanical Garden

(Kyiv, Ukraine) during March, 2015. The whole collection of tropical and subtropical plants at M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) (including *Ficus* spp. plants) has the status of the National Heritage Collection of Ukraine. Collected leaves 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 disk diffusion method [5]. Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922), as well as Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* (ATCC 49619) were used as test organisms.

Antimicrobial activity of crude extract of the plant sample was evaluated by the paper disc diffusion method. Cultures of Gram-positive and Gram-negative bacteria were suspended in sterile solution of 0.9% normal saline and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. All the cultures were inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter paper discs (diameter 8 mm) impregnated with 50 μ L of extract dilutions were applied over each of the culture plates. Isolates of bacteria were then incubated at 37°C for 24 hrs. A negative control disc was impregnated with 50 μ L of sterile ethanol used in each experiment. The antimicrobial activities of tested extracts were evaluated at the end of the inoculated period by measuring the inhibition zone diameter around each paper disc in millimeters. The presence of inhibition zones around each of the well after the period of incubation was regarded as the presence of antimicrobial action, whereas the absence of any measurable zone of inhibition was interpreted as absence of antimicrobial effect. For each extract six replicate trials were conducted against each organism. Zone diameters were determined and averaged. Results for the antimicrobial activities are presented as mean \pm standard error of the mean ($M \pm S.E.M.$).

All statistical calculation was performed on separate data from each bacterial strain.

RESULTS AND DISCUSSION

The results of antimicrobial activity of ethanolic extract obtained from the leaves of *F. lyrata* are presented in Figs 1 and 2.

Our results showed that the ethanolic extract of *F. lyrata* leaves exhibited moderate activity against the Gram-positive bacteria (11.3 mm of inhibition zone diameter for *Staphylococcus aureus*) (Fig. 2), and the Gram-

negative bacteria (10.3 mm for *Escherichia coli*). *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* appeared to be less sensitive to the extracts, the inhibition zone were 8.9 mm, 8.5 mm, 8.9 mm, and 8.4 mm, respectively (Fig. 1).

Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy. *F. lyrata* has been reported to have numerous bioactive compounds such

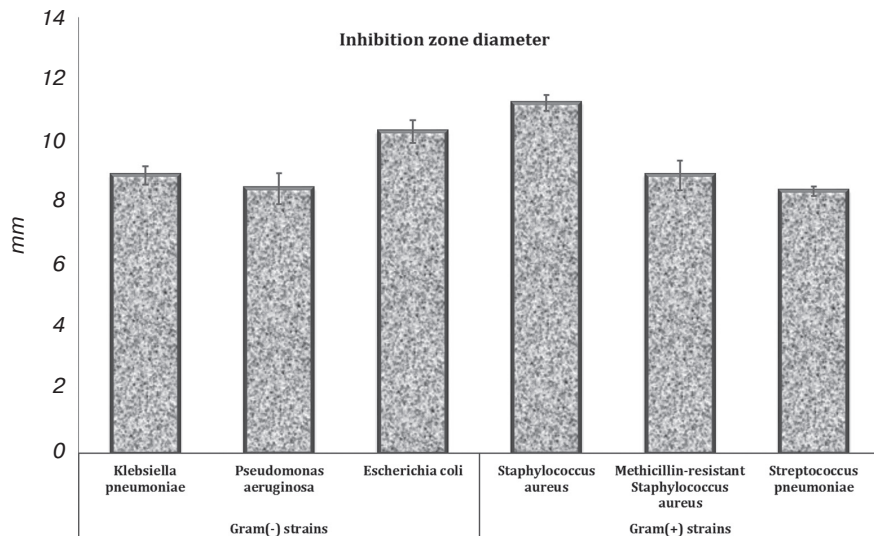


Fig. 1. Antimicrobial activity of ethanolic extract obtained from the leaves of *Ficus lyrata* against bacterial strains measured as inhibition zone diameter ($M \pm m$, $n = 6$). Error bars represent standard errors of the mean (S.E.M.)



Fig. 2. Antimicrobial activity of ethanolic extract obtained from the leaves of *Ficus lyrata* against *Staphylococcus aureus* measured as inhibition zone diameter

as arabinose, β -amyrins, β -carotenes, glycosides, β -setosterols and xanthotoxol [6]. Farag and co-workers [7] have elucidated the secondary metabolite profiles of *F. lyrata* leaves and fruits. A total of 72 metabolites were evaluated. Seventeen flavonoids have been characterized and identified with the main constituents being catechins/procyanidins, O- and C-linked flavonoid glycosides. The major procyanidins were dimers and trimers comprising (epi)catechin and (epi)afzelechin units, whereas the predominant flavones were C-glycosides of luteolin and apigenin. Aside from these major flavonoid classes, a group of benzoic acids, caffeoylquinic acids, fatty acid and sphingolipids have been also found [7].

The broad antibacterial activities of this extract, apparently, could be explained as a result of the plant secondary metabolites. Previously it has been reported [1], that the therapeutic properties of *F. species* may be attributed to the presence of a wide range of phytochemical compounds.

To identify the active principles responsible for the antibacterial activity, Rizvi and co-workers [8] screened the isolated pure compounds (FL-1 – FL8) from *F. lyrata* for their inhibitory effect against the growth of various bacterial strains. They observed that only two compounds, i.e. ursolic acid (FL-1) and acacetin-7-O-neohesperidoside (FL-2) showed antibacterial activity. The Minimal Inhibitory Concentrations (MICs) were significantly lower not only of those bacterial strains sensitive to the crude extract but also to *Salmonella typhi*, *S. paratyphi* A, *S. typhimurium*, *Vibrio cholerae*, *E. coli*, *K. pneumoniae* and extended-spectrum beta-lactamases (ESBL) producing *E. coli* and *K. pneumoniae*. Thus the range of activity against Gram-negative bacteria was greatly enhanced on testing with pure compounds [8]. Ursolic acid was found to be more potent than Acacetin-7-O-neohesperidoside. Ursolic acid is a triterpenoid sapogenin from the ursan group, whereas Acacetin-7-O-neohesperidoside is a flavonoid glycoside [8]. Findings of Rizvi and co-workers [8] suggest that ursolic acid has excellent antibacterial activity against several problematic bacteria like MRSA and ESBL producing

bacteria, *Pseudomonas*, *Salmonella*, *Shigella* and *Vibrio cholerae* and other known pathogens with drug resistance. Ursolic acid and acacetin-7-O-neohesperidoside contributes significantly to the antimicrobial activity of the crude extract of *F. lyrata* [8]. Ahmad and co-workers [9] revealed that glycosides and saponins extracted from leaves using alcohol displayed biological effects but they had no effects on *C. albicans*, *S. aureus* and *E. coli*.

Our recent studies have also revealed that other *Ficus* species possess antibacterial activity. The ethanolic extract from leaves of *F. carica* showed potent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [10]. *S. aureus* demonstrated susceptible test for five of the ethanolic leaf extracts: *F. erecta* var. *sieboldii*, *F. rubiginosa*, *F. benjamina*, *F. septica*, and *F. erecta* [10]. *E. coli* ATCC 25922 demonstrated susceptible test for nine of the ethanolic leaf extracts: *F. villosa*, *F. benjamina*, *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 was demonstrated for 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 the most effective as an antibacterial agent against Gram-negative *E. coli* [11].

Moreover, latex extract of *F. lyrata* also possessed antifungal activity against 65 clinical isolates of *Candida albicans* from *Vulvovaginal candidiasis* and standard strain of *C. albicans* (ATCC 5027). As shown by Bidarigh and co-workers [4], *F. lyrata* extract has inhibitory effect on clinical isolates and type strain of *C. albicans* in lower concentrations than Nystatin drug with the diameters of inhibition zones ranging from 22 to 26 mm and 30 to 32 for clinical isolates and standard strains of *C. albicans*, respectively.

The diameter of inhibition zones for Nystatin was between 16 to 20 mm and 21 to 24 mm for standard strain and clinical isolates of *C. albicans*, respectively. Based on the data analysis (Macrobroth dilution method), the best MIC of *F. lyrata* ethyl acetate latex

extract on clinical isolates and type strain of *C. albicans* were 25 mg/ml and 2.5 mg/ml, respectively. The chemical analysis of latex showed that extract contains alkaloids, flavonoids, phenols, tannins, terpenoid [4]. Ethyl acetate extract of *F. lyrata* latex possesses compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases [4]. The methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*Enterococcus faecalis*, *Citobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*) [4].

CONCLUSION

Thus, the ethanolic extract of *F. lyrata* has moderate antimicrobial activities, apparently,

attributed to its higher triterpenoids, flavonoids, and glycosides content, that confirm the traditional use of this plant for the treatment of diseases caused by pathogens. Yet, this research illustrates that a Gram-positive bacterium was more susceptible to the ethanolic leaf extracts of *F. lyrata* as compared to Gram-negative bacteria species.

Further studies will be focused on the separating and purifying active compounds and detecting mechanisms underlying the pharmacological effects of *F. lyrata*. Screening of medicinal plants for antimicrobial activities is important for finding potential new compounds for therapeutic use. The results of present investigation allow us to suggest that the extracts of *F. lyrata* can be used to discover antibacterial substance for developing new pharmaceuticals to control clinically important pathogens responsible for severe disorders.

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