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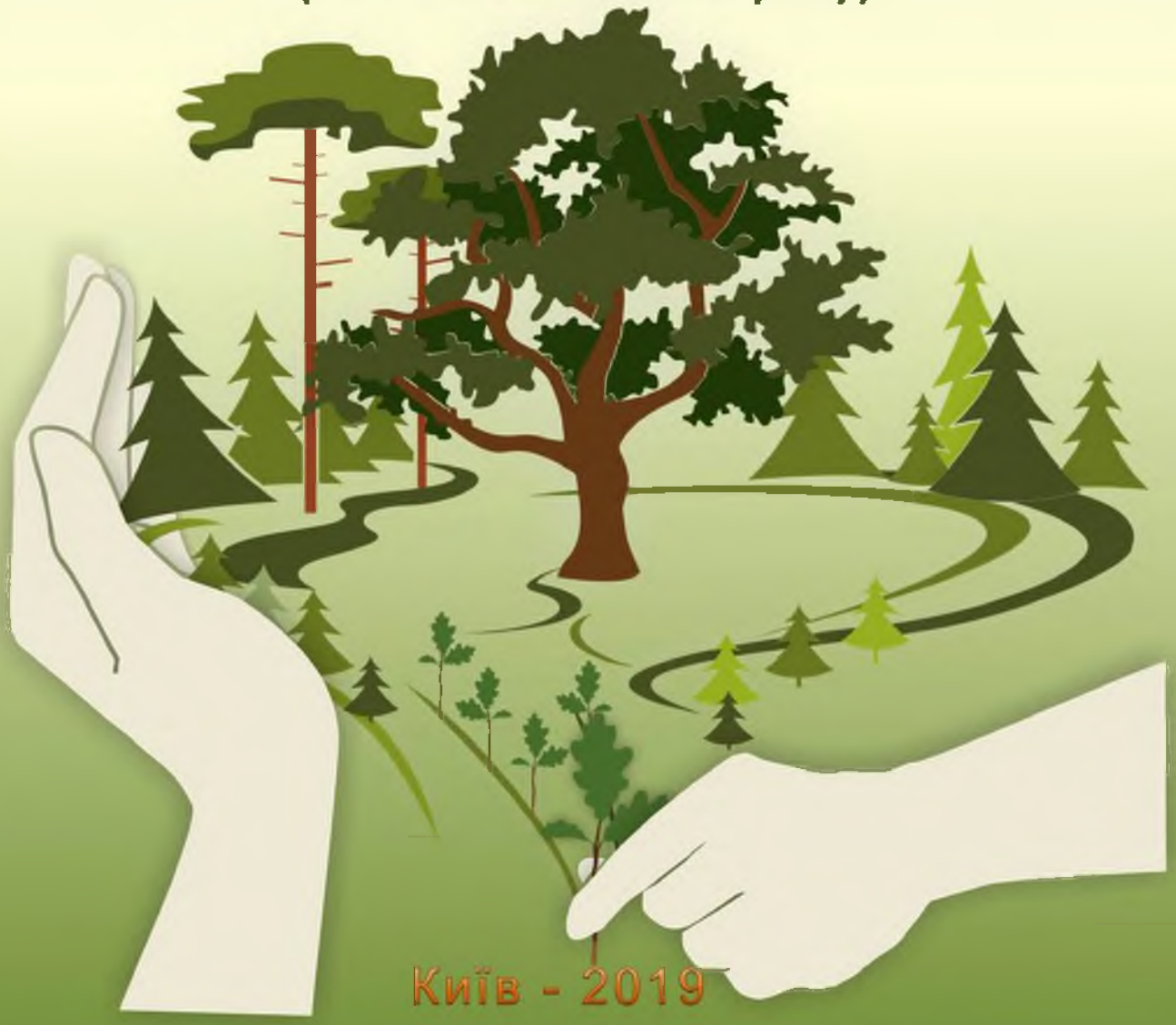

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PRELIMINARY *IN VITRO* STUDY OF THE ANTIMICROBIAL POTENTIAL OF *FICUS MACROPHYLLA* DESF. EX PERS. LEAVES (*MORACEAE*)

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Ficus macrophylla Desf. ex Pers. (Moreton Bay fig) is a monoecious evergreen tree reaching up to 30 m in height, hemi-epiphytic or terrestrial, with glabrous or puberulous leafy twigs, native to eastern Australia. This species normally starts life in the forest as an epiphyte growing on the branch of another tree. As it grows larger, it sends down aerial roots which root into the ground below, providing the plant with extra nutriment and allowing it to out-compete the host tree, eventually smothering it. The trees gradually reach large proportions, with immense buttresses, trunks up to 8 meters or more in circumference, and branches both high and spreading. Aerial roots (if produced) grow mainly from large, framework branches near the ground, and these may produce a few extra trunks or props. Its leaves are 7-30 cm long and 4-13 cm wide, alternate, ovate to elliptic, with acute to the obtuse apex and rounded to obtuse base. The upper leaf surface is glabrous, glossy green, sometimes puberulous on the midvein, while the lower surface is mostly silvery to rusty or brownish, tomentose with weak ferruginous hairs, rarely glabrous. The syconia are pedunculate, spheroid to cubical or oblong, 18-25 mm long and 15-24 mm in diameter, puberulous to pilose, glabrescent, red to red-brown with pale spots when mature (Dixon, 2001).

Moreover, Australian Aborigines found a myriad of uses for the *F. macrophylla* long before European settlement. The most obvious use is the year-round fruit, the figs. Next, the inner bark or roots were used to make a sturdy cloth and cord for bags as well as woven fishing nets. Also, the

branches, as well as the bark, were used to make waterproof dug-out canoes. Lastly, the milky sap, which exudes when the tree is cut was prepared as a medicine to treat infections and to dress small wounds (http://hpathy.com/).

The literature survey indicates that genus of *Ficus* has multiple pharmacological actions that include antidiabetic, antioxidant, antidiarrhoeal, anti-inflammatory, antipyretic, antifungal, antibacterial, hypolipidemic, anti-filarial, and hepatoprotection (Yadav et al., 2015). Moreover, their leaves are used for alleviation of infectious and inflammatory conditions in many countries (Yadav et al., 2015). Widespread medicinal use and significant biological activities of the extracts from the plants of genus *Ficus* justified a continued investigation of *F. macrophylla*. Based on the above considerations, the aim of this study was to test the efficacy of ethanolic extract prepared from *F. macrophylla* leaves against Gram-positive and Gram-negative bacteria to evaluate the possible use of this plant in the prevention of bacterial infections.

Material and methods. Plant materials and Preparing Plant Extracts. The leaves of *F. macrophylla* were sampled in M.M. Gryshko National Botanic Garden (Kyiv, Ukraine) and Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine). The whole collections of tropical and subtropical plants both at M.M. Gryshko National Botanic Garden (Kyiv, Ukraine) and Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine) (including *Ficus* spp. plants) have the status of a National Heritage Collection of Ukraine and are supported through State funding (Buyun et al., 2018). At the NBG the plant of *F. macrophylla* has been in cultivation since 1950. The species author abbreviations were followed by Brummitt and Powell (1992). The sampled leaves of *F. macrophylla* were brought into the laboratory for antimicrobial studies. Freshly collected leaves were washed, weighed, and homogenized in 96% ethanol (in proportion 1:10) at room temperature. The extract was then filtered and investigated for their antimicrobial activity. The extract was stored at 4°C until use.

Bacterial strains. The testing of the antibacterial activity of the plant extract was carried out *in vitro* by Kirby-Bauer disc diffusion technique (Bauer et al., 1966). 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* (NEQAS 3679) and *Streptococcus pneumoniae* (ATCC 49619), as well as fungus *Candida albicans* locally isolated were used as test organisms. The clinical strain of *C. albicans* was also used in this study. *C. albicans* were differentiated from other *Candida* and *Cryptococcus* species by its ability to grow on the Levine formula of EMB agar and to produce germ tubes within 3 h, and pseudohyphae and budding cells at 18-24 h when incubated at 35 °C in 5%-10% CO_2. The addition of tetracycline to the Levine formulation aids in the selection of *C. albicans* from clinical sources that are contaminated with bacteria. Susceptibility testing of the isolate was performed by disk diffusion according to the Guidelines of Clinical and Laboratory Standard Institute (CLSI).

Evaluation of Antibacterial Activity of Plant Extracts by the Disk Diffusion Method. Strains tested were plated on TSA medium (Tryptone Soy Agar) and incubated for 24 hr at 37°C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was done on Muller-Hinton agar by disc diffusion method (Kirby-Bauer disk diffusion susceptibility test protocol). Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (10^8 CFU/mL) of the bacterium and spread with sterile swabs. Growth from freshly subcultured *C. albicans* isolates was suspended in 10 mL of sterile saline to obtain turbidity of 0.5 McFarland standard. Using a sterile swab, the Sabouraud dextrose agar plates were evenly inoculated with the *C. albicans* suspension. The plates were then incubated at 27°C for 48 h. The antifungal activity was evaluated by measuring the diameter of inhibition zones (mm). Each test was repeated eight times. Sterile filter paper discs impregnated by extract were applied over each of the culture plates, 15 min after bacteria suspension was placed. A negative control disc impregnated by sterile ethanol was used in each experiment. After culturing bacteria on Mueller-Hinton agar, the disks were placed on the same plates and incubated for 24 hr at 37°C. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the disks. The diameters of the inhibition zones were measured in millimeters and compared with those of the control and standard susceptibility disks. The activity was evidenced by the presence of a zone of inhibition surrounding the well.

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 extract tested. All statistical calculation was performed on separate data from each bacterial and fungal strains.

Results and conclusions. Our results revealed that the ethanolic extract of *F. macrophylla* leaves possessed mild activity against the Gram-positive bacteria (14.06±0.67 mm inhibition zone diameter for *S. aureus* and 12.5±1.61 mm methicillin-resistant *S. aureus*), the Gram-negative bacteria (10.88±0.54 mm for *K. pneumoniae*, 10.19±0.25 mm for *P. aeruginosa*) and fungus strain (10.13±0.74 mm for *C. albicans*) (Fig. 2). However, *S. pneumoniae* and *E. coli* appeared to be less sensitive to the *F. macrophylla* extract; the inhibition zones were 9.75±0.58 mm for *E. coli* 9.0 mm and 9.5±0.49 mm for *S. pneumoniae*, respectively.

In agreement with the results obtained from the present study, previous studies undertaken by numerous researchers have found that various *Ficus* species possess noticeable antibacterial activity against bacterial and fungus strains. In general, *Ficus* species are rich sources of polyphenolic compounds. In particular, flavonoids and isoflavonoids are responsible for the extract's strong antioxidant activity that may be useful in preventing diseases involving oxidative stress (Sirisha et al., 2010). All the detected phenolic acids are known to have antimicrobial and antioxidant properties (Jaafar et al., 2012). The antimicrobial property of *F. macrophylla* extract may be due to its constituents. As it was suggested by Kumar and Pandey (2013), antibacterial flavonoids might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form a complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. In addition, lipophilic flavonoids may also disrupt microbial membranes (Kumar and Pandey, 2013).

It was evidenced that phytochemicals are able to inhibit peptidoglycan synthesis, damage microbial membrane structures, modify bacterial membrane surface hydrophobicity and also modulate quorum-sensing (QS) (Rasooli et al., 2008).

To conclude, the ethanolic extract obtained from *F. macrophylla* leaves showed varying inhibitory activities against all the test organisms. *F. macrophylla* possesses the medicinal potential for the therapy of bacterial infections induced by *S. aureus* and may be used as a natural antiseptic and antimicrobial agent in medicine. Moreover, the antibacterial activity of this plant would help for the development of a new alternative medicine system which has no adverse effects. Further investigation is necessary to identify those bioactive compounds, which will be a platform for clinical applications.

Additionally, we have to keep in mind that, even though we have revealed potent *in vitro* antimicrobial activity of *F. macrophylla* extract for certain bacteria, it may not be expressed *in vivo*.

Finally, these findings are important in order to evaluate the significance of collections of tropical plants maintained under glasshouse conditions at Botanic Gardens worldwide and to plan the conservation strategy by the establishment of national collections of plants with valuable characteristics with the prospects of their use as sources of antimicrobial agents.

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