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EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT DERIVED FROM LEAVES OF *FICUS CYATHISTIPULA* WARB. (*MORACEAE*)

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ОЦІНКА АНТИМІКРОБНОЇ АКТИВНОСТІ ЕТАНОЛЬНОГО ЕКСТРАКТУ ЛИСТЯ *FICUS CYATHISTIPULA* WARB. (*MORACEAE*)

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ABSTRACT

Purpose: We continue our investigations regarding assessing the antibacterial and antioxidant properties of extracts derived from the leaves of various plants belonging to the *Ficus* genus. In the current study, we aimed to assess the antibacterial properties of ethanolic extract prepared from leaves of *Ficus cyathistipula* Warb. against some Gram-positive and Gram-negative bacteria to evaluate the possible use of this plant in the prevention and treatment of bacterial infections caused by these bacteria.

Methodology. The leaves of *F. cyathistipula* were sampled at M.M. Gryshko National Botanic Garden (NBG, Kyiv, Ukraine) and the Botanic Garden of Ivan Franko National University in Lviv (Lviv, Ukraine). Freshly collected leaves were washed, weighed, and homogenized in 96% ethanol (in the proportion of 1:9, w/w) at room temperature. The extract was then filtered and investigated for its antimicrobial activity. The testing of the antibacterial activity of the plant extract was carried out *in vitro* by the Kirby-Bauer disc diffusion technique. Gram-negative bacteria, *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC®27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®35218™), and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™), as well as Gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* strain (ATCC®25923™), *Staphylococcus aureus* subsp. *aureus* strain (ATCC®29213™) and methicillin-resistant *Staphylococcus aureus* (NEQAS 3679™), as well as the fungus *Candida albicans* locally isolated, were used as test organisms. 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 ≥ 15 mm, Intermediate = 10–15 mm, and Resistant ≤ 10 mm.

Scientific novelty. The ethanolic extract derived from the leaves of *F. cyathistipula* exhibited varying inhibitory activities against all the test strains. More sensitive for this extract was *C. albicans* strain. *S. aureus* subsp. *aureus* strain (ATCC® 25923™), *S. aureus* subsp. *aureus* strain (ATCC® 29213™), methicillin-resistant *S. aureus* (NEQAS 3679™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), and *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains were more resistant to *F. cyathistipula* extract. The results are encouraging enough to pursue bioactivity-guided fractionation of this extract and structure elucidation of the active phytoconstituents from the *F. cyathistipula* extract as a possible anti-bacterial agent.

Conclusions. *S. aureus* and *C. albicans* appeared to be more sensitive to the *F. cyathistipula* extract. The antibacterial activity may be associated with the presence of secondary metabolites. The results of this study provide baseline information on *F. cyathistipula* potential validity in the treatment of fungus-induced and bacterial infections, caused by *Candida albicans* and *Staphylococcus aureus*.

Key words: *Ficus cyathistipula* Warb, Gram-negative bacteria, Gram-positive bacteria, susceptibility or resistance of bacteria, Kirby-Bauer disc diffusion technique

АНОТАЦІЯ

Мета: Ми продовжуємо наші дослідження щодо оцінки антибактеріальних та антиоксидантних властивостей екстрактів, отриманих з листя різних рослин, що належать до роду *Ficus*. У цьому дослідженні ми мали на меті дослідити антибактеріальні властивості спиртового екстракту, отриманого з листя *Ficus cyathistipula* Warb. щодо деяких грампозитивних і грамнегативних бактерій, щоб оцінити можливе використання цієї рослини для профілактики та лікування бактеріальних інфекцій, викликаних цими бактеріями.

Методологія. Зразки листя *F. cyathistipula* відбирали для досліджень у Національному ботанічному саду імені М.М. Гришка (НБС, Київ, Україна) та Ботанічному саду Львівського національного університету імені Івана Франка (Львів, Україна). Свіжозібране листя промивали, зважували та гомогенізували в 96% етанолі (у пропорції 1:9, мас./мас.) при кімнатній температурі. Потім екстракт фільтрували та досліджували його антимікробну активність. Тестування антибактеріальної активності рослинного екстракту проводили in vitro методом дискової дифузії Кірбі-Бауера. Грамнегативні бактерії, *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC®27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®35218™) і *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™), а також грампозитивні бактерії *Staphylococcus aureus* subsp. *aureus* (ATCC®25923™), *Staphylococcus aureus* subsp. *aureus* (ATCC®29213™) і метицилін-резистентний *Staphylococcus aureus* (NEQAS 3679™), а також локально виділений гриб *Candida albicans* використовували як тест-організми. Визначали та усереднювали діаметри зон інгібування росту бактерій. Наступні критерії діаметра зон інгібування використовувалися для визначення чутливості або резистентності бактерій до досліджуваних фітохімічних речовин: чутливі ≥ 15 мм, проміжні = 10–15 мм і стійкі ≤ 10 мм.

Наукова новизна. Етанольний екстракт, отриманий з листя *F. cyathistipula*, виявляв різну інгібуючу дію щодо всіх досліджуваних штамів. Більш чутливим до цього екстракту виявився штам *C. albicans*. Штами *S. aureus* subsp. *aureus* (ATCC®25923™), *S. aureus* subsp. *aureus* (ATCC®29213™), метицилін-резистентний *S. aureus* (NEQAS 3679™), *P. aeruginosa* (Schroeter) Migula (ATCC®27853™), *E. coli* (Migula) Castellani and Chalmers (ATCC®25922™) і *E. coli* (Migula) Castellani і Chalmers (ATCC®35218™) були більш стійкими до екстракту *F. cyathistipula*. Результати є досить обнадійливими, щоб продовжити фракціонування цього екстракту, кероване біоактивністю, і з'ясувати структуру активних фітокомпонентів екстракту *F. cyathistipula* як можливого антибактеріального агента.

Висновки. Штами *S. aureus* і *C. albicans* виявилися більш чутливими до екстракту *F. cyathistipula*. Антибактеріальна активність цього екстракту може бути пов'язана з наявністю вторинних метаболітів *F. cyathistipula*. Результати цього дослідження надають базову інформацію про потенційну роль *F. cyathistipula* в лікуванні інфекцій індукованих грибками *Candida albicans* та бактеріальних інфекцій, спричинених *Staphylococcus aureus*.

Ключові слова: *Ficus cyathistipula* Warb, грамнегативні бактерії, грампозитивні бактерії, чутливість або резистентність бактерій, методика дискової дифузії Кірбі-Бауера.

Introduction

Among 37 genera of Moraceae comprising 1050-1100 species in total, Ficus L. is the largest one with ca 750 species of tropical and subtropical distribution worldwide. Its characteristic features include the presence of waxy glands on vegetative plant parts, heterostyly, and prolonged protogyny, i.e., the anthesis of staminate flowers in already mature fruits. These features are functionally linked to the unique pollination mode in Ficus, which involves mutualistic relationships with agaonid wasps (order *Hymenoptera*) and has attracted much research interest over the last two centuries [9, 5]. In the review by Y. Shi and coworkers [37], it was noted that Ficus is a large genus of flowering plants with wide distribution in tropical and semi-tropical temperate zones. Plants in this genus have occupied many ecological niches and can be deciduous or evergreen trees, shrubs, herbs, climbers or creepers, and life forms can be free-standing trees, epiphytes or semi-epiphytes in crevices, rheophytes or lithophytes [37].

Ficus trees have widely been used by humans over their history in a variety of industries and fields of activity. Virtually all parts of their body are utilized by local people in various medicinal practices to cure wounds, sores, stomach and eye problems, headaches and toothaches, and even tumours and cancer, etc. A number of species are known helpful in healing disorders of digestive and respiratory systems, parasitic infections, and also as painkillers, tonics, and ecbolics [24].

Ficus species have been used in Indian ayurvedic and African traditional medicine [1, 20, 11]. Over the past decades, the medicinal properties of the genus Ficus have been extensively investigated through both ethnobotanical field surveys and pharmacological studies [37]. Many Ficus species have been used in traditional medicine in the treatment of a variety of ailments and diseases such as convulsive disorder [7], wound healing [30], gonorrhea [11], tuberculosis [3], diabetes [12], diarrheal infections [18], dysentery [36], malaria [8] and HIV [17]. These plants possess antioxidant [29], anti-diabetes and anti-hyper-

glycemic [27], antibacterial [28], antifungal [43], antiviral [13], anti-protozoal [8], anticancer [25], cytotoxic [32], anti-ulcer [16], anti-inflammatory [26], anti-diarrhea [18], hepato-protective [34], muco-protective and gastro-protective activities [35]. These plants to be rich sources of flavonoids, lignans, terpenoids, alkaloids, coumarins steroids, and ceramides [3, 10].

Antimicrobial resistance is the phenomenon causing a challenge in new drug development through conventional methods. Therefore, the requirement of alternative medicine, such as phytotherapy, is in high demand [15]. Several developing countries use medicinal plants as a first medicinal response to certain diseases including bacterial infection diseases [19]. There were reports showing that medicinal plants have antibacterial activity against some pathogenic and opportunistic bacteria [38]. The knowledge of traditional medicines hidden and lost should be researched and the loss of natural resources used as traditional medicines should be prevented [19].

We continue our investigations regarding assessing the antibacterial and antioxidant properties of extracts derived from the leaves of various plants belonging to the *Ficus* genus. In the current study, we aimed to assess the antibacterial properties of ethanolic extract prepared from leaves of Ficus cyathistipula Warb. against some Gram-positive and Gram-negative bacteria to evaluate the possible use of this plant in the prevention and treatment of bacterial infections caused by these bacteria. A study of Ficus species focusing on antibacterial aspects may provide an understanding of a wider range of health benefits of the genus *Ficus*.

Ficus cyathistipula Warb. is a monoecious evergreen tree reaching up to 8 m in height, hemi-epiphytic or terrestrial, native to Africa. Its leaves are 6-20 cm long and 3-7 cm across, oblanceolate to obovate, with acuminate apex and acute to attenuate base, coriaceous and glabrous. The syconia are born solitary (or up to 3 together) in the leaf axils; they are globose to obovoid or pyriform, sessile or pedunculate, 3-5 cm in diameter, often somewhat scabrous, at maturity pale green to pale yellow [6].

Material and methods

Plant materials and Preparing Plant Extracts. The leaves of *F. cyathistipula* were sampled at M.M. Gryshko National Botanic Garden (NBG, Kyiv, Ukraine) and the 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.

The sampled leaves of *F. cyathistipula* were brought into the laboratory at the Department of Biology, Institute of Biology and Earth Sciences, Pomeraniam University in Słupsk (Poland) for antimicrobial studies. Freshly collected leaves were washed, weighed, and homogenized in 96 % ethanol (in the proportion of 1:9, w/w) at room temperature. The extract was then filtered and investigated for its antimicrobial activity. The extract was stored in the glass bottles with dark walls at 4 °C until use.

Bacterial strains. Gram-negative bacteria, *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC®27853™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) and *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™), as well as Gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* strain (ATCC®25923™), *Staphylococcus aureus* subsp. *aureus* strain (ATCC®29213™) and methicillin-resistant *Staphylococcus aureus* (NEQAS 3679™), as well as the fungus *Candida albicans* locally isolated, were used as test organisms. *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% CO2. 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 Technique. Strains tested were plated on TSA medium (Tryptone Soy Agar) and incubated for 24 h at 37 °C. Then the suspension of microorganisms was suspended in sterile PBS and the turbidity was adjusted to 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) [4]. Muller-Hinton agar plates were inoculated with 200 µl of standardized inoculum (108 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. 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 control disc impregnated with sterile 96 % ethanol was used in each experiment. The disks were incubated for 24 h at 37 °C. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the disks (mm). The diameters of the inhibition zones were measured in millimeters and compared with those of the control disks. 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 [33].

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).

Results and discussion

The antibacterial activity induced by the ethanolic extract derived from the leaves of *F. cyathistipula* estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains was presented in Figures 1 and 2.

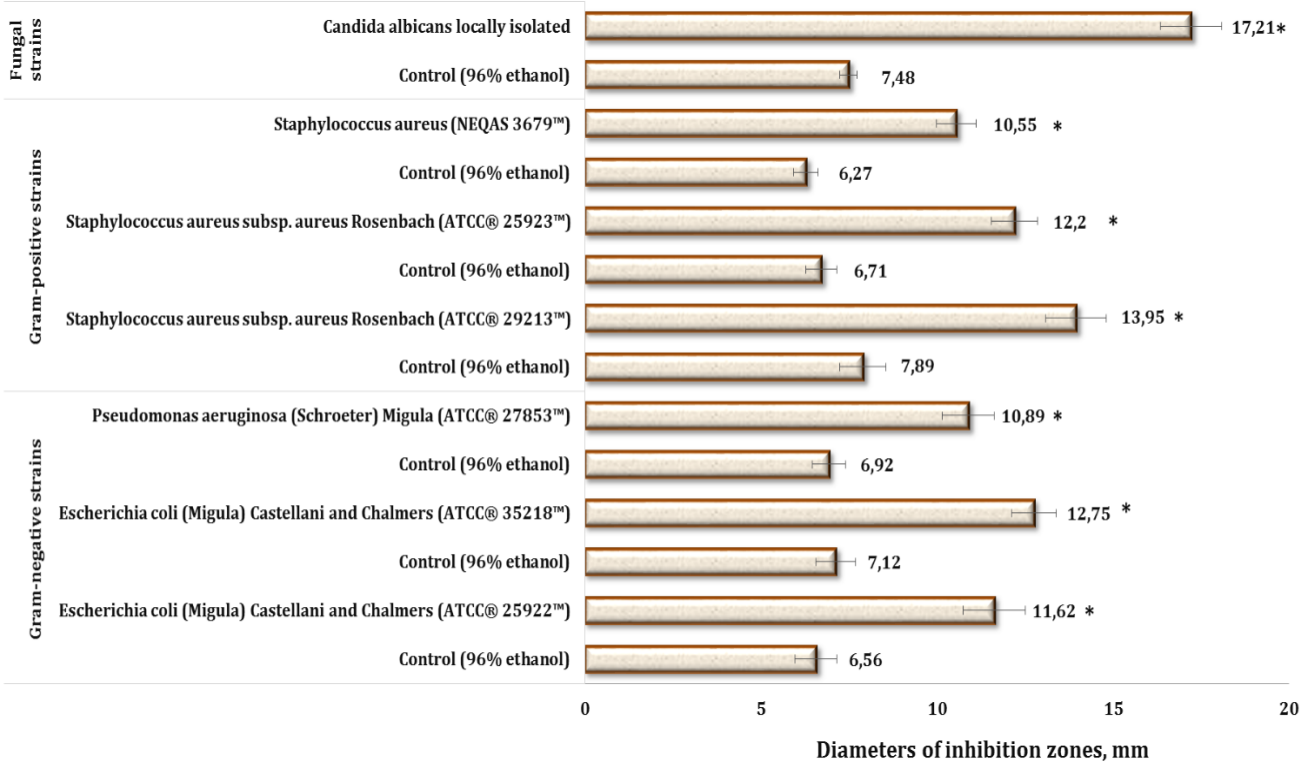
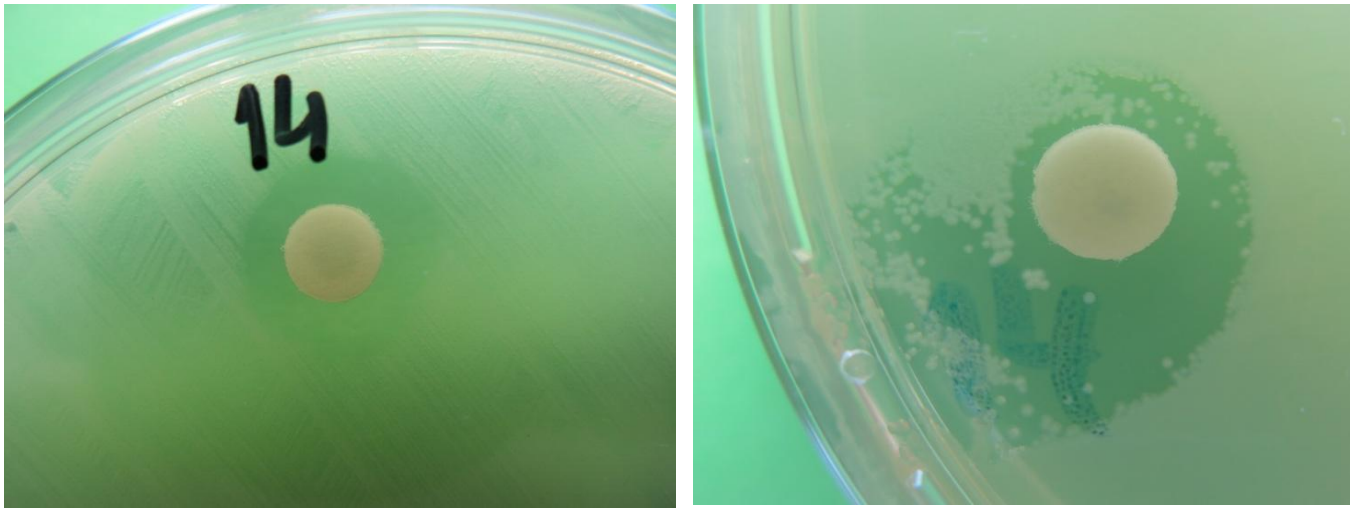


Fig. 1. The antibacterial activity of the ethanolic extract derived from the leaves of *Ficus cyathistipula* estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains. The data were presented as the mean ± the standard error of the mean (S.E.M.). Note: * – significant differences between the control (96 % ethanol) and *Ficus cyathistipula* extract ($p < 0.05$)

Our results revealed that the ethanolic extract derived from leaves of *F. cyathistipula* possessed intermediate activity against the Gram-positive bacteria, i.e. (12.2 ± 0.65) mm inhibition zone diameter for *S. aureus* subsp. *aureus* strain (ATCC®25923™), (13.95 ± 0.86) mm inhibition zone diameter for *S. aureus* subsp. *aureus* strain (ATCC®25923™), and (10.55 ± 0.56) mm for methicillin-resistant *Staphylococcus aureus* (NEQAS 3679™) compared to the 96 % ethanol – (6.71 ± 0.44) mm, (7.89 ± 0.65) mm, and (6.27 ± 0.35) mm, respectively. Also, the ethanolic extract derived from leaves of *F. cyathistipula* possessed mild activity against the Gram-negative bacteria, i.e. (11.62 ± 0.88) mm for *E. coli* (Migula) Castellani and Chalmers (ATCC®25922™), (12.75 ± 0.64) mm for *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), and (10.89 ± 0.74) mm for *P. aeruginosa* (Schroeter) Migula (ATCC®27853™) compared to the ethanolic controls 6.56 ± 0.59 mm and 6.92 ± 0.47 mm, respectively). The ethanolic extract derived from leaves of *F. cyathistipula* possessed significant antifungal activity against *C. albicans* strain (17.21 ± 0.87 mm for *C. albicans* compared to the controls 7.48 ± 0.24 mm). Thus, *S. aureus* and *C. albicans* appeared to be more sensitive to the *F. cyathistipula* extract.

Detailed photos regarding the zones of inhibition induced by the *F. cyathistipula* extract against Gram-positive and Gram-negative bacterial strains were recorded and presented in Figure 2.



A **B**

Fig. 2. Inhibition growth zones induced by the *F. cyathistipula* extract against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC®25922™) (A) and *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) (B)

This study is part of a continued investigation of the antibacterial properties of some species belonging to the *Ficus* genus. In our previous study [41], *in vitro* antimicrobial activity of ethanolic extract prepared from *F. benghalensis L.* leaves against both Gram-positive (*Staphylococcus aureus*, methicillin-resistant *S. aureus* locally isolated, and *Streptococcus pneumoniae*) and Gram-negative bacterial strains (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*), as well as fungus *Candida albicans*, were demonstrated to evaluate the possible use of this plant in preventing infections. The ethanolic extract derived from leaves of *F. benghalensis* showed moderate antibacterial activity against *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*, while no significant antibacterial activity against *Klebsiella pneumoniae* and *Streptococcus pneumoniae*, methicillin-resistant *S. aureus*, and *Candida albicans* were demonstrated. Among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi. This trend is not unusual because the plant extracts often displayed better bactericide than fungicide activities. For

example, this tendency was revealed for crude plant extracts, derived from the aerial parts of 25 plants belonging to four plant families (*Asteraceae, Euphorbiaceae, Rubiaceae*, and *Solanaceae*) [31]. Moreover, the antibacterial activity was more pronounced in the Gram-positive bacteria (*S. aureus*) than the Gram-negative bacteria (*E. coli* and *P. aeruginosa*). The pronounced antibacterial activities of this extract could be a result of the plant's secondary metabolites (carbohydrates, reducing sugars, sterols, glycosides, phenolic compounds, tannins, saponins, flavonoids, etc.). Therefore, *F. benghalensis* has great medicinal potential for the therapy of infections induced by Gram-positive and Gram-negative bacteria and may be used as a natural antiseptic and antimicrobial agent in medicine [31]. Considering the increasing rate of antibiotic resistance throughout the world, this species of plant can be considered a potential source of antibacterial agents.

Also, we have screened the antimicrobial activity of ethanolic extract obtained from *Ficus lyrata* Warb. leaves against the standard and locally isolated strains of Gram-negative bacteria *Klebsiella pneumonia* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922), as well as Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *S. aureus*, and *Streptococcus pneumoniae* (ATCC 49619) [39]. Our results showed that the ethanolic extract derived from leaves of *F. lyrata* exhibited moderate activity against the Gram-positive bacteria (11.3 mm of inhibition zone diameter for *S. aureus*), and the Gram-negative bacteria (10.3 mm for *E. coli*). *K. pneumonia*, *P. aeruginosa*, methicillin-resistant *S. aureus*, and *S. pneumoniae* appeared to be less sensitive to the extracts, the inhibition zone was 8.9 mm, 8.5 mm, 8.9 mm, and 8.4 mm, respectively. The ethanolic extract of *F. lyrata* has moderate antimicrobial activities attributed to its higher triterpenoids, flavonoids, and glycosides content, which confirms 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 [39].

In our previous study [40], we also assessed the in vitro antibacterial activity of ethanolic extract prepared from *Ficus sur* Forssk. leaves against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains, clinically important bacteria, which are indicator organisms commonly used in programs to monitor antibiotic resistance. For this study, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* NCTC 12493, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27583 were used. The results of antibacterial activity clearly showed that the extract has shown antibacterial activity against the entire tested organisms. The extract has shown better activity against *S. aureus* strains compared to the *E. coli* and *P.* aeruginosa strains. The ethanolic extract exhibited mild antibacterial activity against *E. coli*. The results of this investigation suggest that the leaf extracts of *F. sur* can be used to isolate antibacterial agents for developing new pharmaceuticals to control pathogenic bacteria responsible for infective diseases in humans and animals [40].

Also, other researchers revealed the antibacterial activity of many Ficus species. For example, Kuete and co-workers (2008) have evaluated the antimicrobial activity of the methanol extracts from *Ficus chlamydocarpa* Mildbr. & Burret (FCR), *Ficus cordata* Thunb. (FCB), mixture of the two plants (FCM), as well as that of the isolated flavonoids Alpinumisoflavone (2), Genistein (3), Laburnetin (4), Luteolin (5) (isolated from FCR), Catechin (7) and Epiafzelechin (8) (isolated from FCB). Mycobacteria, fungi, Gram-positive and Gram-negative bacterial species were tested for their susceptibility to the above samples. The microplate dilution and radiometric respiratory methods were used to determine the susceptibility testing of the samples against *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*, respectively. The disc diffusion assay was used to determine the sensitivity of the samples, whilst the microdilution method was used for the determination of the minimal inhibition concentration (MIC) and the minimal microbicidal concentration (MMC) against fungi, Gram-positive and Gram-negative bacterial species. All the samples except compound 7 were found to be active to *Mycobacterium smegmatis* and the MIC ranged from 0.61 to 312.50 microg/ml. Compound 4 showed the best activity against *Mycobacterium tuberculosis* exhibiting a MIC of 4.88 microg/ml. The results of the diffusion test indicated that the crude extract from FCB, FCM as well as compounds 5 and 8 were able to prevent the growth of all tested (fungi, Gram-positive and Gram-negative

bacteria) organisms. The inhibition effect of the crude extract from *Ficus chlamydocarpa* was observed on 10 (62.5 %) of the 16 tested microorganisms (excluding mycobacteria) whereas that of compounds 4, 2, and 3 was respectively noted on 14 (87.5 %), 8 (50.0 %) and 7 (39.9 %) of the tested microbial species. FCB was found to be more active than FCR in most of the tested organisms. The results of Kuete V. and co-workers [22] provided evidence that the studied plants extract, as well as some of the isolated compounds might be potential sources of the new antimicrobial drugs.

The effect of ethanolic extract of *F. religiosa* fruits extract was studied by Kumar Goyal A. and co-workers [23] against two Gram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Pseudomonas vulgaris* and *Klebsiella pneumonia*). The minimum inhibitory concentration of extract effective against *S. epidermidis* and *K. pneumonia* was 15 mg/ml while the minimum inhibitory concentration for *S. aureus* and *P. vulgaris* was 30 mg/ml. At 15 mg/ml concentration of extract, *K. pneumonia* showed more sensitivity (inhibition zone 21 mm) than *S. epidermidis* (inhibition zone 19 mm). At 30 mg/ml concentration, *P. vulgaris* showed more sensitivity (inhibition zone 12 mm) than *S. aureus* (inhibition zone 9 mm). Present observations indicate that the extracts possess antibiotic activity. Further scientific evaluation is needed to screen active phytochemicals from this important plant to use it for the production of new antibiotics [23].

H. Usman and co-workers [42] tested stem bark crude methanolic extract and its n-butanol and residual aqueous portions from Ficus thonningii Blume against clinical isolates of Gram-negative (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Salmonella typhi*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus* spp.) bacteria and carried out a qualitative phytochemical analysis of the extracts. All tested organisms were susceptible and the inhibition efficacy depended on the bacterial species, extract and portion type, and concentration, with no significant difference between the effects on Gram-positive and Gram-negative bacteria in general. Overall inhibition increased in the sequence crude extract – residual aqueous portion – n-butanol portion. Based on the disc diffusion assay, *S. aureus* was most strongly inhibited by the extract's n-butanol portion (inhibition zone diameter ranged from 20.0 to 26.33 mm), while the residual aqueous portion appeared the weakest (13 to 17 mm). Nutrient broth dilution essay showed MIC 2.5 mg/ml for the crude extract and n-butanol portion and MIC 1.25 mg/ml for the residual aqueous portion against *S. aureus*. Although phytochemical analysis showed quite rich chemical content of crude extract and both its portions (with alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, and tannins present), no difference between these three reagents tested were found that would potentially account for their variation in bacterial inhibition efficacy.

The phytochemical test of the crude extracts of *Ficus* species revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, and tannins [21, 2, 44]. These classes of secondary metabolites, such as alkaloids, saponins, tannins, anthraquinones, and flavonoids are known to have curative activity against several pathogens and therefore could suggest the use traditionally for the treatment of various illnesses [42]. The antimicrobial activity of secondary metabolites present in *F. cyathistipula* extract can be responsible for the antibacterial properties of this extract.

Phytochemical investigation of the bioactive extracts of the leaves of *F. cyathistipula* was assessed by F. El-Sakhawy and co-workers [14]. Ethanolic and aqueous leaf extracts of *F. cyathistipula* significantly reduced blood glucose levels, and improved triglycerides and cholesterol levels of dyslipidemia in diabetic rats. They similarly reduced the inflammation of paw edema and stomach ulcers in rats. Fractions obtained by the successive partition of ethanolic extract were assessed by F. El-Sakhawy and co-workers [14] for their cytotoxicity, antioxidant and antimicrobial activities. Petroleum ether fraction was the most cytotoxic (IC50 = 4.43 ± 0.2, 17.3 ± 2.22, and 15.5 ± 3.67 μg/ml on MCF7, HepG2 and HeLa cell lines, respectively). Ethyl acetate fraction was the strongest antioxidant in the DPPH assay (IC50 = 100 μg/ml). All samples exhibited low to strong antimicrobial activity. Chemical investigation of leaf extracts led to the isolation of α-amyrin palmitate (1), lupeol acetate (2), taraxerol (3), β-sitosterol (4), protocatechuic acid (5) and 3-O-caffeoyl quinic acid (6) that were identified via spectral and chromatographic analyses. Metabolite profiling was performed via UPLC-PDA-MS and revealed the

presence of flavonoid glycosides, phenolic acids, isoflavones, coumarins, and fatty acids. Quantitative determination revealed 593 ± 0.5 mg BSE, 348.1 ± 0.09 mg GAE, 238.7 ± 0.5 mg rutin, and 9 ± 0.5 g tannins per 100 g d.wt. of leaves. GLC analysis of lipid fraction revealed the identification of phytosterols (15.6 %), saturated (51.71 %), and unsaturated (41.9 %) fatty acids. Galactose, glucose, arabinose, and glucuronic acid (36.98 %, 28.86 %, 22.56 %, and 1.06 %, respectively) were identified by HPLC analysis of mucilage-hydrolysate [14].

Conclusions

The ethanolic extract derived from the leaves of *F. cyathistipula* exhibited varying inhibitory activities against all the test strains. More sensitive for this extract was *C. albicans* strain. *S. aureus* subsp. *aureus* strain (ATCC® 25923™), *S. aureus* subsp. *aureus* strain (ATCC® 29213™), methicillin-resistant *S. aureus* (NEQAS 3679™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), and *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strains were more resistant to *F. cyathistipula* extract. The results are encouraging enough to pursue bioactivity guided fractionation of this extract and structure elucidation of the active phytoconstituents from the *F. cyathistipula* extract as a possible antibacterial agent. The results of this study provide baseline information on *F. cyathistipula* potential validity in the treatment of fungus-induced infections, caused by *Candida albicans*.

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