

EXPERIMENTAL PAPER

Antimicrobial activity of *Ficus sycomorus* L. (*Moraceae*) leaf and stem-bark extracts against multidrug resistant human pathogens

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The present work was conducted to investigate antibacterial activity of methanol and acetone in leaf (LE) and stem-bark (SBE) of *Ficus sycomorus* L. crude extracts against sensitive and resistant species of *Staphylococcus aureus* and *Acinetobacter baumannii* pathogens. Antimicrobial activity expressed by disc-diffusion method (zone of inhibitions – ZIs), minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBC) were measured as reported for many investigations. Similar study with 6 commercial antibiotics as a reference drug was undertaken. Based upon the estimated ZIs, MIC and MBC values, acetone LE exhibited higher antimicrobial activity than that of methanol one. Otherwise, standard antibiotics have lower effectiveness (ZIs, MICs and MBC) on all tested bacteria as compared to the SBE and LE. The highest antibacterial activity was recorded in sensitive *A. baumannii* isolate with MICs 2.5, 4.9 mg/ml and MBC 3.8, 9.7 mg/ml for acetone LE and SBE, respectively. Our data indicated that the lowest antibiotics antibacterial activity was recorded for resistant *A. baumannii* pathogen. It was lower than those of the both plant fractions extracts.

Key words: antibacterial activity, *Ficus sycomorus* L., minimum inhibitory concentrations (MICs), minimum bactericidal concentration (MBC)

INTRODUCTION

The use of plant as an antimicrobial may inhibit bacteria by a mechanism different from that of antibiotic. Thus, may contribute to the treatment of resistant

microbial pathogens. As a result of this, a number of investigations have been reported by authors on the screening of plants as natural antimicrobials [1-5]. Medicinal plants have a great positive impact on the treatment of gastroenteritis and other infectious diseases caused by bacteria. Exploration of newer antimicrobials in plants brings about a different approach in minimizing antibiotic resistance, and thus offers potential benefits.

The potential antibacterial activity of *Ficus* species has been extensively reported in many investigations [2, 6-11]. *Ficus* is a genus of about 800 species and 2000 varieties of *Ficus* of woody trees, shrubs and vines in the family *Moraceae* occurring in most tropical and subtropical forests worldwide [12]. Many *Ficus* species have been used for a long time in pharmacological studies to delineate the antibacterial activities. The antibacterial activities of ethanolic extracts of *F. sycomorus* L. and *F. platyphylla* Del. in the treatment of ailments have been previously reported [6]. The antibacterial activity of *F. sycomorus* L. could be related to the presence of bioactive compounds, such as flavonoids [6], alkaloids, tannins, saponins and setroids [11].

Other scientists, however, reported that the *F. benghalensis* and *F. racemosa* ethanolic extract of roots at different concentrations (25, 50 and 75 mg/ml) showed moderate anti-bacterial activity against *S. aureus*, *P. aeruginosa* and *K. pneumonia* isolates [7]. Other study stated that the acetone, methanol and ethyl acetate SBE of *Ficus* spp. showed good antibacterial activity against *P. aeruginosa*, *E. coli*, *P. vulgaris*, *B. subtilis*, and *S. aureus* pathogens [8]. While, other investigation reported that the leaf acetone *F. tsiela* extract showed maximum inhibitory activity (11 mm) against *S. aureus*, *P. aeruginosa* and *K. pneumonia* isolates [9].

Antibacterial therapeutic failure due to the emergence of resistant bacterial strain is a worldwide phenomenon. So, many efforts have been done to enhance antibacterial effectiveness from sources such as plants that have become a necessity to overcome emergent of bacterial resistance in clinical practice. *S. aureus* began the development of penicillin-resistant strains not long after the introduction of penicillin. Recently, resistant strains of *S. aureus* as well as other *Enterobacteriaceae* such as *Salmonella* species that colonize the intestines is a global health problem in hospitals [13].

The emergence of multidrug resistance (MDR) among *A. baumannii* and *S. aureus* strains has been described worldwide. *S. aureus* is a Gram-positive, true facultatively anaerobic, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. *S. aureus* is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms [14]. *S. aureus* causes disease by infecting tissues typically creating abscesses and/or by producing toxins. *S. aureus* is commonly found in the environment (soil, water and air) is also in nose and on skin of humans [15]. Whereas, *Acinetobacters* spp. are non-fermenting Gram-negative coccobacilli, non-motile, strict aerobic, oxidase negative and catalase positive. Its cells are often found in diploid formation or in clusters of variable length [16]. *Acinetobacters* are also found as a part of human

skin flora. Up to 43% of the individuals in the community carry them on their skin and mucous membrane. The various forms of infections by *A. baumannii* include bacteremia, urinary tract infection, meningitis, wound and burn infections, and most importantly nosocomial pneumonia, particularly in ventilated patients [17]. The main factors thought to play a role in the persistence of *A. baumannii* in the hospitals are their resistance to antibiotics and disinfectants and the capacity to survive desiccation [18].

Thereby, the current study aimed to investigate the antimicrobial spectrum of methanol and acetone of *F. sycomorus* L. LE and SBE against *S. aureus* and *A. baumannii* pathogens that belonged to antibiotic resistant bacterial nosocomial infections and causing a problem in intensive care units (ICU).

MATERIALS AND METHODS

Collection and preparation of plant material

Plant materials fresh leaves and stem-barks of the medicinal plant *F. sycomorus* L. were collected from their natural habitat from Lattakia city located in the coastal regions of Syria. The plants were identified by taxonomical study in the Division of Plant Biotechnology at the AECS in Damascus, Syria. Sampling was carried out in Spring from trees spread on clay soil and annual rainfall ranging from 650 to 700 mm. Further, stem-barks (cut into small pieces) and leaves fractions were shade dried for one week, powdered by special electric mill and stored separately in polyethylene bags until analysis.

Extraction of plant material

For the methanolic and acetonetic extracts: 500 g of shade-dried pulverized plant material were subjected to extraction in a Soxhlet apparatus successively with solvent 10 times the volume of plant extract. The extraction was conducted until no more coloured matter was extracted. Solvent from each extracted mixture was evaporated to dryness using a rotary evaporator under reduced pressure at 40°C. All dried extracts were then kept in tightly fitting stopper bottles and stored in 4°C. The concentration of extract was considered 100 mg/ml.

Microorganisms and growth conditions

The pure clinical isolates of *S. aureus* and *A. baumannii* were collected from the Microbiology and Immunology Division, Department of Molecular Biology

and Biotechnology, Atomic Energy Commission of Syria (AECS), in Damascus city, Syria.

The cultures were started from the transference of stock cultures for trypticase soy broth (TSB, Difco, BD, Sparks, MD) at 37°C for *S. aureus* and *A. baumannii*, for a period of 24 h. After growth, the samples were centrifuged (1000 xg/15 min/4°C) and resuspended in sterile phosphate-buffered saline (PBS). Prior to antimicrobial sensitivity test, a bacterial suspension was obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). The bacterial cultures standardize to approximately 10⁶ CFU/ml [12]. The exact counts were assessed retrospectively by viable counts on trypticase soy agar plates (TSA, Difco, BD, Sparks, MD) at 37°C for 18 h.

The disc-diffusion method

The disc-diffusion method was adopted to test the antibacterial activity where ciprofloxacin was used as a standard drug to compare the results of experimental plant. Filter paper discs (Whatman no.1) of 6 mm in diameter were prepared and sterilized. The discs impregnated with 100 µl of extract dilutions (100 mg/ml) and reconstituted in minimum amount of methanol or acetone were applied over each of the culture plates previously seeded with the 10⁶ CFU/ml cultures of bacteria. Bacterial cultures were then incubated at 37°C for 18 h, while the paper discs impregnated with 20 µl of a solution of 10 mg/ml of ciprofloxacin were used as standard antimicrobials for comparison. Negative control was prepared using methanol or acetone (final concentration of the solvent in the highest concentration of plant extract was tested). Diameter of inhibition zones (ZIs in mm) was measured after incubation at 37°C for 18-24 h. For each extract, duplicate trials were conducted against each organism.

Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The following antimicrobial agents were used in this study: chloramphenicol (Merck, Germany), rifampicine (Sigma-Aldrich, USA), erythromycin (Merck, Germany), tetracycline (Sigma-Aldrich, USA), levofloxacin (Sigma-Aldrich, USA) and ciprofloxacin (Bayer, Istanbul, Turkey). Stock solutions of antibiotics were prepared according to manufacture. Determinations of MICs and MBCs by the microdilution broth method were carried out according to NCCLS approved standards. Microdilution broth susceptibility assay was used [19]. Three replicates of serial dilutions of extract (100 mg/ml) or of antibiotics (128 µg/ml) were prepared in TSB medium in 96-well microtiter plates. One hundred microlitres

of freshly grown bacteria standardized 10^6 CFU/ml in TSB were added to each well. Positive control was achieved with the same conditions but without extract or antibiotics, negative control was also made with the same conditions but without adding the bacteria. The MIC was defined as the lowest concentration of each antimicrobial agent that inhibited visible growth of the tested isolate that was recorded and interpreted as the MIC₁₀₀. The MBC was determined by plating 0.010 ml from the wells showing no visible growth on Mueller-Hinton agar plates (Oxoid) and incubating for 18–24 h. The MBC was defined as the concentration at which there was a 99.9% reduction in CFU compared with the original inoculum.

Statistical methods

Results were expressed as mean \pm standard deviation (SD). The data was analyzed using the Student's t-test. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Antibacterial activity of methanol and acetone LE and SBE of *F. sycomorus* L. crude extracts against sensitive and resistant species of *S. aureus* and *A. baumannii* pathogens was investigated based on ZIs, MICs and MBC parameters.

Zone of inhibition (ZIs) was varied according to the tested bacteria pathogens and plant fraction extract (tab. 1). This value ranged between 15–23.5 mm and 16–27 mm for methanol and acetone extracts, respectively. It was noticed that sensitive *A. baumannii* isolate was the pathogen most inhibited by the both extracts. In this respect, this value was recorded to be 26, 27 mm for acetone LE and SBE; while it was recorded to be 23, 23.5 mm for methanol LE and SBE; respectively.

Table 1.

Antimicrobial activity of the leaf and stem-bark methanol and acetone extracts of *F. sycomorus* L. against *S. aureus* and *A. baumannii* using disc-diffusion method (zone of inhibition in mm)

Bacteria	Methanol		Acetone	
	LE	SBE	LE	SBE
Sensitive <i>S. aureus</i>	16.5 \pm 0.4	18.0 \pm 0.3	18.0 \pm 0.5	19.5 \pm 0.6
Resistant <i>S. aureus</i>	15.0 \pm 0.2	16.0 \pm 0.0	16.0 \pm 0.0	18.0 \pm 0.3
Sensitive <i>A. baumannii</i>	23.0 \pm 0.7	23.5 \pm 0.4	26.0 \pm 0.4	27.0 \pm 0.0
Resistant <i>A. baumannii</i>	18.5 \pm 0.5	20.0 \pm 0.5	22.0 \pm 0.2	23.5 \pm 0.7

Observations are expressed as mean \pm standard deviation (SD), n=3.

SE – leaf extract; SBE – Stem-bark extract

This investigation shows that the LE acetone possesses a higher antibacterial activity than that of LE methanol which may be a result of differences in photochemical compounds (*F. sycomorus* L.). LE methanol phytochemical screening test shows the presence of flavonoids, saponins, terpenoids and tannins which have been previously reported for their antibacterial activity (unpublished); while, that of LE acetone, were flavonoids and phenol where these compounds constitute also an important class of phytochemical which were reported to display strong antimicrobial activity (unpublished)].

It was found that the ZIs by methanol *F. sycomorus* L. ranged between 11.5 and 21.5 mm, while that of *F. platyphylla* was from 17.0 to 22.0 mm [2]. Whereas, other investigation reported that LE 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) [9]. The antimicrobial activity of methanolic extract of *F. carica* against five bacterial strain *B. cereus*, *E. aerogens*, *K. pneumoniae*, *B. subtilis*, and *S. epidermidis* at different concentration (30, 40, 50, 60 µg/ml) was recently reported [10]. The latter study revealed that the estimated ZIs values ranged between 12.5–14.0 mm for the five pathogens examined.

Overall, antibiotic antibacterial activity revealed that applied antibiotics were less potential than methanol and acetone LE against major pathogens examined (tab. 2).

Table 2.

Antimicrobial activity of the commercial antibiotics against *S. aureus* and *A. baumannii* using disc-diffusion method (zone of inhibition in mm)

Bacteria	Chl	Rif	Ery	Tetra	Levo	Cip
Sensitive <i>S. aureus</i>	17.0±0.4	33.0±0.24	23.0±0.07	26.0±0.15	27.0±0.14	24.0±0.17
Resistant <i>S. aureus</i>	13.0±0.2	17.0±0.09	16.0±0.02	14.0±0.08	15.0±0.09	13.0±0.07
Sensitive <i>A. baumannii</i>	18.0±0.05	23.0±0.12	13.0±0.12	19.0±0.22	27.0±0.3	32.0±0.24
Resistant <i>A. baumannii</i>	11.0±0.1	20.0±0.32	ND	ND	19.0±0.2	ND

Observations are expressed as mean ± standard deviation (SD), Chl: Chloramphenicol; Rif: Rifampicine; Ery: Erythromycin; Tetra: Tetracycline; Levo: levofloxacin; Cip: Ciprofloxacin. ND: not determined

Experimental assay showed that the two *S. aureus* and *A. baumannii* pathogens were resistant to different antibiotics, e.g. penicillin, chloramphenicol, tetracycline, amoxicillin, trimethoprin sulfamethoxazol, and erythromycin (data not shown here).

Our data indicated that the lowest antibiotic activity was recorded for rifampicine against all the tested bacteria isolates. No effect was observed against resistant *A. baumannii* isolate by erythromycin, tetracycline and ciprofloxacin antibiotics (tab. 2). Other study reported that the ZIs recorded by ofloxacin and gentamicin against *S. aureus* were 17 and 18 mm, respectively [2].

Minimum inhibitory concentrations (MICs) was also evaluated to establish the pathogens susceptibility to the methanol and acetone LE and SBE. Estimated MICs values were ranged between 3.7–17.3 mg/ml and between 2.5–13.5 mg/ml for methanol and acetone LE and SBE, respectively (tab. 3). The highest antibacterial activity was recorded in sensitive *A. baumannii* isolate (2.5 and 4.9 mg/ml for acetone LE and SBE, respectively), while it was 3.7 and 6.7 mg/ml for methanol LE and SBE, respectively.

Table 3.

Minimum inhibition concentrations (MIC₅₀) values of leaf and stem-bark methanol and acetone extracts of *F. sycomorus* L against the pathogenic bacteria

Bacteria	Minimum inhibitory concentration (MIC) [mg/ml]			
	Methanol		Acetone	
	LE	SBE	LE	SBE
Sensitive <i>S. aureus</i>	8.7	13.5	6.6	11.2
Resistant <i>S. aureus</i>	9.2	17.3	7.3	13.5
Sensitive <i>A. baumannii</i>	3.7	6.7	2.5	4.9
Resistant <i>A. baumannii</i>	5.7	8.9	4.3	6.9

SE: leaf extract; SBE: Stem-bark extract

It has been previously reported that MIC values by ethanol LE of *F. exasperate* against *E. coli* was 300 mg/ml, while that of *S. albus* was 700 mg/ml [20].

Other investigation, however, found that MIC values by ethanol *F. sycomorus* L. extract ranged between 1.95-31.3 mg/ml; while, that of *F. platphylla* was from 1.95-7.81 mg/ml [6]. Whereas, it has been found that isolated lupenol from *F. deltoidea* leaves showed antibacterial activities against *E. coli*, *B. subtilis* and *S. aureus*. The MIC against *E. coli*, *B. subtilis* and *S. aureus* are 150, 220 and 130 µg/ml, respectively [21]. It has been however, reported that MIC values ranged between 3-7 µg/ml by methanolic extract of *F. carica* against five bacterial strains examined [10].

As shown in table 3, LE exhibited a better antibacterial activity compared to the SBE one. This activity could be related to the higher flavonoids content present in LE compared to SBE. These findings were in agreement with earlier observations [2, 6].

All over, plant extracts were more effective against examined bacteria, as compared to the commercial antibiotics tested in this study (tab. 4). In this respect, the range of MIC exhibited by examined antibiotics revealed that all the bacteria isolates were resistant to the tested antibiotics, except for sensitive *S. aureus* isolate which was revealed as resistant only against chloramphenicol. Otherwise, resistant *A. baumannii* isolate revealed highest level of resistance against all tested antibiotics in different degrees. The lowest effect was found against previous isolate by rifampicine and levofloxacin. No effect was observed against the same isolate by erythromycin, tetracycline, chloramphenicol and ciprofloxacin.

Table 4.

Minimum inhibition concentrations (MIC₅₀) values of the examined commercial antibiotics against the pathogenic bacteria

Bacteria	Chl	Rif	Ery	Tetra	Levo	Cip
Sensitive <i>S. aureus</i>	32	1	2	4	4	8
Resistant <i>S. aureus</i>	64	16	32	32	32	32
Sensitive <i>A. baumannii</i>	8	32	128	64	16	2
Resistant <i>A. baumannii</i>	128	64	ND	ND	64	ND

Chl: Chloramphenicol; Rif: Rifampicine; Ery: Erythromycin; Tetra: Tetracycline; Levo: levofloxacin; Cip: Ciprofloxacin. ND: not determined

Otherwise, *F. sycomorus* effectiveness in killing the bacteria has been established by minimum bactericidal concentration (MBC) estimation. In this respect, the highest antibacterial activity was recorded in sensitive *A. baumannii* isolate (3.8 and 9.7 mg/ml for acetone LE and SBE, respectively) (tab. 5), while, it was 4.2 and 10.5 mg/ml for methanol LE and SBE, respectively. It has been previously reported that the MBC by ethanol *F. sycomorus* L. extracts ranged between 3.9-250 mg/ml; while, that of *F. platphylla* was from 3.9 to 62.5 mg/ml against *S. aureus* and *S. typhi* isolates [6]. While, it has been found that MBC values ranged between 6-11 µg/ml by methanolic extract of *F. carica* against five bacterial strains examined [10]. Overall, the effectiveness of plant extracts was higher against bacteria examined compared to the commercial antibiotics tested in this study (tab. 6).

Table 5.

Minimum bactericidal concentrations (MBC) values of leaf and stem-bark methanol and acetone extracts of *F. sycomorus* L against the pathogenic bacteria

Bacteria	Minimum bactericidal concentration (MBC) [mg/ml]			
	Methanol		Acetone	
	LE	SBE	LE	SBE
Sensitive <i>S. aureus</i>	13.3	24.2	11.2	21.2
Resistant <i>S. aureus</i>	18.9	30.4	16.2	27.5
Sensitive <i>A. baumannii</i>	4.2	10.5	3.8	9.7
Resistant <i>A. baumannii</i>	6.9	13.8	5.8	11.7

SE: leaf extract; SBE: stem-bark extract

CONCLUSION

In current investigation, LE of *F. sycomorus* was observed to be more effective against sensitive and resistant tested pathogens compared to the SBE one. Otherwise, acetone LE of *F. sycomorus* L. (2.5, 3.8 mg/ml for MICs and MBC, respectively)

was more potent than the LE methanol one (3.7, 4.2 mg/ml for MICs and MBC, respectively) against sensitive *A. baumannii* isolate. The difference in observed biological activity between acetone and methanol LE could be attributed to the difference in phytochemical compounds; where flavonoids, saponins, terpenoids and tannins were presented in *F. sycomorus* LE methanol; while, that of LE acetone, was flavonoids and phenol. The highest antibacterial activity was recorded in sensitive *A. baumannii* isolate with MICs 2.5, 4.9 mg/ml and MBC 3.8, 9.7 mg/ml for acetone LE and SBE, respectively.

Results from the MBC assay supported the data obtained from the MIC determination assay. Otherwise, chloroamphenic has the lowest antibacterial activity against all the examined pathogens.

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DZIAŁANIE ANTYBAKTERYJNE WYCIĄGÓW Z LIŚCI I KORY *FICUS SYCOMORUS* L. (MORACEAE) NA PATOGENY LUDZKIE OPORNE NA WIELE LEKÓW

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Streszczenie

W niniejszej pracy badano aktywność antybakteryjną wyciągu metanolowego i acetonowego z liści (LE) i kory (SBE) *Ficus sycomorus* L. przeciwko wrażliwym i opornym gatunkom patogenów *Staphylococcus aureus* i *Acinetobacter baumannii*. Działanie antybakteryjne oznaczone za pomocą metody dyfuzji dyskowej (strefa zahamowań – ZIs), minimalne stężenia hamujące (MICs) i minimalne stężenia bakteriobójcze (MBC) mierzono w wielu innych ba-

daniach. Przeprowadzono podobne badania z użyciem sześciu antybiotyków obecnych na rynku jako leków referencyjnych. Na podstawie oznaczonych wartości ZIs, MIC i MBC stwierdzono, że wyciąg acetonowy z liści silniej działał antybakteryjnie niż metanolowy. Natomiast standardowe antybiotyki miały niższą skuteczność (ZIs, MICs i MBC) dla wszystkich testowanych bakterii w porównaniu z testowanym wyciągiem z kory i liści. Najsilniejsze działanie antybakteryjne zanotowano dla wrażliwego izolatu *A. baumannii* z wartościami MICs 2.5, 4.9 mg/ml i MBC 3.8, 9.7 mg/ml odpowiednio dla wyciągu acetonowego LE i SBE. Nasze dane wskazują, że najslabsze działanie bakteriobójcze antybiotyków zanotowano dla opornego patogenu *A. baumannii*. Było ono słabsze niż dla obu frakcji wyciągów roślinnych.

Słowa kluczowe: działanie bakteriobójcze, *Ficus sycomorus* L., minimalne stężenie hamujące (MICs), minimalne stężenie bakteriobójcze (MBC)