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### ***In vitro* Antibacterial Activity of Crude Extract of Leaves and Stem-bark *Ficus sycomorus* L. (Moraceae)**

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#### Abstract

The current study was carried out to investigate the activity of the crude methanol extracts of *Ficus sycomorus* L. from stem-bark and leaves were examined *in vitro* against nine bacteria isolates pathogens to estimate the inhibitory effect of *F. sycomorus* L. Susceptibility and minimum inhibitory concentration (MICs) was inspected against 9 bacterial isolates using standard antibiotics ciprofloxacin as a control. The phytochemical test of the methanolic crude extracts of both stem-bark and leaves of *F. sycomorus* L. revealed that the flavonoid content was higher in leaves compared to bark. Our data proved that the aqueous extract of leaves and stem bark has no inhibitory effect against all tested bacteria in the present investigation. In addition, for methanolic extract, it has been concluded that the methanolic extract of *F. sycomorus* leaves had antibacterial activity better than the stem bark extract against all tested organisms. Furthermore, it was noticed that *Salmonella typhimurium* was the most sensitive pathogen, with the lowest MICs values of 92.3 & 33.6 µg/mL and the maximum zone of inhibition of 15 & 16.5 mm with the methanolic extracts of stem bark and leaves respectively; contrary to *Klebsiella pneumoniae*. The present study somewhat permits the detection of the activity of *F. sycomorus* L. as a potential medicinal plant used in biological laboratories.

**Keywords:** *Ficus sycomorus* L., Antibacterial activity, Minimal inhibitory concentration, Antimicrobial resistance, Pharmacological effects.

#### Introduction

*Ficus sycomorus* L. species is classified as a subgenus of fig belonging to the *Moraceae* family. Approximately 400 monoecious and 800 gynodioecious [1]; species exist within the genus *Ficus*. The name *sycomorus* came from the Greek *Syca-Morus* which means Mulberry fig. The trees are not tolerating cold as the common fig *F. carica* L. They are usually grown in the Middle East and Africa in warmer regions. *F. sycomorus* L. is a rare sub-tree or perennial tree. It was originated from Ethiopia and Central Africa. It has been planted since the antiquity period in Egypt, Palestine, Lebanon and Syria. It has become rare because of urban development, e.g. the rest of this species could be found in Sida and Syrian littoral [2]. It is known as Sycamore as a common English name and commonly in Arabic as Al-Joumayz, and this species are in danger of extinction as a plant genetic resource in Syria.

However, there are some efforts for characterization and conservation of the genetic variety and further utilization to prevent its potential extinction. This species shows a good tolerance for an abiotic stress. Furthermore, it is highly appreciated traditionally for its ripe fruit. In Syria, and other region of the world, *F. sycomorus* is used traditionally to treat many diseases and ailments. About 70% of the human population is dependent (wholly or partially) on plant-based medicines [3]. The active ingredients of many drugs are found as secondary metabolites in plants. These secondary metabolites which isolated from different parts of plants have been formed an important source of the pharmaceutical drugs. The phytochemical analysis of *F. sycomorus* and *F. platyphylla* showed the presence of secondary metabolites such as tannins, anthraquinones, flavonoid, saponins, steroids, alkaloids which have been reported for their antimicrobial activities previously [4, 5]. Other investigation reported the possibility of aqueous extract from stem bark of *F. sycomorus* L as remarkable antidiabetic on Alloxan-induced diabetic mice [6].

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The appropriate utilization of local resources play a basic role in medicinal plants research to cover our needs of drugs, play a basic role in medicinal plants research. Several investigations have mentioned the importance of stem bark and leaves of *F. sycomorus* L. in pharmacological studies [6, 7, 8]; are presented in many recent findings of importance of this plant.

Antimicrobial resistance is the durability of bacteria to an antibiotic medicine to which it was originally sensitive. Usually resistant microorganisms that don't respond to the standard treatment extend the illness and increase the possibility of death. The development of resistant strains happens when bacteria are exposed to antibiotic drugs, and resistant phenomenon can be exchanged between strains of bacteria.

When infections become resistant to first-line medicines, more expensive therapies must be used. The prolonged duration of treatment and illness, often in hospitals, increases the economic burden to societies. Therefore, this study was designed to estimate the methanolic and the aqueous extract of stem bark and leaves inhibitory effectiveness on different pathogenic bacteria. A pharmacological effect is presented in many recent findings on the importance of this plant.

## Material and Methods

### Collection and Preparation of Plant Material

Plant materials: fresh stem barks and leaves of the medicinal plant *F. sycomorus* L. were harvested in April 2012 from their natural habitat in Lattakia city located in the coastal regions of Syria (Latitude: 35°31'54" N, Longitude: 35°47'24" E, Elevation above sea level: 29 m = 95 ft) Latakia has a hot-summer Mediterranean climate [9] and the average receives around 760 millimetres of rainfall annually. The plants were identified based on taxonomical study in the Division of Plant Biotechnology at the AECS in Damascus, Syria.

Samples were collected from trees grow on clay soil in spring with 650 to 700 mm annual rainfall ranging. Further, stem barks (were divided to small parts) and leaves fractions were shade dried for one week, powdered by special electric mill and stored separately in polyethylene bags until needed for analysis.

### Extraction of Plant Material

For the aqueous extract: 200 gr of each powdered sample was dissolved in 500 ml of sterile distilled water, shaken manually, and allowed to extract for 48 hours, before each extract was filtered using filter paper Whatmann No 1. The filtrates were evaporated

in a water bath at 50°C to dryness. The extracts were stored at 4°C until needed.

In the case of methanol extraction: 500 gr of shade-dried pulverized plant materials were subjected to extraction in a Soxhlet apparatus successively with methanol (SIGMA- Germani ) 10 times the volume of plant extract. The extraction was conducted until no more coloured matter was extracted. The methanol extracts were evaporated at 40°C under reduced pressure using a rotary evaporator, then stored at 4°C in tightly fitting stopper bottles. The concentration of extract was considered 100 mg/ml.

### Microorganisms and Growth Conditions

The pure clinical isolates of *Escherichia coli* O157, *Brucella melitensis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* were collected from the Microbiology and Immunology division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus City, Syria.

Bacterial cultures were cultured on 2YT agar (peptone, 16 gr/l; yeast extract, 10 gr/l; NaCl, 5 gr/l; agar, 13 gr/l [Difco, BD, Spars, MD]) and were incubated at 37°C for 24-48 hours. Prior to antimicrobial sensitivity test, 0.2 ml of overnight culture of each bacteria was cultured into 20 ml of sterile Mueller Hinton Broth (Hi-media Laboratory Pvt. Ltd., Mumbai, India) and then incubated for about 16-24 hours to standardize the cultures to approximately  $1 \times 10^6$  cfu/ml [10]. The bacteria were dissolved in a sterile phosphate-buffered saline (PBS) and monitored by the optical density (OD) at 590 nm. The accurate counts were estimated by viable counts on 2YT agar plates.

### Determination of Antimicrobial Activity

#### The disc diffusion

The disc diffusion antibacterial activity was evaluated by the paper disk diffusion method on Mueller-Hinton agar (MHA) plates [11]; Ciprofloxacin was used as a positive control. Filter paper discs of 6 mm diameter (Whatman no.1) were prepared and sterilized. Discs were saturated with 100 µl of extract dilutions (100 mg/ml) and applied over each of the culture plates previously cultured with  $1 \times 10^6$  cfu/ml of bacteria, 20 µl of Ciprofloxacin (10 mg/ml) was used as positive control. All plates were incubated for 18 hours at 37°C, then antimicrobial activity was determined by measurement the zone of inhibition around each paper disc (in mm). One duplicate was done for each extract.

#### *Determination of minimum inhibitory concentrations (MICs)*

Microdilution broth susceptibility assay was used [12]. Serial dilutions of extract were prepared at three replicates in 2YT broth medium in microtiter plates (96-well), using a range of concentrations for methanol and aqueous extracts of both stem-bark and leaves of *F. sycomorus* L. 100 µl of freshly grown bacteria standardized  $1 \times 10^6$  cfu/ml in 2YT broth were added to each well. The same conditions without extract were done for positive control, and without bacteria for negative control. Plates were incubated with shaking for 24 hours at 37°C. The lowest concentration that completely inhibited visual growth was recorded and interpreted as the MICs.

#### **Phytochemical Screening of Extracts**

To determinate the presence of secondary plant metabolites, qualitative assay was carried out on the methanolic extract of the stem bark and leaves of *F. sycomorus* L. for the presence of phytochemicals such as saponins, alkaloids, flavonoids, terpenoids, and tannins using the standard procedures previously described [13,14].

#### *Determination of alkaloids content*

Alkaloids content was determined as follows: 5% ethyl ether was used for defatting 0.5 gr of the accurately weighted sample for 15 min, and was extracted it with 5.0 ml of 2 M HCl on a steam bath for 20 minutes. The resulting mixture was centrifuged at 3000 rpm for 10 minutes to remove supernatant. The presence of alkaloids was tested by adding few drops of Mayer's reagent to one ml of the filtrate, and Dragendorff's reagent to a second 1.0 ml portion. The observed coloured precipitates in the test tubes for either of these reagents, was taken as a proof for the presence of alkaloids.

#### *Determination of saponins content*

The presence of saponins was tested using the capability of saponins to produce frothing in aqueous solution by shaking 0.5 gr of dried extract with water in a test tube, frothing which persevere on warming, was taken as proof for the presence of saponins.

#### *Determination of tannins content*

For tannins test: 5.0 gr of dried extract was shaking with 10.0 ml of distilled water. After that it was filtered and added ferric chloride reagent (Ferric chloride hexahydrate: 7.50 gr, concentrated hydrochloric acid: 1.0 ml, water to 100 ml) to it. The formation of green pellet was proof of the presence of tannins.

#### *Determination of flavonoids content*

Flavonoids test was done as follows: 1.0 ml of 10% lead acetate was added to 1.0 ml of the extract

contained in a test-tube. The formation of a yellow pellet was proof of the presence of flavonoids.

#### *Determination of terpenoids content*

As for terpenoids test: 0.5 ml of the chloroform extract of the dried extracts was dried by evaporation on water bath and heated for 10 minutes with 3 ml of concentrated sulphuric acid on a water bath. Development of a grayish color indicates the presence of terpenoids.

#### **Statistical methods**

Results were expressed as mean  $\pm$  standard deviation (SD). The data was analyzed using two way ANOVAs using statistical program SPSS 21.0 software. Significance level for the differences was set at  $p < 0.05$ .

#### **Results and Discussion**

The phytochemical test of the crude methanol extracts of both stem-bark and leaves of *F. sycomorus* L. revealed the presence of terpenoids, flavonoids, saponin and tannins in both previous fractions (Table 1). Whereas, it has been reported the abundance of glycosides, tannins and flavonoids with absence of saponins and alkaloids in *F. sycomorus* L. stem bark extracts [7].

Alkaloids were not detected in the methanol extracts for both stem-bark and leaves of *F. sycomorus* L.

The antibacterial activities such as susceptibility and MICs were concluded using appropriate methods. The antibacterial susceptibility test showed that stem bark and leaves extracts of *F. sycomorus* L. were different according to the examined bacterial genus (Figure 1). The estimated MICs values obtained for the methanolic extracts against the tested organisms varied among the bacterial tested genus. As mentioned in Table 2, the leaves extract of *F. sycomorus* L. was observed to be higher against all tested organisms than the stem bark extract of the same plant.

The flavonoids were higher in leaves compared to stem bark part. The mechanisms of action of some phytochemicals are [15, 16,17]: (i) flavonoids are complex with cell wall, they inhibit the release of autocoids and prostaglandins and they inhibit GI release of acetylcholine (ii) terpenoids is membrane disrupter that inhibits the release of autocoids and prostaglandins (iii) saponin possesses membrane permeabilizing properties, it leads to vacuolization and disintegration of teguments (iv) tannins are enzyme inhibitor, substrate depricator, they are complex with cell wall, leading to membrane disruption and metal ion complexation. It was noticed that, *L. monocytogenes*, *S. typhimurium*, *S. aureus*, *P. mirabilis* and *B. melitensis* pathogens were

effectively inhibited by both methanolic stem bark and leaves extracts. Furthermore, *S. typhimurium* was the most sensitive pathogen with the largest zone of inhibition of 15 & 16.5 mm for the methanol stem bark and leaves extracts respectively; contrary it was clear that, no inhibition was recorded by either stem bark or leaves extracts in *K. pneumoniae* pathogen. Further, *E. coli* O:157, *P. aeruginosa* and *B. cereus* had the greatest resistance to the stem bark extracts (Figure 1). No inhibition was recorded by either stem bark or leaves extracts in *K. pneumoniae* pathogen. Due to the small standard deviation values (0.0-0.25), they were not presented in Figure 1. Other study stated the antibacterial activity of ethanolic extracts from 19 Zimbabwean plants on five bacterial strains [18]. The previous investigation indicated that *F. sycomorus* L. leaves extracts had an inhibition effect on *P. aeruginosa* and *B. cereus* with no inhibition recorded for *E. coli*, *S. aureus* and *B. subtilis* bacteria. The higher effect observed in leaf extract against previous pathogenic bacteria compared to the stem bark part could be related to the higher flavonoid content recorded in leaves compared to stem bark. This observation is in agreement with previous findings reported on the same plant [5, 19]. However, the extract *F. sycomorus* L. stems bark and leaves aqueous extracts had no effect on all bacterial strains used in our study (data not shown).

The methanolic extract with the greatest antibacterial activity was recorded for *S. typhimurium* (92.3 and 33.6 µg/ml for stem bark and leaves respectively), whereas the lowest one was pronounced for *K. pneumoniae* (644.3 and 360 µg/ml for stem bark and leaves respectively; Table 2).

On the other hand, standard antibiotic ciprofloxacin has more effectiveness (MICs) on all examined bacteria compared to the stem bark and leaves extract. Our findings are accordance with other scientists [5]; who reported that the leaves extract of *F. sycomorus* L. was found to be less efficient against Ciprofloxacin-sensitive and resistant test organisms than the stem bark extract of the same plant.

Our results are also accordance with other observations [19]; who reported the microbial inhibitory activities of *F. capensis* against *P. mirabilis* and *Streptococcus faecalis*. The phenomenon of antibacterial activity could be related to the presence of flavonoids in plant extracts [20, 21]. While, other investigation [8]; on phytochemical constituents and effects of aqueous root-bark extract of *F. sycomorus* L. on anaesthetic, muscular relaxation and sleeping time on laboratory animals showed the presence of alkaloids, tannins, reducing

compounds, saponins, flavonoids, steroids, terpenoids and anthracenoside. Similarly, the absence of alkaloids and saponins in the stem bark of *F. sycomorus* L. has also been previously demonstrated [7]. Our results revealed that, the stem bark methanol extracts have inhibitory effects on *S. typhimurium*, *L. monocytogenes*, *P. mirabilis*, and *E. coli* O157; whereas other researchers reported that the stem bark ethanol extracts of the same plant have no effect on the previous bacterial genus tested in our study [7]. Overall, the sensitivity of the test bacterial isolates to the *F. sycomorus* L. plant extracts was different according to the tested isolates.

On the other hand, the extract of *F. sycomorus* L. is relatively well documented with antibacterial activity as well as for the management of some diseases; e.g. previous reports have shown that *F. sycomorus* L. possesses antimalaria potentials against *Plasmodium falciparum* *in vitro*, [15]. *F. sycomorus* L. have been suspected to possess anti-diarrhoeal activities [22]. It has been reported that the ethanol and anthraquinone glycosides extracts of *F. sycomorus* L. at higher doses (617 to 1229 mg/kg) might be possibly toxic to the rat liver and kidney [23].

In addition, other scientists investigated the phytochemical screening of the *F. sycomorus* for the chemical constituents studied [8]. This paper showed that leaves and root-bark extract promotes muscle relaxation and increased aminobarbitone sleeping time in rats. It has been stated that the methanol extracts of *F. sycomorus* L. and *A. indica* and their derived ethyl acetate and butanol fractions reduced the oxidative damage and improved the liver function in *Schistosoma mansoni* infected mice [24]. Recently, scientists assessed the aqueous stem bark extract of *F. sycomorus* L. for its antidiabetic possible along with evaluating its preliminary *in vivo* toxicity in alloxan-induced diabetic mice, [6]. Phenolic compounds from *F. sur Forssk* and *F. sycomorus* L. on sickle cell were assessed in Burkina Faso [25]. This investigation indicated that extracts of *F. sycomorus* L. produce the highest antiradical activity compared to *F. sur Forssk*. Correspondingly, the same study mentioned also, that the latex of *F. sycomorus* L. presented the lowest MICs against *S. aureus* (0.13 mg/ml) and *E. coli* (0.25 mg/ml), than *F. sur Forssk*.

### Conclusion

This investigation enabled us to detect the antibacterial activities of *F. sycomorus* L. plant extracts against different bacterial isolates. It was noticed that, inhibitory bacteria effect was higher in leaves compared to bark, where the sensitivity of the

tested bacterial isolates to the *F. sycomorus* L. plant extracts different according to the tested isolate. Based upon antibacterial activity against selected pathogenic bacteria and the estimated MICs values, it was found that the greatest antibacterial activity was recorded for *S. typhimurium*, whereas the lowest one was pronounced for *K. pneumoniae*. The present study somewhat permits the detection of the biological activity of *F. sycomorus* L. as a potential medicinal plant used in biological laboratories.

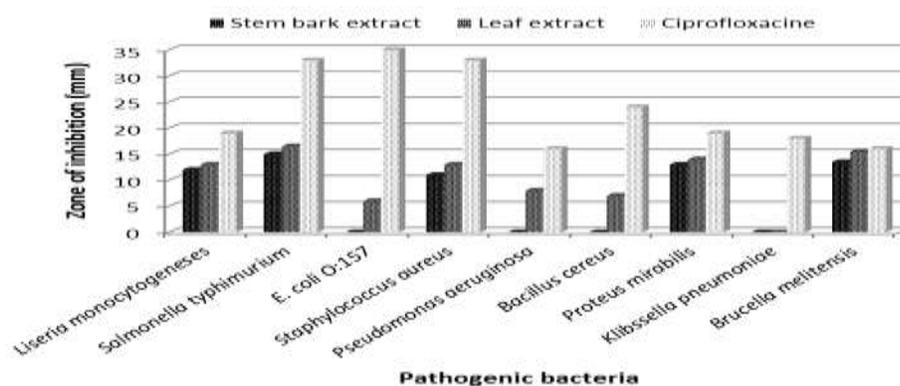
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**Fig. 1:** Susceptibility of the test bacterial isolates to the stem bark and leaf methanol extract of *F. sycomorus* L. (100 mg/ml)

Table 1: Phytochemical components of leaf and stem bark methanol extract of *F. sycomorus* L.

Chemical component	Leaf	Stem bark
Alkaloids	-	-
Flavonoids	++	+
Saponins	+	+
Terpenoids	+	+
Tannins	++	++

Key: (+) Present; (++) Higher presence; (-) Absent

Table 2: Minimum inhibition concentrations (MIC<sub>s</sub>) values of stem bark and leaf methanol extract of *F. sycomorus* L. and Ciprofloxacin hydrochloride against the pathogenic bacteria.

Pathogenic Bacteria	Minimum inhibitory concentration values (µg/ml)*		
	Stem bark extract	Leavs extract	Ciprofloxacin
<i>L. monocytogenes</i>	102.3 ±26.5	67.0 ±17.3	8.3 ±3.6
<i>S. typhimurium</i>	92.3 ±17.3 <sup>b</sup>	33.6 ±38.3	4.0 ±1.8
<i>E. coli</i> O:157	155.3 ±38.6	50.8 ±11.1	10.4 ±3.6
<i>S. aureus</i>	177.6 ±38.6	117.6 ±26.5	16.6 ±7.2
<i>P. aeruginosa</i>	235.6 ±61.8	102.3 ±26.5	16.6 ±7.2
<i>B. cereus</i>	270.6 ±61.2	140.0 ±56.8	10.4 ±3.6
<i>P. mirabilis</i>	140.0 ±56.8	50.66 ±10.9	8.3 ±3.6
<i>K. pneumoniae</i>	644.3 ±153.6 <sup>a</sup>	360.0 ±61.2 <sup>c</sup>	16.6 ±7.2
<i>B. melitensis</i>	130.0 ±71.5	60.6 ±24.7	16.6 ±7.2

\*Significant at  $p < 0.05$ ; according to Stem bark extract, a: for *K. pneumoniae* compared to all tested organisms, b: for *S. typhimurium* compared to *B. cereus*, *P. aeruginosa* and *K. pneumoniae*; according to Leavs extract: c: for *K. pneumoniae* compared to all tested organisms.

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