

Phytochemical constitutes of *Ficus sycomorus* L. and inhibitory effect of their crude extracts against bacterial pathogens

Basel Saleh*, Ayman Al-Mariri

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), P.O. Box 6091, Damascus-Syria

*Corresponding Author

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ABSTRACT

Etheric and acetic leaf (LE) and stem-bark (SBE) *Ficus sycomorus* L. extracts were evaluated phytochemically and for their antibacterial activity by disc-diffusion method (zone of inhibition ZI) and minimum inhibitory concentration (MIC), against 10 clinical bacterial isolates. Phytochemical test showed that phenol compounds were followed similar tendency either in etheric and acetic of LE and SBE; with their abundance occurrence in SBE than LE. Our data revealed that etheric SBE and LE showed no antibacterial activity against all the examined bacterial isolates. Whereas, acetic SBE and LE revealed inhibitory effects. Based on estimated ZI and MIC values, *Salmonella typhimurium* was the most sensitive pathogen by showing the highest ZI (22 and 19 mm) combined with the lowest MIC (32.5 and 52 mg/ml) for SBE and LE, respectively. Overall, acetic SBE found to be more potent than LE against both Gram-negative and Gram-positive bacteria, thus showing it to possess broad spectrum activity.

Key words: Antibacterial activity; *Ficus sycomorus*; Minimum inhibition concentration.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents, and have a positive impact on gastroenteritis treatment and other infectious diseases caused by the bacteria. Exploration of newer antimicrobials in plants brings about a different approach in minimizing antibiotic resistance (Kubmarawa et al., 2007; Anowi et al., 2012; Adebayo-Tayo and Odeniyi, 2012; Josephs et al., 2012; Kashani et al., 2012; Alves et al., 2013). Hence, a more detailed search for new antimicrobial drugs is needed.

F. sycomorus L., a medicinal plant belonging to the class *Moraceae*, is used worldwide to treat various ailments (Saleh et al., 2015). It was originated from Ethiopia and Central Africa. It becomes rare because of urban development, e.g. the rest of this species could be found in Sida and Syrian littoral (Mouterde, 1966).

A large number of their secondary metabolites such as *e.g.* alkaloids, flavonoids, saponins, terpenoids, tannins and coumarins compounds and its antibacterial activities have been successfully identified in *F. sycomorus* plant extracts (Ahmadu et al., 2007; Zaku et al., 2009; Adeshina et al., 2010; Saleh et al., 2015). Some of these compounds like terpenoids and tannins have been revealed to exert their antibacterial activity through membrane perturbations.

Previously, Ramde-Tiendrebeogo et al. (2012) demonstrated that the difference in biological activity of *F. sur Forssk* and *F. sycomorus* L. on sickle cell could be related to the observed difference in their phenolic compounds from. Other investigation reported the antibacterial compounds from *F. deltoidea* lack leaves (Suryati et al., 2011).

Thereby, this study was undertaken to investigate the phytochemicals (alkaloids, flavonoids, tannins, terpenoids, tannins and phenols) screening test in the SBE and LE and assess the antibacterial effects of the mentioned extracts of *F. sycomorus* L. on some selected bacterial isolates using ether and acetone solvents.

MATERIALS AND METHODS

Collection and preparation of plant material: *F. sycomorus* L. samples were collected from Lattakia-Syria, identified by Saleh (2013) and their voucher specimen number is 10. Plant materials fresh leaves and stem-bark were shade dried for 1 week.

Extraction of plant material: Etheric and acetonic SBE and LE were extracted according to Saleh et al. (2015).

Phytochemical Screening: Phytochemical test was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as tannins, flavonoids, saponins, alkaloids, phenols, coumarins and fatty acids. The presences of these phytochemicals were determined as previously described by some investigations (Farnsworth, 1966; Fadeyi et al., 1989; Odebiyi and Sofowora, 1990; Evans, 1996). The color intensity or the precipitate formation was used as analytical responses to these tests.

Microorganisms and growth conditions: Ten pure clinical *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O:157, *Salmonella typhimurium*, *Brucella melitensis*, *Proteus mirabilis*, *Yersinia enterocolitica* O:9, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates were obtained from the Microbiology and Immunology division, Department of Molecular Biology and Biotechnology of Atomic Energy Commission of Syria (AECS) in Damascus City, Syria. The cultures and growth conditions were performed according to Saleh et al. (2015) as described in many researches.

Antibacterial activity

The disc diffusion method: To evaluate the antibacterial activity of *F. sycomorus* L. crude extracts, the disc diffusion method was adopted with Ciprofloxacin as a standard drug. Filter paper discs (Whatman no.1, from England) of 6mm diameter were prepared and sterilized. The test was performed by impregnating discs with 100µl of extract dilutions (100 mg/ml) and reconstituted in minimum amount of ether or acetone were applied over each of the culture plates previously seeded with the 10⁶ CFU/ml cultures of bacteria. Cultural bacterial was incubated at 37°C for 18h, while the paper discs impregnated with 20µl of a solution of 10 mg/ml of ciprofloxacin were used as standard

antimicrobial for comparison. Negative control was also prepared using ether or acetone (final concentration of the solvent in the highest concentration of plant extract was tested). Diameter of inhibition zone (ZI in mm) was measured after incubation at 37°C for 18-24h. For each extract, duplicate trials were conducted against each organism.

Minimum Inhibitory Concentration (MIC): Six standard antibiotics were applied in the current investigation: Ciprofloxacin (Bayer, Istanbul, Turkey); Tetracyclines; (Sigma-Aldrich, USA); Gentamicin (Sigma-Aldrich, USA); Cefazolin (Bristol-Myers Squibb, New-York, USA); Cefotaxime (Sigma, St. Louis, USA) and Ofloxacin (Sigma, St. Louis, USA). Their stock solutions were prepared according to manufacture. Determination of MICs by the microdilution broth method was carried out according to NCCLS approved standards. Microdilution broth susceptibility assay was used (Saleh et al., 2015). Three replicates of serial dilutions of extract (100mg/ml) or of antibiotics (128µg/ml) were prepared in TSB medium in 96-well microliter plates. One hundred microliters 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 was recorded and interpreted as the MIC₁₀₀.

Statistical analysis: Results were expressed as mean of 3 replicates. The data were analyzed using the Student's t-test. $P \leq 0.05$ was considered to be significant. Data were analysed by one way ANOVA to test significance of differences among variables.

RESULTS AND DISCUSSION

Qualitative determination of phytochemical components in *F. sycomorus* L. LE and SBE was investigated with etheric and acetonic extracts. Their antibacterial activity against 10 clinical bacterial pathogens was evaluated. Phytochemical test of LE and SBE ether and acetone crude extracts of *F. sycomorus* L. was presented in table 1.

Table-1: Phytochemical components of ether, acetonic leaf and stem bark extracts of *F. sycomorus*.

Chemical components	Ether extract		Acetone extract	
	SBE	LE	SBE	LE
Alkaloids	+	+	-	-
Flavonoids	-	-	+	++
Saponins	-	-	-	-
Terpenoids	+	+	-	-
Tannins	-	-	-	-
Phenol	++	+	++	+
Coumarins	+	+		
Fatty acids	+	+		

- SBE= Stem-bark extract; LE = Leaf extract; + Present; ++ Higher presence; - Absent

Phytochemical analysis proved the presence of alkaloids, terpenoids, coumarins and fatty acids either in LE and SBE. As for acetone extract, it was observed that phenol content presented in the same trends with ether extract, in an inverse tendency to flavonoids ones. Whereas, alkaloids, saponins, terpenoids and tannins were not detected either in LE or

SBE acetonetic extracts (table 1). As shown in table 1, alkaloids and terpenoids were presented in both plant parts for ether extract, inversely to acetone one.

Antibacterial activity of *F. sycomorus* L. crude extracts was evaluated against ten bacterial pathogens based on ZI and MIC values. For ZI, our data presented herein showed that no antibacterial activity with etheric SBE and LE has been recorded against all the tested pathogens (Data not shown here). As for acetonetic SBE and LE showed varying degree of antibacterial activities against the tested bacterial pathogens (table 2).

Table -2: Antibacterial activity of the LE and SBE acetone of *F. sycomorus* against tested bacteria.

Sample No.	Tested organisms	Zone of inhibition in mm	
		SBE	LE
1	<i>L. monocytogenes</i>	14	10
2	<i>S. aureus</i>	12	9
3	<i>B. cereus</i>	15	10
4	<i>E. coli O:157</i>	19	17
5	<i>S. typhimurium</i>	22	19
6	<i>B. melitensis</i>	19	15
7	<i>P. mirabilis</i>	23	18
8	<i>Y. enterocolitica O:9</i>	20	17
9	<i>P. aeruginosa</i>	17	11
10	<i>K. pneumoniae</i>	14	13

- SBE, Stem-bark extract; LE, Leaf extract.

Statistical variance analysis revealed that the effect of acetonetic plant part extracts on ZI values was significantly ($P \leq 0.05$) different.

Antibacterial activity of the tested six antibiotics against examined bacteria was also evaluated (table 3). In order to evaluate the plant extracts antibacterial inhibitory efficiency, their effect was compared to 6 antibiotics as presented in table 3.

Table-3: Antibacterial activity of the commercial antibiotics against tested bacteria.

Sample No.	Tested organisms	Zone of inhibition in mm					
		Ciprofloxacin	Tetracyclines	Gentamicin	Cefazolin	Cefotaxime	Ofloxacin
1	<i>monocytogenes</i>	19	20	13	5	9	17
2	<i>S. aureus</i>	24	23	15	6	12	21
3	<i>B. cereus</i>	21	20	14	4	8	16
4	<i>E. coli O:157</i>	27	20	16	7	6	17
5	<i>S. typhimurium</i>	34	22	16	9	7	18
6	<i>B. melitensis</i>	17	27	19	0	0	19
7	<i>P. mirabilis</i>	33	18	15	5	9	20
8	<i>Y. enterocolitica O:9</i>	30	17	14	0	5	18
9	<i>P. aeruginosa</i>	15	9	7	0	0	13
10	<i>K. pneumoniae</i>	18	16	13	0	0	15

Statistical variance analysis showed that applied commercial antibiotics have significant ($P \leq 0.05$) effect on ZI values against tested bacterial isolates.

Moreover, MIC of crude plant extracts was also determined in order to detect the antibacterial activities. The SBE and LE effectiveness against the tested isolates in the current study was shown in the table 4. The antimicrobial activities of the partitioned fractions against tested isolates showed different degrees of activity at 100mg/ml.

Table-4: MIC values of SBE and LE acetone of *F. sycomorus* against the tested bacteria.

Sample No.	Tested organisms	Minimum inhibitory concentration values (mg/ml)		
		SBE	LE	Ciprofloxacin
1	<i>L. monocytogenes</i>	91.1	104.2	14.6
2	<i>S. aureus</i>	104.12	130.2	14.6
3	<i>B. cereus</i>	130.2	182.3	12.5
4	<i>E. coli O:157</i>	45.5	52	20.7
5	<i>S. typhimurium</i>	32.5	52	16.7
6	<i>B. melitensis</i>	65.1	104.2	20.7
7	<i>P. mirabilis</i>	45.5	91.1	8.2
8	<i>Y. enterocolitica O:9</i>	45.4	143	27.1
9	<i>P. aeruginosa</i>	84.5	162.6	20.83
10	<i>K. pneumonia</i>	91	156.1	25

- SBE, Stem-bark extract; LE, Leaf extract.

Statistical variance analysis showed that the effect of acetonic plant part extracts on MIC values was significantly ($P \leq 0.05$) different.

Indeed, MIC of the six tested antibiotics was also estimated (table 5). The SBE and LE antibacterial activity was also compared with 6 antibiotics. The application of the tested antibiotics had an adverse effect against the tested isolates (table 5). Based upon the results obtained herein, it was noticed that, the higher antibacterial activity was recorded for Ofloxacin and Tetracyclines (8.2mg/ml) against *B. melitensis* isolate; Gentamicin and Ciprofloxacin (8.2mg/ml) against *P. mirabilis*. Statistical variance analysis revealed that the applied commercial antibiotics have significant ($P \leq 0.05$) effect on MIC values against studied bacterial isolates.

Table-5: Minimum inhibition concentration values of the tested antibiotics against studied bacteria.

Sample No.	Tested organisms	Minimum inhibitory concentration values (mg/ml)					
		Ciprofloxacin	Tetracyclines	Gentamicin	Cefazolin	Cefotaxime	Ofloxacin
1	<i>L. monocytogenes</i>	14.6	16.7	20.73	33.2	25	16.7
2	<i>S. aureus</i>	14.6	12.5	16.7	41.7	33.2	14.6
3	<i>B. cereus</i>	12.5	16.7	20.7	50	41.7	16.7
4	<i>E. coli O:157</i>	20.7	14.63	14.6	58.2	50	10.3
5	<i>S. typhimurium</i>	16.7	10.37	10.3	66.7	58.2	12.5
6	<i>B. melitensis</i>	20.7	8.2	10.3	75	66.7	8.2
7	<i>P. mirabilis</i>	8.2	10.3	8.2	41.7	33.2	10.3
8	<i>Y. enterocolitica O:9</i>	27.1	18.75	18.75	58.2	66.7	10.3
9	<i>P. aeruginosa</i>	20.83	25	20.7	83.2	75	16.7
10	<i>K. pneumoniae</i>	25	20.7	20.7	83.2	75	20.7

In the current study, it was noticed that, flavonoids content was more abundant in LE than in SBE. This result was in agreement with Adeshina et al. (2010), who reported the same findings in the crude ethanolic extracts of *F. sycomorus* and *F. platyphylla*.

While, phenol content was inversely found in the previous fractions. Similar findings were also reported by Adebayo-Tayo and Odeniyi (2012). Whereas, alkaloids, saponins, terpenoids and tannins were not detected either in LE or SBE. Other investigation however reported the presence of alkaloids, terpenoids and tannins either in *F. sycomorus* L. LE or SBE; presence of flavonoids and saponins in LE and not found in SBE. While, phenol compound was disappeared either in LE or SBE in the same plant species (Zaku et al., 2008). Previously, Ahmadu et al. (2007) reported the presence of tannins and the disappearance of both alkaloids and flavonoids in *F. sycomorus* L. LE n-butanol. As for ether extracts, some phytochemical components were appeared in the two plant parts. Where, alkaloids, terpenoids, coumarins and fatty acids were found either in LE and SBE. Whereas, phenol content was higher in SBE compared to the LE part. While, flavonoids, saponins and tannins were not detected either in LE or SBE. It was noticed that, phenol content was presented in the same amount with the two extracts (acetone and ether extracts).

Inhibitory effect expressed as ZI proved that *S. aureus* was the most resistant isolate among the 10 tested isolates either by SBE and LE (12 and 9mm, respectively). Whereas, *P. mirabilis* (23 and 18mm) followed by *S. typhimurium* (22 and 19mm) were the most sensitive isolates with SBE and LE, respectively. It was noticed that ZI values varied between 9-19mm and 12-23mm for LE and SBE, respectively. While other study showed that this value was ranged between 11.5-21.5mm for *F. sycomorus* and between 17.0-22.0mm for *F. platyphylla* extracts (Adeshina et al., 2010). Whereas, Shamila et al. (2012) stated that among the different LE of *F. tsiela*, diethyl ether exhibited better inhibitory effect against *K. pneumoniae* (20 mm) followed by *E. coli* (12mm), *P. aeruginosa* (12mm) and least activity was noted against *S. aureus* (10mm).

Our data proved that, *P. aeruginosa* was the most resistant isolate to Gentamicin, Tetracyclines, Ofloxacin and Ciprofloxacin (7, 9, 13 and 15mm, respectively) antibiotics. Similar findings were reported by Adebayo-Tayo and Odeniyi (2012), who observed the same findings in *F. capensis* ethanolic extracts against the previous pathogen using 8 antibiotics. Otherwise, *S. typhimurium* (34mm) was the most sensitive isolates to Ciprofloxacin. Whereas, *S. aureus* was pronounced as the most sensitive pathogen to both Ofloxacin and Cefotaxime (21 and 12mm, respectively). However, Adebayo-Tayo and Odeniyi (2012) reported that ZI recorded by Ofloxacin and Gentamicin against *S. aureus* were 17 and 18mm, respectively.

Overall, it worth noting that out of the 6 tested antibiotics, two (Cefazolin and Cefotaxime) showed little or no activity against the tested isolates.

Our data showed that the higher antibacterial activity was recorded against *S. typhimurium* (32.5 and 52mg/ml for SBE and LE, respectively). While, the lowest one was pronounced in *B. cereus* isolate (130.2 and 182.3mg/ml, for SBE and LE, respectively). Other study however reported that with LE and SBE ethanolic *F. sycomorus* extracts, MIC were ranged between 1.95-31.3mg/ml against the tested microorganisms (Adeshina et al., 2010). In the present investigation, MIC values against *S. typhimurium* isolate (varying from 32.5 to 52 mg/ml for SBE and LE, respectively)

were comparable with those previously reported by Adeshina et al. (2010) who stated that these values ranging from 1.95 to 15.9mg/ml and from 3.91 to 15.6 mg/ml for *F. sycomorus* LE and SBE, respectively. Moreover, estimated MIC values herein against *S. aureus* pathogen (ranging from 104.12 to 130.2mg/ml for *F. sycomorus* LE and SBE, respectively) were also comparable with those reported by Adeshina et al. (2010). Where, the latter investigation indicated that this value varied between 7.81-15.6 and 15.6-31.3mg/ml for LE and SBE, respectively in the same plant species. This observed difference in antimicrobial activities of *F. sycomorus* extract against *S. typhimurium* and *S. aureus* isolates could be related to the geographical location where the samples were collected. Geographical location has been reported to influence the chemical constituents of plant extracts of the same genus found in different environment (Adeshina et al., 2010). Whereas, investigation of antibacterial compound from *F. deltoidea* lack leaves proved that the MIC against *E. coli* and *S. aureus* pathogens were 150 and 130µg/ml, respectively (Suryati et al., 2011).

Moreover, the greatest antibacterial activity was recorded for Cefazolin (33.2mg/ml) against *L. monocytogenes*. Whereas, the lowest one was pronounced for Cefazolin (83.2mg/ml) against both the *P. aeruginosa* and *K. pneumoniae* isolates. While the lowest one was pronounced in the case of Ofloxacin (20.7mg/ml) against *K. pneumoniae*; Gentamicin (20.7mg/ml) against *L. monocytogenes*, *B. cereus*, *P. aeruginosa* and *K. pneumoniae*.

The current study could suggested that the LE and SBE had superior microbial inhibitory activities compared to the tested antibiotics applied herein e.g. SBE had a greatest effect (45.5mg/ml) against *E. coli* O:157 compared to Cefotaxime (50mg/ml); LE (52mg/ml) against the same isolate compared to Cefazolin antibiotic. The difference in phenolic content recorded in LE and SBE could explain the difference in their biological activity. The highest phenolic content recorded in SBE compared to LE could explain their potential compared to LE. In this respect, El-Sayed et al. (2009) previously reported that the antioxidant activities of the methanolic LE *F. sycomorus* were highly correlated with their total phenolic contents. Furthermore, Kashani et al. (2012) reported that phenolics are responsible for color development, pollination and protection against UV radiation and pathogens. Moreover, Ramde-Tiendrebeogo et al. (2012) reported phenolic compounds from *F. sur Forssk* and *F. sycomorus* L. on sickle cell. The latter investigation stated that the difference in phenolic content could explain the difference in biological activity between the two *Ficus* species. Moreover, Alves et al. (2013) reported that the phenolic compounds in wild mushrooms had higher activity against the majority of Gram-negative and Gram-positive bacteria. Thereby, phenolic compounds could be used as antimicrobial agents, namely against some micro-organisms resistant to antibiotics. Furthermore, Kutama et al. (2013) reported that antibacterial activity of morula (*Sclerocarya birrea*) SBE and LE against some selected bacterial isolates in Kano, Nigeria could be related to the presence of soluble phenolic and polyphenolic compound. More recently, Saleh et al. (2015) reported the inhibitory effects of methanolic and acetonc SBE and LE against both the sensitive and resistant *Staphylococcus aureus* and *Acinetobacter baumannii* isolates. The two pathogens were considered as dangerous bacteria in intensive care units (ICU). The previous study revealed that the highest inhibitory effect was observed in sensitive *A. baumannii*

pathogen with MIC of 2.5 and 4.9mg/ml and minimal bactericidal concentration (MBC) of 3.8 and 9.7mg/ml for acetonic LE and SBE, respectively.

CONCLUSION

Phytochemical screening of crude acetone extract showed the occurrence of flavonoids and phenol in contrary tendency. Our data proved that ether extract has no inhibitory effect against all the tested bacterial pathogens. Whereas, adverse effect was noticed with acetone extracts. The higher phenol content in the SBE than the LE probably account for their high observed antibacterial activity. Based upon the estimated MIC values, it could suggest that *B. cereus* was the most resistant pathogen followed by *S. aureus* pathogen. Whereas, *S. typhimurium* was pronounced as the lowest resistant isolate. Overall, susceptibility test proved that the Cefotaxime and Cefazolin antibiotics showed little or no activity against the tested isolates compared to the other antibiotics tested in this study.

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