

Synergistic effect of *Ficus sycomorus* (Moraceae) leaf and stem-bark extracts against Some Selected Pathogens

Mr. Mohamed M. Jouda^{*}, Dr. Tarek Elbashiti^{**}, Dr. Atef Masad^{***}, Mr. Zuhair Dardona^{****}

^{*}M. Sc. Microbiology, Faculty of intermediate studies" Al Azhar University of Gaza"

^{**}Department of Biology. Islamic University of Gaza.

^{***} Ph. D. Biomedicine.

^{****} M. Sc. Microbiology

Abstract- The aim of the study was to assess the antibacterial effect of *Ficus sycomorus* extracts and their synergistic antibiotics against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extract of medicinal plants were prepared using Soxhlet apparatus for alcoholic extract, and water reflux for aqueous extracts. The results of this study showed that ethanolic extracts of *Ficus* were showed against *E. coli*, *S. aureus* and *P. aeruginosa*, While Aquatic extract showed synergistic effect with some antibiotics better than organic solvent. The results of this study showed that there is a decrease in MIC in case of methanolic extract of *F. sycomorus* extracts against selected bacteria.

Thereby, our results indicate the possibility of using these extracts in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo. These results was revealed the importance of plant extracts when associated with antibiotic and in control of bacteria.

Index Terms- Plant extracts, Synergistic effects, Antimicrobial, Microdilution method

I. INTRODUCTION

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin [8].

Phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity [10].

In Palestine, there are numerous medicinal plants described for treatment of many diseases. Herbal medicine is considered an integral part of the Palestinian culture and plays a pivotal and indispensable role in the current public healthcare. The hills and mountains of Palestine are covered with more than 2600 plant species of which more than 700 are noted for their uses as medicinal herbs or as botanical pesticides [5].

In this study we investigated of antibacterial and Synergistic effect of *Ficus sycomorus*. The Sycamore Fig Belongs to family Moraceae (Table 1) is one of the old and historic plant species in

the Palestine coastal valley and the study area as well. The trees have some medicinal values as the sap extracted from the trunk can cure some skin diseases [1].

Kingdom:	<i>Plantae</i>
Division:	<i>Magnoliophyta</i>
Class:	<i>Magnoliopsida</i>
Order:	<i>Urticales</i>
Family:	<i>Moraceae</i>
Genus:	<i>Ficus</i>
Species:	<i>sycomorus</i>

Table 1: Classification of *Ficus sycomorus*

The antibacterial activity of *F. sycomorus* could be related to the presence of bioactive compounds, such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars. *Ficus sycomorus* have been suspected to possess anti-diarrhoeal activities and sedative and anticonvulsant properties of this plant have also been reported [11].

Reported different solvent extracts of some plants to have different pharmacological properties. Reported organic stem extracts of *F. sycomorus* with higher antifungal activity than aqueous extracts [4].

Thereby, the current study aimed to investigate the antimicrobial spectrum of methanol, ethanol and water extracts of *F. sycomorus* (Leaves and barks) against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

II. MATERIALS AND METHODS

Collection and preparation of plant material

Plant materials fresh leaves and stem-barks of the medicinal plant *F. sycomorus* were collected from their natural habitat from Gaza city that located in the coast regions of Palestine. Sampling was carried out in April from trees. Further, stem-barks (cut into small pieces) and leaves were dried in the shade for one week, Then grinded by special electric mill.

Preparation of plant extract

Uniform weights (20g) of pulverized *F. sycomorus* plant parts were subjected to a 150ml solvent extraction using ethanol

and methanol by a soxhlet extractor. Aqueous extraction was done by boiled on slow heat for 2 hours. Then the extracts were filtered and allowed to evaporate in oven (45 °C). The dried extract was dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator for further use [6,12].

Preparation of inocula

According to Jayaraman *et al.*, stock cultures were maintained at 40C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37 °C for 24 hours.

Paper Disk Diffusion Assay

A suspension of testing microorganisms were spread on MHA medium. The filter paper discs (5mm in diameter) was placed on the agar plates which was inoculated with the test microorganisms and then impregnating with 20µl of plant extract (concentration 200 mg/ml). The plates were subsequently incubated at 37° C for 24 Hrs. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm [9].

Determination of MIC of plant extract by Micro-dilution Method

The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth, into each well. A 50 µl from the stock

solution of tested extracts (concentration of 200 mg/ml) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 0.1953 mg/ml. And then added 10 µl of inocula to each well except a positive control (inocula were adjusted to contain approximately 1.5X10⁸ CFU/mL). Plant extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37 °C for 18 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active microorganisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth [2, 3, 13].

The Synergistic Effect

Commercially available antimicrobial disks (Table. 2) were applied on the surface of inoculated MHA by pressing slightly, and then 20µl from the extracts was carefully and slowly dispensed on the antibiotic disk. The plates were incubated at 37°C for 24 h. At the end of the period, the inhibition zone formed on the media was measured with a transparent ruler in mm. The diameters of inhibition zones were measured and compared with that of the plant extracts alone.

Antibiotics	Antibiotics potency	Manufactured by
Cefotaxime	30 µg	Bioanalyse, Turkey
Ofloxacin	5 µg	Himedia, Indian
Ceftriaxone	30 µg	Himedia, Indian
Ceftazidime	30 µg	Himedia, Indian
Amikacin	30 µg	Bioanalyse, Turkey
Neomycin	30 µg	Himedia, Indian
Ceflexin	30 µg	Himedia, Indian

Table 2 list of antibiotic potency

III. RESULT AND DISCUSSION

The antibacterial activity of the stem bark and leaf extracts of *F. sycomorus* and MIC value

The methanol and ethanol extracts of *Ficus sycomorus* bark showed the highest effect towards *S. aureus* (with a 15 mm zone

of inhibition), While only methanol extract of *F. sycomorus* (bark) was showed effect against *E. coli* with a zone of inhibition = 9 mm. The largest zone of inhibition against *P. aeruginosa* was observed with the ethanol and methanol extracts of *F. sycomorus* bark with a zone of inhibition (10 mm) as shown in Table3.

	Ethanol extract		Methanol extract		Water extract	
	LE*	SBE*	LE*	SBE*	LE*	SBE*
<i>S. aureus</i>	11	15	12	15	0	0
<i>E. coli</i>	8	8	0	9	0	0
<i>P. aeruginosa</i>	7	10	0	10	0	0

Table 3. Antibacterial activity of the leaf and stem-bark ethanol , methanol and water extracts of *F. sycomorus* against *S. aureus*, *E. coli* and *P. aeruginosa* using disc-diffusion method (zone of inhibition in mm) *LE – leaf extract; SBE – Stem-bark extra.

It was also noted that alcoholic extract has greater effect in the inhibition from aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts. The difference in antibacterial activity of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors

(temperature, light water), time of harvesting of plant materials and drying method used before the extraction process.

As shown in Table4. The methanol and aquatic extract of *F. sycomorus* (leaves) was significantly active exhibiting the highest potency with MIC from 6.25-3.125 mg/mL against *S. aureus*. This activity may be attributed to the rich plant contents of active components such as tannins, saponins, alkaloids and flavone aglycones.

	Ethanol extract		Methanol extract		Water extract	
	LE*	SBE*	LE*	SBE*	LE*	SBE*
<i>S. aureus</i>	25	12.5-6.25	6.25-3.125	6.25	6.25-3.125	25
<i>E. coli</i>	12.5	12.5	12.5-6.25	25-12.5	25	25
<i>P. aeruginosa</i>	25	50	25	25	50	50

Table4. Minimal inhibitory concentrations (MIC) of the plants extracts (mg/ml)

Synergistic activity of Plants Extracts and Antibiotics

In our study, the plant extracts had different synergistic ability to inhibit the growth of microorganism depending on the method of extraction.

As shown in **Table 5,6 and 7.** The strongest effect against *S. aureus* was recorded when aqueous extract of *F. sycomorus*

(leaves and bark) were mixed with Ceftriaxone. And the strongest effect on *E. coli* was observed when *F. sycomorus* (leaves and bark) were mixed with Ofloxacin.

The strongest effect against *P. areuginosa* was observed when Ceftazidime was combined with *F. sycomorus* (leaves and bark).

Antibiotics		Ethanol extract		Methanol		Water	
		LE	BSE	LE	BSE	LE	BSE
CTX	11*	15**	20	17	18	15	14
OF	20	25	19	28	22	25	20
CTR	12	14	16	15	16	11	14
CTZ	0	14	16	13	14	13	14
AK	20	24	24	22	24	24	26
N	20	19	20	18	20	18	16
CN	10	23	24	22	27	24	12

Table5. Synergistic effects of Antibacterial drugs with plant extracts on *S. aureus* (mm)

* antibiotics only, ** extract+ antibiotics.

Antibiotics		Ethanol extract		Methanol		Water	
		LE	BSE	LE	BSE	LE	BSE
CTX	8*	15**	13	12	14	12	14
OF	0	20	18	20	18	19	17
CTR	9	15	0	0	0	16	0
CTZ	11	9	7	7	0	0	7
AK	10	19	20	19	20	18	18
N	14	14	13	15	15	16	15
CN	0	0	0	0	0	0	0

Table6. Synergistic effects of Antibacterial drugs with plant extracts on *E. coli* (mm)

* antibiotics only, ** extract+ antibiotics.

Antibiotics		Ethanol extract		Methanol		Water	
		LE	BSE	LE	BSE	LE	BSE
CTX	0*	0**	0	0	0	0	0
OF	0	0	0	0	0	0	9
CTR	0	0	0	0	0	0	0
CTZ	9	14	14	14	13	13	14
AK	17	18	20	20	20	20	19
N	0	8	0	10	0	0	8
CN	0	0	0	0	0	0	0

Table7. Synergistic effects of Antibacterial drugs with plant extracts on *P. aeruginosa*

(mm). * antibiotics only, ** extract+ antibiotics. CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime; AK: Amikacin; GN: Gentamicin; N: Neomycin; CN: Ceflexin (Cephalexin).

IV. CONCLUSION

On the basis of the antibacterial assay of this study *S. aureus* was found the more (susceptible to the employed *F. sycomorus* extracts) than *E. coli* and *P. aeruginosa*.

Ethanol plant extracts were showed antimicrobial and synergistic activity with antibiotics better than methanolic and aquatic extracts, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts.. Otherwise, aqueous extract showed synergistic effect with most tested antibiotics against selected bacteria.

REFERENCES

[1] Abd Rabou. A, Yassin. M, Al- Agha. M, Madi. M, Al-Wali. M, Ali. A and Hamad. D (2008). Notes on some common flora and its uses in Wadi Gaza, Gaza Strip. The Islamic University Journal Vol.16 (1), 31-63

[2] Abou Elkair. E, Fadda. H and Abu Mohsen (2010). Antibacterial Activity and Phytochemical Analysis of Some Medicinal Plants from Gaza Strip-Palestine. Journal of Al Azhar University-Gaza, Vol. 12, 45-54.

[3] Abu-Shanab. B, Adwan. G, Abu-Safiya. D, Jarrar. N and Adwan. K (2004). Antibacterial Activities of Some Plant Extracts Utilized in Popular Medicine in Palestine. Turkish Journal of Biology Vol. 28, 99-102.

[4] Hassan. S, Lawal. M, Muhammad. B, Umar. R, Bilbis. L, Faruk. U and Ebbo. A (2007) Antifungal Activity and Phytochemical Analysis of Column Chromatographic Fractions of Stem Bark Extracts of *Ficus sycomorus* L. (Moraceae). Journal of Plant Sciences, Vol.2: 209-215.

[5] Jaradat. N (2005). Medical Plants Utilized in Palestinian Folk Medicine for Treatment of Diabetes Mellitus and Cardiac diseases Journal of Al-Aqsa university Vol.9.

[6] Jameela. M, Mohideen. A., Sunitha. K and Narayanan. M (2011) Antibacterial Activities of Three Medicinal Plant Extract against Fish Pathogens. International Journal of Biological Technology Vol.2(2):57-60.

[7] Jayaraman. S, Manoharan. M.S and Illanchezian. S (2008). In-vitro Antimicrobial and Antitumor Activities of *Stevia Rebaudiana* (Asteraceae) Leaf Extracts. Tropical Journal of Pharmaceutical Research; Vol.7 (4): 1143-1149.

[8] Kirbag. S, Zengin. F and Kursat. M (2009). Antimicrobial Activities of Extracts of some Plants. Pakistan Journal of Botany Vol.41(4): 2067-2070.

[9] Kumara. M, Agarwala. R, Deyb. K, Raib. V, Johnsonc. B (2009) Antimicrobial Activity of Aqueous Extract of *Terminalia chebula* Retz. on Gram positive and Gram negative Microorganisms. International Journal of Current Pharmaceutical Research Vol. 1 (1): 56-60.

[10] Madhuri S.and Pandey G (2009) Some anticancer medicinal plants of foreign origin. Current Science, Vol. 96, No. 6, 25.

[11] Olusesan. A, Ebele. L, Onwuegbuchulam. O, Olorunmola. E (2010) Preliminary in-vitro Antibacterial Activities of Ethanolic Extracts of *Ficus sycomorus* Linn. and *Ficus platyphylla* Del. (Moraceae). African Journal of Microbiology Research Vol. 4 (8), pp. 598-601.

[12] Parekh. J and Chanda. S (2006) In-vitro Antimicrobial Activities of Extracts of *Launaea procumbens* Roxb. (Labiatae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). African Journal of Biomedical Research, Vol. 9: 89 -93.

[13] Radojević. I, Stanković. O, Topuzović. M, Čomić. L and Ostojić. A (2012). Great Horestail (*Equisetum telmateia* Ehrh.): Active Substances Content and Biological Effects. Experimental and Clinical Sciences International Journal, Vol. 11:59-67.

AUTHORS

First/ Correspondence Author - Mr. Mohamed M. Jouda, .
M.Sc. Microbiology, Faculty of intermediate studies" Al Azhar
University of Gaza", mjouida87@hotmail.com,
00972598158169.

Second Author -Dr. Tarek Elbashiti, Assoc. prof. of
Biotechnology, Department of Biology and Biotechnology,
Islamic University of Gaza.
Third Author - Dr. Atef Masad, Assist. prof. of Biomedicine.
Fourth Author - M. Sc. Microbiology.