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In vitro Antibacterial Activity of *Ficus carica* L. (*Moraceae*) from Libya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BD and FIMA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors BD and SMBG managed the literature searches; analyses of the study performed the spectroscopy analysis. Authors MBS and FIMA done the analyses of the study with help of statisticians. Authors BD, NE and FIMA done and supervised the laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to assess the antibacterial effect of *Ficus carica* extracts against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, & Staphylococcus aureus*.
Study Design: Evaluation of antimicrobial activity using Cup-cut agar method.
Place and Duration of Study: Microbiology Research Laboratory, Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli, from October 2015 to March 2016.
Methodology: The leaves and stem part extracts of *Ficus carica* plant were prepared using

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maceration method. The antibacterial activities of the extracts were evaluated using Cup-cut agar method to determinate inhibitory zone diameters in millimeters of the plant extracts against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The measurement of exponential bacterial growth curves was used to determine the type of growth pattern spectrophotometrically at 600 nm. Furthermore, plate count methods were also used to enumerate the bacterial count and to determine the percentage of inhibition as well as IC_{50}.

Results: The results of this study showed that *Ficus carica* extracts used, exhibited antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The maximum zone of inhibition against *S. aureus* (27± 0.04mm, *p<0.05*) of methanol extract of stem part, while the minimum zone of inhibition was against *Klebsiella pneumoniae* (6 ± 0.04 mm) for methanol extract of leaf. The methanol extracts of stem part inhibited the *S. aureus* (27 ± 0.04 mm *p<0.05*), more than methanol extract of leaf (*S. aureus* 15 ± 0.06 mm). Latex had lower IC_{50} (1.69 ± 0.5w/v%) against *S. aureus* than *P. aeruginosa* (3.54 ± 0.2 w/v%); *E. coli* (8.24 ± 0.1w/v%), Leaf extract (0.79 ± 0.1 mg/ml) and stem part extract (0.204 ± 0.08mg/ml) against *S. aureus.*

Conclusion: *Ficus carica* methanolic extract was more effective against most of the tested bacteria than n-hexane extract, the stem part extract was more active as antibacterial than leaf extract against most of the tested bacteria except in case of *Klebsiella peneumonia* the leaf methanolic extract only inhibited it at zone of inhibition (6 ± 0.04 mm), while the n-hexane extract of leaf and stem part methanolic and n-hexane extract had no effect at all. Thereby, our results indicate that leaf and stem part of *Ficus carica* plant also latex has a strong antibacterial activity against Gram-positive and Gram-negative tested bacteria.

Keywords: Ficus carica; Escherichia coli; Klebsiella pneumoniae; Pseudomonas aeruginosa; Staphylococcus aureus.

1. INTRODUCTION

In the world, failure of treatment of various bacterial infectious diseases as multi-drug resistant bacteria is increasing. Therefore an urgent need to investigate an alternative source with established antimicrobial activity to overcome such problems [1]. Medicinal plants were useful to use as sources of antioxidant and antimicrobial compounds, and it is important to carefully investigate their composition and activity and their validate to use. There are various plants, which are traditionally used in medicine system for their medicinal and therapeutic potentials worldwide [2]. *Ficus carica* is one of the medicinally important plants that belong to the mulberry tree (*Moraceae*) and also one of the oldest fruits in the world. The common fig (*Ficus carica* L.) belongs to the *Moraceae*, a family with over 1400 species distributed in about 40 genera. The genus *Ficus* L. contains about 750 species of woody trees, epiphytes and shrubs, mainly of tropical and subtropical distribution, divided into six subgenera [3]. Traditional *Ficus carica* L are used for healing various diseases like, diabetes, ulcer, cancer, fever[4]. The roots of *Ficus carica* L are used in treatment of leucoderma and ringworms and its fruits, which are sweet, have antipyretic, purgative, aphrodisiac properties and have shown to be useful in the treatment of inflammations and paralysis [5]. *F. carica* has been reported to have antiviral, antibacterial, hypoglycemic, and anthelmintic effects [6]. The latex of *Ficus carica* L fruit has been used in several traditional herbal medicine remedies, most of them aimed to treat skin viral infections [7]. Leaves of *Ficus carica* show high degree of antimicrobial activity of methanol extract against oral bacteria. The combination effects of methanolic extract with ampicillin or gentamicin are synergistic against oral bacteria [8]. In *F. carica* leaves the two photoactive furanocoumarins, Psoralen and bergapten, were found. Psoralens are used in the treatment of skin diseases as dermatitis and eczema [9]. *F. carica* latex extracts contain strong anti-angiogenic and anti-proliferative activities that could be used as a potential agent for the prevention of angiogenesis in cancer [10]. This study was aimed at the evaluation of antibacterial activity of leaves, stem and latex of *F. carica* collected from the west of Libya.

2. MATERIALS AND METHODS

2.1 Plant Material

Latex, leaves and stem parts of *Ficus carica* L were collected in October 2015 from the area of

Tripoli city in Libya. Latex was collected and kept at 4°C, -20°C and -40°C until use, while leaves and stem were shade dried at room temperature for fifteen days to be ready for work.

2.2 Bacterial Strains

Standard bacterial strains used in this study were *Pseudomonas aeruginosa* NCTC 12903/ATCC*, Escherichia coli* NCTC 12241/ATCC 25922*, Staphylococcus aureus* NCTC 12973/ATCC, 14153 and *Klebsiella pneumoniae*, NCTC 9633/ATCC. The standard bacterial strains were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C.

2.3 Preparation of Plant Extracts

Ficus carica leaves or stem part were dried at room temperature and then ground to coarse powder. In order to prepare the extracts, 50g of the sample was separately extracted with 250mL methanol, after stirring for one week by using magnetic agitator, then the extractions were filtrated, evaporated in vacuum at 40°C, concentrated to dryness and the residue was kept at 4°C.

Methanol extract was added to the separation funnel and mixed with n-hexane and shaken well, then each layer was collected separately, the solvent was removed by using a rotary vacuum evaporator at 40°C to obtain a concentrated extract (methanol extract and n-hexane extract were then stored at 4°C until use).

2.4 Antimicrobial Assays

2.4.1 Cup-cut agar method

Antimicrobial susceptibility was tested using Cup-cut agar method [11], this method was used throughout this study to find out if the extract has the ability to inhibit bacterial growth. This method was performed using freshly prepared Mueller Hinton agar with overnight culture of bacteria inoculum, which in turn was prepared by suspending the freshly grown bacteria in sterile normal saline, and adjusted to a 0.5 McFarland standard. On each plate wells (5mm in diameter) were made using sterile cork borer. Each well was filled with 100µL of the tested extract and the plates were then re-incubated for 24hr at 37°C. The diameter of zones of inhibition were measured[12]. Inhibition zones were then measured to the nearest millimeter. Inhibition zones were indicated by a lack of microbial growth due to inhibitory concentrations. The antibiotics Ciprofloxacin (5µg) and A 5% (v/v) phenol were used as standards to compare the activity of extracts in inhibiting the growth of bacteria. Each experiment was carried out three times.

Latex extracts, methanolic & n-hexane extracts were diluted in 20%DMSO (dimethyl sulfoxide) directly before used.

2.4.2 Minimum inhibitory concentration (MIC)

All the materials and equipment used were sterilized by using autoclave. Inoculates were prepared by growing each strain of tested microorganism in nutrient broth [Difco, Detroit, Mich.] adjusted to a turbidity equal to that of a No. 0.5 McFarland standard, by using a blank Nutrient Broth (in order to get the bacteria number about $1x10^8$ CFU /ml). The extracts were prepared by taking the calculated weight of cured extracts and adding 1ml of 2% DMSO to give a final needed concentration followed by a serial two fold dilution of extracts was prepared in normal saline, the latex was prepared as 100%v/v (latex only), 50%v/v (latex diluted to half in 2% DMSO). A positive control tube was also inoculated in the same manner, all tubes were incubated at 37°C. Control tubes were tested after 24hr to determine whether the extract-containing tubes were ready to be read. This was accomplished by adding 0.02ml of Alamar blue at concentration (0.0125% (w/v) resazurin salt in PBS solution) to the positive control tube and incubating it for 10min at 37°C. the colour in the control tube changed from blue to pink after 10 mints of incubation, also Alamar blue was added to the extract-containing tubes and these were incubated for 10min at 37°C. The absorbance was measured at 570nm using UV spectrophotometer. The results were expressed as percentage reduction in bacteria viability compared to controls and concentrations that gave the 50% inhibition (IC_{50}) were calculated by Probit analysis[13]. The mean value was calculated from three separate experiments.

2.4.3 Optical density measurements and bacterial viable count tests

Twenty ml of an overnight bacterial culture was prepared, as mentioned above, and diluted to 1:50, to give an OD_{600} (600 nm) ≈ 0.05. The stem extract, as well as leaf extract at 1mg\ml

and the Latex at concentration 50%v/v were added respectively. The inoculated cultures were incubated at 37°C in an orbital shaker. Growth was measured, in triplicate, at an optical density of 600 nm using a UV spectrophotometer (Biochrom UK) at 0.4 every 4 hrs for 24 hrs. The Plate count method was used to enumerate the microbial count. One ml of each sample was serially diluted (10^{-1} to 10^{-8}) with 9.0 ml of 0.1% peptone water. Then, 0.1 ml of the diluent was inoculated onto Plant Count Agar (Oxoid). All the plates were incubated at 30ºC for 24-48 hrs. The Colony counts were converted to CFU per ml according to the criteria specified by ISO, 2003 [14].

2.5 Statistical Analysis of Data

The data was tested for normality using a QC Analyses/K-S Normality Test. Normally distributed data was analyzed by student's t-test or one-way analysis of variance (ANOVA) combined with Fisher's LSD test post-hoc, using the Statview® version 5.0.1 software package (SAS Institute Inc, Abacus Concept, *Inc.,* Berkeley, CA, USA). A p value of < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

The results in Table 1 show that methanol extract of leaves was the most active, it was active against all strains tested, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with another polar solvent [15]. Since polyphenols and flavonoids are described as the active compounds in *Ficus carica* this was expected they don't dissolve in n-hexane [16]. The results were found to have a higher zone of inhibition for methanol extract of stem than leaf extracts. The maximum zone of inhibition against *S. aureus* (27 ± 0.04 mm *p<0.05*) for the stem part methanol extract, while the minimum zone of inhibition was against *Klebsiella pneumoniae* (2 ± 0.04 mm) for leaf methanol extract and no inhibition against it when n-hexane and methanol extract of stem and n-hexane extract of leaf were used. The methanol extracts of stem inhibited the *S. aureus* (27 ± 0.04 mm *p<0.05*), more than leaf methanol extract (*S. aureus* =15±0.06 mm). This coincides with other work findings, in that methanol stem part extract has higher effect on *S. aureus* (18 ± 0.3 mm) than leaf methanol extract *S. aureus* (16 ± 0.4 mm) [17].

In this study the methanol extract of both stem part and leaf had more antibacterial activity than n-hexane extract. A pervious study reported that difference in biological activity between acetone and methanol extracts could be attributed to the difference in phytochemical compounds; where flavonoids, saponins, terpendois and tannins were present in Fig methanol extract; while, that of acetone extract was flavonoids and phenol [17].

The extracts of stem and leaf showed activity against gram positive more than Gram-negative. The higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of outer membrane lipopolysaccharides [15]. Also these observations are likely to be the consequences of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, thus the Gram-negative outer membrane can act as a barrier against many environmental substances, including antibiotics [18].

It was noted that in the latex of *Ficus carica*, almost 91% of the active constituents found in it were coumarins. It was also noticed that the *Ficus carica* latex exerted powerful anti-bactericidal properties against several species of bacteria [19]. The results of antibacterial activity of latex of *Ficus carica* are indicated in Table 2. It showed that the fresh collected latex of *F. carica* exhibited strong activity against the gram positive bacteria (*S. aureus* 32±0.03mm in diameter as inhibition zone), and the gram negative bacteria (*E. coli* 29 ± 0.06mm, *P. aeruginosa 25* ± 0.02mm*)* as compared with control treatment (35mm±0.1 when treated with the antibiotic ciprofloxacin or phenol 34mm±0.09), the activity of latex was reduced by dilution to more than the half against tested bacteria. On storage of latex of *F. carica* for one week at 4°C, the antibacterial activity against tested bacteria was completely lost, while when stored at -20°C the activity was reduced to less than activity of fresh collected latex. The antibacterial activity of latex of *F. carica* stored at -40°C is as strong as fresh collected. The solvents control "normal DMSO" had no inhibitory effect on bacterial growth.

The antibacterial activity of latex could be related to the presence of flavonoids, terpenes and steroids, alkaloids, saponins and tannins which possess diverse biological effect like antioxidant, anti-inflammatory and antibacterial activities [20]. In another study that used the

green fruit latex which was collected from Chott Mariam Souse, Middle East coast of Tunisia, the antimicrobial activity of the extracts were evaluated and based respectively on the inhibition zone using the disc-diffusion assay, the methanolic extract had no effect against bacteria except for Proteus mirabilis while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*Enterococcus fecalis, Citobacter freund*ei, *Pseudomonas aeruginosa, Echerchia coli* and *Proteus* mirabilis) [21].

When the methanolic extract of stem and leaf were compared with Latex 100%w/v (Table 3), the results showed that stem part extract and latex had higher activity against tested bacteria compared to leaf extract. The latex had the highest zone of inhibition 27±0.8 mm, 23±0.7 mm and 20±0.2 mm against *S. aureus, E. coli* and *P. aeruginosa* respectively. A pervious study demonstrated that only glycosides and saponin extracted from *F. carica* leaves using alcohol as solvent had biological effects but they had no effects on, *S. aureus* and *E. coli* [7], compared to this study in which latex extracts are more active than leaf extracts on human pathogenic bacteria. Latex had no effect on *Klebsiella pneumonia* under all conditions.

The effect of latex, leaf extract and stem extract on the growth curve of bacteria shows that the latex and methanolic stem extract reduced the OD value of bacteria culture, which is considered a significant reduction compared to negative control. In *S. aureus,* the latex had the highest effect on the reduction of the bacteria growth curve, then stem and leaf extract (Fig. 1) and the percentage of growth inhibition (Fig. 2). Latex had less effect on *P. aeruginosa, E. coli* (Fig. 1), as well as the % of growth inhibition (Fig. 2). Latex had less IC_{50} (1.69±0.5v/v%) against *S. aureus* than *P. aeruginosa* (3.54 ± 0.2v/v%), *E. coli* (8.24 ±0.1v/v%), Leaf extract (0.79 ± 0.1 mg/ml) and stem part extract (0.204 ± 0.08 mg/ml) against *S. aureus* (Table 4).

Table 1. Evaluation the antimicrobial activity of methanol or n-hexane extracts of *Ficus carica* leaves and stems

Bacteria	Zone of inhibition (mm ± SE)					
	***Ficus carica* leaves**		***Ficus carica* stem**		**Phenol**	**Ciprofloxacin**
	Methanol extract (0.1mg/ml)	**n-hexane Extract (0.1mg/ml)**	**Methanol extract (0.1mg/ml)**	**n-hexane Extract (0.1mg/ml)**		
P. aeruginosa	14 ±0.02	6±0.06	20±0.03	10±0.01	30±0.01	35±0.02
K. pneumoniae	2±0.04	0±0	0±0	0±0	28±0.01	33±0.05
E. coli	7±0.02	6±0.02	23±0.03	10±0.05	25±0.01	35±0.01
S. aureus	15±0.06	6±0.03	27±0.04	13±0.03	29±0.01	30±0.02

Table 2. Evaluation of the effect of storage temperature on antimicrobial activity of *Ficus carica* latex using cup cut agar method

Bacteria	Zone of inhibition (mm ± SE)						
	Fresh collected Latex		**Stored Latex**			**Phenol**	**Ciprofloxacin**
	100%v/v	**50% v\v**	**4°C**	**-20°C**	**-40°C**		
P. aeruginosa	25±0.2	10±0.4	0±0	10±0.2	24±0.2	34±0.09	35±0.1
K. pneumonia	0±0	0±0	0±0	0±0	0±0	28±0.01	33±0.05
E. coli	29±0.06	17±0.1	0±0	13±0.1	20±0.4	30±0.1	25±0.7
S. aureus	32±0.03	25±0.1	0±0	10±0.2	27±0.1	30±0.3	27±0.5

Table 3. Antibacterial activity of plant extracts and freshly collected latex against *P. aeruginosa, E. coli,* and *S. aureus*

Bacteria	Zone of inhibition (mm ± SE)		
	Latex	**Leaf extract**	**Stem part extract**
P. aeruginosa	25±0.2	14±1	20±0.2
K. pneumoniae	0±0	2±0.04	0±0
E. coli	29±0.1	7±0.5	23±0.7
S. aureus	32±0.5	15±1	27±0.8

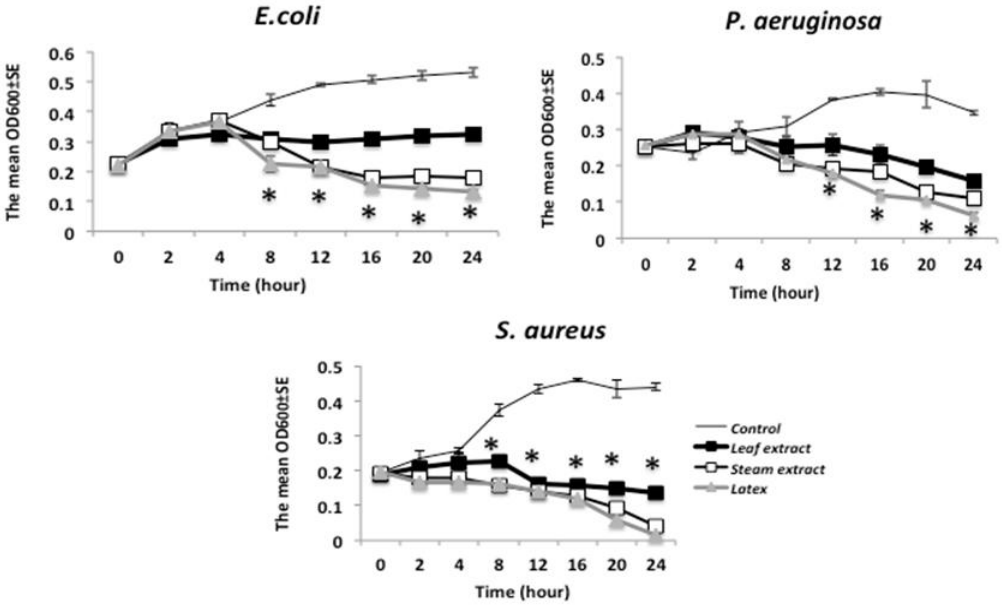

Fig. 1. Effect of plant-derived drugs on the bacteria growth according to the incubation time
(p<0.05 comparing to negative control)*

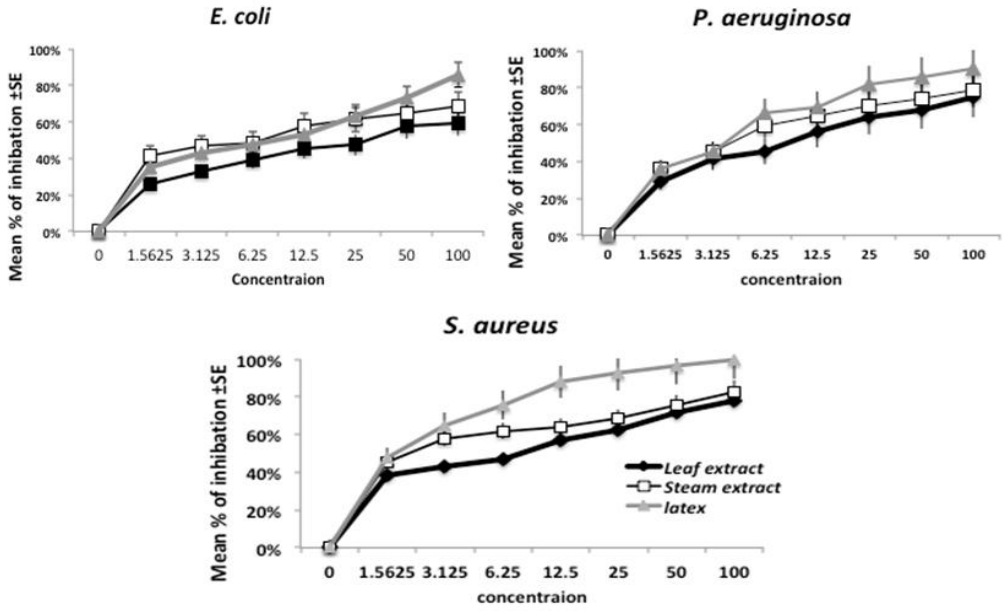

Fig. 2. Bacterial inhibitory effect of plant-derived drugs

Table 4. Antibacterial activity of plant-derived drugs: Methanol extract of leaf and stem (mg/ml ±SE) and freshly collected latex (% v/v ± SE)

Bacteria	IC_{50} (concentration ±SE)		
	Latex	Leaf extract	Stem extract
P. aeruginosa	3.54±0.2	0.865±0.1	0.396±0.02
E. coli	8.24±0.1	3.2±0.5	0.755±0.07
S. aureus	1.69±0.5	0.79±0.1	0.204±0.08

Previous study investigated the antimicrobial activity of methanol extract of figs against oral bacteria. Showed that the results of the antibacterial activity that the methanolic extract of *F. carica* leaves exhibited strong activities against, *P. aeruginosa,* and *P. gingivalis* (MIC, 0.156 to 0.625 mg/ml; MBC, 0.313 to 0.625 mg/ml), while *E. coli* , *S. aureus* appeared to be less sensitive (MIC, 2.5 to 10 mg/ml; MBC, 2.5 to 10 mg/ml). The MIC and MBC for ampicillin were found to be either 0.5/0.5 or 256/256 µg/ml; for gentamicin, either 2/2 or 256/512 µg/m l[22]. The study was done on the *Ficus carica* leaves and latex in Baghdad, they found that *S. aureus* was the most sensitive to the latex and ethanol extracts, while *P. aeruginosa*. E*scherichia coli* showed resistance to the extracts [23]. This study indicates that fig latex has antimicrobial activity against some bacteria.

4. CONCLUSION

The present study was designed to obtain information on the antimicrobial effect of stem, leaf extracts and latex of *Ficus carica* plant against *Escherichia coli, Klebsiella pneumoniae , Pseudomonas aeruginosa, & Staphylococcus aureus*. The results showed that *Staphylococcus aureus* was more sensitive to the fresh collected latex, with the activity of Latex deminishing when stored. The methanolic extract of leaves was active against all strains tested, which may be due to the active compounds extracted from the plant being dissolved better when methanol was used as a solvent rather than other solvents. On the basis of this study *S. aureus* was found to be more susceptible to the employed fig extracts than other bacteria strains tested, while, *K. pneumoniae* was the most resistant to most extracts.

The result of these studies indicate the importance of *Ficus carica* plant extracts as antibacterial agents which would be helpful in future study for synthesizing or developing the plant based antibacterial agent that could be used for preventing and curing the common diseases of human; to reduce the pathogen population.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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