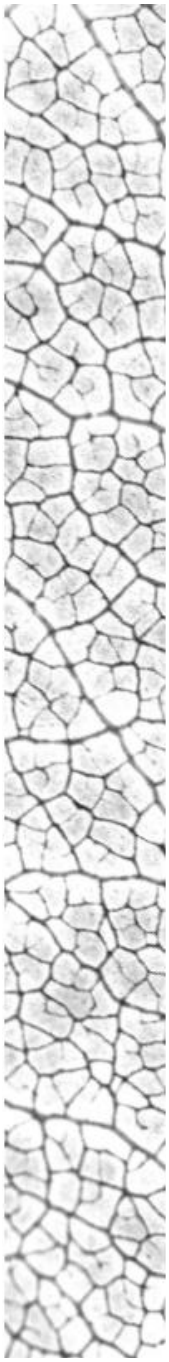

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Antibacterial activity of phytochemical compounds extracted from *Ficus carica* Linn. leaves against human pathogenic bacteria

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Abstract
The current study was accompanied to investigate the influence of phytochemical compounds extracted from leaves of *Ficus carica* Linn.by using three different solvents such as Ethanol, Acetone, and Chloroform against some human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections in Iraq, such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella sp,* and *Klebsiella sp*. The antibacterial activity was performed by using agar well diffusion method against some human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections by preparing three concentrations (300, 400, and 500 mg/ml) for each extract. Dimethyl sulfoxide 10% was used as a negative control and different types of antibiotics based on the types of pathogenic bacteria as a positive control. Phytochemical compounds extracted by ethanol solvent at (400 and 500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics when applied to *Klebsiella* sp. Phytochemical compounds extracted by acetone solvent at (500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics when applied to *Klebsiella* sp. Whereas, phytochemical compounds extracted by chloroform solvent at (300, 400, and 500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics when applied to *Klebsiella* sp. And also, phytochemical compounds extracted by chloroform solvent at (500 mg/ml) showed significant superiority at (P≤ 0.05) over the Penicillin and Tetracycline antibiotics when applied to *Enterobacter aerogenes*. Finally phytochemical compounds in leaves of *Ficus carica* L. regard a good source for controlling human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections.

Keywords: Ficus carica Linn., antibacterial activity, pathogenic bacteria

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INTRODUCTION

Ficus genus form Moraceae family, It is a large genus of trees, shrubs or climbers which covers over 800 species and is distributed throughout the tropical countries. Ficus is spread in Mediterranean area and Southwest of Asia, in a region extending from Turkey to eastern Spain and ending in western of Portugal; it is also implants commercially in portions of United State of America and Chile and to small degree, in India, Arab countries, Iran, Japan, and China (Sastri, 1950; Adeyeye, et al, 2016). The species are particularly widespread in the Indo-Malayan region and in the islands of Pacific Polynesia. Six species of Ficus have been recorded from Iraq: Ficus benghalensis Linn, Ficus cunia Buch-Ham., Ficus clastica Roxb, Ficus hispida Linn, Ficus nitida Blume and Ficus carica Linn. Common Fig F. carica Linn. (Arabic name Tin) is a small deciduous tree, leaves petioled, scabrous at the upper surface, pubescent or velvety at lower, ovate or cordate at the base, 3-5 lobed (Townsend and Guest, 1974). The main cause of human morbidity and mortality has always been pathogenic bacteria. Although drug companies have in recent years produced a number of new antibacterial agents, drugs resistance has augmented and is now converted a global issue (Adwan and Mhanna, 2008). The global appearance of multi-drug resistant (MDR) microorganisms is progressively restraining the efficiency of present medicines and expressively causing treatment disappointment

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Table 1. Types of antibiotic, and concentration per disc in this study

Antibiotic	Concentration (Disc/µg)	Bacteria
Tetracycline	30	*Staphylococcus aureus*
Tetracycline	30	*Enterococcus faecalis*
Penicillin	10	*Enterobacter aerogenes*
Ciprofloxacin	5	*Pseudomonas aeruginosa*
Tetracycline	30	*Escherichia coli*
Ciprofloxacin	5	*Shigella sp.*
Ciprofloxacin	5	*Klebsiella sp.*
Penicillin	10	
Clindamycin	15	
Trimethoprim	30	
Amoxicillin	5	
Tetracycline	30	

(Hancock, 2005). Phytochemicals play an important role in the routine healthcare systems worldwide. The major classes of phytochemicals like alkaloids, phenolics, terpenoids and tannins have potential to prevent diseases and act as anti-microbial, anti-inflammatory, antioxidant, anti-cancerous, detoxifying agent, Potential immunity agent and neuropharmacological agent. Each class of these functional agents comprises of a broad variety of compounds with differing effectiveness. Some of these phytochemicals are found to be multifunctional (Koche, et al, 2016). Undoubtedly, the resistance of bacteria to antibiotics is a global problem, so there is an urgent need to search for new sources used in the manufacture of new antibodies that are capable of facing the threat of bacteria. However, the aimed of this study to investigate the biological activity of phytochemical compounds extracted from leaves of (*Ficus carica* Linn.) against to human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections.

MATERIALS AND METHODS

Plant material: Fig (*Ficus carica* Linn.) leaves were collected from gardens at Hillah City in Babil Governorate, during October 2019. Plants were identified at University of Babylon, Faculty of Science for women. The leaves were cleaned, dried, and kept according to (Harborne et al. 1975).

Plant materials extraction: Phytochemical compounds were extracted by using digestion methods, by using 50 gm of plants materials powder instant in 250 ml of Ethanol, Acetone, and Chloroform solvents separately, then shake it well for 1 hour, after that leave it for 72 hours in water path at 40C˚ to complete the process of extraction, and then dried (Handa et al. 2008). The stock solutions of (500 mg/ml) have been prepared in 10% Dimethyl Sulfoxide solvent (DMSO), and then, sterilized by Millipore filter, pore size (0.22µm) and stored at (-20C˚) till usage (Al-Jassani, 2017).

Antibacterial Efficacy: The anti-bacterial activity of the bioactive compounds extracted from the leaves of (*Ficus carica* Linn.) was tested against the isolated bacteria by using agar-well diffusion method (Perez *et al*., 1990). Wells were made by using cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control and different types of antibiotics based on the types of pathogenic bacteria as a positive control (**Table 1**).

Table 2. Types of Bacterial Isolates and their sources

No	Bacteria isolate	Type of specimen
1	*Staphylococcus aureus*	Urinary tract infections
2	*Enterococcus faecalis*	Intistitis infections
3	*Enterobacter aerogenes*	Intistitis infections
4	*Pseudomonas aeruginosa*	Urinary tract infections
5	*Escherichia coli*	Intistitis infections
6	*Shigella sp*	Intistitis infections
7	*Klebsiella sp*	Intistitis infections

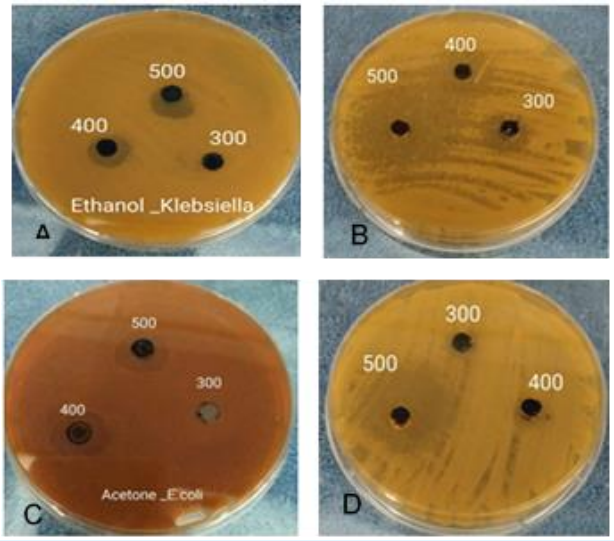

Fig. 1. (A) Antibacterial activity of ethanolic extract of (*Ficus carica*) at (300, 400, and 500mg/ml) against *Klebsiella sp* (B) Antibacterial activity of chloroform extract of (*Ficus carica*) at (300, 400, and 500mg/ml) against *Klebsiella sp*. (C) Antibacterial activity of acetone extract of (*Ficus carica*) at (300, 400, and 500mg/ml) against *E. coli.* (D) Antibacterial activity of chloroform extract of (Ficus carica) at (300, 400, and 500mg/ml) against E. aerogenes.

Bacterial Isolates: All isolates used in this study was isolated from Urinary tract infections and Intistitis infections at Hillah city, Iraq (**Table 2**).

Statistical analysis: All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at P≤ 0.05.

RESULTS

The antibacterial activity of phytochemical compounds extracted from (*Ficus carica* Linn.), leaves by using different types of solvents like (Ethanol, Acetone, and Chloroform) against human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections is presented in **Tables 3**-**5**. Activity of the plant was screened by agar well diffusion methods (**Fig. 1A**-**1D**). The results revealed that, the extracts of ethanolic, acetone, and chloroform of (*Ficus carica*

Table 3. Antibacterial activity of the crude Ethanolic extract of *Ficus carica* Linn. against some human pathogenic bacteria

Concentration	Pathogenic bacteria						
	S. aureus	***E. faecalis***	***E. aerogenes***	***P. aeruginosa***	***E.coli***	***Shigella sp.***	***Klebsiella sp.***
	Inhibition zone/mm						
Control negative DMSO (10%)	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
300mg/ml	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
400mg/ml	0± 0	12± 1	0± 0	0± 0	0± 0	0± 0	11± 1
500mg/ml	0± 0	13± 1	14± 1	0± 0	0± 0	16± 1	13.6± 0.57
Control positive	33± 0	25± 0	15± 0	32± 0	15± 0	30± 0	0± 0
	Tetracycline	Trimethoprim	Penicillin	Ciprofloxacin	Tetracycline	Ciprofloxacin	Resistant
L.S.D	0	1.15	0.81	0	0	0.82	1.15

*Mean± standard deviation

Table 4. Antibacterial activity of the crude acetone extract of Ficus carica Linn. against some human pathogenic bacteria

Concentration	Pathogenic bacteria						
	S.aureus	***E.faecalis***	***E.aerogenes***	***P.aeruginosa***	***E.coli***	***Shigella sp.***	***Klebsiella sp.***
	Inhibition zone/mm						
Control negative DMSO (10%)	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
300 mg/ml	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
400 mg/ml	11± 1	0± 0	0± 0	0± 0	13.3± 0.57	0± 0	0± 0
500 mg/ml	15± 1	0± 0	0± 0	13± 1	15.6±0.57	0± 0	15± 1
Control positive	33± 0	25± 0	15± 0	32± 0	15± 0	30± 0	0± 0
	Tetracycline	Trimethoprim	Penicillin	Ciprofloxacin	Tetracycline	Ciprofloxacin	Resistant
L.S.D	1.15			0.81	0.66		0.81

*Mean± standard deviation

Linn.) leaves showed significant reduction at P≤ 0.05 in the growth of pathogenic bacteria. Antibacterial activity was applied at (300, 400, and 500 mg/ml), and then, compared with 10% dimethyl sulfoxide (DMSO) as a negative control and different types of antibiotic based on pathogenic bacteria as a positive control, Inhibitory zone diameter increase significantly at (P≤ 0.05) by increasing concentration from 300 to 500 mg/ ml. The results also revealed that, phytochemical compounds extracted by ethanol solvent at (400 and 500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics (**Table 6**) as the inhibition diameter reached to (11± 1 and 13.6± 0.57) in the plant extract compared with (0± 0) in all antibiotics under study when applied to *Klebsiella sp.* pathogenic bacteria. Also, phytochemical compounds extracted by ethanol solvent at (500 mg/ml) showed a similar effect (There in no significant effect at P ≤ 0.05) between medicinal plant and the Penicillin antibiotic as the inhibition diameter reached (14± 1) in the plant extract compared with (15±1) in the antibiotic when applied to *E. aerogenes* pathogenic bacteria. On other hand, *S. aureus, P. aeruginosa,* and *E. coli* were showed completely resistance to ethanolic extract in different concentrations under study (**Table 3**).

The present investigation also revealed that, there are significant decrease in the growth of pathogenic bacteria with the increasing of concentration of phytochemical compounds extracted by acetone solvent compared with the negative control DMSO 10% (**Table 4**). In the same context, The results also revealed that, phytochemical compounds extracted by acetone solvent at (500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics (**Table 6**) as the inhibition diameter reached to (15± 1) in the plant extract compared with (0± 0) in all antibiotics under study when applied to *Klebsiella sp.* pathogenic bacteria. Also, phytochemical compounds extracted by ethanol solvent at (500 mg/ml) showed a similar effect (There in no significant effect at P ≤ 0.05) between medicinal plant and the Tetracycline antibiotic as the inhibition diameter reached (15.6±0.57) in the plant extract compared with (15± 0) in the antibiotic when applied to *E. coli* pathogenic bacteria. In contrast, E. faecalis, E. aerogenes, and Shigella sp. were showed completely resistance to acetone extract in different concentrations under study (**Table 4**).

The current study also uncover that, phytochemical compounds extracted by chloroform solvent at (300, 400, and 500 mg/ml) showed significant superiority at (P≤ 0.05) over the Ciprofloxacin, Penicillin, Clindamycin, Trimethoprim, Amoxicillin, and Tetracycline antibiotics (**Table 6**) as the inhibition diameter reached to (15± 1, 20.3± 0.57, and 25± 1) in the plant extract compared with (0± 0) in all antibiotics under study when applied to *Klebsiella sp.* pathogenic bacteria. In the same context, phytochemical compounds extracted by chloroform solvent at (500 mg/ml) showed significant superiority at (P≤ 0.05) over the Penicillin and Tetracycline antibiotics as the inhibition diameter reached to (30± 1) in the plant extract compared with (15± 0) in Penicillin antibiotic when applied to E. aerogenes. Whereas, reached to (20.6 ± 0.57) in the plant extract compared with (15± 0) in Tetracycline antibiotic when applied to E. coli pathogenic bacteria. (**Table 5**).

Table 5. Antibacterial activity of the crude Chloroform extract of Ficus carica Linn. against some human pathogenic bacteria

Concentration	Pathogenic bacteria						
	S.aureus	***E.faecalis***	***E.aerogenes***	***P.aeruginosa***	***E.coli***	***Shigella sp.***	***Klebsiella sp.***
	Inhibition zone/mm						
C Negative DMSO (10%)	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0	0± 0
300 mg/ml	0± 0	0± 0	11± 1	0± 0	13.3± 0.57	0± 0	15± 1
400 mg/ml	0± 0	0± 0	15± 1	0± 0	16± 1	11± 1	20.3± 0.57
500 mg/ml	0± 0	0± 0	30± 1	0± 0	20.6± 0.57	13.3± 0.57	25± 1
Control positive	33± 0	25± 0	15± 0	32± 0	15± 0	30± 0	0± 0
	Tetracycline	Trimethoprim	Penicillin	Ciprofloxacin	Tetracycline	Ciprofloxacin	Resistant
L.S.D			1.40		1.04	0.93	1.24

*Mean± standard deviation

Table 6. Types of antibiotic, and their effect in the Bacterial isolates

Bacteria	Types of antibiotic					
	Cip 5µg	**Pen10 µg**	**Clin 15 µg**	**Trim 25 µg**	**Amo 30 µg**	**Tetra 30 µg**
	Inhibition zone/mm					
Staphylococcus aureus	33± 0	20± 0	R	20± 0	15± 0	33± 0
Enterococcus faecalis	R	R	R	25	R	22± 0
Enterobacter aerogenes	10± 0	15± 0	R	R	R	15± 0
Pseudomonas aeruginosa	32± 0	R	R	22± 0	R	30± 0
Escherichia coli	R	R	R	R	R	15± 0
Shigella sp	30± 0	22± 0	R	R	15± 0	20± 0
Klebsiella sp	R	R	R	R	R	R

DISCUSSION

Until such time as the pharmaceutical industry is able to manufacture medical drugs economically, medicinal plants will remain an important source of the pharmaceutical drugs industry. The Fig leaves comprise numerous active phytochemical compounds for example, alkaloids, flavonoids, saponins, tannins, and sesquiterpenes (Tchombe and Louajri, 2015) that possess biological actions as antibacterial, antioxidant, anticancer, antiviral, and anti-inflammation (Badgujar et at. 2014). The extracts of Fig leaves comprise; flavonoids, alkaloids, tannins, terpenes, saponins and steroids (Rashid et al. 2014). The phytochemical detecting of Fig leaves extract exhibited positive results of flavonoids, terpenoids, and tannins but, exhibited negative results of saponins and alkaloids (Nirwana et al, 2018). Bioactive compounds such as phenolic, terpenoids, and alkaloids extracted from several medicinal plants like *(Lactuca serriola L., Lepidium sativum L., Myrtus Communis L., Cassia senna L., Ricinus communis L., Cassia didymobotrya* (Fresenius) *Irwin & Barneby, Melia azedarach L. and Dianthus caryophyllus L.)* have antibacterial efficacy against different pathogenic microorganisms (Al-Marzoqi et al. 2015; Al-Marzoqi et al. 2016; Hussein et al. 2017; Hussein et al. 2018a; Hussein et al. 2018b; Hussein et al. 2019; Hussein and Al-Marzoqi, 2020; Kamil et al. 2020). Hussein et al. (2018c) were used primitive plant like Chlorella vulgaris as antibacterial against pathogenic microorganisms. Kamal et al. (2019) were used Hibiscus sabdarifa extracts against members of Enterobacteriaceae microorganisms. Al Masoodi et al. (2020) were used *Curcuma longa L.* and Boswellia carteri Birdwood against Fusarium species isolated from maize seeds. The methanolic extract of Fig leaves showed a strong antibacterial activity against *Streptococcus gordonii, Streptococcus anginosus, Prevotella intermedia, Porphyromonas gingivalis, and Aggregatibacter actinomycetemcomitans* oral bacteria (Jeong et al. 2009). The aqueous extract was active counter to Gram positive bacteria more than Gram-negative bacteria but not active against yeast strains (Al Askari et al. 2013). Methanolic extract of F. carica leaves exhibited the antibacterial effect against pathogenic as well as non-pathogenic test bacteria (Ahmad et al. 2013). The ethanolic extract of leaves showed strong action in contradiction of the bacteria *S. aureus* and *S. typhi* (Rashid et al. 2014). Fig leaves extract at fifty percent concentration has antibacterial action counter to E. faecalis (Nirwana et al, 2018). In contrast, natural bioactive compounds extracted from medicinal plants make their effects by many mechanisms, for example polyphenols binding with polysaccharides and proteins (Macromolecules), thus inhibiting their roles in biochemical metabolites. Terpenoids and flavonoids make their effects by disruption of microbial membranes and Polypeptides embarrassment of linkage of bacterial proteins to host polysaccharide receptors and alkaloids complexes make their effect by inhibiting of efflux pump (Okusa et al. 2009). For the meantime, terpenoids compounds are also recognized to be active counter to microorganisms, the antibacterial action of terpenoids compounds are thought to involve microorganism membrane disruption triggered by the lipophilic compounds (Działo et al. 2016). Antibacterial strategy of flavonoids was to inhibit synthesis of nucleic acid, inhibit energy metabolism and disturb cytoplasmic membrane function and bacterial membrane damage (Cushnie and Lamb, 2005). Additionally, tannins considered as toxic compounds for microorganisms can bind their cell walls, and stop their growth and protease enzyme activity or

denaturation of proteins as a result to formation of hydrogen bonds between tannins and proteins of pathogenic bacteria and this leads to a weakening of metabolic processes within the bacterial cell. Finally, antibacterial efficacy of (Ficus carica Linn.) might be belonging to phytochemical compounds and their effect in proteins and polysaccharides and disruption in membranes permeability or inhibiting of efflux pump.

CONCLUSION

Phytochemical compounds extracted from *Ficus carica* Linn. by using different types of organic solvents such as (Ethanol, Acetone, and Chloroform) regard a good source for controlling human pathogenic bacteria isolated from Urinary tract infections and Intistitis infections.

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