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Antimicrobial activity of fig (*Ficus carica* Linn.) leaf extract as compared with latex extract against selected bacteria and fungi

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Abstract

Ethanolic leaf extract and latex of fig (*Ficus carica* L.) were investigated for their antimicrobial activity against six bacterial strains, two gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and four gram negative (*Klebsiella pneumonae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*), and three fungal strains (*Candida albicans*, *Fusarium oxysporum*, *Aspergillus nigar*), using agar well diffusion method for determination of inhibitory zone diameters (IZD). The detection of some active compounds was carried out by chemical methods.

The results obtained showed that the extracts of fig contain; flavonoids, tannins, terpenes and steroids, alkaloids and saponins. The ethanolic extract of leaves exhibited strong activity against the bacteria *Staph. aureus* and *Salm. typhi* (13 mm, 14 mm IZD),and the fungi *Fusarium oxysporum* (16 mm IZD), whereas The latex showed higher activity against these bacteria (15 mm IZD) for each of them, and the fungi *Aspergillus nigar* (18 mm IZD). *Kleb. pneumonae* and *E. coli* seemed to be resistant to both extract which showed (8 mm, 9 mm) and (11 mm, 10 mm IZD) with ethanolic extract and latex respectively. This study was indicated that fig leaf and latex extracts have antimicrobial activity against some pathogenic infections.

الخلاصة

فحصت الفعالية المايكروبية لأوراق وحليب التين ضد ستة انواع من البكتريا، اثنتان موجبة لصبغة جرام (*Staphylococcus aureus*، *Streptococcus pyogenes*) واربعة سالبة للصبغة (*Klebsiella pneumonae*، Pseudomonas aeruginosa، Salmonella typhi، *Escherichia coli*) وثلاثة انواع من الفطريات (*Candida albicans*، *Fusarium oxysporum*، *Aspergillus nigar*)، باستعمال طريقة الانتشار وتحديد اقطار مناطق التثبيط. لقد تم اجراء الكشف عن المركبات الفعالة باستعمال الطرائق الكيمياوية.

بينت النتائج ان مستخلصات التين تحتوي على : flavonoids و tannins و terpenes و steroids و alkaloids و saponins. اظهرت الخلاصة الايثانولية للأوراق فعالية قوية ضد بكتريا *Staph. aureus* و *Salm. typhi* (قطر منطقة التثبيط ١٣ ملم و ١٤ ملم على التوالي) والفطر *F. oxysporum* (قطر منطقة التثبيط ١٦ ملم) في حين اظهر حليب التين اكثر فعالية ضد انواع البكتريا ذاتها حيث بلغ قطر منطقة التثبيط (١٥ ملم) لكلاهما، وبلغ للفطر*Fusarium oxysporum* (١٨ ملم). ابدت البكتريا *Kleb. pneumonae* و *E. coli* مقاومة لكلا المستخلصين اذ بلغ قطر مناطق التثبيط (٨ ملم، ٩ ملم) و (١١ ملم، ١٠ ملم) للمستخلص اليثانولي وحليب التين على التوالي. اكدت هذه الدراسة وجود فعالية مايكروبية لمستخلصات وحليب التين ضد بعض الاصابات المرضية.

Introduction

Fig (*Ficus carica* Linn.) belongs to the family Moraceae which is one of the oldest fruits in the world. Ficus constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees, and shrubs. Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important (Jander and Machado, 2008). The fig is a very nourishing food and used in industrial products. It is rich in vitamins,

mineral elements, water, and fats. Figs are one of the highest plant sources of calcium and fiber (Vinson, 1999).

The dried figs produced a significant increase in plasma antioxidant capacity and also used in various disorders such as gastrointestinal respiratory inflammatory cardiovascular disorders, ulcerative diseases, and cancers (Vinson et al., 2005; McGovern, 2002). Some active constituents found in *Ficus carica latex include natural furocoumarins,* phytosteroids, 18 fatty acids, and certain amino acids, phytosterols, polyunsaturated fatty acids and phenolic acids (Robnov etr al., 2001; canal et al., 2000).

In traditional medicine the roots of fig 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 inflammations and paralysis (Ross and Kasum, 1995; Nadkarni and nadkarni, 2002). *F. carica* has been reported to include antiviral, antibacterial, hypoglycemic, and anthelmintic effects (Wang et al., 2004; Solomon, 2006; Jeong et al., 2005). The latex of fig fruit has been used in several traditional herbal medicine remedies, most of them aimed to treat skin viral infections (Houda et al., 2010).

In a study performed on the latex of *Ficus carica*, it was observed that almost 91% of the active constituents found on it were coumarins**.** It was also notice that the *Ficus carica* latex exerted powerful anti-bactericidal properties against several species of bacteria (Mi-Ran et al., 2009).

The recent studies suggest that the anti-inflammatory and antioxidant activity of *Ficus carica* latex could be due to the presence of steroids and flavonoids, due to its free radical scavenging activity, more present in darker fruits than in lighter ones. *Ficus carica* leaves have been commonly used to cure hemorrhoids (Vaya and Mahmood, 2006).

This study was aimed to present an overview of bioactive compounds present in this plant and the antimicrobial activity of leaf and latex extracts against some bacteria that are the causative agents of nosocomial infections and an important fungi.

Materials and Methods

This study was carried out in September / 2011 in the laboratories of Research Biotechnology Center - Al-Nahrain University.

Plant material and extraction

Ficus carica leaves were collected in May / 2011 from Baghdad nurseries. The leaves were dried at room temperature and then reduced to coarse powder. In order to prepare the extracts, 25 g of the sample was separately extracted with 125 ml of ethanol, after stirring for 24 hours, then the extraction solvent was evaporated in vacuo at 40 C°. Latex extract of the leaves were prepared from the green leaves without drying (Galal et al., 2012).

Detection of active compounds

Detection of tannins

Ten gram of plant powder was mixed with 50 ml distilled water in a magnetic stirrer. The mixture was boiled in a boiling water bath for few minutes, then filtered and the filtrate was treated with few drops of 1% lead acetate solution. The development of greenish-blue precipitate is an indicator for the presence of tannins (Evans, 1989).

Detection of saponins

Five milliliters of aqueous extract of the plant was added to 1-3 drops of 3% ferric chloride solution, a white precipitate was developed which indicates a positive result (Alsereita and Abu-Amer, 1996).

Detection of terpenes and steroids

One milliliter of ethanol extract was participated in a few drops of chloroform, then a drop of acetate anhydride and drop of concentrated sulfuric acid were added, brown precipitate appeared which representing the presence of terpene, and the appearance of dark blue color after few minutes would represent the present of steroids (Harborne, 1984).

Detection of flavonoids

Ethanol extract was patitioned with petroleum ether; the aqueous layer was mixed with the ammonia solution. The appearance of dark color is an evidence for the presence of flavonoids (Harborne, 1984).

Detection of alkaloids

Ten gram of the extract was boiled with 50 milliliters of distilled water and 4% of hydrochloric acid was added, then the solution was filtered and cooled. 0.5 ml of the supernatant was tested with Mayer solution, appearance of white precipitate indicates the presence of alkaloids (Harborne, 1984).

Microbial strains

The test organisms used in this study included, 2 gram positive staining bacteria (*Staphylococcus aureus, Streptococcus pyogenes*) and 4 gram negative staining bacteria (*Klebsiella pneumonae*, *Pseudomonas aeruginosa*, *Salmonella typhi, Escherichia coli*)

and 3 fungi (*Candida albicans, Fusarium oxysporum, Aspergillus nigar*). These strains were collected from laboratories of College of Science / Baghdad University.

Antimicrobial assay

Well diffusion method

Mueller-Hinton agar (MH) and Sabouraud Dextrose agar (SDA) medium were respectively used for bacteria and fungi growth. Microbial cultures, freshly grown at 37°C/30°C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 105 CFU: ml. To evaluate Antimicrobial activity, an agar well diffusion method was used as described by Baur et al. The organisms were spread on MH and SD agar plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 100 µl of fig latex and ethanol extract (200 mg/ml). The plates were incubated for 24 h at 37°C for bacteria and 72 h at 30°C for fungi. Antimicrobial activity was evaluated by measuring the inhibition zone diameter against the test organisms. Gentamicine (30 µg/ml) was used as positive reference standard to determine the sensitivity of one strain/isolate in each tested microbial species (Bauer et al., 1966).

Results and discussion

The chemical test of the active compounds in fig (*Ficus carica*) ethanol and latex extracts showed in table 1 indicated that this medicinal plant contains; favonoids, tannins, terpenes and steroids, alkaloids, and saponins.

Table (1): Detection of some active compounds in ethanol and latex extracts of fig (*Ficus Carica* Linn.)

Phytochemical compound	Ethanol extract	Latex extract
Flavonoids	+	+
Tannins	+	+
Terpenes and steroids	-	+
Alkaloids	-	+
Saponins	-	+

(+) means present, (-) means absent.

The results of antimicrobial activity (antibacterial and antifunjal activity) of leaf ethanol extract and latex of fig are indicated in table 2. It showed that the ethanol extract of *F. carica* leaves exhibited strong activity against the gram positive bacteria (*staphylococcus aureus* 13 mm in diameter as inhibition zone), and the gram negative bacteria (*Salmonella typhi* 14 mm) as compared with control treatment (18 mm when treated with the antibiotic gentamycin), while it showed moderate activity against (*Streptococcus pyogenes, Pseudomonas aeruginosa*) which recorded 12 mm, 11 mm respectively, control treatment with gentamycin recorded 16 mm in diameter for each of them . *Klebsiella pneumonae* and *Escherichia coli* appeared to be less sensitive to the extracts, the inhibition zone were 8 mm, 9 mm respectively (It was recorded 15 mm, 16 mm with gentamycin.

The antibacterial activity of latex extract as shown in table 2 was examined by the presence and absence of inhibition zone diameter. These results revealed that latex extract of fig had inhibition effect on the growth of all bacterial species used in this study.

Latex extract was more active than ethanol extract in inhibit bacterial growth as compared to control treatment (gentamycin antibiotic).

Table (2): Antimicrobial activity of leaf ethanol extract and latex against bacterial and fungal strains measured as inhibition zone diameter

Microorganisms	**Inhibition zone diameter (mm)**		
Bacteria strain	**Leaf extract**	**Latex**	**Gentamycin**
Staphylococcus aureus	13	15	16
Streptococcus pyogenes	12	14	15
Klebsiella pneumonae	8	11	13
Pseudomonas aeruginosa	11	13	14
Salmonella typhi	14	15	16
Escherichia coli	9	10	12
Fungi strain			
Candida albicans	15	16	19
Fusarium oxysporum	16	17	18
Aspergillus nigar	14	18	19

Staphylococcus aureus and *salmonella typhi* were the most sensitive to the latex and ethanol extracts, it was recorded 15 mm in diameter as inhibition zone with latex, while it was recorded 14 mm, 13 mm, mm for each of *Sterptococcus pyogenes, Pseudomonas aeruginosa*. E*scherichia coli* and K*lebsiella pneumonae* showed resistance to the extracts. Fig latex showed 10 mm, 11 mm respectively as inhibition zone diameter for *E. coli* and *Kleb. pneumonae* as compared with ethanolic extract.

On the other hand, the antifungal activity of leaf ethanol and latex extract of fig showed high inhibitory effect against all fungi strains. It was recorded inhibition zone diameter ranged from 14 mm to 16 mm with ethanol extract and 16 mm to 18 mm with fig latex. The highest inhibition zone diameter was recorded against *Fusarium oxysporum* (16 mm), while the lowest inhibition zone diameter was recorded against *Aspergillus niger* (14 mm) with ethanol extract as compared with latex which was recorded 18 mm as the higher inhibition zone diameter for the fungus *Aspergillus nigar,* and 16 mm as the lowest value for *Candida albicans*. The results in table 2 showed that latex of fig was more active than ethanol extract of fig as compared with the control treatment (gentamycin antibiotic). The pharmacological properties are probably in part due to the high content of enzymes, flavonoids, and furanocomarines from fig latex (Chevallier, 2001).

The detection of ethanolic extract and latex of fig revealed the presence of flavonoids, terpenes and steroids, alkaloids, saponins and tannins which possess diverse biological effect like antioxidant, antiinflammatory and antibacterial activities (Solomon et al., 2006). It is generally considered that the flavonoids in *F. carica* may be related, to the antibacterial effects observed in this study. The high antimicrobial activity may perhaps due to leaves content of rutin, quercetin, luteolin, phenolic acids and phytosterols (Ross and Kasum, 2002).

Mi-Ran et al., found the same results with methanolic extracts of fig (*F. carica*) leaves against oral bacteria, they indicated that the latex and extracts were high against gram positive bacteria as compared with gram negative bacteria (Mi-Ran et al., 2009).

These results are in agreement with other studies on *Ficus carica* which showed beneficial properties and was even able to inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogens, Salmonella enterica serovar* Typhi, *pseudomonas aeruginosa, Escherichia coli,* and *Proteus mirabilis* (Jeong et al., 2009).

The results indicated the therapeutic virtue of fig leaves as an antimicrobial agent against some microbial infections, such as *Staphylococcus aureus, pseudomonas aeruginosa,* and E. coli which recognized as a global nosocomial problem.

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