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*Full Length Research Paper*

# Study of antibacterial effects of ripped and raw fig alone and in combination

**Hosainzadegan H.[1], Alizadeh M[2], Karimi F[4] and Pakzad P.[2,3]***

[1]Department of Microbiology, Maragheh Faculty of Nursing and Midwifery, Maragheh, Tabriz University of Medical Sciences, Tabriz/Iran.
[2]Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam/ Iran.
[3]Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam/ Iran.
[4]Department of Infectious Diseases,Faculty of Medicine, Lorestan University of Medical Sciences,Khoramabad/Iran.

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**In recent years many of researchers were studying antimicrobial and other chemical and biological characteristics of medicinal and nonmedicinal plants seeking for better drug alternatives for treatment of diseases. The antimicrobial activity of metanolic extracts of ripped and unripped figs was studied alone and in combination *in vitro*. Minimum inhibitory concentration (MIC) values were determined by broth microdilution on standard microorganisms. Two different lower concentrations of MIC were used for analysis of combinational antibacterial activities. Findings showed that ripped Fig extract in concentrations of ≥ 13 to ≥6.5 mg/ml have MIC values on tested bacteria, but unripped Fig extract showed a MIC value in ≥7 mg/ml against all of the studied bacteria. Sub MIC concentrations (1:4 and 1:4 MIC) of combination of ripped and unripped extracts indicated synergic and additive effects on bacteria, respectively. In this study ripped and unripped Fig extracts in selected concentrations(1:4 and 1:2 MICs) have indicate synergic and additive antibacterial activity on bacteria, in addition antibacterial activity of unripped Fig extract was higher than ripped Fig extract and was significant(p=0.002). Since Fig has been used from ancient eras by humans, this kind of extracts could be a potential candidate for combination therapy of infections**.

**Key words:** Ripped fig extract, unripped fig extract, synergy, additive, MIC.

## INTRODUCTION

Holy Quran in first verse of Sura Tien have been mentioned of two fruits Fig and olive with blessing and swearing to them. Islam in this verse swears to two important meals of human that without them the life is impossible (Kislev et al., 2009). Therapeutic effects of medicinal plants have been recognized several years ago (Valero and Salmeron, 2003). Different studies have shown that medicinal plants can have a potential activity against drug resistant strains of bacteria (Kone et al., 2004; Aref et al., 2010; Odunbaku et al., 2008), especially medicinal plants rich in various antimicrobial compounds such as tannins, flubatanins, trepenoides, saponin, steroids, alkaloids and flavonoids (Uma et al., 2009; Adebayo et al., 2009). One of the important plants in this regard is the Fig, which recently is discussed due to high amounts of calcium as a major fruit for the survival of other plants and animals (O'Brien et al., 1998). Fig is one of the oldest medicinal plants that have been used by humans so researches show that more than 11,000 years is grown. A number of Fig species has been used as food source and medicinal properties in traditional Chinese medicine. Fig genus has more than 800 species and this genus is one of about 40 genera of the family *Moraceae* (Ephraim et al., 2008). Fig in Arabic and religious texts is mentioned with Tien, the fruits of the Prophet Muhammad that have wished for heaven. Fig is one of the oldest fruits that have been cultivated. Therapeutic applications of figs have been reported from Sumerian, in the literatures since 2900 BC. In the ancient Olympic Games, Fig was given as gift to first winners as prize (Goor, ,

*Corresponding author. E-mail: pakzad_i2006@yahoo.com.

**Table 1.** MIC and FIC of extracts on bacteria.

| | MIC of extracts in mg/ml | | |
|---|---|---|---|
| | **Raw Fig** | **Ripped Fig** | **Combination (FIC)** |
| *S.aureus* ATCC 25923 | 7≤ | 13≤ | 1.75+3.25(0.5) |
| *E.coli* ATCC 25922 | 7≤ | 13≤ | 1.75+3.25(0.5) |
| *P. aeruginisa* ATCC 27853 | 7≤ | 6.5≤ | 3.5+3.25(1) |
| *K. pneumoniae* ATCC 700603(K6) | 7≤ | 13≤ | 3.5+6.5(1) |

1964).

Fruits, leaves and roots of fig have been used in traditional medicine to treat various digestive disorders respiratory, inflammatory and ulcerative, cardiovascular diseases and cancer (Mi-Ran et al., 2009). Flavonoids of fig acting as antioxidants and neutralize free radicals of metabolism (Adebayo et al., 2009). Dried fig has nearly 0.5% of coumarin (Innocenti et al., 1982). Fig posse's large amounts of polyphenolic, benzaldehyde and coumarin compounds that has anti-cancer properties (Kelloff et al., 1994). Benzaldehyde have been used successfully to treat human terminal carcinoma (Kochi et al., 1980) Coumarin is also used to treat prostate cancer. Figs containing highest concentration of polyphenols among other foods and vegetables. Only certain species of sorghum and beans have the same amounts of polyphenolic compounds as figs (Maucher et al., 1993; Berkarda, 1993). Study of protective properties of methanolic extracts of fig leafs on liver function showed effects comparable to Silymarin (Krishna et al., 2007). In traditional Indian medicine, fruit and roots of figs, have been used as treatments of leucoderma, ring worms infestations, paralysis, and anti-inflamatory agent (Kirthikar and Basu, 1996; Nadkarni and Nadkarni, 1995). Different studies indicated that figs have antimicrobial effects on various positive and gram-negative bacteria as well as drug resistant bacteria, yeasts and mold (Friedman et al., 2002; Ojala et al., 2000). Extracts of fig leaves are also used for treatment of gingivitis, cancer , asthma, sneezing and coughing, abscesses, diabetes and constipation (Rubnov et al., 2001; Canal et al., 2000).

Therefore, according to the recommendations of nutritionists on therapeutic effects of traditional figs of Lorestan province, we conducted this study to evaluate the antimicrobial effects of ripped and unripped (raw) figs alone and in combination.

## MATERIALS AND METHODS

A black variety of fig (Ficus carica; type species Zivdar) was used in this study after identification and confirmation by botanist. Triptic Soy Broth (TSB, Merck) and nutrient agar (NA, Merck) were used . In this study, four standard bacterium *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603 (K6) were used for antimicrobial activities.

### Preparation of methanolic extraction

Values of about 2 kg of raw and ripped figs from the identified varieties of black figs randomly picked and collected from Poledokhtar city gardens. After washing, ripped and raw fruits were dried for 45 h at a temperature of 60°C .Then extraction was done by maceration using 100% methanol as follow. Methanol was added to separate containers containing dried fruits of ripped and raw figs and after 12 h of intense shaking and filtered twice. After that the solvent (methanol) was evaporated by rotary evaporator. Net weight of dried extracts and the extraction efficiency was calculated. Aliquots of dried extracts were sterilized by 0.2 µm filtration and dissolved in TSB and stored in refrigerator until antimicrobial study (Rajesh et al., 2008).

### Determination of minimum inhibitory concentration (MIC) with microdilution

At first 18 h culture of bacteria was prepared. The next day, 3 h cultures of bacteria in fresh TSB medium was prepared. Then a dilution of twentieth (equivalent to 0.5 Mac Farland turbidity) in TSB was prepared and used for inoculation of culture media and anti-bacterial study. In the second step, 180 µL of each bacterial suspension were inoculated in separate rows of the microplate (from 1 to $7^{th}$ well of row). After that, 20 µL of 1 to 1:64 dilutions of filtered extracts added to wells of each row. Microplates incubated for 18 h at 37°C, and growth or lack of growth of bacteria were observed macroscopically against a light source. MIC of the extracts was determined by inspection of turbidity (Thrupp, 1986). All tests were done as triplicate and mean of the semi-quantitative results reported finally.

### Antimicrobial effects of combined extracts

Combination effects of tow sub-MIC concentrations (1:4 and 1:2 MIC of extracts) were evaluated on standard bacteria as checkerboard on microplates. Growth of bacteria in different concentrations recorded and FIC values were calculated. Values of FIC ≤0.5, FIC=0.5-1, FIC =1-4 and FIC ≤4 interpreted as synergistic, cumulative, indifferent and antagonism, respectively (Climo et al., 1999).

## RESULTS

Ripped and raw figs extracts calculated about 45.77 and 36.04% of their dried weights, respectively. Antimicrobial effects of the extract are indicated in Table 1.

In fact, we have no comprehensive goals on study of synergisim, antagonism or indifference between extracts. So Combination study has been done by checkerboard of

two concentrations only (1:4 and 1:2 of ripped and raw extracts). As indicated in Table 1, in studied concentrations 2 extracts showed synergistic and cumulative (FICs of 0.5 and 1) effects on 4 standard bacteria. In addition antibacterial activity of unripped fig extract was higher than ripped fig extract and was significant in comparison (p=0.002).

## DISCUSSION

Antimicrobial effects of medicinal plant extracts taken with different methods have been studied extensively worldwide .This study also showed that methanolic extracts of raw and ripped figs have antimicrobial effects on studied bacteria. Meanwhile, 1:4 and 1:2 concentrations of extracts have exert synergistic activity on bacteria.

Antimicrobial activity of fig extracts has been studied in various studies. For example, in a study antimicrobial activity of watery, methanol, chloroform, petroleum ether and hexane extracts were taken from *Ficus bengalensis* and compared with amikacin (10 µg) on Enterotoxigenic *E. coli*. (Uma et al., 2009). In another study, extracts of garlic and figs (*F. carica*) have been used for protection of potato species from invasion of important pathogens including *P. syringa*, *X. vesicatoria* and *C. mishiganensis* successfully (Balestra et al., 2009). Oral consumption of fig leafs methanolic extracts in laboratory mice with induced hepatotoxicity indicated high protective effects comparable to the effects of known drug silymarin. (Krishna et al., 2007). Adebayo et al. (2009) in their study indicated that methanol extracts of fig have potent antibacterial activity on pathogenic bacteria, the estimated MICs on *P. aeruginisa*, *S. aureus* and *E. coli* was comparable to our results. This study also showed that extracts of various parts of fig including roots, leaves, and bark of fig stems have strong antimicrobial activities (Adebayo et al., 2009). Mi-Ran et al. (2009) study of antimicrobial activity of fig leaves, methanolic extracts on different oral pathogens and some standard bacteria indicated MIC and MBC amount comparable to our study.

## Conclusion

The present study shows that methanolic extracts of raw and ripped figs are suitable source of antibacterial materials. Moreover, the combinations of the two extract has synergistic effects on bacteria which can be used as adjuvant with antibiotics in cases of infections, or as an additive in mouthwashes and a safe preservative for foods without any complications. On the other hand using natural antimicrobials in environments such as hospitals could decrease the rate of emerging resistant organisms, and in turn helping to decrease mortality of infections and increasing hospitals and public health profits. Raw fig extract has higher antibacterial effects than ripped fig, and this could explain why ripped fruits and specially figs are decaying very faster than raw fruits in routine life and storing process, and also this means that raw fruits are likely good sources of antimicrobial agents for bulk production.

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