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Antibacterial Effects of Iranian Fennel Essential Oil on Isolates of *Acinetobacter baumannii*

N.H. Jazani, M. Zartoshti, H. Babazadeh, N. Ali-daiee, S. Zarrin and S. Hosseini
Department of Microbiology, Immunology and Genetics, Faculty of Medicine,
Urmia University of Medical Sciences, Urmia, Iran

Abstract: The aim of the present study was the evaluation of the antibacterial activity of Fennel essential oil on isolates of *Acinetobacter baumannii*. Forty eight isolates were collected from clinical specimens from burn wards of hospitals in Tehran, Iran between April and September, 2006. The susceptibility of isolates was determined using a broth microdilution method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of isolates to Fennel essential oil were determined. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. The rates of resistance were determined to antibiotics as follows: cefazolin 100%, ciprofloxacin 100%, ofloxacin 95.8%, kanamycin 95.8%, carbenicillin 93.7%, ticarcillin 93.7%, piperacillin 88.9%, co-trimoxazole 79.1%, ceftizoxime 75%, gentamicin 70.8%, cefalotin 60.4%, amikacin 52% and imipenem 14.6%. Fennel essential oil possessed antibacterial effect against all isolates of *A. baumannii*. These results suggest the potential use of the Fennel essential oil for the control of multi-drug resistant *A. baumannii* infections. However, more adequate studies must be carried out to verify the possibility of using it for fighting bacterial infections in human.

Key words: Herbal medicine, minimum bactericidal concentration, minimum inhibitory concentration, *Foeniculum vulgare*

INTRODUCTION

Medicinal plants contain biologically activity compounds, many of which have been shown to have antibacterial properties (Mohsenzadeh, 2007). *Foeniculum vulgare* (Fennel) belonging to the family Apiaceae is a perennial herb native to the Mediterranean Region. It is widely cultivated and extensively used as a culinary spice. The plant is aromatic and is used as a pot herb. The leaves have diuretic properties and the roots are regarded as purgatives. Dried fruits of Fennel possess a pleasant aromatic taste and used for flavouring soups, meat dishes and sauces. The fruits are considered to be useful in treatment of diseases of the chest, spleen and kidney (Singh and Kale, 2008).

The antiinflammatory, analgesic and antioxidant activities of the fruit of Fennel have been reported previously by Choi and Hwang (2004). Oral administration exhibited inhibitory effect against acute and subacute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect. It significantly decreased the high density lipoprotein-cholesterol level along with a decrease in the peroxidative damage (Choi and Hwang, 2004). The antibacterial activity of

essential oil of Fennel has been reported previously by Ruberto *et al.* (2000).

Anethole is the principal active component of Fennel seeds which has exhibited anticancer activity (Aggarwal *et al.*, 2008). Also, chemopreventive potential of Fennel against carcinogenesis has been shown earlier by Singh and Kale (2008).

Acinetobacter baumannii is a Gram-negative, nonmotile, nonfermentative and oxidase-negative bacillus, whose natural reservoir has not been clearly determined. It is found in many hospital environments and can be colonize in human body in the hospital environments. The combination of its environmental colonization and its very high resistance to antimicrobial renders it as a successful nosocomial pathogen (Nordmann, 2004). There are many reports of Multi Drug Resistant (MDR) *A. baumannii* from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea and many other areas (Barbolla *et al.*, 2003; Houang *et al.*, 2001; Lee *et al.*, 2004; Liu *et al.*, 2006; Naas *et al.*, 2006; Nishio *et al.*, 2004; Quale *et al.*, 2003; Van Looveren and Goossens, 2004; Yu *et al.*, 2004). These MDR strains often spread to cause outbreaks throughout hospital wards. *Acinetobacter* sp. is usually considered to be

opportunistic pathogens. They cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, especially in immunocompromised patients. MDR *A. baumannii* infections tend to occur in immunosuppressed patients, in patients admitted in intensive care and burn units and in those subjected to invasive procedures and treated with antibiotics. In respect of its very high resistance to antimicrobials, introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections (Perez *et al.*, 2007).

In this study we evaluated the antibacterial activity of Fennel on 48 hospital isolates of MDR *A. baumannii*.

MATERIALS AND METHODS

Essential oil: Fennel essential oil from Barije Essence Pharmaceutical Company, Iran (commercial producer of plant essential oils and aromatic substances) were used in this study. The oil was selected based on literature survey and its use in traditional medicine. Quality of the oil ascertained to be more than 80% pure. The main effective components of the Fennel essential oil were anethole (50-60%) and fenchone (10-20%).

Bacterial strains and culture media: A total of 48 isolates were collected from clinical specimens from burn wards of hospitals in Tehran, Iran during a 6 months period between April and September, 2006. The isolates were further processed by the standard methods to identify as the *A. baumannii* (Baron and Finegold, 1990). Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -60°C, cultures were maintained for daily use on nutrient agar (BBL) slants at 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of strains. *Acinetobacter calcoaceticus* PTCC 1318 has been used as reference strain.

Determination of antimicrobial activity of Fennel essential oil: The susceptibility of *Acinetobacter* isolates to Fennel essential oil was determined using a broth microdilution method based on CLSI guidelines. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Fennel essential oil for isolates were determined. Muller-Hinton Broth (MHB; Oxoid) was supplemented with 0.002% (v/v) Tween 80 (Sigma) (MHB-T) to enhance dispersion of the Fennel oil (Papadopoulos *et al.*, 2006). The initial concentration of Fennel essential oil in the first tube contains MHB-T was

1/2. This was used to prepare serial doubling dilutions over the range 0.03-25% (v/v). 1.5×10⁶ inoculums of the isolates were added to each concentration in MHB-T. A tube containing growth medium without essential oil and an un-inoculated tube were used as a positive and negative growth control, respectively. Antibacterial activity was measured by determining MICs and MBCs. The MIC was the lowest concentration of essential oil that resulted in a clear tube. Ten microlitres from each tube was spot-inoculated onto Nutrient Agar (NA) and incubated overnight at 37°C to determine the MBC. The highest dilution that inhibits bacterial growth on nutrient agar after overnight incubation was taken as MBC (Baron and Finegold, 1990; Papadopoulos *et al.*, 2006). Experiments were repeated for three times and the modal value calculated.

Determination of the strains sensitivity to antibiotics:

The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. To represents the different classes of antimicrobial agents commonly used for the treatment of *Acinetobacter* sp. infections, we used piperacillin, ciprofloxacin, gentamicin, ofloxacin, cephalotin, ticarcillin, kanamycin, imipenem, amikacin, co-trimoxazole, ceftizoxime, cefazolin and carbenicillin (Hi-media, Mumbai, India).

RESULTS

The rates of resistance to different antibiotics for 48 isolates of *Acinetobacter baumannii* have been shown in Table 1. Cefazolin (100%), ciprofloxacin (100%) ofloxacin (95.8%) and kanamycin (95.8%) showed the highest rate of resistance and amikacin (52%) and imipenem (14.6%) demonstrated the lowest (Table 1). 45.8% of isolates showed resistance to the 11 tested antimicrobials. Results showed that Fennel essential oil possessed antibacterial effect against all isolates of *Acinetobacter* sp. (Table 2), 39 isolate were sensitive to all

Table 1: The rates of resistance to different antibiotics for 48 burn wound isolates of *Acinetobacter baumannii*

Antibiotics	Resistance (%)
Cefazolin (Cz)	100.0
Ciprofloxacin (Cf)	100.0
Ofloxacin (Of)	95.8
Kanamycin (K)	95.8
Carbenicillin (Cb)	93.7
Ticarcillin (Ti)	93.7
Piperacillin (Pe)	88.9
Cotrimoxazole (Co)	79.1
Ceftizoxime (Ce)	75.0
Gentamicin (G)	70.8
Cefalotin (Ch)	60.4
Amikacin (Ak)	52.0
Imipenem (I)	14.6

Table 2: Antibacterial activity of Fennel essential oil against 48 burn isolates of *Acinetobacter baumannii*

MBCs for each isolate ($\text{mm}^3 \text{mm}^{-3}$)	No. of isolates
Sensitive to all tested dilutions	39
1.56×10^{-2}	4
3.9×10^{-3}	5
Total	48

tested dilutions and the remaining nine isolates showed MIC and MBC values in the range of 3.9×10^{-3} to $1.56 \times 10^{-2} \text{ mm}^3 \text{mm}^{-3}$ (Table 2). Also *Acinetobacter baumannii* PTCC1318 was sensitive to all tested dilutions.

There was complete growth on the positive growth control tube (containing growth medium without essential oil) and no growth on negative growth control (un-inoculated tube).

DISCUSSION

Acinetobacter baumannii play an important role in colonization and infection of patients admitted to hospitals. They have been implicated in a variety of hospital acquired infections, including bacteremia, urinary tract infection, meningitis and pneumonia (Perez *et al.*, 2007). Antibiotic resistance is a major problem in treating infection with *A. baumannii*, which has become resistant to almost all available antibacterial drugs (Longo *et al.*, 2007). The use of broad spectrum antibiotics in hospital environments for treating such infections results in promoting infections by multi-antibiotic resistant isolates. Present finding showed that the most useful antibiotics for infections caused by *A. baumannii* were imipenem, amikacin and cefalotin. Resistance to some antibiotics such as gentamicin, ciprofloxacin and co-trimoxazole showed very high increases in comparison with earlier studies by Guardabassi *et al.* (1998). Also, in present study burn wound isolates of *Acinetobacter baumannii* showed high resistance to other tested antibiotics (Table 1), so it seems reasonable to explore new sources of natural compounds with antibacterial activity against *Acinetobacter* sp.

The antimicrobial properties of essential oils obtained from *Foeniculum vulgare* have been investigated in different trails. The oil has been shown to be effective against *Streptococcus haemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* species, *Staphylococcus epidermidis* (Mohsenzadeh, 2007), *Bacillus cereus* and *Bacillus brevis* (Ozcan *et al.*, 2006). Another study has shown an activity against the food-borne pathogens like *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* but in Sagdic's study Fennel extract was inactive against some pathogenic bacteria (Sagdic and Yasar, 2005).

In present study results showed that Fennel essential oil possessed antibacterial effect against all isolates of *Acinetobacter baumannii*, furthermore, beside the confirmation of the popular use, the obtained results demonstrate that this herbal drug could represent a new source of antimicrobial agents, for the control of *Acinetobacter* infections. However, more adequate studies must be carried out to verify the possibility of using it for fighting these bacteria in human body infections.

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