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Antibacterial Effects of Iranian *Mentha pulegium* Essential Oil on Isolates of *Klebsiella* sp.

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Abstract: The aim of the present study was the evaluation of the antibacterial activity of *Mentha pulegium* essential oil on isolates of *Klebsiella*. Thirty nine isolates were collected from urine specimens submitted to two educational hospitals in Urmia, Iran. The susceptibility of isolates was determined using a broth microdilution method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of isolates to *Mentha pulegium* 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: gentamicin 46.1%, tobramycin 48.7%, ceftizoxime 41%, co-trimoxazole 46.1%, amikacin 33.3%, cephtazidime 51.3%, ciprofloxacin 30.8%, kanamycin 53.8%, nalidixic acid 30.8% ampicillin 79.5% and nitrofurantoin 41%. *Mentha pulegium* essential oil possessed antibacterial effect against all isolates of *Klebsiella* sp. with MIC and MBC values in the range of 1.9×10^{-3} to 4.9×10^{-4} mm^3 mm^{-3}. In this study clinical isolates of *Klebsiella* sp. showed very high resistance to tested antibiotics. These results suggest the potential use of the *Mentha pulegium* essential oil for the control of multi-drug resistant *Klebsiella* sp. infections. However, more adequate toxicological study must be carried out to verify the possibility of using it for fighting microorganisms in human.

Key words: Herbal medicine, minimum bactericidal concentration, minimum inhibitory concentration, pennyroyal

INTRODUCTION

The use of plants for healing purposes forms the origin of modern medicine (Wargovich *et al*., 2001). Many drugs originate from herbal sources: a century ago, most of the effective drugs were plant based. The development of drugs from plants continues, with drug companies engaged in pharmacological screening of herbs (Cetin *et al*., 2006; Jarema, 2008; Vickers and Zollman, 1999).

Mentha pulegium is one of the Mentha species known as pennyroyal. It is native species of Asia and near East (Chalchat *et al*., 2000). *Mentha pulegium* L. has been traditionally used as antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis (Zargari, 1990) and also as antiflatulent, carminative, expectorant, diuretic, antitussive, menstruate (Newall *et al*., 1996). Some pharmacological effect of *Mentha pulegium* L. essential oil such as abortifacient effect in rat myometrium (Soares *et al*., 2005), cytotoxic activity against different human cell lines and its antioxidant effect (Mahboubi and Haghi, 2008) were confirmed previously. Analysis of the Iranian essential oil of *Mentha pulegium* revealed the presence of piperitone (38.0%), piperitenone (33.0%), α-terpineol (4.7%) and pulegone (2.3%) as the major components. The antibacterial effects of *Mentha pulegium* essential oil on limited strains of bacteria have been investigated in earlier studies (Mahboubi and Haghi, 2008). However there is no studies in literature about the antibacterial effects of this herb or its essential oil on *Klebsiella* sp.

Klebsiella sp. is a group of gram negative rods and they can cause different kinds of infections especially in immunocompromised hosts. Multi-drug resistant *Klebsiella* has been recognized as a cause of hospital acquired infections worldwide. They are resistant to numerous antibiotics. Their resistance to antibiotics restricts the choice of antibiotics for therapy (Gonzalez and Schaeffer, 1999; Keynan and Rubinstein, 2007). So, introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections. However, there is no study on investigation the antibacterial effects of *Mentha pulegium* on *Klebsiella* sp. In this study we evaluated the antibacterial activity of *Mentha pulegium* essential oil on isolates of *Klebsiellae* sp.

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MATERIALS AND METHODS

Essential oil: *Mentha pulegium* essential oil from Barij Essence Pharmaceutical Company, Iran (commercial producers of plant essential oils and aromatic substances) were used in this study. This oil was selected based on literature survey and its use in traditional medicine. Analysis of the essential oil by GC/MS revealed that the prominent components were piperitone (38.0%), piperitenone (33.0%), α-terpineol (4.7%), 1,8-cineole (4.0%), piperitone oxide (3.4%), menthone (3.1%), borneol (2.9%) and pulegone (2.3%) (Mahboubi and Haghi, 2008).

Bacterial isolates: A total of 39 isolates were collected from urine specimens submitted to two educational hospital clinical laboratories in Urmia, Iran during a three months period from December 2006 until March 2007. The isolates were further processed by the standard methods to identify as the *Klebsiella* sp. (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 on 4°C. *Klebsiella pneumoniae* ATCC 10031 has been used as reference strain.

Susceptibility testing: The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) media (Merck) were used for detection of antibiotic resistance of isolates. The susceptibility of isolates to different antibiotics was tested using agar disk diffusion method (Akram *et al*., 2007). To represents different classes of antibacterial agents commonly used for treatment of *Klebsiella* infections, gentamicin, tobramycin, ceftizoxime, co-trimoxazole, amikacin, cephtazidime, ciprofloxacin, kanamycin, nalidixic acid, ampicillin and nitrofurantoin were used in present study (Hi-media, Mombay, India).

Determination of antimicrobial activity of *Mentha pulegium* essential oil: The susceptibility of *Klebsiella* isolates to *Mentha pulegium* essential oil was determined using a broth microdilution method based on CLSI guidelines. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of isolates to *Mentha pulegium* essential oil were determined. Mueller-Hinton Broth (MHB; Oxoid) was supplemented with 0.002% (v/v) tween 80 (Sigma) (MHB-T) to enhance dispersion of the *Mentha pulegium* essential oil (Papadopoulos *et al*., 2006). The initial concentration of *Mentha pulegium* 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). 1.5×10^6 inoculums of the isolates were added to each concentration in MHB-T. A tube containing growth medium without *Mentha pulegium* 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 *Mentha pulegium* 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 performed at least three times and the modal value selected.

RESULTS

The rates of resistance to different antibiotics for 39 isolates of *Klebsiella* have been showed in Table 1. Ampicillin (79.5%) and kanamycin (53.8%) showed the highest rate of resistance and ciprofloxacin and nalidixic acid (30.8%) demonstrated the lowest. Twenty percent of isolates showed resistance to the 11 tested antimicrobials.

Results showed that *Mentha pulegium* essential oil possessed antibacterial effect against all isolates of *Klebsiella* sp. with MIC and MBC values in the range of 1.9×10^{-3} to 4.9×10^{-4} mm^3 mm^{-3} (Table 2).

Also MIC and MBC amounts for *Klebsiella pneumoniae* ATCC 10031 was 4.9×10^{-4} mm^3 mm^{-3}.

DISCUSSION

Klebsiella is an opportunistic pathogen and is a causative agent of several kinds of infections in humans. It is one of the major pathogens in nurseries, intensive care units and in hospital wards in spite of many effective antibiotics now available.

Table 1: The rates of resistance to different antibiotics for 39 isolates of *Klebsiella* from two educational hospitals in a three months period

	Antibiotics										
	Am	K	CAZ	Tob	Ct	SXT	Gm	AN	Cp	NA	FN
Resistance (%)	79.5	53.8	51.3	48.7	41	46.1	46.1	33.3	30.8	30.8	41

Gm: Gentamicin, Tob: Tobramycin, Ct: Ceftizoxime, SXT: Co-trimoxazole, AN: Amikacin, CAZ: Cephtazidime, Cp: Ciprofloxacin, K: Kanamycin, NA: Nalidixic Acid, Am: Ampicillin and FN: Nitrofurantoin

Table 2: Antibacterial activity of *Mentha pulegium* essential oil against 39 isolates of *Klebsiella*

MBC for each isolate (mm^3 mm^{-3})	No. of isolates
1.9×10^{-3}	8
9.7×10^{-4}	8
4.9×10^{-4}	23
Average of MBCs±SD = $8.8\times10^{-4}\pm 5.6\times10^{-4}$ mm^3 mm^{-3}	Total = 39

*MIC amount for each isolate was equivalent to MBC

The use of broad spectrum antibiotics in hospital environments exerts selective pressure on bacteria, results in promoting infections by multi-antibiotic resistant isolates. Present finding showed that the most useful antibiotics for infections caused by *Klebsiella* sp. were amikacin, nalidixic acid and ciprofloxacin. Resistance to some antibiotics such as ampicillin, gentamicin, kanamycin and nitrofurantoin showed increases in comparison with previous studies in different countries (Randrianirina *et al*., 2007; Akram *et al*., 2007).

Iranian people used the *Mentha pulegium* plant against infectious diseases and find it to be efficacious against these problems without any scientific base to explain of this action. The antibacterial effects of *Mentha pulegium* essential oil has been investigated previously and significant antimicrobial activity against Gram-positive bacteria especially *Staphylococcus aureus* has been shown (Mahboubi and Haghi, 2008). Other study showed piperitone, one of the main components of *Mentha pulegium* essential oil completely inhibited *Aspergillus flavus* at low concentrations (Cardenas-Ortega *et al*., 2005), however the antibacterial effects of *Mentha pulegium* essential oil is much lower on *E. coli* and *Salmonella typhimurium* (Mahboubi and Haghi, 2008).

These results suggest the potential use of *Mentha pulegium* essential oil for the control of *Klebsiella* infections. However, more adequate toxicological study must be carried out to verify the possibility of using it for fighting microorganisms in human body.

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