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Antibacterial Effects of Iranian *Cuminum cyminum* Essential Oil on Burn Isolates of *Pseudomonas aeruginosa*

N. Hosseini Jazani, M. Zartoshti and S. Shahabi
Department of Microbiology, Immunology and Genetics, Faculty of Medicine,
Urmia Medical Sciences University, Urmia, Iran

Abstract: The aim of this study is the evaluation of the antibacterial activity of cumin essential oil on burn isolates of *P. aeruginosa*. Fifty two burn isolates of *P. aeruginosa* were obtained from burn wards of two hospitals at Tehran, Iran. The susceptibility of isolates was determined using a broth microdilution method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of isolates to cumin essential oil was determined. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. The rates of resistances were determined to antibiotics as follows: Gentamicin 96%, ceftazidime 100%, tobramycin 100%, kanamycin 100%, amikacin 73%, ceftizoxime 100%, piperacillin 94.2%, imipenem 50% and ciprofloxacin 71%. Cumin essential oils possessed antibacterial effect against all isolates of *P. aeruginosa*, with MIC and MBC values in the range of 0.015 to 0. 25 ml mL^{-1}. These results suggest the potential use of the cumin essential oil for the control of *P. aeruginosa* infections.

Key words: Cumin, *Cuminum cyminum*, essential oil, *Pseudomonas aeruginosa*, burn wound infections

INTRODUCTION

Cumin is a flowering plant in the family Apiaceae, native from the East Mediterranean to East India. It is an herbaceous annual plant, with a slender branched stem 20-30 cm tall. The leaves are 5-10 cm long, pinnate or bipinnate, thread-like leaflets. The flowers are small, white or pink and borne in umbels. The fruit is a lateral fusiform or ovoid achene 4-5 mm long, containing a single seed. Cumin seeds are similar to fennel seeds, but are smaller and darker in color. Cumin seeds are used as a spice for their distinctive aroma, popular in North African, Middle Eastern, western Chinese, Indian, Cuban and Mexican cuisine. Cumin's distinctive flavour and strong, warm aroma is due to its essential oil content. Historically, Iran has been the principal supplier of cumin, but currently it is also cultivated in India, Sri Lanka, Syria, North Africa, China, Indonesia, Pakistan and Turkey (Ishikawa *et al*., 2002).

Cumin has a broad antibiotic spectrum against both gram-positive and gram-negative bacteria. In particular the sensitivity of *Helicobacter pylori*, *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia*, *Agrobacterium* and *Pseudomonas* have been shown to cumin essential oil previously (Nostro *et al*., 2005; Iacobellis *et al*., 2005). And also in some reports it has been shown that the essential oil of cumin is equally or more effective when compared with standard antibiotics, at a very low concentration (Singh *et al*., 2002).

P. aeruginosa is an opportunistic gram negative bacilli and one of the most important causes of nosocomial infections especially in patients with burns, cystic fibrosis and neutropenia. Especially burn isolates of *P. aeruginosa* show high resistance to different classes of antimicrobial agents (Algun *et al*., 2004).

P. aeruginosa is an opportunistic pathogen found along with other *Pseudomonas* sp. as part of the normal flora of the human skin (Larson and Ramphal, 2002). When the host is immunocompromised, this opportunistic bacterium can quickly colonize and infect the burn and wound sites. *P. aeruginosa* plays a predominant role as an etiological agent involved in serious infections in burned patients. Since *P. aeruginosa* can rapidly disseminate from the wounds into other organs via the bloodstream and can produce a number of virulence factors that induce endotoxic shock, the clinical outcome in these patients can lead to sepsis which is often fatal. In case studies of burn patients who developed *P. aeruginosa* septicemia, the mortality rate was more than 75% (Holder, 1985; Wurtz *et al*., 1995). Antibiotics are generally ineffective against most serious infections especially burn wounds infections by *P. aeruginosa*, treatment of these infections is frequently complicated by antibiotic resistance, a problem that is increasing in recent years, so introducing of the new antimicrobial agents

Corresponding Author: N. Hosseini Jazani, Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia Medical Sciences University, Urmia, Iran
Tel: +98-441-3449548, +98-914-3464234 Fax: +98-441-2780801

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 cumin essential oil on *P. aeruginosa*.

In this study we evaluated the antibacterial activity of cumin essential oil on 52 burn isolates of *P. aeruginosa*.

MATERIALS AND METHODS

Essential oil: Cumin 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. Quality of the oil was ascertained to be more than 98% pure. The green fruit of the cumin contains at least 2.5% essential oil. This essential oil obtained from distillation of crushed fruit under the steam water. The resulting product is a colorless sticky fluid gradually converts to yellow/brown. Complete separation of the components of essential oils which contain tens or even hundreds of chemical components, are difficult to achieve even if rigorous conditions are imposed on the chromatographic separation process, however major constituents in cumin are gamma-terpinene, 2-methyl-3-phenyl-propanal, myrtenal, *para*-Cymene, alpha-pinene, beta-pinene, alpha and beta-phellandrene and myrcene (Jalali-Heravi *et al*., 2007).

Bacterial strains and culture media: During a period of one year (2005), 52 burn isolates of *P. aeruginosa* were obtained from burn wards of two hospitals at Tehran, Iran. The isolates were further processed by the standard methods to identify as *P. aeruginosa*. 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 slants on 4°C (Jazani *et al*., 2007).

Determination of antimicrobial activity of cumin essential oil: The susceptibility of *Pseudomonas* isolates to *Cuminum cyminum* 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 cumin essential oil was determined. Mueller-Hinton Broth (MHB; Oxoid) was supplemented with 0.002% (v/v) tween 80 (Sigma) (MHB-T) to enhance dispersion of the cumin essential oil (Papadopoulos *et al*., 2006). The initial concentration of cumin 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 cumin 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 cumin 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 (Sahm and Weissfeld, 2002; Papadopoulos *et al*., 2006). Experiments were performed at least three times and the modal value selected.

Determination of the strains sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method (Bauer *et al*., 1966). *P. aeruginosa* ATCC 27853 was used as reference strain. To represents the different classes of antimicrobial agents commonly used for the treatment of *P. aeruginosa* infections, we used tobramycin (10 mcg), ciprofloxacin (5 mcg), ceftazidime (30 mcg), piperacillin (100 mcg), gentamicin (10 mcg), kanamycin (30 mcg), imipenem (10 mcg), amikacin (30 mcg), ceftizoxime (30 mcg), carbenicillin (100 mcg) (Hi-media, Mombay, India).

RESULTS AND DISCUSSION

A total of 52 *P. aeruginosa* isolates were collected from burn wound specimens submitted to the hospital clinical microbiology laboratories of two hospitals in Tehran, Iran. The rates of resistances to different antibiotics for studied isolates were showed in Table 1 63.4% of isolates showed resistance to all tested antibiotics.

Results showed that cumin essential oil possessed antibacterial effect against all isolates of *P. aeruginosa*, with MIC and MBC values in the range of 0.015 to 0. 25 ml mL^{-1} (Table 2).

P. aeruginosa is notorious for its involvement in nosocomial infections and its incidence of resistance to antibiotics. Alternative treatments for *Pseudomonas* infections especially burn infections that fall outside

Table 1: The rates of resistance to different antibiotics for 52 burn wound isolates of *P. æruginosa*

Antibiotics	Resistance (%)
Ceftazidime (Ca)	100.0
Tobramycin (Tb)	100.0
Kanamycin (K)	100.0
Ceftizoxime (Ck)	100.0
Gentamicin (G)	96.0
Piperacillin (Pc)	94.2
Imipenem (I)	50.0
Amikacin (Ak)	73.0
Ciprofloxacin (Cf)	71.0

Table 2: Antibacterial activity of cumin essential oil against 52 burn isolates of *P. aeruginosa*

MBCs for each isolate (ml mL^{-1})	No. of isolates
1.5×10^{-2}	3
3.1×10^{-2}	21
6.2×10^{-2}	22
12.5×10^{-2}	5
25×10^{-2}	1
Average of MBCs*±SD = $5.7\times10^{-2}\pm4\times10^{-2}$ ml mL^{-1}	Total = 52

*MIC amount for each isolate was equivalent to MBC

the realm of conventional antibiotics are needed (Papadopoulos *et al*., 2006). In this study burn wound isolates of *P. aeruginosa* showed very high resistance to tested antibiotics (Table 1). Herbs and spices have been used for generations by humans as food and to treat ailments. Scientific evidence is accumulating that many of these herbs and spices do have medicinal properties that alleviate symptoms or prevent disease (Srivastava, 1989; Jalali-Heravi *et al*., 2007). Cumin is one of the popular spices regularly used as a flavoring agent in a number of ethnic cousins. In Iranian ancient medicine, the fruits of the plant have been used for treatment of toothache, diarrhea and epilepsy. Cumin is also emerging as an alternative antimicrobial agent that is safe for human applications (Janahmadi *et al*., 2006). It has been shown that some of essential oils extracted from medicinal plants have no antibacterial activity on *P. aeruginosa* isolates (Pereira *et al*., 2004), however in the present study we evaluated the antibacterial effects of cumin essential oil against 52 burn isolates of *P. aeruginosa* and the bactericidal activity of it was satisfactory (Table 2). These results suggest the potential use of the cumin essential oil for the control of *P. aeruginosa* 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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