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The Study of Antibacterial Effects of Thyme Essence Against Multidrug-Resistant Strains of *Acinetobacter baumannii* Selected Hospitals in Tehran

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***Acinetobacter baumannii* is very highly resistant to chemical antimicrobial agents. These bacteria are one of ESBL producing that great potential have for the rapid development of antibiotic resistance. The indecorous use of antibiotics is often associated with adverse effects on the human health. Because of Thyme essence has antimicrobial properties so they can be used against infections caused by MDR *Acinetobacter baumannii*. Thyme essence has inhibitory properties against Thyme. In this study the antimicrobial effects of Thyme essence on drug-resistant strains of *Acinetobacter baumannii* were investigated using antimicrobial analysis with CLSI 2013 and Kirby Bauer method. The agar dilution method results revealed that Thyme essence had strong inhibitory effects against Multidrug-resistant strains of *A. baumannii*. Because of proper effects of plant essential oils, with a broader range of studies can be used as a complementary therapy.**

Key words: *Acinetobacter baumannii*, Multidrug-resistant, Thyme essence, MIC.

Acinetobacter baumannii is Gram-negative, non-fermentative, coccobacillus, belonging to the family Moraxellaceae that has increased attention to it as a nosocomial pathogen[1-2]. This bacterium causes outbreaks of infections and associated to nosocomial infections, including bacteremia, pneumonia, meningitis, urinary tract infection, and wound infections[3-4]. Use of medicinal herbaceous drugs recommended for treatment for far ancient period for the treatment. Humans have been used and realize their beneficial effects. With the increase in population and urban growth, and reduced use of synthetic drugs, many of these of medicinal herbs have been replaced. The usage problems such as the increasing resistance of microorganisms and reduce the impact of the continuing application is reported[5]. Thyme is one of the Lamiaceae plants in the Mediterranean and some parts of Asia[6]. Thyme is Included 0.8 -2.6% essence and phenol (20-80%), monoterpene hydrocarbons (p-cymene), alcohol (linalool, β-terpinen)[7]. Thymol and carvacrol as the main part of the major phenolic compounds in the herb[8-9]. Thyme oil has anti-fungal effects, properties anti spasmodic, carminative, Capriccio ,spectrum, antifungal disinfectant, antioxidant and anti-rheumatism is a natural food preservative. This essence plant also is used in the beverage industry,

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pharmaceutical and cosmetic care[10-11]. Thymol and carvacrol is an effective compound in toothpaste and mouthwash. It contain plenty of soap and disinfectant products. The present study was aimed to investigate the inhibitory effects Thyme essence against Multidrug-resistant strains of *Acinetobacter baumannii* Selected hospitals in Tehran.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The number of 60 *Acinetobacter baumannii* strains was isolated and identified using standard microbiological methods. (Culture, biochemical tests, catalase, oxidase, morphology). Susceptibility testing of antibiotics neomycin, gentamicin, amikacin, kanamycin, and oxacillin was performed. The resistant strains were used to investigate in order to antimicrobial effect of thyme. To evaluate the antimicrobial effects of thyme essential oil diffusion method (disk diffusion) were used. We use dimethyl sulfoxide (DMSO) for dissolve thyme essential oil. Culture carried out with sterile swab and microtube suspension was cultured for 24 h and then inoculated onto Mueller Hinton agar blank discs with a diameter of 6 mm and containing 30 µl of the concentration of essential oil was placed on Muller Hinton agar medium. After 24 h incubation at 37°C, zones of growth inhibition were measured. Each concentration was repeated 3 times for each of the bacteria and the average was documented. Disks containing 30 µl of dimethyl sulfoxide were used as a negative control. To determine the minimum inhibitory concentration (MIC) for the thyme essential oil to 100 µl (100% concentration) was dissolved in 900 µl DMSO.

Then the 1, 3, 5, 7, 9, 11, 13, 15 µg of this suspension was added to 1 ml Muller Hinton broth. After mixing, 1 ml of bacterial suspension (1.5×10^8 cfu / ml) Was added to the medium after incubation at 37 °C for 24 hours and the results were evaluated. *Acinetobacter baumannii* ATCC 19606 was employed in this study as a model reference strain.

Plant material and extraction procedure

First, thyme flower at full bloom were collected in June 2010 in Najaf Abad, Isfahan, Iran. Essential oil by steam distillation of the aerial parts of the plant was prepared aerial. Oil after drying with sodium sulfateat 4 °C until the GC injection system was kept. Analysis and identification of constituent composition of the oils was performed by GC mass. The results have a high percentage of antimicrobial compounds in the essential oil of this plant show. Thymol (28.8%) and carvacrol (23.46%) on the essential oil is a sign antimicrobial. STEM: Beifen 3420 capillary gas chromatographs. Column: BP-5 (5%phenyl: 95% polydimethyl siloxane) fused silica capillary column (30mm×0.25mm Internal diameter, 0.25µm film thickness Kind of gas: Helium with purification 99.999%.

RESULTS AND DISCUSSION

Based on the results of *Acinetobacter baumannii* for measuring the zone of growth inhibition at a concentration of 30µg/ml thyme essential oil were found to have inhibitory effects on bacteria. Antibiotic susceptibility test results

Table 1. Results MIC Zataria*multiflora* essential oil the standard strain and Pathogen strain *Acinetobacterbaumannii*

	Zatariamultiflora *Essence*			Antimicrobial agents					
				MIC(µg/ml)			Zone of growth inhibition(mm)		
Pathogenic strains	Average MIC (µg/ml)	Average MBC (µg/ml)	Average Zone of growth inhibition(mm)	CIP	GM/IMP	AMK/CRO/OX/K/N	CIP	GM/IMP	AMK/CRO/OX/K/N
A.baumannii	0.44	0.6	18.6	>4	>16	>64	0	0	0
A.baumannii ATCC 19606	0.5	0.7	24	>4	>16	>64	0	0	0

CIP(Ciprofloxacin),GM(Gentamaicin),IMP(Imipenem),AMK(Amikacin),CRO(Ceftriaxone),OX(Oxacillin), K(Kanamaycin),N(Neomycin).

are as follows. Oxacillin (100%) , Amikacin (91%), Kanamycin (61.6)% , Gentamicin(55%) and (86.6%) were resistant Neomycin. Inhibitory effect of thyme on drug-resistant strains were found (Table 1). Increasing of antibiotic resistance is a serious problem in worldwide. Some medications and antibiotics have completely lost their medicinal effects, this problem causes to the development and creation of multiple disease resistance.

Medicinal plants in the last decade used as natural reservoirs of drug and because of this have been discussed. Their advantages in relation to plant extracts are natural reservoir and laboratory experiments have shown no adverse effects.

In this research In this study, satureja essence were used to assess their antibacterial activity against important pathogens by inserting some minor changes to the CLSI recommended agar dilution method that have been originally developed for analyzing the conventional antimicrobial agents activity, so it could be used to analyze plant extracts and essential oils for their antimicrobial activity (12-13-14-15). Ciprofloxacin and Ceftriaxone is used to treat infections caused by *Acinetobacter baumannii*. According to all the above results Ceftriaxone and Ciprofloxacin resistant strains are sensitive to the *Zataria multiflora* essential oil with low Mic. This shows the power of *Zataria multiflora* essential oil antimicrobial drug-resistant strains of *Acinetobacter baumannii* are resistant.

In this study using satureja essence against *Acinetobacter baumanii* resulted in these which can be effective enough to reduce the rate of infection transmission. Due to the high resistance to most drugs and disinfectants in *Acinetobacter baumannii* and high prevalence of nosocomial infections and enormous economic costs and the restrictions on the use of broad-spectrum drugs in people with weakened immune systems and according to the antimicrobial effect of thyme essential oil It is hoped the research laboratory to the clinic with a wider range be used as a complementary therapy.

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