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Research Article

Antibacterial Effects of Herbal Compounds against *Acinetobacter baumannii* Isolated from Hospital of Tehran, Iran

Abstract

Increasing isolation of multidrug-resistant *Acinetobacter baumannii* (MDR) has been reported in worldwide and it is recently one of the most difficult nosocomial acquired gram-negative pathogens to control and treatment. These bacteria are one of ESBL producing that great potential have for the rapid development of antibiotic resistance. Use of medicinal herbaceous drugs recommended for treatment since ancient periods. Humans have been used and realize their beneficial effects. With the increase in population and urban growth, reduced use of synthetic drugs, many of these of medicinal herbs have been replaced. Because of *Satureja, Origanum vulgare* and *Thyme* essence has antimicrobial properties so can be used against infections caused by MDR *Acinetobacter baumannii*. In this study the antimicrobial effects of *Satureja, Origanum vulgare* and *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 *Satureja* essence compare with *Origanum vulgare* and *Thyme* 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.

Introduction

Acinetobacter baumannii is a gram-negative bacteria and important opportunistic pathogen, causes a variety of nosocomial infections especially in Intensive Care Units (ICU's) [1,2]. These infections include bacteremia, surgical-site infections, secondary meningitis, urinary tract infections and ventilator associated pneumonia. These organisms have been implicated in a diverse range of infections such as respiratory tract, blood stream, skin and soft tissue. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and demonstrates its successful adaptation to the 21st century hospital environments [3]. Use of medicinal herbaceous drugs recommended for treatment since ancient periods. 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 [4]. The usage problems such as the increasing resistance of microorganisms and reduce the impact of the continuing application is reported.

Materials and Methods

Extraction, isolation and identification of the oils

Aerial parts of *Satureja* and *Thyme* were collected from Barij Essence research farm in May 2010. Plant material and extraction procedure flowering aerial parts of *O. vulgare* was collected from Khoramabad, Lorestan, Iran, in June 2010. Essential oils by steam distillation of the aerial parts of the plant were prepared aerial. Oil after drying with sodium sulfate at 4°C until the GC (gas chromatography) injection system was kept. Analysis and identification of constituent composition of the oils was performed by Barij Essence Company. The fresh flowering aerial parts of *Satureja*, *Origanum vulgare* and *Thyme* were subjected to hydro distillation and a yield of 0.27% (v/w) was obtained. The GC /MS (gas chromatography–mass spectrometer) results indicated that these oils have many antibacterial compounds. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60m×0.25mm, film thickness 0.25_m). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230°C at a rate of 3°C/min held for 10min. Helium was used as the carrier gas at a flow rate 1.0 ml/min.

Bacterial strain and culture conditions

The number of seventy five *Acinetobacter baumannii* strains was isolated and identified using standard microbiological methods. Susceptibility testing of antibiotics neomycin, gentamicin, amikacin, kanamycin, imipenem and oxacillin were performed. The resistant strains were used to investigate in order to antimicrobial effect of *Satureja, Origanum vulgare* and *Thyme* essence. To evaluate the antimicrobial effects *Satureja, Origanum vulgare* and *Thyme* essential oil diffusion method (disk diffusion) were used. We use dimethyl sulfoxide (DMSO) for dissolve essential oil. Culture carried out with sterile swab and micro tube 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 oils were 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 *Satureja, Origanum vulgare* and *Thyme* essential oil to 100 µl (100% concentration) was dissolved in 900 µl DMSO. Then the 1, 2, 3, 4, 5, 6, 7, 8,9,10 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 h and the results were evaluated. *Acinetobacter baumannii* ATCC 19606 was employed in this study as a model reference strain.

Results and Discussion

Antibiotic susceptibility test results the number of seventy five *Acinetobacter baumannii* strains are as follows. Oxacillin (100%), Amikacin (75%), Kanamycin (68%), Gentamicin (60%), Imipenem (60%) and (89%) were resistant Neomycin. Inhibitory effects of *Satureja* on drug-resistant strains were found. Results MIC (µg/ml) *Satureja, Origanum vulgare* and *Thyme* essential oils and Zone of growth inhibition (mm) pathogen strain *Acinetobacter baumannii* (Table 1) (Figure 1). The major components of *Satureja* essence were carvacrol (90.88%), ρ-cymene (3.11%), γ - trepanned (1.24%), linalool (0.91%), *Origanum vulgare* are Pulegone (68.59%), Piperitenone Oxide and Piperitone (7.8%) and the *Thyme* are thymol (28.8%) and carvacrol (23.46%).

Conclusions

Nosocomial infections caused by Multidrug resistant strains of *Acinetobacter baumannii* (MDR-AB) are currently among the most difficult to treat, and they continue to present serious challenges to clinicians' empirical and therapeutic decisions in burned patient [5]. Outbreaks of extensively, and pan drug-resistant *A. baumannii* (XDR, and PDR, respectively) currently has been reported from worldwide.

Table 1: Results MIC *Satureja, Origanum vulgare and Thyme* essential oils the standard strain and Pathogen strain *A. baumannii.*

Pathogenic strains		*Acinetobacter baumannii*	*A.baumannii ATCC 19606*
Satureja Essence	Average MIC (µg/ml)	0.3	0.5
	Average MBC (µg/ml)	0.48	0.7
	Average Zone of growth inhibition(mm)	25.5	24
Origanum vulgare	Average MIC (µg/ml)	2.6	2.5
	Average MBC (µg/ml)	3.2	3
	Average Zone of growth inhibition(mm)	10.8	15
Thyme Essence	Average MIC (µg/ml)	0.44	0.5
	Average MBC (µg/ml)	0.6	0.7
	Average Zone of growth inhibition(mm)	18.6	24
MIC(µg/ml)	CIP	>4	>4
	GM/IMP	>16	>16
	AMK/CRO/OX/K/N	>64	>64
Zone of growth inhibition(mm)	CIP	0	0
	GM/IMP	0	0
	AMK/CRO/OX/K/N	0	0

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

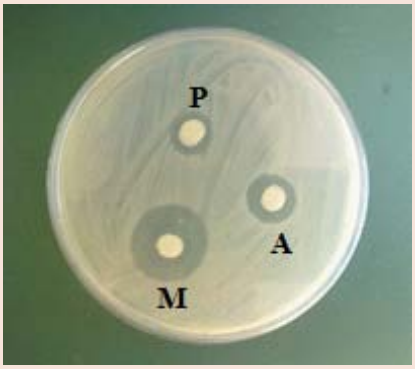

Figure 1: Zone of Growth Inhibition of the *Satureja, Origanum vulgare and Thyme* essence against Multidrug-Resistant *Acinetobacter baumannii .M (Satureja), A (Thyme), P (Origanum vulgare*).

Health problems have been caused by *Acinetobacter spp*. and the possibility of transition between living and nonliving things as well as long-term survival in hospital environments enhance the appearance of this bacterium in hospital environments and its consequent infections [4]. In this study, the high prevalence of XDR and PDR *A. baumannii* isolates (37.1% and 8.1%, respectively) from patients is consistent with previous reports [6,7]. Increasing prevalence of XDR and PDR *A. baumannii* strains and limited treatment options has prompted the use of antibiotics combinations like tigecycline and colistin as therapeutic regimens [8,9]. There are several studies on the effects of antibacterial essential oils of *Thyme* and *Satureja* including carvacrol and thymol. The major antimicrobial properties of these plants are dependent carvacrol and thymol [10]. In the study by Kim and et al., antibacterial effects of carvacrol on *Salmonella typhimurium* were studied. Carvacrol had a strong antibacterial effects [11]. In a study, *Satureja* inhibitory effect on the enzyme S, exotoxin a, secretory systems and efflux pumps of *Pseudomonas aeruginosa* was investigated by semi-quantitative RT-PCR technique, against this genes has an inhibitory effects [12]. The antimicrobial essential oil of *Origanum vulgare* against a range of bacteria including: *Staphylococcus species, Pseudomonas, Bacillus* and *E. coli* as well as some fungal species such as *Aspergillus, Fusarium* and *Penicillium* been found [13].In this research, *Satureja, Origanum vulgare* and *Thyme* essence were used to assessment their antibacterial activity against important pathogen 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. Due to the high resistance to more drugs and disinfectants in *A. baumannii* and high prevalence of nosocomial infections and enormous economic costs and the restrictions on the use of broad-spectrum drugs in persons with Immune compromised applications of native compounds against these pathogens resulted in these which can be effective enough to reduce the rate of infection transmission. According to results of current research we hope in future be used it to the clinic with a wider range as a complementary therapy. Additional clinical research and trials are necessary to completely confirm the above results for medical purposes.

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