



Investigation of Aqueous and Alcoholic Extract of *Thymus daenensis* against methicillin Resistant *Staphylococcus aureus*

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ABSTRACT: Considering the increase in resistance to antibiotics, study of medicinal plant and their mechanism of effect can be of considerable help in appropriate use of these compounds in treatment of infectious diseases. In this study the effect of aqueous and alcoholic extract of *Thymus daenensis* has been explored on methicillin resistant *Staphylococcus aureus*. The antimicrobial effect of extract of *Thymus daenensis* were examined against two species of clinical and standard methicillin resistant *Staphylococcus aureus* and standard *Staphylococcus aureus* by agar well diffusion and serial dilution methods. The results indicate that the aqueous extract of *Thymus daenensis* effective on the growth of species studied. The antimicrobial effects of alcoholic extract were increased by increasing their concentration. Considering the antibacterial effect of aqueous and alcoholic extracts of *Thymus daenensis* on methicillin resistant *Staphylococcus aureus* (MRSA), more extensive studies on its antibacterial effect at *in vivo* condition is proposed for clinical usage.

Keywords: Antibiotic resistance, methicillin resistant *Staphylococcus aureus*, *Thymus daenensis*

INTRODUCTION

The herbal medicine has been used for long in treatment of diseases (Yadegar *et al*, 2009 and Moshafi *et al*, 2006). In spite of chemical drugs, the effective elements are accompanied by other elements with biologic state which prevents the accumulation and side effect of these medicine (Talei *et al*, 2008). According to increasing of antibiotic resistance, study of medicinal plant and their mechanism can be of considerable help in appropriate use of these compounds in treatment of infectious diseases (Sadeghzadeh *et al*, 2006). *Thymus* is aromatic plant, leaves for a few years and belongs to the Lamiaceae family. *Thymus* has anti-spastic, antifungal and antibacterial effects (Godarzi *et al*, 2006 and Akbarinia *et al*, 2010 and, Barazandeh and Bagherzadeh, 2007). *Thymus daenensis* is a type of thymus that is exclusive to Iran (Nikavar *et al*, 2004). The infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) are drug resistant infections. This infection is one of the important causes of nosocomial infections (Yadegar *et al*, 2009 and Cunha, 2005). Mupirocin (pseudomonic acid) which is produced by *Pseudomonas fluorescens* is used for control of MRSA infections. The most important problem in treatment of MRSA infections is the increase in resistance to most antibiotics (Saderi *et al*,

2006 and Cutler and Wilson, 2004). Therefore, this is necessary to research in the field of herbal medicine use in treatment of infections. In this study, the effect of aqueous and alcoholic extracts of *Thymus daenensis* on methicillin resistant *Staphylococcus aureus* is explored.

MATERIAL AND METHODS

Extraction: *Thymus daenensis* is collected mostly from Fereydounshahr. Confirming the species has taken place in the agricultural research center and natural resources in the province of Isfahan. 10 grams of leaves was added to 250 ml of distilled water for 72 hours on rotary shaker (Pandey *et al*, 2014). 10g of leaves powder were extracted with 250 ml methanol by Soxhlet extraction for 72 hours (Samsamshariat, 2007). Condensation of the extracts were done by rotary evaporator. The extracts were dissolved in 5%, dimethylsulfoxide (DMSO). The resulting extracts were kept in a dark and cold place in sterile vials for further studies (Amjad *et al*, 2012).

Test organisms: In this study the clinical and standard species of methicillin resistant *Staphylococcus aureus* (PTCC 1764) and the standard species of *Staphylococcus aureus* (PTCC 1112) were obtained from the Institute of Scientific and Industrial Researches, Iran.

In order to study the antibacterial effect of extracts, a suspension provide from bacteria (1.5×10^8 CFU/mL, 0.5 McFarland) in Mueller Hinton Broth medium. Antibacterial assay: The extracts were diluted in 5% DMSO at the concentrations of 5, 10, 20, 50 and 150 mg/ml of alcoholic extract and also 150 mg/ml of aqueous extract. Antibacterial activities were studied by the agar well diffusion method. Mueller Hintonagar was used as the bacteriological medium. The suspension, equivalent to 0.5 McFarland, was cultured. Wells were prepared in the seeded agar plates and 30 μ l from each dilution of aqueous and alcoholic extracts was added separately to the wells. DMSO 5% was used as the negative control and ciprofloxacin was used as positive control (Amjad *et al.*, 2011). The plates were incubated overnight at 37°C. Plates were then examined for the presence of zones of inhibition, and the diameters were measured. The minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration

(MBC) were determined. From the alcoholic extract serial dilutions of 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml and from the aqueous extract, serial dilutions of 200, 100, 50 and 25 mg/ml use for each species on the Mueller Hinton Broth medium. After incubation in the temperature of 37°C, the results were explored.

RESULTS

The aqueous extracts of Thymus leaves have effected on growth of tested organisms and zones of inhibition was observed around them (Table 1). The inhibitory effects of alcoholic extract against different tested organisms are shown in Fig.1. MBC of aqueous extract for each species was more than 200 mg/ml and the least dilution of MBC of alcoholic extract was related to the standard methicillin resistant Staphylococcus aureus species (Table 2). The statistical analysis was done by SPSS software.

Table 1: Zone of Inhibition (ZOI) produced by aqueous extract against pathogens.

Tested bacteria	Zone of Inhibition(ZOI) (mm)		
	Extract concentration (150mg/ml)	Negative control	Positive Control
MRSA	13.6	-	29
MRSA (PTCC1764)	13	-	24
<i>S. aureus</i> (PTCC1112)	13	-	25

Cotrol (+): Ciprofloxacin, Cotrol (-): DMSO 5%

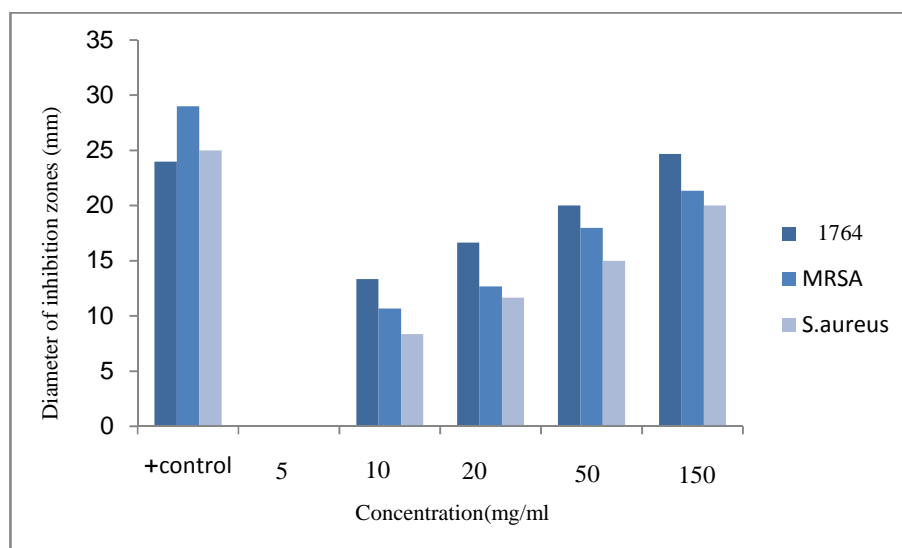


Fig.1. ZOI of different concentrations of ethanol extract of thyme.

Table 2: MIC, MBC.

Bacteria	Aqueous extract		Alcoholic extract	
	MBC	MIC	MBC	MIC
MRSA	>200	50	100	100
MRSA (PTCC1764)	>200	100	50	50
<i>S. aureus</i> (PTCC1112)	>200	100	>200	100

DISCUSSION

God has created the treatment of any disease of any area in the plants growing there. Exploring the effects of different types of plants in Iran on treatment of diseases can be an important step forward in identification and optimal use of this precious resource (4). Previous studies have shown that the extract of *Thymus daenensis* has impact on growth of *Candida albicans* and *Listeria monocytogenes* (Ghasemi Pirbalouti *et al*, 2010 and Ghasemi Pirbalouti *et al*, 2009). The components of *Thymus daenensis* extract include thymol, carvacrol, para-cymene, gamma-terpinenebetacaryophyllene as the major ingredients (Arzani *et al*, 2010 and Barazandeh and Bagherzadeh, 2007). A study on the extract of this plant has shown that this extract has impact on gram positive bacteria but not the gram negative bacteria (Mojab *et al*, 2008). The results of the current study shows that the aqueous and ethanol extract of leaves of *Thymus daenensis* have antimicrobial effect on methicillin resistant *Staphylococcus aureus*.

CONCLUSION

Considering the antibacterial effect of aqueous and alcoholic extracts of *Thymus daenensis* on methicillin resistant *Staphylococcus aureus* (MRSA), more extensive studies on its antibacterial effect for clinical purposes in treatment of MRSA seems to be essential.

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