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EVALUATION OF ANTIBACTERIAL ACTIVITY OF DECOCTION, INFUSION AND ESSENTIAL OIL OF *ORIGANUM VULGARE* ON METHICILLIN RESISTANT AND METHICILLIN SENSITIVE *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Methicillin resistance of 163 strains of *Staphylococcus aureus* isolated from nasal cavity was determined by disc diffusion method. The incidence of methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *Staphylococcus aureus* (MRSA) was noted as 128 (78.5%) and 35 (21.5%) respectively. The inhibition efficacy of decoction, infusion and essential oil of *Origanum vulgare* (oregano) was assayed against all isolates, using well diffusion assay. All the isolates were found resistant to decoction and infusion whereas the essential oil of oregano exhibited antibacterial activity against 124 (96.88%) MSSA and 28 (80%) MRSA isolates. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oregano oil against MSSA and MRSA was same. It was noted as 0.31-2.5%,(v/v).

Key words: *Origanum vulgare*, methicillin resistance, *Staphylococcus aureus*, antibacterial activity.

INTRODUCTION

Staphylococcus aureus is one of the most frequently isolated pathogens from clinical specimens (Gakuu, 1997; Sakoulas *et al*., 2001). Its main habitats are the nasal membranes and the skin of humans and warm blooded animals (Oliveira *et al*., 2002; Fang and Hedin, 2003). It causes a variety of serious diseases associated with a high mortality (Gakuu, 1997). *S. aureus* acquire antibiotic resistance with remarkable proficiency, and strains for which vancomycin is the only effective therapeutic agent have emerged (Fang and Hedin, 2003).

Methicillin resistant *S. aureus* (MRSA) is an important pathogen and a major cause of nosocomial infections (Watanabe *et al*., 2000; Sakoulas *et al*., 2001). Resistance to methicillin was first described in 1960, shortly after the introduction of the drug into clinical practice. Since then, MRSA has become a widely recognized cause of morbidity and mortality throughout the world (Hookey *et al*., 1998). Data from around the world verify the escalating incidence of infections caused by MRSA. Infections with MRSA are serious and often life-threatening (Wenzel *et al*., 1991).

The incidence of infections caused by MRSA continues to increase in many countries world-wide (Simor *et al*., 2001). The percentage of nosocomial *S. aureus* isolates that are methicillin resistant rose from 14.3% in 1987 to 39.7% in 1997 (Sakoulas *et al*., 2001). Since methicillin resistance is now wide spread in hospitals all over the world, therapy has become cumbersome. A new and even more threatening development is the emergence of strains with a reduced susceptibility to glycopeptides. Therefore, control of *S. aureus* in hospitals has now become more important than ever before (Gakuu, 1997).The growing concern over the advent of increasingly antibiotic resistant organisms has prompted many individuals to look for alternative treatments. One such alternative is the herbal remedies, which are being marketed with mounting zeal. Scientific experiments since the late 19^{th} century have documented the antimicrobial properties of some spices, herbs and their components (Zaika, 1988). Researchers have been testing herbs for activity against pathogenic organisms.

The leaves and dried herb of oregano as well as oil are used medicinally (Hammer *et al*., 1999). Mohacsi-Farkas *et al.* (2003), tested antimicrobial activity of essential oil and supercritical fluid extracts (SCFEs) of *Origanum vulgare* and *Satureja montana* and concluded that microorganism strains were more sensitive to *Origanum vulgarum* extracts than to *Satureja montana* extracts, and essential oils were more effective than SCFEs added in the same concentrations. In a similar study conducted by Hammer *et al.* (1999) oregano oil showed a strong anti-microbial action against a wide number of bacteria, including *Escherichia coli, Klebsiella pneumoniae, Salmonella enterica* and *Staphylococcus aureus.* Other studies demonstrated sensitivity of *Staphylococcus aureus, Pseudomonas* spp, *Proteus mirabilis, Klebsiella pneumoniae* and group B *Streptococci*, *Escherichia coli, Aerobacter aerogluess* and *Bacillus subtilis* (Anonymous, 2003) *Listeria monocytogenes, Escherichia coli* O157:H7, *Yersinia enterocolitica, Lactobacillus plantarum, Aspergillus niger, Geotrichum* and *Rhodotorula* (Elgayyar *et al*., 2001) to oregano. . These results support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives. In view of this, it was aimed to conduct the study to evaluate the antibacterial activity of

decoction, infusion and essential oil of *Origanum vulgare* against methicillin resistant and methicillin sensitive *Staphylococcus aureus*.

MATERIALS AND METHODS

A total number of 163 strains of *S.aureus* isolated from the nasal cavity. All cultures were maintained on tryptic soy agar medium (Sonnenwirth and Jarett, 1980). Methicillin resistance test was performed using disc diffusion assay according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (Cheesbrough, 1994; Brooks *et al*., 2002).Mueller-Hinton broth was used for the preparation of inoculum and Mueller-Hinton agar medium for the determination of antibiotic resistance pattern. Zone of inhibition was measured to the nearest mm. Isolates exhibiting ≤ 9mm and ≥ 14mm zone of inhibition were considered resistant and sensitive, respectively.Mueller-Hinton broth was used for the preparation of inoculum and Mueller-Hinton agar medium for the determination of antibacterial activity of oregano. Infusion of oregano was prepared by steeping 10gm and 20gm of oregano in 50mL of sterile distilled water for 24 hrs., yielding 20 and 40% concentration. The contents were filter sterilized.Decoction of oregano was prepared by boiling 10gm and 20gm of oregano in 50mL sterile distilled water over low flame for 15-20minutes, yielding 20 and 40% concentration. Decoction was cooled and filter sterilized. Essential oil of oregano (Planter) was purchased from the market. It was considered as 100% concentration. Screening of antibacterial activity was performed by well diffusion assay (Kivanc and Kunduhoglu, 1997). All the tubes and plates were incubated at 35-37°C for 24 hours. Zone of inhibition was measured to nearest mm. Minimal inhibitory concentration (MIC) of sensitive strains was determined by using tube dilution assay (Baron *et al*., 1994). Minimum bactericidal concentration (MBC) of sensitive strains was determined by using tube dilution assay (Baron *et al*., 1994).

RESULTS AND DISCUSSION

Antibacterial activity of infusion, decoction and oil of oregano was evaluated against methicillin sensitive and methicillin resistant *S. aureus. S. aureus* is one of the most significant human pathogens (Fang and Hedin, 2003) and methicillin resistant *S. aureus* is responsible for an increasing number of serious nosocomial and community acquired infections (Sakoulas *et al*., 2001). One hundred and sixty three strains of *Staphylococcus aureus* were isolated from nasal cavity. Methicillin resistance was determined among the isolates and thirty five (21.5%) isolates were found resistant to methicillin (Table 1).

Table 1. Pattern of methicillin susceptibility of *Staphylococcus aureus.*

Organisms	Isolates		Average zone of inhibition in mm ±S.D.
	No.	%age	
Methicillin sensitive *S.aureus* (MSSA)	128	78.5	23±2.58
Methicillin resistant *S.aureus* (MRSA)	035	21.5	8.7±1.15
TOTAL	163	100	19.8±6.3

S.D.= Standard Deviation, Resistant breakpoint used was ≤ 14 mm.

The antibacterial activity of essential oils and their derivatives has been recognized for a long time (Baydar *et al*., 2004). In recent years, oil of oregano has been largely investigated for its antimicrobial properties and has been reported to kill at least 30 different harmful bacteria, such as *S.aureus* as well as other microorganisms, including corona viruses (Meschino, 2005). Lin *et al.* (2005), have reported the antibacterial activity of oil of oregano against *Vibrio parahaemolyticus*. Baydar *et al*. (2004), also reported inhibitory activity of oregano oil in concentration <1/100 against *Aeromanas hydrophila, Bacillus amyloliquefaciens, B. brevis, B. cereus, B. subtilis,*

Corynebacterium xerosis, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Mycobacterium smegmatis, Proteus vulgaris, Staphylococcus aureus and *Yersenia enterocolitica*. Another study also reported the antimicrobial activity of essential oil of oregano against *Escherichia coli* O157:H7 (Moreira *et al*., 2005). In view of this, the screening of antibacterial activity of decoction, infusion and essential oil of *Origanum vulgare* against MSSA and MRSA was determined. None of the isolates used in this study showed sensitivity towards infusion and decoction of oregano. However, oil of oregano was very effective against both MSSA and MRSA, as 96.88% of MSSA and 80% of MRSA isolates were found sensitive to oregano oil. The average zones of inhibition for MSSA and MRSA against oregano oil were recorded as 11.3mm and 10.9mm, respectively (Table 2).

Table 2. Screening of antibacterial activity of decoction, infusion and essential oil of *Origanum vulgare* against *Staphylococcus aureus.*

Origanum vulgare	TOTAL ISOLATES	ISOLATES SUSCEPTIBLE		ISOLATES RESISTANT	
		No.	%age	No.	%age
INFUSION	163	Zero	0	163	100
DECOCTION	163	Zero	0	163	100
ESSENTIAL OIL	163	152	93.3	11	6.7

Table 3. Determination of minimum inhibitory concentration (mic) of oil of *Origanum vulgare* against methicillin sensitive and methicillin resistant *Staphylococcus aureus.*

ORGANISMS	TOTAL NO.OF ISOLATES	No. of isolates inhibited at conc.%							MIC
		0.16	0.31	0.63	1.25	2.5	5.0	10.0	
METHICILLIN SENSITIVE S.*AUREUS*	28	Zero (0)	02 (7.1)	05 (17.8)	21 (75)	28 (100)	28 (100)	28 (100)	0.31-2.5%
METHICILLIN RESISTANT S.*AUREUS*	124	Zero (0)	13 (10.4)	40 (32.2)	89 (71.8)	124 (100)	124 (100)	124 (100)	0.31-2.5%

Numbers in parenthesis are percentages.

In the present study, MIC and MBC of essential oil of *Origanum vulgare* against MSSA and MRSA was also determined. The MIC and MBC of oregano oil against both MSSA and MRSA was recorded as 0.31-2.5% (Table 3 & 4). The results of the present study demonstrate that MSSA and MRSA showed a similar kind of sensitivity pattern. These results are in harmony with that of Hammer *et al*.(1999), who reported the MIC for oregano oil ≤2.0% and Nostro *et al.*, (2004) who reported the MIC values for oregano oil as 0.06-0.125% v/v against methicillin sensitive and methicillin resistant *staphylococci*. These results suggest that essential oil of oregano can be used effectively against both MSSA and MRSA.

Table 4. Determination of minimum bactericidal concentration (mbc) of oil of *Origanum vulgare* against methicillin sensitive and methicillin resistant *Staphylococcus aureus.*

ORGANISMS	TOTAL NO.OF ISOLATES	No. of isolates inhibited at conc.%							MBC
		0.16	0.31	0.63	1.25	2.5	5.0	10.0	
METHICILLIN SENSITIVE S.*AUREUS*	28	Zero (0)	02 (7.1)	05 (17.8)	21 (75)	28 (100)	28 (100)	28 (100)	0.31-2.5%
METHICILLIN RESISTANT S.*AUREUS*	124	Zero (0)	13 (10.4)	40 (32.2)	89 (71.8)	124 (100)	124 (100)	124 (100)	0.31-2.5%

Numbers in parenthesis are percentages.

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