ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS AGAINST MRSA STRAINS ISOLATED FROM SURGICAL WOUND INFECTIONS

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Methicillin resistance in *Staphylococcus aureus* is one of the most important antibiotic resistance. In this study, we aimed to investigate the antibacterial activities of some medicinal plants *(Laurus nobilis, Salvia officinalis, Thymbra spicata)* against MRSA strains isolated from surgical wound infections. Firstly, non-cytotoxic concentrations were determined in cellculture. In order to determine the non-cytotoxic concentrations of essential oils, HEp-2 cell line was selected. Antimicrobial activity studies were carried out under the non-cytotoxic concentrations for cells. Mueller-Hinton broth was selected to test the bacterium strain. The inoculum density was 1x 10^6 cfu/ml. The essential oils of plants were dissolved in absolute ethanol. The ratio of essential oils in the test medium furnished the required concentration ranging from 1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml. The plates were incubated at 37°C and visually read after 48 hours. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity. The antibiotic susceptibilities of MRSA isolates were determined by microdilution method according to the CLSI (Clinical and Laboratory Standards Institute) criteria. MIC for essential oils were investigated against both standard and clinical isolates of Methicillin-resistant *S.aureus*. In this study, the essential oils of these three plants have been confirmed the antibacterial effect against methicillin resistant *S. aureus*. Also, while the essential oils of *L. nobilis* and *S. officinalis* were found to exhibita significant synergistic activity with antimicrobial drugs, *T. spicata* showed limited synergistic activity compared the others.

Keywords: *S.aureus*, methicillin, resistance, essentialoils, cellculture.

INTRODUCTION

Antibiotic resistance is a major public health problem. *S. aureus* is an opportunistic pathogen microorganism that is asymptomatically carried on different parts of the human body including skin and nasal passages. In recent years, the significance of Gram-positive microorganisms in surgical site infections has come to the fore worldwide. Among Gram-positive bacteria is *S. aureus*, one of the most significant infectious agents, whose infections range from mild to life threatening degree (CDC, 2006; Wasserman and Taljaard 2011; Jonson, 1998). Especially, *S. aureus* in patients who undergo surgery may lead to life-threatening infections. Methicillin resistance in this microorganism is reported to be more difficult to treat than ordinary staph infections. MRSA ratio was reported to vary from 10 to 65% in hospitals. This rate is reported to be significantly higher in intensive care units. MRSA is resistant to many conventional drugs. It is resistant to most beta-lactams in addition to aminoglycosides, erythromycin, clindamycin, fluoroquinolones, co-trimoxazole and rifampin (Fraise, 1998; Alexander *et al.*, 2011; CLSI, 2010).

Despite effective antimicrobial options in staphylococcal infections, increasing drug resistance leads to serious problems for the treatment of staphylococcal infections. To this end, new drug research is underway. Nowadays, natural products continue to be an important source of novel drugs. Among these natural products, are *L. nobilis, S. officinalis* and *T. spicata*, which have various pharmacological properties (Derwich *et al.*, 2009; Lawrence, 2005; Alarcon *et al.*, 2002; Akin *et al.*, 2010; Kilic, 2006).

In this study, we aimed to search the antibacterial activities of some medicinal plants *(L. nobilis, S. officinalis, T. spicata)* against MRSA strains isolated from surgical wound infections.

MATERIALS AND METHODS

Microorganisms

In this study, MRSA ATCC and clnical MRSA strains isolated from surgical wound infections were used. MRSA strains were obtained from Mustafa Kemal University culture collections, Hatay. For culture of microorganisms Mueller-Hinton broth (Difco, USA) and Blood agar (Difco, USA) were used. The essential oils of the *L. nobilis, S. officinalis* and *T. spicata* were obtained from Department of Field crops, Agriculture Faculty of Mustafa Kemal University. Firstly, non-cytotoxic concentrations were determined in cell culture. In order to determine the non-cytotoxic concentrations of essential oils, HEp-2 cell line was selected. Antimicrobial activity studies were carried out under non-cytotoxic concentrations for cells. Mueller-Hinton broth was selected to test the bacterial strains. The inoculum density was 1x 10^6 cfu/ml.

Cell Culture

Firstly, non-cytotoxic concentrations were determined in cell culture. In order to determine the non-cytotoxic concentrations of essential oils of *L. nobilis, S. officinalis* and *T. spicata*, HEp-2 cell line (human epithelial cells derived from a larynx carcinoma) was selected. Antimicrobial activity studies were carried out studied under thenon-cytotoxic concentrations for cells. As a culture medium RPMI-1640 was selected. The cell culture medium consisted of RPMI-1640 with fetal calf serum (Seromed, Germany) at a ratio of 10% as the growth factor. The cells were incubated in an atmosphere of 5% carbon dioxide at 37°C.

The Effect of Ethanol

Ethanol was used as a solvent for the essential oils of *L. nobilis, S. officinalis* and *T. spicata.* To determine the non-toxic concentration of ethanol, 1×10^6 cells were inoculated into each well of flat-bottomed plates containing RPMI-1640 and incubated for an 48 h in the presence of decreasing amounts of ethanol (15%, 12.5%, 10%, 7.5%, 5%, 2.5%, 1%). The non-toxic concentration was determined up to 10%. The 1-20% concentrations of ethanol did not affect the growth of the cells. Therefore, the essential oils were dissolved in 5% ethanol in experiments.

The essential oils of plants were dissolved in absolute ethanol. The ratio of essential oils in the test medium furnished the required concentration ranging from 1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml. The plates were incubated at 37°C and read visually after 48 hours. The MIC (minimal inhibitory concentrations) values were recorded as the lowest concentrations of the substances that had no visible turbidity. The antibiotic susceptibilities of MRSA isolates were determined by the microdilution

method according to the CLSI (Clinical and Laboratory Standards Institute) criteria. Minimal inhibitory concentrations for essential oils were investigated against both standard and clinical isolates of methicillin-resistant *S. aureus* (CLSI, 2008).

Cell Culture Tests for Cytotoxicity

In order to test the effect of the essential oils of *L. nobilis, S. officinalis* and *T. spicata* on HEp-2 cells, 5×10^5 cells were seeded into each well of flat-bottomed plates, cultured for 6 h at 28°C and the cells were allowed to grow for an additional 48 h. The essential oils of the plants were placed per well at decreasing amounts (1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000 µg/ml). The cytotoxicity of the essential oils was determined using a conventional haemocytometer and the trypan blue-exclusion method (Burlesson *et al.*, 1992). The highest noncytocidal (on HEp-2 cells) concentration of the The essential oils of *L. nobilis***,** *S. officinalis* and *T. spicata* were determined to be 125, 250 and 250 µg/ml, respectively. Therefore, up to 250 µg/ml was used for the determination of the antimicrobial activities.

Antimicrobial Activity

The essential oils of *L. nobilis*, *S. officinalis* and *T. spicata* were prepared by dissolving in ethanol and then diluting in Mueller-Hinton broth to give an initial concentration of 1000 µg/mL. Further dilutions of the essential oils and standard drug in the test medium were prepared at the required quantities at concentrations of 7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml µg/ml. The MIC for each compound was investigated against standard (methicillin resistant *S. aureus* (MRSA, ATCC 33591) and clinical methicilline resistant bacteria. The cultures were prepared in Mueller-Hinton broth (Difco, USA) for methicilline resistant bacteria. Antimicrobial activity was evaluated by using broth microdilution method. The MIC range was determined according to the CLSI guidelines. The lowest concentration that showed no growth of microorganism was recorded as the MIC expressed in µg/mL. These experiments were triplicated to determine the MIC values. Ampicillin/Sulbactam was used as the control drug.

Statistical Analysis

The statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS for Windows, version 17.5 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The essential oils were evaluated *in-vitro* for antimicrobial activity against both standart strains of MRSA and clinical strains of isolated from surgical wound infections. As the control drug, ampicillin/sulbactam was employed. Ethanol was used to solve the essential oils.

The cell cultures were incubated with ethanol alone (without any essential oils) in the ethanol control group. In the experiment, we compared the effects of ethanol with cell control group on the viability of HEp-2 cells. As given in Figure 1, ethanol concentration up to 10% (w/v) did not inhibit the growing of cells. At the end of incubation, there were not statistically significant difference in the cell number between the cell control and the ethanol containing groups ($p>0.05$). Nor were any cytopathological changes observed in ethanol group compared with the cell control group (Figure 1).

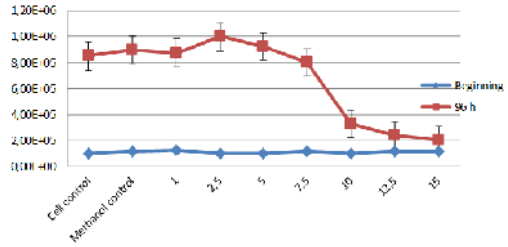

Figure 1. HEp-2 cell viability with different concentrations of ethanol

The cytotoxicity results of the essential oils were given in Figures 2-4. As can be seen from these figures, non-cytotoxic concentrations for *L. nobilis*, *S. officinalis* and *T. spicata* are up to 125 µg/ml, 250 µg/ml and 250 µg/ml, respectively.

Antimicrobial test results were given in Figures 5 and 6. As illustrated by these figures, while standart drugs inhibited the microorganisms growth between 2 and 8 µg/ml, the essential oils proved to inhibit the growth of bacteria with MIC values ranging between from 15.6 to 62.5 µg/ml (Figures 5 and 6).

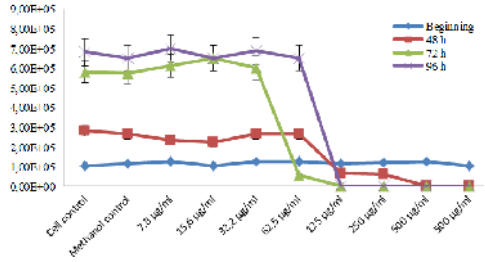

Figure 2. HEp-2 cell viability with different concentrations of the essential oils of *L. nobilis*

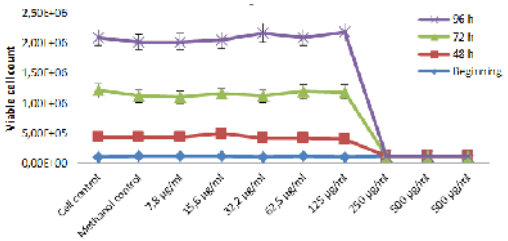

Figure 3. HEp-2 cell viability with different concentrations of the essential oils of *S. officinalis*

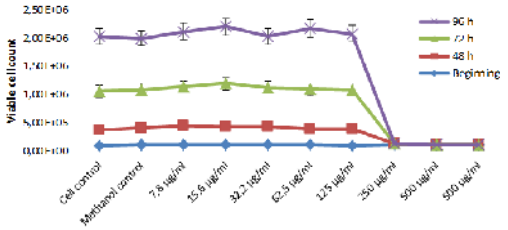

Figure 4. HEp-2 cell viability with different concentrations of the essential oils of *T. spicata*

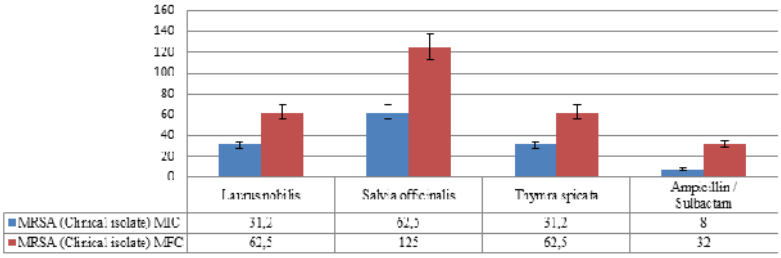

Figure 5. MIC values of the essential oils against MRSA isolated clinically

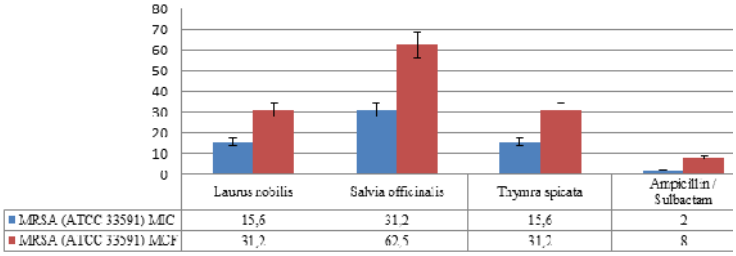

Figure 6. MIC values of the essential oils against standart MRSA strains

In this study, essential oils of these three plants have been confirmed to have antibacterial effect against methicillin resistant *S. aureus*. Also, while the essential oils of *L. nobilis* and *S. officinalis* were found to exhibit a significant synergistic activity with antimicrobial drugs, *T. spicata* showed limited synergistic activity compared with the others (Tables 1-4).

Table 1. MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) values (µg/ml) of the essential oils and Ampicillin/Sulbactam for MRSA

	MRSA (Clinical isolate)		*MRSA (ATCC 33591)*	
	MIC	MFC	MIC	MCF
Laurus nobilis	31.2	62.5	15.6	31.2
Salvia officinalis	62.5	125	31.2	62.5
Thymbra spicata	31.2	62.5	15.6	31.2
Ampicillin/Sulbactam	8	32	2	8

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 2. Synergistic effects of *L. nobilis* and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Laurus nobilis (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	NG	NG	NG	NG
4	G	G	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 3. Synergistic effects of *S. officinalis* and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Salvia officinalis (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	NG	G	NG	NG
4	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 4. Synergistic effects of T. spicata and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Thymbra spicata (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	G	G	G	NG
4	G	G	NG	NG	NG	NG
8	G	G	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

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