EFFECTS OF N-ACETYLCYSTEINE (NAC), FERMENTED SUMACH and EAU DE ROSE ON THE FORMATION OF SLIME LAYER OF *STAPHYLOCOCCUS* SPP.

by

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ABSTRACT

EFFECTS OF N-ACETYLCYSTEINE (NAC), FERMENTED SUMACH and EAU DE ROSE ON THE FORMATION OF SLIME LAYER OF *STAPHYLOCOCCUS* SPP.

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Implant associated infections are reported to cause very important problems in hospitalized and immunocompressed patients in worldwide. The genus *Staphylococcus* spp., mostly known with its severe pathogenic strains are responsible to secrete glycocalyx reported to be a polysaccharide coating, attach bacteria to prosthetic surfaces and after colonization the bacteria can spread out of the infected area to entire body forming the slime layer. In this study, N-acetyl cystein (NAC), a mucolytic and an antioxidative agent, fermented sumach (*Rhus coriaria*); widely used in south eastern Turkey as a salad dressing; and Eau de rose, an antiseptic was used as in solely and/or in combination to investigate the effect on the slime formation of *Staphylococcus* spp. A total number of 89 *Staphylococcus* strains were studied and 41 of them were found as slime producing strains. Significant differences between various concentrations of sole treatments were observed in the methicillin resistant Staphylococci (MRS) and methicillin sensitive Staphylococci (MSS) ($p<0.05$). It was found that the slime formation was decreased due to the increased concentrations of the fermented sumach, NAC and eau de rose treatments respectively, and the effects of combination treatments were not found to be better effective than the sole treatment.

Keywords: *Staphylococcus*, Slime layer, NAC, Fermented Sumach (*Rhus coriaria*), Eau de rose.

ÖZET

N-ASETİL SİSTEİN (NAC), SUMAK EKŞİSİ ve GÜL SUYU' NUN *STAPHYLOCOCCUS* TÜRLERİNDE BİYOFİLM TABAKASI OLUŞUMUNA ETKİLERİ

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İmplant ilişkili infeksiyonların, dünyanın her yerinde hastaneye yatırılmış ve immunitesi baskılanmış hastalarda çok önemli problemlere sebep olduğu bildirilmiştir. Çoğunlukla şiddetli patojenik suşlar olduğu bilinen Stafilokoklar, polisakkarit yapısında olduğu bildirilen, bakteriyi protez yüzeye yapıştıran glikokaliksi salgılar ve kolonizasyondan sonra bakteri biyofilm tabakasını oluşturarak vücudun her yerine infekte olmuş alandan yayılabilir. Bu çalışmada, mukolitik ve antioksidan bir ajan olan N-acetylcysteine, çoğunlukla salatalarda Türkiye' nin güney doğusunda geniş çapta kullanılan sumak ekşisi (Rhus coriaria); ve bir antiseptik olan gül suyu yanlız başlarına ve bileşik olarak Stafilokokların biyofilm oluşumuna etkilerini araştırmak için kullanıldı. Toplam 89 Stafilokok suşu çalışılmış ve bunların 41'inin slime oluşturduğu bulunmuştur. Kontrol ve eklentiler ile ilgili çalışmalardan sonra, metisilin dirençli Stafilokoklar (MRS) ve metisilin duyarlı Stafilokoklar (MSS)' da her bir eklentinin çeşitli konsantrasyonları arasındaki farklılıklar incelendi ($p<0.05$). Sumak ekşisi, NAC ve gül suyu eklentilerinin konsantrasyon artışlarından dolayı biyofilm oluşumunun azaldığı ayrıca bileşik uygulamaların etkisinin yalın uygulamalardan daha etkili olmadığı bulunmuştur.

Anahtar Kelimeler: Stafilokok, Biyofilm tabakası, N-asetil sistein, Sumak ekşisi (Rhus coriaria), Gülsuyu.

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LIST OF ABBREVIATIONS

CG : Control Group

CoNS : Coagulase Negative *Staphylococcus*

EGP : Eau de rose Group

ERK : Extracellular signal Regulated Kinase

GSH : Glutathione

IL : Interleukine

IUD : Intra Uterine Device

IV : Intravenous

MRS : Methicillin Resistant *Staphylococcus*

MRSA : Methicillin Resistant *Staphylococcus aureus*

MRSE : Methicillin Resistant *Staphylococcus epidermidis*

MSS : Methicillin Sensitive *Staphylococcus*

MSSA : Methicillin Sensitive *Staphylococcus aureus*

MSSE : Methicillin Sensitive *Staphylococcus epidermidis*

NAC : N-acetylcysteine

NACGP : NAC Group

NAPQI : N-acetyl-p benzoquinone imine

NF-κB : Nuclear Factor Kappa-light-chain-enhancer of activated B cells

NO : Nitric oxide

PBP : Penicilin Binding Protein

PIA : Polysaccharide Intercellular Adhesin

PJI : Prosthetic joint infections

PSA : Polysaccharide Adhesin

PVE : Prosthetic Valve Endocarditis

S. aureus : *Staphylococcus aureus*

S. epidermidis : *Staphylococcus epidermidis*

SGP : Sumach Group

TNF : Tumor Necrosis Factor

TSB : Triptic Soya Broth

WES : Water Extracted Sumach

PBS : Phosphate-Buffered Saline

OD : Optical Density

MIC : Minimum Inhibitory Concentration

SI : Slime Index

CHAPTER 1

INTRODUCTION

Staphylococcus epidermidis and other coagulase-negative Staphylococci (CoNS) are the most common causes of prosthetic device and catheter-related infections (Kloos and Bannerman, 1994).

The rates of resistance of pathogenic microorganisms to antimicrobial agents are increasing with an alarming frequency. The emergence of bacterial resistance to antibiotics has consequently become a worldwide concern (Edmond et al., 1999; Ge et al., 2002). Therefore, combination therapy is often beneficial for patients with serious infections caused by drug-resistant pathogens (Meletiadis et al., 2003; Pankey et al. 2005). The use of combination therapy can broaden the spectrum of antibacterial activity, minimize the emergence of resistant bacterial variants and can sometimes result in synergic interaction (Eliopoulos and Eliopoulos, 1998).

Salicyclic acid and certain other non-steroidal anti-inflammatory drugs decrease the production of slime and therefore prevent the formation of biofilms and the adherence of *S. epidermidis* to medical polymers (Farber and Wolff, 1992). Subinhibitory concentrations (sub-MICs) of some antimicrobial agents may also modify slime production (Carsenti-Etesse et al., 1993; Pérez-Giraldo et al., 1994).

The increasing occurrence, particularly in hospitals, of *S. aureus'* resistance including methicillin and a wide range of antimicrobial agents like all kinds of β-lactams, has made therapy more difficult (Fluit et al., 2001; Adwan et al., 2005; Fridkin et al., 2005; Schito, 2006). Although strategies have been proposed in an attempt to

control the spread (Blatnik and Lesnicar, 2006), the search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections.

In a biofilm, bacteria are well protected from the host immune defense. An increase in antibiotic resistance is the consequence (von Eiff, 1999; Darouiche, 2004; Costerton et al., 2005) even high local concentrations of antibiotics do not completely eradicate bacteria in biofilms (Dunne et al., 1993; Darouiche, 2004).

Aiming at the identification of new therapeutic and preventive targets the pathogenesis of staphylococcal biomaterial-related infections has gained much attention. Today, it is anticipated that the implant is colonized at the time of implantation due to the introduction of commensal skin bacteria (Frank, 2004).

N-acetylcysteine (NAC) is a non-antibiotic drug that has antibacterial properties. It is a mucolytic agent that disrupts disulphide bonds in mucus and reduces the viscosity of secretions (Pérez-Giraldo et al., 1997).

Sumach (*Rhus coriaria*) is commercially available in the local markets (Turkey and the Middle East) prepared by grinding with up to 20% salt in the fermented form. In folk medicine, it is used for treatment of indigestion, anorexia, diarrhea, hemorrhagia and hyperglycemia (Wetherilt and Pala, 1994). Spices and herbs, in addition to imparting flavor, exhibit antimicrobial activity and may help preserve the food (Beuchat and Golden, 1989).

It is known that Rosaceae is rich in corilagin and tellimagrandin (Shiota, 2004) and this class of compounds has antimicrobial activity.

1.1 N- acetylcysteine (NAC)

1.1.1 The Functional Properties of NAC

N-acetylcysteine (NAC), a derivative of the aminoacid cysteine, acts as a sulfhydryl donor for glutathione synthesis, as surrogate glutathione, and may increase the nontoxic sulfation pathway resulting in conjugation of NAPQI (N-acetyl-p benzoquinone imine) (Rakel and Bope, 2004).

N-acetylcysteine is a synthetic precursor of GSH, which stimulates the intracellular synthesis of GSH, acts as a nucleophile to conjugate with reactive metabolites and enhances glutathione S-transferase (GST) activity (Tylickiet al., 2003).

N-Acetyl-L-cysteine, used in medical treatment of chronic bronchitis, cancer, and paracetamol intoxication (Riise et al., 2000; Stey et al., 2000), is one of the smallest drug molecules in use (Noszal et al., 2000), and it has antibacterial properties. The molecule is a thiol containing antioxidant that disrupts disulfide bonds in mucus (Sheffner, 1963; Blanco et al., 1997) and competitively inhibits amino acid (cysteine) utilization (Zygmunt and Martin, 1968; Ventura et al., 1999).

N-acetylcysteine decreases biofilm formation by a variety of bacteria (Pe´rez-Giraldo et al., 1997; Marchese et al., 2003; Schwandt et al., 2004) and reduces the production of extracellular polysaccharide matrix (Olofsson et al., 2003) while promoting the disruption of mature biofilm (Marchese et al., 2003; Schwandt et al., 2004). NAC is widely used in medical practice via inhalation and oral and intravenous routes (Yip et al., 1998; Oldemeyer et al., 2003; Marzullo, 2005), and it has an excellent safety profile (Kao et al., 2003).

NAC also suppresses the activation of neutrophils and macrophages (Kharazmi et al., 1988), attenuates leukocyte–endothelial cell adhesion and capillary leakage

(Kharazmi et al., 1988), and blocks the release of tumour necrosis factor alpha and IL-8, probably by modulating gene expression of these mediators at the transcriptional level (Patterson et al., 2003).

NAC can rescue neurons from apoptotic death in the absence of growth factors by activation of the Ras-Extracellular signal regulated kinase (ERK) pathway, an effect due to direct action on transcription factors by the thiol group, rather than anti-oxidant effects (Yan, 1998). GSH (NAC or whey) can promote immune cell clonal expansion, restore natural killer cell activity and induce p53-dependent apoptosis in cancer cells (Gustavo, 2003). NAC can prevent insulin resistance due to high blood glucose, and this effect was attributed to NAC's anti-oxidant action (Haber, 2003).

NAC can reduce the inflammatory symptoms of chronic obstructive pulmonary disease by direct inhibition of the pro-inflammatory transcription factor NF-κB (increases transcription of genes coding for TNF−α and IL−1), in addition to its GSH-boosting action (Dekhuijzen, 2004). NAC blocks the inducible form of Nitric Oxide (NO) synthetase from producing inflammatory cytokines, by inhibition of NF-κB activation (Pahan, 1998).

NAC is the antidote of choice for paracetamol overdose (Vale and Proudfoot, 1995). Reports suggests prompt IV therapy with acetylcysteine may help to minimize hepatorenal damage in acute poisoning with carbon tetrachloride (Mathieson et al., 1985; Ruprah et al., 1985).

Local installation of acetylcysteine into the cavity containing the fungus ball has been used to treat aspergilloma (Kauffman, 1996). There is some evidence in vitro that acetylcysteine has inhibitory properties against *Aspergillus* and *Fusarium* spp (De Lucca et al., 1996).

NAC has been used to regenerate oxidative phosphorylation complexes in mitochondria from age-related decline in function by sulfhydryl group action, rather than antioxidant effect (Miquel, 1995).

1.1.2 The Structure and Biological Components of NAC

N-Acetyl Cysteine (NAC, N-Acetyl-L-Cysteine) is the amino acid L-Cysteine plus an acetyl (-CO-CH_3) group attached to the amino (NH_2) group (URL 1).

Acetylcysteine have thiol (-SH) groups; if this group is free it may be substituted for disulfide bonds in mucus and therefore break the mucus chain (Sweetman, 2002) and act as an electron for neutralizing free-radicals (URL 1). So, NAC is a mucolytic agent and a precursor of L-cysteine and reduced glutathione (Rieutord, 1999).

L-cysteine is neither easily water soluble, nor it is absorbed well by the intestine. Cystine competes with glutamate for transport into cells such that conditions of elevated extracellular glutamate can lead to glutathione depletion, worsened oxidative stress and cell death (Sato, 1999).

Cysteine can significantly decrease binding of copper ions (97% inhibition) to LDL-cholesterol. But cysteine can also reduce copper (Cu^{2+} to Cu^{+}) and iron -- resulting an increase in free-radical damage (Patterson, 2003).

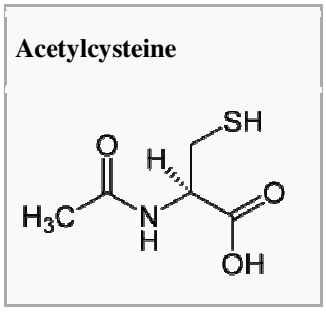


Figure 1 The Structure of NAC (URL 2)

1.2 Sumach

Sumach is a member of approximately 250 species of flowering plants in the genus *Rhus* and related genera, in the family Anacardiaceae (URL 8).

Sumach grows wild in the region extending from the Canary Islands to the Mediterranean and southeastern Anatolian region of Turkey. The ground spice is used as a condiment and sprinkled over kebabs, grilled meats, soups, and some salads (Wetherilt and Pala, 1994; Digrak et al., 2001; Nasar-Abbas and Halkman, 2004).

1.2.1 The Functional Properties of Sumach

Food antimicrobials are mostly synthetic chemicals, and some are restricted in foods, because they may cause adverse effects on public health and reluctance by consumers. Therefore, much attention in recent years has been focused on extracts from herbs and spices, which have been used for many centuries to improve the sensory characteristics and to extend the shelf life of foods. Various tanniniferous plants, including sumach (*Rhus coriaria* L.), have been known to contain naturally occurring compounds with antimicrobial activities (Wetherilt and Pala, 1994; Cowan, 1999; Nasar-Abbas and Halkman, 2004).

The bacteriostatic and bactericidal effects of water extracted sumach (WES) on foodborne bacteria, including pathogens, have been demonstrated in broth and agar media (Digrak et al., 2001; Nasar-Abbas and Halkman, 2004).

Rhus coriaria and some other species of Rhus brought powdered leaves and fruits that they have antibacterial properties have also been reported by other researchers (Saxena et al., 1994; Mc Cutcheon et al., 1994) and this species in the Black Sea region, by people who were used as wound healing was determined (Sezik, 1991).

1.2.2 The Biological Components of Sumach

The main compounds in sumach are hydrolyzable tannins and substantial amounts of flavonoids. It has been demonstrated that gallotannins in sumach leaves are decomposed by heating above 50°C (Zalacain et al., 2003).

Nasar-Abbas and Halkman (2004) have demonstrated that not only the organic acids but also other substances in WES are effective antimicrobial agents.

It is generally believed that the fully protonated species of organic acids can diffuse into the bacterial cells, and cause cell death (Booth and Kroll, 1989; Stradford and Anslow, 1998; Brul and Coote, 1999). Other factors affecting the antimicrobial activity of organic acids include pH, acid concentration, and ionic strength as well as the bacterial strains and environment (growth phase, induced acid resistance, and temperature) of the microbial cultures (Conner and Kotrola, 1995; Buchanan and Edelson, 1996; Entani et al., 1998; Cheng et al., 2003).

1.2.2.1 Tannin

Sumach is rich in water-soluble tannins, and the antimicrobial activity of tannins is well documented (Chung et al., 1998).

Tannins dissolve better in water than in methanol and ethanol (Pansera et al., 2004). Tannins have molecular weights ranging from 500 to over 3,000 (Bate-Smith and Swain, 1962).

Anti-HIV activity of hydrolysable tannin was demonstrated to be mediated, at least in part, by inhibition of HIV adsorption to the cells (Nakashima et al., 1992).

Transaminases and nitrate reductase activities of the cyanobacterium were inhibited by all tannin compounds in a concentration dependent manner (Zaki and Fathy, 2000).

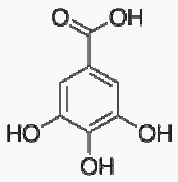
Base Unit:	 Gallic Acid
Class/Polymer:	Hydrolyzable tannins

Figure 2 The Structure Unit of Hydrolyzable Tannins (URL 3)

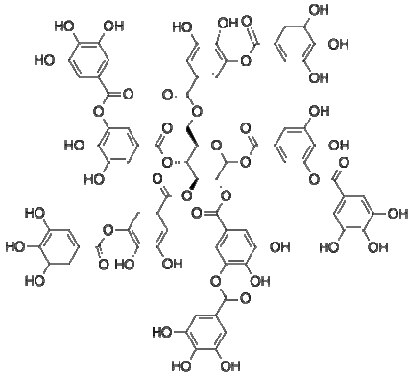


Figure 3 The Structure of Tannin (URL

1.2.2.2 Gallic acid

Gallic acid is an organic acid, known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumach, witch hazel, tea leaves and other plants. It exists as free acid and as a part of tannins (URL 5).

Structure-activity relationship analysis showed that the o-dihydroxy group of gallic acid is important for the inhibitory activity in vitro (Kroes et al., 1992).

A few researchers believe that gallic acid may benefit people at risk of neural degeneration. Basically, gallic acid induced apoptosis in a dose-dependent manner as evidenced by analyses of DNA fragmentation, changes in cell morphology and loss of viability (Ohno et al., 1999).

In vitro studies have shown its anti-cancer activities against leukemia HL-60RG, as well as certain prostate, colon and lung cancer cells (Inoue et al., 1994; Ohno et al., 1999; Yoshioka et al., 2000; Raina et al., 2008). An in-vitro study showed that gallic acid inhibited on Abeta (25-35) (10 microM)-induced apoptotic neuronal death (Ban et al., 2008).

Improved formulations of pharmaceutical compounds include the gallic acid ester to enhance the bioavailability of the active ingredient of the pharmaceutical compounds (Wacher, 2000).

1.2.2.3 Flavonoids

Flavonoids (specifically flavanoids such as the catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants" (Spencer, 2008).

Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2001), anti-microbial (Cushnie and Lamb, 2005) and anti-cancer activity (De Sousa et al., 2007).

In 2007, research conducted at the Linus Pauling Institute and published in *Free Radical Biology and Medicine* indicates that inside the human body, flavonoids themselves are of little or no direct antioxidant value (Lotito and Frei, 2006). Flavonoids could also induce mechanisms that help kill cancer cells and inhibit tumor invasion (URL 9). Flavonoids and related polyphenols possess promising anti-HIV activity. A number of flavonoids inhibit reverse transcriptase (RT), induce interferons and inactivate viral protease (Havsteen, 2002).

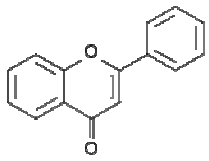


Figure 4 The Structure of Flavonoid (URL 5)

1.2.2.4 Malic acid

Malic acid is widely distributed in small amounts in many natural food products. It is the predominant acid in many fruits, being known as "apple acid" because it is found in high concentration in apples, apricots, peaches, grapes as well as various citrus fruits, berries, figs, carrots, peas, beans and tomatoes (Gardner, 1973).

1.2.2.5 Citric acid

Its antibacterial effect is probably by a mechanism different than that for lipophilic acids. The antibacterial effect is partially due to its ability to chelate divalent cations (Baird-Parker, 1980; Doors, 1993).

1.2.2.6 Methyl gallate (ester of 3,4,5-trihydroxybenzoic acid)

It was reported to be a strong antimicrobial agent (Ahn et al., 1998).

1.2.2.7 Other compounds

4-methoxy-3,5-dihydroxybenzoic acid was also present in fermented sumach extract (Saxena et al., 1994).

1.3 Eau de rose

Rose (*Rosa damascena* Mill.) is a species being used to produce attar of rose by distilling volatile oils from the flowers (Tosun et al., 2002).

In recent years, antioxidant, antibacterial and antimicrobial activities of *R. damascena* essential oil have been demonstrated (Ardogan et al., 2002; Achuthan et al., 2003; Basim and Basim, 2003; Ozkan et al., 2004). Also rose oil is famous not only with its wide application in parfumery and cosmetics, but also, along with its aroma properties, it is a valuable natural drug agent possessing bacteriostatic, antihistological, gall curative, antispasmodic and relaxing etc. (Basim and Basim, 2003). Avicenna showed that rose oil has use in aroma-therapy for treatment of cardiac disease (Abdolhammid, 1982).

1.3.1 Biological Components of Eau de rose

It has a solid component (Stearoptene) and a liquid one (Oleoptene). The nice odor of rose oil is due to the component which itself is composed of Geraniol (45-75%) and Citronellol (20-40%) (Momeni and Shahrokhi, 1991). It is known that Rosaceae is rich in corilagin and tellimagrandin (Shiota et al., 2004) and this class of compounds has remarkable antimicrobial activity.

The major constituents of rose oil are (-)-citronellol, certain specific paraffines, geraniol and nerol, phenethyl alcohol, and methyleugenol - in decreasing order. Some of the important minor- and trace constituents are (-)-cis- rose oxide, beta-damascenone, beta-ionone, beta-damascone, 1-p-menthen-9-al, and rose furan. More than 350 compounds have been identified (Müler et al., 1991; Jirovetz et al., 2005; Joichi et al., 2005).

1.3.1.1 Corilagin

Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose) is a novel member of the tannin family which has been discovered in many medicinal plants such as Phyllanthus species etc. (Shen et al., 2003). The molecular formula of corilagin is $C_{27}H_{22}O_{18}$ (Duan et al., 2005). It has been reported that Corilagin has strong antioxidative (Kinoshita et al., 2007), thrombolytic (Shen et al., 2003), hepatoprotective (Kinoshita et al., 2007), antiatherogenic (Duan et al., 2005) and antihypertensive (Cheng et al., 1995) effects and has potential activity on beta-lactams against methicillin-resistant *Staphylococcus aureus* (Shiota et al., 2004). A preliminary study has reported that Corilagin is a TNF-α inhibitor (Okabe et al., 2001).

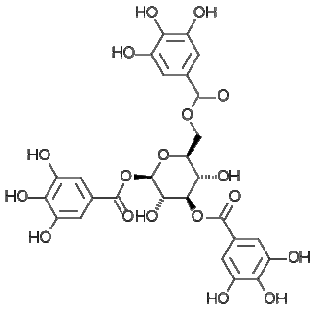


Figure 5 The Structure of Corilagin (URL 6)

1.3.1.2 Tellimagrandin

Observed synergistic effects of certain polyphenols such as tellimagrandin I have been suggested as a means to restore the effectiveness of β-lactam antibiotics against MRSA. When used together with these tannins, the MICs of oxacillin against MRSA strains were markedly lowered to 1/250 or 1/500 (Hatano et al., 2005).

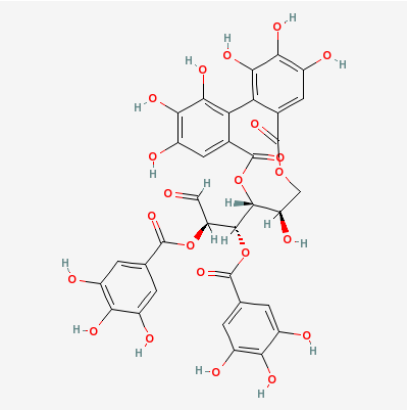


Figure 6 The Structure of Tellimagrandin (URL 7)

1.3.1.3 Other compounds

Geraniol and Citronellol was reported to be the other compounds in eau de rose (URL 10).

1.4 Staphylococcus

Staphylococcus aureus

Staphylococcus aureus is a leading cause of nosocomial and community-acquired infections. Although the types and severity of diseases produced by this opportunistic pathogen vary, it is a frequent cause of infections associated with indwelling medical devices (e.g., catheters and artificial heart valves) (Vuong and Otto, 2002).

Staphylococcus epidermidis

Staphylococcus epidermidis normally colonizes the human skin and mucous membranes and represents a major component of the normal bacterial flora of this habitat. In predisposed hosts, usually with an indwelling medical device, *S. epidermidis* has become a significant nosocomial pathogen (Souvenir at el.,1998; Vermont, 1998; de Viedma, 2000; O'Gara and Humphreys, 2001).

At present, coagulase-negative staphylococci(CoNS), mostly *Staphylococcus epidermidis*, represent the most frequent cause by far of nosocomial sepsis and are the most prominent organisms responsible for infections associated with implanted biomaterials like intravascular catheters, peritoneal dialysis catheters, cerebrospinal fluid shunts, prosthetic heart valves, and prosthetic joints, resulting in substantial morbidity and mortality (Pfaller and Herwaldt, 1988**;** Emori and Gaines, 1993; Kloos and Bannerman, 1994; Rupp and Archer, 1994). The prevalence of methicillin-resistant *S. epidermidis* (MRSE) strains (Jarlov, 1999; Giacometti et al., 2000; Tammelin et al., 2000) and the emergence of vancomycin resistance in this species further complicate treatment of biomaterial infections (Raad, 1998; Villari, 2000).

1.4.1 The Methicillin Resistance of Staphylococcus

The increasing numbers of multidrug-resistant Gram-positive pathogens have generated worldwide concern in the medical community. The emergence and spread of MRSA has been shown to be associated with both hospital- and community-acquired infections. Effective treatment options for these infections are limited and the situation may become more severe soon. For these reasons, a proactive management of MRSA in healthcare facilities is needed (Harbarth et al., 2006; Kluytmans, 2007).

The use of different types of antibiotics over the years has led to the emergence of multi-resistant MRSA strains (Livermore, 2000).

Methicillin resistance in clinical isolates has been reported to arise from expression of a chromosomal or plasmid mediated methicillin-hydrolysing β-lactamase (Montanari, 1996) and is conferred by the *mecA* gene (Wu et al., 1996), which acquired from another species (Chambers, 1997) and encodes a penicillin-binding protein (PBP2A) with decreased affinity for β-lactam antibiotics (Wu et al., 1996). This has resulted in the development of multidrug resistance against β-lactam and other antibiotics.

1.4.2 The Pathogenesis

1.4.2.1 The Slime Production

Indwelling device-associated infections commonly involve the formation of a bacterial biofilm on an uncoated plastic surface or on a plastic surface coated with host proteins (Vuong and Otto, 2002).

The major virulence factor associated with this organism's ability to cause infections is dependent on adherence to medical devices and formation of a biofilm (Vuong and Otto, 2002). Microbial adhesion to surfaces has been shown to be a complex process, involving physicochemical, protein and polysaccharide factors (Busscher and Weerkamp, 1987; Jucker, 1996; Busscher et al., 1997; Cunliffe et al., 1999; Mohamed et al., 1999; Azeredo and Oliveira, 2000; Bruinsma et al., 2001; Gross et al., 2001; Koerner et al., 2002; Dunne, 2003).

After their ''race to the surface'' (Gristina, 1987) staphylococci establish persistent infections due to their capability to grow as a biofilm (Mack et al., 2006):

multilayered communities on the surface of the implant that protect the bacteria from the host immune system (Vuong, 2004) and renders them less susceptible to antibiotics (Knobloch, 2002).

Biofilm formation is a two-step process in which the staphylococci first adhere to the foreign-body surface and then accumulate into a complex biofilm architecture (Mack et al., 2006). A variety of different mechanisms and factors which can contribute to primary attachment of *S. epidermidis* cells to polymer surfaces, including nonspecific van der Waals and hydrophobic interactions (Costerton et al., 1987). Specific binding mediated by a capsular polysaccharide adhesin (PSA) (Tojo, 1988) or a 220-kDa cell wall-associated protein (Timmerman, 1991). Specific interaction with plasma proteins which is adsorbed to the polymer surface (Russell et al., 1987; Herrmann et al., 1988; Vaudaux et al., 1989), binding to activated platelets on the surface (Wang et al., 1993), and lipoteichoic acid-mediated binding to fibrin-platelet clots (Chugh et al., 1990).

In the second, accumulative phase of biofilm formation most Staphylococci have no direct contact with the surface but remain in the biofilm via expression of intercellular adhesive mechanisms. One of the first factors described mediating biofilm accumulation in *S. epidermidis* was the polysaccharide intercellular adhesin (PIA), synthesized by icaADBC encoded proteins (Heilmann, 1996; Mach et al., 1996; Trautner and Darouiche, 2004). Later it was demonstrated that PIA mediates biofilm formation in *S. aureus* too (Cramton, 1999; McKenney, 1999).

1.4.2.2 The Structure of Biofilm

Costerton et al. (1978) observed that communities of attached bacteria in aquatic systems were found to be encased in a “glycocalyx” matrix that was found to be polysaccharide in nature, and this matrix material was shown to mediate adhesion.

Polysaccharide intercellular adhesin (PIA)/polysaccharide adhesin (PSA) produced by *S. epidermidis* is composed primarily of N-acetyl-glucosamine in β-1,6-glycosidic linkages containing deacetylated amino groups and succinate and phosphate substitutions (Marc et al., 1994; Mack et al., 1996; McKenney et al., 1998).

1.4.3 The Resistance To Antimicrobial Agents Due To Biofilm

Mechanisms responsible for resistance may be due to one or more of the following: (i) delayed penetration of the antimicrobial agent through the biofilm matrix, (ii) altered growth rate of biofilm organisms, and (iii) other physiological changes due to the biofilm mode of growth (Donlan and Costerton, 2002).

In the first case (i), Hoyle et al. (1992) found that dispersed bacterial cells were 15 times more susceptible to tobramycin than were cells in intact biofilms. DuGuid et al. (1992) examined *Staphylococcus epidermidis* susceptibility to tobramycin and concluded that the organization of cells within biofilms could in part explain the resistance of this organism to this antimicrobial agent. Souli and Giamarellou (1998) demonstrated that not all antimicrobial agents were equally affected; glycopeptides such as vancomycin and teicoplanin were significantly affected, whereas agents such as rifampin, clindamycin, and the macrolides were either unaffected or minimally affected.

In (ii) biofilm-associated cells grow significantly more slowly than planktonic cells and, as a result, take up antimicrobial agents more slowly. DuGuid (1990) showed that *S. epidermidis* biofilm growth rates strongly influenced susceptibility; the faster the rate of cell growth, the more rapid the rate of inactivation by ciprofloxacin.

In (iii) the conditions that elicit the slowing of bacterial growth, such as nutrient limitation or build-up of toxic metabolites, favor the formation of biofilms (Donlan and Costerton, 2002).

1.4.4 Indwelling Medical Device Associated Biofilm Infections

1.4.4.1 The Prosthetic Valves

The surgical implantation of the prosthetic valve results in tissue damage, leading to the accumulation of platelets and fibrin at the suture site and on the device. There is a greater susceptibility for initial microbial colonization in these locations (Douglas and Cobbs, 1992).

Illingworth et al. (1998) noted that prosthetic valve endocarditis (PVE) is predominantly caused by colonization of the sewing cuff fabric of the prosthetic valve by microorganisms. Karchmer and Gibbons (1994) added that the microorganisms will commonly invade the valve annulus into which the prosthetic valve has been sewn, potentially leading to a separation between the valve and the tissue and resulting in leakage.

CoNS are the predominant early colonizers (Hancock, 1994; Karchmer and Gibbons, 1994), probably resulting from initial contamination of the surgical site during the procedure. For late PVE, which by definition is from 12 months onward following the valve replacement, the organisms responsible may be streptococci, CoNS, enterococci, *S. aureus*, gram-negative coccobacilli, or fungi (Karchmer and Gibbons, 1994).

1.4.4.2 The Prosthetic Joints and Knees

Today, it is anticipated that the implant is colonized at the time of implantation due to the introduction of commensal skin bacteria (Frank et al., 2004). Consequently, *Staphylococcus* species, especially *Staphylococcus aureus* (22–23.6%) and

Staphylococcus epidermidis (19–37.5%), are isolated most frequently in prosthetic joint infections (PJI) (Lentino, 2003).

The increased use of implanted prosthetic joints has provided a physiological niche for pathogenic organisms to cause septic arthritis (Bengtson and Knutson, 1991).

Implanted medical devices produce local immune compromise through frustrated phagocytosis (Roisman et al., 1983). In this case, professional phagocytes may undergo apoptosis when encountering a substrate of a size that is beyond their phagocytic capability. The resulting release of reactive products of oxygen and lysosomal enzymes may cause accidental host tissue damage and local vascular insufficiency, thereby increasing the predisposition of osteomyelitis development. Some of the normal phagocytic processes are also devoted to removal of the implant foreign material (particularly with metals, methylmethacrylate, and polyglycolic acid), thereby utilizing the energy and resources of the immune system that would normally be used to fight infection (Santavirta et al., 1990; Santavirta et al., 1991; Wang et at., 1997).

1.4.4.3 Central Venous Catheters

Catheters may be inserted for administration of fluids, blood products, medications, nutritional solutions, and hemodynamic monitoring (Flowers et al., 1989).

The characteristic flora migrating to the catheter surface from the skin site include coagulase-negative staphylococci and *Staphylococcus aureus* (Raad and Hana, 2002).

Because device is in direct contact with the bloodstream; the surface becomes coated with platelets, plasma, and tissue proteins such as albumin, fibrinogen, fibronectin, and laminin (Raad, 1998). These materials act as conditioning films; *S.*

aureus adheres to proteins such as fibronectin, fibrinogen, and laminin, and *S. epidermidis* adheres only to fibronectin (Raad, 1998).

Although epidemics of infusate related sepsis do occur, these cases are very rare in comparison with the numbers of cases of bacteremia arising from primary catheter infection (Raad and Bodey, 1992).

Colonization and biofilm formation may occur within 3 days of catheterization (Anaissie et al., 1995). Raad et al. (1993) also showed that catheters in place for less than 10 days tended to have more extensive biofilm formation on the external surface of the catheter; for longer-term catheters (up to 30 days), biofilms were more extensive on the internal lumen.

1.4.4.4 Urinary (Foley) Catheters

Urinary catheters are tubular, latex, or silicone devices that are inserted through the urethra into the bladder to measure urine output, collect urine during surgery, prevent urinary retention, or control urinary incontinence (Kaye and Hessen, 1994).

The organisms that attach to the catheter and develop the biofilm originate from one of several sources: (i) organisms are introduced into the urethra or bladder as the catheter is inserted, (ii) organisms gain entry through the sheath of exudate that surrounds the catheter, or (iii) organisms travel intraluminally from the inside of the tubing or collection bag (Kaye and Hessen, 1994).

Initially, catheters are colonized by single species, such as *S. epidermidis*, *Enterococcus faecalis*, *E. coli*, or *Proteus mirabilis* (Stickler, 1996).

1.4.4.5 Intrauterine Devices (IUD)

IUD use has been shown to result in pelvic inflammatory disease (Wolf and Kreiger, 1986; Chesney, 1994; Lewis, 1998). IUDs removed from asymptomatic women have been shown to be heavily contaminated with *S. epidermidis*, enterococci, and anaerobic lactobacilli (Wolf and Kreiger, 1986). In addition, IUDs removed from women with pelvic inflammatory disease may also contain beta-hemolytic streptococci, *S. aureus*, *E. coli*, and some anaerobic bacteria (Wolf and Kreiger, 1986).

One study determined that contamination was heaviest on the distal portions of the tail, which is directly exposed to the vaginal flora (Bank and Williamson, 1983).

1.4.5 Clinical Practice & Prevention

There are 4 main intervention strategies in clinical practice for biofilm-associated infections. The first strategy is to prevent initial device contamination through maintaining optimal aseptic techniques and minimizing duration of catheter placement. Second, steps are taken to minimize initial microbial cell attachment, for example, the use of antimicrobial-coated central venous catheters (Darouiche et al., 1999; Darouiche, 1999). Third, for an established infection, agents are used to penetrate the biofilm matrix and kill the embedded organisms, such as high dose of antibiotics or ethanol in a catheter lock solution (Sherertz et al., 2006). Lastly, removal of the infected device is the definitive treatment strategy (O'Grady et al., 2002).

The silver cuff has been found to reduce the risk of extraluminal contamination in short-term catheters (Maki et al., 1988).

Antibiotics already incorporated in controlled-release devices include vancomycin, tobramycin, cefamandol, cephalothin, carbenicillin, amoxicillin and gentamicin (Price, 1996; Stigter, 2004).

1.4.6. Treatment

Once a staphylococcal biofilm has formed on an implanted medical device or damaged tissue, it is difficult to disrupt. A biofilm-infected implant often must be removed and replaced, placing the patient at increased risk for complications due to these additional procedures (Raad et al., 1998; Chamis et al., 2001).

Although many strains of MRSA are susceptible to trimethoprim sulfamethoxazole, treatment with trimethoprim-sulfamethoxazole has been associated with clinical failure, especially in the presence of significant tissue damage (Proctor, 2008).

β-lactam antibiotics are the preferred drugs against *S. aureus* infections. Although, *S. aureus* has developed resistance to the β-lactam antibiotics (Dennesen et al., 1998; Bachi and Rohrer, 2002). The incidence of methicillin-resistant *S. aureus* is increasing in most hospitals, vancomycin seems, at this stage, the most appropriate antibiotic for empiric therapy (Mylotte and McDermott 1987; Widmer, 1997).

Vancomycin-resistant isolates have been reported; isolates with an increased minimum inhibitory concentration (MIC) to vancomycin are becoming more common. Increased dosing of vancomycin (through >15 mcg/mL) may be required to treat infections with these isolates (Hidayat, 2006).

CHAPTER 2

THE AIM AND THE SCOPE OF THE STUDY

The main objective of the study is to investigate the effects of NAC, fermented sumach and eau de rose on the slime layer of *Staphylococcus* spp. and its serotypes of MRSA, MSSA, MRSE, MSSE. Also, the effects of NAC, fermented sumach and eau de rose on the growth of *Staphylococcus* spp. were investigated. The inhibition of strains due to the pH and temperature changes of the NAC, fermented sumach and eau de rose were also examined by disc diffusion method.

CHAPTER 3

MATERIALS AND METHOD

3.1 The microorganisms

89 Staphylococci isolates were investigated in this study and 41 of them were found to be the slime producer isolates, following the growth in triptic soy broth (TSB) agar at 37° C for a period of 24 hours. The isolates were previously recruited from the samples of the patients from the microbiology laboratory of the hospital of Abant Izzet Baysal University, The Faculty of Medicine, Bolu, Turkey.

3.2 The Treatment with The Supplements

In this experiment, there were three supplements. These were the NAC (Merck ^{TM}), fermented sumach, obtained from a local vendor in Gaziantep, Turkey and eau de rose (Gülbirlik A.Ş. Isparta, Turkey).

The supplements were mixed with TSB and the relevant isolates were treated with the related supplements which is in the TSB at 24 hours and 37^{o} C in the incubator with respect to the controls of isolates, which were studied in non-treated TSB at 24 hours and 37° C in the incubator.

3.3 The Experimentation

The slime production capacity of 89 isolates were tested with TSB. 41 slime positive isolates were selected for further experiments. Then, each, treatment including four different concentrations were added to each microtiter plate containing TSB and analyzed separately.

The concentrations were as follows: NAC: 0.03, 0.12, 0.5 and 2.0 mg/mL, fermented sumach: 0.1, 0.2, 0.5 and 1 µl/mL, eau de rose: 0.1, 0.2, 0.5 and 1 µl/mL. In all cuvettes TSB was used and the process was repeated in triplicates. These processes are summarized in Table 3.1.

Table 3.1 The Treatments in The Experiment

Without Treatment	The Controls of Isolates
1. Treatment	0.03 mg/ml NAC
	0.12 mg/ml NAC
	0.5 mg/ml NAC
	2.0 mg/ml NAC
2. Treatment	0.1 µl/ml Sumach
	0.2 µl/ml Sumach
	0.5 µl/ml Sumach
	1 µl/ml Sumach
3. Treatment	0.1 µl/ml Eau de rose
	0.2 µl/ml Eau de rose
	0.5 µl/ml Eau de rose
	1 µl/ml Eau de rose
4. treatment	Eau de rose-NAC combination
5. treatment	Sumach-NAC combination
6. treatment	Sumach-Eau de rose combination
7. treatment	Sumach-Eau de rose-NAC combination
8. treatment	Control

Isolates were inoculated to cuvettes (LP Italiana SPA ™) which contained treated and non-treated groups.

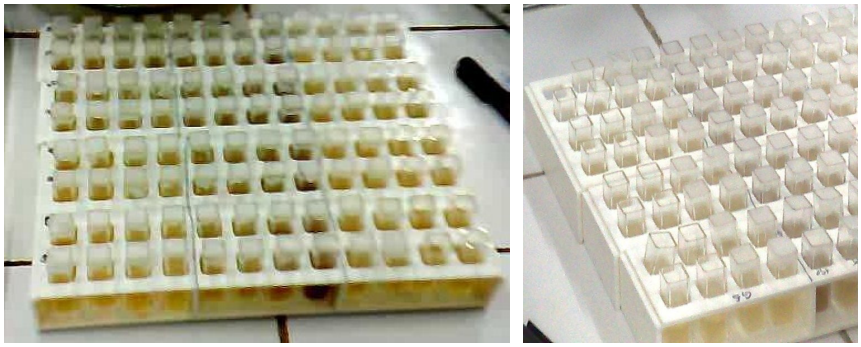


Figure 7 Isolate inoculated cuvettes which contained treatment groups and TSB.

3.4 The Qualitative Determination of Slime

For screening biofilm formation by the *S. epidermidis* and *S. aureus*, bacteria were grown on Congo red agar (Merck ™) as described by Freeman et al. (1989). Colony morphology was examined after 24 h at 37°C. Also, the biofilm formation by bacteria was detected by another method described by Christensen et al. (1985) by overnight cultures of *S. epidermidis* and *S. aureus,* inoculated in polystyrene test tube which contained TSB as an alternative.

3.5 The Quantitative Determination of Slime

Non-treated TSB were used for controls. TSB (Merck ™), supplemented with the different concentrations of NAC, sumach and eau de rose were used. The OD value of the inoculum was approximately 0.600 (Hitachi ™). 200 µl of bacterial suspension were inoculated into 96-well flat-bottomed sterile polystyrene microplate (LP Italiana SPA ™) cuvettes which contained TSB. Some wells were left free of NAC, sumach and eau de rose as controls and incubated for 24 h at 37°C.

The biofilm formation by bacteria was detected by the method described by Christensen et al. (1985) as follows. The biofilms formed on the plates were washed

twice with phosphate-buffered saline (PBS) to remove the planktonic cells. Then, the cells were stained with saphranin for 1 hour. After removal of saphranin from microplate, microplate was washed twice with PBS and followed the air drying of the wells. Adherent bacterial films were measured spectrophotometrically at 540 nm in a microplate reader (Thermo Instruments ^{TM}). This process was repeated with 0.03, 0.12, 0.5, 2.0 mg/mL concentrations of NAC treated TSB, 0.1, 0.2, 0.5 and 1 µl/mL concentrations of sumach treated TSB and 0.1, 0.2, 0.5 and 1.0 µl/mL of eau de rose treated TSB to determine the effects of NAC, sumach and eau de rose on slime production of isolates.

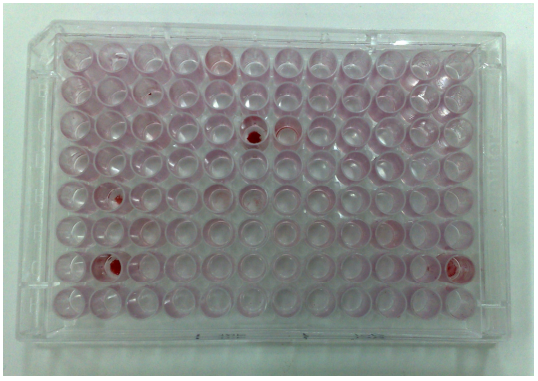


Figure 8 Stained slimes of isolates in 96-well flat-bottomed microplate

3.6 The Determination of The Slime Index (SI)

Following a period of 24 hours' incubation of isolates which are treated with the different concentrations of NAC, the fermented sumach and eau de rose, the growth of *Staphylococcus* were confirmed with the microelisa reader instrument (Thermo Instruments ^{TM}).

The OD value of the biofilm was corresponding with the value in OD of bacterial growth determined spectrophotometrically, before the aspiration of the culture in order to compensate the partial inhibition in growth caused by NAC, sumach and eau de rose

and this was termed as the slime index (SI). The result was expressed as percentage relative to the control without NAC, fermented sumach and eau de rose. For this purpose the following formula was applied: SI = 100 x (mean density of biofilm with supplement/mean growth with treatment)/(mean density of biofilm without treatment/mean growth without treatment) (C. Pérez-Giraldo et al., 1997).

3.7 The Effect of pH and Temperature on the Inhibitory Activity of the Treatment Groups

The pH of the treatment groups were adjusted to 2, 7 and 10 by HCl and NaOH (Gül Biyoloji ™) respectively. The temperature of the treatment groups were adjusted to 4, 25, 50 and 100° C. Then, by using disc diffusion method (Bauer et al. 1966) inhibitions of isolates by the treatment groups were determined on Mueller-Hinton agar (Gül Biyoloji ™). The pH of the treatment groups were checked by a pH meter (Hanna Instruments ™).

3.8 The Effects of The Combined Treatments on the Inhibitory Activity of *Staphylococcus*

The combinations of supplements were studied by disc diffusion method (Bauer et al. 1966). For combination treatments, 10 grams of NAC was diluted with 10 ml distilled water. Pure eau de rose and sumach was used. 30 µl of each of these supplements were added to discs on the Mueller-Hinton agar (Gül Biyoloji ™).

3.9 The Statistical Evaluation

The data were shown as means of standard deviation and analyzed by the SPSS program. Friedman test was used to detect the existence of differences in different concentrations of each treatment group. If significant differences were found, the comparison between the concentrations of each treatment group was carried out by the two related sample test (Wilcoxon test). The significant level was set for $p<0,05$ at the beginning of the study.

CHAPTER 4

THE RESULTS AND DISCUSSION

In all of the experiments, significant differences were observed between the concentrations of each treatment group.

4.1 The Effects of NAC on Biofilm formation and Growth of *Staphylococcus*

The 41 isolates of *S. epidermidis* and *S. aureus* were found to be biofilm-producing among the analyzed 89. The results of biofilm formation and growth in the presence of different concentrations of NAC, determined by microelisa assay are shown in the Table 4.1.1.

Table 4.1.1 The Friedman test results which show the effects of different concentrations of NAC on the growth and biofilm formation of 41 isolates

	NAC (mean ± std.deviation)				**df**	**N**	**p**
	0,03 mg/mL	**0,12 mg/mL**	**0,5 mg/mL**	**2,0 mg/mL**			
SI	83,99 ±28,97	75,61 ±24,37	71,60 ±2,51	73,69 ±3,56	3	41	,000*
Slime	80,54 ±32,56	73,94 ±31,95	66,67 ±2,58	57,23 ±2,39	3	41	,000*
Growth	97,48 ±32,96	94,91 ±25,30	88,70 ±2,25	79,22 ±2,33	3	41	,000*

*** p < 0,05**

There were significant differences in biofilm formation and growth between the concentrations of NAC (Table 4.1.1). As seen from the table 4.1.1 the significant

reduced formation of biofilms and growth were observed between different concentrations ($p < 0,05$).

Table 4.1.2 The Wilcoxon test results which show the effects of different concentrations of NAC on the growth and biofilm formation of 41 isolates

		Concentrations of NAC (mg/mL - mg/mL)					
		0,12 –0,03	**0,5 – 0,03**	**2,0 – 0,03**	**0,5 – 0,12**	**2,0 – 0,12**	**2,0 – 0,5**
p	**SI**	,008 *	,001 *	,001 *	,161	,065	,214
	Slime	,000 *	,000 *	,000 *	,000 *	,000 *	,000 *
	Growth	,368	,002 *	,000 *	,009 *	,000 *	,000 *

*** p < 0,05**

In all of the concentrations (0,03–2,0 mg/mL) dose dependent reduced formation of biofilms was detected as statistically significant ($p < 0,05$) (Table 4.1.2). SI was found to be significant with the concentrations of 0,03 and 0,12–2,0 mg/ml ($p < 0,05$). On the other hand, with the concentrations of 0,12 and 0,5 mg/ml, 0,12 and 2,0 mg/ml, 0,5 and 2,0 mg/ml, the decrease in the formation of biofilms were not statistically significant according to SI ($p > 0,05$).

The growth was significantly reduced with the increased NAC concentrations ($p < 0,05$), but these data were found to be significant except with the concentrations of 0,03 and 0,12 mg/ml. The growth with the concentrations of 0,03 and 0,12 mg/mL were not significantly reduced ($p > 0,05$). The growth reduction with the concentration of 0,03 and 0,12 mg/ml was not found to be dose dependent ($p > 0,05$), but growth reduction with the other concentrations were found to be dose dependent. The findings were consistent with those suggested by C. Pérez-Giraldo et al. (1997) that NAC may

prevent the formation of biofilms and adherence of *S. epidermidis* to prosthetic and intravascular devices, as well as the NAC-tigecycline combination consistently decreased viable biofilm-associated bacteria with relative to the control (Alsam et al., 2007).

4.2 The Effects of Sumach on Biofilm formation and Growth of *Staphylococcus*

The results in biofilm formation and growth in the presence of different concentrations of sumach determined by microelisa assay are shown in the Table 4.2.1. Probably the decrease in the OD of the biofilms was directly proportional to the sumach concentration.

Table 4.2.1 The Friedman test results which show the effects of different concentrations of sumach on the growth and biofilm formation of 41 isolates

	Sumach (mean±std.deviation)				**df**	**N**	**p**
	0,1 µl/ml	**0,2 µl/ml**	**0,5 µl/ml**	**1,0 µl/ml**			
SI	85,98 ± 3,30	79,13 ± 3,13	73,68 ± 2,98	54,60 ± 2,39	3	41	,000 *
Slime	7,65 ± 3,08	7,12 ± 3,12	6,37 ± 2,55	5,40 ± 2,33	3	41	,000 *
Growth	92,43 ± 2,28	91,06 ± 2,49	88,62 ± 2,35	1,02 ± 2,90	3	41	,000 *

*** p < 0,05**

There were significant differences in biofilm formation and growth between concentrations of sumach (Table 4.2.1).

Table 4.2.2 The Wilcoxon test results which show the effects of different concentrations of sumach on the growth and biofilm formation of 41 isolates

		Concentrations of sumach intervals (µl/ml)					
		0,2µl/ml – 0,1µl/ml	**0,5µl/ml – 0,1µl/ml**	**1,0µl/ml – 0,1µl/ml**	**0,5µl/ml – 0,2µl/ml**	**1,0µl/ml – 0,2µl/ml**	**1,0µl/ml – 0,5µl/ml**
p	**SI**	,001 *	,000 *	,000 *	,007*	,000 *	,000 *
	Slime	,000 *	,000 *	,000 *	,000 *	,000 *	,000 *
	Growth	,464	,043 *	,001 *	,131	,000 *	,000 *

*** p < 0,05**

Sumach demonstrated a dose-dependent slime reducing activity (Table 4.2.2). Slime formation significantly decreased with the increased sumach concentration ($p < 0,05$).

The growth was significantly reduced with the increased sumach concentration ($p < 0,05$), but these data were found to be significantly different except the concentrations of 0,1 and 0,2 µl/mL, and between the concentrations of 0,2 and 0,5 µl/mL ($p > 0,05$). So, growth reduction in this group was not found to be dose dependent ($p > 0,05$).

The sumach extract displayed a variable degree of antimicrobial activity on different bacteria. *L. monocytogenes* was found to be the most resistant among Gram positive isolates followed by *S. aureus* (Nasar-Abbas et al., 2004). In general, Gram negative bacteria were found to be more resistant to sumach treatment than Gram positive bacteria except *P. vulgaris* and *C. freundii*. Studies by the other researchers reveal same type of results (Shelef et al., 1980; Marino et al., 1999).

The water extracted sumach (WES) exerted an antibacterial effect against coliforms and presumptive fecal coliforms. WES can be used at each decontamination point. Nasar-Abbas and Halkman (2004) have demonstrated that not only the organic acids but also the other substances in WES are effective antimicrobial agents and they were implied that the inhibitory action was not only due to its acid content but also due to some antimicrobial substances present in sumach. The results indicate that the effect of sumach to all of the bacterial isolates tested was not only bacteriostatic but it was also bactericidal because it caused an irreversible damage (death) to the test organisms (Nasar-Abbas et al., 2004).

Sumach is rich in water-soluble tannins, and the antimicrobial activity of tannins is well documented (Chung et al., 1998).

Dığrak et al. (2001) determined antibacterial and antifungal activities of some medical plants grown in Turkey which include *Rhus coriaria.* A diameter of 35-51 mm inhibition zones against bacteria was determined in all trials with *Rhus coriaria.*

The other researchers determined that *Rhus coriaria's* leaves and fruits which were in powdered form had antibacterial effects (Saxena et al., 1994; Mc Cutcheon et al., 1994).

4.3 The Effects of Eau de rose on Biofilm formation and Growth of *Staphylococcus*

The 40 isolates of *S. epidermidis* and *S. aureus* included in this study were biofilm-producing. The results in biofilm formation and growth in the presence of different concentrations of eau de rose determined by microelisa assay are shown in the Table 4.3.1

Table 4.3.1 The Friedman test results which show the effects of different concentrations of eau de rose on the growth and biofilm formation of 40 isolates

	Eau de rose concentrations (mean±std.deviation)				df	N	P
	0,1 µl/ml	**0,2 µl/ml**	**0,5 µl/ml**	**1,0 µl/ml**			
SI	8,13 ± 3,32	8,27 ± 3,19	8,20 ± 3,32	7,51 ± 3,91	3	40	,000 *
Slime	7,44 ± 2,85	7,27 ± 2,52	8,26 ± 8,50	6,94 ± 2,56	3	40	,037 *
Growth	8,91 ± 1,76	8,96 ± 1,87	8,80 ± 1,98	9,71 ± 1,94	3	40	,000 *

*** p < 0,05**

There were significant differences in the reduction of biofilm formation and growth between the concentrations of eau de rose (p< 0,05) (Table 4.3.1).

Table 4.3.2 The Wilcoxon test results which show the effects of different concentrations of eau de rose on the growth and biofilm formation of 40 isolates

		Concentration intervals of Eau de rose (µl/ml)					
		0,2µl/ml – 0,1µl/ml	**0,5µl/ml – 0,1µl/ml**	**1,0µl/ml – 0,1µl/ml**	**0,5µl/ml – 0,2µl/ml**	**1,0µl/ml – 0,2µl/ml**	**1,0µl/ml – 0,5µl/ml**
P	**SI**	,844	,260	,002 *	,181	,001 *	,001 *
	Slime	,545	,077	,057	,051	,101	,050
	Growth	,600	,582	,000 *	,819	,000 *	,000 *

*** p < 0,05**

SI was found to be significantly different only with the concentrations of 1,0 and 0,1–0,5 mg/ml (p < 0,05) (Table 4.3.2). But between the concentrations of 0,1 and 0,2 mg/ml and 0,2 and 0,5 mg/ml; a decrease in biofilm formation were not found to be statistically significantly different (p > 0,05). Although, between the concentrations of

0,2 and 0,5 mg/ml, a reduction of biofilm formation was detected, these data were not statistically significant according to SI ($p > 0,05$).

4.4 The Effects of Supplements on Biofilm formation and growth of MRS and MSS

The 41 isolates of *Staphylococcus* included 9 MSSA, 4 MRSA, 13 MSSE and 15 MRSE. The choice of drugs, to be used against MRSA, is shrinking day by day as susceptibility of MRSA to drugs is decreasing by target site alteration, enzyme modification and permeability changes (Brumfitt and Miller, 1989).

The results of biofilm formation and growth in the presence of different concentrations of NAC, sumach and eau de rose was determined according to methicillin sensitivity of *Staphylococcus*.

Table 4.4 The effects of supplements on the growth and biofilm formation of 22 MSS and 19 MRS

		SI	Slime	Growth
NAC	**0,03mg/ml**	,537	,647	,513
	0,12mg/ml	,386	,774	,583
	0,5 mg/ml	,217	,539	,937
	2,0 mg/ml	,211	,565	,210
Sumach	**0,1 µl/ml**	,803	,676	,937
	0,2 µl/ml	,646	,896	,657
	0,5 µl/ml	,833	,937	,513
	1,0 µl/ml	,619	,784	,875
Eau de rose	**0,1 µl/ml**	,684	,774	,490
	0,2 µl/ml	,870	,464	,507
	0,5 µl/ml	,860	,896	,409
	1,0 µl/ml	,645	,308	,394

*** p < 0,05**

The supplements which include four different concentrations had the same effect on biofilm formation and growth of MSS and MRS ($p > 0,05$) (Table 4.4). These data were not statistically significant ($p > 0,05$).

4.4.1 The Effects of NAC on Biofilm Formation and Growth of MRS and MSS

Table 4.4.1.1 The Friedman test results which show the effects of different concentrations of NAC on the growth and biofilm formation of MSS and MRS

		(mean ± std. deviation)			df	N
		SI	**Slime**	**Growth**		
MSS	**0,03mg/ml**	80,18±31,47	81,18±39,54	101,62±38,54	3	22
	0,12mg/ml	72,55±27,91	73,75±35,34	96,42±26,51		
	0,5 mg/ml	67,34±2,86	65,81±2,87	89,72±2,77		
	2,0 mg/ml	66,30±2,40	56,35±2,60	82,43±2,40		
	p	,002 *	,000 *	,001 *		
MRS	**0,03mg/ml**	88,39±25,88	79,80±23,02	92,68±25,22	3	19
	0,12mg/ml	79,15±19,65	74,15±28,50	93,17±24,43		
	0,5 mg/ml	76,53±1,99	67,70±2,27	87,53±1,49		
	2,0 mg/ml	82,24±4,48	58,25±2,19	75,50±2,25		
	p	,111	,000 *	,000 *		

*** p < 0,05**

There were significant differences in biofilm formation and growth of MSS and in growth of MRS between concentrations of NAC ($p < 0,05$) (Table 4.4.1.1). It seemed that there were differences in biofilm formation of MRS between concentrations of NAC but in contrast the data of SI indicated that there were no significant differences in biofilm formation of MRS between concentrations of NAC ($p > 0,05$).

Table 4.4.1.2 The Wilcoxon test results which show the effects of different concentrations of NAC on the growth and biofilm formation of MSS

		Concentration intervals of NAC (mg/ml - mg/ml)					
		0,12 –0,03	**0,5 – 0,03**	**2,0 – 0,03**	**0,5 – 0,12**	**2,0 – 0,12**	**2,0 – 0,5**
p	**SI**	,070	,008 *	,001 *	,296	,144	,327
	Slime	,000 *	,000 *	,000 *	,000 *	,000 *	,000 *
	Growth	,211	,005 *	,003 *	,053	,007*	,013*

*** p < 0,05**

There were significant differences in biofilm formation of MSS with all of the concentrations of NAC, but SI was found to be significantly different between the concentrations of 0,03 and 0,5 mg/ml, 0,03 and 2,0 mg/ml ($p < 0,05$) (Table 4.4.1.2). There were significant dose dependent reduction of biofilm formation of MSS between the concentrations of 0,03 and 0,5 mg/ml, 0,03 and 2,0 mg/ml ($p < 0,05$). There were significant differences in growth of MSS with all of the concentration intervals ($p< 0,05$) except with the concentration of 0,12 and 0,03 mg/ml and 0,12 and 0,5 mg/ml ($p >0,05$). Dose dependent reduction of growth of MSS was observed with all of the concentration ($p < 0,05$) except with the concentration 0,03 and 0,12 mg/ml and 0,12 and 0,5 mg/ml ($p > 0,05$).

Table 4.4.1.3 The Wilcoxon test results which show the effects of different concentrations of NAC on the growth and biofilm formation of MRS

		Concentration intervals of NAC (mg/ml - mg/ml)					
		0,12 –0,03	**0,5 – 0,03**	**2,0 – 0,03**	**0,5 – 0,12**	**2,0 – 0,12**	**2,0 – 0,5**
p	**SI**	**-**	-	-	-	-	-
	Slime	,002 *	,001 *	,000 *	,006 *	,000 *	,000 *
	Growth	,904	,107	,006 *	,070	,000 *	,005 *

*** p < 0,05**

There were significant differences in biofilm formation of MRS with all of the concentrations of NAC (Table 4.4.1.3), but in table 4.4.1.1 SI had indicated that there were no significant differences in biofilm formation of MRS between all of the concentrations of NAC ($p > 0,05$). Also, Table 4.4.1.3 indicated that there were a significant dose dependent reduction of growth of MRS, with the concentrations of 2,0 and 0,03-0,5 mg/ml ($p < 0,05$).

4.4.2 The Effects of Sumach on Biofilm Formation and Growth of MRS and MSS

Table 4.4.2.1 The Friedman test results which show the effects of different concentrations of sumach on the growth and biofilm formation of MSS and MRS

		(mean ± std. deviation)			df	N
		SI	**Slime**	**Growth**		
MSS	**0,1 µl/ml**	83,65±3,54	73,09±2,66	93,18±2,78	3	22
	0,2 µl/ml	76,80±3,37	68,40±2,64	93,21±2,97		
	0,5 µl/ml	72,54±3,10	62,87±2,46	91,16±2,56		
	1,0 µl/ml	52,94±2,06	52,66±1,93	1,03±3,45		
	P	,000 *	,000 *	,061		
MRS	**0,1 µl/ml**	88,68±3,07	80,38±3,55	91,55±1,59	3	19
	0,2 µl/ml	81,82±3,01	74,38±3,65	88,57±1,84		
	0,5 µl/ml	75,00±2,92	64,63±2,71	85,68±2,12		
	1,0 µl/ml	56,51±2,77	55,51±2,77	1,01±2,18		
	p	,000 *	,000 *	,005 *		

*** p < 0,05**

There were significant differences in biofilm formation of MSS and MRS and in growth of MRS between concentrations of sumach ($p < 0,05$) (Table 4.4.2.1). No significant differences in growth of MSS were observed between varying concentrations of sumach ($p > 0,05$).

Table 4.4.2.2 The Wilcoxon test results which show the effects of different concentrations of sumach on the growth and biofilm formation of MSS

		Concentrations of Sumach (µl/ml - µl/ml)					
		0,2 µl/ml – 0,1 µl/ml	**0,5 µl/ml – 0,1 µl/ml**	**1,0 µl/ml – 0,1 µl/ml**	**0,5 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,5 µl/ml**
p	**SI**	,019 *	,003 *	,000 *	,063	,000 *	,000 *
	Slime	,001 *	,000 *	,000 *	,001 *	,000 *	,000 *
	Growth	-	-	-	-	-	-

*** p < 0,05**

There were significant differences in biofilm formation of MSS with all concentrations of sumach ($p < 0,05$), but SI was not showed significant differences between the concentration of 0,2 and 0,5 µl/ml ($p > 0,05$). A significant dose dependent reduction of biofilm formation of MSS was present with all of the concentrations except with 0.2 and 0.5 µl/ml (Table 4.4.2.2).

There were no significant differences in growth of MSS between concentrations of sumach ($p > 0,05$) (Table 4.4.2.1).

Table 4.4.2.3 The Wilcoxon test results which show the effects of different concentrations of sumach on the growth and biofilm formation of MRS

		Concentration intervals of Sumach (µl/ml - µl/ml)					
		0,2 µl/ml – 0,1 µl/ml	**0,5 µl/ml – 0,1 µl/ml**	**1,0 µl/ml – 0,1 µl/ml**	**0,5 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,5 µl/ml**
p	**SI**	,013 *	,004 *	,000 *	,047 *	,000 *	,000 *
	Slime	,001 *	,000 *	,000 *	,000 *	,000 *	,001 *
	Growth	,227	,091	,027 *	,084	,010 *	,001 *

*** p < 0,05**

There were significant differences in biofilm formation of MRS with all concentrations of sumach ($p < 0,05$). A significant dose dependent reduction of biofilm formation of MRS was present with all of the concentrations ($p < 0,05$). There were a significant dose dependent reduction of growth of MRS with the concentrations of 1,0 and 0,1-0,5 µl/ml ($p < 0,05$) (Table 4.4.2.3).

4.4.3 The Effects of Eau de rose on Biofilm Formation and Growth of MRS and MSS

Table 4.4.3.1 The Friedman test results which show the effects of different concentrations of eau de rose on the growth and biofilm formation of MSS and MRS

		(mean ± std. deviation)			Df	N
		SI	**Slime**	**Growth**		
MSS	**0,1 µl/ml**	8,40±3,27	7,18±2,43	8,63±1,41	3	22
	0,2 µl/ml	8,18±3,26	6,99±2,31	8,62±1,71		
	0,5 µl/ml	8,11±3,07	8,86±1,12	8,44±1,91		
	1,0 µl/ml	7,48±4,56	6,47±2,05	9,37±2,11		
	p	,000 *	,003 *	,002 *		
MRS	**0,1 µl/ml**	7,83±3,42	7,73±3,31	9,23±2,07	3	19
	0,2 µl/ml	8,37±3,20	7,60±2,77	9,34±2,01		
	0,5 µl/ml	8,31±3,66	7,57±3,78	9,19±2,03		
	1,0 µl/ml	7,54±3,17	7,49±3,01	1,01±1,70		
	p	,060	,875	,001 *		

*** p < 0,05**

There were significant differences in biofilm formation of MSS between the concentrations of eau de rose ($p < 0,05$) (Table 4.4.3.1). But there were no significant differences in biofilm formation of MRS between the different concentrations of eau de rose ($p > 0,05$). The biofilm formation of MRS was reduced by eau de rose but this reduction was not dose dependent. The biofilm formation was found to be almost the same with an increase in the eau de rose concentration. There were significant

differences in growth of MSS and MRS between concentrations of eau de rose ($p < 0,05$) (Table 4.4.3.1).

Table 4.4.3.2 The Wilcoxon test results which show the effects of different concentrations of eau de rose on the growth and biofilm formation of MSS

		The concentration intervals of Eau de rose (µl /ml - µl /ml)					
		0,2 µl/ml – 0,1 µl/ml	**0,5 µl/ml – 0,1 µl/ml**	**1,0 µl/ml – 0,1 µl/ml**	**0,5 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,5 µl/ml**
p	**SI**	,276	,107	,004 *	,058	,003 *	,010 *
	Slime	,390	,054	,030 *	,035 *	,025 *	,366
	Growth	,768	,986	,009 *	,498	,012 *	,003 *

*** p < 0,05**

It was observed that there were significant differences in biofilm formation of MSS between the concentrations of 0.1 and 1 µl/ml, 0,2 and 1 µl/ml, 0,5 and 1 µl/ml of eau de rose ($p < 0,05$) (Table 4.4.3.2). The growth of MSS was lowered by all of the concentrations of eau de rose but a significant reduction of biofilm production of MSS was not observed between the concentration of 0,1 and 0,2 µl/ml, 0,1 and 0,5 µl/ml, 0,2 and 0,5 µl/ml ($p > 0,05$). In table 4.4.3.2 it was inferred that there were significant differences in growth of MSS between the concentrations of 1,0 and 0,1-0,5 µl/ml ($p < 0,05$), this difference was also confirmed by the table 4.4.3.1 that showed an increase in the growth of MSS with the concentration of 1,0 µl/ml when compared with the concentrations of 0,1 µl/ml , 0,2 µl/ml and 0,5 µl/ml.

Table 4.4.3.3 The Wilcoxon test results which show the effects of different concentrations of eau de rose on the growth and biofilm formation of MRS

		The concentration intervals of Eau de rose (µl /ml - µl /ml)					
		0,2 µl/ml – 0,1 µl/ml	**0,5 µl/ml – 0,1 µl/ml**	**1,0 µl/ml – 0,1 µl/ml**	**0,5 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,2 µl/ml**	**1,0 µl/ml – 0,5 µl/ml**
p	**SI**	-	-	-	-	-	-
	Slime	-	-	-	-	-	-
	Growth	,658	,334	,008 *	,445	,010 *	,007 *

*** p < 0,05**

There were significant differences in growth of MRS with the concentrations of 1,0 and 0,1-0,5 µl/ml ($p < 0,05$) (Table 4.4.3.3). There were significant reduction of growth of MRS with the concentration of 1,0 and 0,1-0,5 µl/ml.

Table 4.4.3.1 had indicated that there were no significant differences in the biofilm production of MRS between the different concentrations of eau de rose ($p > 0,05$).

4.5 The Effects of pH on the Growth of *Staphylococcus*

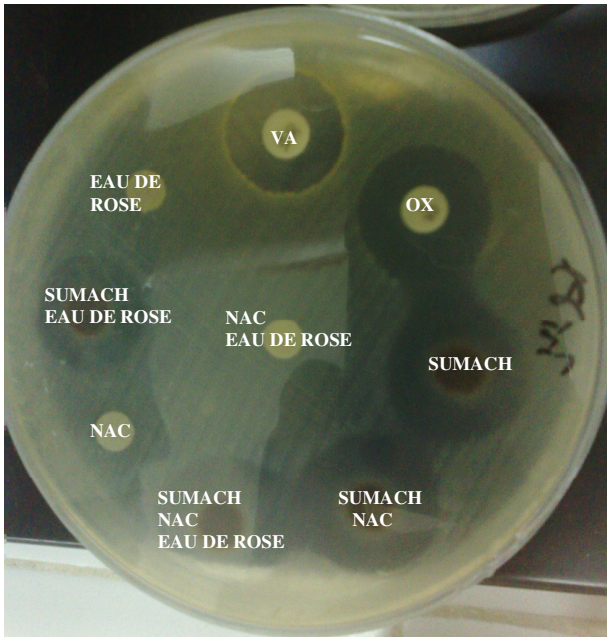
NAC and eau de rose effects were not varied much by changing the values of pH 7 and 10. NAC and eau de rose did not inhibit the isolates by disc diffusion method. Following an increase in the pH of the sumach (pH=7 and pH=10) the diameter of the inhibition zone was decreased. The best inhibition of isolates by sumach was observed at its original pH (pH=1.5).

4.6 The Effects of Temperature on the Growth of *Staphylococcus*

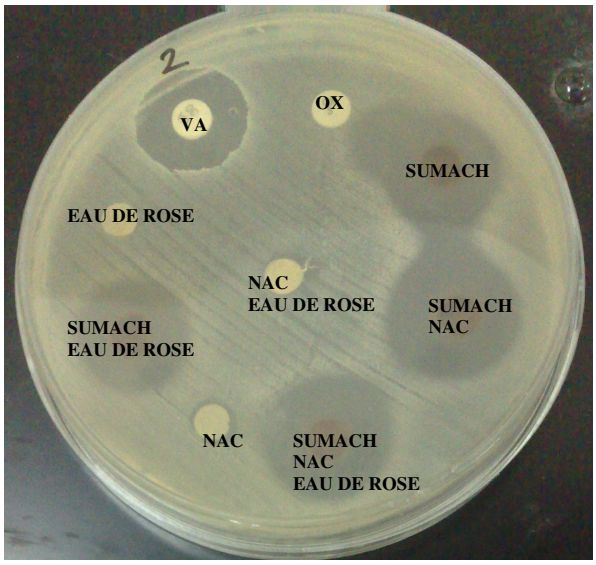
The changes in temperature (2 ^{o}C, 50 ^{o}C, 100^{o}C) were not reduced the effects of NAC and eau de rose. The best inhibition of isolates by sumach was observed at 2 ^{o}C and 25 ^{o}C and the zone diameters were larger than those 50 ^{o}C and 100 ^{o}C application.

4.7. The Effects of The Combined Treatments on The Growth and Biofilm formation of all types of *Staphylococcus aureus* and *Staphylococcus epidermidis*

It was found that the combined treatments (Eau de rose-NAC combination, Sumach-NAC combination, Sumach-Eau de rose combination and Sumach-Eau de rose-NAC combination) had no difference on the inhibition of the isolates compared with the sole treatments of Sumach, Eau de rose and NAC, Therefore the combined effects of these sole treatments were found to be negligible.



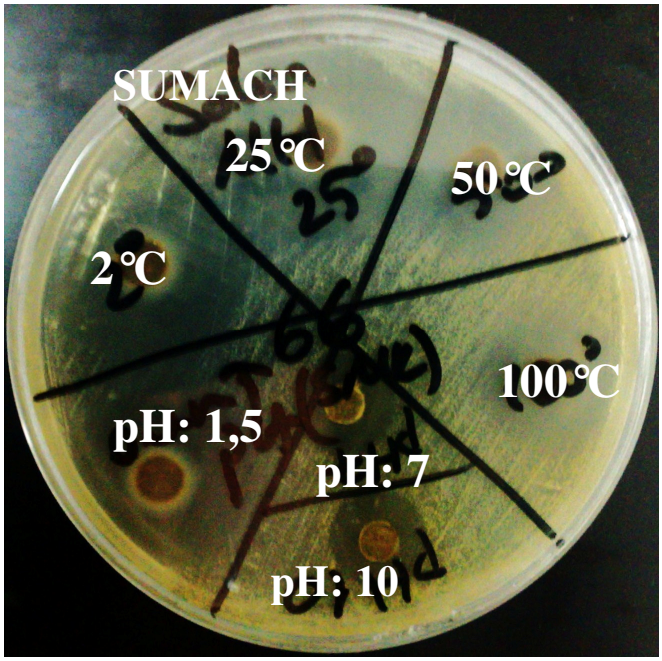
a. MSSA



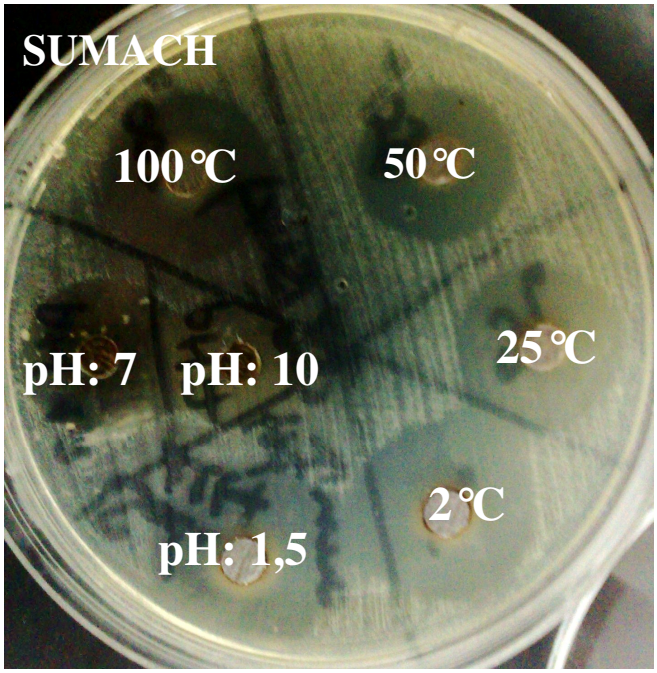
b. MRSE

Figure 9 Combination zones by disc diffusion method. **a.** MSSA, **b.** MRSE**.**

(By starting from the top to the bottom) Discs were placed due to following the order: Vancomycin, oxacillin, sumach, sumach-NAC, sumach-NAC-eau de rose, NAC, sumach-eau de rose, eau de rose, NAC-eau de rose was added to the disc located at the middle of the plate.



a. MSSA



b. MRSE

Figure 10 The effect of the temperature (2°, 25°, 50° and 100° C) and pH (2, 7 and 10).

a. MSSA, **b.** MRSE.

CHAPTER 5

CONCLUSION

It is urgently desirable to discover or synthesize new drugs that are effective against drug-resistant microorganisms. Spices not only provide flavor and aroma but also retard microbial growth in food. In these experiments it was observed that sumach and eau de rose showed a remarkable reduction of the biofilm and growth of MRS and MSS.

It can be concluded that the effect of NAC, sumach and eau de rose statistically affect and decrease biofilm formation and growth of *Staphylococcus* including MRS and MSS.

By disc diffusion method, the bacteriostatic effect of sumach on the isolates were sensitive to pH and temperature. The diameters of the inhibition zones around discs were decreased by increased pH and temperature of sumach. The effects of NAC and eau de rose were not changed by increasing the pH and temperature.

Consequently this study was demonstrated that the higher the concentrations of NAC, sumach and eau de rose, the lower the OD values of the biofilm and growth.

This study may form an opinion that NAC, sumach and eau de rose may be used in medicine and food industry. NAC, sumach and eau de rose coated catheters and prostheses may be produced to prevent implant associated infections. They may be used for decontamination of industrial machines too.

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7. APPENDICES

1. The SI of isolates which was treated with NAC

Bacteria no	Bacteria	NAC (mg/ml)			
		0.03	**0.12**	**0.5**	**2.0**
30	**MSSE**	43.478	50	41.667	43.478
72	**MRSE**	37.037	33.333	35.714	37.037
50	**MRSE**	58.824	62.500	50	52.632
83	**MSSA**	90.909	83.333	71.429	71.429
27	**MSSA**	100	100	76.923	55.556
53	**MRSE**	90.909	76.923	76.923	66.667
31	**MSSE**	100	83.333	11.111	62.500
84	**MSSA**	43.478	43.478	38.462	32.258
45	**MSSE**	125	125	100	83.333
23	**MRSE**	90.909	71.429	76.923	52.632
62	**MRSE**	142.857	76.923	50	66.667
2	**MRSE**	111.111	111.111	90.909	76.923
11	**MSSE**	58.824	50	55.556	76.923
12	**MRSE**	71.429	90.909	83.333	83.333
17	**MSSE**	71.429	76.923	62.500	71.429
35	**MSSE**	83.333	66.667	76.923	76.923
37	**MSSE**	100	100	111.111	90.909
49	**MSSE**	37.037	37.037	37.037	25.641
86	**MSSA**	26.316	23.810	22.222	24.390
66	**MSSA**	41.667	47.619	40	35.714
60	**MSSA**	62.500	58.824	71.429	66.667
13	**MRSA**	43.478	37.037	45.455	40
25	**MRSE**	83.333	90.909	83.333	250
38	**MRSE**	100	90.909	90.909	83.333
10	**MRSE**	83.333	100	100	90.909
51	**MSSA**	111.111	125	111.111	111.111
3	**MRSE**	71.429	71.429	71.429	83.333

5	**MSSE**	71.429	100	90.909	76.923
33	**MRSE**	76.923	76.923	83.333	83.333
28	**MSSE**	90.909	83.333	83.333	83.333
26	**MSSA**	125	90.909	111.111	100
16	**MRSA**	100	71.429	100	83.333
63	**MSSE**	111.111	55.556	76.923	71.429
76	**MRSE**	100	100	83.333	83.333
4	**MSSE**	100	58.824	71.429	76.923
75	**MRSE**	90.909	76.923	66.667	55.556
19	**MSSA**	125	90.909	76.923	83.333
34	**MRSA**	125	90.909	83.333	90.909
1	**MRSE**	111.111	90.909	111.111	111.111
18	**MSSE**	45.455	45.455	43.478	38.462
20	**MRSA**	90.909	83.333	71.429	71.429

2. The slime formation of isolates which was treated with NAC

Bacteria no	Bacteria	NAC (mg/ml)			
		0.03	**0.12**	**0.5**	**2.0**
30	**MSSE**	52.151	48.925	45.161	35.215
72	**MRSE**	28.846	26.573	24.301	22.378
50	**MRSE**	43.182	42.532	41.234	32.468
83	**MSSA**	229.070	201.163	163.953	144.186
27	**MSSA**	90.395	79.096	68.362	45.198
53	**MRSE**	89.655	82.069	72.414	52.414
31	**MSSE**	75.309	72.840	70.370	61.728
84	**MSSA**	47.619	43.810	42.857	39.048
45	**MSSE**	97.059	89.706	83.824	75
23	**MRSE**	85	80	70	61.250
62	**MRSE**	74.016	51.969	42.520	38.583
2	**MRSE**	90	77.273	73.636	56.364
11	**MSSE**	91.176	72.059	67.647	58.824
12	**MRSE**	93.590	91.026	79.487	61.538
17	**MSSE**	73.494	73.494	71.084	65.060
35	**MSSE**	65.487	62.832	59.292	53.097
37	**MSSE**	94.186	91.860	88.372	60.465
49	**MSSE**	45.638	39.262	38.591	20.470
86	**MSSA**	26.168	21.807	20.249	19.315
66	**MSSA**	95.876	86.598	74.227	58.763
60	**MSSA**	69.307	65.347	61.386	55.446
13	**MRSA**	38.817	37.789	35.990	30.848
25	**MRSE**	90.805	80.952	77.143	70.115
38	**MRSE**	99.048	89.524	80.952	68.571
10	**MRSE**	120.270	159.459	125.676	118.919
51	**MSSA**	96.386	93.976	90.361	83.133
3	**MRSE**	98.765	96.296	81.481	79.012
5	**MSSE**	97.368	94.737	81.579	76.316

33	**MRSE**	100	97.436	84.615	76.923
28	**MSSE**	100	98.667	73.333	68
26	**MSSA**	86.667	78.095	66.667	59.048
16	**MRSA**	69.412	68.235	82.353	64.706
63	**MSSE**	86.957	56.522	43.243	37.838
76	**MRSE**	71.552	60.345	55.172	44.828
4	**MSSE**	71.910	60.674	56.180	48.315
75	**MRSE**	77.778	61.438	50.324	38.562
19	**MSSA**	52.147	51.534	44.172	42.945
34	**MRSA**	97.297	75.676	71.622	67.568
1	**MRSE**	77.333	64	73.333	66.667
18	**MSSE**	41.611	39.597	36.913	32.215
20	**MRSA**	70.787	66.292	64.045	55.056

3. The growth of isolates which was treated with NAC

Bacteria no	**Bacteria**	**NAC (mg/ml)**			
		0.03	**0.12**	**0.5**	**2.0**
30	**MSSE**	119.649	98.270	109.486	80.541
72	**MRSE**	78.956	80.995	67.210	60.522
50	**MRSE**	72.595	66.431	81.838	63.162
83	**MSSA**	75.514	79.806	61.111	63.316
27	**MSSA**	86.066	82.355	90.385	82.557
53	**MRSE**	99.867	109.329	93.028	80.578
31	**MSSE**	72.224	84.953	62.504	96.057
84	**MSSA**	111.247	100.746	112.020	121.775
45	**MSSE**	75.892	69.158	87.778	89.899
23	**MRSE**	89.299	108.052	89.351	116.338
62	**MRSE**	49.282	70.035	86.737	56.867
2	**MRSE**	85.437	72.480	84.348	74.054
11	**MSSE**	156.611	143.549	119.383	74.968

12	**MRSE**	133.147	102.552	97.727	75.559
17	**MSSE**	101.666	95.636	110.526	94.261
35	**MSSE**	77.455	93.506	74.758	71.406
37	**MSSE**	97.582	92.078	81.164	66.879
49	**MSSE**	125.509	105.940	105.308	80.057
86	**MSSA**	100.661	91.076	91.152	78.617
66	**MSSA**	231.401	181.438	182.098	161.849
60	**MSSA**	112.055	109.415	88.263	84.447
13	**MRSA**	91.173	100.740	83.343	78.588
25	**MRSE**	104.685	92.033	90.074	30.995
38	**MRSE**	97.236	87.742	86.494	80.254
10	**MRSE**	138.588	152.824	123.176	135.176
51	**MSSA**	89.124	75.756	79.869	77.438
3	**MRSE**	136.321	130.245	111.685	77.097
5	**MSSE**	134.065	94.091	90.959	99.257
33	**MRSE**	126.547	130.302	104.559	89.609
28	**MSSE**	113.632	119.183	88.640	79.494
26	**MSSA**	70.299	84.401	61.738	58.876
16	**MRSA**	68.743	92.100	85.834	83.137
63	**MSSE**	75.583	67.723	55.888	53.915
76	**MRSE**	69.185	62.941	66.375	52.451
4	**MSSE**	73.803	106.026	78.261	63.511
75	**MRSE**	86.400	80.160	74.160	70.480
19	**MSSA**	44.217	56.989	56.045	50.197
34	**MRSA**	81.908	83.479	83.711	74.113
1	**MRSE**	71.187	70.718	62.884	59.032
18	**MSSE**	91.492	89.113	86.518	84.102
20	**MRSA**	80.364	77.072	90.476	76.573

4. The SI of isolates which was treated with Sumach

Bacteria no	Bacteria	Sumach (µl/ml)			
		0.1 µl/ml	**0.2 µl/ml**	**0.5 µl/ml**	**1.0 µl/ml**
30	**MSSE**	17.544	18.182	13.159	13.333
72	**MRSE**	27.027	27.778	20.408	11.111
50	**MRSE**	19.231	20.408	21.739	10
83	**MSSA**	100	83.333	76.923	55.556
27	**MSSA**	90.909	100	76.923	41.667
53	**MRSE**	58.824	58.824	50	32.258
31	**MSSE**	142.857	111.111	90.909	62.500
84	**MSSA**	47.619	45.455	40	40
45	**MSSE**	125	142.857	142.857	71.429
23	**MRSE**	83.333	71.429	76.923	47.619
62	**MRSE**	83.333	76.923	62.500	38.462
2	**MRSE**	90.909	90.909	58.824	37.037
11	**MSSE**	66.667	66.667	83.333	71.429
12	**MRSE**	83.333	71.429	71.429	76.923
17	**MSSE**	71.429	66.667	71.429	66.667
35	**MSSE**	62.500	62.500	62.500	58.824
37	**MSSE**	111.111	100	100	71.429
49	**MSSE**	40	38.462	33.333	28.571
86	**MSSA**	35.714	28.571	26.316	23.810
66	**MSSA**	52.632	45.455	50	22.727
60	**MSSA**	100	111.111	83.333	62.500
13	**MRSA**	50	41.667	41.667	19.231
25	**MRSE**	100	100	90.909	90.909
38	**MRSE**	111.111	90.909	76.923	62.500
10	**MRSE**	142.857	142.857	90.909	76.923
51	**MSSA**	100	100	76.923	58.824
3	**MRSE**	90.909	76.923	71.429	55.556
5	**MSSE**	100	100	90.909	76.923

33	**MRSE**	83.333	90.909	90.909	76.923
28	**MSSE**	111.111	90.909	90.909	90.909
26	**MSSA**	111.111	100	90.909	62.500
16	**MRSA**	100	111.111	111.111	90.909
63	**MSSE**	100	58.824	71.429	43.478
76	**MRSE**	100	100	90.909	71.429
4	**MSSE**	142.857	125	125	71.429
75	**MRSE**	76.923	76.923	71.429	37.037
19	**MSSA**	66.667	58.824	58.824	41.667
34	**MRSA**	125	111.111	125	100
1	**MRSE**	125	111.111	125	83.333
18	**MSSE**	40	35.714	40	28.571
20	**MRSA**	100	83.333	76.923	55.556

5. The slime formation of isolates which was treated with sumach

Bacteria no	**Bacteria**	**Sumach (µl/ml)**			
		0.1 µl/ml	**0.2 µl/ml**	**0.5 µl/ml**	**1.0 µl/ml**
30	**MSSE**	18.280	18.011	16.935	16.398
72	**MRSE**	19.755	18.706	16.958	11.888
50	**MRSE**	17.532	14.935	13.636	10.390
83	**MSSA**	88.764	79.775	67.416	58.427
27	**MSSA**	95.480	83.616	63.277	46.328
53	**MRSE**	55.862	51.724	48.276	44.138
31	**MSSE**	79.012	76.543	70.370	61.728
84	**MSSA**	54.286	51.429	50.476	47.619
45	**MSSE**	102.941	100	95.588	66.176
23	**MRSE**	82.500	77.500	71.250	53.750
62	**MRSE**	70.866	58.268	34.646	33.858
2	**MRSE**	74.545	70	69.091	40.909
11	**MSSE**	72.059	75	86.765	76.471

12	**MRSE**	92.647	79.412	83.824	92.647
17	**MSSE**	67.470	65.060	63.855	56.627
35	**MSSE**	53.982	51.327	50.442	47.788
37	**MSSE**	96.512	91.860	81.395	62.791
49	**MSSE**	44.631	39.597	33.221	26.175
86	**MSSA**	33.022	25.545	25.545	22.118
66	**MSSA**	98.969	92.784	81.443	55.670
60	**MSSA**	98.020	96.040	89.109	74.257
13	**MRSA**	36.761	35.476	33.162	16.710
25	**MRSE**	94.253	88.506	81.609	68.966
38	**MRSE**	98.095	80.952	63.810	49.524
10	**MRSE**	179.730	186.486	124.324	118.919
51	**MSSA**	95.181	91.566	81.928	66.265
3	**MRSE**	96.296	90.123	82.716	65.432
5	**MSSE**	96.053	93.421	88.158	78.947
33	**MRSE**	98.718	97.436	87.179	75.641
28	**MSSE**	98.667	96	85.333	80
26	**MSSA**	80.952	68.571	59.048	48.571
16	**MRSA**	98.824	95.294	83.529	76.471
63	**MSSE**	63.587	39.674	34.239	32.609
76	**MRSE**	68.103	56.897	53.448	52.586
4	**MSSE**	97.753	95.506	92.135	70.787
75	**MRSE**	64.052	62.092	56.863	39.216
19	**MSSA**	36.810	39.877	34.356	33.129
34	**MRSA**	97.297	94.595	87.838	79.730
1	**MRSE**	89.333	84	70.667	66.667
18	**MSSE**	35.570	33.557	32.215	29.530
20	**MRSA**	92.135	70.787	65.169	57.303

6. The growth of isolates which was Treated with Sumach

Bacteria no	Bacteria	Sumach (µl/ml)			
		0.1 µl/ml	**0.2 µl/ml**	**0.5 µl/ml**	**1.0 µl/ml**
30	**MSSE**	105.027	98.324	128.378	123.541
72	**MRSE**	73.899	68.108	82.518	107.314
50	**MRSE**	91.946	72.568	62.622	104.054
83	**MSSA**	78.454	89.830	72.840	97.149
27	**MSSA**	105.938	81.680	84.379	109.683
53	**MRSE**	92.696	86.786	95.817	134.562
31	**MSSE**	58.146	68.765	79.142	96.783
84	**MSSA**	113.726	110.901	128.571	118.683
45	**MSSE**	78.721	66.633	69.327	90.337
23	**MRSE**	96.468	106.571	94.935	114.442
62	**MRSE**	86.620	73.263	56.655	87.845
2	**MRSE**	82.652	79.988	114.260	111.353
11	**MSSE**	109.788	116.078	104.397	106.418
12	**MRSE**	98.776	99.895	100.944	101.713
17	**MSSE**	91.351	98.598	91.325	83.549
35	**MSSE**	84.839	80.492	82.194	79.393
37	**MSSE**	86.446	90.710	78.715	86.001
49	**MSSE**	110.846	103.013	99.799	92.310
86	**MSSA**	92.449	88.228	97.915	92.906
66	**MSSA**	192.517	208.291	165.444	244.974
60	**MSSA**	99.141	89.917	102.863	115.967
13	**MRSA**	74.943	84.909	78.559	86.304
25	**MRSE**	95.077	89.254	86.818	77.131
38	**MRSE**	87.252	90.104	84.555	77.647
10	**MRSE**	132.647	138.588	137	159.765
51	**MSSA**	97.195	88.376	99.346	110.284
3	**MRSE**	107.451	115.342	115.370	118.257
5	**MSSE**	98.805	89.441	99.677	100.097

33	**MRSE**	119.441	104.559	93.385	100.514
28	**MSSE**	93.558	109.922	90.394	86.425
26	**MSSA**	72.794	66.702	64.811	75.762
16	**MRSA**	96.164	88.682	72.655	87.885
63	**MSSE**	62.762	66.019	53.497	75.493
76	**MRSE**	69.622	57.196	56.978	72.338
4	**MSSE**	71.491	79.224	74.491	98.459
75	**MRSE**	85.120	81.760	76.880	105.640
19	**MSSA**	56.491	66.509	58.169	81.039
34	**MRSA**	80.628	82.635	71	79.901
1	**MRSE**	72.176	78.214	61.999	82.015
18	**MSSE**	89.510	92.934	79.849	104.326
20	**MRSA**	95.944	84.362	84.891	101.117

7. The SI of isolates which was treated with Eau de rose

Bacteria no	**Bacteria**	**Eau de rose (µl/ml)**			
		0.1 µl/ml	**0.2 µl/ml**	**0.5 µl/ml**	**1.0 µl/ml**
30	**MSSE**	83.333	66.667	52.632	43.478
72	**MRSE**	38.462	58.824	27.027	28.571
50	**MRSE**	45.455	38.462	34.483	27.778
83	**MSSA**	83.333	76.923	76.923	76.923
27	**MSSA**	62.500	62.500	58.824	55.556
53	**MRSE**	45.455	55.556	76.923	76.923
31	**MSSE**	100	111.111	100	62.500
84	**MSSA**	58.824	41.667	58.824	38.462
45	**MSSE**	142.857	125	111.111	111.111
23	**MRSE**	66.667	76.923	76.923	58.824
62	**MRSE**	71.429	111.111	83.333	58.824
2	**MRSE**	83.333	83.333	58.824	71.429
11	**MSSE**	90.909	83.333	71.429	55.556

12	**MRSE**	76.923	76.923	62.500	52.632
17	**MSSE**	62.500	66.667	62.500	58.824
35	**MSSE**	66.667	55.556	52.632	45.455
37	**MSSE**	100	90.909	76.923	71.429
49	**MSSE**	20.408	21.277	21.739	30.303
86	**MSSA**	29.412	37.037	142.857	250
60	**MSSA**	83.333	71.429	66.667	66.667
13	**MRSA**	23.810	22.727	38.462	37.037
25	**MRSE**	16.667	111.111	166.667	142.857
38	**MRSE**	142.857	142.857	142.857	125
10	**MRSE**	100	83.333	125	76.923
51	**MSSA**	111.111	125	111.111	90.909
3	**MRSE**	90.909	111.111	111.111	111.111
5	**MSSE**	100	111.111	125	100
33	**MRSE**	111.111	111.111	111.111	90.909
28	**MSSE**	111.111	90.909	90.909	83.333
26	**MSSA**	142.857	142.857	125	76.923
16	**MRSA**	66.667	62.500	66.667	62.500
63	**MSSE**	125	111.111	90.909	83.333
76	**MRSE**	125	71.429	76.923	71.429
4	**MSSE**	76.923	111.111	100	83.333
75	**MRSE**	76.923	62.500	52,632	52,632
19	**MSSA**	62.500	66.667	58.824	52.632
34	**MRSA**	111.111	90.909	90.909	100
1	**MRSE**	111.111	142.857	100	111.111
18	**MSSE**	50	50	47.619	34.483
20	**MRSA**	83.333	76.923	76.923	76.923

8. The slime formation of isolates which was treated with Eau de rose

Bacteria no	Bacteria	Eau de rose (µl/ml)			
		0.1 µl/ml	0.2 µl/ml	0.5 µl/ml	1.0 µl/ml
30	**MSSE**	75.269	69.355	58.065	42.742
72	**MRSE**	27.622	45.804	31.469	27.972
50	**MRSE**	44.805	32.792	29.870	30.195
83	**MSSA**	92.135	87.640	80.899	76.744
27	**MSSA**	55.932	56.497	56.497	54.802
53	**MRSE**	57.931	79.310	94.483	89.655
31	**MSSE**	72.840	80.247	75.309	71.605
84	**MSSA**	60.952	44.762	60	44.762
45	**MSSE**	98.529	95.588	91.176	98.529
23	**MRSE**	60	80	67.500	61.250
62	**MRSE**	55.906	89.764	54.331	59.843
2	**MRSE**	67.273	70.909	49.091	77.273
11	**MSSE**	97.059	88.235	76.471	69.118
12	**MRSE**	83.333	79.487	73.077	57.692
17	**MSSE**	57.831	62.651	57.831	59.036
35	**MSSE**	54.867	47.788	45.133	42.478
37	**MSSE**	77.907	79.070	66.279	76.744
49	**MSSE**	20.134	29.195	23.826	27.517
86	**MSSA**	27.103	36.137	41.433	67.913
60	**MSSA**	81.881	73.267	63.366	81.881
13	**MRSA**	23.650	23.650	39.332	38.046
25	**MRSE**	139.080	101.149	139.080	142.529
38	**MRSE**	102.857	117.143	105.714	110.476
10	**MRSE**	152.703	110.811	177.027	114.865
51	**MSSA**	100	97.590	87.952	74.157
3	**MRSE**	97.531	116.049	109.877	104.938
5	**MSSE**	94.737	111.842	102.632	92.105
33	**MRSE**	101.282	100	98.718	88.462

28	**MSSE**	106.667	84	82.667	82.667
26	**MSSA**	89.524	84.762	77.143	60.952
16	**MRSA**	62.353	61.176	62.353	67.059
63	**MSSE**	80.435	61.413	62.500	59.783
76	**MRSE**	62.931	46.552	41.379	50
4	**MSSE**	77.528	78.652	79.775	77.528
75	**MRSE**	73.203	56.209	56.209	54.248
19	**MSSA**	36.810	34.356	33.742	32.515
34	**MRSA**	93.243	64.865	68.919	87.838
1	**MRSE**	92	101.333	72	80
18	**MSSE**	44.295	42.953	40.268	35.570
20	**MRSA**	71.910	66.292	68.539	80.899

9. The growth of isolates which was treated with Eau de rose

Bacteria No	**Bacteria**	**Eau de rose (µl/ml)**			
		0.1 µl/ml	**0.2 µl/ml**	**0.5 µl/ml**	**1.0 µl/ml**
30	**MSSE**	73.203	56.209	56.209	54.248
72	**MRSE**	36.810	34.356	33.742	32.515
50	**MRSE**	93.243	64.865	68.919	87.838
83	**MSSA**	92	101.333	72	80
27	**MSSA**	44.295	42.953	40.268	35.570
53	**MRSE**	71.910	66.292	68.539	80.899
31	**MSSE**	90.973	101.486	111.757	100.189
84	**MSSA**	73.002	77.379	115.552	97.336
45	**MSSE**	97.486	85.703	87	93.135
23	**MRSE**	89.359	78.953	82.569	86.390
62	**MRSE**	92.072	91.903	98.043	100.270
2	**MRSE**	126.594	144.157	119.323	116.336
11	**MSSE**	71.602	70.875	72.224	117.606
12	**MRSE**	104.078	107.010	104.451	115.192

17	**MSSE**	65.017	75.960	80.606	93.064
35	**MSSE**	90.753	104.519	85.091	105.818
37	**MSSE**	79.623	77.880	66.808	99.859
49	**MSSE**	77.990	83.409	85.377	108.840
86	**MSSA**	110.045	109.692	105.551	123.748
60	**MSSA**	93.766	99.873	96.915	119.275
13	**MRSA**	100.028	104.499	101.993	104.641
25	**MRSE**	87.533	91.345	86.713	97.327
38	**MRSE**	83.107	83.441	75.262	88.924
10	**MRSE**	146.765	133.588	138.647	150.588
51	**MSSA**	85.198	80.804	80.960	89.031
3	**MRSE**	110.531	105.004	94.556	92.246
5	**MSSE**	94.446	95.124	85.760	91.799
33	**MRSE**	95.263	93.095	85.721	100.067
28	**MSSE**	91.947	95.370	88.697	100.345
26	**MSSA**	66.702	61.187	63.524	76.917
16	**MRSA**	92.176	96.658	95.215	108.204
63	**MSSE**	65.660	53.347	69.845	72.714
76	**MRSE**	53.387	67.281	54.262	68.467
4	**MSSE**	100.798	73.528	78.178	93.451
75	**MRSE**	94.680		92.720	104.080
19	**MSSA**	105.120	57.330	52.872	56.727
34	**MRSA**	63.441	80.134	74.316	76.702
1	**MRSE**	88.482	73.660	66.346	71.161
18	**MSSE**	74.206	87.058	86.518	85.004
20	**MRSA**	104.182	83.510	87.478	89.065

10. The controls of slime formation of 41 isolates

Bacteria no	Bacteria	O.D. values
30	**MSSE**	0.372
72	**MRSE**	0.572
50	**MRSE**	0.308
83	**MSSA**	0.089
27	**MSSA**	0.177
53	**MRSE**	0.145
31	**MSSE**	0.081
84	**MSSA**	0.105
45	**MSSE**	0.068
23	**MRSE**	0.08
62	**MRSE**	0.127
2	**MRSE**	0.11
11	**MSSE**	0.068
12	**MRSE**	0.078
17	**MSSE**	0.083
35	**MSSE**	0.113
37	**MSSE**	0.086
49	**MSSE**	0.298
86	**MSSA**	0.321
60	**MSSA**	0.101
13	**MRSA**	0.389
25	**MRSE**	0.087
38	**MRSE**	0.105
10	**MRSE**	0.074
51	**MSSA**	0.083
3	**MRSE**	0.081
5	**MSSE**	0.076
33	**MRSE**	0.078

28	**MSSE**	0.075
26	**MSSA**	0.105
16	**MRSA**	0.085
63	**MSSE**	0.184
76	**MRSE**	0.116
4	**MSSE**	0.089
75	**MRSE**	0.153
19	**MSSA**	0.163
34	**MRSA**	0.074
1	**MRSE**	0.075
18	**MSSE**	0.149
20	**MRSA**	0.089

11. The controls of growth of 41 isolates

Bacteria no	**Bacteria**	**Incubation Density**	**Growth after incubation**
30	**MSSE**	0.314	0.989
72	**MRSE**	0.353	1.127
50	**MRSE**	0.287	0.889
83	**MSSA**	0.31	0.901
27	**MSSA**	0.338	0.876
53	**MRSE**	0.313	0.814
31	**MSSE**	0.321	0.807
84	**MSSA**	0.31	0.987
45	**MSSE**	0.298	0.759
23	**MRSE**	0.311	0.015
62	**MRSE**	0.305	1.087
2	**MRSE**	0.325	0.926
11	**MSSE**	0.28	0.737
12	**MRSE**	0.335	0.839

17	**MSSE**	0.292	0.926
35	**MSSE**	0.279	0.885
37	**MSSE**	0.344	0.944
49	**MSSE**	0.293	0.862
86	**MSSA**	0.289	0.949
60	**MSSA**	0.327	0.891
13	**MRSA**	0.347	1.058
25	**MRSE**	0.28	0.88
38	**MRSE**	0.253	0.912
10	**MRSE**	0.287	0.443
51	**MSSA**	0.322	0.892
3	**MRSE**	0.287	0.875
5	**MSSE**	0.332	0.894
33	**MRSE**	0.268	0.977
28	**MSSE**	0.322	0.961
26	**MSSA**	0.293	0.936
16	**MRSA**	0.274	0.617
63	**MSSE**	0.281	0.79
76	**MRSE**	0.3	0.82
4	**MSSE**	0.31	0.958
75	**MRSE**	0.326	0.719
19	**MSSA**	0.272	0.857
34	**MRSA**	0.338	1.006
1	**MRSE**	0.273	0.867
18	**MSSE**	0.25	0.58
20	**MRSA**	0.31	0.901

12. The OD values of sterile treatments

Treatments	**O.D. values (read in 540nm)**	
	Before incubation	**After incubation**
0.03 mg/ml NAC	0.07	0.07
0.12 mg/ml NAC	0.07	0.07
0.5 mg/ml NAC	0.07	0.07
2.0 mg/ml NAC	0.07	0.07
0.1 µl/ml Sumach	0.075	0.08
0.2 µl/ml Sumach	0.089	0.097
0.5 µl/ml Sumach	0.094	0.104
1 µl/ml Sumach	0.235	0.260
0.1 µl/ml Eau de rose	0.07	0.07
0.2 µl/ml Eau de rose	0.07	0.07
0.5 µl/ml Eau de rose	0.07	0.07
1 µl/ml Eau de rose	0.07	0.07