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Research Article / Araştırma Makalesi

Evaluation of Antibacterial Activities of Stryax Liquidus on Staphylococcus aureus on Stainless Steel Surface

Sığla Yağının Paslanmaz Çelik Yüzeyde Staphylococcus aureus Üzerine Antibakteriyel Aktivitesinin Değerlendirilmesi

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Abstract: Sığla tree (*Liquidambar orientalis* Miller) is an endemic species in Turkey. Styrax liquidus obtained from Sığla tree and it has antiparasitic and antibacterial properties. *Staphylococcus aureus* is an important agent of mastitis. This pathogen can be found in milk and on the milk collection tank surfaces and it can produce some types of toxins. Staphylococcal enterotoxins may cause food poisoning. In this study; it was aimed to investigate the effects of the styrax liquidus solution on the bacterial culture applied to the stainless-steel surface, on different periods, experimentally. As a result of the study, it was determined that styrax liquidus was effective on *S. aureus* on the surface of the bulk tank milk at 5%, 10%, 15% and 20% concentrations. It was determined that styrax liquidus was reduced population of *S. aureus* level of 7.94 log cfu/ml. Styrax liquidus could be used as a natural antimicrobial agent in industrial surface cleaning. This is the first study to determine the disinfection properties of styrax liquidus.

Keywords: Styrax liquidus, Sığla tree, *Staphylococcus aureus*, Milk.

Öz: Sığla ağacı (*Liquidambar orientalis* Miller) Türkiye'de endemik bir ağaç türüdür. Bu ağacın özünden elde edilen sığla yağının antiparaziter ve antibakteriyel özellikleri bulunmaktadır. *Staphylococcus aureus* önemli bir mastitis etkenidir. Süt ve süt toplama tank yüzeylerinde bulunması ve salgıladığı toksinler gıda zehirlenmeleri oluşturabilir. Bu çalışmada; deneysel olarak paslanmaz çelik yüzeye uygulanan bakteri kültürü üzerine farklı sürelerde uygulanan sığla yağı içerikli solüsyonun etkilerinin araştırılması amaçlanmıştır. Çalışma sonucunda sığla yağının %5, %10, %15 ve %20 konsantrasyonlarda süt toplama tankı yüzeyinde tüm konsantrasyonlarda *S. aureus* üzerinde etkili olduğu tespit edilmiştir. Sığla yağının *S. aureus* varlığı üzerinde 7,94 log cfu/ml inaktivasyon gerçekleştirdiği belirlenmiştir. Sığla yağı endüstriyel anlamda yüzey temizliğinde doğal bir antimikrobiyal ajan olarak kullanılabilir. Bu çalışma sığla yağının dezenfeksiyon özelliklerinin belirlenmesi bakımından ilk çalışma özelliğindedir.

Anahtar Kelimeler: Sığla Ağacı, Sığla Yağı, *Staphylococcus aureus*, Süt.

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Introduction

Milk is one of the most important food in human nutrition (Haug et al., 2007). Especially for children in their growing age, it is necessary to have sufficient milk in their diets for adequate and balanced nutrition (Kandpal et al., 2012). Microbial contamination of milk is a concern for the food industry (Keyvan et al., 2018; Turkoglu and Keyvan, 2019; Keyvan et al., 2020). Especially some microorganisms that are capable of forming biofilms can maintain their vitality on these surfaces and cause contamination (Engel et al., 2017). Surface properties also play an important role in the occurrence of microbial contamination. Stainless steel is a preferred material for the food industry due to its physicochemical properties, low cost, resistance to corrosion, processing advantage and mechanical durability. In addition to these good properties, stainless steel surfaces also have a high free surface energy that causes free bacterial binding and hydrophilic properties in favor of biofilm formation (Chmielewski and Frank, 2003; Van Houdt et al., 2010).

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Staphylococcus aureus is a gram-positive bacterium and it can produce some types of toxin. Foodborne intoxications may occure due to consumption of contaminated food with these toxins (Le Loir et al., 2003). Meat products, poultry and egg products, milk and dairy products are food sources responsible for staphylococcal food poisoning (Normanno et al., 2005). In addition, *S. aureus* has the ability to adhere to equipment surfaces such as stainless steel in food processing units and to generate biofilms (Hamadi et al., 2005; Marques et al., 2007; Oulahal et al., 2008). Equipment-related contamination may occure in milk and dairy products through the biofilm created in milk processing equipment. Food poisoning may occur as a result of these contaminations (Hamadi et al., 2014). Anatolia Sığla Tree (*Liquidambar orientalis* Miller) is an endemic species in Turkey. Styrax liquidus contains alcohol, ether, acid, phenolic and volatile compounds. Antimicrobial and antioxidant properties of extracts obtained from Anatolian Sığla tree leaves were determined (Arslan and Şahin, 2016). This study is aimed to determine the antibacterial activities of styrax liquidus on *S. aureus* at different application time.

Materials and Methods

Material

Styrax liquidus was purchased from local producers. Styrax liquidus dissolved in absolute ethanol (Sigma, 1.02428) to prepare stock solution concentration of at 5%, 10%, 15% and 20%.

Bacterial Strain

American Type Culture Collection (ATCC) standard *S. aureus* (ATCC 25923) was used for the assessment of antibacterial activities of styrax liquidus.

Table 1: Experimental groups used in this study.

Experimental groups	Solution (%)	Application time (min)	Application time (min)	Application time (min)	Application time (min)
Control	-	5	10	20	60
Group I	5	5	10	20	60
Group II	10	5	10	20	60
Group III	15	5	10	20	60
Group IV	20	5	10	20	60

Decontamination of Bulk Milk Tank Surface

The surface to be used to determine the effect of styrax liquidus on *S. aureus* on the surface of the milk collection tank was produced from the milk collection tank material. For this purpose, the stainless steel surface (AISI type 304 standard stainless steel) was divided into 20x20 cm^2 pieces and five different regions were marked with a template. Before experimental bacterial contamination, the sterile steel plate surfaces were sterilized at 121 degrees for 15 minutes and covered with aluminum foil. The suspension level of *S. aureus* was adjusted 0.5 McFarland, it was applied as 1 ml a spray to the previously sterilized surface. It was waited for 30 minutes to ensure the bacterial attachment. Experimental groups were designed as group I, II, III and IV (concentration of at 5%, 10%, 15% and 20%) and control at 5 min, 10 min, 20 min and 60 min. In order to determine the effect of styrax liquidus applied to surfaces contaminated with *S. aureus* at different concentrations and durations (Table 1), samples were taken from the surfaces with sterile swabs and then placed in tubes containing peptone water (Oxoid, CM0009). Samples prepared in 6 different dilutions were inoculated on Baird Parker agar (Oxoid, CM1127) and incubated at 37 °C for 24-48 hours to determine the growth rates (ISO, 2003). Colonies that grew after incubation were counted and the effect of the styrax liquidus-

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containing solution on *S. aureus* was determined. Experimental study applied in duplicate.

Results

In the current study, 0.5 McFarland *S. aureus* was detected as an average level of 7.94 log cfu/ml. In addition, the antimicrobial effect of ethanol used to dissolve styrax liquidus was investigated. It was determined in all experimental groups that ethanol used in dissolving styrax liquidus did not have any antimicrobial effect by ethanol effect group test. In this study, the styrax liquidus has inactivated at a concentration of 5%, 10%, 15% and 20% on *S. aureus* on stainless steel surfaces. Antibacterial activities of styrax liquidus on *S. aureus* were determined as 5% concentration in at least 5 min.

Discussion

Foodborne pathogens cause significant illness and death in humans (Painter et al., 2013). Milk is a suitable environment for the growth of most pathogens (Ding et al., 2016) Factors such as the health of dairy animals, udder diseases, udder hygiene, post-pasteurization contamination, milking conditions and cleaning and disinfection of tools and equipment are effective in the occurrence of milk-borne pathogens (Oliver et al., 2005; Dhanashekar et al., 2012). Various type of microorganisms can survive on stainless steel surfaces. It may pose significant public health hazards. It has been reported that *S. aureus* survived for 4 days after contamination of stainless steel surfaces (Kusumaningrum et al., 2003). This pathogen may contaminate milk as a result of its adherence to stainless steel surfaces and by its presence on the surfaces.

In recent years, the resistance created by bacteria against antibiotics is a worldwide concern (Gootz, 2010; Nathan and Cars, 2014). There are studies reporting that natural antimicrobial agents can be used as an antibiotic alternative (Keyvan and Tutun, 2019). It has been reported that many natural antimicrobial agents are effective on pathogens (Cabarkapa et al., 2019; Porter et al., 2020). In this study, it was aimed to determine the antimicrobial effect of styrax liquidus obtained by using Sığla tree (*Liquidambar orientalis*) which is an endemic tree species on stainless steel surfaces. It is reported that Anatolian styrax liquidus can also be used as an antimicrobial in the leather industry (Bayramoğlu, 2010). Similarly, Okmen et al. (2014) determined the antioxidant and antimicrobial effects of extracts obtained from leaves of Anatolian Sığla tree. It can also be used as ointment for skin diseases and for healing wounds with its antibacterial and scatrizant effect (Aydıngöz and Bulut,2014).

In this study, the effect of styrax liquidus in different time parameters was investigated in the experimental group. In a study on the antimicrobial effects of styrax liquidus; it is emphasized that the activity occurring at 10% concentration is more effective than other concentrations (0.1%, 0.2% and 0.4%) (Sağdıç et al., 2005). In the current study, similar results were found in all time parameters. As a result of examining the effect of styrax liquidus concentrations; antimicrobial effect has been detected at all concentrations. In conclusion, it was determined that the solution prepared within the scope of this study was an effective antimicrobial agent due to the adsorption property of *S. aureus* on the surface. Studies on the effect of styrax liquidus at lower concentrations should be planned. However, due to the risk of leaving residues on surfaces, studies should be planned for solutions prepared with different solvents.

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References

Arslan, M.B., Şahin, H.T., 2016. Unutulan Bir Orman Ürünü Kaynağı: Anadolu Sığla Ağacı (*Liquidambar Orientalis* Miller). *Journal Of Bartin Faculty Of Forestry* 18(1), 103-117.

Aydıngöz, M., Bulut, S., 2014. Egenin Gizli Kalmış Şifa İksiri: Sığla. Afyon Kocatepe University Journal of Science & Engineering 14(1), 1-6

Bayramoğlu, E.E., 2010. Soaking with Storax-Possibility of Using Sığla Tree (*Liquidambar orientalis Mill. Var orientalis*) Storax as Bactericide in the Soaking Float. Jalca. Journal of the American Leather Chemists Association 105, 62-68.

Cabarkapa, I., Colovic, R., Duragic, O., Popovic, S., Kokic, B., Milanov, D., Pezo, L., 2019. Anti-biofilm activities of essential oils rich in carvacrol and thymol against *Salmonella* Enteritidis. Biofouling 35 (3), 361-375.

Chmielewski, R.A.N., Frank, J.F., 2003. Biofilm formation and control in food processing facilities. Comprehensive Reviews in Food Science and Food Safety 2, 22–32.

Dhanashekar, R., Akkinepalli, S., Nellutla, A., 2012. Milk-borne infections. An analysis of their potential effect on the milk industry. Germs *2*(3), 101.

Ding, T., Yu, Y.Y., Schaffner, D.W., Chen, S.G., Ye, X.Q., Liu, D.H., 2016. Farm to consumption risk assessment for *Staphylococcus aureus* and staphylococcal enterotoxins in fluid milk in China. Food Control 59, 636-643.

Engel, J.B., Heckler, C., Tondo, E.C., Daroit, D.J., da Silva Malheiros, P., 2017. Antimicrobial activity of free and liposome-encapsulated thymol and carvacrol against *Salmonella* and *Staphylococcus aureus* adhered to stainless steel. International Journal of Food Microbiology *252*, 18-23.

Gootz, T.D., 2010. The global problem of antibiotic resistance. Critical Reviews in Immunology, 30(1), 79-93.

Hamadi, F., Asserne, F., Elabed, S., Bensouda, S., Mabrouki, M., Latrache, H., 2014. Adhesion of *Staphylococcus aureus* on stainless steel treated with three types of milk. Food Control 38, 104-108.

Hamadi, F., Latrache, H., Mabrrouki, M., Elchmari, A., Outzourhit, A., Ellouali, M., 2005. Effect of pH on distribution and adhesion of *Staphylococcus aureus* to glass. Journal of Adhesion and Science of Technology 19, 73-85.

Haug, A., Høstmark, A.T., Harstad, O.M., 2007. Bovine milk in human nutrition–a review. Lipids in Health and disease 6(1), 1-16.

International Standart Office, 6888-1-ISO, 2003. Microbiology of food and animal feedingstuffs – Horizontal method for the enumeration of coagulasepositive staphylococci (*Staphylococcus aureus* and other species).

Kandpal, S.D., Srivastava, A.K., Negi, K.S., 2012. Estimation of quality of raw milk (open & branded) by milk adulteration testing kit. Indian Journal of Community Health 24(3), 188-192.

Keyvan, E., Tutun, H., 2019. Effects of Carvacrol on *Staphylococcus aureus* isolated from bulk tank milk. Medycyna Weterynaryjna 75(04), 238-241.

Keyvan, E., Yurdakul, O., Sen, E., 2020. Staphylococcal Enterotoxins and Enterotoxigenic *Staphylococcus aureus* in Raw Milk: A Screening Study. Kocatepe Veteriner Dergisi 13(2), 104-109.

Keyvan, E., Yurdakul, O., Kocasarı, F., Tutun, H., Demirtaş, A., Kahraman, H.A., Sen, E., 2018. Detection of ochratoxin A in bulk tank milk. Kocatepe Veteriner Dergisi, 11(3), 255-259.

Kusumaningrum, H.D., Riboldi, G., Hazeleger, W.C., Beumer, R.R., 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. International Journal of Food Microbiology 85(3), 227-236.

Le Loir, Y., Baron, F., Gautier, M., 2003. *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research 2(1), 63-76.

Marques, S.C., Rezende, J.D.G.O.S., Aparecida de Freitas Alves, L., Silva, B.C., Alves, E., Ronaldo de Abreu, L., 2007. Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surface and its resistance to some selected chemical sanitizers. Brazilian Journal of Microbiology 38, 538-543.

Nathan, C., Cars, O., 2014. Antibiotic resistance--problems, progress, and prospects. New England Journal of Medicine 371 (19), 1761-1763

Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Celano, G. V., 2005. Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. International Journal of Food Microbiology 98(1), 73-79.

Okmen, G., Turkcan, O., Ceylan, O., Gork, G., 2014. The Antimicrobial Activity of *Aliquidambar orientalis* mill. Against Food Pathogens and Antioxidant Capacity of Leaf Extracts. African Journal of Traditional, Complementary and Alternative Medicines 11(5), 28-32.

Oliver, S.P., Jayarao, B.M., Almeida, R.A., 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathogens & Disease 2(2), 115-129.

Oulahal, N., Brice, W., Martial, A., Degraeve, P., 2008. Quantitative analysis of survival of *Staphylococcus aureus* or *Listeria innocua* on two types of surfaces: polypropylene and stainless steel in contact with three different dairy products. Food Control 19, 178-185.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. Emerging Infectious Diseases Journal 19(3), 407-415.

Porter JA, Morey A, Monu EA., 2020. Antimicrobial efficacy of white mustard essential oil and carvacrol against *Salmonella* in refrigerated ground chicken. Poultry Scicence 99 (10): 5091-5095.

Sağdıç, O., Özkan, G., Özcan, M., Özçelik, S., 2005. A Study on inhibitory effects of sığla tree *(Liquidambar orientalis Mill. var. orientalis*) storax against several bacteria. Phytotherapy Research 19(6), 549-551.

Turkoglu, C., Keyvan, E., 2019. Determination of aflatoxin M1 and ochratoxin A in raw, pasteurized and UHT milk in Turkey. Acta Scientiae Veterinariae 47(1), 1626.

Van Houdt, R., Michiels, C.W., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology 109, 1117–1131.

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