BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF THE GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

THE INVESTIGATION OF ANTIMICROBIAL EFFECTS OF SILVER FLAKES, STRAWBERRY TREE AND ROSE WATER COMBINATION

MASTER OF SCIENCE

EKİM BOYRAZ

BOLU, JUNE 2020

APPROVAL OF THE THESIS

THE INVESTIGATION OF ANTIMICROBIAL EFFECTS OF SILVER FLAKES, STRAWBERRY TREE AND ROSE WATER COMBINATION submitted by **Ekim BOYRAZ** and defended before the below named jury in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, The Institute of the Graduate Studies of Bolu Abant Izzet Baysal University in 03.06.2020** by.

Examining Committee Members	**Signature**
Supervisor Prof. Dr. Seyhun YURDUGÜL ABANT İZZET BAYSAL UNIVERSITY	.....
Member Prof. Dr. Arzu TÜRKER ABANT İZZET BAYSAL UNIVERSITY	.....
Member Assoc.Prof Dr. Arzu ÜNAL IĞDIR UNIVERSITY	.....

Prof. Dr. Osman GÖRÜR ……

Director of the Institute of Graduate Studies

To my family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ekim BOYRAZ

ABSTRACT

THE INVESTIGATION OF ANTIMICROBIAL EFFECTS OF SILVER FLAKES, STRAWBERRY TREE and ROSE WATER COMBINATION
MSC THESIS
EKİM BOYRAZ
BOLU ABANT IZZET BAYSAL UNIVERSITY INSTITUTE OF THE GRADUATE STUDIES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. SEYHUN YURDUGÜL)

BOLU, JUNE 2020

An antimicrobial agent is termed as any of a large variety of chemical compound and physical agent, capable of destroying microorganisms or to prevent their development, either designed as natural or synthetic. Antimicrobial agents that are used in the treatment of disease include synthetic and semi-synthetic chemicals as well as chemical substances or metabolic products made by microorganisms and chemical substances derived from plants. In this study silver flakes was mixed with rose water (Eau de rose) and strawberry tree fruit (*Arbutus unedo*) in order to form an antimicrobial combination. The studies were performed on the determination of its antimicrobial properties. The effect of the combination was tested on two Gram positive and two Gram negative pathogenic bacteria, therefore the stability of different pHs and temperatures on the combination was analyzed. It was found to be effective at the lowest pH and temperature values on all bacteria investigated in our study. However, there was no statistically significant effect of the combination on bacteria ($p > 0.05$). This is thought to be due to the fact that bacteria are too resistant.

KEYWORDS: Strawberry tree, Silver flakes, Rose water, Antimicrobial

ÖZET

GÜMÜŞ TOZU, KOCA YEMİŞ VE GÜL SUYU KOMBİNASYONUNUN ANTİMİKROBİYAL ETKİLERİNİN ARAŞTIRILMASI
YÜKSEK LISANS TEZI
EKİM BOYRAZ
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
BIYOLOJI ANABILIM DALI
(TEZ DANIŞMANI: PROF. DR. SEYHUN YURDUGÜL)

BOLU, HAZİRAN - 2020

Antimikrobiyal ajan, mikroorganizmaları yok edebilecek veya gelişimini önleyebilecek, doğal ya da sentetik olarak tasarlanmış herhangi bir büyük çeşit kimyasal bileşik ve fiziksel ajan olarak tanımlanır. Hastalıkların tedavisinde kullanılan antimikrobiyal ajanlar sentetik ve yarı sentetik kimyasalların yanı sıra mikroorganizmalar tarafından yapılan kimyasal maddeleri veya metabolik ürünleri ve bitkilerden türevlenen kimyasal maddeleri içermektedir. Bu çalışmada yeni bir antimikrobiyal kombinasyon (bileşim) oluşturmak için gümüş tozları, gül suyu ve kocayemiş meyvesi karıştırılmıştır. Mikrobiyolojik etkilerin belirlenmesi üzerine çalışmalar gerçekleştirilmiş olup bu etkiler farklı iki Gram negatif ve iki Gram pozitif patojen bakteri üzerinde test edilmiş, bununla birlikte çeşitli pH ve sıcaklık derecelerinin inhibitörün kararlılığı üzerine etkisi saptanmıştır. Fakat kombinasyonun bakteriler üzerinde istatistiki olarak bir etkisi gözlenmemiştir ($p>0.05$). Bunun nedeni bakterilerin çok fazla dirençli olmasından dolayı kaynaklandığı düşünülmektedir.

ANAHTAR KELİMELER: Koca yemiş, Gül suyu, Gümüş tozları, Antimikrobiyal

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS AND SYMBOLS

(A/H5N1)	: Highly Pathogenic Avian Influenza A (H5N1)
ATCC	: American Type Culture Collection
ATP	: Adenosine 3'- triphosphate
BM4454	: *Acinetobacter baumannii* strain BM4454
CFU	: Colony-Forming Units
CO_2	: Carbondioxide
C.violaceum	: *Chromobacterium violaceum*
cm	: Centimeter
E.coli	: Escherichia coli
EU	: European Union
g	: Gram
Gram(+)	: Gram positive
Gram(-)	: Gram negative
HCL	: Hydrochloric acid
H_2O_2	: Hydrogen peroxide
kHz	: kilohertz
K.pneumoniae	: *Klebsiella* pneumoniae
L. monocytogenes	: *Listeria monocytogenes*
m	: Meter
mg	: Milligram
mL	: Milliliter
MRSA	: Methicillin-Resistant Staphylococcus aureus
NaOH	: Sodium hydroxide
N	: Normality
°C	: Centigrade
pH	: Power of Hydrogen
µL	: Microliter
S. aureus	: *Staphylococcus aureus*
S.epidermidis	: *Staphylococcus epidermidis*
spp.	: Species pluralis
TMAB	: Total Mesophilic Aerobic Bacteria
XLD agar	: Xylose Lysine Deoxycholate Agar
Vol/vol	:Volume/volume
W/m^{-2}	:Watt per meter square
%	: Percent

ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to his supervisor Prof. Dr. Seyhun YURDUGÜL for their guidance, advice, criticism, encouragements and insight throughout the research.

The author would also like to thank Prof. Dr. Hakan TÜRKER and Res. Assist. Şeyda KARABÖRK for their suggestions and comments.

Finally, I am grateful to my family and my husband for their encouragement, support and love during my life; especially to my father, Talip MOR, to my mother, Hayriye MOR, to my brother, Murat MOR who provides financial and moral support and my sister, Ümit YALÇIN, who allows studying me at this university and to my husband, İbrahim BOYRAZ who adds meaning to my life and encouraging me and never missing his love, helped me in all conditions.

1. INTRODUCTION

An antimicrobial is defined as a substance that inhibits the growth of or kills microorganisms. These substances may be not only in a form of a chemical compound, but also it can exist as a physical agent. They are capable of interacting with the growth and reproduction of a wide range of organisms such as bacteria, parasites, viruses and molds etc. (El Fane et al., 2017).

These agents are able to show their inhibitory or lethal effect even at low concentrations by suppressing or inhibiting the growth of microorganisms. They are mainly divided into the antibiotics (natural substances produced by yeast, molds, and other microorganisms and the semi-synthetic group, formed via modification of the natural substance produced by microbial means) and the chemotherapeutics (those are chemically synthesized agents). In addition, some elements existed in nature, such as minerals like copper, silver, bromine etc. show antibacterial properties when present in high concentrations. These agents find their use in many fields such as veterinary, medicine, home appliances etc. For example, antimicrobials have been mainly used in feed additives for swine since 1950s. The promotion of growth, improvement of feed usage, decreasing death rate, and improving the reproduction capacity in these animals were aimed even at low (subtherapeutic) levels, reaching an approximate amount of 88 % in total (IOM 1988). In addition, at moderate- to –high (therapeutic) levels for the disease management in swine was reported (Miller et al., 1991).

When dated back, infectious diseases comprise a very great proportion of human diseases as a whole. It was not until the last half of the 19th century that microorganisms were regarded as the causative agents of the infectious diseases that had been threatening human life from the past. In accordance with the causative organisms, the chemotherapy was aimed as the primary therapeutic strategy. Salvarsan was reported as the first antimicrobial substance in the world, a cure for syphilis that was discovered by Ehrlich in 1910. Sulfonamides followed this discovery, which were of text are acceptable only on non-textual pages, such as those

presenting tables and illustrations. developed by Domagk's team in 1935. These agents were synthetic and their safety and efficacy were limited (Saga and Yamaguchi, 2009).

Fleming's discovery of penicillin in 1928 was considered to be a milestone. He found that a clear zone was formed around a blue mold culture (a fungus from the Penicillium genus) surrounded by *Staphylococcus aureus* colonies, and thought that a microorganism can be able to produce agents that could also inhibit the growth of another microorganism. After then, this antibiotic was called penicillin, and it was used in clinical field in the 1940s, which would then become as one of the most important antibiotics in the world, together with its safe and efficient use initiated the antimicrobial chemotherapy period by decreasing the mortality rate of a great number of wounded army members during World War II.

Following the next twenty years, novel antimicrobial drugs were introduced one after another, including streptomycin's discovery in 1944, an aminoglycoside antibiotic, isolated from *Streptomyces griseus*. Moreover, chloramphenicol, tetracyclines, macrolides, and glycopeptides (such as vancomycin) were isolated from soil bacteria and launched into the pharmaceutical market. A synthetic antimicrobial substance, nalidixic acid, a quinolone, was discovered in 1962. Research and development studies have gained importance in improving each class of antimicrobial drugs leading to a broader antimicrobial spectrum and greater antimicrobial ß-lactams can be a good example including the penicillins, cephems, carbapenems, and monobactams (Saga and Yamaguchı, 2009).

Most of the drugs that target bacteria show cationic property and disrupt the anionic lipid part of the bacterial membrane. Since different bacteria have different lipid components in the cell membrane, those bacteria bearing anionic and zwitterionic or neutral lipids can be able to form domains in the existence of antimicrobial peptides possessing several cationic charges. The disruption of anionic and zwitterionic lipids into domains can eventually lead to the growth inhibition or death. Such agents mainly show their toxicity on Gram-negative bacteria, than on the gram-positive. That's why the lipid composition of bacterial membranes is regarded as one of the crucial cell characteristics in determining the effect of some

antimicrobial drugs against the susceptible microorganisms (Richard and Raquel, 2010).

1.1 Silver

Human beings were intensively effected by the re-emerging and emerging infections in last years, from the health and economy point of view. This is mainly related with the poor hygienic and sanitary measures, inadequate access to clean water and the uncontrollable increase of population; leading to the increase in the number of pathogenic microorganisms. The pathogenic microorganisms may easily spread between individuals and cause epidemics of diseases such as influenza (A/H5N1), diarrhea (Escherichia coli), typhoid fewer (Salmonella typhi, Salmonella paratyphi) etc. worldwide. The thorough application of surfaces containing pathogenic microorganisms by intensive disinfectant nanomaterials has been proposed for elimination of the epidemics. A typical example is the silver nanoparticles (Ag-NPs) with unique properties of intensive antimicrobial action, have gained much interest from the scientific and technologic communities to develop nanosilver-based disinfectant products (Tran et al., 2013).

The helpful characteristics of silver in medicine have been known for over two thousand years. Silver ions have been preferred in many antimicrobial treatments since the nineteenth century and considered in the nanoparticles concept. Nanoparticles have been known to be used for various pharmaceutical, biological and physical applications. Silver nanoparticles are preferred as antibacterial substances in various public places such as railway stations, elevators, the texture of garments such as antimicrobial cotton fabrics (Xu et al., 2017) around the world, and it is regarded as powerful against pathogen microorganisms. It is reported that silver ions, as well as silver-originated substances, are intensively toxic to bacteria including sixteen important bacterial species. These properties of silver enable it an excellent choice for different tasks in the medical applications. The nitrate form of silver is mainly used to trigger antibacterial action, but when silver nanoparticles are preferred, there is a huge increase in the treated areas suitable for the growth of microorganisms. Though silver nanoparticles are preferred in many antimicrobial

applications, the action of this metal on microorganisms is still not yet determined. Silver nanoparticles show their action on microorganisms either by cell lysis or inhibiting the cell transduction. Different mechanisms are proposed in the cell lysis and growth inhibition of silver (Prabhu and Poulose, 2012). Since one of the main nanomaterial preferred in various industries is silver, its easy incorporation into other substances or its use for coating allows silver an available element for medical purposes by its antibacterial and disinfection properties. Nanosilver is used in the production of dressings, surgical instruments or implants. It is also applicable in environmental engineering, for example, water treatment and purification processes. It finds its application in the production of textiles, underwear, and cosmetics (Chojniak et al., 2016). Silver ions are used in different areas including the formulation of dental resin composites; coatings of medical devices; bactericidal coatings in water filters; antimicrobial agent in air sanitizer sprays, soaps, wet wipes, pillows, socks, respirators, detergents, shampoos, toothpaste, washing machines, and many other consumer products; as bone cement; and in many wound dressings to name a few. Though there are various benefits of silver nanoparticles, there is also the problem of nanotoxicity of silver. There are various studies that suggest that the nanoparticles can lead to different environmental and health problems due to their possible toxicity, although there is a requirement for more work to be conducted to end up that there is a real problem with silver nanoparticles. Silver nanoparticles can penetrate into the microbial cell by attaching to the cell wall and subsequently destroy it, then cause structural changes in the membrane like the changing of the permeability and death of the cell. The occurrence of 'pits' and the accumulation of the nanoparticles form at the cell surface. Based on the electron spin resonance spectroscopy studies, bacterial death can be intensively related by the free radical formation of silver nanoparticles. They occur through contact with the bacteria, subsequently damaging the cell membrane through opening pores and result in death. The thiol groups of many essential enzymes are inactivated by the releasing of silver ions interacting with them. When the silver ions are accepted into the cytoplasm, it disrupts several functions and give harm to the cell. Afterwards, the silver ions help generation of the reactive oxygen species through inhibiting a respiratory enzyme and the cell is targeted. Silver displays a soft acidic character, and naturally it can easily react with a soft base, as since the cells which are mainly composed of sulfur and phosphorus bearing a soft base character. The action of these nanoparticles on

the cell can react with these molecules and eventually lead to cell death. The other mechanism of these nanoparticles is based on the disruption of the soft bases of DNA containing sulfur and phosphorus and resulting of cell death. When silver nanoparticles react with the sulphur and phosphorus, the DNA replication of the microorganisms is negatively affected and thus inhibit the bacteria (Prabhu and Poulose, 2012).

Even though the antimicrobial and anti-inflammatory characteristics allow silver nanoparticles as perfect candidates for various applications in the medical field, it was found that they can cause adverse effects not only on humans as well as the environment. The environmental pollution of silver is based on the tons of industrial waste disposal, and the free silver ions in the aqueous phase is the main reason. These free silver ions on human beings and other organisms can lead to permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and hepatotoxicity, nephrotoxicity; irritations of the eye, skin, respiratory and intestinal tract; and problems in blood cells. Nanosilver has been widely studied from the end of twentieth century. The effect of nanosilver on the different strains of microorganisms is found to be different and non-pathogenic microorganisms are also inhibited by these particles. The toxicity of nanosilver is only evaluated in very few studies. In vitro studies consisted up of rat liver cells indicated that silver nanoparticles in low concentration caused mitochondrial disruption by generation of oxidative stress and impaired mitochondrial function. In addition, mouse germline stem cells were negatively affected by these nanoparticles, showing toxicity on lowering the function of mitochondria and releasing the cytoplasmic content from the cell membranes. The toxicity of these particles is found to be stronger and effective than asbestos. Silver ions can alter the permeability of the cell membrane to potassium and sodium ions at concentrations that do not even block sodium, potassium, ATP, or mitochondrial activity. Nanosilver particles can cause toxic effects on the proliferation and cytokine expression by peripheral blood mononuclear cells. The male reproductive organs are negatively affected by the silver ions, since they can cross the blood-testes barrier and accumulated in the testes and the sperm cells are destroyed. Experiments showed that commercially available silver-based dressings displayed cytotoxic effects on different organisms. Rat liver is found to be the organ that is mostly effected by the intake of these particles. Histopathological

studies showed that bile duct hyperplasia with or without necrosis, fibrosis and pigmentation was observed in animal trials. If the nanoparticles stay inside the tissue that was accumulated, it can release after some time, thus it indicates its toxicity in more severe form when aged. Up to now, the experiments are mostly carried out in vitro, so in order to evaluate high concentrations of nanosilver particles, it was proposed that more research should be done to understand in vivo toxicity effects of these nanoparticles (Prabhu and Poulose, 2012).

On the other hand some researchers include silver into environment friendly substances which display bactericidal characteristics, thus nowadays, for this reason it has been studied for disinfection of water. The investigation of antimicrobial characteristics of silver was initially carried out by Gibbard (1937). The ionic, colloidal, metal or nanoparticle form of silver ions have been studied to eliminate the pathogenic microorganisms (Yoshida et al., 1999; Furno et al., 2004; Lee et al., 2006). Also food packaging is another field of application(Quintavalla and Vicini, 2002; Tankhiwale and Bajpai, 2009) and in water treatment (Bandyopadhyaya et al., 2008; Maioli et al., 2009). Not only silver ions but also all heavy metals inactivate the proteins by interacting with thiols on the bacterial membrane and subsequently end up with death (Lehninger et al., 1993; Liau et al., 1997; McDonnell and Russell, 1999).

Small particles of silver ions were found to display strong antibacterial properties, since they have an increased specific surface area (Yoon et al.,2007). Nanotechnology helps emerging antimicrobial action of silver during its development. These particles were found to be size and shape dependent against pathogenic microorganisms. Shortened triangle-shaped silver particles were shown to have a strong antimicrobial activity, when its similarity assessment is done with spherical and rod-shaped form (Pal et al.,2007), and silver ions with a diameter of one to ten nanometer can easily attach onto the cell membrane and its subsequent penetration through the bacteria give harm to the DNA (Morones et al., 2005).

The remnants of silver in the industry was also said to be a problem from the environmental point of view. Due to this reason, thin metallic silver flakes were screened out for their antimicrobial characteristics in aqueous phase. The flakes used in that study were provided from a company producing silver films, as waste

materials. By this way the cost of silver will be kept low in screening assays. Their easy application can allow them to be involved in filters, devices or formulations without using any supporting material. This is the first instance that this type of silver is used as antimicrobial agent (Anzano et al., 2011).

1.1.1.1 *Arbutus unedo* (Strawberry Tree)

The biological substances display a wide variety in plant kingdom and most of them are known to have antimicrobial characteristics to fight with pathogenic microorganisms (Palombo & Semple, 2001). Aromatic compounds are one important group among these substances and show biological activity (Cowan, 1999). The importance of these compounds increased the potential and demand of the extraction of them from plants to obtain novel medicinal agents (Ferreirab et al., 2012).

Strawberry tree (*Arbutus unedo* L.) is a typical example to these kind of plants; its leaves and fruits are used in folk medicine for treating inflammation, hypertension, and diabetes (Jurica et al., 2017). It contains many different antioxidative compounds and display antimicrobial property (Ülgen, 2013). Other than medicine, in food production and nutritional sciences, the bioactive compounds not only contain antioxidative activity, but also the reactive oxygen species and halogenated hydrocarbons generating free radicals can be neutralized by these biological molecules, avoiding serious pathological disorders (Gupta et al., 1992).

Arbutus unedo L., the strawberry tree, is a member of Ericaceae family and an evergreen shrub (Figure 1.1), widely present in Mediterranean basin. Mediterranean countries are the main homeland of this fruit and growth is reported in Portugal, Spain, France, Italy, Albania, Greece, Bosnia and Herzegovina, Croatia, Macedonia, Montenegro, Serbia, Slovenia, Algeria, including some islands (Balear, Corsica, Sardinia, Sicily and Crete), mainly in coastal and inland areas where climate is available for its growth. Interestingly, the south west of Ireland was also reported to be a habitat of this tree. Even though it can rarely reach a height of twelve meters, its normal height ranges between an interval of one-half to three meters. When ripened, its fruits are notable, globular-shaped, slightly red, and they can grow up to two centimeters in diameter. Its flower is made up of little cream-colored lanterns.

The strawberry tree fruit mature in two different periods in a year, at first starting in the midst of October and ending at the first days of December, then the second is around the beginning of January. Its leaves show alternate and simple characteristic with oblanceolate form. It has a dark green color, leathery, short-stalked and toothed. It is characterized as an ornamental, ecological, economic, therapeutic and medicinal plant. This plant flowers during the winter with a pinkish-white color. The strawberry tree has numerous benefits to nature, regarded as ecologically important. These benefits include the maintenance of animal diversity, preventing soil erosion, helping the reestablishment of the forest flora after fire, able to grow in poor soil texture and it is helpful in phytoremediation, especially preferred in the arsenic-related contamination of soil.. The strawberry tree fruits are edible and used to make jam, marmalade and liquor. Strawberry-tree honey gains attention by strong and distinctly bitter taste and has also been investigated in recent years. This honey is also believed to contain organic acids, phenolics, together with physicochemical and melissopalynological characteristics. These products imply that the strawberry tree fruits are economically important and rural people benefit from this shrub. The use of different components of strawberry tree was reported in traditional medicine. Fruits are preferred in folk medicine since they display antiseptic, diuretic and laxative property. In addition, the strawberry tree leaf is consumed as an infusion for its astringent, diuretic, urinary antiseptic, antidiarrheal and depurative characteristics. Moreover, the leaves are used as a curative agent in hypertension, diabetes and inflammatory disorders. The roots and bark of strawberry tree is considered as valuable in folk medicine like gastrointestinal, urological and dermatological disorders; via decoction of the roots the “drug” is ready to drink. Other than these findings, the strawberry tree is not yet to be exactly discovered, extremely due to the high heterogenic flora. The content of biologically active substances present in the different parts of this tree is related with its medicinal and therapeutical performance. The composition of fruits, bark and roots, as well as its biological activity and positive healthcare effects of extracts of the strawberry tree was characterized by Alarcão-e-Silva and coworkers in 2001 (Oliveira et al., 2011).

According to Rosato and coworkers (2001), an aqueous extract of strawberry tree leaves displayed antihypertensive and vasorelaxant characteristics. Another study, carried out by in vitro assays showed that diethylether and ethyl acetate

extracts of strawberry leaves displayed an antiaggregating property on human thrombocytes through antioxidative activity (Bouyahya et al., 2016).

Figure 1.1. *Arbutus unedo* and its fruits.

Intensive research has been carried on strawberry tree fruits, especially on essential oils with particular attention to characterize their ingredients (Alarçao-e-Silva et al., 2001, Kivack et al., 2001, Barros et al., 2010). Other studies are focused on to find out its nutritional value, antioxidative and antimicrobial properties (Pallauf et al., 2008, Fortalezas et al., 2010, Benzeggouta,2005, Dib et al., 2010). The inhibition of strawberry tree fruit and its essential oils on pathogenic Gram-negative strains; *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) was reported by Alarçao-e-Silva and coworkers in 2001. Currently, no study was devoted to the antimicrobial effect of *Arbutus unedo* L. fruit juice. All studies highlighted the antimicrobial effects of roots and leaves only. However, studies on antibacterial effect have rarely been reported on its essential oils (Koula and Souhila, 2014).

1.1.1.1.1 Rose Water

Plants have been widely consumed as a curative agent in many disorders for centuries and up to date, they display a crucial function to cover the basic health requirements in developing countries. A blend of plant materials and phytochemicals have been observed to display powerful antibacterial effect. Rose is a perennial plant belonging to the genus Rosa, within the family Rosaceae. Rose has got significant

effect on civilizations in artistic, economical, clinical, scientifical, psychological and religious point of view because of its fragrance (Bahl et al.,2016).

The distillation of its petals with steam lead to the production of rose water. Rose water has an attractive fragrance, and alternatively used instead of chemical containing perfumes (https://www.healthline.com/health/rose-water-benefits /Rose Water: Benefits and Uses – Healthline, 25 October 2017). It has been in use since the period of Middle Ages. Its origin was considered to be Iranian and its use is not only limited to the field of cosmetics but also in various food and beverages. Many health benefits were reported for rose water (www.dailymail.co.uk/.../Rosewater-benefits-make-home.htm/Rosewater benefits and how to make it at home | Daily Mail Online, 25 October 2017). Its synonym is rose syrup, composed of the hydrosol part of rose petal distillate, a side product of its oil added to perfumes. Another potential use finds its way as food flavoring, since some components are thought to have beneficial properties (www.healthguidance org › Fitness Wellness › Beauty Rose Water Benefits - Health Guidance, 05 November 2017).

Rose water is believed to stop and cure infectious disorders by its antiseptic characteristics. Due to this property, rose water is frequently preferred in various natural and medical treatments. A common eye disorder, conjunctivitis was found to be treated by rose water, in eye drop form depending on its antiseptic and analgesic characteristics, reported by a case study (www.dailymail.co.uk/.../Rosewater-benefits-make-home.htm/Rosewater benefits and how to make it at home | Daily Mail Online, 25 October 2017).

Rose petals and oil has a variety of strong antioxidative agents and these substances help protect cellular damage by inhibiting the lipid peroxidation activity (www.dailymail.co.uk/.../Rosewater-benefits-make-home.htm/Rosewater benefits and how to make it at home | Daily Mail Online, 25 October 2017). Too many rose species are reported to be present worldwide, but a few has the desired fragrance that is required by the cosmetics industry. *Rosa damascena* Mill is a typical example to these species, having a highly qualified aromatic oil, preferred by pharmacy and cosmetics industry. Turkey is the leading country for the production of *Rosa damascena* in the world by the growth of an approximate of ten thousand tonnes of roses in order to produce essential oil (Özkan et al., 2004).

The fresh roses which are steam distilled, are exposed to extract their essential oil, the consumed flower portion of the stills was either remediated or dried to form compost. Therefore, a great amount of distilled flower residues is partially used, so in last years, a thoughtful attraction has appeared on the possible utilization of these kind of plants, containing essential oils presenting antioxidative and antibacterial properties in the food industry. Most of the spices and their derivatives have been extensively investigated against many microorganisms but the antioxidative effect of herbal and spice extracts are not so frequently investigated. The main rose water essential oil parts such as citronellol, geraniol and nerol showed powerful antimicrobial effect against some bacteria. The potential antimicrobial and antioxidative characteristics of the fresh and used flower extracts of *Rosa damascena* was investigated too (Arıdoğan et al. 2002; Achuthan et al. 2003; Özkan et al., 2004).

As both an eyewashing agent and a moisturizer, rose water displays its antiseptic characteristics. By using the bioactive substances, antimicrobial and antioxidative action of rose water is maintained, attributing to the presence of flavonoids and the phenolics (Bahl et al., 2016). In addition, roses are greatly appreciated when the inimitable aroma, extensive uses, and their beauty are considered. The different damask rose products such as oil, water, concrete, and gulkand, its dried petals are also produced for protective healthcare (Verma et al. 2011). Roses have solid (stearoptene) and liquid (oleoptene) components, as well as they contain high amount of corilagin and tellimagrandin (Shiota et al. 2004). In general rose water showed antimicrobial activity in fruit juices too (Yurdugul et al., 2016).

1.1.1.1.2 The Pathogenic Bacteria

Many pathogenic bacteria are present in different environment and they can display their pathogenicity when they find their way, such as weak immune system, unclean water, unhygienic conditions etc. in living organisms. Some of these pathogens are frequently present in poor hygienic conditions such as *Listeria* spp., *Salmonella* spp., *Escherichia coli*, *Staphylococcus* spp. etc. *Listeria monocytogenes* is a typical example to these pathogens, displaying a short, gram positive, non

sporeformer rod characteristic. It is catalase positive and found to be motile at 22–28°C but not at 37°C, by a tumbling end-over-end characteristic. By testing its motility, *Listeria* spp. is easily differentiated from diphtheroid bacteria, an inhabitant of normal skin flora. *L. monocytogenes* can lead to severe disorders, both in animals and human; capable of growth and survival over a wide range of environmental conditions. Moreover, *L. monocytogenes* shows facultative anaerobe and esculin hydrolysis positive characteristic (Ryan KJ and Ray, 2004).

Listeriosis is the main disease caused by this bacteria and especially newborns, the elder, patients with impaired immune system and women who has pregnancy are mainly affected, as well as healthy individuals may suffer from this disorder. The symptoms of invasive listeriosis are listed as abortion in pregnancy, septic infection and meningoencephalitis. Moreover, it can show febrile gastroenteritis. Most species of vertebrates are reported to be affected by this bacteria (Vazquez-Boland et al. 2001)

S. aureus is a gram positive bacteria, sized in regular cocci, linked together in clusters such as a chain. In some conditions such as an aged culture, a resolved lesion, and if there is an antibiotic, the cellular structure is mostly reported to be varied in size, and interestingly most cultures are no longer to be a gram positive bacteria. *S. aureus* contains teichoic acid, showing antigenic and relatively specific for this bacteria such as a regular gram positive peptidoglycan including a ribitol.

Staphylococci is reported to infect healthy tissues in many ways, causing skin lesions (boils, styes) and localized abscesses in other sites. *S aureus* can also cause important problems in bone and heart, such as osteomyelitis and endocarditis and a kind of severe skin infection, termed as furunculosis. The main cause of hospital related (nosocomial) infections after surgery is *S epidermidis*, also reported to cause infectious problems related to indwelling medical devices. Many serious food poisoning cases are mainly associated with *S aureus* by its enterotoxin releasing mechanism to food. Toxic shock is another problem associated with *S aureus* by the delivery of superantigens into blood. Another important species *S. saprophiticus* is the causative agent of infectious urinary tract problems, during puberty in girls. Other staphylococci (*S. lugdunensis, S. haemolyticus, S. warneri, S. schleiferi, S. intermedius*) are rarely seen organisms regarding to the others above (Baron,1996).

Acinetobacter baumannii is a gram negative, non-motile, obligate aerobic coccobacillus having effective virulence factors, including its attachment to and resisting on solid and dry places, its effort to supply vital elements like iron, the attachment to and sequential epithelial cell destruction, and the productivity of gelatinases and proteinases that can give harm to related tissues in some strains. *A. baumannii* can be able to live in the skin of patients or even healthy people without causing any disease. *A. baumannii* has commonly defined as an important nosocomial pathogen, being easily resistant to common antibiotics abundant in the market (Ryan KJ and Ray, 2004, Boucher and Talbot et al.2009).

Its epidemiology is mainly related with being a hospital pathogen as since it can survive in the hospital environment, through developing resistance to multiple antimicrobial agents synchronously (Villegas, 1977-2000). The epidemiology of *A. baumannii* is not easy to find out as sporadic and epidemic clones, as well as different sites such as environmental and patient-based, may be present, struggling against efficient infection control tactics (Rodriguez and Bonomo,2008).

Klebsiella pneumoniae is one of the most prevalent gram negative bacteria encountered by physicians in the world. Likewise *Staphylococcus* and *Acinetobacter*, it is a widespread nosocomially acquired microorganism, leading to infectious problems of urinary tract, hospital acquired pneumonia, and intraabdominal infections. *K. pneumoniae* is also reported to be a potential society acquired bacteria. An internationally collaborated research showed the geographic differences and tendencies of society acquired *Klebsiella* infection, indeed *K. pneumoniae* has been reported as a bacteria which can cause pulmonary infections (Ryan and Ray, 2004).

2. AIM AND SCOPE OF THE STUDY

The aim of our study is to observe the effect of the combination of the silver flakes, strawberry tree and rose water to kill or inhibit two gram positive and two gram negative pathogenic bacteria. Subsequently following the preparation of the combination, its antimicrobial characteristic was investigated. The combined agent might be potentially available to be used in the structure of a cleaning agent in future as well, depending upon its possible antimicrobial activity.

3. MATERIALS AND METHOD

3.1 Materials

3.1.1 Silver Flakes

Silver flakes were obtained from a local vendor in Bolu, TURKEY.

3.1.2 Rose Water

Rose water (Eau de rose) was purchased from Gülbirlik A.Ş., Isparta, TURKEY.

3.1.3 Strawberry Tree (*Arbutus Unedo* L.)

Arbutus unedo L. (Strawberry tree) fruits were harvested from the environs of Akçakoca, Düzce, and Karadeniz Ereğli, Zonguldak, TURKEY.

3.1.4 The Bacteria

Two reference bacteria, tested in this study were recruited from the microbiology laboratory of the hospital of Abant Izzet Baysal University, Faculty of Medicine, Department of Microbiology, Bolu, TURKEY. They were listed as follows: *A. baumannii* and *K. pneumoniae*. *L. monocytogenes* and *S.aureus* were provided as a generous gift from Food Engineering Department, Faculty of Engineering of Abant Izzet BAYSAL University. All necesary standard biosafety procedure was maintained while working in a laminar hood (Nüve, MN 090, Ankara, Turkey) by sterile masks and gloves.

3.2 Method

3.2.1 The Freeze-Drying Process of The Strawberry Tree Fruits

Strawberry tree fruits, kept frozen at -80° C was lyophilized by a freeze-drier (Christ, Germany) with a temperature of -57^{o} C for 24 hours.

3.2.2 The Determination of The Best Effective Dose

The dose determination was carried out by Kirby-Bauer disc diffusion method. For this purpose, 5, 10, 20 and 50 µL/mL of the rose water (vol/vol) was used against the microorganisms mentioned in Section 3.1.4. according to Kırmusaoğlu, 2010; with modified amounts. From 67 mg/mL stock solution of the rehydrated strawberry tree fruit, 10, 20, 30 and 40 µL was tested on the reference bacteria and the other species according to Orak et al.(2011); with modified amounts. The silver flakes was tested as 10, 20, 30 and 40 mg/mL, on the reference organisms and the other bacteria according to Anzano et al.(2011); with slightly modified amounts.

3.2.3 Agar Disc Diffusion (Kirby-Bauer) Method

All bacteria were first inoculated into 10 mL Mueller-Hinton broth (MHB) containing test tubes by 1-2 sterile loops and adjusted to 0.5 McFarland (approximately 1.5×10^{8} cfu/mL) by a McFarland densitometer (Biosan, Model Den-1, Latvia). From this stock, individually, a sterile cotton swab was dipped into each of the standardized bacteria and it was used to streak the entire surface of the plates containing Mueller-Hinton agar (MHA). Sterile filter paper discs (Glass Microfiber filters, Whatman, 6 *mm* in diameter) were treated with the appropriate amounts of rose water, rehydrated strawberry tree fruit powder and silver flakes reported in Section 3.2.2, individually. These disc containing inoculated plates were incubated at 37º C for a period of 24-48 hours and the diameters (*mm*) of the inhibition zones were measured after incubation (Bauer et al., 1966).

3.2.4 Testing The Antimicrobial Activity of The Combination

The antimicrobial activity of the combination against the strains *A. baumannii*, *K. pneumoniae*, *L.monocytogenes* and *S. aureus* were tested in this assay.

For this purpose, individually, all bacteria were first cultured in a Mueller-Hinton Broth (MHB) (Merck, Germany). The reference bacteria were adjusted to a starting population of approximately 1.5×10^{8} cfu/mL concentration (0.5 Mc Farland). Afterwards, for the preparation of 10 mL bacterial suspension, 1 mL bacteria were transferred to 9 mL sterile distilled water in control and homogenized by thorough vortexing, in combination, 1 mL bacteria was thoroughly homogenized with 1 mL antimicrobial combination to obtain $1.5x10^{-7}$ cfu/mL concentration(Table 3.2.1) and 8 mL sterile distilled water containing test tubes and serial dilutions were completed to 10^{-5}. From the dilutions of 10^{-3}, 10^{-4} and 10^{-5}, 0.1 mL was spreaded onto plate count agar (PCA) (Merck, Germany) and incubated at 37° C for 24-48 hours to reach the final bacterial concentration of $1.5x10^{-6}$ cfu/mL. The composition of the combination medium and the final concentration of the ingredients during the inhibition tests was tabulated in Table 3.2.1, and 3.2.2 respectively. Susceptibility tests were made according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2007).

Table 3.1. The amount of bacteria in test tubes.

Final bacterial concentration (cfu/mL)	Bacteria	Gram staining	Amount of combination (mL)	Amount of sterile distilled water (mL)
$1.5x10^{-6}$	*A. baumannii*	G(-)	1	8
$1.5x10^{-6}$	*K. pneumoniae*	G(-)	1	8
$1.5x10^{-6}$	*L.monocytogenes*	G(+)	1	8
$1.5x10^{-6}$	*S. aureus*	G(+)	1	8
	Control			
$1.5x10^{-6}$	*A. baumannii*	G(-)	-	9
$1.5x10^{-6}$	*K. pneumoniae*	G(-)	-	9
$1.5x10^{-6}$	*L.monocytogenes*	G(+)	-	9
$1.5x10^{-6}$	*S. aureus*	G(+)	-	9

3.2.5 The Composition of The Combination

Two different treatment groups, including the control and the combination were prepared according to the formula tabulated in Table 3.2.2. The main

components of the combination are freeze-dried strawberry tree fruits in freeze-dried form, rose water and silver flakes, adjusted to their final concentrations of 6.7 g/100 mL, 5 mL/100 mL and 40 mg/mL against all of the bacteria used in this study respectively and ste

Table 3.2. The main composition of the combination and the control groups.rile distilled water were served as control.

Control	Combination Group
Sterile distilled water (100 mL)	6.7 g/100 mL rehydrated strawberry tree fruits powder 5mL/100 mL rose water 40 mg/ mL (4 g/100 mL) silver flakes. Completed to 100 mL sterile distilled water.

3.2.6 The Effect of pH on The Combination

The combination was subjected to pH stability assay by adjusting to a pH interval of 2-6 via 0.1 N HCl (pH=2.0) (Merck) and 7-10 via 0.1 N NaOH (pH=13.5) (Merck) in 100 mL containing beakers. The activity of the combination was measured by transferring an aliquot of 40 µL onto sterile filter paper discs placed on lawn cultures of 0.1 mL of the bacteria mentioned in Section 3.1.4, with a final concentration of 1.5×10^{-7} cfu/mL, swabbed onto Mueller-Hinton agar(Merck) containing plates, incubated at 24-48 hours at 37°C.

3.2.7 The Effect of Temperature on The Combination

The combination was subjected to temperature stability assay in 10 mL containing test tubes at 4°, 25°, 37° and 100° C for a period of 30 minutes. Subsequently, the activity of the combination was tested by transferring an aliquot of 40 µL onto sterile filter paper discs placed on lawn cultures of 0.1 mL of the bacteria mentioned in Section 3.1.4, with a final concentration of 1.5×10^{-7} cfu/mL, swabbed onto Mueller-Hinton agar(Merck) containing plates, incubated at 24-48 hours at 37°C.

3.2.8 The Statistical Analysis

Analysis of variance (ANOVA) and Duncan's Multiple Range Tests using SPSS version 15 (SPSS Inc., Chicago, II, USA) were performed for data analysis. The significant level was set to 0.05.

4. RESULT AND DISCUSSION

4.1 The Individual Effect of The Components of The Combination

4.1.1 The Effect of Silver Flakes

When the effect of the silver flakes was independently evaluted on *S. aureus, K. pneumoniae, A. Baumannii* and *L. monocytogenes* by using Kirby-Baurer disc diffusion method the silver flakes was found to be effective on all species mentioned above (Figure 4.1, 4.2, 4.3 and 4.4.)

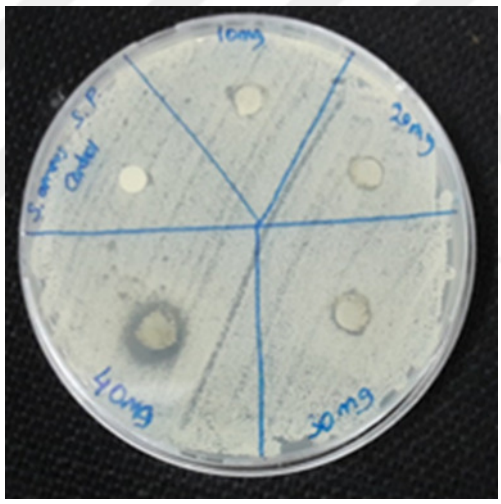

Figure 4.1. The effect of silver flakes against *S. aureus* tested by Kirby-Bauer disc diffusion method.

The action of silver nanoparticles on pathogenic strains isolated from wounds and burns, including methicillin-resistant and sensitive *Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumannii*, a multi-drug-efflux-positive *Acinetobacter baumannii* (BM4454), *Staphylococcus aureus* (MRSA), *Enterobacter cloacae, Proteus vulgaris, Escherichia coli* were studied as growing them in broth cultures by the research group of Ip et al. (2006). The fastness and scope of elimination of these pathogenic strains under in vitro conditions were analyzed. One of the silver-containing dressings showed a high extent of bactericidal activity not only against the Gram-positive but also negative bacteria, indicating a very quick

bactericidal effect and reached a decrease of ≥10 000 cfu/mL after thirty minutes for *Acinetobacter baumanii, Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (Ip et al., 2006).

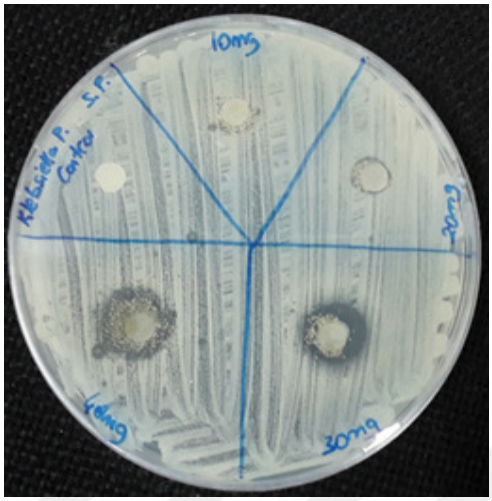

Figure 4.2. The effect of silver flakes against *K. pneumoniae*, tested by Kirby-Bauer disc diffusion method.

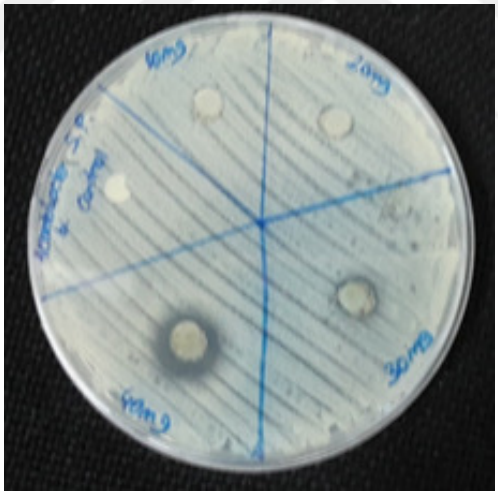

Figure 4.3. The effect of silver flakes against *A. baumannii*, tested by Kirby-Bauer disc diffusion method.

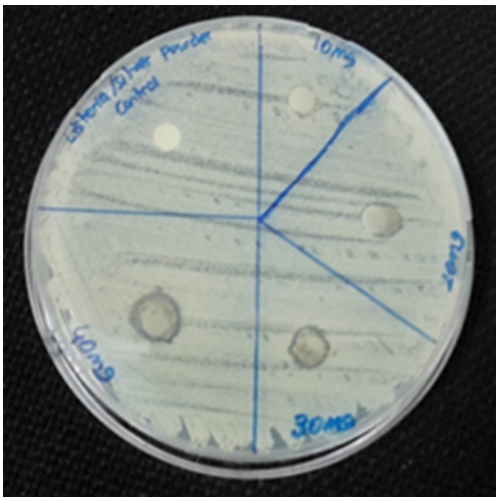

Figure 4.4. The effect of silver flakes against *L. monocytogenes*, tested by Kirby-Bauer disc diffusion method.

Table 4.1. The mean and standard deviation results of the diameters of inhibition of zones of silver flakes against different bacteria to determine the best effective dose of antimicrobial combination.

	Mean± SD*			
Concentration	***S. aureus***	***L. monocytogenes***	***K. pneumoniae***	***A.baumannii***
10 mg/mL	7.33±0.57[b]	7.00±0.00[b]	7.67±0.57[b]	7.00 ±0.00[b]
20 mg/mL	8.00±1.00[b]	7.67±0.57[b]	9.33±1.52[b]	7.33±0.58[b]
30 mg/mL	8.67±1.52[b]	9.00±2.00[ab]	12.33±1.52[a]	8.00±1.73[ab]
40 mg/mL	13.00±3.61[a]	10.67±1.15[a]	14.00±1.00[a]	12.67±0.57[a]

*Means with the different letter indicates no significant difference among the treatments for *S.aureus*, *L. monocytogenes, K. pneumoniae* and *A.baumannii* within columns (p>0.05)

10, 20, 30 and 40 mg/mL silver flakes were used in order to observe the effect of silver on bacteria by using Kirby-Bauer disc diffusion method (Figures 4.1, 4.2, 4.3 and 4.4.). When the amount of silver was increased, the diameter of the zones around *K. pneumoniae*, *A. baumannii*, *L. monocytogenes* and *S. aureus* got bigger, probably because increased silver flakes' content showed its toxic effect to these microorganisms. The diameters in 40 mg/mL were found to be significantly different from the other treatments (p>0.05) (Table 4.1). In addition, the effect of 30 and 40 mg/mL silver flakes against *K.pneumoniae* was found to be significantly different from the other treatments (p>0.05) (Table 4.1). These results were found to be in line with the findings of Wakshlak et al. (2015). A novel mechanism of crucial continuous effect of antimicrobials was demonstrated, which leads to the access and persistence of these substances in inactivated bacteria and its reversible action. Likewise another antimicrobial substance, silver was mentioned to show not only

bactericidal but also a bacteriostatic effect, related to concentration and its surroundings in Wakshlak and coworkers' studies. It is also valid for wounds, meaning that, their microbial infections can be evaluated as the level of almost complete eradication of the pathogens. Wakshlak's study implied that silver showed not only a bactericidal, but also bacteriostatic effect in the growth medium at various silver concentrations, and that in model wound fluid, eradication of bacteria is still observed at very high concentrations (Wakshlak et al., 2015).

In our study, the antimicrobial effect of the silver flakes against two selected Gram(+) and Gram (-) bacteria were analyzed. For this purpose, the zones of inhibition were measured in milimeters. The silver flakes were found to be effective against Gram negative and positive bacteria. 40 mg/mL of the silver flakes was found to be the most effective dose, when compared with the other doses against all bacteria that was investigated in our study. Depending upon this finding, 40 mg/mL silver flakes was used in the antimicrobial combination. According to Table 4.1., silver flakes were found to be more effective on *K. pneumoniae* than *A.baumannii*, *L. monocytogenes* and *S. aureus*. While the zone diameter of *K. pneumoniae* had a mean value of 14 *mm* at 40 mg/mL concentration, the inhibition on *S. aureus* had 13 *mm* but, the diameters on *A.baumannii* and *L. monocytogenes* was found to be 12.7 and 10.7 *mms*. respectively. *A.baumannii* and *L. monocytogenes* were found to be more resistant to silver flakes than *K. pneumoniae* and *S. aureus.* The minimum inhibition zone was recorded at 10 mg/mL silver flake concentration. The average lowest mean value of diameter zone was between 7 *mm*-7.3 *mm*, recorded at *A. baumannii* and *S.aureus* respectively. In control, silver flakes were not used so no inhibition zones were observed. According to the studies performed by Chulovskaya et al (2008), the action of silver on a microbial cell is carried out in two stages. Initial stage was consisted up of the sorption of silver on the surface of cells, then it penetrates into the cell that leads to inactivation of enzymes, damage of nucleotides and ends up with cell lysis. Silver, in metal form is reported to show a well known antimicrobial activity in chemical means, but its ionic form and ionogenic compounds are more bioactive.

The bacteriostatic and bactericidal effect of the silver ions can be attributable to the interactions among nanoparticles and biomembranes, which increase attention

in today's nanomedicine, and exhibit the main task, not only in nanotoxicology, but also utilizing nanomaterials in diagnostic field, the delivery of drugs, biomaterial science, moreover in combination of them such as theranostics. Therefore, nanomaterials act as antimicrobials due to the intensive utilization of them, depending on the increasing resistance to traditional antibiotics. Different nanomaterials gain interest because of their increased functionalities to fight against pathogens. There is a complex interplay between the nanomaterials and if present, the properties of included drugs affecting their performance; and the interaction between the biological systems and nanoparticle-membrane display the key initial step and a duty for the following biological response. The membrane interactions and antibacterial action of these substances are related with the characteristics of the nanoparticles, the structure of membranes, and as well as the light and magnetic fields (Häffner and Malmsten, 2017).

According to Davis (1971), the zone diameters of inhibition during an antimicrobial activity test was evaluated as 'no inhibition zone', 'very weak', 'moderate', 'strong' and 'very strong'. (-) shows no inhibition zone, 0-5 *mm* (or ++) is very weak, 5-10 *mm* (or +++) zone is found to be moderate, 10-15 *mm* (or ++++) is strong and 15- 20 *mm* and more (or +++++) is considered as very strong. So, in our study, if an amount of 40 mg/mL is considered, the effect of the silver flakes against *L. monocytogenes, A. baumannii*, *K.pneumoniae* and *S. aureus* were found to be strong (Table 4.2).

Table 4.2. The antibacterial activity evaluation criteria of the silver flakes against different microorganisms in our study. √: indicates a strong antimicrobial effect according to Davis (1971) and - : indicates no effect.

	Antibacterial activity criteria			
Bacteria	**Very weak**	**Moderate**	**Strong**	**Very strong**
S. aureus	-	-	√	-
L.monocytogenes	-	-	√	-
A.baumannii	-	-	√	-
K.pneumoniae	-	-	√	-

4.1.2. The Effect of Strawberry Tree (Arbutus Unedo)

When the effect of the strawberry tree *(A. unedo)* was evulated on *A. baumannii, K. pneumonia, L. monocytogenes* and *S. aureus* by using Kirby- Bauer disc diffusion method independently, the rehydrated strawberry tree fruit was found to be effective against *S. aureus, L. monocytogenes* and *A. baumannii* but not against *K. pneumonia* (Figure 4.5, 4.6, 4.7 and 4.8).

Figure 4.5. The effect of rehydrated strawberry tree fruit (*A. unedo*) against *S. aureus* tested by Kirby-Bauer disc diffusion method.

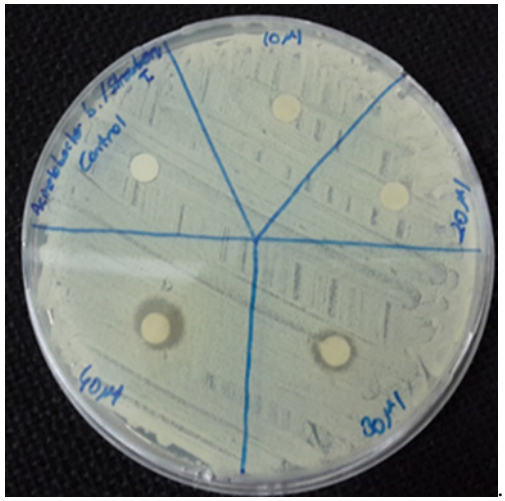

Figure 4.6. The effect of rehydrated strawberry tree fruit (*A. unedo*) against *K.pneumoniae* tested by Kirby-Bauer disc diffusion method.

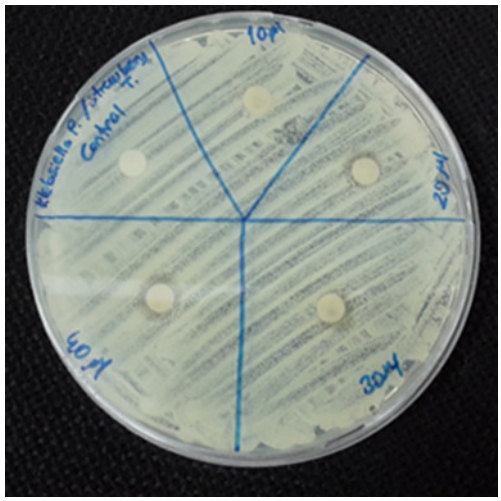

Figure 4.7. The effect of rehydrated strawberry tree fruit (*A. unedo*) against *A. baumannii*, tested by Kirby-Bauer disc diffusion method.

Figure 4.8. The effect of rehydrated strawberry tree fruit (*A. unedo*) against *L. monocytogenes*, tested by Kirby-Bauer disc diffusion method.

In 2011, a study was conducted on determining the antimicrobial activity of the roots of *A. unedo* against *E. coli, P.aeruginosa* and *S. aureus* by El Amine Dib et al. The root extracts were prepared by using distilled water. The diameters of the inhibition zones of *E. coli* were found to be 30, *P.aeruginosa* was 9 and *S. aureus* was 12 *mm* in distilled water (El Amine Dib et al. 2011). In this study, *A. baumannii*, *L. monocytogenes,* and *S. aureus* were found to be strongly sensitive to 40 µL/mL rehydrated strawberry tree (*A. unedo*) fruit. The zone diameters of the bacteria mentioned above was found to be ranging between 7-10 mm at 40 µl/mL dose(Table 4.3). *K. pneumoniae* was found to be more resistant against rehydrated strawberry tree (*A. unedo*) fruit than *A.baumannii*; even though they are gram negative species. This might be possibly due to the capsule structure surrounding

K.pneumoniae, which is a very strong layer, providing resistance by carbohydrates inside it. It was also said to be resistant when compared to the mean value of zone diameters recorded during the evaluation of inhibitory action of *A. unedo against L. monocytogenes* and *S. aureus.*

Several studies were carried out in order to monitor the effect of strawberry tree on different microorganisms. By the rise in the occurrence of high resistivity to antibiotics, the different choices of natural plant products could be attractive. Various extracts of plants and phytochemical substances are shown to possess antibacterial characteristics, which could be regarded as important in the therapeutic cases. At present, certain studies have been carried out in various countries, showing the effect of the treatment (Coutinho et al., 2008). The existence of different substances like quinones, anthraquinones, reducteur compounds, anthocyanins, flavonoids and tannins in strawberry tree fruit gained importance and a thorough interest. By the phytochemical research on these extracts, it was feasible to find the existence of various types of secondary metabolites that display a wide range of antimicrobial (Djipa et al., 2000; Esquenazi et al., 2002), antioxidative (Barreiros and David, 2006), antitumorogenic and antiophidic activity (Okuda et al., 1989). According to the work of El Amine Dib et al. (2011), *A. unedo* was effective against *E.coli* than *P.aeruginosa* and *S.aureus*. In our study, *A. unedo* was found to be effective against *A. baumannii* than Gram(+) bacteria (*L. monocytogenes,* and *S. aureus)* and, *K. pneumoniae*, another G(-) bacteria that we used as reference strains. It was reported that microorganisms differ significantly according to the resistance to physical or chemical substances. For example, many Gram(+) are found to be more resistant to high temperatures than Gram(-) bacteria; on the other hand many Gram(-) species are found to be more resistant than Gram(+) (Pelczar et al.,2010). The inhibition of *A. unedo* fruits against Gram positive species is regarded to be effective, because of its complex peptidoglycan nature it is very hard to break down the cell wall of these bacteria, even by using non-thermal technologies. But on the other hand, in our study, when an interval of 30-40 µl/mL was considered, no significant difference between the treatments against *S. aureus and L.monocytogenes* was observed ($p>0.05$) (Table 4.3).

Table 4.3. The mean and standard deviation results of the diameters of inhibition of zones of strawberry tree fruits (*Arbutus unedo*) against different bacteria to determine the best effective dose of antimicrobial combination.

	Mean± SD*			
Conc.	***S.aureus***	***L.monocytogenes***	***K.pneumoniae***	**A. baumannii**
10 µl /mL	6.33 ± 0.58^{b}	6.33 ± 0.58^{b}	6.0 ± 0.0^{b}	6.33 ± 0.58^{b}
20 µl/mL	8.33 ± 1.15^{ab}	6.66 ± 1.15^{b}	6.0 ± 0.0^{b}	7.0 ± 1.13^{b}
30 µl/mL	9.33 ± 1.53^{a}	8.67 ± 0.58^{ab}	6.33 ± 0.58^{ab}	7.33 ± 1.15^{b}
40 µl/mL	9.33 ± 1.53^{a}	9.0 ± 2.0^{a}	7.67 ± 1.53^{a}	10.67 ± 0.58^{a}

*Means with the different letter indicates no significant difference among the treatments for *S.aureus*, *L. monocytogenes, K. pneumoniae* and *A.baumannii* within columns ($p>0.05$).

In this study, according to Davis (1971) the effect of the rehydrated strawberry tree fruit against *L. monocytogenes, K. pneumoniae* and *S. aureus* were found to be moderate, but on the other hand it was found to be stronger against *A. baumannii* than those mentioned above (Table 4.4).

Table 4.4. The antibacterial activity criteria of the rehydrated strawberry tree fruit against different microorganisms. √: indicates a strong antimicrobial effect according to Davis (1971) and - : indicates no effect.

	Antibacterial Activity Criteria			
Bacteria	**Very weak**	**Moderate**	**Strong**	**Very strong**
S. aureus	-	√	-	-
L.monocytogenes	-	√	-	-
A.baumannii	-	-	√	-
K.pneumoniae	-	√	-	-

4.1.2 The Effect of Rose Water

In this study, 50 µLs of rose water was found to be the best dose, only effective on *Staphylococcus aureus* by using Kirby-Bauer disc diffusion method. As this was the only species found to be inhibited by rose water in our study, almost similar findings were observed in 9 MSSA, 4 MRSA, 13 MSSE and 15 MRSE strains studied between the range of 0.1-1 µL/mL by Kırmusaoğlu (2010).

When the effect of the rose water was tested against *A. baumannii*, *K. pneumoniae*, *L. monocytogenes* and *S.aureus* by using Kirby-Bauer disc diffusion method, independently, in only one trial, the rose water was found to be slightly effective(8 mm) against *S. aureus*. The effect of rose water was found to be lower than the silver flakes and strawberry tree in our study. Rose water found its way to be

used in different areas, such as fruit juices, which have a tendency to spoil due to improper processing and storage problems. In a study, conducted by Yurdugul et al.(2016); *Salmonella* spp., *E. coli* 0157:H7 and *L. monocytogenes* were studied for their inhibition potential in peach juices by using rose water. *E. coli* 0157:H7, *Salmonella* spp. and *L. monocytogenes* were all found to be completely inhibited when 20 µL/10 mL of rose water was used in combination with a power of 10^{-3} W/m^{-2} at 24 kHz frequency of ultrasonication. No any chemical or physical change was recorded in peach juices treated with ultrasonication and rose water. However, in our study, rose water was found to be not effective against *L. monocytogenes, A. baumannii* and *K.pneumoniae,* as well as *S. aureus*. Possibly the strains used in this study may be more resistant compared to the study of Yurdugul et al. (2016). Moreover, *S. aureus* and *L.monocytogenes* are gram (+) and contain a thick layer of peptidoglycan. Based on this property, rose water was not found to be effective against these bacteria, especially on *L.monocytogenes*. No significant difference was observed between the treatments in Section 3.2.2; reported against the bacteria mentioned in Section 3.1.4 ($p>0.05$). Indeed, *K.pneumoniae* has a capsule layer surrounding itself, so one of the reasons may be due to this structure.

A study performed by Ulusoy et al.(2009) on the rose absolute and essential oil implied that the high amount of phenolic substances inherent showed strong antimicrobial action against *Escherichia coli* (ATCC 25922), Pseudomonas *aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Chromobacterium violaceum* (ATCC 12472) and *Erwinia carotovora* (ATCC 39048) strains. The antibacterial action of rose products was analyzed by agar diffusion and minimal inhibitory concentration assays against six important pathogens. The control containing hexane showed no inhibition, therefore, rose oil and absolute indicated strong antibacterial action on the strains. The rose oil and absolute was found to be sensitive against *C. violaceum* 12472, having the biggest zone of inhibition (>25 mm) and the smallest minimal inhibitory concentration values of 0.25 and 0.5%, respectively. *E. coli* 25922 was also found to be sensitive to rose essential oil and absolute with the minimal inhibitory concentration values of 0.25 and 0.5%, respectively. Referring to the minimal inhibitory concentration values, the rose essential oil demonstrated stronger efficiency compared to the absolute. Antibacterial activities and the minimal inhibitory concentration levels of

rose essential oil and absolute were implied that the antibacterial activity of the tested extracts was mainly due to their phenolic contents. Due to the strong antimicrobial action of rose oil and the high tocopherol and carotene amount of rose absolute; it could be preferred as natural preservation agents in food and medical area as well as an antimicrobial substance for the disinfection of different surfaces.

4.2 The Effect of The Combination on Various Pathogenic Bacteria

In order to determine the best dose for preparing an antimicrobial combination by using rose water, strawberry tree fruit and silver flakes; 10, 20, 30 and 40 µL/mL rehydrated strawberry tree fruit (*A. unedo*) was used and its effect was tested against Gram(+) and Gram(-) bacteria mentioned in Section 3.1.4. by using Kirby-Bauer disc diffusion method. The zones of inhibition were measured in milimeters. The best doses of the rose water, rehydrated strawberry tree fruit and the silver flakes which displayed inhibitory action in an individual manner were combined and the following results were obtained in Table 4.5 as log cfu/mL:

Table 4.5. The mean and standard deviation values of the effect of the combination against the bacteria mentioned in Section 3.1.4. with respect to control, given in log cfu/mL.

	Mean± SD*			
	S.aureus	***L.monocytogenes***	***K.pneumoniae***	***A. baumannii***
The control	6.34±0.06[a]	5.95±0.10[a]	6.23±0.06[a]	6.16±0.13[a]
Combination	5.87±0.03[a]	5.43±0.31[a]	5.84±0.08[a]	5.25±0.07[a]

* Means with the different letter indicates no significant difference among the treatments for *S.aureus*, *L.monocytogenes* ,*K.pneumoniae* and *A. baumannii* within columns (p>0.05).

The bacteria mentioned in Section 3.1.4 was treated by the combination group for a growth period of 24-48 hours at 37° C. The inhibition were detected by the plate count agar (PCA) counts for all bacteria mentioned in Section 3.1.4. for the combination group with respect to control and then the results were expressed in log cfu/mL. In this study no significant difference (p>0.05) was observed between the control and the combination against *K. pneumoniae* and *S.aureus* as well as *A.baumannii* and *L. monocytogenes* (p>0.05). When *L. monocytogenes* is considered, this is mainly due to the resistant structure of containing a thick layer of peptidoglycan (Table 4.5.).

When statistical analysis was considered, the antimicrobial combination was found to be slightly effective against the growth of all four species mentioned in Section 3.1.4. when compared with control. The microbial growth was reduced approximately up to 0.5 log cfu/mL. As since *K.pneumoniae* was found to contain a thick capsule structure, this reduction is found to be important(Table 4.5).

In general, the antimicrobial combination was found to be slightly effective against all bacteria in our experiment. The freeze-dried strawberry tree fruits in freeze-dried form, rose water and silver flakes were found to be effective by their final concentrations of 6.7 g, 5 mL and 4 g against bacteria, which were completed to 100 mL sterile distilled water respectively. The effect of these ingredients, even at very low doses, was found to be adequate. According to this table, when statistical differences was not considered, the antimicrobial combination was found to be the most effective against *A.baumannii*. Multidrug-resistant *Acinetobacter baumannii* is reported to be among the most difficult antimicrobial resistant gram negative bacilli to control and resist to treatments. An increase in antimicrobial resistance among *Acinetobacter* isolates has been reported, therefore definitions of multidrug resistance differ in the literature. *A. baumannii* has been viable for longer periods under different environmental conditions, and its resistance among *Acinetobacter* species has greatly emerged in the past decade (Lockhart et al. 2007). The increase of *Acinetobacter* species for extensive antimicrobial resistance is found to be partially related to the relatively impermeable external membrane structure of the bacteria and its environmental exposure to a large reservoir of resistance genes (Eliopoulos et al. 2008). In our study, the antimicrobial combination contains silver flakes, rehydrated strawberry tree fruit and rose water. Probably, silver flakes display their toxic effect against bacteria by accumulation in the cytoplasm, moreover in addition to the toxic effect of silver flakes, *A. baumannii* may also be easily affected by the antimicrobial effect of the rehydrated strawberry tree in the combination, the acidic nature of this compound may easily disrupt its lipopolysaccharide membrane structure, synergistically. But, interestingly, since no statistical difference was found, the effect of combination on *A.baumannii* was negligible.

The lowest antimicrobial activity of the combination was observed against *K.pneumoniae* in our study. Bacteria in the genus *Klebsiella* often lead to human

nosocomial problems. Mainly, the most important *Klebsiella* species, *Klebsiella pneumoniae* was frequently isolated from hospitals and accounts for an important proportion of hospital related urinary tract infections, pneumonia, septicemias, and soft tissue disorders. The main pathogenic reservoirs for transmission of *Klebsiella* are the digestion system and the hands of hospital staff. Since this bacteria can quickly multiply in the hospital environment, they have a tendency to cause nosocomial outbreaks. These outbreaks of multidrug resistant *Klebsiella* spp., particularly those in maternity wards, are often caused by novel strains, called extended-spectrum-β-lactamase (ESBL) producers. The existence of this enzyme producer strains among clinical *Klebsiella* isolates has been persistenly increasing over the last years. The consequent limitations on the therapeutic alternatives require new measures for coping with *Klebsiella* hospital problems. *Klebsiella* acquires virulence factors and these structures may fortify its pathogenicity, therefore the control of infections of *Klebsiella* species require different typing methods as epidemiological tools to manage this control, relevant to these factors. During vaccine development for *Klebsiella* species, capsules or lipopolysaccharides should be seriously regarded as promising candidates; serving immunological infection control measures (Podschun and Ullmann, 1998), so our antimicrobial combination may not show a desired effect against *K.pneumoniae* regarding to its probable resistance problem due to these structures mentioned above, in line with the study of Podschun and Ullmann (1998). It was also supported by the results tabulated in Table 4.5.

According to the study of Loza et al. (2014), the silver nanoparticles are accepted to the cell by endocytotic mechanism and follow the endosomal and lysosomal route, and the acidic medium of the cytoplasm allow the release of the silver particles at the low pH (Liu and Hurt, 2010). According to these researchers, the mechanism of this release is simply based on the quick dissolution when these particles are taken up by the endosome. The uptake time of these ions by the endosome is said to be more than the lysosome and the silver particles are prone to oxidation after their release, so it has resulted in the formation of reactive oxygen species (Loza et al., 2014).

Another cell-based mechanism proposed for silver nanoparticles was reported by Sondi and Salopek-Sondi (2004). The antimicrobial action of silver nanoparticles against *E. coli* displayed the accumulation of silver nanoparticles and the occurrence of "pits" in cell wall causing to cell death. The small silver nanoparticles with a large surface volume ratio in *E. coli* demonstrated a more potent antimicrobial activity than the large ones. The antimicrobial action of the silver nanoparticles is said to be both size and shape related. An approximate 25 nanometer silver nanoparticles, synthesized by four different types of saccharides displayed high antibacterial action against Gram-positive and Gram-negative pathogenic species, like methicillin-resistant *S. aureus*. The importance of shape in the effect of these nanoparticles is related to the shape-dependent interactions with the Gram-negative organisms. Moreover, studies on the efficiency of the inhibitory effects of silver nanoparticles against yeast, *E. coli*, and *S. aureus* reflected that at low concentrations of the silver nanoparticles, the growth of yeast and *E. coli* was completely inhibited, whereas a slight effect was seen in *S. aureus*. Individually; in our studies silver was found to be moderately effective against *S. aureus*, but in combination, on the contrary, almost similar effect was observed in our study, in line with these findings reported by Sondi and Salopek-Sondi, where silver concentration were decreased with respect to its individual effect. The silver nanoparticles, naturally released from the supernatants of *K. pneumoniae* were analyzed; the efficacy of different antibiotics, including penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin against *S. aureus* and *E. coli* were enhanced by the existence of silver nanoparticles. When compared to silver nanoparticles, hydrogel–silver nanocomposites displayed a potent antimicrobial action against *E. coli*. The chitosan containing silver nanoparticle structure was found to show more antibacterial action than its components at their respective concentrations since it enables the occurrence of small silver nanoparticles linked to the polymer that may scatter at a pH less than or equal to 6.3. The silver nanoparticle microbial product isolated from the supernatants of *S. aureus* displayed important antibacterial action against methicillin-resistant *S.aureus*, following methicillin resistant *Staphylococcus epidermidis* and *Streptococcus pyogenes*, on the other hand only mild antibacterial action was seen against *Salmonella typhi* and *K. pneumoniae*. The mechanisms of silver nanoparticles were reported to trigger the bactericidal action through the reducing sugar and protein release in *E. coli*. (Feng- Zhang et al. 2016).

4.2.1 The pH of The Combination

The pH of the combination group was measured in order to evaluate the effect on different pathogenic bacteria. The pH of the combination was found to be 3.55 and the control was 7.0. The difference was possibly due to the presence of strawberry tree fruits (*Arbutus unedo*) and rose water in the combination, since they contain acidic compounds.

4.2.2 The Effect of Temperature on The Combination

The temperature effect on the combination was studied on all of the microorganisms used in our study. There are no detailed studies concerning the effect of temperature on the action of disinfectants as well as such kind of antimicrobial substances. Various chemical and physical treatments are preferred for isolation of proteins from microorganisms, studied in different fields. On the other hand, most of these treatments have severe disadvantages. Since different criteria affect the efficiency of isolated proteins negatively, the research team of Haberl-Meglic et al. (2016) aimed to analyze the effect of temperature and microbial growth phase on the efficiency of the protein extracts. In addition, microbial viability was investigated. The results pointed out that the temperature has a great effect on the isolation of proteins, and after the treatment, the optimum temperature is kept at 4°C. There is no microbial viability during all of the temperature assays. No effect of microbial growth phase was claimed related to the yield of protein isolation or microbial viability. Based on these experiments Haberl-Meglic et al. (2016) pointed out that the temperature is a key element for the bacterial membrane to stay in a permeabilized state, so more proteins flow out of bacteria into surrounding media. The bacterial proteins such as bacteriocins display inhibitory effect against pathogenic species like the disinfectants. The antimicrobial action of most disinfectants was reported to increase when the temperature is elevated and this is mostly related to the treatment time and temperature coefficient (Russell and Chopra, 1990), partially supporting our findings for the combination in our study.

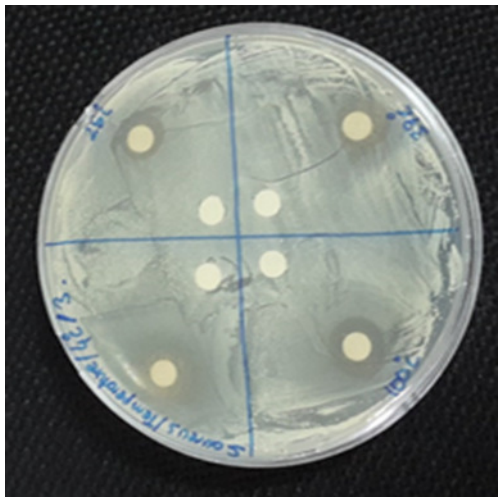

Figure 4.9. The effect of different temperatures on the combination against *S. aureus.*

In our study, the effect of different temperatures on the antimicrobial combination against the pathogenic bacteria mentioned in Section 3.1.4 was tested by using Kirby-Bauer disc diffusion method. These pathogenic bacteria were tested by the inhibitory effect of the combination treated with different temperatures (4°C, 25°C, 37°C and 100°C). No significant difference among the treatments against *S.aureus*, *L.monocytogenes* and *K. pneumoniae* and *A.baumannii* was observed ($p>0.05$) (Table 4.6).

The combination was found to show its most strongest effect at +4 °C against *S. aureus*, the refrigeration temperature. The zone diameter of *S. aureus* was approximately 30 *mm* (Figure 4.9) and the diameter was found to be 11 *mm* at 25°C, 16 *mm* at 37°C and 26 *mm* at 100°C, respectively. When temperature was increased, interestingly, a subsequent increase of inhibition was observed by raising the temperatures between 25-100° C interval. The mean value of zone diameter of *S. aureus* was recorded as 20.7 at 4°C, 13 *mm* at 25°C, 14.3 *mm* at 37°C and 17.7 *mm* at 100°C with respect to the control.

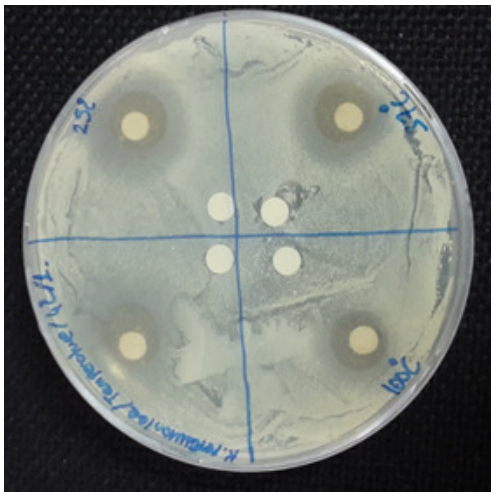

Figure 4.10. The effect of different temperatures on the combination against *K. pneumoniae.*

The combination was found to be effective at 4°C against *K.pneumoniae*. The average zone diameter recorded against *K. pneumoniae* was 24 mm (Figure 4.10) and the diameter was measured as 24 *mm* at 25°C, 19 *mm* at 37°C and 16 *mm* at 100°C. The mean value of diameters of *K.pneumoniae* was found to be 18.2 at 4°C, 17.2 *mm* at 25°C, 14.7 *mm* at 37°C and 13.7 *mm* at 100°C, respectively.

The antimicrobial combination was negatively affected at the treatment by high temperatures, as expected when tested against *K. pneumoniae.* Probably the natural antioxidative and antimicrobial compounds present in the antimicrobial combination, mainly in strawberry tree and rose water was partially destroyed when temperature was elevated.

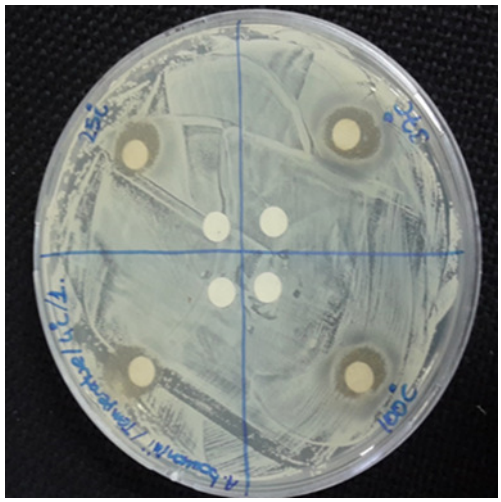

Figure 4.11. The effect of different temperatures on the combination against *A. baumannii*.

The combination was found to be effective at 4°C against *A. baumannii*. The average zone diameter of *A. baumannii* was 12 *mm* at 4 °C (Figure 4.11) and the zones of diameter were measured as 12 *mm* at 25°C, 12 mm at 37°C and 9 *mm* at 100°C, respectively. The mean values of zones of *A. baumannii* was 13.3 *mm* at 4°C, 12.3 *mm* at 25°C, 10.7 *mm* at 37°C and 9.5 *mm* at 100°C. The case was similar to *K.pneumoniae* but, indeed, *A. baumannii* was found to be significantly different when compared between the interval of 4 °C-100°C (Table 4.6.).

Table 4.6. The mean and standard deviation results of the diameters of inhibition of zones of different temperatures against different bacteria to determine the best effective dose of antimicrobial combination.

	Mean±SD			
Temperatures	***S.aureus***	***L.monocytogenes***	***K. pneumoniae***	***A. baumannii***
4 °C	20.66±8.32[a]	14.16±3.17[a]	18.16±6.60[a]	13.33±2.30[a]
25 °C	13.00±2.64[a]	10.66±0.76[a]	17.16±6.52[a]	12.33±2.51[ab]
37 °C	14.33±2.08[a]	12.16±2.56[a]	14.66±4.50[a]	10.66±1.15[ab]
100 °C	17.66±7.37[a]	13.00±3.46[a]	13.66±3.21[a]	9.50±0.50[b]

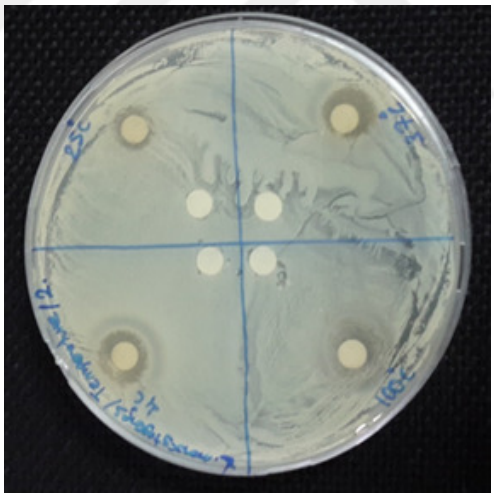

Figure 4.12. The effect of different temperatures on the combination against *L. monocytogenes***.**

The combination was found to be the most effective at 4 °C against *A. baumannii* according to the mean values(Table 4.6). The average zone diameter was 13 *mm* at 4 °C (Figure 4.12) and the zones of diameter were measured as 12 *mm* at 25°C, 10 mm at 37°C and 9.5 *mm* at 100°C. The activity of the antimicrobial combination was negatively affected by the elevation of temperature against *A. baumannii*, likewise in other bacteria tested in our study. This may be due to the

possible action of the degradation of the substances present in rehydrated strawberry tree fruit and rose water into the combination medium following the exposition of the combination to elevated temperatures.

4.2.3 The Effect of pH on The Combination

Kirby-Bauer disc diffusion method was used to observe the effect of pH on bacteria in different pHs of 2, 3, 4, 5, 6, 7, 8, 9 and 10. The adjustment of the pH of the combination was done by using 0.1 N HCl (pH=2.0) and 0.1 N NaOH (pH=13.5). 40 µL of the combination was added onto the sterile filter plates and they were incubated at 37° C for 24-48 hours. The inhibition zones were recorded in diameters and it was found to be effective against *L. monocytogenes, S.aureus, A. baumannii* and *K. pneumoniae* (Figures 4.13, 4.14, 4.15, 4.16, 4.17, 4.18, 4.19 and 4.20, respectively).

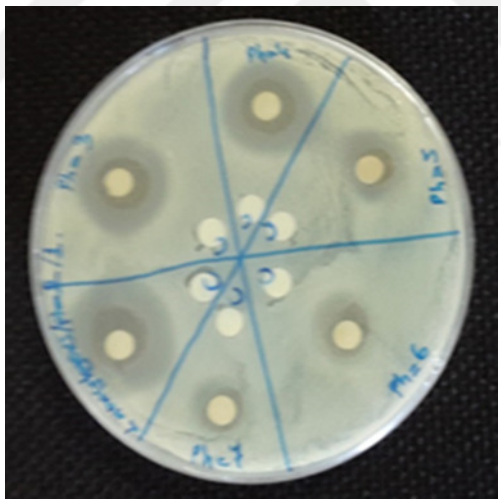

Figure 4.13. The effect of pH (2-7) against *L.monocytogenes*, tested by Kirby-Bauer disc diffusion method. The discs located in the center of the plate serve as controls.

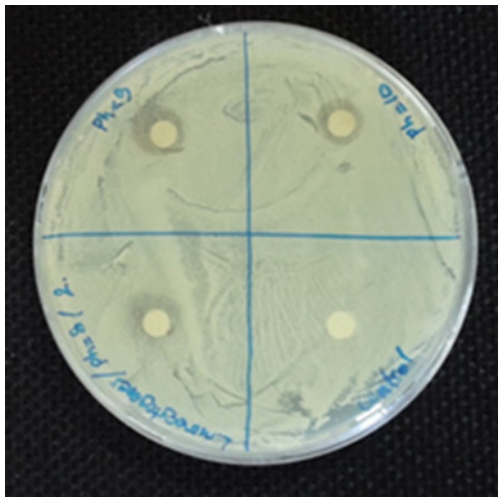

Figure 4.14. The effect of pH (8-10) against *L.monocytogenes*, tested by Kirby-Bauer disc diffusion method.

In Figures 4.13 and 4.14, the zone diameters were measured as 23 *mm* in pH=2, 20 *mm* in pH=3, 19 *mm* in pH=4, 13.5 *mm* in pH=5, 11 *mm* in pH=6, 10 *mm* in pH=7, 10 *mm* in pH=8, 11 *mm* in pH=9 and 10 *mm* in pH=10 against *L.monocytogenes* when the effect of pH on the stability of the combination was investigated. It was observed that the antimicrobial combination was found to be more effective at low pH values than the alkaline; with respect to the control. Mainly, at pH=2 the combination reached its most effective capacity compared to other pH. Acidification of the combination probably caused the activation of the acidic substances alreadily present in the rose water and strawberry tree. A reduction of zone diameters was related with the decrease of activity up to pH=10. Even more or less, a stability was achieved between the pH interval of 6-10.

The mean values were measured as 21.5 *mm* in pH=2, 18.5 *mm* in pH=3, 17.0 *mm* in pH=4, 11.8 *mm* in pH=5, 10.8 *mm* in pH=6, 10.0 *mm* in pH=7, 8.5 *mm* in pH=8, 10.5 *mm* in pH=9 and 10.0 *mm* in pH=10 against *L.monocytogenes* (Table 4.7).

The pH stability of the combination was investigated against *S.aureus* in Figures 4.15 and 4.16(Figure 4.15, Figure 4.16).

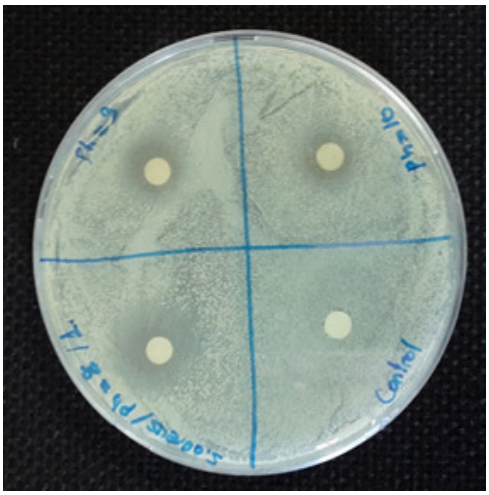

Figure 4.15. The effect of pH (2-7) against *S. aureus*, tested by Kirby-Bauer disc diffusion method. The discs located in the center of the plate serve as controls.

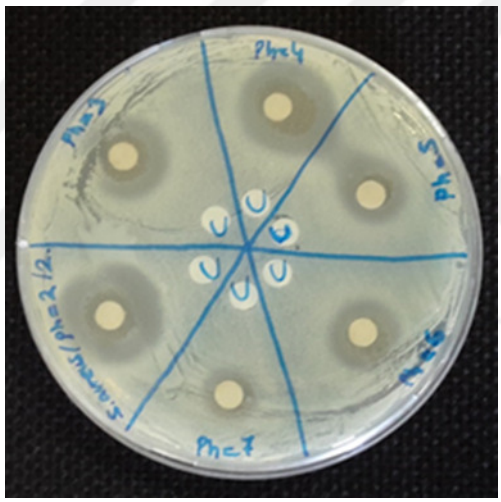

Figure 4.16. The effect of pH (8-10) against S.aureus, tested by Kirby-Bauer disc diffusion method.

It was found that the zone diameters were 20 *mm* in pH=2, 17 *mm* in pH=3, 18 *mm* in pH=4, 13 *mm* in pH=5, 12 *mm* in pH=6, 10 *mm* in pH=7, 17.5 *mm* in pH=8, 11 *mm* in pH=9 and 10 *mm* in pH=10. In general the antimicrobial combination was found to be highly active at low pH values than high pH, when compared to the control. At pH=2 the combination showed its strongest effect when compared to other different pH values. If the growth conditions of *S. aureus* is considered, it is able to grow in a range of pH 4.0-9.8, with an optimum of pH 6-7 (Baird-Parker, 2000, Asperger and Zangerl 2003 and Jay 2000). Our findings support this by observing the strongest inhibition at low pH. The minimal values of pH for growth are influenced by other environmental factors. The growth of *S. aureus* is

inhibited by 0.1% of acetic acid and also by the presence of lower (C1-C4) fatty acids. Moreover, *S. aureus* is found to be more sensitive to acidification when salt concentration is increased, although it is a halotolerant microorganism (Normanno et al. 2007). The large zones of inhibition of *S. aureus* in this study reflects its sensitivity to combination which has an acidic nature. When pH rised up, the sensitivity was found to be decreased. The mean values were measured as 18.3 *mm* in pH=2, 16.0 *mm* in pH=3, 20.0 *mm* in pH=4, 12.0 *mm* in pH=5, 12.8 *mm* in pH=6, 11.0 *mm* in pH=7, 16.3 *mm* in pH=8, 10.5 *mm* in pH=9 and 10.3 *mm* in pH=10 against *S.aureus* (Table 4.7).

The pH stability of the combination was investigated against *A.baumannii* (Figures 4.17 and 4.18).

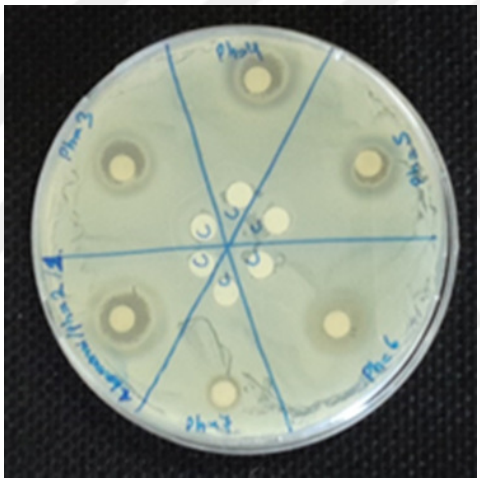

Figure 4.17. The effect of pH (2-7) against A.baumannii, tested by Kirby-Bauer disc diffusion method. The discs located in the center of the plate serve as controls.

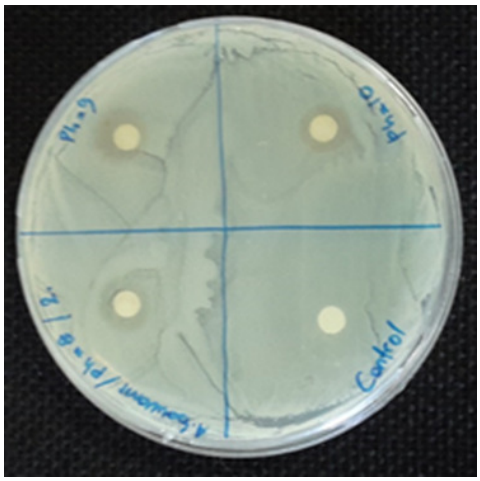

Figure 4.18. The effect of pH (8-10) against *A.baumannii*, tested by Kirby-Bauer disc diffusion method.

The zone diameters were measured as 17 *mm* in pH=2, 14 *mm* in pH=3, 16 *mm* in pH=4, 13 *mm* in pH=5, 14 *mm* in pH=6, 8 *mm* in pH=7, 10 *mm* in pH=8, 11 *mm* in pH=9 and 10 *mm* in pH=10. Low pH values of the antimicrobial combination were also found to be effective against *A.baumannii* according to the control. It was in line with the results observed in *L. monocytogenes* and *S. aureus* in this study. The combination was found to be the most effective at pH=2 when compared to other pH values studied in our assay. The mean values were measured as 14.8 *mm* in pH=2, 12.0 *mm* in pH=3, 13.5 *mm* in pH=4, 10.0 *mm* in pH=5, 12.5 *mm* in pH=6, 8.5 *mm* in pH=7, 10.0 *mm* in pH=8, 11.0 *mm* in pH=9 and 10.3 *mm* in pH=10 against *A.baumannii* (Table 4.7).

For *K.pneumoniae* the same procedure was repeated (Figure 4.19 and 4.20).

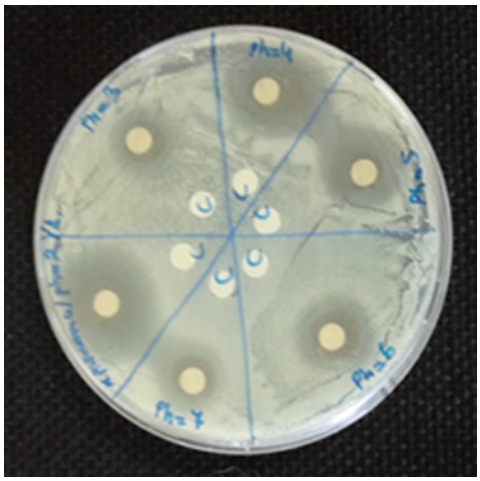

Figure 4.19. The effect of pH (2-7) against *K.pneumoniae*, tested by Kirby-Bauer disc diffusion method. The discs located in the center of the plate serve as controls.

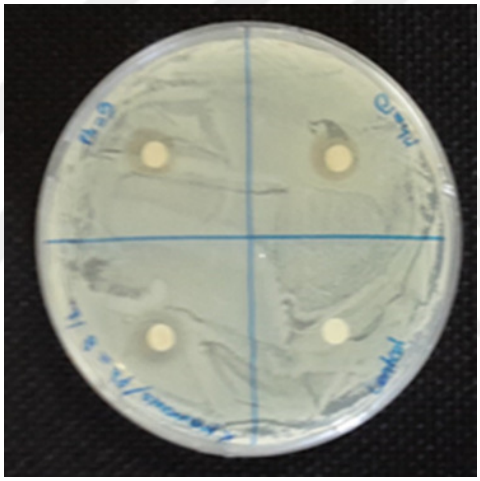

Figure 4.20. The effect of pH (8-10) against *K.pneumoniae*, tested by Kirby-Bauer disc diffusion method.

When the pH stability of the combination was observed in Figures 4.20 and 4.21 against *K.pneumoniae*, it was found that the zone diameters were *23 mm* at pH=2, 20 *mm* at pH=3, 19 *mm* at pH=4, 13.5 *mm* at pH=5, 11 *mm* at pH=6, 10 *mm* at pH=7, 10 *mm* at pH=8, 11 *mm* at pH=9 and 10 *mm* at pH=10. Similar to the findings reported for the other bacteria tested in this study, the combination was found to be effective at low pH values against *K.pneumoniae* with respect to the control. The combination was most effective at pH=2 similar to the other bacteria investigated in our study. The mean values were measured as 17.8 *mm* in pH=2, 13.5 *mm* in pH=3, 22.5 *mm* in pH=4, 16.8 *mm* in pH=5, 13.5 *mm* in pH=6, 12.0 *mm* in pH=7, 5.0 *mm* in pH=8, 5.3 *mm* in pH=9 and pH=10 against *K.pneumoniae* (Table 4.7).

Consequently, a significant difference was found to be present against *S.aureus* and *L. monocytogenes* (pH=2-10 interval) ($p>0.05$) but on the other hand no significant difference against *K. pneumoniae* and *A.baumannii* ($p>0.05$) was observed (Table 4.7).

There are no detailed studies concerning the effect of pH on the action of such kind of combinations. It was reported that the existence and structure of *S. aureus* and *S. epidermidis* within different biofilms were related to mainly on physical surroundings and the dose of antibiotics. There is few information regarding their activities in communities made up of many species, even though they show their infectious properties on similar devices like orthopedic implants. The optimum pH for the prevalence of *S. aureus* is found to be 7 and its growth at elevated temperatures such as 45°C leads to the formation of porous biofilms. The change in pH showed that the existence of *S. epidermidis* is mostly seen at a low pH of 5 and 6, while *S. aureus* is the dominant species at an alkaline pH of 8 and 9. The species differ as they undergo physical treatment (Stewart et al., 2017). In addition our findings showed that, the combination mainly showed its effect around the pH range of 2-6 against all pathogenic species used in our assays (Figures 4.13, 4.15, 4.17 and 4.19). The combination has an average pH of 3.55 owing to an acid nature, even though the pH of the disinfectant is either increased or decreased by adding the appropriate amount of HCl and NaOH, the effect on the pathogens is mostly observed in acidic pH range. Basically the solubility of acidic c compounds in the rehydrated strawberry tree fruit was increased in 0.1 N hydrochloric acid, therefore it is compatible with the acidic environment. But if the alkaline treatment was considered, the increase in pH by NaOH, more or less disrupts the structure of the combination, therefore we observed smaller zone diameters in that pH range (Figures 4.14, 4.16, 4.18 and 4.20) with respect to the control. Interestingly, the inhibitory action of the combination against *S.aureus* was also possible even in high pH (Figure 4.15). In general, a tolerance of *S. aureus* to pH values more than 5.5 is observed due to the maintenance of the intracellular pH by the sequestering or releasing protons from cytoplasm, in addition by the expression of genes responsible for cytoplasm buffering, in line with our findings (Medveďová and Valík, 2012).

If *S. aureus* and *S. epidermidis* are to be considered, they are mainly responsible for the resistive biofilm formation on medical devices, a high-temperature environment and a low and high pH does not disrupt the structure of these biofilms as well as they do not decrease the prevalence of these two organisms (Stewart et al., 2017).

The powerful inhibitory effect of the combination at low pHs was mostly due to the possible positive influence, not only on the antioxidative compounds of the rehydrated strawberry tree fruit; as well as the rose water inherent, as these compounds present in these ingredients may be solubilized and find their way to show their effect on the pathogens easily than approximately two pH units above or below the original pH (3.55) of the combination. It was reported by Russell and Chopra (1990) that the action of many antimicrobial substances, including organic acids, quaternary ammonium compounds, chlorhexidine, glutaraldehyde, various halogens, and phenols is severely affected by the changes in pH, in line with our study. This is mostly based either on the ascending pH; increasing the dissociation of organic acids, leading the main reason of killing bacteria or as pH rises, the surface of the bacteria become more negatively charged. From the economical point of view, the ingredients of the disinfectant are kept to a limited amount. If the content of the rehydrated strawberry tree fruit and rose water may be increased, the expectancy of the possible increasing effect of this combination is under question and remains to be further investigated.

Table 4.7. The mean and standard deviation results of the diameters of inhibition of zones of different pH values against different bacteria to determine the best effective dose of antimicrobial combination.

	Mean±SD			
pH Values	***S.aureus***	***L.monocytogenes***	***K.pneumoniae***	***A.baumannii***
2	18.25±2.47^{a}	21.50±2.12^{a}	17.75±9.54^{ab}	14.75±3.18^{a}
3	16.00±1.41^{ab}	18.50±2.12^{ab}	13.50±3.53^{ab}	12.00±2.82^{ab}
4	20.00±2.82^{a}	17.00±2.82^{bc}	22.50±1.41^{a}	13.50±3.53^{ab}
5	12.00±1.41^{c}	11.80±1.41^{d}	16.75±1.76^{ab}	10.00±4.24^{ab}
6	12.75±1.06^{bc}	10.75±0.35^{d}	13.50±3.53^{ab}	12.50±2.12^{ab}
7	11.00±1.41^{c}	10.00±0.00^{d}	12.00±4.24^{ab}	8.50±0.70^{b}
8	16.25±1.76^{ab}	8.50±2.12^{d}	5.00±7.07^{b}	10.00±0.00^{ab}
9	10.50±0.70^{c}	10.50±0.70^{d}	5.25±7.42^{ab}	11.00±0.00^{ab}
10	10.25±0.35^{c}	10.00±0.00^{d}	5.25±7.42^{ab}	10.25±0.35^{ab}

*Means with the different letter indicates no significant difference among the treatments for *S.aureus L.monocytogenes, K. pneumoniae and A. baumanniii* within columns (p>0.05).

5. CONCLUSION

In this study, when the amount of the silver flakes was increased, the diameter of the zones got bigger around *K.pneumoniae, A. baumannii, L. monocytogenes* and *S. aureus*. This may indicate that silver flakes has a toxic effect on microorganisms. When the effect of rehydrated strawberry tree fruits on bacteria is observed before forming the antimicrobial combination, a moderate effect was observed against *K.pneumoniae, L. monocytogenes* and *S. aureus* but it was found to be stronger against *A. baumannii*. Strawberry tree fruit probably contains antimicrobials such as alcohol in its structure. Hovever, its moderate efficacy indicates that bacteria may be somewhat resistant to this fruit. Moreover, in our study, rose water was only found to be slightly effective against *S.aureus* by using disc diffusion method. It has been observed that rose water is not effective on these bacteria. We can talk about resistance of *S.aureus* in this extent.

The bacteria used in our studies, as previously mentioned in Section 3.1.4. was inhibited by the combination as well as individual inhibition of the components was observed and they were found to be more sensitive to the combination treatments. Kirby-Bauer disc diffusion method indicated zones against the lawn cultures of the pathogens on the appropriate agars, plate count agar was used to monitor and determine the effect of the combination and it was indicated an average of 0.4-1.0 logarithmic cfu/mL decrease. Even though the rehydrated strawberry tree-rose water and silver flakes was dissolved in sterile distilled water; a good inhibitory effect was observed. For future prospects different solvents, such as ethanol, isopropanol or methanol may be used to increase the effect of this combination. Moreover, the effect of pH and temperature on the combination was found to be effective too, mostly at pH 2-6 and +4° C, respectively. Hovewer, in this study, it was thought that the combination did not have a statistical effect on bacteria due to the possible antagonistic effect between the inhibitory substances in the combination.

In this study, our aim was to prepare a natural antimicrobial combination. This may support some studies in the future, especially in the field of biotechnology

and medicine. In addition to silver, low-cost elements such as nickel or copper, which are thought to have an effect on bacteria, may be used.

6. REFERENCES

Achuthan CR, Babu BH and Padikkala J (2003) ”Antioxidant and Hepatoprotective Effects of *Rosa damascene*”, Journal of Pharmaceutical Biology, 41: 357–361.

Alarcão-e-Silva MLCM, Leitão AEB, Azinheira HG and Leitão MCA (2001) “The Arbutus berry: Studies on Its Color and Chemical Characteristics at Two Mature Stages”, J. Food Compos. Anal, 14: 27–35.

Amorim de AM and Nascimento JDS (2017) “Acinetobacter: An Underrated Foodborne Pathogen?”, Journal of Infection in Developing Countries, 11: 111-114.

Anzano M, Tosti A, Lasagni M, Compiglio A, Pitea D and Collina E (2011) “Antimicrobial Activity of Thin Metalic Silver Flakes, Waste Products of a Manufacturing Process”, Journal of Environmental Science, 23: 1570-1577.

AOAC. (1995). pH of acidified foods AOAC Method No. 981.12. AOAC, Washington, pp. 2.

Aryana KJ and Olson DW (2017) “A 100-Year Review: Yogurt and Other Cultured Dairy Products”, Journal of Dairy Science, 100: 9987-10013.

Asperger H and Zangerl P (2003) *Staphylococcus aureus*. In: Roginski H, Fuquay J, Fox P, editors. Encyclopedia of Dairy Science. San Diego, Academic Press pp. 2563-2569.

Bagge-Ravn D, Ng Y, Hjelm M, Christiansen JN, Johansen C and Gram L (2003) “The Microbial Ecology of Processing Equipment in Different Fish Industries – Analysis of the Microflora During Processing and Following Cleaning and Disinfection”, Int J Food Microbiol, 87: 239–250.

Bahl D, Chakravarthy A, Mutalik S. and Devkar R (2016) “Determination of Antibacterial and Antifungal Properties of Rose Extract- An In Vitro Study”, International Journal of Pharmacognosy and Phytochemical Research, 8: 1695-1697.

Baird-Parker T (2000) *Staphylococcus aureus*. In: Lund B, Baird-Parker T, Gould G, editors. The Microbiological Safety and Quality of Food. Gaithersburg: Aspen Publishers. pp. 1317-1330.

Baker-Austin C and Dopson M (2007) “Life in acid: pH Homeostasis in Acidophiles.” Trends Microbiol, 15: 165-171.

Baron S. (1996) Medical Microbiology, Fourth Edition, Chapter 12 Staphylococcus, University of Texas Medical Branch at Galveston.

Bauer, AW, Kirby WMM, Sherris JC and Turck M (1966) “Antibiotic Susceptibility Testing by a Standardized Single Disk Method”, Amer J.Clin. Pathol, 45: 493-496.

Benzeggouta, N. (2005). “Etude de l’activité Antibactérienne des Huiles Infusées de Quatre Plantes Médicinales Connues Comme Aliments”, Mémoire de Magister en Pharmacochimie. Université de Constantine (Algeria), 56- 62. (in French)

Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B and Bartlett J (2009) “Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America”, Clin Infect Dis, 1; 48: 1-12.

Bouyahya A, Moussaoui NE, Abrini J, Bakri Y. and Dakka N, (2016) “Determination of Phenolic Contents, Antioxidant and Antibacterial Activities of Strawberry Tree (Arbutus unedo L.) Leaf Extracts”, British Biotechnology Journal, 14: 1-10.

Bridier A, Briandet R, Thomas V and Dubois-Brissonnet F, (2011) “Resistance of Bacterial Biofilms to Disinfectants”, The Journal of Bioadhesion and Biofilm Research, 27: 1017-1032.

Brooks GF, Carroll KC, Butel JS, Morse SA and Mietzner TA (2013) Jawetz, Melnick & Adelberg’s Medical Microbiology -A lange medical book, Twenty-Sixth Edition, McGraw Hill Book, New York.

Camp C and Tatum OL (2010) “A Review of Acinetobacter baumannii as a Highly Successful Pathogen in Times of War”, Laboratory Medicine, 41: 649–657.

Charlier C, Cretenet M, Even S and LeLoir Y (2009) “Interactions Between Staphylococcus Aureus and Lactic Acid Bacteria: An old story with new perspectives.” Int. J. Food Microbiol, 131: 30-39.

Chojniak J, Jalowiecki L, Biedron I and Plaza G (2016) “Antibacterial Properties of Biological Synthesized Silver Nanoparticles (AgNPs)”, International Multidisciplinary Scientific Geo Conference, 1: 391-396.

Chulovskaya SA, Garas’kob EV and Parfenyuk VI (2009) ”Electrochemical Preparation and Properties of Ultradisperce Silver Powder”, Russian Journal of Applied Chemistry, 82: 1396-1400.

CLS, 2007. Performance Standards for Antimicrobial Susceptibility Testing, Clin. Lab. Stand. Inst, (1-182) 1-56238-525-5.

Cooper, IR, White J, Mahenthiralingam E and Hanlon GW (2008) ” Long-term Persistence of a Single Legionella pneumophila Strain Possessing the Mip gene in a Municipal Shower despite Repeated Cycles of Chlorination”, J Hosp Infect, 70: 154–159.

Coutinho HDM, Costa JGM, Siqueira Jr, JP and Lima E.O (2008) " In Vitro Anti-Staphylococcal Activity of Hyptis martiusii Benth Against Methicillin-Resistant Staphylococcus aureus-MRSA strains", Rev. Bras. Farmacogon, 670-675.

Cowan, MM (1999) "Plant Products as Antimicrobial Agents", Clinical Microbiology Reviews, 12: 564–582.

Çon AH, Gökalp HY (2000) "Laktik Asit Bakterilerinin Antimikrobiyal Metabolitleri ve Etki Şekilleri", Türk Mikrobiyol Cem Dergisi 30: 180-190.

Dettenkofer M. and Spencer, RC (2007) "Importance of Environmental Decontamination--a Critical View", Journal of Hospital Infection, 65: 55-57.

Deva AK, Vickery K, Zou J, West RH, Selby W, Benn, RA, Harris JP and Cossart YE (1998) "Detection of Persistent Vegetative Bacteria and Amplified Viral Nucleic Acid from in-Use Testing of Gastrointestinal Endoscopes", J Hosp Infect, 39: 149– 157.

Dib ME, Allali H, Bendiabdellah A, Meliani N and Tabti B (2013) "Antimicrobial activity Phytochemical Screening of *Arbutus unedo L.*" Journal of Saudı Chemıcal Socıety, 17: 381-385.

Djipa CD, Delmee M and Quentin-Leclercq J (2000) " Antimicrobial Activity of Bark Extracts of Syzygium Jambos (L.) Alston (Myrtaceae)", Journal of Ethnopharmacology, 71: 307–313.

El Amine Dib M, Allali H , Bendiabdellah A , Meliani N and Tabti B (2011) "Antimicrobial Activity and Phytochemical Screening of *Arbutus unedo* L", Journal of Saudi Chemical Society,17: 381-385.

El Fane M, Sodqi M, Chakib A, EL Filali KM (2017) "Prevention of Infection in Lupus Patients", Journal Antimicrobial Agents (ISSN: 2472-1212) 3: 131.

Eliopoulos GM , Maragakis LL and Perl TM (2008) **"***Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options", Clinical Infectious Diseases, 46, 8, 1254–1263.

Epand RM and Epand RF (2010) "Bacterial Membrane Lipids in the Action of Antimicrobial Agents", Journal of Peptide Science,17: 5: 298–305.

Esquenazi D, Wigg MDM, Miranda MFS, Rodrigues HM, Tostes JBF, Rozental S, Silva A. JR da and Alviano CS (2002) "Antimicrobial and Antiviral Activities of Polyphenolics from Cocos nucifera Linn. (Palmae) Husk Fiber Extract", Research in Microbiology, 153: 647-652.

European Parliament & European Council (2012). Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 Concerning the Making Available on the Market and use of Biocidal Products. Off. J.Europ Union, L, 167: 1-123.

Ferreiraab S, Santosb J, Duarteb A, Duartea AP, Queirozab JA and Domingues FC (2012) "Screening of Antimicrobial Activity of Cistus ladanifer and Arbutus Unedo Extracts", Journal Natural Product Research, 26: 1558–1560.

Fortalezas S, Tavares L, Pimpao R, Tyagi M, Pontes V, Alves PM, McDougall G, Stewart D, Ferreira RB and Santos CN (2010) "Antioxidant Properties and Neuroprotective Capacity of Strawberry tree fruit (*Arbutus unedo*)", 2: 214–229.

Gosling RJ, Breslin M and Davies RH (2017) "Efficacy of Disinfectants and Detergents Intended for a Pig Farm Environment where *Salmonella* is present", Veterinary Microbiology, 204: 46-53.

Gupta VK, Mallika V, Yashik G and Srivastava DK (1992) " Oxygen derived free radicals in clinical context", Ind J Clin Biochem, 7: 3–10.

Haberl-Meglic S, Levicnik E, Luengo E, Raso J and Miklavcic D (2016) "The Effect of Temperature and Bacterial Growth Phase on Protein Extraction by means Ofelectroporation", Bioelectrochemistry, 112: 77-82.

Hajipour MJ, Fromm KM, Ashkarran AA, Jimenez de Aberaturi D, de Larramendi IR, Rojo T, Serposhan V, Parak WJ and Mahmoudi M (2012) "Antibacterial Properties of Nanoparticles", Trends in Biotechnology, 30: 499-511.

Häffner S.M and Malmsten M (2017) "Membrane Interactions and Antimicrobial Effects of Inorganic Nanoparticles", Advances in Colloid and Interface Science, 248: 105-128.

Iniguez-Moreno M, Avila-Novoa MG, Iniguez-Moreno E, Guerrero-Medina PJ, Gutierrez-Lomeli M (2017) "Antimicrobial Activity of Disinfectant Commonly Used in the Food Industry in Mexico", Journal of Global Antimicrobial Resistance, 10: 143-147.

Ip M, Lui SL, Poon VKM, Lung I and Burd A (2006) "Antimicrobial Activities of Silver Dressings: an in Vitro Comparison", Journal of Medical Microbiology, 55: 59-63.

Jay J (2000) *Staphylococcal Gastroenteritis*. In: Jay J, editor. Modern Food Microbiology. Gaithersburg: Aspen Publishers. pp. 441-459.

Jose JK and Kuttan R (1995) " Antioxidant Activity of Emblica offi- cianalis", J Clin Biochem Nutr, 19: 63–70.

Jung WK, Koo HC, Kim KW, Shin S, Kim SH and Park YH (2008) "Antibacterial Activity and Mechanism of Action of the Silver Ion in Staphylococcus aureus and Escherichia coli ", American Society For Microbiology, 74: 2171–2178.

Jurica K, Gobin I, Kremer D, Cepo DV, Grubesic RJ, Karaconji IB and Kosalec I (2017) "Arbutin and its metabolite hydroquinone as the main factors in the antimicrobial effect of strawberry tree (Arbutus unedo L.) leaves", Journal of Herbal Medicine, 8: 17-23.

Kırmusaoğlu S (2010) Effect of N-Acetylcysteine (NAC), Fermented Sumach and Eau de Rose on the Formation of Slime Layer of *Staphylococcus* spp., Master's thesis, AIBU, Graduate School of Natural and Applied Sciences, Bolu.

Koula D and Souhila T (2014) "Antibacterial effect of *Arbutus unedo* L. Fruit and Its essential oils on *Salmonella Typhii* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853)", International Journal of Applied and Natural Sciences, 3: 25-30.

Ko WC, Paterson DL, Sagnimeni AJ, Hansen DS, Gottberg AV, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, McCormack JG, and Yu VL (2002) "Community-Acquired Klebsiella pneumoniae Bacteremia: Global Differences in Clinical Patterns", Emerging Infections Diseases, 8: 160–166.

Kozacinski, L, Hadziosmanovic M, (2001) "The occurrence of *Listeria Monocytogenes* in Home-made Dairy Products", Tıerarztlıche Umschau, 56: 590-594.

Liu JY and Hurt RH (2010) "Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids", Environmental Science & Technology, 44: 2169-2175.

Lockhart SR, Abramson MA, Beekmann SE,et al. (2007), "Antimicrobial Resistance among Gram Negative Bacilli as Causes of Infections in Intensive Care Unit Patients in the United States Between 1993 and 2004", J Clin Microbiol, 45: 3352-9.

Loza K, Diendorf J, Sengstock C, Ruiz-Gonzalez L, Gonzalez-Calbet JM, Vallet-Regi M, Köller M and Epple M (2014) " The Dissolution and Biological Effects of Silver Nanoparticles in Biological Media", Journal of Materials Chemistry, 2: 1634-1643.

MedCalc Statistical Software version 15.8 (2015) MedCalc Software bvba, Ostend, Belgium; (https://www.medcalc.org 2016).

Medveďová A and Valík Ľ (2012) Chapter 4, *Staphylococcus aureus*: Characterisation and Quantitative Growth Description in Milk and Artisanal Raw Milk Cheese Production, Page: 74.

Mijnendonckx K, Leys N, Mahillon J, Silver S and Van Houdt R (2013) "Antimicrobial Silver: Uses, Toxicity and Potential for Resistance Biometals", 26: 609-621

Miller ER, Ullrey DE and Lewis AJ (1991) Swine Nutrition (A book) 17. Chapter, SF396.5.S95- copyright by Butterworth- Heinemann in the United Cited of America.

Normanno G, LaSalandra G, Dambrosio A, et al. (2007) "Occurence, Characterization and Antimicrobial Resistance of Enterotoxigenic Staphylococcus Aureus Isolated from Meat and Dairy Products". Int. J. Food Microbiol, 115: 290-296.

O'Gara JP and Humphreys H (2001) "Staphylococcus Epidermidis Biofilms: Importance and Implications", The Pathological Society of Great Britain and Ireland, 50: 582-587.

Okuda T, Yoshiba T and Hatano T (1989) "Ellagi Tannins as Active Constituents of Medicinal Plants", Planta Med, 55: 117-122.

Oliveira I, Baptısta P, Bento A, Pereıra JA (2011) Arbutus unedo L. and Its Benefits on Human Health", Journal of Food and Nutrition Research, 50: 73–85.

Özkan G, Sağdiç O, Baydar NG and Baydar H (2004) "Antioxidant and Antibacterial Activities of Rosa Damascena Flower Extracts", Food Science and Technology International, 10: 277–281.

Pallauf K, Rivas-Gonzalo JC, del Castillo MD, Cano MP and de Pascual-Teresa S (2008) "Characterization of The Antioxidant Composition of Strawberry Ttree (*Arbutus unedo L.*) fruits", J. Food Compos. Anal, 21: 273–281

Palombo EA and Semple SJ (2001) "Antibacterial Activity of Traditional Australian Medicinal Plants", Journal of Ethnopharmacology, 77: 151–157.

Pelczar, MJ, Chan ECS, Krieg NR (2010) Microbiology, An Application Based Approach, Tata Mc Graw Hill Education Private Ltd, New Delhi.

Podschun R and Ullmann U (1998) "*Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors", Clin Microbiol Rev, 11: 589–603.

Prabhu S and Poulose E (2012) "Silver Nanoparticles: Mechanism of Antimicrobial Action, Synthesis, Medical Applications, and Toxicity Effects", International Nano Letters, 2: 32.

Rodriguez-Bano J, Bonomo RA (2008) "Multidrug-resistant *Acinetobacter Baumanni*: "Eyes Wide Shut"? Enferm Infecc Microbiol Clin, 26:185–186.

Rosato JA, Porras T, Conde M and Sage SO (2001) "Cyclic Nucleotides Modulate Storemediated Calcium Entry Through The Activation of Protein-Tyrosine Phosphatases and Altered Actin Polymerization in Human Platelets", J. Biol. Chem, 276: 15666– 15675.

Russell AD (1999) "Bacterial Resistance to Disinfectants: Present Knowledge and Future Problems", J Hosp Infect, 43:57-68.

Russell AD (2003) "Similarities and Differences in the Responses of Microorganisms to Biocides", J Antimicrob Chemother, 52: 750–763.

Russell and Chopra (1990) “Understanding Antibacterial Action and Resistance”, Ellis Horwood Limited, Chichester, England, 16: 188 (p.99).

Ryan KJ and Ray CG, Sherris Medical Microbiology (2004), Fourth edition -An Introduction to Infectous Diseases, McGraw-Hill Companies, United States of America.

Saga T and Yamaguchı K (2009) “History of Antimicrobial Agents and Resistant Bacteria”- JMAJ 52: 103–108.

Sondi I and Salopek-Sondi B (2004) “Silver Nanoparticles as Antimicrobial Agent: a Case Study on E. coli as a Model for Gram-Negative Bacteria, J Colloid Interface Sci, 1;275:177-82.

Siegel J, Rhinehart E, Jackson M and Chiarello L (2007) “Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings”, The Healthcare Infection Control Practices Advisory Committee (HICPAC), Am J Infect Control, 35: 65-164.

Shohayeb M, Abdel-Hameed ESS, Bazaid SA and Maghrabi I (2014) “Antibacterial and Antifungal Activity of Rosa Damascena MILL, Essential Oil, Different Extracts of Rose Petals”, Global Journal of Pharmacology 8: 01-07.

Stewart EJ, Payne DE, Ma TM, VanEpps JS, Boles BR, Younger JG and Solomon MJ (2017) “Effect of Antimicrobial and Physical Treatments on Growth of Multispecies Staphylococcal Biofilms”, Applied and Environmental Microbiology, 83: e03483-16.

Tran QH, Nguyen VQ, Le A.T (2013) “Silver Nanoparticles: Synthesis, Properties, Toxicology, Applications and Perspectives”, Advances in Natural Sciences: Nanoscience and Nanotechnology, 4: 20.

Ulusoy S, Boşgelmez-Tınaz G, Seçilmiş-Canbay H, (2009) “Tocopherol, Carotene, Phenolic Contents and Antibacterial Properties of Rose Essential Oil, Hydrosol and Absolute”, Curr Microbiol, 59: 554–558.

Ülgen C (2013) Certain Chemical, Physical and Microbiological Properties of The Freeze-Dried (Lyophilized) Apple of Cain / Cain Apple (Arbutus Unedo) Fruit, Master’s thesis, AIBU, Graduate School of Natural and Applied Sciences, Bolu.

Villegas MV. Acinetobacter outbreaks (1977-2000). Infect Control Hosp Epidemiol, 24: 284–295.

Wakshlak RBK, Pedahzur R, Avnir D (2015) “Antibacterial Activity of Silver-Killed Bacteria: The "Zombies" Effect”, Scientific Reports 5, Article Number: 9555.

Weese JS and Rousseau J (2006) “Survival of Salmonella Copenhagen in Food Bowls Following Contamination with Experimentally Inoculated Raw Meat: Effects of Time, Cleaning, and Disinfection”, Can Vet J, 47: 887–889.

Xu QB, Xie LJ, Diao HLN, Li F, Zhang YY, Fu FY and Liu XD (2017) "Antibacterial Cotton Fabric with Enhanced Prepared Using Silver Nanoparticles and Carboxymethyl Chitosan", Carbohydrate Polymers, 177: 187-193.

Yurdugül S, Aydın Ş and Kirmusaoğlu S (2016) "Elimination of Foodborne Pathogens in Peach Juice by Combination of Ultrasonication and Eau de Rose", Philippine Agricultural Scientist, 99: 391–400.

Zhang XF, Liu ZG, Shen W and Gurunathan S (2016) "Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches", Int. J. Mol. Sci, 17:1534.

https://www.healthline.com/health/rose-water-benefits /Rose Water: Benefits and Uses – Healthline, 25 October 2017.

www.dailymail.co.uk/.../Rosewater-benefits-make-home.htm/Rosewater benefits and how to make it at home | Daily Mail Online, 25 October 2017.

www.healthguidance.org › Fitness Wellness › Beauty Rose Water Benefits - Health Guidance, 05 November 2017.

7. CURRICULUM VITAE

Name SURNAME	**:** Ekim BOYRAZ
Place and Date of Birth	**: 05.02.1992**
Universities	**: ABANT IZZET BAYSAL UNIVERSITY**
Bachelor's Degree	**: 3,27**
e-mail	**: ekim.mor92@gmail.com**
Address	**:**
List of Publications	**:**
Awards	**:**
Hobbies (Optional)	**:**