

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

**INVESTIGATION OF POTENTIAL ANTIMICROBIAL AND
ANTIBIOFILM ACTIVITIES OF ROSEHIP (ROSA CANINA L.)
EXTRACTS**

DİLARA ŞAHİN
THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF BIOTECHNOLOGY

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2022

GTÜ BİYOTEKNOLOJİ Enstitüsü Yönetim Kurulu'nun 08/06/2022 tarih ve 2022-13/02 sayılı kararıyla oluşturulan jüri tarafından 16/06/2022 tarihinde tez savunma sınavı yapılan DİLARA ŞAHİN'İN tez çalışması BİYOTEKNOLOJİ Anabilim Dalında YÜKSEK LİSANS tezi olarak kabul edilmiştir.

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SUMMARY

In this thesis, the antimicrobial and antibiofilm effects of rosehip fruits (*Rosa canina* L.) extract were investigated. An extract of rosehip was prepared the use of a Microwave-Assisted Extraction (MAE) method. The total phenolic content and the antioxidant activity of the extract was determined by the Folin-Ciocalteu assay and Brand-William method. The antimicrobial activities of the rosehip extract with different dilutions were investigated against food-borne pathogenic bacteria (*E. coli*, *L. monocytogenes*, *S. aureus*, and *B. cereus*) molds and yeasts. The antibiofilm activity of rosehip extract was investigated for the early and the late-stage biofilms (24h and 72h biofilms). The anti-quorum sensing activities of the extract with different dilutions were investigated using model strain *Chromobacterium violaceum* Bergonzini ATCC 12472.

The inhibition zones obtained by the agar well diffusion method was measured in the range of 10-24 mm for all tested bacteria by use of the extract without dilution. Moreover, the extract at 1/32 dilution (0.070 mg/mL) also showed an inhibitory effect of up to 90%. The antifungal effects of the extract with different dilutions were obtained up to 100%. The antibiofilm activities of the extract with 1/2 and 1/4 dilutions were up to 67% and 57% on 24h biofilms. The rosehip extract showed inhibitory activity on quorum sensing at a 1/2 dilution ratio. As a result, it was determined that rosehip extract prepared by using ethanol and MAE can be used as a natural antimicrobial and antibiofilm agent to eliminate foodborne pathogens in the food industry.

Key Words: Rosehip, *Rosa canina* L, food industry, antimicrobial, antibiofilm, natural preservatives

ÖZET

Bu tez çalışmasında kuşburnu (*Rosa canina* L.), meyvesi ekstraktının antimikrobiyal ve antibiyofilm etkileri araştırılmıştır. Kuşburnu ekstraktı Mikrodalga-Bazlı Ekstraksiyon yöntemi ile ekstrakte edilmiştir. Kuşburnu ekstraktının total fenolik bileşen analizi Folin-Ciocalteu yöntemi ile yapılmış ve antioksidant aktivitesi ise Brand-William metodu kullanılarak saptanmıştır. Kuşburnu ekstraktının ve farklı sulandırmalarının antimikrobiyal aktiviteleri ise gıda patojeni bakteriler (*E. coli*, *L. monocytogenes*, *S. aureus* ve *B. cereus*), küfler ve mayalar üzerinde araştırılmıştır. Kuşburnu ekstraktının antibiyofilm aktivitesi genç ve olgun biyofilmler (24 ve 72 saatlik) üzerinde incelenmiştir. Kuşburnu ekstraktının ve farklı sulandırmalarının anti-quorum aktiviteleri *Chromobacterium violaceum* Bergonzini ATCC 12472 suşu kullanılarak incelenmiştir.

Kuşburnu ekstraktının direk kullanımı ve agar kuyu difüzyon yöntemi ile kullanılan bütün bakteriler üzerinde 10-24 mm aralığında inhibisyon zon çapları ölçülmüştür. Ayrıca, ekstraktın 1/32 sulandıma oranında (0.070 mg/mL) dahi %90 oranında inhibisyon belirlenmiştir. Kullanılan ekstraktın antifungal etkisi incelendiğinde %100 etkinliği olduğu gözlenmiştir. Ekstraktın 24 saatlik biyofilmlerde antibiyofilm aktivitesinin 1/2 ve 1/4 sulandıma oranlarında sırasıyla %67 (1.125 mg/mL) ve %57 (0.5625 mg/mL) olduğu belirlenmiştir. Kuşburnu ekstraktının 1/2 oranında quorum sensing üzerinde inhibitör aktivitesi olduğu belirlenmiştir. Sonuç olarak bu tez çalışması ile, etanol ve MAE metod ile hazırlanan kuşburnu ekstraktının doğal bir antimikrobiyal ve antibiyofilm ajan olarak gıda endüstrisinde patojen mikroorganizmaları ortamdaki uzaklaştırmak amacıyla kullanılabileceği gösterilmiştir.

Anahtar Kelimeler: Kuşburnu, *Rosa canina* L, gıda endüstrisi, antimikrobiyal, antibiyofilm, doğal koruyucu.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations</u>	<u>Explanations</u>
<u>Acronyms</u>	
°C	: Centigrade degree
µL	: Microliter
µm	: Micrometre
Dsu	: Distilled water
EtOH	: Ethanol
g	: Gram
CFU	: Colony-forming unit
L	: Litre
MeOH	: Methanol
mg	: Milligram
MHz	: MegaHertz. 10 ⁶ hertz
mL	: Millilitre
mm	: Millimeter
NaCl	: Sodium Chloride
nm	: Nanometer
OD	: Optical Density
ppm	: One in per million
PSW	: Physiological Saline Water
TPC	: Total Phenolic Content
TSA	: Tryptic Soy Agar
TSB	: Tryptic Soy Broth
TSB-L	: TSB Medium w/ Lactose 4%
UAE	: Ultrasound-Assisted Extraction
MAE	: Microwave-Assisted Extraction
UV	: Ultraviolet
V	: Volume

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1. INTRODUCTION

Based on the information from the World Health Organization's data every year, almost 10% of people suffer from foodborne illness, especially children [WHO, Fact Sheets, Food Safety, 2021]. Due to the insufficient and expensive preservation methods for the preparation of foodstuffs, pathogenic bacteria are one of the main issues in the food industry [Monu et al., 2015, Qadri et al., 2015, FDA, Foodborne Illness and Contaminants, 2016].

The use of synthetic chemicals as food preservatives can cause severe damage to human health and nature, such as the development of resistance in target microorganisms, acute or chronic poisoning symptoms in humans, inducing cancer risk, soil, and water source contamination, etc. [Kourkoutas et al., 2020]. Thus, researchers mainly concentrate on natural preservatives and bioactive compounds such as essential oils and plant extracts containing phenolic compounds and bioflavonoids for decades. The low toxicity and potential biological activity of essential oils and plant extracts can be recommended as potential natural preservatives and antimicrobial and antibiofilm agents. [Gutiérrez-del-Río et al., 2018, Foong-Cunningham et al., 2012, Beya et al., 2021]. The biological activity of these plants' extracts is based on their chemical components such as secondary metabolites i.e., phenolic antioxidants, lipids, and bioflavonoids [Stralsjo et al., 2003, Larsen et al., 2003, Olsson et al., 2005].

One of the potential natural bioactive compounds' sources of plants, rosehip [*Rosa canina* spp.], is a pseudo fruit widely found in Central Europe, the Middle East, and Asia [Ercisli, 2007]. According to the literature, rosehip oils are used traditionally for medicinal purposes such as preventing influenza, inflammation, acne, chronic pain, wound healing, etc. as marmalade, herbal tea, and powder forms [Ahmad et al., 2016, Dąbrowska et al., 2019]. Additionally, rosehip is considered a good source of nutrients and phytochemicals due to its high level of biochemical compounds such as vitamin c, polyunsaturated fatty acids, antioxidants, tannins, linoleic acid, etc. [Leahu A. et al., 2014, Ahmad et al., 2016, Mármol et al., 2017].

Therefore, this thesis aims to investigate the antimicrobial, antibiofilm, and anti-quorum sensing activities of rosehip fruit extract on different microorganisms.

1.1 Aim and Contribution of Thesis

According to the literature, different rosehip (*Rosa canina* L.) seed and leaf materials had been studied for their antimicrobial, and antioxidant properties [Concha et al., 2006, Živković et al., 2015, Ghendov-Moşanu et al., 2018]. The characterization of bioactive compounds of *Rosa canina* L. had been studied [Ercisli 2007, Ilyasoglu et al., 2013, Murathan et. al., 2018, Turan et al., 2018, Guney et al., 2020].

However, the antimicrobial and antibiofilm activities of the rosehip pseudo fruits extract obtained by microwave-assisted extraction (MAE) method against foodborne pathogen bacteria have not been studied.

The data from this thesis will show that the extract of rosehip obtained by using MAE with the antimicrobial and antibiofilm effect could be used as an alternative food preservative or disinfectant in the food industry.

2. GENERAL INFORMATION

2.1. Food Industry And Food Safety

The global human population will be around 9.7 billion people by 2050, Therefore, it would be more difficult to ensure that everyone has access to adequate, nutritional, and healthy food all around the world. Therefore, the need for food has carried some perspectives to the food industry such as availability, safety, and quality control of food products [FDA, 2016].

Food security means the ability to reach food at all times while food safety refers to preservations of food to prevent foodborne illnesses. Nowadays food safety has been covered by legal regulations. However, due to the insufficient decontamination methods in the food industry and food supply chain, one out of ten people in the world suffer from contaminated food consume and over 400.000 people die every year for this reason [FDA 2016, WHO, 2021].

Consumption of food that contains detrimental bacteria, viruses, parasites, and chemical substances which do not comply with the safety and quality regulations is associated with more than 200 diseases ranging from diarrhoea to cancers. Especially, children under 5, elders, pregnant, and people who suffer from immune diseases carry 40% of the foodborne diseases which causes over 200.000 deaths each year. Also, more than 110 billion USD was spent for foodborne diseases in medical aspect [WHO, 2020].

Food contamination is possible at every point of the food supply chain like at a farm/field, during harvesting, processing, preparation, transportation, and storage. The most common causes of foodborne diseases are bacteria (Salmonella, Listeria, Campylobacter, and Pathogenic Escherichia coli strains, Yersinia), bacterial toxins (such as Staphylococcus aureus, Clostridium botulinum, Bacillus cereus, and Clostridium perfringens toxins), viruses (Calicivirus -including norovirus), rotavirus, hepatitis A virus, hepatitis E virus), parasites (Trichinella, Toxoplasma, Cryptosporidium, Giardia), prions and moulds (Aspergillus spp., Cladosporium spp.) [Verran et al. 2008, Hernandez et al., 2018, Ismail et al., 2013, Kaczmarek et al., 2019].

2.2. Biofilm

Biofilms are known as microbial communities that adhere to biotic and abiotic solid surfaces and are surrounded by a matrix of extracellular polysaccharides, DNA and proteins produced by the cells [Sadiq et al., 2017b; Wingender et al., 2001]. At the same time, biofilms are life forms in industrial areas where microorganisms can multiply and produce thermally stable enzymes and spores on the surface [Sadiq al., 2017b].

Biofilm formation stages in general; surface attachment, small colony formation, matrix formation, and biofilm maturation are given in Figure 2.1 [edited from Banerjee et al., 2019].

Biofilm formation and maturation stages basically consist of four stages:

- Connecting; free bacteria adhere to the surface.
- Formation of small colonies; With the proliferation of cells, a small colony is formed, and the movement of the cells is prevented.
- Matrix formation: With the inhibition of movement and more EPS formation, quorum sensing and mature biofilm formation are provided.
- Mature biofilm: Some of the cells are released from the biofilm layer and spread to the environment.

The nutrient content of the environment where microorganisms are found affects biofilm formation [Sar and Akbas, 2019; Karaca, 2018; Tolker-Nielsen, 2015]. Different biofilms exhibit different physical and chemical properties [Tolker-Nielsen, 2015; Heydorn et al., 2000]. In addition, gene expressions of bacteria in the free state and bacteria in the biofilm are different from each other [Kot, et al., 2018; Tolker-Nielsen, 2015]. Cells communicate between cells through biochemical signaling molecules and adapt to each other [Solano et al., 2014; Borlee et al., 2010; Flemming and Wingender, 2010; De Kievit, 2009; Hammer and Bassler, 2003]. Mechanism of intercellular communication [sufficient majority sensing] and secondary messenger cyclic di-GMP molecules play a role in biofilm and matrix production [Sadiq et al., 2017a; Solano et al., 2014; Borlee et al., 2010; De Kievit, 2009; Hammer and Bassler, 2003].

Microorganisms in biofilms live in the exopolysaccharide [EPS] matrix that they produce, which is located outside the cell and makes up about 90% of the biofilm

[Flemming and Wingender, 2010]. EPS mainly consists of water, polysaccharide, protein, nucleic acids and lipid [Flemming and Wingender, 2010]. EPS is necessary for biofilm formation and provides a different way of life to free-living bacteria [Flemming and Wingender, 2010]. Differences between EPS components that bind bacteria in biofilms lead to the formation of different biofilm structures [Tolker-Nielsen, 2015].

EPS has many functions in biofilms and benefits biofilms [Flemming and Wingender, 2010]. These:

- To ensure that the cells adhere and adhere to the surface.
- Facilitating communication between cells, providing cell-cell recognition.
- To create the dimensional structure of the biofilm.
- Protecting the biofilm against water loss by maintaining a highly hydrated micro-environment around the bacteria.
- Confer resistance to cells against infection and resistance to various antimicrobial substances (eg, disinfectants.),
- To protect bacteria against factors such as UV, radiation, different pH conditions, osmotic pressure.
- Allowing nutrients from the environment to accumulate in the biofilm.
- To release cells from biofilms by digesting exogenous macromolecules for nutrient absorption and breaking down structural EPS.
- It can be listed as facilitating horizontal gene transfer between biofilm cells.

Bacteria tend to form biofilms on different surfaces such as stainless steel, polystyrene, glass, rubber, wood, and foods [Srey et al., 2013; Djordjevic et al., 2002]. Bacteria can adhere to surfaces more easily or more difficult due to different reasons such as surface roughness, surface chemistry, surface charge and degree of hydrophobicity [Sadiq et al., 2017b]. Cell surface hydrophobicity is directly related to the binding of bacteria [Jindal and Anand, 2018]. In general, hydrophobic materials and rough surfaces are preferred by bacteria over hydrophilic and smooth ones in biofilm formation [Donlan, 2002].

2.3. Decontamination Methods Used In The Food Industry And Limitations

2.3.1. Non-Chemical Methods and Limitations

The surfaces of meats, fish, fruit, vegetables, etc., or food processing surfaces can be contaminated by both pathogenic and spoilage bacteria. There is no disinfection application to remove pathogenic organisms from many raw products until they are bought by consumers [Scheerlinck et al., 2004].

2.3.1.1. Surface Pasteurisation

Any potential or increased risk to human health from food safety would be reduced by the application of effective pasteurization methods to prevent such contamination. However, standard pasteurization yields a "cooked" product from such items. There has been a lot of interest in creating thermal pasteurization processes that just target the bacteria on the food's surface without denaturing the surface because microbial contamination on such raw materials often occurs on the surface. Heat treatments for fruits and vegetables are at lower temperatures than those employed for treating meats [Purnell et al., 2012]. This is principal because they are aimed at controlling insect pests and fungal spoilage rather than thermotolerant foodborne pathogens. Typically, a temperature below 55°C is applied for several minutes [Scheerlinck et al., 2004].

The practical implementation of such methods in processing facilities at industrial production rates, however, poses numerous engineering difficulties. The biggest problem with using surface thermal pasteurization treatments is how difficult it is to apply enough heat to all of the food's surfaces for long enough to significantly reduce bacteria populations without actually cooking the food's surface.

2.3.1.2. Ultraviolet (UV)

When thermal methods are not suitable for temperature-sensitive products alternative methods should be developed and evaluated for decontamination. Among these methods, UV has gained attention in recent years due to its low cost and ease [Scheerlinck et al., 2004]. UV is generated by mercury lamps and its wavelength range from 100 to 400 nm. The UV-C [200-280 nm] is highly bactericidal causing photochemical changes in microbial DNA which inactivate the microorganisms [Singh et al., 2021].

Numerous studies have shown that UV light is excellent at killing harmful bacteria, fungi, viruses, and protozoans that are present in water and food [Singh et al., 2021]. Nowadays UV light is generally used for the decontamination of drinking water with chlorine, in Europe and US. [Keklik et al., 2012]. In the food industry, UV light is also used to sanitize food and other surfaces that come into contact with food, as well as packaging materials like bottles, caps, and films. It is also used to extend the shelf life of fruits and vegetables after harvest by inactivating spoilage fungi [Begum et al., 2009].

Various researchers are still examining how UV light affects the microbial content in meals. The use of pulsed UV light technology enables faster, safer, and more effective decontamination than non-pulsed UV light, but it still has limitations in terms of its capacity to permeate foods and accumulate [Shama et al., 1999; Seiler et al., 1998].

2.3.2. Chemical Methods and Their Limitations

Most of the chemical disinfection agent is ozone. Ozone is used as an effective disinfection agent in both gaseous and aqueous forms and, it has been applied to sterilisation of drinking water since 1906. It is known as the strongest oxidizing sanitizer that could be used in food processing steps. [Hakguder et al., 2009].

Rather than that, ozone has limited solubility in water. Aqueous applications of ozone will be performed by efficient gas injection systems in a closed treatment.

In 2001, ozone usage in food industries have been approved by FDA. [FDA, 2001]. There are many reports on the potential applications of ozone as an

antimicrobial agent in the food industry [e.g., Kim et al., 1998, Niveditha et al., 2021, Shezi et al., 2020]. It is applied on a closed treatment vessel, to many foods including meat, fish, poultry, fruits, and vegetables. However, ozone gas cannot be stored. Moreover, ozone generators are relatively inexpensive according to similar agents, and it is toxic to humans. Human exposure to ozone above 0.2–0.5 ppm can affect human health [Folinsbee et al., 1985].

Chlorine dioxide another agent, has been used as a bleaching agent and disinfectant [Simpson, 2005]. The food industry uses ClO_2 as an extraction solvent and a potent antibacterial solution, while the textile and paper industries utilize it extensively as a bleaching agent. ClO_2 is an oxidizing chemical that responds by removing electrons from a number of cellular components, rupturing molecules, and ultimately killing bacteria. Its disinfecting activity was said to impair protein synthesis by Benarde et al. (1967) and Olivieri (1968). In addition, Roller et al. [1980] revealed that impaired protein synthesis was one of the factors contributing to ClO_2 -induced cell death. Inhibition of amino acid synthesis, inactivation of messenger RNA (and consequently the absence of coded information for protein translation), and ribosome degradation have all been suggested as additional mechanisms of ClO_2 action [Byun et al., 2021]. Inhalation by those exposed to ClO_2 is the primary safety concern. When ClO_2 is inhaled or exposed in excess, it can have harmful health effects such irritated eyes, noses, and throats, which can lead to severe respiratory damage [Byun et al., 2021].

2.4. Plant-Based Natural Compounds

Since ancient times the preservation of food and food derivatives has been important for humankind. Therefore, many conventional and novel methods have been developed, such as salting, ozone, sulfide derivated chemicals, etc. Their limitations regarding allergenicity, carcinogenicity, and based on the type of food applications lead to the researchers' mainly naturally occurring molecule discovery.

For decades plants and their extracts have been used in traditional medicine in different local communities, in China, Turkey, Mexica, Ethiopia, Japan, India, Spain, etc [Wu et al 2022]. From the beginning of the 2000s researchers demonstrated that medicinal plants have antimicrobial [Lin et al., 2019], antifungal [Gishen et al., 2020],

anti-yeast [Parham et al., 2020], antiulcer [Gadekar et al., 2010], insecticide and larvicide [Niroumand et al., 2016], anticarcinogen [Desai et al., 2008], and antiviral [Shabat et al., 2020], antioxidant and antiinflammatory [Ravipati et al., 2012].

The main feature of the plant-derived molecules is decreasing lipid oxidation and suppressing microbial growth could be the possible options in the food preservation industry [Lin et al. 2019].

Most of the plant-based antimicrobial compounds belong to the secondary metabolites which are widely related to plant growth promotion or natural defense mechanisms. These secondary metabolites can be classified as terpenes, phenolics, and nitrogen/sulfur-containing molecules according to their chemical structures [Twahij et al., 2022].

2.4.1. Classification of Plant-Based Natural Compounds

Plant secondary metabolites were given in figure 2.3.1 according to their biosynthetic pathway [Twahij et al., 2022].

Limonene, terpinene, thymol, eugenol, linalool, myrcene, and carvacrol are the most studied compounds for their antimicrobial activities among the terpene family. Their antimicrobial, antifungal, and antioxidant activities were evaluated against *S. aureus*, *B. cereus*, *E. coli* O157:H, *A. niger*, and *P. italicum* [Zengin et al., 2014, Mahizan et al., 2019; Masyita et al., 2022].

Plant phenolics are also classified as flavanols, flavonoids, anthocyanins, tannins, and phenolic acids. Gallic acid, ferulic acid, caffeic acid, and quercetin are one of the most studied antimicrobial compounds in plant phenolics. Several studies have demonstrated that phenols have antimicrobial activity against *L. monocytogenes*, *S. aureus*, *P. auregonisa*, and *E. coli* species [Tako et al., 2020]. They also prevented biofilm formations of *L. monocytogenes* and *S. aureus* [Slobodnikova et al., 2016]

N/S contains compounds mainly synthesized from amino acids, such as allicin, defensins, thionins, and alkaloids. One of the important cases is malaxin, found in the orchids that are used as an antimalarial alkaloid [Teoh et al 2016].

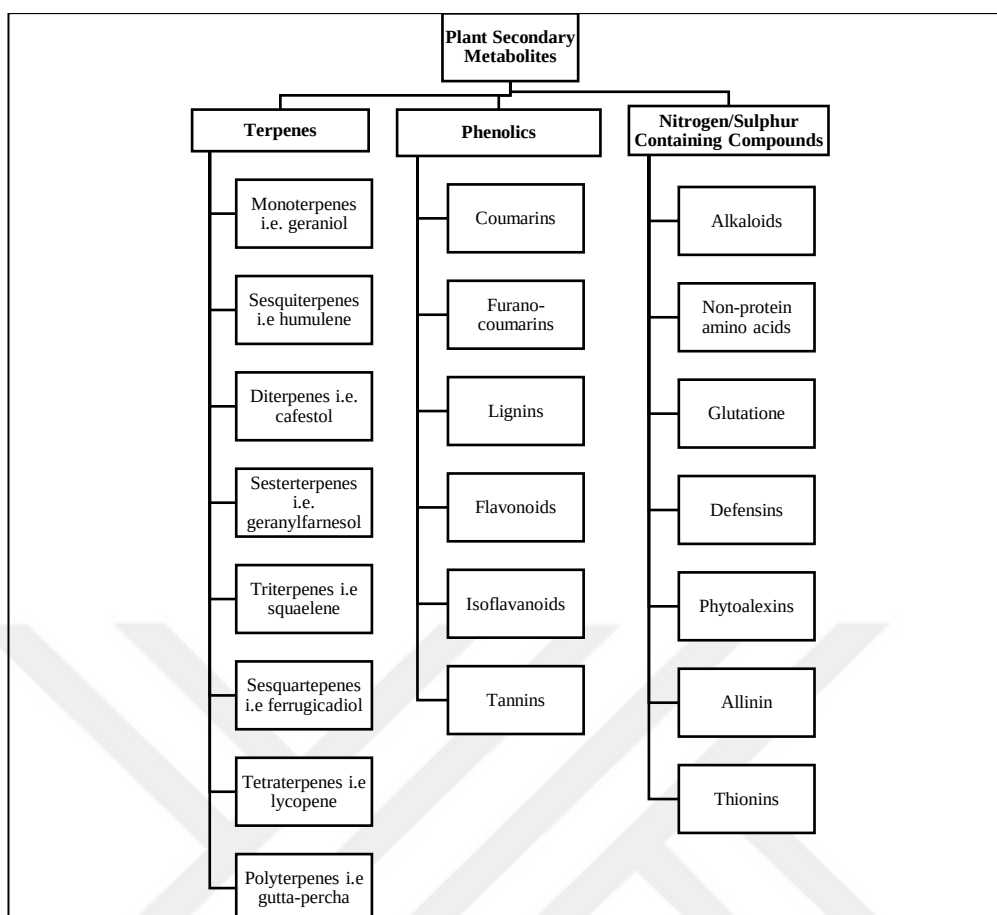


Figure 2.1: Classification of Plant Secondary Metabolites, potential natural preservatives [Twaij et al., 2022].

2.4.2. The Extraction Methods of Plants

Extraction is the primary process of the procedure for obtaining active molecules from plants. According to the extraction principal methods, the extraction can be divided into solvent extraction, distillation, pressing, and sublimation. Due to its ease of use, solvent extraction is the most used method for extraction [Abubakar et al., 2020, Zhang et al., 2018, Handa et al., 2008].

One of the simple methods of extraction is maceration, due to its long extraction time and low efficiency of the desired bioactive molecules, novel technological extraction methods have been developed. Cujic et al., [2016] have demonstrated the high yield of total phenols from chokeberry fruits by maceration, with 50% ethanol.

Soxhlet extraction method has advantages like less processing time and presenting more efficiency than the maceration method. Since it is an automatized continuous extraction, it continuously extracts the compounds from the plant materials

by using a fresh solvent. On the other hand, the Soxhlet extraction method can increase thermal degradation due to the use of high temperatures and long extraction time [Abubakar et al., 2020, Azwanida et al., 2015].

Ultrasound extraction allows for faster extraction and a low-cost extraction process. In addition, ultrasound extraction is widely used in combination with other methods to increase extraction efficiency. [Bakirtzi et al., 2016, Santos et al., 2014]

Microwave-assisted extraction (MAE) generates heat which induces the efficiency of extraction and increases the yield of extract from the plant materials. MAE is also complying with the green technology extractions, which ensures the reduction of energy consumption and high yield and quality of the plants [Du et al., 2009, Routray et al., 2011, Bagade et al., 2019].

2.5. ROSEHIP

2.5.1. Characteristics of Rosehip

Rosehip (*Rosa canina* L). berry-like fruits belong to the Rosa genus in the Rosaceae family. The Rosa genus which generally grown in Central Europe, Africa, and Western Asia [Dąbrowska et al. 2019]. Rosehip is well known in Turkey with different names, such as yabangülü, şillan, gül elması [Rehder et al., 1949]. Especially endemic species of rosehip are grown in Tokat and Gümüşhane, Turkey. Since rosehip bushes are rooted to 4 m under the soil, the plant is resistant to heat and tough environmental conditions. [Guler and Ercisli et al., 1996] The bush type of *Rosa canina* L. can be high as 3.5 m and is also seen in different leaf types, the *Rosa canina* L. bushes are grown in cliffs and forested areas [Oz et al., 2018]. The taxonomical information of *Rosa canina* L. is listed as [PFAF Plant Database, 2022]:

The taxonomy of *Rosa canina* L.

Superorder: Rosanae

Order: Rosales

Family: Rosaceae-roses

Genus: Rosa L. -wild rose

Species: *Rosa canina* L.

Rosa canina L. contains a wide range of vitamins and phytochemicals such as anthocyanin, flavonoids, phenolic acids, tannins, organic acids, lipids, quercetin, B, C, and E vitamins, carotenoids, etc.

Most of the rosehip research mainly focuses on the oil content and fatty acid composition of rosehip species [Celik et al., 2010, Kazaz et al., 2009, Ercisli et al., 2007, Yilmaz et al., 2011]. According to several studies, *R. canina* L contains linoleic acid as the main fatty acid by up to 33-55% and oleic acid in the range of 12-34% [Celik et al., 2010, Kazaz et al., 2009, Nowak et al., 2005, Adamczak et al., 2011, Sharma et al., 2012].

2.5.2. Usage of Rosehip

Rosehip, a medicinal plant, is generally used medically, cosmetically, and for food purposes. [Ayati et al., 2018; Patel, 2017] In traditional medicines rosehip was used for preventing influenza, inflammation, acne, chronic pain, wound healing, etc. as a marmalade, an herbal tea, or powder forms [Ahmad et al., 2016, Dąbrowska et al., 2019]. In addition to traditional medicine, rosehip was studied from a scientific perspective. Studies showed that rosehip has anti-inflammatory activity in muscles [Chrubasik-Hausmann et al., 2014, Rein et al., 2004], anticancer activity, anti-cardiac activity [Rein et al., 2004, Fu et al., 2006], antioxidant activity [Jemaa et al., 2017], antimicrobial and antifungal activities on *Helicobacter pylori*, *E. coli*, *L. monocytogenes*, *C. albicans*, *S. aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Acinetobacter baylyi*, *A. niger*, and *Fusarium culmorum* and *Alternaria alternata* species [Montazeri et al., 2011, Živković et al., 2015, Ghendov-Moşanu et al., 2018, Živković et al., 2016, Rovna et al., 2015]. Martinez-Romero et al., [2019] also evaluated that rosehip oils can be used for the preservation of plum cultivars increasing 20 days the shelf life of fruits after post-harvesting. Moreover, due to its health-promoting activity, the rosehip and its components are used as an ingredient in soups, yogurts, probiotics, and drinks [Gao et al., 2000; Chrubasik et al., 2008].

3. MATERIAL AND METHODS

3.1. Materials

In this thesis, agar, Luria Bertani Broth (LB), Tryptic Soy Broth (TSB), Potato Dextrose Broth [PDB], Yeast Extract Dextrose Agar (YPDA), and ethanol (EtOH) were obtained from HiMedia (Mumbai, India) and Alkomed (Plovdiv, Bulgaria). The 96-well microplates were obtained from TPP (Darmstadt, Germany).

The dried rosehip fruits were obtained from a local shop in Istanbul, Turkey.

3.1.1. Chemicals

A culinary grinder (Sinbo, SCM-2934, Düzce, Turkey) was used to pulverize the dried rosehip fruit. Extraction was done in a microwave (Arçelik MD-524S, Istanbul, Turkey) and water bath (Isolab, Ultrasonic Cleaner 621.05.001, Bavaria, Germany), and solids separation was done with a centrifuge device (Gyrozen 1248R, Daejeon, Korea). Removal of solvent and excess water in the extracts was carried out using a rotary evaporator (Heidolph Hei-VAP, Bavaria, Germany).

A precision balance (Kern, Balingen, Germany) was used to weigh the powdered rosehip and media contents, and a pH meter (Hanna Instruments HI 2211 pH/ORP, Bedfordshire, England) was used to measure the pH of the media and extracts. Sugar analysis of the extracts was performed with an HPLC device (Shimadzu, Kyoto, Japan) containing the Refractive Index (RID-10A, Shimadzu, Kyoto, Japan) detector.

A heated magnetic stirrer (IKA RH basic 2, Deutschland, Germany) was used during the preparation of the medium. Bacterial cells were incubated under suitable conditions using a shaker incubator (New Brunswick Innova 42, Hamburg, Germany). A spectrophotometer (Shimadzu UV-1280, Duisburg, Germany) was used for the optical density of the growing bacterial cultures and the total phenolic content of the extracts. A vortex (IKA Vortex Genius 3, Deutschland, Germany) was used to spread the bacteria that settled to the bottom of the container evenly in the medium and to distribute the extracts evenly before use. During the biofilm formation stage, an oven [Thermo Scientific Heratherm IGS 60, Langenselbold, Germany] was used as an incubator for culture growth, and an ELISA reader [BMG LABTECH FLUOstar

Omega, Offenburg, Germany] was used to measure the absorbance value of biofilm formation at 570 nm. All test materials were cleaned with the help of an autoclave device (Nüve, OT 90L, Akyurt, Ankara, Turkey) before the experiment.

3.1.2. Microorganisms

All bacteria strains, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* KUEN 133, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Penicillium italicum*, and fungi, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 1001 were obtained from Nanomik Biotechnology Co. Turkey.

3.2. Methods

3.2.1. Sterilization of Media

The media were sterilized at 121 °C for 20 minutes in an autoclave (Nüve, OT 90L, Akyurt, Ankara) and stored at 4 °C until use after cooling at room temperature. The sugar content of the media was autoclaved separately. When used, the medium and sugar content were combined under sterile conditions. The media containing agar were transferred to Petri dishes in 17 mL volume under sterile conditions. After the solidification of the media was completed, it was stored at 4 °C until the time of use.

3.2.2. Storage of Media

Bacterial strains were inoculated on sterile Luria Bertani Agar medium [LBA, containing 15 g/L agar] and yeast strains were inoculated on sterile YPDA, filamentous fungi strains were inoculated onto PDA, using the streak plate method with the help of loops. Petri dishes containing microorganisms were incubated overnight for bacteria and yeast, and overnight incubated at 28 °C for fungi in the incubator [Thermo Scientific Heratherm IGS 60, Langenselbold, Germany]. Fresh cultures were stored at +4 °C and subcultured weekly.

3.2.3. The Preparation of Rosehip Extracts (RFE)

The microwave-assisted extraction process from rosehip [*Rosa canina L.*] fruits was carried out according to Rovna *et al.* 2015. The rosehip fruits were bought from a local shop and purged from seed and fruit dust, then the fruits were rounded. For the extraction, 10g powder of rosehip and 50% ethanol as 1:10 [w/v] were mixed and exposed to 120W, 3min microwave. [Arçelik, MD574S Microwave] The extracts were centrifugated at 4000 rpm x 15 min x +4°C. The supernatants were collected and evaporated [Heidolph Rotary Evaporator-Core] at 100 rpm, 55°C, and 60 min. The extracts are stored at -20 °C for further usage.

The stages of extraction, analytical methods, and efficacies of rosehip extract were summarized in Figure 3.2.

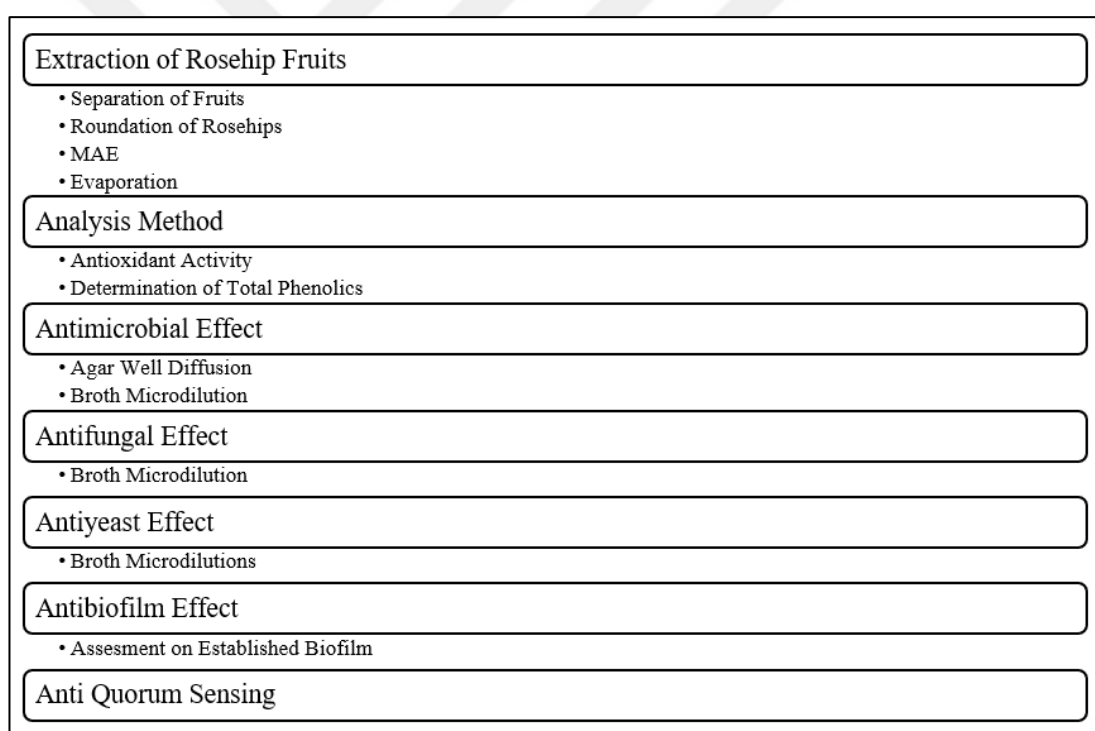


Figure 3.2: The stages of extraction, analytical methods, and effects of the rosehip extract.

3.2.4. Antioxidant Activity and Total Phenolic Content (TPC) Analysis of Rosehip Extracts

The antioxidant activity [DPPH] scavenging activity measurements were optimized from the Brand-William method using DPPH. The 100 μ L plant extracts were mixed with 200 μ L DPPH [50 ppm] in a 96-well plate. The plate was incubated at 25°C, 120 rpm, and 20min. After the incubation, the absorbance value was measured at 515nm. The antioxidant activity was calculated with the following formula:

$$\text{Antioxidant Capacity Percentage (\%)} = \left[\frac{AC - AS}{AC} \right] \times 100 \quad (3.1)$$

AS indicates the absorbance value of the sample; AC indicates the absorbance value of control (DPPH), [Saeed et al., 2012].

The total phenolic content was determined with the Folin-Ciocalteu assay according to Singleton and Rossi et al., [1965]. The Folin-Ciocalteu method determines phenolic content. The extraction solvent choice affects both the yield of plant extract and overall phenolic content of plant extracts. The calibration curve was done using different concentrations of gallic acid i.e., 0- 0.5mM. Briefly, 500 μ L of extracts and 2.5 mL of Folin-Ciocalteu reagent (1:1 v/v with water) were added and incubated at 5 min. then vortexed. The solution was left for 5 min and then 2 mL of Na₂CO₃ was added to the mixture. The whole mixture was incubated for 90 min at room temperature. Absorbance was measured with a UV–Vis spectrophotometer at 765 nm. The total phenolic content was calculated as mM gallic acid equivalent (mM GAE) by using the gallic acid calibration curve.

3.2.5. Antimicrobial Activity of Rosehip Extracts (Agar Well Diffusion and Broth Microdilution)

Agar well diffusion assay protocol was examined according to Balaban et al. 2021. All the microorganisms were overnight incubated in Tryptic Soy Broth. The grown cultures were diluted in physiologic saline solution till reached 0.5 MF $\cong$ 1 x 10⁸ CFU/mL (OD₆₀₀= 0.08-0.1). The diluted bacteria solutions were spread onto LBA media. A well for each plate was prepared in the plates with the help of a cork borer (8 mm). The serial dilutions of rosehip fruit extract are loaded into the wells as 40 μ L

and plates were incubated overnight. Microbial growth was investigated by measuring the diameter of the zone of inhibition of bacteria. The results were obtained with zone diameters. The experiment was done five times to ensure.

The rosehip extract that previously showed antimicrobial activity was investigated with determination of MIC and MBC by the broth microdilution method against the same foodborne pathogen microorganisms. MIC-MBC test protocols were performed according to Adnan et., al 2020. The bacterial inoculations were adjusted to 0.5 MacFarland turbidity in 96 well microtitre plates. The 100µg/mL of Gentamicin was used as a negative control. The plates were incubated at 24h and 37 °C, and during incubation in the absorbance value of the plate was recorded every 5 min, at OD₆₂₀ nm. By subculturing 100 L from each negative well and the growth control onto a nutrient agar plate, minimum bactericidal concentrations were found. The minimum inhibitory concentration was determined as the lowest concentration of each extract at which no growth could be seen (MIC). However, MBCs were described as the lowest concentration of the extract that could kill 99.9% of the strains. The growth inhibition percentage was determined with the following formula [Rekha et al., 2018]:

$$\text{Percentage of Growth Inhibition} = \frac{(\text{OD Experiment} - \text{OD of Sample Control})}{\text{OD Negative Control}} \times 100 \quad (3.2)$$

OD Experiment indicates the absorbance value of the sample; OD of sample control indicates the absorbance value of sample control [only extract containing wells], OD Negative control indicates the Negative control, the well which contains only medium. [Rekha et al., 2018].

3.2.6. Antifungal Activity of Rosehip Extract

The activity of plant extract against fungi was investigated against *Penicillium italicum* and *Aspergillus niger* ATCC 16404 was determined with broth microdilution assay in 96-well plates with some modifications [Perimal et al., 2012].

3.2.7. Preparation of antifungal plant extract

200 µL of a plant extract and PDB were prepared in the first well of the column in a 96-well microtitre plate. 100 µL PDB was added to the rest of the wells of the 96-well plate, and 100 µL mixed solution was transferred from the first well to the second one and so on (to obtain serial dilution of plant extract).

3.2.8. Preparation of spore suspension

For fungal bioassays, the fungi species were cultivated in PDA and incubated at 28°C for 7 days. The spores were harvested by scraping the sporulating colonies and suspended in sterile distilled water containing 1.0% Tween80 (v/v aqueous solution, as wetting agent) [Hicks, et al., 2001]. The content of suspension tubes was filtered with a Whatman filter model 40 (pore size 8µm) which keeps hyphae and allows transfers only of filamentous fungi spores. The densities of suspensions were adjusted with a spectrophotometer (Biotek, Epoch 2) at 520nm wavelength to a transmittance level of 78-82% and were diluted 1:50 with PDB-broth medium.

3.2.9. Experimental Procedure

The spore suspension (10^5 CFU/mL) was added to each well and mixed thoroughly to obtain final concentrations of plant extract ranging from, 31.26-1000 mg/mL extract concentration. The 96-well plates were covered and incubated at 28 °C for 72 h. Experiments of each sample were carried out in triplicate. PDB mixed with the conidial suspension was used as the negative control, while the blank included only the PDB medium. Amphotericin B (50µg/mL) antibiotic was used as a positive control. The minimum inhibitory concentration (MIC) was described as the lowest concentration showing no visual growth of mould. 100 µL of the mixture from the well with no visual growth of the fungus was inoculated onto a PDA plate and cultured for 72 h. The minimum fungicidal concentration (MFC) was described as the lowest concentration showing no fungal growth on the PDA plate.

3.2.10. Anti-Yeast Activity of Rosehip Extract

The MIC and MFC methods were performed by the broth microdilution method [CLSI, 2006; Krishnamoorthy et al., 2020]. 200 µL of a plant extract and YPD were prepared in the first well of the microtitration plate column. 100 µL YPD was added to the rest of the wells of the 96-well plate, and 100 µL mixed solution was transferred from the first well to the second one and so on to obtain serial dilution of plant extract. The Mid-log-phase of *Candida* strains adjusted to the final inoculum

0.5×10^3 - 2.5×10^5 cells/mL. The 96-well plates were incubated overnight at 37 °C. Amphotericin B (50µg/mL) antibiotic was used as a positive control.

3.2.11. Assessment of Established Biofilms of Rosehip Extracts

This protocol was performed according to an established method by Şar et al. 2018. Biofilms of all bacterial strains were formed on a 96-well microtitre plate (polystyrene surface plate) filled with TSB (2% glucose) and cells for 72h at 37 °C. After 72h of incubation, the planktonic cells were removed, and the wells will be washed three times with ultrapure water. The crude extract (at MIC value) as 200 µL was added to wells and incubated for 3 to 5 min at 37 °C. The plate was stained with 200 µl crystal violet [0.5%] for 20 min. Then, the cells were decanted and treated with ethanol: acetic acid for 10 min. The biofilm removal of plant extract was measured at 570 nm. (As 100 µl)

$$\text{Biofilm Removal Percentage (\%)} = \frac{(OD_{NC} - OD_{PC}) - [OD_S - OD_{NC}]}{[OD_{NC} - OD_{PC}]} \times 100 \quad (3.3)$$

OD NC is the mean absorbance of the negative control, OD PC is the mean absorbance of the positive control, and the OD S is the mean absorbance of the extracts [Balaban et al., 2021].

3.2.12. Anti-Quorum Sensing Activity of Rosehip Extracts

Anti-quorum sensing activity was studied as the control of violacein pigment. 100 μ L of stock *Chromobacterium violaceum* Bergonzini ATCC 12472 strain was overnight incubated at 30°C, for 24-30h in LB Broth medium. After incubation 100 μ L of microorganisms are mixed with the LBA medium by pour plate method. Moreover, 100 μ L of microorganisms are spread on the LBA plates and the wells on each plate were prepared with the help of a sterile cork borer (7 mm). The diluted extract solutions were added to the wells as 40 μ L. The plates were incubated at 30°C for 24h. Microbial growth was determined by measuring the diameter of the zone of inhibition. The result was obtained by measuring the zone diameter.

3.2.13. Statistical Analysis

In this thesis study, all experiments were repeated at least three times. Statistical analyzes were determined with Microsoft Office, Excel 2019 program. The p-values were determined by comparing the antimicrobial effects of rosehip extracts with gentamicin antibiotics, and the removal efficiency of biofilm formation with the application of chlorine solution by a one-tailed t-test.

4. RESULTS

4.1. The Antioxidant Activity and Total Phenolic Content of Rosehip Extracts

The antioxidant (DPPH scavenging) activities of rosehip extracts were performed for each serial dilution from 1/2 to 1/64. ($p < 0.05$) The antioxidant activity of 1/2 diluted extract was measured as 100%, while the most diluted extract's activity was 83% was shown in Table 4.1. It was detected that the antioxidant activity of 1/32 diluted extracts activity was higher than the gallic acid solution was shown in Table 4.1.

Table 4.1: The dry weight of rosehip extract with dilutions and their DPPH scavenging activities (%).

Dry weight of extract [mg/mL] (dilution rate)	Antioxidant Activity (%)
1.125 [1/2]	100
0.562 [1/4]	100
0.280 [1/8]	100
0.140 [1/16]	100
0.070 [1/32]	100
0.035 [1/64]	83

The total phenolic acid concentration of the extract was investigated according to the Folin Ciocalteu assay according to Singleton and Rossi et al., [1965]. The total phenolic acid of fruit extract was determined as 122 mg GAE/g.

4.2. The Antimicrobial Activity of Rosehip Extract against Food-Borne Pathogens

The antimicrobial activities of rosehip fruit extract with different dilutions were evaluated on both agar well diffusion and broth microdilution methods against *Escherichia coli* ATCC 25922, *Listeria monocytogenes* KUEN 133, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 25923 according to Adnan et al.,

2020. The experiments were replicated at least 3 times. The visual representation of agar well diffusion is shown in Figure 4.1., each bacterium was cultivated on different plates, and the activities of six serial dilutions of rosehip extract were examined for 4 different bacterial strains.

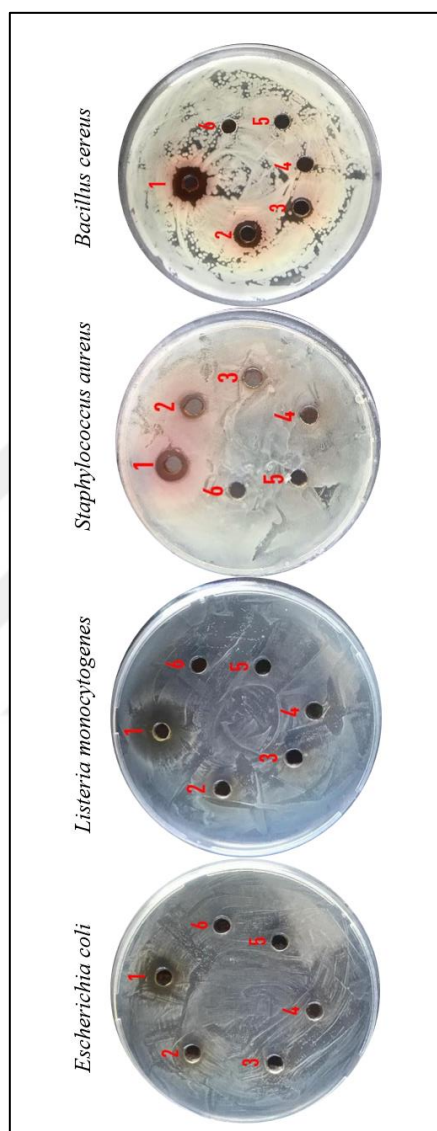


Figure 4.1: The Agar Well Diffusion Visuals of Antimicrobial Activity of Rosehip Extract against *E. coli*, *L. monocytogenes*, *B. cereus*, and *S. aureus*. (1-6 numbers indicate the serial dilutions to 1-1/32).

According to zones of bacterial growth inhibitions (mm), the antibacterial activity of rosehip extract was significantly effective as Gentamicin against *L. monocytogenes* KUEN 133 and *E. coli* ATCC 25922 measured as $14,3 \text{ mm} \pm 1.15$, $12 \text{ mm} \pm 0.71$ respectively was shown in Table 4.2.

Except for *B. cereus* ATCC 11778, against three foodborne bacteria, the inhibition zones were not observed in lower serial dilutions from 1/4 to 1/32 as shown in Table 4.2.

Table 4.2: The measured inhibition zone diameters on pathogenic bacteria by rosehip extract with different dilutions according to the agar well diffusion method.

Microorganism Name / Extract Dilutions	Inhibition zones [mm]			
	<i>Escherichia coli</i> ATCC 25922	<i>Listeria monocytogenes</i> KUEN 133	<i>Staphylococcus aureus</i> ATCC 25923	<i>Bacillus cereus</i> ATCC 11778
1	10 ± 0	14,3 ± 1.15	10 ± 0	12 ± 0.71
1/2	8 ± 0	10 ± 0	8 ± 0	10 ± 0
1/4	NI	NI	NI	8.66 ± 0.57
1/8	NI	NI	NI	NI
1/16	NI	NI	NI	NI
1/32	NI	NI	NI	NI
Gentamicin	10 ± 0	15 ± 0	20 ± 0	20 ± 0

** NI indicates, not identified, ± standard deviations of three experiments, for each replicate, the diameter of the agar well is 5 mm.

Broth microdilution assay determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Escherichia coli* ATCC 25922, *Listeria monocytogenes* KUEN 133, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 25923. The gentamicin antibiotic was used as a negative control group, the experiment was performed with 96-well plates, and the absorbance value was measured every 5 min, 24h at OD₆₂₀.

The Drying Process for the rosehip plant extract was done at 60 °C For, 3days. After drying 4.9g extract was obtained, and the mg/mL calculations were done for a dry extract for this evaluation.

According to MIC-MBC values of rosehip extract against foodborne pathogenic bacteria, the MIC value is around 0.07-0.280 mg/mL of the extract concentration. Despite the MIC values, the MBC values of the extract are around 2.25 mg/mL

concentration for 99.9% growth inhibition was shown in Table 4.3, Figures 4.2 to Table 4.6. The dry weight of rosehip extract with dilutions was presented in Table 4.3.

Table 4.3: The dry weight of rosehip extract with dilutions.

Dilution rate of rosehip extract	Dry weight of rosehip extract [mg/mL]
1	2.25
1/2	1.125
1/4	0.562
1/8	0.280
1/16	0.140
1/32	0.07
1/64	0.035

Table 4.4: MIC and MBC values of rosehip extract against foodborne pathogenic bacteria.

Microorganism Name	<i>Escherichia coli</i> ATCC 25922	<i>Listeria monocytogenes</i> KUEN 133	<i>Staphylococcus aureus</i> ATCC 25923	<i>Bacillus cereus</i> ATCC 11778
MIC	0.280 mg/mL	0.280 mg/mL	0.07 mg/mL	0.140 mg/mL
MBC	2.25 mg/mL	2.25 mg/mL	2.25 mg/mL	2.25 mg/mL

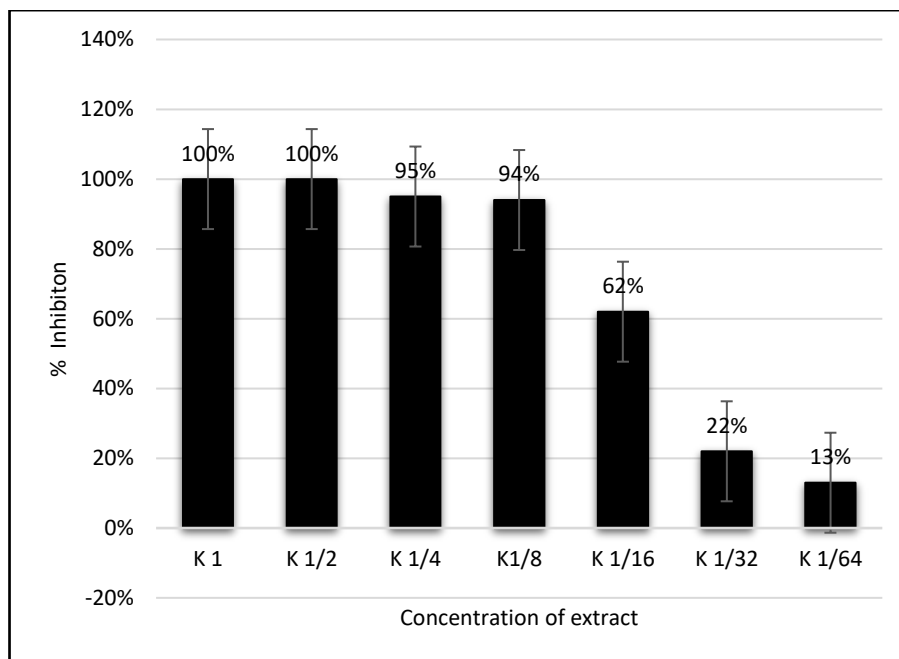


Figure 4.1: The Inhibitions (%) obtained by Rosehip Extract with different dilutions [1- 1/64] on *E. coli*.

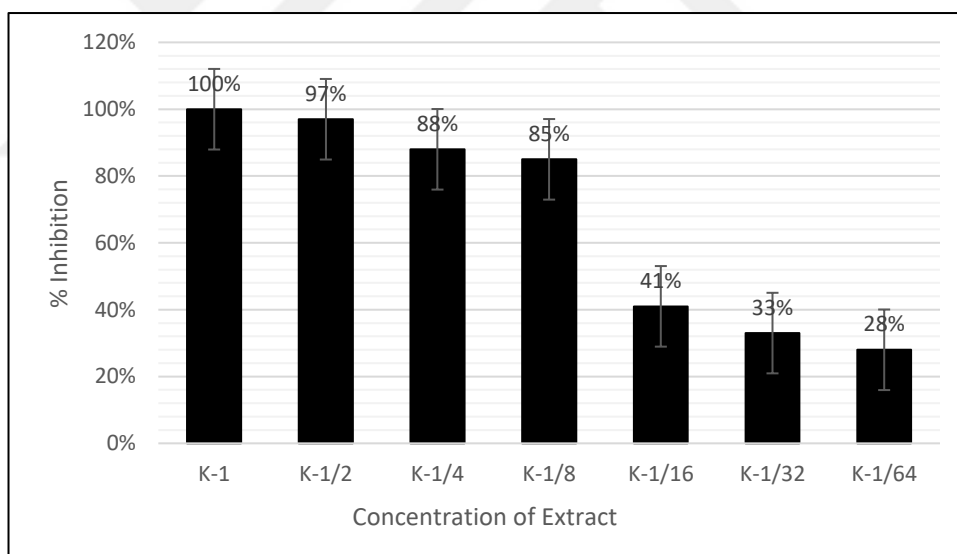


Figure 4.2: The Inhibitions (%) obtained by Rosehip Extract with different dilutions [1- 1/64] on *L. monocytogenes*.

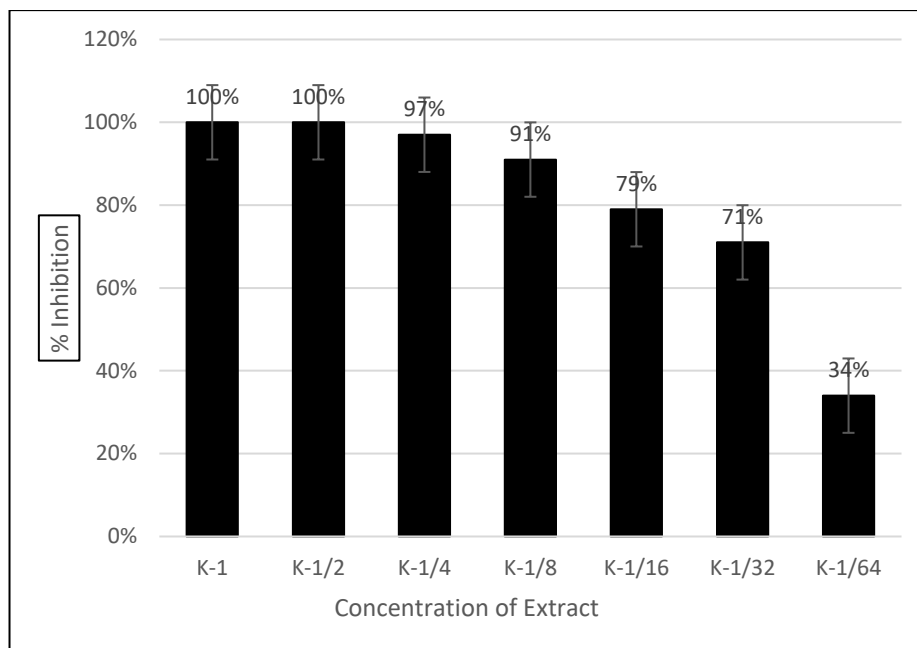


Figure 4.3: The Inhibitions (%) obtained by Rosehip Extract with different dilutions [1- 1/64] on *S. aureus*.

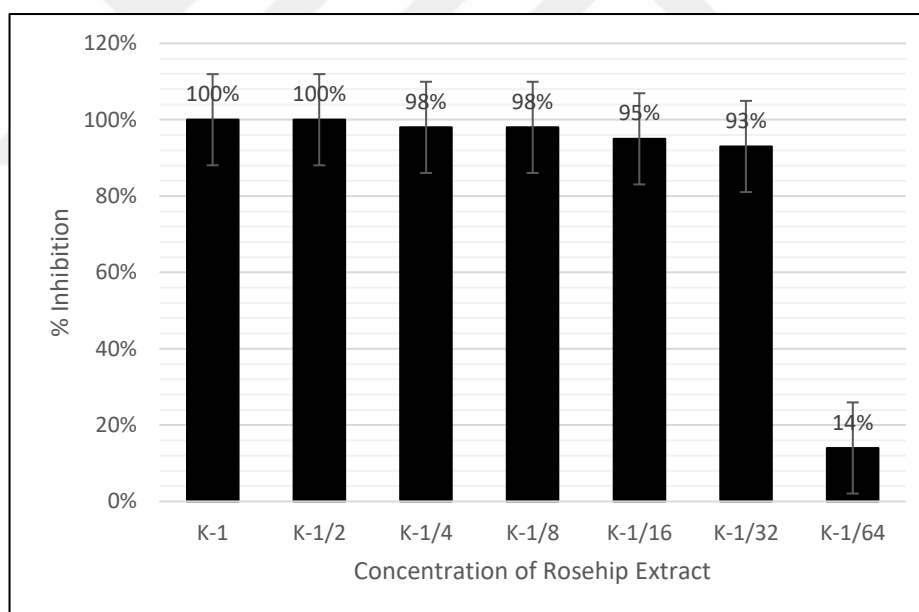


Figure 4.4: The Inhibitions (%) obtained by Rosehip Extract with different dilutions [1- 1/64] on *B. cereus*.

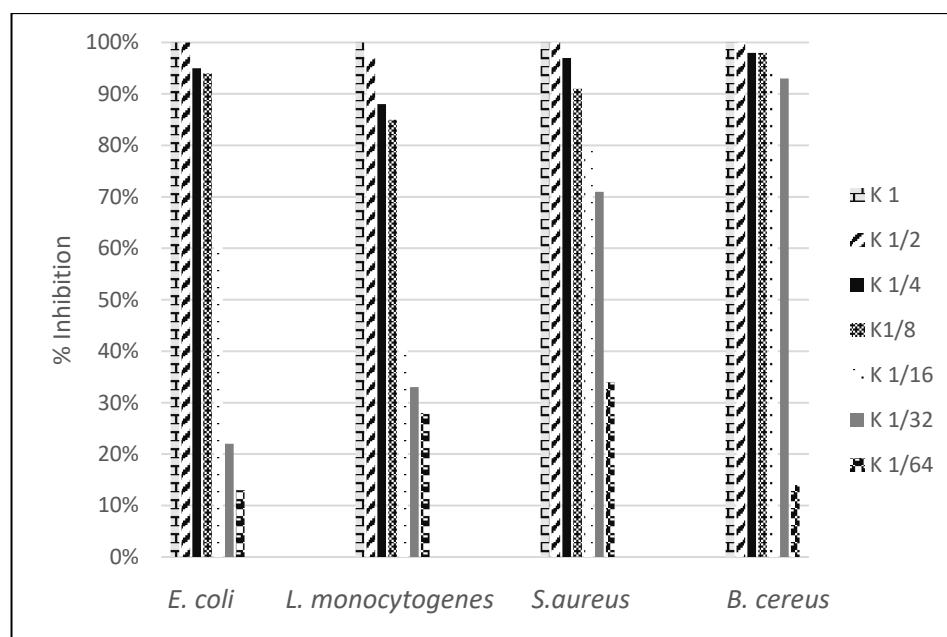


Figure 4.5: The Inhibitions obtained by rosehip extract with different dilutions (K-1, K-2, K-4, K-8, K-16, K-32) on foodborne pathogenic bacteria.

4.3. The Antifungal & Antiyeast Activity of Rosehip Extract

The antifungal activity of the extract was determined according to Perimal et al. 2012 with slight modifications. Amphotericin B (50µg/mL) antibiotic was used as a positive control. The MIC value was defined as the lowest concentration showing no visual growth of mould. The lowest concentration of the extract required to prevent fungal growth on plate is known as the minimum fungicidal concentration (MFC).

According to experiments, the MAE-based rosehip extract did not show antifungal activity against *Penicillium italicum* and *Aspergillus niger* strains was shown in Figure 4.6.

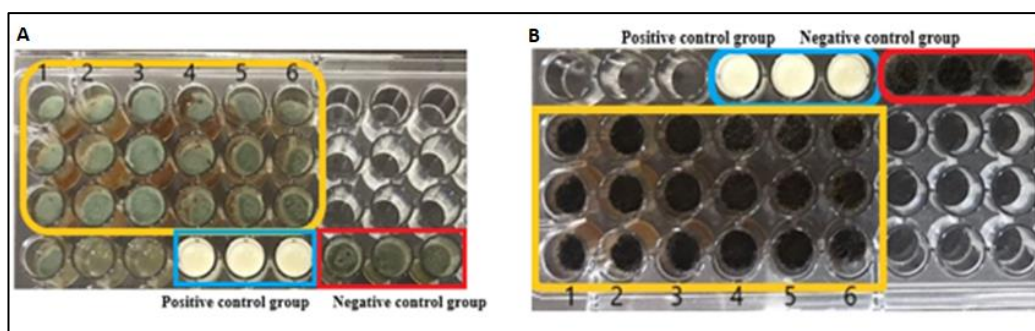


Figure 4.6: The antifungal activity of rosehip extract with different dilutions against A: *Penicillium italicum*, B: *Aspergillus niger* strains. Amphotericin B was used as positive control while media was used as negative control. The wells of 1-6 were dilutions of the rosehip extract ranged from 1-1/32.

According to results of anti-yeast activity, rosehip fruit extract does not show 100% inhibition against *C. albicans*, only showed 50% growth inhibition. The anti-yeast activity of rosehip extract against *C. albicans*, and the yeast growth inhibition against *C. kruseii* in the rosehip extract showed 90% inhibition at 0.28mg/mL and 100% inhibition at 1.125 mg/mL was shown in Table 4.4 and Figure 4.7, serial dilutions were performed as antimicrobial assay against bacteria.

Table 4.5: MIC and MFC values of rosehip extract against *C. albicans* and *C. kruseii* strains.

Microorganism	MIC	MFC
<i>Candida albicans</i>	2.25mg/mL	ND*
<i>Candida kruseii</i>	0.07mg/mL	0.562 mg/mL

* ND indicates, Not Determined. The results are presented as means $\pm$ standard deviations of three experiments.

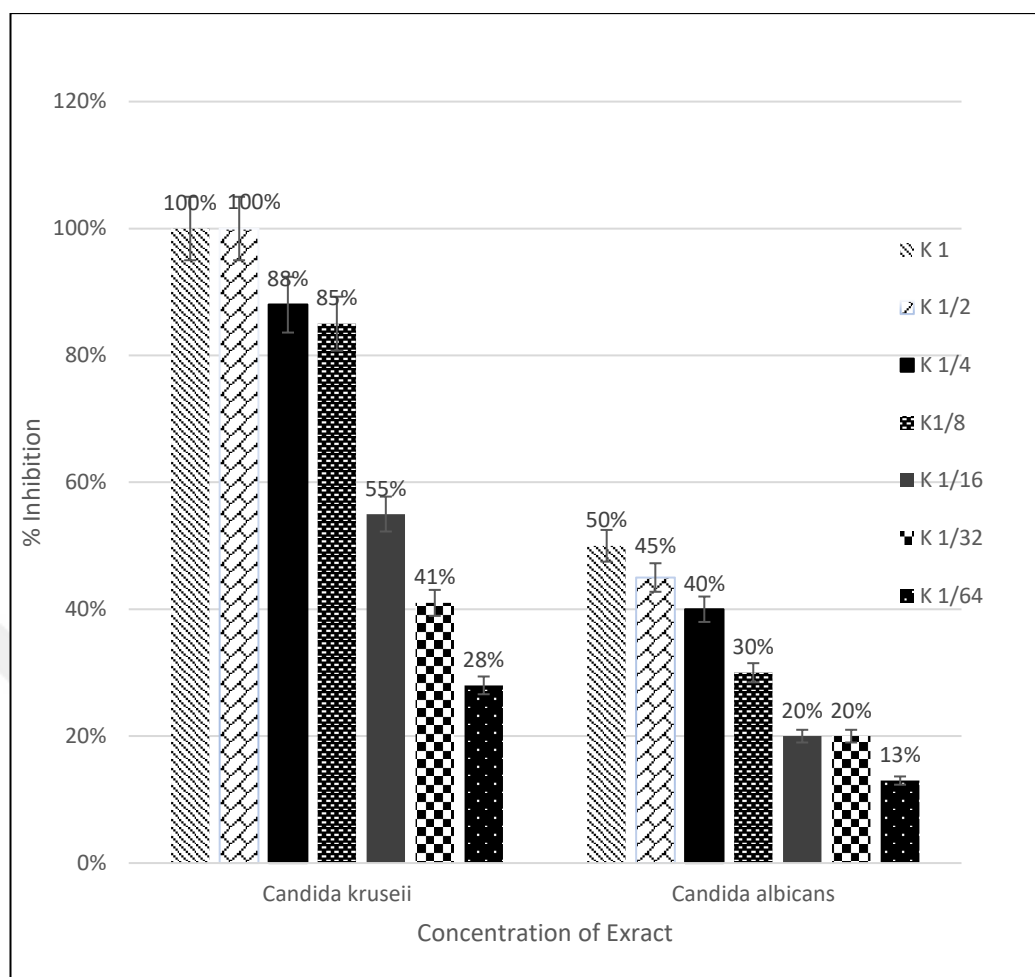


Figure 4.7: The Inhibitions obtained by rosehip extract with different dilutions [K-1, K-2, K-4, K-8, K-16, K-32] on pathogenic yeasts.

4.4. The Antibiofilm Activity of Rosehip Extract Against Foodborne Bacteria

The antibiofilm activity of rosehip extracts on the 72h biofilms did not show significant activity against *E. coli*, *L. monocytogenes*, and *S. aureus*. Moreover, even 2x MIC dilutions did not remove biofilms except for *B. cereus* was shown in Table 4.6.

In, 24h biofilm tests, the rosehip extract showed 49% antibiofilm activity against *E. coli*, 37% against *L. monocytogenes*, 66% against *S. aureus* and 67% against *B. cereus* biofilms. The activity of antibiotic (Gentamicin) showed the highest antibiofilm activities was shown in Table 4.7.

Table 4.6: The biofilm (72h) removal rates (%) of rosehip extract against *E. coli*, *L. monocytogenes*, *S. aureus* and *B. cereus* biofilms.

Extract Concentration	Biofilm removal (%)			
	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>B. cereus</i>
1.125 mg/mL [1/2]	0	0	0	100
0.5625 mg/mL [1/4]	0	0	0	100
0.281 mg/mL [1/8]	0	0	0	100
Gentamicin [100 µg/mL]	70	75	73	100

Table 4.7: The biofilm [24h] removal rates (%) of rosehip extract against *E. coli*, *L. monocytogenes*, *S. aureus* and *B. cereus* biofilms.

Extract Concentration	Biofilm removal (%)			
	<i>E. coli</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>B. cereus</i>
1.125 mg/mL [1/2]	49	37	66	67
0.5625 mg/mL [1/4]	36	25	57	13
0.281 mg/mL [1/8]	24	0	0	0
0.140 mg/mL [1/16]	15	0	0	0
Gentamicin [100 µg/mL]	95	79	85	100

4.5. Anti-Quorum Sensing Activity of Rosehip Extract

Anti-quorum sensing activity was studied as the control of violacein pigment of *Chromobacterium violaceum* Bergonzini ATCC 12472 strain. According to the results of pour plate and streak plate methods, the rosehip extract had inhibitory activity on quorum sensing on only at 1/2 dilution ratio of the extract. In addition, the zone

diameter was significantly in the pour plate method was larger ($14\text{mm} \pm 2$) than that of obtained by streak plate method, $12\text{mm} \pm 1.5$ was shown in Figure 4.9.

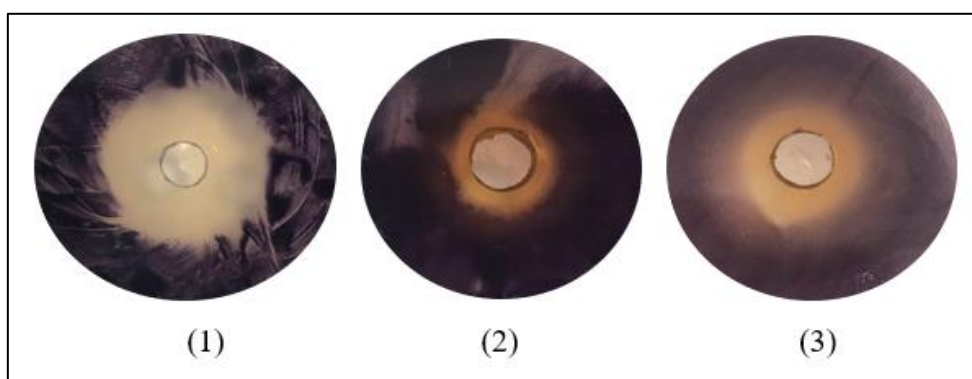


Figure 4.9: The Anti-Quorum Sensing activity of Rosehip Extract by pour plate and streak plate method. (1), inhibition zone obtained by the control group, gentamicin. (2) inhibition zone obtained by streak plate method by using rosehip extract with 1/2 dilution and [3] inhibition zone obtained by pour plate method by using rosehip extract with 1/2 dilution.

5. DISCUSSION AND CONCLUSION

According to the World Health Organization's data every year, almost 10% of people suffer from foodborne illness, especially children [WHO, Fact Sheets, Food Safety, 2021]. Pathogenic bacteria are one of the biggest problems in the food industry due to the insufficient and expensive preservation methods for the preparation of foodstuffs [Barth et al., 2010, Qadri et al., 2015, FDA, Foodborne Illness and Contaminants, 2016]. Therefore, researchers mainly focus on the natural bioactive components that could be used as a preservative in the food industry. One of these potential natural preservative sources among the plants, rosehip [*Rosa canina* L.] is pseudo fruit that is widely found and used in Central Europe, for ethnomedical purposes.

According to the literature, different *Rosa canina* L. extracts had been studied for their antimicrobial, and antioxidant properties [Concha et al., 2006, Živković et al., 2015, Ghendov-Mošanu et al., 2018]. The characterization of bioactive compounds of *Rosa canina* L. had been studied. [Ercisli 2007, Ilyasoglu et al., 2013, Murathan et. al., 2018, Turan et al., 2018, Guney et al., 2020]. However, the extraction of bioactive compounds from *Rosa canina* L. pseudo fruits with microwave-assisted extraction [MAE] method and the effect of this extract on rosehipfoodborne pathogenic bacteria has not been studied yet.

In this thesis study, the antimicrobial and antibiofilm activities of MAE-based rosehip extract were investigated on *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* KUEN 133, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Penicillium italicum* wild type (isolated from a tangerine, Mersin/Turkey), and *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 1001 strains. The antioxidant and total phenolic activity of the MAE-based rosehip extract was also investigated. In addition, the anti-quorum sensing activity of rosehip extract was investigated for the first time.

The MAE-based extracts of the rosehip showed 100% activity as a possible antioxidant agent with a higher antioxidant capacity which correlates to the findings of Ghendov-Mošanu et al., 2018 and Fascella et al., 2019. In both studies, the antioxidant capacity of *Rosa canina* L. extract has been found 80% with Brand-

William Method. Therefore, we assume that the MAE-based extraction can reveal the capacity of antioxidants in rosehip fruits.

The total phenolic content (TPC) of MAE-based rosehip fruit extracts was determined as 122 mg GAE/g. According to different research articles, the amount of total phenolic compounds (TPC) of rosehip extracts can be varied by solvent type and amount of water. [Okan et al., 2019, Tahirovic and Basic et al., 2017, İlbay et al., 2013]. Specifically, İlbay et al., 2013 pointed out that the method of extraction can increase the amount of TPC from 7.54 mg GAE/g [100% ethanol-based extraction] to 48.59 mg GAE/g [100% water-based extraction]. Therefore, the extraction method and solvent type diversity can affect the antioxidant capacity and total phenolic content of the rosehip fruits [Koczka et al., 2018; Demir et al., 2014; İlbay et al., 2013].

The antimicrobial activities of rosehip extract with dilutions were investigated against foodborne pathogenic bacteria (*Escherichia coli* ATCC 25922, *Listeria monocytogenes* KUEN 133, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 25923) by using both agar-well diffusion assay and broth microdilution assay. By agar well-diffusion assays the MAE-based direct extract showed a 10 mm zone of inhibition while gentamicin and, 1/2 dilution of the extract showed 10 inhibition zones on the *E. coli* strain, respectively. The higher dilutions of extract (1/4, 1/8, 1/16, and 1/32) showed no inhibition zones on the *E. coli* strain. The direct use of MAE-based extracts showed a 14.3 mm inhibition, and the 1/2 diluted extract showed a 10 mm zone on *L. monocytogenes* strain, whereas the antibiotic gentamicin exerted a 15 mm inhibition zone. For *S. aureus* and *B. cereus* strains, the direct extract application of extract caused 10 mm and 12 mm inhibition zones, respectively. It was determined that the MAE-based extracts showed a similar antimicrobial effect on *E. coli* strain and *L. monocytogenes* strains.

To investigate the antimicrobial and anti-yeast activity of MAE-based rosehip extracts the broth microdilution assay was also performed in 96-well microtiter plates. Since the dilution methods are more efficient to determine the MIC/MBC/MFC values of the extracts in a simple application, dilution methods are also preferred as a fundamental method for antimicrobial activity [Balouiri et al., 2015]. In this thesis, the growth of microorganisms was measured in the 96-well plate for 24h, and the results were interpreted as growth inhibition percentages against four different foodborne bacteria. For the *E. coli* strain, the extract was found 100% effective in both direct and 1/2 dilution concentrations, and the inhibitions of, 95% for 1/4, 94% for 1/8, 62% for

1/16, 22% for 1/32, and 13% for 1/64 dilution concentrations were determined. The MIC value was determined as 1/16 whereas the MBC value was 1/2. For the *L. monocytogenes* strain, the extract was found to be almost 100% effective in both direct and 1/2 diluted extract concentrations and the inhibitions of 88% for 1/4, 85% for 1/8, 41% for 1/16, 33% for 1/32 and 28% for 1/64 dilution concentrations were measured. The MIC value was determined as 1/8 whereas the MBC value was 1/2. For the *S. aureus* strain, the extract was also found as 100% effective in both direct and 1/2 diluted concentrations, and the inhibitions of 97% for 1/4, 91% for 1/8, 79% for 1/16, 71% for 1/32, and 34% for 1/64 dilution concentrations were determined. The MIC value was 1/32 whereas the MBC value was 1/2 diluted extract. For the *B. cereus* strains, the extract was 100% effective in both direct and 1/2 diluted concentrations, and the inhibitions of 98% for 1/4, 98% for 1/8, 95% for 1/16, 93% for 1/32, and 14% for 1/64 dilution concentrations of were obtained. The MIC value was determined as 1/32 whereas the MBC value was 1/2 diluted extract.

For the *Candida albicans* strain, the MIC value was determined as 1/2 whereas the MFC value was not determined. For the *Candida krusei* strain, the MIC value was found as 1/32, whereas the MFC value was 1/4 dilution concentration of extract.

By broth microdilution methods, the 1/2 diluted extract was significantly effective on all four bacteria strains and *C. krusei* strain. The broth microdilution antimicrobial assay results were different from the agar-well diffusion assay results. The reason could be the diffusion of the MAE-based rosehip extract on the agar [Gebrechistos et al., 2020]. The antimicrobial activity of MAE-based rosehip fruit extract assays is similar to findings obtained by Demirkol and Ertürk et al., 2019, Rovna et al., 2015, Ghendov-Moşanu et al., 2018. Rovná et al. [2015] investigated the effect of rosehip flower extracts using ethanol and methanol solvents on Gram-negative *Escherichia coli*, and *Pseudomonas aeruginosa*. As a result of the study, the ethanolic extract had an inhibition zone diameter of 3.33 mm on *E. coli*, and an inhibition zone diameter of 4.33 mm on *P. aeruginosa*; It was determined that the methanolic extract formed a 4.33 mm inhibition zone diameter on *E. coli* and 1.66 mm inhibition zone diameter on *P. aeruginosa*. In another study performed by Rovná et al. [2020], they examined the antimicrobial effect of rosehip extracts collected from different locations and prepared with ethanol solvent on *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes*. They used gentamicin (10 µg/disc) and

distilled water as controls. As a result of the study, inhibition zone diameters between 14.33-15.67 mm on *E. coli*, between 10.67-12.00 mm on *K. oxytoca*, and between 9.33-10.67 mm on *P. auroginosa*, 8.00-8.67 mm on *B. cereus*. Inhibition zone diameters between 4.00-5.33 mm on *C. perfringens* and between 5.67-6.67 mm on *L. monocytogenes* were determined. As a result of this study, it was determined that the extract of rosehip with ethanol solvent was effective on *E. coli* but not on *C. perfringens*, and it was determined that the environment in which rosehip was grown affects the antimicrobial potential of its genome on bacteria. As a result of these two studies, it was assumed that rosehip flowers have a less antimicrobial effect than rosehip fruit, and the superiority of the antimicrobial effect of rosehip extract prepared using ethanol or methanol on bacteria is thought to depend on the genotypic properties of rosehip and the type of bacteria.

Ghendov-Moşanu et al. [2018] prepared rosehip and hawthorn fruit extracts using 50% ethanol-water solvent and examined their antimicrobial effects on *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* strains. As a result of the study, it was determined that rosehip extract formed an inhibition zone diameter of 16 mm on *S. aureus*, an inhibition zone diameter of 10 mm on *E. coli*, and an inhibition zone diameter of 9 mm on *K. pneumonia* which were than those obtained in this study although 50% ethanol-water was used as a solvent in both studies. The amount of water in the solvent used is thought to be one of the factors affecting the antimicrobial effect of rosehip.

The antifungal activity of MAE-based rosehip fruit extracts was investigated against *Penicillium italicum* and *Aspergillus niger* ATCC 16404, with broth microdilution method in 96-well plate as 72h. At the end of 72h, there was no significant antifungal activity against filamentous fungi.

Rovna et al. 2015 investigated the antifungal activity of rosehip flower extracts using methanol as a solvent against *Aspergillus niger*, *Fusarium culmorum*, and *Alternaria alternata* strains. The extracts formed a 1.33 mm diameter inhibition zone for three different fungi. The extraction solvent and the difference of intact material from rosehip could be reasons for variabilities in antifungal activities obtained by Rovna et al. 2015 and this thesis study.

The antibiofilm activity of MAE-based rosehip fruit extracts were investigated on four different foodborne pathogen bacteria on 72h-old and 24h-old-established biofilms. The gentamicin antibiotic was used as a control group. In 72h old-established

biofilms, the rosehip fruit extract at 1/2 dilution concentration was only found effective on *B. cereus* strain. In 24old-established biofilms the extracts were found to be effective as 49% by 1/2, 36% by 1/4 diluted extract concentrations on *E. coli* strain, 37% by 1/2, and 25% by 1/4 diluted extract concentrations on *L. monocytogenes* strain 66% by 1/2, 57% by 1/4 diluted extract concentrations on *S. aureus* strain and 67% by 1/2 diluted extract concentrations on *B. cereus* strain.

Quave et al., 2008, investigated the antibiofilm activity of different Italian ethnomedical plants including *Rosa canina* var. *canina* against methicillin-resistant *Staphylococcus aureus* on 40h old-established biofilms. Ten plant extracts were shown $IC_{50} \leq 32 \mu\text{g/ml}$ for biofilm inhibition, including rosehip fruit extract which was extracted using ethanol. Živković et al., 2015 investigated the antimicrobial and antibiofilm activity of methanolic extracts of rosehip leaves against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The MIC and MBC values of the extract against both bacteria were 0.0009 mg/ml. This research article also evaluated that the methanolic rosehip leaf extract inhibited the in vitro biofilm growth in *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Leishmania monocytogenes*, and *Escherichia coli* at sub-MIC-levels which is 1/4 and 1/8. The biofilm growth of *P. aeruginosa* and *E. coli* was 90% inhibited. Živković et al., 2015 also evaluated the flavonoid components in the methanolic extract of rosehip leaves, which consists of rutin, hyperoside, and isoquercetin.

By generating polymers such extracellular polysaccharides, bacteria commonly exploit the process of biofilm formation to proliferate and adhere to surfaces [Donlan et al., 2001]. Additionally, it is well-known that the production of biofilms influences the evolution of bacterial species' drug resistance. Quorum sensing, also known as cell-to-cell signalling, is a part of this process [Singh et al., 2017]. Small diffusible compounds known as autoinducers, which are often oligopeptides and N-acyl homoserine lactones in Gram-positive and Gram-negative bacteria, respectively, mediate this signalling. Once a certain concentration has been reached, autoinducers created by bacteria diffuse back into the bacterium and control the transcription of particular genes. Autoinducers are molecules that bacteria produce that disperse out and accumulate in the environment. Biofilm production [McLean et al. 1997], bioluminescence [Nealson 1977], and swarming [Eberl et al. 1996] are examples of outcomes of this multicellular behaviour that have been proven to significantly contribute to bacterial pathogenicity. Using its autoinducer N-hexanoyl homoserine

lactone for quorum sensing (HHL). When a quorum is attained, the autoinducer synthase CviI releases HHL into the environment, which then diffuses back into the bacteria. The autoinducer functions to regulate the expression of particular genes, including violacein, by binding to the transcriptional regulator CviR. Violacein production is a precondition for quorum sensing which leads the biofilm formation process. Since the reference strain *C. violaceum* CV026 lacks the autoinducer synthase CviI, it cannot perform quorum sensing or manufacture violacein without the exogenous addition of HHL. [Edited from Choo and colleagues, 2006].

According to the literature, the anti-quorum sensing activity of rosehip extracts has not been investigated. The data on anti-quorum sensing activity showed that MAE-based rosehip extracts could be also used as an anti-quorum sensing agent. Further studies on the mode of action of rosehip fruit extracts on anti-quorum sensing activity and antibiofilm activity should be performed.

As reported by Martinez-Romero et al., 2019 rosehip oils can delay postharvest decay on plum cultivars by increasing 20 days the shelf life of fruits. In this study, Martinez-Romero used a commercially available rosehip fruit oil as a coating material. In addition to Martinez-Romero's studies, this thesis study also showed that MAE-Based rosehip extracts can be used as a coating material for post-harvest fruit delay.

This thesis showed that MAE-based rosehip extract can be used as an antimicrobial and antibiofilm agent in the food industry.

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BIOGRAPHY

Dilara ŞAHİN achieved her bachelor's degree from the Department of Molecular Biology and Genetics, Istanbul University in 2019. She started her Master of Science degree in the Institute of Biotechnology at Gebze Technical University. She made a poster presentation at the 6th Academic Student Congress, 2022 namely “Investigation of Antifungal Activities of Rosehip (*Rosa canina* L.) Extract.”



APPENDICES

Appendix A: Publications Related to the Thesis

Sahin D., Yesilcimen-Akbas M., [2022], “Investigation of antifungal activities of rosehip (*Rosa canina* L.) “ 6th Academic Student Congress, Online, April 2022.



