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SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DETERMINATION OF ANTIBACTERIAL ACTIVITY MECHANISM OF RED ROSE PETAL EXTRACT

MSc. THESIS

Kazi Jannatul MARDIA

Food Engineering Department

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The thesis work titled “DETERMINATION OF ANTIBACTERIAL ACTIVITY MECHANISM OF RED ROSE PETAL EXTRACT” prepared By Kazi Jannatul MARDIA was accepted by the following jury on 02/08/2024 by unanimously/majority of votes as a Masters THESIS in Sakarya University Graduate School of Natural and Applied Sciences, Food Engineering Department.

Thesis Jury

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STATEMENT OF COMPLIANCE WITH THE ETHICAL PRINCIPLES AND RULES

I affirm that the thesis titled "Determination of Antibacterial Activity Mechanism of Red rose petal Extract " conforms to the ethical guidelines outlined by the Higher Education Institutions Scientific Research and Publication Ethics Directive, as well as the regulations stipulated by the Graduate School of Natural and Applied Sciences at Sakarya University. I have diligently adhered to all guidelines and directives throughout my academic pursuit. The content of this thesis is entirely original and represents the culmination of my independent research efforts. I have meticulously followed all protocols and procedures, ensuring that the methodologies employed are both rigorous and compliant with academic standards. I have conducted comprehensive literature reviews and have duly acknowledged and referenced all sources utilized during this investigation. Importantly, this thesis has yet to be submitted to any other academic institution to obtain a degree. I affirm that this thesis is a product of my own scholarly endeavors, conducted with integrity and by the highest ethical standards of academic research.

(02.08.2024)

Kazi Jannatul MARDIA

I dedicate my thesis to my parents and my husband

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Kazi Jannatul Mardia

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ABBREVIATIONS

◦C	: Centigrade degree
DPPH	: 1,1-diphenyl-2-picrylhydrazyl
DMSO	: Dimethyl sulfoxide
FCR	: Folin-Ciocalteu Reagent
GAE	: Gallic Acid Equivalent
g	: gram
h	: Hour
IC_{50}	: Half-maximal inhibitory concentration
L	: Liter
MIC	: Minimum Inhibitory Concentration
Min	: Minute
MFU	: McFarland unit
MHA	: Mueller-Hinton Agar
MHB	: Mueller-Hinton Broth
mg/mL	: Milligrams per Milliliter
mg	: Milligram
mL	: Milliliter
nm	: Nano meter
rpm	: Revolutions per minute
TSA	: Tyriptic Soy Agar
TSB	: Tyriptic Soy Broth
TCA	: Tricarboxylic acid
UV	: Ultraviolet light
µg	: Microgram
µL	: Microliter
µg/mL	: Microgram per Milliliter

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DETERMINATION OF ANTIBACTERIAL ACTIVITY MECHANISM OF RED ROSE PETAL EXTRACT

SUMMARY

Roses have been celebrated for centuries for their beauty, fragrance, and potential health benefits. Among the many bioactive compounds in red rose petals, anthocyanins stand out for their vibrant color and wide range of physiological effects. Anthocyanins, known for their antiatherogenic, anticancer, antidiabetic, anti-inflammatory, and antioxidant activities, are the primary pigments in red rose petals. Extracting these compounds can provide a visually appealing natural coloring agent suitable for various food products. Each component of a plant encompassing the trunk, bark, stems, leaves, fruits, roots, flowers, and seeds harbors a variety of chemical compounds, including phenolic acids, flavonoids, anthocyanins, and carotenoids. These compounds function as natural antioxidants, thereby mitigating the risk of plant diseases by delaying or preventing oxidative processes. This study focuses the antimicrobial properties of red rose petal extract and it is potential as natural preservative in the food industry. The extraction process involved freeze-drying the petals to preserve their bioactive constituents, followed by 70% ethanol extraction to obtain the desired extract. Frozen leaves were subjected to the vacuum freeze-dryer for a duration of 48 h. Moisture content was determined and the moisture content of the rose petal powder obtained in this study was found to be 4.65±0.05%.

The antioxidant properties of red rose petal extract contribute to preserving food quality by preventing oxidative degradation and extending shelf life. Rose extract is used in medicinal chemistry to treat bacterial illnesses, targeting both Gram-positive and Gram-negative bacteria. This study assesses the antimicrobial activity and antioxidant potential of the extracted red rose petal extract. Through well diffusion assays and determination of Minimum Inhibitory Concentration (MIC) values, the study aims to identify susceptibilities of common foodborne pathogens against the extract as well as its antibacterial activity mechanism. The inherent antimicrobial properties of the rose extract position it as a promising candidate for utilization as a preservative in food products, thereby diminishing the risk of spoilage and ensuring food safety. Utilizing well diffusion assays, the efficacy of the extract against a spectrum of foodborne pathogens, including *S.* Typhimurium (31 mm), *S.* Enteritidis (23 mm), *B. cereus* (23 mm), *S. aureus* (25 mm), *E. coli* O157:H7 (23.5 mm), *E. coli* Biotype I (25mm), and *L. monocytogenes* (32.5 mm), was assessed. The discernible formation of zones of inhibition around the extract-treated discs signified inhibition of microbial growth, underscoring the extract's potential as an antimicrobial agent. Furthermore, MIC (Minimum Inhibition Concentration) values ranged from 114.07 mg/mL to 57.03 mg/mL. The determination of MIC values revealed *S.* Typhimurium and *E. coli* Biotype I had the highest MIC values and *S.* Enteritidis and *B. cereus* had the lowest MIC values.

The petals of flowers are known as an ideal natural source of antioxidants from plant fiber due to their high flavonoid compound element level which includes anthocyanins.

the red coloration of roses is attributed to the presence of carotenoids and anthocyanin. Plant based anthocyanins and carotenoids are valuable for food, nutrition, and pharmaceutical preparations due to low toxicity. The extract's total phenolic content (23.10 ± 0.81 mg GAE/g) and antioxidant activity were determined using established methods, including the FCR and DPPH assay, respectively. The observed antiradical activity ranged from 16.60 % to 74.15 %, indicating a dose-dependent response. The potential applications of red rose petal extract in food preservation are diverse. Its antioxidant activity can help retard lipid oxidation and preserve the quality of fats and oils in food products.

Several studies were carried out to assess the time-dependent decrease in bacterial cell viability in the presence of rose petal extract. Initially, 24-hour bacterial cultures were prepared and supplemented with the rose petal extract, followed by incubation at 37°C to simulate optimal bacterial growth conditions. Sampling was conducted at predetermined intervals (0, 2, 4, 6, 24, and 48 hours) to monitor changes in bacterial cell viability over time. Each sampled culture was plated onto a Tryptic Soy Agar (TSA) medium to facilitate bacterial enumeration. A control group consisting of extract-free bacterial cultures was maintained to establish baseline bacterial growth patterns without the extract. The number of viable cells in the control tubes increased during the 24-hour incubation period for all bacterial cultures. In contrast, in the extract-added culture tubes, the number of viable cells reduced gradually, revealing the bactericidal effect of the red rose extract.

A series of experiments assessed the potential damage to bacterial cytoplasmic membranes induced by rose petal extract. The extract was added to bacterial cultures at twice the Minimum Inhibitory Concentration (MIC × 2) and then incubated at 37°C for 24 hours. After the incubation period, the number of viable cells was determined by seeding onto two types of agar media: Tryptic Soy Agar (TSA) and TSA supplemented with 3% NaCl. Adding 3% NaCl to TSA created an osmotically stressful environment to challenge bacterial growth. Petri dishes containing the seeded agar were subsequently incubated at 37°C for 48 hours to facilitate colony formation. Following the incubation period, colonies were manually counted, and the difference in colony counts between TSA and TSA with 3% NaCl represented the number of cells with damaged membranes. The reduction percentages varied significantly among the tested strains: *E. coli* O157:H7 exhibited the highest reduction percentage at (80.83%), followed by *S.* Enteritidis at (64.52%), *S. aureus* at (34.51%), *E. coli* Biotype I at (14.86%), *L. monocytogenes* at (19.38%), *S.* Typhimurium at (11.5%) and *B. cereus* (6.72%).

The investigation into the impact of rose petal extract on the tricarboxylic acid (TCA) cycle activity of pathogenic bacteria involved a series of experimental procedures. The extract was subsequently introduced into the suspension, adjusting bacterial cell concentration to 10^8 colony-forming units per ml (CFU/mL) during the logarithmic growth phase. Following a one-hour incubation period at 37°C, the mixture underwent centrifugation at 8000×g for ten minutes, forming a pellet. This pellet was then re-suspended in a 0.9% NaCl solution. Iodonitrotetrazolium chloride (INT) was then added to the suspension at a concentration of 1 mmol/L, and the mixture was further incubated for 30 minutes at 37°C. After the incubation, the maximum absorbance at 630 nm was measured using a spectrophotometer. By quantifying the maximum absorbance at 630 nm, changes in metabolic activity associated with the TCA cycle could be determined. Of note, the extract had a relatively minimal impact on the

metabolic activity of *E. coli* O157:H7. However, it demonstrated a more pronounced effect on the TCA cycle of *Salmonella* Typhimurium.

Roses are emblematic of beauty, bravery, passion, and love, often revered as the king or queen of flowers. Their extensive range of applications offers significant advantages over most other flowers. The polyphenols from rose petals can enhance the value of by-products in fruit processing. Utilizing an enzyme-assisted extraction followed by spray drying, this green technology offers an eco-friendly alternative to traditional methods like organic solvent extraction. This natural oil and its constituents offer safe alternatives for incorporation into various applications within the food sector. Specifically, they may serve as additives in dietary supplements or functional food products.

This study emphasizes the potential of red rose petal extract as a natural preservative and antimicrobial agent in food products. It highlights its promising applications in the food industry, suggesting it as a natural and health-promoting ingredient. Further research is needed to explore its effectiveness in different food matrices and ensure its safety for consumption, paving the way for its integration into various food products.

KIRMIZI GÜL YAPRAĞI EKSTRAKTININ ANTİBAKTERİYEL AKTİVİTE MEKANİZMASININ BELİRLENMESİ

ÖZET

Güller yüzyıllardır güzellikleri, kokuları ve potansiyel sağlık faydalarıyla kullanılmaktadır. Kırmızı gül yapraklarındaki birçok biyoaktif bileşen arasında, canlı renkleri ve geniş fizyolojik etkileri ile öne çıkan antosiyaninler, kırmızı gül yapraklarının temel pigmentleridir. Antiaterojenik, antikanser, antidiyabetik, antiinflamatuar ve antioksidan aktiviteleri ile bilinen antosiyaninlerin ekstraksiyonu, çeşitli gıda ürünleri için görsel olarak çekici bir doğal renklendirici sağlayabilir . Bir bitkinin her bileşeni gövde, kabuk, saplar, yapraklar, meyveler, kökler, çiçekler ve tohumlar fenolik asitler, flavonoidler, antosiyaninler ve karotenoidler gibi çeşitli kimyasal bileşenler içerir. Bu bileşenler doğal antioksidanlar olarak işlev görerek, oksidatif süreçleri geciktirerek veya önleyerek bitki hastalıklarının riskini azaltırlar. Bu çalışma, kırmızı gül yaprağı ekstraktının antimikrobiyal özelliklerine ve gıda endüstrisinde doğal bir koruyucu olarak potansiyeline odaklanmıştır. Bu amaçla öncelikle kırmızı gül yaprakları dondurarak kurutulmuş, ardından etanol (%70) ile ekstrakte edilmiştir. Donmuş yapraklar, 48 saat süresince vakumlu dondurucuya tabi tutuldu. Nem içeriği belirlendi ve bu çalışmada elde edilen gül yaprağı tozunun nem içeriğinin %4,65±0,05 olduğu tespit edildi.

Kırmızı gül yaprağı ekstraktının antioksidan özellikleri, oksidatif bozulmayı önleyerek gıda kalitesini korumaya ve raf ömrünü uzatmaya katkıda bulunabilir. Gül ekstresi, hem Gram-pozitif hem de Gram-negatif bakterileri hedef alarak bakteriyel hastalıkları tedavi etmek amacıyla tıbbi kimyada kullanılmaktadır. Gül ekstresi, antibakteriyel özellikleri nedeniyle çeşitli enfeksiyonların tedavisinde etkili olabilir. Bu özellik, gülün içerdiği aktif bileşenler, özellikle fenolik asitler, flavonoidler, ve antosiyaninler gibi antioksidan ve antimikrobiyal maddeler sayesinde elde edilir. Bu bileşenler, bakteriyel hücrelerin büyümesini engelleyerek veya öldürerek enfeksiyonların tedavi edilmesine yardımcı olabilir. Ayrıca, gül ekstresi, anti-inflamatuar ve analjezik etkileri nedeniyle de tıbbi alanda çeşitli uygulamalarda kullanılmaktadır.

Bu nedenle çalışmada kırmızı gül yaprağı ekstraktının antimikrobiyal aktivitesi yanında antioksidan potansiyelini de değerlendirilmiştir. Gıda kaynaklı patojenlere üzerine antimikrobiyel etkisi kuyu difüzyon yöntemi ile belirlenmiş, ayrıca MİK (Minimum İnhibitör Konsantrasyonu) değerleri ve antibakteriyel aktivite mekanizması araştırılmıştır. Gül ekstraktının doğal antimikrobiyal özellikleri, gıda ürünlerinde koruyucu olarak kullanımını bir aday olarak konumlandırmakta, böylece bozulma riskini azaltmakta ve gıda güvenliğini sağlamaktadır. Kuyu difüzyon testi ile ekstraktın *S.* Typhimurium (31 mm), *S.* Enteritidis (23 mm), *B. cereus* (23 mm), *S. aureus* (25 mm), *E. coli* O157:H7 (23.5 mm), *E. coli* Biotype I (25 mm) ve *L. monocytogenes* (32.5 mm) karşı antimikrobiyel etki gözsterdiği belirlenmiştir. MİK (Minimum İnhibitör Konsantrasyon) değerleri 114.07 mg/mL ile 57.03 mg/mL arasında değişmektedir. *S.* Typhimurium ve *E. coli* Biyotip I'nin en yüksek MİK değerlerine sahip olduğunu, *S.* Enteritidis ve *B. cereus*'un ise en düşük MİK değerlerine sahip olduğunu ortaya koymuştur.

Çiçeklerin taç yaprakları, yüksek flavonoid bileşen düzeyi, özellikle antosiyaninler içerdiği için bitki liflerinden gelen ideal bir doğal antioksidan kaynağı olarak bilinir. Güllerin kırmızı renginin, karotenoidler ve antosiyaninlerin varlığına atfedildiği söylenir. Bitki bazlı antosiyaninler ve karotenoidler, düşük toksisite nedeniyle gıda, beslenme ve farmasötik hazırlıklar için değerli kabul edilir.Ekstraktın toplam fenolik içeriği 23.10 ± 0.81 mg GAE/g olarak belirlenmiştir. DPPH radikalini süpürme aktivitesi ise %16.60 ile %74.15 arasında değişerek doza bağlı bir yanıt göstermiştir. Kırmızı gül yaprağı ekstraktının gıda muhazasında kullanılabilir ve antioksidan aktivitesi sayesinde gıda ürünlerindeki yağ ve yağların oksidasyonunu yavaşlatarak gıda ürünlerinin kalitesini koruyabilir.

Gül yaprağı ekstraktı varlığında bakteriyel hücre canlılığının zaman bağlı azalmasını değerlendirmek için birkaç çalışma yapılmıştır. Öncelikle 24 saatlik bakteriyel kültürler hazırlanmış ve gül yaprağı ekstraktı ilave edilmiştir. Ardından optimal bakteriyel büyüme koşullarını simüle etmek için 37°C'de inkübasyona alınmıştır. Belirlenen aralıklarda (0, 2, 4, 6, 24 ve 48 saat) bakteriyel hücre canlılığındaki değişiklikleri izlemek için Triptik Soya Agar (TSA) besiyerine ekim yapılmıştır. Kontrol grubu olarak ekstrakt ilave edilmemiş bakteri kültürleri kullanılmıştır. Kontrol tüplerindeki canlı hücre sayısı, tüm bakteri kültürleri için 24 saatlik inkübasyon süresi boyunca arttı. Buna karşılık, özüt eklenmiş kültür tüplerinde canlı hücre sayısı kademeli olarak azalmış olup, kırmızı gül özütünün bakterisidal etkisini ortaya koydu.

Gül yaprağı ekstraktının sitoplazma membranına potansiyel zararını değerlendirmek için bir dizi deney yapılmıştır. Ekstrakt, Minimum İnhibitör Konsantrasyonunun iki katı (MİK × 2) miktarda bakteriyel kültürlere eklenmiş ve ardından 24 saat boyunca 37°C'de inkübe edilmiştir. İnkübasyon süresi sonrasında, canlı hücre sayısı seçici olmayan (Triptik Soya Agar; TSA) ve seçici (TSA + %3 NaCl) iki farklı besiyerine ekim yapılarak belirlenmiştir. TSA'ya ilave edilen NaCl bakteriyel büyümeyi zorlayan osmotik olarak stresli bir ortam oluşturmuştur. İnkübasyon süresi sonrasında koloniler manuel olarak sayılmış ve TSA ile %3 NaCl içeren TSA arasındaki koloni sayılarındaki fark, zarar görmüş zarlara sahip hücre sayısını temsil etmiştir. Buna göre, *E. coli* O157:H7 en yüksek azalma yüzdesine (%8 0.83) sahip olmuş, ardından *S.* Enteritidis (% 64.52), *S. aureus* (% 34.51), *E. coli* Biotype I (% 14.86), *L. monocytogenes* (% 19.38), *S.* Typhimurium (% 11.5) ve *B. cereus* (% 6.72) gelmiştir.

Patojenik bakterilerin trikarboksilik asit (TCA) döngüsü aktivitesi üzerindeki gül yaprağı ekstraktının etkisinin araştırılması için deneysel işlemler uygulanmıştır. Bu amaçla 10^8 log CFU/mL düzeyinde logaritmik faz hücrelerini içrene kültüre ekstract ilave edilmiş ve 37°C'de bir saat inkübe edilmiştir. İnkübasyon ardından, on dakika boyunca 8000×g'de santrifüj edilmiştir. Elde edilen pelet %0.9'luk NaCl çözeltisinde süspanse edilmiş ve üzerine 1 mmol/L İodonitrotetrazolyum klorür (INT) eklenmiş ve karışım, 37°C'de 30 dakika inkübe edilmiştir. İnkübasyon sonrasında, maksimum absorbans 630 nm'de bir spektrofotometre kullanılarak ölçülmüştür. Ekstraktın *E. coli* O157:H7'nin metabolik aktivitesi üzerinde nispeten minimal bir etki yarattığı, buna karşın *S.* Typhimurium'un TCA döngüsü üzerinde daha belirgin bir etki gösterdiği ortaya konuldu.

Güller, güzellik, cesaret, tutku ve aşkın sembolleri olarak kabul edilir ve sıklıkla çiçeklerin kralı veya kraliçesi olarak saygı görürler. Güllerin geniş uygulama yelpazesi, birçok diğer çiçekten önemli avantajlar sunar. Gül yapraklarındaki polifenoller, meyve işleme süreçlerinde yan ürünlerin değerini artırabilir. Enzim yardımlı ekstraksiyon ve ardından sprey kurutma kullanılarak gerçekleştirilen bu yeşil teknoloji, organik çözücü ekstraksiyonu gibi geleneksel yöntemlere çevre dostu bir

alternatif sunar. Bu doğal yağ ve bileşenleri, gıda sektöründe çeşitli uygulamalar için güvenli alternatifler sağlar. Özellikle, diyet takviyeleri veya fonksiyonel gıda ürünlerinde katkı maddesi olarak kullanılabilirler. Ayrıca, aromatik özellikleri sayesinde hem gıda hem de parfümeri endüstrilerinde aroma artırıcı olarak kullanılmaya uygun olup, böylece koku deneyimini zenginleştirebilirler.

Bu çalışma, kırmızı gül yaprağı ekstraktının gıda ürünlerinde doğal bir koruyucu ve antimikrobiyal ajan olarak kullanım potansiyelini vurgulamaktadır. Ekstraktın gıda endüstrisinde umut verici uygulamalarını öne çıkararak, doğal ve sağlığı destekleyici bir bileşen olarak önerilmektedir. Farklı gıda matrislerindeki etkinliğini araştırmak ve tüketim güvenliğini sağlamak amacıyla daha fazla bilimsel çalışmaya ihtiyaç duyulmaktadır; böylece çeşitli gıda ürünlerine entegrasyonunun yolu açılacaktır.

1. INTRODUCTION

Red rose petals' visual appeal and pleasant aroma have made them popular in traditional medicine for centuries. Red rose petals have drawn interest for their alleged therapeutic characteristics, antibacterial activity, and aesthetic and aromatic features. The rose (*Rosa hybrida* L) is a captivating flower among the *Rosaceae* family. Widely distributed across the Northern Hemisphere, this species boasts over 200 variations (Raymond et al., 2018; Szołtysik et al., 2020). Extensive reticulate variety has occurred in roses as a result of multiplication, introgression, and cross-species crossing. The complex hybrid rose varieties today, such as (*Rosa hybrida*), are considered to have originated from only 8 to 20 rose species (De Vries and Dubois, 1996). Roses are locally found in Asia and North America, and a small number of roses are available in Europe. Due to anthocyanin pigments, the rose exhibits diverse colors, from delicate shades of white, yellow, and pink to vibrant purple, orange, and red hues (Lu et al., 2021).

The rose is the most prominent ornamental flower cultivated worldwide. States like Kenya and Ecuador are among the top providers, contributing around 75% of the world's total flower production (Laws, 2005). The red rose is one of several edible flowers that have been used in the culinary sector for a long time. In order to enhance the aesthetic appeal or the aroma of food, flowers have been used for food preparation in the ancient Roman and Greek civilizations as well as in eastern nations like China (Gonçalves et al., 2020). Since ancient times, people have used rose petals to make jams, cakes, tea, flavor food, and cure certain diseases naturally (Roberts, 2003).

Additionally, it may be possible to use it as a natural food preservative because of its antimicrobial properties. The most significant commercial crop is *Rosa hybrida,* which gets its color from anthocyanins and carotenoids (Lee et al., 2011). The key component of pigments that dissolve in water and are present across several berries, blossoms, and greens, including purple grain such as maize, red radish, purple sweet carrot, black grapefruit, black carrot, elder Berry, and red grape that is anthocyanin.

Rosa hybrida is highly significant for its color attributed to the presence of anthocyanins and carotenoids (Eugster and Märki- Fischer, 1991). Red flowers are a fantastic source of anthocyanins. Rose petals embrace numerous substances that consist of anthocyanins, proanthocyanidins, tellimagrandin I, rugosin B, carotenoids, plant acids, and essential oils. (Charan and Gupta, 2013). Food manufacturing seems to have a great deal of interest in using these organic pigments because of their appealing colors and advantageous effects on wellness, which include anti-cancer, anti-inflammatory, antidiabetic, and antioxidant capabilities (Lee et al., 2011). A study found that red rose can be used as a dietary source with anti-inflammatory, antioxidant, and cyclooxygenase properties (Deliorman et al., 2007; Wenzig et al., 2008). Numerous researches have been conducted to determine red rose petal extract's antibacterial activity and its efficacy against a variety of microorganisms.

It was demonstrated that the extracts from white, red, and pink rose petals act effectively against both the Gram-positive (*Bacillus cereus, Bacillus subtilis, Micrococcus luteus, and Staphylococcus aureus*) and Gram-negative bacteria *(Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Serratia marcescens, Pseudomonas aeruginosa*) (Zhang et al., 2011). An analysis of leaf extracts from *Rosa damascena* (Isparta rose), had an antibacterial impact on all other examined bacteria except *E. coli* O157:H7 but *Salmonella* Enteritidis *and Mycobacterium smegmatis* both showed the most susceptibility (Özkan et al., 2004).

According to Saati et al., (2018) ethanol and ethanol/water extracts made from red rose leaf were successful in inhibiting *E. coli*, *Salmonella* Typhi, and *Pseudomonas* sp. They determined that it had an antibacterial impact on pathogens. Another research by Rovna et al. (2015) showed, utilizing *Rosa canina* (rosehip) the ethanolic extract has a more substantial antibacterial impact on *P. aeruginosa* and the methanolic extract on *E. coli*. Red rose petal extract has also exhibited decisive antifungal action against a range of fungal infections (RovnÃ et al., 2015). Another study with three different kinds of mold (*Aspergillus niger, Fusarium culmorum, and Alternaria altarnata*) revealed antifungal effects. white rose leaf extracts made with ethanol and butanol had antibacterial efficiency, especially against *Propionibacterium acnes* and *Helicobacter pylori* (Park et al., 2016).

Researchers use a range of methodologies to determine the red rose petal extract's antibacterial functions. These include the determination of the minimum inhibitory

concentration (MIC), disc diffusion assays, and agar diffusion assays. These techniques entail exposing the pathogens to various extract concentrations or dilutions and assessing how much the extract inhibits the pathogen's capacity to grow or reproduce. The researchers found that the rose petal extracts had antimicrobial activity against *E. coli, Acinetobacter baumanii, K. pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Enterococcus faecalis, Listeria monocytogenes, and S. aureus,* with the (MIC) ranged from 1.25 to 10 mg/mL. Saati et al. (2022) found that *Pseudomonas* sp. had a bactericidal effect on the rose petal extract, and prolonged the shelf life of a fish to two days (Saati et al., 2022).

Different studies were found on the antimicrobial activities of flower petals extracts of different rose varieties in the literature. However, no study was found on the antimicrobial effect mechanism of red rose petal extract. The current study intended to look at an extract from red rose petals' antibacterial and antioxidant capabilities across an array of bacterial infections, including, *Escherichia coli, Salmonella* Enteritidis, *Bacillus cereus, Listeria monocytogenes,* etc.

2. LITERATURE REVIEW

2.1. Origin of Rose

The rose, revered for its beauty and symbolic significance, stands as the most widely cultivated and beloved flower in the global floral production sector. It belongs to the *Rosaceae* family and genus and is well known for its depiction of inspiration, love, and aesthetic delight., encompassing over 18,000 cultivars and 200 species (Gudin, 2000).Taxonomically, roses are classified as being of the genus *Rosa* and the family *Rosaceae*. They are woody perennial plants that are widely used in parks and gardens as significant agricultural and commercial crops (Ge and Ma, 2013).

There are 120 species in the genus *Rosa*, according to the generally accepted classification. The species are widespread throughout the world's subtropical and northern temperate climate zones. This comprises the region from the arctic circle to New Mexico, Ethiopia, the Himalayas, Bengal, and the southern region of China in the Far East. Only eight of the numerous species, originating in the Far East, Europe, and the eastern Mediterranean, helped form the present rose varieties (De Hoog, 2001).

The majority of rose species are indigenous to Asia, with a smaller number found in North America, some in Europe, and very few in the northwest region of Africa. It is difficult to distinguish between the fundamental species of rose since roses from different parts of the world hybridize easily. Giving rise to kinds that are overlapping the paternal forms. Fewer than ten species that were collected from the Asian species were crossed together to create numerous varieties of garden roses. The Northern Hemisphere's temperate and subtropical climates are the place of a variety of *Rosa L. taxa.* There are approximately 200 rose species in the genus, and each species has about 18000 variations. Four subgenera were identified: *Hulthemia* (Dumort) Focke, *Platyrhodon* (Hurst) Rehder, *Hesperhodos* Cockerell, and *Eurosa* Focke (Wissemann and Ritz, 2005).

According to S. Gudin, (2000) the allotetraploid species *R. damascena Mill.* is a crossed species of *R. gallica L*. and *R. moschata J.Herm* (for the summertime Damask roses) or *R. gallica L*. and *R. phoenicia Boiss* (for the autumn season Damask roses). According to Iwata et al., (2000) it had been claimed that *R. gallica L*., *R. moschata J. Herm.,* and *R. fedtschenkoana Reg*. are probably triparental ancestors. Duplication, introgression, and interspecific breeding have all made essential contributions to the enormous reticulate creation of roses. Only 8 to 20 species of rose, specifically the (*Rosa × hybrida)* are known to have given to the multilayered hybrid rose varieties that exist nowadays (De vries and Dubois, 1989).

Turkey is acknowledged as a critical gene source for a diverse array of commercial plants (Mustafa and Hasan, 2011). The country holds the key to world economics in plants that are both aromatic and medicinal. Among the foremost centers for the production and development of these commercially significant plants is the city of Isparta, located in the Lake Region (Göller Yöresi).

However, these species of plants exist in this country in a number of hybrid species, variants, and farmed developments, the majority of which are still unidentified. Nearly 25 species of the genus were known to be growing in Turkey (Davis, 1970). These variables crossings created hybrid tea rose breeds, the ancestors of contemporary roses with incredibly changed features (Martin et al., 2001).

2.2. History of Rose

In the Ottoman Empire during the 19th century, growing roses in specific gardens was a daily routine and tradition. In literally every region of the Empire, rose water and oil was produced, but usually only in small amounts that were used just locally. The one zone where rose oil was produced for sale was the Balkans. There were numerous rose gardens in this area, especially in the surrounding areas of Edirne, Filibe, Kazanlık, Karlo, Çorpan, Tatar, and Pazarcık. Many tons of rose oil were collected each year from these rose gardens in the Ottoman imperial time. The other regions of the Ottoman Empire, aside from the Balkans, observed the rise of new rose gardens for various reasons. Still, these gardens were often modest in size and of minimal economic significance. Aydın, Konya, Adana, Diyarbakır, and the cities of Syria (all inside the Ottoman Empire) were established as places for planting new rose gardens after the 1850s (Alp et al., 2015).

Here is evidence of rose cultivation for the manufacture of rose water dates back to the 17th century in European Turkey, Bulgaria, which at the time was a province of the Ottoman Empire, received the development of rose cultivation from a Turkish trader toward the end of that era midway through the 18th century (Baytop, 1990). One of the most diverse rose-growing regions in Turkey. In Turkey, over 35% of the world's rose species are grown, and 25% of its taxa are native to Turkey and its surroundings. Old garden rose production is heavily influenced by the provinces of İzmir, Manisa, İstanbul, Bursa, Antalya, Gaziantep, Kahramanmaraş, Gümüşhane, Sivas, Tokat, Erzurum, Erzincan, Malatya, Çorum, Konya, Isparta, and Burdur. Only *R. damascena* takes contemporary manufacturing procedures now for genus production. Cities in Turkey including Isparta, Trabzon, Rize, Kastamonu, Gümüşhane, Erzurum, Erzincan, Sivas, Ankara, Çankırı, Niğde, and Amasya are significant provinces of diversity for wild roses (Özçelik et al., 2012).

China is one of few Asian countries with an ancient civilization. Most of recorded history said that Chinese people have been producing plants and flowers since the ancient period. The first roses were grown 2000 years ago, during the Han Dynasty (141–87 BC), when wild roses bloomed across the Imperial Palace lawns. *Rosa* genus is thought to have originated in China. The north and west of the country include more than 100 natural wild rose species. Chinese roses were present at least 40 million years ago (during the Eocene epoch), according to fossilized rose leaflets found in Fushun, Liaoning province. Two different species of wild roses' fossils were found in the Shanwang Paleobotanic Region of Shandong Province in 1940 (Wang, 2007). Around 150–200 different species of roses can be found worldwide. *Rosa* is a genus that has of 82 species found to exist in China before 1985.

The history of rose cultivation in Lithuanian gardens is mainly unexplored. *Rosa* species seeds from the 16th century were discovered in the Vilnius Lower Castle's archeological layer (Steponavičienė, 2006). Roses were recognized in Lithuania as a Christian sign during the era of the Middle Ages and the Renaissance. They were discovered in a number of religious artworks or decorations in churches (Rylien, 2018). In the gardening sector, roses did not have importance until the 19th century. The variety of rose cultivars available in Europe quickly increased thanks to Josephine, Napoleon's wife, who inspired local plant farmers to launch intensive breeding efforts

in France. The number of available rose varieties nearly doubled in (1814–1829) fifteen years (Elliott, 2014).

According to Maia and Venard (1976) *R. moschata; R. gallica; R. alba; and R. damascena* are only summer-flowering roses. Growing in Europe up until the 13th century, Non-Regel exhibits periodic repetitive flowering and was recognized in southern Europe until the 14th era. There is considerable evidence to argue that *R. chinensis* was cultivated in Italy as early as the fifteenth century. This species and *R. x odorata (Andr.)* were introduced in England at the end of the eighteenth century and early in the nineteenth century, bringing the recurring flowering feature to modern varieties. In the seventeenth century, the diploid Chinese rose *R. chinensis* was brought to Europe (Fougère-Danezan et al., 2015).

The top three countries in the world producing damask rose are Iran, Turkey, and Bulgaria. However, *R. damascena* plants are taken from Iran and its nearby regions, including Pakistan. It showed extensive genetic variability according to DNA markers (Kiani et al., 2010). Due to the rose's enormous popularity and attraction, as well as the growing heritage values associated with its traditional growing and the creation of luxurious rose products, Bulgaria is renowned as the land of roses. According to Kovatcheva, (2011) the Bulgarian rose evolved during a period of the previous 340 years and is a member of the *Rosa Damascena Mill.* The Rose Valley, which is around 130 km long, 1 to 16km wide, and has an elevation range of 375 to 711 m, is where the Bulgarian rose is grown. Here the mild, temperate continental climate has warmer winters, colder summers, frequent heavy rains, and greater humidity levels (Loulanski, 2014). Around one hundred year ago, the Indian Roses were shipped from India to Europe. This group is divided into two distinct classes. The Tea Rose is one of them, and another is the Bengal Rose, with its mild and sweet scent (Alp et al., 2015).

The Rose was favored over all other plants by the ancient Greeks, who called it as the "Queen of Flowers." There are passages from S. Sappho's writings, who lived 600 years before the advent of Christianity, in which the rose is given a high rank. *Rosa bifera* is a further variety. It is most likely that the rose was first grown in Babylon's famed gardens, the creation of which is credited to Semiramis several 1200 years before the birth of Christ. From the diaries of modern tourists, it is assumed that different varieties of roses entered Persia (Parsons, 1860).

2.3. Taxonomy of Rose

In the rose family (*Rosaceae*), the genus *Rosa* contains about 100 species of perennial shrubs. The Northern Hemisphere's temperate zones are where roses are native. Most roses have a great aroma that differs based on their species and the conditions of the climate, and sometimes they are cultivated for their attractive blossoms, which range in color from white through different tones of yellow and pink to dark burgundy and maroon. Botanists started working to improve systematic classification immediately after Linnaeus published Systema Naturae (1735). Michel Adanson (1763) was the first to use "*Rosaceae*" as the name for the rose family. However, Antoine Laurent de Jussieu (1789) is now recognized by the International Code of Botanical Nomenclature (ICBN) (2006) as the author (Hummer and Janick, 2009).

Rehder (1947) It has been suggested that approximately more than 120 species in the category *Rosa,* which has been split into four subgenera, three of which (*Hesperhodos*, *Platyrhodon,* and *Hypothermia*) only have one or two species apiece. Based on the size of the styles, the sorts of flower buds, the sheer number of booklets, and the shapes of thorny structures, the 115 species that define the subgenus Eurosa as a whole are grouped into 10 groups. (Rehder, 1947).

The variants of *Cinnamomea* and *Caroline* should be regarded as a single species, according to research by Ratsek et al. (1939) and Lewis and Basye (1961) on the pollen viability of intersectional and inter-hybrid plants. Characters used to distinguish between different species of roses used to depend on anatomy, including hip structure besides the existence and arrangement of the prickles, roots, and glands (Nybom et al., 2005).

According to Wissemann (2003) there is a suggestion that traits such as growth type, sepal activity, and orifice size may be primarily inherited from the paternal lineage, indicating a close association among these characteristics. A study by (Hutchınson, 1964). *Rosaceae* is a descendant of the antique woody magnolias and is related to orders with more specialized inflorescences like Leguminales (Fabales), Araliales (Umbellales), Fagales, and Juglandales via standard genetic line. Rose taxonomy benefited greatly from cytology investigations, however, rose chromosomes are difficult to find using typical techniques (Millan et al., 1996; Rowley, 1967).

An RAPD (Random Amplified Polymorphic DNA)-based phenetic analysis of 119 accessions defining 36 species was done by (Jan and Byrne, 1999) which included 34 species from a total of eight categories of the subgenus Eurosa and one of species from every of the subgenera *Platyrhodon* and *Hesperhodos*. When compared to samples from other sections, species in the taxonomically distinct section *Caninae* form a small, closely connected group with low levels of general variation (Debener et al., 1996; Millan et al., 1996).

2.4. Characteristics of *Rosa Hybrida*

Species of the *Rosaceae*, or rose family, hybrid roses are decorative flowering trees with leaves and bushes. It is also known as 'Tea Rose'. Large, rising buds and tall, upright branches are features of this shrub (Figure 2.1). Usually, they only have one flower per stalk. They grow quickly and within three to four years are 3 to 8 feet in height and 2 to 3 feet in width. The cultivars, frequently called *Rosa hybrida* of them are being cultivated nowadays as many as 30,000 and 35,000. most of the cultivars are to be tetraploid (Esselink et al., 2003).

During the growing season, this shrub will continue to bloom, and the majority of them have an appealing fragrance. The stems are upright, rigid, and green. The leaves are complex, oval, green to reddish-green, and have serrated margins. The blooms come in a variety of hues, including shades of pink red, yellow, white, purple, and bright orange. Usually exclusive, the flowers have more than 20 petals. Single, semi-double, or multiple blooms can be available (Brun and Settembrino, 1996).

According to (De vries and Dubois, 1989) the Rose plant prefers full light and grows best in sandy texture and acidic soils. Roses need at least six hours in direct sunlight each day. Although they can blossom best in full sun and be more disease resistant, they can tolerate mild shade. Daily irrigation and fertilization at the shrub's root made it grow up. Researchers have investigated both greenhouse-cut roses and rose rootstocks that how they reacted to salt pressures(Cabrera and Perdomo, 2003). Perhaps the most vital climatic condition for the growth of roses is light. High light intensities during the summer, however, may be problematic for cut flower production in open fields and greenhouse settings in tropical, subtropical, and certain temperate environments. High light intensity from April to November is a problem in most

regions of Iran. which is involved cutting rose production and is related to producing high light stress and heat stress (Bredmose, 1993).

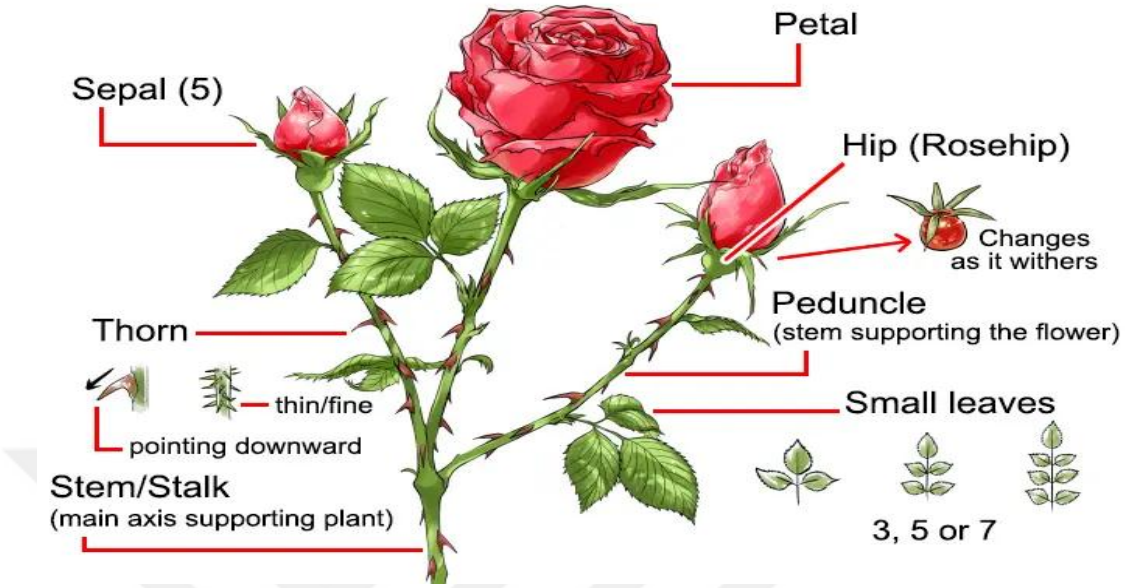

Figure 2.1. Anatomy of hybrid rose (www.clipstudio.net).

The optimal temperature for rose blooming is (20-25°C) during daylight and (13-16°C) at night, having 8 hours of sunlight and 75% humidity in the air (Shin et al., 2001). Roses can be cultivated at temperatures below 15°C, but the gap between blooms increases longer, and bullheads are formed. When temperatures increase above 30°C, poor-quality blooms with fewer petals grow (Desta et al., 2022).

In a study by (Shin et al., 2001) *Rosa* × *hybrida* 'Kardinal's bud-to-flowering period raised from 21 to 63 days as the temperature dropped from 30 to 15°C. Although the highest quality stems were found at 18°C, the area of leaves, branch length, photosynthetic levels, and diameter of stem all grew with warmer temperatures. The flower's dry mass grew from 0.7 to 3.0 g when the temperature dropped from 30 to 15°C. The dry mass of the flowers was shown to rise when plants switched to lower temperatures, they were in the open bud phase.

(Ferrante et al., 2010) showed that flower shelf life is determined by tissue aging, which differs from species to species and between genotypes. Flower shelf life (aesthetic quality) is connected to the genes that drive the aging process and that aging causes staining and the breakdown of tissues, which causes petal abscission. Van Doorn, (2002),contends that roses are extremely susceptible to ethylene, and their

susceptibility varies between species and genotypes. The ambient temperature has an important impact on anthocyanin production. Roses grown with cooler temperatures increased anthocyanin content, especially throughout the summertime (Z. Plaut 1979). Every plant's concentration of chlorophyll fluctuates in response to environmental variables in order to maximize photon absorption.(Lazar, 2003) found that light intensity influences phenolic respiration, including anthocyanin production.

2.5. Antioxidant Properties of Rose

Scientists are constantly seeking plant-derived antioxidants for their biological analysis and as superior substitutes for natural antioxidants that promote carcinogenesis by inhibiting reactive oxygen species in cell (Kim, 2019). Naturally generated antioxidants such as phenolic acids, flavonoids, anthocyanins, and carotenoids are found in all aspects of the plant itself, notably the branch, tree bark, leaves, stalks, berries, roots, flowers, and pods, and minimize the risk of different diseases of plants by postponing or preventing oxidation (Krishnaiah et al., 2011).

The petals of flowers are known as an ideal natural source of antioxidants from plant fiber due to their high flavonoid compound element level which includes anthocyanins (Elzaawely et al., 2007). According to Eugster et al. (1991), suggest that the red coloration of roses is attributed to the presence of carotenoids and anthocyanin. Plant based anthocyanins and carotenoids are valuable for food, nutrition, and pharmaceutical preparations due to low toxicity (Akhmadieva et al., 1993; Dougall et al., 1998).

The high number of anthocyanins in rose petals demonstrates their importance because they have strong antioxidant, antibacterial, and anti-inflammatory properties. Flower water is also suitable for use as a cleaning solution and hand sanitizer (Akhmadieva et al., 1993). Rose flowers are popular for their diverse colors and pleasant scents in the production of commercial flowers that are edible (Friedman et al., 2010).

Rosa de Castillo (VanderJagt et al., 2002), *R. moschata* (Speisky et al., 2006), *R. hybrida* (Vinokur et al., 2006), and *R. odorata* (Rop et al., 2012), When compared to other edible flowers, roses exhibit superior antioxidant capabilities. However, the correlation between various related compounds such as phenols, flavonoids, vitamins, and antioxidant activity remains unclear (Fu et al., 2009). Antioxidants and their activities play an essential role in rose flower function. To gain deeper insights into

antioxidant metabolic alterations and phytochemical synthesis, this study examined the impact of growth stages on floral characteristics, antioxidant content, and DPPH activity in two distinct-colored cultivars, namely "Carola" and "Iceberg." (Luo and Jing , 2011).

According to(Weisburger, 1999) which are thought to be harmful and dangerous to human health, they are synthetic additives. The application of natural antioxidants in place of artificial agents is becoming more popular. Damask Rose is rich in antioxidants like phenolics, flavonoids, carotenoids, and anthocyanins, acting as reducing substances and hydrogen donors due to their inhibitory qualities (Kähkönen et al., 1999).

Antioxidants from natural plant sources, such as those found in Damask roses, are being utilized to cure illnesses, improve nutrition, provide healthcare, and prevent collagenase activity (Liu et al., 2020; Mohsen et al., 2020). The phenol compounds, b-carotene, lycopene, vitamin C, tocopherol, bioflavonoids, citrus acids, tannins, pectin, glucose, natural acids, amino acids, and essential oils which are in rose petals are a merged combination (Demir and Özcan, 2001; Ercisli, 2007, p. 2; Uggla et al., 2005).

Flavonoids are a major subgroup of the vast array of chemical molecules that make up phenolic compounds together. Anthocyanins are a subclass of flavonoids. One of the most prominent natural sources of pigments, such as purple, blue, violet, and bright red is anthocyanin (Dai and Mumper, 2010).

“Antho," meaning flower, and "cyanin," which indicates blue, are the roots of the word "anthocyanin". The primary colors blue, purple, red, and orange exhibit a direct correlation with the quantity of hydroxyl groups present, while the number of methoxyl groups demonstrates an inverse correlation with the hydroxyl group count. The pigments called anthocyanins, which give colors like red and purple, are found in a lot of natural objects. Vibrant and precise, red anthocyanin pigments are frequently used in various sectors, including the food coloring and beverage industries (Yao et al., 2004).

Red roses had the highest percentage of anthocyanin concentration, at 1.03 mg/mL with 1.14% yield, compared with other anthocyanin sources like grapes, canna flowers, leafy greens such as spinach, dragon fruit, purple cabbage, broccoli.

According to Kumar et al. (2009) and Schmitzer et al. (2012),anthocyanidins compose primarily glycosylated cyanidins, pelargonidins, and peonidins in roses. Within these, cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside serve as the primary pigments in both red and pink rose varieties, respectively (Schmitzer et al., 2012).

According to, Ge and Ma,(2013) the potent antioxidant properties of anthocyanins stem from the phenolic hydroxyl groups they contain. By supplying a hydrogen atom, this set of molecules may be able to neutralize reactive oxygen species and halt the oxidation chain process. The hydroxyl group in phenol can successfully inhibit peroxidation. The pigments designated as anthocyanins offer no hazards and are not toxic.

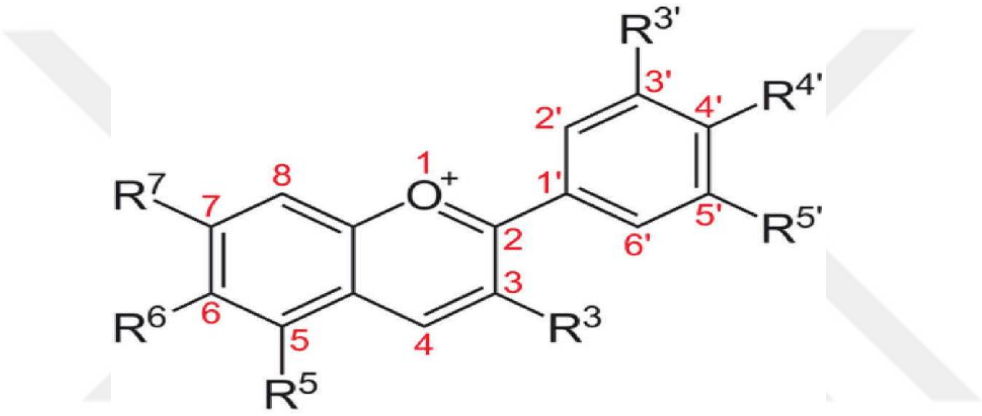

Figure 2.2. Basic structure of anthocyanin (Dabas, 2016).

According to Enaru et al. (2021), anthocyanins are dissolved in water and an aglycone also referred to as anthocyanidin, constitutes the structural component of anthocyanins. These substances exist as glucose of 2-phenylbenzopyrylium salt polyhydroxy acids and polymethoxy derivatives (Enaru et al., 2021). Fruits, flowers, and vegetables have beautiful color combinations because of these pigments. Co-pigments, metallic ions, and alkalinity all affect the color tone of the material. More than 635 anthocyanins have been characterized, with six major aglycones identified: cyanidin, peonidin, pelargonidin, malvidin, delphinidin, and petunidin (Castañeda-Ovando et al., 2009; Fleschhut et al., 2006; Seeram et al., 2001).

(Ng et al., 2010) established a correlation between the presence of a gallic acid derivative, a phenolic compound, and the antioxidant activity observed in a water-soluble extract of Rosa rugosa. Additionally, phenolic components found in extracts from *Rosa rugosa* and *Rosa davurica* flowers were linked to detoxifying properties.

(VanderJagt et al., 2002) noticed the polysaccharide-based antioxidants in rose flowers, but their activity was less. They demonstrated the presence of polysaccharide-based antioxidants in rose blossoms, although their efficacy was diminished. Essential oils were shown to enhance the antioxidant activity of oil containing rose blossoms and their extracts. Essential oils also contributed to the antioxidant capacity of rose petals that contain oil and their extracts (Achuthan et al., 2003; Lee et al., 2011; Ulusoy et al., 2009). (Wei and Shibamoto, 2007) discovered that rose oil's citronellol content (34.2%) and excellent antioxidant potential of over 50% at a low concentration. (Ulusoy et al., 2009)suggest using rose absolute with high tocopherol and carotene as natural antioxidants for industrial use.

2.6. Antimicrobial Activity of Rose Petals

An antimicrobial agent is a compound that inhibits pathogens including bacteria, fungi, protozoa, or viruses. These compounds are responsible for destroying or stopping the formation of other pathogens (Kumar et al., 2009). Recent investigations have demonstrated that *R. canina* extracts can effectively prevent the *Staphylococcus aureus* (MRSA) strain from growing and forming bacterial biofilms (Quave et al., 2008; Serteser et al., 2008). A study conducted by RovnÃ¡ et al. (2015), *Rosa canina* flower ethanolic extract exhibited notable antibacterial activity in opposition to Gram-negative pathogens such as *Pseudomonas aeruginosa* and *Escherichia coli*, as well as microscopic fungi including *Aspergillus niger, Fusarium culmorum, and Alternaria alternata*. Additionally, Rossnagel and Willich (2001a) observed that extracts derived from *Rosa canina* (red rose) petals significantly potentiated the antibacterial efficacy of various medications opposition to methicillin-resistant *Staphylococcus aureus.*

The antibacterial efficacy of rose hip seed extracts opposed to 11 pathogenic bacterial species, encompassing both Gram-positive and Gram-negative strains, was evaluated across methanol, dichloromethane, and n-hexane solvents. Among the tested extracts, The only extract with antibacterial action was methanol, albeit modest, with discernible inhibition observed solely against *E. coli* 8110 (Kumarasamy et al., 2002). Extraction solvents and flower shelf life may have an impact on the antibacterial power of red roses (Saati et al., 2018).

The antibacterial properties of the WRPE (White Rose Petal Extract) ethanol (WRPE-EtOH) and butanol extract (WRPE-Bu OH) from *R. hybrida* against a variety of

microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi, were investigated by Park et al. (2016). Rose petals and tubers contain organic compounds that offer natural protection against pathogenic attacks. Methanol and methylene chloride extracts derived from red, yellow, and white rose petals exhibit antibacterial activity against diverse strains of bacteria and fungi (Olech et al., 2015; Zhang et al., 2011).

WRPE's n-hexane fraction exhibits antibacterial and anti-inflammatory properties potentially benefiting gastroenteritis by combating *Escherichia coli* (Joo et al., 2010; Lee et al., 2011). It has been noted that a few popular flowers, like *Hibiscus rosa-sinensis* and *Ixora cocconea*, contain antibacterial properties that are effective against microbe (Gauthaman et al., 2006). According to Scalbert (1991), *Hibiscus rosa-sinensis* flower petals show antibacterial properties against various microbes, including *E. coli, B. subtilis, P. aeruginosa, S. aureus, Streptococcus* sp. and *Salmonella* sp. Plant materials as alternative sources of antimicrobial compounds are increasingly being studied due to antibiotic-resistant organisms' rapid development. Raw flower extracts contain specific chemical substances, including flavonoids, tannins, alkaloids, and triterpenoids, which can suppress bacterial growth in vitro individually or together. These phenolic chemicals, like tannins, are excellent antibacterial agents.

According to Sharmin et al. (2015) ethanolic extracts of *R. kordesii* demonstrated inhibitory effects on both Gram-positive and Gram-negative bacteria. The ethanol extract of *G. hybrid* displayed the most pronounced activity against *Klebsiella* and *Bacillus* species (Kalemba and Kunicka, 2003). Demonstrated rose extracts have antibacterial and antifungal properties. Rose extract is used in medicinal chemistry to treat bacterial illnesses, targeting microorganisms that are equally Gram-positive and Gram-negative. On the other side, the Bulgarian rose is one of numerous rose species that lacks antibacterial characteristics. While rose extract was prepared with various solutions, it became clear that petroleum solvent and rose extract exhibited stronger antimicrobial capabilities than rose extract made using water or alcohol (Hirulkar and Agrawal 2010).

Additionally, according to Yilmaz and Ercisli (2011), Rosa fruits possess antibacterial properties against various pathogens. Moreover, an extract from *Rosa pisiformis* was

observed to inhibit the growth of *Salmonella* typhimurium, *Bacillus cereus*, *Streptococcus aureus,* and *Yersinia enterocolitica*.

Research conducted by Shohayeb et al. (2014), the antibacterial characteristics of rose oil and various petal extracts were assessed against eleven bacterial species encompassing Gram-positive, Gram-negative, and acid-fast bacteria. The findings indicated that rose oil and its extracts exhibited broad-spectrum antibacterial activity against the tested microorganisms. Gram-positive bacteria, specifically *Streptococcus pyogenes*, *Bacillus subtilis*, and *Staphylococcus aureus*, exhibited greater sensitivity to the extracts compared to Gram-negative bacteria. The antifungal activity of the extracts was observed in decreasing efficacy against *Penicillium notatum*, *Aspergillus niger*, and *Candida albicans*. Among the extracts, the ethyl acetate fraction demonstrated the maximum degree of action opposed to the microbes under test.

Research by Balkan et al. (2016) demonstrated that *Staphylococcus aureus* and *Escherichia coli* were susceptible to rose oil. Disk diffusion tests further revealed the in vitro antibacterial properties of essential oil from *Rosa damascena* against *E. coli, S. aureus,* and *Pseudomonas aeruginosa*. In multiple trials, *Rosa damascena* exhibited significant antibacterial efficacy specifically against *S. aureus*. Kosakowska et al. (2018) demonstrated the antibacterial and antioxidant properties of various products derived from *R. rosea*, including aqueous and ethanolic dry extracts and subterranean organs. Based on the study, the water and ethanol extracts of *R. rosea* demonstrated higher bacteriostatic activity for the majority of isolates of Gram-positive as well as Gram-negative bacteria when compared to the powdered raw material. In particular, the development of two isolates was suppressed using the extract in water. of *Bacillus cereus, Bacillus subtilis, Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Escherichia coli* twice as effectively as the powdered raw material, and inhibited *Listeria monocytogenes* four times more effectively.

The antibacterial and antifungal effectiveness of iron oxide nanoparticles (NPs) synthesized using rose plant extract was evaluated against four different bacterial isolates, including Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative *(Klebsiella pneumoniae* and *Candida)* species. To assess the efficacy, the restraint areas were determined in mm. The study found that while iron oxide NPs exhibited significant inhibition percentages, the biosynthesized NPs from rose plant extract were more effective than the extracts alone. The primary mechanism for the

antibacterial activity of iron oxide NPs is the release of iron ions, which are attracted to the bacterial cell wall by electrostatic forces. The iron oxide nanoparticles (Fe0.911O) synthesized with rose plant extract demonstrated notable efficacy against *Klebsiella pneumoniae* (Abid et al., 2021).

Nowak (2014), evaluated the antibacterial and cytotoxic effects of *R. rugosa* petals. The study assessed antibacterial efficaciousness to enforce eight bacterial species: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis*, and *Proteus mirabilis*. The findings revealed that *R. rugosa* petals possess significant antibacterial and antifungal properties. A comprehensive analysis of the extracts' activity was performed, determining the (MBC) and comparing it with the (MIC) values. Low MBC/MIC ratios indicated strong bactericidal activity.

Karimi et al. (2015) revealed a quick, easy, environmentally a sustainable and economical process for producing nanoparticles of silver (AgNPs) which employs rose flower pollen extract as a reducing and stabilizer. The synthesized AgNPs had an ideal dimension of 12 nm and were predominantly spherical in shape. Utilizing the disk diffusion method with Mueller-Hinton Agar, the AgNPs exhibited noteworthy antimicrobial efficaciousness over *S. aureus, E.coli, B.cereus,* and *Enterococcus faecalis*. These findings suggest that green-synthesized AgNPs have potential applications on food, medical, and cosmetic industries.

Rossnagel and Willich (2001) observed that the extract from the petals of *Rosa canina L.* enhances the efficacy of various medications opposed to methicillin-resistant *Staphylococcus aureus.* Khan and Saurabh (2011) researched the antibacterial activity of various components of rose plants, contrasting the roots leaves, and petals of red and yellow rose species by means of their methanol as and ethanol-based extracts. The analysis highlighted the superior antibacterial properties of the red rose petals. The extract demonstrated significant efficacy against cultures of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli.*

Pandey et al. (2012) reported that the comparison of ethanol based extracts from the petals and leaves of red and orange roses exhibited the highest activity against *Pseudomonas aeruginosa.* Abdelbaky et al. (2021) investigated the antibacterial and antioxidant properties of five leaf extracts from *Rosa gallica var*. *aegyptiaca*, a species within the *Rosaceae* family. The study assessed the extracts' efficacy against

Salmonella Enteritidis, *Candida albicans, Bacillus subtilis, Staphylococcus aureus,* and *Listeria monocytogenes.* Andoğan et al. (2002) investigated the antibacterial properties of essential oil from *Rosa* × *damascena* using the disk diffusion method against strains of *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Escherichia coli*. The study's findings demonstrated that the volatile oil from this plant was effective against *Staphylococcus aureus.*

2.7. Applications of Rose Extract

Roses are emblematic of beauty, bravery, passion, and love, often revered as the "king" or "queen" of flowers. Their extensive range of applications offers significant advantages over most other flowers. The structures that are multilayered of rose petals possess potential for use in various electronic products. The aesthetic appeal of roses provides psychological comfort, their fragrance has calming effects on both the body and mind and their constituents are valuable sources of nutrients and medicinal compounds.

Dinkova et al. (2022) demonstrated that polyphenols from rose petals can enhance the value of by-products in fruit processing. Utilizing an enzyme-assisted extraction followed by spray drying, this green technology offers an eco-friendly alternative to traditional methods like organic solvent extraction. The study highlights the role of colorless polyphenols from rose petals as co-pigments that intensify strawberry color. Understanding the interaction between co-pigments and matrix components, such as cell wall material, is crucial for optimizing the efficacy of rose petal polyphenols in complex food systems.

Slavov et al. (2017) addressed the ecological risks posed by the significant waste generated by the rose oil industry, attributed to the low essential oil content in fresh rose petals. To mitigate this issue, various methods for utilizing rose waste have been developed. Increased rose oil production has driven interest in managing this waste, focusing on enhancing essential oil yield and extracting biologically active substances such as aroma compounds, flavonoids, and polyphenols. These initiatives have led to new products and waste valorization technologies. The study suggests that further integration and development of methods for comprehensive utilization of rose waste are needed to fully exploit its potential.

Hegde et al. (2022) highlighted the high value of edible flowers, particularly roses, in the the food and pharmaceutical sectors due to their rich bioactive components. Roses, used in various cuisines for centuries, contain flavonoids, phenolics, and carotenoids, which provide numerous health benefits, including antioxidant, anti-inflammatory, anti-aging, anti-obesity, anti-diabetic, hepatoprotective effects. The increasing use of edible rose flowers in the global food and pharmaceutical sectors presents significant growth opportunities for the edible flower industry. Cultivating roses for food can generate income, create jobs, alleviate hunger, and boost economic development.

Nasery et al. (2016) noted that the essential oil extracted from *Rosa damascena*, commonly known as the Damascus rose, exhibits inherent antibacterial, antifungal, and antioxidant attributes. This natural oil and its constituents offer safe alternatives for incorporation into various applications within the food sector. Specifically, they may serve as additives in dietary supplements or functional food products. Furthermore, their aromatic properties render them suitable for utilization as flavor enhancers in both the food and perfume industries, thereby elevating the olfactory experience. The potential application areas of rose extracts were mentioned in Table 2.1.

Table 2.1. The potential application areas of Rose extracts.

Goods made using rose petals	Rose species	Essential Phytochemicals	Benefits	Reference
Rose Petal Tea	*R. damascena*	Total phenolics	Enhance antioxidant activity	Vinokur et al. (2006)
Rose syrup	*R. damascena*	Anthocyanin	Enhanced sensory scores	Kumar et al. (2021)
Rose Juice	*R. rugosa*	Anthocyanin and total phenols	Increased anthocyanin activity	Dan-min and Zhi-long (2019)
Liqueurs	*R. rugosa*	Flavonoids	Increased antioxidant activity	Shen et al. (2007)
Wine	*R.hybrida var. Colorado*	Anthocyanin, Resveratrol	Increased antioxidant activity	Seong et al. (2017)
Strawberry beverage fortified with rose petal extracts	*R. damascena*	Polyphenolic co-pigments	Reduced thermal degradation of anthocyanin	Mollov et al. (2007)
Yoghurt	*R. damascena*	Anthocyanin	Antioxidant enriched	Chanukya and Rastogi (2016)
Jam (mixed jams of rose petals)	*Rosa. sp (cv. Carola)*	Total phenolics	Increased total phenolic content and antioxidant activity	Moreira et al.,2019

Roses represent a notable category of ornamental plants with extensive applications in the food industry, nutritional products, and the cosmetics sector. Historically, these plants were also utilized as therapeutic herbs in traditional medicine. Particularly significant is the volatile oil extracted from the *Rosa damascena* flower, which is highly valued in the cosmetics industry for its use in fragrances (Sadraei et al., 2013). Rose water, a well-known essential oil product derived from *Rosa damascena* contains varying concentrations of rose volatile oil depend on the quality of the product. According to Boskabady et al. (2011), rose water is utilized in the culinary industry and for various traditional practices.

Rivas-García et al. (2021) explored the therapeutic potential of *Rosa hybrida* extract beyond its conventional applications. The study investigated its efficacy in addressing liver disease, gastrointestinal disorders, and neurological illnesses. Moreover, the antiproliferative effects of a methanolic extract of *Rosa hybrida* were evaluated in ovarian cancer cells, demonstrating its potential to induce autophagy and apoptosis, thus exhibiting antiproliferative properties against ovarian cancer cells. Furthermore, the study suggests the possibility of developing novel treatments for Alzheimer's disease utilizing this extract.

Schieber et al. (2005) investigated the antioxidant properties of flavonol glycosides isolated from essential oil obtained through industrial distillation of *Rosa damascena* petals. Their findings underscored the presence of antioxidant properties in distilled rose petals, suggesting their potential utilization as culinary additives. Consequently, it is plausible to consider their application within the food sector as a safe, plant-derived natural antioxidant.

Adeel et al. (2022) highlighted the increasing preference for natural dyes in the textile industry due to concerns regarding the environmental and health impacts of synthetic counterparts. Their study focused on the anthocyanin pigment present in rose blossoms, which serves as a significant natural dye source for fabric dyeing. Through controlled wool dyeing processes, involving precise regulation of pH, temperature, duration, and salt concentrations, researchers observed the efficacy of chemical and biological mordant agents in achieving desired color variations and enhancing colorfastness properties in natural textiles.

Jamal et al. (2019) conducted an animal study utilizing the acute restraint paradigm in mice to assess the possibilities of *Rosa moschata* extract in reducing stress. Stress,

characterized by emotional or mental strain, disrupts the body's physiological and psychological equilibrium and may necessitate pharmacological intervention. The observation revealed that *Rosa moschata* extract significantly mitigated stress levels, indicating its potential as a stress-reducing agent with implications for pharmacotherapy.

The existing gap in research underscores the necessity for in-depth investigation into the potential antimicrobial properties of *Rosa hybrida* rose petal extracts. Detailed exploration of the antimicrobial activity of these extracts could yield significant insights, paving the way for innovative applications in both medicine and natural product development. This line of assurances of an inquiry to enhance our understanding of the therapeutic potential of these natural compounds, possibly resulting in the realization of new treatments and products that leverage their antimicrobial effects.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Red rose

The flowers were procured from a nearby wholesale florist in Sakarya province and were allowed to bloom for a maximum of two days at room temperature. Fresh *Rosa hybrida* (red rose) flowers were specifically obtained for the study. Subsequently, fresh flower leaves were transported to the laboratory and preserved by freezing at -45°C.

3.1.2. Microorganisms

Seven Gram-positive and Gram-negative bacterial strains were utilized in this investigation. Gram-negative bacteria include *Salmonella* Typhimurium, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* O157:H7, *Escherichia coli* Biotype I. Gram-positive bacteria include *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* ATCC 7644. All the microorganisms were kept in nutrient broth and 25 % glycerol with 1:1 (v/v) at –45°C.

3.1.3. The laboratory instruments

The instruments that were used in this study are listed in Table (3.1.)

Table 3.1. Laboratory instruments, company, and origin used in the study.

Instrument	Manufacturer Company
Autoclave	WiseClave WAC-80 (Korea)
Burner	Shandon (England)
Centrifuge	Hettich Universal 320 R (Germany)
Vortex	VELP Scientifica ZX3 (Italy)
Spectrophotometer	Shimadzu UVmini-1240 (Japan)
Lyophilizer	Freezone 6 litter Benchstope model no:7753027 (Germany)
Water bath	WiseBath WSB-30 (Korea)
Vacuum packaging machine	CromPack (Turkey)
Refrigerator	Profilo (Turkey)
Deep freeze Refrigerator	DF 590 (Turkey)

Table 3.1. (Continued) Laboratory instruments, company, and origin used in the study.

Instrument	Manufacturer Company
Precision scale	Weight lab instruments WL-303L(Germany)
Magnetic stirrer	IKA CMAG HS 7 (Italy)
Incubator	Micro test brand MIN-120 D(Turkey)
Shaking Incubator	Wisd brand WSB-30 (Korea)
Coffee Grinder	Fakir brand (Turkey)
Micropipette	Hamilton (USA)
Densitometer	Biosan Brand DEN 1B (Germany)
Rotary Vacuum Evaporator	BUCHI brand Rotavapor R-215 Advanced (V-850)

3.1.4. Chemical and biological materials

The materials used in the study are given in Table (3.2.)

Table 3.2. Chemicals used in the study.

Material	Company
Alcohol (Ethanol)	Merck-Germany
DPPH	Sigma-Aldrich, Germany
Folin–Ciocalteu reagent	Sigma-Aldrich, Germany
Galic Acid	Sigma-Aldrich, USA
Dimethyl sulfoxide $(CH_3)_2SO$ (DMSO)	Merck, Darmstadt, Germany
Mueller Hinton Agar (MHA)	Merck, Darmstadt, Germany
Mueller Hinton Broth (MHB)	OXOID, Hampshire, England
Tryptic Soy Agar (TSA)	Merck, Darmstadt, Germany
Tryptic Soy Broth (TSB)	Merck, Darmstadt, Germany
Iodonitrotetrazolium chloride (INT)	Sigma-Aldrich, USA
Sodium chloride (NaCl)	Merck, Darmstadt, Germany
Methanol	Honeywell-Poland

3.1.5. Culture media

Tryptic soy Agar (TSA): TSA was used to grow pathogenic microorganisms. TSA was prepared by mixing 40 g of Tryptic Soy Agar (TSA) with 1 L of distilled water and then sterilized at 121°C for 15 min (pH 7.3±0.2).

Tryptic soy Broth (TSB): TSB was used to activate pathogenic microorganisms for antimicrobial activity testing, another method was employed. In this process, 30 g Tryptic Soy Broth (TSB) was dissolved in 1 L distilled water and sterilized at 121°C for 15 min (pH 7.3±0.2).

Mueller-Hinton Agar: It was used for antimicrobial activity tests. A mixture of 1 Liter of distilled water and 32 g of Mueller-Hinton Agar was sterilized in the autoclave at 121°C for 15 minutes, then added to Petri dishes after cooling to 50°C (pH 7.4 ± 0.2).

Mueller-Hinton Broth (MHB): It was used for antimicrobial activity tests. A mixture of 1 L of distilled water and 21 g of Mueller-Hinton Broth powder was sterilized in an autoclave at 121°C for 15 minutes (pH 7.4 ± 0.2).

Double Strength MHB: It was used for Time-Kill Efficacy Assay. For this aim, 42 g of Mueller- Hinton Broth was diluted together in 1 L of distilled water. After completely dissolving it was autoclaved at 121°C for 15 minutes (pH 7.4 ± 0.2).

3.1.6. Solutions

Rose petal extraction solvent: 70% concentration of ethanol (Ethanol: Water,70:30) solution was used as the solvent.

DPPH (1,1-diphenyl-2-picrylhydrazyl) solution: 2 mg DPPH with 80% methanol was filled up in 100 mL baker with distilled water and stirred on a hot plate for 1 hour without any heating.

Preparation of stock solution:1^{st} stock→100 µL extract+900 µL distilled water

2^{nd} stock→100 µL (1:20) diluted extract +900 µL distilled water

Gallic Acid Stock Solution: 25 mg of Gallic acid was mixed with 25 mL of distilled water.

Iodonitrotetrazolium chloride (INT) solution: The 0.04 mol/L INT was created by combining methanol and water (1:1).

3.2. Methods

3.2.1. Freeze-drying rose petals

A freeze-dryer was employed for the drying process. The freeze-drying procedure involves freezing the product at atmospheric pressure during the initial phase, followed by controlled drying at a regulated temperature. Frozen leaves were subjected to the vacuum freeze-dryer for a duration of 48 h (-50°C, 0.0045 mbar). The dried petals were subsequently pulverized into a fine powder using a coffee grinder.

3.2.2. Extraction

Anthocyanins were extracted using the technique described by Barani et al. (2020) with a few minor modifications. Seventy percent concentration of ethanol solution was used as the solvent. One g of the dried and powdered sample was weighed and transferred to the centrifuge tube. Ten mL of ethanol solution was added and kept in water bath for 15 minutes at 50°C. The solution was centrifuged for 10 min and the sediment was removed. Then the solvent is removed by keeping it in the rotary evaporator and rotate it 3 minutes at 50°C (Saati et al., 2018).

3.2.3. Calculation of extraction yield

The rose petals were weighed before and after freeze-drying. After the rotary evaporator, the extract was weighed. The calculation of yield was calculated using the following equation 3.1.

$$\text{Yield } \% = \frac{Weight\ of\ dried\ extract}{weight\ of\ the\ dried\ plant} \times 100\% \quad (3.1)$$

3.2.4. Determination of moisture content

Moisture determination analysis of freeze-dried rose petal powder samples was performed according to the method written in AOAC 930.15 (AOAC, 2012). In the experiment, 5 g of the samples were weighed in glass Petri dishes brought to constant weight. After being kept in the oven at 135°C for 2 h, it was left in the desiccator to cool. The final weighing of the samples at room temperature was carried out, and the moisture content % was calculated with the help of the equation in 3.2.

$$\text{Moisture } \% = \frac{m-(M2-M1)}{m} \times 100\% \quad (3.2)$$

M1= Weight of drying vessel + weight of the sample (g)

M2= Weight of drying vessel + dried sample (g)

m = sample weight (g)

3.2.5. DPPH Radical scavenging activity

The ethanolic extraction of *Rosa hybrida* was observed for its capacity to scavenge DPPH radical. Methanol solution at 80% concentration was used to prepare a DPPH solution (0.05%). To make the stock solution, 100 µL rose extract was dissolved in

900 µL distilled water and serially diluted to 20, 40, 60, 80 and 100 µL/mL concentrations using distilled water. Two hundred µL of diluted extract was transferred in a tube and added with 3 mL of 0.05 mmol/L DDPH solution. After vortexing, mixture was incubated for 30 minutes in dark. Subsequently, the absorbance of the samples was measured at 517 nm using a UV-VIS spectrophotometer, where methanol solution was used as blank (Shah, 2015). The antioxidant activity of the samples was calculated using the following equation 3.3.

$$\% \text{ DPPH} = \frac{A\,(control) - A\,(sample)}{A\,(control)} \times 100\% \quad (3.3)$$

A (control): absorbance of the control

A (sample): indicates the absorbance of the extract

Using Microsoft Excel, plots showing extract concentrations vs. percentage inhibition of DPPH radical were used to determine the IC_{50} values. IC_{50} values were determined by averaging the three values obtained from each duplicate experiment. Using the three experiment values, the standard deviation was determined for every concentration.

3.2.6. Total phenolic content analysis (TPC)

The Folin-Ciocalteu (FC) method described by Gao et al., (2000) was used to determine the total phenolic content of the floral extract. A hundred µL of diluted extract (1:40; 1:60), 200 µL Folin-Ciocalteu Reagent (FCR) and 7 mL distilled water were mixed and incubated for 3 min at ambient temperature. After that, 1 mL Na_2CO_3 (20%) solution was added and incubated for 30 minutes at dark. Then, absorbance was measured at 765 nm using a UV-VIS spectrophotometer. A standard calibration curve was created using Gallic acid solutions from 0 to 1000 ppm. Gallic acid equivalents (GAE) per gram of material were used to express all result (Mak et al., 2013). The total phenolic content of rose petal extract was calculated using the equation 3.4.

$$\text{GAE (mg Gallic acid/g sample)} = c \times \frac{v}{m} \quad (3.4)$$

c: concentration of Gallic acid obtained from calibration curve

m: Weight of extract (g)

v: Amount of extract used in the analysis (mL)

3.2.7. Antimicrobial activity by well-diffusion method

Seven bacterial strains, both Gram-positive and Gram-negative, were utilized in this investigation. Gram-negative bacteria include *S.* Typhimurium, *S.* Enteritidis ATCC 13076, *E. coli* O157:H7, and *E. coli* type I, and Gram-positive bacteria including *B. cereus*, *S. aureus*, and *L. monocytogenes* ATCC 7644 and three replications were utilized to test them.

Slightly modified, the previously described well-diffusion assay was used for examining the antimicrobial activity (Özkan et al., 2004). Two-fold dilutions of rose extracts (1, 1/2, 1/4, 1/8, 1/16) were prepared using 1% DMSO. DMSO used for negative control. The wells with 6 mm in diameter were opened using sterile pipet tips on MHA, and then 10 µL of soft MHA (0.6% Agar) was added to cover the bottoms of the wells. Thereafter the wells were loaded with 50 µl of diluted extract. The Petri dishes were stored at 4°C for 24 h (Özkan et al., 2004).

The bacterial strains were activated in TSB at 37°C for 24 h. Then the active cultures were streak plated on TSA and Petri dishes were incubated at 37°C for 24 h. From the colonies grown on TSA, inoculums were prepared in physiological saline (0.85 % NaCl) and adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL) using densitometer. A 100 µL portion of the inoculum was transferred into soft MHA (0.6% Agar) and vortexed. Then soft MHA was poured onto MHA containing wells loaded with rose extracts. Petri plates were incubated at 37°C for 24 h (Safdar & Malik, 2020). 1% DMSO (Merck, Darmstadt, Germany) and the extract were filtered through a filter (0.22 µm) before being utilized in the analysis (Sharmin et al., 2015). For S. aureus TSB, TSA, and soft-TSA media (Merck, Darmstadt, Germany) were used in the analysis, as well as the controls (+)76% ethanol (50µL) and (-)1% DMSO (50µL) were utilized (Umesh et al., 2012). Petri plates were incubated at 37°C for 24 h and inhibition zones around the wells were measured.

3.2.8. MIC Analysis

The MIC determination was performed according to Shohayeb et al. (2014) and two replications were utilized to test them. For this, two-fold dilutions of the extract (1 – 1/256) were prepared using 1% DMSO (Merck, Darmstadt, Germany). From each dilution, 10 µL portions were transferred onto MHA surface ad left to dry for a while. The bacterial cultures were first activated in MHB and then streaked on MHA at 37°C

for 24 h. The culture suspensions corresponding to 0.5 McFarland were prepared in FTS and 100 µL portions were inoculated into soft-MHA. The extract inoculated soft MHA that had been inoculated were moved to the proper Petri plates and left there for 24 hours of incubation (Bayoub et al., 2010; Mishra et al., 2011; Quave et al., 2008). The results were determined as the lowest extract dilution amount that stops bacteria from multiplying.

3.2.9. Time-Kill Efficacy Assay

The approach outlined by Appiah et al. (2017) was utilized to conduct time-kill kinetics of ethanol extracts of *Rosa hybrida*. One ml of double-strength MHB was inoculated with 100 µL the bacteria suspension adjusted to 0.5 McFarland turbidity to achieve an initial concentration of bacteria 5 log CFU/mL. Then, 1 mL of extract solution prepared with DMSO (%1) was added to the MHB tubes. The final extract concentration in tubes were 2 × MIC for each bacterium. The procedure was the same for the positive control groups; the extract solution was substituted with 1 mL of DMSO in the tubes. The tubes were incubated at 37°C for 24 h. At predetermined times (0, 2, 4, 8, and 24 h) 1 mL culture serially diluted culture was plated on TSA and plates were incubated at 37°C for 24 h. After incubation colonies were counted manually and the counts were expressed as log CFU/mL.

3.2.10. Determination of Membrane Damage

One mL of double strength MHB were added with 1 mL of extract solution with the concentration of 2 × MIC. Pathogenic bacteria were activated twice in 5 mL TSB at 37°C for 18-24 h. The active cultures are centrifuged at 6000 rpm for 10 min at 4°C. After centrifugation, the supernatant was discarded. Five mL of sterile peptone water (0.1% peptone) was added to the remaining pellet in the tube, vortexed, and centrifuged again under the same conditions. Washing with sterile physiological saline was repeated once again and the residues of the medium were completely removed. As a final process, sterile physiological saline was added to the pellet and the original volume was (5 mL). The bacterial suspensions were adjusted to 0.5 McFarland turbidity (1.5×10^8 CFU/mL). The tubes containing MHB added with extract were inoculated individually bacterial cultures at a level of 10^5 log CFU/mL. After incubation at 37°C for 24 h, cultures were diluted and pour plated on simultaneously on TSA (non-selective medium) and TSA with 3 % NaCl (selective medium). The

difference between counts obtained non-selective and selective media showed the counts of membrane-damaged cells (Espina et al., 2016).

3.2.11. Effect on Tricarboxylic Acid (TCA) Cycle

Effect of red rose extract on the tricarboxylic acid cycle (TCA) method was determined according to (Sun et al., 2018). Pathogenic cultures in the logarithmic phase were centrifuged at 9000 rpm for 10 min, washed twice with sterile normal saline, and supernatant was discarded. Each pellet was added with saline and rose extract to reach 2 × MIC. The tubes were vortexed and incubated at 37°C for an hour. Following incubation, the bacterial cultures were centrifuged (8000 rpm, 10 min), washed twice and final pellets were resuspended in 2.5 mL of sterile saline. The 0.04 mol/L iodonitrotetrazolium chloride (INT) was prepared by combining methanol and water (1:1) in a separate step. The 250 µL INT was added to 2.25 µL of bacterial suspension to produce final concentrations of INT in solution of 1 mmol/L. After incubating at 37°C for 30 minutes the absorbance was measured at 630 nm using UV-VIS spectrometer (Guo et al., 2021).

4. RESULT AND DISCUSSION

4.1. Moisture Content of Rose Petal Powder

The percentage of water content in the powder sample is shown by the results of the moisture content analysis of rose petal powder. Figure 4.1 shown the freeze-dried rose petal and its powder. Accordingly, the moisture content of the rose petal powder obtained in this study was found to be 4.65±0.05%. According to literature data, for the dry flowers to remain solid and retain their freshness for over six months, they should have a moisture level of between 8-11.5%. Petals dropped upon handling when flowers were too dried (Singh and Dhaduk, 2004). A study by Ran et al., (2021) also found that fresh roses had an initial moisture content of 4.71–4.85% which is a similar result to our investigation.

Figure 4.1. Freeze dried rose petal and powder.

4.2. Extraction yield

The rose petals were weighed before and after freeze-drying. After the rotary evaporator, the extract was weighed. The result of extraction yield was 41.5%.

4.3. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

DPPH radical scavenging activity test was performed to determine the antioxidant capacity of red rose extract (Figure 4.2). Table 4.1 presents the evaluation of rose petal extracts' capability to scavenge DPPH radicals, a widely employed method to assess antioxidant activity. Extracts derived from rose petals were prepared specifically for antioxidant studies, aiming to quantify their antioxidative potential. IC_{50} value, the concentration of the extract that shows 50% radical scavenging activity, was calculated from the graphic (r^2 = 0.9826; Figure 4.3). Our investigation yielded an IC50 value of 46.53 µL/mL for the extract, indicating its capacity to scavenge DPPH radicals.

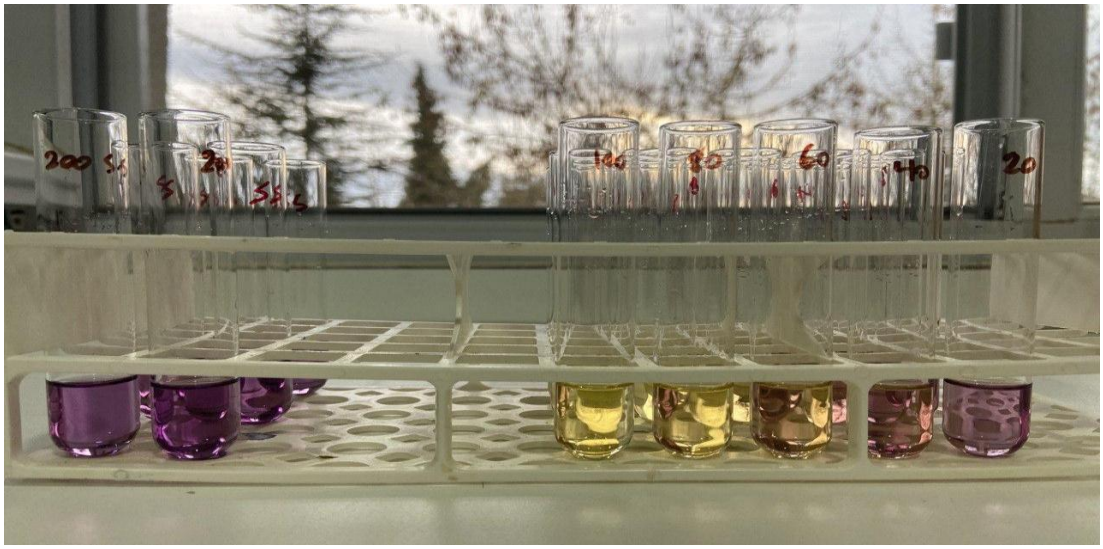

Figure 4.2. DPPH radical scavenging activity test.

According to research conducted by Pal et al., (2018) the water extraction of rose petal extract exhibited the highest IC_{50} value, measured at (25.66 µg.) Additionally, the study reported a notable association (r^2 = 0.9188) between flavonoid content and antioxidant activity. Interestingly, the water extraction demonstrated the highest free radical scavenging activity based on IC_{50} values. From these findings, it can be inferred that the enhancement of antiradical activity in the evaluated extracts is most effectively achieved through methanolic or aqueous extractions. The efficacy of the methanol extract in scavenging free radicals can be attributed to the presence of

flavonoids and polyphenols, which facilitate the preservation of essential metabolites in the extract.

Table 4.1. DPPH radical scavenging activity (%) of rose extract.

Extract concentration (µL/mL)	DPPH (%)	IC_{50} (µL/mL)
20	16.60 ± 1.65	
40	42.73 ± 0.69	
60	63.86 ± 0.75	46.53
80	74.15 ± 0.47	
100	73.89 ± 1.77	

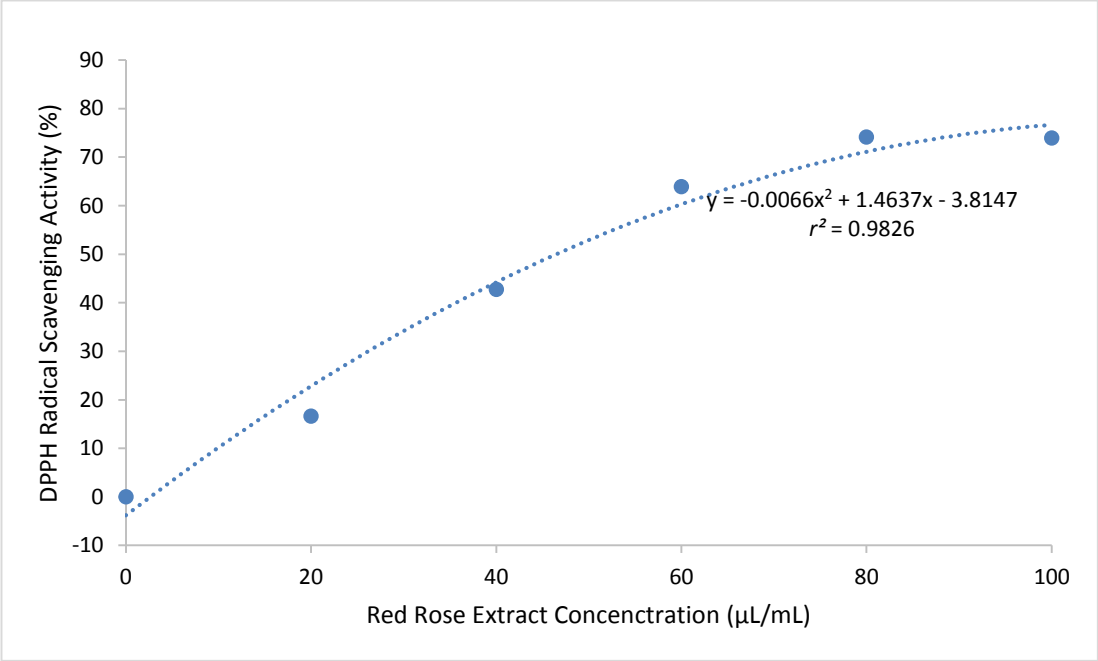

Figure 4.3. DPPH radical scavenging activity (%) of red rose extract.

In a study by Özkan et al., (2004) an examination was undertaken to assess the antiradical activity of extracts derived from newly bloomed and withered flowers of *R. damascena*. Specifically, at a concentration of 100 ppm, it was determined that the fresh flower extract manifested an antiradical activity of (75.51%), while the spent flower extract exhibited a marginally elevated antiradical activity of 75.94%.

By comparing the extract's percentage of inhibition of the DPPH radical's production, the radical-scavenging activity was ascertained. At 50 µg/mL, the acidic methanol extract exhibited high DPPH scavenging activity (76.5%). In general, it is well known that the DPPH scavenging capacity has been widely utilized in the screening of antioxidants, including carotenoids, phenolic compounds, and anthocyanins from

fruits, vegetables, crops, and natural goods. The study employed the (DPPH) assay to evaluate the antioxidant activity of compounds and extracts, focusing on red rose petal extract (Lee et al., 2005; Prior and Wu, 2006).

A study conducted by Shafirany et al., (2021) evaluated the DPPH radical-scavenging activity of red and purple *Hibiscus sabdariffa* petal extracts at various concentrations. The results indicated that the extracts exhibited strong antioxidant activity, with IC_{50} values of 63.77 μg/mL for red petals and 37.19 μg/mL for purple petals. Phytochemical screening revealed the presence of flavonoids and polyphenols in both red and purple petal extracts of *H. sabdariffa*. In a study by Baydar and Baydar (2013), the antiradical properties of synthetic antioxidants and methanolic extracts at concentrations of 50, 100, and 150 μg/mL were examined. The study revealed that the antiradical activity of extracts varied significantly depending on the extraction method and concentration. Specifically, cold extraction of fresh flowers at 150 μg/mL exhibited an antiradical activity of 89.86%, while hot extraction of spent flowers at 50 μg/mL demonstrated a lower activity of 65.88%.

According to Simin et al. (2024) all rose petal extracts tested this study showed significant antioxidant activity. The results of the two antioxidant assays conducted showed a strong correlation (r^2 = 0.983). The IC_{50} values in the DPPH assay for each genotype examined fell within a relatively narrow range 17.7–27.8 μg/mL, with *Rosa* × *hybrida* extracts exhibiting the highest level of activity. When compared to other Turkish variations of *Rosa damascena*, which had IC_{50} values ranging from 3.9 μg/mL to 32.0 μg/mL, the newly examined cultivars in this study displayed similar antioxidant activity. The antioxidant activity of rose petal extracts from all genotypes studied by Kumar et al. (2009) was significantly higher than that of vitamin C, which has an IC_{50} of 30.0 μg/mL in the DPPH assay. The methanol extracts *of Rosa brunonii*, *Rosa bourboniana*, and *Rosa damascena* exhibited IC_{50} values of 35.2 μg/mL, 25.0 μg/mL, and 21.4 μg/mL, respectively, indicating comparable antioxidant activity. Through a color-removing assay conducted at concentrations ranging from 20, 40, 60, 80and 100 μL/mL, a comprehensive profile of antiradical activity was delineated. Notably, a discernible trend emerged, indicating a progressive increase in antiradical activity with ascending extract concentration. The respective values obtained at different concentrations yielded radical scavenging activity between 16.60-73.89 %. Moreover,

to increase concentration from 80 to 100 µL/mL did not cause any further increase in antioxidant activity (Table 4.1).

4.4. Total Phenolic Content (TPC) Analysis

The total phenolic content of the extract derived from rose petals was quantified utilizing the Gallic acid calibration curve shown in Figure 4.4. Therefore, total phenolic content of ethanol extract of red rose was calculated as 23.10±0.81 mg GAE/g.

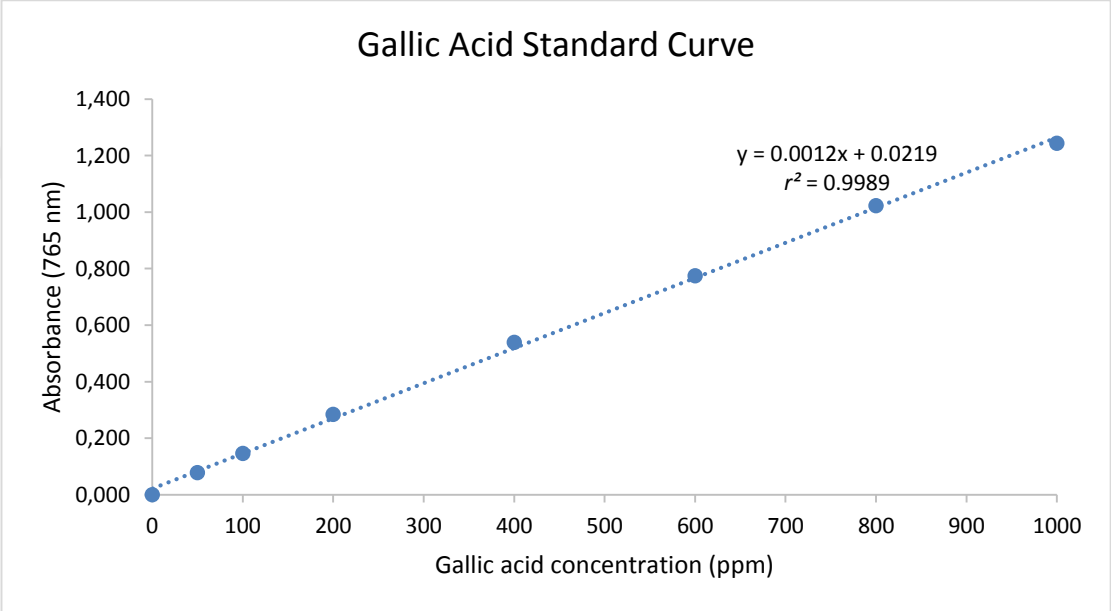

Figure 4.4. Gallic acid standard curve.

Owing to their diverse biological functions impacting human health, vitamins and phenolic compounds characterized by varying molecular structures, such as phenols, flavonoids, and anthocyanins, have elicited considerable scientific interest (Kim, 2018, 2019). According to research conducted by (Baydar and Baydar, 2013) this study investigates the potential of edible roses as a source of antioxidant compounds beneficial to human health. Nine cultivars of edible roses harvested in Jincheon, Chungbuk, were subjected to examination. Extracts derived from the flower petals of these edible roses were prepared, and the constituent antioxidant compounds, along with their antioxidant activity, were analyzed. Significantly, higher concentrations of total anthocyanins and total flavonoids were observed in the Mister Lincoln cultivar compared to others. Furthermore, the cultivars Mister Lincoln and Orange Meillandina exhibited significantly higher levels of total phenolic compounds and total antioxidant

activity compared to other cultivars ($p < 0.05$). A strong positive correlation was observed between total anthocyanin content and flavonoid content ($R = 0.927$), while a similarly strong correlation was noted between total phenolics and DPPH radical scavenging activity ($R = 0.915$). Overall, the study underscores the superior antioxidant compounds and activity found in edible roses compared to fruits and leafy vegetables. Consequently, edible roses emerge as a promising natural source of antioxidants with significant potential for application in functional food production and the cosmetic industry.

In a study conducted by Yang & Shin (2017), notable variations were observed in the total phenolic contents of 9 different edible rose cultivars. The total phenolic contents ranged from 798.67mgGAE/g to 2978.89 mgGAE/100 g across the cultivars, indicating considerable diversity in the phenolic composition among the varieties investigated. Similarly, research by Kumar et al., (2009) reported total phenolic contents of roses within the range of 14.5 g to 25.4 g GAE/100g. These findings underscore the variability in phenolic composition among different studies and highlight the need for comprehensive investigation into the factors influencing phenolic content in roses.

Contrastingly, a study conducted by Rop et al. (2012) revealed total phenolic concentrations ranging from 253 mg to 528 mg GAE/100g across 12 cultivars of edible flowers. These levels were found to be comparable to those observed in certain fruits such as raisin, plum, and blueberry, albeit significantly higher than those recorded in vegetables like cabbage, cucumber, and onion. Such comparisons emphasize the potential significance of roses as a rich source of phenolic compounds, which contribute to their nutritional and health-promoting properties. In a comprehensive investigation conducted by Kumari et al. (2022) fifty distinct varieties of roses were examined, revealing a broad spectrum of total phenolic content ranging from 5.21 ± 0.39 mg GAE/100g to 427.59 ± 3.47 mg GAE/100g. The results elucidated a correlation between petal color and phenolic content, with roses exhibiting vibrant red hues demonstrating higher phenolic concentrations compared to those with lighter colorations.

This observation aligns with prior findings by Zheng et al.(2018) who observed that, with the exception of white roses, red and pink-colored rose species exhibited the highest total phenolic content. Furthermore, Roman et al.(2013) conducted a study

focusing on *Rosa canina,* revealing a variation in total phenolic content ranging from 326 mg/100g frozen extract to 575 mg/100g frozen extract.

This investigation underscores the diversity in phenolic composition among different rose species, highlighting the importance of species-specific analysis in elucidating phenolic profiles. Similarly, Ge and Ma, (2013) reported a total phenolic content of 2087.43 ± 17.37 mg gallic acid equivalents (GAE) per 100g fresh weight (FW) in rose petals. Notably, certain cultivars of Rosa hybrida exhibited particularly elevated levels of phenolic compounds compared to others within the study cohort. For instance, the orange Meillandina cultivar demonstrated a robust total phenolic content of 2805.56 mg/100 g, while the red-colored Mister Lincoln cultivar exhibited an even higher concentration of 2978.89 mg/100 g

These findings underscore the substantial variability in phenolic composition among different rose cultivars, with specific varieties displaying notably higher phenolic content compared to others. In a study conducted by Pal et al.(2018), it was revealed that the methanol extract exhibited the high (TPC) value among the extracts examined. Specifically, the TPC value of the methanol extract was measured at 296.60 µg GAE / mg. This finding suggests that the methanol extract contained a significant concentration of phenolic compounds compared to other extracts evaluated in the study.

4.5. Antimicrobial Analysis

In this experiment, antibacterial activity of fresh rose petals ethanol (70%) extract of *Rosa hybrida* was assessed against seven bacterial isolation, encompassing over Gram-positive and Gram-negative types. The bacterial strains included *B. cereus, S. aureus, L. monocytogenes* (ATCC 7644), *S.* Typhimurium, *S.* Enteritidis (ATCC 13076), *E. coli* O157:H7, and *E. coli* Biotype I. Three replications were performed to evaluate the antibacterial effects against each strain. The assessment was based on the measurement of the diameter of the clear zone observed around the bacterial cultures in Petri plates, serving as an objective indicator of the extract's antibacterial sensitivity and efficacy.

The objective of this study was to evaluate the antibacterial activity exhibited by rose petals. The findings revealed a notable difference in the antimicrobial effect of the ethanolic extract between low and high dilutions. Specifically, the antibacterial activity

was observed to be more pronounced at lower dilutions compared to higher dilutions. The well diffusion assay results were shown in Figures 4.5, 4.6 and Table 4.2.

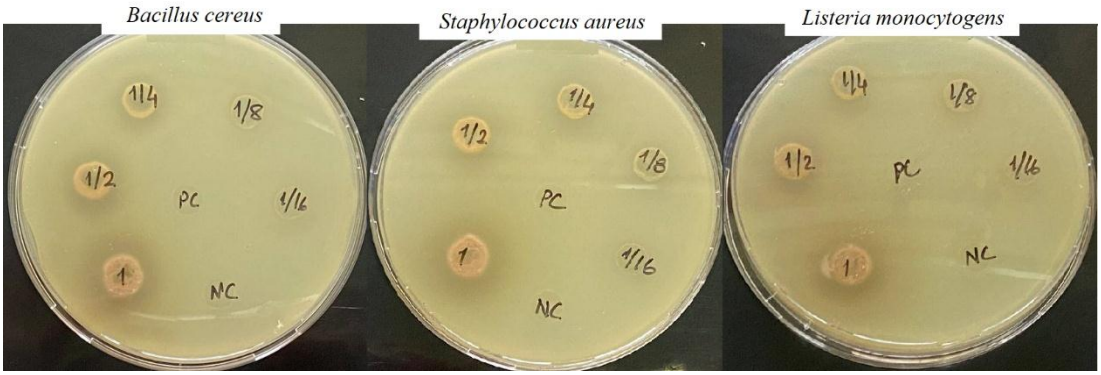

Figure 4.5. Well diffusion assay test results of Gram-positive bacteria.

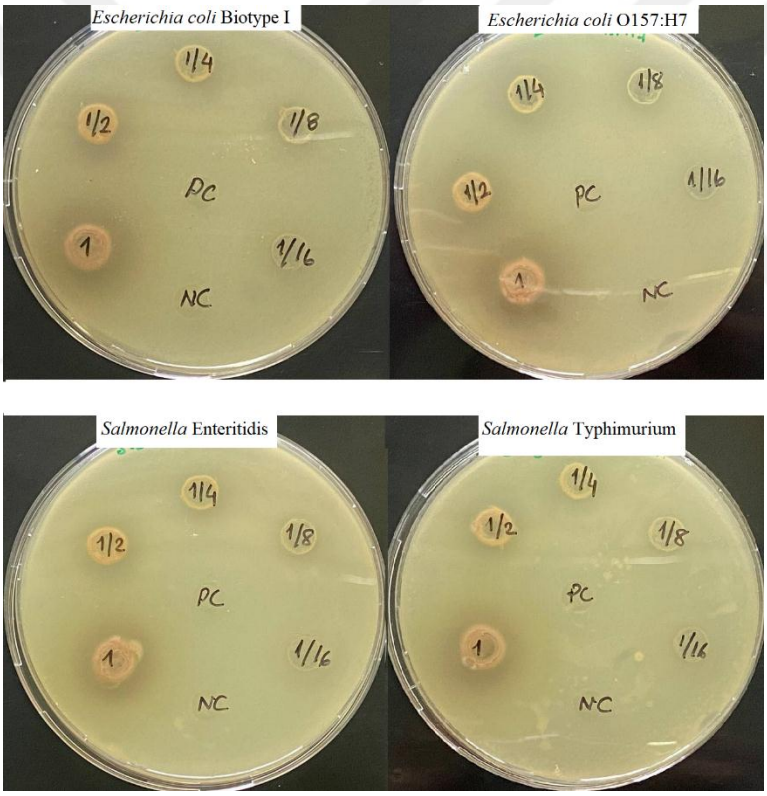

Figure 4.6. Well diffusion assay test results of Gram-negative bacteria.

The size of inhibition area ranged from 23 to 32.5 mm. Particularly noteworthy was the observation that the ethanol extract demonstrated enhanced inhibition against *L. monocytogenes*, *S.* Typhimurium, *S. aureus*, *E. coli Biotype* I very well, while *E. coli O157:H7*, *B. cereus* and *S.* Enteritidis were less sensitive. Moreover, analysis revealed that the zone of inhibition was larger at higher concentrations when comparing the antibacterial activity of rose petals with the control group. The findings of the study

conducted by Safdar and Malik, (2020) revealed the inhibitory effect of rose extract on the growth of various bacterial strains, encompassing both Gram-positive and Gram-negative bacteria. Specifically, they observed significant inhibition against *B. cereus* (30 mm), *S. aureus* (20 mm), and *E. coli Biotype* I (24 mm).

Table 4.2. Inhibition zone diameters (mm) measured in well diffusion assay (*n*=2).

Microorganism	Red rose extract concentrations					Positive control	Negative control
	1	1/2	1/4	1/8	1/16		
Gram-positives							
L. monocytogenes	32.50 ± 3.54	28.00 ± 2.83	22.00 ± 2.83	13.50 ± 4.24	11.50 ± 2.12	30 ± 1.41	-
S. aureus	25.00 ± 0.00	22.50 ± 0.71	19.00 ± 1.41	16.00 ± 0.00	12.00 ± 0.00	30 ± 1.41	-
B. cereus	23.00 ± 3.54	23.00 ± 4.24	20.50 ± 0.71	16.00 ± 1.41	11.50 ± 0.00	30 ± 0.00	-
Gram-negatives							
S. Typhimurium	31.00 ± 1.41	28.00 ± 2.83	22.50 ± 3.54	18.50 ± 2.12	12.00 ± 2.12	28 ± 2.83	-
E. coli Biotype I	25.00 ± 0.00	23.00 ± 0.00	20.00 ± 0.00	14.50 ± 0.71	12.50 ± 0.71	28 ± 1.41	-
E. coli O157:H7	23.50 ± 4.95	23.50 ± 2.12	20.50 ± 0.71	13.50 ± 3.54	12.50 ± 2.83	28 ± 2.83	-
S. Enteritidis	23.00 ± 3.54	22.00 ± 0.00	19.00 ± 1.41	17.00 ± 1.41	10.00 ± 2.12	30 ± 2.83	-

This suggests the potential of rose extract as a broad-spectrum antibacterial agent, capable of targeting bacteria with diverse cell wall compositions and structural characteristics. The observed inhibition zones provide valuable insights into the effectiveness of rose extract against these pathogenic bacteria. The study conducted by Sivaraj et al. (2019) investigated the inhibitory capacity of *Rosa damascena* extract against various bacterial strains, including *S. aureus* and *E. coli* Biotype I. Their findings indicated that the extract exhibited an inhibition area of 17 mm against *S. aureus*, which was comparatively lower than the inhibition observed in our study using *Rosa hybrida* extract. Conversely, for *E. coli* Biotype I, Sivaraj et al. (2019) also reported an inhibition zone of 34 mm, which was higher than the inhibition observed in our study. These differences in inhibition capacities between the two studies could potentially be attributed to variations in factors such as the extraction method, concentration of the extract, bacterial strains tested, and environmental conditions. Differences in the composition of the rose extracts, as well as variations in the susceptibility of bacterial strains to the active materials present in the extracts, and may be contribute to the observed variations in inhibition capacities. The discrepancy in inhibition zones underscores the importance of considering multiple studies and evaluating various factors when interpreting and comparing experimental results. Further research is necessary to elucidate the specific mechanisms underlying the antimicrobial activity of various rose extracts and to optimize their effectiveness against specific bacterial strains.

The investigation conducted by Özkan et al., (2004) focused on evaluating the antibacterial activity of extracts derived from *Rosa damascena* against various bacterial strains. The results revealed distinct variations in the inhibition zones observed for different bacterial species upon exposure to the *Rosa damascena* extract. Specifically, notable inhibition zones were observed for *E. coli* (17 mm), *B. cereus* (16 mm), *S. aureus* (12 mm), *S.* Enteritidis (21 mm), and *S.* Typhimurium (17mm) indicating a degree of antibacterial efficacy associated with the extract. However, it is noteworthy that no inhibition zone was detected for *E. coli O157:H7*, suggesting potential differences in susceptibility among bacterial strains to the antimicrobial effects of *Rosa damascena* extract.

Interestingly, our study underscored the superior antibacterial activity of *Rosa hybrida* petal extract compared to other rose species investigated. This was evidenced by the

larger inhibition zones observed for the tested bacterial strains, indicating that *Rosa hybrida* may possess distinct bioactive compounds or higher concentrations there of that confer enhanced antibacterial properties. Additionally, the significant inhibition zone observed for *L. monocytogenes* upon exposure to *Rosa hybrida* petal extract highlights its potential as an effective inhibitor of this pathogenic strain. The results showed that *Rosa hybrida* petal extract may serve as a promising antibacterial agent, particularly against *L. monocytogenes* (Table 4.2).

In a study conducted by Yilmaz and Ercisli, (2011) the antibacterial efficacy of methanolic extracts derived from various *Rosa* taxa fruits against a range of bacteria was investigated. The results revealed variations in antibacterial activity among the different taxa tested. Specifically, the methanolic extract of *Rosa* taxa exhibited inhibitory effects on the growth of *Yersinia enterocolitica*, *Streptococcus aureus*, *Bacillus cereus*, and *Salmonella* Typhimurium, with inhibition zones ranging from 9 to 13 mm. Furthermore, the extract from *Rosa canina* demonstrated antibacterial activity against *B. cereus*, *Enterococcus faecalis*, and *Yersinia enterocolitica*, as evidenced by inhibition zones measuring (9–11 mm). These findings underscore the potential of *Rosa* taxa extracts as antibacterial agents, with distinct effects observed across different bacterial strains and *Rosa* species. The *Rosa villosa* extract, *Bacillus cereus* and *Enterococcus faecalis* exhibited inhibitory zones measuring 10–12 mm. Similarly, the extract from *Rosa dumalis* subsp. *antalyensis* demonstrated suppressive effects on the growth of *B. cereus, Y. enterocolitica a*nd *Klebsiella pneumoniae*, resulting in inhibition areas ranging from 10 to 14 mm.

The bactericidal properties of *Rosa indica* (red) against pathogenic bacteria were evidenced in a study by Mishra et al (2011) They showed the methanolic extract of *Rosa indica* flowers exhibited the widest zone of inhibition, measuring 18 mm, followed by leaf extracts with a zone of 14 mm and stem extracts with a zone of 17 mm. Specifically, an ethyl acetate extract of *Rosa indica* stem demonstrated a notable 13 mm zone of inhibition against *Pseudomonas aeruginosa*. However, this extract showed no inhibitory effects on *Escherichia coli* or *Staphylococcus aureus*.

4.6. Minumum Inhibitory Concentration (MIC)

The low concentration of antimicrobial substances needed to prevent the apparent growth of bacteria during an overnight incubation period is known as the Minimum

Inhibitory Concentration (MIC). In our study, the MIC values for various pathogens, including *S.* Typhimurium, *S.* Enteritidis, *B. cereus, S. aureus, E. coli* O157:H7, *E. coli* Biotype I, and *L. monocytogenes,* were determined. MIC values (mg/ml) are shown in Table 4.5. Upon analysis, it was found that the MIC values varied among the tested pathogens. *S.* Typhimurium exhibited a MIC value of 8mg/ml, indicating that the antimicrobial agent was effective at a relatively low concentration against this pathogen. Similarly, *E. coli O157:H7* and *E. coli* Bio*type I* also displayed a MIC value of 8mg/ml, suggesting susceptibility to the antimicrobial agent at this concentration. In contrast, *S. Enteritidis, B. cereus, S. aureus, and L. monocytogenes* exhibited slightly higher MIC values, ranging from 16mg/ml to 8mg/ml.

Table 4.3. The minimum inhibitory concentration (MIC) values of rose extract against Gram-positive and Gram-negative bacteria.

Microorganism		MIC value (mg/mL)
Gram-positives	*L. monocytogene*s	95.05
	S. aureus	76.04
	B. cereus	57.03
Gram-negatives	*S.* Typhimurium	114.07
	E. coli Biotype I	114.07
	E. coli O157:H7	95.05
	S. Enteritidis	57.03

In our study MIC (Minimum Inhibition Concentration) values ranged from 114.07 mg/mL to 57.03 mg/mL. *S.* Typhimurium and *E. coli* Biotype, had the highest MIC values, and *S.* Enteritidis and *B. cereus* had the lowest MIC values. Another study by Chroho et al., (2022) investigated that the minimum inhibitory concentration (MIC) of the Rosa damascena extract against each tested bacterium ranged from 20.83 ± 0.12 to 41.66 ± 0.15 mg/mL. Notably, the extract showed a bacteriostatic effect against *E. coli,* indicated by an MBC/MIC value of 8. Conversely, it demonstrated a bactericidal effect against *Salmonella* Typhimirium, *S. aureus*, and *L. monocytogenes*, as evidenced by an MBC/MIC value of 4.

Shohayeb et al. (2014) showed that the antimicrobial efficacy of rose oil and diverse extracts was evaluated through the determination of MIC values against a spectrum of tested microorganisms. MIC values ranging from 0.125 to 8 mg/ml were observed across the various formulations. Notably, Gram-positive bacteria demonstrated a

higher susceptibility compared to Gram-negative bacteria. Gram-negative strains, with the exception of *A. baumannii*, exhibited inhibition at doses ranging between 2 and 8 mg/ml, while complete microbial eradication was achieved at concentrations ranging from 4 to greater than 8 mg/ml of extract. In contrast, Gram-positive bacteria were inhibited by lower concentrations, ranging from 0.125 to 2 mg/ml of extracts, and effectively eradicated at doses between 0.5 and 4 mg/ml.

4.7. Kill Time Assay

The time-kill kinetics profile of the *Rosa hybrida* extract against various bacterial strains, including *E. coli* Biotype I, *S. aureus, B. cereus, S.* Typhimurium, *S.* Enteritidis, *E. coli* O157:H7, *and L. monocytogenes,* demonstrated a substantial decrease in viable cell counts within specific time frames. For *E. coli* Biotype I, *S. aureus, and B. cereus*, this reduction occurred within the initial 8, 2, and 2 hours, respectively. Similarly, against *S.* Typhimurium, *S.* Enteritidis, *E. coli* O157:H7, and *L. monocytogenes*, viable cell counts decreased within the initial 8, 8, 2, and 8 hours, respectively, following exposure to the rose extract at test doses. These findings indicate the potential of *Rosa hybrida* extract as an antimicrobial agent, showing efficacy against a spectrum of bacterial strains within relatively short time periods. In the control groups, which were devoid of any antimicrobial agents, the bacterial strain numbers exhibited gradual increases in viability over specific time intervals. Specifically, for *E. coli* Biotype I, the viable cell count increased from 4 hours, while for *S. aureus, B. cereus, S.* Typhimurium, and *S.* Enteritidis, this increase was observed up from 4 hours. Similarly, for *E. coli* O157:H7 and *L. monocytogenes*, the viable cell count showed a gradual up from 2 hours. These observations underscore the natural growth dynamics of the bacterial strains under investigation in the absence of antimicrobial intervention. Figure 4.6 (continued) shown the kill time kinetics profile of tested microorganisms.

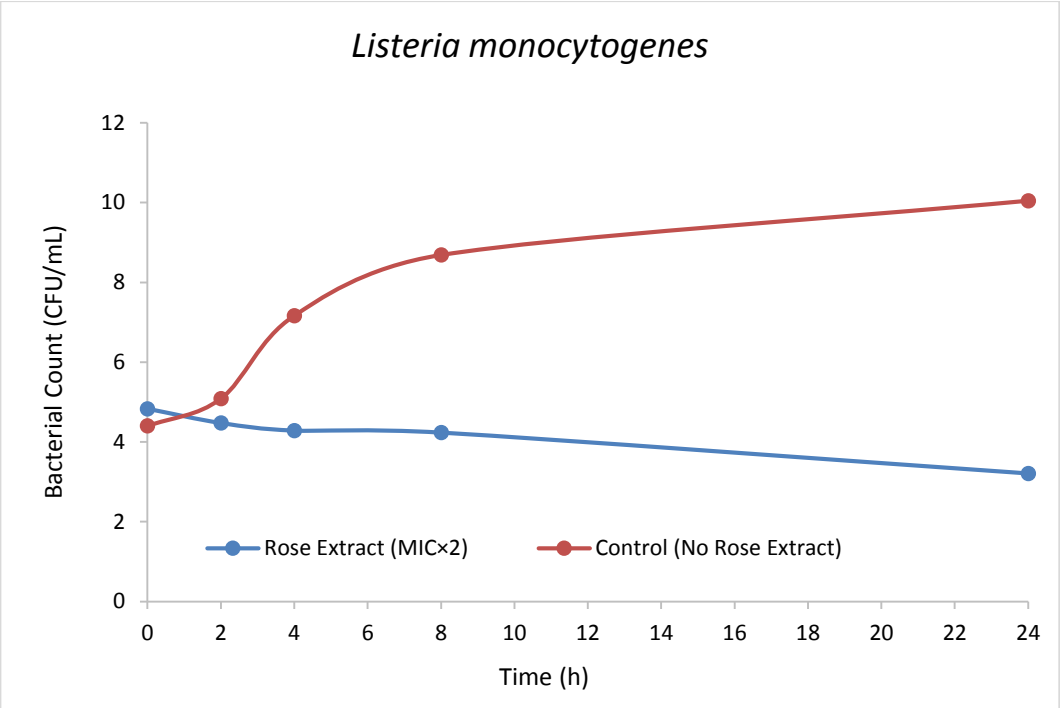

Figure 4.7. The time-kill kinetics profile of *Listeria monocytogenes.*

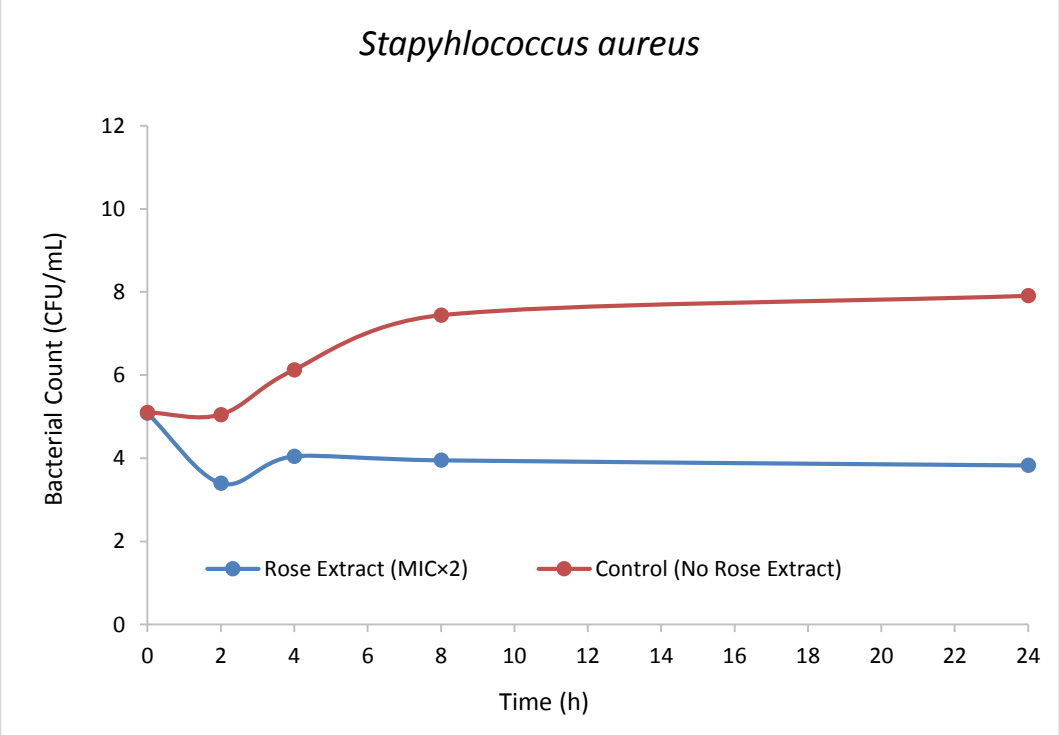

Figure 4.8. The time-kill kinetics profile of *Staphylococcus aureus.*

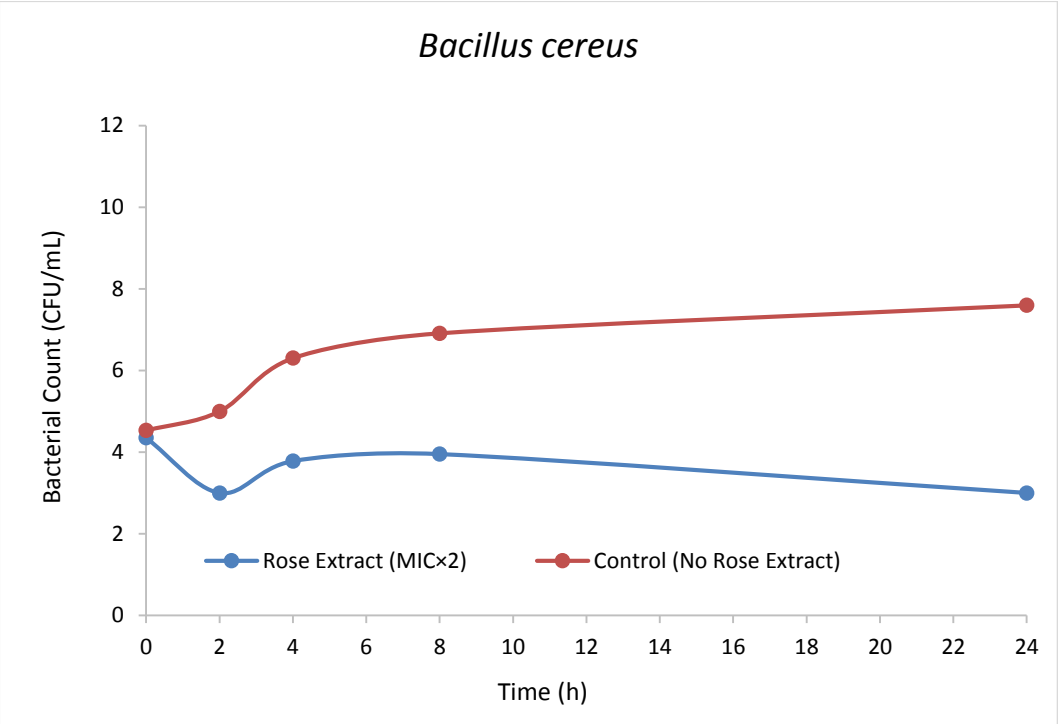

Figure 4.9. The time-kill kinetics profile of *Bacillus cereus.*

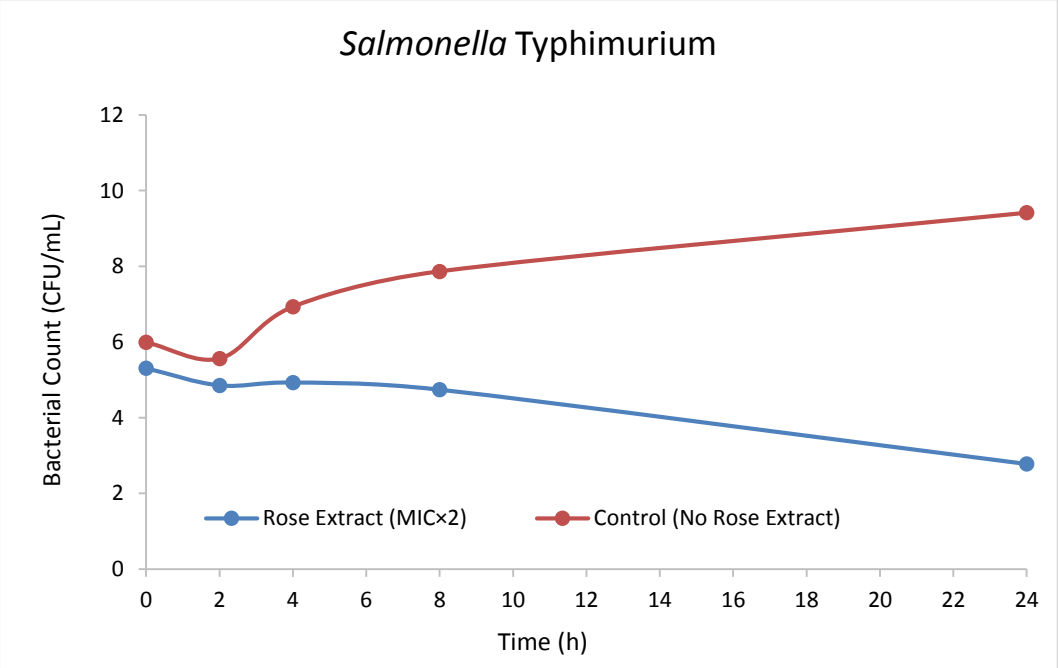

Figure 4.10. The time-kill kinetics profile of *Salmonella* Typhimurium.

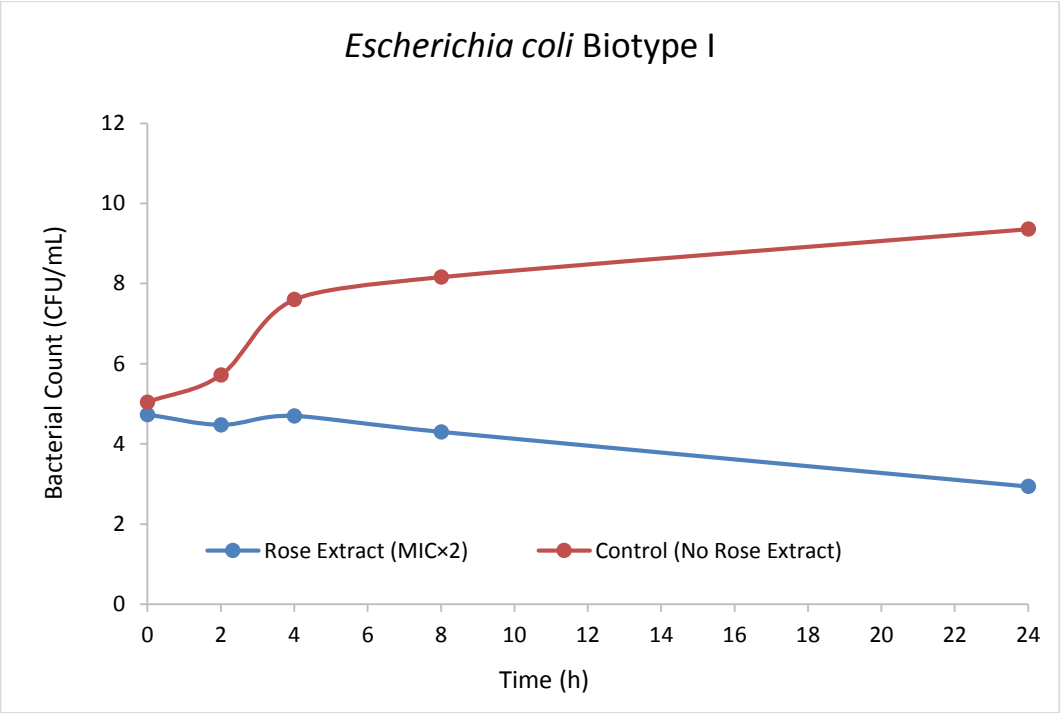

Figure 4.11. The time-kill kinetics profile of *Escherichia coli* Biotype I.

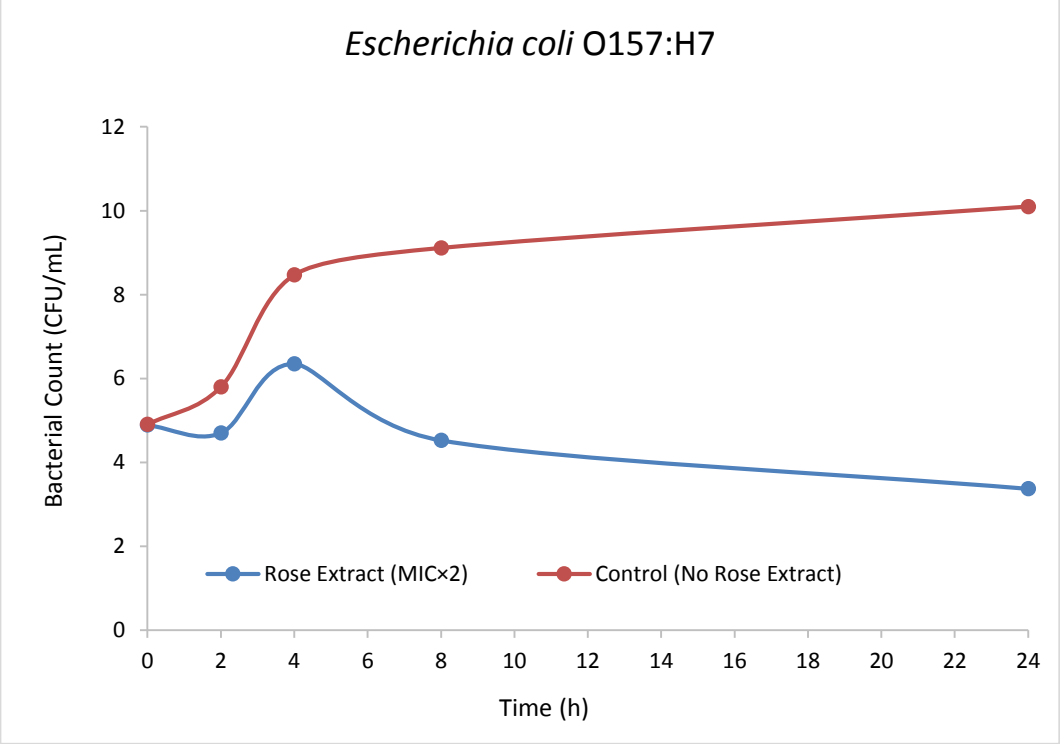

Figure 4.12. The time-kill kinetics profile of *Escherichia coli* O157:H7.

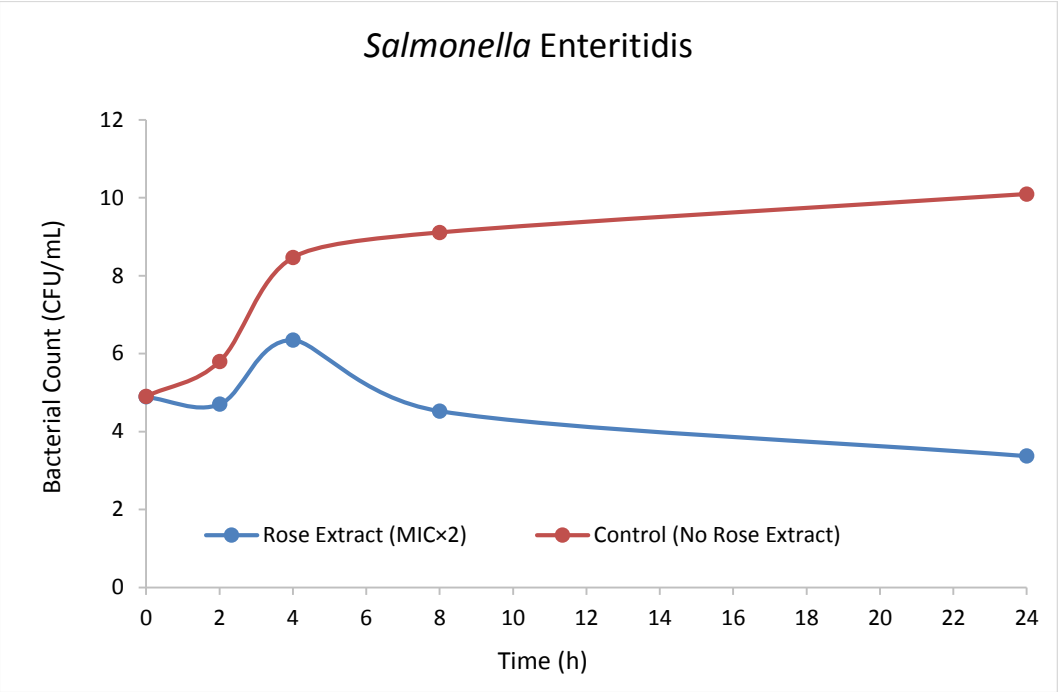

Figure 4.13. The time-kill kinetics profile of *Salmonella* Enteritidis.

4.8. Determination of Membrane Damage

Membrane damage analysis constitutes a critical examination aimed at elucidating the mechanism through which rose extract exerts its inhibitory effect on bacterial growth within the cytoplasm or cells of bacteria. This investigative endeavor seeks to discern both the extent of bacterial survival and the nature of damage inflicted upon bacterial cells upon exposure to rose extract. Integral to this analysis is the evaluation of NaCl influence on bacterial growth inhibition. Given its known inhibitory properties against bacterial proliferation, understanding the survival rates of bacteria following exposure to NaCl in conjunction with rose extract is paramount. This holistic approach endeavors to unravel the synergistic or antagonistic interactions between NaCl and rose extract and their collective impact on bacterial cell viability and integrity(Espina et al., 2016). The number of cells with damaged membranes was determined from the difference between cells incubated in the media. Figure 4.14 shows the number of survivors after exposing red rose extract.

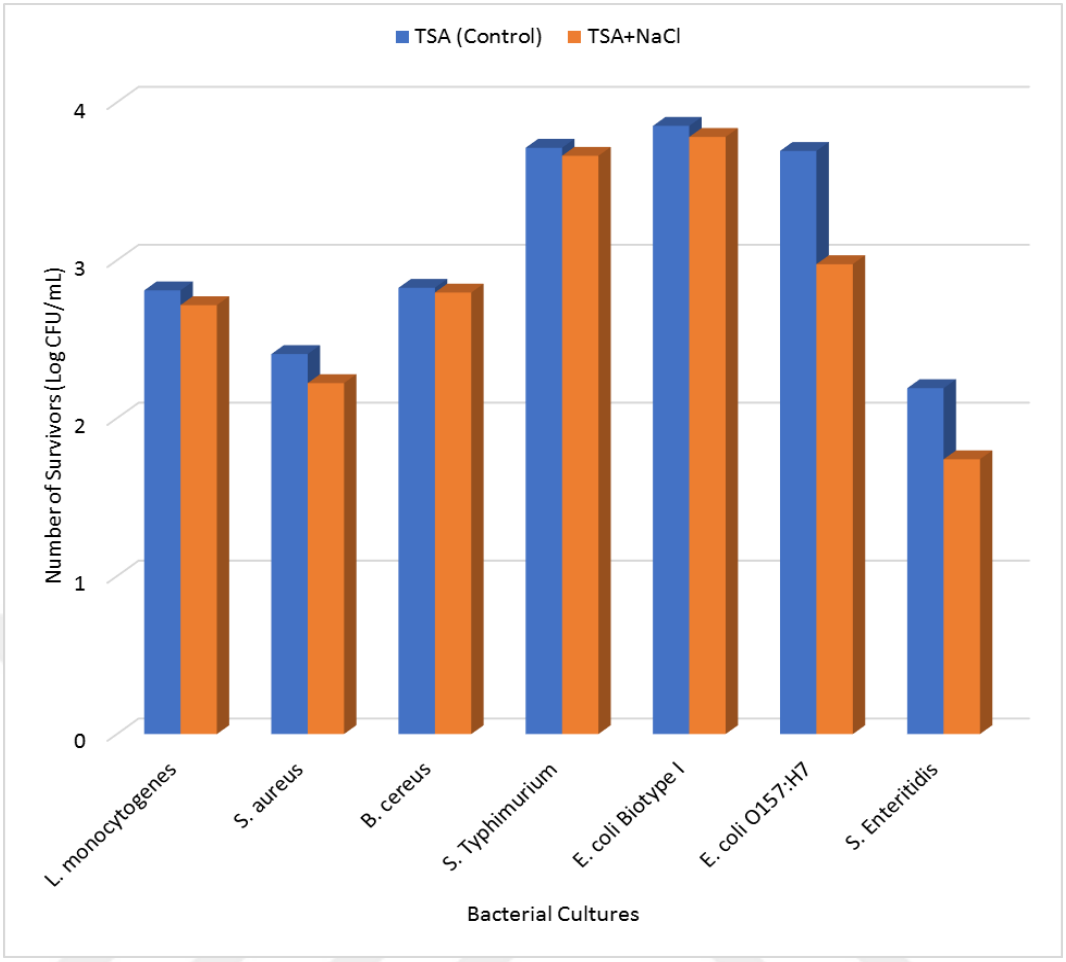

Figure 4.14. The bacterial counts determined on non-selective (TSA) and selective (TSA with 3% NaCl) media.

The study investigated the impact of rose extract and sodium chloride (NaCl) on bacterial injured cell counts, aiming to elucidate their effectiveness in inhibiting bacterial growth and inducing cell damage. The results unveiled distinct reductions in bacterial cell counts when exposed to TSA control and TSA+NaCl conditions across different bacterial strains. Specifically, the reduction percentages varied significantly among the tested strains as demonstrated in Figure 4.15. *E. coli* O157:H7 exhibited the highest reduction percentage (80.83%), followed by *S.* Enteritidis (64.52%), *S. aureus* (34.51%), *L. monocytogenes* (19.38%), *E. coli* Biotype I (14.86%), *S.* Typhimurium (11.5%) and *B. cereus* (6.72%). Of particular note, the highest level of reduction was observed in *E. coli* O157:H7 injured cell, indicating its heightened susceptibility to the combined action of rose extract and NaCl. Conversely, *B. cereus* demonstrated the lowest reduction percentage, suggesting a comparatively lower vulnerability to the antimicrobial effects of the tested agents. Moreover, the combined treatment of rose extract and NaCl exhibited notable efficacy in inducing

cell injury, particularly against *E. coli* O157:H7, *S.* Enteritidis, and *S. aureus*. However, its effectiveness appeared to be less pronounced against *B. cereus*, as evidenced by the comparatively lower reduction percentage observed in this strain. These findings underscore the differential susceptibility of various bacterial strains to the antimicrobial properties of rose extract and NaCl.

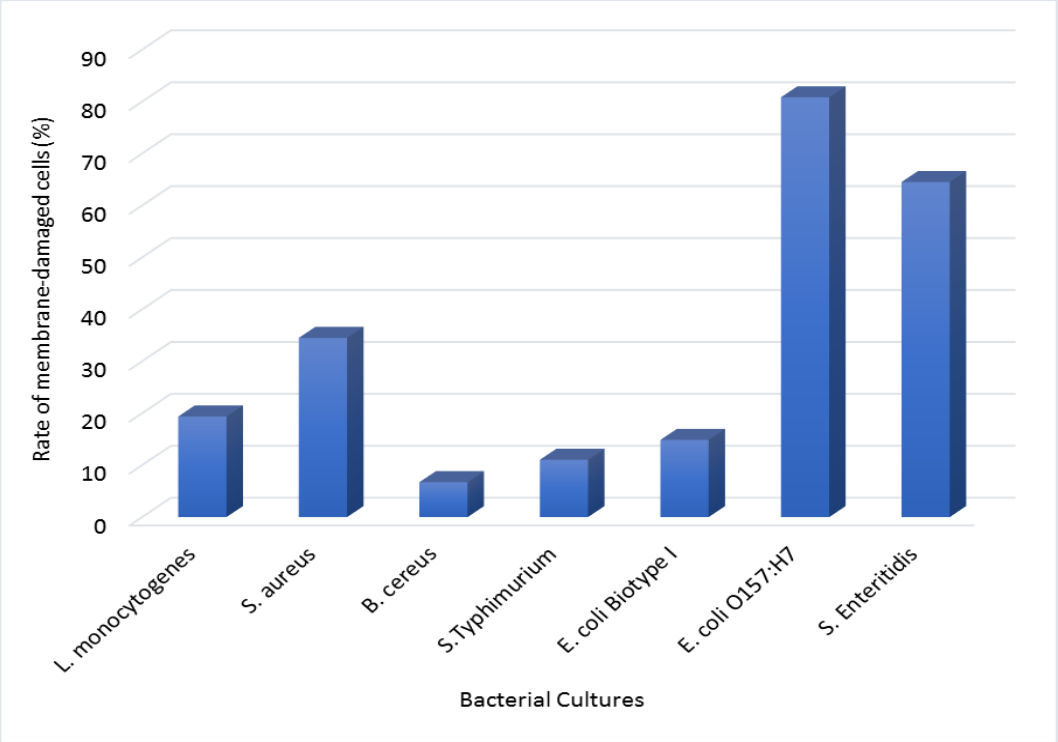

Figure 4.15. The rate of Membrane damage cells in TSA control.

4.9. Effect of Rose Petal Extract on Tricarboxylic Acid (TCA) Cycle

The utilization of iodonitrotetrazolium chloride (INT) in assessing the metabolic activity of live cells relies on its conversion to a stable red formazan when it reacts with the H+ ions generated by dehydrogenases involved in the electron transfer mechanism of the tricarboxylic acid (TCA) cycle. The bacterial cultures were treated with the extract at a concentration of two-fold MIC value. The resultant restoration of INT to its red formazan signifies the metabolic activity of the cells. Upon treatment with the (2× MIC) extract, the observed reductions in metabolic activity varied across the tested bacterial strains. Figure 4.16 and Figure 4.17 show the test tubes after incubation.

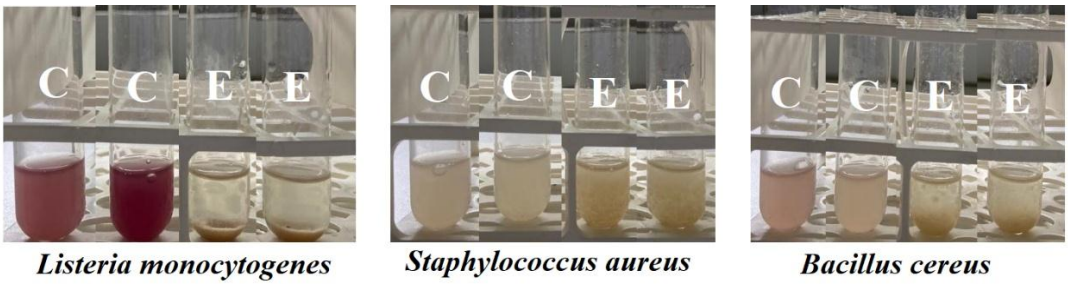

Figure 4.16. Effect on TCA cycles of Gram-positive bacteria (C: control, E: Rose extract).

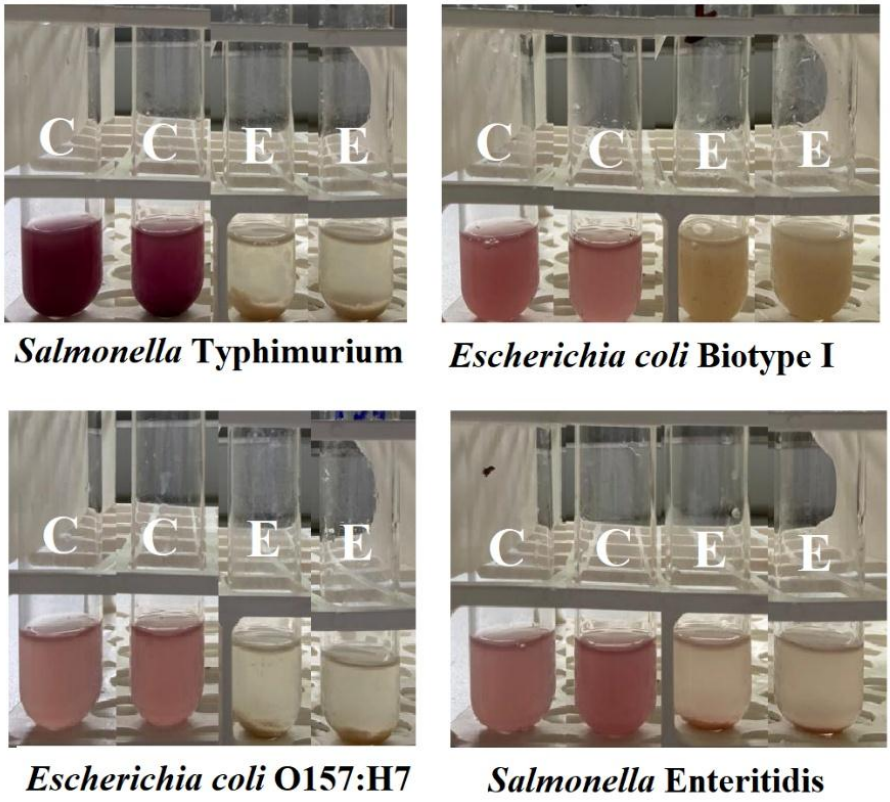

Figure 4.17. Effect on TCA cycles of Gram-negative bacteria (C: control, E: Rose extract).

Figure 4.18 displays the absorbance values at 630 nm measured after 30-min incubation in the presence of INT. Of note, the extract exerted a relatively minimal impact on the metabolic activity of *Escherichia coli O157:H7*, whereas it demonstrated a more pronounced effect on the TCA cycle of *Salmonella* Typhimurium. These findings underscore the differential susceptibility of bacterial strains to the extract's influence on metabolic activity, with potential implications for understanding the mechanism of action and therapeutic efficacy of the extract against specific bacterial pathogens. At the termination of the concluding 30-minute

incubation period, the coloration of the control tubes manifested a transformation to a purple hue, in accordance with the anticipated outcome (Guo et al., 2021).

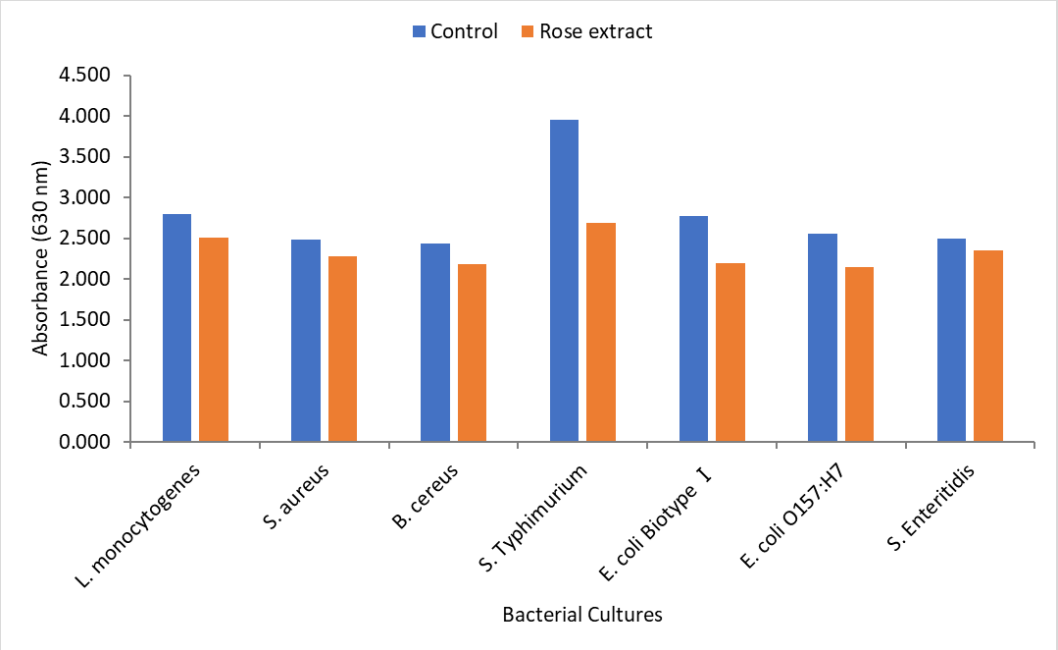

Figure 4.18. The absorbance values at 630 nm (*n*=2).

5. CONCLUSIONS

In conclusion, this study demonstrates red rose petal extract's significant antimicrobial and antioxidant properties, making it a promising natural preservative in the food industry. Utilizing freeze-drying and ethanol extraction, the extraction process effectively retained critical bioactive compounds, particularly anthocyanins, known for their diverse physiological effects. The extract exhibited inhibitory effects against various foodborne pathogens, including *S.* Typhimurium, *S.* Enteritidis, *B. cereus*, *S. aureus*, *E. coli* O157:H7, *E. coli* Biotype I, and *L. monocytogenes*, as evidenced by healthy diffusion assays and determination of Minimum Inhibitory Concentration (MIC) values.

Furthermore, the extract showed significant antioxidant activity, which can contribute to preserving food quality by preventing oxidative degradation and extending shelf life. Time-dependent studies revealed a rapid decrease in bacterial cell viability upon exposure to the extract, highlighting its potential as an effective antimicrobial agent. Additionally, experiments assessing damage to bacterial cytoplasmic membranes and alterations in the tricarboxylic acid (TCA) cycle activity further supported the antimicrobial mode of action of the extract. Overall, the findings underscore the potential of red rose petal extract as a natural and effective preservative and antimicrobial agent in food products. Further research is warranted to explore its efficacy in different food matrices and its safety for consumption, paving the way for its integration into various food products as a natural and health-promoting ingredient.

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