T.R.
SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *EQUISETUM ARVENSE L.*

MSc. THESIS

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Biyoloji Department

DECEMBER 2023

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Thesis Advisor: Prof.Dr. Şule BARAN

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The thesis work titled "INVESTIGATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *EQUISETUM ARVENSE L."* prepared by Sarah Luay ALAZZAWI was accepted by the following jury on 01/12 /2023 by unanimously/majority of votes as a MSc THESIS in Sakarya University Graduate School of Natural and Applied Sciences, Biology.

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Sarah Luay ALAZZAWI

***To my family, who are my ammunition and to my country Iraq*.**

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Sarah Luay ALAZZAWI

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ABBREVIATIONS

%	: Percentage
µg	: Micrograms
µm	: Mikrometre
Bs	: *Bacillus subtilis*
CFU	: Colony forming unit
cm	: Centimeter
DPPH	: The 2,2-Diphenyl-1-picrylhydrazyl
Ec	: *Escherichia coli*
EDX	: Energy Dispersive X-Ray
Ef	: *Enterecoccus faecalis*
g	: Gram
m	: Metre
mg	: Miligram
mL	: Mililitre
mm	: Milimetre
Na2CO3	: Sodium Carbonate
ºC	: Degrees centigrade
Sa	: *Staphylococcus aureus*
Se	: *Staphylococcus epidermidis*
SEM	: Scanning Electron Microscope
St	: *Salmonella typhimurium*
TPC	: Total phenolic content
TSA	: Tyriptic Soy Agar
TSB	: Tyriptic Soy Broth

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INVESTIGATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *EQUISETUM ARVENSE* L.

SUMMARY

To date, the search for bioactive compounds from new natural sources has been shown to improve food quality, general standard of living and public health. Among bioactive compounds, especially phenolic compounds constitute the most studied group.Many studies have proven that plant-derived polyphenols have anti-aging, anti-inflammatory and anti-proliferative properties and are effective in reducing the risk of developing cardiovascular diseases, cancer and diabetes.

In addition, because of the antioxidant activities of phenolic compounds, they can prevent oxidative damage by neutralizing free radicals, clearing oxygen or breaking down peroxides.

It is estimated that between fifty thousand and seventy thousand different plant species are used in various medicinal practices around the globe, including both traditional and contemporary approaches. The cosmetics and botanicals businesses, which are both developing rapidly, utilize an uncountable number of other species.

The vast majority of these components are obtained via the collecting of samples from various natural resources. Wild collection is expected to remain the primary source for the majority of medicinal and aromatic plant species in the majority of the world's regions, based on the ecological, economic, and social variables that are involved in the matter. This is the case despite the increased interest in cultivation.

The issue of bacterial resistance is a prevalent concern that has garnered significant attention in the research community. Numerous efforts are currently underway to identify viable solutions and explore potential chemical or natural alternatives that possess the requisite therapeutic properties. These alternatives should exhibit potent antibacterial and antifungal efficacy while minimizing adverse effects and economic burden. Additionally, the inclusion of efficacious antioxidants is highly desirable in such alternatives.

Herbs have long been used to counteract all kinds of microbial infections. Many types of microorganisms can develop multiple resistance to standard antibiotics. New molecules isolated from plants can break the bacterial resistance mechanism and inhibit bacterial growth. For such reasons, in the face of the emergence of antibiotic-resistant forms of bacteria, it has become a necessity to search for new active molecules with a wide antibacterial spectrum.

Natural compounds, such as those found in medicinal plants, are receiving more research interest due to their antibacterial capabilities. There is reason to believe that microbial resistance to plant compounds might develop at a far slower rate, if at all, than chemical resistance, which reflects well for the prospects of such investigations.

The biological activities of plant extracts largely depend on the extraction efficiency of bioactive components and the composition of the extracts. Extraction with solvents is frequently used for the isolation of antioxidant compounds, and both the extraction efficiency and antioxidant activity of extracts have a strong relationship with the solvent used, mainly due to the different polarities of the compounds obtained.

Organic solvents (petroleum ether, hexane, chloroform, methanol, etc.) are widely used, especially for the extraction of phenolic compounds to be used as antioxidants. The choice of the most suitable solvent is a determining factor on the extract properties, and due to the different structure and composition of the matrix, each matrix-solvent system exhibits a certain unpredictable behavior.

Due to the fact that the extracts of *Equisetum arvense* L. contain a wide variety of pharmacological activities, they are of major value in the area of drug development. This is something that has been acknowledged in a number of different countries. *Equisetum arvense* has a long history of use in traditional medical practices, where it has been used to treat a variety of diseases, including brittle fingernails, hair loss, and rheumatic disorders.

This plant has been recognized as a potential source of medicine in a number of different countries due to the ease with which it may be collected, its widespread availability, and its significant biological features.

This study was conducted on 100 swabs (burn, ear, abscess, nose), stool, and urine samples, isolated and previously diagnosed in the laboratory. They were brought to the laboratory from Sakarya Research Hospital in 2023, a period between the months of 8-12.

In our study, it was aimed to determine the solvent and extraction method that reveals the biological activities and phenolic content of the *Equisetum arvense* plant at the maximum rate. Leaf and stem extracts of *Equisetum arvense* were prepared using the soxhlet and maceration method in the presence of 5 different solvents.

Total phenolic content (TPC) of the extracts was determined by using the Folin-Ciocalteu method, and antioxidant activities were determined by using the DPPH radical scavenging test.

The *Equisetum arvense* plant was examined in this research using scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDX) data. The SEM images show the surface of the leaf, while the EDX data show the inside of the leaf. The stem of the plant was also examined. The results of this study revealed that there are differences in the morphological images of each component that was employed, as well as differences in the elemental composition that was shown in the results of the EDX spectroscopic analysis.

The antibacterial activity of *Equisetum arvense* leaf and branch extracts on test bacteria *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028 and Enterecoccus faecalis ATCC 29212 was investigated using the disk diffusion method.The bacteria that are most likely to be resistant to antibiotic treatment were selected. These germs are the ones that cause sickness.

When the total phenolic contents of *Equisetum arvense* stem and leaf extracts were examined, it was determined that the highest total phenolic compound in the leaf was in the acetonic extract prepared by the maceration method (507.61 mgGA/g), and in

the stem it was found in the acetonic extract prepared by the soxhlet method (466 mgGA/g). It was observed that leaf and branch extracts prepared with petroleum ether had the lowest phenolic content.

It was determined that the methanolic extract prepared from leaf pieces by the soxhlet method was 85.1%, the acetonic extract was 84.5%, the methanolic extract prepared by the maceration method was 83% and the acetonic extract was 84.1% scavenged DPPH radicals, and as a result of the study, it was determined that the maceration method gave better results. It was determined that the extracts obtained from the branch showed lower DPPH radical scavenging activity than the leaf in both methods.

Stem surface of *Equisetum arvense* on EDX spectroscopy has concetrations of elements, with oxygen being the most abundant, followed by carbon, potassium, and aluminum, and the elements on the inner side of the leaf showing a similar pattern, with carbon having the highest percentage, followed by oxygen, sulfur, silicon, sodium, and calcium.

When the antibacterial activity results were examined, the methanolic leaf extract prepared by the maceration method showed an inhibition zone of 14.5 mm on *S. aureus*, 14.2 mm on *S. epidermidis* and 14 mm on *E. faecalis*.Acetonic leaf extract created an inhibition zone diameter of 9.5 mm on *S. aureus* and *E. faecalis* bacteria. Stem methanolic extract prepared by the maceration method showed an inhibition zone diameter of 8 mm on *S. aureus*, and acetonic extract showed an inhibition zone diameter of 11 mm on *S. aureus*. It has been determined that leaf extracts show higher antibacterial activity than branch extracts.

It was determined that the ethyl acetate extract prepared by the soxhlet method creates an inhibition zone diameter of 9.5 mm on *E. faecalis*, and the methanol and acetonic extracts creates an inhibition zones diameter of 8 mm. The methanolic extract obtained from the stem showed antibacterial activity 10mm only on *E. faecalis*.

It has been determined that extracts prepared by the Soxhlet method for *Equisetum arvense* plant generally exhibit lower antibacterial activity than extracts prepared by the maceration method.

As a result of the study, it was determined that the method and solvent used in the preparation of the extract were important in revealing the chemical content and displaying the activity.While the extracts produced by the maceration method exhibited higher antibacterial activity, the extracts obtained by the soxhlet method showed higher antioxidant activity. It was determined that qualitative efficiency was not directly related to extract yield values.

The highest antioxidant and antibacterial activity was found in methanolic leaf extract. Methanol and acetone were determined to be ideal solvents for TPC antioxidant and antimicrobial activity studies with *Equisetum arvense*.

In addition, the importance of pre-experimental optimization to find the appropriate method and solvent while investigating the biological activities of herbal extracts was revealed in our study.

In this investigation, the biological activities of the leaves and stems of the *Equisetum arvense* plant were studied separately with 5 different solvents and compared in detail for the first time.

These biological activities include antibacterial and antioxidative, and we did not find this work in previous studies. For example, this study was the first time that antibacterial and antioxidative activities were examined separately with solvents.

EQUISETUM ARVENSE L.'NİN ANTİBAKTERİYEL VE ANTİOKSİDAN AKTİVİTELERİNİN İNCELENMESİ.

ÖZET

Bugüne kadar yeni doğal kaynaklardan biyoaktif bileşiklerin araştırılması, gıda kalitesini, genel yaşam standardını ve halk sağlığını geliştirdiği görülmüştür. Biyoaktif bileşikler içerisinde özellikle fenolik bileşikler en çok çalışılan grubu oluşturur. Birçok çalışma, bitki kaynaklı polifenollerin yaşlanmayı önleyici, iltihap önleyici ve çoğalmayı önleyici özelliklere sahip olduğunu ve kardiyovasküler hastalıklar, kanser ve diyabet gelişme riskini azaltmada etkili olduğunu kanıtlamıştır. Ayrıca fenolik bileşikler antioksidan aktiviteleri sayesinde serbest radikalleri nötralize ederek, oksijeni temizleyerek veya peroksitleri parçalayarak oksidatif hasarları önleyebilirler.

Hem geleneksel hem de çağdaş yaklaşımlar dahil olmak üzere dünya çapında çeşitli tıbbi uygulamalarda elli bin ile yetmiş bin arasında farklı bitki türünün kullanıldığı tahmin edilmektedir. Her ikisi de hızla gelişen kozmetik ve botanik işletmeleri, sayılamayan sayıda başka tür kullanmaktadır.

Bu bileşenlerin büyük çoğunluğu, çeşitli doğal kaynaklardan örneklerin toplanmasıyla elde edilmektedir. Yabani toplamanın, konuyla ilgili ekolojik, ekonomik ve sosyal değişkenlere dayanarak, dünya bölgelerinin çoğunda tıbbi ve aromatik bitki türlerinin çoğunluğu için birincil kaynak olmaya devam etmesi beklenmektedir. Ekime olan ilginin artmasına rağmen durum böyledir.

Bakteriyel direnç sorunu, araştırma topluluğunda önemli ilgi gören yaygın bir sorundur. Uygulanabilir çözümleri belirlemek ve gerekli terapötik özelliklere sahip potansiyel kimyasal veya doğal alternatifleri araştırmak için şu anda çok sayıda çaba gösterilmektedir. Bu alternatifler, olumsuz etkileri ve ekonomik yükü en aza indirirken güçlü antibakteriyel ve antifungal etkinlik göstermelidir. Ek olarak, bu tür alternatiflere etkili antioksidanların dahil edilmesi oldukça arzu edilir.

Bitkiler uzun süredir her türlü mikrobiyal enfeksiyona karşı koymak için kullanılmaktadır. Pek çok mikroorganizma türü kullanılan standart antibiyotiklere çoklu direnç geliştirebilmektedir.

Bitkilerden izole edilen yeni moleküller bakteriyel direnç mekanızmasını kırabilmekte ve bakteri gelişimini inhibe edebilmektedir.Bu gibi nedenlerden dolayı bakterilerin antibiyotiklere dirençli formlarının ortaya çıkması karşısında, geniş antibakteriyel spektrumuna sahip yeni aktif moleküllerin araştırılması bir zorunluluk haline gelmiştir.

Şifalı bitkilerde bulunanlar gibi doğal bileşikler, antibakteriyel yetenekleri nedeniyle daha fazla araştırmaya ilgi duymaktadır. Bitki bileşiklerine karşı mikrobiyal direncin, kimyasal dirençten çok daha yavaş bir oranda gelişebileceğine inanmak için nedenler vardır, bu da bu tür araştırmaların umutlarını iyi yansıtır.

Hem geleneksel hem de çağdaş yaklaşımlar dahil olmak üzere dünya çapında çeşitli tıbbi uygulamalarda elli bin ile yetmiş bin arasında farklı bitki türünün kullanıldığı tahmin edilmektedir. Her ikisi de hızla gelişen kozmetik ve botanik işletmeleri, sayılamayan sayıda başka tür kullanmaktadır.

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Bitki ekstraktlarının biyolojik aktiviteleri büyük ölçüde biyoaktif bileşenlerin ekstraksiyon verimliliğine ve ekstraktların bileşimine bağlıdır. Çözücülerle ekstraksiyon, antioksidan bileşiklerin izolasyonu için sıklıkla kullanılır ve ekstraktların hem ekstraksiyon verimi hem de antioksidan aktivitesi, esas olarak elde edilen bileşiklerin farklı polaritelerinden dolayı kullanılan solvent ile güçlü bir ilişkiye sahiptir.

Özellikle antioksidan olarak kullanılacak fenolik bileşiklerin ekstraksiyonu için organik çözücüler (petrol eteri, hekzan, kloroform, methanol vb.) yaygın olarak kullanılmaktadır. En uygun çözücünün seçimi, ekstrakt özellikleri üzerinde belirleyici bir faktördür ve matrisin farklı yapısı ve bileşimi nedeniyle, her matris-çözücü sistemi tahmin edilemeyen belirli bir davranış gösterir.

Bu çalışma, laboratuvarda izole edilen ve önceden teşhis edilen 100 sürüntü örneği (yanık, kulak, apse, burun), dışkı ve idrar örnekleri üzerinde gerçekleştirildi. 2023 yılı 8-12 ayları arasında Sakarya Araştırma Hastanesi'nden laboratuvara getirildiler.

Çalışmamızda *Equisetum arvense* bitkisinin biyolojik aktivitelerini ve fenolik içeriğini ortaya çıkaran çözücü ve ekstraksiyon yönteminin maksimum oranda belirlenmesi amaçlanmıştır. *Equisetum arvense'in* yaprak ve gövde ekstraktları, 5 farklı çözücü varlığında soxhlet ve maserasyon yöntemi kullanılarak hazırlandı. Ekstraktların toplam fenolik içeriği (TPC) Folin-Ciocalteu yöntemi, antioksidan aktiviteleri DPPH radikal süpürücü testi kullanılarak belirlenmiştir.

Equisetum arvense L.'nin ekstraktlarının çok çeşitli farmakolojik aktiviteler içermesi nedeniyle, bunlar ilaç geliştirme alanında büyük öneme sahiptir. Bu, birçok farklı ülkede kabul edilen bir şeydir.

Equisetum arvense, kırılgan tırnaklar, saç dökülmesi ve romatizmal bozukluklar dahil olmak üzere çeşitli hastalıkları tedavi etmek için kullanıldığı geleneksel tıbbi uygulamalarda uzun bir kullanım geçmişine sahiptir.

Bu bitki, toplanabilme kolaylığı, yaygın bulunabilirliği ve önemli biyolojik özellikleri nedeniyle birçok farklı ülkede potansiyel bir ilaç kaynağı olarak kabul edilmiştir.

Equisetum arvense tesisi bu araştırmada taramalı elektron mikroskobu (SEM) görüntüleri ve enerji dağıtıcı X-ışını spektroskopisi (EDX) verileri kullanılarak incelenmiştir. SEM görüntüleri yaprağın yüzeyini gösterirken, EDX verileri yaprağın içini gösterir.

Bitkinin gövdesi de incelendi. İnceleme, kullanılan her bileşenin morfolojik görüntülerinde farklılıkların yanı sıra EDX spektroskopik analiz sonuçlarında gösterilen element kompozisyonunda farklılıklar olduğunu ortaya koydu.

Equisetum arvense yaprak ve dal ektraktlarının *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028 ve *Enterecoccus faecalis* ATCC 29212 test bakterileri üzerindeki antibakteriyel aktivitesi disk difizyon metodu kullanılarak araştırılmıştır.Antibiyotik tedavisine dirençli olması en muhtemel bakteriler seçildi. Bu mikroplar hastalığa neden olanlardır.

Equisetum arvense dal ve yaprak ekstraktlarının toplam fenolik içerikleri incelendiğinde, yaprakta en yüksek toplam fenolik bileşik maserayon yöntemiyle hazırlanan asetonik ekstrakta (507.61 mgGA/g), dalda ise soksklet metoduyla hazırlanan asetonik ekstrakta (466 mgGA/g) olduğu belirlendi.

Petrol eteri ile hazırlan yaprak ve dal ekstraktlarının en düşük fenolik içeriğe sahip olduğu görüldü. Yaprak parçalarından soxhlet yöntemi ile hazırlanan metanolik ekstraktın %85.1, asetonik ekstraktın %84.5, maserasyon yöntemi ile hazırlanan metanolik ekstraktın %83 ve asetonik ekstraktın %84.1 süpürülmüş DPPH radikali olduğu ve çalışma sonucunda maserasyon yönteminin daha iyi sonuçlar verdiği belirlenmiştir. Daldan elde edilen ektraktlar her iki yöntem içinden yapraktan daha düşük DPPH radikali süpürme aktivitesi gösterdiği tespit edilmiştir.

EDX spektroskopisinde *Equisetum arvense*'in gövde yüzeyi, oksijenin en bol olduğu, ardından karbon, potasyum ve alüminyumun ve yaprağın iç tarafındaki elementlerin benzer bir model gösterdiği, karbonun en yüksek yüzdeye sahip olduğu elementlerin konsantrasyonlarına sahiptir. oksijen, kükürt, silikon, sodyum ve kalsiyum.

Antibakteriyel aktivite sonuçları incelendiğinde, maserasyon yöntemi ile hazırlanan metanolik yaprak ekstraktı *S. aureus* üzerinde 14.5 mm, *S. epidermidis* üzerinde 14.2 mm ve *E. faecalis* üzerinde 14 mm'lik bir inhibisyon bölgesi göstermiştir.Asetonik yaprak ekstraktı ise *S. aureus* ve *E. faecalis* bakterileri üzerinde 9.5 mm inhibisyon zon çapı oluşturmuştur.

Maserassyon yöntemiyle hazırlan dal metanolik ekstraktı *S. aureus* üzerinde 8 mm, Asetonik ekstrakt *S. aureus* üzerinde ise 11 mm inhibisyon zon çapı göstermiştir. Yaprak ekstraktlarının dal ekstraktlarına göre daha yüksek antibakteriyel aktivite gösterdiği tespit edilmiştir.

Sokslet metoduyla hazırlanan etil asetat ekstraktını *E. faecalis* üzerinde 9.5 mm, metanol ekstraktının ise 8 mm inhibisyon zon çapı oluşturduğu belirlenmiştir. Daldan elde edilen metanolik ekstrak ise sadece *E. faecalis* üzerindee antibakteriyel aktivite göstermiştir.*Equisetum arvense* bitkisi için Sokslet metoduyla hazırlanan ekstraktlarının genel olarak maserasyon yöntemiyle hazırlanan ekstraktlara göre daha düşük antibakteriyel aktivite sergilediği belirlenmiştir.

Çalışma sonucunda ekstraktın hazırlanmasında kullanılan yöntem ve solventin kimyasal içeriğin ortaya çıkarılmasında ve aktivitenin sergilenmesinde önemli olduğu belirlenmiştir. Maserasyon yöntemiyle üretilen ekstraktlar daha yüksek antibakteriyel aktivite sergilerken, soxhlet yöntemiyle elde edilen ekstraktlar daha yüksek antioksidan aktivite göstermiştir. Kalitatif etkinliğin ekstrakt verim değerleri ile doğrudan ilişkili olmadığı belirlenmiştir.

En yüksek antioksidan ve antibakteriyel aktivite metanolik yaprak ekstraktında bulundu. *Equisetum arvense* ile yapılacak TPC antioksidan ve antimikrobiyal aktivite çalışmaları için metanol ve asetonun ideal çözücüler olduğu belirlenmiştir. Bu araştırmada *Equisetum arvense* bitkisinin yaprak ve gövdelerinin biyolojik aktiviteleri çözücülerle ayrı ayrı incelenerek ilk kez detaylı olarak karşılaştırılmıştır.

Bu biyolojik aktiviteler antibakteriyel ve antioksidatif içerir ve bu çalışmayı önceki çalışmalarda bulamadık.Örneğin bu çalışma, antibakteriyel ve antioksidatif aktivitelerin 5 farklı çözücü ile ayrı ayrı incelendiği ilk çalışma olmuştur.

1. INTRODUCTION

Plants are the main source of food and the first medicines that people used. People have learned through trial and error since the beginning of time which plants are safe to eat and which are poisonous or useful. Using simple methods and medicinal plants, they were able to get the main active ingredient of the plant Numerous biological benefits, including antibacterial, anti-allergic, antiviral, antioxidant, anti-inflammatory, and even anti-aging capabilities, have been associated with phenolic compounds, including phenolic acids and flavonoids. Flavonoids and phenolic acids are examples of other phenolic chemicals [1].

The World Health Organization (WHO) says that traditional medicine based on medicinal and aromatic plants is used by most people, especially in developing countries. Between fifty thousand and seventy thousand plant species are cultivated for their medicinal value across worldwide [1].

Biological characteristics, such as antibacterial, antifungal, and antioxidative activities, and their active components were used to categorize plants and plant extracts. Among them, phenolic acids and flavonoids have been examined for the antibacterial and antioxidative effects they possess in a number of different research. Plant *Equisetum arvense* is well-known for its beneficial effects on human health as a result of the presence of several vital compounds and elements.The secondary metabolite concentration of horsetail includes phenolics (such as flavonoids, styrylpyrones, and phenolic acids), alkaloids (including equisetin, nicotine, palustrine, and palustrinine), phytosterols (specifically campesterol), bitter principle, and minerals (such as silica, calcium, magnesium, selenium, iron, potassium, zinc, and others) [2].

It has been used for many years to treat a variety of medical issues, including tuberculosis, kidney and bladder catarrh, nosebleeds, stomach bleeding, brittle nails, hair loss, gout, rheumatism, poor wound healing, ulcers, swelling, and fractures, and frostbite [3].

Aromatic herbs and spices have significant relevance within the realms of the culinary, cosmetics, and pharmaceutical sectors. The use of natural items has been seen since ancient civilizations, and while some have been replaced by synthetic alternatives, there is a growing need for these natural substances. The essential oils of many different herbs, such as basil, celery, dill, horsetail, lovage, marjoram, milfoil, oregano, parsley, rosemary, and thyme, have been shown to have powerful antioxidant capabilities after being extracted from the leaves and/or flowers by stem distillation [4].

The paper was to provide evidence that *Equisetum arvense* has the potential to be used as a therapeutic agent. According to the research that has been conducted, *Equisetum arvense* has a variety of pharmacological effects, including those that are anti-inflammatory, analgesic, anticonvulsant, antihyperglycemic, antioxidant, anticancer, sedative, antibacterial, and antifungal [5].

The aim of this study was to investigate at the total phenolic content of horsetail *(Equisetum arvense L.)* and develop a non-toxic, bio-synthesized antibacterial and antioxidant agent that only acts on certain types of bacteria. The phenolic component concentration of the extracts studied was related to their antioxidative activity. For the purpose to determine whether or not extracts have antibacterial properties, bacteria were subjected to the procedures of disc diffusion and microbroth dilution.

1.1. Literature Review

In the summer, the plant *Equisetum arvense* L. (Equisetaceae, sometimes known as "Horsetail") has sterile green branched buds, but in the spring and early summer, the buds are a rich brown color and do not branch [4]. The manufacturing of plant-based pharmacological supplements results in the generation of a significant quantity of waste that retains its biological activity . Numerous studies has been conducted on the chemical and biological properties of the Equisetum genus in a variety of places as a result of the widespread use of this particular species in traditional medicinal practices. as well as the commercialization of derivative goods that include this species in their formulations. There are already a number of products available in the market that make use of extracts from species belonging to this genus. In addition, polyphenols that have been isolated from the plant have a wide array of therapeutic qualities, including anti-

allergic, anti-inflammatory, antioxidant, antibacterial, anticoagulant, and hepatoprotective effects [4].

There are several places in Europe, North America, Central America, and South America where horsetail may found growing [5]. It is used to treat tuberculosis as well as renal disorders. As a hemostatic treatment for severe menstruation, nose and lung bleeding, splitting and hair loss, rheumatic disorders, gout, poor wound healing, and ulcers, swelling, and fractures [5].

The consumption of food that has been supplemented with antioxidants is an efficient method for preventing the formation of a variety of off-flavors and unpleasant chemicals caused by lipid peroxidation. Natural antioxidants originated from biological sources, especially herbal plants, are of considerable interest in both the area of preventive medicine and the food industry [6].

Phenolic compounds, such phenolic acids and flavonoids, have been credited with a wide range of biological advantages, including those related to fighting pathogens, allergies, viruses, oxidative damage, inflammation, and the aging process. In addition to phenolic acids, flavonoids are another kind of phenolic substance [7].

Several horsetail (*Equisetum arvense*) extracts were tested for their ability to inhibit the proliferation of human cancer cell lines HeLa, HT-29, and MCF7 using the sulforhodamine B colorimetric assay [8].

The inhibitory effect of the extracts on cell proliferation was shown to be depending upon the specific cell line, kind of extract used, and the concentration of the extract employed. Ethyl acetate extract stopped human tumor cell lines from growing the most, and it did this without making the cells grow faster. The utilization of electron spin resonance research revealed that, conditional with the dosage, the extracts effectively inhibited the generation of lipid peroxyl radicals in both of the examined systems. The results show that extracts of n-butanol, methanol, ethyl acetate, and water were very good at getting rid of peroxyl radicals [8].

The goal of this study was to assess the antioxidant activity and phenolic composition of extracts derived from field horsetail (*Equisetum arvense*) using different solvents, namely ethanol, n-butanol, and water. The evaluation of antioxidant activity was conducted through various methods, including the determination of total reducing power (expressed as ascorbate equivalent antioxidant capacity - AEAC), inhibition of

lipid peroxidation, and free radical scavenging capacity (RSC) towards 2,2-diphenyl-1-picrylhydrazyl (DPPH radical) and nitric oxide [9].

It was revealed from the study [10] that the extract's ability to scavenge free radicals (against both DPPH and hydroxyl radicals) varied with the extract type and concentration used, with the highest DPPH (EC50 = 0.65 mg/ ml) and hydroxyl radical scavenging activities (EC50 = 0.74 mg/ ml) obtained in the case of n-butanol extract. Total phenolic levels substantially linked with the radical scavenging activity of extracts.The hydroalcoholic extract of *Equisetum arvense* (HAE) tested at doses of 200 and 400 mg/kg on mices showed significant activity on the open-field, increased the number of falls in the rotarod, decreased the time of permanence in the bar, and increased the sleeping time (46% and 74%). In the sleeping time, it decreased the severity of convulsions, reduced the percentage of animals that developed convulsion (50% and 25%), and protected animals from death.Phytochemical analysis detected the presence of tannins, saponins, sterols and flavonoids [10].

Various methodologies are used to assess the capacity of antioxidants in scavenging free radicals. The utilization of the DPPH method is highly advised due to its expeditious, uncomplicated, and dependable nature, as well as its lack of need for specific reactions or apparatuses. DPPH is a synthetic radical that is stable and does not dissolve in water, methanol, or ethanol [11].

The *Equisetum arvense* extract has a capacity to scavenge free radicals. Consequently, it works in the capacity of an antioxidant. The present study aimed to investigate the antioxidative activity of water and ethanol extracts derived from the top and body sections of field horsetail (*Equisetum arvense*) [12].

Additionally, it should be noted that the phenolic contents of the extracts are capable of change based on the choice of extraction solvent. Moreover, it is important to recognize that not only does the phenolic content play a role, but also the specific properties of these compounds have an impact on the activities shown by different extracts. The use of high-polar solvents is considered the most effective method for extracting active components [13]. The microorganisms mentioned include *Staphylococcus aureus, Escherichia coli (E. coli), Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Salmonella enteritidis.* Additionally, the fungal species *Aspergillus niger* and *Candida albicans* are included.

It was revealed from the study [14] the antibacterial activity of *Equisetum arvense L.* essential oil was shown to be very strong against all microbes tested, when diluted at a ratio of 1:10 [14].

The antibacterial activity of the methanolic extract derived from the aerial parts of *Equisetum arvense* was shown against *Escherichia coli* at a significant concentration of 1 g/ml. The antibacterial activity of *Equisetum arvense* extracts was shown against *Staphylococcus epidermidis* and *Escherichia coli*, but no significant effect was seen against *Candida albicans*. The antibacterial activity of volatile components of *Equisetum arvense* against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella enteritidis* was investigated using a diffusion approach [15].

1.2. The Antibacterial Activity of *Equisetum arvense*

Using the disc diffusion technique, the antibacterial activity of ethanolic and aqueous extracts of *Equisetum arvense* was tested against selected urinary tract pathogens (*E.coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, and Enterococcus faecalis*). [8] An inhibitor of HIV-1-induced cytopathy is produced by applying an extract of the aerial parts of the *Equisetum arvense* plant [16].

When given at higher concentrations, both gram-positive and gram-negative bacteria exhibited a bigger rise in their respective mean zones of inhibition for the extract. The average size of the zone of inhibition against *Escherichia coli* was determined to be 32 millimeters when it was tested [12].

1.3. The Clinical Effects of *Equisetum arvense*

Previous research conducted by [17] investigated the effects of a 5% solution of *Equisetum arvense* on wound healing in rabbits, and compared these effects to those of povidone iodine and sodium chloride. On the dorsal portion of their bodies, skin wounds were produced. After the surgery, a macroscopical examination of the wound surfaces was performed, and the healing process. In addition, the expansion, contraction, and epithelization rates of the lesion were examined [17].

Previous research [18] on the extract of the plant's stems and its experiment on leukemia diseases showed positive results that it has a damaging impact on tumor cells, as seen in the figure below.

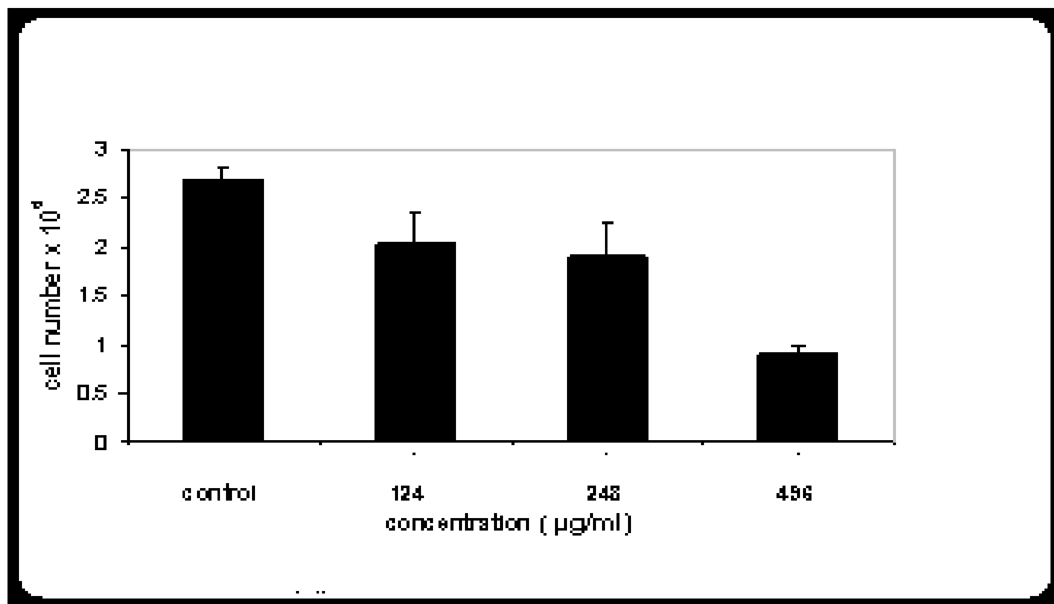

Figure 1.1. The effects of different doses of stem extraction on growth rate of cancer cells [18].

The antinociceptive and anti-inflammatory properties of hydroalcoholic stem extract of *Equisetum arvense* were evaluated in mice. 10mg/kg ip extract reduced acetic acid-induced writhing by 49, 57, 93, and 98%. Ip formalin reduced licking activity by 80% and 95%, respectively, although only the greater dose reduced licking time by 35%. Naloxone failed to counteract the analgesic effect in both phases. Hot-plate licking and jumping delay had no effect. The extract of 50mg/kg reduced carrageenan-induced paw oedema at 2 h (25%) and 4 h (30%) later. Carrageenan at 100mg/kg reduced paw oedema by 29% [19].

According to the findings in this study [20] Equisteum species should be studied in the dentistry field since they have anti-inflammatory and antibacterial activities without being cytotoxic. They also have antioxidant and antinociceptive properties.

Because it stimulates blood coagulation, the plant's juice is beneficial for anaemia caused by internal bleeding such as stomach ulcers. Horsetail is indicated for anemia and general debility due to its mineral concentration. It possesses anti-aggregant properties for platelets [21]. Horsetail's local astringent and antihaemorrhagic impact explains why it's used to treat illnesses including bleeding from the mouth, nose, and

vagina, as well as diarrhoea, dysentery, and intestinal bleeding, additional to conjunctivitis, chilblains, and slow-healing sores. *Equisetum arvense* functions as a potent genitourinary system analgesic. Because of its high silica content, it can be used to treat illnesses such as urethritis or cystitis with haematuria, lowering haemorrhagic and mending wounds [3].

Equisetum arvense preparations have no documented interactions.There have been reports of mild digestive complaints and allergic responses.The frequency has not been determined.Because there are no data on reproductive and developmental harm, usage during pregnancy and breastfeeding is not advised It is not suggested for people with disorders that need a reduced fluid intake (e.g., cardiac or renal disease), or for those who have a known intolerance to horsetail. *Equisetum arvense* preparations should not be given to children under the age of 12 [22].

2. RESOURCES RESEARCH

2.1. Prokaryotes and Eukaryotıc

Prokaryotes are microorganisms that belong to the bacterial and archaeal domains, which are two of the three primary domains of life. Bacteria and archaea are the two major domains of life. (The third kingdom is called Eukarya, and it includes all eukaryotic organisms, such as animals, plants, and fungi.) The majority of eukaryotic organisms are multicellular, in contrast to the unicellular nature of bacteria and archaea. Fossils demonstrate that prokaryotes are believed to have inhabited Earth around 3.53 billion years ago, and it is widely accepted among scientists that these ancestral prokaryotes served as the ancestors for the wide range of species that exist today on Earth [23].

Prokaryotes and eukaryotes are very different from each other in numerous respects. Because their nucleus is not encased in a membrane, prokaryotes are typically single-celled creatures with a diameter of 1-5 µm. Oftentimes, metabolic tasks in prokaryotes are carried out by membranes that have been modified specifically for the purpose. These structures develop when the plasma membrane folds inward. They differ from eukaryotes in that their genomes are less complex and more compact. Their DNA is circular and double-stranded [23].

Some prokaryotes have DNA loops called plasmids that contain their own set of genes apart from the main chromosome. Bacteria and archaea are the two primary categories of prokaryotic organisms. The vast majority of prokaryotes are bacteria. Form and protection are provided by a cell wall in most prokaryotes. The bacterial cell wall contains an organic substance called peptidoglycan, while the archaeal cell wall does not. Prokaryotes are essential to the ecology because they produce chemicals and recycle many different materials. Due to their fundamental features, they have an essential function within the framework of biotechnology [24].

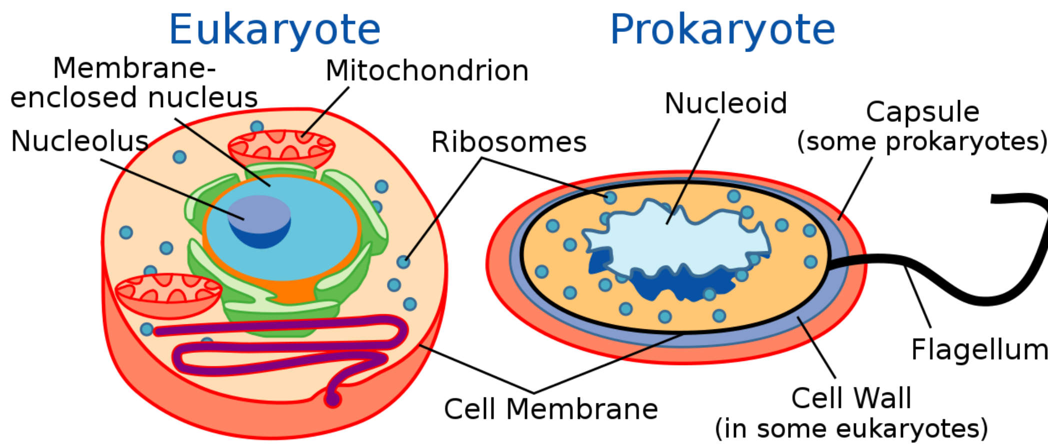

Figure 2.1. Prokaryotes and eukaryotıc cells.[URL-1]

Table 2.1. Comparation between prokaryotes vs. eukaryotes [25].

Prokaryotes	Eukaryotes
➢ Free DNA in The Cytoplasm	➢ DNA is Surrounded By A Membrane
➢ Chromosome is Usually Haploid, Single And Circular.	➢ Chromosome İs Usually Diploid, Multiple And Linear
➢ DNA Complexed With Histone-Like Proteins	➢ Complex With DNA Histone Protein
➢ Transfer Of Genetic information Occurs By Conjugation, Transduction And Transformation.	➢ The Transmission Of Genetic İnformation Occurs Only Through Sexual Reproduction.
➢ Energy Production is Provided By The Cell Membrane.	➢ Energy Production is Provided By Mitochondria And Chloroplasts.
➢ Small Ribosomes 70s	➢ Ribosomes 80s

Bacteria often range in size from 1 to 5 µm. However, the length of spiral-shaped bacteria is much more than the length of their axis. They may, for instance, aggregate in the shape of lengthy chains, agglomerations, or even crystal-like orderliness [26].

Cell wall structure gives staining bacteria their classification. Gram staining is the most used approach. Waxy mycomic acid-containing bacteria may be identified by resistive staining.

Cell walls composed of peptidoglycan are unique to Gram-positive bacteria. Many gram-positive organisms have peptidoglycan covalently bound to taichoic acids, polymers of glycerol units connected by phosphodiester linkages.

Gram-negative bacteria have outside and interior membranes. The peptidoglycan layer is in the periplasmic space. Enzymes and other compounds are in periplasm. Thin Peptidoglycan makes cells vulnerable to physical harm [26].

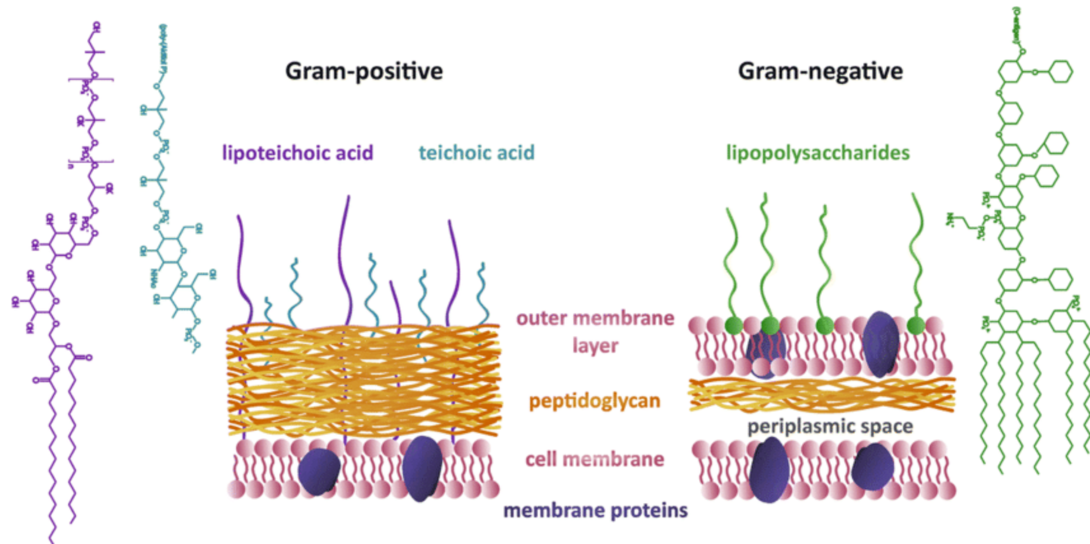

Figure 2.2. Differences between gram-positive and gram-negative bacterial cell walls [24] [URL-2]

2.2. Test Microorganisms Used in the Study

2.2.1. *Escherichia coli*

Is a rod-shaped, gram-negative, facultative anaerobic, motile, non-spore-forming bacteria. Different strains of pathogenic *E. coli* are categorized based on the disease they're responsible for spreading. Bacterial infections caused by *E. coli* may be classified as either enteric (extratestinal) or gastrointestinal (intestinal) [27].

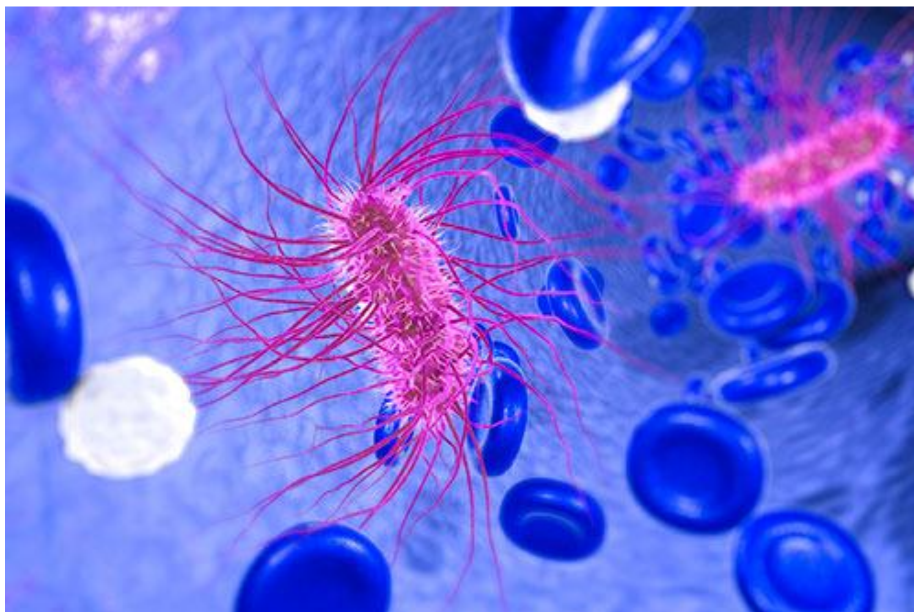

Figure 2.3. *Escherichia coli [URL-3]*

Even "nonpathogenic" strains of *E. coli* may cause infection in a weak or immunocompromised host, or when gastrointestinal barriers have been overcome. *E. coli* is generally harmlessly confined to the intestinal lumen. In addition, even the healthiest individuals of our species might fall prey to infection from any of a number of *E. coli* clones that have become highly adapted and have the potential to induce a variety of human diseases [27]. Infections induced by pathogenic Escherichia coli may appear as either localized mucosal infections or disseminate to several systemic sites inside the body. Infection with *E. coli* strains that are fundamentally pathogenic may lead to three different types of general clinical syndromes.These include urinary tract infections, sepsis and meningitis, and enteric and diarrheal diseases [28] [29].

2.2.2. *Staphylococcus epidermidis*

Staphylococcus epidermidis the most common coagulase-negative organism found in isolation. Differentiating them from *Staphylococcus aureus* is their inability to produce coagulase. In the past few years, *S. epidermidis* has gained a lot of attention due to the fact that it may spread throughout hospitals and cause nosocomial infections Once considered relatively safe, *S. epidermidis* is now widely recognized as a dangerous infection. *S. epidermidis*, on the other hand, needs a genetically susceptible host in order to go from being a normal part of the human skin to being an important pathogen. Because of this, it is clear that *S. epidermidis* is opportunistic [30].

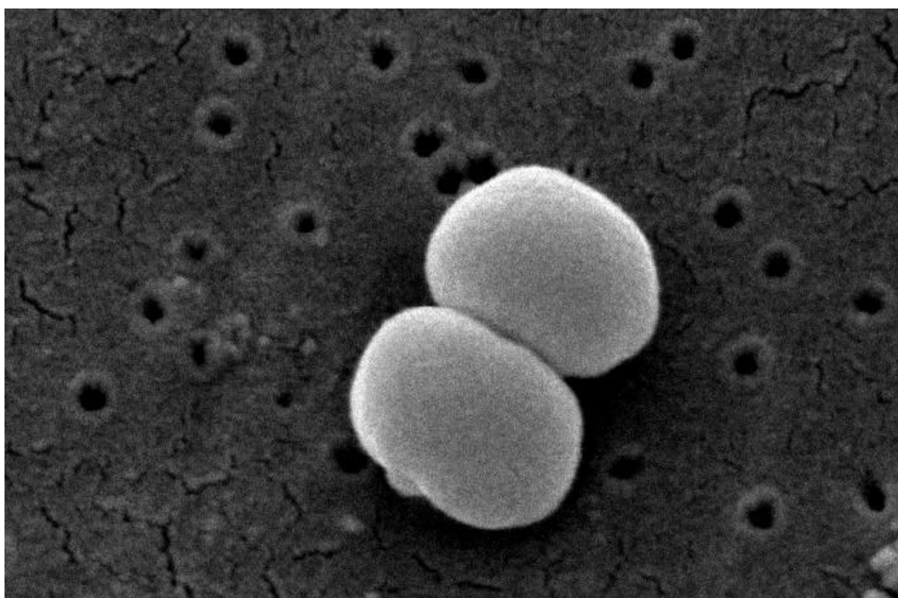

Figure 2.4. *Staphylococcus epidermidis [URL-4]*

2.2.3. *Staphylococcus aureus*

Staphylococcus aureus is a species of gram-positive bacteria that forms irregular clusters with a grape-like shape. It is immobile, does not produce spores, and releases catalase. Under aerobic circumstances, it exhibits fast growth and proliferates abundantly. *Staphylococcus aureus* lives on human skin and in the nasal cavity and throat. Infections of the skin, soft tissues, endovascular locations, and internal organs are all possible. *Staphylococcus aureus* Biofilms consist mostly of water, accounting for about 97% of their composition, along with organic materials such as extracellular polymeric substances (EPS) and microcolonies. This composition is similar to that of bacterial biofilms comprised of different bacterial species. High morbidity and mortality due to *S. aureus* infections persist both in the community and in healthcare settings. Diseases caused by *S. aureus* are usually caused by two types of virulence factors: cell surface-associated proteins and toxin proteins located outside the cell. *Staphylococcus aureus* makes a lot of cell surface and extracellular proteins, some of which could be involved in the development of disease. It can make a number of proteins on its surface that can bind to parts of the extracellular matrix, blood clots, and damaged tissue [31] [32].

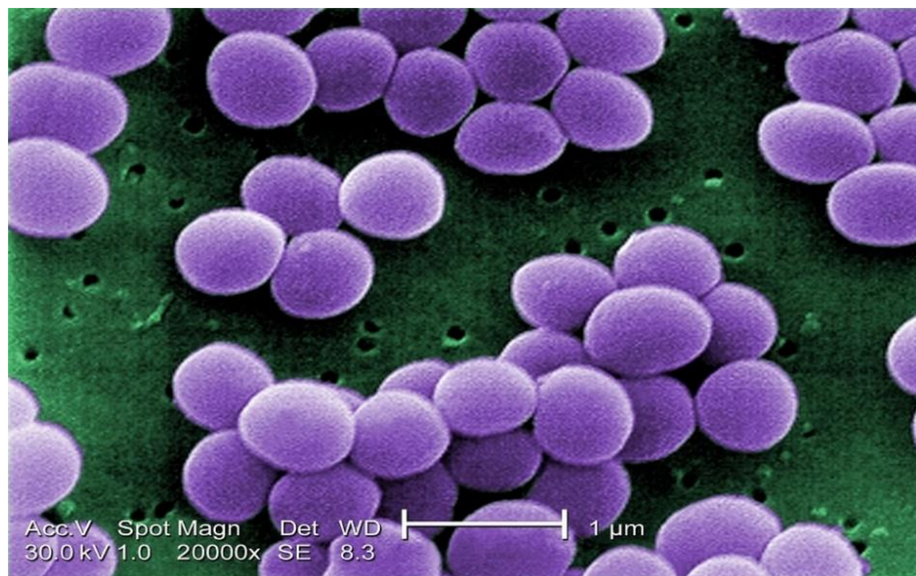

Figure 2.5. *Staphylococcus aureus [URL-5]*

2.2.4. *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, catalase-positive bacteria lives in soil and the digestive tracts of ruminants, humans, and marine sponges. subtilis looks like a rod

and can make a protective endospore that helps it survive in harsh environments. *Bacillus subtilis* may be found in almost any environment, including the air, dust, plants, and salt water, and that can contaminate clinical specimens. Even though bacteria are saprophytes, they can cause eye burns like panophthalmitis and iridocyclitis when they get into the tissue and especially the eye. People think that they are to blame for some food poisoning. They make the bread go bad and get soft. A substance called subtilin, which prevents some bacterial growth was also found in the filtrates of cultures [33].

Figure 2.6. *Bacillus subtilis* [URL-6]

2.2.5. *Salmonella typhimurium*

Widespread in nature, it may be discovered in the gastrointestinal tracts of both domestic and wild animals, reptiles, birds, and insects. In both people and animals, they spread several illnesses. On medium made from simple components like agar and broth, they develop M or S type colonies in 24 to 48 hours. The range of acceptable breeding temperatures is 10 to 42 ^{o}C, with 37 ^{o}C being the ideal. Cockroaches and house flies may mechanically transfer bacteria to food products.The risk of getting *salmonella* by consuming milk, meat, eggs, creamy foods, and drinks, especially in the summer when these goods may be safely stored at room temperature, is high. *S. typhimurium* is a bacteria that lives in mice, it can readily spread from mice to people via contaminated food or other goods. Also, this bacteria develops resistance to

treatment and spreads rapidly, particularly in newborn clinics, across healthcare facilities [34].

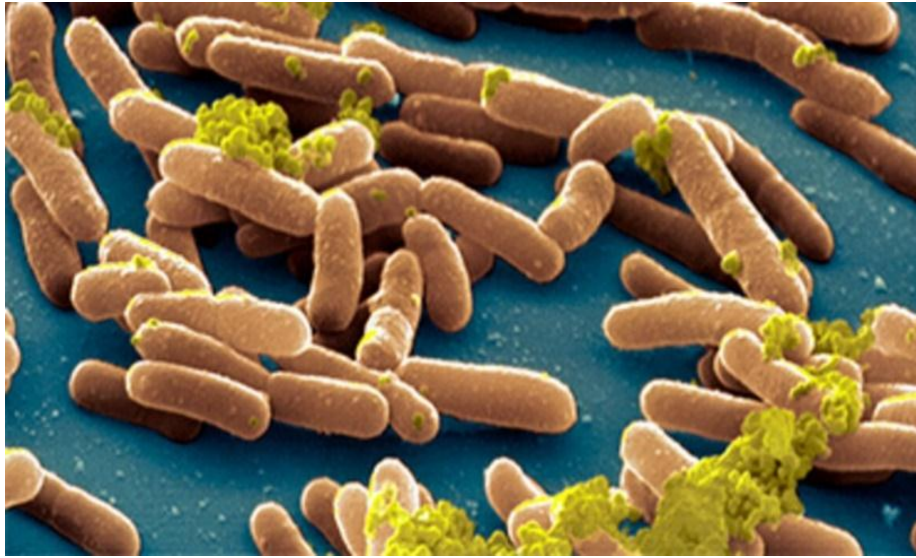

Figure 2.7. *Salmonella typhimurium.*[URL-7]

2.2.6. *Enterecoccus faecalis*

Enterococcus faecalis is a Gram-positive, nonspore-forming, fermentative, facultatively anaerobic coccus. They are typically extended in the chain's orientation. The majority of strains are nonhemolytic and nonmotile. Surface coloniesoid with a diameter of 0.5 to 1 mm. They appear singly or in pairs and are round, smooth, and complete on blood agar. Identifying enterococci in various locations has always been difficult. Culture techniques have been used. In fact, the incidence of enterococci in primary endodontic infections and chronic infections has nearly entirely been documented by cultivation experiments [35] [36] .

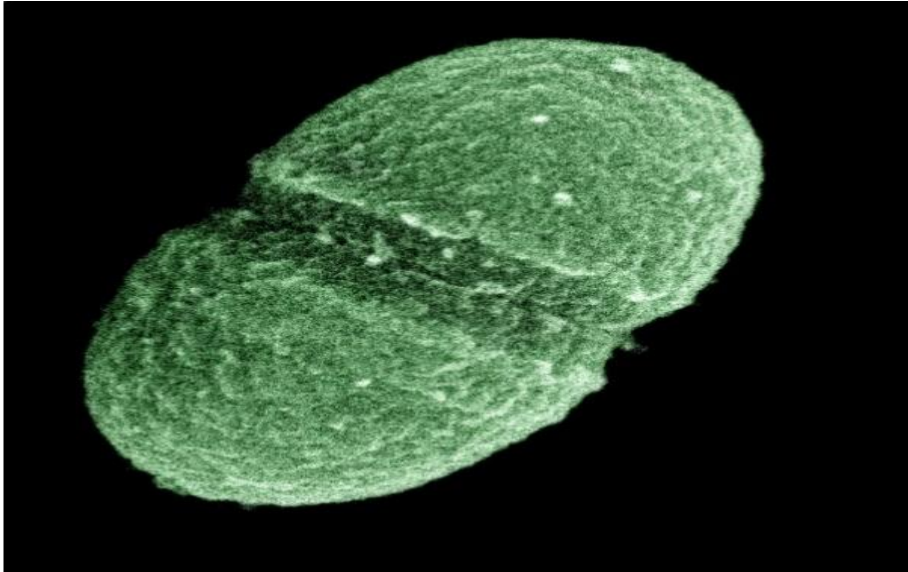

Figure 2.8. *Enterecoccus faecalis.*[URL-8]

2.3. Plant Used in the Study

2.3.1. *Equisetum arvense* L. (commen name: Horsetail)

The plant Horsetail, as it is commonly called, is a bushy perennial that is indigenous to the northern hemisphere. The Equisetum species may be found over a large portion of the world, including Canada, the U.S.A. (with the exception of the southeast), Europe and Asia to the south, including Turkey, Iran, and the Himalayas, as well as China (with the exception of the southeast), Korea, and Japan. The horsetail plant has distinct characteristics, including a spreading rhizome and filamentous structure, along with the presence of roots at nodes that give rise to many hollow stems. The stems of horsetail are also hollow [37] .

Equisetum arvense is the unique surviving member of the Equisetaceae family, which is part of the order Equisetales and has no other genera. There are (30) different species of rush-like, noticeably jointing, perennial plants that belong to the genus Equisetum. Horsetail is a peculiar-looking plant with a creeping, string-like rhizome and roots at the nodes that create multiple hollow stems. The stems of horsetail are also hollow [37].

Table 2.2. Taxonomy of *Equisetum arvense L.*

Kingdom	***Plantae***
Subkingdom	*Tracheobionta*
Division	*Equisetophyta*
Class	*Equisetopsida*
Order	*Equisetales*
Family	*Equisetacaea*
Genus	*Equisetum*
Species	*Equisetum arvense*

Researchers have found antioxidant components in *Equisetum arvense* (i.e. caffeic acid, chlorogenic acid, ferulic acid, kaempferol,quercetin, isoquercetin, apigenin, and luteolin). Horsetail is also an excellent source of a wide variety of vitamins and minerals, including B1, B2, B6, nicotinic acid, folic acid, pantothenic acid, vitamins C, E, and K, silicic acid, saponins, and trace elements sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium, and tin.This herb's potential health benefits include providing protection against a wide range of diseases and conditions [8].

Field horsetail has been used medicinally for a wide variety of conditions ever since it was first discovered, due to its numerous health advantages. Steril stems are used to cure conditions this includes anemia, inflammation, diabetes, ulcers, cancer, convulsions, anxiety, and depressive disorders [38].

Figure 2.9. Arieal parts of *Equisetum arvense L. [URL-9]*

2.3.2. The Aim Of This Study

The total phenolic content of horsetail (*Equisetum arvense* L.) was the primary aim of this investigation. and to create a non-toxic, bio-synthesized antimicrobial and antioxidant agent that selectively acts only on certain types of the most common bacteria*(E.coli,S.epidermidis,B.subtilis,S.aureus,E.faecalis,S.typhimurium).*

The antioxidative activity of the investigated extracts has been connected with the quantity of phenolic components present. The investigation of the antibacterial properties of extracts has been conducted using disc diffusion and microbroth dilution methods against bacteria.

3. MATERIALS AND METHODS

This study was conducted on 100 swabs (burn, ear, abscess, nose), stool, and urine samples, isolated and previously diagnosed in the laboratory. They were brought to the laboratory from Sakarya Research Hospital in 2023, a period between the months of 8-12, and the plant samples were also collected (*Equisetum arvense*) from (Serdivan/Sakarya/Türkiye). It was noted that the previously isolated bacteria include (*Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhimurium, and Enterococcus faecalis*) and were chosen for this study, to detect the effect of biological activities and the phenolic content of the *Equisetum arvense* plant at the maximum rate.*Equisetum arvense* leaf and stem extracts were prepared using the Soxhlet and maceration method in the presence of 5 different solvents. The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu method, and antioxidant activities were determined using the DPPH radical scavenging assay. In this research, *Equisetum arvense* was examined using scanning electron microscope (SEM) images and energy-dispersive X-ray spectroscopy (EDX) data. In this study, antimicrobial activity against the six isolates was verified using the disk diffusion method. Bacteria that were likely to be resistant to antibiotic treatment were selected as it is considered one of the species most responsible for causing these diseases.

3.1. Sample collection

The sample of the letter consisted of 100 clinical swab samples and isolated and previously diagnosed urine and stool samples coming to the laboratory from Sakarya Research Hospital. Laboratory samples are listed in Table 3.1 according to their source and gender status.

Table 3.1. Laboratory samples and their details that utilized in the study.

Bacteria name	Source of sample	Number of samples	Gender	
			Female	Male
Staphylococcus epidermidis	**Nasal Swab**	17	**11**	**6**
Staphylococcus aureus	**Ear swab**	30	**18**	**12**
Bacillus subtilis	**Stool sample**	**10**	**2**	**8**
Escherichia coli	**Urine sample**	**9**	**5**	**4**
Salmonella typhimurium	**Stool sample**	16	**9**	**7**
Enterococcus faecalis	**Stool sample**	18	**8**	**10**
Total: 6	**Total: 6**	**Total: 100**	**Total: 53**	**Total: 47**

3.2. Plant material Collection

Plant samples were collected by Şule Baran 2022 (Serdivan/Sakarya/Turkey). After the samples were sorted and cleaned, for 7 days, they remained in the shade at room temperature for drying. The dried stem and leave samples were grinded separately in an electric grinder.

Figure 3.1. The dried samples of *Equisetum arvense L.*

Figure 3.2. The dried and grinded samples of *Equisetum arvense L.*

3.3. Morphologıcal and Elemental Analyzes of the Leaf and Stem of *Equisetum arvense* Plant by SEM

Samples were collected from Northern west Turkiye (Kazımpaşa, Sakarya) in September 2022 and air dried after washing with water. Examination and analysis of the species carried out under The JEOL JSM-6060 high-performance, compact, scanning electron microscope & energy dispersive x-Ray (EDX) spectroscopy in Sakarya University, Metallurgy and Materials Engineering Department, Sakarya, Turkiye.

3.4. Chemicals and Reagents

All of the chemicals (petroleum ether, ethyl acetate, chloroform, methanol, and acetone) and reagents (2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocalteu, gallic acid, Mueller Hinton Agar, ascorbic acid) used in this study were of analytical grade and were acquired from Merck Company, Germany.

3.5. Microorganisms Used in Experiments

B. subtilis ATCC 6633, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. typhimurium* ATCC 14028, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228 that were isolated from the microbiology laboratories and accurately diagnosed previously in the laboratory of Sakarya Research Hospital.

3.6. Tools And Equipment Used In Experiments

Tools and materials used during the experiments Sakarya University Department of Biology Microbiology Research Laboratory . These tools and equipment are given in Table (3.1.)

Table 3.2. Materials and equipment utilized in the study.

1.	Incubator (Friocell MMM)	2.	Digital Caliper (Stainless Hardened)
3.	Micropipette 5-50 Ml 5-50 Ml (ISOLAB)	4.	Micropipette Tip
5.	Autoclave (Alp Cl-32l)	6.	Baguette
7.	Electronic Precision Weighing	8.	Magnetic Stirrer (IKA RCT Classic)
9.	Petri Dish and Carrier Oz	10.	Loop
11.	Uv Spectrometer Glass Balloon	12.	Glass Tube
13.	Densitometer (Biosan Den-1)	14.	Rotary Evaporator Beaker
15.	Pens	16.	Collet Grinder (Premier PRG 259)
17.	Foil	18.	Micropipette 100-1000 Ml
19.	Erlenmeyer	20.	Filter Paper
21.	Pipette	22.	Rotary Evaporator Beaker

3.7. Consumables Materials that Used In Experiments

Materials for this experiment were obtained from the Sakarya University's Biology Department's Microbiology Research Laboratory as found in Table 3.2.

Table 3.3. Consumables materials that used in study.

1.	Methanol(MERCK)	2.	Ethyl acetate(MERCK)
3.	DPPH(MERCK)	4.	Acetone(MERCK)
5.	Petroleum ether(MERCK)	6.	Autoclave bag
7.	Chloroform	8.	Mueller Hinton Agar (MERCK)
9.	Distilled water,	10.	Sterile glass petri dish
11.	Sterile plastic petri dish	12.	Antibiotic Discs (MERCK)
13.	Stretch film	14.	Gentamisin
15.	Swab Sticks	16.	Tryptic Soy Broth (MERCK)

3.8. Preparation of Plant Extracts

The dried samples were extracted by two methods:

- Maceration Method.
- Soxhlet Method

3.8.1. Maceration method

Equisetum arvense L. Weighing 15 grams of powder of leaves and stem parts, transferred to a glass jar with a screw cap. Then, 150 ml of solvent (petroleum ether, ethyl acetate, chloroform, methanol, and acetone) was added to it, kept in the dark for 72 hours, and the extract was filtered with filter paper.

Figure 3.3. Preparation of the plant extract of *Equisetum arvense* L. By maceration method.

3.8.2. Soxhlet method

After grinding the plant's stem and leaves separately , they were put in filter paper envelopes that looked like tea envelopes. All such envelopes were made by hand in the lab. Powdered samples (15 g) were put into Soxhlet cartridges and extracted in 150 mL of solvents (acetone, methanol, petroleum ether, ethyl acetate, chloroform)for 8 hrs.

Figure 3.4. Preparation extract of *Equisetum arvense L.* By soxhlet method.

3.8.3. Removal of solvents

All extracts were evaporated under vacuum at 45 ◦C, and the obtained extracts were stored at -20 ◦C until performing chemical and biological analyses. The yield of the dry weight of the extract to the weight of the source materials was used to determine the yield of the produced extracts: yield **(%)=m/M×100** (where m is the dry weight of the extract (g) and M is the raw material weight (g)).

3.9. Determining of Total Phenolic Content

Total phenolic content (TPC) determination was determined using the Folin-Ciocalteu method. 100 µL of the prepared extract (1mg/mL) was taken, 200 µL of 50% Folin-Ciocalteu reagent was added and left for 2 minutes. 1 mL of 2% Na_2CO_3 solution was added to it and it was left to rest in the dark for 1 hour. By using a spectrophotometer, absorbances at 760 nm were measured at the ending of the time period. A calibration curve was employed for gallic standard, and TPC extract was expressed as mg per gr (mg GA/g).

3.10. Antioxidant Activity

The modified Blois method was used to investigate the antioxidant activity of *Equisetum arvense* extracts. (Blois, 1958). Briefly, 1 mL of 0.004% solution of DPPH radical in ethanol was mixed with 1 mL of extract solution (100 µg/mL). This mixture was kept in a dark place for 30 minutes and then optical density was measured at 517 nm using a spectrophotometer. Ethanol was used as a blank.

In order to calculate the % DPPH radical scavenging activity, the following equation was utilized: %DPPH radical scavenging = [(control absorbance - extract absorbance)/control absorbance] x 100

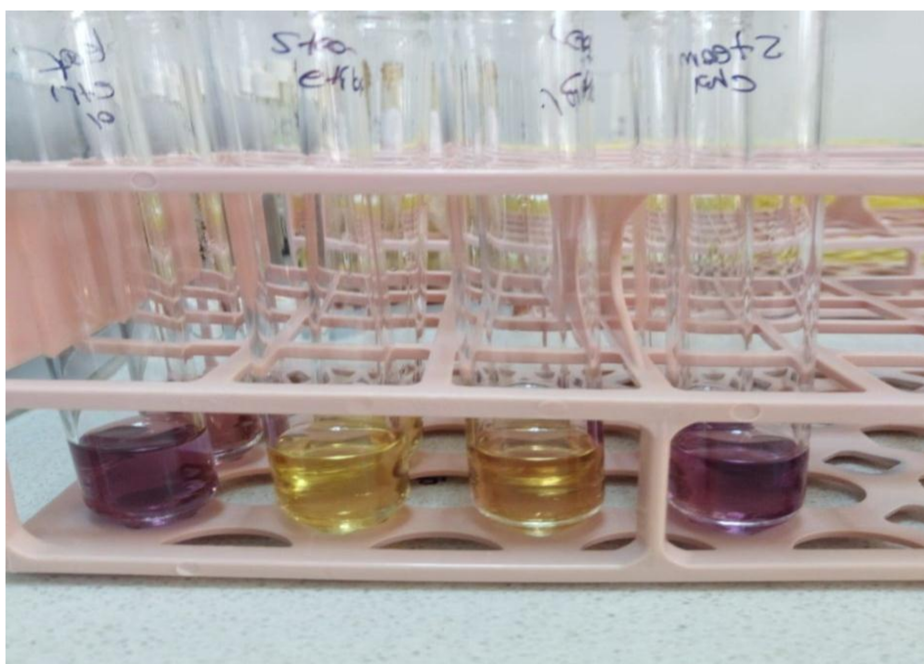

Figure 3.5. Determining of DPPH radical scavenging activity.

3.11. Preparation of Media

The media employed were Mueller Hinton Agar and Tryptic Soy Broth. In the lab, aseptic procedures were followed to make Mueller Hinton Agar and dehydrated Tryptic Soy Broth. The Tryptic Soy Broth medium was made by adding 30 g of powdered medium to 1000 mL of distilled water. At 121°C and 1 atm, 5 mL was transferred to each of the short screw-capped test tubes. For 15 minutes, it was sterilized under pressure. The autoclaved media were taken out, their lids sealed, and they were stored in a +4°C refrigerator until they were needed.To make Mueller Hinton Agar medium, 34 g of powder medium were combined with 1000 ml of distilled water. After thoroughly combining the Erlen Mayer's medium solution, the vials were sealed shut with aluminum foil at 121°C and 1 atm. It was sterilized for 15 minutes under pressure. After being taken out of the autoclave, the media were cooled to 50°C, put onto sterile petri dishes with a 4 mm thickness, and left to harden. Until usage, the prepared media were kept in a refrigerator at +4°C.

Figure 3.6. Preparation of media in microbiology lab.

3.12. Preparation of Microorganisms

To prepare for the experiment, the bacteria strains were placed in Tryptic Soy Broth medium and incubated at 37 degrees Celsius for 24 hours to reach full viability. Bacteria were diluted to a concentration of 0.5 McFarland (108 CFU/mL) by inoculating 9 mL of Tryptic Soy Broth medium into test tubes containing bacteri from the fresh cultures produced.

Figure 3.7. Preparation and activation of microorganisms .

3.13. Antibacterial Activity Testing

To prepare the bacterial suspensions, the overnight bacterial cultures were used. A densitometer was then used to adjust the suspensions to 0.5 McFarland. In the disc diffusion method, 20 µL of the previously prepared extracts (1 mg/mL concentration) were absorbed into sterile discs (6 mm in diameter).Solvents (petroleum ether, ethyl acetate, chloroform, methanol and acetone) impregnated discs were used as negative control and commercial antibiotic discs (Gentamicin (10 mcg) were utilized as positive control. Sterile swabs were used for applying density-adjusted microbe suspensions to Mueller Hinton agars. Occasionally impregnated discs were positioned on inoculated Mueller Hinton Agar.

Figure 3.8. Preparation of antibacterial activity tests.

3.14. Measuring Zone Diameters

At the end of 24 hours incubation at 37ºC, incubation zone diameters (mm) around the disc were measured with a digital caliper to determine whether the plant extracts had antibacterial activity against microorganisms. By looking at the inhibition zone diameters, it was determined whether the plant extracts had antibacterial activity on the bacteria used in the study (Figure 3.9).

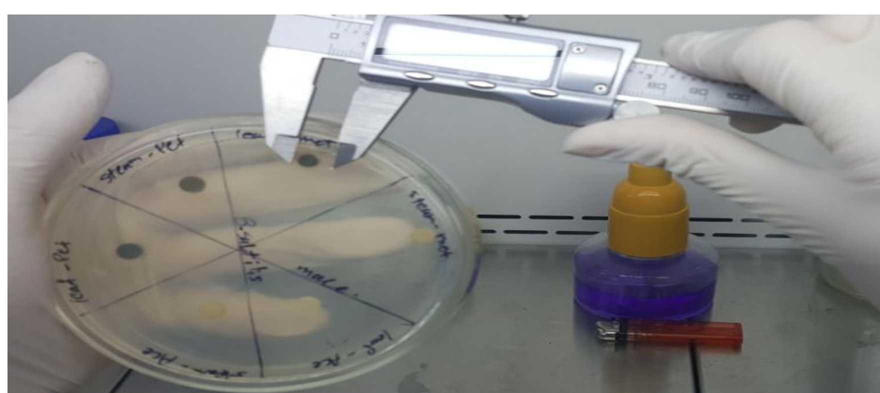

Figure 3.9. Measuring zone diameters with a digital caliper.

3.15. Statistical Analysis

The findings of each analysis, which were carried out in triplicate, are shown as mean ± SD(standard deviation) . The SPSS 20.0 software was used to conduct statistical analyses. One-way ANOVA and the Duncan-test (P 0.05) were used to perform statistical analysis on the data.

4. RESULTS AND DISCISION

4.1. Sampling distribution and percentages

In this study, six previously identified isolates were obtained. The distribution of the isolates and their percentages were studied, as shown in Table 4.1 and figure 4.1.

Table 4.1. Numbers and percentages of bacteria among the 100 isolates previously obtained.

Bacteria name	Source of sample	Number of samples
Staphylococcus epidermidis	**Nasal Swab**	17
Staphylococcus aureus	**Ear swab**	30
Bacillus subtilis	**Stool sample**	**10**
Escherichia coli	**Urine sample**	**9**
Salmonella typhimurium	**Stool sample**	16
Enterococcus faecalis	**Stool sample**	18
Total: 6	**Total: 6**	**Total: 100**

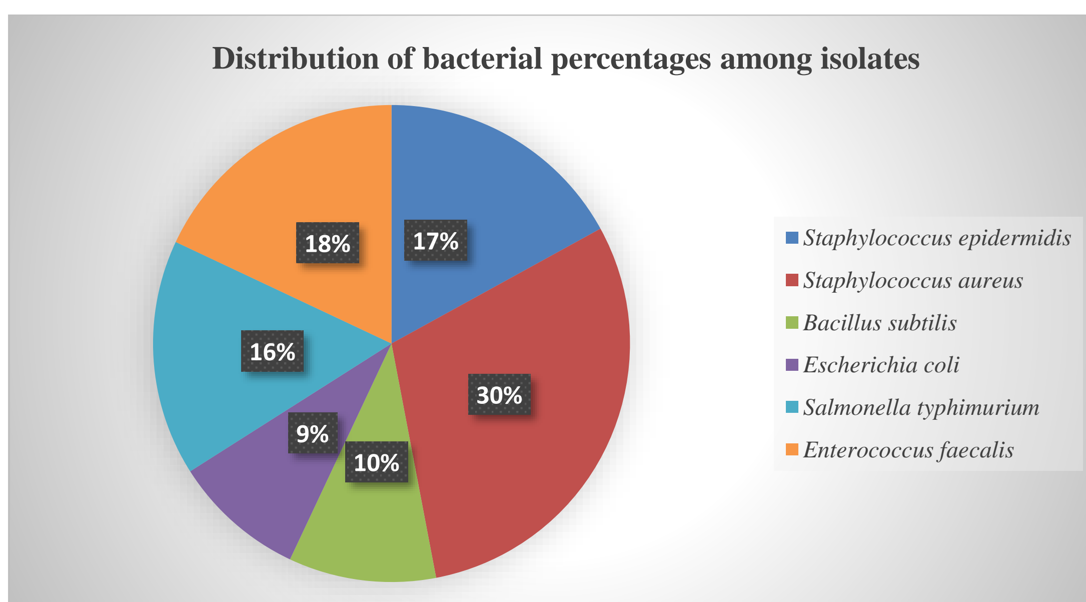

Figure 4.1. Numbers and percentages of bacteria among the 100 isolates previously obtained.

4.2. Results of SEM

SEM images and EDX spectroscopy results of leaf surface, leaf interior and stem of *Equisetum arvense* plant are shown in Figure 4-1.

As a result of the study, it has been shown that the morphological images of each part used are different, as well as the amount of elements in the EDX spectroscopy results.

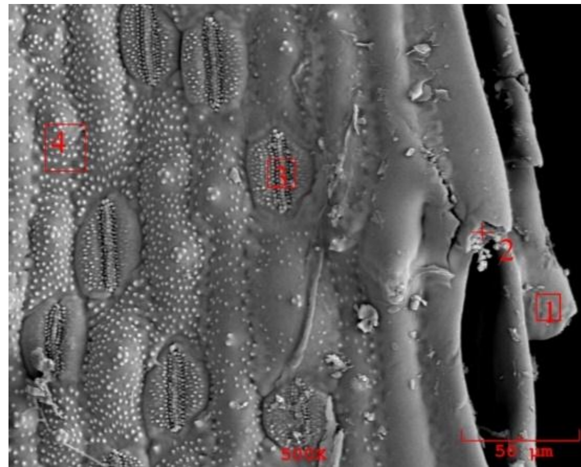

Figure 4.2. SEM image of *Equisetum arvense* leaf surface. (X500)

As shown in Figure 4.1. SEM image of *Equisetum arvense* leaf surface (x500) the image of the microscope shows the stomata significantly.

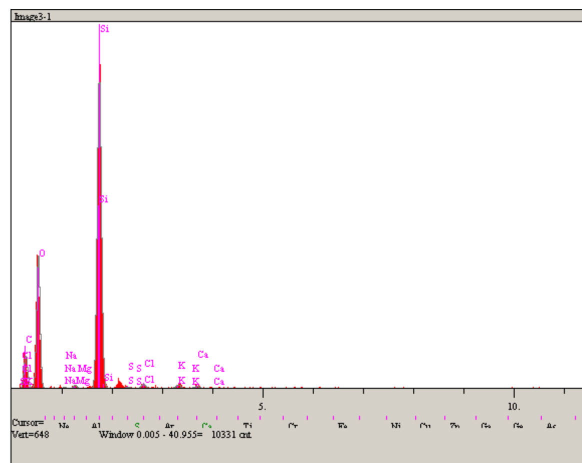

Figure 4.3. *Equisetum arvense* leaf surface EDX spectroscopy.

The upper Figure 4.2. shows the elements on the surface of the leaf and shows that the highest percentage is for the element silicon, and then oxygen, potassium, calcium and sodium.

Table 4.2. Concetration of elements *Equisetum arvense* leaf surface EDX spectroscopy.

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc(%wt)	
C	Ka	54.29	4.659	22.796	
O	Ka	201.89	8.985	**42.319**	
Na	Ka	1.95	0.884	**0.160**	
Mg	Ka	5.26	1.450	0.328	
Si	Ka	573.10	15.138	32.097	
S	Ka	3.50	1.183	0.255	
Cl	Ka	9.42	1.941	0.724	
K	Ka	8.65	1.860	0.734	
Ca	Ka	6.54	1.617	0.587	
				100.000	Total

As shown in Table 4.1. the concetration of elements *Equisetum arvense* leaf surface edx spectroscopy .oxygen was highst(42.319),then silicon (32.097),carbon (22.796) but the lowest was sodium(0.160).

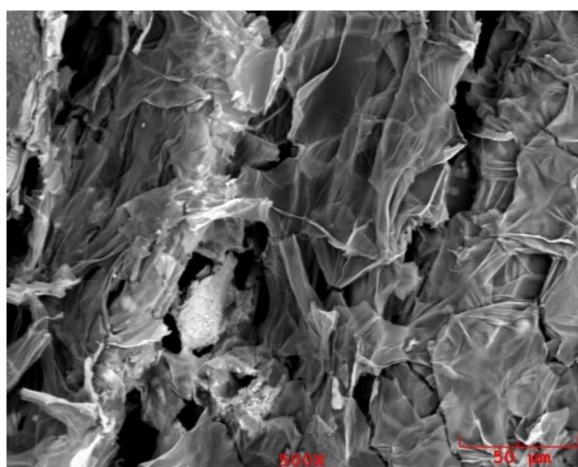

Figure 4.4. SEM image of *Equisetum arvense* leaf inner side. (X500)

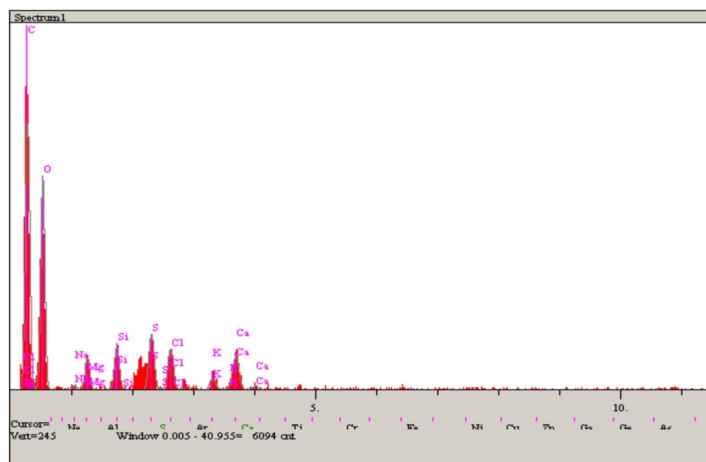

Figure 4.5. *Equisetum arvense* inner side of leaf EDX spectroscopy.

The upper Figure 4.2. shows the elements on the inner side of the leaf and shows that the highest percentage is for the element Carbon, and then oxygen, sulfur, silicon and sodium while the lowest element was calcium.

Table 4.3. Concetration of elements *Equisetum arvense* inner side of leaf EDX spectroscopy.

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc(%wt)	
C	Ka	165.08	8.126	**43.572**	
O	Ka	120.35	6.938	**41.730**	
Na	Ka	2.86	1.069	**0.325**	
Mg	Ka	20.99	2.898	1.793	
Si	Ka	30.06	3.467	2.249	
S	Ka	41.60	4.079	3.311	
Cl	Ka	30.76	3.508	2.760	
K	Ka	9.81	1.980	1.020	
Ca	Ka	28.87	3.398	3.240	
				100.000	Total

Table (4.2.) that showed the concetration of elements *Equisetum arvense* inner side of leaf edx spectroscopy and the concetrations were different from leaf surface .The highest was carbon (43.572) and oxygen (41.730) then sulfur (3.311) and the lowest was sodium (0.325) .

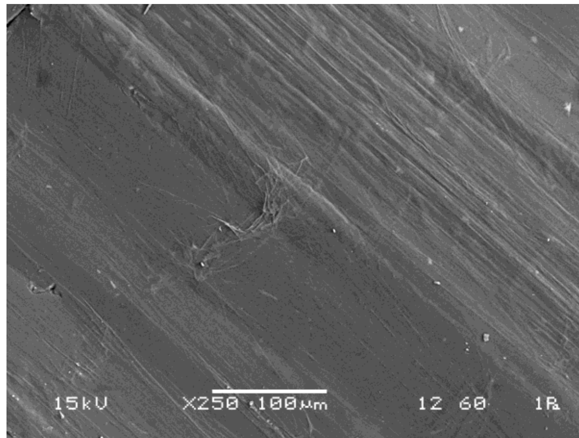

Figure 4.6. SEM image of *Equisetum arvense* stem surface. (X250)

As shown in Figure 4.5. SEM image of *Equisetum arvense* stem surface (x500) the image of the microscope shows the stem significantly.

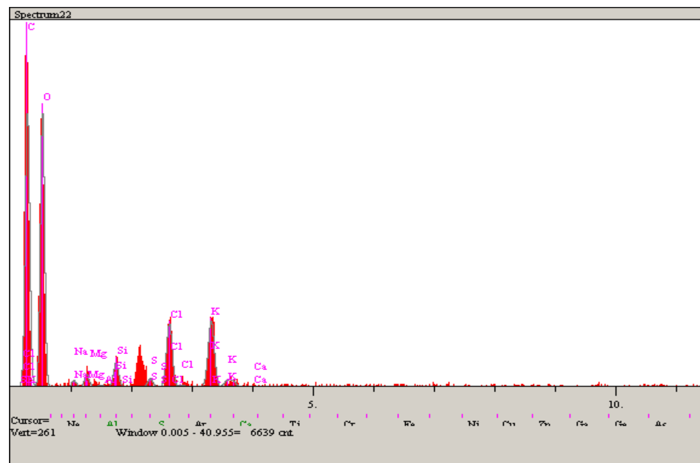

Figure 4.7. *Equisetum arvense* stem surface EDX spectroscopy.

In Figure 4.6. shows the elements on the stem surface of the plant and shows that the highest percentage is for the element Carbon, and then oxygen, chlorine, potassium and sodium while the lowest element was calcium.

Table 4.4. Concetration of elements *Equisetum arvense* stem surface EDX spectroscopy

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc(%wt)	
C	Ka	181.02	8.505	**38.347**	
O	Ka	167.21	8.174	**48.697**	
Na	Ka	4.28	1.308	0.465	
Mg	Ka	8.19	1.809	0.663	
Al	Ka	1.33	0.729	**0.099**	
Si	Ka	16.99	2.605	1.175	
S	Ka	6.34	1.592	0.460	
Cl	Ka	51.94	4.556	4.176	
K	Ka	58.06	4.817	**5.555**	
Ca	Ka	3.48	1.180	0.363	
				100.000	Total

Table 4.3. show that stem surface *of Equisetum arvense on* EDX spectroscopy has concetrations of elements and the highest element was oxygen (48.697),carbon (38.347) then potassium was (5.555) and the lowest aluminium (0.099)**.**

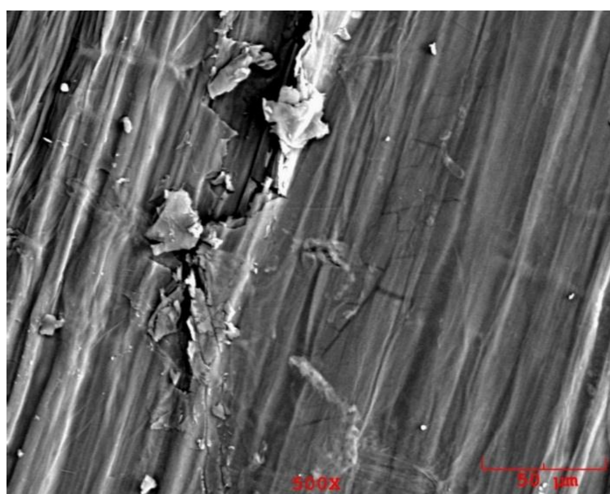

Figure 4.8. SEM image of *Equisetum arvense* stem inner side. (X500)

As shown in Figure 4.7. SEM image of *Equisetum arvense* stem inner side (x500) the image of the microscope shows the stem from inner side

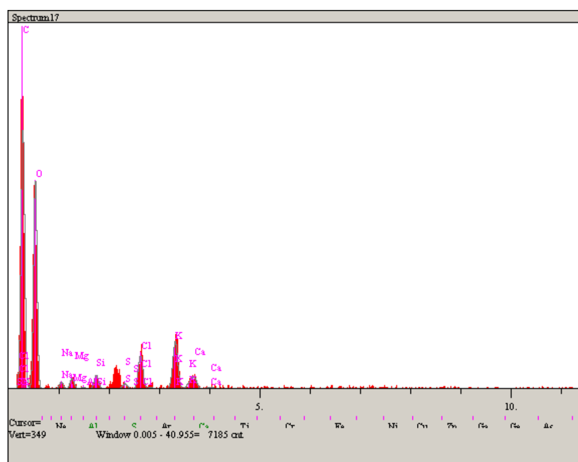

Figure 4.9. *Equisetum arvense* inner side of stem EDX spectroscopy.

The concetration of elements *Equisetum arvense* inner side of stem EDX spectroscopy show that stem surface *of Equisetum arvense on* EDX spectroscopy has concetrations of elements and the highest element was oxygen then carbon , potassium and the lowest aluminium .

Table 4.5. Concetration of elements *Equisetum arvense* inner side of stem EDX spectroscopy.

Elt.	Line	Intensity (c/s)	Error 2-sig	Conc(%wt)	
C	Ka	229.42	9.579	**40.166**	
O	Ka	170.55	8.259	**47.980**	
Na	Ka	6.15	1.569	0.633	
Mg	Ka	9.90	1.990	0.761	
Al	Ka	1.03	0.642	**0.073**	
Si	Ka	12.98	2.278	0.852	
S	Ka	5.40	1.470	0.371	
Cl	Ka	36.59	3.825	2.779	
K	Ka	55.78	4.723	5.009	
Ca	Ka	14.01	2.367	1.376	
				100.000	Total

Table 4.4. Concetration of Elements *Equisetum arvense* inner side of stem EDX spectroscopy show that stem surface *of Equisetum arvense on* EDX spectroscopy has concetrations of elements and the highest element was oxygen (47.980),carbon (40.166) then potassium was (5.009) and the lowest aluminium (0.073).

4.3. Expirmental Results

The results showed that the plant has an inhibitory ability for some types of bacteria in both the whole plant and in the case of leaves and stem separately. Depending on the method used to extract natural compounds, one might expect a wide range of results and accuracy. The outcomes of our extraction, which were evaluated with *Equisetum arvense* stem and leaf parts using 2 different extraction methods and 5 different solvents, are shown in figure 4.10. The results are summarized in the tables and figures below.

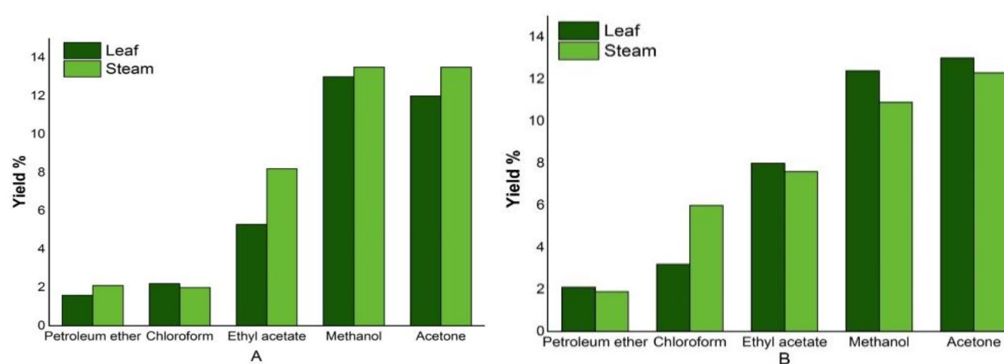

Figure 4.10. A- % yield of applications with maceration method, B- % yield of applications with soxhlet method**.**

Plant phenolic compounds are well recognized to have a substantial impact in shaping the biological properties of the plant, including antioxidant. The total phenolic content of extracts originate from the leaf and stem of *Equisetum arvense* are given in the Table 4.5.

Table 4.6. Totle phenolic contenets of extracts obtained by maceration and soxhelt methods

Extract	TPC (*mgGA/g*)			
	Maceration		Soxhlet	
	Leaf	Stem	Leaf	Stem
P. ether	$18.3^{a}\pm1.2$	$24.5^{a}\pm0.2$	$20.47^{a}\pm0.5$	$23.8^{a}\pm4.8$
Chloroform	$156.21^{b}\pm2$	$59.1^{b}\pm0.8$	$134.04^{b}\pm2.20$	$63.09^{b}\pm0.2$
Ethyl acetate	$219.76^{c}\pm1.6$	$114.28^{c}\pm1.6$	$213.3^{c}\pm4.6$	$150.47^{c}\pm2.9$
Methanol	$234.04^{c}\pm0.9$	$238.09^{d}\pm4.3$	$139.52^{b}\pm0.7$	$135.23^{c}\pm3.2$
Acetone	$507.61^{d}\pm8.3$	$471.42^{e}\pm0.5$	$252.1^{d}\pm6.2$	$466.6^{d}\pm4.6$

Data were represented as mean ± SD of three measurements. Different letters symbolized significant differences (P <0.05) by mean of the ANOVA Duncan-test.

As a result of the study, the highest total phenolic content of acetone leaf extract was (507.61) and the lowest was P. ether(18.3) has been found in maceration method

while in Soxhlet method the highest total phenolic content of acetone stem extract was(466.6^{d}±4.6)) and the lowest was P. ether(20.47^{a}±0.5) It was seen in Table 4.4. that the extracts using the maceration method contained higher TPC values. Maceration is an extraction method in which no heat is used throughout the process. Our research suggests that it may be effective in halting the breakdown of phenol, but it did not lead to particularly high yields. However, although the efficiency of the soxhlet method is high, the extracted phenolic substances may have been adversely affected since it was extracted under reflux at temperature. Depending on the increasing polarity seen in our study results, it is seen that the phenolic substance increases. In addition, it was determined that the extracts prepared with petroleum ether from the plant *Equisetum arvense* were not suitable for TPC isolation. The impact of various procedures on the content of phenolic components (PCs) depends via numerous criteria, including treatment dosage and duration, plant species, PC subtype, and other characteristics of the product, such as metabolic stage and initial PC content [39].

Each plant includes a unique combination of phenolic chemicals with varying levels of antioxidant action [40].

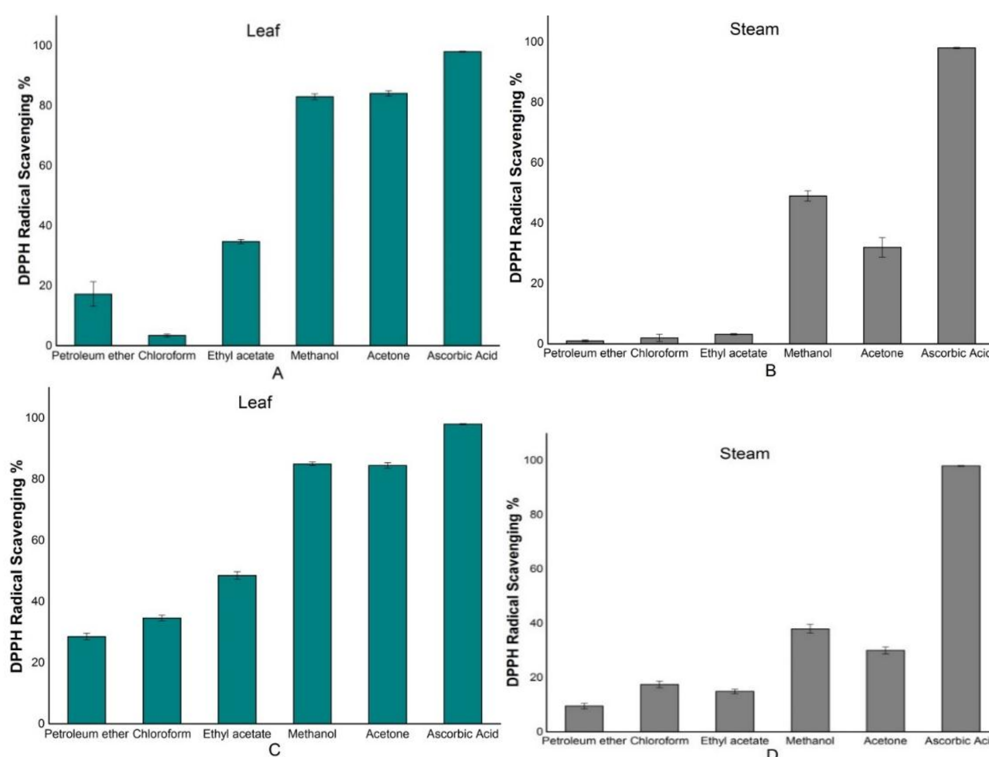

Figure 4.11. DPPH scavenging activity (%) of different extracts prepared from *Equisetum arvense*

A-B: Maceration methods, C-D:Soxhlet methods

DPPH scavenging activity of different extracts prepared from *Equisetum arvense in* our study the highest percentage of DPPH scavenging activity was in maceration method whith leaf extract 84.1% with acetone and 83% methanol and the lowest was with chloroforom 5% while in stem the highest percentage with methanol was 40% and as comparative with the soxhelt method results the highest percentage of DPPH scavenging activity of leaf with methanol was 85.1% and acetone was 84.5% , the lowest was with p.ether was 25% while in stem the highest was methanol 40% and the lowest was p.ether 15% . Using a methanol solution of the DPPH reagent, they measured the antioxidant activity of *Equisetum telmateia* plant extracts, which showed values (33.4 ± 1.2) , acetone (115.7 ± 1.6) ethyl acetate (982.2 ± 2.3) [13].

The antioxidant and antibacterial activity of the soxhlet-extracted extracts of this plant was not investigated in any of the studies that were identified to have been published. In the literature, there are studies evaluating the DPPH scavenging activity and antioxidant activity of the plant *Equisetum arvense,* some of which are given in Table 4.6.

Table 4.7. DPPH scavenging activities of *Equisetum arvense* extracts in the literature.

Used of parts	Methods	Solvents	Antioxidant activity(DPPH methods)	Reference
Stem	Maceration	Ethyl acetate Butanol	IC50=2.37µg/mL IC50=7.16 µg/mL	[9]
Stem	Modified of Maceration	Methanol	1 mg/mL for * %87	[38]
Aerial parts	Maceration	Ethyl acetate Butanol	1mg/mL for* %10 %70	[38]
All parts	Microwave assisted extraction	Ethanol-water	1 mg/mL for* %33	[41]
All parts	Maceration	Methanol	0.2 mg/mL for* %52.4	[42]

*:%DPPH radical scavenging, IC50: concentratıon requıred for 50 percent DPPH radical scavenging

In the given literature, it has been reported that *Equisetum arvense* plant shows antioxidant activity and as in our study, extracts prepared with alcohol groups such as methanol are more effective in revealing antioxidant activity (Table 4.5.). Depending on the place where the plants are collected, the collection time, the environmental conditions of the area where they are collected, secondary metabolites of different

types and rates can be produced, and accordingly, there may be differences in biological activities.

There has been a growing interest in herbal products and compounds derived from plants in recent years. It is known that many diseases, including infectious diseases, have been treated with herbal medicines throughout human history. Standardized and reliable antimicrobial activities are needed to examine the potential antimicrobial properties of plant-derived phytochemicals [43].

In order to obtain bioavailable compounds such as phenolic compounds in herbal extracts at maximum rate and with minimum degradation, an effective extraction method and solvent selection should be made.

In our study, the antibacterial activity results of the extracts obtained from the plant *Equisetum arvense* by Maceration and Soxhlet methods are given in Table 4.6. and Table 4.7. When the results of the study were examined, it was seen that the highest antibacterial activity was in the methanolic leaf extract prepared by the Maceration method, and this extract had a broad spectrum effect on the test bacteria used (*S. aureus* 14.5 mm, *S. epidermidis* 14.2 mm, *E. faecalis* 14 mm, *B. subtilis* 13.5 mm inhibition zone diameter). It was determined that this extract was comparable to gentamicin, a standard antibiotic on *S. aureus*, *E. faecalis* and *S. epidermidis*. Its strong antibacterial activity on *S. aureus*, one of the most common Gram-positive bacteria in food poisoning, is a valuable data for the literature. It was observed that the extract obtained from the leaf part of the *Equisetum arvense* plant was more effective on the test bacteria than the stem extracts. It was determined that the acetonic extract prepared by the Maceration method among the extracts obtained from the stem produced the highest activity in *S. aureus* with an inhibition zone diameter of 11 mm. In addition, it was determined that only methanolic and acetonic stem extracts prepared by the Maceration method showed activity on *S. aureus*. In the Soxhlet method, it was observed that only the methanolic extract formed a 10 mm inhibition zone on *E. faecalis*. Like in another study, *Equisetum arvense* extract was shown to be ineffective on *E.coli* [6].

Table 4.8. Antibacterial activity of extracts obtained by maceration method.

Extracts		Test bacteria (Inhibition Zones (mm) (±SD))					
		Bs	Ec	Ef	Sa	Se	St
Leaf	P. ether	0	0	0	8	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	13.5	0	14	14.5	14.2	0
	Acetone	8	0	9.5	9.5	8	0
Stem	P. ether	9	0	8	0	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	0	0	0	9	0	0
	Acetone	0	0	0	11	0	0
Gentamicin 10 mcg		20	18	19	22	21	20
N. control		0	0	0	0	0	0

Ec-*Escherichia coli*, Se-*Staphylococcus epidermidis*, Bs-*Bacillus subtilis,* Sa-*Staphylococcus aureus*, Ef-*Enterecoccus faecalis,* St-*Salmonella typhimurium.*

As showed in Table 4.7. the results of antibacterial activity of extracts obtained by maceration method were the highest inhibition zone with methanol on *Staphylococcus aureus (*14.5 mm *), Staphylococcus epidermidis (*14.2 mm*), Enterecoccus faecalis* (14mm) and on *Bacillus subtilis (*13.5 mm). And Aceton has less effects on bacteria than methanol as comparasion the inhbition zones were (9.5 mm) *on Enterecoccus faecalis* and *Staphylococcus aureus ,* (8 mm) on *Bacillus subtilis* and *Staphylococcus epidermidis.* P. ether considered the lowest due to these results (8mm) on *Enterecoccus faecalis* and *Staphylococcus aureus* ,(9mm) on *Bacillus subtilis* only while *Escherichia coli* and *Salmonella typhimurium* there is none inhbition zones with all solvents, this antibacterial activity agree with studies that showed activity of the plant extract against *S. epidermidis* with inhibition zone (3.3 mm) and but none for *E.coli* [44].

In study of *Melissa officinalis L.* Extracts that showed inhibition zone arond *S.aureus*(16.33 mm) but no antibacteiral activity to *E.coli* also [45]. While this antibacterial activity not agrees with previous studies that showed activity of the plant extract against *E.coli* [46].

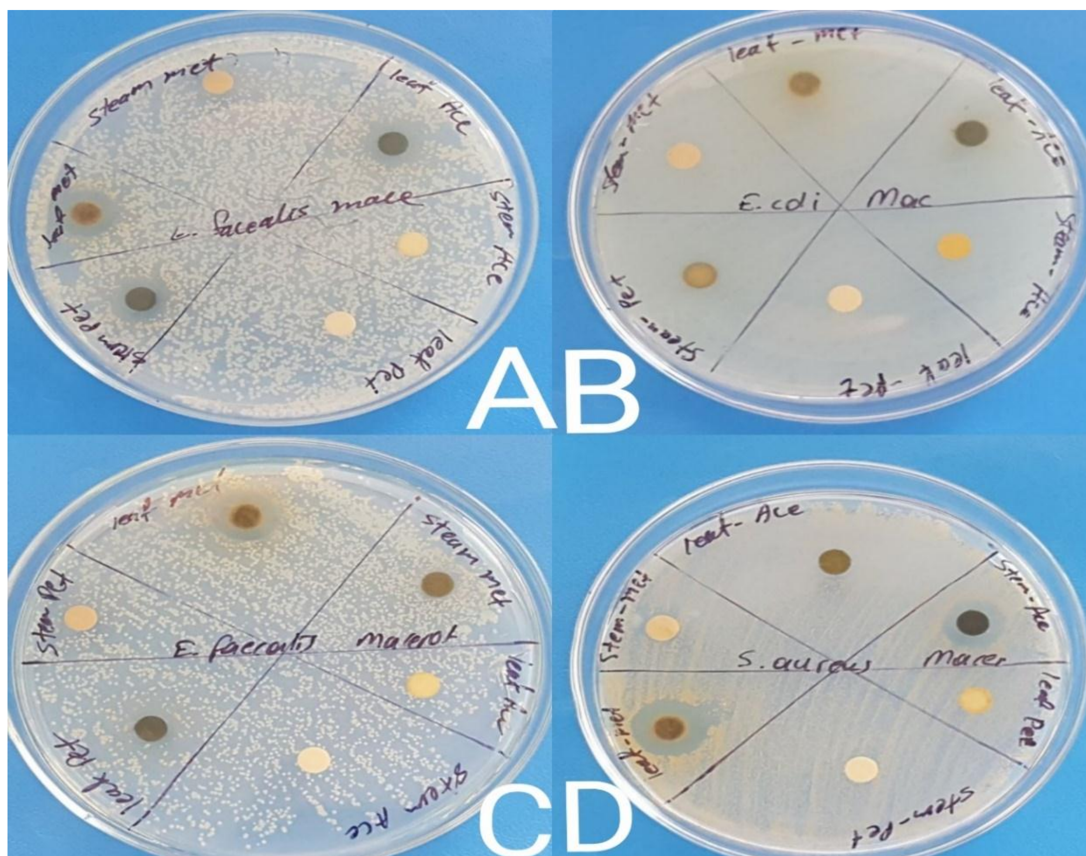

Figure 4.12. Inhibtion zones of extracts obtained by maceration method.

A-*Enterecoccus faecalis* ***B-*** *Escherichia coli* ***C-****Bacillus subtilis* ***D-****Staphylococcus aureus*

Table 4.9. Antibacterial activity of extracts obtained by soxhlet method.

Extracts		Test bacteria (Inhibition Zones (mm) (±SD))					
		Bs	Ec	Ef	Sa	Se	St
Leaf	P. ether	0	0	0	0	0	0
	Ethyl acetate	0	0	9.5	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	9	0	8	0	0	0
	Acetone	0	0	8	0	0	0
Stem	P. ether	0	0	0	0	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	0	0	10	0	0	0
	Acetone	0	0	0	0	0	0
Gentamicin 10 mcg		20	18	19	22	21	20
N. control		0	0	0	0	0	0

Ec-*Escherichia coli*, Se-*Staphylococcus epidermidis*, Bs-*Bacillus subtilis,* Sa-*Staphylococcus aureus*, Ef-*Enterecoccus faecalis,* St-*Salmonella typhimurium.*

As showed in Table 4.8. the results of antibacterial activity of extracts obtained by soxhelt method were the highest inhibition zone with leaf Ethyl acetate extraction on

Enterecoccus faecalis (9.5 mm) and with leaf methanol extraction and actone on *Enterecoccus faecalis (*8 mm) while stem methanol extraction was (10mm).

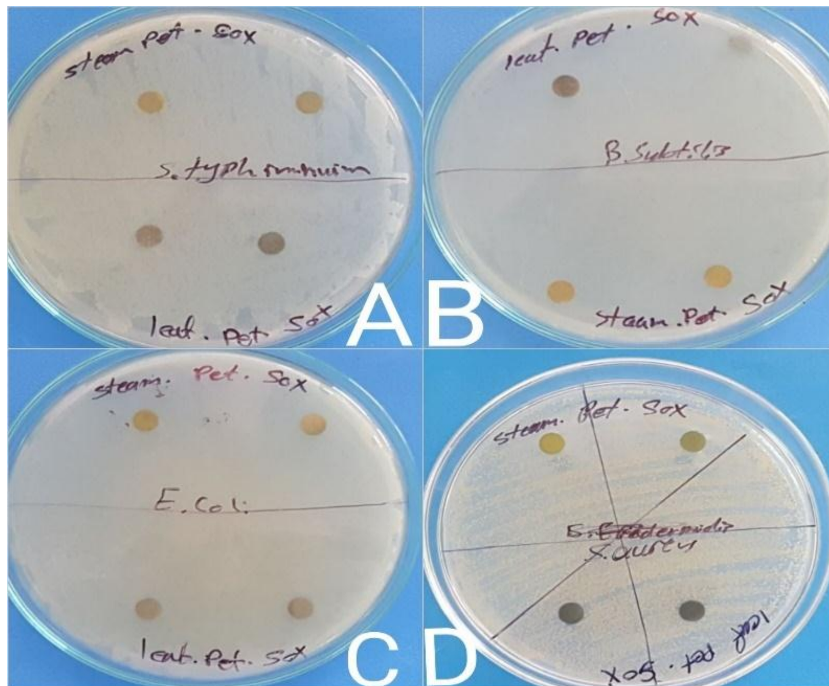

Figure 4.13. Inhibtion zones of extracts obtained by soxhelt method

A*-Salmonella typhimurium* ***B-*** *Bacillus subtilis* ***C-*** *Escherichia coli* ***D-****Staphylococcus aureus.*

The difference in cell wall structure and permeability of Gram-positive and Gram-negative bacteria causes different sensitivities .In Gram-negative bacteria, the outer membrane is a barrier to many substances, including antibiotics. Gram-negative bacteria have an outer phospholipidic membrane that carries the lipopolysaccharide components [46].

Gram-positive bacteria, on the other hand, only have an outer peptidoglycan layer that does not have an effective permeability barrier [45] [47].

Recent studies [48] have confirmed the in vitro antimicrobial activity of the plant *Equisetum arvense*. It has also been reported in studies that Petroleum ether and chloroform solvents are not suitable for antibacterial activity studies [49]. On the other hand, ethyl and methyl extracts were more effective in this investigation [50].

5. CONCLUSION AND RECOMMENDATIONS

As a result of the study, it was determined that the method and solvent used in the preparation of the extract are important in revealing the chemical content and exhibiting the activity. Extracts produced by the maceration method exhibited higher antibacterial activity, while the extracts obtained by the soxhlet method showed lower antibacterial activity may be due to the heating but showed higher antioxidant activity.It was determined that the qualitative efficiency was not directly related to the extract result values. In addition, the importance of pre-experiment optimization in order to find the appropriate method and solvent while investigating the biological activities of herbal extracts has been demonstrated in our study as showed the solvents that used in our study were diffirent in their effect on the plant . From our study, it depends on the parts of the plant used that give different results, as it was observed that the leaf and the stem were used separately, and once again they gave completely different results, and the results were higher always when the leaf was used.

Our study is considered to be a remarkable research that reveals the effect of solvent diversity and different extract densities on antimicrobial activity.

It was determined that the highest antibacterial activity in leaf extracts of *Equisetum arvense* was on *S. aureus* and *Enterecoccus faecalis* bacteria.

By disseminating such studies, it can be seen that plants can be an alternative to synthetic antimicrobial substances as natural sources of antimicrobial substances. We think that the isolation and identification of substances responsible for antimicrobial activity in plants is an important phenomenon for their use in medicine, food and industry. In other words, the drug diagnostic properties investigated in this work can be used as a tool for plant identification, raw material validation, and formulation standardization.

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