T.C.
KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME *CUPRESSUS* AND *EUCALYPTUS* SPECIES

ZHALA BAQI TAHA (KURACHI)

PhD THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ 2022

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Prepared for the Degree.

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DECLARATION

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

Zhala Baqi Taha (KURACHI)

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BAZI *CUPRESSUS* VE *EUCALYPTUS* TÜRLERİNİN KİMYASAL BİLEŞİMİ VE BİYOLOJİK AKTİVİTELERİ

(DOKTORA TEZİ)

ZHALA BAQI TAHA (KURACHI)

ÖZET

Bu çalışmada bazı *Cupressus* (*C.sempervirens L.* var. *pyramidalis*, *C.sempervirens* var. *horizontalis*, *C.arizonica*) ve *Eucalyptus* türlerinin (*E.camaldulensis*, *E.sideroxylon*) kimyasal bileşenleri ve biyolojik aktiviteleri belirlenmesi amaçlanmıştır. Numuneler Irak'ın farklı yerlerinden toplanarak (Erbil, Shaqlawa, Hiran) yaprak ve dal bölümlerine ayrılmıştır. Örneklerin etkinliği biyolojik aktivite, kondanse tanen, toplam fenolik miktarı, toplam flavonoid miktarı, antosiyanin, antioksidan kapasitesi (DPPH, ABTS, CUPRAC) ve kimyasal bileşenleri (alkaloid, glikozit bağlı, fenoller ve flavonoid bileşikler) HPLC yöntemine göre değerlendirilmiştir.

Antimikrobiyal aktivite testlerinde 4 patojenik bakteri (*S.aureus*, *E.faecalis*, *E.coli*, *K.pneumonia*) ve 2 yaygın mantar (*C.albicans*, *A.niger*) kullanılmıştır. Örnekler su, etanol ve metanol ile ekstrakte edilmiştir. Antibakteriyel ve antifungal analizlerde üç farklı konsantrasyon (%25, 50, 75) test edilmiştir. En iyi sonuçlar %75 konsantrasyonda metanol ekstrelerinde bulunmuştur.

Cupressus türleri antibakteriyel aktivite özellikleri bakımından genel olarak şu şekilde sıralanmıştır: *C.arizonica*>*C.sempervirens* L. var. *horizontalis*>*C.sempervirens* L. var. *pyramidalis*. Okaliptüs türlerinde ise *E.camaldulensis* ve *E.sideroxylon* sonuçları birbirine yakın çıkmıştır. En yüksek sonuçlar *Cupressus* ve *Eucalyptus* türlerinin yaprak kısımlarında tespit edilmiştir.

Cupressus örneklerinde toplam kondanse tanen miktarı 209.1-247.5 mg/kg aralığında belirlenmiştir. En yüksek toplam fenolik (TPC) (3.96-13.43 mg GAE/g DW) ve toplam flavonoid (TFC) (3.39-6.89 mg QE/g DW) miktarı *C.sempervirens* L. var. *horizontalis* yaprak kısımlarında bulunmuştur.

Antioksidan testlerinde konsantrasyon oranı arttıkça DPPH %inhibisyon değerleri de yükselmiştir. En yüksek süpürücü aktivite değerleri etanol ekstrelerinde (%53.6-97.8) bulunmuştur.

En yüksek alkaloid bileşik (berberine=344.16 ppm), glikozit bağlı bileşik (cupressuflavone= 1200.26ppm) ve fenol-flavonoid bileşik (gallik asit=307.50 ppm) sırasıyla *E.camaldulensis*, *C.sempervirens* L. var. *horizontalis* and *C.sempervirens* L. var. *pyramidalis* kısmında tespit edilmiştir.

Anahtar Kelimeler: *Cupressus*, *Eucalyptus*, antifungal, antibakteriyel, antioksidan

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME *CUPRESSUS* AND *EUCALYPTUS* SPECIES

(PhD THESIS)

ZHALA BAQI TAHA (KURACHI)

ABSTRACT

In this study, it was aimed to determine the chemical components and biological activities of some *Cupressus* (*C.sempervirens L.* var. *pyramidalis*, *C.sempervirens* var. *horizontalis*, *C.arizonica*) and *Eucalyptus* (*E.camaldulensis*, *E.sideroxylon*) species. The samples were collected from different places in Iraq, namely Erbil, Shaqlawa and Hiran. The biological activities, condensed tannin, phenolic compounds, flavonoid contents, anthocyanin, antioxidant capacity (DPPH, ABTS, CUPRAC), and chemical components (alkaloid, glycosidically bound, phenols, and flavonoids compounds) of the samples were determined using the HPLC method to evaluate the effectiveness of extract samples.

In the antimicrobial activity test trials, involved using four pathogenic bacterial strains (*S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumonia*) and two important strains of the common pathogenic fungi (*C.albicans* and *A.niger*) were used. The samples were extracted with water, ethanol, and methanol. Three different concentrations (25%, 50%, and 75%) of the extracts were tested in biological activity tests. Based on these tests, the best results were found in the methanol extract at concentration of 75%.

In general antibacterial activity of *Cupressus* spp. is as follows: *C.arizonica*>*C.sempervirens* L. var. *horizontalis*>*C.sempervirens* L. var. *pyramidalis*. As for *Eucalyptus* spp., the results of *E.camaldulensis* and *E.sideroxylon* were close to each other. The highest results were exhibited in the leaf parts of *Cupressus* and *Eucalyptus* species.

The amount of TCT was in the range of 209.1-247.5 mg/kg in *Cupressus* samples. The highest TPC (3.96-13.43 mg GAE/g DW) and TFC (3.39-6.89 mg QE/g DW) values were determined in *C.sempervirens* L.var. *horizontalis* leaf samples.

This study discovered that the DPPH % inhibition values increased with an increase in concentration in the antioxidant test. In addition, the highest scavenging activity values were found in ethanol extract (53.6-97.8%).

The alkaloid, glycosidically bound, and phenol-flavonoid contents of *Cupressus* and *Eucalyptus* samples were determined using the HPLC method. Berberine (344.16 ppm), the highest alkaloid compound, was found in *E.camaldulensis*. The highest glycosidically bound (cupressuflavone= 1200.26ppm) and phenol-flavonoid (rutin=367.82 ppm) compounds were detected in *C.sempervirens* L. var. *horizontalis*.

Keywords: *Cupressus*, *Eucalyptus*, antifungal, antibacterial, antioxidant capacity

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Zhala Baqi Taha (KURACHI)

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SYMBOLS AND ABBREVIATIONS

A.niger	: *Aspergillus niger*
ABTS	: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
AGC	: Accelerated gradient chromatography
B.cereus	: *Bacillus cereus*
B.subtilis	: *Bacillus subtillis*
C.albicans	: *Candida albicans*
C.arizonica	: *Cupressus arizonica*
C.sempervirens	: *Cupressus sempervirens*
Conc	: Concentration
CUPRAC	: Cupric reducing antioxidant capacity
CVD	: Cardiovascular disease
DPPH	: 2, 2-diphenyl-1, 1-picrylhydrazyl
DW	: Dry weigth
E.camaldulensis	: *Eucalyptus camaldulensis*
E.coli	: *Escherichia coli*
E.faecalis	: *Enterococcus faecalis*
E.sideroxylon	: *Eucalyptus sideroxylon*
EOs	: Essential oils
GAE	: Gallic acid equivalents
GC	: Gas chromatography
GC-MS	: Gas chromatography-mass spectrometry
GLM	: Generalized linear model
HD	: Hydrodistillation
HIV	: Human immunodeficiency virus
HPLC	: High -performance liquid chromatography
ICU	: Intensive Care Units
ITMS	: GC-Ion trap mass spectrometry
K.pneumonia	: *Klebsiella pneumoniae*
MBC	: Minimum bactericidal concentration
MCC	: Minimal cidal concentration

MIC : Minimum inhibitory concentration
ml : Milliliter
mm : Millimetre
P.aeruginosa : *Pseudomonas aeruginosa*
PBPs : Penicillin-binding proteins
pH : Potential of hydrogen
PYP : Pyrrolidony 1 arylamidase
ROS : Reactive oxygen species
S.aureus : *Staphylococus aureus*
S.typhymurium : *Salmonella typhymurium*
SFE : Supercritical fluid extraction
SPF : Specific Pathogen-Free
TAC : Total antioxidant capacity
TCT : Total condensed tannin
TFC : Total flavonoid content
TPC : Total phenolic content
UV : Ultraviolet
VLC : Vacuum liquid chromatography
VRE : Vancomycin resistant *enterococci*
WHO : World Health Organization

1. INTRODUCTION

1.1. Traditional Medicine

Plants as the basis of pharmaceutical compounds have continuosly played a key role in the maintenance of human health from the earliest period. The World Health Organization (WHO) reported that herbal extracts or their active components were used as folk medicine in ancient treatments involving 80% of the global population. Over 50% of all contemporary clinical drugs are of natural product derivative (Kirbag et al., 2009).

Vitamins (A, C, E, and K), terpenoids, carotenoids, polyphenols, flavonoids, alkaloids, saponins, tannins, enzymes, pigments, and minerals with the antioxidant and antibacterial properties are examples of phytochemicals (Madhuri and Pandey, 2009). Although many functions of phytochemicals in particular are still unknown, numerous studies have revealed that they play a role in plant-pest and plant-disease interactions. Therefore, the reference point for the development of antimicrobial drugs is the antimicrobial screening of plant extracts and phytochemicals. Plant scientists have been interested in phytochemical research as a result of the advancement of cutting-edge methods. These methods were very important in the search for new raw materials in the pharmaceutical industry (Shakeri et al., 2012).

The antibacterial actions are produced by the medicinal plants' immunomodulatory and antioxidant characteristics. They are known to stimulate both non-specific and specific immunity, exhibiting diverse immunomodulatory functions (Shukla et al., 2022). Utilizing plant extracts and phytochemicals, both of which have recognized the antibacterial characteristics, can be extremely important in therapeutic procedures. Numerous studies have been carried out recently from various nations to demonstrate their effectiveness. Due to their antibacterial properties, which are brought about by the substances created in plant secondary metabolism, many plants have been utilized (Nascimento et al., 2000).

1.2. Phytochemicals in Plants

Phytochemicals are natural chemical compounds that are present in plants. They give food its colour, taste and aroma. Overall, herbal compounds are referred to as phytochemicals when they protect plant cells from environmental threats such pollution, drought, harmful UV rays and pathogenic attack (Saxena et al., 2016).

Numerous secondary metabolites found in plants (e.g., tannins, alkaloids, flavonoids, and terpenoids) exhibit biological functions, such as antimicrobial, antioxidant, anticancer and others. These compounds can be very important in therapeutic procedures (Gislene et al., 2000).

Secondary metabolites are substances that play a role in the way organism is interacting with its atmosphere, however, are not required for the organism to live. These substances frequently help to protect plants from biotic and/or abiotic stresses (Shitan, 2016). Secondary metabolites belong to many groups of metabolites and can be strongly induced in response to stressors. By taking part in nutrition and reproduction, primary metabolites play crucial metabolic roles. Some secondary metabolites have great economic importance since they are employed as chemicals, particularly in producing medicines, flavours, perfumes, insecticides, and dyes. Continuous use of higher plants as sources of chemicals, particularly therapeutic compounds, will be extended and enhanced by new technologies (Beran et al., 2019).

Secondary metabolites are low-molecular-weight chemicals often formed in certain organs, tissues, and cells (Isah et al., 2018). In terms of quantity and quality, these chemicals frequently vary between members of the same plant populations. They shield plants from biotic (e.g., bacteria, fungi, nematodes, insects, or animal grazing) and abiotic stresses (environmental factors) (e.g., high temperature and moisture, shading, injury, presence of heavy metals). Due to their high economic value, secondary metabolites are particularly employed by humans as chemicals in medicines, flavors, perfumes, pesticides, and colors (Keller, 2019).

1.2.1. Phenolic and tannin compounds

There are more than 300.000 documented plant species in the world, from which a wide variety of chemicals are produced. Additional classifications for these substances include main and secondary metabolites (Fiehn, 2002). Primary metabolites encompass substances that are essential for the development and growth of all plants, including fatty acids sugars, nucleic acids, and amino acids. Secondary metabolites are substances found in specialized cells that are not directly essential for the basic photosynthesis process or respiratory metabolism. However, this type of substances are necessary for the continued existence of the plants in the atmosphere. Secondary metabolites are structurally and chemically much more diversified than primary metabolites (Wu and Chappell, 2008).

Plant phenolics and "polyphenols" are secondary natural metabolites that develop biogenetically from either the shikimate/phenylpropanoid pathway, which provides phenylpropanoids directly, or the "polyketide" acetate/malonate pathway, which can produce simple phenols, or both. These secondary natural metabolites result in monomeric and polymeric phenols and polyphenols, which have a wide range of physiological functions in plants. Higher plants produce thousands of distinct phenolic chemicals that are known to exist (Campos et al., 2022).

1.2.2. Flavonoids

Flavonoids are secondary metabolites found in a variety of plant tissues. They are mostly identified for their distinctive red, blue, and purple pigments, including the seeds, berries, nuts, bark, roots, stems, and flowers. Plants have been shown to contain over 4000 structurally distinct flavonoids (Brahmachari et al., 2011). Due to their high ability to give electrons or hydrogen atoms, many of them have been shown to outperform numerous well-known antioxidants, such as ascorbate (vitamin C) and -tocopherol (vitamin E) based on in vitro antioxidant assays (Hernández et al., 2009).

Phenolic compounds, found in plant foods, have become increasingly important. Long known to have anti-inflammatory, antioxidant, hepatoprotective, anti-allergic, antithrombotic, antiviral, and anticarcinogenic properties, flavonoids also have other beneficial properties (Shaik et al., 2018). It is challenging to provide a precise scientific definition for the complex collection of naturally occurring polymers known as plant polyphenols (tannins).

The molecular composition of flavonoids determines their ability to function as antioxidants. Free radical scavenging behaviors and the antioxidant properties of flavonoids are influenced by the arrangement of hydroxyl groups and other molecular features in their chemical structure (Saxena et al., 2012). Plant-derived flavonoids are consumed in the amount of one gram daily by humans (Kühnau, 1976), with drinks, such as tea, chocolate, red wine, and alcohol comprising a great quantity of flavonoids, and herbal remedies containing flavonoids being utilized all over the world (Gurib-Fakim, 2006).

1.2.3. Anthocyanin

An important group of secondary metabolites found in plants, called anthocyanins, is a significant class of flavonoids. Plants, particularly flowers, fruits, and tubers, contain blue, red, or purple pigments called anthocyanins. Red and blue anthocyanin are found in acidic and alkaline environments, respectively (Khoo et al., 2017).

The intravacuolar environment affects the hue of anthocyanins, which are water-soluble pigments that are mostly found in cell vacuoles. In nature, more than 600 anthocyanins have been found (Smeriglio et al., 2016). The six widely distributed anthocyanidins (namely, cyanidin, pelargonidin, delphinidin, petunidin, peonidin and malvidin) are sources of the majority of anthocyanins found in plants. Partly because of their powerful antioxidant effects, anthocyanins protect plants from various biotic and abiotic stress factors. Additionally, anthocyanin-rich food items are more well-liked for their appealing hues and potential health advantages (Liu et al., 2019). Figure 1.1. depicts the overall molecular structure of anthocyanin.

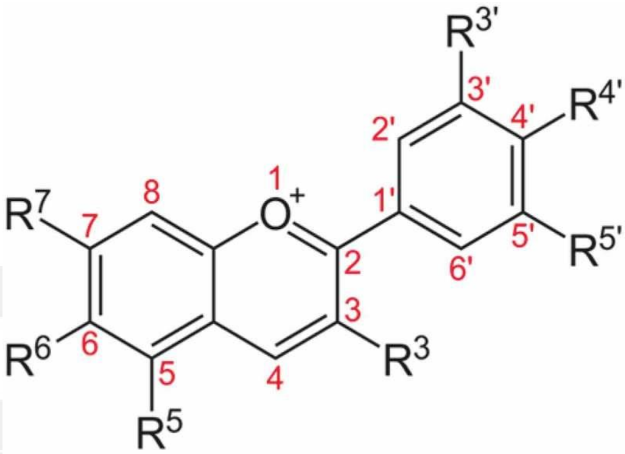

Figure 1.1. Basic anthocyanin structure (Khoo et al., 2017)

Plant-based alkaloids are basic while having one or more nitrogen atoms (often in a heterocyclic ring), and typically have a pronounced physiological effect. The functions of alkaloids in plants are unknown, as is the reason for their existence. There are a variety of opinions held by various authorities, including the idea that they are unimportant and can be simply seen as byproducts of plant metabolism (Roberts et al., 2016). They might serve as protein production reservoirs or as defense against insect or animal attacks. They may operate as plant stimulants or regulators of processes including growth, metabolism, and reproduction, similar to hormones. Although they can act as detoxifying agents by methylating, condensing and circulating substances that can harm the plant (Moudi et al., 2013).

1.2.4. Alkaloids

Alkaloids from plant sources are characterized as basic, containing one or more nitrogen atoms (usually in a heterocyclic ring), and generally having a pronounced physiological effect. The functions of alkaloids in plants are unclear. There are various views by different authorities, such as they are of no importance and may be regarded as byproducts of plant metabolism (Roberts et al., 2013). They may act as protective substances against animal or insect attacks in addition to reservoirs for protein synthesis. Like hormones, they may function as plant stimulants or regulators in activities like growth, metabolism, and reproduction. Although it functions as a detoxifying agent by methylating, condensing and cyclizing compounds that can harm the plant (Moudi et al., 2013).

1.2.5. Glycosides

A sizeable subclass of secondary metabolites is glycosides. Gycosides have a wide range of structural characteristics. Due to their well-established bioactivities and long history of usage, they are crucial to the practice of pharmacognosy. However, much remains to be understood about the functions and characteristics of gycosides (Bartnik and Facey, 2017). Glycosides are acetals of carbohydrates. They are created when alcohol and sugar hemiacetal react. In water, glycosides are stable, and an open chain form is not in equilibrium with them (do not show mutarotation). By hydrolyzing them with aqueous acid, they can be transformed back into the original monosaccharide. Many biologically significant compounds, which include glycosidic connections and glycosides, are extensively distributed in nature (Cui, 2005).

1.2.6. Total Antioxidant Capacity

In recent years, medicinal plants have undergone more thorough screening in order to be taken into consideration as a unique therapeutic option and potential replacement for chemical-based food preservatives. In fact, plants are a major source of many medicinal substances and biological processes, particularly those with antioxidant, antibacterial, and antiparasitic capabilities (Guendouze-Bouchefa et al., 2015).

An excess of oxidants, which include reactive oxygen species and free radicals, may develop in the human body as a result of "oxidative stress" conditions, which are defined as an imbalance of oxidants over antioxidants. This imbalance may ultimately lead to various diseases, such as like mutagenic changes, cell aging, cardiovascular, cancer, and neurodegenerative diseases (Özyürek et al., 2011).

Antioxidants are classified into three categories: Blocking antioxidants, hunting antioxidants, and replenishing novo antioxidants.They all perform essential roles throughout the body's protection mechanism (Niki, 2010). The preventing antioxidants, which inhibit the manufacture of responsive oxygen and nitrogen species (ROS/RNS), is the first line of protection. Active organisms are easily removed by scavenging antioxidants, therefore they can harm biologically important molecules. The antioxidant activity of a biological substance can be measured using many methods. The ABTS+ and DPPH radicals are the most often used radicals. DPPH is a free radical that can be obtained without any preparation, whereas ABTS+ requires enzymatic or chemical reactions (Brand-Williams et al., 1995; Miller and Rice-Evans, 1997).

1.3. Plants Used in This Study

1.3.1. *Cupressus sempervirens* L. var *pyramidalis*

This tree, also known as the Italian cypress, is found in most areas of the Mediterranean basin and the Middle East. It is an evergreen conical tree with 20-30 meters height, and grows in gravel and clay soils that are fertile and have good drainage. These trees are conical upright, having dense dark green-gray leaves of small size, the stem stands, and their branches dense upright (Tümen, 2012). The other features of this tree include the striped foreskin turning gray when the tree is ripeand, and small reproduct, as well as, conical fruits. This tree also reproduces seeds and branches, it endures heat and drought, and responds well to summer watering every month or two (Giovanelli et al, 2007). In the past, these trees were of great importance in people's lives. They are attractive samples in official gardens for their straight stems. They use high walls or windbreaks and are planted on the sides of streets and highways. Excess irrigation and fertilizers may disturb the coordinated conical shape. The appropriate location for this tree should be chosen as it grows enormously (Brofas, 2006).

Figure 1.2. *C.sempervirens L.* var *pyramidalis* (Z. Baqi Taha, 2022)

1.3.2. *Cupressus sempervirens* L. var *horizontalis*

It is one of the evergreen trees. It is distinguished by its branches which grow upward, and this species was produced by a boom. This particular tree is recommended to grow at moderate temperature environments, preferably at high mountainous areas, knowing that cypress trees have a great capability to adapt to the surrounding situations. It is preferable to grow cypress trees using exchangeable soil. Planting this type of trees helps to reduce the problem of soil erosion. Cypress trees are known to be able to withstand drought and lack of water, as they have branched roots that are able to penetrate deep into the soil to reach water.

Figure 1.3. *C.sempervirens L.* var *horizontalis* (Z. Baqi Taha, 2022)

1.3.3. *Cupressus arizonica*

These coniferous trees are evergreen and vary in their forms from conical to round crown when they reach a height of 10-20 m. They are from the wild plants in southwestern America in the state of Arizona. They are found naturally in dry areas and barren rock slopes with poor soil and in the sides of valleys. Leaves are semi-flat, light green to silvery blue. With regard to their branches, they are dense and parallel to the stem. In addition, the modern branches are dark red while the older branches are dark brown. This type of trees tolerate sunny sites, heat and wind. They have an average growth rate, and tolerates drought at the beginning of their cultivation. Moreover, they prefer plowing every month, which is not suitable for soils with high moisture content.

Sometimes they are planted as a windbreak, but this species is not suitable for a shield as its branches are scattered at the beginning of growth. These trees also do not tolerate attacking insects when they are old or under environmental pressures. Leaves are affected when they are planted as an ornamental plant in external locations. They are often planted in forests or as garden plants, as they are suitable as single plants.

Figure 1.4. *C.arizonica* leaves (Z. Baqi Taha, 2022)

1.3.4. *Eucalyptus camaldulensis*

Eucalyptus camaldulensis (E.camaldulensis), which belongs to the Myrtaceae family, is an important ethnomedical plant. This genus has over 700 species and the majority are endemic to Australia. It is widely grown in tropical regions, especially in Asia, Africa and Central America (Brooker, 2000).

Chinese traditional medicine uses *E.camaldulensis* trees to treat a range of illnesses. Hot water extract of dried leaves is used for analgesic, anti-inflammatory and antipyretic treatment of respiratory tract infection symptoms such as flu, cold and sinus congestion. In addition, it is considered to contain bioactive components with antibacterial, antifungal, analgesic, anti-inflammatory and antioxidant properties (Cheng et al., 2009). It is used to treat bacterial infections of the urinary and respiratory systems, as well as sore throats. While volatile oils from the leaves are used as an expectorant, essential oils from the leaves are utilized to treat lung problems. Traditional aboriginal healers have also employed eucalyptus oil-based topical ointments to treat fungal infections and wounds. The active ingredient of eucalyptus oil is eucalyptol (1,8-cineole), which gives it broad pharmacological action. Eucalyptus oil is produced by steam distillation and rectification of fresh leaves (Adeniyi and Ayepola, 2008). Studies have shown that *Eucalyptus* spp. oil and leaf extracts have antifungal and repelling properties. *C.albicans* is claimed to be inhibited by a crude methanolic extract of *E.camaldulensis*. In addition, it has been reported that the ethanol leaf extract of *E.camaldulensis* is highly effective in killing clinical isolates of fungi, such as *Microsporium gypseum* and *Trichophyton mentagrophytes* (Falahati et al., 2005).

1.3.5. *Eucalyptus sideroxylon*

Eucalyptus sideroxylon (E.sideroxylon) is an evergreen, tall, single-stemmed tree with black deeply fissured bark on the trunk and branches. Its leaves are lanceolate and bluish gray on both sides. When the sun hits the leaves, the contrast between the bluish leaves and dark bark is quite striking. The tree can reach a height of 35 meters.

1.4. The Microorganisms Tested in This Study

1.4.1. *Staphylococus aureus*

The micrococcaceae family includes *Staphylococus aureus* (*S.aureus*). The organisms are seen under a microscope as clusters of gram-positive cocci. Due to the gold coloring of the colonies and the good outcomes of the coagulase, mannitol-fermentation, and deoxyribonuclease tests, *S.aureus* is distinguishable from other staphylococcal species (Lowy, 1998). A circular chromosome of around 2800 base pairs makes up the staphylococcal genome, together with transposons, plasmids, and prophages. The chromosome and extrachromosomal components both include genes that control pathogenicity and antibiotic resistance. These genes are spread by the extrachromosomal elements across staphylococcal strains, species, or other gram-positive bacterial species. Microcapsules are produced by most staphylococci (DeLeo et al., 2010). Types 8 and 5 account for 75% of human infections out of the 11 different microcapsular polysaccharide serotype types have been found. Certain structural characteristics are shared by many staphylococcal surface proteins. These characteristics include an N-terminal secretory signal sequence, positively charged amino acids that extend into the cytoplasm, a carboxyl terminus hydrophobic membrane-spanning domain, and a cell wall-anchoring region. Protease, lipase and hyaluronidase can be given as examples of enzymes formed by staphylococci and degrading tissue. Although their significance in the pathophysiology of illness is unclear, these bacterial products may help an infection spread to nearby tissues (Nishimura et al., 2014).

S.aureus is a gram positive, non-motile bacterium. Under the microscope, they appear as irregular balls resembling bunches of grapes. The reason for the term "golden" is that it can completely analyze red blood cells, grow as yellow colonies on blood agar. It is anaerobic optional that it can live in the absence or presence of oxygen (Kobayashi et al., 2015).

S.aureus usually lives naturally on human skin, in the respiratory system or in the nasal cavity. However, it can cause a range of diseases, from minor skin infections (e.g., folliculitis, pimples, boils, cellulitis, impetigo, scalded skin syndrome and abscesses) to life-threatening diseases (i.e., osteomyelitis, pneumonia, and bacteremia (Septicemia)). It is one of the most common causes of hospital-based diseases. *S.aureus* bacterium is an opportunistic pathogen responsible for many purulent infections in animals and humans (Peton and Le Loir, 2014).

S.aureus bacteria produce a number of toxins that distinguish it in food circles under certain conditions, including hemolysin alpha, which breaks down red blood cells in rabbits. In addition, hemolysin beta leads to toxins that lead to the breakdown of red blood and white blood cells in sheep (Argudín et at., 2010).

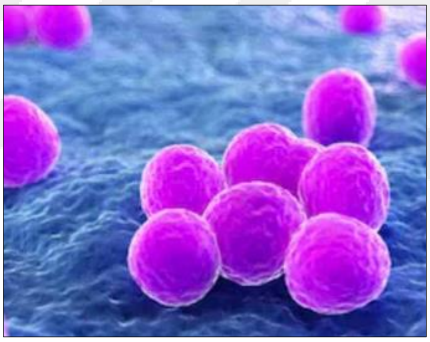

Figure 1.5. *S.aureus* (URL 1, 2022)

1.4.2. *Enterococcus faecalis*

This bacterial species is considered anaerobic and has the ability to grow at temperatures ranging from 10-45 °C with a concentration of 6.5% NaCl and a pH of 9.6 pH. It is able to substitute esculin in the presence of 40% of bile and to produce the enzyme pyrrolidony 1 arylamidase (PYP) which distinguishes it from the bacterial genus *Steptococci*. Its cells are spherical in shape, positive for Gram stain, and are found in binary or in the form of short chains. This bacterial type is not preservative and most of its types are immobile. The Lancefield group D of the Lansfield classification includes all types of *Enterococcal spp* and the bacterium *Sreptococcus bovis*.

This group is naturally present in the human and animal digestive system. It can also be found in the mouth, on the genitals, in the upper respiratory system, especially in genital children, and in the upper respiratory system, especially in infants. However, it is considered an opportunistic bacterium that may cause diseases. Common as urinary tract infections. This bacterial type is sensitive to the antibiotic amoxicillin and may show resistance to the antibiotic penicillin. Some strains show resistance to many antibiotics, including the antibiotic vancomycin called vancomycin resistant *enterococci* (VRE), which causes many serious hospital infections and infections.

Enterococcus faecalis (*E.faecalis*) (formerly known as *Streptococcus faecalis*) is the most important pathogen of this bacterial genus, as it is responsible for about 95% of the infections caused by this bacterial type, including urinary tract infection, cholecystitis, wound inflammation, specifically abdominal wounds and bed sores. It may also have a role in endocarditis and meningitis, which is a natural flora of the vagina and digestive tract. Also, the bacterial type *E.faecium* is responsible for about 10% of infections.

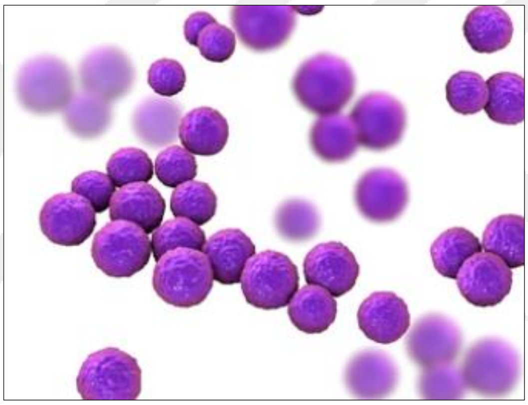

Figure 1.6. *E.faecalis* (URL 2, 2022)

1.4.3. Escherichia coli

Escherichia coli (*E.coli*) is a bacterium that often dwells in the intestines of healthy humans and animals. Most *E.coli* strains are either innocuous or very briefly cause diarrhea. There are a few harmful types, such as the *E.coli* O157:H7 strain, which can result in a severe stomachache, diarrhea, and vomiting (Li et al., 2010).

Warm-blooded creatures' big intestines are frequently home to the bacteria *E.coli*. Although certain *E.coli* strains can result in life-threatening food poisoning, they are moslty benign. It could consume tainted water or infected food, particularly raw or undercooked ground meat and vegetables. Healthy individuals often recover from an *E.coli* O157: H7 infection within a week. On the other hand, children and the elderly are more likely to get hemolytic uremic syndrome, a kind of kidney failure that can be fatal (Kim et al., 2012).

This *E.coli* produces the toxin that is known as the shiga toxin for it is similar to the toxin produced by the bacterium Shigella dysentery. *E.coli*, which produces shiga toxin, can grow at temperatures from 7 to 50 °C, and its optimum growth temperature is 37 °C. Some species of *E.coli* producing shiga toxin can grow in acidic foods with a pH of at most 4.4 and in foods with a water activity of 0.95 as a minimum (Köhler and Dobrindt, 2011).

Abdominal cramps and diarrhea, which in rare cases might turn into bloody diarrhea, are signs of the sickness caused by the Shiga toxin-producing *E.coli* (hemorrhagic colitis). There may also be a fever and vomiting. The incubation phase lasts, on average, three to four days and can last up to eight days. The majority of patients recover within 10 days, however in a small number of them, particularly infants and the elderly. An infection might result in a life-threatening condition, such as hemolytic uremic syndrome. Hemolytic anemia, thrombocytopenia, and acute renal failure are symptoms of hemolytic uremic syndrome (low platelet count) (Vincent et al., 2010).

Due to its easy biochemical differentiation from other E.coli strains, the O157:H7 serotype has been reported to be the subject of the majority of material currently available and the primary reservoir of this pathogen is cattle. Additionally, infection has been seen in other animals (e.g., pigs, horses, rabbits, dogs, and cats), birds, and various ruminants, including sheep, goats, and deer (e.g., chickens and turkeys) (Ferens and Hovde, 2011).

Figure 1.7. *E.coli* (URL 3, 2022)

1.4.4. *Klebsiella pneumoniae*

Klebsiella is a gram-negative bacillus of the intestinal family Enterobacteriaceae, which is common in nature. It has two types of common habitats. The first is environmental, as it spreads in various environments, especially surface waters, and the other habitat is the mucous surfaces of the building. In this respect, *Klebsiella* is similar to *Citrobacter* and *Enterobacter,* however unlike *Shigella* spp or *E.coli* which is common in humans instead of environment (Paczosa and Mecsas, 2016).

K.pneumonia was first described by the German microbiologist and pathologist, Karl Friedlander in 1882. Also known as Friedlander bacillus, *K.pneumonia* is the pathogen in the human respiratory system that causes pneumonia (Navon-Venezia et al., 2017). Disease except in patients with underlying medical problems such as chronic lung disease and alcoholism often arises as a nosocomial infection (David, et al., 2019).

While some *Klebsiella* infections can be effectively treated with a single agent containing penicillin or a similar antibiotic, the emergence of organisms resistant to these drugs requires the development of new therapeutic approaches. The resistance of *K.pneumoniae* to beta-lactam antibiotics (e.g., penicillins, carbapenems and cephalosporins) can be given as an example. Drug-resistant *K.pneumonia* usually requires combination treatment with structurally diverse agents, an antibiotic such as beta-lactam and an aminoglycoside (Bengoechea and Sa Pessoa, 2019).

Community acquired pneumonia occurs if an individual contracts it in a community environment, such as a mall or subway. Hospital-acquired pneumonia may occur if the patient becomes infected at a hospital or in a nursing home. In rare cases, *K.pneumonia* can cause bacterial meningitis, or inflammation of the membranes that cover the brain and spinal cord. It occurs when bacteria infect the fluid around the brain and spinal cord. Most cases of *K.pneumonia* meningitis occur at hospitals (Lee et al., 2017).

Figure 1.8. *K.pneumoniae* (URL 4, 2022)

1.4.5. *Aspergillus niger*

Aspergillus is one of the groups of fungi. It is found in all parts of the world, especially in the fall and winter seasons in the northern hemisphere. A filamentous fungus as one of the types of fungi, except a few types of mushrooms, can cause diseases in animals and humans. If an individual has natural immunity against the fungus, *Aspergillus* does not cause diseases in humans. However, when a person becomes ill from *Aspergillus*, the disease takes many forms (Cairns et al., 2018).

Aspergillus spp. this genus includes about 120 species, the most important or most famous of which is A. niger. This fungus is widespread due to the huge quantities of spores produced by asexual reproduction by conidia carried on unbranched carriers that arise from a cell called Foot cell (Espinel-Ingroff et al., 2017). The mycelium is dense and divided by barriers. The carrier ends with a vesicle bubble, which is a bulging structure surrounded by one or two rows of Sterigmata, the first is short and the second is bottle-shape (Rivero-Menendez et al., 2016).

Aspergillus spp. usually spend a life cycle in the asexual phase and some species reproduce sexually and form fruiting structures of Cleistothecium which is called Eurotium. The vesicle is spherical, the tails consist of two rows like a bottle. The first row is short and the second row is branched. The colonies are black in color (Bongomin et al., 2018).

Aspergillus does not commonly cause human and animal diseases unless a large amount of spores are inhaled and thus causes lung problems called aspergillosis. This disease is particularly prevalent among orchard workers, as they are exposed to inhalation of peat dust, which is rich in *Aspergillus* spores. It is one of the most important causes of otomycosis which is associated with pain including injury to the auditory canal and the helix membrane (Seyedmousavi et al., 2015).

Figure 1.9. *A.niger* (URL5, 2022)

1.4.6. *Candida albicans*

Candida, a yeast fungus, causes the most common infections among fungi. There are about 20 different types of Candida, with the most common type in humans being *C.albicans*. This type of fungus lives on the whole body, especially in hot and humid areas

(Nobile and Johnson, 2015). The fungi, in different cases and under special conditions, will multiply very large and cause inflammation. The oral cavity, vagina, skin and diaper rash in babies are instances of the parts most likely to be infected with candida fungus. In rare cases, the ovaries penetrate the body and enter the bloodstream. Thus, this leads to general pollution. This situation is dangerous, as about 45% of cases lead to death (Kashem et al., 2015).

Infection can infect numerous body organs, including the kidneys, brain, eyes, heart, liver, spleen, and intestines, in persons with weakened immune systems, such as those who take steroidal medications, patients with specific forms of cancer, or AIDS patients. About 15% of persons with compromised immune systems get this illness (Bennett, 2015).

Using specific antibiotics, it results *Candida* to spread easily for fewer competitors for the nutrients in the body. The use of a catheter or intravenous injection is another factors for the fast spread of these fungi into the body. With regard to *Candida* that affects the intestine, the possibility of its infection can be identified through symptoms, such as inflammation and pain in the abdominal area. Poor eating habits indicated by consuming sugar and products that contain white flour fasten the growth and reproduction of *Candida* (Prasad, 2017).

There are different forms of treatments for fungal infections depending on the infection type and the severity level. However, the treatments depend mainly on anti-fungal drugs. Oral infections react well to topical antifungals (e.g., clotrimazole and nystatin) and systemic antifungals (e.g., fluconazole and itraconazole), which may be used if the inflammation also involves the pharynx, especially if it is not cured using the topical medications (Prasad, 2017).

Figure 1.10. *C.albicans* (URL 6, 2022)

2. LITERATURE REVIEW

2.1. *Cupressus* spp.

The cypress plant is considered one of the most important plants with cosmetic uses. The oil is extracted through steam distillation from the virgin branches. Mercenine, terpineolene and linalool, alpha terpinene, alpha pinene, beta pinene, carene, bornyl acetate, cedrol, camphene, sabinene, cadinene, terpinolene, myrcene and linalool, which enhance the health and medicinal properties of the plant as it astringes the body tissues, treat varicose veins and hemorrhoids, and serve to reduce bleeding. It limits the secretion of perspiration, functions to heal breast swelling, and calms the body due to its refreshing properties (Okino et al., 2008).

Cypress oil helps to strengthen the gums and prevents permanent gum bleeding. It tightens the muscles in all parts of the body. It also prevents hair loss, as it strengthens the hair follicles. The antiseptic properties of the oil make it the first in cleansing wounds, infections, and bruises. The oil is used in creams and cosmetics manufacturing, and it is also useful for treating burns caused by exposure to sunlight. The oil is applied daily to the skin to unify the colour due to the presence of the active substance camphene (Shaheen et al., 2020).

It is useful to treat all types of cramps with oils. It dries out the respiratory system spasms, stomach and intestinal cramps, muscle spasms, and spasms. Once the oil is massaged on the affected area, the active substances function to reduce the severity of spasm. One of the important properties of the oil is that it increases the process of urination, thus the body gets rid of toxins, fat and fluids. Thus, excess weight is lost easily and safely, by stopping the gases produced by the intestine, the swelling of the body decreases, the kidneys are cleaned and high blood pressure decreases (Price, 2003). The oil also prevents blood circulation and thus results in blood clots due to the astringent properties that contract the blood vessels. Oil increases the efficiency of lung and prevents accummulation of cough and phlegm in the respiratory system. It also treats congestion, which makes breathing easier, especially for those with colds and influenza. Sudorific is the substance that causes sweating. It works to clean the pores of the skin, increasse the secretion of sebum and expels toxins from the skin. Oil is not recommended to be used by those with skin disease, as it increases the secretion of the sebaceous glands and thus acne increases. Another benefit of oil is it is used ti treat anti-inflammatory, rheumatic, anti-arthritis. However, oil should be avoided during pregnancy (Sueng et al., 2015).

Infectious viruses or germs are often the cause for cold, rhinolaryngitis, bronchitis, and other infections that affect the ear, nose and throat. Since antibiotics do not cure these diseases, the most appropriate solution is to ensure complete comfort for the patient. This is to make sure this stage passes smoothly. Here comes the role of the cypress tree, which is resistant to various types of respiratory infections. The female cypress cones contain acidic squamous seeds that are firstly green in color and then turns brown when ripe, as they contain polyphenolic antiviral compounds including flavonoids, phenolic acid and tannins in particular (Leigh-de Rapper et al., 2020).

Certain concentrated tannins, known as proanthocyanidols, prevent viruses from attaching to the cells, and penetrating them as well as their reproduction. Cypress essential oil also disrupts the membranes that protect germs, thus weakening immune defenses. As for the best method of use, it is to resort to the ready-made and approved drugs in pharmacies (note that the dosages differ between adults and children, which requires consulting the doctor or pharmacist). Cypress essential oil can also be used with Echinacea to further stimulate the immune defenses (Vasey et al., 2018).

Cypress essential oil is known to be an anti-cough and expectorant. These are two complementary effects that contribute to sterilization of the ear, nose, and throat area. Therefore, cypress essential oil helps to lighten the respiratory airways through its containment of monoterpene, sesquiterpene and other diterpenoid acids. The best way to use it is to dissolve a drop of cypress essential oil in a spoon full of honey and drink this mixture two to three times a day. Two thousand years ago the Greeks treated asthma and hemorrhagic cough with cypress extract. Cypress essential oil contains mono terpenes that help to calm bronchospasms and soothe a tired cough. Hafsin supplements the action of this mono terpene by preventing the bleeding of damaged blood vessels in the bronchi. It also helps to get rid of bronchospasms, especially if a person has Broncopneumopathy. As for the best method of use, it is by rubbing cypress essential oil on the chest or mixing it with a conical tree oil, such as pine nuts to enhance its effect. This treatment can be applied to both adults and children (Al-Snafi, 2018).

One of the benefits of cypress is the acceleration of wound healing. It is one of the benefits of cypress tree, as camphene in the volatile oils, extracted from cypress helps to heal external as well as internal wounds. Camphene helps prevent infections. Volatile oils extracted from the cypress tree has antimicrobial properties that help to prevent the growth of bacteria. Studies also indicate that the oils extracted from the cypress tree can be used in the manufacturing of soaps and other cosmetic materials, due to their ability to kill bacteria and treat ulcers (Dhar and Dhar, 2019).

The other benefit of cypress is that the extracted volatile oils facilitate the exit of toxic substances from the body. These oils increase sweating, which helps the body to get rid of toxic substances, salts and excess fluids quickly. The volatile oils extracted from cypress play a role in cleaning the liver and naturally reducing cholesterol levels in the body (Valnet, 2015).

S.aureus (ATCC6538), *Bacillus subtillis* (ATCC6633), *P.aeruginosa* (ATCC6643), *E.coli* (ATCC15224), *K.pneumonia* (MTCC618), and *S.typhimurium* were tested for the antibacterial activity of methanol, ethanol, and ethyl acetate extracts of the aerial sections of *C.sempervirens* (ATCC13048). Eight different concentrations (1, 2, 3, 5, 7.5, 10, 12.5 and 15 mg/ml) of 48 extracts were tested. To all of the studied bacteria, all *C.sempervirens* extracts inhibited the bacterial growth (Boukhris et al., 2012). *B.subtilis*, *Proteus vulgaris*, *S.aureus* (Gram-positive), *E.coli*, *Pseudomonas aeruginosa*, and Salmonella typhi were the six bacterial strains tested for the antibacterial properties of water and chloroform extracts of *C.sempervirens* (Gram-negative). The zone of inhibition for *C.sempervirens*' activity in contradiction of Gram positive bacteria was large (9-14 mm for water extract and 9-12 mm for chloroform extract), while the zone of inhibition for Gram negative bacteria was low (1-6 mm for water extract and 1-5 mm for chloroform extract) (Abdulaziz et al., 2012).

Ethyl acetate, ethanolic and methanolic extracts of leaves part of *C.sempervirens* were assessed in the antibacterial activity of against six bacteria (*K.pneumoniae, Bacillus subtilis, S.aureus, Pseudomonas aeruginosa, E.coli and Salmonella typhimurium*) expending agar disk diffusion method. A significant antimicrobial effect was obtained with the methanolic extract. This was followed by ethylacetate and ethanol extracts (EL-Kamali and EL-Amir, 2010). The methanolic extract showed maximum inhibitory activity against *S.aureus, K.pneumonia* and *B.subtilis*. The ethanolic extract showed good antimicrobial activity against *P.aeruginosa*. Greater inhibitory activity in contradiction of *E.coli and S.typhimurium* was possessed by ethyl acetate extract of *C.sempervirens* (Rguez et al., 2019).

Essential oil showed moderate *in vitro* antimicrobial activity against bacteria including Gram positive (*Serratia marcescens, S.aureus, Bacillus cereus*, *E.feacalis*), and Gram negative (*K.pneumonia, Proteus vulgaris, Salmonella indica, Aeromonas hydrophila*, *E.coli, Pseudomonas aeruginosa*) with diameter zones of inhibition 4 to 12 mm, with MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values ranging from 62.5 to 250 µg/ml. *C.sempervirens* methanol extract effectively inhibited the growth of all tested bacteria (Silva et al., 2021).

S.aureus, E.coli, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis, Halomonas elongate, and *Enterococcus hirae* were examined for *C.sempervirens* essential oil's antimicrobial activity. According to the results, *C.sempervirens* oil inhibited resistant bacteria from growing. The MIC and MCC values showed the effectiveness of *C.sempervirens* essential oil. The essential oil's bactericidal activity was further supported by the MIC/MCC ratio. However, the essential oils of *C.sempervirens* have higher antibacterial effects against Gram-positive than Gram-negative bacteria (Khadri et al., 2010).

The inhibition zone 2 and 4 µl/disc of essential oil of *C.sempervirens* against the established microorganisms were respectively: *S.aureus* 7-8mm; *Micrococcus luteus* 10-13mm, *Yersinia enterolitica* 8-9mm; *Pseudomonas pyocyaneus* 9-11mm; *Mycobacterium simegmatis* 10-11mm; *Aeoromonas hydrophila* 7-10mm; *E.faecalis* 7-9mm; *Bacillus megaterium* 7-9mm; *S.faecalis* 7-9mm and *Bacillus brevis* 7mm- 8mm (Al-Snafi, 2018).

Three strains of *Micrococcus luteus, Bifidobacterium lactis* and *E.coli* were tested by using essential oil of *C.sempervirens*. After 96-hours incubation against *E.coli,* the inhibition zones for essential oils were 16.11 mm, 11.90 mm for *Micrococcus luteus*, and 24.05 mm for *Bifidobacterium lactis*. Diterpenes from *C.sempervirens* cones (fruits), 6-deoxytaxodione (11-hydroxy-7, 9(11), 13-abietatrien-12-one) and taxodione showed high antibacterial activity (IC50 values of 0.80 and 0.85 g/ml) against methicillin-resistant *S.aureus* (Al-Snafi, 2016).

S.aureus ATCC 25923, *S.aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *E.coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *K.pneumoniae* RSKK574, and *C.albicans* ATCC 10231 were examined against the extracts from *C.sempervirens* var. *pyramidalis*. Compared to the standards, the extracts showed weak antibacterial activity against some bacteria (Eryılmaz et al., 2016).

The powdered cones (fruits) of *C.sempervirens* L. var. *horizontalis* (Mill.), collected from Oxford, Mississippi in 2010 in the fall, showed significant antibacterial and antiparasitic effects in an ethanol extract. Six-deoxytaxodione (11-hydroxy-7, 9(11), 13-abietatrien-12-one) and taxodione, along by ferruginol (3) and sugiol (2), were obtained using bioassay-guided fractionation utilising a centrifugal preparative tinny-layer chromatography. Mixtures 1-2 displayed effective antileishmanial activity by half-maximal inhibitory concentration (IC50) values of 0.077 µg/mL - 0.025 µg/mL, respectively, in contradiction of Leishmania donovani promastigotes, related to the standard antileishmanial medicines, pentamidine (IC50 1.62 µg/mL) and amphotericin B (IC50 0.11 µg/mL). In THP1 macrophage cells, compounds 1-3 were also examined against *L. donovani* amastigotes (Zhang et al., 2012).

Commercially obtainable oils of cypress (*C.sempervirens* L.) was estimated for their antimicrobial activity. Evaluation of the oils by GC-FID and GC/MS showed that the major residents of these oils was α-pinene (31.1%) then δ-3-carene (18.6%) in cypress oil. The antimicrobial motion of the essential oils was estimated on existence and growth of several microorganisms hypothetically dangerous to the care of foods (*E.coli, Listeria innocua, S.aureus*). Antimicrobial tests were carried out in solid and liquid media. *E.coli* was the best sensitive organism to the inhibition influence of the oils (Romeo et al., 2008).

Through spectrometric analysis and the Folin-Ciocalteu method, the amounts phenolics in the extracts of several medicinal plants was estimated as Gallic acid equivalents (GAE). Determined by 2, 2-diphenyl-1, 1-picrylhydrazyl (DPPH) and reducing antioxidant power (FRAP). The results of TPC of plant extracts were ranged between 62.16- 261.8 mg/g. The highest amount was in *C.sempervirens* cones (261.8 mg /g) followed by *Punica granatum* peels (243 mg /g). It can be concluded that *Punica granatum* peels possess significant antioxidant activity (Al-Qaraleh and Tarawneh, 2016).

For a variety of conditions, including viral illnesses, herbal preparations have been employed as traditional treatments. Since antiviral medications are becoming less effective at treating viral infections. Ethanol extracts of *C.semipervirens*, *C.semipervirens* cv. *cereiformis* and *C.semipervirens* var. *horizontalis* were used to determine their impact on herpes viruses (HSV-1). The HSV-1 virus is resistant to all three plants' antiviral properties. The extract of *C.semipervirens* was the most effective. The fruit extract has the highest anti-HSV activity (Emami et al., 2009).

The survival and growth of various bacteria possibly harmful to the safety of foods were investigated for the antimicrobial activity of *C.sempervirens L.* essential oil (*E.coli*, *S.aureus* and *Listeria innocua*). Both solid and liquid media were used for the antibacterial tests. The organism studied that was most sensitive to the oils' inhibitory effect was *E.coli* (Nogueira et al., 2017).

In a different investigation, the cypress cones were divided into two portions and extracted with ethanol and hexane, respectively. The stain was then enhanced with acetone or water. Chemical reactions were used to conduct the phytochemical investigation. The well-spread agar way was used to determine the antibacterial properties of cone extracts. The presence of tannin, glycoside, alkaloid, terpenes, and flavonoids were discovered through phytochemical examination of an ethanolic extract of a *C.sempervirens* cone. The cone extracts also depict the antibacterial and oxidative activities compared to ciprofloxacin and acid. The ethanolic extract produced the best results in both situations in comparison with ascorbic acid. Further research is required to understand the mechanisms underlying the antibacterial and antioxidant activities of various cone extracts from *C.sempervirens*, which is crucial in the treatment of many disorders (Eldalawy et al., 2020).

The antimicrobial activity of extracts of some types of forest trees (*Olive europea*, *C.sempervirens*, *Thuja oreintalis*) in different culture medium concentrations (1.0, 0.5, and 0.25 g/100 ml) against two fungi pathogens of (*Rhizopus stolonifer* and *A.niger*) was investigated. The test showed the presence of the inhibitory effectiveness of the extracts against the fungi used, as it reached the lowest rate of fungal growth when using concentration of 5.0 g / 100 ml cultivated medium for each of the extract, leaf of green olive, green cypress, and thuya, respectively (2.2, 1.2, and 3.2 cm) with a rate of inhibition of fungal growth of 8.72, 4.73 and 3.69% against fungi (Al-zaidbaqy and Hmawndi, 2019).

Commonly known as Mediterranean cypress or Italian cypress, *C.sempervirens* grows naturally in the Eastern Mediterranean regions, North America and subtropical Asia. *C.sempervirens* have antibacterial, aromatherapeutic, acerbic, balsamic or anti-inflammatory, antispasmodic, astringent, antiseptic, deodorizer, and diuretic actions, according to a literature review on its pharmacology. According to a literature review on chemistry, this species contains monoterpenes, diterpenes, flavonoid glycosides, and flavonoid compounds (Hussain et al., 2019).

It has been reported in the literature that *C.sempervirens* contains a wide variety of chemicals. Polyphenols, flavonoids, and flavone glycosides were discovered in *C.sempervirens* by chemical analyses. This plant has also been linked to the bioflavonoids amentoflavone, cupressoflavone, 4′′-mono-Omethyl amentoflavone, hinokiflavone, isocryptomerin, and podocarpusflavone. According to various investigations, volatile monoterpenes and sesquiterpenes were the primary constituents of the essential oils from fruits and cones (Rawat, 2010).

The essential oils of *C.sempervirens* L. cv. *cereiformis*, a plant occurring in Iran, were extracted from garden-fresh fruits and lethal branchlets with close leaf. The essential oils were found to have thirteen components. α-pinene, α-terpenyl acetate, Δ-3-Carene and terpinolene were the primary components of both fruits and leaves (Miloš et al., 1998).

Cold and hot ethyl acetate extracts were used to separate the glycosides from fresh cones of *C.sempervirens*. 18 aglycones were liberated during enzymatic hydrolysis using βglucosidase. Volatile substances that were glycosidically bound made up 7-8 mg/kg. The main aglycones were 3-hydroxybenzoic acid methyl ester (15.5%) and thymoquinone (5-isopropyl-2-methyl-1,4-benzoquinone: 3.7-9.7%). Other important aglycones were perilla alcohol (3.6-8.2%), p-cymen-8-ol (5.3-6.4%), 2-phenylethanol (2.7-6.9%) and carvacrol (2.5-6.3%). There was no similarity between the glycosidically bound aglycones and the corresponding free compounds found in the essential oil (Krishnaveni et al., 2013).

The antibacterial activity of the ethanol, methanol and ethyl acetate extracts of the aerial parts of *C.sempervirens* were studied against *S.aureus* (ATCC6538), *B.subtilis* (ATCC6633), *P.aeruginosa* (ATCC6643), *E.coli* (ATCC15224), *K.pneumonia* (MTCC618) and *S.typhimurium* (ATCC13048). There were eight different concentrations of the extracts (1, 2, 3, 5, 7.5, 10, 12.5 and 15 mg/ml). Among all of the studied bacteria, totally *C.sempervirens* extracts caused amount-dependent bacterial growth inhibition (Asiaei, 2019).

The gram positive bacteria (*B.subtillis, P.vulgaris, S.aureus, E.coli, P.aeruginosa, S.typhi*), and the fungi (*A.niger* and *C.albicans*) have been tested against the antibacterial and antifungal properties of chloroform. Water extracts of *C.sempervirens* demonstrated higher activity against gram positive bacteria of inhibition zones 9-14 mm for water extract and 9-12 mm for chloroform extract), while less activity against gram negative bacteria inhibition zones 1-5 mm for chloroform extract and 1-6 mm for water extract was observed. Water extract, on the other hand, had no effect on fungus, whereas chloroform extract had a negligible impact on *C.albicans* (3mm) (Rguez et al., 2018).

The early phytochemical investigation revealed *C.sempervirens* included a high concentration of biologically active compounds, including tannin (0.31%), phenols (0.067%), flavonoids (0.22%), alkaloids (0.7%), saponins (1.9%), and essential oils. Earlier pharmacological research showed that *C.sempervirens* had a wide range of beneficial effects, including those that were antifungal, antibacterial, antiparasitic, antiviral, insecticidal, wound-healing, anticancer, anticoagulant, and estrogenic. In a research paper, the pharmacological significance of *C.sempervirens* spp. and its chemical components were demonstrated. The antimicrobial activity of *C.sempervirens* essential oil was studied against ten bacteria and fungi (*E.coli, S.aureus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimurium, Halomonas elongate, Enterococcus hirae, C.albicans, A.niger, and Trichoderma reesei*). The results revealed that the oil of *C.sempervirens* inhibited the growth of susceptible bacteria, filamentous fungi and yeasts. The MIC and MCC values demonstrated the great efficacy of *C.sempervirens* essential oil. The essential oil's bactericidal and fungicidal efficacy was further supported by the MIC-MCC ratio. However, the essential oils from *C.sempervirens* have stronger antibacterial effects against Gram-positive than Gram-negative bacteria (Al-Snafi, 2016).

By using agar well disc diffusion, the antimicrobial effect of leaves extract from *C.sempervirens* L. against six bacteria, including *Pseudomonas aeruginosa*, *S.aureus*, *B.subtilis*, *E.coli*, *Salmonella typhimurium,* and *K.pneumoniae* was assessed. Methanolic extracts from plants had the strongest antibacterial activity among some of the plant extracts, following by ethyl acetate and ethanol extracts. Maximum inhibitory activity was depicted by the methanol extracts against *K.pneumoniae*, *B.subtilis*, and *S.aureus*. The ethanolic extract had greater anti-*P.aeruginosa* activity. The ethyl acetate extract of *C.sempervirens* has stronger inhibitory activity against *S.typhimurium* and *E.coli*. The results showed that the examined bacterial strains were more inhibited by *C.sempervirens*, which can be used to treat pathogenic diseases and enhance crop yields (Chaudhary et al., 2012).

2.2. *Eucalpytus ssp.*

Over 300 of *Eucalyptus* leaves that are commercially exploited to produce volatile essential oils for both the pharmaceutical and cosmetic industries. Despite being widely planted to produce the pulp, plywood, and solid wood, eucalyptus has a surprising range of biological properties, including antioxidant, antimicrobial, antiseptic, chemotherapeutic, wound healing, respiratory and gastrointestinal disorder treatment, insecticidal or insect repellent, antitumor, acaricidal, perfumes, antimalarial, as well as used in soap making and fats removal (Dhakad et al., 2018).

E.camaldulensis trees could be a potential source of antimicrobial components since they inherently possess defense mechanisms and antimicrobial agents in the form of androgens due to their unique nature. Alkaloids, phenolics, flavonoids, terpenes, pigments, starches, corticosteroids, and essential oils make up the majority of these plants' active components. Their antimicrobial properties against fungal, parasitic, bacterial, and viral pathogens have been demonstrated (Ghasemian et al., 2019).

GLC-MS was used to analyze qualitatively and statistically volatile oil contents of *E.camaldulensis* var. *brevirostris* leaf produced by hydrodistillation (HD) and supercritical fluid extraction methods (SFE). Ninety different components were determined, as well as the majority of them had been identified. The primary components of both extracts discovered included β-phellandrene (8.94, 4.09%), p-cymene (24.01, 10.61%), cryptone (12.71, 9.82%), and spathulenol (14.43, 13.14%). In comparison to SFE extract (0.242 g/100 g fresh leaves), the yield of monoterpene hydrocarbons in HD oil (0.288 g/100 g fresh leaves) was marginally greater. Terpenoids, light oxygenated compounds, and heavy oxygenated mixture were all present in larger amounts in the SFE extract than in the HD oil. The relationship between the antioxidant activity and chemical composition of the extracted oils was investigated (Fadel et al., 1999).

The leaf of *E.camaldulensis* is frequently used in traditional medicine for treating a variety of illnesses. In a research, three distinct solvents (methanol, chloroform, and hexane) extracts of *E.camaldulensis* leaves were investigated for their chemical composition and biological (in vitro antioxidant and anticancer) efficiencies. By using the colorimetric tests, the total phenolic and total flavonoid contents of each extract were investigated. By using the free radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) assays, antioxidant activity and total antioxidant levels of various extracts was measured. The anticancer potential of each extract was calculated using the potatoes disc assay. The results of eucalyptol was discovered to be a significant component of the methanol (31.86%) and chloroform (45.94%) extracts using GC/MS analysis. Hexane extract was primarily composed of palmitic acid (31.06%) (Ashraf et al., 2015).

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques were used to determine the chemical content of the essential oils extracted from the leaves and fruits of *E.camaldulensis* growing in Mersin, Turkey. The disc diffusion method was used to assess the biological activity (e.g., antibacterial and/or antifungal). The main leaf oil constituents were *p*-cymene (42.1%), eucalyptol (1,8-cineole) (14.1%), α-pinene (12.7%), and α-terpinol (10.7%). The main constituents of the fruit oil were eucalyptol (1,8-cineole) (34.5%), *p*-cymene (30.0%), α-terpinol (15.1%), and α-pinene (9.0%). The results indicated that both types of oils are abundant in monoterpene hydrocarbons and oxygenated monoterpenes. Additionally, against *Candida tropicalis* and *C.globrata*, the oils showed fungicidal activity. Due to their higher p-cymene concentration, leaves extract essential oils demonstrated more activity than fruit essential oils (Doğan et al., 2017).

E.camaldulensis essential oils and plant extracts have been extensively studied, as the plant's traditional medical use suggests it has strong antibacterial capabilities. *E.camaldulensis* essential oil has antibacterial activity against a variety of Gram positive (0.07–1.1%) and Gram negative bacteria (0.01–3.2%). *E.camaldulensis* essential oil and leaves extract are more effective against bacteria in comparison with the other species belonging to the genus Eucalyptus. The antibacterial effect is confirmed for bark and leaf extracts (conc. from 0.08 g/mL to 200 mg/mL), with significant variations according to extraction procedure. *E.camaldulensis* essential oil has a sensitivity range of 0.125-1.0% for the majority of fungal model organisms. The extracts are effective against a number of dermatophytes, including *C.albicans* (0.2-200 mg/mL leaf extracts and 0.5 mg/mL bark extracts) (Sabo and Knezevic, 2019; Abu-Jafar and Huleihel, 2017).

The antibacterial activity of *E.camaldulensis* was examined using the disc diffusion method, and the antifungal activity was determined by computing the percentage decrease in mycelia's radial growth. The %inhibition by *E.camaldulensis* crude extracts varied significantly ($p \leq 0.01$). Acinetobacter baumannii's growth was significantly suppressed by the capsule's crude extract (30% acetone) (35 mm clear zone). Rhizopus stolonifer had the strongest antifungal activity, with a 96% reduction in radial growth. The bacterial MICs against *E.coli* and *Bacillus subtilis* ranged from 20 to 0.5 mg/mL, respectively. For fungi, the MIC ranged from 18 mg/mL (Mucor sp.) to 4 mg/mL. The antibacterial abilities of *E.camaldulensis* were significantly influenced by the solvent's type and concentration (Nasr et al., 2019; Yugandhar et al., 2017).

Biofilm-producing microorganisms are responsible for many problems in industry and medicine. In a research the antimicrobial influence of *E.camaldulensis* alcoholic extracts against planktonic form and biofilm of six bacteria, namely *S.aureus, B.cereus, Streptococcus pneumoniae, Pseudomonas aeruginosa, E.coli* and *K.pneumoniae*. The results confirmed the strong ability of *E.camaldulensis* extracts against the biofilm of tested bacteria and their free-living forms. *E.camaldulensis* methanol extract at concentration 20 mg/ml was the most efficient in the inhibition of biofilm formation of *S.aureus* (84.42%). These extracts had the ability to remove more than 50% of stabilized biofilms. In *K.pneumonia*, however, only 29.20% eradication of biofilms was observed. The highest decrease in methabolic activity was observed in *Streptococcus pneumoniae* biofilms (82.13%) treated with 20 mg/ml ethanolic extract. The antimicrobial activity of *E.camaldulensis* extracts against the selected microorganisms was demonstrated. Thus, these extracts are recommended against the selected isolates (Mohsenipour et al., 2015; Abubakar, 2010).

In a research, the antifungal activity of *E.camaldulensis* Dehnhessential's oil (EO) was assessed against five *Fusarium* spp. that are frequently found in maize. Using the disc diffusion method, the antifungal efficacy of various EO concentrations was assessed. 1,8-cineole (16.2%), α-pinene (15.6%), α-phellandrene (10.0%), and p-cymene (8.1%) have been discovered the most prevalent substances found in the EO. After five days of incubation, the EO completely inhibited mycelial development in all test pathogens at a dosage of 7-8 L/mL. On the test fungi, the EO's low inhibitory concentration and low fungicidal concentration fell between 7-8 μL/mL and 8-10 μL/mL, respectively. These results support the fungicidal abilities of *E.camaldulensis* essential oils, their prospective application in the control of economically significant *Fusarium* species, and their possibility as synthetic antifungal alternatives (Gakuubi et al., 2017).

E.camaldulensis leaf extracts from the Caspian shoreline and the Ghalegardan regions in the Mazandaran province of Iran and dried to analyze the chemical structure of the essential oil and its antibacterial properties. By hydro-distillation, the essential oils were separated, and GC-MS was used to study them. The essential oil of Ghalegardan sample had greater oxygenate to hydrocarbon compound ratios (MO to MH and SO to SH), according to the results. 37 chemicals were found in the Ghalegardan sample. Eucalyptol (29.2%), α-phellanderene (17.43%), α-pinene (7.1%), arommadenderene(allo) (5.75%), and terpineol-4-ol (4.92%) were the main ingredients in Ghalegardan. 36 chemicals were found in Seashore samples. Eucalyptol (46.74%), arommadenderene(allo) (12.1%), terpineol-4-ol (7.6%), and α-pinene (6.35%) were the main ingredients in Seashore. The results of the antibacterial experiment indicated that the essential oils of both samples had antibacterial activity, and they were more potent against *S.aureus* than *E.coli*. In addition, the antibacterial activity of Ghalegardan sample is slightly higher than Seashore sample. In conclusion, the antibacterial activity of the *E.camaldulensis* essential oils suggested its clinically useful potentials, although more research need to be carried out (Miranda et al., 2013; Ghalem and Mohamed, 2014).

To investigate the essential oil from *E.globulus* leaves' *in vitro* antibacterial properties, the hydrodistillation process was used to extract the essential oils from this plant. Using agar disc diffusion and dilution broth techniques, the inhibitory properties of this essential oil were examined against *E.coli* and *S.aureus*. The findings demonstrated that essential oil from *E.globulus* leaf had the antibacterial activity against both gram positive and gram negative microorganisms, including *E.coli* and *S.aureus*. The findings suggest that the essential oil of *E.globulus* leaves may be utilized as a natural antibiotic for the treatment of a number of infectious disorders caused by these two pathogens and may also be helpful in understanding the relations between traditional cures and current medicines (Bachir and Benali, 2012).

Essential oils (EOs) are odoriferous and volatile substances produced by numerous plant species. These mixtures have been used by humans for a variety of reasons, including medications, agrochemicals, aromatherapy, and food flavorants, and they play significant functions in nature. More than 3000 EOs have been described in the literature, with about 300 of them being used commercially, including EOs from *Eucalyptus* species. The majority of the EOs from Eucalyptus species are high in monoterpenes, and many of them have been used in perfumes, agrochemicals, food flavorings, and pharmaceuticals. These usages are connected to their numerous biological and organoleptic characteristics. The most recent data on the chemical composition and biological properties of essential oils from various *Eucalyptus* species were evaluated. Among 900 species and subspecies of *Eucalyptus*, 68 species were examined. Studies on these species were carried out in 27 different nations. The data may help justify the use of essential oils as ingredients for agrochemical and pharmaceutical applications, as well as flavorants and food supplements (Barbosa et al., 2016; Ghaffar et al., 2015).

The use of *Eucalyptus* as a medicinal plant is well known throughout the world. The chemical composition, antibacterial, and antioxidant properties of seven commonly encountered *Eucalyptus* species in South East Asia (Pakistan) were investigated. Hydrodistillation was used to extract EOs from the leaves of *E.citriodora*, *E.crebra*, *E.melanophloia*, *E.tereticornis*, *E.camaldulensis*, *E.globulus*, and *E.microtheca*. The chemical composition of the EOs was determined through GC-MS-FID analysis. Based on the analysis, 31 compounds were detected in *E.citriodora* and *E.melanophloia*, 27 in *E.crebra*, 24 in *E.tereticornis*, 10 in *E.globulus*, 13 in *E.camaldulensis* and 12 compounds in *E.microtheca*. The highest primary components in these EOs were 1,8-cineole (56.5%), α-pinene (31.4%), citrinyl acetate (13.3%), eugenol (11.8%), and terpenen-4-ol (10.2%). *E.citriodora* had the most antimicrobial activity against *S.aureus*, *B.subtilis*, *E.coli*, *A.niger* and *Rhizopus solani*). Gram positive bacteria were discovered to be more sensitive to all EOs than Gram negative bacteria. *E.citriodora* had the highest diphenyl-1-picrylhydazyl (DPPH) radical scavenging activity and percentage suppression of linoleic acid oxidation (82.1% and 83.8%, respectively), whereas *E.camaldulensis* came in second (81.9% and 83.3%, respectively). Eucalyptus essential oils have a wide range of chemical compositions, which highlight their potential use in nutraceutical and medical products (Ghaffar et al., 2015 and Neelam et al., 2014).

Steam distillation was used to extract the EO from the aerial sections of the *E.camaldulensis* Dehnh. plant, which was found growing wildly throughout Sardinia (Italy). The oil was then evaluated using gas chromatography (GC), FID, and GC-ion trap mass spectrometry (ITMS). The EO yields (v/dry wt) varied from 0.2 to 0.5%. To investigate the seasonal chemical variations of the EO, samples were collected between April and

December. Depending on the various origins, the chemical composition changed significantly during the vegetative stage. Thirty-seven compounds, representing at least 97.7% of the total EOs, were identified. The main constituents were 1,8-cineole (4.1-39.5%), p-cymene (27.8-42.7%), β-phellandrene (3.9-23.8%), cryptone (3.2-10.2%), and spathulenol (2.1-15.5%). Cuminal and phellandral, two rare aldehydes, were present in the oils in moderate concentrations (1.4-4.7%). Through the paper disk diffusion method, the essential oils have been tested for their antifungal effects against major phytopathogenic fungi, and they displayed activity at low concentrations against the tested pathogens. The antioxidant activity, assessed by DPPH-test and expressed as trolox equivalent antioxidant capacity, showed values ranging between 0.5 and 5.8 mmol/L (Barra et al., 2010; Siramon and Ohtani 2007).

The essential oil extracted from *E.camaldulensis* plant leaves that were collected from five locations along the Montenegrin coast was analyzed. The oil yield ranged from 0.63% (Kotor) to 1.59% (Tivat). The chemical composition of the essential oil was analyzed according to the GC-MS method. Among them, the main compounds were p-cymene (17.38-28.60%), ß-phellandrene (12.35-14.47%), and ß-pinene (0.94-11.48%). The second largest group was oxygenated monoterpenes with cryptone (4.97-7.25) and terpinene-4-ol (2.75-4.21%) as predominant. Moreover, high content of sesquiterpene alcohol spathulenol (7.83-14.15%) was found (Grbović et al., 2010).

By using the agar dilution method, the *in vitro* microbiological activity of methanolic extracts of *Terminalia catappa* and *E.camaldulensis* leaves was investigated. The phytochemical analysis of the crude extracts of the medicinal plants revealed the presence of saponin, tannins, saponin glycosides, steroid, phenols, cardiac glycoside, volatile oils, and balsam (gum). The methanolic extracts of the two plants inhibited the growth of *B.subtilis* (ATCC 103207) and *S.aureus* (Clinical strain), however had no inhibitory effects on *Pseudomonas aeruginosa*, *Salmonella typhi*, and *E.coli*. *C.albicans* was inhibited by the crude extracts of *E.camaldulensis* only. Accelerated gradient chromatography (AGC) produced fractions of the *T.catappa* extract that were more effective against *C.albicans* and *E.coli* than the crude extract. The extracts' minimum inhibitory concentration (MICs) ranged from 1.25 to 5g/ml. The results revealed that *T.catappa* and *E.camaldulensis* can be utilized to treat illnesses caused by the tested organisms (Babayi et al., 2004).

A plasmodium berghei-induced albino mouse model of malaria infection was investigated, and the bioactive elements of *E.camaldulensis* aqueous leaf extracts and their protective effects on liver and kidney function were investigated. The findings demonstrated an abundance of phytochemicals present in *E.camaldulensis* extracts, including flavonoids, phenols, saponin, terpenes, and tannin. At doses of 200 and 300 mg/kg, *E.camaldulensis* had its best therapeutic benefit. These results support the use of *E.camaldulensis* as an antimalarial remedy in traditional medicine by showing protection against malaria-induced abnormalities in liver and renal function including anti-malarial activities (Anigboro et al., 2020).

Two grades of *E.sideroxylon* leaves were found across this tree's range. The polyphenol aglycones in the leaves of various samples of the normal form of the tree were fairly comparable in nature and quantity, however the glycoside composition was typically regional in nature. The latter chemicals included p-coumarylquinic acid, catechin, epicatechin, shikimic, chlorogenic, and 3-glucoside, 3-rhamnoside, 3-rutinoside, and 3-rhamnoside of quercetin, 3-rhamnoside, and 3-rutinoside of kaempferol, as well as other unexplained products. Large concentrations of stilbenes, chlorogenic and p-coumarylquinic acids, as well as occasionally dihydrokaempferol 3-rhamnoside, were present in leaves of the second class, along with some of the first class' polyphenols. In two regions, trees of both kinds have been seen growing close to one another. The leaves of the second class were taken from the trees that were identified as variant forms. Leucocyanidin and esters of gallic, ellagic, and gentisic acids are present in both forms. A different sample yielded a novel flavone, 5,4′-dihydroxy-7-methoxy-6,8-dimethylflavone, and both forms of the 4′-methoxy derivative were found. While the flavonoid content of many variant forms of leaves fluctuates, the predominant stilbene in leaves of the second class however does not, and it can be 3-glucoside of either 3,4′,5-trihydroxy-, or 3,3′,5-trihydroxy-4′-methoxy- or another hydroxy-stilbene (Hillis and Isoi,1965; Hillis and Hasegawa, 1962).

The essential oil yield was seven times heigher in *E.sideroxylon* than *E.gomphocephala*'s. The quantitative and qualitative difference in essential oil between the two species studied was confirmed by the sensitivity of the different phytopathogenic fungi to the essential oils of *Eucalyptus* which differs from one species to another. In fact, the EO of *E.sideroxylon* showed very potent inhibitory effect against Penicillium and Fusarium. However, the examined strains of phytopathogenic fungi are unaffected by the EO of *E.gomphocephala* (El-Kaoua et al., 2017).

Leaves from eucalyptus trees have long been used to treat wounds and fungus infections. Some Eucalyptus species' essential oils and extracts have antibacterial and anticancer effects. The antibacterial and cytotoxic properties of the extracts and oils from the leaves, flowers and stems of Egyptian-grown *E.torquata* and *E.sideroxylon* plants were investigated. The antibacterial effects of *Eucalyptus* essential oils and extracts against therapeutically significant gram-positive and gram-negative bacteria were examined using the agar diffusion method. Oils and extracts were tested for their *in vitro* cytotoxic effects on human breast adenocarcinoma cell line and human hepatocellular carcinoma cell line (HEPG2) using a sulphorhodamine B assay (MCF7). The oils and extracts from both eucalyptus species were discovered quite effective at killing gram-positive bacteria. Gram-negative bacteria were resistant to extracts, with the exception of *E.coli*, however were vulnerable to the oil extracted from at least one organ of *E.sideroxylon* and *E.torquata*. Although *Aspergillus flavus* and *A.niger* were resistant to the extracts, *C.albicans*, *Aspergillus flavus*, and *A.niger* were all susceptible to the essential oils of *E.sideroxylon* and *E.torquata*. This was folowed by oils of *E.torquata* stems, *E.torquata* leaves, and *E.sideroxylon* leaves, oils of *E.torquata* demonstrated cytotoxic effects on MCF7 cells. However, neither species' oils had any cytotoxic effects on HEPG2 cells (Ashour, 2008; Takahashi et al., 2011).

3. MATERIALS AND METHODS

3.1. Materials

The plants utilised in this study are shown in Table 3.1.

Table 3.1. Samples of plants

No	Samples Type	Location
1.	*C.sempervirens var. pyramidalis*	Erbil - Shaqlawa- Hiran
2.	*C.arizonica*	Erbil- Samiabdulrahman's Park
3.	*C.sempervirens var. horizontalis*	Erbil- Samiabdulrahman's Park
4.	*E.sideroxylon*	Erbil- Samiabdulrahman's Park
5.	*E.camaldulensis*	Erbil - Shaqlawa

Fungal and bacterial species (positive and negative) pathogenic for humans used in this study are as follows:

A- Gram Negative Bacteria:

- *E.coli ATCC 25922*
- *K.pneumoniae ATCC 13883*

B- Gram Positive Bacteria

- *S.aureus* ATCC 43300
- *E.faecalis* ATCC 29212

Two Type of Fungi:

- *C.albicans ATCC 10231*
- *A.niger ATCC 16404*

3.2. Methods

3.2.1. Samples preparation

The samples of this study were collected at different parts in Erbil from mid-March 2015 to the end of June 2015 and were transferred to the laboratory. After the samples were washed with water, they were divided into two groups, namely leaves and branches. It was then dried at room temperature and in a dark environment to prevent optical oxidation. After drying, the samples were ground using an electric grinder and stored in the refrigerator. Plants have been identified based on the flora encyclopedia of Iraq, Turkey and Iran.

3.2.2. Plant extracts preparation

While the samples used in the extract yield, antimicrobial activity and antifungal activity were extracted as stated in Section 3.2.2.1; the samples used in total condensed tannin, total phenolic, total flavonoid, total anthocyanin and antioxidant analyses (DPPH, ABTS, CUPRAC) were prepared according to Section 3.2.2.2.

3.2.2.1. Hot water, ethanol and methanol extraction

Using hot water, ethanol, and methanol, this study employed the extraction method by Rispail et al. (2005) with some modifications (Table 3.2.).

Table 3.2. Experimental plan

No	Plant	Plant Part	Extraction Methods
1.	*C.sempervirens var. pyramidalis*	Leaf Branch	Hot water Ethanol Methanol
2.	*C.arizonica*		
3.	*C.sempervirens var. horizontalis*		
4.	*E.sideroxylon*		
5.	*E.camaldulensis*		

3.2.2.1.1. Hot water extraction

In the hot water extraction process, 40 g of sample was mixed with 160 ml of distilled water (1:4 w/v), heated at 80-90 ^{o}C and left to cool at the end of the period. Filtering was done with the help of a Buchner funnel using Whatmann filter papers No.1. Then, the evaporation process was carried out in a rotary evaporator, not exceeding 40 °C and after that the extract was placed in a shaker incubator at 30-35 °C. After the extract dried, it-was placed in a lidded container and stored at -4°C until used.

3.2.2.1.2. Ethanol extraction

The ethanol extraction process involved 20 g of sample was mixed with 200 ml of pure ethanol (1:10 w/v), and left for 24 hours to soak. After filtering task was done using the Whatmann filter paper No.1, alcohol was evaporated in a rotary evaporator at 40 ^{o}C and then the extract was placed in a shaker incubator at 25- 30°C. After the extract dried, it was stored at -4°C until used.

3.2.2.1.3. Methanol extraction

In the methanol extraction process, 20 g of sample was mixed with 200 ml of pure methanol (1:10 w/v), and left for 24 hours to soak. After filtering using Whatmann filter paper No.1, alcohol evaporated in a rotary evaporator at 40 ºC and then the extract was placed in a shaker incubator at 25- 30°C. After the extract dried, it was stored at -4°C until used.

3.2.2.2. Extraction process by maceration method

25 g of the grinded samples following the maceration method were taken and 200 ml of solvents (pure water, ethanol, hexane) was added to it. It was then closed and kept in a dark environment at room temperature for 24 hours. Pure water, ethanol and hexane with different polarities were used as solvents. At the end of the period, the plant extracts were filtered using a coarse filter paper.

3.2.3. Antimicrobial activity tests

3.2.3.1. Sterilization of extracts and their concentrations used in the study

1 g of dry plant extract was mixed with 10 ml of distilled water (100 mg/ml). Whatman No.1 filter paper and 0.2 micron disc filter were used for sterilization (Mukhtar, 2009). This prepared solution is the stock solution. The solutions (25%, 50% and 75%) used in the biological tests were prepared from this stock solution.

3.2.3.2. Agar media preparation

The following agars were used as growth media:

1. Nutrient Agar : for bacterial growth
2. Potato Dextrose Agar : for fungi growth
3. Nutrient Broth : for bacterial growth
4. Mueller Hinton Agar : for sensitivity test of bacteria

The medium was prepared according to instructions on the package, and then was sterilized for 15 minutes at 121°C and 15 lb/kg^2 (Atlas, 1995). The medium was dispensed into petri dishes (diameter 9 cm) and the dishes were allowed to cool at room temperature under sterile fume hood until the medium hardened. Some distilled water instead of the extract was also sterilized as a control sample.

3.2.3.3. Bacterial and fungal isolation

The bacterial and fungal species used in this study are as follows:

3.2.3.3.1. Bacterial isolation

The bacteria employed in this study were obtained from Media Center located in Erbil, Iraq.

A- Gram Negative Bacteria:

- *E.coli ATCC 25922*
- *K.pneumoniae ATCC 13883*

B- Gram positive Bacteria

- *S.aureus* ATCC 43300
- *E.faecalis* ATCC 29212

3.2.3.3.2. Fungal isolation

The following fungi were taken from the Media Center in Erbil, Iraq:

- *C.albicans* ATCC 10231
- *A.niger* ATCC 16404

3.2.3.4. Preparation of petri-dishes to test plant extracts

3.2.3.4.1. Preparation of petri-dishes to test extracts against bacteria

Three methods were employed for the antibacterial activity tests: Holes in the growth media, mixing the extract with the agar medium, and disk preparation. Positive results were not obtained from the other 2 methods except the disk diffusion method. For each bacterium, 3 petri dish replicates and 5 replications (4+1 control) were made in each petri dish. The hole drilling method was used (Adıgüzel et al., 2009) and 5 holes of 6 mm diameter were drilled into each petri dish (Girish and Satish, 2008). In the disk diffusion method, 6 mm diameter disks were prepared from Whatman No.1 filter papers and 5 disks were placed in each petri dish. Bacterial isolates of the bacterial suspension were prepared in nutrient broth with a glass diffuser at approximately 610 cells/ml. 50 µl of each extract concentration was added using a micropipette. A new micropipette tip was used for each extract and concentration. Petri dishes were then incubated at 37±2 ^{o}C for 18-24 hours. Diameter measurements are made in millimeters.

3.2.3.4.2. Preparation of petri-dishes to test extracts against fungus

Two methods, namely the disk diffusion method and mixing with the medium method, were used to determine the antifungal activity tests of the samples. Due to the long incubation period, the disc diffusion method did not provide good results. For this reason, the method of mixing with the medium, which resulted in better outcomes in the antifungal trials, was employed. Fungal isolation was planted in potato dextrose agar on the petri dishes. 3 petri dishes were replicated for each fungi species. The prepared petri dishes were incubated at 25-37±2 ^{o}C. The incubation process continued for ten days. Measurements were made and noted every three days (Jethinlakhosh and Lathika, 2012; Adıgüzel et al., 2009).

3.2.4. Determation of total condensed tannin

The total condensed tannin content (TCT) of the extracts was analyzed according to the method determined by Karaoğul et al. (2017).

First, tannin solution was prepared for analysis. The tannin solution was prepared by mixing 0.05 g of Fe_2SO_4, 95 ml of n-butanol, and 5 ml of HCl (35%). To measure the condensed tannin, 0.01 g of plant powder was inserted into a test tube and 10 ml of tannin solution was added. The test tubes were kept in boiling water in a water bath for 1 hour. The absorbance values were measured at 580 nm wavelength in SHIMADZU UV-1280 UV-VIS model spectrophotometer.

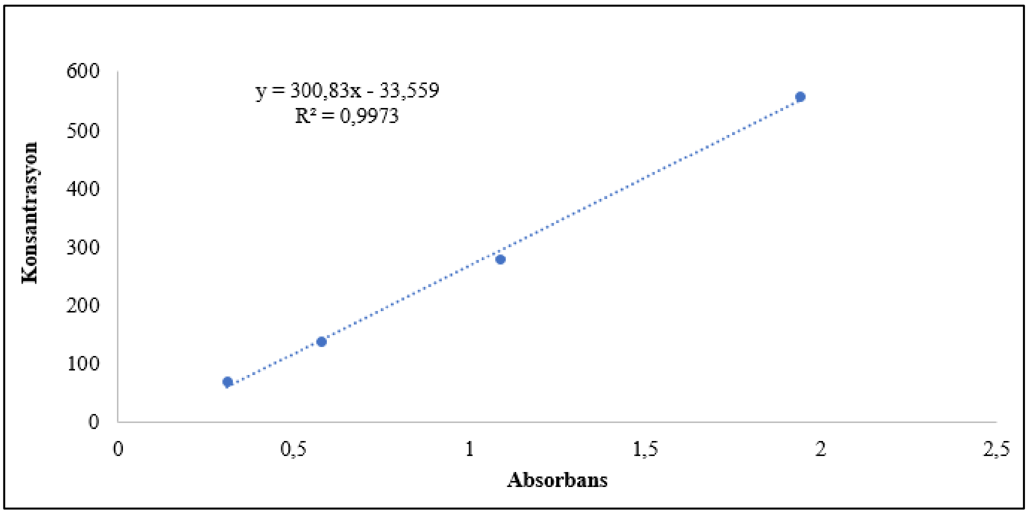

Figure 3.1. Calibration curve of total condensed tannin analysis

3.2.5. Determation of total phenolic compounds (TPC)

Phenolic compounds are aromatic secondary plant metabolite compounds found in the plants. It has been reported that they are natural antimicrobial compounds used in foods to extend shelf life and stop the growth of pathogenic microorganisms. It was also discovered that they have many biological effects, such as antioxidant, anti-mutagenic and scavenging activity on free radicals (Proestos and Komaitis, 2008; Cevallos et al. 2006; Özcan et al., 2014).

In this study, the total phenolic compounds (TPC) were analyzed using the method developed by Singleton & Rossi (1965) and modified by Liet. TPC was calculated in gallic acid. Accordingly, solutions of gallic acid at different concentrations were prepared, their absorbance was measured, and a calibration curve was created. As seen in Figure 3.2, the regression value of the calibration curve was greater than 0.99 and had a high linearity.

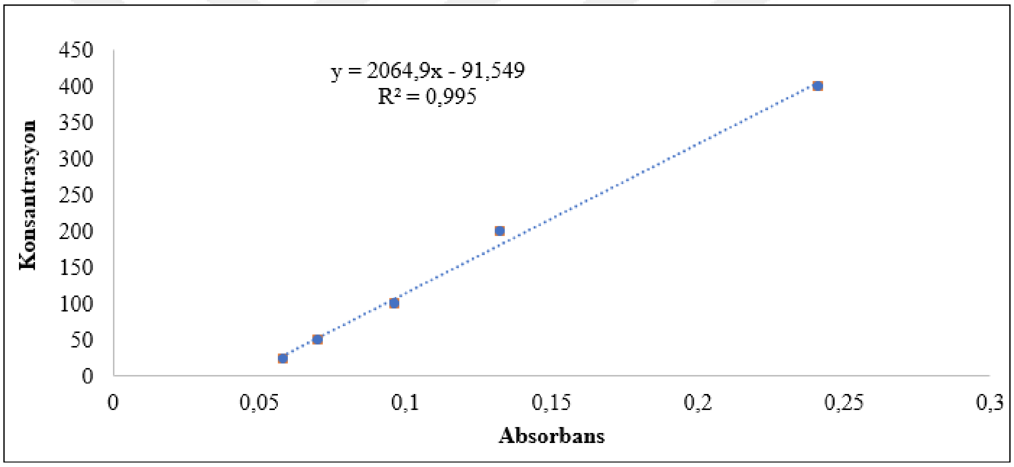

Figure 3.2. Gallic acid calibration curve of TPC analysis

To determine the phenolic content of the samples used in the thesis, Folin & Ciocalteau reagent was diluted 1:9 with distilled water and a 7.5% Na_2CO_3 solution was prepared. 0.4 ml of extract was added to the test tubes. 2 ml of folin and 1.6 ml of Na_2CO_3 solution were placed on it and mixed with vortex. After it was kept in the dark for 1 hour at room temperature, its absorbance was measured at 765 nm wavelength in the SHIMADZU UV-1280 UV-VIS model spectrophotometer. All samples were repeated three times.

3.2.6. Determation of total flavonoid contents (TFC)

It was reported that flavonoids exhibit multiple bioactive effects such as antiviral, antibacterial, anti-inflammatory, vasodilator, anticancer and anti-ischemic properties as well as antioxidant properties (Prochazkova et al., 2011). The flavonoid content of the samples used in the thesis was determined using the method by Zhinsen et al. (1999). Quercetin reference was used to determine the total flavonoid value. In addition, a calibration curve was created by measuring the absorbance of the quercetin reference at different concentrations and as seen in Figure 3.3. It was found that the regression value of the created calibration curve was greater than 0.99 and its linearity was high.

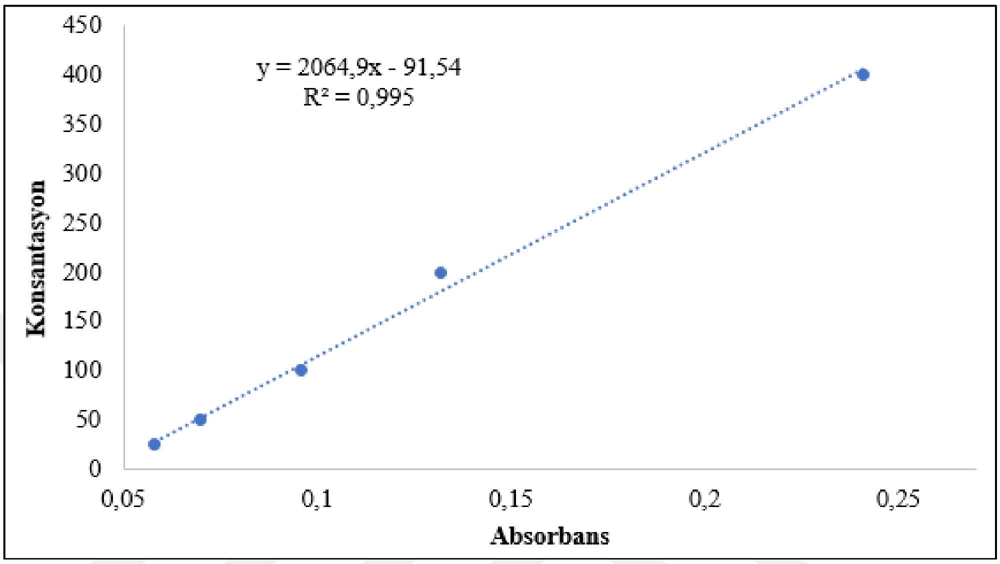

Figure 3.3. Quercetin calibration curve generated for TFC

To determine the flavonoid content of the extracts, 5% $NaNO_2$ solution, 10% $AlCl_3$ solution and 1 M NaOH solutions were prepared. 1 ml of extract was placed in test tubes and 4 ml of distilled water was added to it and mixed with vortex. Then, 0.3 ml of 5% $NaNO_2$ solution and 0.3 ml of 10% $AlCl_3$ solutions were added. The mixture, which was mixed by vortexing again, was left for 5 minutes, and then 2 ml of 1 M NaOH solution was added. After waiting for 6 minutes, the volume of the tubes was filled to 10 ml with distilled water. The absorbance of the extracts was read on the SHIMADZU UV-1280 UV-VIS brand model spectrophotometer at a wavelength of 510 nm. Three replicates were made for each sample.

3.2.7. Determation of total anthocyanin

The differential method of Wrolstad (1976) using pH1 and pH4.5 buffers was used to determine the total anthocyanin content of the sample extracts. In this method, 1.86 g of KCl was weighed and 980 ml of distilled water was added to it. Then the pH of the buffer was adjusted to 1 using acetic acid. Weigh 54.43 g of sodium acetate trihydrate for buffer pH4.5. 960 ml of pure water was also added to it. The pH of the buffer was adjusted to 4.5 using HCl.

4 ml of pH1 was added to the test tube and 1 ml of extract was added and mixed with vortex. The absorbance values were measured at 520 and 700 nm in SHIMADZU UV-1280 UV-VIS brand model spectrophotometer. The same process is done for the pH4.5 buffer. The amount of anthocyanin was calculated based on the following formula (Güven et al., 2019).

Total amount of anthocyanins (mg/L) = $\frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$

A = (A520-A700) $_{\text{pH 1}}$ - (A520-A700) $_{\text{pH 4.5}}$

DF = Dilution factor

Molecular weight (MW) (g/mol)= 449.2 g/mol for Cy-3-glc (Cyanidin-3-glucoside),

Extinction coefficient (ε) = 26.900 A×MW×DF×1000

ε is the extinction coefficient (L× cm−1× mol−1) = 26.900 for Cy-3-glc, where L (path length in cm) = 1.

3.2.8. Total antioxident capacity

3.2.8.1. DPPH radical scavenging activity

For analysis purpose, a solution containing DPPH radical was firstly prepared. The DPPH radical scavenging capacity should increase in direct proportion to the concentration of the sample. For this reason, experiments with different concentrations were made and linear increasing amounts were determined with minimum scavenging activity. For the extracts, the sample at three different concentrations was placed in a test tube and filled to 4 ml with DPPH solution. The tubes, which were mixed by vortexing, were left in the dark for half an hour at room conditions. At the end of half an hour, the absorbances of the samples were read on the SHIMADZU UV-1280 UV-VIS brand model spectrophotometer at a wavelength of 517 nm. The results were calculated as percentages and their linear graphs were created. The regression curves and R^2 values of the generated linear graphs were determined (Figure 3.4).

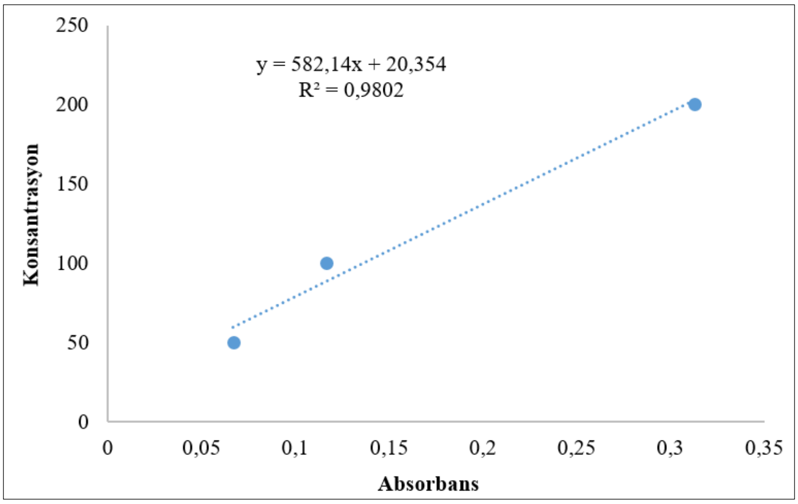

Figure 3.4. Trolox calibration curve generated for DPPH and ABTS

3.2.8.2. ABTS radical scavenging activity

ABTS+ cation radical scavenging activity analysis was conducted based on the method utilized by Re et al. (1999). 7 M ABTS+ cation aqueous solution was mixed with 0.00245 M potassium persulfate ($K_2S_2O_8$) dissolved in water. Then, the prepared solution was kept in the dark at room temperature for 12 hours. Before using the ABTS+ solution, it was diluted with distilled water until its absorbance at 734 nm reached 0.708±0.025 in a spectrophotometer.

To calculate the analysis results for trolox, solutions of Trolox reference at 50, 100 and 200 ppm concentrations were prepared and their absorbance ability was measured. Calibration chart was drawn with the absorbance and concentration values. It was observed that the regression value of the obtained calibration curve was greater than 0.98 and its linearity was high (Fig. 3.4).

For ABTS antioxidant capacity analysis of the extracts used within the scope of the study, the amounts in which the minimum radical scavenging activity of the sample at different concentrations increased linearly in direct proportion to the concentration were firstly determined. The values determined for the extracts were put into a test tube and filled with 4 ml of ABTS+ solution. It was determined that as the concentration increased, the scavenging capacity increased. Three replications were made for each sample. The absorbance values were measured at 734 nm wavelength in Shimadzu UV-1800 UV-VIS Spectrophotometer. The results were calculated as trolox equivalents and the R_2 value was calculated by plotting their linear graphs.

3.2.8.3. CUPRAC method

Antioxidant substance analysis with CUPRAC was performed following the method developed by Apak et al. (2004). In this method, Cu(II)-neocuproin(Nc) reagent, which can respond to hydrophilic and lipophilic antioxidant compounds, was used. It is known in the literature as CUPric Reducing Antioxidant Capacity = CUPRAC [copper (II) ion reduction] antioxidant capacity analysis. The method is based on the reduction of copper(II) ion and the formation of Cu(I)-Nc chelate, resulting in a darker yellow color compared to the initial and maximum absorbance at 450 nm. For analysis purpose, 1.0×10^{-2} M $CuCl_2.2H_2O$ solution was prepared with distilled water. In addition to this, 1 M ammonium acetate solution and 7.5×10^{-3} M Neocuproin (2.9-dimethyl-1.10-phenanthroline) solution were prepared.

The absorbance of troloxs reference at 25, 30, 50, 62.5, 100 and 120 ppm concentrations was measured and a calibration curve was created. The regression value of the created calibration curve was found to be greater than 0.99, and its linearity was high (Figure 3.5.).

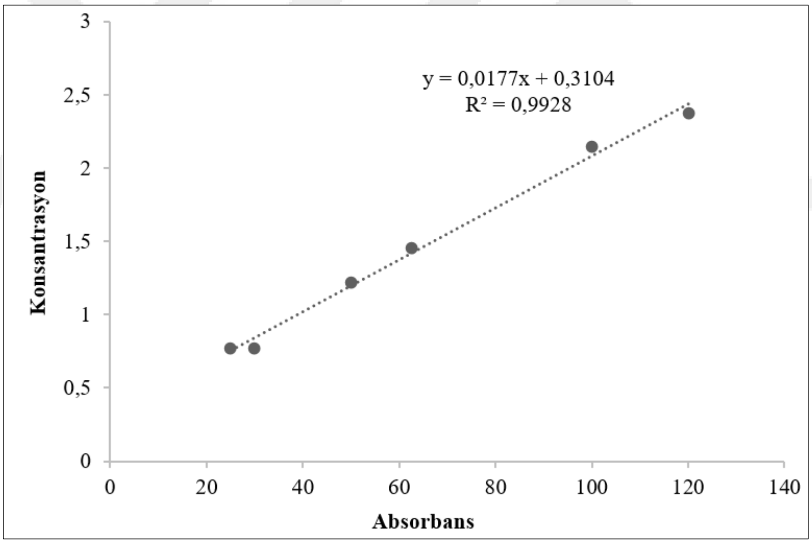

Figure 3.5. Trolox calibration curve generated for CUPRAC

For the antioxidant capacity analysis using the CUPRAC method, the minimum antioxidant capacity and the increased linearly amounts of the sample were determined at different concentrations. The extracts were diluted at three different concentrations according to the determined amounts. Three parallel runs were made for each sample. 1.1 ml of diluted samples were taken and put into test tubes. 1 ml of 1.0×10-2 M $CuCl_2.2H_2O$ solution, 1 ml of 1 M ammonium acetate solution and 1 ml of 7.5×10^{-3} M Neocuproin (2.9-dimethyl-1.10-phenanthroline) solution were added to them, respectively. The test tubes were mixed by vortexing. It was kept in the dark for half an hour at room temperature. The absorbance values of the samples were measured at 450 nm wavelength in Shimadzu UV-1800 UV-VIS Spectrophotometer. Results were calculated as trolox equivalent.

3.4. Determination the Chemical Compounds of Plant Extracts by HPLC Method

Based on the antimicrobial and antifungal activities of the samples, the leaf part was solely tested for the chemical composition in this study for better results were demonstrated in the leaf than the branch part. The alkaloid, glycosidically bound, phenol, and flavonoid contents of the plant extracts were determined by the HPLC method.

1 g of the dried plant sample was taken and dissolved in 10 mL of high-performance chromatography methanol. After the samples were mixed in an ultrasonic bath for 10 minutes, liquid nitrogen was applied up to the final volume of 1 ml and then filtered (0.27 µm diameter). 16534K Equipped with Supelco Corporation. Compounds were determined using Shimadzu LC-10A type liquid chromatography. 20 microliters plant extracts were injected into the injection column (Rispail et al., 2005).

3.4.1. HPLC analysis conditions for the determination of alkaloids

- C18 (50 x 4.6mm I.D. 3µm) HPLC column
- Mobile phase: [0.01M phosphate buffer pH 6.2: acetonitrile (75:25, V / V)]
- 220 nm wavelength
- 1 ml/min flow rate
- 30 ^{o}C temperature
- 25 mg/ml standard concentration

The concentrations of derived alkaloids were estimated by comparing the confined area under the columns to that of standard alkaloids which were adopted simultaneously the minutes were calculated.

3.4.2. HPLC analysis conditions for the determination of glycosidically bound compounds

- HPLC column: Lichrospher C18 (50 x 4.6mm, 3µm).
- The mobile phase: Acetonitrile: water (20:80, v / v)
- 203 nm wavelength
- 31 ml/min flow rate
- 30 ^{o}C temperature
- 25 mg/ml standard concentration

The concentrations of the glycosidically bound compounds were estimated by comparing the confined area under the columns to that of the standard glycosidically bound compounds that were adopted at the same time as the minutes calculated.

3.5. Statistical Analysis

GLM-Univariate (at 95% confidence interval) was applied to the test results obtained using the SPSS program. In case of difference ($p \leq 0.05$), homogeneous groups were identified using the post hoc Tukey-B test. The analytical test was done separately for each solvent type (hot water, ethanol, and methanol), fungi (*C.albicans, A.niger)* and bacteria (*S.aureus, E.faecalis, E.coli, K.pneumoniae*). Thus, the effect of plant part (leaf, and branch) and extract concentration (25%, 50%, and 75%). The t-test was used instead of Tukey-B test was used when there were no enough factors to compare some experimental results.

4. RESULTS

4.1. Extract yield results

The solubility results of hot water, ethanol and methanol of some *Cupressus* and *Eucalyptus* species are presented and explained in this chapter.

4.1.1. Extract yield results of *Cupressus* spp.

Table 4.1 shows the hot water, ethanol and methanol solubility results of leaf and branch parts of *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, and *C.arizonica* species.

Table 4.1. Extract yield results of *Cupressus* spp.

Plant	**Plant Part**	**Extract yield (%)**		
		Hot Water	**Ethanol**	**Methanol**
C.sempervirens L. var. *pyramidalis*	Leaf	41.33 (0.33)	47.32 (0.02)	64.53 (0.32)
	Branch	41.79 (0.68)	47.29 (0.45)	52.13 (0.45)
C.sempervirens L. var. *horizontalis*	Leaf	24.30 (0.01)	36.18 (0.74)	51.72 (0.32)
	Branch	32.44 (0.43)	39.81 (0.26)	42.40 (0.59)
C.arizonica	Leaf	17.83 (0.47)	27.99 (0.68)	44.95 (0.60)
	Branch	13.44 (0.63)	22.94 (0,04)	38.51 (0.45)

Note: Values in brackets are standard deviation

Based on Table 4.1, methanol extraction exhibited the highest extract yield results in all *Cupressus* spp.

4.1.2. Extract yield results of *Eucalyptus* spp.

The extract yield results of *E.camaldulensis* and *E.sideroxylon* are shown in Table 4.2.

Table 4.2. Extract yield results of *Eucalyptus* spp.

Plant	**Plant Part**	**Extract yield (%)**		
		Hot Water	**Ethanol**	**Methanol**
E.camaldulensis	Leaf	18.09 (0.14)	24.45 (0.09)	43.05 (0.76)
	Branch	43.55 (0.54)	49.11 (0.45)	54.54 (0.24)
E.sideroxylon	Leaf	18.42 (0.03)	25.81 (0.09)	47.15 (0.45)
	Branch	12.32 (1.00)	20.27 (0.87)	37.22 (0.37)

Note: Values in brackets are standard deviation

The highest extract yield was obtained in the methanol extraction. The yield of methanol was 54.54% for *E.camaldulensis* (branch part) and 47.15% for *E.sideroxylon* (leaf part).

4.2. Antimicrobial Activity Results

4.2.1. Antimicrobial activity results of *Cupressus* spp.

The antimicrobial activity results of the *Cupressus* spp. extracts (hot water, ethanol and methanol) against *S.aureus, E.faecalis, E.coli,* and *K.pneumoniae* at different extract concentrations (25%, 50%, and 75%) are depicted in Table 4.3.

4.2.2. Antimicrobial activity results of *Eucalyptus* spp.

On the other hand, Table 4.4 shows the antimicrobial activity results of the *Eucalyptus* spp. hot water, ethanol and methanol extracts at different concentrations (25%, 50%, and 75%) against *S.aureus, E.faecalis, E.coli*, and *K.pneumoniae*.

4.3. Antifungal activity results

4.3.1. Antifungal activity results of *Cupressus* spp.

Table 4.5. provided the antifungal activity results of the *Cupressus* spp. hot water, ethanol and methanol extracts at different concentrations (25%, 50%, and 75%) against *A.niger* and *C.albicans.*

4.3.2. Antifungal activity results of *Eucalyptus* spp.

The antifungal activity results of *Eucalyptus* spp. against *A.niger* and *C.albicans* are shown in Table 4.6 with different concentrations (25%, 50%, and 75%) and extraction solvents, such as hot water, ethanol and methanol.

Table 4.3. The antimicrobial activity test results of *Cupressus* spp.

Plant	Plant Part	Strains	Inhibition Diameter (mm)								
			Hot Water Concentration (%)			**Ethanol** Concentration (%)			**Methanol** Concentration (%)		
			25	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
C.sempervirens L. var. *pyramidalis*	Leaf	*S.aureus*	-	1.0 (0.0)	2.0 (0.8)	-	2.0 (0.8)	3.0 (0.8)	1.0 (0.8)	2.0 (0.8)	3.0 (0.8)
		E.faecalis	-	1.0 (0.0)	2.0 (1.4)	-	1.0 (0.0)	3.0 (0.8)	1.0 (0.0)	1.0 (0.8)	3.0 (0.0)
		E.coli	-	2.0 (0.8)	3.0 (0.8)	1.0 (0.8)	2.0 (0.8)	4.0 (0.8)	2.0 (0.8)	4.0 (0.8)	6.0 (0.8)
		K.pneumonia	-	1.0 (0.8)	2.0 (0.8)	1.0 (0.0)	1.0 (0.0)	3.0 (0.8)	1.0 (0.0)	1.0 (0.8)	3.0 (0.0)
	Branch	*S.aureus*	-	-	-	-	-	1.0 (0.0)	-	-	1.0 (0.8)
		E.faecalis	-	-	-	-	-	-	-	-	1.0 (0.0)
		E.coli	-	-	-	-	-	1.0 (0.8)	-	1.0 (0.8)	2.0 (0.8)
		K.pneumonia	-	-	-	-	-	1.0 (0.0)	-	-	1.0 (0.8)
C. sempervirens L. var. *horizontalis*	Leaf	*S.aureus*	-	1.0 (0.8)	1.0 (0.0)	1.0 (0.0)	1.0 (0.8)	2.0 (0.0)	-	1.0 (0.8)	3.0 (0.8)
		E.faecalis	-	-	1.0 (0.0)	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.0)	3.0 (0.8)
		E.coli	-	1.0 (0.8)	2.0 (0.8)	-	1.0 (0.0)	3.0 (0.8)	1.0 (0.0)	2.0 (0.8)	4.0 (0.8)
		K.pneumonia	-	1.0 (0.0)	2.0 (1.4)	-	1.0 (0.0)	3.0 (0.8)	-	1.0 (0.0)	3.0 (0.8)
	Branch	*S.aureus*	-	-	1.0 (0.0)	-	1.0 (0.0)	2.0 (1.4)	-	-	-
		E.faecalis	-	-	-	-	-	1.0 (0.0)	-	-	1.0 (0.8)
		E.coli	-	-	-	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	-	-	1.0 (0.8)
		K.pneumonia	-	-	1.0 (0.0)	-	-	1.0 (0.8)	-	-	1.0 (0.8)
C.arizonica	Leaf	*S.aureus*	-	1.0 (0.0)	2.0 (0.8)	1.0 (0.8)	1.0 (0.0)	3.0 (0.8)	1.0 (0.0)	2.0 (0.8)	4.0 (0.8)
		E.faecalis	-	1.0 (0.8)	2.0 (0.8)	-	1.0 (0.8)	3.0 (0.8)	1.0 (0.8)	2.0 (0.8)	4.0 (0.8)
		E.coli	-	1.0 (0.0)	2.0 (0.0)	1.0 (0.0)	2.0 (0.8)	4.0 (0.0)	1.0 (0.0)	3.0 (0.8)	6.0 (0.0)
		K.pneumonia	-	1.0 (0.0)	2.0 (0.0)	1.0 (0.0)	1.0 (0.8)	3.0 (0.0)	1.0 (0.0)	2.0 (0.8)	4.0 (0.8)
	Branch	*S.aureus*	-	1.0 (0.0)	1.0 (0.8)	-	1.0 (0.8)	1.0 (0.0)	-	-	1.0 (0.8)
		E.faecalis	-	-	-	-	-	1.0 (0.8)	-	1.0 (0.0)	2.0 (0.8)
		E.coli	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.8)	2.0 (0.0)
		K.pneumonia	-	1.0 (0.0)	2.0 (0.0)	-	-	1.0 (0.0)	-	-	1.0 (0.8)

(-) No inhibition

Table 4.4. The antimicrobial activity test results of *Eucalyptus* spp.

Plant	Plant Part	Strains	Inhibition Diameter (mm)								
			Hot Water Concentration (%)			Ethanol Concentration (%)			Methanol Concentration (%)		
			25	50	75	25	50	75	25	50	75
E.camaldulensis	Leaf	*S.aureus*	-	1.0 (0.0)	3.0 (1.2)	1.0 (0.0)	3.0 (0.8)	4.0 (0.8)	1.0 (0.0)	3.0 (0.8)	6.0 (0.8)
		E.faecalis	1.0 (0.0)	2.0 (0.8)	3.0 (0.0)	1.0 (0.0)	2.0 (0.8)	4.0 (0.0)	1.0 (0.0)	4.0 (0.8)	6.0 (0.0)
		E.coli	-	1.0 (0.8)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	3.0 (0.8)	1.0 (0.0)	2.0 (0.8)	4.0 (0.0)
		K.pneumonia	1.0 (0.8)	1.0 (0.0)	2.0 (0.8)	1.0 (0.8)	2.0 (0.8)	4.0 (0.8)	2.0 (0.8)	4.0 (0.8)	6.0 (1.2)
	Branch	*S.aureus*	-	-	1.0 (0.8)	-	-	1.0 (0.0)	-	1.0 (0.0)	2.0 (0.8)
		E.faecalis	-	-	1.0 (0.8)	-	-	1.0 (0.8)	1.0 (0.8)	1.0 (0.0)	2.0 (0.8)
		E.coli	-	-	1.0 (0.0)	-	-	1.0 (0.0)	-	-	1.0 (0.0)
		K.pneumonia	1.0 (0.0)	-	-	-	-	1.0 (0.8)	1.0 (0.0)	2.0 (1.4)	3.0 (0.8)
E.sideroxylon	Leaf	*S.aureus*	-	1.0 (0.0)	2.0 (0.0)	1.0 (0.0)	2.0 (0.8)	3.0 (0.8)	-	1.0 (0.0)	3.0 (0.8)
		E.faecalis	-	1.0 (0.0)	3.0 (0.8)	3.0 (1.2)	2.0 (0.8)	5.0 (0.8)	1.0 (1.2)	3.0 (1.4)	6.0 (0.8)
		E.coli	-	1.0 (0.0)	2.0 (0.8)	1.0 (0.0)	1.0 (0.0)	3.0 (0.0)	1.0 (0.0)	2.0 (0.8)	4.0 (0.8)
		K.pneumonia	-	1.0 (0.0)	3.0 (0.8)	1.8 (0.9)	3.0 (0.8)	5.0 (0.8)	2.0 (1.4)	3.0 (0.8)	6.0 (0.8)
	Branch	*S.aureus*	-	-	1.0 (0.0)	-	-	1.0 (0.0)	-	-	1.0 (0.8)
		E.faecalis	-	-	-	-	1.0 (0.0)	2.0 (0.8)	1.0 (0.0)	1.0 (0.8)	2.0 (0.8)
		E.coli	-	-	-	-	-	1.0 (0.0)	-	-	-
		K.pneumonia	-	-	1.0 (0.0)	-	1.0 (0.0)	1.0 (0.8)	-	1.0 (0.8)	2.0 (0.8)

(-) No inhibition

Table 4.5. The antifungal activity test results of *Cupressus* spp.

Plant	**Plant Part**	**Strains**	**Growth Diameter (mm)**								
			Hot Water Concentration (%)			**Ethanol Concentration (%)**			**Methanol Concentration (%)**		
			25	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
C.sempervirens L. var. *pyramidalis*	Leaf	*C.albicans*	79.5 (2.1)	78.0 (3.7)	75.8 (2.5)	78.8 (1.3)	77.5 (1.7)	73.3 (1.3)	73.3 (2.9)	71.0 (1.4)	69.3 (1.7)
		A.niger	81.5 (1.9)	80.0 (1.6)	77.0 (1.1)	80.5 (1.3)	79.5 (1.9)	77.8 (2.2)	77.5 (1.9)	75.8 (3.9)	74.3 (2.2)
	Branch	*C.albicans*	82.0 (1.6)	80.3 (2.6)	79.0 (2.2)	83.8 (2.2)	82.8 (1.3)	82.8 (1.3)	83.3 (1.7)	81.8 (2.2)	81.0 (0.8)
		A.niger	83.0 (2.6)	81.0 (2.9)	78.3 (2.6)	84.3 (1.7)	83.5 (1.3)	83.5 (2.4)	83.0 (1.6)	81.8 (0.9)	82.5 (1.3)
C.sempervirens L. var. *horizontalis*	Leaf	*C.albicans*	82.0 (2.8)	81.5 (1.9)	80.5 (1.3)	81.3 (1.3)	80.0 (0.8)	79.8 (2.2)	80.3 (0.9)	78.8 (1.3)	78.3 (2.8)
		A.niger	83.5 (1.3)	82.5 (2.1)	80.5 (1.3)	82.0 (0.8)	82.5 (1.3)	81.3 (1.7)	80.5 (0.6)	80.8 (1.7)	79.3 (1.7)
	Branch	*C.albicans*	84.0 (1.6)	82.3 (1.7)	80.5 (1.9)	83.8 (1.7)	82.8 (1.3)	82.0 (1.8)	82.0 (1.2)	81.5 (1.3)	80.8 (1.7)
		A.niger	83.8 (1.7)	82.0 (0.8)	81.5 (1.3)	83.3 (2.1)	82.8 (2.1)	82.8 (2.1)	81.8 (1.5)	81.8 (0.9)	81.3 (0.5)
C.arizonica	Leaf	*C.albicans*	83.5 (1.9)	83.3 (2.1)	81.3 (1.5)	80.8 (0.9)	80.0 (1.8)	79.3 (1.7)	79.8 (0.9)	79.0 (0.5)	79.3 (1.7)
		A.niger	84.5 (1.3)	82.5 (1.3)	82.3 (1.7)	82.8 (1.7)	81.3 (1.3)	80.8 (1.7)	80.8 (0.9)	79.8 (1.6)	79.3 (1.7)
	Branch	*C.albicans*	85.0 (1.4)	84.0 (1.4)	82.0 (1.2)	84.0 (1.2)	82.3 (0.9)	81.8 (2.2)	81.8 (1.5)	80.8 (1.0)	80.8 (1.0)
		A.niger	85.0 (2.6)	83.5 (1.3)	82.5 (1.3)	84.3 (1.5)	82.5 (1.9)	82.8 (2.1)	81.8 (1.7)	81.5 (0.9)	81.5 (1.7)

Note: 1.) Values in brackets are standard deviation

2.) The fungi growth was determined as 90 mm for control petri dishes in both fungi species

Table 4.6. The antifungal activity test results of *Eucalyptus* spp.

Plant	Plant Part	Strains	Growth Diameter (mm)								
			Hot Water Concentration (%)			Ethanol Concentration (%)			Methanol Concentration (%)		
			25	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
E.camaldulensis	Leaf	*C.albicans*	83.0 (1.4)	82.8 (0.9)	82.3 (1.7)	82.8 (1.7)	82.0 (1.8)	81.5 (1.3)	81.5 (1.3)	80.0 (0.8)	80.0 (0.8)
		A.niger	83.8 (1.3)	82.3 (2.6)	81.3 (1.5)	81.8 (1.5)	81.3 (1.3)	80.3 (0.9)	81.3 (1.7)	80.0 (0.8)	79.8 (2.5)
	Branch	*C.albicans*	85.3 (1.7)	83.8 (0.9)	83.0 (1.6)	84.0 (1.8)	82.0 (1.8)	82.5 (1.9)	82.3 (0.9)	81.3 (1.3)	81.0 (0.8)
		A.niger	85.3 (1.5)	83.0 (1.6)	82.5 (1.9)	83.8 (2.5)	82.0 (1.8)	82.8 (2.1)	82.0 (1.8)	81.3 (1.3)	82.0 (1.4)
E.sideroxylon	Leaf	*C.albicans*	84.0 (0.8)	84.0 (1.4)	82.3 (1.7)	82.3 (1.5)	81.5 (1.3)	80.5 (1.3)	80.0 (0.8)	80.3 (0.9)	79.5 (0.6)
		A.niger	80.0 (0.8)	79.0 (1.6)	77.3 (2.1)	79.3 (1.7)	77.8 (2.2)	76.0 (2.6)	79.5 (0.6)	76.5 (2.5)	73.8 (1.5)
	Branch	*C.albicans*	85.5 (1.3)	84.5 (1.9)	84.3 (1.7)	83.8 (2.5)	82.3 (2.1)	82.8 (3.1)	82.8 (2.1)	80.3 (0.9)	81.5 (2.5)
		A.niger	84.3 (1.3)	82.5 (3.4)	81.8 (1.7)	83.0 (2.2)	82.5 (3.1)	82.5 (1.9)	82.8 (1.7)	81.8 (2.5)	82.0 (2.6)

Note: 1.) Values in brackets are standard deviation
2.) The fungi growth was determined as 90 mm for control petri dishes in both fungi species

4.4. Total condensed tannin results of *Cupressus and Eucalyptus* spp.

According to the antimicrobial activity and antifungal activity test results, only the total condensed tannin (TCT) content of the leaf samples was determined. The experiment was repeated three times for each sample and the average results was taken. The TCT results are shown in Table 4.7.

Table 4.7. Total condanse tannin results (mg/kg)

Plant	Plant Part	TCT (mg/kg)
C.sempervirens L. var. *pyramidalis*	Leaf	247.5 (±0.21)
C.sempervirens L. var. *horizontalis*	Leaf	209.1 (±0.12)
C.arizonica	Leaf	229.4 (±0.34)
E.camaldulensis	Leaf	82.9 (±0.16)
E.sideroxylon	Leaf	37.6 (±0.07)

4.5. Total phenolic content results

4.5.1. Total phenolic content results of *Cupressus* spp.

Only the total phenolic content (TPC) of the leaf samples was analysed based on the antimicrobial activity and antifungal activity test results. The TPC results of The sample extracts obtained by the maceration method using pure water, ethanol, and hexane compared to the dry sample weight are shown in Table 4.8.

Table 4.8. TPC analysis results of some *Cupressus* spp (mg GAE /g DW)

Plant	Solvent Type		
	Water	Ethanol	Hexane
C.sempervirens L. var. *pyramidalis*	10.10 (0.24)	12.77 (0.18)	2.77 (0.33)
C.sempervirens L. var. *horizontalis*	9.76 (0.11)	13.43 (0.04)	3.96 (0.16)
C.arizonica	10.48 (0.09)	11.47 (0.49)	1.79 (0.12)

4.5.2. Total phenolic content results of *Eucalyptus* spp.

The TPC results of the *E.camaldulensis* and *E.sideroxylon* leaf part are presented in Table 4.9. As shown fom this table, the water and hexane extracts of *E.camaldulensis* generally depicted higher TPC content.

Table 4.9. TPC analysis results of some *Eucalyptus* spp (mg GAE /g DW)

Plant	**Solvent Type**		
	Water	Ethanol	Hexane
E.camaldulensis	11.7 (0.09)	10.36 (0.57)	4.00 (0.26)
E.sideroxylon	6.47 (0.19)	11.15 (0.18)	3.28 (0.08)

4.6. Total flavonoid content results

4.6.1. Total flavonoid content results of C*upressus* spp.

Table 4.10 shows the total flavonoid content (TFC) results of the leaf part of *Cupressus* spp. The highest TFC results (4.92-6.89 mg QE/g DW) were revealed in the ethanol extract.

Table 4.10. TFC analysis results of some *Cupressus* spp. (mg QE /g DW)

Plant	**Plant Part**	**Solvent Type**		
		Water	Ethanol	Hexane
C.sempervirens L. var. *pyramidalis*	Leaf	4.12 (±0.08)	6.53 (±0.01)	3.01 (±0.13)
C.sempervirens L. var. *horizontalis*	Leaf	5.48 (±0.17)	6.89 (±0.16)	3.39 (±0.39)
C.arizonica	Leaf	2.46 (±0.06)	4.92 (±0.26)	3.10 (±0.25)

4.6.2. Total flavonoid content results of *Eucalyptus* spp.

The TFC results of the water, ethanol, and hexane extracts of the *E.camaldulensis* and *E.sideroxylon* leaf part are shown in Table 4.11. Among these extracts, the ethanol extract exhibited the highest TFC results (3.91-4.71 mg QE/g DW).

Table 4.11. TFC analysis results of some *Eucalyptus* spp. (mg QE /g DW)

Plant	**Plant Part**	**Solvent Type**		
		Water	Ethanol	Hexane
E.camaldulensis	Leaf	2.11 (±0.20)	4.71 (±0.14)	1.47 (±0.23)
E.sideroxylon	Leaf	1.35 (±0.17)	3.91 (±0.30)	2.89 (±0.07)

4.7. Total Anthocyanin Results

4.7.1. Total anthocyanin results of *Cupressus* spp.

The total anthocyanin results of the leaf part of *Cupressus* spp. are provided in Table 4.12. Based on this table, the highest total anthocyanin results for *C.sempervirens L.* var. *horizontalis* were indicated in the ethanol extract (3.64 mg/L).

Table 4.12. Total anthocyanin results of *Cupressus* spp. (mg/L)

Plant	**Plant Part**	**Solvent Type**	
		Water	**Ethanol**
C.sempervirens L. var. *pyramidalis*	Leaf	0.24 (±0.05)	2.42 (±0.18)
C.sempervirens L. var. *horizontalis*	Leaf	0.49 (±0.06)	3.64 (±0.30)
C.arizonica	Leaf	0.90 (±1.65)	2.17 (±1.04)

4.7.2. Total anthocyanin results of *Eucalyptus* spp.

As shown in Table 4.13, anthocyanin was detected at the rate of 0.89 mg/L solely in *E.sideroxylon* leaf water extract. Anthocyanin could not be determined in other samples.

Table 4.13. Total anthocyanin results of *Eucalyptus* spp. (mg/L)

Plant	**Plant part**	**Solvent Type**	
		Water	**Ethanol**
E.camaldulensis	Leaf	ND	ND
E.sideroxylon	Leaf	0.89 (±1.40)	ND

ND: Not Determined

4.8. Total Antioxident Capacity Results

Based on the other data obtained within the scope of the thesis, the antioxidant capacities of only the leaf parts of the samples were determined. DPPH radical scavenging activity, ABTS, and CUPRAC antioxidant substance analysis were performed. The results from these experiments are described in this Section 4.8.

4.8.1. DPPH radical scavenging activity results of *Cupressus and Eucalyptus* spp.

Section 4.8.1 presents the DPPH % inhibition, trolox, linearity and IC50 value (maximum scavenging activities) results of the leaf extracts of *Cupressus* and *Eucalyptus* spp.

4.8.1.1. DPPH radical scavenging activity results of *Cupressus* spp.

The DPPH % inhibition (scavenging activity), trolox, linearity and IC50 value results of the *Cupressus* spp. leaf extracts used in the study are shown in Table 4.14. Based on this table, a linear graph was drawn with the results in which the linear increase was observed. The table also provided information about the R^2 factor and the equation formed by the line. In addition, IC50 value, which is the half-inhibition activity obtained from the equation, was also calculated. The table showed that the R^2 factors are generally close to 1. It was observed that when scavenging activity values increased in parallel with the increase in concentration, trolox values however decreased inversely. Trolox value shows the amount of DPPH remaining in the medium after the free radical scavenging activity of the sample. As can be seen from the table, there is an increase in DPPH % inhibition rates with increasing concentration in all samples. The highest scavenging activity was indicated in the ethanol extracts (53.6-97.8%).

The IC50 results suggested that the ethanol extract of *C.arizonica* has the highest antioxidative properties (14.9 µg/100g).

Table 4.14. DPPH %inhibition, trolox and linearity of *Cupressus* spp.

Sample	**Conc (µg/100g)**	**Solvent**	**%Inhibition**	**Trolox Equivalent (mg/kg)**	**Equation**	**R²**	**IC50 (µg/100g)**
C. sempervirens L. var. *pyramidalis*	55	Water	32.0 (0.53)	280.3 (2.04)	y = 0.4995x + 4.2783	0.9986	91.5
	80		44.9 (0.70)	231.1 (2.67)			
	110		59.5 (1.30)	172.6 (2.04)			
	55	Ethanol	53.6 (2.03)	197.7 (2.75)	y = 0.4833x + 28,74	0.9537	43.9
	80		71.9 (0.15)	127.5 (0.58)			
	110		80.2 (1.85)	96.0 (2.06)			
	55	Hexane	4.52 (1.70)	385.6 (2.49)	y = 0.0333x + 3.1683	0.5461	1406.4
	80		4.95 (0.23)	383.9 (0.87)			
	110		7.31 (0.15)	374.9 (0.58)			
C. sempervirens L. var. *horizontalis*	55	Water	30.2 (0.38)	287.3 (1.46)	y = 0.4x + 7.7667	0.9952	105.6
	80		39.9 (0.85)	250.3 (3.24)			
	110		52.2 (1.22)	203.2 (4.66)			
	55	Ethanol	65.6 (1.07)	151.9 (4.07)	y = 0.5853x + 36.568	0.8964	22.9
	80		91.2 (0.46)	54.1 (1.75)			
	110		97.8 (0.23)	28.8 (1.23)			
	55	Hexane	10.9 (0.58)	361.3 (2.20)	y = 0.024x + 9.5067	0.9924	1687.2
	80		11.4 (0.00)	359.2 (0.00)			
	110		12.2 (0.02)	356.3 (0.00)			
C. arizonica	55	Water	32.1 (1.07)	279.9 (4.07)	y = 0.3338x + 13.017	0.9807	110.8
	80		39.1 (0.58)	253.4 (2.20)			
	110		50.5 (0.88)	209.7 (3.36)			
	55	Ethanol	67.4 (0.99)	145.2 (3.78)	y = 0.4709x + 42.97	0.9603	14.9
	80		84.9 (1.90)	78.3 (2.28)			
	110		93.3 (1.22)	46.2 (4.67)			
	55	Hexane	7.76 (0.95)	373.1 (3.64)	y = 0.1146x + 1.3991	0.999	424.6
	80		10.5 (0.47)	362.9 (1.78)			
	110		14.1 (0.72)	349.1 (2.75)			

Conc.: Concentration

4.8.1.2. DPPH radical scavenging activity results of *Eucalyptus* spp.

The DPPH % inhibition, trolox, linearity and IC50 values of the leaf water, ethanol and hexane extracts of *E.camaldulensis* and *E.sideroxylon* are shown in Table 4.15. In this table, the DPPH %inhibition values increased in direct proportion with an increase in the concentration ratio, as in the *Cupressus* samples, while trolox data decreased inversely. In general, the %inhibition values of water extracts were found the highest followed by the ethanol extracts. However, the %inhibition values of the hexane extracts were identified the lowest.

4.8.2. ABTS radical scavenging activity results of *Cupressus and Eucalyptus* spp.

4.8.2.1. ABTS radical scavenging activity results of *Cupressus* spp.

The ABTS %inhibition (scavenging activity), trolox, linearity and IC50 value results of the *Cupressus* spp. leaf extracts are provided in Table 4.16. This table depicted that the R^2 factors were seen to be close to 1 in general. The table also revealed that the scavenging activity values increased simultaneously with an increase in concentration. On the other hand, the trolox values decreased inversely. In addition, the ABTS %inhibition values were identified the lowest in the hexane extracts (1.66-34.8%). As shown in Table 4.16, *C.arizonica* ethanol extract had the best antioxidative properties (IC50 = 1.62 μg/100g).

4.8.2.2. ABTS radical scavenging activity results of *Eucalyptus* spp.

Table 4.17 presented the ABTS %inhibition, trolox, linearity and IC50 values of the water, ethanol and hexane extracts of the *E.camaldulensis* and *E.sideroxylon* leaf. The table showed that the %inhibition values increased in direct proportion with an increase in concentration ratio compared to the trolox data that decreased inversely. The best IC50 value was achieved in *E.camaldulensis* in hexane (5.48 μg/100g) and in *E.sideroxylon* in water extracts (39.9 μg/100g).

Table 4.15. DPPH %inhibition, trolox and linearity data of *Eucalyptus* spp.

Sample	Conc (µg/100g)	Solvent	%Inhibition	Trolox Equivalent (mg/kg)	Equation	R^2	IC50 (µg/100g)
E.camaldulensis	55	Water	70.5 (0.30)	133.3 (1.16)	y = 0.5175x + 42.263	0.9991	14.9
	80		85.5 (2.36)	75.9 (2.02)			
	110		98.9 (0.46)	24.4 (1.75)			
	55	Ethanol	65.2 (1.29)	153.4 (4.95)	y = 0.6033x + 34.323	0.9462	25.9
	80		88.7 (0.84)	63.7 (3.20)			
	110		98.4 (0.38)	26.5 (1.46)			
	55	Hexane	8.30 (0.38)	372.7 (3.21)	y = 0.0658x + 4.37	0.9191	693.5
	80		9.18 (0.32)	367.7 (1.21)			
	110		11.9 (0.49)	357.2 (1.87)			
E.sideroxylon	55	Water	45.9 (1.24)	227.2 (4.74)	y = 0.582x + 12.115	0.9636	65.1
	80		56.5 (0.08)	186.6 (0.29)			
	110		77.9 (0.46)	107.5 (5.02)			
	55	Ethanol	38.2 (0.15)	256.7 (0.58)	y = 0.4525x + 13.095	0.9991	81.6
	80		50.0 (0.53)	211.6 (2.04)			
	110		63.1 (0.23)	161.5 (0.87)			
	55	Hexane	5.68 (0.47)	381.1 (1.78)	y = 0.0655x + 1.8867	0.9665	734.6
	80		6.90 (0.70)	376.4 (2.69)			
	110		9.28 (0.15)	367.3 (0.58)			

Conc.: Concentration

Table 4.16. ABTS %inhibition, trolox and linearity data of *Cupressus* spp.

Sample	Conc (μg/100g)	Solvent	%Inhibition	Trolox Equivalent (mg/kg)	Equation	R^2	IC50 (μg/100g)
C.sempervirens L. var. *pyramidalis*	50	Water	65.8 (3.05)	164.1 (2.40)	y = 0.428x + 43.27	0.9682	15.7
	75		73.1 (0.55)	133.3 (2.33)			
	100		87.2 (0.62)	74.2 (2.62)			
	50	Ethanol	37.6 (0.56)	283.2 (0.87)	y = 1.058x + 13.063	0.9844	34.9
	75		69.8 (1.70)	143.2 (1.16)			
	100		90.5 (0.35)	60.3 (1.47)			
	50	Hexane	1.71 (2.84)	433.7 (0.82)	y = 0.2106x - 8.5483	0.9889	196.8
	75		7.89 (3.15)	407.5 (2.47)			
	100		13.5 (1.38)	389.4 (2.50)			
C.sempervirens L. var. *horizontalis*	50	Water	55.5 (1.42)	210.7 (1.75)	y = 0.615x + 25.772	0.9867	39.4
	75		73.9 (0.55)	129.8 (2.33)			
	100		86.2 (1.26)	75.4 (2.04)			
	50	Ethanol	89.1 (2.46)	60.5 (2.33)	y = 0.2105x + 73.384	0.9995	7.68
	75		94.1 (0.07)	45.1 (0.29)			
	100		99.6 (0.14)	22.1 (0.58)			
	50	Hexane	6.09 (2.08)	415.0 (8.73)	y = 0.5744x + 22.69	0.999	47.6
	75		20.3 (0.29)	355.5 (1.23)			
	100		34.8 (3.47)	294.3 (2.5)			
C.arizonica	50	Water	57.6 (0.32)	198.7 (1.34)	y = 0.6118x + 28.022	0.9863	35.9
	75		75.9 (0.80)	121.3 (3.36)			
	100		88.2 (0.76)	70.1 (2.26)			
	50	Ethanol	66.7 (1.27)	162.9 (1.75)	y = 0.6094x + 49.017	0.9297	1.62
	75		74.7 (1.13)	129.5 (1.46)			
	100		97.1 (0.35)	32.4 (1.47)			
	50	Hexane	14.9 (2.71)	377.9 (4.53)	y = 0.1548x + 6.7133	0.9599	279.6
	75		17.4 (4.38)	367.5 (2.88)			
	100		22.7 (1.06)	347.9 (4.45)			

Conc.: Concentration

Table 4.17. ABTS %inhibition, trolox and linearity data of *Eucalyptus* spp.

Sample	Conc (µg/100g)	Solvent	%Inhibition	Trolox Equivalent (mg/kg)	Equation	R^2	IC50 (µg/100g)
E.camaldulensis	50	Water	54.6 (0.81)	211.1 (3.41)	y = 0.9048x + 35.953	0.9161	15.5
	75		89.1 (1.12)	66.2 (4.71)			
	100		99.9 (1.88)	20.9 (0.38)			
	50	Ethanol	50.9 (0.69)	226.4 (2.91)	y = 0.9696x + 30.037	0.9471	20.6
	75		85.1 (1.16)	80.0 (0.29)			
	100		99.5 (0.60)	21.2 (0.29)			
	50	Hexane	69.8 (1.08)	140.6 (3.20)	y = 0.0988x + 65.459	0.8528	5.48
	75		74.1 (0.49)	129.4 (2.04)			
	100		74.8 (1.66)	119.0 (2.95)			
E.sideroxylon	50	Water	56.4 (0.69)	203.7 (2.91)	y = 0.664x + 23.487	0.9989	39.9
	75		73.9 (0.29)	129.9 (1.21)			
	100		89.6 (0.76)	62.6 (1.46)			
	50	Ethanol	49.4 (0.62)	233.1 (3.70)	y = 0.6236x + 18.21	1	50.9
	75		65.0 (2.26)	172.9 (1.16)			
	100		80.6 (1.32)	102.1 (3.70)			
	50	Hexane	36.9 (1.87)	285.5 (3.90)	y = 0.2702x + 21.725	0.8443	104.6
	75		38.6 (0.28)	278.2 (1.16)			
	100		50.4 (4.02)	228.8 (2.56)			

Conc.: Concentration

4.8.3. CUPRAC results of *Cupressus and Eucalyptus* spp.

4.8.3.1. CUPRAC results of *Cupressus* spp.

The CUPRAC trolox equivalent values of *C.sempervirens L.* var. *pyramidalis, C.sempervirens* L. var. *horizontalis*, and *C.arizonica* leaf samples are provided in Table 4.18. The table showed that the trolox values increased in parallel with an increase in extract concentration in all samples and solvents.

Table 4.18. CUPRAC trolox data of *Cupressus* spp.

Sample	Conc (mg/L)	Solvent	Trolox Equivalent (mg/kg)
C.sempervirens L. var. *pyramidalis*	50	Water	2.32 (0.27)
	75		8.72 (0.04)
	100		14.5 (0.87)
	50	Ethanol	8.74 (0.24)
	75		18.9 (0.24)
	100		29.3 (1.54)
	50	Hexane	17.8 (0.06)
	75		19.1 (0.61)
	100		28.5 (1.36)
C.sempervirens L. var. *horizontalis*	50	Water	5.81 (1.68)
	75		13.3 (1.37)
	100		18.1 (1.25)
	50	Ethanol	12.1 (0.53)
	75		23.5 (0.72)
	100		34.7 (0.50)
	50	Hexane	6.26 (1.69)
	75		11.3 (0.69)
	100		16.1 (0.59)
C.arizonica	50	Water	0.00 (0.00)
	75		3.69 (0.17)
	100		7.04 (0.03)
	50	Ethanol	10.6 (0.11)
	75		19.3 (0.68)
	100		28.9 (1.04)
	50	Hexane	7.85 (0.61)
	75		12.2 (0.71)
	100		22.2 (2.12)

Conc.: Concentration

4.8.3.2. CUPRAC results of *Eucalyptus* spp.

The Trolox equivalent values of some *Eucalyptus* leaf samples according to the CUPRAC method are depicted in Table 4.19. As in the *Cupressus* samples, the trolox values increased when the extract concentration increased. The Trolox value was found in the range of 10.5-38.2 µg/100g in *E.camaldulensis* and 0-21.3 µg/100g in *E.sideroxylon.*

Table 4.19. CUPRAC trolox data of *Eucalyptus* spp.

Sample	Conc (mg/L)	Solvent	Trolox Equivalent (mg/kg)
E.camaldulensis	50 75 100	Water	14.6 (0.83) 29.1 (0.34) 38.2 (0.47)
	50 75 100	Ethanol	10.5 (0.42) 20.2 (0.64) 30.1 (1.08)
	50 75 100	Hexane	19.5 (1.08) 24.4 (10.75) 31.3 (0.08)
E.sideroxylon	50 75 100	Water	5.37 (0.42) 6.20 (1.37) 9.75 (0.50)
	50 75 100	Ethanol	0.00 (0.00) 2.76 (0.95) 8.11 (1.01)
	50 75 100	Hexane	12.9 (3.73) 16.5 (3.14) 21.3 (1.96)

Conc.: Concentration

4.9. Results of Chemical Compounds by HPLC

Considering the results of the biologicial activities, the leaf part of the samples were solely determined by HPLC for the alkaloid, glycosidically bound, phenols and flavonoids compounds. The results obtained were analysed in this section.

4.9.1. Results of chemical compounds by HPLC in *Cupressus* spp.

In HPLC analysis with regard to *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, and *C.arizonica*, the alkaloid, glycosidically bound, phenols & flavonoids compounds of the leaf part of these plants were only analysed. The results for the alkaloid, glycosidically bound and phenols & flavonoids groups were shown.

4.9.1.1. Identification and quantification of alkaloid compounds in *Cupressus* spp.

Table 4.20 presented the alkaloid compounds of the *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, and *C.arizonica* leaf part.

Table 4.20. Alkaloid compounds of *Cupressus* spp.

		Alkaloid Compounds (ppm)			
Plant	**Plant Part**	**p-carboline**	**Quinine**	**Quinolone**	**Coiine**
C. sempervirens L. var. *pyramidalis*	Leaf	111.47	114.14	110.59	317.88
C. sempervirens L. var. *horizontalis*	Leaf	60.11	66.69	106.95	-
C. arizonica	Leaf	89.17	33.15	43.84	-

In this study, p-carboline, quinine, quinolone and coiine were determined as alkaloid compounds in *Cupressus* samples by using the HPLC method. As shown in Table 4.20, the highest chemical compounds were identified in *C.sempervirens L.* var. *pyramidalis* with p-carboline content (111.47 ppm), quinine (114.14 ppm), quinolone (110.59 ppm), and coiine (317.88 ppm).

4.9.1.2. Identification and quantification of glycosidically bound compounds in *Cupressus* spp.

Table 4.21 displayed the glycosidically bound compounds through the HPLC analysis of the leaf part in all species for *Cupressus* spp.

Table 4.21. Glycosidically bound compounds of of *Cupressus* spp.

		Glycosidically Bound Compounds (ppm)				
Plant	**Plant Part**	quercetin-3-o -glycosides	Apigenin-3-o –glycoside	Cupressuflavone	Amentoflavone	a-terpenyl acetate
C.sempervirens L. var. *pyramidalis*	Leaf	449.43	588.00	497.69	316.87	375.09
C.sempervirens L. var. *horizontalis*	Leaf	264.62	565.85	1200.26	504.57	272.94
C. arizonica	Leaf	341.15	776.29	367.93	695.9	271.69

In the HPLC analysis, five glycosidically bound compounds in *Cupressus* spp. leaf part were identified. The highest and lowest chemical compounds were indicated in cupressusflavone (1200.26 ppm) and quercetin-3-o –glycosides (264.62 ppm) in *C.sempervirens* L. var. *horizontalis*, respectively.

4.9.1.3. Identification and quantification of phenols & flavonoids compounds in *Cupressus* spp.

The phenolic and flavonoid compounds in *C.sempervirens* L. var. *pyramidalis, C.sempervirens* L. var. *horizontalis*, and *C.arizonica* (only leaf part) were determined by the HPLC analysis, as shown in Table 4.22.

Table 4.22. Phenols and flavonoids compounds of *Cupressus* spp.

		Phenols and Flavonoids (ppm)									
Plant	**Plant Part**	Gallic acid	Eiiagic acid	Catafeic acid	Taxifolin	Rutin	Quercetin	Kaemferol	Castalagin	Apigenin	**??**
C.sempervirens L. var. *pyramidalis*	Leaf	307.5	193.04	207.04	111.97	242.33	110.06	84.03	107.48	76.43	170.68
C.sempervirens L. var. *horizontalis*	Leaf	194.1	101.52	133.23	116.97	368.82	57.40	119.46	101.58	93.00	172.00
C. arizonica	Leaf	245.9	59.62	135.38	66.15	392.34	116.35	95.40	97.56	125.80	267.81

Through the HPLC method, the phenols and flavonoids compounds of *Cupressus* spp. leaf parts were identified (Table 4.22). Based on Table 4.22, rutin was discovered as the highest chemical compound (392.34 ppm) in *C.arizonica*.

4.9.2. Results of chemical compounds by HPLC in *Eucalyptus* spp.

The results of the alkaloid, glycosidically bound, phenols and flavonoids compounds in the leaf part of *E.camaldulensis* and *E.sideroxylon* plants according to the HPLC analysis are described in this Section 4.9.2.

4.9.2.1. Identification and quantification of alkaloid compounds in *Eucalyptus* spp.

Table 4.23 showed the alkaloid compounds in *E.camaldulensis* and *E.sideroxylon* leaf part based on the HPLC analysis.

Table 4.23. Alkaloid compounds of *Eucalyptus* spp.

		Alkaloid Compounds (ppm)		
Plant	**Plant Part**	**Chelythrine**	**Berberine**	**Sanquinarine**
E.camaldulensis	Leaf	98.59	344.16	134.01
E.sideroxylon	Leaf	66.76	118.21	39.43

Using the HPLC method, chelythrine, berberine, and sanquinarine were determined as alkaloid compounds in *Eucalyptus* sample. The highest alkaloid compounds were found in *E.camaldulensis* with chelythrine (98.59 ppm), berberine (344.16 ppm), and sanquinarine (134.01 ppm), as shown in Table 4.23.

4.9.2.2. Identification and quantification of glycosidically bound compounds in *Eucalyptus spp.*

The glycosidically bound compounds results of the *Eucalyptus* spp. leaf part are shown in Table 4.24.

Table 4.24. Glycosidically bound compounds of *Eucalyptus* spp.

		Glycosidically Bound Compounds (ppm)		
Plant	**Plant part**	**Qurercetin-3-arabinoside**	**Quercetin -3,7-di-arhamanose**	**Kaempferol-3-arabinoside**
E. camaldulensis	Leaf	211.87	318.59	431.31
E. sideroxylon	Leaf	272.14	345.46	442.89

Based on Table 4.24, kaempferol-3-arabinoside (442.89 ppm) in *E. sideroxylon* was identified as the highest compound.

4.9.2.3. Identification and quantification of phenols and flavonoids compounds in *Eucalyptus* spp.

Phenols and flavonoids compounds of the leaf part of *E.camaldulensis* and *E.camaldulensis* were determined by the HPLC analysis.

Table 4.25. Phenols & flavonoid compounds of *Eucalyptus* spp.

		Phenols and Flavonoids (ppm)						
Plant	**Plant Part**	**Tannin**	**Gallic acid**	**Gentisic acid**	**Ellagic acid**	**Rutin**	**Quercetin**	**Eucalyptone**
E. camaldulensis	Leaf	210.58	209.90	99.01	41.73	182.87	109.75	207.60
E. sideroxylon	Leaf	180.40	81.11	117.35	107.5	125.98	207.31	245.03

HPLC analysis detected seven phenols and flavonoid compounds in *Eucalyptus* spp. leaf part. As shown in Table 4.25, eucalyptone (245.03 ppm) in *E.sideroxylon* and ellagic acid (41.73 ppm) in *E.camaldulensis* were the higest and lowest chemical compounds, respectively.

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5. DISCUSSION

5.1. Evaluation of extract yield results

The effect of plant part (stem and branch) and solvent type (hot water, ethanol, and methanol) on the extract yield results of *Cupressus* and *Eucalyptus* spp. was assessed using multiple variance using the SPSS program. When a substantial difference ($p \leq 0.05$) was observed, the Tukey B test was used to define the homogenous groups. The Tukey post hoc (B) test could not be carried out in this study for the plant part, since the factors are less than three. For this reason, the t-test was employed in this study to identify a significant difference between the results of the plant part.

5.1.1. Evaluation of the effects of plant part and solvent type on the extract yield of *Cupressus* spp.

Tables 5.1 and 5.2, and Figure 5.1 show the extract yield results of *Cupressus* spp. based on the GLM-Univariate analysis results. The impact of plant part (leaf, branch), solvent type (hot water, ethanol, and methanol), and the relationship between these two factors on the extract yield results of all *Cupressus* spp. which was significant at the 95% confidence range (p=0.000), are depicted in Table 5.1.

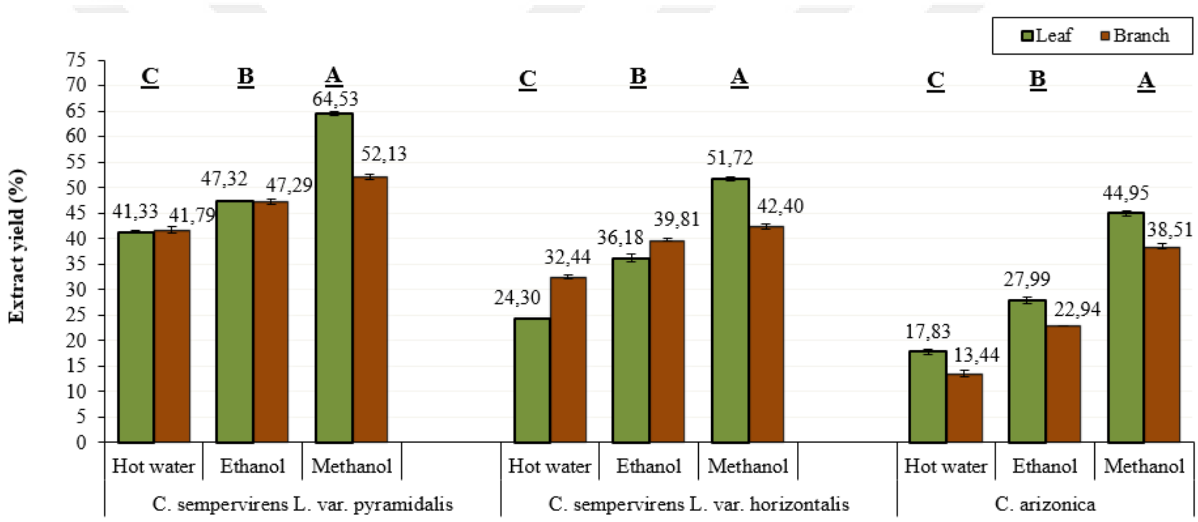
Table 5.1. GLM - Univariate analysis of *Cupressus* spp. extract yield results

Plant	GLM - Univariate Analysis		
	Tests of Between-Subjects Effects	**Sig.**	**Difference**
C.sempervirens L. var. *pyramidalis*	*Part*	0.000	+
	Solvent type	0.000	+
	Part Solvent type*	0.000	+
C.sempervirens L. var. *horizontalis*	*Part*	0.000	+
	Solvent type	0.000	+
	Part Solvent type*	0.000	+
C.arizonica	*Part*	0.000	+
	Solvent type	0.000	+
	Part Solvent type*	0.001	+

Based on the Tukey B analysis results, the maximum extract yield was exhibited in methanol (as in Table 5.2 and Figure 5.1), followed by ethanol and hot water in all *Cupressus* species. The highest yield in methanol extraction was found in the leaf. Accordingly, the extact yield of the leaf of *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, and *C.arizonica* was 64.53%, 51.72%, and 44.95%, respectively.

Table 5.2. Post Hoc analysis of *Cupressus* spp. extract yield results

Plant	Plant Part	Solvent Type		
		Water	**Ethanol**	**Methanol**
C. sempervirens L. var. *pyramidalis*	Leaf	41.33 (0.33) **A**	47.32 (0.02) **A**	64.53 (0.32) **A**
	Branch	41.79 (0.68) **A**	47.29 (0.45) **A**	52.13 (0.45) **B**
	Tukey B	**C**	**B**	**A**
C.sempervirens L. var. *horizontalis*	Leaf	24.30 (0.01) **B**	36.18 (0.74) **B**	51.72 (0.32) **A**
	Branch	32.44 (0.43) **A**	39.81 (0.26) **A**	42.40 (0.59) **B**
	Tukey B	**C**	**B**	**A**
C.arizonica	Leaf	17.83 (0.47) **A**	27.99 (0.68) **A**	44.95 (0.60) **A**
	Branch	13.44 (0.63) **B**	22.94 (0.04) **B**	38.51 (0.45) **B**
	Tukey B	**C**	**B**	**A**



Note: Different letters in each plant species represent statistical differences at the 95% confidence level

Figure 5.1. Extract yield of different plants parts of *Cupressus* spp. in different solvents

With regard to t-test analysis of the effect of plant part on the extract yield results, it was found that the best yield results in all solvents were exhibited in leaf of *C.arizonica*. For instance, extract yield results were between 17.83 to 44.95% in leaf compared to 13.44 to 38.51% in branch.

In Tümen et al.'s (2012) study, the extract yield of *C.sempervirens* L. var. *pyramidalis* and *C.sempervirens* L. var. *horizontalis* leaves were found in methanol 9.21-13.38%. Such findings are lower than the findings of the current study (51.72% - 64.53%).

A previous study by Rahmani (2019) showed that the extract yield of *C.sempervirens* leaves was found in water 10.43%, and in methanol 27.71%. The current study, however reveal higher results than of Rahmani's (2019) study.

5.1.2. Evaluation of the effects of plant part and solvent type on the extract yield of *Eucalyptus* spp.

GLM-Univariate analysis results based on *Eucalyptus* extract yields are shown in Table 5.3. The effect of plant part (leaf, branch), solvent type (hot water, ethanol, methanol), and the interaction between these two factors on the extract yield results of all *Eucalyptus* spp. which was significant at the 95% confidence range (p=0.000).

Table 5.3 GLM - Univariate analysis of *Eucalyptus* spp. extract yield results

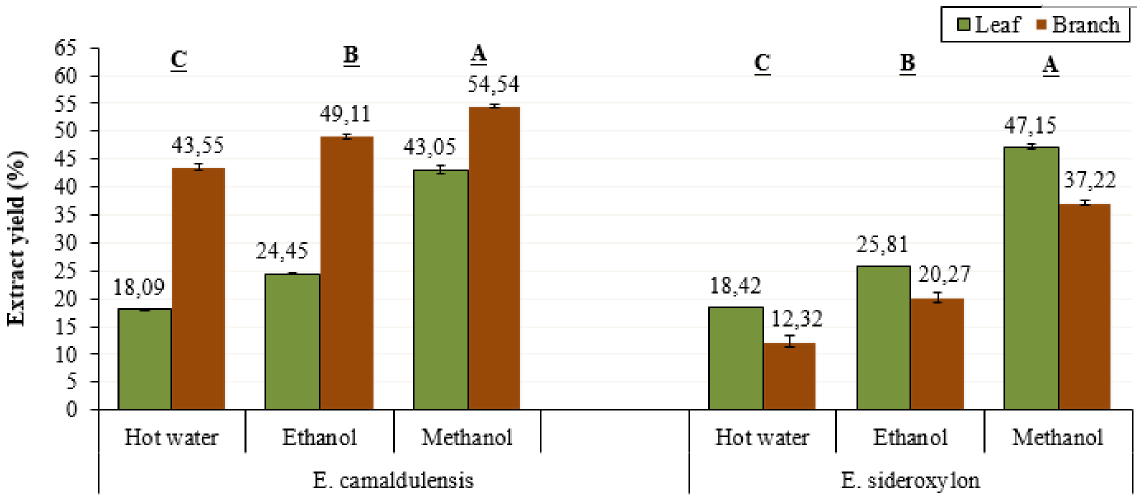
Plant	GLM- Univariate analysis		
	Tests of Between-Subjects Effects	Sig.	Difference
E.camaldulensis	*Part*	0.000	+
	Solvent type	0.000	+
	Part Solvent type*	0.000	+
E.sideroxylon	*Part*	0.000	+
	Solvent type	0.000	+
	Part Solvent type*	0.000	+

Among all *Eucalyptus* spp., the largest extract yield was exhibited in methanol, as depicted in Table 5.4. *E.camaldulensis* and *E.sideroxylon* had a methanol extraction yield of 43.05 to 54.54% and 37.22 to 47.15%, respectively.

Table 5.4. Post Hoc analysis of *Eucalyptus* spp. extract yield results

Plant	Plant Part	Solvent Type		
		Water	Ethanol	Methanol
E.camaldulensis	Leaf	18.09 (0.14) **B**	24.45 (0.09) **B**	43.05 (0.76) **B**
	Branch	43.55 (0.54) **A**	49.11 (0.45) **A**	54.54 (0.24) **A**
	Tukey B	**C**	**B**	**A**
E.sideroxylon	Leaf	18.42 (0.03) **A**	25.81 (0.09) **A**	47.15 (0.45) **A**
	Branch	12.32 (1.00) **B**	20.27 (0.87) **B**	37.22 (0.37) **B**
	Tukey B	**C**	**B**	**A**

According to Post Hoc analysis as shown in Table 5.4 and Figure 5.2, the best extract yield (43.55 - 54.54%) was exhibited in the branch of *E.camaldulensis* for all types of solvents. As for *E.sideroxylon*, the highest extract yield (18.42 - 47.15%) was indicated in the leaf for all types of solvents.



Note: Different letters in the each plant species represent statistical differences at the 95% confidence level

Figure 5.2. Extract yield of different plants parts of *Eucalyptus* spp. in different solvents

The results from Gullón et al.'s (2017) study showed that the extraction yield of *E.globulus* leaves (35.7%) was achieved at 50 °C, 300 min and 80% ethanol concentrations. Such results in Gullón et al.'s (2017) study were higher compared to the findings of the current study (24.45-25.81%).

In addition, the total ethanol and methanol extraction yield of *E.globulus* leaves according to Rodrigues et al.'s (2018) study was 25.90% wt and 30.34% wt, respectively. These findings are consistent with the results of the current study.

The results of the current study are also close to the previous study by Alghoraibi et al. (2020). For instance, the yield percentage (17.0%) of the *E.camaldulensis* leaves for water was depicted in Alghoraibi et al.'s (2020) study.

In a study by Alghamdi et al. (2019), the extract yield of *E.camaldulensis* leaves was indicated with 40.2, 34.8, and 22.2g for methanol, ethanol, and water extracts, respectively.

The extract yield percentage of *E.camaldulensis* leaves was also discovered in Dawoud et al.'s (2015) study. According to Dawoud et al.'s (2015) study, the highest extract yield (9.34%) was exhibited in the ethanolic extract, followed by water extract (6.54%). However, the chloroform and petroleum ether extracts yield were indicated with 3.82% and 1.45%, respectively. In relation to the present study, the water and ethanol extracts showed great yield results (18.09-24.45%).

5.2. Evaluation of the antimicrobial activity results

The hot water, ethanol, and methanol extracts of the leaf and branch parts of *Cupressus* and *Eucalyptus* at concentrations 25%, 50% and 75% were employed in the antimicrobial activity test in this study. Moreover, *S.aureus*, *E.faecalis, E.coli*, and *K.pneumonia* were the bacteria used in the study. The effect of antimicrobial activity arithmetic means obtained from each bacterium, leaf, branch and extract solution at concentrations 25%, 50%, 75% was assessed using the SPSS program, at 95% confidence intervals in GLM-Univariate analysis. The Post Hoc Tukey-B test to evaluate antimicrobials was employed to generate homogeneous groups arithmetic average at the 95% confidence intervals when a significant (p=0.000) statistical result indicated in this study. The statistical evaluations for *Cupressus* and *Eucalyptus* spp. are discussed in this section.

5.2.1. Evaluation of *Cupressus* spp. antimicrobial activity results

The antimicrobial activity results of *Cupressus* spp. based on the GLM-Univariate analysis results are presented in Table 5.5. The impact of plant part (leaf, branch), extract concentrations (25%, 50%, and 75%), and the interaction between these two factors on the antimicrobial activity results of all *Cupressus* spp. which was significant at the 95% confidence range (p=0.000-0.048), are discussed in this subsection.

5.2.1.1. Evaluation of *C.sempervirens* L. var. *pyramidalis* antimicrobial activity results

Post Hoc analysis of the *C.sempervirens* L. var. *pyramidalis* antimicrobial activity results are provided in Table 5.6.

5.2.1.1.1. Evaluation of the effects of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* L. var. *pyramidalis* hot water extracts

Tables 4.3, 5.6, and Figure 5.3 illustrate the antimicrobial activity results of the hot water extracts *of C.sempervirens* L. var. *pyramidalis*. According to the GLM Univariate analysis results, the effect of plant part (leaf, branch), extract concentrations (25%, 50%, 75%), and the interaction between these two factors at the 95% confidence range, the antimicrobial results of *S.aureus*, *E.faecalis*, *K.pneumonia*, and *E.coli* were significant (p=0.0-0.01) (Table 5.5).

Based on the Tukey B homogeneity analysis results, in the antimicrobial tests using four different bacteria, the best results were shown at concentration 75% (2-3 mm).

Table 5.5. GLM - Univariate analysis of *Cupressus* spp. antimicrobial activity results

			S.aureus		***E. faecalis***		***E.coli***		***K. pneumonia***	
Plant	**Solvent Type**	**Tests of Between-Subjects Effects**	**Sig.**	**Difference**	**Sig.**	**Difference**	**Sig.**	**Difference**	**Sig.**	**Difference**
C.sempervirens* L. var. *pyramidalis	**Water**	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.010	+	0.000	+	0.002	+
		Part Concentration*	0.000	+	0.010	+	0.000	+	0.002	+
	Ethanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.000	+	0.000	+	0.026	+	0.010	+
	Methanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.001	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.025	+	0.010	+	0.048	+	0.075	-
C.sempervirens* L. var. *horizontalis	**Water**	*Part*	0.025	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.010	+	0.000	+	0.000	+	0.000	+
	Ethanol	*Part*	0.236	-	0.000	+	0.025	+	0.000	+
		Concentration	0.001	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.250	-	0.000	+	0.000	+	0.002	+
	Methanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.000	+	0.002	+	0.010	+	0.002	+
C.arizonica	**Water**	*Part*	0.100	-	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.002	+	0.000	+	0.000	+
		Part Concentration*	0.075	-	0.002	+	0.000	+	0.000	+
	Ethanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.010	+	0.010	+	0.010	+	0.010	+
	Methanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.010	+	0.025	+	0.000	+	0.010	+

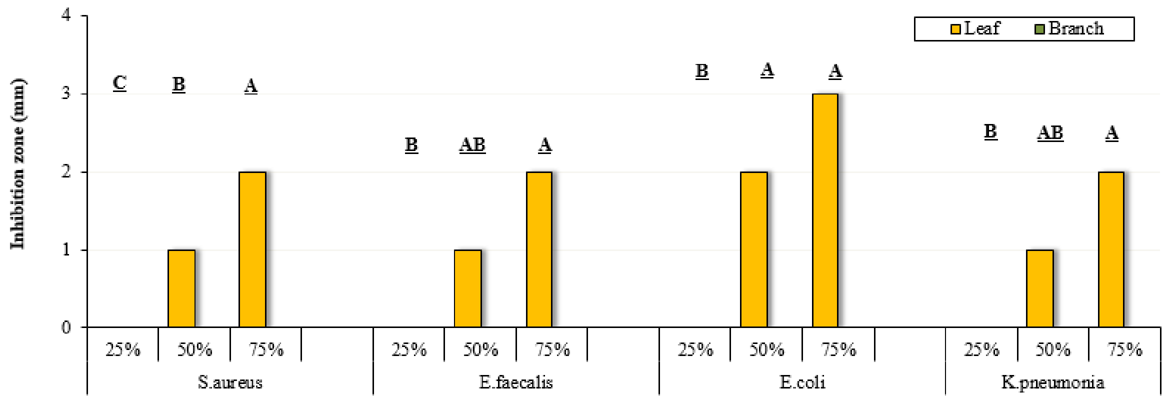
Table 5.6. Post Hoc analysis of *C.sempervirens* L. var. *pyramidalis* antimicrobial activity results

		S.aureus			***E. faecalis***			***E.coli***			***K. pneumonia***		
		Concentration			**Concentration**			**Concentration**			**Concentration**		
	Plant Part	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**
WATER	**Leaf**	-	1.0 (0.0)	2.0 (0.8) **A**	-	1.0 (0.0)	2.0 (1.4) **A**	-	2.0 (0.8) **A**	3.0 (0.8) **A**	-	1.0 (0.8) **A**	2.0 (0.8) **A**
	Branch	-	-	- **B**	-	-	- **A**	-	- **B**	- **B**	-	- **B**	- **B**
	Tukey B	**C**	**B**	**A**	**B**	**AB**	**A**	**B**	**A**	**A**	**B**	**AB**	**A**
ETHANOL	**Leaf**	-	2.0 (0.8) **A**	3.0 (0.8) **A**	-	1.0 (0.0)	3.0 (0.8) **A**	1.0 (0.8) **A**	2.0 (0.8) **A**	4.0 (0.8) **A**	1.0 (0.0)	1.0 (0.0)	3.0 (0.8) **A**
	Branch	-	- **B**	1.0 (0.0) **B**	-	-	- **B**	- **B**	- **B**	1.0 (0.8) **B**	-	-	1.0 (0.0) **B**
	Tukey B	**C**	**B**	**A**	**C**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**
METHANOL	**Leaf**	1.0 (0.8) **A**	2.0 (0.8) **A**	3.0 (0.8) **A**	1.0 (0.0)	1.0 (0.8) **A**	3.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**	6.0 (0.8) **A**	1.0 (0.0)	1.0 (0.8) **A**	3.0 (0.0) **A**
	Branch	- **B**	- **B**	1.0 (0.8) **B**	-	- **B**	1.0 (0.0)	- **B**	1.0 (0.8) **B**	2.0 (0.8) **B**	-	- **B**	1.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**C**	**B**	**A**	**B**	**B**	**A**

(-) No inhibition

Based on the t-test analysis results as shown in Table 5.6, the findings revealed that the leaf part of the plant was more effective in the *S.aureus, E.faecalis, E.coli,* and *K.pneumonia* trials. This value was in the range of 2-3 mm.

In a study, the antimicrobial activity of *C.sempervirens* L. *pyramidalis* leaf and branch against *S.aureus* and *E.coli* was investigated. The findings in the *S.aureus* tests depicted the inhibition values of 6 to 8 mm and 6.3 to 12.4 mm of the leaf and branch, respectively compared to the inhibition values of 13.7 mm and 8.7 to 11.7 mm of the leaf and branch, respectively in the *E.coli* tests (Zouaghi et al., 2015). With regard to the findings of the current study, it was revealed that inhibition against bacteria was shown solely in the leaf.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.3. Antimicrobial activity of *C.sempervirens* L. var. *pyramidalis* hot water extract

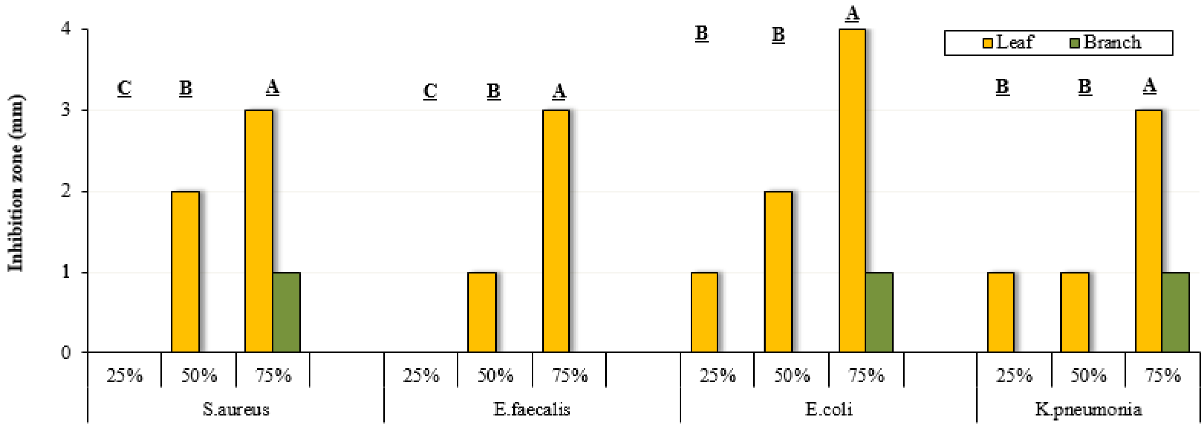
5.2.1.1.2. Evaluation of the effects of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* L. var. *pyramidalis* ethanol extracts

This subsection discusses the effect of plant part (leaf, branch), extract concentrations (25%, 50%, and 75 %), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.026) in the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumoniae*, based on the GLM Univariate analysis result (Table 5.5). Tables 4.3, 5.6, and Figure 5.4 illustrate the antibacterial activity of the *C.sempervirens* L. var. *pyramidalis* ethanol extracts.

Based on the Tukey-B test, the best results were achieved at extract concentration of 75%. This was depicted with the value 3.0 mm was achieved for *E.faecalis*, *S.aureus*, and *K.pneumoniae*, whereas the value 4.0 mm was achieved for *E.coli*.

In general, the effect of plant part on the antimicrobial activity in t-test analysis showed the highest inhibition value against *E.coli* was detected in the leaf. This value was in the range of 1 to 4 mm.

A study by Chaudhary et al. (2012) investigated the ethanol extract of *C.sempervirens* L. var. *pyramidalis* leaf against *S.aureus* and *E.coli* strains. This study found the inhibition values of 23.25 mm and 18.5 mm in *S.aureus* and *E.coli*, respectively.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.4 Antimicrobial activity of *C.sempervirens* L. var. *pyramidalis* ethanol extract

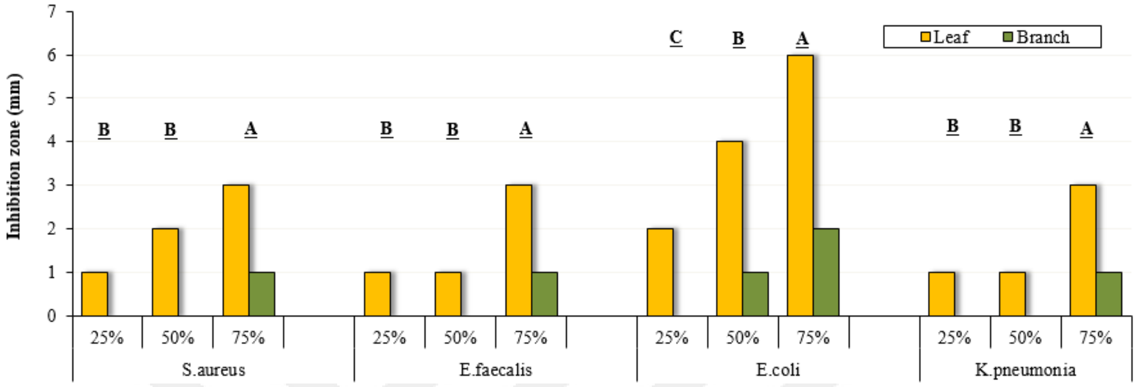
5.2.1.1.3. Evaluation of the effects of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* L. var. *pyramidalis* methanol extracts

The antimicrobial activity results *of C.sempervirens* L. var. *pyramidalis* methanol extracts are shown in Tables 4.3, 5.6, and Figure 5.5. The effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors on the antimicrobial activity results of all testes bacterias were significant at the 95% confidence range (p=0.000-0.0048).

Referring to Table 5.6 and Figure 5.5 which show the antimicrobial activity of *C.sempervirens* L. var. *pyramidalis* according to the Tukey-B test, the best results (around 3-6 mm inhibition zone) were achieved at concentration 75% with methanol extraction against all bacteria.

The results of the leaf and branch plant parts of each bacterium were compared using the t-test. The results depicted a difference between the two parts. Overall, the leaf part was more effective against bacteria (1-6 mm).

In a previous study by Chaudhary et al. (2012), the activity of the leaf part of *C.sempervirens* L. var. *pyramidalis* methanol extracts against each of *S.aureus* and *E.coli* strains was assessed. The results of Chaudhary et al.'s (2012) study showed inhibition values of 23.25 mm and 18.5 mm in *S.aureus* and *E.coli,* respectively. Such findings are different from the current study which showed lower values.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.5. Antimicrobial activity of *C.sempervirens* L. var. *pyramidalis* methanol extract

5.2.1.2. Evaluation of *C.sempervirens* var. *horizontalis* antimicrobial activity results

The results of the antimicrobial activity of *C.sempervirens* var. *horizontalis* against *S.aureus, E.faecalis, E.coli, and K.pneumonia* are shown in Table 5.7

5.2.1.2.1. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* var. *horizontalis* hot water extracts

Tables 4.3, 5.7 and Figure 5.6 illustrate the antimicrobial activity results of *C.sempervirens* var. *horizontalis* hot water extracts. According to the GLM Univariate analysis result, the effects of plant part (leaf, branch), extract concentrations (25, 50, 75 %), and the interaction between these two factors at the 95% confidence range on antimicrobial results of S.aureus, E.faecalis, K.pneumonia, and E.coli were significant (p=0.00-0.025) (Table 5.5).

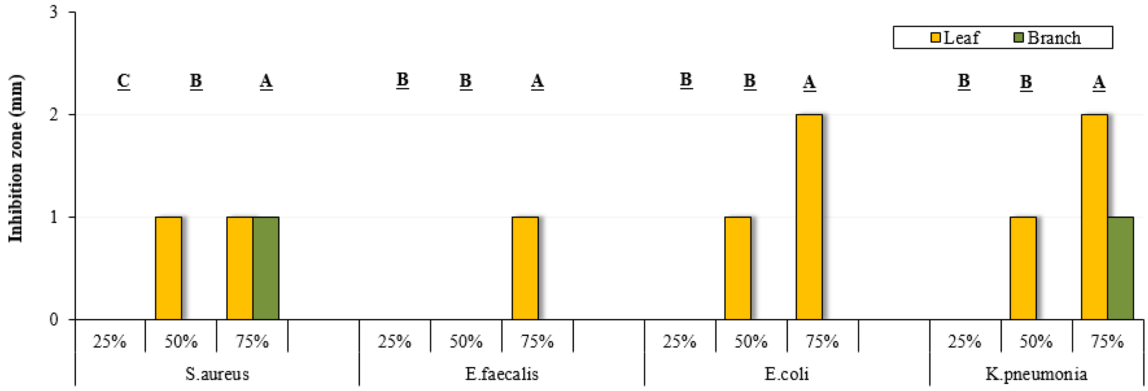
Based on the Tukey-B homogeneity analysis results, in the antimicrobial tests using four different bacteria, it was discovered that the best results were at 75% extract concentration (1-2 mm).

According to the t-test analysis results provided in Table 5.7, it was found that leaf part of the plant was more effective. This value was in the range of 1 to 2 mm.

Table 5.7. Post Hoc analysis of *C.sempervirens* L. var. *horizontalis* antimicrobial activity results

		S.aureus Concentration			***E. faecalis*** Concentration			***E.coli*** Concentration			***K. pneumonia*** Concentration		
	Plant part	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**
WATER	**Leaf**	-	1.0 (0.8) **A**	1.0 (0.0)	-	-	1.0 (0.0)	-	1.0 (0.8) **A**	2.0 (0.8) **A**	-	1.0 (0.0)	2.0 (0.0)
	Branch	-	- **B**	1.0 (0.0)	-	-	-	-	- **B**	- **B**	-	-	1.0 (0.0)
	Tukey B	**C**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**
ETHANOL	**Leaf**	1.0 (0.0)	1.0 (0.8) **A**	2.0 (0.0) **A**	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.0)	3,.0 (0.8) **A**	-	1.0 (0.0)	3.0 (0.8) **A**
	Branch	-	1.0 (0.0) **A**	2.0 (1.4) **A**	-	-	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0) **B**	-	-	1.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**B**	**B**	**A**
METHANOL	**Leaf**	-	1.0 (0.8) **A**	3.0 (0.8) **A**	-	1.0 (0.0)	3.0 (0.8) **A**	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**	-	1.0 (0.0)	3.0 (0.8) **A**
	Branch	-	- **B**	- **B**	-	-	1.0 (0.8) **B**	-	- **B**	1.0 (0.8) **B**	-	-	1.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**

(-) No inhibition



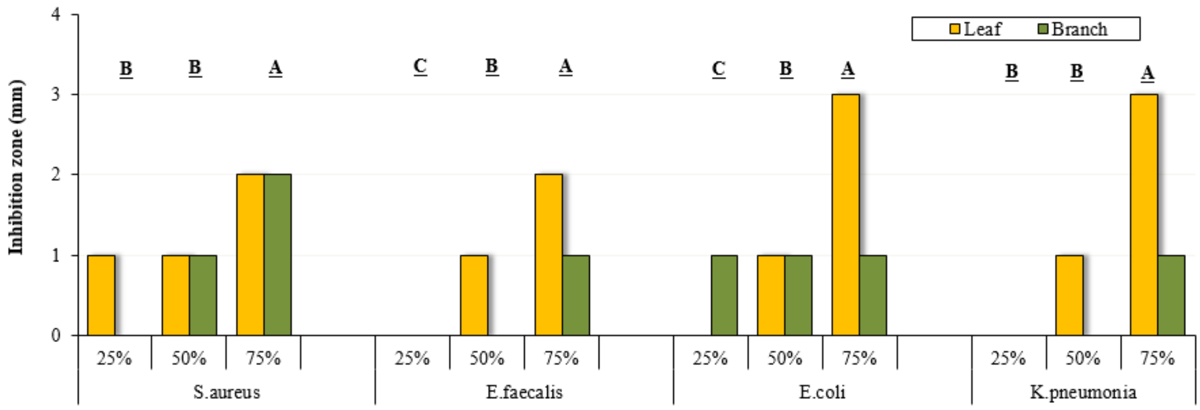
Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.6. Antimicrobial activity of *C.sempervirens* var. *horizontalis* hot water extract

Afsharzadeh et al. (2013) investigated the antibacterial effect of *C.sempervirens* var. *horizontalis* essential oil. In Afsharzadeh et al.'s (2013) study, the results depicted the inhibition values of 18.63 mm and 20.5 mm in *E.coli* and *S.aureus*, respectively.

5.2.1.2.2. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* var. *horizontalis* ethanol extracts

This section discusses the effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.025) on the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, *and K.pneumoniae* according to the GLM Univariate analysis result (Table 5.5). Tables 4.3 and 5.7, and Figure 5.7 presented the antibacterial activity results of *C.sempervirens* var. *horizontalis* ethanol extract.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.7. Antimicrobial activity of *C.sempervirens* var. *horizontalis* ethanol extract

The best results (between 1-3 mm) were achieved at 75% extract concentration as in the Tukey-B test.

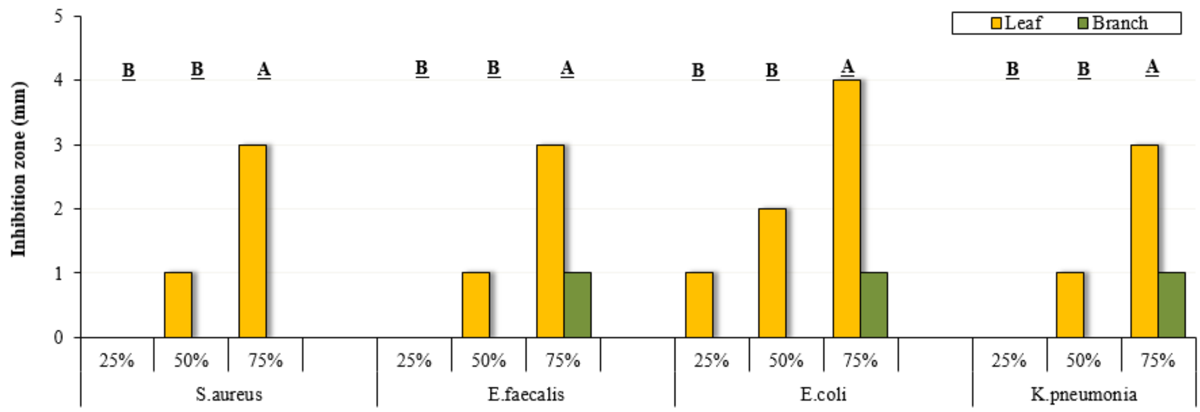
In general, the effect of plant part on the antimicrobial activity was assesed using the t-test. The findings show the highest inhibition zone against *E.coli* and *K.pneumoniae* was detected in the leaf. This value was in the range of 1 to 3 mm.

A study by Eryılmaz et al. (2016) showed the activity of the leaf part of *C.sempervirens L*. var. *horizontalis* ether extracts against each of *S.aureus*, *E.coli*, and *K.pneumoniae* strains. It was found that the inhibition value 7 mm was for *S.aureus*, and *E.coli* while 8 mm for *K.pneumoniae*. These values in Eryılmaz et al. (2016)'s study were found to be higher compared to the value 3mm depicted in the current study.

5.2.1.2.3. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.sempervirens* var. *horizontalis* methanol extracts

The antimicrobial activity results of *C.sempervirens* var. *horizontalis* extracts are shown in Tables 4.3 and 5.6, and Figure 5.5. This subsection discusses the GLM Univariate analysis results (Table 5.5), effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range, which was in general significant on the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumoniae* (p=0.000-0.010).

According to the Tukey-B test results as in the Table 5.7 and Figure 5.8, the best results were achieved at concentration 75% in methanol solution against all bacteria, which was around 1 to 4 mm inhibition value.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.8. Antimicrobial activity of *C.sempervirens* var. *horizontalis* methanol extract

The results of the leaf and branch plant parts of each bacterium were compared using the the t-test. The results showed that there was a difference between the two parts. Overall, the leaf part was more effective against all bacteria (1-4 mm).

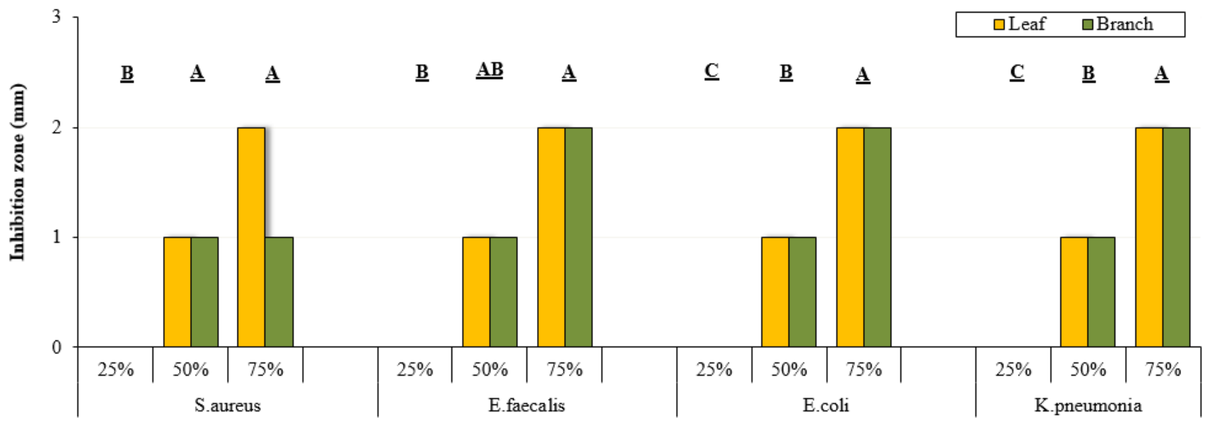
The findings of Ulukanli et al.'s (2014) study showed that the antibacterial activity of *C.sempervirens* var. *horizontalis* had a significant antibacterial activity against most tested *E.coli* and *S.aureus*. The results showed the inhibition values of 15.66 mm and 29.0 mm for *E.coli* and *S.aureus*, respectively. On the other hand, the present study revealed that the leaf part was less effective against bacteria.

5.2.1.3. Evaluation of *C.arizonica* antimicrobial activity results

The antimicrobial activity and Tukey-B statistical analysis results of *C.arizonica* are shown in Table 5.8. The antimicrobial activity test results of the hot water, ethanol and methanol extracts are disscussed in this subsection.

5.2.1.3.1. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.arizonica* hot water extracts

Tables 4.3 and 5.8, and Figure 5.9 presented the antimicrobial activity results of *C.arizonica* hot water extract. This subsection discusses GLM Univariate analysis result, the effects of plant part (leaf, branch), extract concentration (25, 50, 75%), and relationshion between these two factors at the 95% confidence range on the antimicrobial results of *S.aureus*, *E.faecalis*, *K.pneumonia*, and *E.coli*, which was significant (p=0.00-0.002) (Table 5.5).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.9. Antimicrobial activity of *C.arizonica* hot water extract

Table 5.8. Post Hoc analysis of *C.arizonica* antimicrobial activity results

		S.aureus			***E.faecalis***			***E.coli***			***K. pneumonia***		
		Concentration (%)			**Concentration (%)**			**Concentration (%)**			**Concentration (%)**		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
WATER	**Leaf**	-	1.0 (0.0)	2.0 (0.8) **A**	-	1.0 (0.2) **A**	2.0 (0.8) **A**	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.0)	2.0 (0.0)
	Branch	-	1.0 (0.0)	1.0 (0.8) **A**	-	- **B**	- **B**	-	-	1.0 (0.0)	-	-	1.0 (0.0)
	Tukey B	**B**	**A**	**A**	**B**	**AB**	**A**	**C**	**B**	**A**	**C**	**B**	**A**
ETHANOL	**Leaf**	1.0 (0.8) A	1.0 (0.0) **A**	3.0 (0.8) **A**	-	1.0 (0.8) **A**	3.0 (0.8) **A**	1.0 (0.0)	2.0 (0.8) A	4.0 (0.0)	1.0 (0.0)	1.0 (0.8) **A**	3.0 (0.0)
	Branch	- B	1.0 (0.2) **A**	1.0 (0.0) **B**	-	- **B**	1.0 (0.8) **B**	-	1.0 (0.0) B	2.0 (0.0)	-	- **B**	1.0 (0.0)
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**C**	**B**	**A**	**B**	**B**	**A**
METHANOL	**Leaf**	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**	1.0 (0.8) **A**	2.0 (0.8) **A**	4.0 (0.8) **A**	1.0 (0.0)	3.0 (0.8) **A**	6.0 (0.0)	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**
	Branch	-	- **B**	1.0 (0.8) **B**	- **B**	1.0 (0.0) **B**	2.0 (0.2) **B**	-	1.0 (0.8) **B**	2.0 (0.0)	-	- **B**	1.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**B**	**B**	**A**

(-) No inhibition

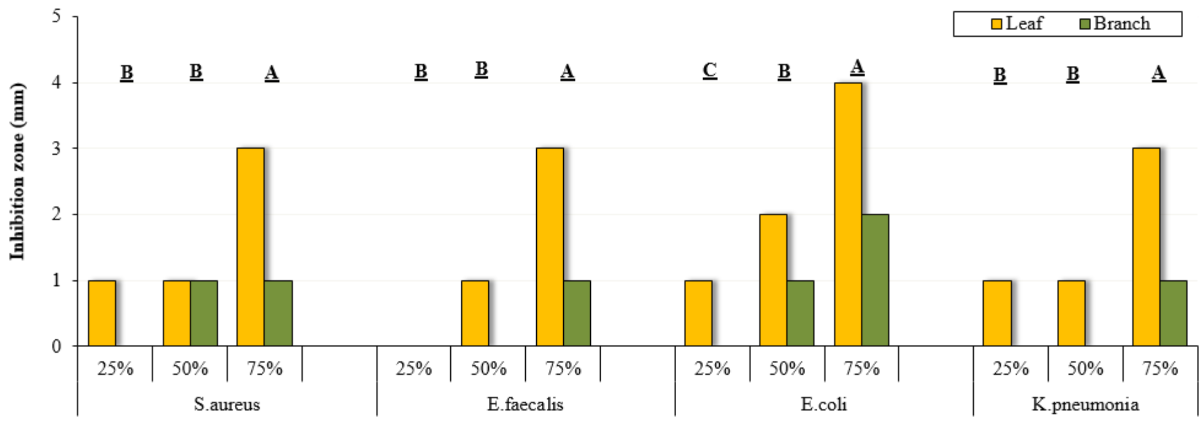
According to Tukey-B homogeneity analysis results, in the antimicrobial tests using four different bacteria, it was revealed that the best results were indicated at 75% extract concentration (1-2 mm).

As per the t-test analysis results in Table 5.8, it was discovered that the leaf part of the plant was generally effective. This value was in the range of 1 to 2 mm.

A study by Chéraif et al. (2007) found that the essential oil of *C.arizonica Greene* has antibacterial effect. The inhibition values 27.0 mm, 18.0mm, 17.0mm and 11.0mm were indicated in *E.coli*, *S.aureus*, *K.pneumoniae*, and *E.faecalis*, respectively.

5.2.1.3.2. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.arizonica* ethanol extracts

This subsection discusses the effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.026) on the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumoniae*, according to the GLM Univariate analysis result (Table 5.5). Tables 4.3 and 5.8, and Figure 5.10 depict the antibacterial activity of the *C.sempervirens* L. var. *pyramidalis* ethanol extract.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.10. Antimicrobial activity of *C.arizonica* ethanol extract

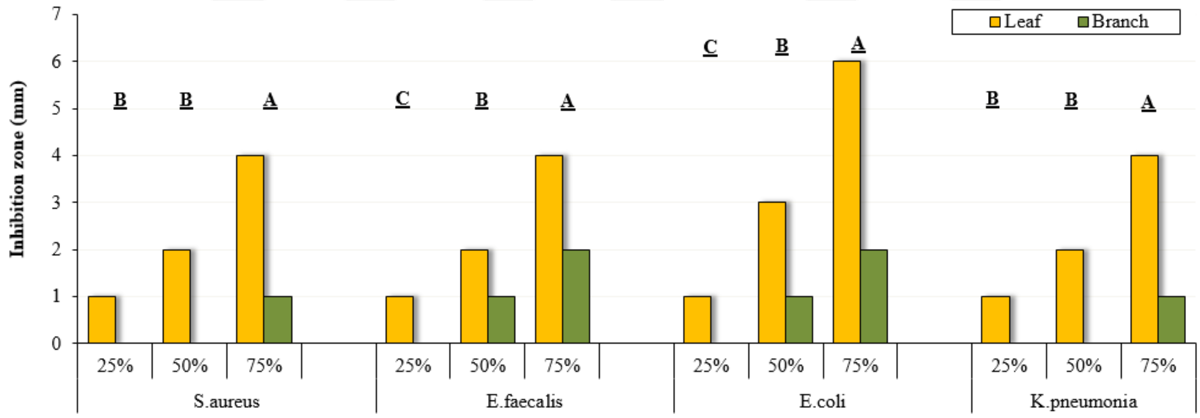
The best results were achieved at 75% extract concentration based on the Tukey-B test, depicted with the values of 2.0-4.0 mm for *E.coli* and 1.0-3.0 mm for *E.faecalis*, *S.aureus*, and *K.pneumoniae*.

Overall, the effect of plant part on the antimicrobial activity results analysed using the t-test. Analysis, showed the highest inhibition zone against *S.aureus* and *E.faecalis* was detected in the leaf. This value was in the range of 1 to 3 mm.

The use of *C.arizonica* extracts leaf as traditional medicine was investigated in Al-Mouhajer et al.,'s (2017) study. Essential oil exhibited a potent inhibitory effect on the growth of different strain of *E.coli*. The results of that study showed 10 mm inhibition zone. These values in Al-Mouhajer et al.,'s (2017) study were found to be higher compared to the value of 1 to 4 mm depicted in the current study.

5.2.1.3.3. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *C.arizonica* methanol extracts

The antimicrobial activity results of the *C.arizonica* methanol extract are shown in Table 4.3, 5.8 and Figure 5.11. This subsection discusses the GLM Univariate analysis result (as in Table 5.5), effect of plant part (leaf, branch), extract concentration (25, 50 and 75%), and the interaction between these two factors at the 95% confidence range, which was significant on the antimicrobial results of *E.faecalis* and *K.pneumoniae*.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.11. Antimicrobial activity of *C.arizonica* methanol extract

Referring to the Tukey-B test (Table 5.8 and Figure 5.11) that showed the antimicrobial activity of *C.arizonica*, it was found that around 4-6 mm inhibition value signified the best results achieved at 75% concentration of methanol extraction against all bacteria.

Using the t-test which compared the results of the leaf and branch plant parts of each bacterium, it was discovered that there was a difference between the two parts. Generally, the leaf part was more effective against bacteria (1-6 mm).

Previous study by Padhaya et al. (2019) showed that the antibacterial activity of the *Cupressus torulosa* leaf extract had significant antibacterial activities against *E.coli* and *S.aureus*. The result showed inhibition zone for *E.coli* (7mm-25mg/ml) and *S.aureus* (9mm-25mg/ml). The findings of Padhaya et al.'s (2019) study are inconsistent with the findings of the current study. For instance, the inhibition value of the methanol extract of the leaf part at 75% concentration was found to be 6mm in *E.coli*, and 4mm in *S.aureus* in the current study.

5.2.2. Evaluation of *Eucalyptus* spp. antimicrobial activity results

The antimicrobial activity results of *Eucalyptus* spp. based on the GLM-Univariate analysis results are presented in Table 5.9. The impact of plant part (leaf, branch), extract concentrations (25%, 50%, and 75%), and the interaction between these two factors on the antimicrobial activity results of all *Eucalyptus* spp. which was in general significant at the 95% confidence range, are discussed in this subsection.

5.2.2.1. Evaluation of *E.camaldulensis* antimicrobial activity test results

The GLM-Univariate analysis (Table 5.9), antimicrobial activity and Tukey-B test statistical analysis results of *E.camaldulensis* are shown in Table 5.10. The antimicrobial activity test results of the hot water, ethanol and methanol extracts are explained in this subsection.

5.2.2.1.1. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.camaldulensis* hot water extracts

Tables 4.4 and 5.10, and Figure 5.12 present the antimicrobial activity results of *E.camaldulensis* hot water extract. Also, this subsection discusses the GLM Univariate analysis result, effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range of the antimicrobial activity results of *S.aureus*, *E.faecalis, K.pneumonia,* and *E.coli*, which was significant (p=0.00-0.025) (Table 5.9).

According to Tukey-B test results for the antimicrobial tests using four different bacteria, the best results were identified at 75% concentration (1-3 mm).

As per the t-test analysis results given in Table 5.10, the findings showed that that the leaf part of the plant was more effective in the *S.aureus, E.faecalis, E.coli* and *K.pneumonia* trials. This value was in the range of 1 to 3 mm.

Table 5.9. GLM - Univariate analysis of *Eucalyptus* spp. antimicrobial activity results

			S.aureus		***E.faecalis***		***E.coli***		***K.pneumonia***	
	Solvent Type	**Tests of Between-Subjects Effects**	**Sig.**	**Difference**	**Sig.**	**Difference**	**Sig.**	**Difference**	**Sig.**	**Difference**
E.camaldulensis	**Water**	*Part*	0.000	+	0.000	+	0.025	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.075	-
		Part Concentration*	0.010	+	0.075	-	0.010	+	0.002	+
	Ethanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.000	+	0.002	+	0.010	+	0.026	+
	Methanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.000	+	0.000	+	0.000	+	0.134	-
E.sideroxylon	**Water**	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.000	+	0.000	+	0.000	+	0.000	+
	Ethanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.075	-	0.021	+	0.000	+	0.009	+
	Methanol	*Part*	0.000	+	0.000	+	0.000	+	0.000	+
		Concentration	0.000	+	0.000	+	0.000	+	0.000	+
		Part Concentration*	0.002	+	0.002	+	0.000	+	0.055	+

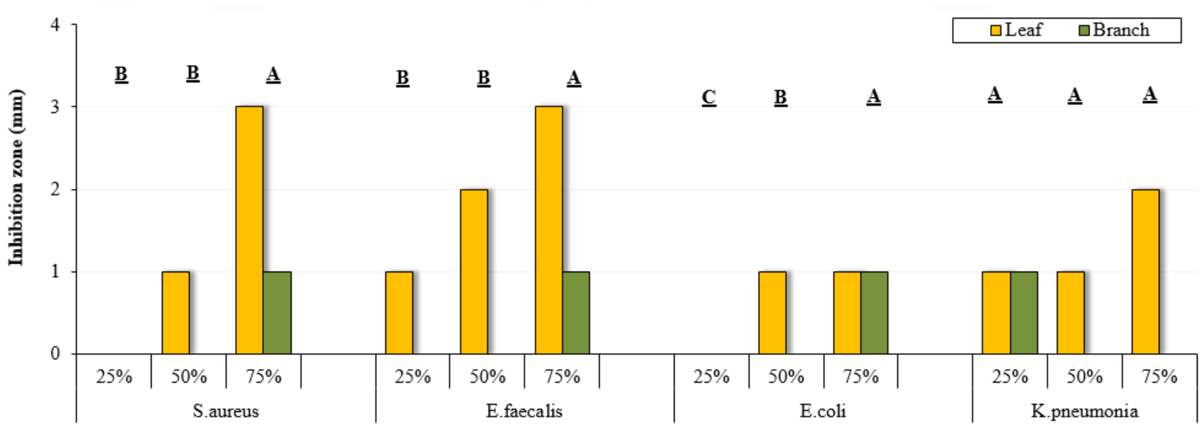
Table 5.10. Post Hoc analysis of *E.camaldulensis* antimicrobial activity results

		S.aureus			***E.faecalis***			***E.coli***			***K.pneumonia***		
		Concentration (%)			**Concentration (%)**			**Concentration (%)**			**Concentration (%)**		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
WATER	**Leaf**	-	1.0 (0.0)	3.0 (1.2) **A**	1.0 (0.0)	2.0 (0.8) **A**	3.0 (0.0) **A**	-	1.0 (0.8) **A**	1.0 (0.0)	1.0 (0.8) **A**	1.0 (0.0)	2.0 (0.8) **A**
	Branch	-	-	1.0 (0.8) **B**	-	- **B**	1.0 (0.8) **B**	-	- **B**	1.0 (0.0)	1.0 (0.0) **A**	-	- **B**
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**C**	**B**	**A**	**A**	**A**	**A**
ETHANOL	**Leaf**	1.0 (0.0)	3.0 (0.8) **A**	4.0 (0.8) **A**	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.0) **A**	1.0 (0.0)	1.0 (0.0)	3.0 (0.8) **A**	1.0 (0.8) **A**	2.0 (0.8) **A**	4.0 (0.8) **A**
	Branch	-	- **B**	1.0 (0.0) **B**	-	- **B**	1.0 (0.8) **B**	-	-	1.0 (0.0) **B**	- **B**	- **B**	1.0 (0.8) **B**
	Tukey B	**C**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**
METHANOL	**Leaf**	1.0 (0.0)	3.0 (0.8) **A**	6.0 (0.8) **A**	1.0 (0.0) **A**	4.0 (0.8) **A**	6.0 (0.0) **A**	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**	6.0 (1.2) **A**
	Branch	-	1.0 (0.0) **B**	2.0 (0.8) **B**	1.0 (0.8) **A**	1.0 (0.0) **B**	2.0 (0.8) **B**	-	- **B**	1.0 (0.0)	1.0 (0.0) **B**	2.0 (1.4) **B**	3.0 (0.8) **B**
	Tukey B	**C**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**

(-) No inhibition

A study by Pandey and Singh (2014) showed that *E.camaldulensis* hot water leaf extract has the potential role as antimicrobial agents against tested *S.aureus, E.coli.* The inhibition value of *S.aureus* and *E.coli* was 24.0mm and 22.0mm, respectively. These values in Pandey and Singh's (2014) study were found to be higher compared to the results exhibited in the current study.

In a study by Alghamdi and Ababutain (2019), it was reported that *E.camaldulensis* leaf water extract showed the inhibition value of 1.2mm for *E.coli* and 2.2mm for *S.aureus*. The current study showed similar inhibition value of 2mm in the leaf water extraction at 75% concentration in *E.coli* and *S.aureus* tests. These findings are consistent with the findings of Alghamdi and Ababutain (2019).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.12. Antimicrobial activity of *E.camaldulensis* hot water extract

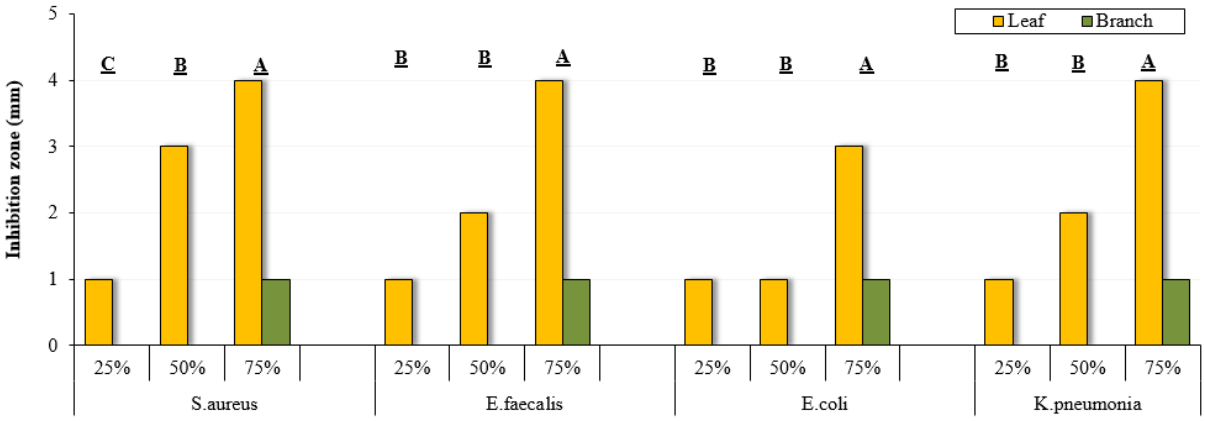
5.2.2.1.2. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.camaldulensis* ethanol extracts

This subsection discusses the effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.026) on the antimicrobial results of *S.aureus, E.faecalis, E.coli, and K.pneumoniae*, according to the GLM Univariate analysis result (Table 5.9). Tables 4.4 and 5.10, and Figure 5.13 depict the antibacterial activity of the *E.camaldulensis* ethanol extract.

The best results were achieved at 75% extract concentration based on the Tukey-B test. The value ranging from 1.0 to 3.0 mm was indicated for *E.coli,* and the value in the range of 1.0 to 4.0 mm was found for *E.faecalis, S.aureus,* and *K.pneumoniae*.

The effect of the plant part on the antimicrobial activity was generally assesed through the t-test. The best results were achieved in leaf. This value was in the range of 1 to 4 mm.

Alghamdi and Ababutain's (2019) study which analysed the antibacterial activity of the *E.camaldulensis* leaves ethanol extracts, showed the inhibition value of 1.8mm and 6mm for *E.coli* and *S.aureus*, respectively.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.13. Antimicrobial activity of *E.camaldulensis* ethanol extract

5.2.2.1.3. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.camaldulensis* methanol extracts

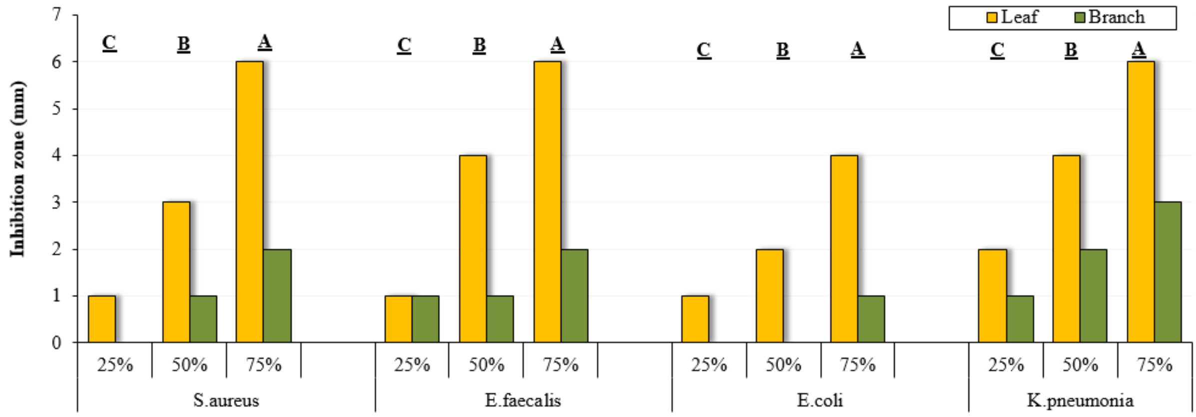
The antimicrobial activity results of the *E.camaldulensis* methanol extracts are shown in Tables 4.4 and 5.10, and Figure 5.14. This subsection discusses the GLM Univariate analysis result (Table 5.9), effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was generally significant on the antimicrobial results of *S.aureus, E.faecalis, E.coli* and *K.pneumoniae* (p=0.000).

According to the Tukey B test results shown in Table 5.10 and Figure 5.14, the best results (4-6 mm inhibition zone) were achieved at concentration 75% for methanol extraction against all bacteria.

The results of the leaf and branch parts of each bacterium, which were compared through the t-test, indicated a difference between these parts. In general, it was discovered that the leaf was more effective against bacteria (1-6 mm).

Pandey and Singh's (2014) study, which used the *E.camaldulensis* leaf methanol extract, depicted the inhibition values of 9 mm and 12.0 mm in *S.aureus* and *E. coli*, respectively. These values in Pandey and Singh's (2014) study are regarded higher compared to the results of the current study which were indicated with the inhibition value of 4-6mm at 75% concentration leaf methanol extraction for all bacteria.

The findings of the current study however showed higher inhibition value in comparison with Alghamdi and Ababutain's (2019) study which investigated the inhibition values against *S.aureus* and *E.coli* using methanol extract of *E.camaldulensis* leaves. The findings of Alghamdi and Ababutain's (2019) study showed the inhibition values of 3.0mm and 4.0mm for *E.coli* and *S.aureus*, respectively.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.14. Antimicrobial activity of *E.camaldulensis* methanol extract

5.2.2.2. Evaluation of *E.sideroxylon* antimicrobial activity results

The antimicrobial activity of *E.sideroxylon* against *S.aureus, E.faecalis, E.coli and K.pneumonia* shown in Table 5.11

5.2.2.2.1. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.sideroxylon* hot water extracts

The antimicrobial activity results of the *E.sideroxylon* hot water extracts are shown in Tables 4.4 and 5.11, and Figure 5.15. This subsection discusses the GLM Univariate analysis results (Table 5.9), effect of the plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was generally significant on the antimicrobial results of *S.aureus, E.faecalis, E.coli*, and *K.pneumoniae* (p=0.000).

According to Tukey-B test results, in the antimicrobial tests using four different bacteria, it was identified that the best results were depicted at 75% concentration (2-3 mm).

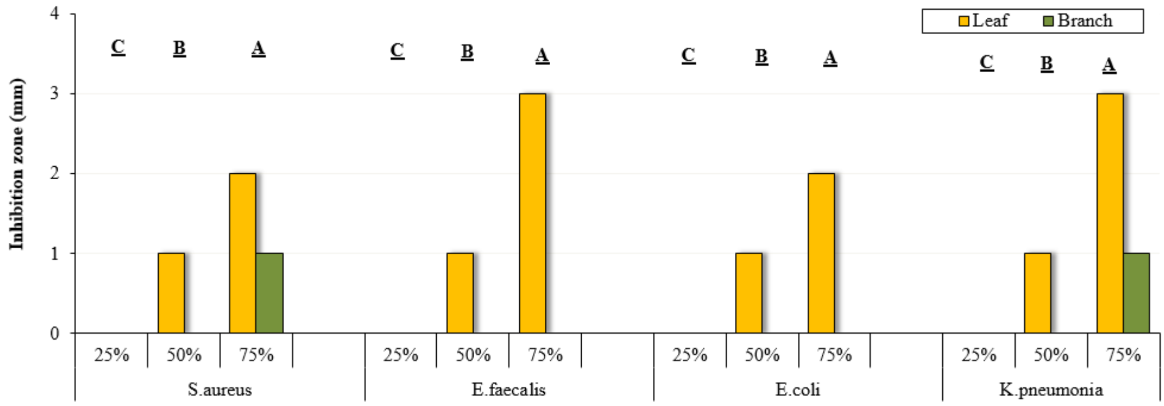
The t-test analysis results in Table 5.11 showed that the leaf part of the plant was more effective in *E.faecalis, E.coli*, and *K.pneumonia* trials. This value was in the range of 1 to 3 mm.

Table 5.11. Post Hoc analysis of *E.sideroxylon* antimicrobial activity results

		S.aureus			***E.faecalis***			***E.coli***			***K. pneumonia***		
		Concentration			**Concentration**			**Concentration**			**Concentration**		
	Plant Part	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**
WATER	**Leaf**	-	1.0 (0.0)	2.0 (0.0)	-	1.0 (0.0)	3.0 (0.8) **A**	-	1.0 (0.0)	2.0 (0.8) **A**	-	1.0 (0.0)	3.0 (0.8) **A**
	Branch	-	-	1.0 (0.0)	-	-	- **B**	-	-	- **B**	-	-	1.0 (0.0) **B**
	Tukey B	**C**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**C**	**B**	**A**
ETHANOL	**Leaf**	1.0 (0.0)	2.0 (0.8) **A**	3.0 (0.8) **A**	3.0 (1.2) **A**	2.0 (0.8) **A**	5.0 (0.8) **A**	1.0 (0.0)	1.0 (0.0)	3.0 (0.0)	1.8 (0.9) **A**	3.0 (0.8) **A**	5.0 (0.8) **A**
	Branch	-	- **B**	1.0 (0.0) **B**	- **B**	1.0 (0.0) **B**	2.0 (0.8) **B**	-	-	1.0 (0.0)	- **B**	1.0 (0.0) **B**	1.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**	**C**	**B**	**A**
METHANOL	**Leaf**	-	1.0 (0.0)	3.0 (0.8) **A**	1.0 (1.2) **A**	3.0 (1.4) **A**	6.0 (0.8) **A**	1.0 (0.0)	2.0 (0.8) **A**	4.0 (0.8) **A**	2.0 (1.4) **A**	3.0 (0.8) **A**	6.0 (0.8) **A**
	Branch	-	-	1.0 (0.8) **B**	1.0 (0.0) **A**	1.0 (0.8) **B**	2.0 (0.8) **B**	-	- **B**	- **B**	- **B**	1.0 (0.8) **B**	2.0 (0.8) **B**
	Tukey B	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**	**B**	**B**	**A**

(-) No inhibition

A study by Ashour (2008) investigated the the antimicrobial activity of the *E.sideroxylon* leaf and branch parts against *S.aureus*, *E.coli* and, *E.faecalis*. While the inhibition values 18.0 mm and 9.00 mm were obtained for the leaf and branch parts in the *S.aureus* tests, respectively, the inhibition values 18.0 mm and 12.0 mm were indicated for the leaf and branch parts in the *E.coli* tests, respectively. The inhibition values 25.0 mm and 21.00 mm were obtained for the leaf and branch parts in the *E.faecalis* tests, respectively (Ashour, 2008). In contrast, the findings of the current study showed that the leaf part only showed inhibition against bacteria.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.15. Antimicrobial activity of *E.sideroxylon* hot water extract

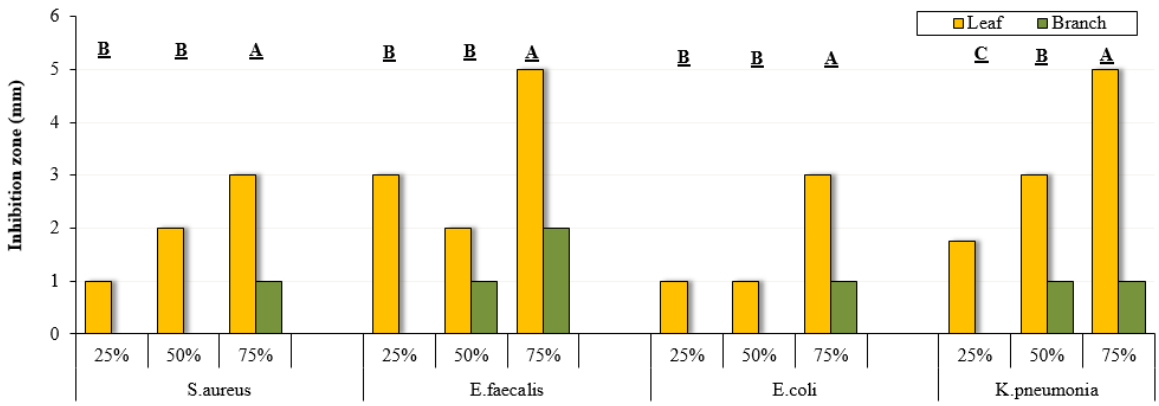
5.2.2.2.2. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.sideroxylon* ethanol extracts

This section discusses the effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.021) on the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumoniae*, according to the GLM Univariate analysis result (Table 5.9). Tables 4.4 and 5.11, and Figure 5.16 illustrate the antibacterial activity of the *E.sideroxylon* ethanol extract.

Referring to the Tukey-B test results for the extract concentration, the best results were achieved at concentration 75%. The inhibition value of 5.0 mm in the leaf part was identified for *E.faecalis* and *K.pneumoniae*, compared to the inhibition value of 3.0 mm for *E.coli* and *S.aureus*.

According to the t-test analysis of the effect of the plant part in the antimicrobial activity, it was discovered that the best results were indicted with the inhibition value in the range of 1.0-5.0 mm in the leaf.

Ashour's (2008) study which assessed the antibacterial activity of the *E.sideroxylon* (leaf and branch) essential oil extract, revealed significant antibacterial activities against *S.aureus*, *E.faecalis* and *K.pneumoniae*. The study showed the inhibition values of 15.0-17.0mm for *S.aureus*, 12.0-16.0mm for *E.faecalis*, and 9.0mm for *K.pneumoniae*. In contrast, the current study showed the inhibition values of 1-3mm, 3.0-5.0mm, and 1.8-5.0mm at 75% concentration of leaf ethanol extraction against *S.aureus*, *E.faecalis*, and *K.pneumoniae*, respectively.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

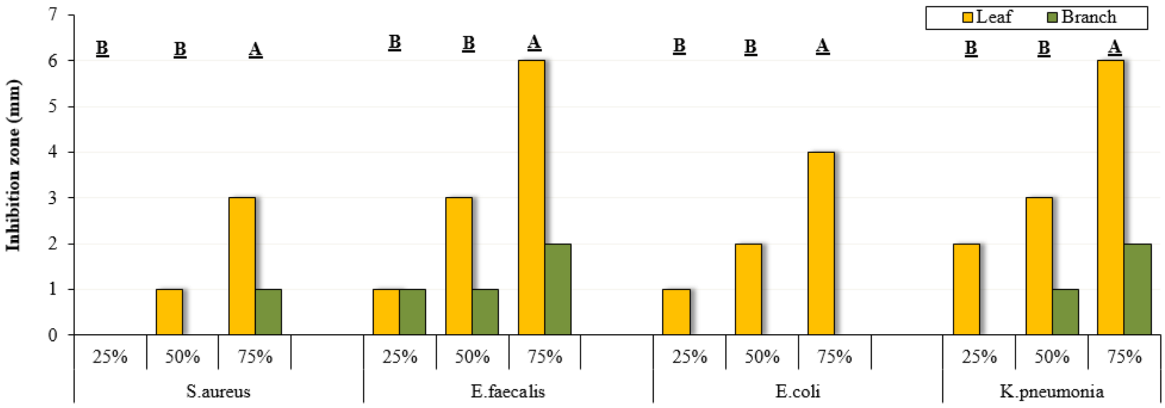
Figure 5.16. Antimicrobial activity of *E.sideroxylon* ethanol extract

5.2.2.2.3. Evaluation of the effect of plant part and extract concentration on antimicrobial activity results of *E.sideroxylon* methanol extracts

The antimicrobial activity results of the *E.sideroxylon* methanol extract are shown in Tables 4.4 and 5.11, and Figure 5.17. This subsection discusses the GLM Univariate analysis results (Table 5.9), effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant on the antimicrobial results of *S.aureus*, *E.faecalis*, *E.coli*, and *K.pneumoniae* (p=0.000-0.055).

According to Tukey-B test (Table 5.11 and Figure 5.17), the best results were indicated at 75% concentration of methanol extraction against all bacteria, which was around 1-6 mm (inhibition value).

The t-test was used to determine the effect of the plant part in the antimicrobial activity. Based to the results obtained, it was shown that the plant part had an influence on the results and the highest value was indicated in the leaf part. The value 6.0mm was indicated in *E.faecalis* and *K.pneumoniae* trials at concentration 75%.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.17. Antimicrobial activity of *E.sideroxylon* methanol extract

Ashour (2008) examined the antimicrobial activity of *E.sideroxylon* methanol extracts (leaf and branch). The value for *S.aureus* was in the range of 11.0 to 15.0mm compared to in the range of 10.0 to 19.0 mm for *E.faecalis*. The findings of the current study, however were not in line with the findings of Ashour's (2008) study. This is due to the findings that the leaf part only showed inhibition against bacteria in the current study.

5.3. Evaluation of the Antifungal Activity Results

The antifungal activity results of *Cupressus* and *Eucalyptus* were disscused in the following section.

5.3.1. Evaluation of *Cupressus* spp. antifungal activity results

The evaluation of the *Cupressus* spp. antifungal test results is described in this subsection.

5.3.1.1. Evaluation of *C.sempervirens* var. *pyramidalis* antifungal activity results

The antifungal activity and Tukey B statistical analysis results of *C.sempervirens* var. *pyramidalis* are shown in Tables 5.12 and 5.13.

Table 5.12. GLM - Univariate analysis of *Cupressus* spp. antifungal activity results

	Solvent Type	Tests of Between-Subjects Effects	*C.albicans* Sig.	*C.albicans* Difference	*A.niger* Sig.	*A.niger* Difference
C.sempervirens* var. *pyramidalis	**Water**	*Part*	0.018	+	0.187	-
		Concentration	0.049	+	0.002	+
		Part Concentration*	0.919	-	0.975	-
	Ethanol	*Part*	0.000	+	0.000	+
		Concentration	0.002	+	0.195	-
		Part Concentration*	0.015	+	0.512	-
	Methanol	*Part*	0.000	+	0.000	+
		Concentration	0.014	+	0.228	-
		Part Concentration*	0.659	-	0.434	-
C.sempervirens* var. *horizontalis	**Water**	*Part*	0.262	-	0.681	-
		Concentration	0.059	+	0.008	+
		Part Concentration*	0.591	-	0.602	-
	Ethanol	*Part*	0.001	+	0.174	-
		Concentration	0.138	-	0.711	-
		Part Concentration*	0.951	-	0.750	-
	Methanol	*Part*	0.003	+	0.013	+
		Concentration	0.162	-	0.253	-
		Part Concentration*	0.818	-	0.717	-
C.arizonica	**Water**	*Part*	0.145	-	0.397	-
		Concentration	0.011	+	0.026	+
		Part Concentration*	0.866	-	0.898	-
	Ethanol	*Part*	0.001	+	0.036	+
		Concentration	0.072	-	0.102	-
		Part Concentration*	0.799	-	0.906	-
	Methanol	*Part*	0.005	+	0.007	+
		Concentration	0.382	-	0.429	-
		Part Concentration*	0.932	-	0.656	-

Table 5.13. Post Hoc analysis of *C.sempervirens* var. *pyramidalis* antifungal activity results

		C.albicans			***A.niger***		
		Concentration (%)			**Concentration (%)**		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**
WATER	Leaf	79.5 (2.1) **A**	78.0 (3.7) **A**	75.8 (2.5) **A**	81.5 (1.9) **A**	80.0 (1.6) **A**	77.0 (1.1) **A**
	Branch	82.0 (1.6) **A**	80.3 (2.6) **A**	79.0 (2.2) **A**	83.0 (2.6) **A**	81.0 (2.9) **A**	78.3 (2.6) **A**
	Tukey B	**A**	**AB**	**B**	**A**	**A**	**B**
ETHANOL	Leaf	78.8 (1.3) **B**	77.5 (1.7) **B**	73.3 (1.3) **B**	80.5 (1.3) **B**	79.5 (1.9) **B**	77.8 (2.2) **B**
	Branch	83.8 (2.2) **A**	82.8 (1.3) **A**	82.8 (1.3) **A**	84.3 (1.7) **A**	83.5 (1.3) **A**	83.5 (2.4) **A**
	Tukey B	**A**	**A**	**B**	**A**	**A**	**A**
METHANOL	Leaf	73.3 (2.9) **B**	71.0 (1.4) **B**	69.3 (1.7) **B**	77.5 (1.9) **B**	75.8 (3.9) **B**	74.3 (2.2) **B**
	Branch	83.3 (1.7) **A**	81.8 (2.2) **A**	81.0 (0.8) **A**	83.0 (1.6) **A**	81.8 (0.9) **A**	82.5 (1.3) **A**
	Tukey B	**A**	**AB**	**B**	**A**	**A**	**A**

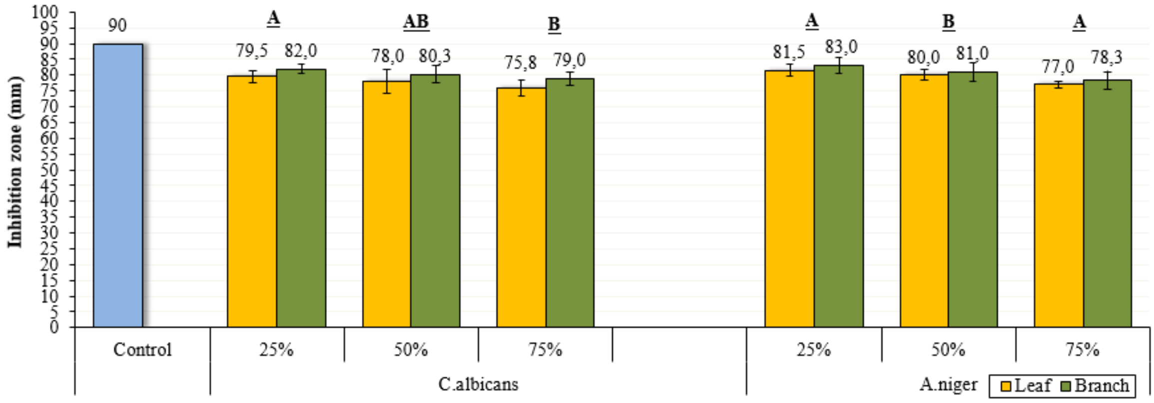
Note: 1.) Values in parentheses are standard deviation
2.) The fungi growth was determined as 90 mm for control petri dishes in both fungi species

5.3.1.1.1. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C. sempervirens* var. *pyramidalis* hot water extracts

Tables 4.5 and 5.13, and Figure 5.18 depict the antifungal activity of *C.sempervirens* L. var. *pyramidalis* hot water extract. This subsection discusses the GLM Univariate analysis results, effect of plant part (leaf, branch) and extract concentration (25, 50, 75%) at the 95% confidence range on the antifungal results of *C.albicans* which was significant (p=0.018-0.049) (Table 5.12).

Based on the Tukey-B test (Table 5.13 and Figure 5.18) that presented the antifungal activity of *C.sempervirens* L. var. *pyramidalis*, the best results (around 75.8-79.0 mm) were exhibited at concentration 75% for water extraction against all antifungal.

Based on the t-test analysis results of the effect of plant part on the antifungal activity testing, it was discovered that the plant part showed no effect in the antifungal activity.



Note: Different letters in the bacteria assay represent statistical differences at the 95% confidence level

Figure 5.18. Antifungal activity of *C.sempervirens* var. *pyramidalis* hot water extract

It was stated in Al-Snafi (2016)'s study that *C.sempervirens* var. *pyramidalis* hot water extract contained some important pharmaceutical potential against fungi, such as *C.albicans* and *A.niger*.

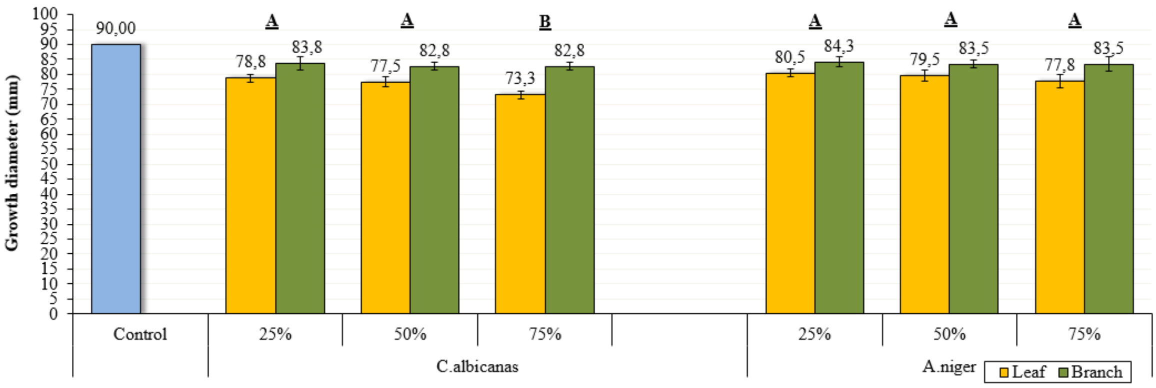
5.3.1.1.2. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.sempervirens* var. *pyramidalis* ethanol extracts

This section discusses the effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the interaction between these two factors at the 95% confidence range which was significant (p=0.00-0.015) on the antifungal activity results of *C.albicans*, according to the GLM Univariate analysis result (Table 5.12). Tables 4.5 and 5.13, and Figure 5.19 illustrate the antibacterial activity of the *C.sempervirens* L. var. *pyramidalis* ethanol extract.

The best results with the values ranging from 73.3 to 82.8mm for *C.albicans* was exhibited at 75% ethanol extract concentration based on the Tukey-B test.

The results of the leaf and branch parts of each fungus were compared using the t-test. It was discovered that there was a difference between the two parts, particularly the leaf part was identified more effective against fungus (73.3-80.5 mm).

In Mahmood et al.,'s (2013) study, 8.0mm was the inhibition value of *C.sempervirens* var. *pyramidalis* leaves ethanol extract against *A.niger*. The results of the current study showed higher inhibition value compared to Mahmood et al.'s (2013) study.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.19. Antifungal activity of *C.sempervirens* var. *pyramidalis* ethanol extract

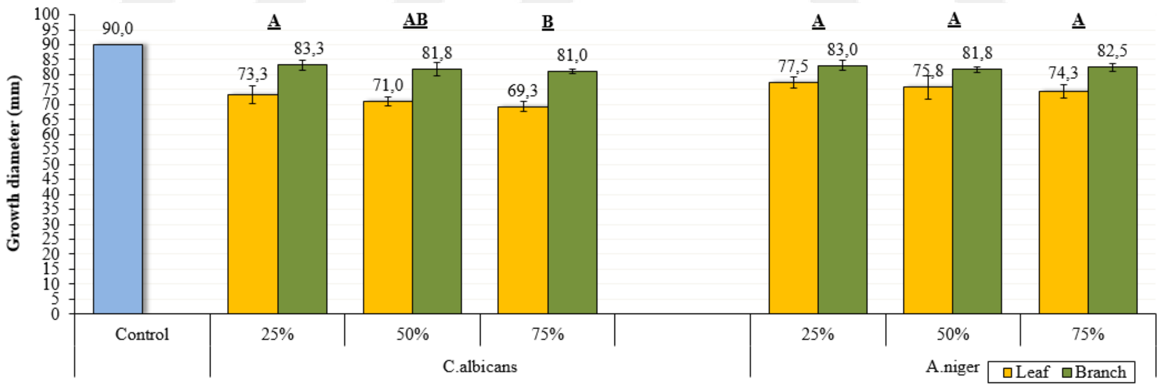
5.3.1.1.3. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.sempervirens* var. *pyramidalis* methanol extracts

The antifungal activity results of the *C.sempervirens* L. var. *pyramidalis* extracts are shown in Tables 4.5 and 5.13, and Figure 5.20. This subsection discusses the GLM Univariate analysis results (Table 5.12), effect of plant part (leaf, branch) and extract concentration (25, 50, 75%) at the 95% confidence range which was generally significant on the antifungal results of *C.albicans* (p=0.000-0.0014).

According to the Tukey-B test results, in the antimicrobial tests using two different fungi, it was shown that the best results were exhibited at concentration 75% of *C.albicans* (69.3 mm).

The t-test analysis results in Table 5.13 exhibited that the leaf part was more effective on the antifungal results. This value was in the range of 69.3 to 77.5mm.

The findings from Afsharzadeh et al.'s (2013) study indicated the presence of antifungal compounds in different solvents of *C.sempervirens* var. *pyramidalis*. The inhibition value of *C.albicans* was (6.44 mm at 200 g L-1). Compared to Afsharzadeh et al.'s (2013) study, the current study showed higher inhibition value.The current study revealed the inhibition value of 69.31mm at concentration 75% methanol extract.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.20. Antifungal activity of *C.sempervirens* var. *pyramidalis* methanol extract

5.3.1.2. Evaluation of *C.sempervirens* var. *horizontalis* antifungal activity results

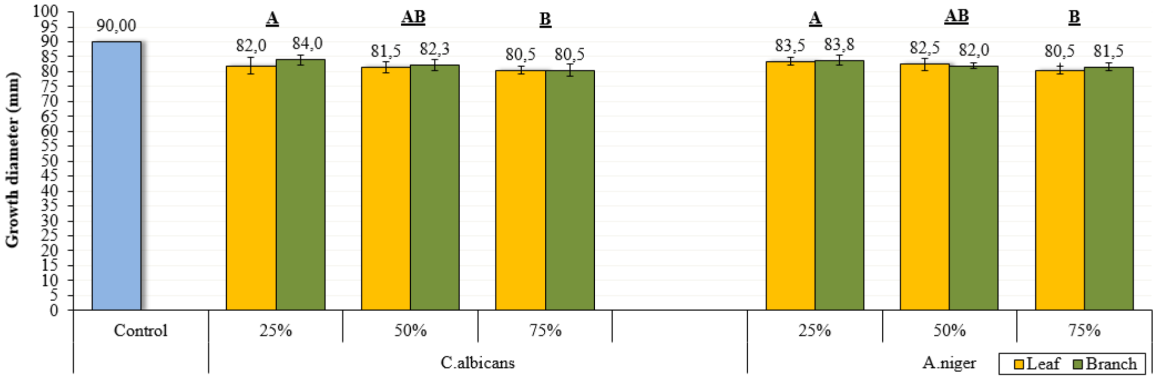
Table 5.14 contains the Post Hoc analysis of the *C.sempervirens* L. var. *horizontalis* antifungal activity results.

Table 5.14. Post Hoc analysis of *C.sempervirens* var. *horizontalis* antifungal activity results

		C.albicans			***A.niger***		
		Concentration			**Concentration**		
	Plant Part	**25%**	**50%**	**75%**	**25%**	**50%**	**75%**
WATER	Leaf	82.0 (2.8) **A**	81.5 (1.9) **A**	80.5 (1.3) **A**	83.5 (1.3) **A**	82.5 (2.1) **A**	80.5 (1.3) **A**
	Branch	84.0 (1.6) **A**	82.3 (1.7) **A**	80.5 (1.9) **A**	83.8 (1.7) **A**	82.0 (0.8) **A**	81.5 (1.3) **A**
	Tukey B	**A**	**AB**	**B**	**A**	**AB**	**B**
ETHANOL	Leaf	81.3 (1.3) **A**	80.0 (0.8) **B**	79.8 (2.2) **A**	82.0 (0.8) **A**	82.5 (1.3) **A**	81.3 (1.7) **A**
	Branch	83.8 (1.7) **A**	82.8 (1.3) **A**	82.0 (1.8) **A**	83.3 (2.1) **A**	82.8 (2.1) **A**	82.8 (2.1) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**
METHANOL	Leaf	80.3 (0.96) **A**	78.8 (1.3) **B**	78.3 (2.8) **A**	80.5 (0.6) **A**	80.8 (1.7) **A**	79.3 (1.7) **A**
	Branch	82.0 (1.2) **A**	81.5 (1.3) **A**	80.8 (1.7) **A**	81.8 (1.5) **A**	81.8 (0.9) **A**	81.3 (0.5) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**

5.3.1.2.1. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.sempervirens* var. *horizontalis* hot water extracts

Tables 4.5 and 5.14, and Figure 5.21 illustrate the antifungal activity of *C.sempervirens* L. var. *horizontalis* hot water extract. This subsection discusses the GLM Univariate analysis result, only the effect of extract concentration (25, 50, 75%) at the 95% confidence range on the antifungal results of *C.albicans* and *A.niger* which was significant (p=0.008) (Table 5.12).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.21. Antifungal activity of *C.sempervirens var. horizontalis* hot water extract

According to Tukey-B test (Table 5.14 and Figure 5.21) which showed the antifungal activity of *C.sempervirens* L. var. *horizontalis* water extract, the best results (around 80.5 mm) were achieved at concentration 75% against to all fungi.

The results of the leaf and branch plant parts of each fungus were compared using the t-test, and the findings revealed that there was no difference between the two parts.

Zouaghi et al. (2010) reported that *C.sempervirens* has some important pharmaceutical agents that fight against the growth of some pathogenic fungi, such as *C.albicans* and *A.niger*. The inhibition value was found between 6.5-9.2mm for *C.albicans*. In contrast, the value of the current study was higher than with the findings of Zousghi et al.'s (2010) study.

5.3.1.2.2. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C. sempervirens var. horizontalis* ethanol extracts

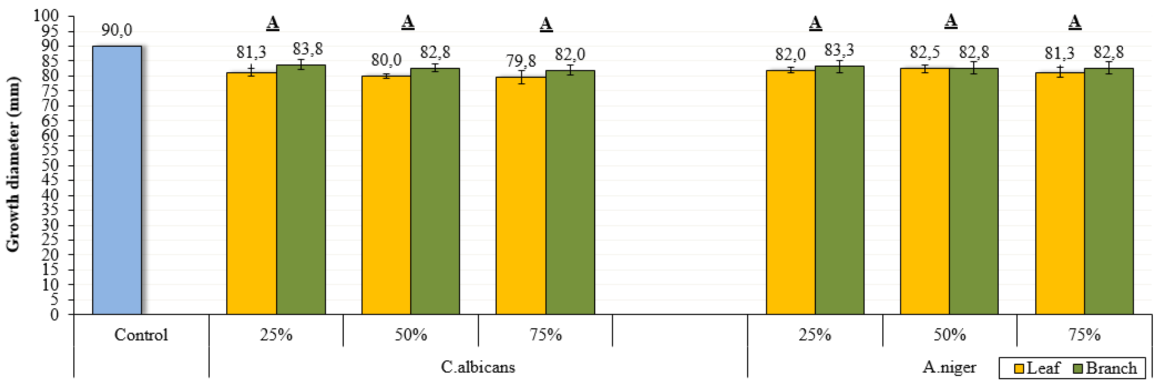
The effect of plant part (leaf, branch) at the 95% confidence range was significant (p=0.001) on the antifungal results of *C.albicans only*, according to the GLM Univariate analysis result (Table 5.12).

Tables 4.5 and 5.14, and Figure 5.22 illustrate the antifungal activity of *C.sempervirens* L. var. *horizontalis* ethanol extract.

According to the Tukey-B test, the extract concentration depicts no effect on the antifungal activity.

The t-test results showed that the highest inhibition value of 80.0 mm against *C.albicans* was detected solely in the leaf part at concentration 50%.

Eryılmaz, et al.,'s (2016) study showed there was no antifungal activity observed against *C.albicans* by using the ethereal extracts of *C.sempervirens* L. var. *horizontalis* cones. Higher results were indicated in the current study in comparison with the results of Eryılmaz, et al.,'s (2016) study.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.22. Antifungal activity of *C.sempervirens* var. *horizontalis* ethanol extract

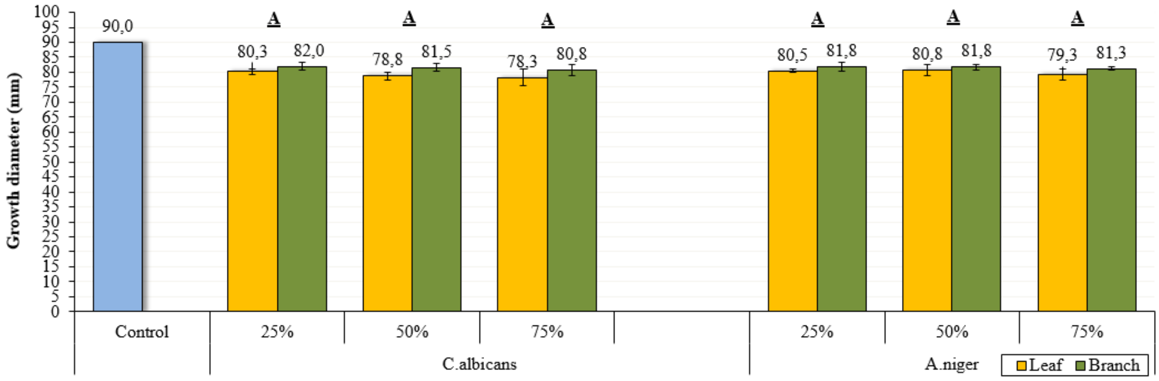
5.3.1.2.3. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.sempervirens* var. *horizontalis* methanol extracts

The antifungal activity results of the *C.sempervirens* L. var. *horizontalis* methanol extracts are shown in Tables 4.5 and 5.14, and Figure 5.23. This subsection discussed the GLM Univariate analysis result (Table 5.12), effect of plant part (leaf, branch) at the 95% confidence range which was significant on the antifungal results *C.albicans* and *A.niger* (p=0.000-0.0013). On the other hand, extract concentrations (25, 50, 75%), and the interaction between these two factors was identified not significant in the antifungal results.

According to the Tukey-B test results as in Table 5.14, the extract concentration showed no effect on the antifungal activity values.

Referring to the t-test analysis results in Table 5.14, the results revealed that the leaf was more effective in *C.albicans* at concentration 50%. This was indicated with the value of 78.8 mm.

Afsharzadeh et al. (2013) reported that the antifungal activity results of the *C.sempervirens* var. *horizontalis* leaf methanol extracts exhibited poor activity for concentrations of 25, 50, 100 and 200 g L-1. The findings of the current study exhibited higher value compared to Afsharzadeh et al.'s (2013) study.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.23. Antifungal activity of *C.sempervirens* var. *horizontalis* methanol extract

5.3.1.3. Evaluation of *C.arizonica* antifungal activity results

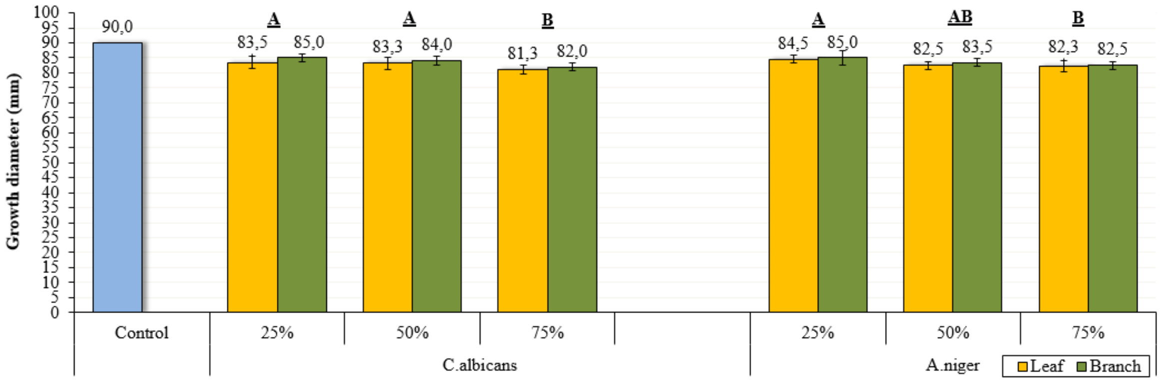
The antifungal activity and Tukey-B test results of *C.arizonica* are shown in Table 5.15.

Table 5.15. Post Hoc analysis of *C.arizonica* antifungal activity results

		C. albicans			***A. niger***		
		Concentration (%)			**Concentration (%)**		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**
WATER	Leaf	83.5 (1.9) **A**	83.3 (2.1) **A**	81.3 (1.5) **A**	84.5 (1.3) **A**	82.5 (1.3) **A**	82.3 (1.7) **A**
	Branch	85.0 (1.4) **A**	84.0 (1.4) **A**	82.0 (1.2) **A**	85.0 (2.6) **A**	83.5 (1.3) **A**	82.5 (1.3) **A**
	Tukey B	**A**	**A**	**B**	**A**	**AB**	**B**
ETHANOL	Leaf	80.8 (0.9) **B**	80.0 (1.8) **A**	79.3 (1.7) **A**	82.8 (1.7) **A**	81.3 (1.3) **A**	80.8 (1.7) **A**
	Branch	84.0 (1.2) **A**	82.3 (0.9) **A**	81.8 (2.2) **A**	84.3 (1.5) **A**	82.5 (1.9) **A**	82.8 (2.1) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**
METHANOL	Leaf	79.8 (0.9) **B**	79.0 (0.5) **A**	79.3 (1.7) **A**	80.8 (0.9) **A**	79.8 (1.6) **B**	79.3 (1.7) **A**
	Branch	81.8 (1.5) **A**	80.8 (1.0) **A**	80.8 (1.0) **A**	81.8 (1.7) **A**	81.5 (0.9) **A**	81.5 (1.7) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**

5.3.1.3.1. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.arizonica* hot water extracts

Tables 4.5 and 5.15, and Figure 5.24 present the antifungal activity results of the *C.arizonica* hot water extract. This subsection discusses the GLM Univariate analysis results, effect of extract concentrations (25, 50, 75%), at the 95% level on the antifungal results of *C.albicans* and *A.niger* which was significant (p=0.000-0.026).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.24. Antifungal activity of *C.arizonica* hot water extract

According to the Tukey-B test results presented in Table 5.15 and Figure 5.24, the best value in the range of 81.3-82.3 mm. was obtained at concentration 75% in both fungi species.

Depending on the t-test analysis results (Table 5.15), the plant part showed no effect in the antifungal activities.

El-Ghorab et al. (2007) reported that *C.arizonica* leaf extract has some important pharmaceutical agents that fight against the growth of some pathogenic fungi, such as *C.albicans* and *A.niger*. The inhibition value of 23.0mm was identified for *C.albicans* while the inhibition value of 26.0mm was depicted for *A.niger*.

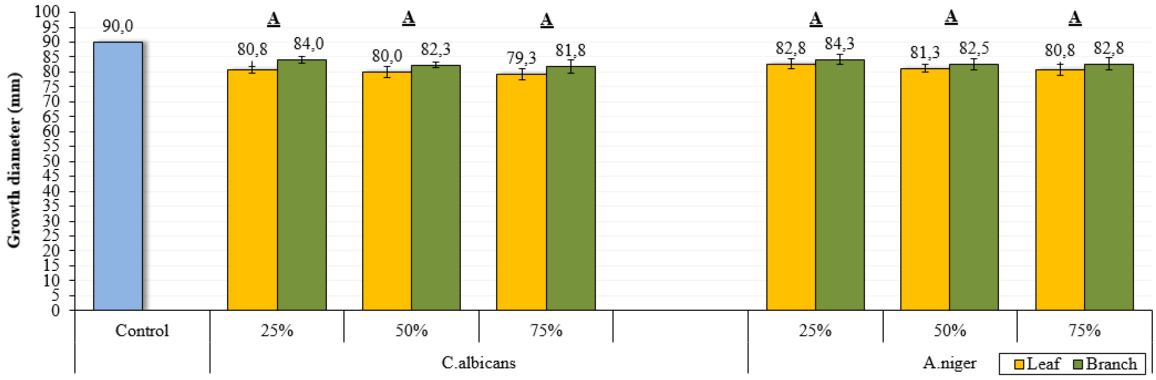
5.3.1.3.2. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C.arizonica* ethanol extracts

Based on the GLM Univariate analysis result (Table 5.12), the effect of plant part (leaf, branch) at the 95% confidence range which was significant (p=0.01-0.036) on the antifungal activity results of *C.albicans* and *A.niger*, are discussed in this subsection. Tables 4.5 and 5.15, and Figure 5.25 depict the antifungal activity of the *C.arizonica* ethanol extract.

According to the Tukey B test analysis results, extraction concentration showed no effect in the antifungal activity (Table 5.15 and Figure 5.25).

The t-test analysis indicated a difference between the leaf and branch parts of *C.albicans* solely at 25% extract concentration. The antimicrobial activity value of 80.8mm was identified for the leaf part, whereas the value of 84.0 mm was found for the branch part.

A study by El-Ghorab et al. (2007) showed the importance of the *C.arizonica* ethanol extracts as an antifungal products and the use of *C.arizonica* in treating some dangerous fungi in different countries in the past, due to the chemical compound that contain antimicrobial and antifungal properties. The inhibition values of 23.0mm and 21.0 mm were identified for *A.niger* and *C.albicans*, respectively. Compared to El-Ghorab et al.'s (2007) study, the findings of the current study revealed lower values.

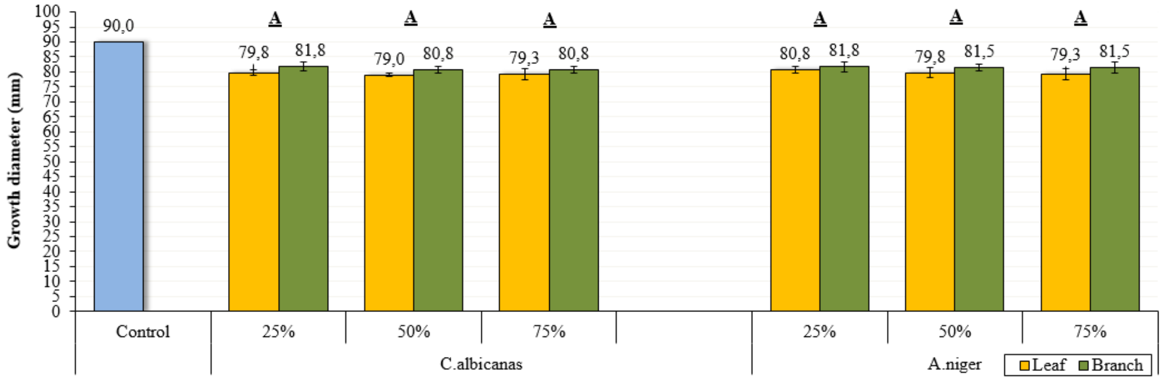


Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.25. Antifungal activity of *C.arizonica* ethanol extract

5.3.1.3.3. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *C. arizonica* methanol extracts

The antifungal activity results of the *C.arizonica* methanol extracts are shown in Tables 4.5 and 5.15, and Figure 5.26. This subsection discusses the GLM Univariate analysis results (Table 5.12), effect of plant part (leaf, branch) at the 95% confidence range which was generally significant on the antifungal results of *C.albicans* (p=0.005) and *A.niger* (p=0.007).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.26. Antifungal activity of *C.arizonica* methanol extract

Moreover, it was found that the extract concentration showed no effect on the antifungal activity results of both fungi based on the Tukey-B test results (Table 5.15 and Figure 5.26).

In general, the effect of the plant part on the antifungal activity which showed the highest inhibition value against *A.niger* and *C.albicans* in the leaf part was indicated at concentrations 50% and 25%, respectively. For both bacteria the inhibition value was 79.8 mm.

El-Ghorab et al.,'s (2007) study examined the use of *C.arizonica* in traditional medicine to treat fungal diseases. *C.arizonica* extract exhibited a potent inhibitory effect on the growth of *C.albicans* and *A.niger*. The inhibition value of 23.0mm was identified for *A.niger*, while the inhibition value of 20.0mm was discovered for *C.albicans* (El-Ghorab et al., 2007).

5.3.2. Evaluation of *Eucalyptus* spp. antifungal activity results

The GLM - Univariate analysis results of the evalutaion of *Eucalyptus* spp. antifungal test are shown in Table 5.16.

Table 5.16. GLM - Univariate analysis of *Eucalyptus* spp. antifungal activity results

	Solvent Type	Tests of Between-Subjects Effects	*C.albicans* Sig.	*C.albicans* Difference	*A.niger* Sig.	*A.niger* Difference
E.camaldulensis	**Water**	*Part*	0.035	+	0.129	-
		Concentration	0.139	-	0.025	+
		Part Concentration*	0.545	-	0.914	-
	Ethanol	*Part*	0.306	-	0.025	+
		Concentration	0.218	-	0.317	-
		Part Concentration*	0.753	-	0.601	-
	Methanol	*Part*	0.027	+	0.052	+
		Concentration	0.026	+	0.475	-
		Part Concentration*	0.886	-	0.665	-
E.sideroxylon	**Water**	*Part*	0.036	+	0.000	+
		Concentration	0.135	-	0.053	+
		Part Concentration*	0.581	-	0.873	-
	Ethanol	*Part*	0.093	+	0.000	+
		Concentration	0.386	-	0.297	-
		Part Concentration*	0.772	-	0.502	-
	Methanol	*Part*	0.018	+	0.000	+
		Concentration	0.311	-	0.016	+
		Part Concentration*	0.193	-	0.071	+

5.3.2.1. Evaluation of *E.camaldulensis* antifungal activity results

The antifungal activity and Tukey-B test results of *E.camaldulensis* are shown in Table 5.17. The antifungal activity test results of the hot water, ethanol and methanol extracts are discussed in this subsection.

Table 5.17. Post Hoc analysis of *E.camaldulensis* antifungal activity results

		C.albicans Concentration (%)			*A.niger* Concentration (%)		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**
WATER	Leaf	83.0 (1.4) **A**	82.8 (0.9) **A**	82.3 (1.7) **A**	83.8 (1.3) **A**	82.3 (2.6) **A**	81.3 (1.5) **A**
	Branch	85.3 (1.7) **A**	83.8 (0.9) **A**	83.0 (1.6) **A**	85.3 (1.5) **A**	83.0 (1.6) **A**	82.5 (1.9) **A**
	Tukey B	**A**	**A**	**A**	**A**	**AB**	**B**
ETHANOL	Leaf	82.8 (1.7) **A**	82.0 (1.8) **A**	81.5 (1.3) **A**	81.8 (1.5) **A**	81.3 (1.3) **A**	80.3 (0.9) **A**
	Branch	84.0 (1.8) **A**	82.0 (1.8) **A**	82.5 (1.9) **A**	83.8 (2.5) **A**	82.0 (1.8) **A**	82.8 (2.1) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**
METHANOL	Leaf	81.5 (1.3) **A**	80.0 (0.8) **A**	80.0 (0.8) **A**	81.3 (1.7) **A**	80.0 (0.8) **A**	79.8 (2.5) **A**
	Branch	82.3 (0.9) **A**	81.3 (1.3) **A**	81.0 (0.8) **A**	82.0 (1.8) **A**	81.3 (1.3) **A**	82.0 (1.4) **A**
	Tukey B	**A**	**B**	**B**	**A**	**A**	**A**

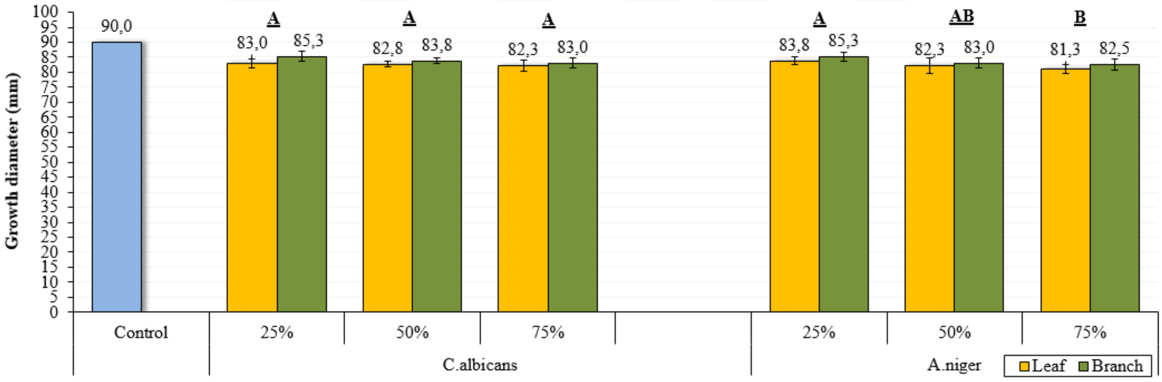
5.3.2.1.1. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E.camaldulensis* hot water extracts

Tables 4.6 and 5.17, and Figure 5.27 present the antifungal activity of *E.camaldulensis* hot water extracts. This subsection discusses the GLM Univariate analysis resulst, effect of plant part (e.g., leaf, stem) for *C.albicans* (p=0.035) and extract concentration (25, 50, 75%) for *A.niger* at the 95% confidence range on the antifungal results which was significant (p=0.025) (Table 5.16).

Based on the Tukey-B test in Table 5.17 and Figure 5.27 that show the antifungal activity of *E.camaldulensis*, the best results with the inhibition value of in the range of 81.3 to 82.5mm was achieved at 75% water extract concentration against *A.niger*.

The results of the leaf and branch parts of each fungi were compared using the t-test. The findings showed that there was no difference between the two parts.

Ghaffar et al.'s (2017) study discovered that the essential oils of *E.camaldulensis* have some important pharmaceutical agents that fight the growth of some pathogenic fungi, such as *A.niger*. The inhibition value was 18.00mm for *A.niger*.



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.27. Antifungal activity of *E.camaldulensis* hot water extract

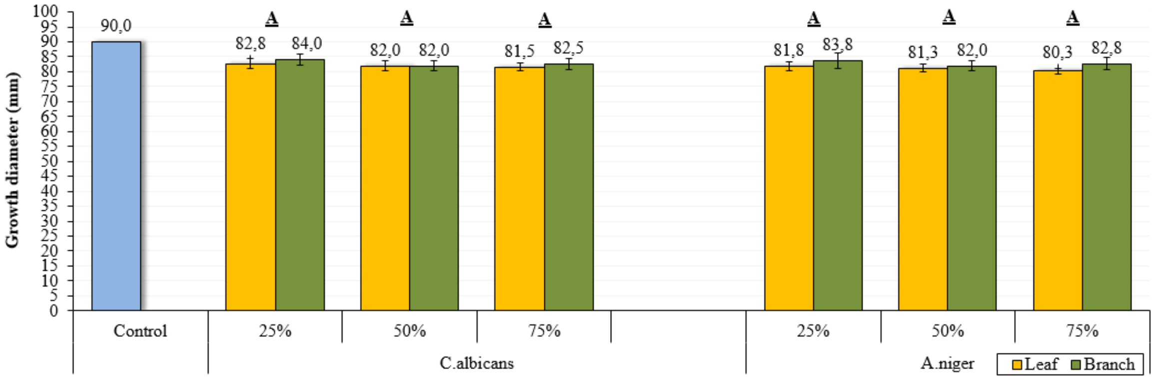
5.3.2.1.2. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E.camaldulensis* ethanol extracts

This subsection discusses the effect of plant part (e.g, leaf, branch) at the 95% confidence range which was significant (p=0.025) on the antifungal results of *A.niger*, according to the GLM Univariate analysis results (Table 5.16). Tables 4.6 and 5.17, and Figure 5.28 illustrate the antifungal activity of the *E.camaldulensis* ethanol extract.

It was discovered that solution concentration has no effect on both *C.albicans* and *A.niger* antifungal results, based on Tukey B test analysis (Tables 5.16 and 5.17).

In addition, the plant part showed no effect on the antifungal activity results (Table 5.17).

A study by Chuku et al., (2016) showed the use of *E.camaldulensis* ethanol extracts as important pharmaceutical agents that fight the growth of some pathogenic fungi, such as *C.albicans*, The inhibition value of 27.0mm was idenfied for *C.albicans* (Chuku et al., 2016). With regard to the results of the current study, the values of the current study were lower.

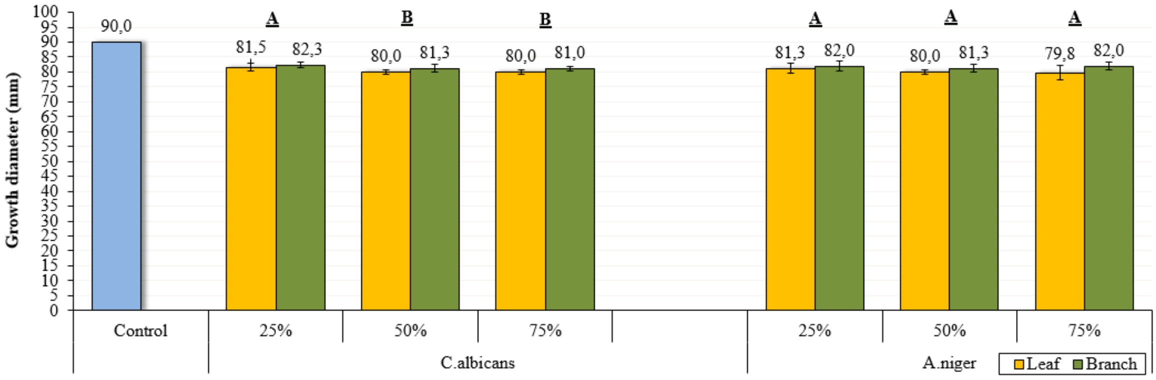


Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.28. Antifungal activity of *E.camaldulensis* ethanol extract

5.3.2.1.3. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E. camaldulensis* methanol extracts

The antifungal activity results of the *E.camaldulensis* methanol extract are shown in Tables 4.6 and 5.17, and Figure 5.29. This section discusses the GLM Univariate analysis results (Table 5.16), effect of plant part (leaf, branch), and extract concentration (25, 50, 75%) at the 95% confidence range which was generally significant on the antifungal results of *C.albicans* (p=0.026-0.027).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.29. Antifungal activity of *E.camaldulensis* methanol extract

According to the Tukey-B test results, in antifungal tests using two different fungi, the best results were exhibited at concentrations 50% and 75% (80.0-81.3mm).

Referring to Table 5.17 which presents the t-test analysis results, the results revealed the plant part was not effective with regard to *C.albicans* and *A.niger*.

The results of the previous study by Chuku (2016) which focused on the antifungal activity of *E.camaldulensis* methanol extracts showed that it had significant antifungal activities against most of tested, *C.albicans*. The result showed the inhibition value of 24.0 mm for *C.albicans*.

A study conducted by Al-Zubairi et al. (2017) showed that leaf methanol extract of *E.camaldulensis* had some important pharmaceutical agents which fight the growth of some pathogenic fungi, such as *C.albicans.* The inhibition value of 18.0mm was discovered for *C.albicans*.

5.3.2.2. Evaluation of *E.sideroxylon* antifungal activity results

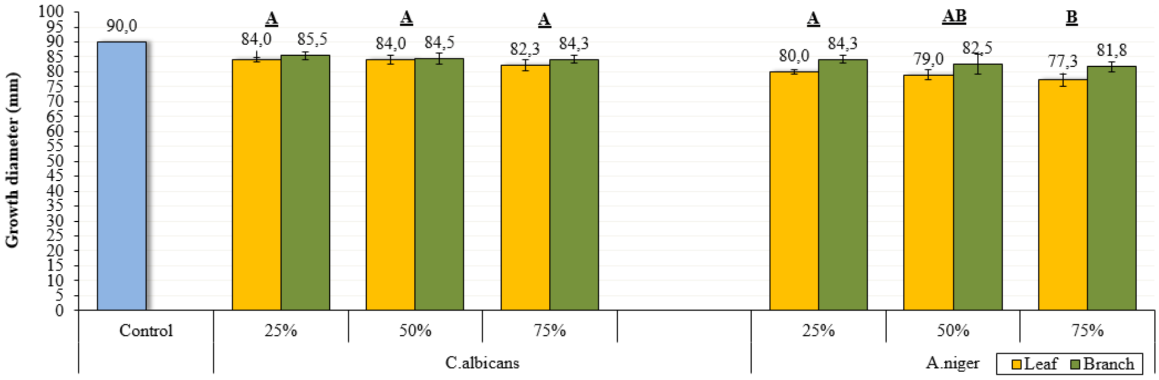
The antifungal activity and Tukey-B test results of *E.sideroxylon* are shown in Table 5.18. The antifungal activity test results of hot water, ethanol and methanol extracts are discussed in this subsection.

Table 5.18. Post Hoc analysis of *E.sideroxylon* antifungal activity results

		C. albicans			***A.niger***		
		Concentration (%)			**Concentration (%)**		
	Plant Part	**25**	**50**	**75**	**25**	**50**	**75**
WATER	Leaf	84.0 (0.8) **A**	84.0 (1.4) **A**	82.3 (1.7) **A**	80.0 (0.8) **B**	79.0 (1.6) **A**	77.3 (2.1) **B**
	Branch	85.5 (1.3) **A**	84.5 (1.9) **A**	84.3 (1.3) **A**	84.3 (1.3) **A**	82.5 (3.4) **A**	81.8 (1.7) **A**
	Tukey B	**A**	**A**	**A**	**A**	**AB**	**B**
ETHANOL	Leaf	82.3 (1.5) **A**	81.5 (1.3) **A**	80.5 (1.3) **A**	79.3 (1.7) **B**	77.8 (2.2) **B**	76.0 (2.6) **B**
	Branch	83.8 (2.5) **A**	82.3 (2.1) **A**	82.8 (3.1) **A**	83.0 (2.2) **A**	82.5 (3.1) **A**	82.5 (1.9) **A**
	Tukey B	**A**	**A**	**A**	**A**	**A**	**A**
METHANOL	Leaf	80.0 (0.8) **B**	80.3 (0.9) **A**	79.5 (0.6) **A**	79.5 (0.6) **B**	76.5 (2.5) **B**	73.8 (1.5) **B**
	Branch	82.8 (2.1) **A**	80.3 (0.9) **A**	81.5 (2.5) **A**	82.8 (1.7) **A**	81.8 (2.5) **A**	82.0 (2.6) **A**
	Tukey B	**A**	**A**	**A**	**A**	**AB**	**B**

5.3.2.2.1. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E.sideroxylon* hot water extracts

Tables 4.6 and 5.18**,** and Figure 5.30 show the antifungal activity of *E.sideroxylon* hot water extract. This subsection discusses the GLM Univariate analysis results, effect of plant part (leaf, branch), and extract concentration (25, 50, 75%) at the 95% confidence range on the antifungal results of *A.niger* which was significant (p=0.00-0.053) (Table 5.16.)



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.30. Antifungal activity of *E.sideroxylon* hot water extract

According to the Tukey-B test (Table 5.18 and Figure 5.30) that showed the antifungal activity of *E.sideroxylon*, the best results with the inhibition value ranging from 77.3 to 81.8 mm were indicated at concentration 75% for hot water extraction against *A.niger* only.

The results from the t-test showed that there was a difference between the leaf and branch part at concentrations 25% and 75% trials in *A.niger*, and the leaf extract showed better results (77.3-80.0 mm).

Previous study by Ashour (2008) showed the use of *E.sideroxylon* extracts leaf and branch as traditional medicine. Hot water extracts in the leaf and branch parts indicated a potent inhibitory effect on the growth of *C.albicans*.

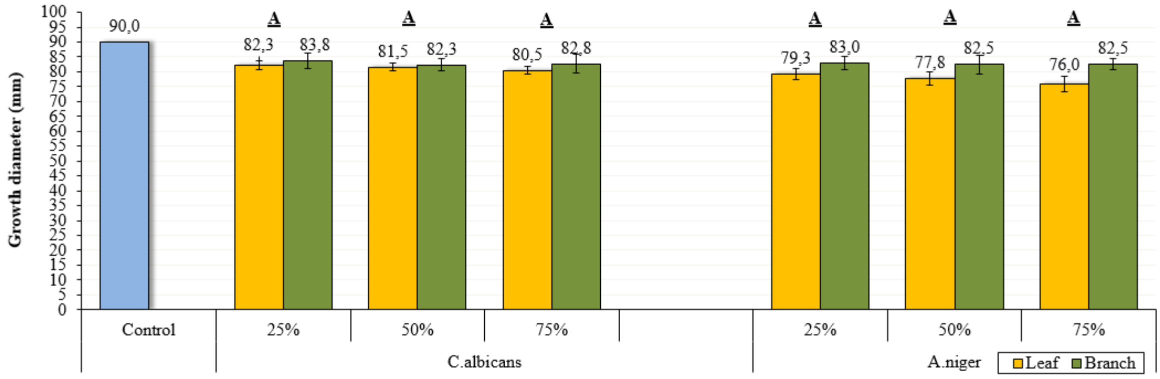
5.3.2.2.2. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E.sideroxylon* ethanol extracts

This subsection discusses the effect of plant part (leaf, branch) at the 95% confidence range which was significant (p=0.00-0.093) on the antifungal results of *C.albicans* and *A.niger*, according to the GLM Univariate analysis results (Table 5.16). Tables 4.6 and 5.18, and Figure 5.31 present the antifungal activity results of the *E.sideroxylon* ethanol extract.

The Tukey-B test results showed the extract concentration did not influence the antifungal activity.

According to the t-test, the effect of solution concentration on the antifungal results was not discovered in *C.albicans*, however it was effective in *A.niger*. This was indicated through the value ranging from 76.0 to 79.3 mm in the *A.niger* trials.

The results of the previous study by Ashour (2008) showed the importance of *E.sideroxylon* leaf and branch as antifungal product. In addition, this study revealed the importance of *E.sideroxylon* as a treatment for some dangerous fungi by different countries in the past, due to the chemical compound of this genus that contain antimicrobial and antifungal properties. The inhibition value for *C.albicans* was identified ranging from 12.0 to 14.0mm, compared to the inhibition value of in the range of 11.0 to 20.0mm was depicted for *A.niger*.

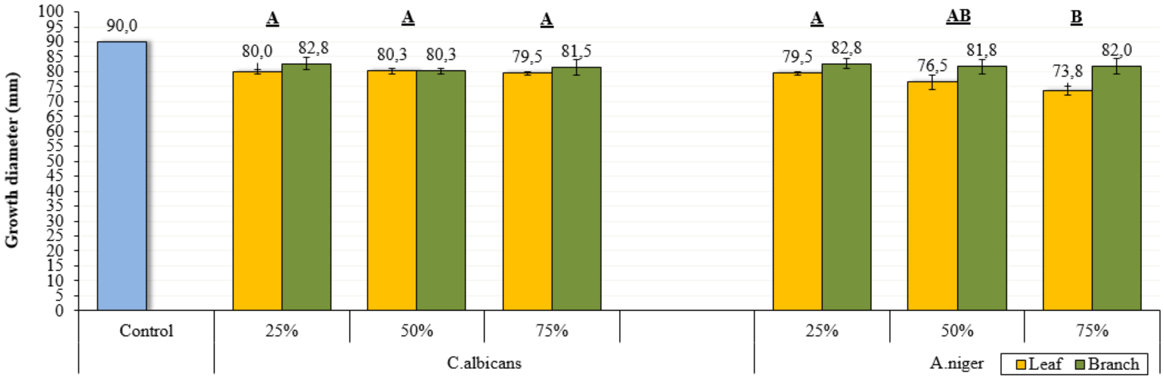


Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.31. Antifungal activity of *E.sideroxylon* ethanol extract

5.3.2.2.3. Evaluation of the effect of plant part and extract concentration on antifungal activity results of *E.sideroxylon* methanol extracts

The antifungal activity results of the *E.sideroxylon* methanol extracts are shown in Tables 4.6 and 5.18, and Figure 5.32. This subsection discusses the GLM Univariate analysis results (Table 5.16), effect of plant part (leaf, branch), extract concentration (25, 50, 75%), and the relationshop between these two factors at the 95% confidence range which was significant on the antifungal results of *A.niger only* (p=0.000-0.071).



Note: Different letters in each bacteria assay represent statistical differences at the 95% confidence level

Figure 5.32. Antifungal activity of *E.sideroxylon* methanol extract

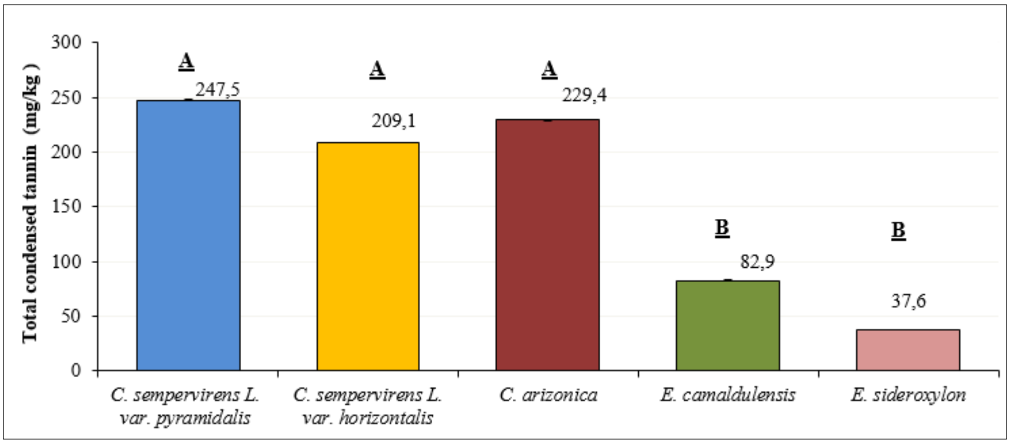
According to Tukey B homogeneity analysis results, in the antifungal tests using two different fungi, the best results were identified with the inhibition value ranging from 73.8 to 82.0 mm at concentration 75% on *A.niger*.

As per the t-test analysis results in Table 5.18, the results revealed that the leaf part of the plant was more effective in *A.niger* trials. This was evident through the value ranging from 73.8 to 79.5 mm.

In a study conducted by Ashor (2008), the *E.sideroxylon* methanol extract showed its antifungal effect against *C.albicans* compared to *A.niger*. The inhibition value (12.0-14.0 mm) was found for *C.albicans* (Ashor, 2008).

5.4. Evaluation of the total condensed tannin (TCT) results

According to the One-Way Anova results applied to the TCT values obtained within the scope of this study at the 95% confidence range, a statistical difference was found between the sample groups (p=0.000). As shown in Figure 5.33, *Cupressus* and *Eucalyptus* spp. were included in two different categories, namely A and B according to the Tukey-B test. The amount of tannin was ranging from 209.1 to 247.5 mg/kg in *Cupressus* samples, whereas it was in the range from 37.6 to 82.9 mg/kg in *Eucalyptus* samples. In general, higher TCT was discovered in the leaf samples of *Cupressus* than *Eucalyptus*.



Note: Different letters represent statistical differences at the 95% confidence level

Figure 5.33. Total condensed tannin results of *Cupressus* and *Eucalyptus* spp.

According to Rahmani (2019), it was reported that the tannin content of *C.sempervirens* was higher in crude (11.02 mg CE/g DW) and water extract (3.47 mg CE/g DW).

A study by Limam et al., (2021) showed the use of *Eucalyptus* spp. leaf extracts to evaluate the TPT results. The maximum level of TCT was detected in the species of *E.lehmannii* (35.11 mg CE/g DW), followed by *E. gomphocornuta* (21.04 mg CE/g DW), *E.angulosa* (20.97 mg CE/gDW), while *E.maidenii* and *E.globulus* showed the lower values 11.38 mg CE/gDW, 6.58 mg CE/gDW (Limam et al., 2021). However, higher values were discovered in the current study which indicate inconsistency with the findings of Limam et al.,'s (2021) study.

5.5. Evaluation of the total phenolic compounds (TPC) results

5.5.1. Evaluation of *Cupressus* spp. TPC results

The GLM - Univariate analysis in Table 5.19 show the plant, solvent type, and the interaction between these two factors, which was significant at the 95% confidence range on the total phenolic results (p=0.000).

Table 5.19. GLM - Univariate analysis of *Cupressus* spp. TPC results

GLM - Univariate analysis		
Tests of Between-Subjects Effects	**Sig.**	**Difference**
Plant	0.000	+
Solvent type	0.000	+
*Plant * Solvent type*	0.000	+

As seen in Table 5.20 and Figure 5.34, the highest TPC values were exhibited in ethanol, followed by water and hexane extracts based on the Tukey-B test.

In terms of the plant species, the best results (3.96-13.43 mg GAE/g DW) were exhibited in *C.sempervirens* L. var. *horizontalis* leaf samples. This was followed by *C.sempervirens* L. var. *pyramidalis* leaf samples (2.77-12.77 mg GAE/g DW), as in Table 5.20 and Figure 5.34.

Table 5.20. Post Hoc analysis of *Cupressus* spp. TPC results (mg GAE/g DW)

Plant	**Solvent Type**			
	Water	Ethanol	Hexane	**Tukey B**
C.sempervirens L. var. *pyramidalis*	10.10 (0.24)	12.77 (0.18)	2.77 (0.33)	**B**
C.sempervirens L. var. *horizontalis*	9.76 (0.11)	13.43 (0.04)	3.96 (0.16)	**A**
C.arizonica	10.48 (0.09)	11.47 (0.49)	1.79 (0.12)	**C**
Tukey B	**B**	**A**	**C**	

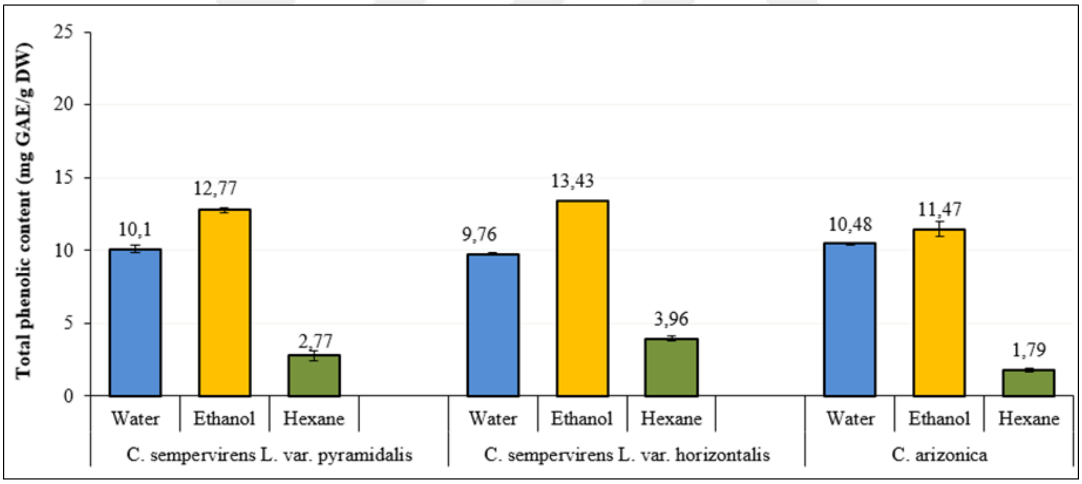


Figure 5.34. Total phenolic content results of *Cupressus* spp. in different solvents

In Rahmani's (2019) study, TPC of sempervirens leaf extracts was determined. The TPC value of 3.096 mg GAE/g DW was discovered in the ethyl acetate extract. On the other hand, higher TPC value (23.81 mg GAE/g DW) was identfied in the water extract (Rahmani 2019). Compared to Rahamani's (2019) study, the current study indicated the values ranging from 11.47 to 12.77 mg GAE/g DW, and in the range of 9.76 to 10.48 mg GAE/g DW of *Cupressus* spp. in the leaf ethanol and water leaf extracts, respectively.

5.5.2. Evaluation of *Eucalyptus* spp. TPC results

According to the GLM - Univariate analysis, the findings depicted the plant, solvent type, and the interaction between these two factors which were significant on the total phenolic results at the 95% confidence range (p=0.000) (Table 5.21).

Table 5.21. GLM - Univariate analysis of *Eucalyptus* spp. TPC results

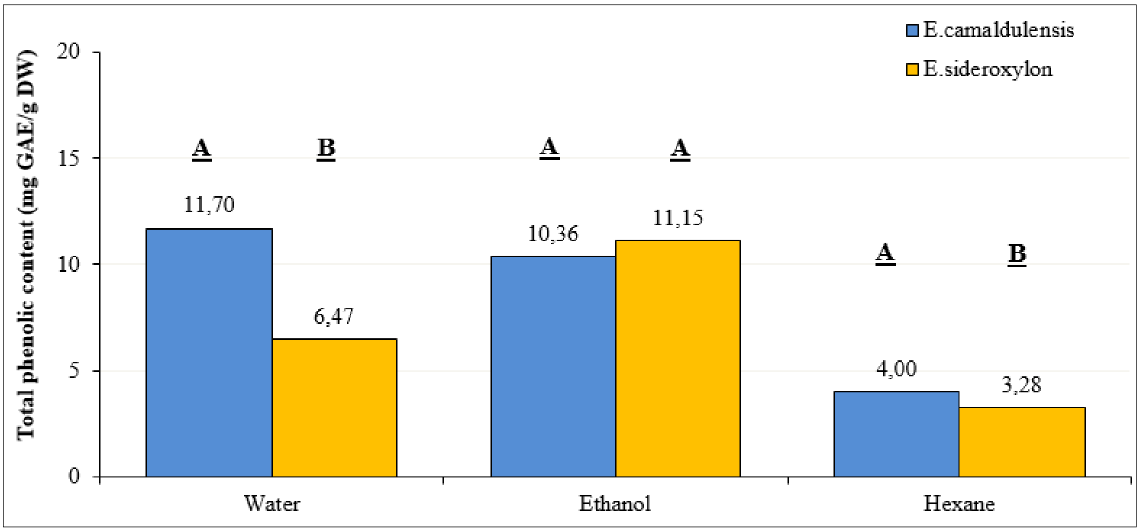
GLM - Univariate Analysis		
Tests of Between-Subjects Effects	**Sig.**	**Difference**
Plant	0.000	+
Solvent type	0.000	+
*Plant * Solvent type*	0.000	+

Referring to the Tukey-B test results (Table 5.22 and Figure 5.35), the best TPC results were exhibited in ethanol, followed by water extract. These results were depicted in the range of 10.36-11.15 mg GAE/g DW and 6.47-11.70 mg GAE/g DW in the ethanol and water extracts, respectively.

Table 5.22. Post Hoc analysis of *Eucalyptus* spp. TPC results (mg GAE/g DW)

Plant	**Solvent Type**		
	Water	Ethanol	Hexane
E.camaldulensis	11.70 (0.09) **A**	10.36 (0.57) **A**	4.00 (0.26) **A**
E.sideroxylon	6.47 (0.19) **B**	11.15 (0.18) **A**	3.28 (0.08) **B**
Tukey B	**B**	**A**	**C**

According to the t-test results (as shown in Table 5.22 and Figure 5.35), a difference was determined between two *Eucalyptus* species in the water and hexane results. The TPC results of *E.camaldulensis* were revealed higher. These results were depicted in the water and hexane extracts with the values of 11.70 mg GAE/g DW and 4.00 mg GAE/g DW, respectively.



Note: Different letters represent statistical differences at the 95% confidence level

Figure 5.35. Total phenolic content and t-test results of *Eucalyptus* spp.

Chahomchuen et al. (2020) reported that TPC of *E.camaldulensis* essential oil leaf methanol extracts was 17.9 mg gallic acid/g. The current study, however showed the value of 10,.36 mg GAE/g DW in the leaf ethanol extract of *E.camaldulensis*.

5.6. Evaluation of the total flavonoid contents (TFC) results

5.6.1. Evaluation of *Cupressus* spp. TFC results

Based on the GLM - Univariate analysis in Table 5.23, it was revealed that the plant, solvent type, and the interaction between these two factors was significant in the 95% confidence range on the TFC results (p=0.000).

Table 5.23. GLM - Univariate analysis of *Cupressus* spp. TFC results

GLM - Univariate Analysis		
Tests of Between-Subjects Effects	**Sig.**	**Difference**
Plant	0.000	+
Solvent type	0.000	+
*Plant * Solvent type*	0.000	+

Based on the t-test results shown in Table 5.24 and Figure 5.36, the highest values were identified in ethanol (4.92-6.89 mg QE/g DW), water (2.46-5.48 mg QE/g DW), and hexane (3.01-3.39 mg QE/g DW) extracts.

Table 5.24. Post Hoc analysis of *Cupressus* spp. TFC results (mg QE/g DW)

Plant	Solvent Type			
	Water	**Ethanol**	**Hexane**	**Tukey B**
C.sempervirens L. var. *pyramidalis*	4.12 (±0.08)	6.53 (±0.01)	3.01 (±0.13)	**B**
C.sempervirens L. var. *horizontalis*	5.48 (±0.17)	6.89 (±0.16)	3.39 (±0.39)	**A**
C.arizonica	2.46 (±0.06)	4.92 (±0.26)	3.10 (±0.25)	**C**
Tukey B	**B**	**A**	**C**	

In terms of the plant species, the highest value (3.39-6.89 mg GAE/g DW) was exhibited in *C.sempervirens* L. var. *horizontalis* leaf samples. This was followed by *C.sempervirens* L. var. *pyramidalis* (3.01- 6.53 mg GAE/g DW), and *C.arizonica* (2.46- 4.92 mg GAE/g DW) leaf samples (Table 5.24 and Fig. 5.36).

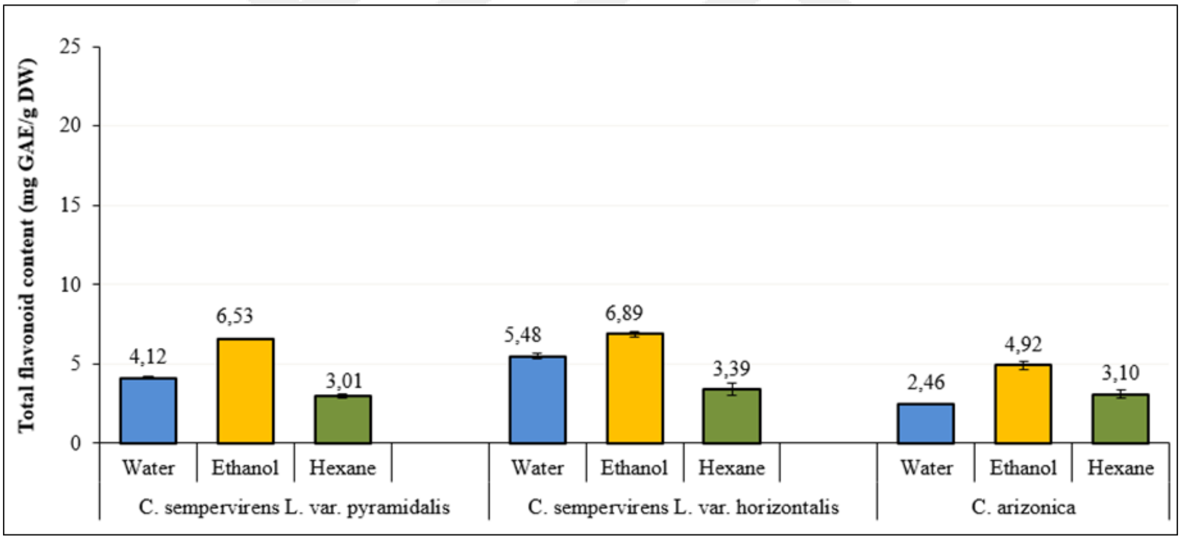


Figure 5.36. TFC results of *Cupressus* spp. in different solvents

Tümen et al. (2012) reported that TFC of the leaf methanol extract of *C.sempervirens var. horizontalis* and *C.sempervirens var pyramidalis* were 11.47mg QE/g and 12.97 mg QE /g, respectively. Lower values were revealed in the water, ethanol and hexane leaf extracts in the present study in comparison with the findings of Tümen et al.'s (2012) study.

5.6.2. Evaluation of *Eucalyptus* spp. TFC results

The GLM - Univariate analysis results showed that the effect of plant species on TFC results at 95% confidence range was not significant (p=0.737). However, solvent type and the interaction between plant and solvent type was discovered significant (p=0.000) (Table 5.25).

Table 5.25. GLM - Univariate analysis of *Eucalyptus* spp. TFC results

GLM - Univariate analysis		
Tests of Between-Subjects Effects	**Sig.**	**Difference**
Plant	0.737	-
Solvent type	0.000	+
*Plant * Solvent type*	0.000	+

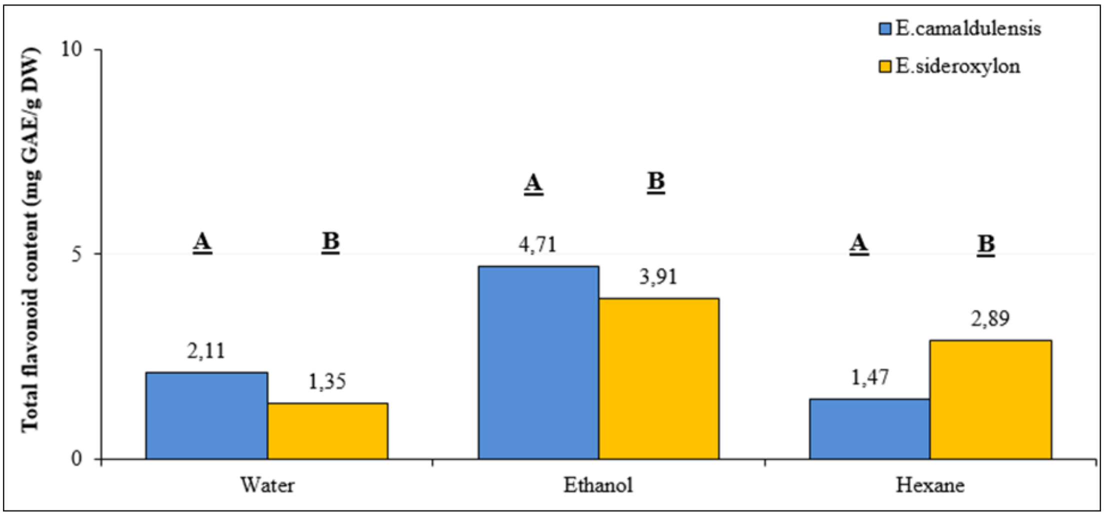
According to Tukey B analysis results (Table 5.26 and Figure 5.37), the best TFC results were discovered in the ethanol (3.91-4.71 mg GAE/g DW), hexane (1.47-2.89 mg GAE/g DW), and water (1.35-2.11 mg GAE/g DW) extracts.

Table 5.26. Post Hoc analysis of *Eucalyptus* spp. TFC results (mg QE/g DW)

Plant	**Solvent Type**		
	Water	Ethanol	Hexane
E.camaldulensis	2.11 (±0.20) **A**	4.71 (±0.14) **A**	1.47 (±0.23) **A**
E.sideroxylon	1.35 (±0.17) **B**	3.91 (±0.30) **B**	2.89 (±0.07) **B**
Tukey B	**C**	**A**	**B**

The t-test analysis showed that there was a difference between *E.camaldulensis* and *E.sideroxylon* in all solvent extracts. On the other hand, *E.camaldulensis* had higher TFC results (Table 5.26 and Figure 5.37).

A previous study by Gullón et al., (2008) discovered that TFC of *E.globulus* ethanol leaf extracts was 53.44 mg RE/g. The current study, however demonstated different results (3.91-4.71 mg GAE/g DW) of *E.camaldulensis* ethanol leaf exctracts.



Note: Different letters represent statistical differences at the 95% confidence level

Figure 5.37. TFC and t-test results of *Eucalyptus* spp.

5.7. Evaluation of the total anthocyanin results

5.7.1. Evaluation of *Cupressus* spp. total anthocyanin results

As seen in Table 5.27, it was discovered that solvent type only had a significant effect on the total anthocyanin results at the 95% confidence range (p=0.000).

Table 5.27. GLM - Univariate analysis of *Cupressus* spp. total anthocyanin results

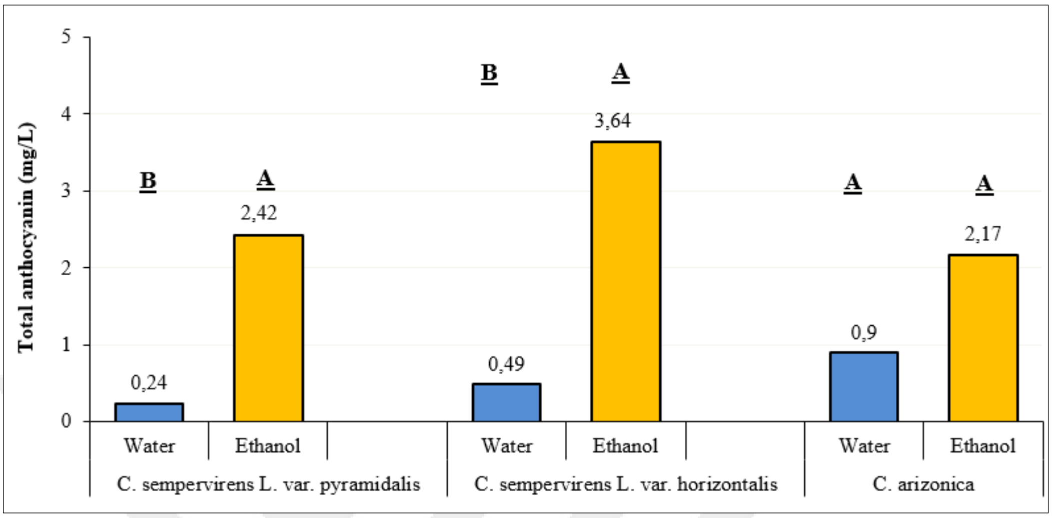
GLM - Univariate Analysis		
Tests of Between-Subjects Effects	**Sig.**	**Difference**
Plant	0.309	-
Solvent type	0.000	+
*Plant * Solvent type*	0.175	-

According to the Tukey-B test analysis (Table 5.27, Table 5.28 and Figure 5.38), it was determined that the *Cupressus* species showed no effect on the total anthocyanidin content.

Table 5.28. Post Hoc analysis of *Cupressus* spp. total anthocyanin results (mg/L)

Solvent Type	**Plant**		
	C.sempervirens L. var. *pyramidalis*	*C.sempervirens* L. var. *horizontalis*	*C.arizonica*
Water	0.24 (±0.05) **B**	0.49 (±0.06) **B**	0.90 (±1.65) **A**
Ethanol	2.42 (±0.18) **A**	3.64 (±0.30) **A**	2.17 (±1.04) **A**
Tukey B	**A**	**A**	**A**

It was revealed through t-test that the type of solvent used had an effect on the anthocyanidin results of *C.sempervirens* L. var. *pyramidalis* and *C.sempervirens* L. var. *horizontalis*. The highest value (2.17-3.64 mg/L) was found in the ethanol extracts (Table 5.28 and Fig. 5.38).



Note: Different letters represent statistical differences at the 95% confidence level

Figure 5.38. Total anthocyanin results of *Cupressus* spp. in different solvents

5.7.2. Evaluation of *Eucalyptus* spp total anthocyanin results

Anthocyanin was determined only in *E.sideroxylon* leaf water extract with the value 0.89 mg/L. However, it was discovered that anthocyanin could not be detected in *E.camaldulensis* (Table 4.13).

Hassine et al.'s (2012) study discovered the total anthocyanin content of *E.gillii* leaves. The results showed that the total of anthocyanin was not detected in ethanol. However, it was identified in water with 1.2 C3GE mg/Kg dry mass.

5.8. Evaluation of the Total Antioxident Capacity results

5.8.1. Evaluation of the DPPH radical scavenging activity results

This subsection discusses the DPPH radical scavening activity results of *Cupressus* and *Eucalyptus*.

5.8.1.1. Evaluation of the *Cupressus* spp. DPPH radical scavenging activity results

The results of GLM - Univariate analysis, which was performed separately for each *Cupressus* species, are given in Table 5.29. As seen in this table, the effect of solvent, concentration, and the interaction between these two factors on DPPH %inhibition and trolox equivalent values of all *Cupressus* species was found to be significant at 95% confidence range (p= 0.00).

Table 5.29. GLM-Univariate analysis of *Cupressus* spp. DPPH radical scavenging activity results

		%Inhibition		Trolox Equivalent (mg/kg)	
	Tests of Between-Subjects Effects	**Sig.**	**Difference**	**Sig.**	**Difference**
C.sempervirens* var. *pyramidalis	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000
C.sempervirens* var. *horizontalis	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000
C.arizonica	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000

The DPPH radical scavening activity results of *C.sempervirens* var. *pyramidalis* are shown in Table 5.30, Figures 5.39 and 5.40. According to the Tukey B test results as in Table 5.30, the highest DPPH %inhibition values in all *Cupressus* spp. were obtained in ethanol>water>hexane extracts with an increase concentration, respectively. The values are 80.2%, 97.8%, and 93.3% in *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, and *C.arizonica*, respectively (Figure 5.39).

According to Tukey B test analysis, the highest trolox equivalent values ranging from 361.3 to 385.6 mg/kg in all *Cupressus* samples were found in the hexane extract at 55 µg/100g concentration (Figure 5.40).

The lowest IC50 value (14.9 - 43.9 µg/100g) was found in the ethanol extract. A low IC50 value indicates a high antioxidant effect (Table 4.14).

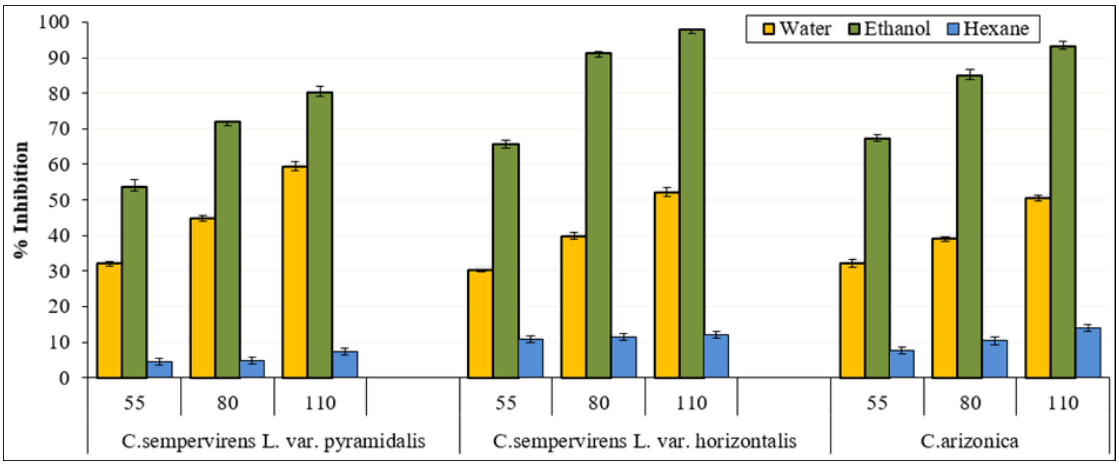


Figure 5.39. DPPH %inhibition of *Cupressus* spp.

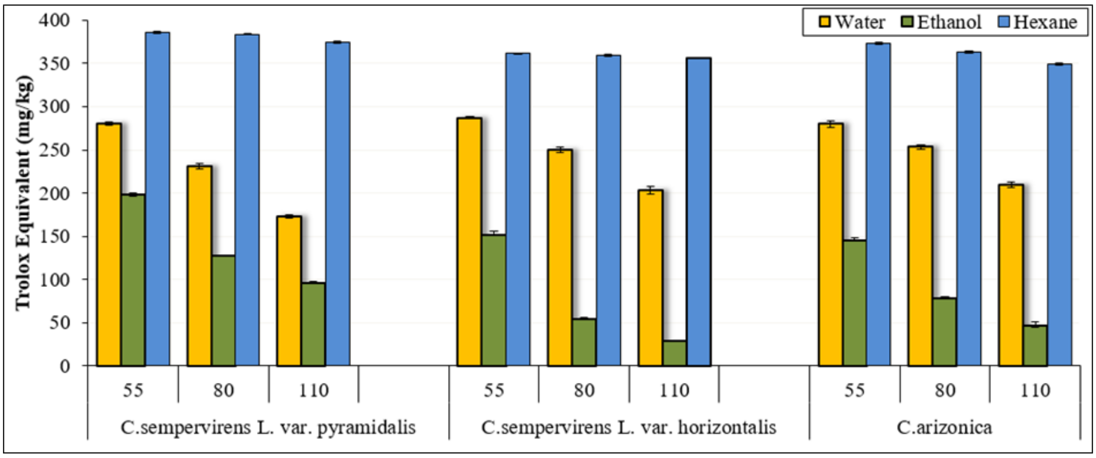


Figure 5.40. DPPH trolox equivalent of *Cupressus* spp.

Al-Snafi (2016) reported that the cone ethyl acetate extract of *Cupressus sempervirens var. horizantalis* displayed the highest DPPH radical scavenging activity (87.53%). With regard to this, higher values were shown in the current study compared to Al-Snafi's (2016) study.

Table 5.30. Post Hoc analysis of *Cupressus* spp. DPPH radical scavenging activity results

		%Inhibition				Trolox Equivalent (mg/kg)			
		Concentration (µg/100g)			**Tukey B**	**Concentration (µg/100g)**			**Tukey B**
	Solvent Type	**55**	**80**	**110**		**55**	**80**	**110**	
C.sempervirens* L. var. *pyramidalis	Water	32.0 (0.53)	44.9 (0.70)	59.5 (1.30)	**B**	280.3 (2.04)	231.1 (2.67)	172.6 (2.04)	**B**
	Ethanol	53.6 (2.03)	71.9 (0.15)	80.2 (1.85)	**A**	197.7 (2.75)	127.5 (0.58)	96.0 (2.06)	**C**
	Hexane	4.52 (1.70)	4.95 (0.23)	7.31 (0.15)	**C**	385.6 (2.49)	383.9 (0.87)	374.9 (0.58)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
C.sempervirens* L. var. *horizontalis	Water	30.2 (0.38)	39.9 (0.85)	52.2 (1.22)	**B**	287.3 (1.46)	250.3 (3.24)	203.2 (4.66)	**B**
	Ethanol	65.6 (1.07)	91.2 (0.46)	97.8 (0.23)	**A**	151.9 (4.07)	54.1 (1.75)	28.8 (1.23)	**C**
	Hexane	10.9 (0.58)	11.4 (0.00)	12.2 (0.02)	**C**	361.3 (2.20)	359.2 (0.00)	356.3 (0.00)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
C.arizonica	Water	32.1 (1.07)	39.1 (0.58)	50.5 (0.88)	**B**	279.9 (4.07)	253.4 (2.20)	209.7 (3.36)	**B**
	Ethanol	67.4 (0.99)	84.9 (1.90)	93.3 (1.22)	**A**	145.2 (3.78)	78.3 (2.28)	46.2 (4.67)	**C**
	Hexane	7.76 (0.95)	10.5 (0.47)	14.1 (0.72)	**C**	373.1 (3.64)	362.9 (1.78)	349.1 (2.75)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	

Note: 1.) Values in parentheses are standard deviation

5.8.1.2. Evaluation of the *Eucalyptus* spp. DPPH radical scavenging activity results

According to the results of GLM - Univariate analysis, the effect of solvent (water, ethanol, hexane), concentration (55, 80, 110 µg/100g) and the interaction between these two factors on the DPPH %inhibition and trolox equivalent values is significant at the 95% confidence range (p= 0.000) (Table 5.31).

Table 5.31. GLM-Univariate analysis of *Eucalyptus* spp. DPPH radical scavenging activity results

		%Inhibition		**Trolox Equivalent (mg/kg)**	
Tests of Between-Subjects Effects		**Sig.**	**Difference**	**Sig.**	**Difference**
E.camaldulensis	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000
E.sideroxylon	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000

Based on the Tukey-B test results as shown in Table 5.32, it is seen that the DPPH %inhibition values increased as the concentration increased from both *Eucalyptus* samples. As presented in Figure 5.41, the highest values were obtained in the water extracts at 110 µg/100g concentration. The values 98.9% and 77.9% were indicated in *E.camaldulensis* and *E.sideroxylon*, respectively. In general, *E.camaldulensis* showed better antioxidant properties than *E.sideroxylon* in the DPHH method.

Trolox equivalent values were found to be the highest in both hexane extracts at 55 µg/100g concentration according to the Tukey-B test analysis (Table 5.32). This was depicted in Figure 5.42 with the values of 372.7 mg/kg and 381.1 mg/kg in *E.camaldulensis* and *E.sideroxylon*, respectively.

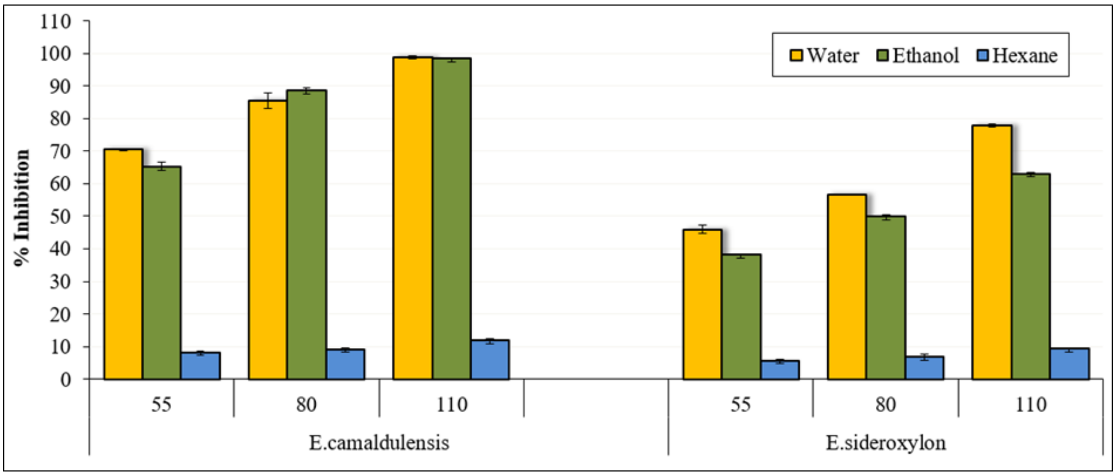


Figure 5.41. DPPH %inhibition of *Eucalyptus* spp.

Table 5.32. Post Hoc analysis of *Eucalyptus* spp. DPPH radical scavenging activity results

	%Inhibition					Trolox Equivalent (mg/kg)			
		Concentration (µg/100g)			Tukey B	Concentration (µg/100g)			Tukey B
	Solvent Type	**55**	**80**	**110**		**55**	**80**	**110**	
E.camaldulensis	Water	70.5 (0.30)	85.5 (2.36)	98.9 (0.46)	**A**	133.3 (1.16)	75.9 (2.02)	24.4 (1.75)	**B**
	Ethanol	65.2 (1.29)	88.7 (0.84)	98.4 (0.38)	**A**	153.4 (4.95)	63.7 (3.20)	26.5 (1.46)	**B**
	Hexane	8.30 (0.38)	9.18 (0.32)	11.9 (0.49)	**B**	372.7 (3.21)	367.7 (1.21)	357.2 (1.87)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
E.sideroxylon	Water	45.9 (1.24)	56.5 (0.08	77.9 (0.46)	**A**	227.2 (4.74)	186.6 (0.29)	107.5 (5.02)	**C**
	Ethanol	38.2 (0.15)	50.0 (0.53)	63.1 (0.23)	**B**	256.7 (0.58)	211.6 (2.04)	161.5 (0.87)	**B**
	Hexane	5.68 (0.47)	6.90 (0.70)	9.28 (0.15)	**C**	381.1 (1.78)	376.4 (2.69)	367.3 (0.58)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	

Note: 1.) Values in parentheses are standard deviation

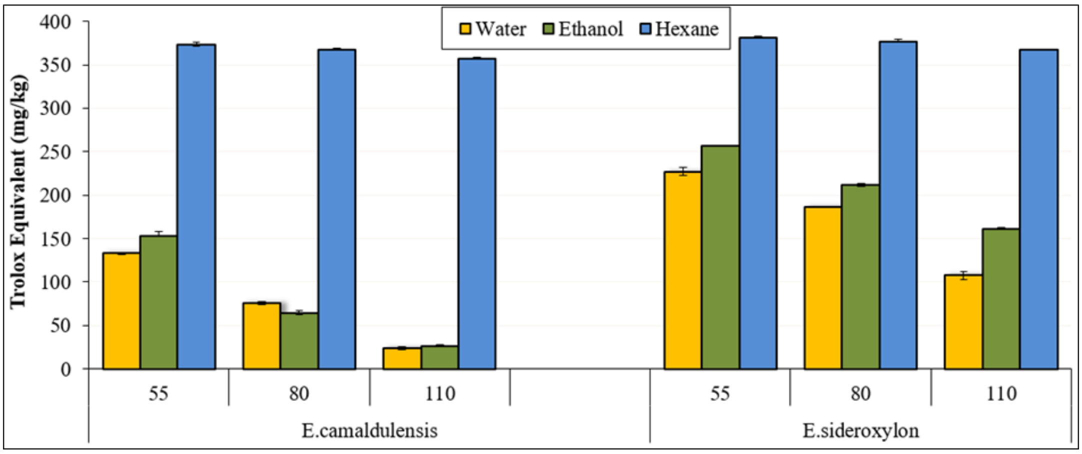


Figure 5.42. DPPH trolox equivalent of *Eucalyptus* spp.

According to the IC50 results as shown in Table 4.15, the best antioxidant properties were found in the water extracts (14.9 - 65.1 µg/100g).

According to Ghaffaret al. (2015), the DPPH scavenging activity showed the highest inhibition result in EO leaves for *E.citriodora* (82.1%), followed by *E.camaldulensis* (81.9%), and *E.microtheca* (81.8%). The results of the current study with 98.9% in *E.camaldulensis* indicated higher value in comparison with the findings of Ghaffaret al.'s (2015) study.

5.8.2. Evaluation of the ABTS antioxidant capacity assay results

This section discusses the ABTS antioxidant capacity assay results of *Cupressus* and *Eucalyptus.*

5.8.2.1. Evaluation of the *Cupressus* spp. ABTS antioxidant capacity assay results

In Table 5.33, the results of GLM - Univariate analysis, which was performed separately for each *Cupressus* species, are given.

Table 5.33. GLM-Univariate analysis of *Cupressus* spp. ABTS antioxidant capacity assay results

		%Inhibition		Trolox Equivalent (mg/kg)	
Tests of Between-Subjects Effects		Sig.	Difference	Sig.	Difference
C.sempervirens var. ***pyramidalis***	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000
C.sempervirens var. ***horizontalis***	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	-	0.334	-	0.230
C.arizonica	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000

The ABTS antioxidant capacity assay results of *Cupressus* spp. are shown in Table 5.34 and Figures 5.43 and 5.44. According to the Tukey B test analysis results in Table 5.34, the highest ABTS %inhibition values in *C.sempervirens* var. *horizontalis* and *C.arizonica*, were obtained in the ethanol>water>hexane extracts, and in *C.sempervirens* var. *pyramidalis* water>ethanol>hexane extracts depending on the concentration increase. The highest ABTS %inhibition values of 87.2%, 99.6% and 97.1% were discovered in *C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. *horizontalis*, and *C.arizonica*, respectively (Figure 5.43).

According to the Tukey B test analysis, the highest trolox equivalent value ranging from 377.9 to 433.7 mg/kg was obtained in the hexane extracts at 50 µg/100g concentration in all *Cupressus* samples (Figure 5.44).

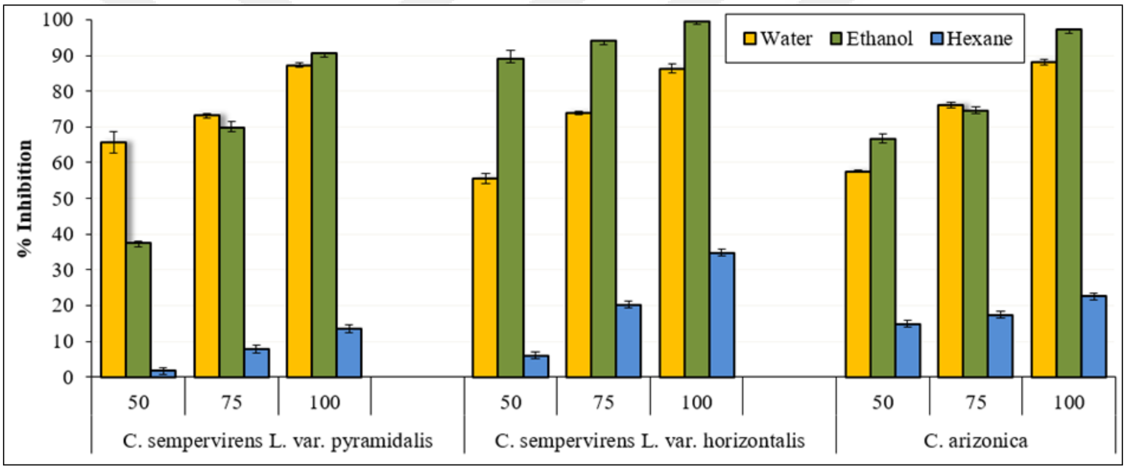


Figure 5.43. ABTS %inhibition of *Cupressus* spp.

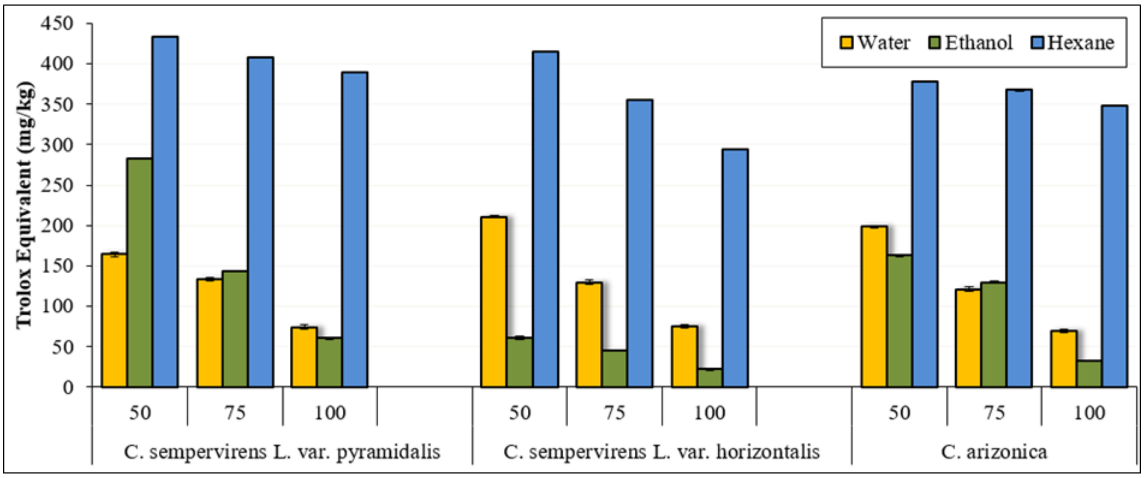


Figure 5.44. ABTS trolox equivalent of *Cupressus* spp.

Table 5.34. Post Hoc analysis of *Cupressus* spp. ABTS antioxidant capacity assay results

		%Inhibition				Trolox Equivalent (mg/kg)			
		Concentration (µg/100g)			**Tukey B**	**Concentration (µg/100g)**			**Tukey B**
	Solvent Type	**50**	**75**	**100**		**50**	**75**	**100**	
C.sempervirens L.* var. *pyramidalis	Water	65.8 (3.05)	73.1 (0.55)	87.2 (0.62)	**A**	164.1 (2.40)	133.3 (2.33)	74.2 (2.62)	**C**
	Ethanol	37.6 (0.56)	69.8 (1.70)	90.5 (0.35)	**B**	283.2 (0.87)	143.2 (1.16)	60.3 (1.47)	**B**
	Hexane	1.71 (2.84)	7.89 (3.15)	13.5 (1.38)	**C**	433.7 (0.82)	407.5 (2.47)	389.4 (2.50)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
C.sempervirens* L. var. *horizontalis	Water	55.5 (1.42)	73.9 (0.55)	86.2 (1.26)	**B**	210.7 (1.75)	129.8 (2.33)	75.4 (2.04)	**B**
	Ethanol	89.1 (2.46)	94.1 (0.07)	99.6 (0.14)	**A**	60.5 (2.33)	45.1 (0.29)	22.1 (0.58)	**C**
	Hexane	6.09 (2.08)	20.3 (0.29)	34.8 (3.47)	**C**	415.0 (8.73)	355.5 (1.23)	294.3 (2.5)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
C.arizonica	Water	57.6 (0.32)	75.9 (0.80)	88.2 (0.76)	**B**	198.7 (1.34)	121.3 (3.36)	70.13 (2.26)	**B**
	Ethanol	66.7 (1.27)	74.7 (1.13)	97.1 (0.35)	**A**	162.9 (1.75)	129.5 (1.46)	32.4 (1.47)	**C**
	Hexane	14.9 (2.71)	17.4 (4.38)	22.7 (1.06)	**C**	377.9 (4.53)	367.5 (2.88)	347.9 (4.45)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	

Note: 1.) Values in parentheses are standard deviation

Khulbe et al., (2016) investgated the ABTS antioxidant capacity assay of *C.torulosa* leaf. The highest ABTS scavenging was recorded in the water extract (83% inhibition; IC50-16.61 µ g/ml), followed by ethanol (77% inhibition; IC50-19.79 µ g/ml), and hexane (61% inhibition; IC50-35.52 µ g/m) (Khulbe et al., 2016). Compared to the findings of Khulbe et al.,'s (2016) study, higher results were exhibited in all *Cupressus* spp. in the current study.

5.8.2.2. Evaluation of the *Eucalyptus* spp. ABTS antioxidant capacity assay results

The results of GLM - Univariate analysis, which was performed separately for each *Eucalyptus* species, are given in Table 5.35. This table shows that the effect of solvent, concentration, and the interaction between these two factors on the ABTS %inhibition and trolox equivalent values of all *Eucalyptus* species was found to be significant at the 95% confidence range (p= 0.00).

Table 5.35. GLM-Univariate analysis of *Eucalyptus* spp. ABTS antioxidant capacity assay results

		%Inhibition		Trolox Equivalent (mg/kg)	
Tests of Between-Subjects Effects		**Sig.**	**Difference**	**Sig.**	**Difference**
E.camaldulensis	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000
E.sideroxylon	*Solvent*	+	0.000	+	0.000
	Concentration	+	0.000	+	0.000
	Solvent Concentration*	+	0.000	+	0.000

Based on the Tukey B test results (Table 5.36), the ABTS %inhibition values were identified increased in both *Eucalyptus* samples, due to an increase in concentration. Referring to Figure 5.45, the highest values of 99.9% and 89.6% were obtained in the water extract at 100 µg/100g concentration in *E.camaldulensis* and *E.sideroxylon*, respectively.

In general, *E.camaldulensis* showed better antioxidant properties than *E.sideroxylon*.

The Trolox equivalent values were found to be the highest in both hexane extracts at 50 µg/100g concentration based on the Tukey B test analysis (Table 5.36). As shown in Figure 5.46, the highest trolox value of 285.5 mg/kg was found in *E.sideroxylon*.

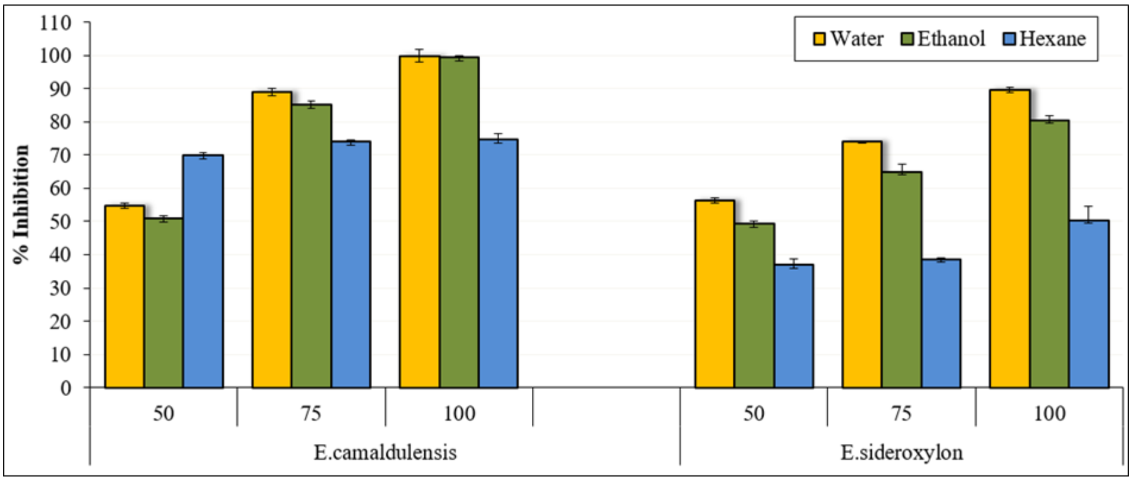


Figure 5.45. ABTS %inhibition of *Eucalyptus* spp.

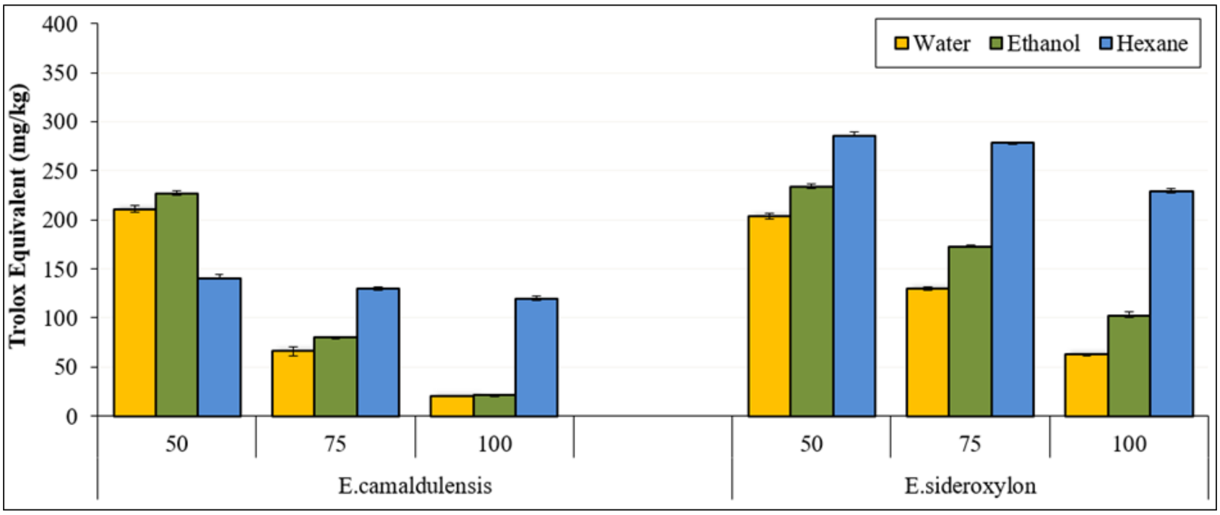


Figure 5.46. ABTS trolox equivalent of *Eucalyptus* spp.

In Noumi et al.,'s (2021) study, the antioxidant activity of *E.camaldulensis* EO leaf samples was measured in terms of radical scavenging ability using the ABTS assay. The highest ABTS %inhibition value of 89.59% were exhibited in the ethanol extracts at <100 µm concentration. In contrast, higher results were discovered in the current study.

Table 5.36. Post Hoc analysis of *Eucalyptus* spp. ABTS antioxidant capacity assay results

		%Inhibition				Trolox Equivalent (mg/kg)			
		Concentration (μg/100g)			**Tukey B**	**Concentration (μg/100g)**			**Tukey B**
	Solvent Type	**50**	**75**	**100**		**50**	**75**	**100**	
E.camaldulensis	Water	54.6 (0.81)	89.1 (1.12)	99.9 (1.88)	**A**	211.1 (3.41)	66.2 (4.71)	20.9 (0.38)	**C**
	Ethanol	50.9 (0.69)	85.1 (1.16)	99.5 (0.60)	**B**	226.4 (2.91)	80.0 (0.29)	21.2 (0.29)	**B**
	Hexane	69.8 (1.08)	74.1 (0.49)	74.8 (1.66)	**C**	140.6 (3.20)	129.4 (2.04)	119.0 (2.95)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	
E.sideroxylon	Water	56.4 (0.69)	73.9 (0.29)	89.6 (0.76)	**A**	203.7 (2.91)	129.9 (1.21)	62.6 (1.46)	**C**
	Ethanol	49.4 (0.62)	65.0 (2.26)	80.6 (1.32)	**B**	233.1 (3.70)	172.9 (1.16)	102.1 (3.70)	**B**
	Hexane	36.9 (1.87)	38.6 (0.28)	50.4 (4.02)	**C**	285.5 (3.90)	278.2 (1.16)	228.8 (2.56)	**A**
	Tukey B	**C**	**B**	**A**		**A**	**B**	**C**	

Note: 1.) Values in parentheses are standard deviation

5.8.3. Evaluation of the CUPRAC results

This section discusses the CUPRAC ile antioksidant antioxidant capacity assay results of *Cupressus* and *Eucalyptus*.

5.8.3.1. Evaluation of the *Cupressus* spp. CUPRAC results

As presented in Table 5.37, the effect of solvent, concentration, and the interaction between these two factors, (except for the solvent*concentration interaction of *C.arizonica*), was found to be significant on the ABTS trolox equivalent values of all *Cupressus* species, at the 95% confidence range (p= 0.000-0.003).

Table 5.37. GLM-Univariate analysis of *Cupressus* spp. CUPRAC results

		Trolox Equivalent (mg/kg)	
Tests of Between-Subjects Effects		Sig.	Difference
C.sempervirens* var. *pyramidalis	*Solvent*	+	0.000
	Concentration	+	0.000
	Solvent Concentration*	+	0.000
C.sempervirens* var. *horizontalis	*Solvent*	+	0.000
	Concentration	+	0.000
	Solvent Concentration*	+	0.003
C.arizonica	*Solvent*	+	0.000
	Concentration	+	0.000
	Solvent Concentration*	-	0.474

The trolox value increased in all *Cupressus* species depending on the concentration. The highes concentration was depicted at 100 µg/100g. In general, the trolox value was determined in the range of 7.04 to 29.3 mg/kg at 100 µg/100g concentrations (Figure 5.47, Table 5.38).

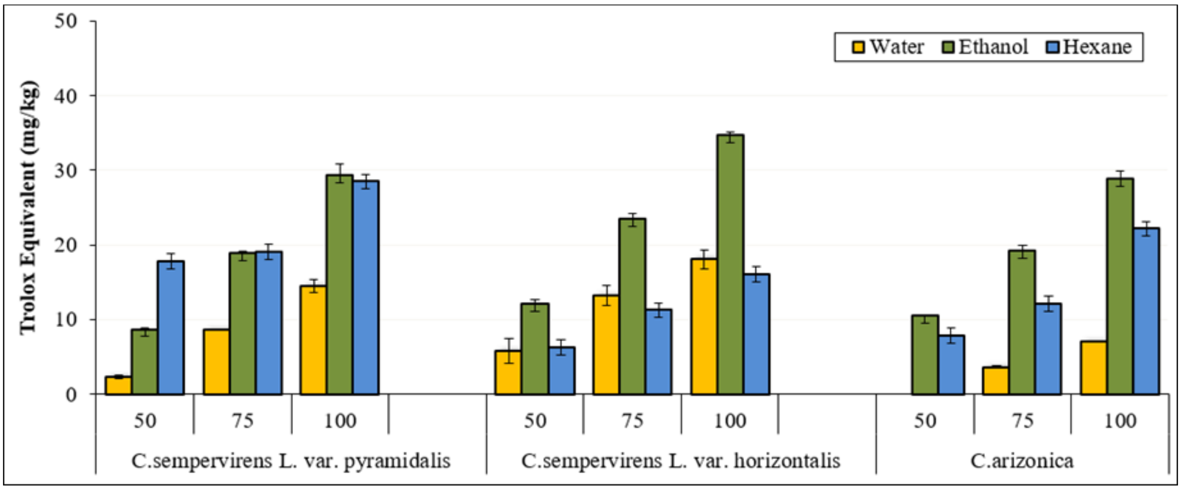


Figure 5.47. CUPRAC of *Cupressus* spp.

Table 5.38. Post Hoc analysis of *Cupressus* spp. CUPRAC results

	Trolox Equivalent (mg/kg)				
		Concentration (μg/100g)			
	Solvent	**50**	**75**	**100**	**Tukey B**
C.sempervirens* L. var. *pyramidalis	Water	2.32 (0.27)	8.72 (0.04)	14.5 (0.87)	**C**
	Ethanol	8.74 (0.24)	18.9 (0.24)	29.3 (1.54)	**B**
	Hexane	17.8 (0.06)	19.1 (0.61)	28.5 (1.36)	**A**
	Tukey B	**C**	**B**	**A**	
C.sempervirens* L. var. *horizontalis	Water	5.81 (1.68)	13.3 (1.37)	18.1 (1.25)	**B**
	Ethanol	12.1 (0.53)	23.5 (0.72)	34.7 (0.50)	**A**
	Hexane	6.26 (1.69)	11.3 (0.69)	16.1 (0.59)	**B**
	Tukey B	**C**	**B**	**A**	
C.arizonica	Water	0.00 (0.00)	3.69 (0.17)	7.04 (0.03)	**B**
	Ethanol	10.6 (0.11)	19.3 (0.68)	28.9 (1.04)	**A**
	Hexane	7.85 (0.61)	12.2 (0.71)	22.2 (2.12)	**A**
	Tukey B	**B**	**B**	**A**	

Note: 1.) Values in parentheses are standard deviation

5.8.3.2. Evaluation of the *Eucalyptus* spp. CUPRAC results

As depicted in Table 5.39, GLM - Univariate analysis was performed for each *Eucalyptus* species separately. In general, the effect of solvent, concentration, and the interaction between these two factors, (except solvent*concentration interaction of *E.sideroxylon*), on ABTS trolox equivalent results of all *Eucalyptus* species was found significant at 95% confidence range (p= 0.000).

Table 5.39. GLM-Univariate analysis of *Eucalyptus* spp. CUPRAC results

		Trolox Equivalent (mg/kg)	
	Tests of Between-Subjects Effects	**Sig.**	**Difference**
E.camaldulensis	*Solvent*	+	0.000
	Concentration	+	0.000
	Solvent Concentration*	+	0.000
E.sideroxylon	*Solvent*	+	0.000
	Concentration	+	0.000
	Solvent Concentration*	-	0.599

The highest trolox value was determined at 100 concentration for both *Eucalyptus* spp. The trolox equivalent values were found to be the highest in the water extract for

E.camaldulensis and in hexane extract for *E.sideroxylon*. As displayed in Table 5.40 and Figure 5.48, the values of 38.2 mg/kg and 21.3 mg/kg were discovered in *E.camaldulensis* and *E.sideroxylon*, respectively.

Table 5.40. Post Hoc analysis of *Eucalyptus* spp. CUPRAC results

	Trolox Equivalent (mg/kg)				
	Solvent	**Concentration (µg/100g)**			
		50	**75**	**100**	**Tukey B**
E.camaldulensis	Water	14.6 (0.83)	29.1 (0.34)	38.2 (0.47)	**A**
	Ethanol	10.5 (0.42)	20.2 (0.64)	30.1 (1.08)	**C**
	Hexane	19.5 (1.08)	24.4 (0.75)	31.3 (0.08)	**B**
	Tukey B	**C**	**B**	**A**	
E.sideroxylon	Water	5.37 (0.42)	6.20 (1.37)	9.75 (0.50)	**B**
	Ethanol	0.00 (0.00)	2.76 (0.95)	8.11 (1.01)	**C**
	Hexane	12.9 (3.73)	16.5 (3.14)	21.3 (1.96)	**A**
	Tukey B	**B**	**B**	**A**	

Note: 1.) Values in parentheses are standard deviation

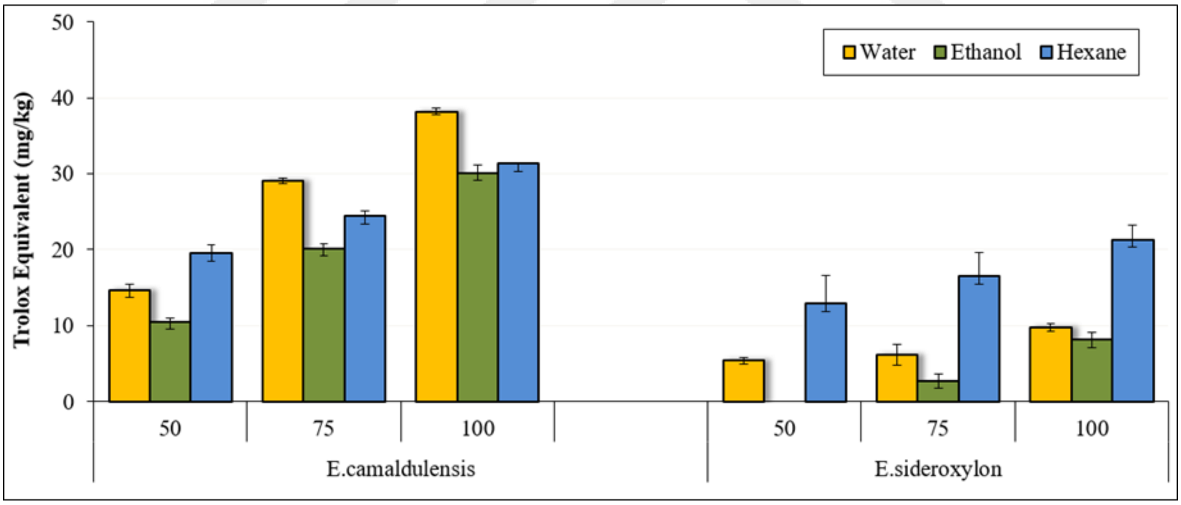


Figure 5.48. CUPRAC of *Eucalyptus* spp.

In a study conducted by Álvarez et al., (2021), the antioxidant capacity properties of some tree species were determined according to the ABTS and CUPRAC method. The antioxidant capacity value of *E.camaldulensis* leaves was found to be 0.33 mmol g−1 DW using the CUPRAC method. The results of the current study were seen higher compared to that findings of Álvarez et al.,'s (2021) study.

5.9. Evaluation of the chemical compound results determined by HPLC

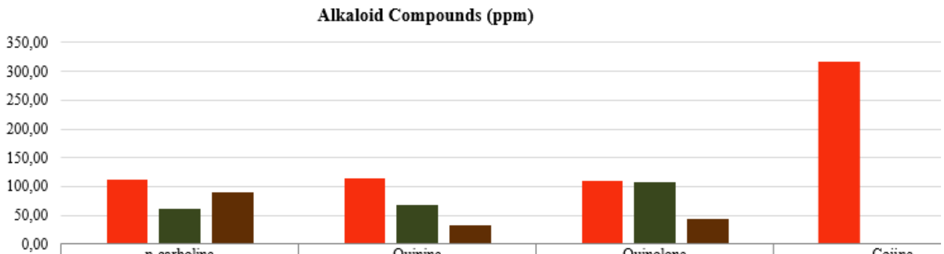
Based on the results of the antimicrobial and antifungal activities of *Cupressus* and *Eucalyptus* species, the chemical components of the leaves were solely assessed. The alkaloid, glycosidically bound and phenols & flavonoids groups were determined by the HPLC method. The results obtained from the HPLC method are disscussed in this section.

5.9.1. HPLC results of *Cupressus* spp.

5.9.1.1 Evaluation of the alkaloid compounds in *Cupressus* spp.

Through the HPLC method, p-carboline, quinine, quinolone and coiine were determined as alkaloid compounds in *Cupressus* samples (Table 4.20).

Table 4.20 and Figure 5.49 show the alkaloid compound for *C.sempervirens* var. *pyramidalis*, *C.sempervirens* var. *horizontalis* and *C.arizonica* leaf as determined by HPLC. The results show different ppm for each compound. The highest alkaloid compounds were found in *C.sempervirens* var. *pyramidalis* species. Accordingly, p-carboline was determined as 111.47 ppm, quinine 114.14 ppm, Quinolone 110.59 ppm, and Coiine 317.88 ppm.



	p-carboline	Quinine	Quinolone	Coiine
C. sempervirens var. pyramidalis	111,47	114,14	110,59	317,88
C. sempervirens var. horizontalis	60,11	66,96	106,95	0
C. arizonica	89,17	33,15	43,84	0

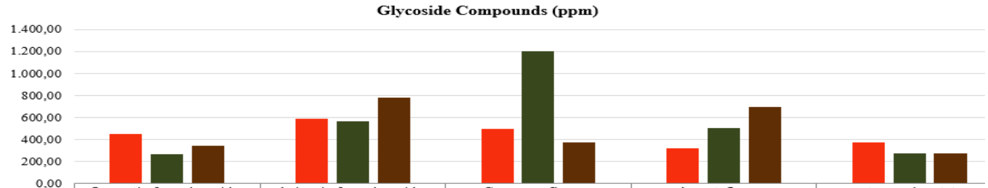
Figure 5.49 Alkaloid compounds in *Cupressus* spp.

A study by Hotti and Rischer (2017) approved that coniine, a polyketide-derived alkaloid, is poisonous to humans and animals. It is a nicotinic acetylcholine receptor antagonist, which leads to inhibition of the nervous system, eventually causing death by suffocation in mammals. Coniine's most famous victim is Socrates who was sentenced to death by poison chalice containing poison hemlock in 399 BC (Hotti and Rischer, 2017).

5.9.1.2. Evaluation of the glycosidically bound compounds in *Cupressus* spp.

Table 4.21 the glycosidically bound compound for *C.sempervirens* var. *pyramidalis*, *C.sempervirens* var. *horizontalis* and *C.arizonica* leaf as determined by HPLC.

Table 4.21 and Figure 5.50 show the glycosidically bound compound for *C.sempervirens* var. *pyramidalis*, *C.sempervirens* var. *horizontalis* and *C.arizonica* leaves identified by HPLC. The highest compound was quercetin-3-o-glycosides (449.43 ppm) and a-terpenyl acetate (375.09 ppm) in *C.sempervirens* var. *pyramidalis*, apigenin-3-o-glycoside (776.29 ppm) and amentoflavone (695.9 ppm) in *C.arizonica*, and cupressusflavone (1200.26 ppm) in *C.sempervirens* var. *horizontalis*.



	Quercetin-3-o -glycosides	Apigenin-3-o -glycosides	Cupressus flavone	Amentoflavone	a-terpenyl acetate
C. sempervirens var. pyramidalis	449,43	588,00	497,69	316,87	375,09
C. sempervirens var. horizontalis	264,62	565,85	1200,26	504,57	272,94
C. arizonica	341,15	776,29	376,93	695,9	271,69

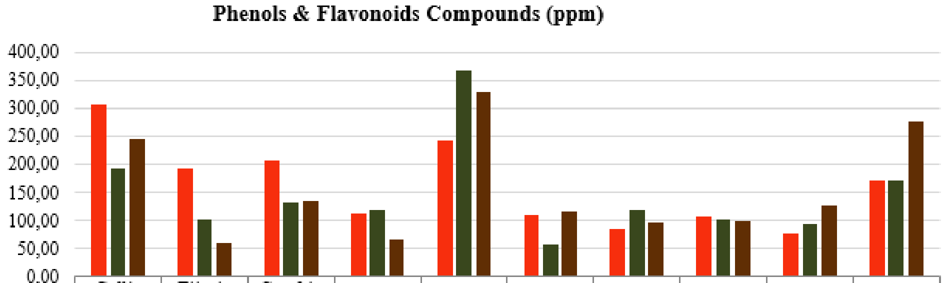
Figure 5.50. Glycosidically bound compound in *Cupressus* spp.

Inflammation is a complex biological process that is generally occurs in response to pathological triggers. Both neurodegenerative diseases and cancer have been linked to inflammation. The analgesic and anti-inflammatory effects of cupressuflavone (CUF) isolated from *Cupressus macrocarpa* were examined. CUF is a potential therapeutic agent showing analgesic and antiinflammatory effects. It is noteworthy that this study is the first to reveal the anti-inflammatory and analgesic mechanism of action of this biflavonoid. The lack of toxicity and potent anti-inflammatory and analgesic activities suggest CUF potential application in dietary supplements and functional foods targeting inflammation-related disease states (Al-Sayed et al., 2018).

5.9.1.3. Evaluation of the phenols & flavonoids in *Cupressus* spp.

According to the HPLC method, gallic acid, eiiagic acid, catafeic acid, taxifolin, rutin, quercetin, kaemferol, castalagin, and apigenin were determined as phenols and flavonoids compounds in *Cupressus* samples (Table 4.22).

Table 4.22 and Figure 5.51 show the phenols and flavonoids compounds for *Cupressus* spp. leaf as detected by HPLC. The results reveal different ppm for each compound. The highest phenols and flavonoids compounds were identified in *C.sempervirens* var. *pyramidalis* specie. Gallic acid was determined as 307.50 ppm, eiiagic acid 193.04 ppm, catafeic acid 207.04 ppm, and castalagin 107.48 ppm. As for *C.sempervirens* var. *horizontalis* taxifolin (116.97 ppm), rutin (368.82 ppm), and kaemferol (119,46 ppm) were the highest compounds. In *C.arizonica*, it was quercetin (116.35 ppm) and apigenin (125.80 ppm).



	Gallic acid	Eiiagic acid	Catafeic acid	Taxifolin	Rutin	Quercetin	Kaemferol	Castalagin	Apigenin	??
C. sempervirens var. pyramidalis	307,50	193,04	207,04	111,97	242,33	110,06	84,03	107,48	76,43	170,68
C. sempervirens var. horizontalis	194,11	101,52	133,23	116,97	368,82	57,40	119,46	101,58	93,00	172,00
C. arizonica	245,90	59,62	135,38	66,15	329,34	116,35	95,40	97,56	125,80	276,81

Figure 5.51. Phenols and flavonoids compound in *Cupressus* spp.

Nowadays, well-known natural products, such as curcumin, rutin, quercetin, resveratrol are beneficial in the biomedicine field for their high safety, cost-effectiveness, and extensive biological effects. Different pharmacological or biological functions of flavonoids, such as reactive oxygen scavenging, antiinflammatory, immunomodulatory, antimicrobial, and cancer treatment activities have transformed them into valuable potential drugs (Negahdari et al, 2021).

Rutin, as a natural flavonoid compound, has revealed an extensive range of therapeutic potentials. The preparation of rutin nanomaterials for the various therapeutic objects confirmed the enhanced aqueous solubility, as well as, enhanced efficacy compared to conventional delivery of rutin. However, more investigations should be conducted to confirm the improved bioavailability of the rutin nanoformulations (Negahdari et al., 2021).

5.9.2. Evaluation of the chemical compound results determined by HPLC in *Eucalyptus* spp.

5.9.2.1. Evaluation of the alkaloid compounds in *Eucalyptus* spp.

Table 4.23 shows the alkaloid compounds. The highest was berberine in *E.camaldulensis*, as detected by the HPLC method.

Table 4.23 and Figure 5.52 depicts the alkaloid compound for *E.camaldulensis* and *E.sideroxylon* leaf, detected by HPLC. The results present different ppm for each compound. The highest compound was identified in *E.camaldulensis* species. Accordingly, chelythrine (98.59 ppm), berberine (344.16 ppm), and sanquinarine (134.01 ppm).

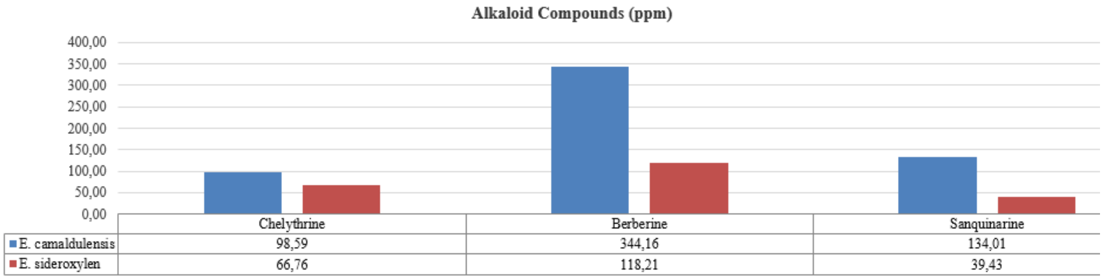


Figure 5.52. Alkaloid compounds in *Eucalyptus* spp.

5.9.2.2. Evaluation of the glycosidically bound compounds in *Eucalyptus* spp.

According to the HPLC method, qurercetin-3-arabinoside, quercetin-3,7-di-arhamanose, and kaempferol-3-arabinoside were determined as glycosidically bound compounds in *Cupressus* samples (Table 4.24).

Table 4.24 and Figure 5.53 display the glycoside compound for *E.camaldulensis* and *E.sideroxylon* leaf detected by HPLC. The higher compound was shown only in *E.camaldulensis* species. For instance; Qurercetin-3-arabinoside (272.14 ppm), quercetin-3,7-di-arhamanose (346.46 ppm), and kaempferol-3-arabinoside (442.89 ppm).

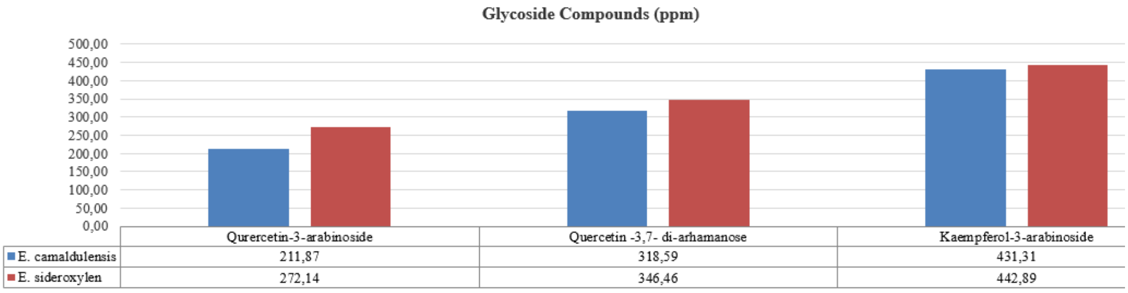
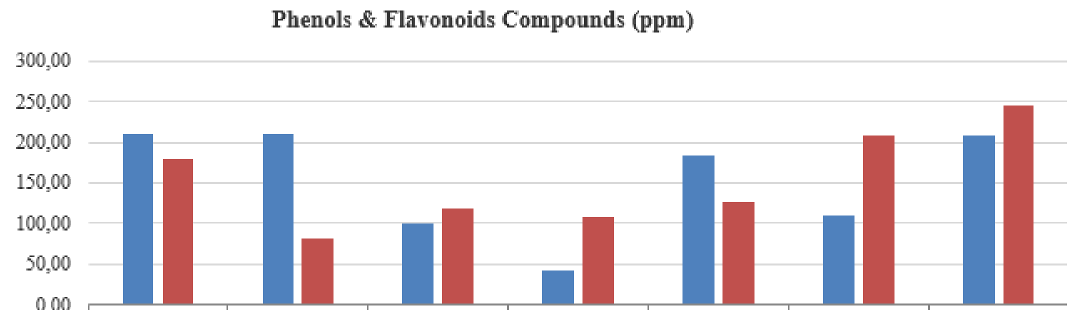


Figure 5.53. Glycosidically bound compounds in *Eucalyptus* spp.

5.9.2.3. Evaluation of the phenols & flavonoids *in Eucalyptus* spp.

Using the HPLC method, several phenols and flavonoids compounds were determined in *Eucalyptus* spp. As shown in Figure 5.54 the highest tannin (210.58 ppm), gallic acid (209.90 ppm) and rutin (182.87 ppm) were determined in *E.camaldulensis.* Genstic acid (117.35 ppm), quercetin (207.31 ppm), and eucalyptone (245.03 ppm) were detected higher in *E.sideroxylon.*



	Tannin	Gallic acid	Genstic acid	Ellagic acid	Rutin	Quercetin	Eucalyptone
E. camaldulensis	210,58	209,90	99,01	41,73	182,87	109,75	207,60
E. sideroxylen	180,40	81,11	117,35	107,5	125,98	207,31	245,03

Figure 5.54. Phenols and flavonoids *in Eucalyptus* spp.

6. CONCLUSION AND RECOMMENDATIONS

In this study, some *Cupressus* and *Eucalyptus* spp. were investigated for their antimicrobial, antifungal, and chemical compositions. The plants (*C.sempervirens* L. var. *pyramidalis*, *C.sempervirens* L. var. *horizontalis*, *C.arizonica*, *E.camaldulensis*, and *E.sideroxylon*) were collected from Erbil, Shaqlawa and Hiran (Iraq) in the year of 2015. Four pathogenic bacterial strains (*E.coli* ATCC 25922, *K.pneumoniae* ATCC 13883, *S.aureus* ATCC 43300, and *E.faecalis* ATCC 29212) and two strains of pathogenic fungi (*A.niger* ATCC 16404 and *C.albicans* ATCC 10231) were used for the antibacterial and antifungal tests, respectively.

Based on the results of this study, the best extract yield was achieved in methanol solvent in all *Cupressus* and *Eucalyptus* spp. Among all extract solution concentrations, the 75% extract concentration exhibited the best results for the antibacterial and antifungal tests.

The findings of this study also revealed that the leaf part of the *Cupressus* and *Eucalyptus* spp. plants showed the highest results in the antibacterial and antifungal tests. The best results from the antibacterial and antifungal tests were discovered in the leaves of *E.camaldulensis*, *C.sempervirens* L. var. *pyramidalis* and *E.sideroxylon*.

The total amounts of condensed tannins were found to be ranged from 209.1 to 247.5 mg/kg for *Cupressus* samples and 37.6 to 82.9 mg/kg for *Eucalyptus* samples. In general, *Cupressus* leaf samples had higher TCT content than *Eucalyptus* leaf samples.

The highest TPC values were demonstrated in *C.sempervirens* L. var. *horizontalis* leaf samples (3.96-13.43 mg GAE/g DW).

The highest DPPH %inhibition values in all *Cupressus* spp. were obtained in ethanol>water>hexane extracts depending on the concentration increase. The findings of this study indicated that the highest values of the DPPH %inhibition of 80.2%, 93.3%, and 97.8% were in *C.sempervirens* L. var. *pyramidalis, C.arizonica*, and *C.sempervirens* L. var. *horizontalis*, respectively.

The HPLC method was used in this study to determine the alkaloid, glycosidically bound, and phenol-flavonoid contents of *Cupressus* and *Eucalyptus* samples. Berberine (344.16 ppm), the highest alkaloid compound, was found in *E.camaldulensis*. The highest glycosidically bound (cupressuflavone= 1200.26ppm) and phenol-flavonoid (rutin=367.82 ppm) compounds were detected in *C.sempervirens* L. var. *horizontalis*.

This study would provide three recommendations for future studies. One of recommendations is more studies involving the use of *Cupressus* and *Eucalyptus* leaves should be carried out. Obtaining extracts with methanol solvent is another suggestion for future studies. Lastly, studies focusing on extract concentration of 75% can also be conducted in the future.

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