TR
KASTAMONU UNIVERSITY
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INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF SOME SPECIES BELONGING TO PINACEAE FAMILY

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APPROVAL

The thesis study entitled "**Investigation of the Antimicrobial Activity of Some Species Belonging to Pinaceae Family**" submitted by **Hend M. E. EMHEMED** has been argued in front of the following examining committee members and accepted as **THE DEGREE OF MASTER OF SCIENCE** in **Department of Forest Engineering**, The Graduate School of Natural and Applied Sciences in Kastamonu University by **unanimity of votes**.

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DECLARATION

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

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TURKISH SUMMARY

Yüksek Lisans Tezi

PINACEAE FAMİLYASINA AİT BAZI TAKSONLARIN ANTİMİKROBİYAL AKTİVİTESİNİN İNCELENMESİ

Hend M.E.EMHEMED

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Özet: Bu çalışma, bazı tıbbi ve aromatik bitkilerden su buharı distilasyonu yöntemi ile elde edilen uçucu yağın patojen mantar ve bakteriler üzerindeki antimikrobiyal etkisini ortaya çıkarmayı amaçlamaktadır.

Bu çalışmada Karaçam (*Pinus nigra* subsp. *pallasiana*), Kazdağı göknarı (*Abies normanniana* subsp. *equi-trojani*), Sarıçam (*Pinus sylvestris*), Doğu ladini (*Picea orientalis*), Kızılçam (*Pinus brutia*), Toros sediri (*Cedrus libani*) taksonlarının her birinden elde edilen uçucu yağlar daha sonra Gram-pozitif bakterilere: *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Enterococcus faecalis, Klebsiella pneumoniae ve Bacillus subtilis* ve Gram-negatif bakterilere: *Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enteritidis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* ve *Pseudomonas fluorescens* patojen bakterilerine ve patojen mantar *Candida albicans'*a karşı test edilmiştir.

Anahtar Kelimeler: Karaçam (*Pinus nigra* subsp. *pallasiana*), Kazdağı göknarı (*Abies normanniana* subsp. *equi-trojani*), Sarıçam (*Pinus sylvestris*), Doğu ladini (*Picea orientalis*), Kızılçam (*Pinus brutia*), Toros sediri (*Cedrus libani*), Antimikrobiyal, Uçucu yağ, GC-MS analizi.

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ABSTRACT

MSc Thesis

INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF SOME SPECIES BELONGING TO PINACEAE FAMILY

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Abstract: This study aims to reveal the antimicrobial effect of the essential oil obtained by steam distillation method from some Pinaceaea species on pathogenic fungi and bacteria.
In this study oil was extracted from *Pinus nigra* subsp. *pallasiana, Abies normanniana* subsp. *equi-trojani, Pinus sylvestris, Picea orientalis, Pinus brutia* and *Cedrus libani* then tested against Gram-positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Enterococcus faecalis, Klebsiella pneumoniae* and *Bacillus subtilis.* Gram-negative bacteria: *Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enteritidis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, and fungi; *Candida albicans*.

Key Words: *Pinus nigra* subsp. *pallasiana*, *Abies normanniana* subsp. *equi-trojani*, *Pinus sylvestris*, *Picea orientalis*, *Pinus brutia*, *Cedrus libani*, Antimicrobial, Essential oil, GC-MS analysis.

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Hend M.E. EMHEMED
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THE SYMBOLS AND ABBREVIATION

%	Percentage
°C	Celsius degree
cfu/mL	Colony forming unit
GC-MS	Gas Chromatography - Mass Spectrophotometers
kg	Kilogram
GI	Gastrointestinal Infections
m	Meter
B.C.	Before Christ
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibition Concentration
mL	Milliliter
subsp.	Subspecies
UTI	Urinary Tract Infections
WHO	World Health Organisation
α	Alpha
β	Beta
γ	Gamma
δ	Delta
μg	Microgram
µL	Microliters

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1. INTRODUCTION

1.1. History of Medicinal Plants

Traditional medicine has remained as a low-cost and easily accessible treatment method in healthcare systems of various societies with limited resources. In addition, the traditional use of plants for medicinal purposes by local communities has a deep-rooted history. The therapeutic use of medicinal plants dates back to 4000–5000 BC. On the other hand, the earliest documentation on the use of herbs as a remedy is in the Rigveda in India. It is said that the book was written between 1600 and 3500 BC. In the later years, different characteristics of medicinal plants and their therapeutic use have been studied in detail and recorded experimentally (in a local medical system) by physicians who were considered to be the founders of early medical science in India. Medicinal plants are crucial components in local medical systems worldwide. Ethnobotany provides a substantial resource for the research and development of natural medicines. The term traditional herbal medicine refers to the use of herbs for therapeutic purposes, which dates back to ancient times. In many developing countries, the majority of the population still relies on traditional practitioners and their treatments with the use of herbal medicines in order to meet their healthcare requirements. In general, traditional herbal medicines continue to be popular because of historical and cultural reasons. Modern medicine may continue to exist alongside these traditional practices. Natural products play an important role in the treatment and prevention of human diseases around the world. Medicines produced from natural products can be manufactured from various raw materials such as plants, animals, and microorganisms, and their importance is discussed in various researches and reports in the modern world. The use of traditional medical knowledge in herbal studies has recently gained importance, and there has been a growing interest in the chemistry of natural products. This growing interest has prompted extensive studies on chemical structure and biological activities of secondary metabolites of natural products. These studies have led to the development of various techniques for the improvement of bioactive compounds, isolation of natural compounds from natural resources, and characterization of the structures of the bioactive compounds. The World Health Organization has recognized the

importance of traditional medicine and created various strategies, guidelines, and standards for herbal remedies. Agricultural technologies should be used for cultivating and processing medicinal plants and producing herbal remedies from these medicinal plants. Medicinal plants are the sources of new drugs, and many modern medicines are indirectly derived from medicinal plants. It is estimated that there are more than 250,000 species of flowering plants around the world. Examination of plants is crucial in understanding plant toxicity and protecting humans and animals from plants that naturally contain poison (Hosseinzadeh et al., 2015).

Essential oils can either be found in the entire plant or stored in specific parts of it, such as the root, trunk, leaves, fruits, flowers, and seeds. They can be stored in secretory trichomes, secretory cavities and ducts, or secretory cells. Essential oils are also found in the epidermal and parenchymal cells. It is known that essential oils are directly formed inside the protoplasm. It is also assumed that because essential oils are not functional in the cell cycle, they are products of detoxification as a result of reaction. However, when an injury occurs to the plant, the essential oils act directly or play a role in the decomposition of resin-like protective products. Depending on their location on the plant, essential oils have an effect on the continuity of photosynthesis in the leaves and enabling pollination of flowers (Ghimire et al., 2015).

The aim of the present study is to investigate the antimicrobial effects of essential oils extracted from various taxa (*Pinus nigra* subsp. *pallasiana*, *P. sylvestris*, *P. brutia*, *Cedrus libani*, *Picea orientalis*, and *Abies nordmanniana* subsp. *equi-trojani*).

1.2. Pinaceae (Pine) Family

Pinaceae family, which can be found all over the world, consists of a total of 225 species in 11 genera (*Abies*, *Cathaya*, *Cedrus*, *Keteleeria*, *Nothotsuga*, *Picea*, *Pinus*, *Larix*, *Pseudolarix*, *Pseudotsuga*, and *Tsuga*). There are five different Pinaceae species in Turkey, including *P. brutia* (Turkish pine), *P. nigra* (Austrian pine), *P. sylvestris* (Scots pine), *P. pinea* (stone pine), and *P. halepensis* (Aleppo pine). Three

of these species (Turkish pine, Austrian pine, and Scotch pine) are used for commercial purposes as wood. The natural distribution area of *P. halepensis* is sparse, and *P. pinea* as a non-timber forest product is used for its seeds. Essentially, studies on the oils extracted from pine species in Turkey are mostly focused on turpentine production. Pine oils are used in cosmetics as fragrance, sweetener additive for food and beverages, and intermediate products in the synthesis of home fragrances and other chemicals in the production of perfumes (Güner et al., 2012). The genus firs (*Abies* spp.) are represented by 56 taxa all around the world. Firs in Turkey consist of four taxa: *A. nordmanniana* subsp. *nordmanniana* (found in the Black Sea region), *A. nordmanniana* subsp. *equi-trojani* (found in Mount Ida), *A. cilicica* subsp. *cilicica* (found in the Taurus region), and *A. cilicica* subsp. *isaurica* (also found in the Taurus region). *A. nordmanniana* and *A. cilicica* are species endemic to Turkey. *A. nordmanniana* subsp. *nordmanniana* and *A. nordmanniana* subsp. *equi-trojani* are scattered in the northern part of Turkey, whereas the Taurus taxa of *A. cilicica* are scattered in the southern part (Güner et al., 2012). The genus spruce is represented by 35 taxa around the world. In Turkey, spruce (*P. orientalis*, Oriental spruce) is represented by one taxon. Spruce wood is used for economic purposes. Its natural area of distribution is in the eastern parts of Melet River in Ordu in the Black Sea region (Güner et al., 2012). The genus cedrus is represented by four taxa throughout the world. In Turkey, it is represented by one taxon (*C. libani*, Taurus cedar). Although cedar wood can be used for economic purposes, its natural area of distribution is limited to the higher mountains (Güner et al., 2012).

1.2.1. *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe (Black pine)

Black pine (*P. nigra* JF Arnold) is a tertiary relictual species belonging to the group of Mediterranean pines. It is one of the most widespread species in Europe with its polymorphic conifers and a natural area of highly fragmented distribution in the North Mediterranean to the eastern parts, stretching from North Africa to the Black Sea region (Güner et al., 2012). The distribution of *P. nigra* subsp. *pallasiana* (Photograph 1.1.) in Turkey is shown in Map 1.1.

Map 1.1. Distribution of *P. nigra* subsp. *pallasiana* in Turkey

Photograph 1.1. The plant *P. nigra* subsp. *pallasiana*

1.2.2. *Pinus sylvestris* L. (Scots pine)

The species *P. sylvestris* is also known as Scots pine. The trees grow up to 25–40 m in height. The tree has a thick, dark grey-brown flaky bark on the lower part and thin, orange flaky bark on the upper part and branches. The needle-shaped leaves are blue-green and 3–5 cm long with two fascicles. The cones are pointed and oval in shape and 3–7 cm long (Güner et al., 2012). Distribution of *P. sylvestris* (Photograph 1.2.) in Turkey is shown in Map 1.2.

1.2.3. *Cedrus libani* A. Rich. (Taurus cedar)

Outside Turkey, the natural area of distribution of Taurus cedar (*C. libani* A. Rich.) is on the mountains located along the coastline of Lebanon (1050-1925 m) and the Atlas Mountains in Algeria and Morocco. In Turkey, *C. libani* is distributed on the Taurus Mountains (530-2000 m), and it is an endemic taxon to the mountains of the Mediterranean region (Güner et al., 2012). The tree has a prized wood with its thick trunk, elaborate branches, and flaking cones on the branches. The acicular leaves are surrounded by 30-40 sheaths. The distribution of *C. libani* (Photograph 1.3.) in Turkey is shown in Map 1.3.

Map 1.2. The distribution of *P. sylvestris* in Turkey

Photograph 1.2. The plant: *P. sylvestris*

Map 1.3. The distribution of *C. libani* in Turkey

Photograph 1.3. The plant *C. libani*

1.2.4. *Picea orientalis* (L.) Peterm. (Oriental spruce)

The species *Picea* is represented by 35 taxa in the world. *P. orientalis* (Oriental spruce) is the sole species of *Picea* endemic to the forest ecosystems of the East Black Sea Region in Turkey. The natural distribution of *P. orientalis* in Turkey stretches from the western part of Melet River in Ordu to the southern parts of Kazdagı Mountains in Georgia. The trunk is usually light-colored and regular, and the branches are arranged in whorls. The pendulous cones usually occur on the upper part of the tree and fall intact when mature. The needle-like leaves are four-sided and can be twirled in hand. Spruce wood is used for economic purposes (Güner et al., 2012). The distribution of *P. orientalis* (Photograph 1.4.) in Turkey is shown in Map 1.4.

Map 1.4. Distribution of *P. orientalis* in Turkey.

Photograph 1.4. The plant *P. orientalis*

Map 1.5. Distribution of *P. brutia* in Turkey

Photograph 1.5. The plant: *P. brutia*

1.2.5. *P. brutia* Ten. (Turkish pine)

P. brutia (Turkish pine) is an important tree in ecological and economical terms for the Eastern Mediterranean region. In addition, it is also an important forest tree in Greece and the Aegean Islands. Because it is acclimated to sun, it is well adapted to regions with dry summer. Turkish pine grows up to 25 m in height and is found in altitudes of 0–1300 m. At early ages, the shoots are red. The needle-shaped leaves are 10–16 cm long and are in pairs enclosed in sheaths (Güner et al., 2012). The distribution of *P. brutia* (Photograph 1.5.) in Turkey is shown in Map 1.5.

1.2.6. *A. nordmanniana* subsp. *equi-trojani* (Asc. & Sint. ex Boiss.) Coode & Cullen (Kazdagı fir)

Kazdagı fir (*A. nordmanniana* subsp. *equi-trojani*) is a taxon endemic to the mountains from the Central Black Sea Region to Mount Ida. It is a shade bearer found at altitudes of 400–1900 m. Its needle-like leaves cannot be twirled in fingers because they are flattened and bifacial. When the cone flakes mature, they disintegrate on the branches (Güner et al., 2012). The distribution of *A. nordmanniana* subsp. *equi-trojani* (Photograph 1.6.) in Turkey is shown in Map 1.6.

Map 1.6. Distribution of *A. nordmanniana* subsp. *equi-trojani* in Turkey

Photograph 1.6. The plant *A. nordmanniana* subsp. *equi-trojani*

2. STUDIES ON ESSENTIAL OILS

The majority of essential oils are lighter than water, and they have a density range of 0.8–1.3. Essential oils are carried with water vapor, and they do not leave a trace on filter paper; these characteristics differ from those of fixed oils. Essential oils form a film on the water surface and are not miscible with water. They dissolve in organic solvents, such as petroleum ether, benzene, and ethanol. Although essential oils are not miscible with water, they dissolve enough to leave their fragrance. Aromatic waters are prepared based on this characteristic of essential oils. The compounds found in essential oils can be divided in four groups: terpenic compounds, aromatic substances (such as benzene derivatives), aliphatic hydrocarbons, and nitrogen- and sulfur-containing compounds. Antimicrobial properties of essential oils involve inactivation of enzyme reaction on microbial metabolism, inhibition of the intake of nutrients, inactivation of enzyme synthesis in the nucleus or at the ribosomal level, and alteration of membrane structure. Some studies conducted on pathogenic bacteria and fungi based on these properties of essential oils are specified below.

Choi et al. (2016) investigated the antibacterial activities of some plant essential oils against *Streptococcus mutans* and *S. sobrinus* using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. ccording to this study, essential oil of *Cinnamomum verum* exhibited the highest antibacterial activity. Gas chromatography (GC)–mass spectrometry (MS) analysis revealed that the major components of *C. verum* essential oil were cinnamaldehyde (56.3%), cinnamyl acetate (7.1%), and phellandrene (6.3%). MIC of cinnamaldehyde was 0.02% against both bacterial strains. MBC of cinnamaldehyde against *S. mutans* and *S. sobrinus* was 0.2% and 0.1%, respectively.

Eryılmaz et al. (2016) investigated the antimicrobial activities of essential oils extracted from the cones of some taxa of Pinaceae and Cupressaceae using the disc diffusion method. In their study, *C. libani* and *P. halepensis* showed no antibacterial activity against the tested bacteria, whereas other species showed weak activity against some bacteria, but no activity was observed against *Candida albicans*.

In their study, Zira and Ghanmi (2016) analyzed the chemical composition of sawdust samples from *C. atlantica* using GC–flame ionization detector (GC-FID) and GC-MS. They tested the antibacterial activities on gram-negative bacteria *Escherichia coli* and *Salmonella*, as well as gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *B. cereus*, using the MIC test. Three bacterial strains (*E. coli*, *B. subtilis*, and *B. cereus*) were found to be sensitive to the essential oil of cedar wood. MIC for both *E. coli* and *B. cereus* was 0.4 µl/mL and that for *B. subtilis* was 0.2 µl/mL.

Using GC-FID and GC-MS in their study, Demirci et al. (2015) analyzed the essential oil of *P. pinea* from different countries extracted using the water distillation method and investigated its antimicrobial effects using the MIC test. Among the 30 components identified, limonene (54.6%), α-pinene (4.0%), myrcene (2.4%), and α-phellandrene (2.4%) were characterized as the major constituents. In the MIC test, the essential oil showed activity against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Salmonella thyphimurium* (MIC > 0.75 µg/mL). The antifungal susceptibility against *C. parapsilosis* was relatively more than that against *C. albicans*, with MIC of 0.375 µg/mL.

In their study, Šarac et al. (2014) extracted essential oils from acicular leaves of the three taxa of *P. nigra* scattered in Serbia (ssp. *nigra*, ssp. *pallasiana* and var. *banatica*) using water distillation method and tested the antimicrobial effects of these oils against *Aspergillus niger*, *S. aureus*, and *B. cereus*. In the antimicrobial assays, one fungal strain, *A. niger*, and two bacterial strains, *S. aureus* and *B. cereus*, showed sensitivity against the essential oils of all three taxa of *P. nigra*. The tested oils were shown to possess inhibitory action in the range of 20–0.62 µg/mL, and var. *banatica* exhibited the highest antimicrobial action.

In another study, Metsämuuronen and Siren (2014) investigated the antibacterial effects of essential oils and phenolic extracts from the stem, bark, roots, and leaves of *P. sylvestris* (Scots pine), *P. abies* (Norway spruce), and *Betula pendula* (white birch) against pathogens. Both essential oils and phenolic extracts were found to be bacteriostatic against several bacteria. The antibacterial phenolic compounds

extracted from the Scots pine effectively inhibited the growth of pathogens such as *B. cereus*, *S. aureus*, and *Listeria monocytogenes* (through pinosylvins). Among other phenolic compounds, lignans appear to possess the lowest bacteriostatic action and flavonoids exhibit lower antibacterial activity than aglycones. Gram-positive bacteria are generally more susceptible to plant-derived bioactive compounds than gram-negative bacteria.

Ghanem and Olama (2014) analyzed the antibacterial and antifungal properties of water and methanolic extracts of the leaves, stems, and pulp of Taurus cedar (*C. libani*) against different pathogens. They evaluated MIC, MBC, and minimum fungicidal concentration (MFC) of pathogens such as *Klebsiella pneumonia*, *E. coli*, *L. monocytogenes*, and *C. albicans* using the disc diffusion method. The tested bacterial and fungal strains exhibited various degrees of sensitivity represented by the inhibition zone diameter: *K. pneumonia* (27 mm), *E. coli* (20 mm), and *C. albicans* (21 mm). *L. monocytogenes* showed the strongest inhibition zone of 70 and 37 mm with methanolic and water extracts of the leaves, respectively. MIC, MBC, and MFC were 5–200 µl/mL and 300 µl/mL, respectively.

Fekih et al. (2014) investigated the antimicrobial activity of essential oil components extracted from the needles, twigs, and buds of *P. halepensis* (Aleppo pine)

by GC-MS using the disc diffusion method. They identified 49 compounds representing 97.9% of the essential oil. It was found that monoterpenes (65.5%) were the most predominant group among hydrocarbon compounds (80.6%) in the essential oil. It was also demonstrated that the essential oil of Aleppo pine had an antimicrobial effect against *L. monocytogenes*, *Enterococcus faecalis*, *P. aeruginosa*, *Acinetobacter baumanii*, *Citrobacter freundii*, and *K. pneumoniae*.

In a study by Rhafouri et al. (2014), the chemical composition of the essential oils extracted from the seeds of Atlas cedar (*C. Atlantica*) with and without wings using steam distillation method was analyzed using GC-MS and the antibacterial and antifungal activities were tested using the MIC test. The yields of essential oils of seeds with and without wings were 2.6% and 3.6%, respectively. The main

constituents of the seeds without wings were α-pinene, manool, and bornyl acetate, whereas the main constituents of seeds with wings were manool and α-pinene. It was found that the fungal strains were more susceptible to essential oils than the bacterial strains.

Koçak and Kiliç (2014) determined the composition of essential oils extracted from the needles of the four taxa of *Picea* (*P. pungens*, *P. mariana*, *P. glauca*, and *P. rubens*) using headspace–solid phase microextraction/GC-MS. It was found that the main constituents were bornyl acetate (29.40%), camphor (26.43%), β-myrcene (7.47%), and camphene (7.01%) in *P. pungens*; camphene (22.03%), bornyl acetate (21.64%), α-pinene (16.62%), and borneol (7.79%) in *P. mariana*; bornyl acetate (31.25%), limonene (17.27%), α-pinene (15.85%), and camphene (13.65%) in *P. glauca*; and borneol (12.38%), α-pinene (10.36%), germacrene D (9.86%), and δ-cadinene (8.25%) in *P. rubens*.

Mimoune et al. (2013) investigated the chemical composition of the essential oil extracted from the needles of *P. pinaster* (maritime pine) and its antimicrobial activity using the disc diffusion method. According to GC-MS results, they identified 23 components in the essential oil. β-caryophyllene (30.9%) and β-selinene (13.45%) were found to be the predominant compounds. It was also demonstrated that the essential oil from the maritime pine exhibited a moderate activity against *S. aureus*, *B. subtilis*, and *E. coli* but did not affect the spread of *Erwinia amylovora*. The oil showed no activity against *A. flavus* and *A. niger*.

Karapandzova et al. (2011) identified that the most abundant constituents of the essential oil extracted from the needles of *P. peuce* (Macedonian pine) using GC-FID and GC-MS were α-pinene (12.89/27.34%), β-pinene (6.16/13.13%), limonene + β-phellandrene (2.09/6.64%), bornyl acetate (2.92/11.67%), trans-(E)-caryophyllene (4.63/7.13%), and germacrene D (8.75/20.14%). The antimicrobial activity of the essential oil was investigated using the MIC test. The most sensitive bacteria against the essential oil of *P. peuce* were *S. aureus, S. epidermidis, S. agalactiae, Acinetobacter* spp., and *S. pyogenes*, whereas MIC of the oil was 7.5–62.5 µl/mL.

A study by Apetrei et al. (2011) analyzed the antioxidant and antimicrobial activities of the essential oils extracted from the bark and needles of *P. cembra*. The antimicrobial effects against *S. aureus*, *Sarcina lutea*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *C. albicans* were tested using the agar diffusion method. It was found that both extracts (4 µg/well) were active against all the microorganisms being tested. In addition, it was found that the essential oil extracted from the bark exhibited a higher inhibition in all strains compared with the essential oil extracted from the needles.

Derwich et al. (2010) performed chemical analysis of the essential oil extracted from the leaves of *C. atlantica* (Atlas cedar). The main constituents of the essential oil were α-pinene (14.85%), himachalene (10.14%), β-himachalene (9.89%), σ-himachalene (7.62%), cis-α-atlantone (6.78%), himachalol (5.26%), α-himachalene (4.15%), germacrene D (3.52%), β-caryophyllene (3.14%), cadinene (3.02%), β-pinene (2.35%), humulene (2.30%), and copaene (2.26%). The antimicrobial activities against gram-negative bacteria such as *E. coli*, *P. aeroginosa*, and *K. pneumonia* and gram-positive bacteria such as *S. aureus*, *E. faecalis*, *B. sphericus*, and *S. intermedius* were tested using the agar disc diffusion method and MIC test. The bactericidal activity was 12–25 mm and 6–22 mm for gram-negative and gram-positive bacteria, respectively.

In their study, Tumen et al. (2010) investigated the yields and composition of the essential oils extracted from the taxa belonging to the family of Pinaceae native to Turkey. The essential oils were extracted using hydrodistillation. Oil yields were 0.13–0.48 mL/100 g for *Pinus* taxa, 0.42–0.59 mL/100 g for *Abies* taxa, 0.36 mL/100 g for *Picea*, and 0.37 mL/100 g for *Cedrus* taxa. When the predominant chemical constituents of the taxa were analyzed, it was found that α-pinene (47.1%–14.8%) was the main constituent of *P. slyvestris*, *P. nigra*, and *P. halepensis*; limonene (62.8%) was the main constituent of *P. pinea*; β-pinene (39.6%) was the main constituent of *P. brutia*; and limonene (22.7%) was the main constituent of *C. libani*. α-pinene (70.6%–53.0%) and β-pinene (10.9%–8.2%) were the main constituents of the fir species, whereas β-pinene (32.7%) was the main constituent of *P. orientalis* contrary to other species.

Dığrak et al. (1999) analyzed the antimicrobial activities of chloroform, acetone, and methanol extracts of the resins, barks, cones, and fruits of the Turkish pine, Black pine, Taurus cedar, Abies, and juniper using the disc diffusion method. The effects of chloroform, acetone, and methanol extracts of the leaves, resins, barks, cones, and fruits of *P. brutia* Ten., *Juniperus oxycedrus* L., *A. cilicia* Ant.&Kotschy Carr., *C. libani* A. Rich., and *P. nigra* Arn. were tested against *B. megaterium*, *B. subtilis*, *B. cereus*, *E. coli*, *K. pneumoniae*, *E. aerogenes*, *S. aureus*, *Mycobacterium smegmatis*, *P. vulgaris*, *L. monocytogenes*, *P. aeruginosa*, *C. albicans*, *C. tropicalis*, and *Penicillium italicum*. None of these extracts showed antifungal effects. In addition, the growth of *E. coli* was not inhibited by any extract. Chloroform and acetone extracts of the leaves of *A. cilicia* showed inhibition zones of 16 mm and 18 mm, respectively. Other plant extracts studied showed no inhibitory effect on the growth of other bacteria.

In their study, Angioni et al. (2003) performed GC-MS analysis of the essential oils extracted from the ripe and unripe cones and leaves of *J. oxycedrus* subsp. *oxycedrus*, *J. phoenicea* subsp. *turbinate*, and *J. communis* subsp. *communis* using water–steam distillation and investigated their antimicrobial activity. The main constituents of the essential oils were α-pinene, β-pinene, δ-3-carene, sabinene, myrcene, β-phellandrene, limonene, and germacrene D. The essential oils and their main constituents were tested against *C. albicans*, *S. aureus*, *E. coli*, and *P. aeruginosa*, and MIC and MBC were determined. The essential oils of *J. oxycedrus* subsp. *oxycedrus* and *J. phoenicea* subsp. *turbinate* exhibited weak activity against *C. albicans* and *S. aureus*.

Benli et al. (2008) observed the antimicrobial activity of six endemic species and found antimicrobial activity in the extracts of *Campanula lyrata* subsp. *lyrata* and *A. nordmanniana* subsp. *bornmuelleriana*. MIC of the extract from *C. lyrata* subsp. *lyrata* (leaf and flower) was ≥29 µg/mL for *B. subtilis* and ≥14.5 µg/mL for *S. aureus*. Moreover, MIC of the extract from *A. nordmanniana* subsp. *bornmuelleriana* (leaf) was found to be >314 µg/mL for *B. subtilis*. No antimicrobial activity was observed in other plant extracts, namely *Onosma bornmuelleri* (leaf and

flower), *Dianthus balansae* (leaf and flower), *Scabiosa columbaria* subsp. *paphlagonica* (leaf), and *Alyssum pateri* subsp. *pateri* (seed).

Dayisoylu et al. (2009) evaluated the antimicrobial activity of the essential oil extracted from the cones of *A. cilicica* subsp. *cilicica* using hydrodistillation. The inhibition zone of all bacteria and fungi except *E. coli* was 4 µl/disc. MIC of 0.5 µg/mL was found to be effective for *Saccharomyces cerevisiae*, *K. pneumoniae*, and *Mycobacterium smegmatis*. The study demonstrated that limonene was the main constituent showing antimicrobial activity, followed by α-pinene, myrcene, and β-pinene, whereas myrcene exhibited the most potent antifungal activity.

Fahed et al. (2017) investigated the chemical compositions and antimicrobial activities of the essential oils extracted from the cones of *A. cilicica*, *Cupressus sempervirens*, *J. excelsa*, *J. oxycedrus*, *C. libani*, and *C. macrocarpa*. While the essential oils were extracted using hydrodistillation, the analysis was performed using GC-MS. MIC tests of the essential oils were performed against an array of bacteria and fungi. The essential oil of *C. libani* was tested on *S. aureus* and *Trichophyton rubrum*, and it exhibited MIC values in the range of 32–64 µg/mL.

Jeong-Ho and Hong (2009) investigated the chemical compositions and antibacterial and antifungal activities of the essential oils extracted from *A. holophylla* and *A. koreana*. GC-MS analysis showed that the main constituents of *A. holophylla* oil were bicyclo-[2.2.1]-heptan-2-ol (28.05%), δ-3-carene (13.85%), α-pinene (11.68%), camphene (10.41%), dl-limonene (7.61%), β-myrcene (7.11%), trans-caryophyllene (5.36%), and α-bisabolol (3.67%). The main constituents of the essential oil extracted from *A. koreana* were bornyl ester (41.79%), camphene (15.31%), α-pinene (11.19%), dl-limonene (8.58%), fenchyl acetate (5.55%), and α-terpinene (2.29%). Both the essential oils exhibited antibacterial activity against several bacteria tested in the range of 2.2–8.8 µg/disc using the agar disc diffusion method and MIC values of 5.5–21.8 µg/mL using the microdilution method. In addition, both the essential oils showed effective antifungal activities against all pathogenic strains with MIC values of 0.5–2.2 µg/mL against *C. glabrata*. It was also demonstrated that

the essential oil extracted from *A. koreana* showed more potent antibacterial and antifungal activities than that extracted from *A. holophylla*.

In their study, Leandro et al. (2014) evaluated the main components of the essential oil extracted from the resin of *P. elliottii*. They also analyzed MIC and MBC values against multiresistant bacteria. It was found that MIC of the resin oil of *P. elliottii* was 25–100 µg/mL and MIC and MBC concentrations were 6.25–50 µg/mL and 6.25–100 µg/mL, respectively.

Marino et al. (2010) investigated the effects of methanol and aqueous branch extracts of different *Juniperus* species on *S. aureus*. The extracts of *J. communis* var. *communis*, *J. communis* var. *saxatilis*, *J. drupacea*, *J. oxycedrus* subsp. *Oxycedrus*, and *J. oxycedrus* subsp. *macrocarpa* were subjected to preliminary phytochemical analysis using thin-layer chromatography. The antimicrobial activities against *S. aureus* were evaluated using MIC and MBC. MICs of all extracts were in the range of 4.88–78.12 µg/mL.

Politeo et al. (2011) investigated the chemical composition and antimicrobial activity of the essential oil extracted from the needles of the Dalmatian Black pine (*P. nigra* ssp. *dalmatica*). The chemical composition of the essential oil was determined using GC-MS analysis, and the main constituents were α-pinene (24.3%), β-pinene (16.0%), germacrene D (14.6%), and β-caryophyllene (9.6%). Antimicrobial studies were performed using the disc diffusion method, and tested on microorganisms as 5–10–20 µL/disc. The essential oil extracted from the Dalmatian Black pine exhibited great potential of antibacterial activity against gram-positive bacteria (MIC = 0.03%–0.50%) and lesser activity against gram-negative bacteria (MIC = 0.12%–3.2%). It was also demonstrated that this essential oil inhibited the growth of all fungi tested.

3. MATERIALS AND METHODS

3.1. Material

3.1.1. Plant Material

The present study investigates the essential oils extracted from the six taxa of Pinaceae family: *P. nigra* subsp. *pallasiana* (Lamb.) Holmboe (Black pine), *P. sylvestris* L. (Scots pine), *P. brutia* Ten. (Turkish pine), *P. orientalis* (L.) Peterm. (Oriental spruce), *Cedrus libani* A. Rich. (Taurus cedar), and *A. nordmanniana* subsp. *equi-trojani* (Asc. & Sint. ex Boiss.) Coode & Cullen (Kazdagı fir). The samples were collected from the border of Kastamonu Province.

3.1.2. Microbial Material (Fungus and Bacteria)

The gram-positive bacterial strains used in the study were *S. aureus* American Type Culture Collection (ATCC) 25923, *S. epidermidis* DSMZ 20044, *E. faecium*, *E. faecalis* ATCC 29212, and *B. subtilis* DSMZ 1971. The gram-negative bacterial strains were *S. typhimurium* SL 1344, *S. kentucky*, *S. infantis*, *S. enteritidis* ATCC 13075, *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *P. aeruginosa* DSMZ 50071, *P. fluorescens* P1, and *K. pneumoniae* ATCC 7544, and the fungus studied was *C. albicans* DSMZ 1386. The characteristics of the gram-positive and gram-negative bacteria are shown in Table 3.1. and Table 3.2.

Table 3.1. *Classification of gram-positive bacteria*

Gram-positive Bacteria			
Bacteria	**Morphology**	**Proliferation**	**Infection**
Staphylococci	Grape-like clusters of cells	Skin, nostrils/endogenous, frontal transmission, and aerobic atmosphere	Soft tissue, bone, and joint infections, endocarditis, and food poisoning
Enterococci	In pairs or short chains	GI tract, endogenous, and frontal transmission	Urinary tract infection, GI tract infection, and catheter-related infections
Bacilli	Rod-shaped, producing endospores	Soil, air, water, animals/aerosol, and transmission	Anthrax, food poisoning, and catheter-related infections

GI, gastrointestinal tract

Table 3.2. *Classification of gram-negative bacteria*

Gram-negative Bacteria			
Bacteria	**Morphology**	**Proliferation**	**Infection**
Enterobacteriaceae (E. coli, Klebsiella, Salmonella, and Shigella)	Rod-shaped	GI tract, animals/endogenous, fecal or oral	Diarrhea, urinary tract infection, food poisoning, and sepsis
Pseudomonas	Rod-shaped	Water, soil/endogenous, and defective skin barrier	Infections in host tissue with immune deficiencies and cystic fibrosis

GI, gastrointestinal tract

3.2. Method

3.2.1. Microorganism Supply and Preparation

The microorganisms used in this study (fungal and bacterial strains) were supplied by the Research Laboratory at the Department of Biology, Faculty of Arts and Sciences, Kastamonu University.

3.2.2. Supply of Plant Taxa and Extraction of Essential Oils

Plant species, localities, collection dates, and the parts used within the scope of the present study are shown in Table 3.3. Herbarium samples of the plant species were prepared and identified by Assistant Professor Dr. Kerim Güney of the Faculty of Forestry, Kastamonu University.

Table 3.3. *Plant species, localities, parts used, and collection dates*

Bitki ismi	**Toplanan İl**	**GPS**	**Kullanılan kısım**	**Toplama tarihi**
Pinus nigra subsp. *pallasina* (Black pine)	Kastamonu	41.422128° 33.769934°	Leaves	03\08\2016
Abies nordmanniana subsp. *equi-trojani* (Kazdagı fir)	Kastamonu	41.067900° 33.733164°	Leaves	30\08\2016
Pinus sylvestris (Scots pine)	Kastamonu	41.422964° 33.769494°	Leaves	22\09\2016
Picea orientalis (Oriental fir)	Kastamonu	41.425113° 33.772207°	Leaves	05\10\2016
Pinus brutia (Turkish pine)	Kastamonu	41.627530° 34.517599°	Leaves	12\10\2016
Cedrus libani (Taurus cedar)	Kastamonu	41.358305° 33.759586°	Branches	14\10\2016

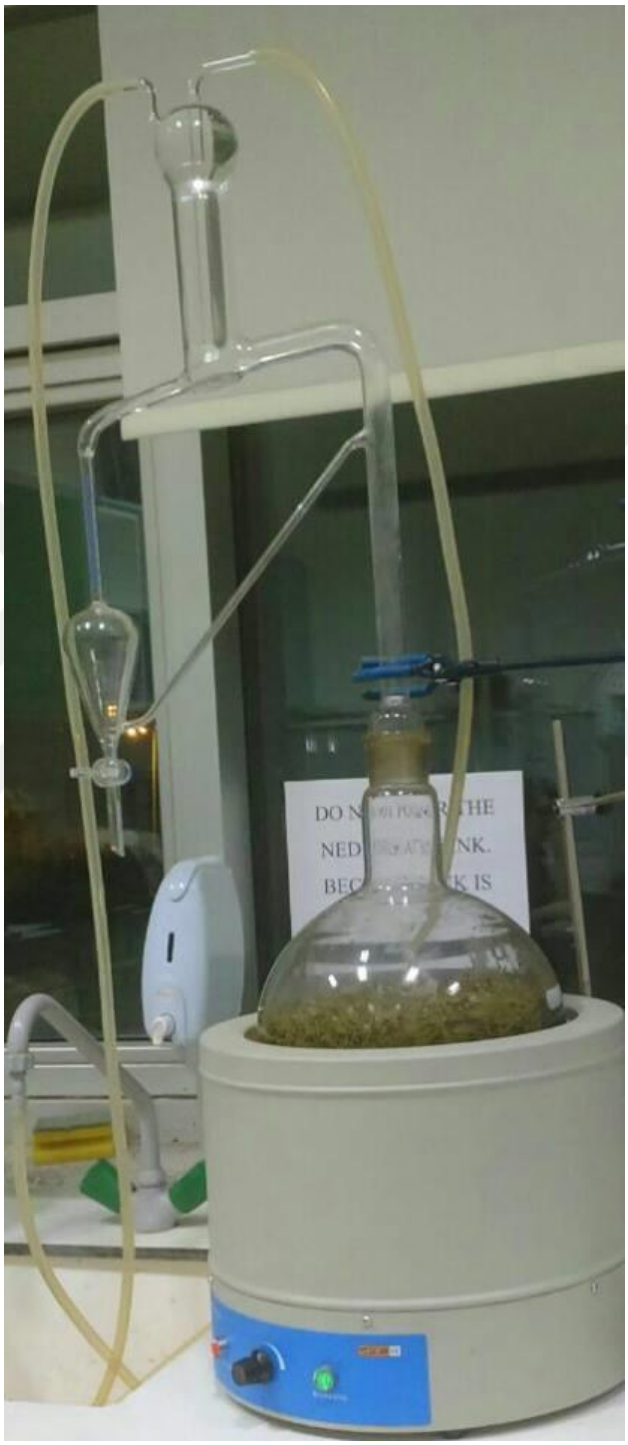
The plants mentioned in Table 3.3 were collected on the specified dates. On the next day, the parts that would be used in the study were sorted, shredded, and preserved inside zip lock plastic bags in a refrigerator at +4°C (Photograph 3.1-3.3.). A day later, their essential oils were extracted using hydrodistillation. The plants were preserved in a refrigerator at +4°C for microbial tests that would be conducted within a period of 1 week.

Photograph 3.1. Collecting of samples

Photograph 3.2. Sorting of samples

Photograph 3.3. Samples collected from the field

The essential oils from leaves and branches were extracted by hydrodistillation using the Clevenger apparatus (Photograph 3.4.).



Photograph 3.4. Clevenger apparatus for extracting essential oils

In terms of essential oil yield, 1.65 mL of the oil was extracted from 1 kg of Black pine leaf (Photograph 3.5.).

Photograph 3.5. Essential oil of Black pine inside Eppendorf tubes

In terms of essential oil yield, 2 mL of oil was extracted from 1 kg of Kazdagı fir neddle (Photograph 3.6).

Photograph 3.6. Essential oil of Kazdagı fir inside Eppendorf tubes

In terms of essential oil yield, 1 mL of oil was extracted from 600 g of Scots pine (Photograph 3.7).

Photograph 3.7. Essential oil of Scots pine inside Eppendorf tubes

In terms of essential oil yield, 1 mL of oil was extracted from 600 g of Oriental spruce leaves (Photograph 3.8).

Photograph 3.8. Essential oil of Oriental spruce inside Eppendorf tubes

In terms of essential oil yield, 4 mL of oil was extracted from 1 kg of Turkish pine leaves (Photograph 3.9).

Photograph 3.9. Sorted Turkish pine leaves and essential oils inside Eppendorf tubes

In terms of essential oil yield, because no essential oil could be extracted from Taurus cedar leaves, 1.3 mL of oil was extracted from 1 kg of the bare branches (Photograph 3.10).

Photograph 3.10. Bare branches of Taurus cedar and essential oil inside Eppendorf tubes

3.2.3. GC-MS Analysis

In order to identify the chemical components, each sample was analyzed using GC-MS QP 2010 Ultra (Shimadzu) equipped with Rtx-5MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm). Analysis conditions were as follows: injector temperature, 250°C; carrier gas, helium (at a flow rate of 1 mL/min); injection method: split ratio, 1:10; injection volume, extracted oil inside 1 μL of hexane; and oven temperature, 4°C/min with the oven set to 40°C–240°C; pressure, 100 kPa; and purge flow, 3 mL/min. MS scan conditions were as follows: transfer line temperature, 250°C; interface temperature, 250°C; and ion source temperature, 200°C. Identification of compounds was based on the comparison of retention times and matching with Wiley database. Where applicable, reference compounds were chromatographed in order to confirm the retention times in GC.

3.2.4. Antimicrobial Activity

Preparation of Microorganisms

In bacterial suspensions prepared particularly for antimicrobial susceptibility testing, the number of bacteria should be within a specific range. The turbidity of the solution adjusted to the number of bacteria in a liquid should be compared to the McFarland turbidity of barium sulfate standard in order to ensure reproducibility of the assessment.

During preparation of the inoculum from bacterial specimens that would be used for the study, colonies with the same appearance were selected from a 24-h culture plate in the agar medium using a loop and were transferred into a sterile serum physiologic solution. The turbidity of the inoculum was adjusted to 0.5 McFarland standard. Therefore, bacterial suspensions were standardized for a microorganism concentration approximately equivalent to 1.0×10^{8} cfu/mL, whereas fungal suspensions were standardized for a microorganism concentration approximately equivalent to 1.0×10^{7} cfu/mL. Thereafter, the names of the bacteria and fungus were written on the tubes and vortexed before use.

MIC

MIC is a test used to determine the effective concentration of the antimicrobial agent. The aim of the test is to determine a concentration range by preparing serial dilutions of main active ingredients and identify the active concentration of the ingredient by observing the range of microbial growth inhibited.

First, the essential oils were sterilized using a 0.45-µm filter. In the MIC test, active concentration was determined using 96-well microplates with the microdilution method. Mueller–Hinton broth (MHB) medium prepared to determine MIC was dripped using 100-µL pipettes into all wells of the microplates. Then, 100 µL of oils extracted from the plants were transferred to the first well, and 10 serial dilutions of each oil were prepared by two-fold dilution at every turn. Subsequently, the 10 wells were inoculated with an equal amount of inoculum. For each serial dilution line, one negative control well (well containing only MHB medium) and one positive control well (MHB + well containing inoculum) were left. Each sample was studied using three parallel experiments. Bacterial specimens were incubated for 24 h at 37°C, whereas fungal specimens were incubated for 48 h at 27°C, and the MIC value was determined by visual inspection as the lowest concentration inhibiting their growth.

1st well: 100 µL medium + 100 µL essential oil + 50 µL pathogenic bacterium
2nd well: 100 µL medium + 50 µL essential oil + 50 µL pathogenic bacterium
3rd well: 100 µL medium + 25 µL essential oil + 50 µL pathogenic bacterium
4th well: 100 µL medium + 12.5 µL essential oil + 50 µL pathogenic bacterium
5th well: 100 µL medium + 6.25 µL essential oil + 50 µL pathogenic bacterium
6th well: 100 µL medium + 3.125 µL essential oil + 50 µL pathogenic bacterium
7th well: 100 µL medium + 1.562 µL essential oil + 50 µL pathogenic bacterium
8th well: 100 µL medium + 0.781 µL essential oil + 50 µL pathogenic bacterium
9th well: 100 µL medium + 0.39 µL essential oil + 50 µL pathogenic bacterium
10th well: 100 µL medium + 0.195 µL essential oil + 50 µL pathogenic bacterium
11th well: 100 µL medium (negative control)
12th well: 100 µL medium + 50 µL pathogenic bacterium (positive control)

MBC and MFC

Bacterial specimens recovered from the wells with a loop that did not show growth in MIC testing were transferred into the nutrient agar, whereas fungal specimens were transferred into Saboraud Dextrose Agar. Bacterial specimens were incubated for 24 h at 37°C, and fungal specimens were incubated for 48 h at 27°C, and the lowest concentration that inhibited their growth was determined as MBC and MFC.

4. FINDINGS

4.1. GC-MS Findings

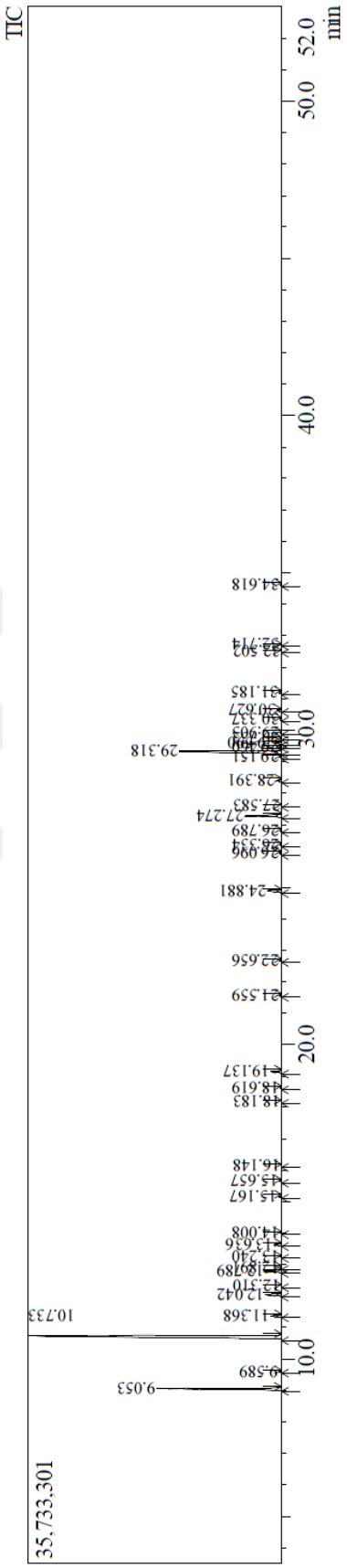
The essential oils of Turkish pine, Black pine, Scots pine, Oriental spruce, Taurus cedar, and Kazdagı fir *(P. brutia*, *P. nigra* subsp. *pallasiana*, *P. sylvestris*, *P. orientalis*, *C. libani*, and *A. nordmanniana* subsp. *equi-trojani*) were analyzed using GC-MS, and the results are shown in Tables 3.7–3.11. The compounds with an availability of >2% were considered as the main compounds.

4.1.1. GC-MS Findings of Turkish Pine

In total, 38 different compounds were identified in the GC-MS analysis of Turkish pine, and seven compounds had an availability of >2%: β-pinene (45.09%), germacrene D (16.94%), (-)-α-pinene (15.93%), caryophyllene (5.48%), sylvestrene (2.17%), δ-3-carene (2.09%), and α-terpinyl acetate (2.03%) (Graphic 4.1.), (Table 4.1.).

4.1.2. GC-MS Findings of Oriental Spruce

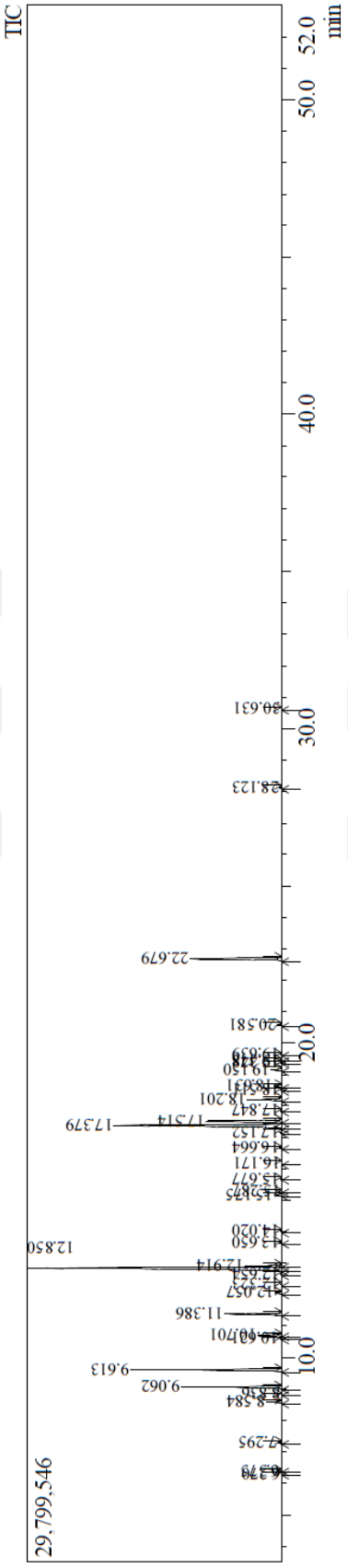
In total, 37 different compounds were identified in the GC-MS analysis of Oriental spruce, and nine agents had an availability of >2%: D-limonene (27.61%), (+)-2-bornanone (18.14%), camphene (12.44%), bornyl acetate (8.39%), (-)-α-pinene (7.87%), camphene hydrate (6.85%), myrcene (4.40%), borneol L (3.24%), and eucalyptol (1,8-cineole) (2.58%) (Graphic 4.2.), (Table 4.2.).



Graphic 4.1. GC-MS chromatogram of the essential oil extracted from Turkish pine

Table 4.1. *GC-MS analysis of Turkish pine*

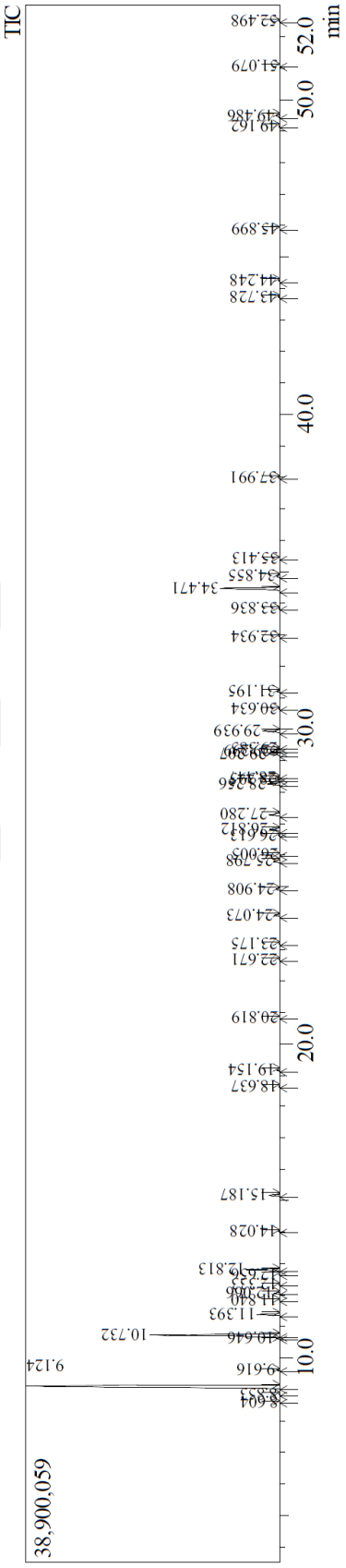
No	% Componenet	Name
1	45.09	β-Pinene
2	16.94	Germacrene-D
3	15.93	α-pinen, (-)-
4	5.48	Caryophyllene
5	2.17	Sylvestrene
6	2.09	δ-3-Carene
7	2.03	α-Terpinyl acetate
8	1.33	Myrcene
9	0.26	Camphene
10	0.03	α-Terpinene
11	0.14	Eucalyptol
12	0.08	trans-β-Ocimene
13	0.91	Z-Ocimene
14	0.08	γ-Terpinene
15	0.48	Terpinolene
16	0.30	Linalool
17	0.07	α-Fenchol
18	0.05	Borneol L
19	0.13	Terpinen-4-ol
20	1.52	α-Terpineol
21	0.20	Linalylacetate
22	0.27	Bornylacetate
23	0.27	β-Bourbonene
24	0.15	β-Elemene
25	0.14	Methyleugenol
26	0.07	Germacrene-D
27	0.92	α-Humulene
28	0.26	α-Amorphene
29	0.18	2-phenylethyl 2-methylbutanoate
30	0.82	Phenethyl isovalerate
31	0.07	α-Bulnesene
32	0.15	α-Muurolene
33	0.17	γ-Cadinene
34	0.65	δ-Cadinene
35	0.32	CIS-α-Bisabolene
36	0.07	Caryophyllene oxide
37	0.08	ethyl-Laurate
38	0.10	α-Cadinol



Graphic 4.2. GC-MS chromatogram of the essential oil extracted from Oriental spruce

Table 4.2. *GC-MS analysis of Oriental spruce*

No	% Component	Name
1	27.61	D-Limonene
2	18.14	(+)-2-Bornanone
3	12.44	Camphene
4	8.39	Bornyl acetate
5	7.87	α-Pinene, (-)-
6	6.85	Camphene hydrate
7	4.40	Myrcene
8	3.24	Borneol L
9	2.58	Eucalyptol (1,8-Cineole)
10	0.38	β-Citronellol
11	0.16	3-methyl-3-butenyl benzoate
12	0.03	δ-Cadinene
13	0.08	Hex-3(Z)-enol
14	0.24	Santene
15	1.14	Tricyclene
16	0.03	α-Thujene
17	0.02	Hex-2(E)-enal
18	0.18	Sabinene
19	1.95	β-Pinene
20	0.56	δ-3-Carene
21	0.06	α-Terpinene
22	0.10	para-Cymene
23	0.14	β-Ocimene
24	0.10	γ-Terpinene
25	0.79	Terpinolene
26	0.05	α-Campholenal
27	0.10	linalool L
28	0.08	α-Fenchol
29	0.17	α-Campholenal
30	0.15	trans-Pinocarveol
31	0.09	(-)-Borneol
32	0.05	Pinocamphone
33	0.66	Terpinen-4-ol
34	0.95	α-Terpineol
35	0.05	Myrtenol
36	0.06	Estragole
37	0.10	Nopol



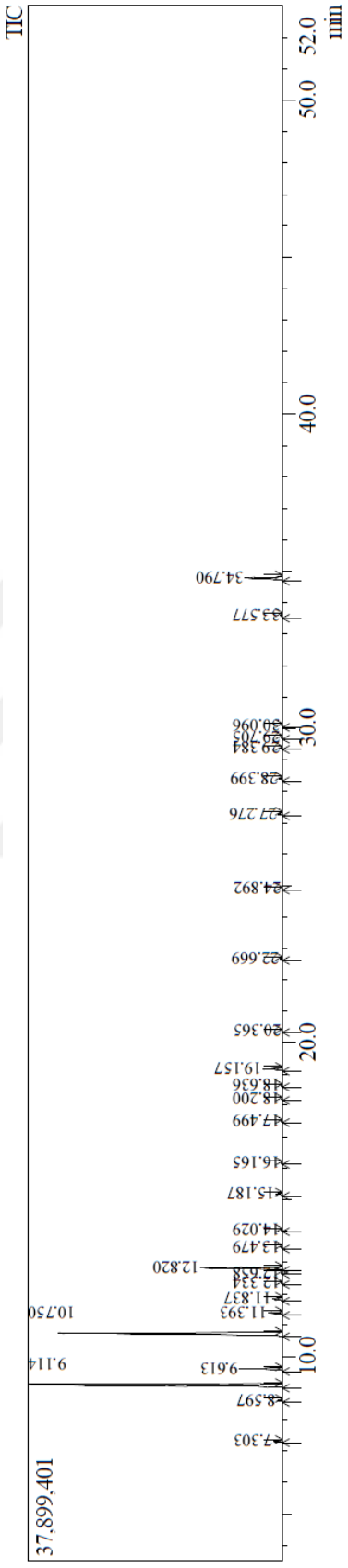
Graphic 4.3. GC-MS chromatogram of the essential oil extracted from Taurus cedar

4.1.3. GC-MS Findings of Taurus Cedar

In total, 47 different compounds were identified in the GC-MS analysis of Taurus cedar, and six agents had an availability of >2%: δ-3-carene (46.70%), β-pinene (16.57%), α-bisabolol (9.35%), β-phellandrene (3.93%), myrcene (2.54%), and β-himachalene (2.51%) (Graphic 4.3.), (Table 4.3.).

Table 4.3. *GC-MS analysis of Taurus cedar*

No	% Component	Name
1	46.70	δ-3-Carene
2	16.57	β-Pinene
3	9.35	α-Bisabolol
4	3.93	β-Phellandrene
5	2.54	Myrcene
6	2.51	β-Himachalene
7	0.09	Methyl isopimarate
8	0.52	β-Phellandrene
9	0.03	Tricyclene
10	0.08	α-Terpinene
11	0.09	para-Cymene
12	0.13	α-Thujene
13	0.18	γ-Terpinene
14	1.37	Terpinolene
15	0.15	Terpinen-4-ol
16	0.45	α-Terpineol
17	0.05	Thymyl Methyl Ether
18	0.18	Bornyl acetate
19	0.09	trans Pinocarveylacetate
20	0.74	Myrtenyl acetate
21	0.63	α-Longipinene
22	0.82	α-Copaene
23	0.06	Myrtanyl acetate
24	0.21	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene
25	1.51	Longifolene
26	1.55	Caryophyllene
27	1.87	α-Himachalene
28	0.29	α-Humulene (CAS)
29	0.35	(E)-β-Famesene
30	1.74	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene
31	1.11	Germacrene D
32	0.08	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene
33	0.34	Camphene
34	0.13	δ-Cadinene
35	0.48	Cis-α-Bisabolene
36	0.10	Longiborneol
37	0.08	l-Phellandrene
38	0.70	δ-3-Carene
39	0.46	Viridiflorol
40	0.08	(-)-Isolongifolol, acetate
41	0.26	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-
42	0.25	Kaur-16-ene
43	0.22	Biformene
44	0.07	Cembrene
45	0.19	Pimara-7,15-dien-3-one
46	0.61	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one
47	0.05	Cycloisolongifolene, 9,10-dehydro-



Graphic 4.4. GC-MS chromatogram of the essential oil extracted from Kazdagı fir

4.1.4. GC-MS Findings of Kazdağı Fir

In total, 28 different compounds were identified in the GC-MS analysis of Kazdagı fir, and six agents had an availability of >2%: δ-3-carene (9.86%), β-pinene (31.70%), β-phellandrene (9.10%), juniper camphor (4.95%), camphene (4.52%), and α-terpineol (2.23%) (Graphic 4.4.), (Table 4.4.).

Table 4.4. *GC-MS analysis of Kazdagı fir*

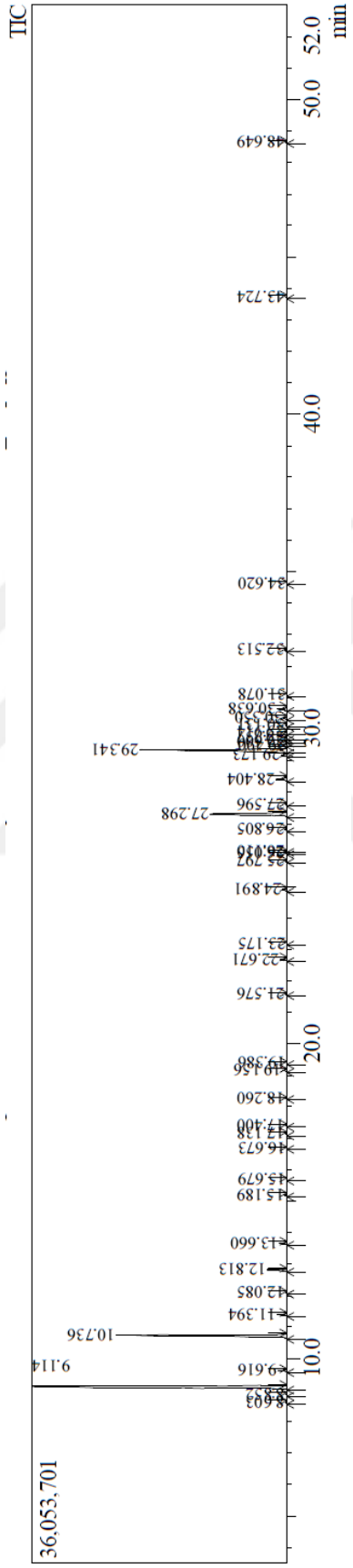
No	% Component	Name
1	39.86	δ-3-Carene
2	31.70	β-Pinene
3	9.10	β-Phellandrene
4	4.95	Juniper camphor
5	4.52	Camphene
6	2.23	α-Terpineol
7	0.86	Santene
8	0.59	Tricyclene
9	1.48	Myrcene
10	1.02	α-Phellandrene
11	0.09	α-Terpinene
12	0.10	para-Cymene
13	0.16	Acetic acid, 5-methylhex-2-yl ester
14	0.10	γ-Terpinene
15	0.70	Terpinolene
16	0.14	α-Fenchol
17	0.08	Camphene hydrate
18	0.08	BORNEOL L
19	0.13	Terpinen-4-ol
20	0.33	Bornyl acetate
21	0.06	α-Terpinyl acetate
22	0.59	Caryophyllene
23	0.38	α-Humulene
24	0.17	4,11-selinadiene
25	0.12	Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-
26	0.14	α-Farnesene
27	0.25	Selina-6-en-4-ol
28	0.03	6-Nonen-1-ol, acetate, (Z)-

4.1.5. GC-MS Findings of Black Pine

In total, 40 different compounds were identified in the GC-MS analysis of Black pine, and five agents had an availability of >2%: δ-3-carene (37.27%), germacrene D (20.39%), β-pinene (19.37%), caryophyllene (9.65%), and sylvestrene (2.09%) (Graphic 4.5.), (Table 4.5.).

Table 4.5. *GC-MS analysis of Black pine*

No	% Component	Name
1	37.27	δ-3-Carene
2	20.39	Germacrene-D
3	19.37	β-Pinene
4	9.65	Caryophyllene
5	2.09	Sylvestrene
6	0.12	Tricyclene
7	0.09	α-Thujene
8	0.87	Camphene
9	0.83	Z-Ocimene
10	0.23	Terpinolene
11	0.07	Linalool L
12	0.04	alpha.-Campholenal
13	0.10	trans-Pinocarveol
14	0.07	Verbenol
15	0.05	p-Mentha-1,5-dien-8-ol
16	0.46	α-Terpineol
17	0.04	(-)-Myrtenol
18	0.11	Linalyl acetate
19	0.74	Bornyl acetate
20	0.06	(-)-trans-Pinocarvyl acetate
21	0.47	α-Terpinyl acetate
22	0.11	α-Copaene
23	0.15	2,6-Octadien-1-OL, 3,7-Dimethyl-, Acetate
24	0.07	β-Bourbonene
25	0.19	Cyclosativene
26	0.12	Hex-3(Z)-enyl acetate
27	0.10	Germacrene-D
28	1.49	α-Humulene
29	0.73	α-Amorphene
30	0.97	Myrcene
31	0.04	α-Guaiene
32	0.16	phenethyl-Isovalerate
33	0.23	α-Copaene
34	0.28	α-Muurolene
35	0.32	δ-Cadinene
36	0.46	γ-Cadinene
37	0.04	α-Muurolene
38	0.21	Caryophyllene oxide
39	0.04	α-Cadinol
40	0.19	epi-13-Manoyloxide



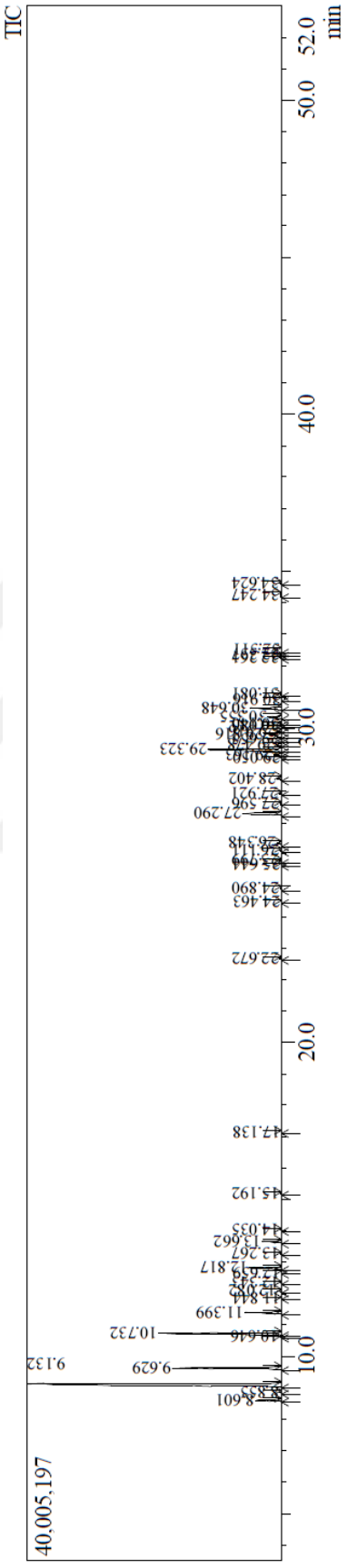
Graphic 4.5. GC-MS chromatogram of the essential oil extracted from Black pine

4.1.6. GC-MS Findings of Scots Pine

In total, different compounds were identified in the GC-MS analysis of Scots pine, and 10 agents had an availability of >2%: δ-3-carene (39.85%), β-pinene (12.08%), camphene (10.15%), germacrene D (8.67%), caryophyllene (4.23%), δ-cadinene (3.54%), myrcene (3.31%), D-limonene (2.99%), bicyclogermacrene (2.68%), and tricyclene (2.34%) (Graphic 4.6.), (Table 4.6.).

Table 4.6. *GC-MS analysis of Scots pine*

No	% Component	Name
1	39.85	δ-3-Carene
2	12.08	β-Pinene
3	10.15	Camphene
4	8.67	Germacrene-D
5	4.23	Caryophyllene
6	3.54	δ-Cadinene
7	3.31	Myrcene
8	2.99	D-Limonene
9	2.68	Bicyclogermacrene
10	2.34	Tricyclene
11	0.15	α-Thujene
12	0.35	α-Cadinol
13	0.07	trans-β-Ocimene
14	1.75	Z-Ocimene
15	0.09	γ-Terpinene
16	0.11	Terpinolene
17	0.04	trans-Pinocarveol
18	0.22	Bornyl acetate
19	0.03	Bicyclogermacrene
20	0.15	α-Cubebene
21	0.04	Ylangene
22	0.21	α-Copaene
23	0.15	β-Bourbonene
24	0.47	β-Elemene
25	0.03	para-Cymene
26	0.08	α-Ionone
27	0.31	Aromadendrene
28	0.59	α-Humulene
29	0.07	trans-Cadina-1(6),4-diene
30	0.94	α-Amorphene
31	0.04	α-Phellandrene
32	0.60	β-Selinene
33	0.09	(+)-epi-Bicyclosesquiphellandrene
34	0.38	Hex-3(Z)-enyl acetate
35	0.53	α-Muurolene
36	0.18	β-Elemene
37	0.07	δ-Cadinene
38	1.60	γ-Cadinene
39	0.23	Sabinene
40	0.05	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
41	0.11	α-Muurolene
42	0.23	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene
43	0.13	Spathulenol
44	0.07	Caryophyllene oxide



Graphic 4.6. GC-MS chromatogram of the essential oil extracted from Scots pine

4.2. Antimicrobial Activity of Essential Oils

The essential oils extracted from six different species by hydrodistillation method were tested against 18 microorganisms (gram-positive and gram-negative bacteria and *C. albicans* strain) at different concentrations, and the MIC, MBC, and MFC values are shown in Tables 4.7 and 4.8.

4.2.1. MIC Values of Plant Samples

Table 4.7 *MIC values of plant taxa* (in µg/mL)

	Bitki Türleri					
Microorganisms	*P. brutia*	*P. n.* subsp. *pallasiana*	*P. sylvestris*	*C. libani*	*P. orientalis*	*A. n.* subsp. *equi-trojani*
B. subtilis	100	50	100	25	6,25	50
C. albicans	-	-	-	-	-	-
E. aerogenes	100	50	100	50	3,125	25
E. coli	100	100	100	50	6,25	6,25
E. durans	100	100	50	25	6,25	12,5
E. faecalis	-	12,5	50	-	-	-
E. faecium	50	3,125	50	100	3,125	3,125
K. pneumoniae	100	100	100	50	6,25	25
L. innocula	50	100	50	50	6,25	50
L. monocytogenes	6,25	100	3,125	3,125	3,125	0,781
P. aeruginosa	3,125	1,562	12,5	6,25	3,125	12,5
P. fluorescens	50	50	50	50	3,125	50
S. aureus	0,39	0,781	0,39	0,39	0,781	0,195
S. enteritidis	50	100	100	50	0,39	12,5
S. epidermidis	25	12,5	25	0,195	0,781	0,781
S. infantis	100	25	50	6,25	1,562	12,5
S. kentucky	50	100	100	100	1,562	12,5
S. typhimurium	100	100	50	50	1,562	100

"-''= No activity

None of the essential oils extracted from the samples were active against the fungus *C. albicans.* MIC values of the taxon *P. orientalis* exhibited the highest activity against the tested microorganisms compared to other taxa.

4.2.2. MBC and MFC Values of Plant Samples

Table 4.8. *MBC and MFC values of plant taxa* (in µg/mL)

Microorganisms	*P. brutia*	*P. n.* subsp. *pallasiana*	*P. sylvestris*	*C. libani*	*P. orientalis*	*A. n.* subsp. *equi-trojani*
B. subtilis	-	-	100	-	6,25	50
C. albicans	-	-	-	-	-	-
E. aerogenes	-	-	100	-	6,25	100
E. coli	100	-	-	-	25	6,25
E. durans	100	100	50	-	6,25	50
E. faecalis	-	50	50	-	-	-
E. faecium	50	100	50	-	6,25	25
K. pneumoniae	100	-	100	-	6,25	50
L. innocula	100	-	100	-	12,5	100
L. monocytogenes	50	-	12,5	50	100	12,5
P. aeruginosa	-	-	100	-	50	50
P. fluorescens	-	-	50	-	12,5	50
S. aureus	50	-	12,5	50	50	12,5
S. enteritidis	100	100	100	-	3,125	12,5
S. epidermidis	50	50	25	-	12,5	6,25
S. infantis	100	-	100	100	1,562	100
S. kentucky	50	-	100	-	3,125	12,5
S. typhimurium	100	-	100	-	3,125	-

"-''= No activity

Because no essential oil from the plant samples exhibited activity against the fungus *C. albicans*, the MFC value could not be obtained. The MBC activity of Black pine and Taurus cedar against the microorganisms was lower than that of the other taxa.

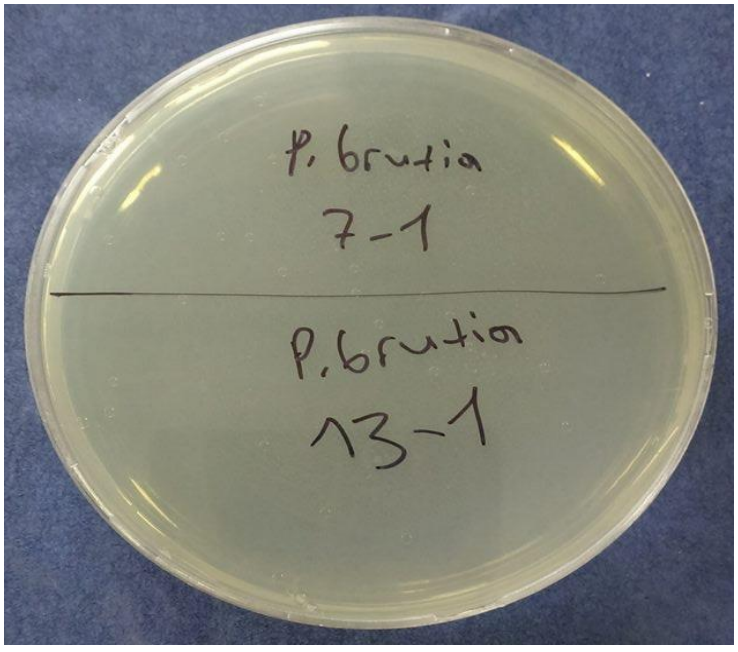
4.2.3. MIC Values of Turkish Pine

The essential oil extracted from *P. brutia* (Turkish pine) yielded the following MIC values: *S. typhimurium*, 100 µg/mL; *E. aerogenes*, 100µg/mL; *S. infantis*, 100 µg/mL; *K. pneumoniae*, 100 µg/mL; *B. subtilis*, 100 µg/mL; *E. coli*, 100 µg/mL; *E. durans*, 100 µg/mL; *S. enteritidis*, 50 µg/mL; *E. faecium*, 50µg/mL; *S. kentucky*, 50 µg/mL; *L. innocula*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *S. epidermidis*, 25 µg/mL; *L. monocytogenes*, 6.25 µg/mL; *P. aeruginosa*, 3.125 µg/mL; *S. aureus*, 0.39 µg/mL. The essential oil exhibited no activity against *C. albicans* and *E. faecalis* (Photograph 4.1.), (Graphic 4.7.).

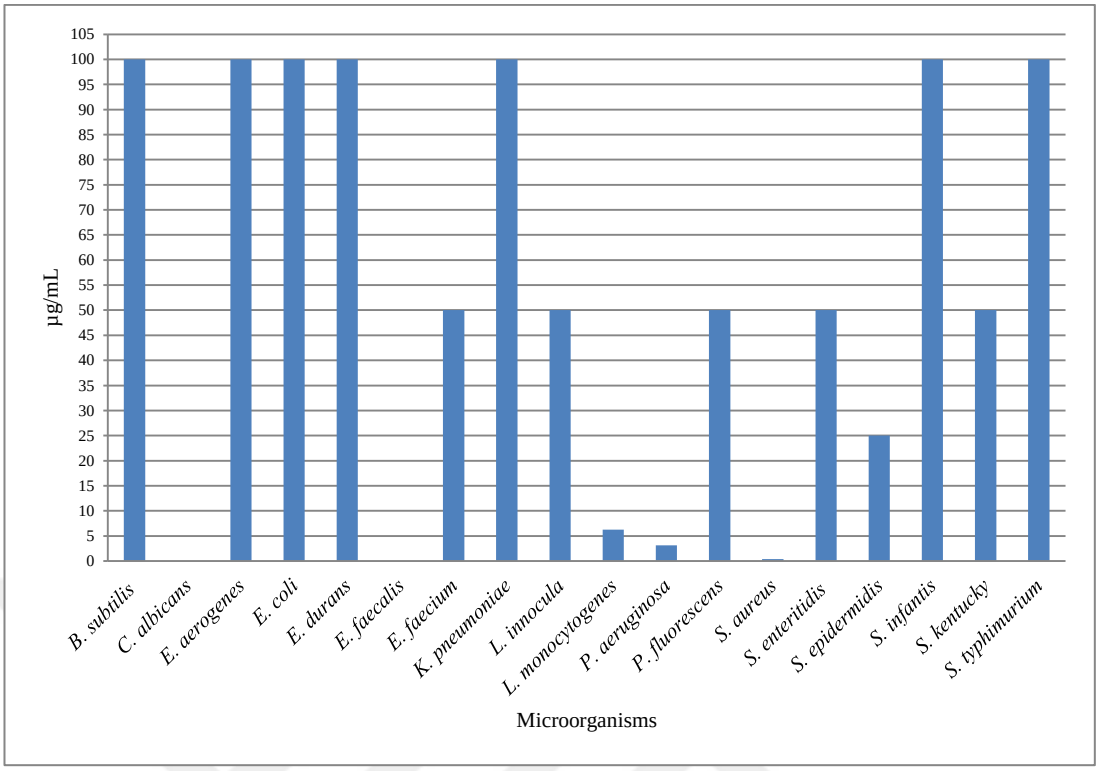
4.2.4. MBC and MFC Values of Turkish Pine

The MBC and MFC values of the essential oil extracted from *P. brutia* are as follows: *S. enteritidis*, 100 µg/mL; *S. typhimurium*, 100 µg/mL; *S. infantis*, 100 µg/mL; *L. innocula*, 100 µg/mL; *K. pneumoniae*, 100 µg/mL; *E. coli*, 100 µg/mL; *E. durans*, 100 µg/mL; *S. aureus*, 50 µg/mL; *E. faecium*, 50 µg/mL; *L. monocytogenes*, 50 µg/mL; *S. kentucky*, 50 µg/mL; and *S. epidermidis*, 50 µg/mL. The essential oil showed no antibacterial activity against *E. aerogenes*, *P. fluorescens*, *B. subtilis*, and *P. aeruginosa* (Photograph 4.2.), (Graphic 4.8.).

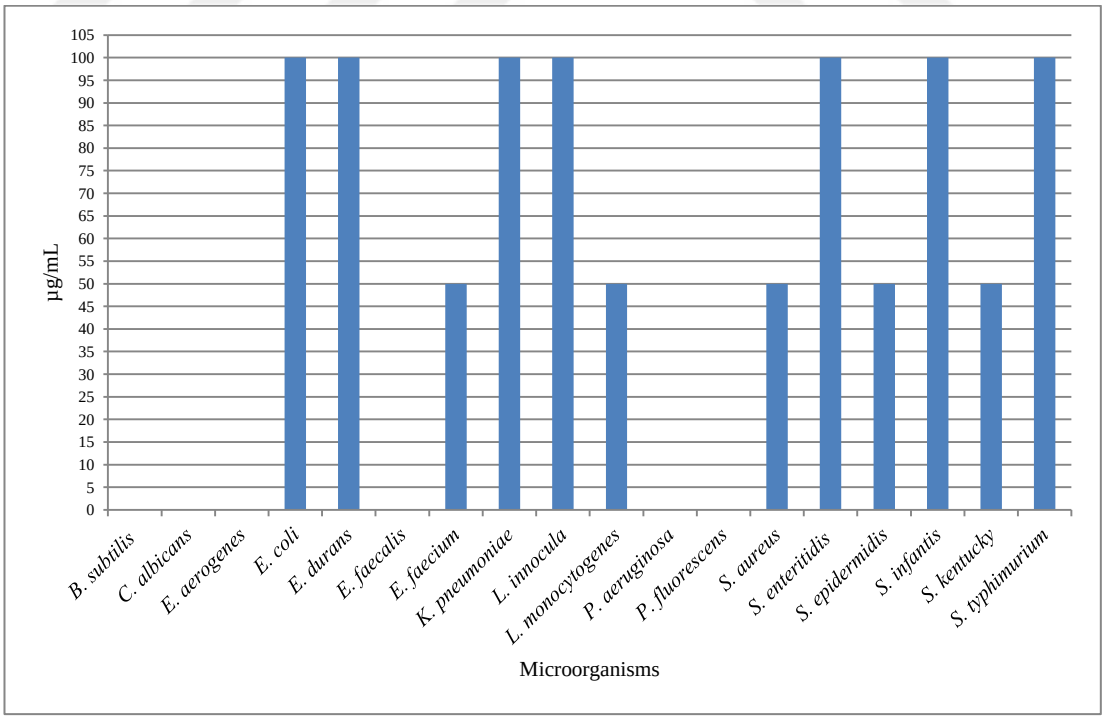
Photograph 4.1. MIC results of Turkish pine



Photograph 4.2. MBC and MFC results of Turkish pine



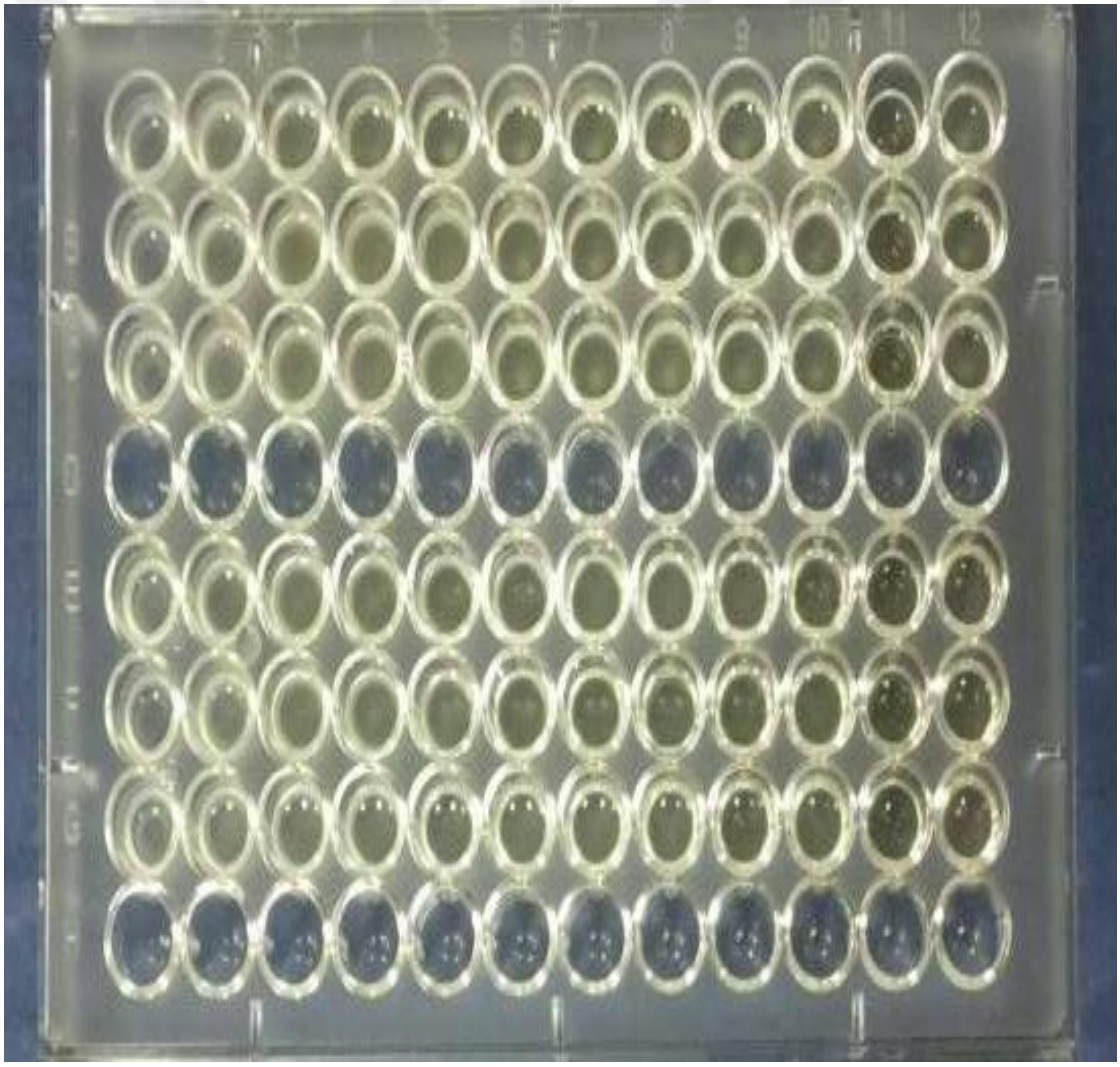
Graphic 4.7. MIC values of Turkish pine



Graphic 4.8. MBC and MFC values of Turkish pine

4.2.5. MIC Values of Black Pine

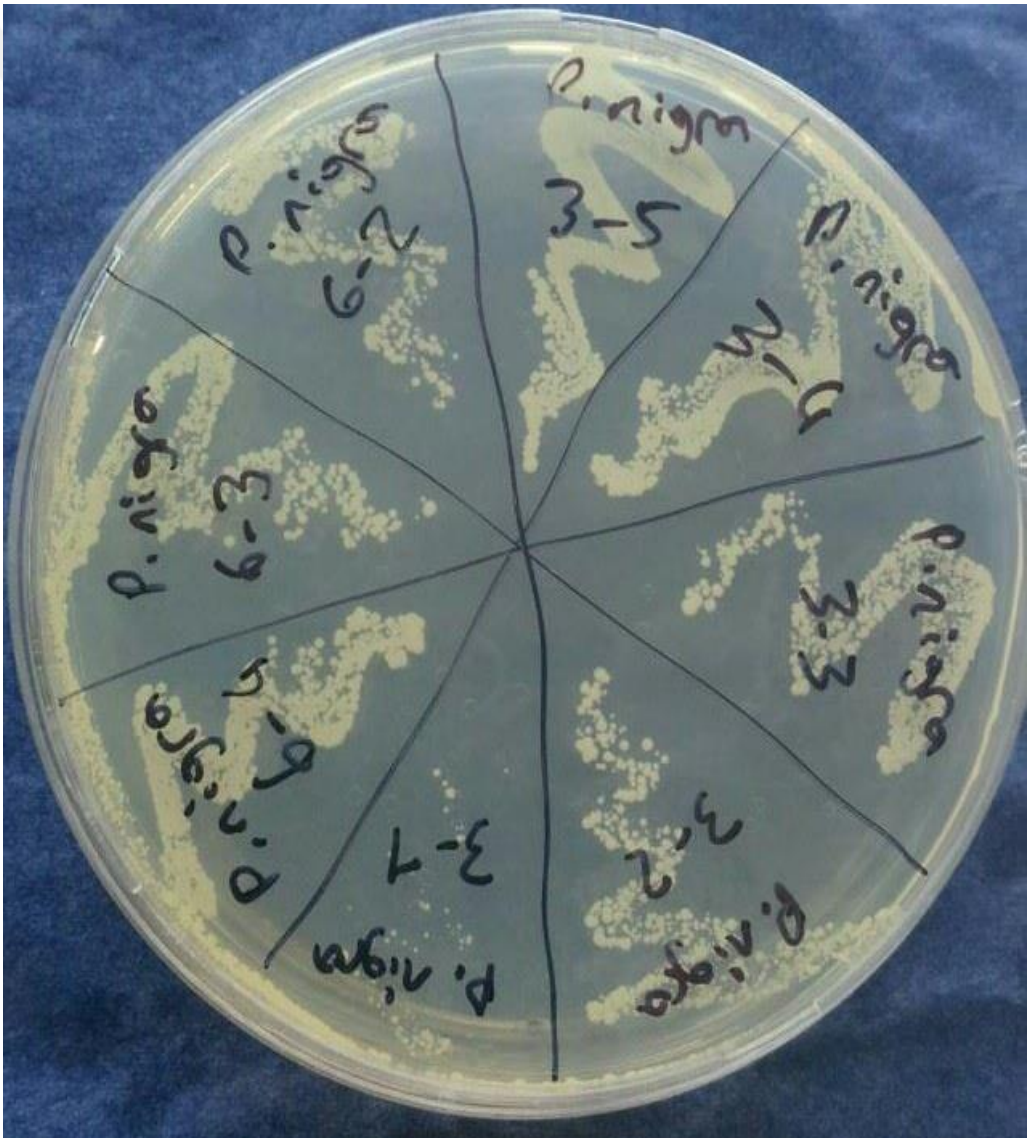
The essential oil extracted from *P. nigra* subsp. *pallasiana* (Black pine) yielded the following MIC values: *S. enteritidis*, 100 µg/mL; *L. monocytogenes*, 100 µg/mL; *S. typhimurium*, 100 µg/mL; *S. kentucky*, 100 µg/mL; *L. innocula*, 100 µg/mL; *K. pneumonia*, 100 µg/mL; *E. coli*, 100 µg/mL; *E. durans*, 100 µg/mL; *E. aerogenes*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *B. subtilis*, 50 µg/mL; *S. infantis*, 25 µg/mL; *E. faecalis*, 12.5 µg/mL; *S. epidermidis*, 12.5 µg/mL; *E. faecium*, 3.125 µg/mL; *P. aeruginosa*, 1.562 µg/mL; and *S. aureus*, 0.781 µg/mL. The essential oil exhibited no activity against *C. albicans* (Photograph 4.3.), (Graphic 4.9.).



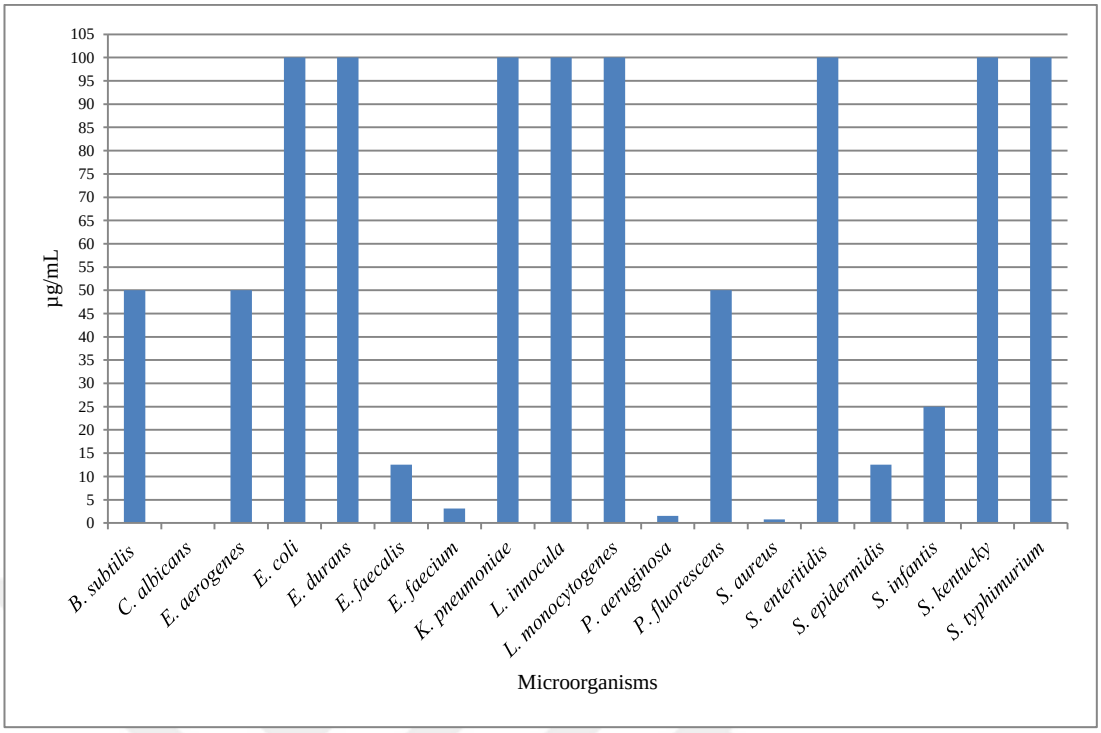
Photograph 4.3. MIC results of Black pine

4.2.6. MBC and MFC Values of Black Pine

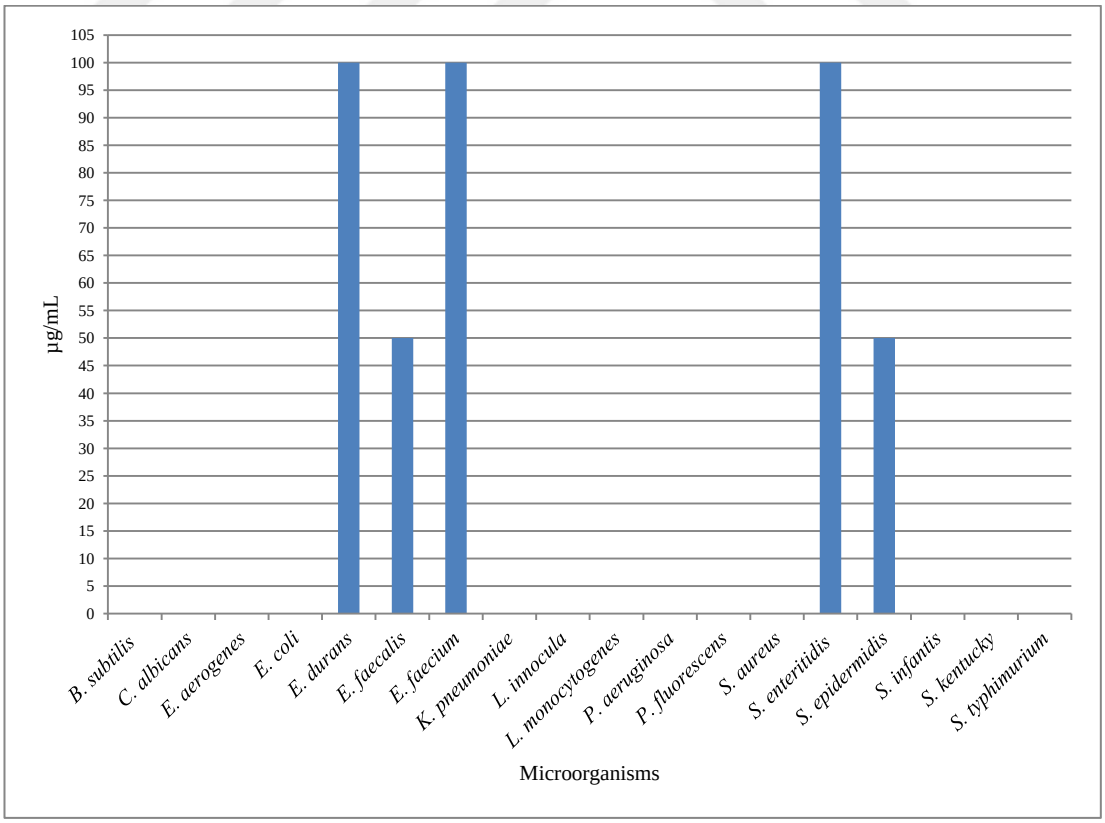
The MBC and MFC values of the essential oil extracted from *P. nigra* subsp. *pallasiana* (black pine) were as follows: *S. enteritidis*, 100 µg/mL; *E. faecium*, 100 µg/mL; *E. durans*, 100 µg/mL; *E. faecalis*, 50 µg/mL; *S. epidermidis*, 50 µg/mL. The essential oil showed no bactericidal activity against *S. aureus*, *L. monocytogenes*, *S. typhimurium*, *E. aerogenes*, *S. infantis*, *S. kentucky*, *L. innocula*, *P. fluorescens*, *K. pneumoniae*, *B. subtilis*, *E. coli*, and *P. aeruginosa* (Photograph 4.4.), (Graphic 4.10.).



Photograph 4.4. MBC and MFC results of Black pine



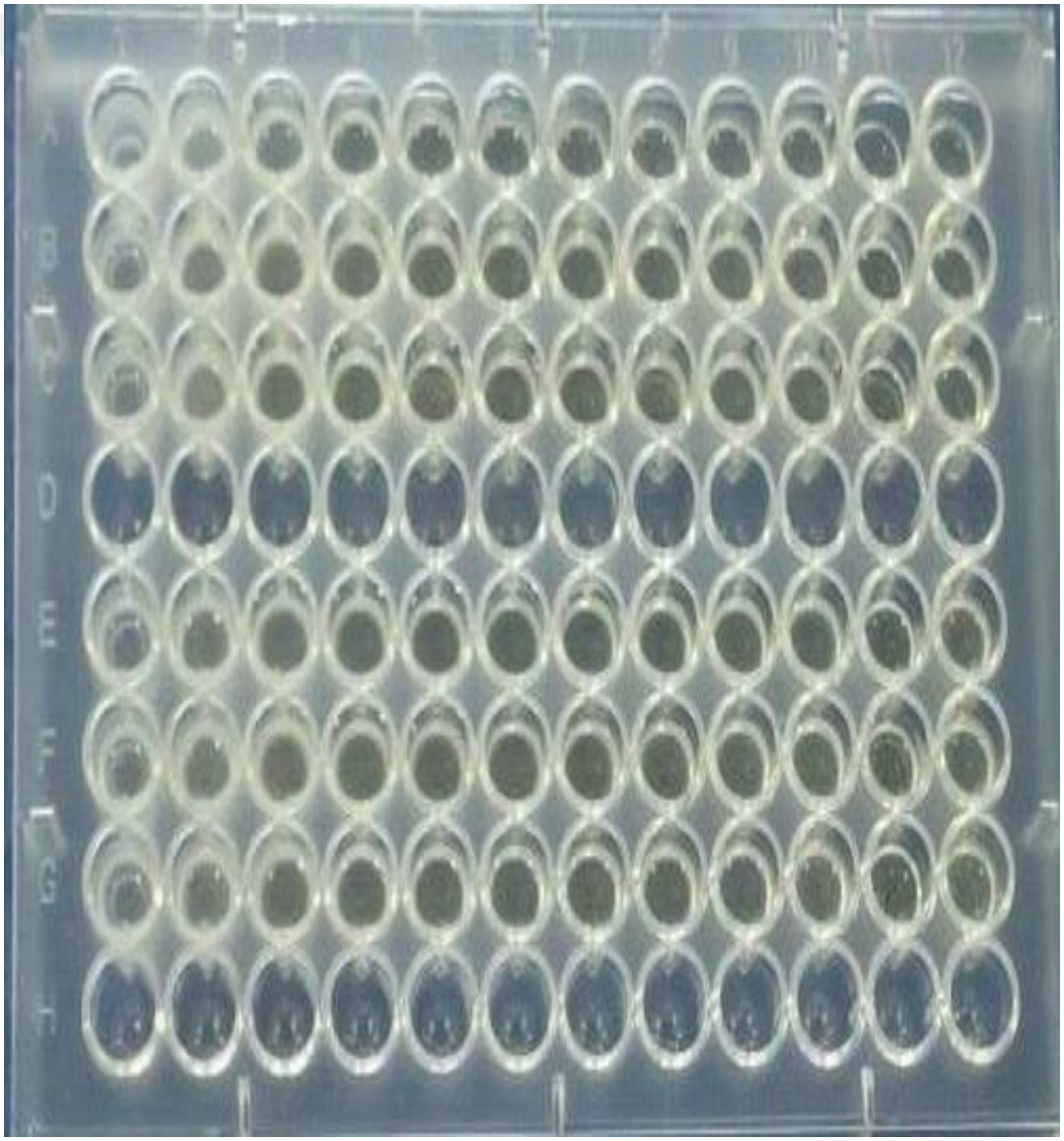
Graphic 4.9. MIC values of Black pine



Graphic 4.10. MBC and MFC values of Black pine

4.2.7. MIC Values of Scots Pine

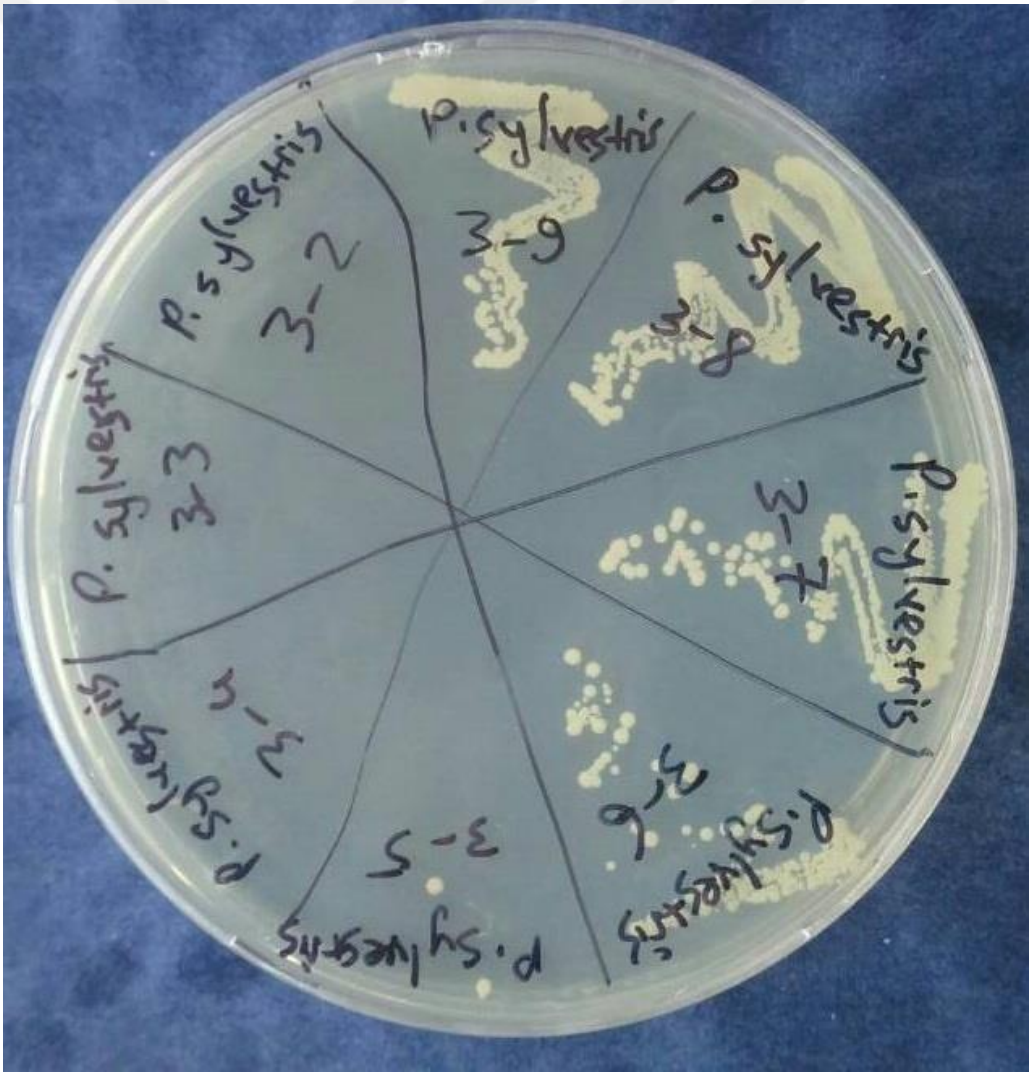
The essential oil extracted from *P. sylvestris* (Scots pine) yielded the following MIC values: *S. enteritidis*, 100 µg/mL; *E. aerogenes*, 100 µg/mL; *S. kentucky*, 100 µg/mL; *K. pneumonia*, 100 µg/mL; *B. subtilis*, 100 µg/mL; *E. coli*, 100 µg/mL; *E. faecium*, 50 µg/mL; *E. faecalis*, 50 µg/mL; *S. typhimurium*, 50 µg/mL; *S. infantis*, 50 µg/mL; *L. innocula*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *E. durans*, 50 µg/mL; *S. epidermidis*, 25 µg/mL; *P. aeruginosa*, 12.5 µg/mL; *L. monocytogenes*, 3.125 µg/mL; and *S. aureus*, 0.39 µg/mL. The essential oil showed no activity against *C. albicans* (Photograph 4.5.), (Graphic 4.11.).



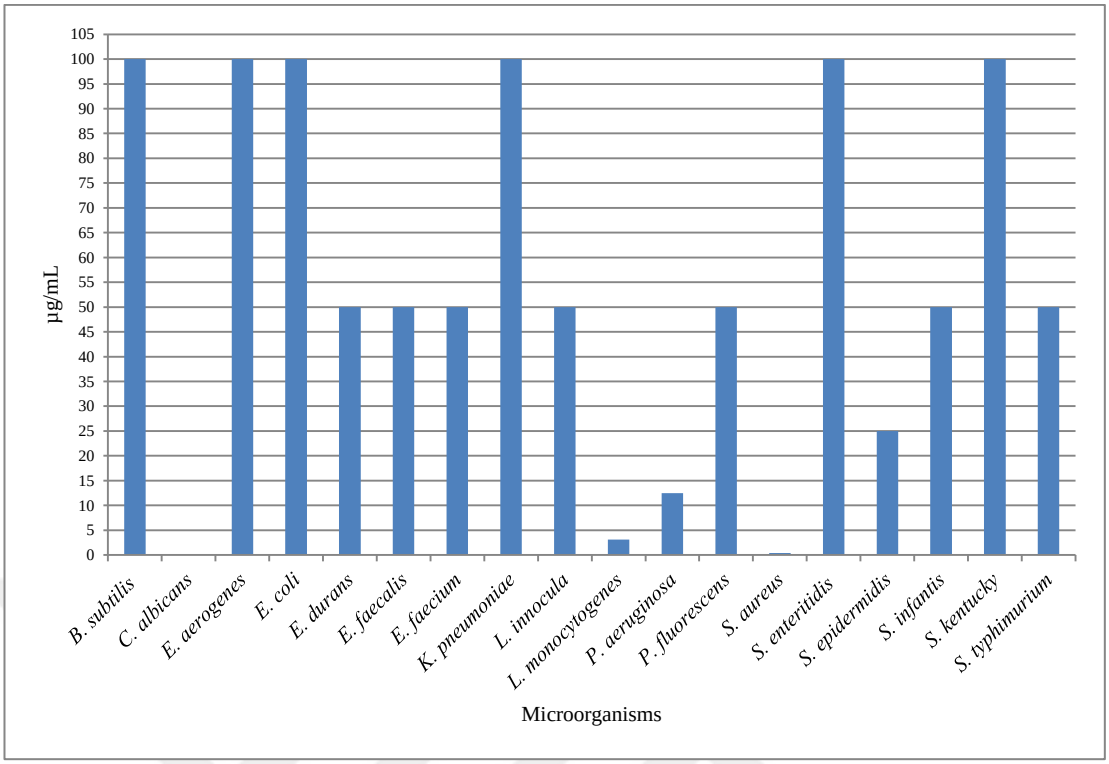
Photograph 4.5. MIC results of Scots pine

4.2.8. MBC and MFC Values of Scots Pine

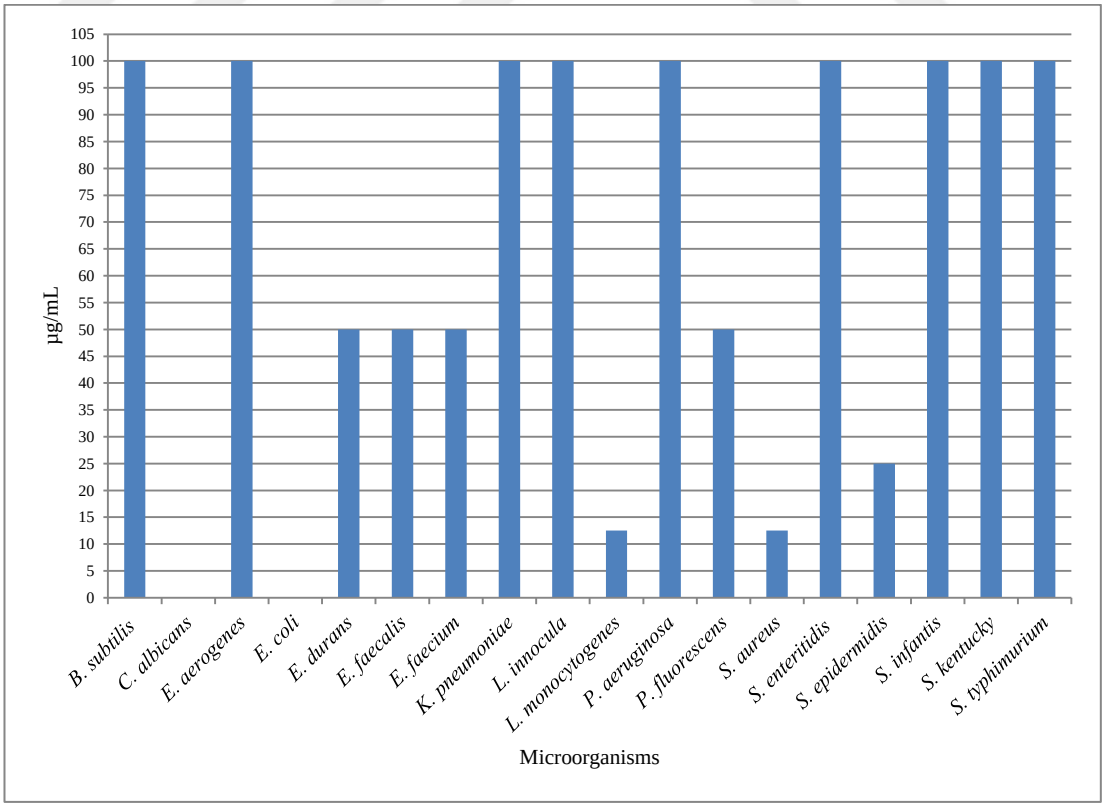
The MBC and MFC values of the essential oil extracted from *P. sylvestris* (Scots pine) are as follows: *S. enteritidis*, 100 µg/mL; *S. typhimurium*, 100 µg/mL; *E. aerogenes*, 100 µg/mL; *S. infantis*, 100 µg/mL; *S. kentucky*, 100 µg/mL; *L. innocula*, 100 µg/mL; *K. pneumoniae*, 100 µg/mL; *B. subtilis*, 100 µg/mL; *P. aeruginosa*, 100 µg/mL; *E. faecium*, 50 µg/mL; *E. faecalis*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *E. durans*, 50 µg/mL; *S. epidermidis*, 25 µg/mL; *S. aureus*, 12.5 µg/mL; and *L. monocytogenes*, 12.5 µg/mL. The essential oil showed no antibacterial activity against *E. coli* (Photograph 4.6.), (Graphic 4.12.).



Photograph 4.6. MBC and MFC results of Scots pine



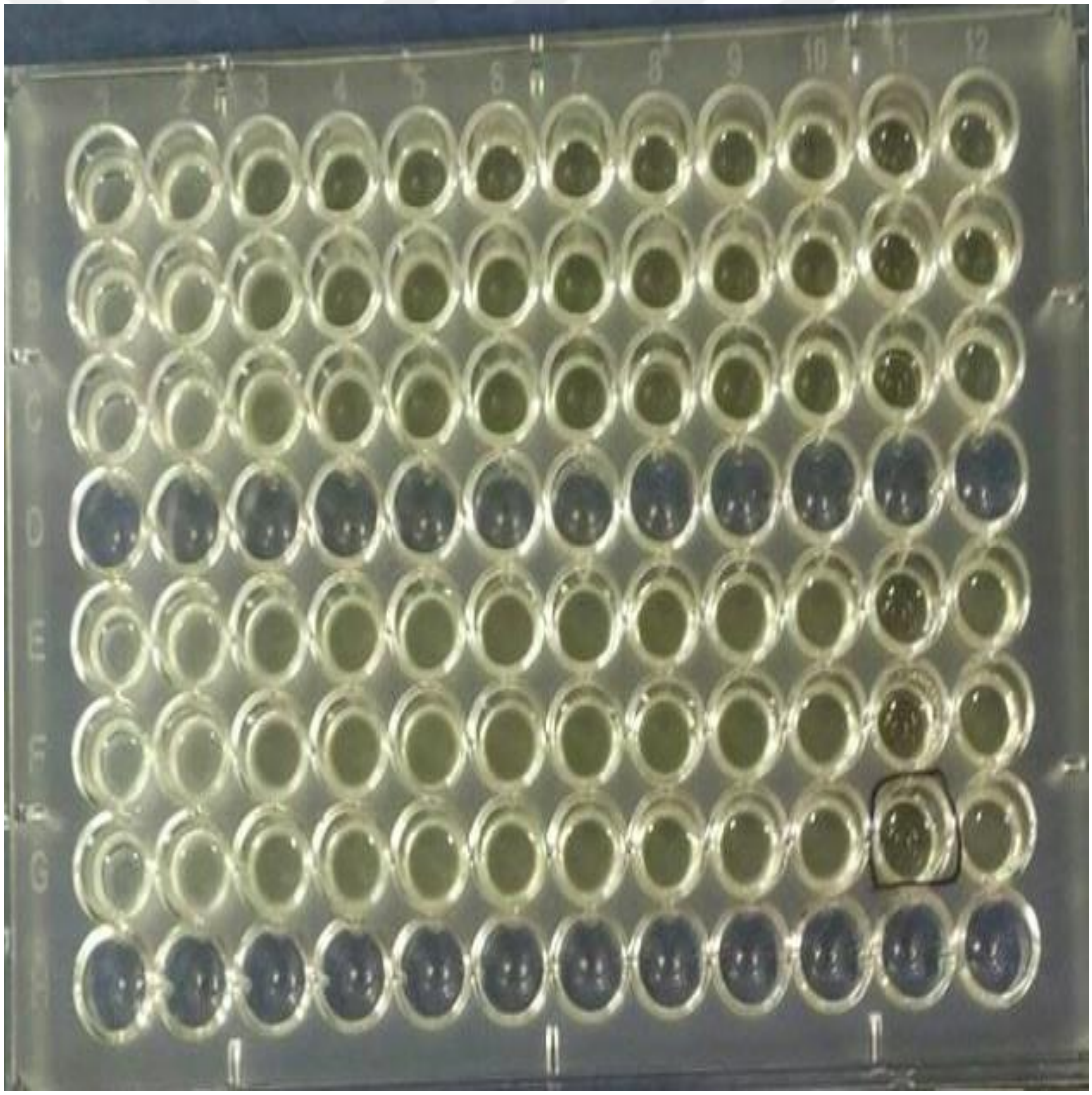
Graphic 4.11. MIC values of Scots pine



Graphic 4.12. MBC and MFC values of Scots pine

4.2.9. MIC Values of Taurus Cedar

The essential oil extracted from *C. libani* (Taurus cedar) yielded the following MIC values: *E. faecium*, 100 µg/mL; *S. kentucky*, 100 µg/mL; *S. enteritidis*, 50 µg/mL; *S. typhimurium*, 50 µg/mL; *E. aerogenes*, 50 µg/mL; *L. innocula*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *K. pneumonia*, 50 µg/mL; *E. coli*, 50 µg/mL; *B. subtilis*, 25 µg/mL; *E. durans*, 25 µg/mL; *S. infantis*, 6.25 µg/mL; *P. aeruginosa*, 6.25 µg/mL; *L. monocytogenes*, 3.125 µg/mL; *S. aureus*, 0.39 µg/mL; and *S. epidermidis*, 0.195 µg/mL. The essential oil showed no activity against *C. albicans* and *E. faecalis* (Photograph 4.7.), (Graphic 4.13.).

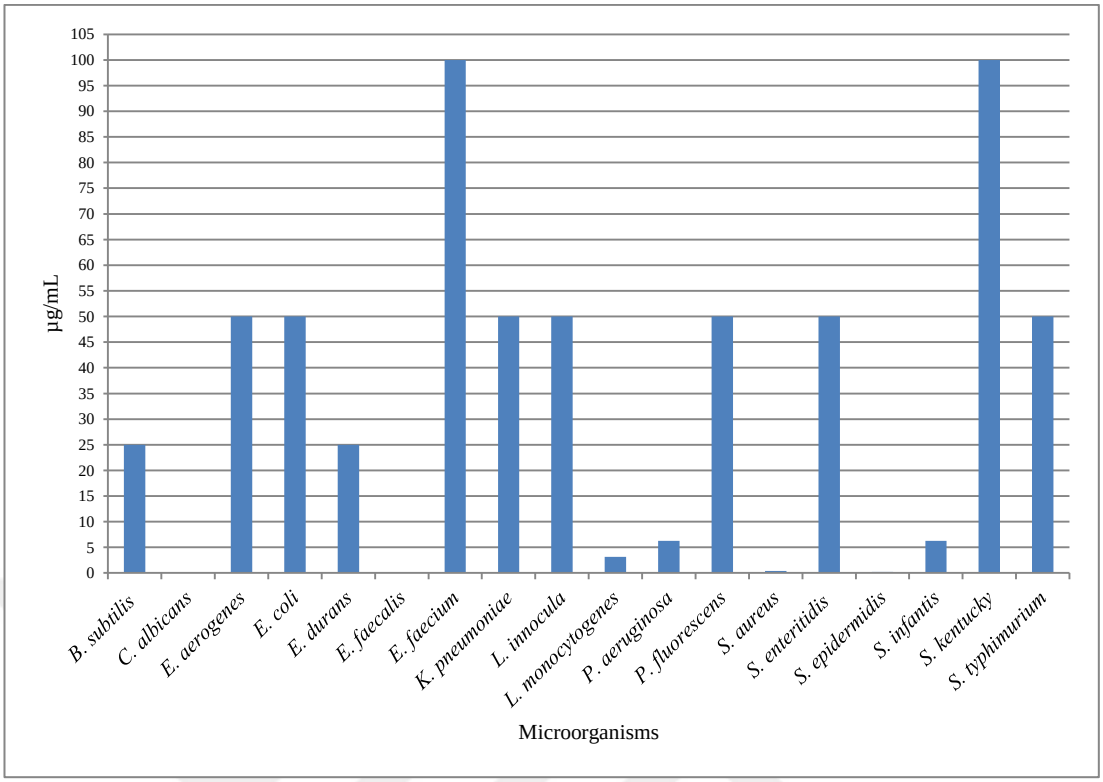


Photograph 4.7. MIC results of Taurus cedar

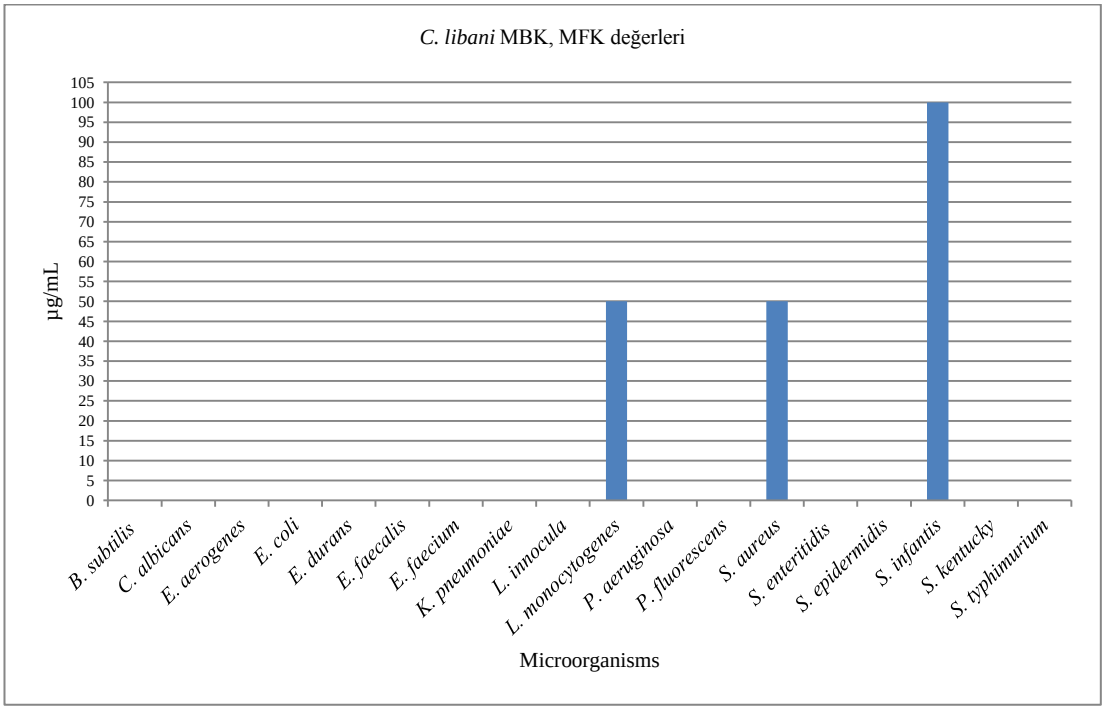
4.2.10. MBC and MFC Values of Taurus Cedar

The MBC and MFC values of the essential oil extracted from *C. libani* (Taurus cedar) are as follows: *S. infantis*, 100 µg/mL; *S. aureus*, 50 µg/mL; and *L. monocytogenes*, 50 µg/mL. The essential oil showed no activity against *S. enteritidis*, *E. faecium*, *S. typhimurium*, *E. aerogenes*, *S. kentucky*, *L. innocula*, *P. fluorescens*, *K. pneumoniae*, *B. subtilis*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, and *E. durans* (Photograph 4.8.), (Graphic 4.14.).

Photograph 4.8. MBC and MFC results of Taurus cedar



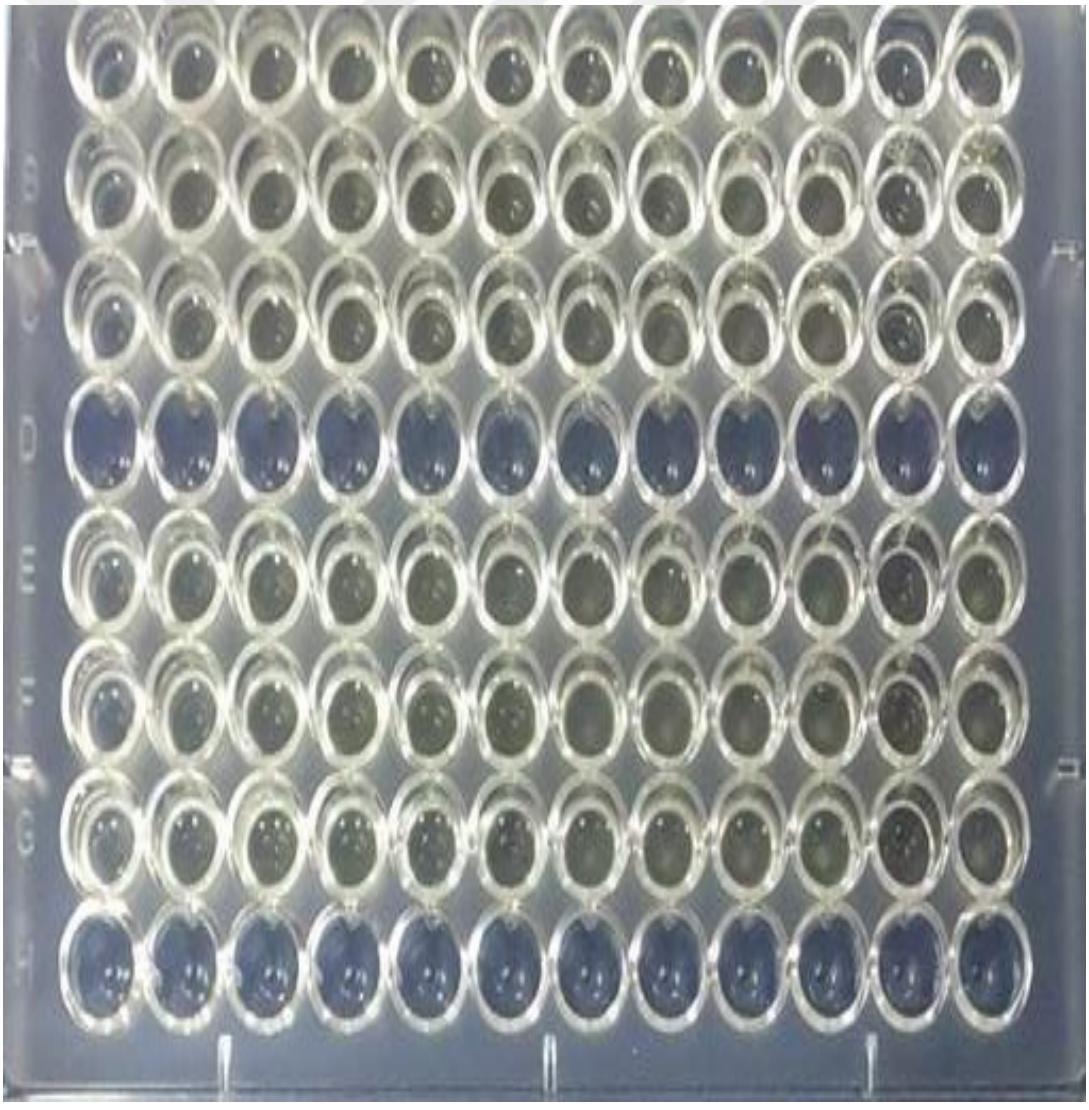
Graphic 4.13. MIC values of Taurus cedar



Graphic 4.14. MBC and MFC values of Taurus cedar

4.2.11. MIC Values of Oriental Spruce

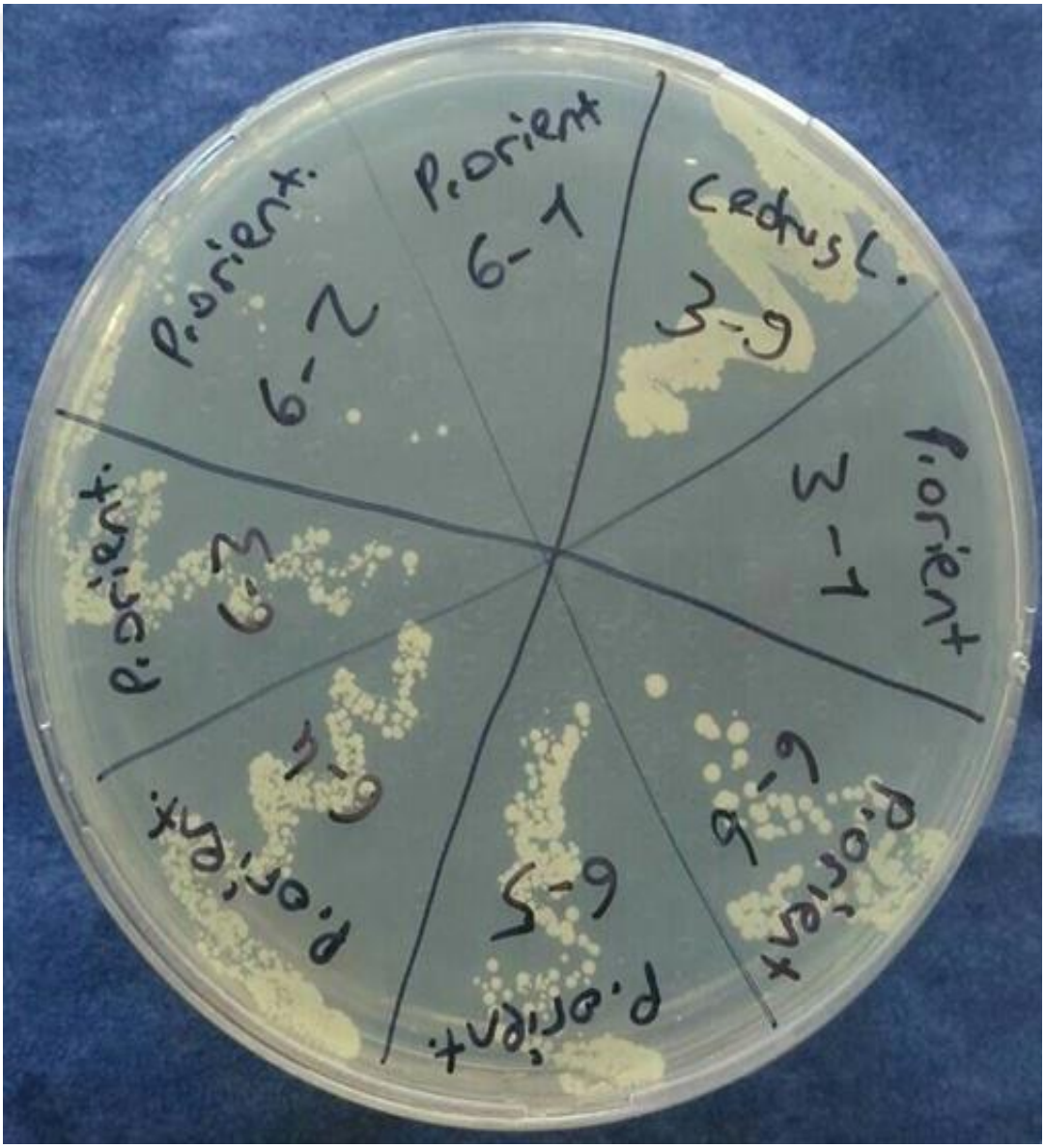
The essential oil extracted from *P. orientalis* (Oriental spruce) yielded the following MIC values: *L. innocula*, 6.25 µg/mL; *K. pneumonia*, 6.25 µg/mL; *B. subtilis*, 6.25 µg/mL; *E. coli*, 6.25 µg/mL; *E. durans*, 6.25 µg/mL; *E. faecium*, 3.125 µg/mL; *L. monocytogenes*, 3.125 µg/mL; *E. aerogenes*, 3.125 µg/mL; *P. fluorescens*, 3.125 µg/mL; *P. aeruginosa*, 3.125 µg/mL; *S. typhimurium*, 1.562 µg/mL; *S. infantis*, 1.562 µg/mL; *S. kentucky*, 1.562 µg/mL; *S. aureus*, 0.781 µg/mL; *S. epidermidis*, 0.781 µg/mL; and *S. enteritidis*, 0.39 µg/mL. The essential oil exhibited no activity against *C. albicans* and *E. faecalis* (Photograph 4.9.), (Graphic 4.15.).



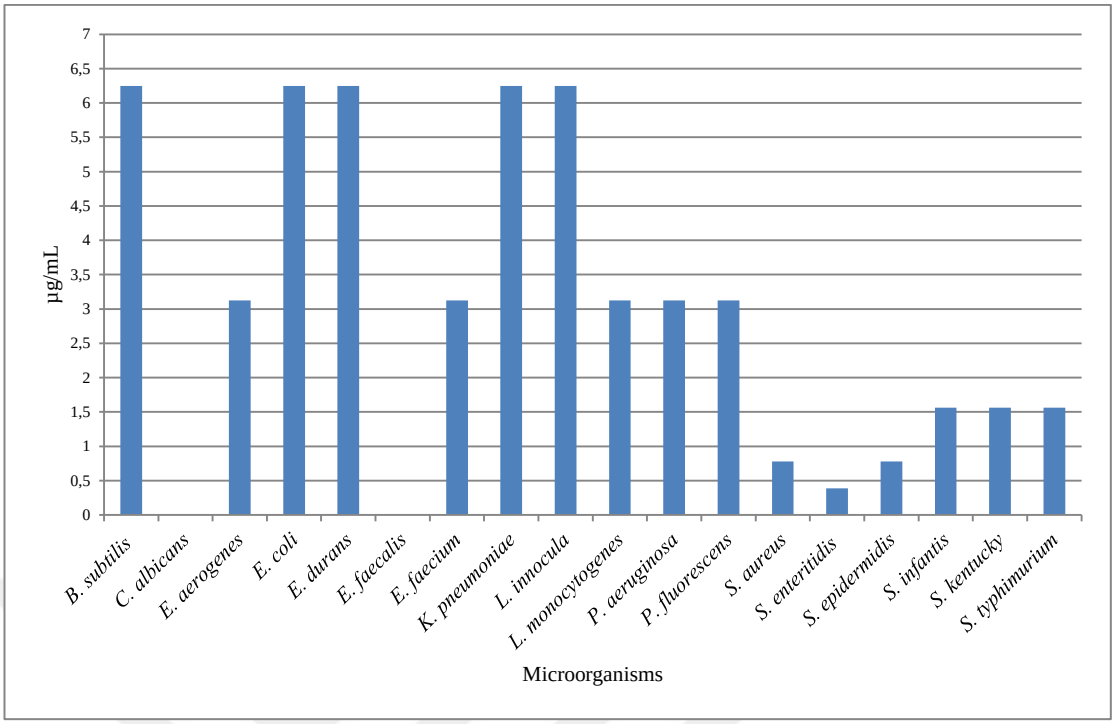
Photograph 4.9. MIC results of Oriental spruce

4.2.12. MBC and MFC Values of Oriental Spruce

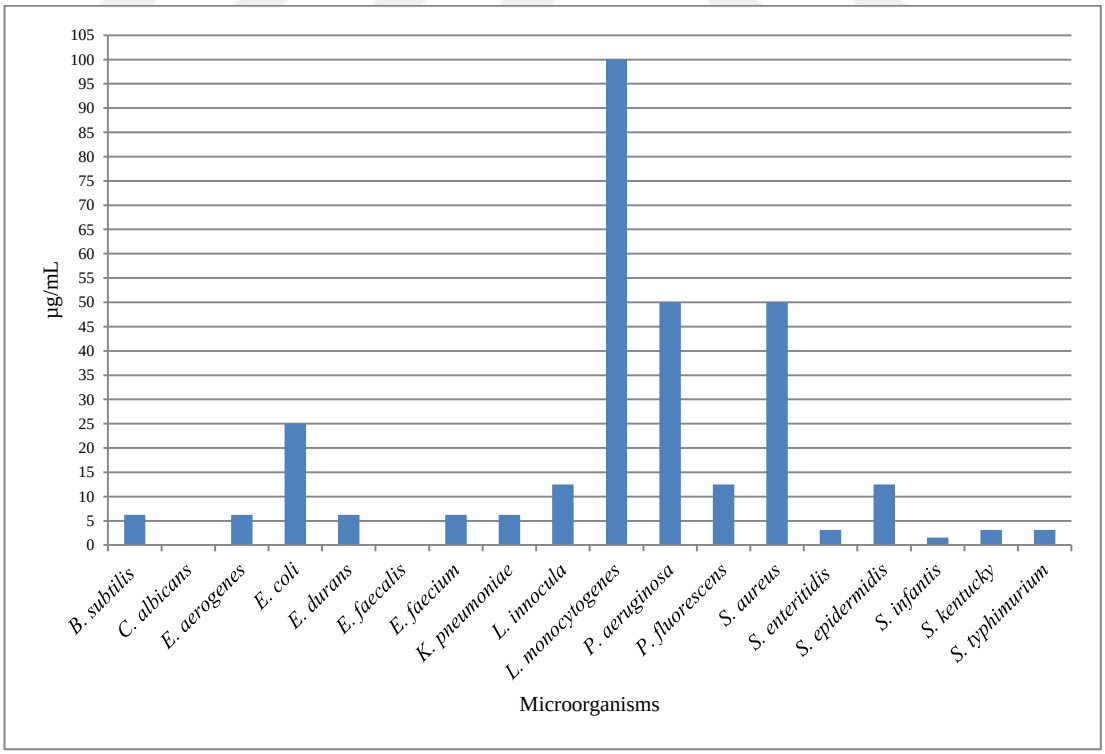
The MBC and MFC values of the essential oil extracted from *P. orientalis* (Oriental spruce) are as follows: *L. monocytogenes*, 100 µg/mL; *S. aureus*, 50 µg/mL; *P. aeruginosa*, 50 µg/mL; *E. coli*, 25 µg/mL; *L. innocula*, 12.5 µg/mL; *P. fluorescens*, 12.5 µg/mL; *S. epidermidis*, 12.5 µg/mL; *E. faecium*, 6.25 µg/mL; *E. aerogenes*, 6.25 µg/mL; *K. pneumoniae*, 6.25 µg/mL; *B. subtilis*, 6.25 µg/mL; *E. durans*, 6.25 µg/mL; *S. enteritidis*, 3.125 µg/mL; *S. typhimurium*, 3.125 µg/mL; *S. kentucky*, 3.125 µg/mL; and *S. infantis*, 1.562 µg/mL. (Photograph 4.10.), (Graphic 4.16.).



Photograph 4.10. MBC and MFC results of Oriental spruce



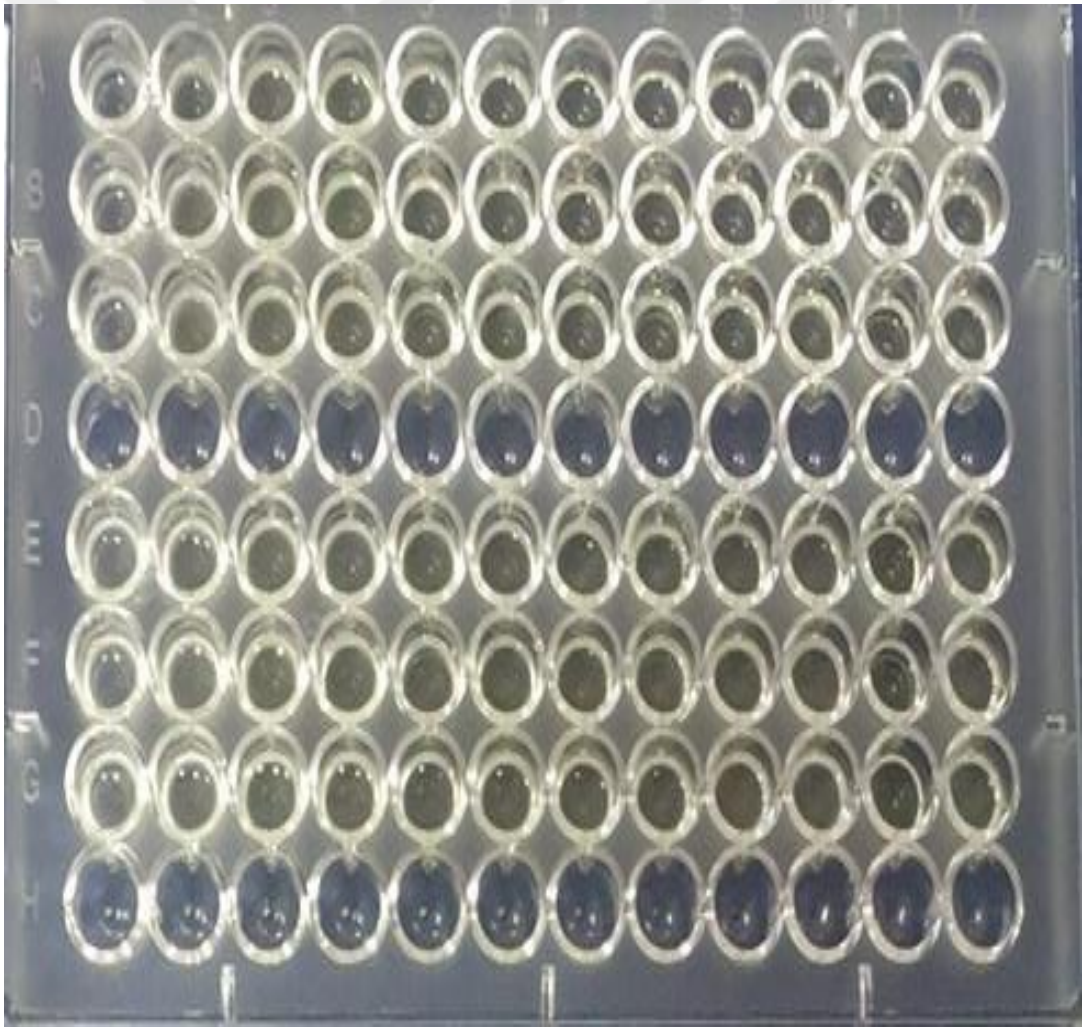
Graphic 4.15. MIC values of Oriental spruce



Graphic 4.16. MBC and MFC values of Oriental spruce

4.2.13. MIC Values of Kazdagı Fir

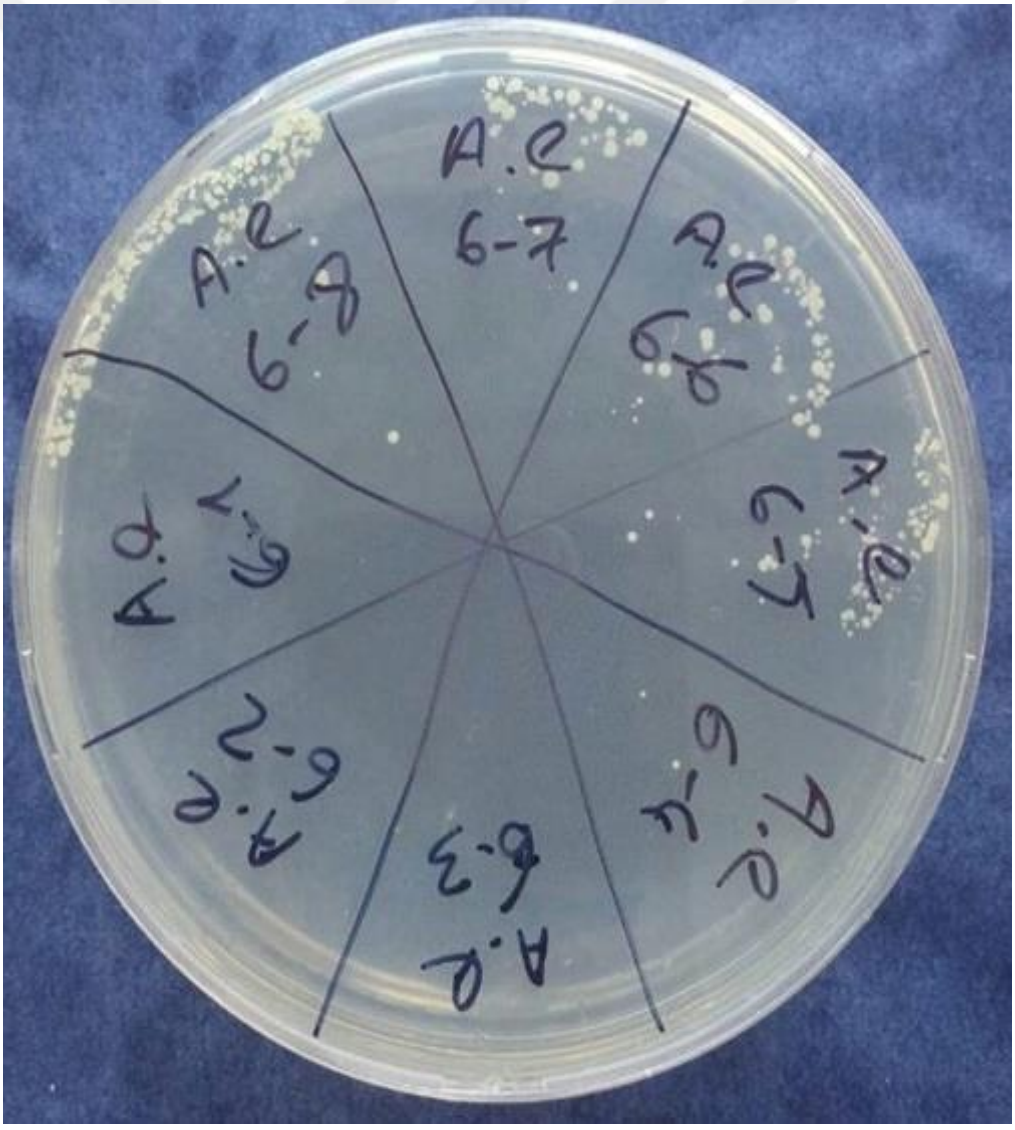
The essential oil extracted from *A. nordmanniana* subsp.*equi-trojani* (Kazdagı fir) yielded the following MIC values: *S. typhimurium*, 100 µg/mL; *L. innocula*, 50 µg/mL; *P. fluorescens*, 50 µg/mL; *B. subtilis*, 50 µg/mL; *E. aerogenes*, 25 µg/mL; *K. pneumonia*, 25 µg/mL; *S. enteritidis*, 12.5 µg/mL; *S. infantis*, 12.5 µg/mL; *S. kentucky*, 12.5 µg/mL; *P. aeruginosa*, 12.5 µg/mL; *E. durans*, 12.5 µg/mL; *E. coli*, 6.25 µg/mL; *E. faecium*, 3.125 µg/mL; *L. monocytogenes*, 0.781 µg/mL; *S. epidermidis*, 0.781 µg/mL; and *S. aureus*, 0.195 µg/mL). The essential oil exhibited no activity against *C. albicans* and *E. faecalis* (Photograph 4.11.), (Graphic 4.17.).



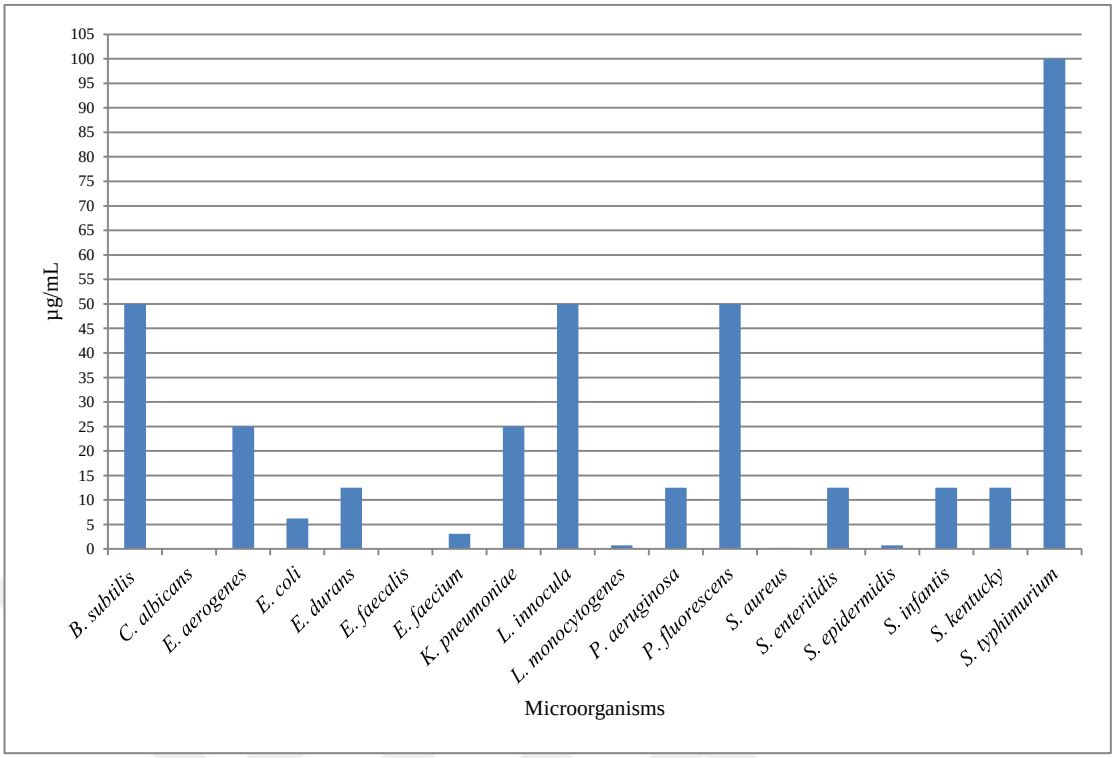
Photograph 4.11. MIC results of Kazdagı fir

4.2.14. MBC and MFC Values of Kazdagı Fir

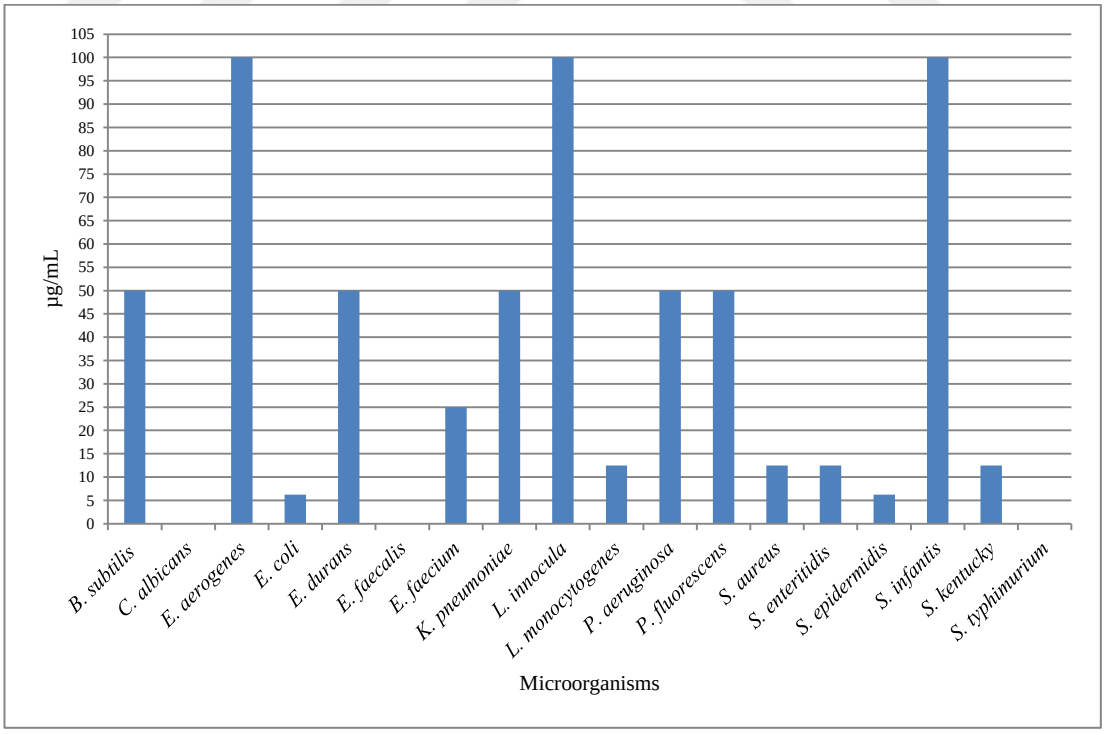
The MBC and MFC values of the essential oil extracted from *A. nordmanniana* subsp. *equi-trojani* (Kazdagı fir) are as follows: *E. aerogenes*, 100 µg/mL; *S. infantis*, 100 µg/mL; *L. innocula*, 100 µg/mL; *P. fluorescens*, 50 µg/mL; *K. pneumoniae*, 50 µg/mL; *B. subtilis*, 50 µg/mL; *P. aeruginosa*, 50 µg/mL; *E. durans*, 50 µg/mL; *E. faecium*, 25 µg/mL; *S. enteritidis*, 12.5 µg/mL; *S. aureus*, 12.5 µg/mL; *L. monocytogenes*, 12.5 µg/mL; *S. kentucky*, 12.5 µg/mL; *S. epidermidis*, 6.25 µg/mL; and *E. coli*, 6.25 µg/mL. The essential oil exhibited no antibacterial activity against *S. typhimurium* (Photograph 4.12.), (Graphic 4.18.).



Photograph 4.12. MBC and MFC results of Kazdagı fir



Graphic 4.17. MIC values of Kazdagı fir



Graphic 4.18. MBC and MFC values of Kazdagı fir

5.1. DISCUSSION

5.1. Regarding GC-MS Values

The seven main compounds according to the GC-MS analysis of the essential oils extracted from the plant species included in the present study are shown in Table 5.1. β-Pinene was the main compound in *P. brutia*, *P. nigra*, *P. sylvestris*, *C. libani*, and *A. nordmanniana* subsp. *equi-trojani*; δ-3-carene was the main compound in *P. nigra*, *P. brutia*, *P. sylvestris*, and *A. nordmanniana* subsp. *equi-trojani*; germacrene D was the main compound in *P. nigra*, *P. brutia*, and *P. sylvestris*; (-)-α-pinene was the main compound in *P. brutia* and *P. orientalis*; camphene was the main compound in *P. sylvestris* and *A. nordmanniana* subsp. *equi-trojani*; and caryophyllene was the main compound in *P. brutia*, *P. nigra*, and *P. sylvestris*. In contrast, the Pineceae taxa showed similarities in terms of the chemical compounds they contain, and cedar and fir showed similarities in terms of predominant compounds. Although spruce showed similarities with the other plant species with the two chemical compounds it contains, it is distinctive in terms of its predominant compounds.

Table 5.1. *Differences and similarities of plant species in terms of predominant compounds*

Takson Name	**Chemical components**					
	1	2	3	4	5	6
P. brutia	% 45.09 β-Pinene	% 16.94 Germacrene-D	% 15.93 α-Pinene,(-)-	% 5.48 Caryophyllene	% 2.17 Sylvestrene	% 2.09 δ-3-Carene
P. nigra	% 37.27 δ-3-Carene	% 20.39 Germacrene-D	% 19.37 β-Pinene	% 9.65 Caryophyllene	% 2.09 Sylvestrene	% 0.12 Tricyclene
P. sylvestris	% 39.85 δ-3-Carene	% 12.08 β-Pinene	% 10.15 Camphene	% 8.67 Germacrene-D	% 4.23 Caryophyllene	% 3.54 δ-Cadinene
C. libani	% 46.70 δ-3-Carene	% 16.57 β-Pinene	% 9.35 α-Bisabolol	% 3.93 β-Phellandrene	% 2.54 Myrcene	% 2.51 β-Himachalene
A. n. equi-trojani	% 31.70 β-Pinene	% 9.86 δ-3-Carene	% 9.10 β-Phellandrene	% 4.95 Juniper camphor	% 4.52 Camphene	% 2.23 α-Terpineol
P. orientalis	% 27.61 D-Limonene	% 18.14 (+)-2-Bornanone	% 12.44 Camphene	% 8.39 Bornylacetate	% 7.87 α-Pinene,(-)-	% 6.85 Camphene hydrate

The findings of the essential oils extracted from Taurus cedar, Scots pine, Black pine, Turkish pine, and Kazdagı fir are specified below. When the current literature on similar taxa is examined, the differences in the percentage of predominant chemical compounds are attributed to ecological conditions and seasonal variations. The fact that some compounds could not be obtained or that different compounds were obtained in the analysis may also be a result of differences in the GC-MS database.

Bilir and Avci (2013) analyzed Taurus cedar in their study. GC-MS analysis identified β-Pinene (29.5%), α-Pinene (21%), 1-hexane-3-yne (5.78%), and bicyclo[2.2.1]heptan-2-o1 (6.08%).

In their study, Venskutonis et al. (2000) investigated the essential oil extracted from Scots pine collected from different locations. Using GC-MS analysis, they identified α-pinene in the range of 18.5%–33%, carene in the range of 9.1%–24.6%, and germacrene D in the range of 9.1%-24.6%.

Judzentiene et al. (2006) analyzed the composition of essential oil extracted from Scots pine in Lithuania, and they identified carene in the range of 58.4%–72.4%, oxygen-containing monoterpenes in the range of 3.2%–5.9%, sesquiterpene hydrocarbons in the range of 14.5%–25.7%, oxygen-containing sesquiterpenes in the range of 4.7%–12.0%, and diterpenoids in the range of 0.4%-4.7%.

In their study, Tumen et al. (2010) investigated the composition of essential oil extracted from Pinaceae species in Turkey. They showed an emission rate of 62.8% for α-pinene in Scots pine and a rate of 47.1%-14.8% in Black pine. In addition, they found that limonene was the main constituent of Taurus cedar, with an emission rate of 22.7%. Furthermore, β-pinene was found to have an emission rate of 43.36% in Black pine, 39.6% in Turkish pine, and 32.67% in Oriental spruce.

5.2. Regarding Antimicrobial Values

In their study, Czerwińska and Szparaga (2015) analyzed the antimicrobial activity of the essential oil extracted from the leaves, flowers, and young sprouts of Scots pine against 12 different microorganisms using disc diffusion method, and they

found an inhibition zone of 12.08 mm for *S. aureus*, 12.08 mm for *L. monocytogenes*, and 8.22 mm for *E. coli*. Consistent with the findings of this research, the present study also demonstrated that the essential oils extracted from Scots pine taxon exhibited similar antimicrobial activity against microorganisms except *C. albicans*.

Another study by Dığrak et al. (1999) investigated antimicrobial activity of chloroform, acetone, and methanol extracts from different parts of various trees (Turkish pine, juniper, Taurus cedar, Taurus fir, and Black pine) against 15 microorganisms. Antifungal activity was not observed in all of the extracts. On the other hand, antibacterial activity was observed in most of the plant extracts, and chloroform and acetone extracts of the Taurus fir showed an inhibition zone of 16 mm and 18 mm against *E.coli*, respectively. In the present study, essential oils extracted from Kazdagı fir, Scots pine, and Oriental spruce exhibited antibacterial activity against *E.coli*.

Eryilmaz et al. (2016) analyzed the antimicrobial activity of essential oils extracted from several taxons of Pinaceae and Cupressaceae species collected in Turkey. They tested the essential oils extracted from *P. nigra* (Black pine), *P. brutia* (Turkish pine), *C. libani* (Taurus cedar), *A. nordmanniana* subsp. *equi-trojani* (Kazdagı fir), and *P. orientalis* (Oriental spruce) against seven microorganisms. Using the disc diffusion method, they identified an inhibition zone of 8 mm against *S. aureus* in Black pine and *P. aeruginosa* in Turkish pine. Taurus cedar showed no antimicrobial activity. It was also demonstrated that Oriental spruce exhibited inhibition zones of 7 mm, 7 mm, 7 mm, 8 mm, and 7 mm against *S. aureus*, *S. aureus*, *E. coli*, *K. pneumonia*, and *P. aeruginosa*, respectively. The essential oils extracted from the species analyzed showed no activity against *C. albicans*. The essential oils extracted from the taxa evaluated in the present study also showed no antifungal activity against *C. albicans*; therefore, the findings of the present study are in line with this study. In addition, the fact that Oriental spruce exhibited antibacterial activity against a greater number of microorganisms in the present study is also consistent with this study.

5. CONCLUSION AND SUGGESTIONS

In the present study, we investigated the antimicrobial activity of essential oils extracted from some of the Pinaceae species distributed in Turkey using hydrodistillation method and found that the essential oils extracted from the plants exhibited no antifungal activity against *C. albicans*. It was also shown that essential oils extracted from Black pine, Kazdagı fir, Scots pine, Oriental spruce, Turkish pine, and Taurus cedar showed antimicrobial activity at certain rates. On the other hand, Oriental spruce showed specifically higher antibacterial activity against bacteria compared with other species. In our study, we identified the composition of essential oils extracted from various species of Pinaceae using GC-MS analysis. The compound α-pinene was observed to have an emission rate of 15.93% and 7.87% in essential oils extracted from Turkish pine and Oriental spruce, respectively. It was also found that the compound β-pinene had an emission rate of 16.57%, 31.70%, 19.37%, and 12.08% in essential oils extracted from Taurus cedar, Kazdagı fir, Black pine, and Scots pine, respectively. The compound camphene had an emission rate of 4.52%, 12.44%, and 10.15% in essential oils extracted from Kazdagı fir, Oriental spruce, and Scots pine, respectively.

Extraction of essential oils using the hydrodistillation method from barks, sub-branches, leaves, and cones of the Pinaceae species that are produced as the by-products of wood processing has a great economic potential in Turkey. The factories collect and use these essential oil-containing parts to generate energy, thereby increasing carbon footprint and triggering global warming. It is important to make use of essential oils, which have found a place in the cleaning and health sector because of their antimicrobial effects, in perfumery for their fragrance, and herbal markets as therapeutic and supportive products. Although the present study only analyzed the leaves of five plant species and the branch of one species, previous studies have shown that other parts of the plant also show similar characteristics in terms of essential oil content. We believe that in countries where wood is produced from coniferous trees containing essential oils, utilization of wood residues by mobile distillation units will provide an efficient method to prevent economic loss.

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