FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

DETERMINING THE ESSENTIAL OIL COMPOSITION OF ENDEMIC ORIGANUM ACUTIDENS (HAND. - MAZZ.) IETSWAART AND ANTIMICROBIAL ACTIVITIES.

ADARİ EL ABDULGANİ

Master's Thesis

DEPARTMENT OF BIOLOGY

DECEMBER 2021

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Author:	ADARİ EL ABDULGANİ
Submission Date:	01 November 2021
Defense Date:	03 December 2021

THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled “DETERMINING THE ESSENTIAL OIL COMPOSITION OF ENDEMIC ORIGANUM ACUTIDENS (HAND. - MAZZ.) IETSWAART AND ANTIMICROBIAL ACTIVITIES.” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

03 December 2021

ADARİ EL ABDULGANİ

PREFACE

At the first I want to thank this great country Türkiye for every thing and Fırat University, because of the opportunity to study and achive this thesis.and also I would like to declare my sincere gratitude to my supervisior Prof. Dr. Eyüp BAĞCI, for the kind patiens and continuous support my study and research of thesis. His guidance helped me to all of the thesis research and writing steps during the study.

I woukl like to thanks to the PhD student Şule İNCİ and Prof. Dr. Sevda KIRBAĞ for the antimicrobial activity studies. And also I would like to thanks to my father and mother and my family supporting to me throughout my life.

ADARİ EL ABDULGANİ
ELAZIG, 2021

TABLE OF CONTENTS

Page

ABSTRACT

DETERMINING THE ESSENTIAL OIL COMPOSITION OF ENDEMIC ORIGANUM ACUTIDENS (HAND. - MAZZ.) IETSWAART AND ANTIMICROBIAL ACTIVITIES.

ADARİ EL ABDULGANİ

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences

Department of Biology

December 2021, Page: xi + 33

In this study the essential oil composition of the *Origanum acutidens* (Lamiaceae) plant, endemic species, and the antimicrobial activity were tested against the some selected bacteria and fungi species. The essential oil of the plant has obtained by using clevenger apparatus and the yield of the oil has determined as 1.5(v/w). The essential oil composition were analysed by using GC and GC – MS system quantittively and qualitatively. Carvacrol (32.11%), P – cymene (19.11%), Gamma terpinene (6.91%), Ethyl benzene (5.28%), Borneol (4.11%) components were found as major compounds in the *Origanum acutidens* essential oil. The antimicrobial activity of the essential oil of *Origanum acutidens* plant was tested against some selected bacteria *Staphylococcus aureus* ATCC25923, *Bacillus megaterium* DSM32; *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* DMS50071, and some fungi and dermatophyte fungi strains, *Candida albicans* FMC17, *Candida glabrata* ATCC66032, *Epidermophyton* sp. and *Trichophyton* sp. by using Disc Diffusion and MIC (Minimal Inhibition Concentration) methods. In the antimicrobial activity of the *Origanum acutidens* essential oil, the oil has shown strong activity on the *Staphylococcus aureus* and middle activities on the *Escherichia. coli, Bacillus megaterium* from bacteria and *Candida albicans* and also *Epidermophyton* sp. Fungi. The results showed that the volatile oil of *Origanum acutidens* will be used as antibacterial and may be antifungal agent.

Keywords: *Origanum acutidens*, Essential oil, Lamiaceae, Antimicrobial activity, carvacrol.

ÖZET

ENDEMİK ORİGANUM ACUTİDENS (HAND. MAZZ.)-IETSWAART'İN UÇUCU YAĞ KOMPOZİSYONUNUN VE ANTİMİKROBİYAL AKTİVİTESİNİN BELİRLENMESİ

ADARİ EL ABDULGANİ

Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ
Fen Bilimleri Enstitüsü

Biyoloji Anabilim Dalı

Aralık 2021, Sayfa: xi + 33

Bu çalışmada, endemik olan *Origanum acutidens* (Lamiaceae) bitkisinin uçucu yağ kompozisyonu ve bu yağın bazı seçilmiş bakteri ve mantarlar üzerindeki antimikrobiayl aktivitesi test edilmiştir. Bitkinin Uçucu yağı Clevenger aparatı ile elde edilmiş ve yağ verimi 1.5 (v/W) olarak saptanmıştır. Bitkinin uçucu yağ kompozisyonu GC ve GC – MS systemi kullanılarak kalitatif ve kantitatif olarak analiz edilmiştir. Karvakrol (%32.11), P – simen (%19.11), Gamma terpinen (%6.91), Ethyl benzene (%5.28), ve Borneol (%4.11) bileşenleri *Origanum acutidens* uçucu yağında major bileşenler olarak bulunmuştur. *Origanum acutidens* bitkisi uçucu yağının antimicroviyal aktivitesi bazı seçilmiş bakteri *Staphylococcus aureus* ATCC25923, *Bacillus megaterium* DSM32; *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Pseudomonas aeruginosa* DMS50071, ve bazı mantar ve dermatofit mantar, *Candida albicans* FMC17, *Candida glabrata* ATCC66032, *Epidermophyton* sp. ve *Trichophyton* sp. türleri üzerinde Disc Diffuzyon ve MIC (Minimal Inhibibisyon konsantrasyonu) metotları kullanılarak test edilmiştir. *Origanum acutidens* uçucu yağı *Staphylococcus aureus* üzerinde yüksek aktivite göstermiş ve *Escherichia. coli, Bacillus megaterium* bakteri türleri ve *Candida albicans* ve *Epidermophyton* sp. mantar türleri üzerinde de orta derecede etkili olmuştur. Sonuçlar *O. acutidens* uçucu yağının Anbakteriyal ve antifungal ajan olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: *Origanum acutidens*, Lamiaceae, Uçucu yağ, Antimikrobiyal aktivite, karvakrol.

LIST OF FIGURES

Page

LIST OF TABLES

Page

1. INTRODUCTION

The evolution of susceptibility and resistance to currently used antimicrobial compounds and their activity and effect mechanism is not a surprising at the moment and not a a new phenomenon; however, infections especially pandemism living in these year are becoming more common unfortunately. It is getting more serious, and more easily transmitted. Many infectious illnesses are now incurable and uncontrolled, and in the future years, they will become untreatable and uncontrollable. The world has need new antimicrobial agents and natural resources to protect the mankind from the different kind illness and microbial infections. Aromatic plants have been known and used from the ancient time and widely used as virucides, fungicides, bactericides and pesticides. Their features has attributed from the consist of volatile oils within the different parts of the plants. These volatile chemicals has a wide chemical diversity, and for these rich diversity it has contribute multidrug processes and resistance infections and alternative solutions Recently, ethnobotanical-based in vitro scanning methods have been extremely effective in finding novel active chemicals and confirming traditional medicinal plant use (Alvino and Alvino, 2009).

A lot of medicinal ad aromatic plants has grown in the fields, home gardens and in plantation landscape in Turkey. 900 plant species has cultivated all over the world and the cultivated plants species and harvested amount is restricted (Arslan et al., 2015). Nowadays the cultivation planning on the aromatic and medicinal plant has improved, the cultivar forms of these plants and the cultivation landscapes has increased day after day. 20 kinds medicinal and aromatic plants has cultivated in 1,3 million da field and red pepper, black tea, cumin, poppy, anise, mint, oregano and oil rose were the first order as production amount. The export ratio of the essential oils are varying according to the years, it is about 33.6 million dollar in 2015, from the 17 species (Temel, et al., .2018).

Turkey is an fatherland of a lot of plants due to the ecological conditions and among the 3 phytogeographic region, Irano – Turan, Mediterrennean and European Siberia. It has more than eleven million plant species and about 3500 plant is endemics to Turkey. The new species number has increased day by day and species nova is published at the moment. Medicinal and aromatic plants are one of the major and important groups in this plant. *Origanum* or kekik with the local name is the significant plant group in the medicinal and aromatic plants in Turkey and in the world, it has also the great proportion in the exported plant group.

Turkey is an important country in view of genetic origin of the Lamiaceae family, and it has represented with the 45 genera and 731 taxa belongs to this family. The endemism ratio in Turkey is 44.2% and it is third of the richest family (Başer 1993; Kocabaş et al., 2001). *Origanum* L. genus belongs to Lamiaceae family is represented by about 22 spp.and totally 32 taxa in Turkey. 21 taxa of them are endemic in Turkish flora. *Origanum* species are known as "kekik, mercanköşk,

merzengüş″. It has been shown antioxidant, antiinflammatory, antifungal, antibacterial, antidiabetic, gastroprotective, hepatoprotective, and insecticidal effect on *Origanum* species (Karaoğlan, 2011). Several members of the Lamiaceae family are eaten as food or condiments and are heavily utilized in traditional medicine to treat a variety of ailments, including microbial infections and folk medicine.

Origanum is an important export products of Turkey. The 90 percent of the origanum export is mostly supply from the *Origanum* species. Among these the most collected and traded and exported species from the nature was *O. onites* (İzmir kekiği). The other exported *Origanum* members were reported as *O. minutiflorum, O. syriacum* var. *bevanii, O. majorana, O. vulgare* subsp. *hirtum* and *O. acutidens* (Karaoğlan, 2011).

Certain plants, particularly those belonging to the Lamiaceae family, such as *Origanum, Stachys, Salvia, Melissa* taxa and others, are harvested from the wild or grown commercially (e.g. oreganos) in various parts of the world and used to make a variety of commercial and pharmaceutical products.

1.1. Essential oils

Essential oils are volatile, natural, complex chemicals produced by various plant species, particularly aromatic plants. They are typically extracted using steam or hydro-.distillation. They were modified and named esans by Arabs in the Middle Ages. They are well-known for their antiseptic qualities, which include bactericidal, virucidal, and fungicidal capabilities, as well as for their therapeutic benefits. The fragrances of the essential oils are used in embalmment of the significant persons in the ancient time, food preservation and antibacterial agents with their different spesification via topical applications, such as analgesic, sedative, anti-inflammatory, and anesthesic treatments. It is important that to detect the characteristics of the essential oil to observe the activities on the bacteria and yeasts and so the characteristics of the essential oils have not changed at the antimicrobial activity mechanism till nowadays (Bakkali et al., 2008).

Plant essential oils has a great roles in the plant chemicals. This essential oils found between the cells, does different duties such as the transportation of the information, stabilizer and have protective effect against the external factor. Significant hormones in the plants takes places within the essential oils. On the other hand the volatile oils has been used for different purposes for a long time. The major usage area are the aromatherapy and phytoterapy. Essential oils serve a variety of critical functions in the preservation of plants and flowers against different insects and harmful orgaisms and pollination of the plants in the nature. They do important works as antibacterials, antifungals, herbicides and antivirals. They also have different duties on the plant kigdom, like attracting of some insects, favouring the distribution of pollens and seeds, or repelling to undesireable organisms and the others.

Essential oils are liquid, volatile, and seldom colored. They are soluble in organic solvents and have a lower density in general. They are derived from a variety of fragrant plants that are usually found in temperate to tropical climates (tropical and Mediterrenean). Essential oils may be extracted and generated by any plant organ. Secretory cells, cavities, epidermal cells, and glandular trichomes secrete and store them. (Bakkali et. al., 2008). They are also extremely complicated natural mixes that may include 20–60 or more components. For example, *Origanum compactum* essential oil has 27% thymol and 30% carvacrol, whereas *Coriandrum sativum* essential oil contains 68% linalol. The majority of essential oils are made up of terpenes and terpenoids, with aromatic and aliphatic components having a low molecular weight (Bakkali, 2008).

There are more groups within the essential oil complex. Terpenes are derived from structurally and functionally distinct groups of compounds. They are manufactured from a mixture of materials known as isoprene. Isoprene is made up of a number of 5-carbon-base (C5) units that are bonded together. One of the main group in the essential oils was terpene and when it is contained oxygen is called as terpenoid. The monoterpenes are the most representative molecules comprised ninety percent of the essential oils and they have a great variation in structures. They make several functions in the different organs of the plants. The sesquiterpenes are a second significant category of essential oils that are produced from three isoprene units (C15) in the essential oil mixture. They are the second most abundant type of essential oils. The function and structure of the sesquiterpenes 15 carbon sceleted are similar to those the monoterpenes:

1.2. The Significance of the Essential oils

Essential oils is a major chemical group found in the medicinal and aromatic plants families. The term of the medicinal and aromatic plant is widely accepted and used for the plant groups, used the prevention of the illness, to live more healthy and to cure the diseases and particularly as drug. Essential oils have been widely used in nature for their many characteristics, such as antifungal, antibacterial, and insecticidal capabilities. Approximately 3000 essential oils are known today, with 300 of them being economically significant. The pharmaceutical, sanitation, agronomy, food, cosmetic, and perfume sectors all benefit from it. The essential oils or some of its components are utilized in fragrances and cosmetics, agriculture, sanitary goods, dentistry, and as food preservers and additives. Creams, soaps, perfumes, flavor additions for food, scents for home cleaning goods, and industrial solvents all include d-limonene, geranyl acetate, or d-carvone (Silva et al., 2003; Bakkali et al., 2008).

It is reported that the extraction process and products of the essential oils may be varied in quality, quantity and in composition according to different ecological conditions, like the climate, soil contents, plant part and ages (Masotti et al., 2003; Angioni et al., 2006). It has more significance to take the oil from the same organ of the plant and grows on the same soil. The same climate

conditions for the picking and harvesting time (Bakkali et al., 2008). Oregano plant is one of the most significant commercial crops in different countries. It is cultivated all over the worlds at different ratios, and it generating export markets at a high prices. In turkey the cultivation amount of the *Origanum* and some aromatic plants has a great attention and importance.

The essential oils are used for different purposes like in massages as mixtures with vegetal oil or in baths. It is a good element of the aromatherapy frequently used. There is now a surge of interest in novel, particularly natural, sources of fragrances, tastes, and biological activity goods. These are critical characteristics for a wide range of applications in the food, pharmaceutical, and fragrance sectors (Gaspar and Leeke, 2004; Juliani et al., 2008).

The essential oils are the ecologically important plant products and chemical mixture. they have also may different effects on the nature (Isman, 2000). There are different ways to control the insect attack and invasion. The chemical, physical and biological control or different combinations of these methods are generally used as common methods for controlling the insect activities (Thomas, 2001). From the Coleoptera: Bruchidae, the Bruchid beetles attacks the seeds of legume and cause serious damages, anf these also has effected the quality and the crops amount (Tozlu et al., 2011).

Essential oils, which are mostly composed of bioactive monoterpenes, may have a variety of impacts, such as attraction or repellence for pollination or other reasons. In certain instances, essential oils have been found to have insecticidal properties (Akhtar and Isman, 2004). Essential oils may also be used as insect repellents against certain bug species (Traboulsi et al., 2005). The vapors and pure elements of essential oils or their components may also be employed as preparatory agents and have effects on the larvae and adults of certain insect groups (Park et al., 2003; Kordali et al., 2006).

Foreign trade and commercial potential of the essential oils is very high and it is day by day increased and takes places significantly in the general trade of Turkey and some other coutries. Those are the red pepper, coffee, tea, cocoa and the other extracts obtained from different kind of plants, and these are on the top of the list (Temel et al., 2018).

According to the WHO data's twenty thousand plant has been used as medicinal purposes. There are big plant drug trade center all over the world, USA, India, China, Germany, France, Italy, Spain, Japan, Hong Kong and England (Faydaoğlu and Sürücüoğlu, 2011).

1.3. Biological Activities of Essential Oils

The essential oils has different features, like volatility, hidrofobic and a special smell affecting the respiration system. These last properties showed that they might be active biologically. The most reported features was to be antimicrobials against the some bacteria and fungi. For these tests, Agar diffusion and Broth dilution techniques generally. Lately except of these techniques, the

one of the other method to observe the inhibiton zones of the essential oils was the disc diffusion techniques widely used (Nccls, 1993). Food spoilages or corruption caused from the microorganisms are common and affects the foods and causes more damages and losing of the quality and quantity in our country, even in the developed countries. In recent years, the use of the volatile oils as beverage, pharmaceutical, functional ingredients, cosmetic, agricultural works, additive for the foods and sanitary has been takes more momentum. These because of the increasing concern to the natuıal products and sources and highly interest to escape harmful synthetic products and additivies (Beyaz, 2014).

Since the Middle Ages, essential oils have been extensively utilized in the pharmaceutical, sanitary, cosmetic, agricultural, and food sectors for their antibacterial, virucidal, fungicidal, insecticidal, therapeutic, and cosmetic properties. From the essential oil compounds. It is known that the thymol and carvacrol are isomeric alcohol class and cinnamaldehyte are the phenyl propanoid class in the essential oils. Both group of the chemical has more antimicrobial activity against *E. coli* O157 and *Salmonella thyphimurium.* While the carvacrol and thymol shred the bacteria membranes, it supply to go out the membrane materials. Thus terpenoids and phenylpropanoids because of the lypophilic properties they pierced the bacteria wall and they delivered the inner parts of the cell.

Pharmaceutical and culinary applications are increasingly prevalent as alternatives to synthetic chemical goods due to their bactericidal and fungicidal characteristics. Although essential oils are cytotoxic to live cells, they are often non-genotoxic (Bakkali et al., 2008).

Cytotoxicity of the essential oils seems to include membrane damages. Essential oils have not specific targets in the cells. Because the oils has more variation in quantity and quality in the composition (Carson et al., 2002; Bakkali et al. 2008). The essential oils has shown cytotoxic effects on some microorganism. The essential oils has inhibited the growth of the pathogenic gram positive and gram negative bacteria. These activities were tested by using the agar diffusion or the dilution methods by using liquid broth or agar cultures (Williams et al., 1998; Kalemba and Kunicka, 2003; Hong et al., 2004; Si et al., 2006).

1.4. *Origanum* L. (Lamiaceae) Genus

Origanum is suffruticose or herbaceous perennials plants and it is represented with different sections like *Amaracus, Anatolicon, Origanum Chilocalyx* as systematically within the *Origanum* genus in Flora of Turkey (Davis, 1982). *Origanum* (oregano) is a Lamiaceae herbaceous perennial and subshrub genus. They are found in open or mountainous areas of Europe, North Africa, and temperate Asia. Several species have naturalized in isolated areas of North America and elsewhere. *Origanum* is a large Lamiaceae genus with around 900 species worldwide. Twenty *Origanum* species are found in the Flora of Turkey (Davis, 1982, Baytop, 1999). Members of the *Origanum*

have historically been used in place of thyme as a spicy culinary ingredient. (Baytop, 1999, Esen et al., 2007). *Origanum* members are known in Anatolia as “Kekik”, and “Keklik otu”.

Historically, *Origanum* species have been employed as sedatives, sweaters, diuretics, degasifiers, and antiseptics, as well as in the treatment of gastrointestinal disorders and constipation (Baytop, 1999). Numerous articles have been published on the chemical composition and biological activity of members of the *Origanum* genus (Daouk et al., 1995, Yildirim et al., 2005, Soylu et al., 2007). *Origanum acutidens* is a rare endemic to northeastern Turkey. This species' essential oil content has been previously described from a variety of locations (Baser et al., 1997, Figueredo et al., 2006). However, there are no further studies on its biological characteristics that have documented the essential oil's and extract's antibacterial and antioxidant capabilities, with the exception of a few (Sokmen et al., 2004).

While *Origanum* is a popular spice, it is also a highly significant taxon in herbal medicine due to its many pharmacological properties. *Origanum* essential oils include phenolic monoterpenoids such as carvacrol and thymol. They demonstrated strong antimicrobial features and activities. Işcan et al., (2020), studied the essential oil of *Origaum boissieri* Ietswaart an endemic species were hydrodistilled and analysed by GC/MS techniques. Thirty compounds were identified. Carvacrol (39%) and *p*-cymene (32% of the essential oil) were identified as the primary components. The methanolic extract and essential oil of both blooming aerial portions of the plant, as well as carvacrol and p-cymene, were tested using the microdilution broth assay for antibacterial and anticandidal activity. The oil has a significant inhibitory effect on *Bacillus subtilis*, *Bacillus cereus, Staphylococcus aureus, Staphylococcus epiderrnidis,* and *Listeria inortocytogenes. O. boissieri* extract and oil had a mild impact on all *Candida* strains tested, with MIC values ranging from 125 to 1000 mg/mL.

1.5. *Origanum acutidens* (Hand.-Mazz.) Ietswaart

Origanum acutidens is subshrub plants to 50 cm., It is distributed in Eastern Anatolian region naturally. It is an Iranian-Turkish endemic species from northeastern Turkey. Related to *O. haussknechtii*, from which it differs by its larger, usually yellowish – green bracts and white or pinkish corolla (Davis, 1982). The essential oil composition of *O. acutidens* from various areas has been investigated. Baser et al., (1997) and Figueredo et al., (2006). Biological characteristics, antioxidant and antibacterial activity of essential oils and compounds have not been studied (Sokmen et al., 2004). **T**he essential oil and various chemicals obtained from *Origanum acutidens* and its methanolic extracts (MeOH) were investigated from callus cultures of the plant. Antimicrobial, antioxidative and antiviral activities of the essential oils and extracts were tested. The essential oil *O. acutidens* showed strong antimicrobial activity at the high ratio against 22 of 35 bacteria and 12 of 18 fungi and a yeast investigated (Sökmen et al., 2004).

1.6. *Origanum* Essential Oils And Antimicrobial Activity Studies

Origanum genus is an significant genus in Lamiaceae family, because of to be medicinal and aromatic plants and the usage for this purposes all over the world. There are more studies on the plant essential oils and their antimicrobial and antifungal activities on different microorganisms. *Origanum* genus from the Lamiaceae family comprises approximately 900 sp., distributed in the world, from these 22 species, totally 32 taxa are grown in Turkey naturally (Davis 1982, Baser 2002). It has also great importance because of the economically product. It is used as spicy additive for food and herbal teas and herb used in the kitchen for the aroma and spicy for flavouring the food products and alcoholic drinks (Kizil et al. 2009).

The essential oil of *Origanum minutiflorum* O. Schwarz et P. H. Davis was studied for its chemical composition and antibacterial and antioxidant properties. The essential oil of *O. minutiflorum* (Lamiaceae) was investigated by Göze et al. (2006) using GC-MS. Leaves were composed of carvacrol 44.96 % while the oil of the flowers was composed of 34.04 % carvacrol. The volatile oils in leaves and flowers has showed antimicrobial activity against to the whole bacteria except *Pseudomonas aeruginosa*. Antioxidant activity was analyzed systems of β-carotene/linoleic acid and DPPH. IC50 values of the essential oils were found 110μg/ml and 105μg/ml respectively. Inhibition percentage of *Origanum minutiflorum* essential oil was found as 66% and 71% respectively.

The essential oils of *Origanum vulgare* ssp. hirtum, *Origanum dictamnus*, and a commercial *Origanum* were analyzed using GC-MS. Carvacrol, thymol, terpinene, and p-cymene make up a large percentage of the oils (73.7, 92.8, and 87.78%, in respective order). Three essential oils also showed antibacterial activity against eight distinct Gram-positive and Gram-negative bacteria strains. Carvacrol and thymol were shown to be the most antibacterial components of these oils, whereas γ- terpinene and p-cymene were inert. *O. vulgare* ssp. *hirtum* essential oil was highly bactericidal at 1/4000 dilution and even at 1/50000 dilution. It also showed cytotoxicity against four immortal animal cell lines, two of which were human malignancies (Sivropoulou et al., 1996).

It is known that the *Origanum* essential oils has different activities on various organism. In the study of the effect of *O. heracleoticum* essential oil and thymol / carvacrol components on the channel catfish (*Ictalurus punctatus*), the growth performance and antioxidant capacity of these *Origanum* feeded fishes has increased (Zheng et al., 2009). There are more studies on the effect of *Origanum* taxa essential oil on fishes. It is reported that the *Origanum onites* essential oil mixtured feed ration has increased the gowth performance of the rainbow traut (*Oncorhynchus mykiss* Walbaum) However, the end of the 8 week applications with the *Lactococcus garvieae* experimental enfections, it is determined that the death rate has decreased (Diler et al., 2016).

GC – MS analysis of the *Origanum minutiflorum* revealed that the major compounds were determined in different ratios as carvacrol (67-84%), p cymene (5.36-8.77%) and gamma terpinene

(0.99 – 3.46%). The major components of the *O. onites* essential oils also has determined that carvacrol (30-71%), p-cymene (3.9-10%) and gamma terpinene (2.25 – 5.07%) in the same study. In the essential oil composition of *O. vulgare* from Alanya (Antalya) region has reported that the oil has contained 81.35 % carvacrol, 10.17 % gamma terpinene and 1.27% p-cymene were determined as major compounds in the oil. Antioxidant activities of these volatile oils were tested for free radical scavenging activity and the antioxidant capacity of the *O. vulgare* has high values in the study of Tekin, 2013.

Asensio et al. (2015) investigated oregano (*Origanum vulgare*) essential oil's antibacterial efficacy against a variety of food-borne pathogens. On the other hand, 4 *Origanum* essential oils were shown to have biological activity. In the essential oils it has determined that *trans*-sabinene hydrate, terpinen - 4 - ol, γ-terpinene, thymol as major components.

The aroma characteristic of the essential oil is important for the oil and other plant extracts. Essential oil smells were classified according to various definitions, including herbaceous, minty, medicinal, resinous, and coniferous overtones, amongst other things. The essential oils from central Argentina were found to have a greater acceptance rate in fragrance tests. Antioxidant profiles revealed that oregano varieties from southern regions were more active than oregano varieties from central Argentina. Anti-yeast and roundworm lethality tests also revealed a similar outcome. The increased biological activity was shown to be linked with decreased scent and increased color acceptability. According to the same research, oils from central Argentina with superior fragrance profiles may be utilized in food flavoring and non-food applications. whereas oils from southern regions have a greater level of bioactivity. This lead to the using these oils as new antioxidants sources for pharmaceutical and food preservation. These aroma and colour properties of the essential oils has an importance in the characterization of the oil and its physical and chemical properties just in the *Origanum* and the other aromatic plants.

Fidan et al., (2020) has studied the *Origanum acutidens* (Hand.-Mazz.) Ietsw. (zemul), and it was extracted using with two different solvents (Water and 80 % ethanol). Some chemical and activities were analysed and compared. The water extract inhibited activity by 77.53%, whereas the ethanol extract inhibited activity by 90.69%. The total phenolic content was likewise found to be 86.48 mg/mL in the water extract and 142.78 mg/mL in the ethanol extract. In both extract, 15 different phenolic compounds and 32 elements were detected in *O. acutidens*. The high amount of the total phenolic compounds of the *Origanum acutidens* plants may be originated or supplied its antioxidant capacities.

Kordali et al. (2008) revealed that the essential oil of *Origanum acutidens* had high levels of carvacrol, p-cymene, linalool acetate, and borneol, all of which were shown to be significant components. The aromatic monoterpenes carvacrol, p-cymene, and thymol, in the essential oil of *O. acutidens*, were identified as the primary constituents. The antifungal activity of *O. acutidens*

oil and its components carvacrol and thymol was evaluated in antifungal tests, where they fully inhibited the mycelial development of 17 phytopathogenic fungus. The antifungal activity of p-cymene was decreased. Carvacrol and thymol in *O. acutidens* essential oil totally prevented seed germination and seedling development in plants. It is also phytotoxic to some plants. These results are important for the usage of this oil and its components as herbicides as well as insecticides. The reduced antifungal activity p-cymene has no phytotoxic impact. Moreover, *O. acutidens* oil killed *Sitophilus granarius* and *Tribolium confusum* with 68.3% and 36.7% mortality, respectively. In the Başer et al., (1997) study on the water distilled essential oil of *Origanum acutidens*, the oil was analysed by GC, GC- MS system. 46 compounds has determined in the oil. In the oil carvacrol and p- cymene were found as major compounds (Başer et al., 1997).

Essential oils may be an effective weapon in the fight against bacterial resistance. Numerous research has shown that essential oils have antiviral, antioxidant, antiseptic, antibacterial, antiparasitic, antifungal, and insecticidal properties (Benjilali & Ayadi, 1986; Burt, 2004; Kaloustian et al., 2008; Stefanakis, 2013).

The eastern Anatolian area is significant for its indigenous plant species and their chemical composition. As a result, it is critical to study the chemical composition and biological activity of antimicrobial agents against microorganisms and fungi. It may provide some insight into the many applications of these plant compounds.

Origanum acutidens is a locally endangered plant species found only in Turkey's south-eastern and eastern Anatolian regions (Bakis et al., 2011). Its essential oil includes large amounts of carvacrol and *p*-cymene, both of which show strong antibacterial action against bacteria (Baser et al., 1997; Figueredo et al., 2006; Cosge et al. 2009). Numerous researchers have conducted extensive research on the antibacterial effects of essential oils and their components. Several essential oil components have been studied extensively, but not all compounds or their methods of action (Chouan, 2017).

Due to their low toxicity, pharmacological activity, and commercial feasibility, therapeutic characteristics of plants have garnered considerable attention (Auddy et al., 2003). These research have concentrated on the advantages of phytochemicals derived from plants and their impact on human health. Compounds, groupings of compounds, or essential oils are all examples of natural plant-derived additives. Antimicrobial activity of an essential oil may be dependent on just one or two of the oil's main components. According to mounting research, the ratio of active components in essential oils may not be the sole factor determining their intrinsic activity; interactions between these and minor compounds in the oils may play a role. Thus, it is critical to identify the major constituents of essential oils as well as their overall content (Chouan et al., 2017).

This research sought to characterize *Origanum acutidens* (Hand Mazz.) Ietswaart essential oil's cytotoxic and radical scavenging capabilities. The essential oil was extracted by

hydrodistillation and analyzed by gas chromatography/mass spectrometry (GC-MS). The cytotoxic impact of the essential oil was determined using the xCELLigence Real Time Cell Analyzer equipment on human colorectal adenocarcinoma (HT-29) and human cervical adenocarcinoma (HeLa) cell lines. Additionally, the oil's radical scavenging ability was evaluated using the DPPH technique. Carvacrol (61.69 percent), p-cymene (17.32 percent), and borneol were identified as the major components of the oil (3.96 percent). At the doses examined, *O. acutidens* essential oil showed a substantial cytotoxic impact. In comparison to butylated hydroxytoluene, the essential oil showed a modest ability to scavenge DPPH radicals (BHT). The results clearly showed that the oil had an inhibiting effect on cancer. The observed high impact may be a consequence of the oil's carvacrol component (Altuntaş and Demirtaş, 2017).

1.7. The Aim of the study

The chemical composition and antibacterial and antifungal properties of *Origanum acutidens* essential oil were investigated utilizing disc diffusion and MIC (Minimal Inhibition Concentration) techniques. The essential oil composition of the *Origanum acutidens* collected from the natural habitats were analysed by GC and GC – MS system. The qualitative and quantitative composition of the oil were also determined. In addition to the chemical analysis, the antimicrobial activity of the *O. acutidens* essential oil were dtermined against the some bacteria and some fungi by using different techniques. The comparison of the essential oil compositon of *Origanum acutidens* from eastern Anatolian region with the other region samples and infrageneric comparison in *Origanum* genus were investigated and evaluated. Determınatıon of qualıtatıve and quantıtatıve contents of the *Origanum acutidens* essentıal oıl and their antibacterial and antifungal actıvıties by usıng dıfferent technıques will give some clues on the usability of the essential oil of this plant at different purposes. Comparıson of the essentıal oıl composıtıon of the *Origanum acutidens* wıth the other patterns from dıfferent regıon will be useful to determıne the chemotypes of the plant. The study is important in view of the determining of the essential oil variation of endemic *Origanum acutidens* species from Eastern Anatolian region, in view of essential oil composition and its antimicrobial and antifungal activities among the other locality patterns. It will be helpful to determine chemical and antimicrobial sensitivity variation by comparing control antibiotic groups and the antimicrobial range of the essential oil commercially.

2. MATERIAL AND METHODS

2.1. Plant Material

O*riganum acutidens* samples were collected from the natural habitats in 2020 years,in June months from the Topalan – Çiriş road, Bingol – Elazığ highway 7. Km. (Figure 2.1). Dr. Azize Demirbağ (From Bingöl University) has collected and identified the plant samples from the natural habitat and stored in the Fırat University Herbarium (FUH).

Figure 2.1. The picture of the *Origanum acutidens* in nature

2.2. Essential oil Analysis

2.2.1. Isolation Of The Essential Oil

Origanum acutidens aerial pieces (Figure 2.2) were hydrodistilled for 3 hours in a Clevenger type equipment. The oil was yellow and had a 1.5 (v/w) essential oil yield. The oil was extracted and analyzed at the Plant Products and Biotechnology Research Laboratory at Fırat University (Figure 2.3).

Fig. 2.2 Has shown that the harvested samples of the Origanum acutidens samples collected from the natural habitats.

Figure 2.2: *Origanum acutidens* hervested samples.

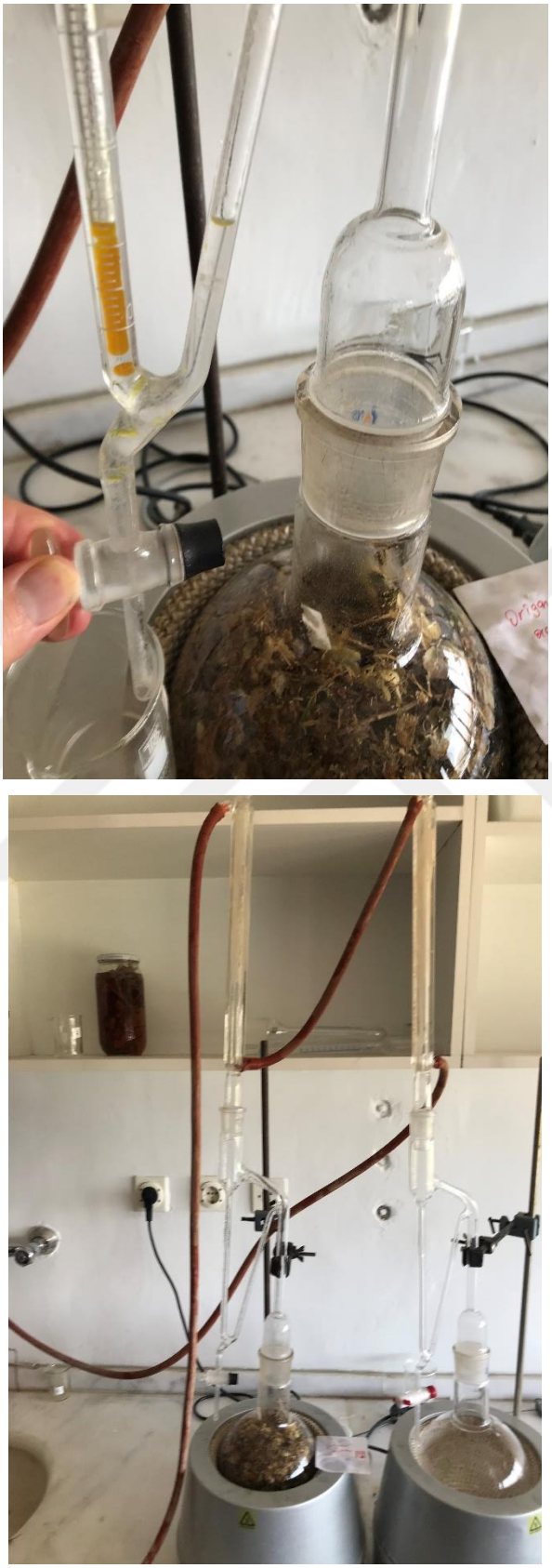

Figure 2.3: Clevenger aparatus for hydrodistillation.

2.2.2. Chemical Analysis

2.2.2.1 Gas Chromatographic (GC.) Analysis

The essential oil was evaluated using an HP 6890 gas chromatograph fitted with a FID detector and an HP- 5 MS capillary column. Column and analytical conditions were identical to those used in GC-MS. The essential oil percentage composition was determined using the GC-FID peak regions. The settings for the GC analysis are identical to those used in previous investigations conducted in this laboratory (Fig. 2.4). As there are more studies on the essential oil analysis in this laboratuary (Kilic & Bagci, 2011), the study conditions has given as the same with the before studies. The Gas Chromatography result as chromatogram is shown in Fig. 3.1.

2.2.2.2 Gas Chromatography-Mass Spectrometry (GC-MS)

The oils were evaluated with the use of a GC-FID-MS analysis system equipped with an HP-Agilent 5973 N GC-MS system and a 6890 GC. In this experiment, helium was used as the carrier gas, and an HP-5 MS column (length 30m, diameter 0.25mm, film tickness 0.25m) was used. When the injector reached 250°C, the split flow rate was adjusted to one milliliter per minute. Following 2 minutes of maintaining 70°C, the temperature of the GC oven was raised to 150°C and then kept constant at 150°C for 15 minutes before being increased to 240°C. At 70 eV and a mass range of 35425, mass spectra were acquired. The components of essential oils were identified by comparing their retention indices (RI), retention times (RT), and mass spectra to authentic WILEY and NIST libraries as well as the literature. The GC – MS analysis is shown in Fig. 2.4.

Figure 2.4. Gas Chromatography (GC – MS) studies

2.3. Antimicrobial activity tests

The antimicrobial activity of the *Origanum acutidens* essential oil were tested on the some selected bacteria and fungi species. For those purpose Disc diffusion method and MIC (Minimal Inhibition Concentration) Methods were used.

2.3.1. Bacteria cultures

2.3.1.1 Test Microorganisms

In this study; five bacteria, two yeasts and fungi species were used as test organism. From the bacteria, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Pseudomonas aeruginosa* DMS50071; From the yeast, *Candida albicans* FMC17, *Candida glabrata* ATCC66032; and from the dermatophyte fungi; *Epidermophyton* sp. and *Trichophyton* sp. were used for the antimicrobial activity tests. The antimicrobial activity studies has performed in the same Faculty, Biology Department, Microbiology Research laboratuary.

2.3.2. Disc Diffusion Assay

2.3.2.1 Preparation Of Microorganism Cultures And Testing Antimicrobial Effect

Disc diffusion was used to test the antibacterial activity of *O. acutidens* essential oil extracts (Collins and Lyne, 1987). Bacterial strains (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, and *Pseudomonas aeruginosa* DMS50071) were seeded into Nutrient Buyyon (Difco) and cultured for 24 hours at 35°C.

Yeast strains (*Candida albicans* FMC17 and *Candida glabrata* ATCC66032) were inoculated in Malt Extract Buyyon (Difco), while dermatophyte fungi (*Trichophyton* sp. and *Epidermophyton* sp.) were inoculated in Glucose Sabouroud Buyyon (Difco) and incubated for 48 hours at 25°C. At a rate of one percent (10^6 bacteria ml, 10^4 yeast ml, 10^4 fungi ml)., a culture of prepared bacteria, yeast, and fungus in broth was injected into Müeller Hinton Agar, Sabouraud Dextrose Agar, and Potato Dextrose Agar. After vigorous shaking, 25 ml was poured onto 9 cm sterile petri plates and evenly distributed.

Agar medium with test microorganisms were injected with 6 mm diameter discs (70 µL extracts each). After that, the petri dishes were maintained at 40 degrees for 2 hours. For bacterial strains, the infected petri dishes were incubated at 37°C for 24 hours, while for yeasts and dermatophyte fungi, the dishes were incubated at 25°C for 72 hours. Standard discs were used as controls for bacteria (Streptomycin sulfate 10 µg disc) and yeasts (Nystatin 30 µg disc), with the concentration of each disc varying. DMSO (dimethyl sulfoxide) was utilized as a negative control in this study. Inhibition zones formed on the medium were measured and evaluated as mm., at the end of the incubation and penetration period. in mm. The results are shown in Table 3.2 and illustration of the studies are shown in Figure 3.2-4.

2.3.2.2 Minimal Inhibitor Concentration Analysis (MIC)

Using the agar dilution technique, antimicrobial activity tests were carried out on the oil hexane extract. The extracts were tested against standard bacteria, yeast, and dermatophyte fungus strains, and the minimal inhibitory concentrations (MIC) for each extract were determined. Additionally, the following bacteria were employed for this purpose: *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25322, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Pseudomonas aeruginosa* DMS50071, *Epidermophyton* species, and *Trichophyton* species. Furthermore, *Candida albicans* FMC17, *Candida glabrata* ATCC66032 were used as yeats strains, *Epidermophyton* sp. and *Trichophyton* sp. were used as dermatophyte fungi. It was decided on the turbidity of bacteria, molds, and dermatophytes on the basis of the McFarland 0.5 scale, in order to obtain standard inoculum. All extracts were tested at 100 - 3.125

µl/mL dilution concentrations. After testing, the minimum inhibitor concentration that stopped bacteria, fungus, and dermatophyte growth was identified (MIC) (Bauer et al., 1966; CLSI, 2012; Matuschek et al., 2014; EUCAST, 2015).

3. RESULTS AND DISCUSSION

In this study, *Origanum acutidens* was collected from the natural habitats from eastern Anatolian region and the essential oil of these plants were hydrodistilled by using Clevenger aparatus for 3 hour. The essential oil composition the *Origanum acutidens* was determined qualitatively and quantitavely and the antimicrobial activities of the oil were tested on the some bacteria and fungi mentioned above. The results of the essential oil chemical analysis were compared with other *Origanum* essential oil and its antimicrobial activity studies. The chemical composition of the *Origanum acutidens* oil was analysed and tested by using GC and GC- MS (Gas chromaography – Mass spectroscopy) system, the results are shown in Table 1. Qualitative and quantitative composition of the essential oil was determined. Twenty five components constituing 96.11% of the total oil were characterizied in the GC – MS analysis. The major components of the oils were determined as carvacrol (32.11%), p cymene (19.40%), gamma terpinene (6.91%), ethyl benzene (5.28%) and borneol (4.11%) relatively. On the other hand, *trans*-caryophyllene, Germacrene D and naphthalene, sesquiterpene were found as minor compounds in the essential oil. As a monoterpene, sabinene, beta pinene, alpha terpinene and Octanone – 3 have determined as lower concentrations in the essential oil. The GC Chromatograms of the *Origanum* essential oil are shown in Figure 3.1. Qualitative and quantitative essential oil composition results are also shown in Table 3.1. Retention times of the components were also shown in the same table.

These results were supported by Başer et al. (1997) study, on the *Origanum acutidens* plant from Bayburt region, They have determined 46 components in the *Origanum acutidens* essential oil. Carvacrol, p-cymene, gamma terpinene were identified as major components (Başer et al., 1997). This results was shown congruency with our study particularly in major compounds determined in the essential oil of *O. acutidens.*

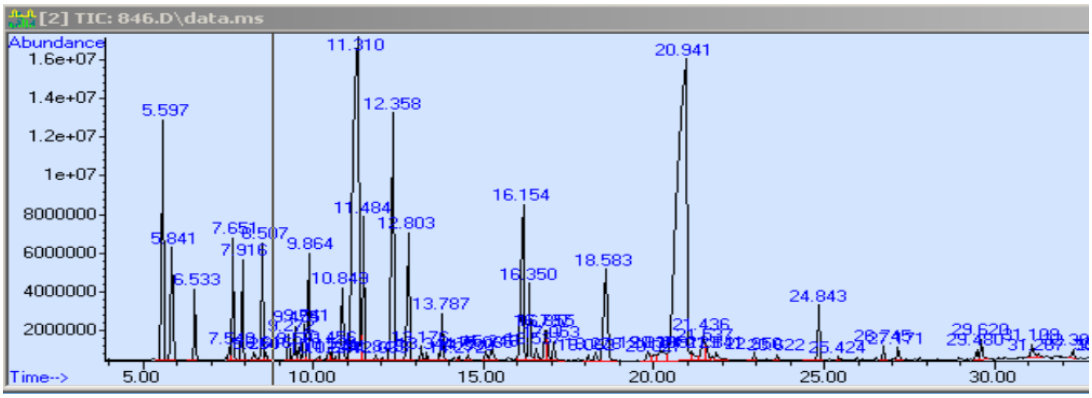

Figure 3.1. GC Chromatogram of the *Origanum acutidens* essential oil.

In the essential oil research of *Origanum boissieri* Ietswaart (İşcan, 2020), thirty compounds comprising 95.1 percent of the total oil were identified using GC and GC/MS analysis. Carvacrol (39%) and p-cymene (32%), have been identified as the oil's primary components. It was necessary to conduct separate investigations into the total methanol extract and essential oil of the aerial portions of the plant. Carvacrol and p-cymene were also evaluated for their growth inhibitory activities against antibacteria and candida using microdilution broth assay methods, which were used to quantify the results. The essential oil composition of the *Origanum boissieri* was found as similar with this study, particularly *O. acutidens* with the high concentrations of the carvacrol and p cymene determined in here. The essential oil composition of *Origanum vulgare* subsp. *gracile* (Kılıç and Bagci, 2008), thymol, gamma-terpinene, alpha terpinolene, carvacrol and p-cymene has determined as major compounds in the oil. It is said that, the *Origanum* essential oils has the carvacrol and p-cymene majority amount in the essential oils of the *Origanum* spp. generally.

It is reported that in the essential oil of *Origanum acutidens*, carvacrol (87.0%), *p*-cymene (2.0%), linalool acetate (1.7%) and borneol (1.6%) were found to be major constituents. In this study, chemical analysis procedures for the essential oil of the *Origanum acutidens* aerial parts were the same used in generally and clevenger apparatus were used for hydrodistillation and the chemical composition of *Origanum* plants oils were detected by using GC – MS system (Kordali et al, 2008).

Table 3.1. Essential oil Composition of the *Origanum acutidens* essential oil.

No	Retention time (Rt.)	Compound name	Concentrations (%)
1	5.57	Ethyl Benzene	5.28
2	5.84	Benzene 1,3 dimethyl	2.44
3	6.53	Benzen 1,2 dimethyl	0.97
4	7.65	Alpha thujene	1.77
5	7.92	Alpha pinene	1.42
6	8.50	Camphene	1.78
7	9.27	Sabinene	0.36
8	9.45	Beta pinene	0.48
9	9.75	Octanone – 3	0.68
10	9.86	**Myrcene**	2.64
11	10.45	Alpha – phellandrene	0.23
12	10.85	Alpha terpinene	1.64
13	11.23	**P – cymene**	**19.40**
14	11.37	Limonene	1.21
15	11.48	1.8 cineole	2.85
16	12.33	**Gamma terpinene**	**6.91**
17	12.79	Sabinene hydrate	2.84
18	16.13	**Borneol**	4.11
19	16.35	Terpinene 4 – ol	1.28
20	16.80	Alpha terpineol	0.82
21	18.39	Thymol	3.48
22	20.71	**Carvacrol**	**32.11**
23	24.84	Trans – caryophyllene	0.96
24	26.75	Germacrene D	0.23
25	36.69	Naphtalene	0.24
		Total	**96.11**

In the study of the antimicrobial activities of the *Origanum acutidens* essential oil, some selected bacteria and yeast were tested to calculate the sensitivity against to the oil. Disc diffusion method and Minimal inhibiton concentrations (MIC) methods for *Origanum* essential oil exttract were applied against some gr. positive and gr. negative bacteria, two yeast and two dermatophyte

fungi strains. The microorganisms strains studied (bacteria, yeats and fungi) and the results of the antimrobial activity are shown in Table 3.2. The control groups tested for the same microorganism were also shown on the same table. 70 l/ml oil was used for the tests on different microorganisms. The ınhibition zones of the essential oil on the pathogenic bacteria and fungi groups were measured and shown in tables following.

Table 3.2. The Inhibition zones of the *Origanum acutidens* essential oil on the microorganism (mm).

Microorganisms	**Oil (70µl/ml)**	**Control**
Staphylococcus aureus	43	14
Escherichia coli	20	15
Klebsiella pneumonia	10	18
Bacillus megaterium	20	10
Pseudomonas aeruginosa	30	14
Candida albicans	20	10
Candida glabrata	15	23
Trichophyton sp.	16	37
Epidermophyton sp.	20	38

The results showed that the *O. acutidens* essential oil has shown different antimicrobial activity against tested bacteria and fungi groups at different ratios. In the antimicrobial activity of the *Origanum acutidens* essential oil, the oil has shown strong activity on *Staphylococcus aureus* (43mm inhibition zone) and middle activities on the *Escherichia. coli, Bacillus megaterium* (20mm) from bacteria. The oil has also inhibited the growth of *Candida albicans* yeast and *Epidermophyton* species at the same inhibition zones (20mm). The antimicrobial activity of the essential oil on the tested bacteria were found as higher than the controls, except *Klebsiella pneumonia* (Table 3.2). At the same time the antimicrobial activit of the essential oil on the yeast species were determined as moderate level and varied according to the yeast strains as well as bacteria. While the oil has shown more activity on the *C. albicans* than control, showed lower activity on the *C. glabrata* than control. It is also determined that the oil has effected the growth of the dermatophyte fungi at lower ratios tha control groups. According to these results, it may be said that O. acutidens essentiall oils has more antimicrobial activity on the bacteria and yeast than fungi strains studied in here.

In the results obtained with this study, it was determined that the inhibition zones of the *Origanum acutidens* essential oil against *S. aureus, E. coli, K. pneumoniae, B. megaterium, P. aeruginosa* bacteria strains at 70µl/ml concentration were 43 mm, 20 mm, 10 mm, 20 mm, 30 mm.

Relatively (Fig. 3.2-4.). The inhibition zones against the yeast and fungi strains (*C. albicans*, *C. galabrata*, *Trichophyton* sp. ve *Epidermaphyto*n sp.) studied was found as 20mm., 15mm., 16mm. and 20mm., respectively (Table 3.2). When the results are compared with the controls, it is seen that the antimicrobial activity of *Origanum acutidens* essential oil has shown high antimicrobial effect especially against *S. aureus* (43mm) and It showed the lowest antimicrobial effect against *K. pneumoniae* (10 mm) (Table 3.2).

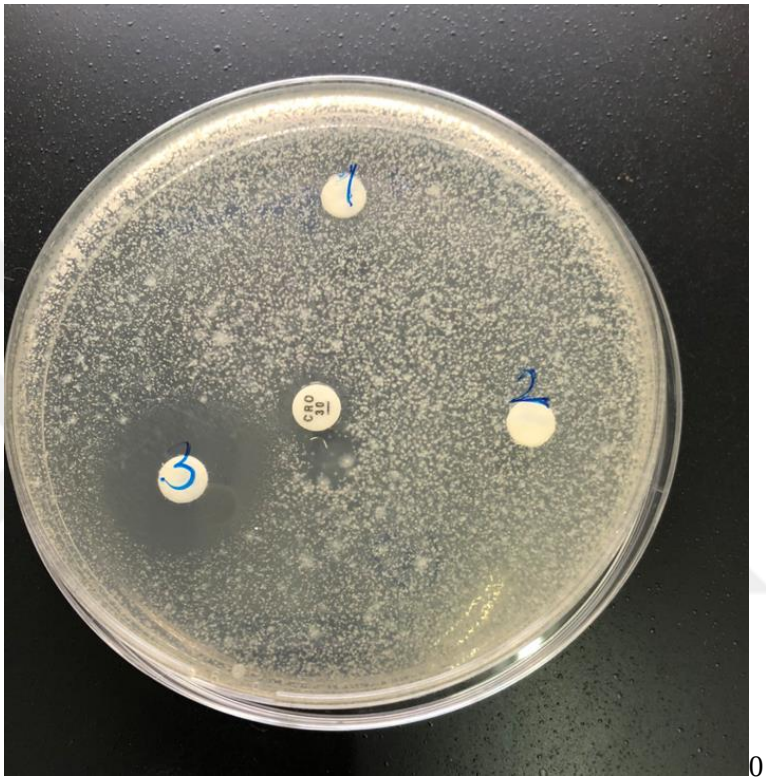

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Figure 3.2. Inhibition zones of Origanum *acutidens* oil (3 number) in *Bacillus megaterium* cultures.

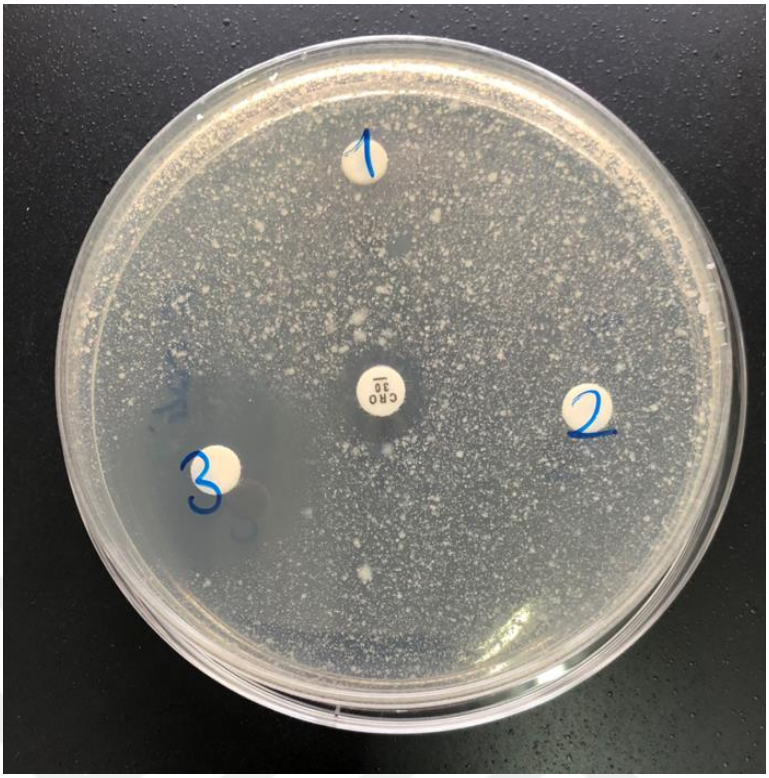

Figure 3.3. Inhibition zones of *Origanum acutidens* oil (3 number) in *Escherichia coli* cultures.

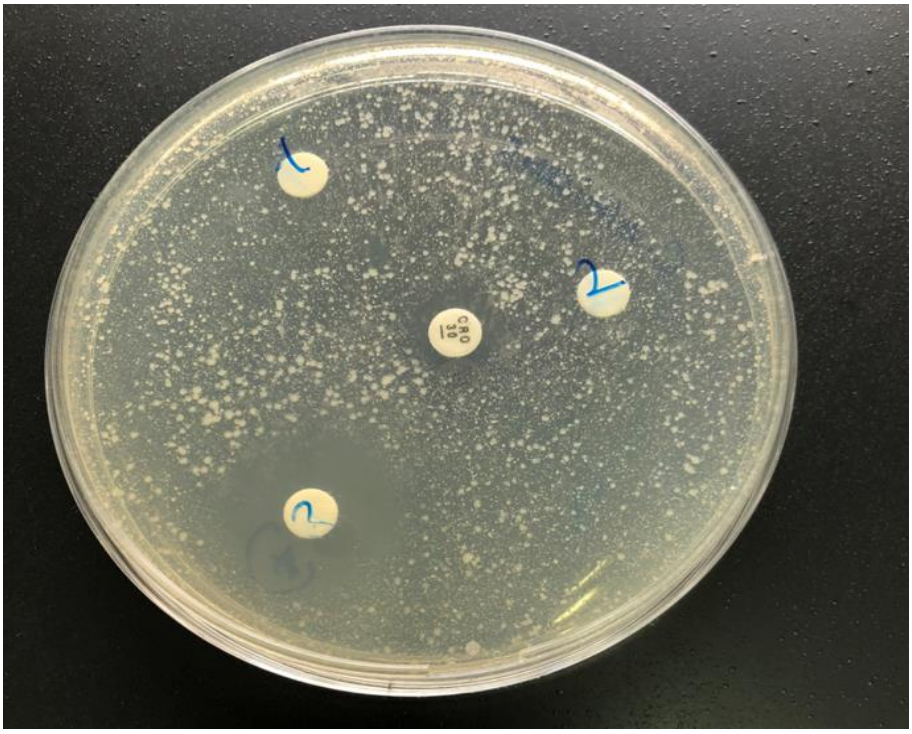

Figure 3.4. Inhibition zones of *Origanum acutidens* oil (3 number) in Candida *albicans* cultures.

While *Staphylococcoccus aureus* bacteria has been the most effected microorganism from the *Origanum* essential oil studied, *Klebsiella pneumoniae* was the lowest effected bacteria from the oil of *O. acutidens.* In general it is said that the essential oil of *Origanum* species studied in here were shown the higher antimicrobial activity on the studied microrganisms than the controls except dermatophyte species (Table 3.2). This means that the essential oil of the *Origanum* essential oil and the components of the oil has more antimicrobial activities or antifungal activities than the control groups (Streptomycin sulfate 10) for bacteria and (Nystatin 40) for yeasts).

Minimal inhibitory concentration (MIC) values of *Origanum acutidens* essential oil also tested in here, against *S. aureus, E. coli, K. pneumoniae, B. megaterium, P. aeruginosa, C. albicans, C. galabrata, Trichophyton sp*. ve *Epidermaphyton* sp.. The results are shown in Table 3.3. and 3.4. The MIC value of the oil against *S. aureus* was determined as 3.125 µl/ml; the MIC value against *E. coli* was 12.5 µl/ml; the MIC value against *K. pneumoniae* was 50 µl/ml; MIC value against *B. megaterium* was 12.5 µl/ml and the MIC value against *P. aeruginosa* was determined as 6.25 µl/ml (Table 3.3).

The MIC values of the essential oil of *Origanum acutidens* against *C. albicans, C. glabrata, Trichophyton* sp. and *Epidermaphyton* sp. were determined as 25 µl/ml (Table 3. 4). In particular, the essential oil of *Origanum acutidens* appears to be effective against *S. aureus* very low concentrations.

Table 3.3. MIC Values of the *Origanum acutidens* Essential oil on the Selected Bacteria (µl/ml).

Bacteria	**Oil**
Staphylococcus aureus	3.125
Escherichia coli	12.5
Klebsiella pneumoniae	50
Bacillus megaterium	12.5
Pseudomonas aeruginosa	6,25

Table 3.4. MIC Values of the Origanum *acutidens* Essential oil on the Selected yeasts (µl/ml).

Yeast sp.	**Oil**
Candida albicans	25
Candida glabrata	25
Trichophyton sp.	25
Epidermophyton sp.	25

There are some studies on the antimicrobial activities used MIC (Minimal Inhibition Concentrations) methods to observe the sensitivity and tha minimal range of the sensation for different plants, particularly timol, carvacrol comprised essential oils. In the previous studies on the MIC values of methanol extracts of *Origanum acutidenson* against some bacteria and yeast strains, some of the were used in this study also, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Candida glabrata* were reported to have 50 µg/ml, 100 µg/ml, 50 µg/ml, 50 µg/ml and 25 µg/ml, respectively (Daştan, 2020). It is reported that essential oil of *O. acutidens* have shown antimicrobial activities against different bacteria and yeasts; *Escherichia coli* A1, two *Klebsiella* strains *(K. pneumoniae* F3 and *K. pneumoniae* A13), three *Pseudomonas* strains *(P. aeruginosa* ATCC902, *P. aeruginosa* ATCC27859 and *P. aeruginosa* F6), *Staphylococcus aureus* A215 were tested as 10-24 mm. ranges (Sökmen et al., 2004). The antimicrobial effects of the essential oils obtained from the aerial parts of *O. acutidens* were tested against *S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae,* it is determined in the range of 56.0±0.0mm, 7.0±0.0 mm (Cosge et al., 2009). In a different study, it was reported that essential oils of the *O. acutidens* collected from Tunceli region has tested on the some fish pathogens microorganisms. The results has shown that the oil has high antimicrobial effect (28.0 ± 1.2 mm) against *S. aureus* (Kucukgul et al., 2014). When the results obtained from this study were compared with the other studies, *Origanum acutidens* essential oil showed high antimicrobial effect against *S. aures*. The study has shown that the O. acutidens essential oil with the high concentrations of the carvacrol and timol has shown high antimicrobial activities on the some fish pathogenic bacteria and fungi.

Essential oil and antimicrobial activity studies of *O. boissiieri* has tested by Işcan et al., (2020). Total methanolic extract and essential oil extracted from the aerial parts of the *O. boissieri* plant with the carvacrol and p-cymene were tested. The antibacterial and anticandidal properties of the oil and methanolic extract of the *O. boissieri* were analysed and screened by using microdilution broth assay. From the bacteria, *Bacillus cereus, B. subtilis, Staphylococcus aureus, S. epiderrnidis* and *Listeria monocytogenes* sensitivities has tested against O. boissieri aplications. It is determined that the oil has strongly inhibited the growth of the microotganims between 15,6 - 62.5 mg/mL. concentration. *O. boissieri* extract and the oil showed moderate activities against tested *Candida* yeast strains with the MIC values of 125-1000 mg/mL.

In the antimicrobial study of some *Origanum* essential oils, *Origanum dictamnus, O. vulgare* ssp. *hirtum* and a commercially available *Origanum* oil, exhibited antimicrobial activity in the high level, against different Gram-positive and Gr.-negative bacteria strains. Different major compounds were tested to measure the sensitivity and resistance of the various microorganism. From the major components of three oils, thymol and carvacrol exhibited the antimicrobial activity at the high level, while their biosynthetic precursors p-cymene and gamma terpinene were not effected the growth

of the microorganism. *O. vulgare* subsp. *hirtum* essential oil was determined as highly bactericidal at 1/4000 dilution. It has also bactericidal activity at dilutions as high as 1/50000. This concentrations has decreased the bacterial growth rates remarkable (Sivropoulou et al., 1996). It may be reported that the antimicrobial activities of the *O. acutidens* essential oil may be originated from carvacrol and thymol compounds, as found at high level in this study.

In general, the effect of the essential oils on the microorganism are reported as different. They can damage to the cell wall and membrane, leading the infiltration of the macromolecules and to lysis (Juven et al., 1994). The essential oils can damage the cell wall and the lipids and proteins (Ultee et al., 2002; Burt, 2004), it is also may coagulate the cytoplasm in the cell (Gustafson et al., 1998).

The essential oil of the *O. acutidens* were evaluated whther usable as insecticide in some studies. Tozlu et al., (2011), has trained and tested the antimicrobial activity and insecticidal activities of some plants. In the different chemical concentrations and insecticidal activities of some plants essential oils from different plant families (*Achillea gypsicola, Hypericum scabrum, Satureja hortensis, Origanum acutidens*) were evaluated on the insecticides against *Bruchus dentipes* (broad bean weevil). From these oils, *O. acutidens* and *S. hortensis* essential oils were characterized and the high contents of carvacrol (86.99% and 55.74%), *p*-cymene (1.95% and 12.30%), α-terpinene (0.13% and 2.04%), γ-terpinene (0.71% and 20.94%) and β-caryophyllene (1.30% and 1.08%) were detected. The essential oils studied in this study have shown toxic activity on the *B. dentipes* adults organism. It is determined that while increased the concentrations of the each oil extracted from these plants has increased the mortality of the insects. However insecticidal activities of the oils from these four plants were found desirable. In the insecticidal activity study, *S. hortensis* and *O. acutidens* oils were found more effective, especially after 6 h treatment. These results have shown congruency with our *O. acutidens* study because of the *O. acutidens* essential oil composition and its own major compounds, carvacrol, p cymene and gamma terpinene. It is possible to say that *O. acutidens,* due to the high essential oil yield and rich carvarcrol and p – cymene compounds may be used as insecticide and different purposes. According to the result of the Tozlu et al., (2011), it may be concluded that the some essential oils, in particular *S. hortensis* and *O . acutidens* oils, may be used as potential insecticides against *Bruchus dentipes (Broadbean weevil)* which is botanically important and may be the other insect species.

Nowadays, the essential oils are currently under investigation for their pest control features at broad-spectrum. It is reported that the essential oils obtained from different plants may have direct or indirect effects on natural enemy organisms. This is important for ecological equilibrium (Isman, 2000). Juven et al (1994) reported that, the thymol has antagonistic effects against *Staphylococcus aureus* and these effect were greater under anaerobic conditions than aerobics. On

the other hand the phenolic compounds of the oil, thymol and carvacrol and gamma-terpinene and p-cymene had not shown the antagonistic activity on *Salmonella typhimurium.*

It may be concluded and suggested that antifungal and herbicidal properties of the *Origanum* oil can be originated to its major components, carvacrol, thymol and p – cymene and these chemical agents have a good potential to use as herbicide and fungicide as well as insecticide. These also reported that by Kordali et al., (2008). Essential oils and their compounds were effected the fish grownment and their enfections sensitivities, using with the feeding ratio. It is reported that the thymol and carvacrol feeded Rainbow traut (*Oncorynchus mykiss*) has increased the growth performance of these fish (Ahmadifar et al., 2011; Giennenas et al., 2012).

Essential oils contained major carvacrol and p- cymene constituents has showed high antimicrobial activities against diffetent gr positive and gr. negative bacteria as determined in here. These results were the congruency with the other studies, especially different bacteria, yeast and fungi strains obtained from various cultures. The results and the other studies has shown that the antimicrobial activity ranges of the *Origanum acutidens* and different *Origanum* species has varied according to the plant species, the parts of the plants, the extraction types from the plants, the harvesting time, and the microorganism strains. Differences in the essential oil composition and their antimicrobial activities of the various plant and especially *Origanum* genus member may be originated from different factors. Such as the seasonal variation, harvesting time, the plant parts, extraction procedures and etc.

CONCLUSION

The essential oil of the *Origanum acutidens* has a good and more oil yield like 1.5 v/w with the yellow colours. The carvacrol and p – cymene has found as major compounds in the oil. These components have shown more inhibitive effect on the some microorganisms such as tested microorganisms and other microorganism groups reported. Former studies on the essential oil has supported these argument. The results of the present study suggest that antibacterial and antifungal features of the *O. acutidens* oil can be originated from its major component, carvacrol and this chemical have a potential for using as herbicide, fungicide and mey be insecticide according to the other study results. On the other hand, according to these results, it may be said that *O. acutidens* essential oils has more antimicrobial activity on the bacteria and yeast than fungi strains studied in here.

The interence and new attractions on natural products like essential oils, despite widely usage and familiar to us as flavor and fragrances, it has significance to develop their biological action models for new applications. Those may be used in agriculture, mankind health and the environment applications. As an endemic species *Origanum acutidens* has also an important role as essential oil and carvacrol potential and also some of them construct effective alternatives or as complements in the pharmaceutical / chemical industry with the synthetic compounds. Those also are important because of the not showing the secondary effects.

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