NEAR EAST UNIVERSITY
GRADUATE INSTITUTE OF HEALTH SCIENCES

EVALUATION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *ORIGANUM MAJORANA* L. ENDEMIC IN NORTHERN CYPRUS.

CHRISTOPHER O. JORADIMETAL GRANT (20193609)

MEDICAL AND CLINICAL MICROBIOLOGY

MASTER THESIS

LEFKOSA (NICOSIA),T.R.N.C 2021

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SUPERVISOR

ASSIST. PROF. DR. GUNER EKIZ

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APPROVAL

This thesis prepared and presented by Christopher O.J Oradimetal Grant entitled as “Evaluation of Antimicrobial Activity of Essential Oil of *OriganumMajorana* L. Endemic In Northern Cyprus”has been accepted by the Examination committee for the degree of Master of science in Medical and Clinical Microbiology.

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According to the relevant articles of the Near East University postgraduate study- Education and examination Regulations, this thesis has been approved by the members of the Thesis Committee and the decision of the Board of the Directors of the Institue.

Prof.Dr. Kemal Hüsnü Can Başer

Director of Institute of Graduate Studies

ACKNOWLEDGMENTS

Firstly, I would to give thanks to the Almighty God, for his continuous blessings throughout my research work to complete successfully.

I would like to express my deep and sincere gratitude to my advisor Assist. Prof.DrGüner. Ekizfor thecontinuous support of my master study and research,for her patience, motivation, enthusiasm, and immense knowledge.Her invaluable guidance helped in all the time of research, methodology and the writing of this thesis. Icould not imagine having a better advisor and mentor for my master study.

Besides my advisor, I would to thank the head of department and all the lecturers in the department for the 2 years spent lecturing me. Iwould like to thank Dr.E.Güler for his knowledge and experiences in the laboratory work.

I am extremely grateful to my parents Mr. and Mrs. Tough for their love, prayers, caring and sacrifices and continuous support to complete this research work. Also, I express my thanks to my sisters, family members, friends and love ones for their support and valuable prayers.

Contents

ABSTRACT

The present work aims to evaluate the antimicrobial activity of *Origanummajorana* essential oil, endemic plant in Northern Cyprus. Essential oils are volatile oils which normally responsible for many sweet smells produced by plants *Origanummajorana*L. which belongs to the mint family (*Lamiaceae)* which includes aromatic plants are extensively used for its medicinal, cosmetic, cultinary and ornamental purposes. The plant material used in this study was collected from Northern Cyprus. The essential oil was obtained by hydrodistillation and analysed by GC-MS, 26 compounds were identified representing 97.6% total of the essential oil. The main compounds found were cis- sabiene hydrate (11.6%,), terpienen-4-ol(24.1) and E- ocimenol.

The antimicrobial activity of the oil was determined using disc diffusion method and it was found out that the essential oil of *Origanummajorana*L. showed significant activity against *Staphylococcousaureus*, *Escherichia coli*, *Pseudomonas aurginosa* and *S. maltophilia*. It was concluded that compound terppiene- 4-ol might be responsible for the antimicrobial activity of endemic *Origanummajorana* L essential oil.

Keywords:*Origanummajorana* L., Antimicrobial Activity, Essential Oils, Northern Cyprus

CHAPTER 1
INTRODUCTION

1.1. Essential Oils

Essential oils are volatile liquids of the secondary metabolism of aromatic plants. They are termed “essential” because they represent the most important part of the plant. They are synthesized by all plant organs such as flowers, leaves, stems, seeds, barks, fruits, roots, peels and are stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes. Essential oils are distributed in all plants kingdom and belonging to the families like Asteraceae, Aristolochiaeceae, Cupressaceae,Fabaceae,Lamiaceae, Lauraceae, Meliaceae,Myrtaceae,Rutaceae, etc. (Shah et al.,Raut et al.,2014). Essential oils are extracted from aromatic plants which are often used in folk medicine for prevention and treatment of different human diseases. Plants essential oils have been studied for their antibacterial activity against microorganisms, includes many pathogens (Dorman and Deans, 2000;Delaquis et al., 2002). In vitro antibacterial, antifungal and milicide activity of the some essential oils have shown effective results in the control of bees pests(Albo et al. el 2003;Dellacasa et al., 2003,Ruffinengo et al., 2006;Fuselli et al., 2007) giving a natural alternative to antibiotics and other synthetic chemical substances.

Essential oils are aromatic oily liquids whichare obtained from plants parts such as;leaves (peppermint), seeds(cardamom), buds (clove), twigs, barks(cinnamon), wood(cedar), bulb(garlic), flower(rose) fruits(fennel) and roots (vetiver)(Tisserand and Young, 2013). They can be produced by expression, fermentation or extraction but the most common method in commercial production is the steam distillation. About 3000 essential oils are known from least at 200 plant species, out of which 300 commercially important in fragrance market(Djilani and Dicko. 2012)

Essential oils are volatile compounds obtained from plants having potent aromatic constituents that are made of the different chemicals such as hydrocarbons, alcohols, phenols,esters,ketones and aldehydes(Younis et al., 2008).Plant essential olis are mixture of hydrophobic and highly volatile secondary metabolites which can physically be separated from other plant components (Grassman and Elstner, 2003,Protzen 1993). Plant essential oils have many important

applications in areas of health, cosmetics, agriculture and food industries.Essential oils may generally constituent 20-100 different metabolites belonging to several chemical classes (Hammmer and Crason,2012).

Table 1.1. Some Essential Oils and their Composition

Botanical Species	Common name	Family	Part	Main Composition (%)	Manufacture
Cinnamomum cassia	Chinese cinnamon	Lauraceae	Leaf-branch	E-cinnamaldehyde (77.90), trans-o-methoxy-cinnamaldehyde (10.50)	Pranarom
CinnamomumVerum	Ceylon Cinnamon	Lauraceae	Bark	E-cinnamaldehyde (63.56), cinnamyl acetate (8.33)	Pranarom
Coriandrumsativum	Coriander	Apiaceae	Fruit	Linalool (70.07), camphor (5.52), α-pinene (4.86)	Pranarom
Cymbopogonflexuosus	Indian lemongrass	Gramineae	Herb grass	NA	Lionel Hitcher
Cymbopogonnardus	Ceylon citronella	Gramineae	Herb grass	Geraniol (24.08), camphene (9.01), geranyl acetate (8.81)	Pranarom
Eugenia caryophyllus	Clove	Myrtaceae	Bud	Eugenol (84.75) eugenyl acetate (7.12), β-caryophyllene (4.60)	Pranarom
Kaempferiagalanga	Aromatic ginger	Zingberaceae	Rhizome	NA	Lionel Hitcher
Origanumcompactum	Oregano	Lamiaceae	Flowering plant	Cavacrol (46.37), thymol (13.70), p-cymene (13.33)	Pranarom

Origanumheracleoticum	Greek oregano	Lamiaceae	Flowering plant	Carvacrol (68.14), thymol (7.47), γ-terpinene (6.06)	Pranarom
Origanummajorana	Sweet marjoram	Lamiaceae	Flowering plant	Terpinene-4-ol (24.21), α-terpinene (8.44), Sabnene (7.12)	Pranoram
Salvia officinalis	Dalmatian sage	Lamiaceae	Flowering plant	NA	Lionel Hitcher
Salvia sciarea	Clary sage	Lamiaceae	Flowering plant	Linalyl acetate (62.38), linalool (21.47), α-terpineol (2.45)	Pranarom
Thymus capitatus	Oregano	Lamiaceae	Flowering plant	NA	Lionel Hitcher
Thymus mastichina	Spanish marjoram	Lamiaceae	Flowering plant	NA	Lionel Hitcher
Thymus vulgaris Thymoliferum	Common thymol Thyme	Lamiaceae	Flowering plant	Thymol (39.74), p-cymene (18.74) γ-terpinene	Pranarom

1.1.1. Sources and physical properties of essential oils

Table 1.2. The various parts of plants where essential oils can be obtained from.

Parts	**Plants**
Fruit	Nutmeg, xanthoxylum,blackpepper
Leaf	Pine, basil,patchouli, bayleaf mint, cinnanmon,citronella,eucalyptus,common sage, lemon grass, melaleuca, oregano, peppermint
Roots	Ginger,plai turmeric, valerian, spikenard, angelica,vetiver
Seed	Fennel, almond, anise, coriander,cardamom,carrot celery, caraway, cumin, parsley, nutmeg
Peels	Bergamot,grapefruit, lemon, lime,orange, kaffir lime,tangerine, mandanrin

Woods	Atlas cedarwood, camphor, rosewood, sandalwood, myrtle,guaiac wood,Himalayan cedarwood, amyris
Flower	Blue tansy, manuka,chamomile,clary sage, clove, cumin, geranium, helichrysum hyssop,lavender, rhododendron anthopogon,marjoram, orange,rose, jasmine,baccharises,palmarosa,patchouli,Rosalina, ajowan,ylang-ylang, marjoram sylvestris, tarragon,immortelle,neroli
Parts	Plants
Barks	Cinnanmon,cassia, katrafay,sassafras
Resins	Myrrth, frankincense
Barriers	Sassafras,allspice,juniper

Essential oils are normally liquid at room temperature but some can also be solid or resinous. The majority of the essential oils colours ranging from pale yellow to emerald green, blue to dark brownish red. They can be synthesized by all plants parts such as flowers, leaves, stems, seeds, bud, barks, wood etc and stored in canals, cavities, tichomes, secretory cells(Bassole and Juliani, 2012).They have characteristic odor which may depends on the the plant organs, plants origin and the species of plants and theydensity less a unity Thus, essential oils float on water with the few exception like cinnamon, sassafras vetiver which sinks at the bottom. They have refractive index and high optical activity as the result of many asymmetrical compounds.Essential oils are soluble in alcohol, ether and fixed oils but insoluble in water, in addition they can readily be oxidized to form resinous products by polymerization (Li et al 2014).

1.1.2.Chemistry of Essential Oils

Essential oils are volatile oils which normally responsible for many sweet smell produced by plants. They can be obtained by plants parts by differential methods such as superficial CO_2extract, hydrom and steam distillation and solvent extraction(El. Mougy et al., 2009)complex mixtures that may contain over 300 differentcompounds (Sell, 2010). Basically there are two main groups of metalbolites that occure in nature, which are primary metabolites and secondary metabolites. Primary metabolites which is found in all living organisms which includes carbohydrates, protieins lipids and nucleic acid. secondary metabolites includes terpenoids, shikimates, polyketides and alkaloids etc. some plants species may contain high

quantity of shikimates which is refereds to as flavoids, phenylpropanoids. They provides specific flavor and colour to the plant (Sangwanet al. 20001).

Terpenes

Terpenes belongs to the the general formula (C5H8)n, it is a 2units of isoprene which is normally isoprenoids, terpenehydrocarbponsare sequinterpenes C15 and monoterpenes C10 other includes diternes C20, triterpenes C30 and tetaterpenes C40.

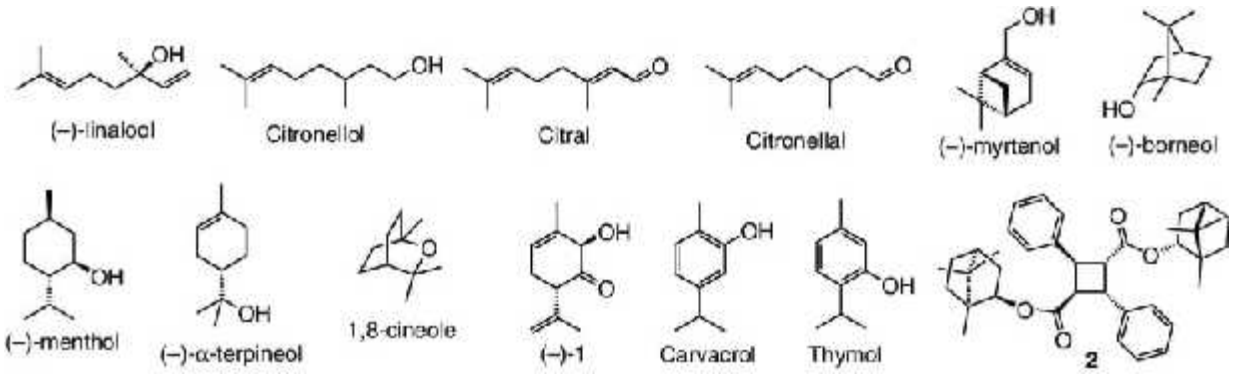


Figure 1.1. Structures ofmonoterpenes(Prena et al., 2015).

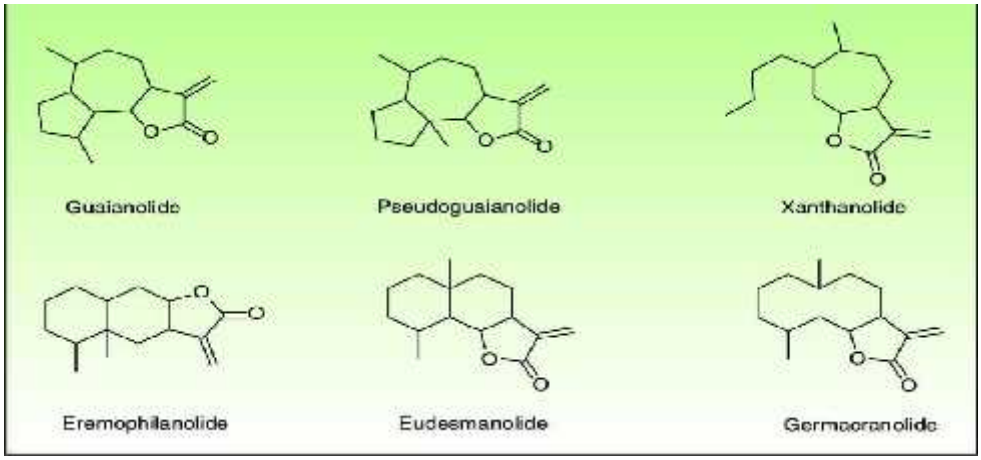


Figure 1.2. Structures of sesquiterpenes(Michele marcus et al., 2007)

Phenylpropanoids

The phenylpropanoids are a family of organic with an aromatic ring and ring 3 carbon propene tail, produced by plants from the amino acids phenylanine and tryrosine(Zhang and Stephanopoulos, 2016). Which is also known as cinnamic acid. This includes phenol, flavonoids, coumarins etc.

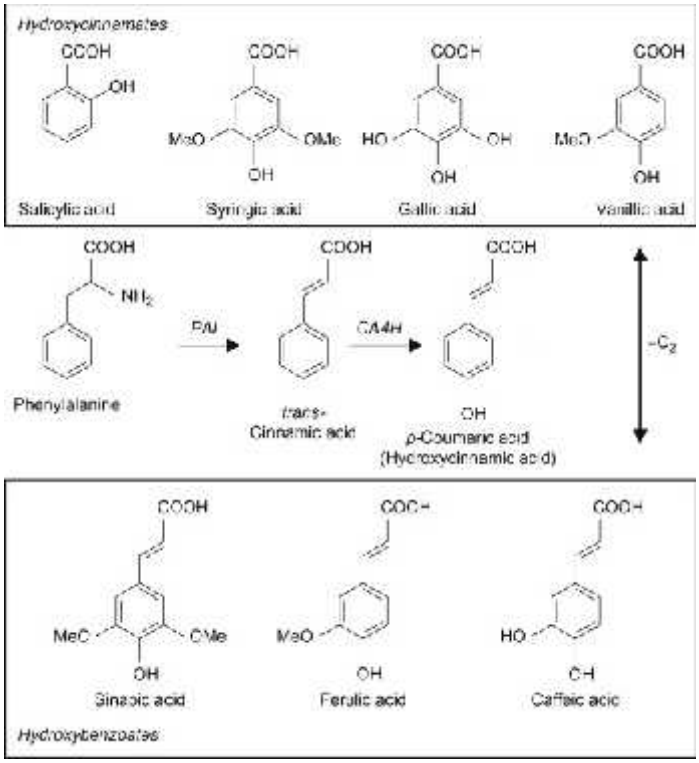


Figure 1.3. Some structures of phenylpropanoids**(**E.Guentheret al.1948)

1.2. Antimicrobial activity of essential oils

Several researches have looked into the secondary metabolites produced by *Origanummajorana*L. Almost all of these investigations have focused on the plant's aerial components. Phytochemical screening of extracts and essential oils of *Origanummajorana L*. has shown the richness of this plant in phenolic acids, flavonoid terpenoidChromatographic analyzes of *Origanummajorana L.* essential oils allowed identifying around thirty terpenoid compounds**.** **(**A.Bouyahya et al.2021)majorana is mainly composed of carvacrol, linalool, thymol, borneol, camphor, terpinen-4-ol, α-pinene, α-thujene, p-cymene, terpinene, α-terpineol, sabinene, myrcene, limonene, camphene, terpinolene, verbenene, β-caryophyllene, 1,8-cineole, eucalyptol,

and phellandrene (Hajlaoui et al., 2016; Muqaddas et al., 2016; Erdogan and Ozkan, 2017; García-Risco et al., 2017; Ben Salha et al., 2017;Al-Fatimi, 2018;da Cunha et al., 2018;Baj et al., 2018;Jan et al., 2018;Partovi et al., 2018;Sefeer and Elumalai, 2018;Abbasi-Maleki et al., 2019;Amor et al., 2019;Benhalilou et al., 2019;Chaves et al., 2019;Della Pepa et al., 2019;Khadhri et al., 2019; Makrane et al., 2019;Ragab et al., 2019;Thanh et al., 2019;Waller et al., 2019;Xylia et al., 2019). Chemical variability is observed in the composition of OMEO extracted by different methods;*Origanum.majorana L.* essential oils are rich in oxygenated monoterpenes and monoterpene hydrocarbons. While, oxygenated sesquiterpenes have the lowest percentage. Terpinene-4-ol is the major compound (Ragab et al., 2019). The work of Chaves et al. (2019) has shown that pegegone (57.05%) is the major compound of *Origanummajorana L*. essential oil followed by verbenone (16.92%), trans-menthone (8.57%), cis-menthone (5.58%), piperitone (2.83%), 3-octanol%), and isopulegol (1.47%). The essential oil of *Origanummajorana L*. aerial parts is rich in terpinoids; whose main component is carvacrol with 52.5%, followed by linalool with 45.4%. This essential oil consists mainly of oxygenated monoterpenes (98.2%), while monoterpene hydrocarbons are poorly represented (1.7%). Terpinene-4-ol is the main component of *Origanummajorana L.* essential oil from Kalocsa, Hungary (Erdogan and Ozkan, 2017). The main compounds identified in *OriganummajoranaL.*. essential oils treated with 75 mMNaCl are sabinene (7.723 µg/g DW) followed by cis-sabinene hydrate (4.857 µg/g DW), and terpinene 4-ol (2.861 µg/g DW) (Olfa et al., 2016). Chemical analysis of two fractions of *OriganummajoranaL.*. essential oil has shown that the colorless fraction is rich in terpinene-4-ol (23.1%) and thymol (16.3%), however these same compounds are also predominant in the yellow fraction, terpinene-4-ol (27.7%) and thymol (24.6%) (Guerra-Boone et al., 2015). The main compounds identified in Origanummajorana L. essential oil are 4-terpineol (34.23%) followed by α-terpinene (14.28%) (Abdalla and Hendi, 2014).

The work of Raina and Negi (2012) showed that the *Origanummajorana L*. essential oil is mainly composed of terpinene-4-ol (31.15%), cis-sabinene hydrate (15.76%), p-cymene (6.83%), sabinene (6.91%), trans hydrate sabinene (3.86%), and α-terpineol (3.71%). In addition, terpinene-4-ol, γ-terpinene, cis-sabinene hydrate, and α-terpineol are the main compounds identified in the essential oil of *Origanummajorana L*. leaves (Jelali et al., 2011). However, the main compounds of OMEO cultivated in China are terpinene-4-ol (33.0%), caryophyllene oxide

(11.9%), p-cymene (6.8%), α-terpineol (6.7%), and spathulenol (6.0%) (Jiang et al., 2011). Chromatographic analysis of OMEO reveals the presence of 27 compounds, namely terpinene-4-ol (36.2%), p-cymene (16.3%), and γ-terpinene (7.31%) (Khanavi et al., 2010). The main compounds of 20 Origanum species are cis-sabinene hydrate and cis-sabinene hydrate acetate, representing a total of 68.5% of the essential oil of each species (Novak et al., 2003). The compounds identified in *Origanummajorana L*. essential oil are represented by cis-sabinenehydrate (cis-thuyanol-4) which reaches 33.3% and terpinene-4-ol with 21.6% (Arnold et al., 1993). The 4-terpineol compound identified in *Origanummajorana L*. essential oil is the major compound with 37%; α-terpineol hydrate and cis- and trans-sabinene made up 50% of this oil (Komaitis et al., 1992). The work of Nyk¨ anen (1986) has shown that cis-sabinene hydrate (8–43% of the oil) and 4-terpineol (21–52% of the oil) are the main compounds of *Origanummajorana L*. essential oil. The essential oil of *Origanummajorana L*. has a very important chemical polymorphism. The content and the nature of the major compounds vary considerably from one sample to another depending on the origin of the plants. In addition to the essential oil, *Origanummajorana L.* also contains phenol acids, flavonoids, sterols, triterpenes, alkaloids, coumarins, tannins, and saponins (Hossain et al., 2011; Benhalilou et al., 2019). have identified three groups of phenolic compounds in *Origanummajorana L.* extracts; phenolic acids group with five compounds: rosmarinic acid, caffeic acid, gallic acid, carnosic acid, and ferulic acid; flavonoids group with eight compounds: luteolin-7-Oglucoside, apigenin-7-Oglucoside, apigenin, hesperetin, luteolin, arbutin, quercetin, and catechin; terpenoids group with five compounds: carnosol, limonene, terpinen-4-ol, linalylacetate, and β-caryophylleneSellami et al 2009)This chemical composition is completely distinct from that of the extracts studied by several authors (Sellami et al., 2009; Kaiser et al., 2013; Taamalli et al., 2015; Makrane et al., 2018; M´eabed et al., 2018). Indeed, the phenolic acids are the main group identified in *Origanummajorana L*. extracts with a dozen compounds, namely gallic acid, caffeic acid, dihydroxy phenolic acid, chlorogenic acid, syringic acid, vanillic acid, p-coumaric acid, ferulic acid, rosmarinic acid, trans-2-dihydroxycinnamic acid, cinnamic acid, lithospermic acid, and pyrogallol. Moreover, several flavonoids have been identified such as epicatechin, rutin, quercetin-3-rhamnoside, luteolin, coumarin, quercetin, apigenin, amentoflavone, hesperidin, taxifolin, and isorhamnetin. On the other hand, the chemical composition of the methanolic extract of *Origanummajorana L.* was determined by reverse phase high performance liquid

chromatography. Amentoflavone is the dominant flavonoid. However, trans 2-hydrocinnamic acid is the main phenolic acid (Baˆ atour et al., 2013). Ayari et al. (2013) have shown that the methanolic extract of different organs of *Origanummajorana L*. studied are rich in phenolic acids, flavonoids, and tannins. Furthermore, the chemical composition of other *Origanummajorana L*. extracts is characterized by the strong dominance of phenolic acids. The results obtained made it possible to identify approximately 8 constituents (catechol, cinnamic acid, gallic acid, ascorbic acid, syringic acid, caffeic acid, p-coumaric acid, and trans-ferulic acid) (Dhull et al., 2016; Makrane et al., 2019). On the other hand, a study was carried out by Adam and Ahmed (2014) on *Origanummajorana L*. extracts and it proved the presence of other secondary metabolites such as sterols, triterpenes, alkaloids, coumarins, tannins, and saponins. The variations encountered in the chemical composition of the essential oil and extract of *Origanummajorana L*., from the qualitative and quantitative point of view, maybe due to certain ecological factors, to the part of the plant used, to the age of the plant and the period of the vegetative cycle, or even to genetic factors.

The antimicrobial properties of fifty plant essential oils against 25 genera of bacteria were evaluated. Thyme, cinnamon, bay, clove, almond (bitter), lovage, pimento, marjoram, angelica and nutmeg essential oils displaced the greatest inhibitory properties. Clove extract showed remarkable antibacterial activity against all organisms tested and oregano and cinnamon exhibited wide inhibitory spectrum (Mohamed et al., 2013).

The antibacterial properties of 14 Essential oils (*clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelica root and angelica)* against four common meat spoilage and pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluores*cens &*Lactobacillus sak*e) was studied and their results showed that individual extracts of *clove, rosemary, cassia bark* and *liquorice* contained strong antibacterial activity (Skrinjar and Nemet, 2009).

In 2007, 46 spice and herb extracts tested by Shan and co-workers, amongst them twelve exhibited high antibacterial activities against the five foodborne bacteria. Methanolic and ethanolic extracts of *Punicagranatum* were effective against *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*. The extracts and essential oils of *Eugenia caryophylata*, *Origaumvulgare*, and *Cinnamomumburmannii* had significant inhibitory properties against

Staphylococcus aureus, *Escherichia coli* and *Listeria monocytogenes*. The ethanolic extracts of *Salvia officinalis* had strong antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*. The extracts of *Pediomelumcuspidatum* strongly inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. *Cassia auriculata* exhibited significant activity against *Escherichia coli* and *Staphylococcus aureus*.

1.3.Microorganisms

Microorganismis a microscopic organism, especaially a bacterium, fungus or virus. These organisms causes infectious diseases and they representa vital pathological state and one in every of the causes of morbility and mortality are include in the list of the 10 leading causes of death worldwide (Menchaacamd etal., 2016 Nunkoo and Mahomodally 2016).

The microorganisms in this study are clinical isolates from Near East University Hospital which are as follows:*Stapyloccocusaureus(MRSA), Escherichia coli,Pseudomonas auerogina*s *and Stenotrophomonasmaltapholia*

1.3.1*Staphylococcus aureus*

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant Staphylococcus aureus. In 1961 there were reports from the United Kingdom of S. aureus isolates that had acquired resistance to methicillin (methicillin-resistant S. aureus, MRSA) (M.P.Jevons 1961), and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States.

Many MRSA isolates are multiply resistant and are susceptible only to glycopeptide antibiotics such as vancomycin and investigational drugs. MRSA isolates that have decreased susceptibility to glycopeptides (glycopeptide intermediately susceptible S. aureus, GISA) (Hiramatsu et al., 1997), reported in recent years, are a cause of great public health concern. Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA). MRSA isolates are generally characterized by pulsed-field gel electrophoresis, a powerful technique for identifying the relatedness of isolateis now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community (F.Hussain et al. 2000)

Characteristics

Gram positive cocci arranged in grapelike clusters, facultative, coagulase positive, catalase positive

(www.labtestguide.com)

Habitat and transmission

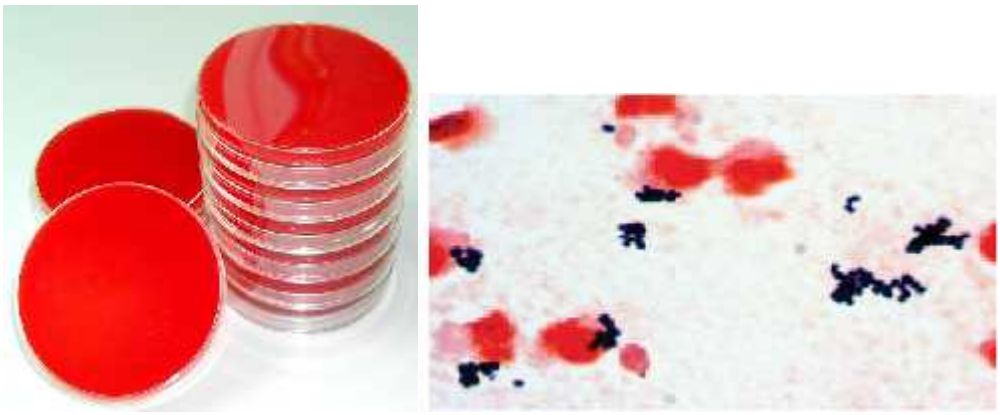
Human nose and skin, transmission via hands

Disease

Abscesses in various organs, sepsis, wound infection, endocarditis, food poisoning, toxic shock syndrome

Laboratory Diagnosis

Gram staining and culture. On blood agar, 1-3 mm golden yellow colonies, often haemolytic, coagulase positive



(www.labtestguide.com)

Treatment, Prevention and Control

Methicillin-resistant *Staphylococcus aureus* (MRSA)

mec A gene ----.> PBP-→> PBP 2A/ PBP 2'-→ Low affinity to ß-lactam antibiotics --→ Peptidoglycan synthesis is not inhibited

Resistant to β-lactam antibiotics (methicillin, oxacillin, penicillin, and amoxicillin)

Also resistant to lincosamides, macrolides, aminoglycosides, etc…

Isolates should be tested for antimicrobial susceptibility

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Glycopeptides (vancomycin and teicoplanin): current mainstay of therapy for MRSA infections

Vancomycin Intermediate *Staphylococcus aureus* (VISA)

Vancomycin Resistant *Staphylococcus aureus* (VRSA)

Quinupristin/dalfopristin, linezolid, tigecycline, daptomycin

Only approved for certain indicatio

Nasal carriage

The most common source of *S. aureus*

~20% persistent carriers, ~30% intermittent carriers, ~50% non-carriers

Prophylaxis consisting of vancomycin and rifampin

Survey of high risk patients for anterior nares colonization

S. aureus can be transferred from nose to the other sites of body via hands

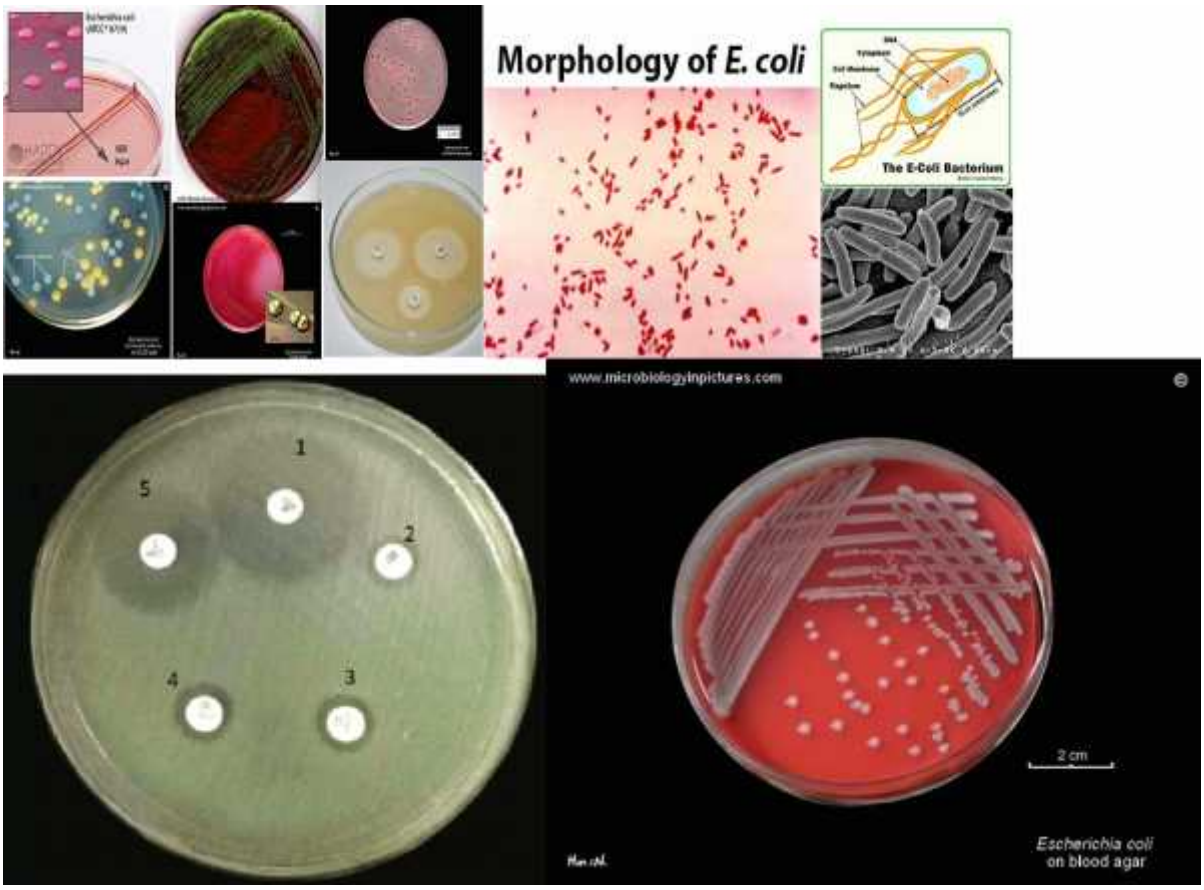
Wearing gloves and **washing hands** before and after patient contact

1.3.2*Escherichia coli*

Escherichia coli, a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens (Kaper, J.B. 2004) As a commensal it lives in a mutually beneficial association with hosts, and rarely causes disease. It is, however, also one of the most common human and animal pathogens as it is responsible for a broad spectrum of diseases. The peculiar characteristics of the *Escherichia coli*, such as ease of handling, availability of the complete genome sequence, and its ability to grow under both aerobic and anaerobic condition, makes it an important host organism in biotechnology. Escherichia coli is used in a wide variety of applications both in the industrial and medical area and it is the most used microorganism in the field of recombinant DNA technology (Yoo, S.H.et al 2009;.) Prior to the identification of specific virulence factors in pathogenic strains, *Escherichia coli* was principally classified on the basis of the serologic identification of O (lipopolysaccharide, LPS) and H (flagellar) antigens. Based on the type of virulence factor present and host clinical symptoms, Escherichia coli strains are classified into pathogenic types (pathotypes are defined as a group of strains of the same species causing a common disease):Intestinal pathogens spread through the faecal-oral route by ingestion of contaminated food or water.

Characteristics

Gram negative motile rods, lactose fermenting



(www.labtestguide.com)

Habitat

Human colon, lower genitourinary tract (normal flora)

Diseases

UTI (ascending infection), sepsis, neonatal meningitis

Laboratory Diagnosis

Culture, lactose fermenting colonies on MacConkey agar

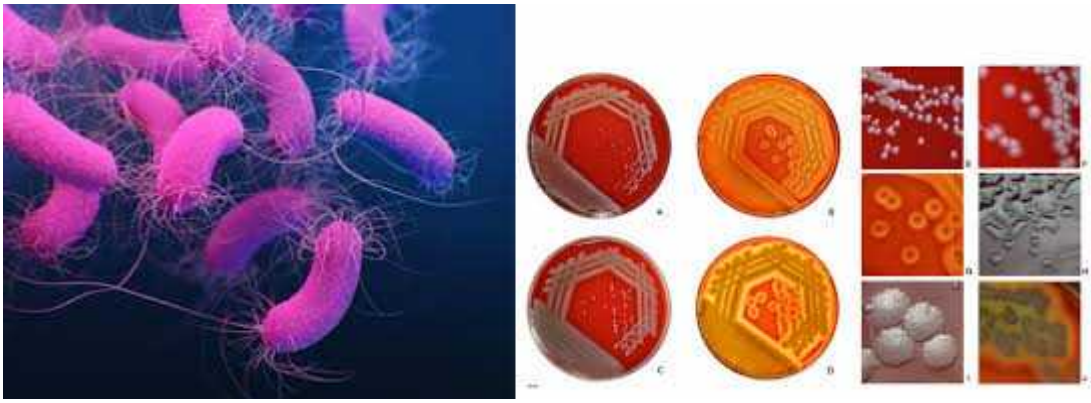
1.3.3.*Pseudomonas aeruginosa*

Pseudomonas aeruginosa (Pseudomonas aeruginosa) is a gram-negative bacillus with cell dimensions of 0.5-0.8 µm by 1.5-3.0 µm. Almost all strains of *Pseudomonas aeruginosa* are motile via a single polar flagellum. *Pseudomonas aeruginosa* can grow well at a range of

different temperatures (25-37 °C) and is resistant to high concentrations of salt, dyes, weak antiseptics and most commonly used antibiotics (Cohen et al ,1986) These characteristics allow it to survive well in many environments resulting in prolific transfer and contamination. The Health Protection Agency (HPA) describes *Pseudomonas aeruginosa* as an opportunistic pathogen which can cause a wide range of infections, particularly amongst the immunocompromised. In a recent study *Pseudomonas aeruginosa* accounted for approximately 93 % of all identified *Pseudomonas spp*. infections reported in UK healthcare facilities England, (Wales, and Northern Ireland, 2004 to 2008. 2009, Health Protection agency: London. p. 10)

Characteristics

Gram negative motile rods, strict aerobe, oxidase positive, non-lactose fermenting on MacConkey agar, green pigment and a sweet smell on blood agar



(www.labtestguide.com)

Habitat

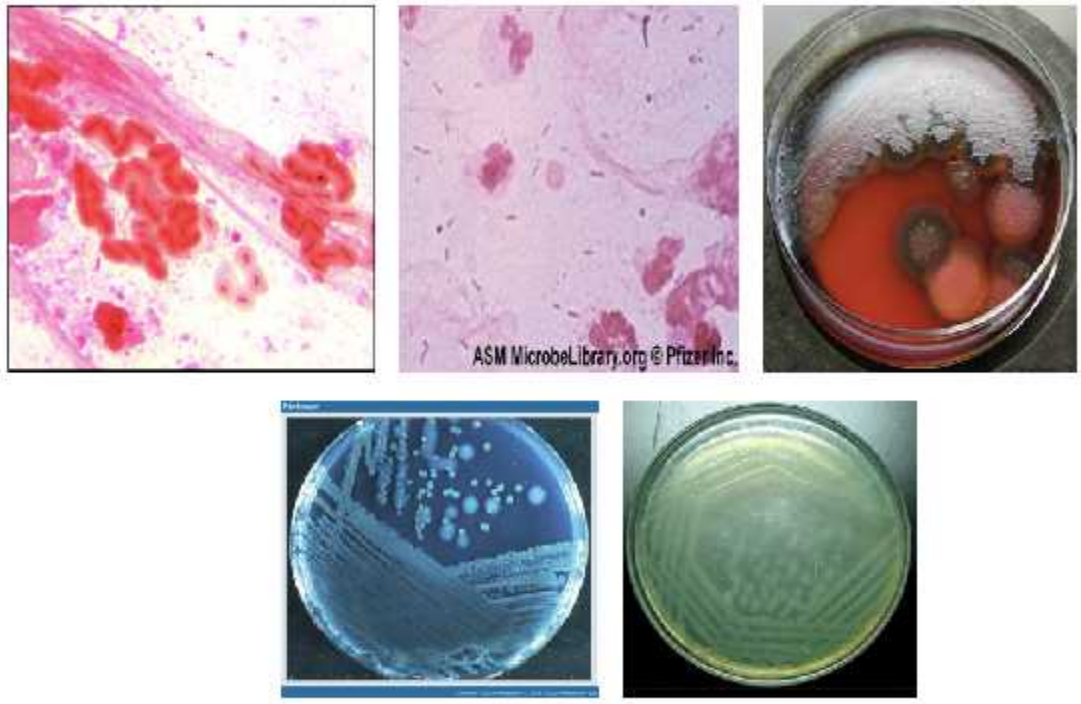
Environment, transmitted via water aerosols, aspiration, contact

Diseases

Wound infections, UTI, sepsis, pneumonia. One of the most important nosocomial pathogen, resistant to many antimicrobials

Laboratory Diagnosis

Culture-greenish pigment on blood agar and nutrient agar with a characteristic sweet smell, oxidase positive, non-lactose fermenting on MacConkey agar.



(www.labtestguide.com)

Treatment

Resistant to many antibiotics

- Extended-spectrum penicillin (piperacillin) + aminoglycoside (tobramycin)
- Other active drugs:
- Aztreonam, carbapenems (imipenem or meropenem), fluoroquinolones, cephalosporins (ceftazidime, cefoperazone, cefepime)
- Primary therapy (especially in patients with neutropenia):
- Ceftazidime + aminoglycoside
- Susceptibility tests should be done

Prevention and control

- Primarily a nosocomial pathogen

- Preventing the contamination of sterile equipment
- Mechanical ventilation equipment and dialysis machine
- Preventing the cross-contamination of patients by medical personnel
- Inappropriate use of broad-spectrum antibiotics should be avoided
- Special attention to moist environments:
- Sinks, water baths, showers, hot tubs, and other wet areas

1.3.4.Stenotrophomonasmaltopholia

*Stenotrophomonasmaltophilia*is an environmental global emerging Gram-negative multiple-drug-resistant organism that is most commonly associated with respiratory infections (J.S Broke et al., 2012)Isolation of*Stenotrophomonasmaltopholia* from human specimens may represent colonization rather than infection. Although not highly virulent,*Stenotrophomonasmaltopholia*can infect immunocompromised hosts and hospitalized patients being predisposed to infection (I.j Abbot et al.,2011).The mortality rates ranged from 14% to 69% in patients with bacteremia by *Stenotrophomonasmaltopholia* (J.N Jang et al 1992l, M.A Victor 1994). *Stenotrophomonasmaltopholia*exhibits high-level intrinsic resistance to a broad spectrums of antibiotics, including β-lactams, quinolones, aminoglycosides, tetracycline, disinfectants, and heavy metals (I Zhang et al., 2000,Alonzo et al., 2009).*Stenotrophomonasmaltopholia* can also acquire resistance through the uptake of resistance genes located integrons, transposons, and plasmids (W J Loney et al., 2009). Therefore, infections caused by *Stenotrophomonasmaltopholia* are particularly difficult to manage because they show resistance to many classes of antimicrobial agents. The recommended therapeutic agents for *Stenotrophomonas. maltophila* infection is trimethoprim-sulfamethoxazole by the evidences of case reports and in vitro susceptibility studies (I J Abbot et al., 2009). Recently, combinations of antimicrobials have been recommended as treatment for*Stenotrophomonasmaltopholia*infection, especially in severe septic, neuropenic, debilitated or immunocompromised patients, or when trimethoprim-sulfamethoxazole cannot be used or tolerated (S I Liaw et al 2012, R RMuder et al 1996)

Characteristics

(www.labtestguide.com)

Gram negative, obligate rod, motile, few polar flagella

Habitat and transmission

Food,animal, water transmission

Diseases

Respiratory infections, endocarditis, ocula, skin and soft tissues

Laboratory Diagnosis

Culture, from body fluids, grows well on commonly used laboratory media, including blood and MacConkey agar

1.4.Essential oil used in this study

1.4.1. *Origanummajorana*L.

Origanummajorana L. which belongs to the mint family (*Lamiaceae)* which includes aromatic plants are extensively used for its medicinal, cosmetic, cultinary and ornamental purposes, which includes*rosemary, basil, orrgano, saga , lavender , thyme and mint*(Raja, 2012).The genus *Origanummajorana L.* is a multipurpose medicinal plant comprises of 42 species and 18 hybrid which is extensively distributed in Eurasia and North Africa(Letswaart, 1980, Duman et al., 1998) , a native to the mountainous areas of Mediterranean and Asia(CHISHT et al, 2012).

OriganummajoranaL.(*sweetmojorana*) is a herbaceous, perennial bushy shrub inhabiting dry slopes and rocky places which is endemic medicinal plant of Cyprus and is commonly known as " Sapsisia.(Johannes et,al 2002) reported sabinenelinalyl acetate and Cis–sabinenehydrate from essential oil of this species. It is used against common cold,as a spasmoltic, antirheumatic, diuretic, and antiasthmatic drug. Dried leaves and flowering tips of this species are used in the formulation and bitters. The essential oil is used for flavoring sausages, sauces, soups , condiments and other products.(de vincenzi et al. 1997). It is ued in perfumery (R.R Vera et al 1997) .The essential oil of marjoram of different origin was analysed for the composition andbiological activities (Teixeira et al.,2013; Hajlaoui et al., 20116);various extracts were studied for antimicrobialactivites(vagi et al., 2005;Leeja and Thoppil, 2007;2007 Abdel- Massih et al., 2010). It is used as diuretic,antiasthmatic and an antiparytic drug in india(yadava and khare, 1995)

In Cyprus *Origanummajaroma L* grows in the southern western and northern eastern part of the island ,in garigues, dry limestones hillside or openings of pine forest at altitude between sea level and it is 90M (R.D Meikle et al., 1985) It is popular among the locals which is known as sapsisia which is use as spice, herbal vingegars and tea with healing properties(R.D Meikle et al 1985).Morever it has been used to treat cancer as well(Johnson et al., 2002;leung et al.,2003). The essential oil extracts from endemic *Origanummjaorana L*as a potential alternative for malarial treatment (Guleret al., 2020)

However, in relation to the antimicrobial activity only a few endemic Cypriotpopulations have been studied to (N.Arnold et al.,1993).

Taxonomic Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Lamiales
Family: Lamiaceae
Genus: Origanum
Species: Origanummajorana

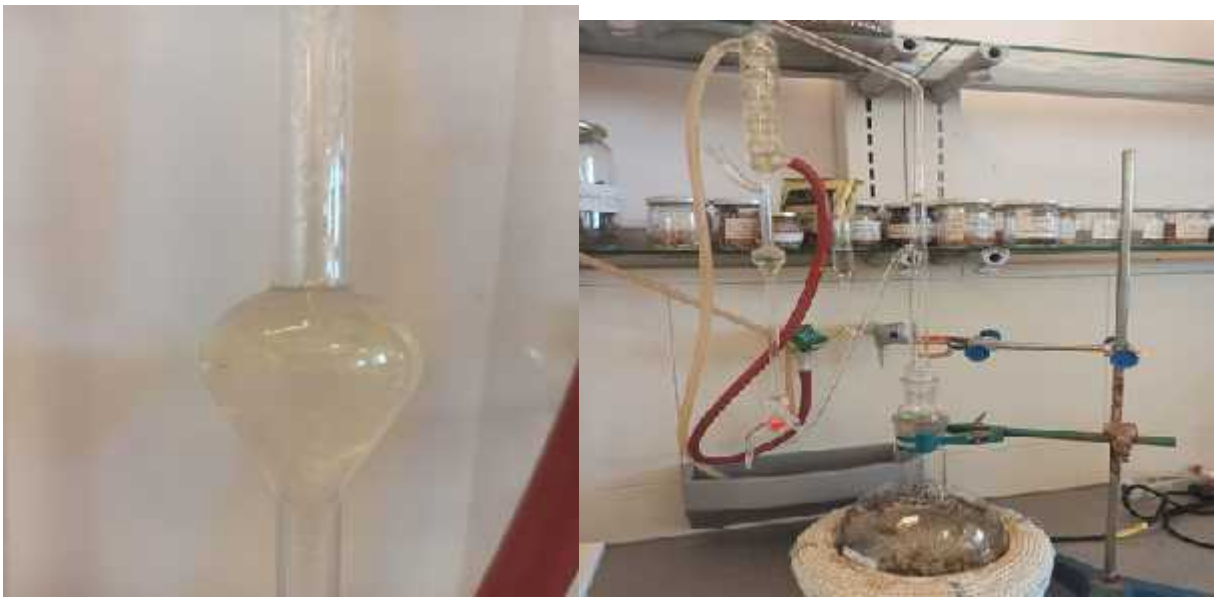
*Origanummajorana*L..in Northern Cyprus(R. D Meikle. et al.,1985 flora of Cyprus)

CHAPTER 2

MATERIALS AND METHODS

2.1 Isolation of Essential Oil

For the isolation of the essential oil of *Origanummajorana*L.samples were hydrodistilled in a Clevenger – type apparatus for 3h. The resulting oils were collected in a coloured bottle and stored at 4℃ until the analysis and activity experiments.



2.2 Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

2.2.1 GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5977B GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

2.2.2 GC analysis

The GC analysis was carried out using an Agilent 7890B GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.3 Identification of compounds

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their linear retention index (LRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, NIST Chemistry WebBook) (F. W. Mclafferty., 1989, S. E. Stein 2021) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification (D. Joulain., 1998, ESO 2000)

2.4 Organism and media

Test organisms used in this study were as, to assess the antimicrobial activites of the test samples 4 differentstrains of clinical isolates from NEAR EAST UNIVERSITY HOSPITAL were used in this study:*Staphylococcus aureus*(MRSA),*Escherichia coli, Pseudomonas aeruginosa*and*Stenotrophomonasmaltophilia*

The antibacterial activity of essential oil of *Origanummajorana*L.was tested against clinical isolates:

Table 2.1. Susceptibility of *Escherichia coli* Clinical Isolates Used in The Study

No	Code	A	Ce	Cf	Ct	Cr	Ci	Er	Fo	Ge	Im	Me	Ni	P/T	X	E
1	1893927	S	R	R	R	R	R	S	S	S	S	S	S	R	R	P
2	1933492	S	R	R	R	R	R	S	S	S	S	S	S	S	R	P
3	2106036	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N
4	2179533	S	-	S	S	S	S	S	-	S	-	S	-	S	S	N
5	2176111	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N
6	2174739	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N
7	2178872	S	R	R	R	R	R	S	S	R	S	S	S	S	R	P
8	2176543	S	S	S	S	S	S	S	S	S	S	S	S	S	R	N
9	2179169	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N
10	2179592	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N

KEYS: A- Amikacin, Ce- Cefixime, Cf-Ceftazidime, Ct-Ceftriaxone, Cr- Cefuroxime- axetil, Ci-Ciprofloxacin, Er-Ertapenem,Fo-Fosfomycin, Ge- Gentamicin,Im- Imipenem,,Me-Meropenem,Ni-Nitrofuration, P/T-Piperracillin/Tazobactam, X- SXT, E-ESBL , S-Ssensivity, R-Resistance ,N-positive,P-Positive

Table 2.2 Susceptibility of *Pseudomonas aeruginosa* Clinical Isolates Used in The Study

No	code	A	Az	Cef	Cf	Ci	Cl	Ge	Im	Le	Me	Ne	Pi	P/T	To	MDR
1	2159728	S	R	S	S	S	S	S	S	S	S	S	S	S	S	N
2	2161159	S	R	R	R	S	S	S	R	S	R	R	R	R	S	P
3	2122646	S	R	S	S	S	S	S	S	S	S	S	S	S	S	N
4	2123669	S	R	R	R	S	S	S	S	S	S	S	R	R	S	P
5	2128442	S	R	S	S	R	S	S	R	R	R	S	R	R	S	P
6	1514182	S	R	S	R	S	R	S	S	S	S	S	S	S	S	N
7	1744787	S	S	S	S	R	S	S	S	R	S	S	S	S	S	N
8	1513731	S	S	S	S	S	S	S	S	S	S	S	R	S	S	N
9	2170607	S	R	S	S	S	S	S	S	S	S	S	S	S	S	N
10	2179123	S	R	S	S	S	S	S	S	S	S	S	R	S	S	N

KEYS: A- Amikacin, Az- Azteronam, Cef- Cefepime, Cf-Ceftazidime, Ci-Ciprofloxacin, Cl- Colistin , Ge-Gentamicin,Im- Imipenem,, Le- Levofloxacin ,Me-Meropenem,Ne-Netilmicin, Pi-Piperacillin, P/T-Piperracillin/Tazobactam, To-Tobramycin, MDR, S- Positive, R- Resistance , P- Positive, N- Negative

Table 2.3. Susceptibility of *Stenotrophomonasmaltophilia*Clinical Isolates Used in The Study

No	Code	A	Az	Cef	Cf	Cr	Cl	Ge	Im	Le	Me	Ne	Pi	P/T	To	MDR
1	2121751	S	\R	S	S	S	S	S	R	R	R	S	R	R	S	P
2	1734242	S	S	S	R	R	S	R	S	S	S	S	S	S	S	N

KEYS: A- Amikacin, Az- Azteronam, Cef- Cefepime, Cf-Ceftazidime, Ci-Ciprofloxacin, Cl- Colistin , Ge-Gentamicin,Im- Imipenem,, Le- Levofloxacin ,Me-Meropenem,Ne-Netilmicin, Pi-Piperacillin, P/T-Piperracillin/Tazobactam, To-Tobramycin , MDR, S-sensivity,R- Resistance, P- Positive, N- Negative

Table 2.4 Susceptibility of *Staphylococcus aureus* Clinical Isolates Used in The Study

No	Code	Be	Cx	Ci	Cn	Da	Fo	Fu	Ge	Le	ld	Mu	Ni	Te	Ty	Tg	X	V	mrsa
1	2125478	R	R	S	R	S	R	S	S	S	S	S	S	R	R	S	S	S	P

KEYS: Be- Benzylpenicillin-Cx-Cefoxitin, Ci-Ciprofloxacin, Cn-Clindamycin, Da-Daptomycin, Fo- Fosfomycin, Fu- Fusidic acid , Ge- Gentamicin, Le- Levofloxacin Ld – Linezolid, Mu-Mupirocin Ni-Nitrofurantion, Te-Teicoplanin, Ty- Tetracyline, Tg- Tigecyline ,X-SXT, V-Vancomycin, MRSA-Meticillin-Resistant S. aureus , S-sensivity, R-Resistance, P-positive, N-Negative.

A total of 23 strains used in this study.All of the microorganism cultures were obtained from the Near East Hospital Microbiology Laboratory, Nicosia, Northern Cyprus. The strains were sub-cultured on an appropriate agar plate 24 hrs.Prior to any antimicrobial test.

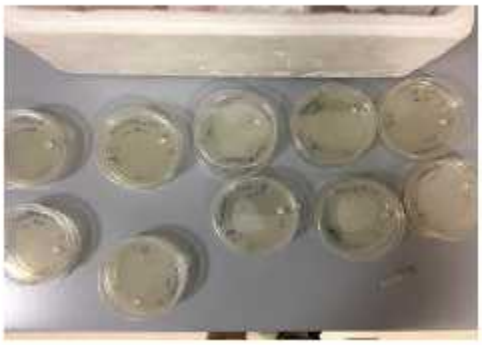
2.5 Culture Media Muller Hinton Agar, Eosin Methylene Blue (EMB), Blood Agar, Muller Hinton Broth(Oxoid)* were used.



2.6 Disc diffusion Assay

Bacterialinoculum was prepared from overnight culture (24h) on tryptone soya blood agar. Colonies were directly suspended in saline to obtain turbidity comparable to that of the 0.5 McFarland standards (approximately 1.5x108 CFU/ml). Aliquots (100µl) of inoculums were spread over the surface of pre-dried Mueller-Hinton agar (NCIPD, Sofia, Bulgaria) plates with a sterile glass spreader. Sterile 6 mm filter paper discs (NCIPD) were placed on the plates and immediately 20 µl portions of the essential oils were added. Sterile PS was used as control. The plates were left for 30 min at room temperature to allow the diffusion of oil and then they were incubated at 35°C for 24h. The inhibition zone was measured in millimeter (mm) and the assay was carried out in triplicate. The scale of measurement was the following (disk diameter included): ≥20mm zone of inhibition is strongly inhibitory;Vancomycin 30 mcg andAmikacin30 mcgantibiotics were used as positivecontrol.




CHAPTER3

RESULTS

3.1. Chemical composition of essential oil

26 compounds were identified representing 97.6% of the total essential oil. The major compounds were identified as *cis*-sabinene hydrate (11.6%), Terpinen-4-ol (24.1%) and (*E*-) ocimenol (12.6%), respectively.

Table 3.1 Percentage Composition of *Origanummajorana* L. endemic in Northern Cyprus

LRI	Compound Name	Relative Percentage Amount (%)
1015	α-pinene	1.3
1019	α-thujene	1.1
1067	Camphene	0.9
1114	β-pinene	0.5
1127	Sabinene	1.8
1167	Myrcene	1.8
1172	α-phellandrene	0.3
1187	α-terpinene	4.7
1206	Limonene	2.5
1216	1,8-cineole	0.4
1218	β-phellandrene	1.8
1255	γ-terpinene	8.5
1283	*p*-cymene	8.7
1294	Terpinolene	1.7
1472	*trans*-sabinene hydrate	4.4
1549	Linalool	0.9
1559	*cis*-sabinene hydrate	**11.6**
1564	Linalyl acetate	0.8
1599	Bornyl acetate	0.6
1618	Terpinen-4-ol	**24.1**
1622	β-caryophyllene	1.0
1640	4-terpinenyl acetate	1.4
1695	(*E*)-ocimenyl acetate	0.5
1711	(*E*)-ocimenol	**12.6**
1721	Borneol	3.2
1759	α-terpinyl acetate	0.7
	Total	97.6

LRI: Linear retention indices calculated against *n*-alkanes, %: calculated from FID data

3.2. Disc diffusion assay

The essential oil (EO) of *Origanummajorana.* L. was tested for its antimicrobial activity against selected clinical isolates by disc diffusion assay.

The results of disc diffusion assayare listed in Tables 3.2 to 3.5.*Origanummajorana*L.showed strong activity on both Gram positive and Gramnegative bacteria.

Table 3.2. Inhibition zone (mm) of essential oil against clinical isolates (*Pseudomonas aeruginosa)*

Test organism	*Origanummajorana*L.EO	Standard (Amikacin)
2128442	8mm	14mm
2123669	9mm	25mm
2122646	9mm	24mm
2161159	10mm	20mm
2159728	9mm	26mm
1514182	10mm	23mm
1744787	10mm	22mm
1513731	11mm	21mm
2179123	10 mm	20 mm
2170607	10 mm	25 mm

Table 3.3. Inhibition zone (mm) of essential oil against clinical isolates (*Escherichia coli*)

Test organism	*Origanummajorana*L.EO	Standard (Amikacin)
1933492	25mm	24mm
2106036	19mm	26mm
1893927	21mm	24mm
2179533	28 mm	25 mm
2176111	7 mm	24 mm
2174739	26 mm	25 mm
2178872	25 mm	25mm
2176543	20 mm	25 mm
2179169	26 mm	25 mm
2179592	26 mm	25 mm

Table 3.4. Inhibition zone (mm) of essential oil against clinical isolate (*Stenotrophomonasmaltophilia)*

Test organism	*OriganummajoranaL.* EO	Standard (Amikacin)
2121751	29mm	25mm
1734242	29mm	19mm

Table 3.5. Inhibition zone (mm) of essential oil against clinical isolate (*Staphylococcus aureus)*

Test organism	*OriganummajoranaL.* EO	Vancomycin
2125478	29 mm	24 mm

CHAPTER 4

DISCUSSION AND CONCLUSION

Search for natural antimicrobials is growing in current studies because of the undesirable health impact of synthetic antimicrobialfood preservatives and the occurrence of pathogenic microorganisms resistant to pharmaceuticals (Tajkarimi et al., 2010). In addition to the flavoring effect, *Origanum* species are proved to have antimicrobial activity on human and plant pathogens (Vági et al., 2005b; Leeja and Thoppil, 2000;Ashraf et al., 2011; Jaber et al., 2012; Chishti et al., 2013; Teixeira et al., 2013).

Table 3.1 shows the percentage composition of *Origanummajorana* fruit, 26 compounds were identified representing 97.6% of the total essential oil. The major compounds were identified as *cis*-sabinene hydrate (11.6%), Terpinen-4-ol (24.1%) and (*E*-)ocimenol (12.6%), respectively. The essential oil from Germany was reported to contain cis-sabinene hydrate, linalool, sabinene and b-caryophyllene as main constituents. French and Italian studies reported similar results (J .Novak et. Al 2004), but the oil from Turkey (K.H.C Baser, 1993) was reported to have a completely different composition, because *Origanummajorana L.* from Turkey contained 78% carvacrol. On the other hand, essential oils from Cuba (J.A Pino),Brazil (C.Busattaet.,et 2008), Hungary (E.Vag et a.,l 2005), and Tunisia (N.Benhamida-B.Ezzeddine et al, 2001) were reported to have terpinen-4-ol, g-terpinene and linalool as main components.

A more recent work conducted inTRNC by Güler et al. (2020) reveals that Origanummajorana .L has a percentage composition of the following: cis -sabinene hydrate 29.1%, Terpinen 4-ol 19.6%, γ-Terpineol 5.8% , and α – Terpienen 5.7%.

With all the studies from various scholars it reveals that Origanummajorana.L which is endemic in the Northern Cyprus has high percentage of Ternpinen- 4 –ol compared to othercountries. In such, it was noted in this research that the percentage chemical compositions of essential oils varies from countries, region, geographical settings and also the origin.

Antibacterial activity was measured using the agar diffusion method. Clinical isolates from Near East University hospital of both gram positive and gram negative bacterial which made total of

23 strains of microorganisms used. The resultswere recorded in Table 3.2 to 3.5Origanummajorana L. showed significant activity against all the tested organisms, namely,*Escherichia coli* (10 isolates),*Staphylococcusaureus* (1 isolate),*Psedomonasauginosa*(10 isolates) and*Stenotrophomonasmaltophilia* (2 isolates). The essential oil was more active than the standard amikacin against *Stenotrophomonasmaltopholia* and vancomycin against *Staphylococusaureus*, respectively. Previous study conducted by Ben et al. (2001) suggests that the essential oil of *Origanummajorana*L possess antibacterial activity. A work conducted by Farooqi and Sreeramu (2004) reveals that the leaves of marjoram have antimicrobial activity against *Bacillus anthracis*, *Proteus vulgaris*, *Salmonella enterica*serovar Stanley, *Salmonellaentericas*erotype Newport, *Streptococcus agalactiae*, and *Aspergillus fumigatus*.

The essential oil of *Origanummajorana*L. endemic in NorthenCyprus shows antimicrobial property against the clinical isolates of *Escherichiacoli, Staphylococcus aureus, Psedomonasauginosa* and *Stenotrophomonasmaltophili*.

The essential oil was more active than the standard amikacin against thetwo clinical isolates of*Stenotrophomonasmaltopholia* and vancomycin against one isolate of*Staphylococusaureus* respectively.Due to its strong microbicidal property and superiority over commercial microbicides, *Origanummajorana* L. essential oil may be an effective herbal protectant against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic and ecofriendly.

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Postgraduate/Special	Medical and Clinical Microbiology	2021
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Undergraduate	Fourah Bay College, University of Sierra Leone	2010
Highschool	Prince Wales School, Sierra Leone	2005

JobExperience

Duty	**Institution**	**Duration(Year -**
Asst.Examiner.	WAEC	2016-2019
Teaching	St. Augustine sch. The Gambia	2015-2019
Teaching	Gambia High School, The Gambia	2014-2015

ForeignLanguag	**Readingcomprehe**	**Speaking***	**Writing***
English	Very good	Very good	Very good
French	Poor	moderate	poor

ForeignLanguageExaminationGrade								
YDS	UDS	IELTS	TOEFLI BT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

	Math	**Equallyweight**	**Non-math**
ALES Grade	N/A	N/A	N/A
(Other) Grade	N/A	N/A	N/A

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Program	**Useproficiency**
N/A	Moderate