GRADUATE INSTITUTE OF HEALTH SCIENCE

ANTIMICROBIAL ACTIVITY *OF SCHINUS MOLLE* L. ESSENTIAL OIL GROWING IN NORTHERN CYPRUS

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SUPERVISOR

Assist. Prof. Dr. GünerEkiz

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APPROVAL

This thesis prepared and presented by **Ighoyinvwin Peter Odogun**entitled as **"Antimicrobial Activity *of Schinusmolle*L. Essential Oil growing in Northern Cyprus"**has been accepted by the Examination committee for the degree of Master of science in Medical and Clinical Microbiology.

Examination committee

Advisor and

Chair of the commiteeAssist. Prof. Dr. GünerEkiz

N.E.U, Faculty of Pharmacy
Department of Pharmaceutical Microbiology

Members: **Assoc. Prof. Dr. Ayse A. Sarıoğlu**

N.E.U, Faculty of Medicine
Department of Medical and Clinical Microbiology

Assist. Prof. Dr. Aye Seyer

C.I.U, Faculty of Medicine
Department of Medical Microbiology

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

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Acknowledgement and Dedication

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Dedication

This work is dedicated to my Late mum Mrs. H.O. Odogun for without her, I won't be where I am today.

ABSTRACT

The recent increase of resistant microorganisms to preexisting synthetic antimicrobial drugs calls for concerns and requires more research to develop novel antimicrobial drugs from natural sources. Secondary metabolites from plant have proven to be effective against microorganism as well as possessing other therapeutic and nutritional functions. This present study is aimed at testing the antimicrobialactivities of essential oil of *Schinusmolle* on Clinical isolates from Near East Hospital.

The essential oil of *Schinusmolle*was providedby Department of Pharmacognosy, Near East University, Faculty of Pharmacy.

The essential oil was evaluated for antimicrobial activity against the clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Stenotrophomonasmaltophilia* using disc diffusion assay. The essential oil of *Schinusmolle* showed strong antimicrobia activity against *Staphylococcus aureus* (MRSA), followed by *Stenotrophomonasmaltophilia* and *Escherichia coli*.

This result indicates the possible use of essential oils of *Schinusmolle* as antibacterial agent however; there is need for further clinical study to ascertain fully its effectiveness and potential toxic effect.

KEYWORDS: *Schinusmolle* L, Antimicrobial Activity, Essential Oils, Ethnomedicine, Northern Cyprus.

1. INTRODUCTION

Plants are a gem from the standpoint of pharmacology. In reality, useful drugs can be found in the plant or its secondary metabolites. They continue to be the primary source of bioactive compounds that can be used directly in therapies or inspire the production of more active derivatives (Cragg, G.M and Newman, D.J 2013). Since ancient times, natural products have been used as essential drug sources. In recent years, there has been growing interest in obtaining biologically active compounds from natural sources (Hassine et al., 2014).Secondary metabolites are a group of chemical compounds that are not specifically necessary for plant survival but are synthesized to improve the plant's resistance to environmental factors, pathogen attacks, and nutrient deprivation. The preservation (antioxidants) and defense (antibiotics, insecticides, and herbicides) activities of these secondary metabolites against external aggressions are their primary functions (Arshad et al., 2017). Chemicals found in small quantities in plants are known as bioactive compounds from plant material. Polyphenols, terpenoids, alkaloids, and carotenoids are among the bioactive groups found in natural plant extracts. Phenols are, without a doubt, one of the most bioactive substances (Kumar, A., Naraian, R., 2019).

Essential oils and their derivatives are widely used in medicine as ingredients in a variety of pharmaceuticals, in the food industry as flavoring additives, and in cosmetics as scents, as well as in the medical and pharmaceutical sectors (Amenu, 2014). Essential oils are aromatic vegetable oily liquids composed of a variety of low molecular weight volatile monoterpenes, sesquiterpenes, and isoprenes (Pawlowski et al., 2012).They contain a range of bioactive metabolites, and their antioxidant, antibacterial, and antifungal properties make them popular in food, cosmetics, and industrial (Diao et al.,

2013, Rosas-Burgos. Cortez-Rocha, Cinco-Moroyoqui et al., 2009, Bettaieb 2010). They also comprise a vast number of plant items that release the scents of the fragrant plants from which they were harvested (Pawlowski et al., 2012).

Herbal medicine's importance cannot be overstated, as it has been instrumental in the development of new medicines (Al- Rimawi et al., 2020). Essential oils have a long history as natural compounds with pharmacological, aesthetic, agrochemical, and nutritional applications in pharmaceutical sciences (Bakkali et al., 2008). The usage of essential oils (EO) in the form of aromatherapy or phytotherapy is widespread, with some of them being utilized as anti-anxiety and anti-stress agents (Setzer, 2009). Aromatherapy, according to (Avato et al., 2017), is a subset of phytotherapy and is defined as the use of essential oils for therapeutic purposes. These products have been used for centuries and are recognized by both traditional and modern medical systems.Many diseases have been treated and prevented with medicinal plants, and have been used as anticancer, analgesic, antipyretic, antibacterial, anti-inflammatory, and antidiabetic agents (Salameh et al., 2020). Integrative herbal medicine, according to the World Health Organization (WHO, P. Health (Ed.),is a major source of primary health care for people living in developing countries. Traditional Medicine, World Health Organization, Geniev, 2013), is a major source of primary health care for people living in developing countries.

2. LITERATURE REVIEW

2.1. Ethnopharmacological Usage of *Schinusmolle*

In traditional medicine, essential oils of*Schinusmolle* L have been used as antibacterial, antiviral, topical antiseptic, antifungal, antioxidant, anti-inflammatory, anti-tumor, antispasmodic, and analgesic. However, pharmacological and toxicological investigations of their effects are limited (Do et al., 2013). It has been used to treat toothaches, rheumatism, menstrual irregularities, and respiratory and urinary tract infections (Barrachina MD et al., 1997 and Bello R et al., 1998).The presence of bicyclogermacrene (20.5%), betacaryophyllene (19.7%), and spathulenol (19.2%) in the current leaf EO, according to researchers, can legitimize the use of *S. molle*in traditional medicine (Doleski MPS, Ferreira CCH, Calil BJ, et al., 2015).

2.2. *Schinusmolle*

Schinusmolle L. belongs to the Anacardiaceae family and is an evergreen plant. *S. molle* is a dioecious plant with compound, imparipinnate, and lanceolate leaves that when crushed have a spicy odor.It is also known as "pink pepper" or "false pepper." The flowers are yellowish-white and arranged in bunches on pendulous branches; the fruits are coral-red and peppercorn-sized (Kasimala and Kasimala, 2012).According to the United States Department of Agriculture, Schinusmolle (Peruvian pepper), sometimes known as American pepper or fake pepper, is a fast-growing evergreen tree that can reach a height and width of 15 meters (50 ft). The tree is found in the arid zone of northern South America, including the Andean deserts of Peru, as well as central Argentina and central Chile (Blood, Kate, 2001).It can also be found in Asia, Australia, and parts of Europe, and in Cyprus and Turkey, it is considered exotic (Orwa et al., 2009). It is distinguished from the closely related S. terebinthifoliusRaddi by its compound leaves (Brazilian pepper tree). *S. molle* fruits are small, spherical berries with a diameter of 5-9 mm that ripen to a bright red color before becoming black (Orwa et al.,

2009). S. molle is a drought-tolerant, enduring, hardy evergreen that has spread over the world as a major invasive weed (Iponga, et Al., 2008).The essential oils isolated from *S. molle* leaves inhibited the growth of *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella setubals, and Candida albicans,* among other bacteria (Simionatto et al., 2011).The antioxidant activity of the essential oil extracted from the fruits was higher than that of the oil extracted from the leaves (Abir et al., 2016). Five terpenes extracted from the bark resin of *S. molle* inhibited the development of human colon cancer cells significantly (Gonzalo et al., 2017).

Table 2.1. Taxonomical classification of *Schinusmolle*

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Anacardiaceae
Genus:	*Schinus*
Species:	***S. molle***

FIGURE 2.1. Fruits and leafs of *Schinusmolle*

2.3. Plant Secondary Metabolites

Secondary metabolites (SM) are substances that aren't required for a cell's (organism's) survival but help the cell (organism) interact with its environment (Pagare*et al*., 2015). Plant secondary metabolites (SMs) are a really useful source of natural products as well as an essential component of the plant's protection mechanism against pathogens and environmental stresses. Plant SMs are increasingly used as medication ingredients and food additives for medicinal, aromatic, and culinary purposes due to their remarkable biological activities (Yang et al., 2018). Plants can generate an infinite number of aromatic secondary metabolites, with phenols and their oxygen-substituted derivatives accounting for the majority. Subcategories of this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins. Because these chemicals have antibacterial characteristics, plants use them to protect themselves from pathogenic bacteria (Gurjar et al., 2012).

2.3.1. Secondary Metabolites of *Schinusmolle*

According to studies carried out by Bendaoud*et al*. (2010)monoterpenoids and sesquiterpenoids make up the majority of essential oils purified from *S. molle*, with -phellandrene and sylvestrene being the most prominent components(Machado et al., 2019)explains that the some studies carried out on the Volatile oil of the aerial part of *Schinismolle*have reported that the major compounds of the volatile oil from leaves and fruits are monoterpenes (Abdel-Sattar et al., 2010, Gomes et al., 2013). However, sesquiterpenes were the main components in different studies by Simionatto*et al*. (2011) and Cavalcanti*et al*. (2015). The main components of VO of *S. molle* were the monoterpenes β-pinene (14.7%), α-pinene (14.1%), limonene (9.4%) and the sesquiterpenemuurolol (11.8%) (Machado *et al*., 2019)

Table 2.2. Chemical composition of Essential oil of *Schinusmolle*(Abrha & Cr, 2014).

Peak No	**RI**	**Compounds Identified**	**% Composition**
1	986	α- Pinene	8.7
2	986	trans-Piperitol	3.5
3	1024	α-Phellandrene	20.6
4	1048	β-Pinene	5.1
5	1030	β –Phellandrene	10.8
6	1130	β –Myrcene	6.9
7	1140	β-Elemene	5.1
8	1390	Copane	6.5

9	1423	Isolidene	1.7
10	1428	Germacrene	5.8
11	1448	α- Cubebene	1.4
12	1480	Aristolene	1.8
13	1520	δ-Cadinene	6.3
14	1560	α-Humulene	5.4
15	1620	α-Gurjunene	1.3
16	1640	β-Caryophyllene	1.4

The preceding result by Abrha & Cr, (2014) is consistent with the result carried out in North Cyprus by Alnawari *et al.*,(2018).

2.3.2. Terpenes

Terpenes are the most abundant secondary metabolites and are free of acetyl-coA or glycolytic intermediates due to their common metabolic origin. Monoterpenes, sesquiterpenes, diterpenes, triterpenes, and polyterpenes are the different types of terpenes. The pyrethroid (monoterpene esters) present in the leaves and flowers of Chrysanthemum species have significant insecticidal effects, while phorbol (diterpene ester) present in plants of the Euphorbiaceae family irritates the skin and causes internal poisons in animals (Pagare et al., 2015).Diterpenes, triterpenes, and tetraterpenes (C20, C30, and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15), share a typical chemical structure of C10H16 and come in a variety of forms (Mohammed et al., 2014). Terpenoids are organic compounds that contain additional elements, the most common of which being oxygen. Terpenoids are similar to fatty acids in that they are built up of acetate units. They are cyclicized and have a lot of branching, which sets them apart

from fatty acids (Vishwakarma, 1990).Common terpenoids include methanol and camphor (monoterpenes), farnesol and artemisin (sesquiterpenoids), and artemisin and its derivative arteether (also known as qinghaosu)(NaseemUllah, Farhat and Ali Khan, 2016). In 1985, the steering committee of the World Health Organization's scientific working group decided to develop the latter drug as a treatment for cerebral malaria.Terpenes, also known as terpenoids, have antibacterial, antifungal, antiviral, and antiprotozoal properties (Chandra et al., 2017). In 1997, it was ascertained that 60% of essential oil derivatives tested so far were fungus inhibitors, whereas 30% were bacteria inhibitors (Chaurasia and Vyas, 1997). Betulinic acid, a triterpenoid, is one of numerous terpenoids that have been found to restrain HIV (Pengsuparp et al., 1995).The exact method of action of terpenes is unknown; however it is thought that the lipophilic molecules disrupt membranes. As a result, Mendoza et al. (1997) discovered that adding a methyl group to kaurenediterpenoids lowered their antibacterial efficacy dramatically. Scientists have discovered that terpenoids found in plant essential oils can help manage Listeria monocytogenes (Pirbalouti&Rahimi, 2010).The ethanol-soluble fraction of terpenoids had shown outstanding effectiveness against *Bacillus subtilis and Staphylococcus aureus, Acinetobacter sp., K. pneumoniae, Proteus sp., Micrococcus sp., Staphylococcus epidermidis, and gram-negative bacteria,* as well as *Candida albicans*, with lesser activity against gram-negative bacteria and *Candida albicans* (Othan et al., 2019).Steglich esterification was used to make esters based on mono- and bicyclic terpenoids with glycine, which were then characterized by 1H-NMR, IR, and mass spectrum analyses. Their analgesic and anti-inflammatory effects were tested on formalin, capsaicin, and AITC-induced pain models after transdermal application. Glycine esters of menthol and borneol had the strongest antinociceptive efficacy, whilst the eugenol derivative successfully suppressed the inflammatory process.The competitive binding of terpenoid esters and TRPA1/TRPV1 agonists has been proposed as a possible reason for the synthesized derivatives' significant analgesic effect. The strong anti-inflammatory activity has been attributed to a competitive inhibition between

terpenoid esters and AITC for binding sites on the TRPA1 ion channel (Nesterkina and Kravchenko, 2017).

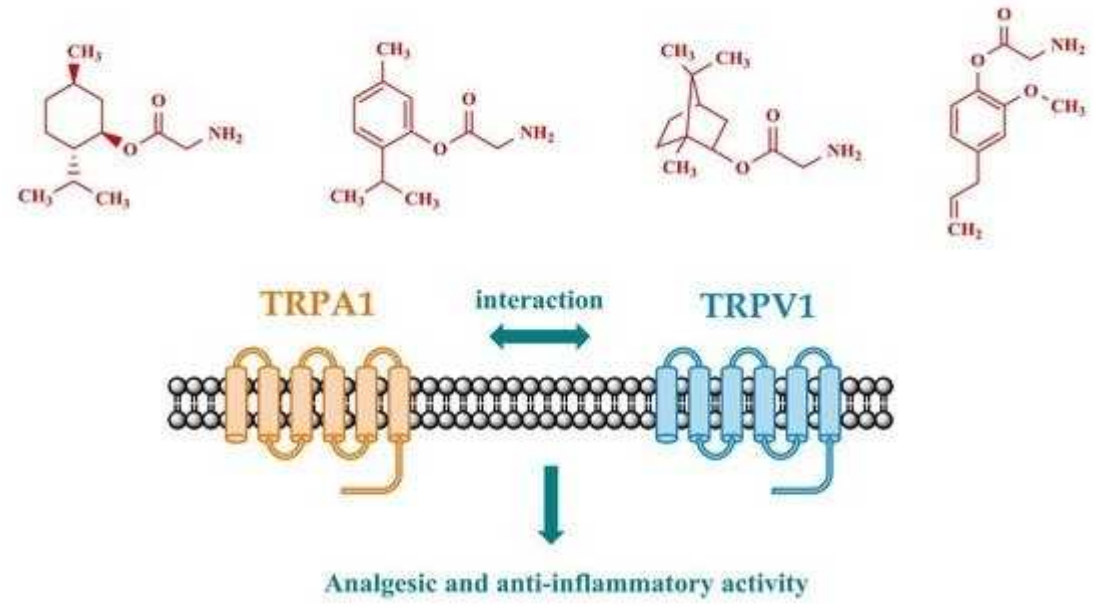

FIGURE 2.2. Analgesic and anti-inflamatoryactivity

2.4. Pharmacological Studies

According to Machado et al (Machado et al., 2019),pharmacological studies have reported several properties such as sedative (Taylor et al., 2016), anti-inflammatory (Yuenqin et al., 2003), antimicrobial activity against Staphylococcus aureus and Streptococcus pyogenes (Guerra-Boone et al., 2013), trypanocidal (Molina-Garza et al., 2014), repellent and insecticidal properties against *Triatomainfestans*, the vector of Chagas' disease (Ferrero et al., 2006). Biological activities have also been described for the volatile oil, such as antibacterial (Pellegrini et al., 2017), antifungal (Martins et al., 2014), cytotoxic (Díaz et al., 2008), and insecticidal against *Haematobia irritans* L. (López et al., 2014). Moreover, the volatile oil is used as an adjuvant in various applications in food products because of its antimicrobial and antioxidant properties or as antiparasitary in cattle and beekeeping (Guala et al., 2016).

2.5. Bioactivity

The term "bioactivity" refers to the ability to produce specific effects after being exposed to a substance; these effects may involve tissue absorption, metabolism, or physiological reaction (Kara *et al.,* 2017). Bioactivity can be determined using in vivo, ex vivo, and in vitro methodologies. However, only in vivo assays can accurately predict a compound's bioactivity responses (Carbonell-Capella *et al.,* 2014).

2.6. Nutritional Value of *Schinusmolle*

In traditional cuisine, as well as in the creation of alcoholic cocktails and beverages, *Schinusmolle* fruits (berries) have been used as a substitute for black pepper (Marongiu et al., 2004).

2.6.1 Usage and Applications in Food

The essential oil of *S. molle* L. is used to reduce the proliferation of ectoparasites in livestock and apiculture, according to (Guala et al., 2016). When utilized in meals, it also possesses antibacterial and antioxidant qualities. Its parasiticide properties ensure the safety of honey, wax, and propolis in apiculture, as well as the absence of contamination of meat and milk in cattle production. It should be noted that contamination of these items can occur when synthetic products are used in excess, as they are difficult to remove.Externally applied *S. molle*derived products induce their effects when the parasite comes into touch with the oil. These items are safe to handle and do not leave any polluting residue.

This essential oil has also been shown to suppress the growth of *Staphylococcus aureus* and *Escherichia coli*, making it a promising antibacterial agent. The results of the experiments done so far as an antioxidant are promising. Because of its high solubility, the oil can be utilized as a substitute for synthetic antioxidants in canned foods and creams. Furthermore, the oil has no effect on the product's organoleptic qualities.

2.7. Microorganisms

Clinical isolates from Near East University Hospitalutilized in this investigation includes; *Escherichia coli*1933492, 2106036, 1893927, *Staphylococcus aureus* 2125478, *Pseudomonas aeruginosa* 2159728, 2161159, 2122646, 2123669, 2128442, 1514192, 1744782, 1513731 and *Stenotrophomonasmaltophilia*2125478.

2.7.1 *Psuedomonas aeruginosa*

The opportunist pathogen *Pseudomonas aeruginosa* belongs to the *Pseudomonas* genus (Mackie and McCartney, 1989). It's a gram-negative, asporogenous, monoflagellated, rod-shaped bacterium having a wide dietary range.It's a 1-5µm long and 0.5-1.0µm high rod. *P. aeruginosa* is an obligate respirer that prefers aerobic (oxygen-rich) to anaerobic (oxygen-depleted) respiration (with nitrate or other alternative electron acceptors). *P. aeruginosa* has the ability to catabolize a wide variety of organic molecules, including benzoate. As a result, *P. aeruginosa is* a very common microorganism with a ubiquitous nature (Lederberg, Joshua et al., 2000). *P aeruginosa* grows best at 37–42 degrees Celsius, and its ability to expand at 42℃ Celsius sets it apart from other Pseudomonas species.While several strains do not ferment carbohydrates, they do oxidize glucose (Brooks et al., 2007). *P. aeruginosa* was chosen as a pathogen based on epidemiological evidence that it is a leading cause of serious sepsis and MODS in the Intensive Care Unit's nosocomial setting (Lodise et al., 2007). According to (Jouda, 2013), *Pseudomonas aeruginosa* is rarely connected with infections in healthy persons, although it has been linked to a number of human infections in persons who are hospitalized or immunocompromised due to other illnesses: • Infection at the surgical site • Infections of the lungs • Burn sepsis • Skin and soft tissue infections (hot tub folliculitis, osteomyelitis), Ocular and ear infections (external otitis, malignant external otitis), HIV/AIDS, cystic fibrosis, chemotherapy-induced neutropenia, and diabetes all increase the risk of infection and complications (Trautmann et al., 2008).

Table 2.3. Taxonomical classification of *P. aeruginosa*

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species	***P. aeruginosa***

FIGURE 2.3.*Pseudomonas aeruginosa* on basic cultivation media

2.7.2. *Escherichia coli*

In the usual colonic flora, the Enterobacteriaceae family's most common member, as well as the most prevalent source of opportunistic infections, is *Escherichia coli* (Sherris, 1984).Dr. Theodor Escherich, who discovered the bacteria, is the name of the genus Echerichia (Gould, 2011).The rod-shaped Gramnegative bacillus lives in the large intestine and is commonly excreted in feces (Woodward, 2015). *Escherichia coli* cells are typically 1.1–1.5-µm-wide, 2–6-µm-long, occurring as single straight rods. They can be either motile or nonmotile. On the cell wall of *Escherichia coli* are strain-specific O lipopolysaccharide antigens (at least 188 O antigens are currently recognized) and, if present, flagella or H antigens (at least 53 H types are recognized). There are several capsular polysaccharide (K) antigens as well. The combination of O, H, and K antigens is used to serotype *Escherichia coli*. (P. Desmarchelier, N. Fegan, 2016) *E. coli* grows best around 37^{o}C (98.6^{o}F). Some laboratory strains, on the other hand, may proliferate at temperatures as high as 49°C (120°F)(Fotadar,2005). Virulent *Escherichia coli* strains cause the majority of diarrheal infections, meningitis, septicemia, and urinary tract infections in children (Makvana and Krilov 2015).

Table 2.4. Taxonomical classification of *E.coli*

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	*Escherichia*

Species ***E. coli***

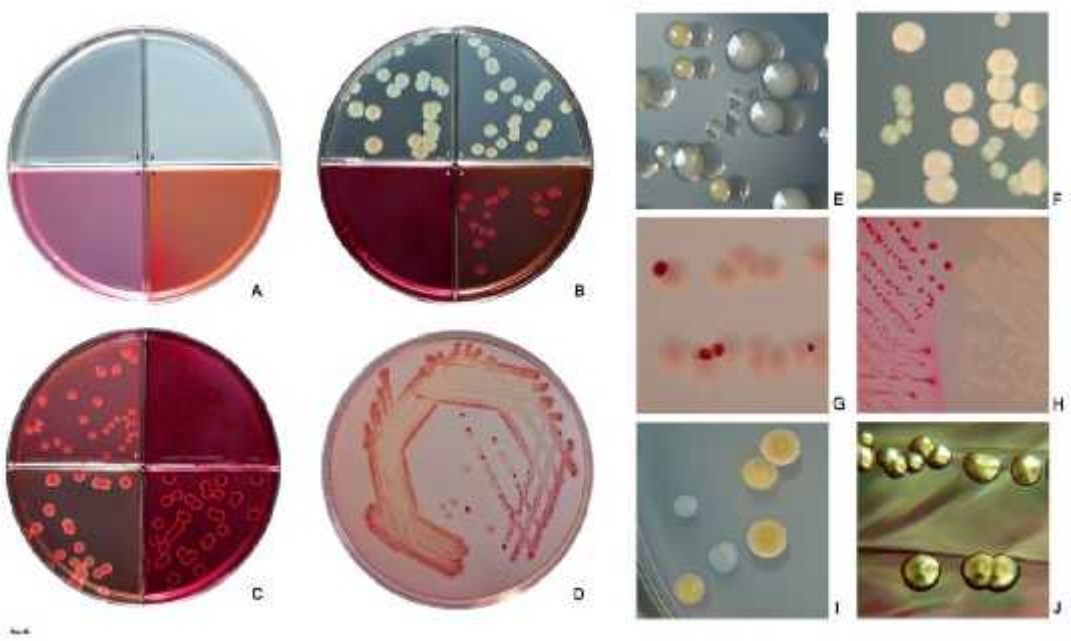

FIGURE 2.4.*E.coli* on basic cultivation media

2.7.3. Staphylococcus aureus

Staphylococci are spherical cells with a diameter of about 1m that form irregular clusters. Liquid cultures may contain single cocci, pairs, tetrads, and chains. Young cocci are gram-positive, but as they age, many of them become gram-negative. Staphylococci are non-motile bacteria that don't produce spores (Brooks et al, 2007).*Staphylococcus aureus* are Gram-positive bacteria that are cocci-shaped and cluster in "grape-like" clusters (stain purple by Gram stain) (Taylor and Unakal, 2020). These species may grow in up to 10% salt on media, and colonies are frequently golden or yellow in color (aureus means golden or yellow). At temperatures ranging from 18 to 40°C, these bacteria can grow aerobically or anaerobically (facultatively). *Staphylococcus aureus* is a gram-positivebacteria that can cause a wide range of illnesses.This pathogen causes infections in both community-acquired and hospital-acquired environments (Taylor and Unakal, 2020). Infections are widespread in both

community and hospital settings, and treatment is difficult to manage because of the advent of multi-drug resistant forms like MRSA (Methicillin-Resistant Staphylococcus aureus) (Centers for Disease Control and Prevention CDC 2002-2003, Boucher HW, Corey GR 2008). *S. aureus* is one of the most common bacterial infections in humans, responsible for infections such as bacteremia, infective endocarditis, and skin and soft tissue infections (Taylor and Unakal, 2020).

Table 2.5. Classification of *Staphylococcus aureus*

Domain	Phylum
Phylum	Bacteria
Class	Firmicutes
Order	Firmicutes
Family	Bacillales
Genus	Staphylococcaceae
Species	*Staphylococcus*

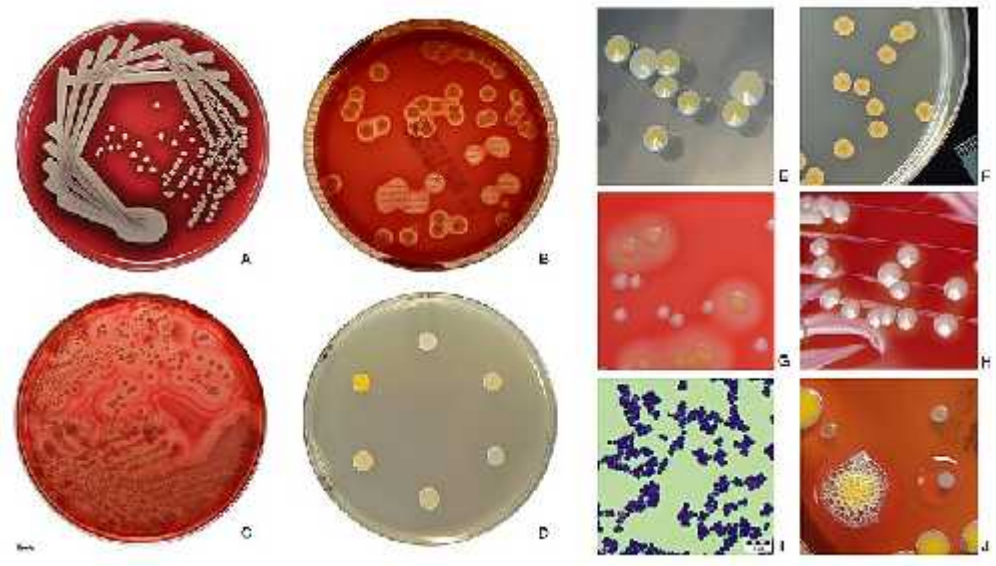

FIGURE 2.5.*Staphylococcus aureus* view on basic cultivation media

2.7.3.1. Methylene resistant *Staphylococcus aereus*

MRSA (Methicillin-resistant *Staphylococcus aureus*) according to (Jouda, 2013) is a typical cause of nosocomial infections. These infections are exceedingly difficult to treat since MRSA strains are resistant to almost all clinically available medicines (Adwan and Mhanna, 2008). People who have not recently been hospitalized or undergone a medical procedure get MRSA infections linked to healthcare (such as dialysis, surgery, or catheters). MRSA (HA MRSA) was first detected in the 1960s and has been associated to health-care-related risk factors such as hospitalization or nursing home care, dialysis, antibiotic therapy, or exposure to invasive devices or procedures. In comparison to infections caused by susceptible strains of *S. aureus*, HA MRSA is a highly resistant and important nosocomial pathogen in both acute care and long-term care settings, causing infections associated with increased morbidity, mortality, and cost (Cuaresma et al., 2008).Community-acquired MRSA (CA MRSA) infections first arose in the 1990s in

patients who had never had any of the MRSA risk factors previously reported. CA MRSA infection is now defined as MRSA infection in a person who has had no prior health-care exposure, such as hospitalization, surgery, permanent intravenous lines or other indwelling devices, or hemodialysis (Davis and Fox, 2005).

In otherwise healthy patients, CA-MRSA infections generally manifest as skin infections such as pimples and boils. They're commonly mistaken for "spider bites," and if they're not treated right once, they can lead to serious infections (www.bop.gov).

2.7.4 *Stenotrophomonasmaltophilia*

Stenotrophomonasmaltophilia is an aerobic, nonfermentative,Gram-negative bacterium. It is an uncommon bacterium and human infection is difficult to treat (Gilligan *et al*., 2003). It is rod shaped and motile with a few polar flagella with optimum growth temp of 35°C and showing no growth at 4°C or 41°C (Brooke, 2012). Initially classified as Bacterium booker (Chang et al., 2005) then renamed *Pseudomonas maltophilia*.*S. maltophilia* was also grouped in the genus *Xanthomonas* before eventually becoming the type specie of the genus *Stenotrophomonas* in 1993 (Denton and Kerry, 1998, and Palleroni and Bradbury 1993).

Table 2.6. Classification of*Stenotrophomonasmaltophilia*

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Xanthomonadales
Family:	Xanthomonadaceae
Genus:	*Stenotrophomonas*

Species: ***S. maltophilia***

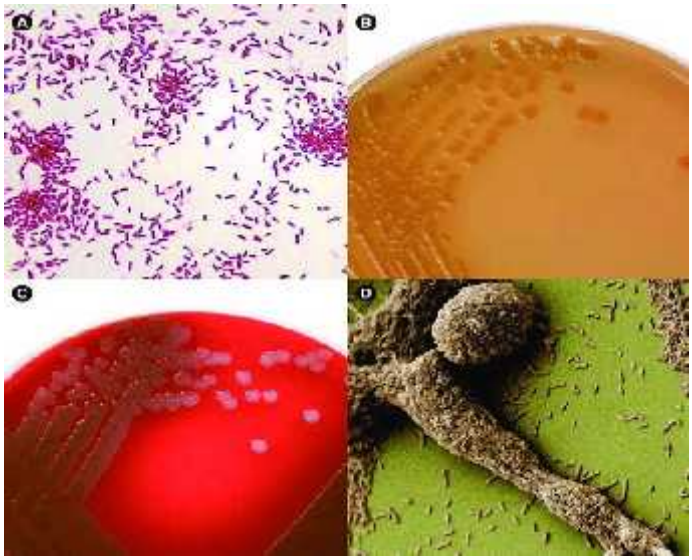

FIGURE 2.6. Laboratory appearance of *Stenotrophomonasmaltophilia*

3. MALTERIALS AND METHOD

3.1 Materials

3.1.1. Plant material collection

Essential oil of *Schinusmolle* was kindly provided by Assist. Prof. Dr. AzmiHanoğlu (Alnawari, 2018).

3.1.2. Identification of components

According to Alnawari *et al.*,(2018), the essential oil components were characterized by comparing their retention periods to those of original samples or by contrasting their Linear Retention Indices (LRI) to a series of n-alkanes. The identification was carried out via computer matching against commercial Wiley GC/MS libraries, MassFinder 3 libraries, and an in-house "Baser Library of Essential Oil Constituents" made up from actual chemicals and components of known oils, as well as MS literature data.

3.1.3. Test Organisms

The antibacterial activity of essential oils was tested against clinical isolates: ***Escherichia coli***1933492, 2106036, 1893927,2179533, 2176111, 2174739, 2178872, 2176543, 2179169, 2179592,***Staphylococcus aureus*** 2125478,***Pseudomonas aeruginosa***2159728, 2161159, 2122646, 2123669, 2128442, 1514192, 1744782, 1513731, 2170607, 2179123 and ***Stenotrophomonasmaltophilia***2121751, 1734242.

All of the microorganism cultures were obtained from the Near East Hospital Microbiology Laboratory, Nicosia, North Cyprus. The strains were sub-cultured on an appropriate agar plate 24 h prior to any antimicrobial test.

Table3.1. Susceptibilityof the clinical isolate used in the study

Pseudomonas aeruginosa

No	Barkod	Cefe	Cefta	Cipro	Col	Gent	Imi	Levo	Mero	Net	Pip	Pip/Taz	Tob	MDR
1	2159728	S	S	S	S	S	S	S	S	S	S	S	S	Negative
2	2161159	R	R	S	S	S	R	S	R	R	R	R	S	Positive
3	2122646	S	S	S	S	S	S	S	S	S	S	S	S	Negative
4	2123669	R	R	S	S	S	S	S	S	S	R	R	S	Positive
5	2128442	S	S	R	S	S	R	R	R	S	R	R	S	Positive
6	1514182	S	R	S	R	S	S	S	S	S	S	S	S	Negative
7	1744787	S	S	R	S	S	S	R	S	S	S	S	S	Negative
8	1513731	S	S	S	S	S	S	S	S	S	R	S	S	Negative
9	2170607	S	S	S	S	S	S	S	S	S	S	S	S	Negative
10	2179123	S	S	S	S	S	S	S	S	S	R	S	S	Negative

Escherichia coli

No	Barkod	Cefta	Ceft	Cefu	Cipro	Erta	Fos	Gent	Imi	Mero	Nit	Pip/Taz	SXT	ESBL
1	1893927	R	R	R	R	S	S	S	S	S	S	R	R	Positive
2	1933492	R	R	R	R	S	S	S	S	S	S	S	R	Positive
3	2106036	S	S	S	S	S	S	S	S	S	S	S	S	Negative
4	2179533	S	S	S	S	S	-	S	-	S	-	S	S	Negative
5	2176111	S	S	S	S	S	S	S	S	S	S	S	S	Negative
6	2174739	S	S	S	S	S	S	S	S	S	S	S	S	Negative
7	2178872	R	R	R	R	S	S	R	S	S	S	S	R	Positive
8	2176543	S	S	S	S	S	S	S	S	S	S	S	R	Negative
9	2179169	S	S	S	S	S	S	S	S	S	S	S	S	Negative
10	2179592	S	S	S	S	S	S	S	S	S	S	S	S	Negative

Stahylococcus aureus

No	Barkod	Ben	Cefo	Cipro	Cli	Dap	Fos	Fus	Gent	Levo	Lin	Mup	Nit	Tei	Tig	SXT	Van	MRSA
1	2125478	R	R	S	R	S	R	S	S	S	S	S	S	R	S	S	S	Positive

Stenotrophomonas maltophilia

No	Barkod	Ami	Azt	Cefe	Cefta	Cipro	Col	Gent	Imi	Levo	Mero	Net	Pip	Pip/Taz	MDR
1	2121751	S	R	S	S	S	S	S	R	R	R	S	R	R	Positive
2	1734242	S	S	S	R	R	S	R	S	S	S	S	S	S	Negative

Key: Ami- Amikacin, Azt- Aztreonam, Ben- Benzylpenicillin, Cefo- Cefoxitin, Cef- Cefixime, Cipro- Ciprofloxacin, Cefta- Ceftazidime, Tet- Tetracycline, Tob- Tobramycin, Cefe- Cefepime, Cefu- Cefuroxime-axetil, Ceft- Ceftriaxone, Dap- Daptomycin, Cli – Clindamycin, Fos- Fosfomycin, Col- Colistin, Gent- Gentamicin, Erta- Ertapenem, Fus- Fusidic acid, Imi- Imipenem, Gent-Gentamycin, Fos- Fosfomycin, Levo- Levofloxacin, Mero- Meropenem, Lin- Linezolid, Net- Netilmicin, Mero- Meropenem, Pip- Piperacillin, Pip/Taz- Piperacillin /Tazobactam, Nit- Nitrofurantion, Mup- Mupirocin, Tei- Teicoplanin, Tig- Tigecycline, Van- Vancomycin, R-Resistant, S-Sensitive.

3.1.4 Culture Media and Chemicals

Mueller Hinton agar, Eosin Methylene Blue (EMB) agar and Blood agar were used to grow bacteria.

Antibiotics used include; Amikacin and Vancomycin and they were used as positive control. Amikacin was used as positive control for *P. aeruginosa* and *E. coli*while Vankomycin was used as positive control for MRSA.

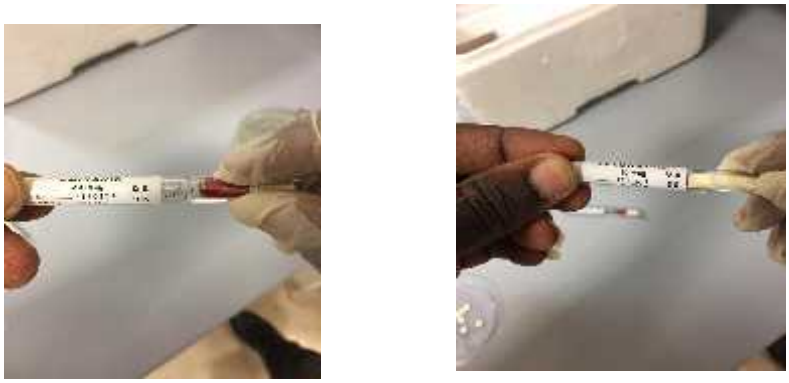

FIGURE 3.1. Antibiotics used as control

3.2 Methods

3.2.1 Essential Oil

Essential oil of *Schinusmolle* was kindly provided by Assist. Prof. Dr. AzmiHanoğlu (Alnawari*et al.*,2018)

3.2.2 Gas chromatography analysis

The essential oils of *S. molle* were analyzed by GC/MS and GC/FID, simultaneously.

GC-MS Analysis

The GC-MS Analysis was carried out with Agilent 5975GC-MS system. Innowax FSC column (60m*0.25mm film thickness) was used with Helium as carrier gas (0.8ml/min).

GC oven was kept at 60℃ for 10mins and programmed 220℃ at a rate of 4℃/min, and kept constant at 220℃ fot 10mins. Then programmed to 240℃ at a rate of 1℃/min. Split ratio was adjusted at 40:1. The injector temperature was set as at 250℃ . Mass spectra were recorded at 70eV. Mass range was from m/z 35 to 450(Alnawari *et al.*,2018).

GC analysis

The GC analysis was carried out using an agilent GC system. FID detector temperature was 300℃to obtain the same elusion order with GC-MS, simultaneously auto injection was done on a duplicate of the same column applying the same operational condition. Relative percentage amounts of the separated compounds where calculated from FID chromatograms. The analysis results are given in the table 3.2 below.

Identification of the essential oil components was carried out by comparison of their retention time with those of authentic samples or by comparison of their Linear Retention Indices (LRI) to a series of n-alkane. Computer matching against commercial (Wiley GC/MS library, MassFinder 3 library) and in house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for identification (Alnawari *et al.*,2018).

Table 3.2.Chemical components of *Schinusmolle* essential oil

LRI	Compound Name	KK% Fruit	NEU% Fruit	KK% Leaf	NEU% Leaf
1020	α – pinene	3.7	4.0	3.9	5.2
1119	β – pinene	0.2	0.2	0.1	0.1
1131	Mabinene	0.1	0.1	0.3	0.2
1173	Myrcene	18.5	19.9	1.9	1.4
1178	α– phellandrene	26.7	36.3	31.5	31.6
1181	Pseudolimonene	0.1	0.1	0.1	0.1
1212	Limonene	12.5	13.3	10.1	11.4
1221	1,8-cineole	0.4	-	-	-
1223	β – phellandrene	10.3	12.2	9.9	10.9
1263	(E)- β – ocimene	-	-	0.1	-
1288	p-cymene	8.1	4.2	2.1	4.2
1299	Terpinolene	0.1	0.2	0.2	0.2
1400	methyl octenoate	0.9	0.5	-	-

1499	Bicycloelemene	-	0.1	1.0	0.5
1555	α – gurjunene	-	-	0.2	-
1556	linalool	0.1	-	-	0.1
1580	cis-sabinene hydrate	-	0.1	-	-
1581	tran-p-menth-2-en-oi	0.4	-	-	-
1605	bornyl acetate	0.1	0.1	-	-
1613	β – elemene	0.1	-	0.7	0.4
1625	terpinen-4-oi	0.2	-	-	-
1627	β – caryophyllene	-	-	0.2	0.2
1638	Aromadendrene	0.1	0.2	0.3	0.4
1661	y-elemene	-	-	0.3	0.2
1685	alloaromadendrene	-	-	0.1	0.1
1688	epi-zonarene	-	-	tr	-
1701	trans-piperitol	0.2	-	-	-
1702	α – humulene	-	-	0.2	0.1
1710	Cryptone	0.3	0.1	-	tr
1715	Carvotanacetone	0.1	-	-	-
1717	y-humulene	-	-	0.1	-
1726	Ledene	-	-	0.2	0.2
1742	Valencene	-	-	-	0.3
1743	germacrene D	-	-	0.3	-
1752	α – muurolene	0.1	-	0.5	0.3
1756	β – selinene	-	-	-	0.2
1759	selina-4,11 diene	-	-	-	0.2
1762	Phellandral	0.1	-	-	-
1769	Bicyclogermacrene	0.1	2.0	12.0	11.1
1786	δ – cadinene	0.3	0.1	1.4	0.6
1692	y-cadinene	-	-	0.2	0.1
1802	cadina-1,4-diene (=cubenene)	-	-	0.1	-
1822	Cuminaidehyde	0.1	-	-	-
1836	p-mentha-1(7),5-dien-2-oi	2.1	0.8	0.2	-
1859	Carveol	0.1	-	-	-
1871	germacrene B	-	-	0.1	-
1873	p-cymen-8-oi	0.1	-	-	-
1916	Epicubebol	-	-	0.1	-
1931	α – phellandrene epoxide	0.5	0.1	-	-
2072	Ledol	-	-	0.1	-
2085	germacrene D-4-oi	-	-	-	0.3
2092	cubenan-11-oi	-	-	0.1	-
2098	Cubenol	-	-	0.1	-
2108	Elemol	0.4	1.4	9.3	6.3
2124	Viridiflorol	-	-	0.6	-

2148	10-epi-y-eudesmol	-	-	0.2	-
2160	Spathulenol	2.0	2.0	2.0	5.3
2205	y-eudesmol	0.6	0.2	1.7	1.1
2217	Eremoligenol	-	-	-	0.2
2222	t-muurolol	0.1	-	0.4	0.2
2226	α – guaiol	0.1	-	0.1	0.2
2231	δ – cadinol	-	-	0.1	-
2244	Thymol	0.7	0.2	0.2	0.2
2261	α – eudesmol	1.2	0.7	1.8	2.2
2268	α – cadinol	0.2	-	0.8	0.3
2273	β – eudesmol	1.4	0.6	1.9	2.4
	Total%	98.4	99.7	97.8	99.0

tr=trace {<0.1%}

3.2.2 Culture preparation

All bacteria where inoculated on EMB and blood agar and Mueller Hilton agar. Active cultures for experiments were prepared by transferring a loopful of culture to EMB and blood agar and incubated at 35^{o}C for 24 hours. The isolates were standardized to Mcfarland turbidity standard and where sub-cultured on MH agar.

FIGURE 3.2. Preparation of culture media

3.2.3 Determination Antibacterial Activity of Essential Oil

3.2.3.1 Disc diffusion assay

The antibacterial activity of *Schinusmolle* was assessed using the disc diffusion method. The inoculum was made according to the Mcfarland method, and the testing microorganism suspension was distributed on MHA medium. The bacteria under investigation were inoculated on MH agar and filter paper discs (5mm in diameter), which were then impregnated with 20ml of essential oil where placed on the inoculated media plate. After that, the plates were incubated for 24 hours at 35°C, and the growth inhibition region was measured in millimeters (Kumar et al., 2019).

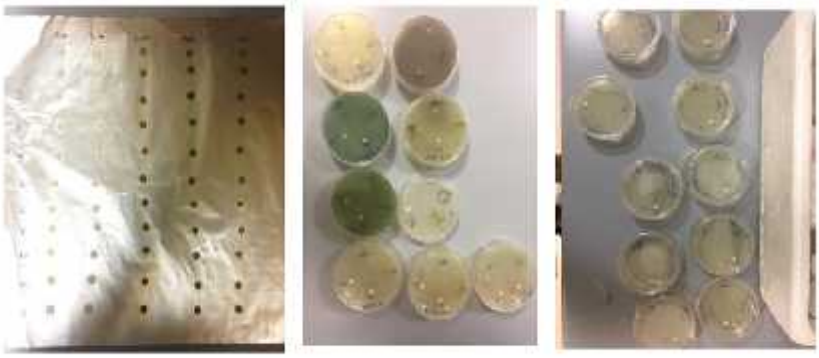

FIGURE 3.3. Disc diffusion assay

4. RESULTS

Antibacterial activity of *Schinusmolle* was ascertained using Essential Oils from *Shinusmolle* against clinical specimens from Near East University. The clear zones of inhibition where used as indicators to show the bioactivity of the EO of *Schinusmolle.*

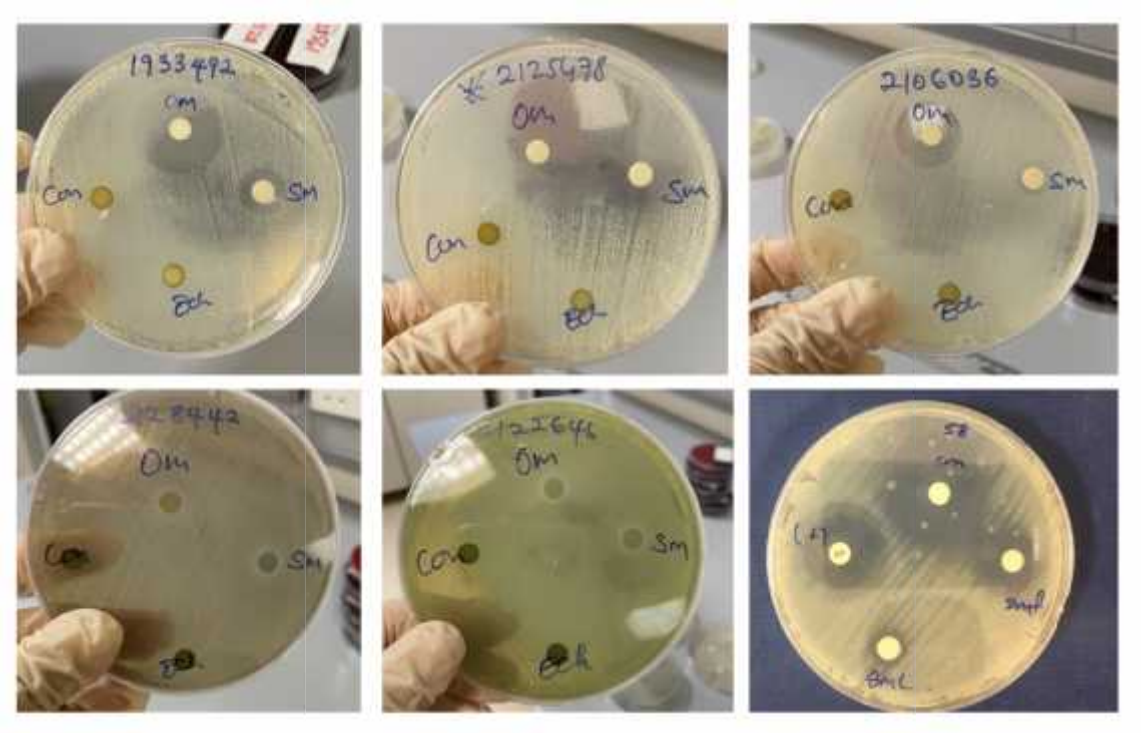

FIGURE 4.1. Agar plates showing inhibition zones

Table 4.1 shows the antibacterial properties of the essential oil of *Schinusmolle*on different clinical isolates and different strains of *Pseudomonas aeruginosa*, *Escherichia coli*, as well as *Stenotrophomonasmaltophilia*and *Staphylococcus aureus*their zone of inhibitionand later minimum inhibitory concentration (MIC).

Table 4.1. Inhibition zone (mm) of essential oil against clinical isolates

Test organism	**Essential oil** **20 μl/disc**	**Amikacin**
2125570	8 mm	25 mm
2128442	7 mm	14 mm
2123669	7 mm	25 mm
2122646	7 mm	24 mm
2161159	9 mm	20 mm
2159728	8 mm	26 mm
1514192	9 mm	23 mm
1744782	9 mm	22 mm
1513731	9 mm	21 mm
1933492	13 mm	24 mm
2106036	10 mm	26 mm
1893927	12 mm	24 mm
2121751	9 mm	25 mm
1734242	15 mm	19 mm
2170607	9mm	25mm
2179123	8mm	20mm

2179592	14mm	25mm
2179169	14mm	25mm
2176543	14mm	25mm
2178872	15mm	25mm
2174739	15mm	25mm
2176111	10	24mm
21795333	13	26mm
2125478*	23 mm	24 mm

. *Vancomycin was used as positive control.

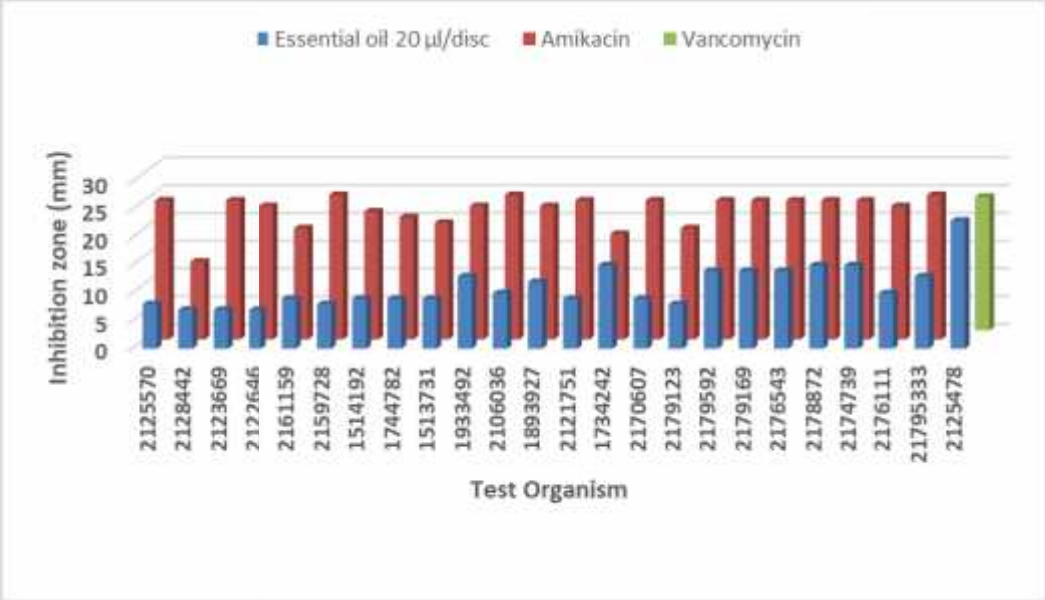

FIGURE 4.2. Graph of Inhibition zone (mm) of essential oil against clinical isolates

5. DISCUSSION AND CONCLUSION

Essential oil from *Schinusmolle* was found to be an active agent against the test bacteria strains. The zone of inhibition ranged from 7-23mm. It showed moderate activity to some strains of *E. coli* while other strains of *E.coli, S. aureus* and *S.maltophilia* showed higher bioactivity. In comparison, inhibition values were lower than those values obtained from standard antibiotics and the zones produced by amikacin and vancomycin range from 14-26mm.

The most sensitive organisms to the essential oil of *Schinusmolle* where: *S. aureus*2125478, *S.maltophilia 1734242 and E. coli*1933492, 2106036,189392, 2176543, 2179169, 2179592, 2174739, 2178872.

According to Abrha & Cr, (2014), antibacterial activity is attributed to the presence of active principles such as phellandrenes, myrcene and pinene in the oil of *S. mole.*

Similar studies done by Do *et al*., (2013) shows the essential oil from the leaf of *Schinusmolle* was more effective against *Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecali*which are all Gram positive bacteria while EO from the fruits of *S. molle* showed antibacterial activity against antibiotic-resistantGram negative bacteria, *Pseudomonas aeruginosa and Salmonella entiritidisserovar Typhimurium*.According to Prado *et al.*,(2018), the *S. molle* EO did not present antibacterial action against the evaluated bacteria. In contrast to the data from his literature which showed that the EO of this species presents action against *P. aeruginosa, E. coli and S. aureus*, however, the chemical constitutions of these EOs were different, which justifies the differentiated biological activities that were found (Rocha et al. 2012). However, in contrast to the result of Abrha & Cr, (2014)*Schinusmolle* oil showed a wide zone of inhibition against gram positive

Staphylococcus aureus while the leaf of *Schinusmolle*showed no bioactivity against the selected clinical isolates in contrast to the result of Do *et al*., (2013).

According to the literature, the result of my study proves that *Schinusmolle* Essential oil has antibacterial effect against *Echerichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, Stenotrophomonasmaltophilia*.

In this investigation, the antibacterial activity of *Schinusmolle* essential oil was found to be effective against clinical isolates and its greatest effect where on the bacteria strain 1933492, 2106036, 1893927, 1734242, 2125478, 2179592, 2179169, 2179592, 2174739 and 2179592. The potency of its antibacterial activities can be further confirmed using MIC.

This study is the first study to show antimicrobial activity of *Schinusmolle* in North Cyrus and it has shown promising lead in its ability to inhibit bacterial growth. However, further studies need to be carried out to ascertain fully its bioactivity and toxicity.

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STATEMENT (DECLARATION)

Hereby I declare that this thesis study is my own study, I had no unethicalbehavior in all stages from planning of the thesis until writing thereof, I obtained all theinformation in this thesis in academic and ethical rules, I provided reference to all of theinformation and comments which could not be obtained by this thesis study and tookthesereferencesintothereferencelistandhadnobehaviorofbreechingpatentrightsandcopyright infringement duringthe studyand writingof this thesis.

Nameand Surname oftheStudent

Ighoyinvwin Peter Odogun

Signature

CURRICULUMVITAE

Name	Ighoyinvwin Peter	**Surname**	Odogun
PlaceofBirth	Benin city	**DateofBirth**	30/07/1990
Nationality	Nigerian	**Tel**	+2348039319529
E-mail	odogunigho@gmail.com		

EducationalLevel

	Name oftheInstitutionwherehe/shewasgraduated	**Graduationyear**
Postgraduate/Specialization		
Masters	Near East University	2021
Undergraduate	University of Benin	2015
Highschool	Unilec international college	2007

JobExperience

	Duty	**Institution**	**Duration(Year-Year)**
Research Intern		Grip Your Life	Sept 2020 – July 2021
Sales Associate		Union Bank Nigeria Plc	Nov 2017 – May 2018
Assistant Lecturer		The Federal Polytechnic Idah	April 2016 – July 2017

ForeignLanguages	**Readingcomprehension**	**Speaking***	**Writing***
English	Fluent	Fluent	Fluent

ComputerKnowledge

Program	**Useproficiency**
MsWord	Intermediate
Ms Excel	Basic

***Evaluateasverygood, good, moderate, poor.

ENCLOSURE: Otherscientificactivities (publication,congressproceedingsetc.)

“Effects of Calcium Supplementation on the Histology of Cardiac Myocytes of Albino Wistar Rats (https://www.ecronicon.com/ecve/ECVE-06-00409.php)”.