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Antimicrobial Activity of Essential Oil of *Origanum Majorana* L. and *Schinus Molle* L. Endemic in Northern Cyprus

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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. Essential oils are volatile oils which normally are responsible for many sweet smells and extensively used in medicinal, cosmetic, cultinary and ornamental purposes. The present work aims to evaluate the antimicrobial activity of Origanum majorana and Schinus molle essential oil endemic plant in Northern Cypruson Clinical isolates from Near East Hospital. 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 Origanum majorana L. and Schinus molle showed significant activity against Staphylococcous aureus (MRSA), Escherichia coli, Pseudomonas aurginosa and Stenotrophomona maltophilia. This result indicates the possible use of essential oils of Origanum majorana L. andSchinus molle as antibacterial agent however; there is need for further clinical study to ascertain fully its effectiveness and potential toxic effect.***

Keywords: *Origanum majorana* L., *Schinus molle*, Antimicrobial Activity, Essential Oils, 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 are aromatic vegetable oily liquids composed of a variety of low molecular weight volatile monoterpenes, sesquiterpenes, and isoprenes (Pawlowski et al., 2012) which are 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 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). 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).

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 plant organs, plants origin and the species of plants and they have low density. Thus, essential oil floats on water with the few exceptions like cinnamon, sassafras and 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).

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. Materials and Methods

2.1 Materials

2.1.1. Plant material collection

Essential oil of *Schinus molle* and *Origanum majorana* L. was kindly provided by Assist. Prof. Dr. Azmi Hanoğlu (Alnawari, 2018).

2.1.2. 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.

2.1.3. 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 ***Stenotrophomonas maltophilia***2121751, 1734242. A total of 23 strains were used in this study.

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.

Table 2.1: Susceptibility of the clinical isolate used in the study

Pseudomonas aeruginosa

No	Barkod	Cefe	Cefta	Clpro	Col	Gent	Iml	Levo	Mero	Net	Plp	Plp/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	Clpro	Erta	Fos	Gent	Iml	Mero	Nit	Plp/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	Clpro	Cll	Dap	Fos	Fus	Gent	Levo	Lln	Mup	Nit	Tel	Tlg	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	Aml	Azt	Cefe	Cefta	Clpro	Col	Gent	Iml	Levo	Mero	Net	Plp	Plp/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.

2.1.4 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.

2.2 Methods

2.2.1 Gas chromatography analysis

The essential oils of *Origanum majorana* L. and *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.

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

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).

2.2.3 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.

2.2.4 Determination Antibacterial Activity of Essential Oil

2.2.4.1 Disc diffusion assay

Bacterial inoculum 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 5mm 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 and Amikacin30 mcg antibiotics were used as positive control.

3. Results

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

Table 4.1 shows the antibacterial properties of the essential oil of *Schinus molle* on different clinical isolates and different strains of *Pseudomonas aeruginosa*, *Escherichia coli*, as well as *Stenotrophomonas maltophilia* and

Staphylococcus aureus their zone of inhibition and later minimum inhibitory concentration (MIC).

Table 3.1: Inhibition zone (mm) of essential oil against clinical isolates

Test organism	Essential oil *Schinus molle*	Essential oil *Origanum majorana* L.	Amikacin
2128442	7 mm	8mm	14 mm
2123669	7 mm	9mm	25 mm
2122646	7 mm	9mm	24 mm
2161159	9 mm	10mm	20 mm
2159728	8 mm	9mm	26 mm
1514192	9 mm	10mm	23 mm
1744782	9 mm	10mm	22 mm
1513731	9 mm	11mm	21 mm
1933492	13 mm	25mm	24 mm
2106036	10 mm	19mm	26 mm
1893927	12 mm	21mm	24 mm
2121751	9 mm	29mm	25 mm
1734242	15 mm	29mm	19 mm
2170607	9mm	10mm	25mm
2179123	8mm	10mm	20mm
2179592	14mm	26mm	25mm
2179169	14mm	26mm	25mm
2176543	14mm	20mm	25mm
2178872	15mm	25mm	25mm
2174739	15mm	26mm	25mm
2176111	10mm	7mm	24mm
21795333	13mm	28mm	26mm
2125478*	23 mm	29mm	24 mm

*Vancomycin was used as positive control.

4. Discussion and Conclusion

Essential oil from *Schinus molle* and *Origanummajorana* L. was found to be an active agent against the test bacteria strains. The zone of inhibition ranged from 7 - 29mm. *Schinus molle* showed moderate activity to some strains of *E. coli* while other strains of *E. coli*, *S. aureus* and *S. maltophilia* showed higher bioactivity as compared to *Origanummajorana* L. which recorded higher antimicrobial activity against the clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Psedomonas auginosa* and *Stenotrophomonas maltophili*.

In comparison, inhibition values of the Essential oil from *Schinus molle* were lower than those values obtained from standard antibiotics and the zones produced by amikacin and vancomycin range from 14 - 26mm while the essential oil of *Origanum majorana* L. was more active than the standard amikacin against the two clinical isolates of *Stenotrophomonas maltopholia* and vancomycin against one isolate of *Staphylococus aureus* respectively. Due to its strong microbicidal property and superiority over commercial microbicides, *Origanum majorana* 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.

The most sensitive organisms to the essential oil of *Schinus molle* 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 *Schinus molle* 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 - resistant Gram negative bacteria, *Pseudomonas aeruginosa and Salmonella entiritidis serovar Typhimurium* while Ben *et al., (*2001) suggests that the essential oil of *Origanum majorana* 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, *Salmonella enterica* serotype Newport, *Streptococcus agalactiae*, and *Aspergillus fumigatus*. 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) *Schinus molle* oil showed a wide zone of inhibition against gram positive *Staphylococcus aureus* while the leaf of *Schinus molle* 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 *Origanum majorana* L. and *Schinus molle* Essential oil has antibacterial effect against *Echerichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, Stenotrophomonas maltophilia*.

In this investigation, the antibacterial activity of *Schinus molle* 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 *Schinus molle* 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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