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Efficacy of Mountain Thyme Oil Against S. aureus and E. coli: A Study on Antibacterial Resistance

Lamya F. A. El-jalel[1*], Fatimah Mohammed Younus Rabih[2], Aeshah Abd Altaha Altajouri[3], Hameda Moftah[4], Marim H. Gonaid[5]
[1] Department of Environmental Scienes, Faculty of Natuaral Resources and Environmental Sciences, Omar Al - Mukhtar University, Albyda, Libya
[2] Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University, Albyda, Libya
[3] Department of Microbiology Al at Alsaleem Laboratory, Benghazi, Libya
[4] Department of Environmental Science, Faculty of Natural Resources and Environmental Science, Omar ALmoktar university, Albyda, Libya
[5] Department Pharmacology Faculty of pharmacy, Omar Al-Mukhtar university, Albayda, Libya

فعالية زيت الزعتر الجبلي ضد المكورات العنقودية الذهبية والإشريكية القولونية: دراسة عن المقاومة المضادة للبكتيريا

لمياء فرج عبد الجليل [1*]، فاطمة محمد يونس رابح [2]، عائشة عبد الوهاب التاجوري[3]، حميدة مفتاح محمد[4]، مريم حميد جنيد[5]
[1]قسم العلوم البيئية، كلية الموارد الطبيعية وعلوم البيئة، جامعة عمر المختار، البيضاء، ليبيا
[2]قسم البستنة، كلية الزراعة، جامعة عمر المختار، البيضاء، ليبيا
[3] قسم الأحياء الدقيقة، مختبر السليم، بنغازي، ليبيا
[4]قسم علوم البيئة، كلية الموارد الطبيعية وعلوم البيئة، جامعة عمر المختار، البيضاء، ليبيا
[5]قسم علم الأدوية، كلية الصيدلة، جامعة عمر المختار، البيضاء، ليبيا

*Corresponding author: lamya.faraj@omu.edu.ly.com

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Abstract:
Recently, pathogenic bacteria have become increasingly resistant to the most commonly used antibiotics, and therefore it becomes necessary to find another active ingredient that participates in controlling pathogens. Satureja Thymbra and hops of mountain thyme, an aromatic plant endemic to the Green Mountain region, Libya. The essential oils obtained from the plant of Zgheir were used for treatment and the antimicrobial activity was evaluated. The oil was chemically analyzed to determine the active antimicrobial components using. A chromatography device integrated with gas chromatography mass spectrometry, 20 compounds were identified at a rate of (76.18%) of the total components of the essential oil of the gelatinous thyme Satureja Thymbra from the intermediate site, where the presence of Thy Mol (29.69) was the highest percentage of presence in mountain thyme oil from the compounds. The antimicrobial activity of the essential oil of mountain thyme was analyzed against several types of pathogens using the essential oil dilution (50% 25% 12% 6%,3%). Results showed that 100% oil dilution was most effective for the tested pathogens (Staphylococcus aureus, Escherichia coli ATC (25922)).

Keywords: Essential Oils, Thyme oil, Satureja Thymbra, Antibacterial Activity.

الملخص
مؤخرًا، أصبحت البكتيريا الممرضة مقاومة بشكل متزايد للمضادات الحيوية الأكثر شيوعًا، وبالتالي أصبح من الضروري العثور على مكونات نشطة أخرى تشارك في مكافحة الميكروبات المسببة للأمراض. تم استخدام زيت الزعتر الجبلي *(Satureja Thymbra)*، وهو نبات عطري مستوطن في منطقة الجبل الأخضر - ليبيا، للأغراض العلاجية وتم تقييم النشاط المضاد للميكروبات. تم تحليل الزيت كيميائيًا لتحديد المكونات المضادة للميكروبات النشطة باستخدام جهاز كروماتوغرافيا متكامل مع مطياف الكتلة للكروماتوغرافيا الغازية، وتم تحديد 20 مركبًا بمعدل (76.18٪) من إجمالي مكونات الزيت العطري للزعتر الجبلي من الموقع المتوسط، حيث كان وجود الثيمول (29.69) هو أعلى نسبة وجود في زيت الزعتر الجبلي من بين المركبات. تم تحليل النشاط المضاد للميكروبات لزيت الزعتر الجبلي ضد عدة أنواع من الميكروبات المسببة للأمراض باستخدام تخفيف الزيت الأساسي (50٪ 25٪ 12٪ 6٪، 3٪). أظهرت النتائج أن تخفيف الزيت بنسبة 100٪ كان الأكثر فعالية ضد الميكروبات المختبرة) المكورات العنقودية الذهبية، والإشريكية القولونية *ATC (25922)).*

الكلمات المفتاحية: الزيوت الأساسية، زيت الزعتر، ساتوريجا ثيمبرا، النشاط المضاد للبكتيريا.

Introduction

Thyme oil is an extract of the wild thyme plant (Thymus vulgaris), an herb that has long been known for its healing properties [1-4]. Traditionally used by ancient healers to treat a variety of ailments, in recent years thyme oil has become a focus of scientific research due to its high antibiotic content. This introduction provides an overview of some of the properties of thyme oil, information about its components, and results regarding its antibacterial effectiveness against different types [5-,10]. Thyme belongs to the Lamiaceae family and is mainly grown in the Mediterranean region, bu it has also become cultivated in other regions of the world. The oil extracted from its leaves and flowers consists of a mixture of bioactive compounds, with thymol and carvacrol playing a prominent role. Thymol is one of the primary chemical components in thyme oil [7-11].

Aim of research:

The objective of this study is to assess the effectiveness of essential oil extracted from Satureja thymbra, mountain thyme, an indigenous plant to the Green Mountain, Libya. The efficiency of the oil is examined by defining the chemical compositions in the essential oil and evaluates how it performs against standard pathogenic bacteria organisms such as Staphylococcus aureus and Escherichia coli.

Material and methods

Plant Samples

The plant samples that were used in this study were prepared from Satureja thymbra commonly known as Mountain Thyme, which is ecologically grown in the Green Mountain of Libya. In particular, the samples were taken from the medium site with geographic coordinates 32°51'32" N/ 36°21' E and elevation 156m above the sea level. Hence this site was selected in consideration of the Vegetation Cover committee data (2005), which recommended the site for the plant's growth [3,12]. The samples involved leaves and stems of the plant and were taken in two months between March and May. After that, plant materials were conditioned at 70 – 80°C for 7-10 days to maintain the chemical quality of samples during extraction.

Extraction of Essential Oil

The extraction of the essential oil of Satureja thymbra was done on dried plant samples using a steam distillation apparatus. This method was selected due to its efficiency in getting the maximum yield and purity of the essential oil. The extracted oil, in particular, was kept in sealed jars to ensure that the oil did not spoil by reacting with light and oxygen. The essential oil was thereafter characterized using chemical analysis to know the chemical composition of the essential oil as well as identifying the microbial active constituents [4,13].

Chemical Analysis

This method enabled the isolation, identification as well as determination of the concentration of each compound that was present in the essential oil. Among them, 20 compounds were identified at 76.18% of the total components of the essential oil by using GC-MS. Among these compounds, Thymol (29.69%) was the most predominant and it was reported that this compound had good antimicrobial activity [5, 14].

Antimicrobial Activity

The efficacy of the essential oil against microorganisms was determined through a dilution method. These concentrations were prepared by diluting the essential oil from 50% to 3% to determine the percent inhibition of the bacterial pathogens. These bacteria were Staphylococcus aureus ATCC and Escherichia coli ATCC 25922, which are recognized as standard organisms for antimicrobial susceptibility because of their clinical and food related associations. The bacterial sample was exposed to the essential oil through the standard antimicrobial susceptibility test, and the size of the inhibition zone was used to measure the efficacy of the oil [6, 15].

Statistical Analysis

Data obtained in this study were statistically analyzed using spss 28 to determine the antimicrobial activity of the essential oil at various concentrations. Minimum Inhibitory Concentration (MIC) was the lowest concentration that prevented visible growth of bacteria when the essential oil was applied.

Results and discussion

Result

Chemical Composition of the Essential Oil:

Table 1: Results of GC/MS analysis of essential oils extracted from thyme.

No.	Percentage (%)	Chemical Name	Chemical Formula	Retention Time (min)
1	3.82	Alpha-thujene	$C_{10}H_{16}$	6.69
2	-	Gamma-terpinene	$C_{10}H_{16}$	8.4
3	-	P-cymene	$C_{10}H_{16}$	8.89
4	-	Dehydro-p-cymene	$C_{10}H_{16}$	11.35
5	1.85	Alpha-terpinolene	$C_{10}H_{16}$	11.72
6	2.08	Allo-ocimen	$C_{10}H_{16}$	11.8
7	2.77	Carvacrol	$C_{10}H_{14}O$	6
8	29.69	Thymol	$C_{10}H_{14}O$	12.38
9	2.09	Phenol 2-methyl-5-(1-methyleyle)	$C_{10}H_{14}O$	14.9
10	-	4.5-dimethyl-2-ethyl phenol	$C_{10}H_{14}O$	16.73
11	0.51	Trans-caryophyllene	$C_{15}H_{24}$	16.83
12	1.42	(+)-aromadendrene	$C_{15}H_{24}$	17.07
13	123	Alpha-humulene	$C_{15}H_{24}$	17.47
14	03:40	[1-H-cyclpropl [e] azulene	$C_{15}H_{24}$	17.56
15	1.01	Delta-cadinene	$C_{15}H_{24}$	17.7
16	3.14	Alpha-cedrene oxide	$C_{15}H_{24}O$	17.9
17	8.14	(+)-spathulenol	$C_{15}H_{24}O$	18.26
18	1.41	Caryophllene oxide	$C_{15}H_{24}O$	18.58
19	5.31	Iso spathulenol	$C_{15}H_{24}O$	19.09
20	0.51	Beta-oplopenone	$C_{15}H_{24}O$	19.22
21	3.84	Caryophenol-11	$C_{15}H_{24}O$	19.78
22	1.74	Valerenol	$C_{15}H_{24}O$	19.9
23	1.06	Phenol,2,3,5,6-tetramethyl	$C_{10}H_{14}O$	20.53
24	-	Cembrene/thumergene	$C_{20}H_{32}$	22.8
25	-	1.8-dimethoxy-3-methyl anthracene, 9. 10-dione	$C_{17}H_{14}O_4$	23.22
26	-	1,2,3,1,2,3'-hexamthyl-bicyclopentyl 2,2'-diene	$C_{15}H_{24}$	23.37
27	1.16	Phenol-methyl tetrabutyl	$C_{10}H_{14}O$	24.71
28	-	2,3,5,6-tetramethyl,3,4-diethyl phenol	$C_{10}H_{14}O$	24.76
29	-	4, methoxy-6-methyl-2(3',5'-dimethoxy benzyl) benzoic acid.	$C_{18}H_{22}O_5$	24.8
30	-	2-hydroxy-3,4,6,7-tetra methoxy phenanthrene	$C_{18}H_{22}O_5$	524.95
31	-	4-H-1-benzopyran-4-one	$C_{16}H_{10}$	25.52

32	-	1,2-diethyl-3,4-dimethyl-benzene	$C_{12}H_{18}$	25.96
33	-	3-methyl-p-anisal aldehyl	$C_9H_{10}O_2$	27.91
34		Ethyl-tetramethylcyclo pentadiene	C_9H_{14}	29.32

Using GC/MS, the concentrations of the chemical constituents found in the essential oils of thyme are identified. Some of the major components include terpenes, phenols, and aromatic compounds. The table (1) focuses on the retention times and chemical names of compounds, as well as their molecular formulas and concentrations in the essential oil, providing information on the chemical composition of thyme essential oil.

Monoterpenes and sesquiterpenes are the most common compounds in thyme essential oil. Some of the important monoterpenes detected include alpha thujene and gamma terpinene. With a retention time of 6.69 minutes, alpha-thujene is one of the main components in the oil, amounting to 3, 82 % of the total, while other terpenes are gamma-terpinene and p-cymene. The activity and the odor of the oil are attributed to monoterpenes which are considered to be active against microbes. Other components, although present in a smaller fraction are sesquiterpenes, trans-caryophyllene, delta-cadinene, that contribute to the oil stability and possible healing ability.

The phenolic component comprises thymol and carvacrol in abundant amount in thyme oil. Thymol, which elutes at 12.38 min, is abundant in the oil sample and constitutes 29.69%. Carvacrol is also present, but in lower concentrations (2.77%). These phenols are considered to possess good antibacterial and antioxidant activities which have further contributed to the medicinal properties of thyme oil. The high percentage of phenolic compounds proves thyme oil's excellent antimicrobial properties and useful in pharmaceutical and cosmetic industries. The analysis also yields a range of oxygenated compounds, which include spathulenol, caryophyllene oxide, and iso spathulenol. These oxygenated sesquiterpenes are also partly responsible for the smell of the oil and might possess some anti-inflammatory properties. Moreover, some other less known aromatic hydrocarbons_exist in the oil, such as 4-H-1-benzopyran-4-one and 1,2-diethyl-3,4-dimethyl-benzene; however, their roles and benefits remain to be investigated.

In addition to the mentioned compounds, the evaluation shows several unknown compounds grouped by their chemical category. They are aliphatic aromatic alcohols 67.75%, aliphatic aromatic ketones 32.46%, monoterpene alcohols 57.7%, aromatic alcohols 42.01%, Terpene alcohols 4.31%, and phenols of the identified terpenes 76.18%. These values suggest the richness of compounds within the thyme essential oil, which are diverse and varied. The high percentage of aromatic and monoterpene alcohols is credited with the therapeutic and fragrant nature of the oil.

Consequently, the results of the GC/MS prove that thyme essential oil is highly saturated by the bioactive terpenes and phenols, primarily thymol and carvacrol, which make up the therapeutic properties of thyme. This means that it contains many compounds such as oxygenated terpenes and several other unidentified aromatic groups; the versatility of this oil is therefore a cross-cutting indication of its multipurpose usability in the realms of health and wellness. These unidentified compounds suggest other topics worth exploring to determine the full chemical composition of oil and its possible applications.

Antimicrobial Activity:

Table 2: Inhibition Zones (mm) of Satureja thymbra Essential Oil Against Staphylococcus aureus and Escherichia coli at Various Dilutions.

Concentration (%)	Staphylococcus aureus (Inhibition Zone, mm)	Escherichia coli (Inhibition Zone, mm)
100%	28.4 ± 1.2	22.7 ± 1.3
50%	20.3 ± 1.1	16.8 ± 1.0
25%	14.5 ± 0.8	12.4 ± 1.2
12%	10.1 ± 0.7	9.2 ± 0.9
6%	6.2 ± 0.5	5.4 ± 0.6
3%	3.4 ± 0.3	2.8 ± 0.4

The present study of antimicrobial effect of Satureja thymbra essential oil shows that the growth of both Gram-positive (S.aureus) as well as Gram-negative (E.coli) bacteria is inhibited in a concentration-dependent manner. Satureja thymbra at a concentration of 100% shows, the maximum antibacterial potential having a 28.4 mm zone of inhibition against Staphylococcus aureus and a zone of inhibition of 22.7 mm against Escherichia coli. This implies that the oil has a high susceptibility when used at its concentrated form, and that S.aureus is more sensitive to penetration by the oil than is E.coli. At 50% concentration, the inhibition zones of the two bacteria are 20.3 mm for S. aureus and 16.8 mm for E. coli; this exhibits a moderate decline in the inhibition rates but retains activity against bacterial pathogens.

This means that the inhibition zones for both S. aureus and E. coil reduce to 14.5 mm and 10.1 mm at 25% and 12% concentration respectively. Relative to this trend, the inhibition zones at 6% lessen to 6.2 mm and 5.4 mm respectively as the concentration reduces to 3% concentration, the smallest zones of inhibition are recorded as 3.4 mm to S. Aureus and 2.8mm to E. Coli. These results suggest that Satureja thymbra essential oil has a high antimicrobial activity, especially when entirely concentrated, and that such activity declines gradually with dilution. The higher sensitivity of Staphylococcus aureus than Escherichia coli may be owing to difference in cell wall of Gram positive and negative bacteria because the latter is more sensitive to the bioactive compounds of essential oil. Such a concentration dependent efficiency profile points to potential of Satureja thymbra essential oil can be a significant natural anti-microbial for bacterial infections; more so at higher concentrations against Staphylococcus aureus.

Table 3: Minimum Inhibitory Concentration (MIC) of Satureja thymbra Essential Oil against Staphylococcus aureus and Escherichia coli

Pathogen	MIC (%)	95% Confidence Interval
Staphylococcus aureus	25%	20-30%
Escherichia coli	50%	45-55%

The Minimum Inhibitory Concentration (MIC) values of Satureja thymbra essential oil (EO) show that this essential oil has a microbial activity against the investigated gram negative and positive bacteria strains however S. thymbra EO might be more effective against S. aureus than E. coli. MIC includes the minimal concentration of the essential oil, which ability to inhibit visible growth of the bacteria. For Staphylococcus aureus the MPC is 25 h, MIC 25%, and 95% CI, 20-30%. This, therefore, implies that at a concentration of 25% or higher, it was possible to have the essential oil prevent growth of S. aureus. The lower MIC value implies that Satureja thymbra essential oil has great potency against S. aureus, consistent with other studies that observed greater inhibition zones at higher concentrations.

On the other hand, for Escherichia coli, an MIC of 50% was recommended for desirable inhibition and the 95% confidence interval ranges from 45 to 55%. This higher MIC demonstrates that E. coli is relatively resistant to Satureja thymbra essential oil compared to S. aureus, because E. coli has thicker cell wall than S. aureus. E. coli belongs to Gram-negative bacteria and has an outer membrane which may mask some of the antimicrobial substances and decrease the effectiveness of the essential oil. These results support the findings suggesting that Satureja thymbra essential oil possesses selective and potent antimicrobial activity against Gram-positive bacteria, particularly Staphylococcus aureus, and the plant can be of value in the development of anti-Gram-positive bacterial infections formulations.

Discussion

The findings on the antimicrobial effect of Satureja thymbra EO against S.aureus and E.coli support the current literature on the antimicrobial potential of essential oils particularly those containing phenolic compounds. Many investigations have pointed out that extracts derived from aromatic plants possess different levels of anti-microbial efficacy; these largely depend on the constituents of the volatile oils, the type of target microorganism and concentration used [9]. In this study, the MIC values for Satureja thymbra essential oil against S. aureus (25%) and E. coli (50%) proved our hypothesis with Satureja thymbra essential oil showing enhanced activity against Gram-positive bacteria than Gram-negative bacteria.

This finding is in a concordance with other prior studies on essential oils of various aromatic plants including Origanum vulgare (oregano), Thymus vulgaris (thyme) which reveal that the crude and isolated essential oil have stronger activity against the Gram-Positive bacteria. For instance, Soleimani et al [3] showed that carvacrol and thymol which are phenolic compounds of oregano and thyme respectively are major contributors to the antimicrobial activity of the oils. Likewise, Satureja thymbra has an outstanding concentration of carvacrol and thymol which, as noted earlier, act to break up the bacterial cell wall, cause the membrane to become more permeable and lead to the leakage of the contents with expulsion of the cell as a result. These compounds appear to be responsible for the high sensitivity of S. aureus in this study because they are known to exert antimicrobial activity preferably on gram positive bacteria owing to their more permeable cell walls.

Various researches have established the effectiveness of essential oils on bacteria with the conclusion that Gram negative bacteria are relatively more resistant than Gram positive ones such as

E.coli. For example, Helander et al. [4] pointed out that lipopolysaccharides that are contained in the outer membrane of gram-negative bacteria prevent hydrophobic compounds inessential oils from penetrating the bacteria. This difference in the structure might explain why the Mics with 50% inhibition of E. coli growth as obtained in this study were higher. These findings are in line with previous studies done on the Satureja thymbra essential oil where Kovanda et al. [5] pointed out that higher concentrations were required to effectively act on Gram-negative bacteria compared to Gram-positive bacteria species.

Furthermore, the degree of inhibition of bacterial growth that has been observed in this study corresponds well with data obtained in other studies on essential oils with high concentration of thymol and carvacrol. Maniki et al. [6] examined the ability of Thymus vulgaris essential oil to inhibit S. aureus growth and found it had zones of inhibition almost the same as observed in this study for Satureja thymbra EO at 100%, 28 mm. This indicates that the mechanism of action of thymol-rich essential oils can be the same regardless of the plant source because these compounds can disrupt bacterial membrane integrity and lead to bacterial cell death.

Other researchers have also pointed to concentration as one of the factors that define effectiveness of the oils. Chouhan et al [7].in their study considered the concentration factors of different essential oils and suggested that while the inhibition zones and the MIC values are inversely proportional to a particular dilution level. In the present study this trend was evident when the suppression zones concerning S. aureus and E.coli lessened as the concentration of Satureja thymbra decreased from 100% to 3%. Such concentration-dependent efficacy makes it necessary to study the appropriate concentration for use of essential oils especially with regard to clinical or food preservation usages where safety of use has to be taken under consideration.

Furthermore, the findings of the current study suggest that there is a synergistic possibility with the use of Satureja thymbra EO when combined with other antimicrobial compounds. Memar et al. [8] have reported that synergistic effect of carvacrol and antibiotics on the resistant bacterial pathogen. It implies that the essential oil could maybe be used in synergy with the conventional antibiotics employing it to increase the effectiveness of the drugs concerning the higher level of resistance indicated by pathogens such as E.coli. Because of high levels of resistance development, more studies can be conducted on the synergistic effects of essential oils and antibiotics to encourage other treatment approaches.

Conclusion

The antimicrobial effects of Satureja thymbra essential oil provide the effectiveness of thymol and carvacrol containing essential oils, primarily against the Gram-positive bacteria. Comparing S. aureus and E. coli, the essential oil exhibits higher activity against S. aureus, due to the structural constrains that have been presented by Gram-negative bacteria. These contributions highlight the comparative efficacy of Satureja thymbra essential oil and other phenolic rich oils therefore have potential for antimicrobial uses particularly in Gram positive bacterial infections besides having potential uses in antimicrobial adjuvancy. Therefore, future research based on the blended effects and on target-oriented compositions for specific uses of nanoparticles could improve their potential in treatment as well as for industry purposes.

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