Antibacterial Activity Evaluations of *Thymus vulgaris* Essential Oil Extract Against Clinically Isolated Gram-Positive and Gram-Negative Pathogens

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Abstract

Thymus vulgaris commonly known as thyme, belongs to the family Lamiaceae which is flowering plant native to Southern Europe, and world widely distributed. Thyme is popular plant that grow as wild plant in the mountains of north Iraq and it has been used for centuries traditionally as herbal tea, flavoring agents, home remedy, drug and perfume. This study investigates the antibacterial effect of thyme essential oil extract. *Thymus vulgaris* collected from Piramagron Mountain 50 km far from Sulaimani city-Iraq. The sample was identified by comparison with a specimen present at the herbarium in College of Agriculture/ University of Sulaimani then the leaves separated, dried, powdered and its essential oil extracted by hydro distillation method. The essential oil of thyme used as antibacterial and tested against five distinct clinically isolated bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus xylosus, and Staphylococcus epidermidis*) in Microbiology lab, at Komar University by Agar well-diffusion method and the results showed strong inhibitory activity of the oil extract that killed both gram-negative and gram-positive bacteria used in this study with a different diameter of inhibition zone (mm). The data of this study suggests that *Thymus vulgaris* essential oil has potential applications as antibacterial agent against different species of pathogenic bacteria and can be used as a safe plant source for discovering new antibacterial agent.

Keywords

Thymus vulgaris, Essential oil extract, hydro distillation, antibacterial, Agar well-diffusion

Introduction

One of our greatest concerns in the fight against bacterial infection and related diseases is the rise of multidrug-resistant bacteria and the resistance of common bacterial pathogens to available antimicrobial medications [1, 2]. Antibiotic resistance in bacteria is a global problem and is associated with a high proportion of common infections (e.g., urinary tract infections, pneumonia, blood-stream infections) [3]. A significant percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), or multidrug-resistant Gram-negative bacteria that are resistant to all b-lactam antibiotics [3].

Patients with infections caused by drug-resistant bacteria are generally at increased risk of worse clinical outcomes and death, and they consume more health-care resources than patients infected with the same bacteria that are not resistant[3, 4]. Although most antibiotics are safe considering their volume of use, like other drugs, there are no antibiotics having utterly no adverse effects [5]. The adverse consequences of antibiotic use include dysbiosis of the gut microbiota, which in turn alters the host immune responses against pathogens [6, 7], hypersensitivity reactions resulting in anaphylaxis [8], and organ toxicity [9 -11].

The past decades have seen a dramatic worldwide increase in human-pathogenic bacteria that are resistant to one or multiple antibiotics. The current lack of successful prevention strategies, shortage of effective medications, and limited number of new antibiotics in the clinical trial necessitate the investigations for development of novel antimicrobial medicines [1]. In order to respond to this emerging crisis, global organizations such as the WHO have urged the scientific community to search for new approaches to combat antibiotic resistance [3].

Phytopharmaceuticals have long been used as a safe and effective alternative to antibiotics. According to recent studies, the use of specific phytodrugs reduces the need for antibiotic prescriptions during the course of the disease and results in significantly shorter sick leaves [12, 13]. On the other hand, phytochemicals can be used in combination with antibiotics. A special synergy effect can occur when antibiotics are combined with an agent that antagonizes bacterial resistance mechanisms [14]. Thyme (Thymus vulgaris), a flowering plant in the mint family Lamiaceae that grows up to 15-30 cm tall by 40 cm wide [15]. It is fairly grown wildly throughout the north of Iraq**.** The aromatic and medicinal properties of the genus Thymus have made it one of the most popular plants all over the world. It has been used for centuries as herbal tea, flavoring agents (condiment & spice), home remedy, drug, perfume and insecticide [15, 16]. Recent studies have shown that Thymus species have antibacterial, antifungal, antiviral, antiparasitic, spasmolytic, antioxidant, and expectorant activities [16-21]. In medicine, it is used as anti-spasmolytic, anti-bacterial, antifungal, secrotolytic, expectorant, antiseptic, anti-helminthic and anti-tussive as reported by other authors [22, 23]. Therefore, investigation of phytochemicals for prevention or treatment of various human infections is reasonable. In this context, the aims of this study were to investigate the antibacterial activity of T. vulgaris leaves oil extracts collected from Piramagron Mountain 50 km far from Sulaimani city-Iraq) against clinically isolated bacterial species: methicillin-resistant *Staphylococcus*

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aureus, *Staphylococcus epidermidis*, *Staphylococcus xylosus* as a genus of gram-positive bacteria, and *Klebsiella* and *Escherichia coli* as a genus of Gram-negative bacteria.

Methods

1.1 Plant row material and extraction of essential oil:

The plant was collected from Piramagron Mountain, 50 km far from Sulaimani City in North of Iraq in June-July, 2021. The identification of *thyme vulgaris* was done in College of Agriculture/ University of Sulaimani. The extract prepared in research laboratory, Department of Food Science and Quality Control at University of Sulaimani. Leaves of thyme separated and dried at 40 c for 48 hrs. and grounded into a powder by using mortars. The extraction of essential oil from plants powder was performed by hydro distillation method according to Aleksovski et al, [24] with a little modification.100 g of powder were soaked in 350 ml of distilled water (20%) in conical flask, and left for 3 hrs. the extract was subjected to steam distillation for 3 hrs. using Clevenger- type apparatus then essential oil was collected after decantation.

1.2 Agar well-diffusion method

Antimicrobial activity was tested in Microbiology lab, Department of Medical Laboratory Science in February 2022 at Komar University of Science and Technology against five distinct clinically isolates bacteria (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus xylosus, and Staphylococcus epidermidis*). All isolates were obtained from Komar University of Science and Technology laboratories. 100 microliter of each bacterium were suspended and adjusted to a density equivalent to (0.5) McFarland density standard. In Mueller-Hinton agar, with sterilized cotton swabs, the suspensions were distributed over the plates, and 50 microliter of the oil extract was applied to a well in the center of the plates. The plates will be incubated at 37°C for 24 hours for bacterial isolates. The diameter of the inhibition zone (mm) is then measured [25].

Results

The results show that the oil extract killed both gram-negative and gram-positive bacteria used in this study, Methicillin resistance *Staphylococcus aureus* and *Staphylococcus xylosus* was the most sensitive to the extract, producing 33 mm and 32 mm inhibition zones, respectively, while *S. epidermidis* had a 28 mm inhibition zone. DMSO was performed as a negative control and produced no inhibition zone, as shown in figure (1, 3) and table 1.

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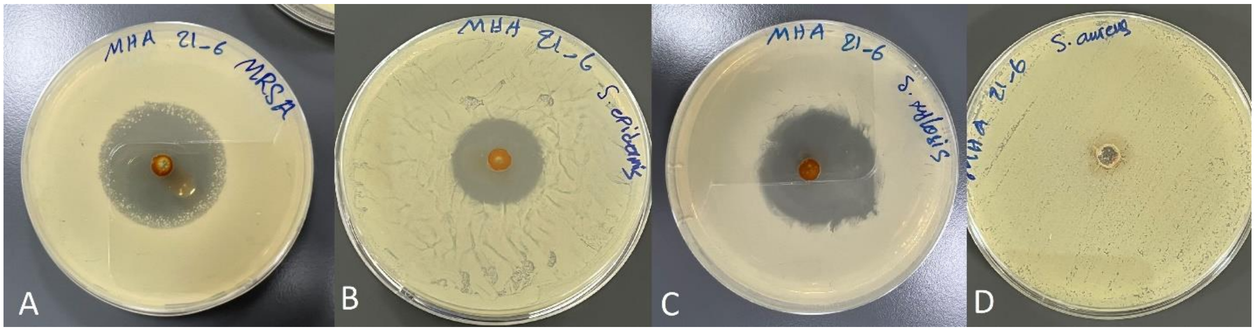

Figure 1: Shows antibacterial activity of Thyme Oil extract against Gram positive bacteria, A: Methicillin resistance *S.aureus*, *B: S.epidermidis*, *C: S.xylosus*, D: Negative Control (DMSO) against Methicillin resistance *S.aureus.*

Followed by *Klebsiella pneumoniae, and E. coli*, produced 24 mm, and 21 mm inhibition zones respectively. The only bacterium that showed resistance to the extract was *Pseudomonas aeruginosa*, also DMSO was applied as a negative control and no response produced. As shown in figure (2, 3) and inn table 1.

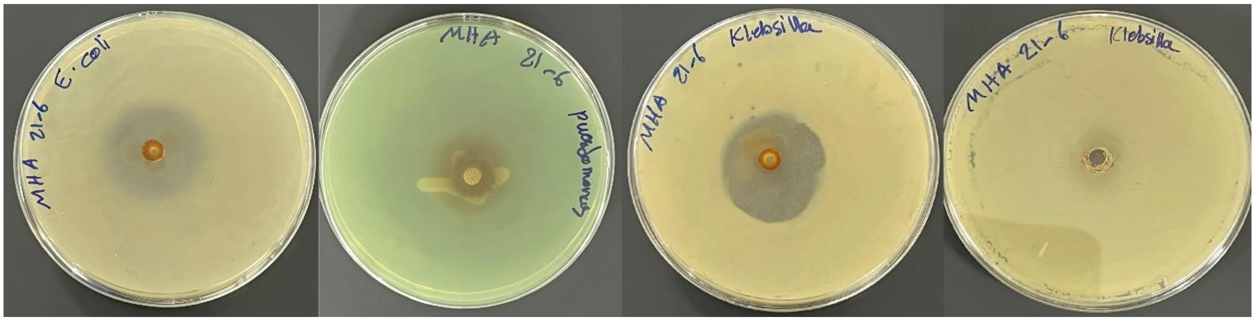

Figure 2: Shows antibacterial activity of Thyme Oil extract against Gram Negative bacteria: A *E. coli, B: Pseudomonas aeruginosa, C: Klebsiella pneumoniae*, D: Negative Control (DMSO) against *Klebsiella pneumonia.*

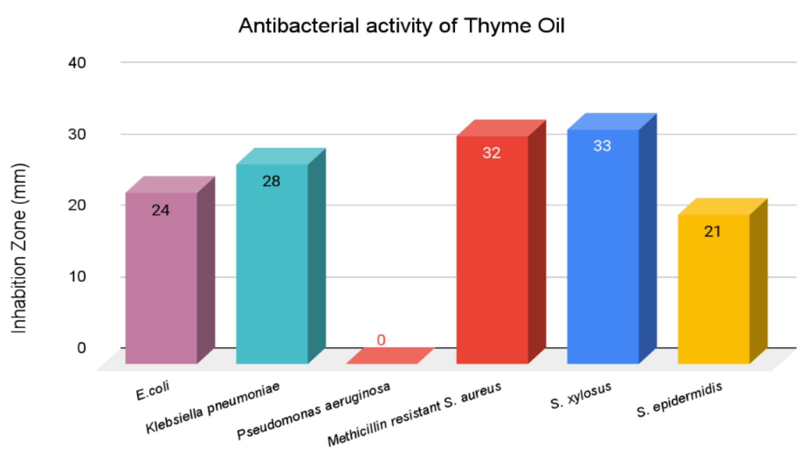

Figure 3: Illustrate the antibacterial activity of Thyme Oil extract against five distinct clinically isolates bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus xylosus, and Staphylococcus epidermidis*).

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Table 1: shows the average of inhibition zone in Millimeter (mm) for each isolate replicates (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus xylosus, and*

Inhibition Zone (mm)	*E. coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Methicillin resistant S. aureus*	*S. xylosus*	*S. epidermidis*
Replicate 1	24	28	0	32	33	21
Replicate 2	24.4	28.5	0	31	34	21
Replicate 3	23.6	27.5	0	33	32	21
Average	24	28	0	32	33	21

Discussion

The present study investigated the antibacterial activity of T. vulgaris leaves oil extracts collected from Piramagron Mountain 50 km far from Sulaimani city-Iraq) against clinically isolated bacterial species: methicillin-resistant *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus xylosus* as a genus of gram-positive bacteria, and *Klebsiella* and *Escherichia coli* as a genus of Gram-negative bacteria.
The results presented in this study showed strong inhibitory activity of Thymus vulgaris oil extract on clinically isolated bacterial species which are common human pathogens, including (*S. xylosus*, Methicillin Resistance *S. aureus, Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, and S. epidermidis*), which was measured by agar well diffusion. The *S. xylosus* and MRSA were the most sensitive strains to *Thymus vulgaris* essential oil by producing the widest growth inhibition zone on the disk (33, 32) mm. A study done by Al-Shuneigat et al, using disc diffusion technique found that the MRSA was highly sensitive to Thymus vulgaris oil and the growth inhibition zone were 24 mm [26]. Other studies also reported similar results [27, 28]. The antimicrobial activity of various thymus species essential oils on MRSA were also reported by Mojab [29]. Similarly, Sienkiewicz et al. [30] investigated the activity of thyme oil against tested clinical strains of *S. aureus*. The authors showed that the clinical strains of *S. aureus* that were resistant to many antibiotics were sensitive to the thyme oil at low concentrations. The authors also observed that the MIC for *S. aureus* was between 0.25 and 1.0 microliter/ml. A study on the antimicrobial activity of Thymus digenesis extract showed strong activity against MRSA by 6.25 mg/ml. The authors attributed the antimicrobial activity potential to flavonoids of the plant [29]. In the present study, the inhibition zone of *T. vulgaris* leaves oil extracts for *Klebsiella pneumonia, S. epidermidis* and *E. coli* were (28, 24, and 21) mm, respectively. The results of the present study was in agreement with Asbaghian et al, [23], which is showed the antibacterial activity of the essential oil of *thymus vulgaris* by determining the minimum inhibitory concentration (MIC) using the broth dilution method against strains of *Escherichia coli* and *Klebsiella pneumonia* and their result indicated that the essential oil of *thymus vulgaris* effectively

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inhibit *E. coli* and *Klebsiella pneumonia*, due to high concentrations of active compounds such as thymol, carvacrol, 1,8-cineol, β-p henchyl alcohol and γ-terpinene [23]. Mustafa Akin & Neslihan Saki [31] used well diffusion assay to determine *Thymus vulgaris* essential oil activity against *E.coli* and noted similar findings.

Mahmoudi et al, [32] investigated the antimicrobial activity of *Thymus vulgaris* essential oil activity against *E.coli* used well diffusion assay and measured the inhibition zone as 10 mm. In another study, Mustafa Akin and Neslihan Saki [33] used disc diffusion method to determine the antimicrobial activities of the ethanol extracts of *Thymus vulgaris* against *Escherichia coli*. The authors showed that the ethanol extracts of *Thymus vulgaris* were effective against *E. coli* in a concentration dependent manner. The mode of action of thyme constituent are not clearly understood, scientist think that due to interactions the hydroxyl group on thymol and carvacrol with the cytoplasmic membrane led to changes its permeability, and affects the stability of its bilayer, leading to burst of cytoplasmic membrane and leakage of cellular contents [34]. Moreover, a study conducted by Kryvtsova et al, demonstrated that the essential oil of thyme vulgaris shows a wide spectrum of antimicrobial activity, which proves especially promising in terms of combatting opportunistic infections microbes [35]. In another study conducted by Mahboubi et al, the authors revealed the antibacterial effect of *thymus vulgaris* against food borne bacteria's such as *E. coli* and attributed it to the total phenolic content of the plant [36]. In the present study, the thyme oil has no antibacterial effect on *Pseudomonas aeruginosa* and this was in contrast with study conducted by Mohsenipour and Hassanshahian [37], Gür et al, [37], and Nzeako et al, [38] in which there was a significant inhibition zone and remarkably decreased the biofilm formation by this bacteria. In contrast to our study, Semeniuc et al, [39] revealed that the Thyme essential oil exhibited strongly (against *E. coli*), moderate (against *Salmonella typhimurium*), and mild inhibitory effects (against *P. aeruginosa* and *S. aureus*). The differences in the antimicrobial activity of *Thymus vulgaris* essential oil in these studies might be attributed to the fact that *Thymus vulgaris* oil chemical composition and active ingredients (thymol, carvacrol, Pcymene, etc.) concentrations are greatly determined by the plant genotype and influence of environmental factors including geographical conditions, nature of soil, temperature, season of collection and harvesting plant, and more important, the oil extraction procedure and culture with sensitivity test procedures [38,40].

Conclusion

Thymus vulgaris essential oil was tested for antimicrobial activities using the agar well method. The essential oil of the thyme showed a significant antimicrobial activity against *S. xylosus*, MRSA, *K. pneumoniae*, *E. coli*, and *S. epidermidis*, which give a promising natural agent for combatting microbial infections. However, the highest antimicrobial effect observed was against *S.xylosus* and MRSA and no effect against *Pseudomonas aeruginosa*. Additionally, this finding could be important for further studies for identifying, purifying and determination of the exact role of bioactive molecules responsible for such effect and discovery of possible uses for both food industry and pharmaceutical applications.

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