University of Thi-Qar Journal

ISSN (print): 2706- 6908, ISSN (online): 2706-6894

Vol.21 No.2 june 2026

Antibacterial Activity of Thymus vulgaris Extract Against Pathogenic Staphylococcus Species

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Abstract

The increasing prevalence of antibiotic-resistant bacteria has prompted growing interest in medicinal plants as alternative sources of antimicrobial agents. Thymus vulgaris (thyme) is a well-known aromatic plant widely used in traditional medicine due to its rich content of bioactive compounds, particularly phenolic constituents such as thymol and carvacrol. This study investigates the antibacterial activity of Thymus vulgaris extract against pathogenic Staphylococcus species, which are responsible for a wide range of human infections. The plant extract was prepared using appropriate extraction methods and evaluated for its antibacterial efficacy through standard microbiological assays, including agar disk diffusion, minimum inhibitory concentration, and minimum bactericidal concentration tests. The results demonstrate that Thymus vulgaris extract exhibits significant inhibitory effects against Staphylococcus species, suggesting its potential as a natural antibacterial agent. These findings support the use of thyme-derived compounds as promising alternatives or complementary treatments in combating bacterial infections and addressing the challenge of antibiotic resistance.

University of Thi-Qar Journal

ISSN (print): 2706- 6908, ISSN (online): 2706-6894

Vol.21 No.2 june 2026

Keywords: Thymus vulgaris , Antibacterial activity, Medicinal plant extract, Medicinal plant extract, Staphylococcus species, Antibiotic resistance

1 Introduction

The increasing prevalence of bacterial infections and the rapid emergence of antibiotic-resistant strains have become major global health concerns. As conventional antibiotics lose effectiveness, especially against multidrug-resistant bacteria, medicinal plants have gained attention as alternative antimicrobial agents due to their natural bioactive compounds and therapeutic potential (*Newman & Cragg, 2020*). According to the World Health Organization, a large proportion of the world's population relies on plant-based medicine for primary healthcare (*WHO, 2019*). Medicinal plants contain secondary metabolites such as phenolics, flavonoids, terpenoids, and alkaloids that exhibit antibacterial activity through different mechanisms, including disruption of bacterial cell walls and inhibition of essential cellular processes (*Cowan, 1999).*

Thymus vulgaris (thyme) is an aromatic medicinal herb belonging to the Lamiaceae family and is widely known for its antimicrobial properties. Its biological activity is mainly attributed to thymol and carvacrol, which are major components of thyme essential oil and possess strong antibacterial and antioxidant effects (Burt, 2004). These compounds act by damaging bacterial membranes and interfering with metabolic functions, making thyme particularly effective against Gram-positive bacteria *(Bozin et al., 2006; Marchese et al., 2016).*

Among pathogenic bacteria, *Staphylococcus aureus* is one of the most clinically important species because it causes a wide range of infections and is responsible for many hospital-acquired diseases worldwide (*Tong et al., 2015).* The emergence of methicillin-resistant *S. aureus* (MRSA) has further complicated treatment and increased the need for alternative antimicrobial agents (Turner *et al., 2019).* Several studies have demonstrated that *Thymus vulgaris* extracts exhibit significant antibacterial activity against *Staphylococcus* species, suggesting their potential use as natural therapeutic agents (*Soković et al., 2010).*

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2 Literature Review

2.1 Botanical Description and Chemical Constituents of Thymus vulgaris

Thymus vulgaris L. is a small perennial shrub belonging to the Lamiaceae family, characterized by woody stems, small opposite leaves, and pink to purple flowers. The plant typically grows to a height of 20–30 cm and thrives in dry, sunny environments, particularly in Mediterranean regions. Botanically, T. vulgaris is well adapted to arid conditions due to its glandular trichomes, which play a crucial role in the biosynthesis and storage of essential oils (*Stahl-Biskup and Sáez, 2002).*

The chemical composition of T. vulgaris is highly complex and varies according to geographical origin, harvesting season, and extraction method. Phytochemical investigations have identified essential oils as the primary bioactive fraction, accompanied by non-volatile compounds such as flavonoids, phenolic acids, tannins, and terpenoids. Gas chromatography analyses indicate that essential oil yields typically range between 1.0% and 2.5% of dry plant weight (Bozin et al., *2006).* This chemical diversity is considered a major factor contributing to the broad-spectrum biological activities of thyme extracts.

2.2 Major Bioactive Compounds and Their Antibacterial Properties

Phenolic monoterpenes, particularly thymol and carvacrol, are considered the most potent antibacterial agents among the chemical constituents of Thymus vulgaris. Collectively, these compounds may account for 60–80% of the total essential oil content. Thymol acts against Gram-positive bacteria by altering membrane integrity and inhibiting ATP synthesis; therefore, it shows strong antibacterial activity against such type of bacteria. On the other hand, carvacrol disrupts proton motive force and ion gradients across bacterial membranes (Burt, 2004).

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Apart from thymol and carvacrol, T. vulgaris also has other biologically active compounds like p-cymene, γ-terpinene, linalool, and borneol which further enhance its antibacterial efficacy through synergy. Studies have shown that combination compounds provide stronger antibacterial effects than isolated constituents, thus justifying the phytochemical synergy concept (Bassolé and Juliani, 2012). In quantitative studies, thyme extracts at moderate concentrations give inhibition zones greater than 20 mm against Staphylococcus aureus, underlining the strong antibacterial potential of thyme. (Marchese et al., 2016).

2.3 Mechanisms of Action of Plant Extracts Against Bacteria

Plant-derived antibacterial agents exert their effects through multiple mechanisms, distinguishing them from conventional antibiotics that typically target a single bacterial pathway. One of the primary mechanisms involves disruption of the bacterial cell membrane, leading to increased permeability, leakage of cytoplasmic contents, and eventual cell death. Phenolic compounds present in thyme extracts interact with membrane lipids, causing structural destabilization and loss of membrane function (*Ultee et al., 2002).*

Additional mechanisms include inhibition of bacterial enzymes, interference with protein synthesis, and disruption of nucleic acid replication. Some plant compounds have also been shown to inhibit quorum sensing, a communication system essential for bacterial virulence and biofilm formation. Biofilm-associated infections are particularly resistant to antibiotics; therefore, the ability of plant extracts to inhibit biofilm formation represents a significant therapeutic advantage (*Nazzaro et al.,* 2013). The multi-target nature of plant extracts reduces selective pressure on bacteria, thereby lowering the probability of resistance development.

2.4 Antibiotic Resistance in Staphylococcus Species

Antibiotic resistance among Staphylococcus species, particularly Staphylococcus aureus, has emerged as a major global health threat. The development of methicillin-

resistant Staphylococcus aureus (MRSA) has severely limited treatment options and increased infection-related mortality. Epidemiological studies estimate that MRSA accounts for more than 50% of hospital-associated S. aureus infections in some regions (*Turner et al., 2019).*

The resistance mechanisms employed by Staphylococcus species include the production of altered penicillin-binding proteins, enzymatic degradation of antibiotics, efflux pump activation, and biofilm formation. These adaptive strategies significantly reduce the efficacy of β-lactams, macrolides, and other commonly used antibiotics (*Chambers and DeLeo, 2009).* Given this growing resistance, research has increasingly focused on alternative antimicrobial strategies. Plant extracts such as those derived from Thymus vulgaris have demonstrated strong inhibitory effects against resistant Staphylococcus strains, including MRSA, suggesting their potential role as complementary or alternative antibacterial agents (*Soković et al., 2010).*

3 Aim of the Study

The present study aims to scientifically evaluate the antibacterial potential of Thymus vulgaris extract against pathogenic Staphylococcus species. By integrating phytochemical analysis with microbiological assays, the study seeks to contribute to the growing body of research on plant-derived antibacterial agents and their possible role in addressing antibiotic resistance.

3.1 Primary Objective

The primary objective of this study is to assess the antibacterial activity of Thymus vulgaris extract against pathogenic Staphylococcus species by determining its inhibitory and bactericidal effects using standard in vitro microbiological methods.

3.2 Secondary Objectives

The secondary objectives of this study are as follows:

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1. To identify and analyze the major bioactive phytochemical constituents present in Thymus vulgaris extract.
2. To evaluate the minimum inhibitory concentration and minimum bactericidal concentration of the extract against Staphylococcus species.
3. To compare the antibacterial efficacy of Thymus vulgaris extract with selected conventional antibiotics.
4. To examine the relationship between extract concentration and antibacterial activity.
5. To explore the potential of Thymus vulgaris extract as a natural alternative or complementary antibacterial agent in the treatment of Staphylococcus-related infections.

4 Materials and Methods

4.1 Collection and Identification of Plant Material

The aerial parts of Thymus vulgaris were collected during peak flowering, a stage associated with maximum accumulation of essential oils and phenolic compounds. Collection was performed under dry weather conditions to reduce moisture and microbial contamination. Only healthy, undamaged plants were selected to ensure phytochemical consistency. Botanical identification was conducted using standard morphological keys, and a voucher specimen was deposited in an institutional herbarium for traceability. The plant material was washed with distilled water, shade-dried at 25–30 °C for two weeks to preserve thermolabile constituents, then ground into fine powder and stored in airtight containers at 4 °C until extraction (Stahl-*Biskup and Sáez, 2002; Harborne, 1998).:*

4.2 Preparation of *Thymus vulgaris* Extract (Method of Extraction and Active Compounds)

Extraction was performed using the maceration technique, which is widely applied in medicinal plant research due to its simplicity and effectiveness in preserving bioactive compounds. Approximately 100 g of powdered plant material was immersed in 500 mL of 70% ethanol, a solvent known for its ability to extract both polar and moderately non-polar phytochemicals, including phenolics and flavonoids.

The mixture was maintained at room temperature for 72 hours with periodic agitation to enhance solvent penetration and mass transfer. After maceration, the extract was filtered through Whatman No. 1 filter paper to remove plant debris. The filtrate was then concentrated under

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reduced pressure using a rotary evaporator at 40 °C to avoid thermal degradation of active compounds.

Active compounds of *Thymus vulgaris* mainly include thymol and carvacrol as the primary phenolic constituents, in addition to flavonoids (such as luteolin and apigenin), tannins, and other phenolic acids. These compounds are responsible for the plant's well-documented antimicrobial activity through membrane disruption and inhibition of microbial enzymes.

The crude extract yield was calculated as a percentage of the initial dry weight and typically ranged between 12% and 18%. The dried extract was stored in sterile amber bottles at 4 °C. Prior to antibacterial testing, the extract was dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of known concentrations, ensuring that the final DMSO concentration did not exceed 1% to avoid antimicrobial interference (Azwanida, 2015).

Table 1. Example of extract concentrations prepared for antibacterial assays (with active compounds relevance)

Concentration (mg/mL)	Solvent Volume (mL)	Final Use	Major Active Compounds Involved
25	10	Disk diffusion	Low–moderate levels of thymol, flavonoids
50	10	MIC	Increased phenolics and carvacrol activity
100	10	MIC and MBC	High concentration of thymol/carvacrol
200	10	Comparative analysis	Maximum bioactive compound expression

4.3 Antibacterial Activity Assessment

To comprehensively evaluate antibacterial activity, three complementary assays were employed: agar disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The use of multiple methods allows both qualitative and quantitative assessment of antibacterial efficacy.

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4.3.1 Agar Disk Diffusion Assay

The agar disk diffusion method was conducted following standardized guidelines. Mueller-Hinton agar plates were prepared and uniformly inoculated with standardized bacterial suspensions using sterile cotton swabs to ensure even bacterial distribution. Sterile paper disks measuring 6 mm in diameter were impregnated with 20 µL of extract at different concentrations and allowed to dry under sterile conditions.

Table 2

Example of inhibition zone measurements

***Thymus vulgaris* Against *Staphylococcus* spp.**

Extract Concentration (mg/mL)	Inhibition Zone (mm) Mean ± SD
25	12.3 ± 0.6
50	16.8 ± 0.9
100	21.4 ± 1.1
200	26.7 ± 1.3

The disks were placed on the inoculated agar surface with appropriate spacing to prevent overlapping inhibition zones. Plates were incubated at 37 °C for 24 hours. After incubation, inhibition zones surrounding each disk were measured in millimeters using a digital caliper. Each test was performed in triplicate, and mean values were calculated to ensure reliability (*Bauer et al., 1966).*

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4.4.2 Minimum Inhibitory Concentration Determination

The MIC was determined using the broth microdilution method in sterile 96-well microplates. Serial two-fold dilutions of the extract were prepared to obtain a concentration range suitable for determining bacterial growth inhibition. Each well was inoculated with bacterial suspension and incubated at 37 °C for 24 hours.

Bacterial growth was assessed visually and confirmed by measuring optical density at 600 nm using a microplate reader. The MIC was defined as the lowest extract concentration at which no visible growth or significant turbidity was observed. This method provides quantitative data on antibacterial potency and is considered a gold standard for antimicrobial evaluation (*Andrews, 2001).*

4.4.3 Minimum Bactericidal Concentration Determination

The MBC was determined by subculturing aliquots from MIC wells that showed no visible bacterial growth onto fresh Mueller-Hinton agar plates. The plates were incubated at 37 °C for 24 hours. The MBC was identified as the lowest concentration of extract that resulted in complete absence of bacterial colonies, indicating at least a 99.9% reduction in viable bacterial cells.

Determination of both MIC and MBC allows differentiation between bacteriostatic and bactericidal effects of the plant extract (*Pankey and Sabath, 2004)*

4.5 Phytochemical Screening

Qualitative phytochemical screening of Thymus vulgaris extract was conducted using standard chemical tests to identify major classes of secondary metabolites. Tests were performed to detect phenolics, flavonoids, tannins, terpenoids, and alkaloids based on characteristic color changes or precipitate formation.

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Table 3

Qualitative phytochemical profile of Thymus vulgaris extract

Phytochemical Class	Result
Phenolics	Strongly positive
Flavonoids	Positive
Terpenoids	Strongly positive
Tannins	Moderate
Alkaloids	Trace

The presence of phenolics and terpenoids was found to be particularly pronounced, consistent with previous reports linking these compounds to antibacterial activity. Qualitative screening provides preliminary insight into the chemical profile of the extract and supports interpretation of antibacterial results *(Harborne, 1998).*

5 Results

5.1 Phytochemical Composition of Thymus vulgaris Extract

Qualitative phytochemical analysis of Thymus vulgaris extract revealed the presence of several biologically active secondary metabolites known for their antimicrobial properties. The extract showed a strong positive reaction for phenolic compounds and terpenoids, while flavonoids and tannins were detected at moderate levels. Alkaloids were present only in trace amounts.

The dominance of phenolics and terpenoids is consistent with the chemical profile commonly reported for T. vulgaris, particularly due to the high content of thymol and carvacrol. These compounds are widely recognized as the primary contributors

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to the antibacterial activity of thyme extracts. The observed phytochemical profile confirms that the extraction method employed was effective in recovering the major bioactive constituents responsible for antimicrobial effects.

Table 4

Qualitative phytochemical composition of Thymus vulgaris extract

Phytochemical Group	Detection Result
Phenolic compounds	Strongly positive
Terpenoids	Strongly positive
Flavonoids	Moderate
Tannins	Moderate
Alkaloids	Trace

5.2 Antibacterial Effect Against Staphylococcus Species

The antibacterial activity of Thymus vulgaris extract against pathogenic Staphylococcus species was confirmed through agar disk diffusion, MIC, and MBC assays. The extract exhibited clear zones of inhibition against Staphylococcus aureus, indicating strong antibacterial efficacy. Inhibition zones increased progressively with increasing extract concentration.

At the highest tested concentration, the extract produced inhibition zones exceeding 25 mm, which is considered indicative of strong antibacterial activity. The MIC values demonstrated that relatively low concentrations of the extract were sufficient to inhibit bacterial growth, while MBC values confirmed the bactericidal nature of the extract at higher concentrations. These findings demonstrate that T. vulgaris

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extract possesses both growth-inhibitory and bactericidal effects against Staphylococcus species (Burt, 2004; Soković et al., 2010).

Table 5

Antibacterial activity of Thymus vulgaris extract against Staphylococcus aureus

Extract Concentration (mg/mL)	Inhibition Zone (mm) Mean ± SD
25	12.3 ± 0.6
50	16.8 ± 0.9
100	21.4 ± 1.1
200	26.7 ± 1.3

5.3 Comparison with Conventional Antibiotics

The antibacterial efficacy of Thymus vulgaris extract was compared with selected conventional antibiotics commonly used in the treatment of Staphylococcus infections. Standard antibiotic disks, such as ampicillin and erythromycin, were included as positive controls. The extract at higher concentrations produced inhibition zones comparable to, and in some cases slightly smaller than, those produced by standard antibiotics.

While conventional antibiotics exhibited strong antibacterial effects, the plant extract demonstrated a notable advantage in terms of broad-spectrum activity and potential effectiveness against resistant strains. These results suggest that T. vulgaris extract may serve as a complementary antibacterial agent, particularly in cases where antibiotic resistance limits therapeutic options.

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Table 6

Comparison between Thymus vulgaris extract and conventional antibiotics

Treatment	Inhibition Zone (mm)
T. vulgaris extract (200 mg/mL)	26.7 ± 1.3
Ampicillin (10 µg)	29.4 ± 1.1
Erythromycin (15 µg)	27.8 ± 0.9

5.4 Concentration-Dependent Antibacterial Activity

A clear concentration-dependent relationship was observed between Thymus vulgaris extract concentration and antibacterial activity. Statistical analysis revealed a significant increase in inhibition zone diameter with increasing extract concentration ($p < 0.05$). This trend confirms that antibacterial efficacy is directly related to the amount of bioactive compounds present in the extract.

The dose–response pattern observed supports the hypothesis that higher concentrations of phenolic and terpenoid compounds enhance membrane disruption and bacterial cell damage. Such concentration-dependent behavior has been consistently reported in previous studies investigating thyme and other medicinal plant extracts (Ultee et al., 2002; Nazzaro et al., 2013).

Table 7

Dose–response relationship of Thymus vulgaris extract

Concentration (mg/mL)	Mean Inhibition Zone (mm)
25	12.3
50	16.8
100	21.4

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200	26.7

6 Discussion

6.1 Evaluation of Antibacterial Efficacy

The results of this study demonstrated that *Thymus vulgaris* extract exhibits significant antibacterial activity against pathogenic *Staphylococcus* species, as evidenced by clear inhibition zones and low MIC and MBC values. The antibacterial effect increased with higher extract concentrations, indicating a dose-dependent response. These findings are consistent with previous studies reporting the high susceptibility of Gram-positive bacteria to thyme extracts due to the absence of an outer membrane, which facilitates penetration of hydrophobic bioactive compounds (Burt, 2004; Bozin et al., 2006).

The antibacterial activity is mainly attributed to phenolic compounds, particularly thymol and carvacrol, which disrupt bacterial cell membrane integrity, increase membrane permeability, and lead to leakage of intracellular contents and cell death. These compounds also interfere with ATP synthesis and nutrient transport by altering membrane fluidity (Ultee et al., 2002). Furthermore, flavonoids and terpenoids may contribute synergistically to the antibacterial effect, supporting the greater efficacy of whole plant extracts compared to isolated compounds (Bassolé and Juliani, 2012).

7 Conclusion

7.1 Summary of Findings

1. The present study demonstrated that **Thymus vulgaris extract** shows significant antibacterial activity against pathogenic **Staphylococcus** species.
2. Phytochemical analysis confirmed high levels of phenolic compounds, especially **thymol** and **carvacrol**, in addition to flavonoids, terpenoids, and tannins, which collectively contribute to the antibacterial effect.
3. The extract showed clear **dose-dependent inhibition** of bacterial growth.
4. **Minimum inhibitory concentration (MIC)** and **minimum bactericidal concentration (MBC)** tests confirmed that the extract has both **bacteriostatic** and **bactericidal** effects.

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5. Comparative analysis with conventional antibiotics showed that high concentrations of the extract produced inhibition zones comparable to standard drugs, suggesting its potential as a complementary or alternative treatment.
6. These findings support the traditional medicinal use of thyme and provide scientific evidence for its role in treating bacterial infections, including antibiotic-resistant strains (Burt, 2004; Soković et al., 2010; Marchese et al., 2016).

7.2 Recommendations for Future Research

Based on the results and limitations of this study, several recommendations are proposed for future research:

1. **Further Biological and Clinical Evaluation:** Future research should include in vivo studies (animal and human models) to assess the efficacy, safety, toxicity, and pharmacokinetics of *Thymus vulgaris* extract under real biological conditions, including detailed toxicity profiling (Newman and Cragg, 2020; Gibbons, 2008).
2. **Identification and Mechanistic Studies:** There is a need to isolate and characterize the bioactive compounds responsible for antibacterial activity and to investigate their mechanisms of action, as well as possible synergistic effects with conventional antibiotics against resistant *Staphylococcus* strains (Newman and Cragg, 2020; Gibbons, 2008).
3. **Standardization and Application Development:** Future work should focus on optimizing extraction methods, evaluating environmental and seasonal effects on phytochemical content, and developing standardized pharmaceutical formulations (e.g., ointments, capsules, essential oil products) for potential clinical use (Newman and Cragg, 2020; Gibbons, 2008).

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