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Evaluation the ethanol and methanol extracts of thymus vulgaris as antibacterial agent against Staphylococcus aureus and Escherichia coli

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ABSTRACT: Plants are rich sources of antibacterial compounds due to their content of flavonoids, phenols, terpenes, alkaloids, saponins, and volatile oils, which are effective against pathogenic bacteria, including strains resistant to conventional antibiotics. This study aims to evaluate the antibacterial activity of ethanol and methanol extracts of Thymus vulgaris collected from Souq Alkhamis Msehel area-Libya, against *staphylococcus aureus* and *Escherichia coli* at various concentrations and comparing the efficacy of the extracts to commonly used antibiotics, using the agar well diffusion technique. The results showed that both extracts possessed antibacterial activity, with the ethanol extract clearly outperforming the methanol extract; the diameters of the inhibition zones for the ethanol extract against *S. aureus* and *E. coli*, recorded results ranged from the lowest to highest concentration as following: (12.10±1.63 - 21.00±7.50mm), (13.10±0.60 - 21.77±1.05mm) respectively. On the other hand, the diameters of the inhibition zones for the methanol extract was varied according to the concentration as no antibacterial activity was observed (0.00±0.00mm) at the lower concentrations (50 and 100mg/ml), and the inhibition zone against *S. aureus* was initiated at concentration (150mg/ml), where recorded 10.47±1.44 reach to 16.07±1.72mm at the highest concentration 250mg/ml, and against *E. coli* the inhibition was initiated at a concentration (200 mg/ml) persisted up to 250mg/ml, reaching their maximum diameters where recorded (10.43±0.94 - 13.23±0.81mm) ranged from 10.43±0.94 to 16.07±1.72mm, reflecting a clear increase in inhibitory activity with increasing concentration. In comparison of the investigated standard antibiotics, Ciprofloxacin maintained absolute superiority and the highest inhibitory activity compared to Azithromycin against *S. aureus*, whereas Ciprofloxacin clearly outperformed Gentamicin against *E. coli.* These results indicate that the ethanol extraction of thyme represents a promising natural source of antibacterial compounds, and could contribute to the development of natural therapeutic preparations or antibiotic adjuvants in the fight against bacterial infections.

Keywords: Antibacterial, *Staphylococcus aureus, Escherichia coli*, Thymus vulgaris, Inhibition zone.

INTRODUCTION

Food-borne pathogens continue to cause major public health problems throughout the world Heredia and **Garcia (2018).** Food-borne diseases cause considerable morbidity, mortality, and economic losses worldwide. The WHO estimates that over 2 billion illnesses resulting in approximately 1 million deaths caused by 22 major foodborne pathogens **Kirk *et al.* (2015).** In the last decades, problem with antibiotic resistant bacteria has emerged. Antimicrobial resistance (AMR) is a major threat for human health world-wide, impairing the capacity to treat an increasing number of infections. Antibiotic resistance is one of the greatest challenges facing the healthcare sector in the modern era **Zhu *et al.* (2022).** In 2023 the World Health Organization (WHO) estimates that millions of infections annually

are caused by multidrug-resistant bacterial strains, this is due to the over use and uncontrolled use of chemical antibiotics, which has led to their ineffectiveness against many pathogenic bacteria, such as *Staphylococcus aureus* and *Escherichia coli* **Patra (2025)**. The highest rates of resistant infections are recorded in Southeast Asia, Africa, and Latin America, with approximately 5 million deaths annually, particularly in low- and middle-income countries due to weak drug regulation and easy access to over-the-counter medications. Highly resistant strains such as methicillin-resistant *S. aureus* (MRSA) and Extended spectrum beta lactamase (ESBL) producing *E. coli* are also spreading in hospitals, making the search for natural alternative treatments, such as thyme extract, a pressing need to curb the spread of infection **WHO (2023)**.

Among the promising approaches to addressing this problem is the use of medicinal plants as a natural source of antimicrobial compounds. Aromatic plants contain volatile oils and phenolic compounds that have proven effective in inhibiting the growth of pathogenic bacteria and fungi **Nazzaro *et al.* (2013); Jamal *et al.* (2026)**. The medicinal plants are important source effective materials, intervention in the preparation of many drugs. It has been proven scientifically that active laboratory manufacturer substance does not perform the same physiological influence role of active derived substance from medicinal plants, also in addition side effects of prepared material on the body, which may not appear until after a period may be long, **Abdi *et al.* (2025)**. Thymus vulgaris (commonly known as thyme) is a widely used medicinal herb with a long history in both traditional and modern therapeutics; Thyme contains active compounds, most notably thymol and carvacrol, both phenolic compounds with antibacterial activity. These compounds work by disrupting the bacterial cell wall, increasing its permeability, and inhibiting vital enzymes **Burt (2004); Marchese *et al.* (2016)**.

Previous studies have shown that T. vulgaris is active against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains such as (MRSA). Its broad-spectrum antimicrobial potential has led to increasing interest in its application in medicine, food preservation, and personal care products, particularly as a natural alternative to synthetic antimicrobials **Aziz *et al.* (2022)**. Therefore, this study aims to evaluate the antibacterial effect of ethanol and methanol thyme extract against pathogenic bacteria. It also seeks to determine the differences in efficacy depending on the solvent used and to compare the results with reference conventional antibiotics, and research results are expected to demonstrate the high efficacy of thyme extract in inhibiting the growth of pathogenic bacteria. This strengthens its potential as a safe and effective natural alternative to chemical antibiotics in both the medical and food industries.

METHODS

This study was conducted in the microbiology lab at the high institute of Souq Alkhamis Msehel area-Libya, from September 2025 to January 2026.

Bacterial strains

Gram-positive and Gram-negative bacterial species were used: *S. aureus* and *E. coli.* The bacterial strains were obtained from Department of Medical Microbiology of the National Animal Health Centre (NAHC), Libya. Bacteria were maintained on nutrient agar slant and stored at 4 °C for subsequent analysis.

Collection of sample

The Thymus vulgaris sample used in this project was collected from Souq Alkhamis Msehel area, which is well known for its environmental suitability for the growth of medicinal and aromatic plants. The samples were carefully selected to ensure their integrity and absence of damage or infestation, and then transported to the laboratory in clean container. A preliminary cleaning process was carried out using distilled water to remove any remaining impurities and air-dried at room temperature then storage under appropriate conditions until use in laboratory analyses and experiments.

Thyme extract preparation

After drying, the leaves were ground into a fine powder using an electric blender to obtain a homogeneous sample. The extract was prepared by soaking 25g of the powder in 100ml (80% of methanol and 80% ethanol) v/v separately for 72 hours with intermittent stirring to promote the transfer of active compounds such as thymol and carvacrol to the solvent. After extraction, the sample was filtered, and the solvent was separated using rotatory evaporator to obtain a concentrated extract. The extract was then stored in dark glass containers at 4 °C to protect the compound from oxidation and degradation according to **Devkota and Das (2015)**.

Thyme extracts concentration

The current study relied on scientific literature confirming that the antibacterial activity of thyme extract is directly related to increasing concentration. Thus, in this study we prepared a different concentration of thyme extract: (50, 100, 150, 200 and 250 mg/ml) were made in both solvents (ethanol and methanol) separately. Ethanol and methanol were used as negative control and the traditional antibiotic introduced as positive control according to **Devkota and Das (2015)**.

Antibacterial activity assay

The Antibacterial activity of the Thymus vulgaris extracts was evaluated using the agar well diffusion method. *S. aureus and E. coli* ware cultured separately in 50 ml nutrient broth and incubated for 24 hrs. Mueller–Hinton agar were prepared and after autoclaving, the media allowed to cooled to approximately 45–50°C then inoculated directly by adding 1% of the tested bacterial pathogen grown in liquid nutrient broth before the agar solidified and was poured into the plates, ensuring a homogenous distribution of the bacteria within the medium. After complete solidification of the agar, 6 mm wells were made using a standard cork borer, and a volume (100 µL) of thyme extract for its different concentrations was introduced into these wells. For comparative purposes and to ensure accurate results, standard antibiotics and the extraction solvents were tested under the same experimental conditions. Following incubation of the plates at 37°C for 24 hours, the antimicrobial efficacy was determined by measuring the diameter of inhibition zone formed around each well in millimeters (mm) **Kowalczyk *et al.* (2021)**.

Data analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance using GraphPad Prism software. Differences were considered statistically significant at $p \geq 0.05$ according to **Ali *et al.* (2024)**.

RESULTS

Two thyme vulgaris extracts (ethanol and methanol) were evaluated using agar well diffusion method at different concentrations, include (250, 200, 150, 100 and 50mg/ml) for their antibacterial activity against *S. aureuse* and *E. coli*.

The results of the study showed that thyme vulgaris extracts prepared using ethanol and methanol exhibited antibacterial activity against the tested pathogenic bacteria. However; the ethanol extract showed significantly higher efficacy compared to the methanol extract at all tested concentrations. While the ethanol extract exhibited clear inhibitory activity that increased gradually with increasing concentration, the methanol extract did not show any antibacterial activity at low concentrations (50 and 100mg/ml), and the inhibitory effect was limited to the higher concentrations (150, 200 and 250mg/ml).

When compared the effect of thyme extract with the best effective standard antibiotics against the tested pathogenic bacteria, the ethanol extract demonstrated similar or superior efficacy at certain concentrations, whereas the methanol extract was less effect than the standard antibiotics, reflecting it's the weak ability to inhibit the growth of the tested bacterial. The results, expressed as mean ± standard deviation (SD) of the inhibition zone diameter in millimetres. The antibacterial activity was assessed by measuring the diameter in millimetre of inhibition zones formed around the wells containing the extract.

Antibacterial activity of thyme vulgaris extracts

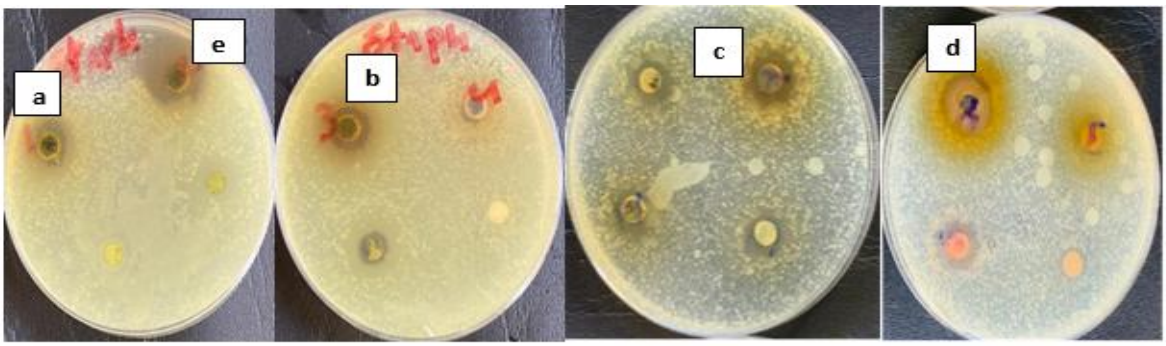
1. Evaluation of the antibacterial activity of ethanol and methanol extracts of thyme vulgaris against *S. aureus*

For *S. aureus,* the obtained results showed that the ethanol extract exhibited antibacterial activity at all tested concentrations. The inhibition zone measured 12.10 ± 1.63mm at (50mg/ml), 13.77 ± 1.19mm at (100 mg/ml), 18.23 ± 4.18mm at (150mg/ml), 20.13 ± 4.42mm at (200mg/ml), and reached its highest value of 21.00 ± 7.50mm at (250mg/ml) Table (1), Figure (1, 4). In comparison, the methanol extract demonstrated inhibitory activity only at the higher concentrations, producing inhibition zones of 10.47 ± 1.44mm at (150 mg/ml), 12.50 ± 1.55mm at (200mg/ml), and 16.07 ± 1.72mm at (250mg/ml), whereas no antibacterial activity was observed at the lower concentrations Table (1), Figure (2, 4). Relative to the standard antibiotics, ciprofloxacin produced the largest inhibition zone (42.90±0.72mm), markedly exceeding that of azithromycin (9.15±0.95mm). Although the antibacterial activity of ciprofloxacin remained substantially higher than that of the ethanol extract, most concentrations of the ethanol extract exhibited greater inhibitory activity than

azithromycin. Meanwhile, the methanol extract produced lower inhibition zones than both ciprofloxacin and azithromycin Table (1), Figure (3, 4).

Table (1): Show the zone of inhibitioni for dfferent Concentration of the thymus vulgaris extracts and conventional antibiotic (mm) against *S. aureus*

Concentrate	N	Ethanol extract	P value	Methanol extract	P value
50 mg/ml	3	12.10±1.63	< 0.431	0.0±0	_
100 mg/ml	3	13.77±1.19	< 0.431	0.0±0	_
150 mg/ml	3	18.23±4.18	< 0.431	10.47±1.44	< 0.0001
200 mg/ml	3	20.13±4.42	< 0.431	12.50±1.55	< 0.0001
250 mg/ml	3	21.00±7.50	< 0.431	16.07±1.72	< 0.0001
ciprofloxacin (5µg/disc)		42.90±0.72	< 0.0001		
Azithromycin (15µ/disc)		9.15±0.95	< 0.0001		



(1): Show the zone of inhibition for the thyme vulgaris ethanol extract at different concentrations against *S. aureus*.

*(a) concentration of 50 mg/ml, (b) concentration of 100 mg/ml, (c) concentration of 150 mg/ml, (d) concentration of 200 mg/ml, (e) concentration of 250 mg/ml.

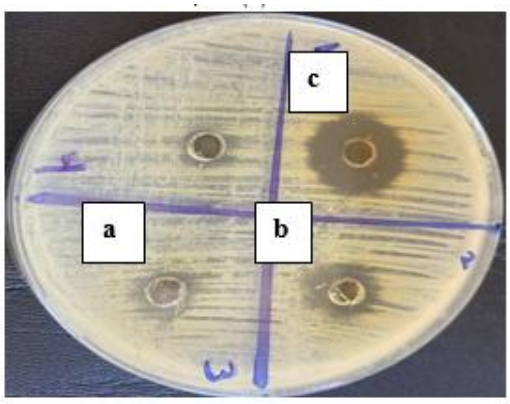


Figure (2): Show the zone of inhibition for the thyme vulgaris methanol extract at different concentrations against *S. aureus*.

*(a) concentration of 150 mg/ml, (b) concentration of 200 mg/ml, (c) concentration of 250 mg/m

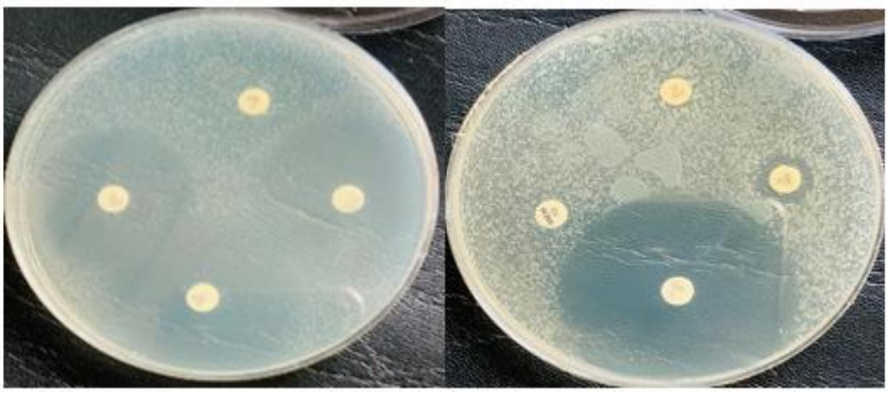


Figure (3): Show the zone of inhibition of conventional antibiotic against *S. aureus.*

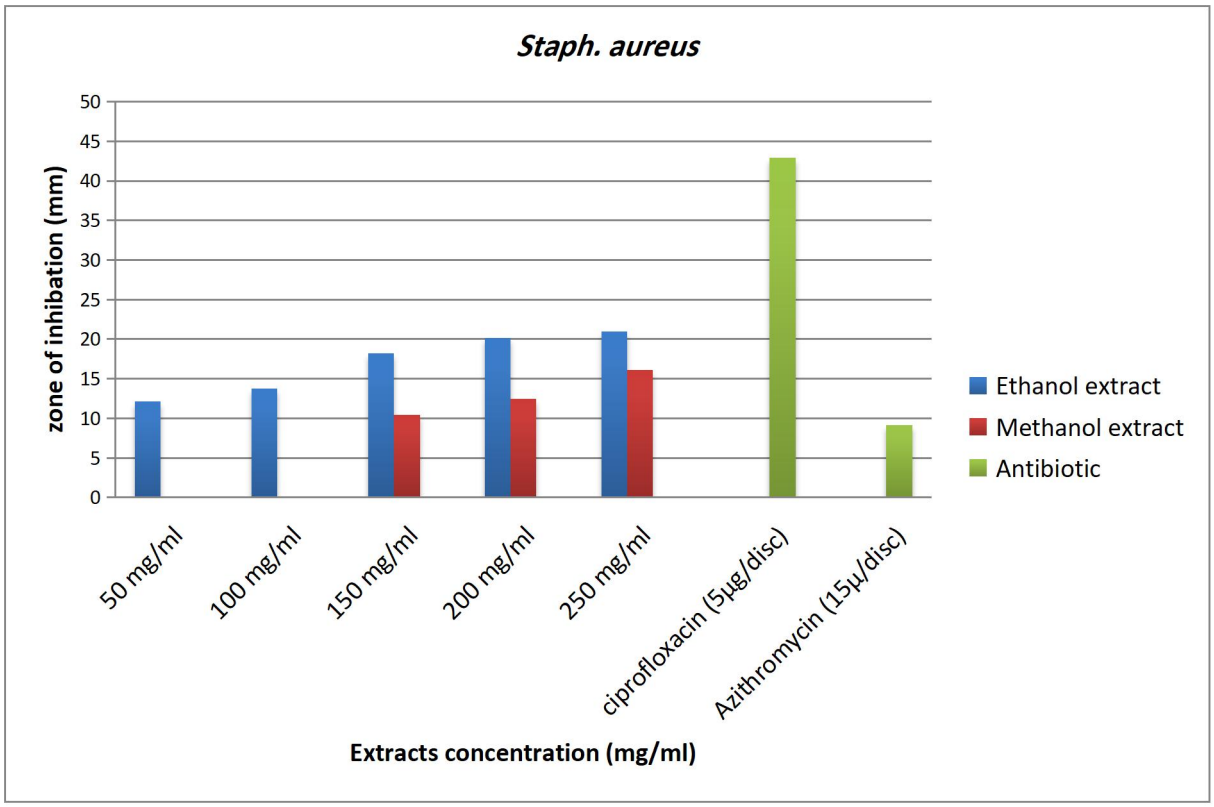


Figure (4): The statistical analysis of inhibition zone for the thyme vulgaris extracts (ethanol and methanol) and conventional antibiotic against *S. aureus.*

2. Evaluation of the antibacterial activity of ethanol and methanol extracts of thymus vulgaris against *E. Coli*

For *E. coli,* the obtained results showed that the ethanol extract exhibited antibacterial activity at all tested concentrations. The inhibition zone measured 13.10 ± 0.60mm at (50mg/ml), 14.13 ± 0.88mm at (100mg/ml), 16.77 ± 0.67mm at (150mg/ml), 20.13 ± 0.92mm at (200mg/ml), and reached its highest value of 21.77 ± 1.05mm at (250mg/ml) Table (2), Figure (5, 8). In comparison, the methanol extract demonstrated inhibitory activity only at the higher concentrations, producing inhibition zones of 10.43 ± 0.94mm at (200mg/ml), and 13.23 ± 0.81mm at (250mg/ml), whereas no antibacterial activity was observed at the lower concentrations (50, 100, and 150mg/ml) Table (2), Figure (6, 8). Relative to the standard antibiotics, ciprofloxacin produced the largest inhibition zone (21.27 ± 0.54mm), markedly exceeding that of gentamicin (15.43 ± 0.41mm). Although the antibacterial activity

of ciprofloxacin remained substantially higher than that of the methanol extract, most concentrations of the ethanol extract exhibited greater or comparable inhibitory activity to ciprofloxacin, and completely outperformed gentamicin. Meanwhile, the methanol extract produced lower inhibition zones than both ciprofloxacin and gentamicin at all active concentrations, as shown in Table (2), Figure (7, 8).

Table (2): Show the zone of inhibition for different concentration for the thymus vulgaris extracts and conventional antibiotic (mm) against *E.coli*

Concentrate	N	Ethanol extract	P value	Methanol extract	P value
50 mg/ml	3	13.10±0.60	< 0.0001	0.0±0	_
100 mg/ml	3	14.13±0.88	< 0.0001	0.0±0	_
150 mg/ml	3	16.77±0.67	< 0.0001	0.0±0	_
200 mg/ml	3	20.13±0.92	< 0.0001	10.43±0.94	< 0.0001
250 mg/ml	3	21.77±1.05	< 0.0001	13.23±0.81	< 0.0001
Ciprofloxacin (5µg/disc)		21.27±0.54	< 0.0001		
Gentamicin (10µg/disc)		15.43±0.41	< 0.0001		

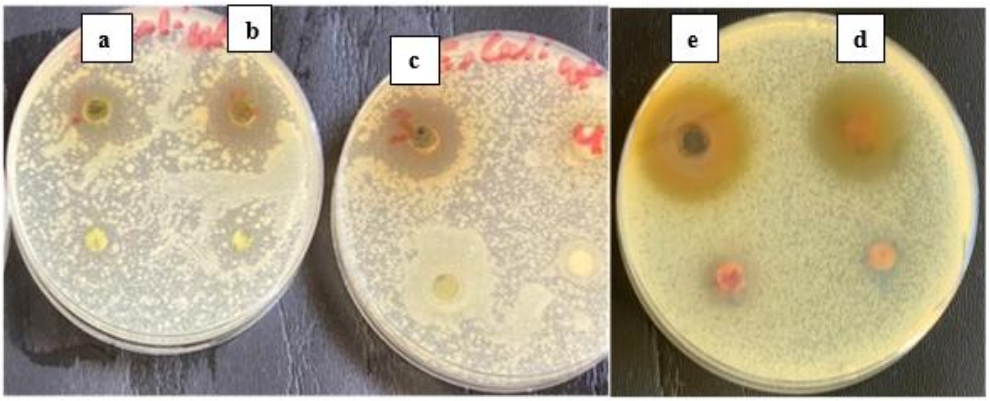


Figure (5): Show the zone of inhibition for the thyme vulgaris ethanol extract at different concentrations against *E. coli*.

*(a) concentration of 50 mg/ml, (b) concentration of 100 mg/ml, (c) concentration of 150 mg/ml, (d) concentration of 200 mg/ml, (e) concentration of 250 mg/ml.

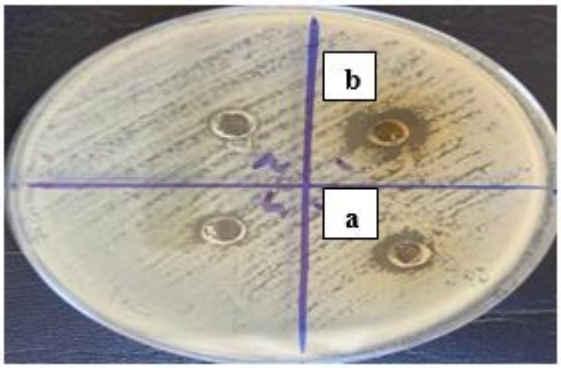


Figure (6): Show the zone of inhibition for the thyme vulgaris methanol extract at different concentrations against *E. coli.*

*(a) concentration of 200 mg/ml, (b) concentration of 250 mg/ml

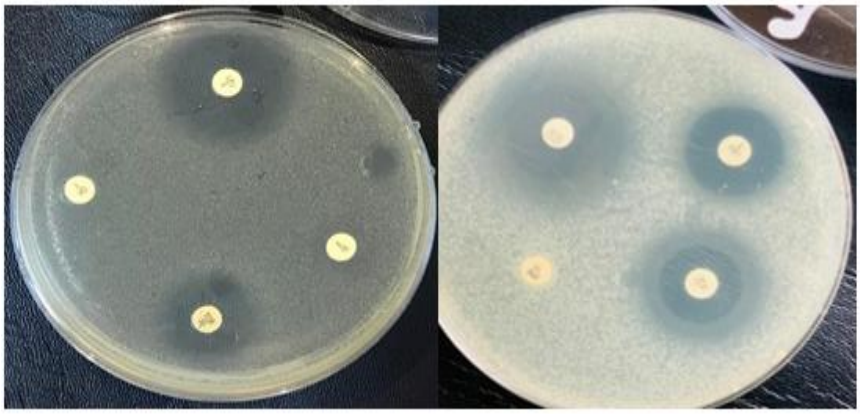


Figure (7): Show the zone of inhibition of conventional antibiotic against *E. coli.*

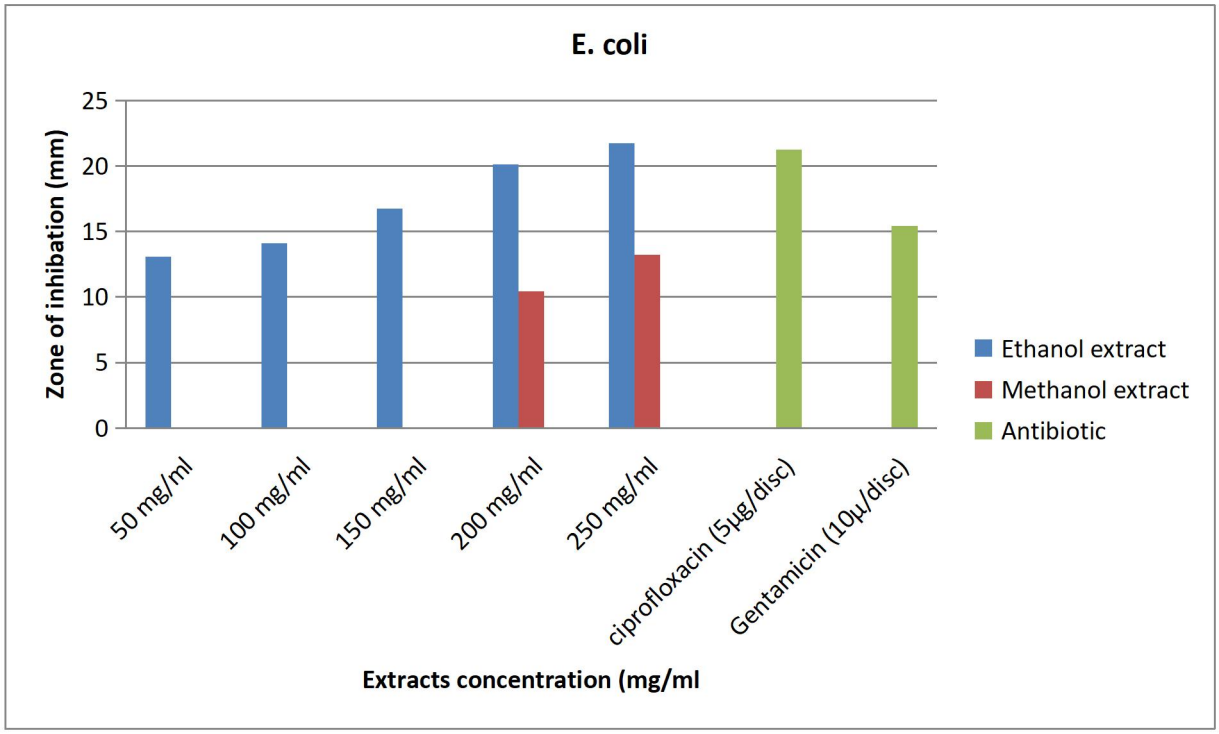


Figure (8): The statistical analysis of inhibition zone for the thyme vulgaris extracts (ethanol and methanol) and conventional antibiotic against *E. coli.*

DISCUSSION

Due to a high incidence of antibiotic resistance, evaluating the antibacterial effect of herbal medicines as potent agents to combat resistance pathogenic bacteria a paramount importance in addressing human as well as animal health problems **Mahima *et al.* (2012), Divya *et al.* (2014).** Natural products contain a range of compounds which may enable the development of novel antimicrobial agents as conventional antimicrobial agents. The secondary metabolites present in a medicinal plant may have different modes of antimicrobial action which help combat the emergence of resistance **Tadesse *et al.* (2016).** Thyme contains numerous bioactive compounds with demonstrated biological activity against a wide range of infectious agents **Hashim *et al.* (2026).** The current study aimed to investigate antibacterial activities of ethanol and methanol thyme extract against two pathogenic bacteria: *S. aureus* and *E. coli.*

In the present study, inhibition-zone diameters for both ethanol and methanol extracts increased proportionally with extract concentration, confirming a clear dose-dependent antibacterial effect **Al-Snafi (2020)**. Moreover, the Gram-positive bacteria (*S. aureus*), exhibited larger inhibition zones at certain concentrations compared to Gram-negative bacteria (*E. coli*), this result agreement with findings of **Jung *et al.* (2022)** who showed that Gram-positive bacteria are more effective to Thymus vulgaris extract as antibacterial agent. This difference is likely related to structural distinctions in bacterial cell envelopes; the outer membrane of Gram-negative bacteria acts as a barrier that restricts the penetration of many antimicrobial compounds **Tavares *et al.* (2020)**. This antibacterial activity of T. vulgaris can be attributed to its rich in phytochemical profile, particularly thymol and carvacrol, which possess strong antibacterial properties. These compounds disrupt bacterial cell membranes, alter membrane permeability, and interfere with essential metabolic processes, ultimately leading to leakage of intracellular components and cell death **Patil *et al.* (2021)**.

Although the inhibition zones produced by the ethanol extract were smaller than those produced by the broad-spectrum antibiotic (ciprofloxacin) against *S. aureus*, the extract notably outperformed ciprofloxacin against *E. coli* at the highest concentration. On the other hand, ethanol extract demonstrated a superior antibacterial activity compared to the methanol extract, which showed no efficacy against the tested pathogens at lower concentrations and only produced a mild inhibitory effect at higher concentrations this result similar to result recorded in study conducted by **Ali *et al.* (2024)**.

In this study, we were able to verify that Gram-positive bacteria (*S. aureus*) were clearly sensitive to different concentrations of the ethanol and methanol extracts of thyme, where the effect started with an inhibition zone recoded 12.10 ± 1.63mm at the minimum concentration of 50mg/ml and increased to reach the maximum value of 21.00 ± 7.50mm at the highest concentration of 250 mg/ml for ethanol extract, and the results for methanol extract was different, as no antibacterial activity was observed (0.00 ± 0.00mm) at the lower concentrations of 50 and 100mg/ml, the inhibitory effect initiated at 150 mg/ml with a zone of inhibition 10.47 ± 1.44mm, and then increased progressively to reach its maximum value of 16.07 ± 1.72mm at the highest concentration 250 mg/ml as shown in Table (1). Our results with agreement the finding of **Al-Zubaidi (2020)** and the study of **Hussein *et al.* (2025)** in proving the high inhibitory efficiency of Thymus vulgaris extracts against *S. aureus* bacteria. This agreement reinforces the promising bioactivity of the phenolic compounds within the plant against Gram-positive bacteria and study conducted by **Dehimi *et al.* (2026)** illustrated that inhibition zone produced by the methanol extract of thyme valugris was less than our results.

The results of the current study no agreement with the study of **Inayatullah *et al.* (2017),** where *E. coli* showed a clear sensitivity towards thyme, and the results recorded a higher zone of inhibition (31 mm) compared to the highest value in our study for the ethanol extract (21.77 ± 1.05 mm). This difference is attributed to the use of essential oil in the previous study, which is characterized by a higher concentration of active compounds. This variation and overall superiority are attributed to the quality and methods of extraction, and the differences in geographical and environmental factors affecting the plant.

The quantitative and qualitative chemical composition of the Thymus vulgaris extract provides a vital explanation for this variation; the crude extracts contain a mixture of bioactive substances such as thymol and carvacrol, which possess strong antibacterial efficacy **Sienkiewicz *et al.* (2012); Marchese *et al.* (2016)**. The superiority of ethanol is due to its optimal polarity, which allows it to penetrate the plant's cell membranes and extract these compounds at higher concentrations and yields compared to methanol **Hammer *et al.* (1999)**.

The difference in the antibacterial efficacy of the thyme extracts can also be explained by the difference in the nature of the solvent used in extraction, in addition to the plant's chemical content being influenced by multiple factors, such as the plant part used, the growth stage, and the environmental and seasonal conditions. These are factors that may affect the concentration of phenolic compounds and volatile oils responsible for the antimicrobial activity, consequently leading to differences in inhibition degrees between the various extracts and concentrations **Marchese *et al.* (2016)**

According to **Irfan *et al.* (2023),** the comparative analysis demonstrated a significant superiority of the ethanol extract of Thymus vulgaris in inhibiting the tested bacterial strains compared to the methanol extract, which remained completely inactive at lower concentrations. Although the majority of literature indicates that methanol often exhibits a slightly higher capacity for extracting total phenolic content due to its elevated polarity, the biological and antimicrobial efficacy in this study clearly favoured ethanol. Furthermore, ethanol holds a critical advantage in pharmaceutical and food applications as a safe,

eco-friendly, and non-toxic solvent for human use, unlike the highly toxic methanol, establishing the ethanol extract as the optimal and safest candidate for therapeutic development.

CONCLUSION

The results of this study showed that the thyme plant (Thymus vulgaris) possesses antibacterial activity, as both the ethanol and methanol extracts demonstrated the ability to inhibit the growth of the tested pathogenic bacteria, with a clear superiority of the ethanol extract in terms of potency and the width of inhibition zones, especially at high concentrations. This activity is attributed to thyme containing active plant compounds, such as phenolic compounds, flavonoids, and volatile oils, which are known for their antimicrobial properties. These results indicate that thyme is a promising natural source of antibacterial compounds and could contribute in the future to developing pharmaceutical preparations or supportive natural products to reduce the problem of antibiotic resistance, with the need to conduct further studies to isolate the active compounds and evaluate their efficacy and safety in vivo studies and clinical applications.

RECOMMENDATIONS

Based on the promising results achieved by the thyme extract, we recommend the following:

- **Isolation of Active Compounds**: Isolate and purify the bioactive constituents within the ethanol extract (e.g. thymol and carvacrol) to precisely identify the specific molecules responsible for the inhibitory effects.
- **In Vivo Studies**: Transition from in vitro laboratory experiments to in vivo animal models to evaluate the efficacy, bioavailability, and safety profile of the extract within a living organism.
- **Development of Pharmaceutical Formulations**: Incorporate the ethanol thyme extract into topical therapeutic formulations (such as ointments and creams) for treating wounds and infections caused by resistant bacterial strains.
- **Investigation of Synergistic Effects**: Test the combination of this extract with standard antibiotics to evaluate its potential to enhance or restore antibiotic activity, thereby reducing required chemical dosages.
- **Broadening the Microbial Spectrum**: Evaluate the efficacy of the extract against a wider range of multi-drug resistant (MDR) bacterial and fungal strains to expand its potential therapeutic applications.

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