

Antimicrobial Activity of Plant Extracts (*Thymus vulgaris* and *Origanum vulgare*) Against Multidrug-Resistant *Staphylococcus aureus*

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ABSTRACT

Antimicrobial resistance (AMR) is spreading quickly, especially in *Staphylococcus aureus*. I discovered that this has seriously reduced the efficacy of traditional antibiotics like erythromycin and dicloxacillin. The antibacterial activity of ethanolic extracts of *Origanum vulgare* (oregano) and *Thymus vulgaris* (thyme) against multidrug-resistant *Staphylococcus aureus* is assessed in this study. Bioactive substances with antimicrobial qualities, such as flavonoids, polyphenols, alkaloids, terpenoids, and saponins, were found through phytochemical screening. Oregano (20 mm) and thyme (18 mm) showed notable inhibitory zones against dicloxacillin-resistant isolates in antimicrobial susceptibility testing (AST) using the disc diffusion method, while oxacillin (positive control) showed decreased activity (7–8 mm), demonstrating resistance. Effective antibacterial activity was shown by minimum inhibitory concentration (MIC) measurement for both extracts at concentrations as low as 12.5 mg/ml. Phytoconstituents like thymol and carvacrol, which damage bacterial cell membranes and obstruct vital cellular processes, are thought to be responsible for the observed antibacterial activity. These results imply that *Origanum vulgare* and *Thymus vulgaris* have strong antibacterial qualities and could be useful adjunctive or alternative treatments for resistant *Staphylococcus aureus* infections.

Keywords: Antimicrobial resistance, *Staphylococcus aureus*, Multidrug-resistance bacteria (MDR), Phytochemicals, Disc diffusion assay, Minimum inhibitory concentration (MIC).

INTRODUCTION

One of the most urgent global health issues is antimicrobial resistance, which severely reduces the efficacy of widely used antibiotics. The therapeutic efficiency of traditional medications has been diminished by the swift emergence of multidrug-resistant (MDR) bacterial strains, increasing morbidity, mortality, and the cost of healthcare. Erythromycin, a macrolide antibiotic extensively used to treat skin and respiratory infections, and Dicloxacillin, a β -lactam

antibiotic commonly given for infections caused by Gram-positive bacteria, are two examples of antibiotics that are frequently impacted by resistance^[1].

Enzymatic degradation, target site alteration, and efflux pump activity are the main processes responsible for the development of resistance to these antibiotics. Bacteria can survive in the presence of antimicrobial medicines thanks to their adaptive responses, which complicates treatment plans and calls for the creation of alternative therapeutic procedures^[2].

Bioactive chemicals originating from plants have garnered increasing attention as possible antibacterial agents in recent years. Phytochemicals with strong antibacterial and antioxidant qualities, such as flavonoids, alkaloids, tannins, and phenolic compounds, are abundant in medicinal plants. Notably, the strong antibacterial properties of *Thymus vulgaris* (thyme) and *Origanum vulgare* (oregano) have been well investigated; these properties are mostly ascribed to substances like thymol and carvacrol. These phytoconstituents have shown efficacy against a wide range of pathogenic bacteria, including strains that are resistant to drugs^[3].

Although there is growing evidence that plant extracts have antibacterial properties, few research have examined how well they work against MDR bacteria that are resistant to certain medicines like erythromycin and dicloxacillin. In order to assess the antibacterial activity of particular plant extracts against these resistant isolates, the current study intends to isolate and describe multidrug-resistant bacterial strains. To determine the bioactive components in charge of the noted antibacterial activities, phytochemical investigation was also carried out^[4].

In addition to potentially laying the groundwork for the creation of innovative therapeutic agents to fight illnesses resistant to multiple drugs, this study aims to add to the expanding corpus of research on plant-based substitutes for traditional antibiotics^[5].

METHODS AND MATERIALS

Sample collection

Plants were obtained in their dried form *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano). Both samples were identified and stored under suitable conditions. Before extraction, the plant samples were grinded and converted into powdered form using a sterile motor pestle and stored in proper containers.

Preparation of Extraction

Plant materials used in this experiment were thyme and oregano. The sample were obtained from reliable sources. The samples were weighed and converted into fine powdered form using mortar and pestle. Samples were then stored in an air tight container. For extraction process ethanolic solvent extraction method was used. Around 5g of each sample was weighed and was soaked in 25ml 70% ethanol each. It was then incubated at 37°C for 24 hours in an incubator. To increase the extraction process efficiency and collect maximum number of phytochemical parts of the plant^[6].

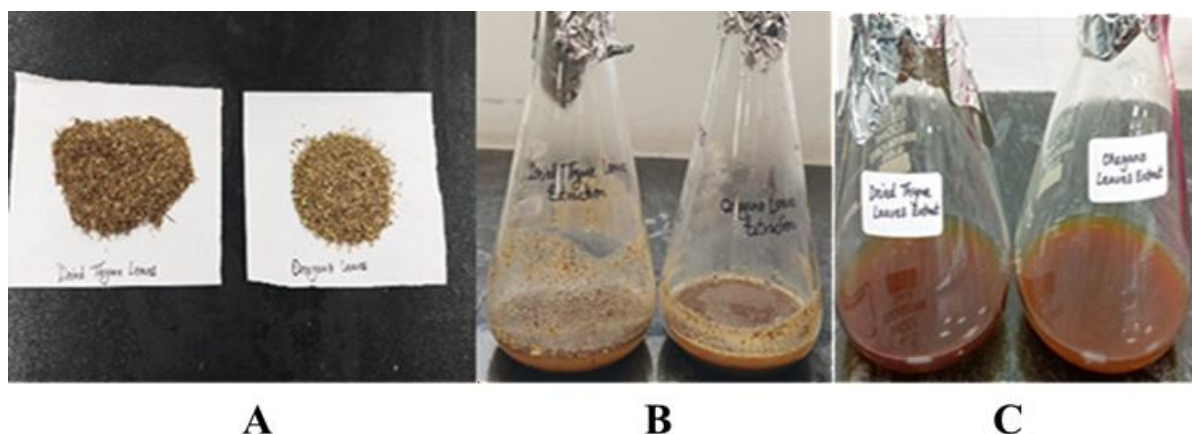


Fig 1: Extraction of Thyme and Oregano Powdered Plant Extracts, (B) Extracted plant sample, (C) Final Filtrate plant Sample.

Filtration

After 24 hours plant extracts were filtered using Whatman filter paper to remove the plant debris and obtain maximum filtrate. The filtrate was stored in clear glass vials at 4°C for further phytochemical test and use^[6].

Qualitative Phytochemical Screening ^[7]

Test for Saponin

The occurrence of froth was determined using the saponin test. In this context, the ethanolic plant extract 2-2ml was added of each sample each test tube with 3ml distilled water. It was Shaken well for about 3-5min. The formation of foam is the positive indication of presence of amphiphilic compounds of saponin^[8].

Test for Polyphenol (Tannin)

Ferric Chloride Test

Polyphenol and tannin were determined by ferric chloride test. In this the extract was added 2-2ml sample in each test tube and 5% ferric chloride solution 2-3 drops, the appearance of bluish-black colour indicates positive result and shows presence of polyphenol and tannin compounds which have the capacity to form complexes with ferric ions resulting in a coloured compound^[9].

Lead Acetate Test

In this polyphenol was determined by using lead acetate. The plant extract was added 2-2ml sample in each test tube and 10% lead acetate solution 2-3 drops was added; the appearance of white precipitate shows the presence of polyphenol and tannin compound and in turn gives positive result^[10].

Test for Flavonoids

Alkaline Reagent test

The presence of flavonoids was determined by alkaline test. 2-2ml of plant extract was taken in each test tube and 1ml of 10% sodium hydroxide along with 2-3 drops of dilute hydrochloric acid was added. The appearance of yellow colour indicates the presence of flavonoids in the sample^[11].

Shinoda Test

Flavonoids were determined using Shinoda test. 2-3 pieces of magnesium ribbon were added along with few drops of concentrated hydrochloric acid in test tube of 2-2ml of plant extract sample in each. The appearance of pink or red colour indicates the presence of flavonoids. This reaction happens due to reduction reaction taking place in the presence of acidic medium to form coloured compound anthocyanidins^[12].

Test for Alkaloids

Mayer's Test

Presence of alkaloids were determined by Mayer's test. 2-2ml of plant extract sample in each test tube were added and few drops of Mayer's reagent were mixed and left for 1-2 min, the formation of cream or pale-yellow precipitate indicated positive result for alkaloids^[13].

Dragendroff's Test

2-2ml of plant extract were added in each test tube and 2-3 drops diluted hydrochloric acid and 2-3 drops of Dragendroff's reagent. Formation of orange or reddish-brown precipitate indicates presence of alkaloids^[14].

Wagner's Reagent Test

2-2ml of plant extract in each test tube and 1-2ml of diluted hydrochloric acid and few drops of Wagner's reagent. Appearance of reddish-brown precipitate shows positive result^[15].

Test for Glycosides

Borntrager's Test (o-glycoside)

O-glycoside was determined by Borntrager's test. 2-2ml of plant extract was taken in each test tube, 1ml of diluted hydrochloric acid was added and kept in water bath for 5-10min for boiling, 2ml of chloroform was added and shaken well and allowed to separate layers. The organic layer was transferred and 10% of ammonia was added and Shaked. The appearance of pink or red colour in lower layer confirms O-glycosides^[16].

Keller-Killiani Test (Cardiac glycoside)

Determined the cardiac glycoside by Keller-Killiani test. 2-2ml of plant extract in each test tube and add 2ml of glacial acetic acid with few drops of ferric chloride solution, followed by addition of 1-2 ml of concentrated sulfuric acid in the mixture and let it settle down at the bottom of the test tube forming a separate layer. Bluish-green or brown or violet ring at the junction shows positive result for C-glycoside^[17].

Test for Resin

Copper Acetate Test

Take 2-2ml of plant extract each in test tube. Add few drops of 1-2% copper acetate solution. Wait for the bluish-green colour to appear^[18].

Test for Quinone

Hydrochloric Acid

2-2ml of plant extract sample in each test tubes, add 2-3 drops of concentrated hydrochloric acid and mix it. Appearance of red colour shows the presence of quinones^[19].

Test for Terpenoids

Salkowski's Test

To detect the presence of terpenoids Salkowski's test was applied. 2-2ml of plant extract was taken in a test tube and 2ml of chloroform was added and mixed well. Later on, 1-2ml of concentrated sulfuric acid was added along the wall of the test tube. Appearance of reddish-brown colour at interface shows the positive result for terpenoids test^[20].

Test Microorganism

Staphylococcus aureus is a gram-positive bacteria causing various diseases in humans. It was used as test organism in this study due to its presence of multidrug-resistant strain. The bacteria were collected from human epidermis and grown on mannitol salt agar media and stored at proper conditions after obtaining pure culture. It was sub-cultured frequently for viability and purity^[21].

Culture Techniques

Spreading Method

Bacterial culture was spread on the MSA media plate using sterile cotton swab. It was kept in incubator for 24hrs at 37°C^[22].

Streaking Method

To obtain pure culture streak plate method technique was used. The colonies were selected from spreading plate and streaked on the sterile MSA media plate containing erythromycin and dicloxacillin antibiotics. It was incubated for 24hrs at 37°C^[23].

Apart from this for confirmation of bacteria standard biochemical test were done.

Gram Staining

Morphological identification was determined by gram staining. Organism shows gram-positive cocci shaped bacteria, found in clusters resembling the feature of *Staphylococcus aureus*. This confirmed the primary identification of the isolate^[24].

Antimicrobial Susceptibility Test (AST)

Antimicrobial activity of plant extracts was checked by using disc diffusion method. The Mueller Hinton Agar (MHA) media was used for this purpose. The plant extract was dried and mixed in dimethyl sulfoxide solution, with plant concentration 250mg/ml. Plate was divided into four parts having positive, negative and both the sample on the plate each. Positive having oxacillin antibiotic, the disc was dipped into antibiotic and plant sample, placed on the plate expect for negative disc. It was done for both the antibiotic erythromycin and dicloxacillin. It was kept for 24hrs in incubation at 37°C. After, incubation the zone of inhibition was measured and antibacterial of the extract against resistant *Staphylococcus aureus* was studied^[25].

Minimum Inhibitory Concentration (MIC)

For determination of minimum inhibitory concentration of plant extracts, serial dilution of plant extracts was done and discs were placed on the MHA media plate along with bacterial culture. The plate was kept for 24hrs in incubation at 37°C. The zone of inhibition on the plate was measured in millimetres at different concentration of plant extracts. Before the MIC procedure due to lower zone of inhibition in erythromycin, we proceeded with dicloxacillin plate only^[26].

RESULT AND DISCUSSION

Saponin were detected in both the plant extracts, while *Origanum vulgare* showing higher foam formation compared to *Thymus vulgaris* indicating greater concentration. It is known for properties and the ability to disrupt the cell membrane, in turn contributing to antimicrobial activity. More concentration in oregano effects the pathogen due to enhancement in its membrane-permeability^[28].

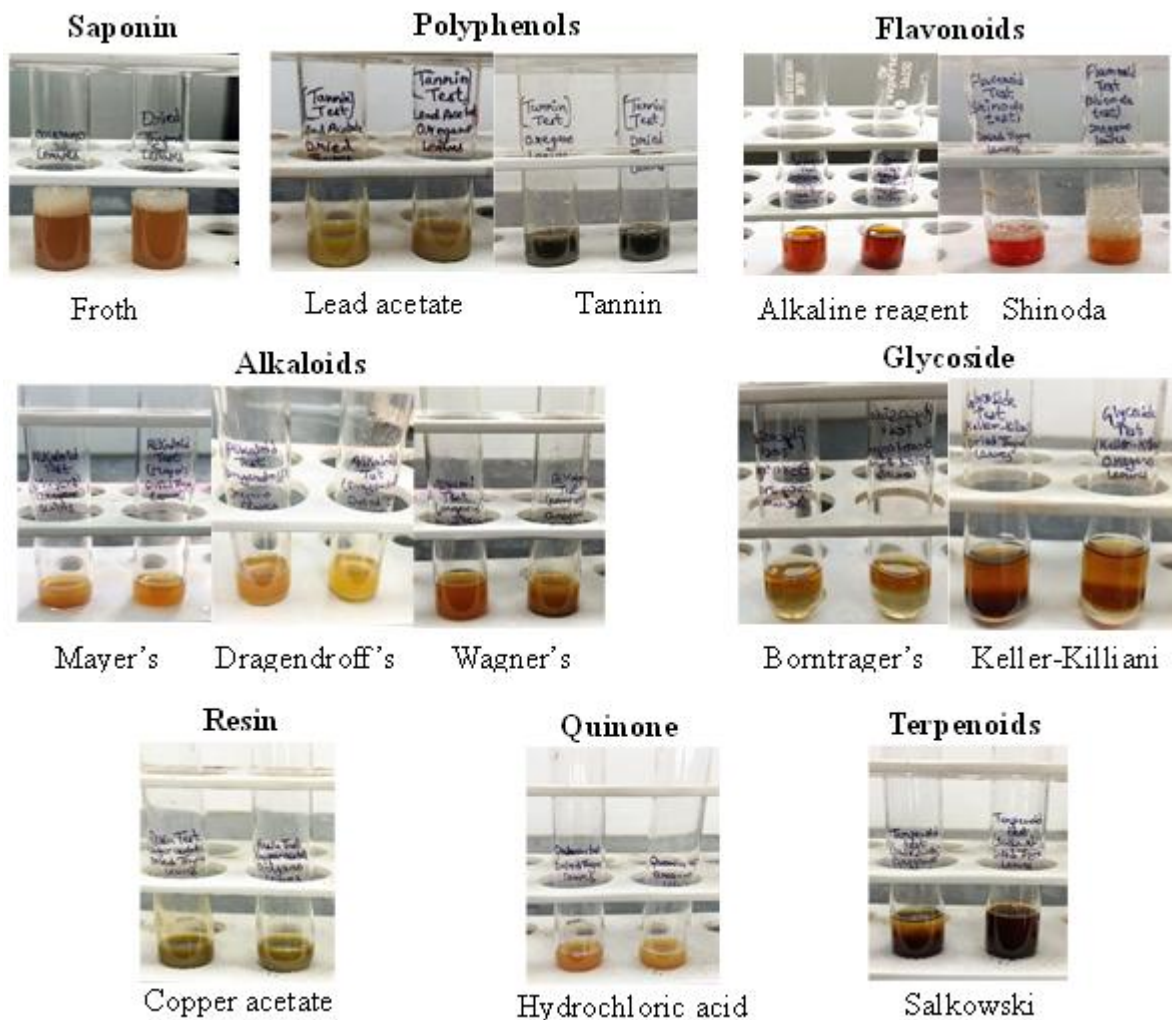


Fig 2: Phytochemical Screening of Thyme and Oregano.

Polyphenols and tannins were found in abundance in both plant extracts, showing the ferric chloride reaction. These phenolic compounds are known for their antioxidant potential and their role in inhibiting microbial growth with the help of enzyme inhibition and protein precipitation. Slightly higher concentration in *Thymus vulgaris* contribute to its protective role against oxidative stress and microbial infections^[29].

Flavonoids showed variation between the two extracts depending on the test used. In alkaline reagent test *Origanum vulgare* gave stronger reaction, while *Thymus vulgaris* in Shinoda test showed higher intensity. Overall, this difference suggests flavonoid composition than the total content. Flavonoids are known for disrupting microbial membranes and inhibiting nucleic acid synthesis which exert antimicrobial effects. This variation can influence the spectrum of antimicrobial activity of each plant^[30].

Table 1: Qualitative Phytochemical Test.

SL.NO.	Phytochemical test	Method	Thymus vulgaris	Origanum vulgare
1	Saponin	Froth	+	++
2	Polyphenols	Tannin	++++	++++
		Lead Acetate	+++	++
3	Flavonoids	Alkaline Reagent	++	++++
		Shinoda	+++	+
4	Alkaloid	Mayer's	++	+++
		Dragendroff's	+	+
		Wagner's	+++	+++
5	Glycoside	Borntrager's	-	-
		Keller-Killiani	++	++
6	Resin	Copper Acetate	++	++
7	Quinone	Hydrochloric Acid	++	+
8	Terpenoid	Salkowski	+++	++

(- = Absent, + = slight present, ++ = moderate present, +++/++++ = Highly present)

Alkaloids were present in both the plant extracts in trace amount, with slightly higher in *Origanum vulgare* as observed. Alkaloids contribute to antimicrobial efficacy by interference with DNA replication and protein synthesis in microorganism. The ability of their existence in both samples is boosted by their consistent detection over several tests^[31].

Glycoside analysis revealed the absence of O-glycosides but confirmed the presence of cardiac glycosides in both plant extracts. Additionally, to their prominent effects on cardiovascular muscle contraction, cardiac glycosides have recently been demonstrated to have cytotoxic and antibacterial qualities. Their presence could boost the extracts' biological activity in an advantageous manner^[32].

Both samples exhibited a moderate number of resins, implying that their chemical properties were similar. As per reports, resins have anti-inflammatory and antibacterial qualities, frequently serving as protective agents in plants^[33].

Thymus vulgaris contained a greater amount of quinones than *Origanum vulgare*. It is known that quinones may disable microorganisms by forming irreversible complexes with nucleophilic amino acids in proteins. This could provide thyme another antimicrobial benefit^[34].

Both extracts comprised terpenoids, with *Thymus vulgaris* displaying a greater intensity. One of the most prominent families of phytochemicals with antimicrobial properties are terpenoids, especially in plants that produce essential oils like oregano and thyme. Strong antibacterial properties of compounds like carvacrol and thymol are widely recognized^[34].

Overall, this study's phytochemical profile validates previous findings about *Thymus vulgaris* and *Origanum vulgare*, demonstrating a large number of beneficial compounds. These metabolites' different concentrations reveal that both plants have a great deal of medicinal promise, especially when it comes to antibacterial uses. Variations in the two plants' biological activity and efficacy may be explained by the observed differences in phytochemical intensity^[35].

Identification of Bacterial Isolates

Culture Techniques

Spreading

Spread plate method resulted in formation of circular, creamy, smooth, whitish to golden-yellow colonies on the mannitol salt agar media surface. The colonies were countable and well-distributed due to uniform spreading.

Staphylococcus aureus typically produces smooth, golden-yellow colonies which is morphological characteristics of the bacteria. This method ensured the correct isolation of the bacteria and for further biochemical analysis and antimicrobial activity^[36].

Streaking

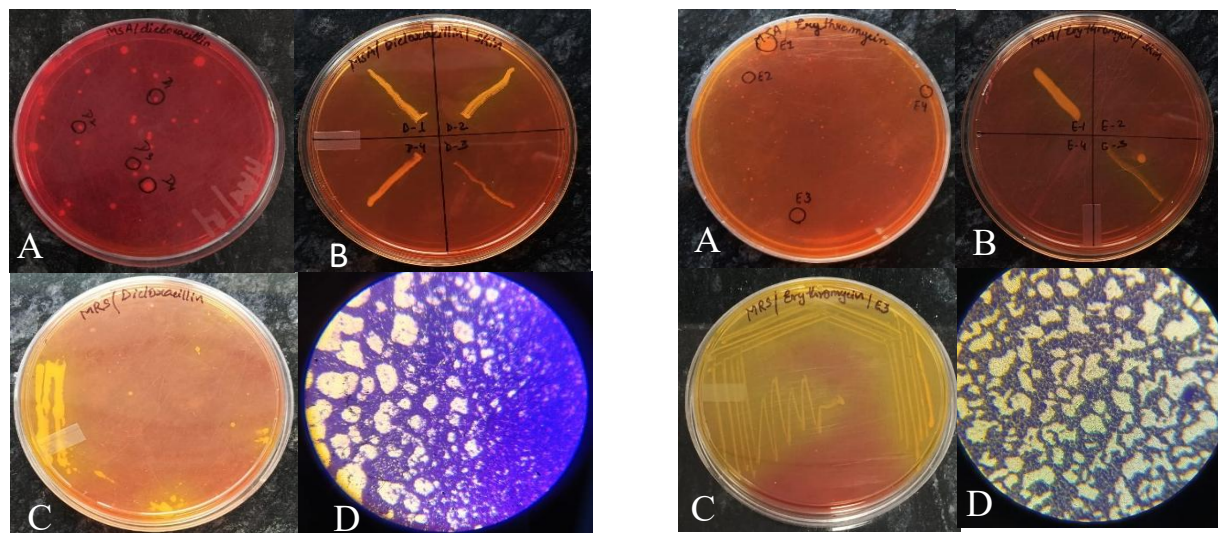
Streak method was used to isolate the pure colonies of *Staphylococcus aureus* reducing the colonies density in the plate. The primary streak showed more bacterial growth but gradually it decreased in subsequent streaks.

Ensuring the pure culture from other colonies, this helps in the identification of *Staphylococcus aureus*. It is essential for further biochemical characterization and antimicrobial susceptibility testing in this study^[37].

Gram Staining

The bacterial isolates were gram positive shows retention of crystal violet stain, imparting blue violet colour. It appeared in clusters having cocci shape under the microscope.

This morphological characteristic indicates that bacteria belong to genus *Staphylococcus*, mainly *Staphylococcus aureus* and indicates the presence of thick peptidoglycan layer^[38].



Dicloxacillin Resistant

Erythromycin Resistant

Fig 3: Identification of Bacterial Isolates. (A= spreading, B= quadrant streaking, C= streaking, D= gram staining).

The bacterial strain's biochemical examination produced a number of results consistent with those predicted for *Staphylococcus aureus*, a facultative anaerobe that is Gram-positive, non-motile, and present in a variety of clinical and environmental samples^[39].

Gram staining revealed that the bacteria was Gram-positive when violet-coloured cocci were present in clusters. Because of their strong peptidoglycan cell wall, which allows them to retain crystal violet, this criterion is one of the most crucial in the identification of *Staphylococci*.

Furthermore, since *S. aureus* lacks flagella, the lack of motility seen during the hanging drop test supported this theory^[40].

The catalase test was determined to be positive in this isolate because hydrogen peroxide caused bubbles to develop. The study's bacteria can differentiate between *Streptococcus* species (negative result) and *Staphylococcus* species (positive result) by reducing hydrogen peroxide to oxygen and water. Nitrate reduction, another encouraging finding, demonstrates that this bacterium may convert nitrate into nitrite or possibly nitrogenous substances^[41].

When dextrose, sucrose, lactose, fructose, maltose, and mannitol produced a yellow tint, tests for carbohydrate fermentation showed favourable outcomes. It should be mentioned that a favourable reaction in mannitol fermentation is one of *S. aureus*'s key characteristics. This feature sets this species of *Staphylococcus* apart from *S. epidermidis*, which is unable to ferment mannitol^[42].

Table 2: Biochemical Test Result.

Sl.No.	Biochemical Test	Result		Observation
		Dicloxacillin Resistance	Erythromycin Resistance	
1	Mannitol Fermentation Test	+	+	yellow colony
2	Gram Staining	+	+	violet colour, cocci shaped
3	Hanging Drop	+	+	Non-motile
4	Catalase	+	+	Bubble formation
5	Nitrate	+	+	No changes after addition of zinc dust
6	Amylase	+	+	Clear zone around growth
7	Dextrose	+	+	yellow colour appearance
8	Sucrose	+	+	yellow colour appearance
9	Lactose	+	+	yellow colour appearance
10	Methyl Red	+	+	Red colour development
11	Voges Proskauer	+	+	Pinkish-red colour development
12	Citrate	-	-	Media remains green
13	Urease	+	+	colour changes to pink
14	Sorbitol	-	-	Media remains red
15	Indole	-	-	No-red ring formed
16	Fructose	+	+	yellow colour appearance
17	Maltose	+	+	yellow colour appearance

(+ = Positive, - = negative)

A clear zone surrounding the bacterial growth indicates a positive amylase test, which indicates that the organism can hydrolyse starch into simpler carbohydrates. This enzymatic activity promotes the availability of nutrients and helps the bacteria survive in a variety of environmental settings. Similar to this, a positive urease test indicates that the organism can convert urea into carbon dioxide and ammonia, which causes an alkaline shift as shown by the formation of a pink hue. In several bacterial species, this trait is frequently linked to pathogenicity^[43].

The organism can use a variety of metabolic pathways, such as mixed acid fermentation and acetoin synthesis, if both the Voges-Proskauer (VP) and Methyl Red (MR) tests are simultaneously positive. This dual outcome may indicate the organism's ability to adapt its metabolism to different environmental situations, even if it is not frequently highlighted^[41].

The organism's identification as *Staphylococcus aureus* is further supported by the negative results for citrate utilization, indole synthesis, and sorbitol fermentation. Indole negativity and the incapacity to use citrate as a single carbon source are consistent with this species' known biochemical traits^[43].

Antibiotic resistance does not alter the biochemical characteristics of the bacteria itself, as evidenced by the comparability of the biochemistry results for all antibiotic-tested strains (Dicloxacillin). The previously mentioned assertions align with other literature sources that say that drug targets are impacted by resistance systems, while metabolism is unaffected^[44].

Overall, the biochemical picture of *Staphylococcus aureus* obtained in this work is completely consistent with the biochemical picture reported in the scientific literature. It should be mentioned that a sophisticated method of bacteriological diagnostics made it possible to identify the pathogen using its morphological and biochemical characteristics with a high degree of reliability^[45].

Antimicrobial Activity of Plant Extracts (AST) Against *Staphylococcus aureus*

Table 3: Antimicrobial Activity of Plant Extracts.

Sl.No.	Treatment	Erythromycin resistant	Dicloxacillin resistant
1	Negative Control	0mm	0mm
2	Positive Control	8mm	7mm
3	<i>Thymus vulgaris</i>	7mm	18mm
4	<i>Origanum vulgare</i>	8mm	20mm

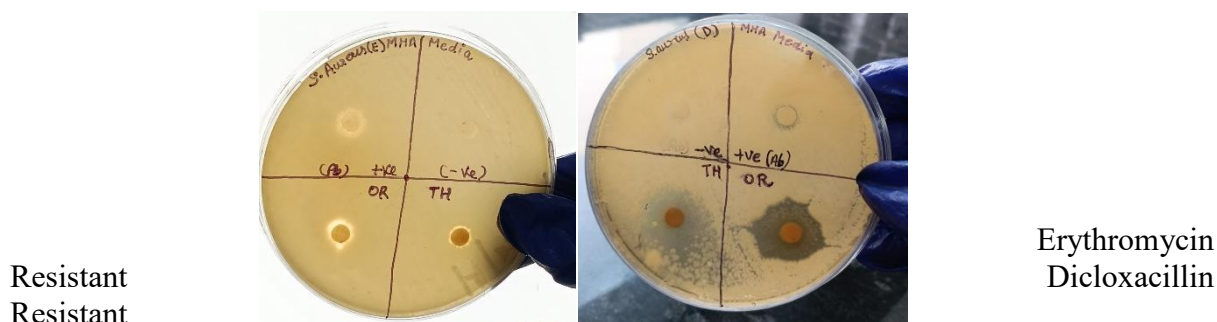
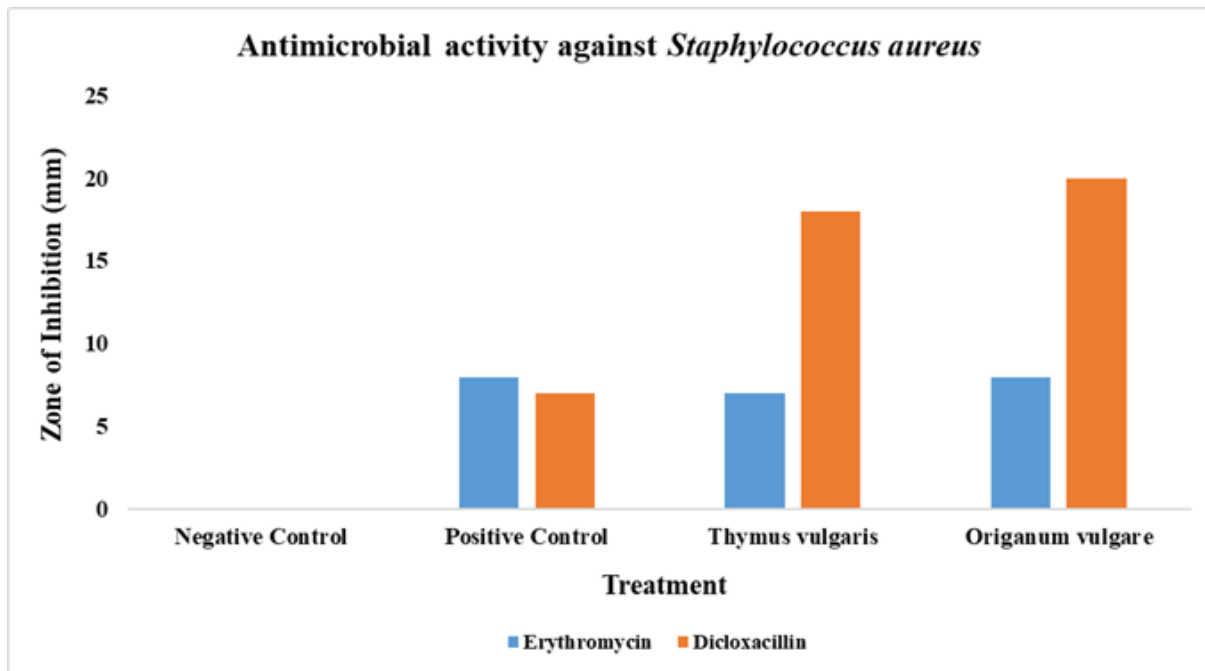


Fig 4: Antimicrobial Activity of Plant Extracts.

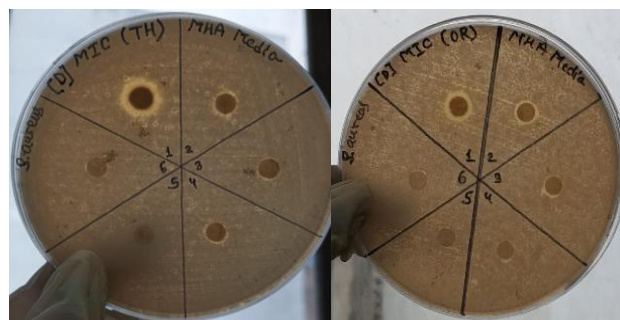


Graph 1: Antimicrobial Activity of Plant Extracts.

Table 4: Minimum Inhibitory Concentration (MIC) of Plant Extract Against *Staphylococcus aureus*.

Sample No.	Concentration (mg/ml)	Thyme(mm)	Oregano(mm)
1	100	10mm	8mm
2	50	8mm	7mm
3	25	7mm	6.5mm
4	12.5	6.5mm	6.5mm
5	6.25	-	-
6	3.125	-	-

Fig 5: MIC Oregano agaist bacteria.



result A. Thyme B. Dicloxacillin resistance

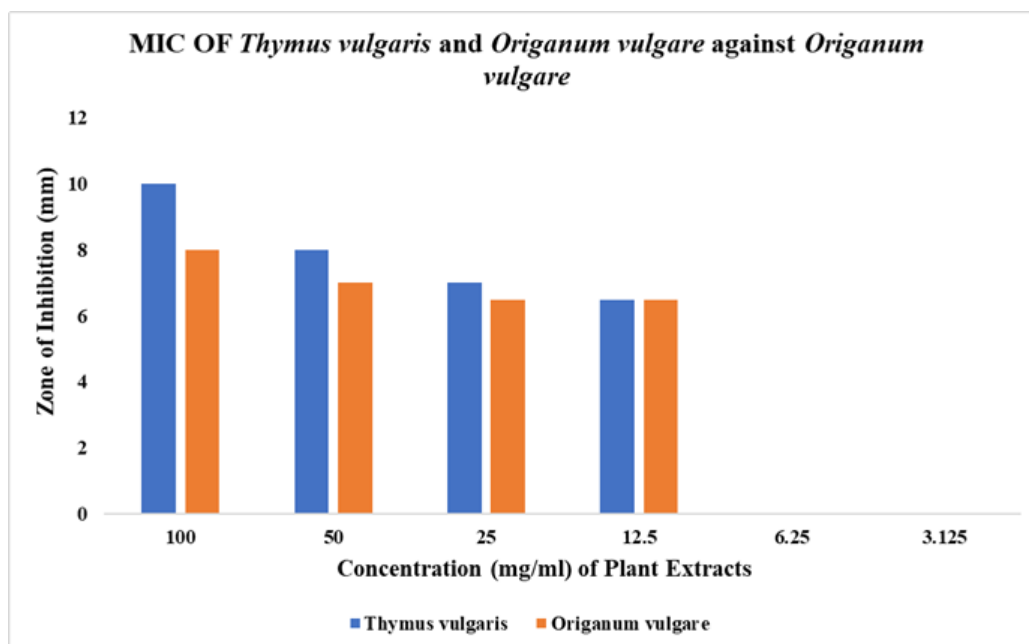


Fig 2: MIC result. A. Thyme B. Oregano against Dicloxacillin resistance bacteria.

The present study used combined disc diffusion (AST) and minimum inhibitory concentration (MIC) techniques to examine the antibacterial activity of extracts from *Thymus vulgaris* and *Origanum vulgare* against multidrug-resistant *Staphylococcus aureus*. The combined method offered a thorough assessment of the antibacterial efficacy in terms of both concentration-dependent activity and inhibition zone^[46].

The absence of a zone of inhibition in the antimicrobial susceptibility test for the negative control (DMSO) validated the experimental design and confirmed that the solvent had no inherent antibacterial activity. Since oxacillin is a common β -lactam antibiotic used to identify methicillin resistance in *Staphylococcus aureus*, it was utilized as the positive control. A positive control should, under normal circumstances, result in a greater zone of inhibition than test samples. Nevertheless, oxacillin showed very tiny zones of inhibition (7–8 mm) in this investigation, which were significantly smaller than those seen for the plant extracts^[47].

This reduced susceptibility to oxacillin can be attributed to the presence of β -lactam resistance mechanisms in the bacterial isolate. In methicillin-resistant *Staphylococcus aureus* (MRSA), resistance is primarily mediated by the *mecA* gene, which encodes an altered penicillin-binding protein, PBP2a. This protein has a significantly lower affinity for β -lactam antibiotics, including oxacillin and dicloxacillin, allowing bacterial cell wall synthesis to continue even in the presence of these antibiotics. so, reduced or minimal zones of inhibition are observed. Because of this, the diminished activity of oxacillin in this study does not indicate experimental error but rather confirms the resistant nature of the isolate. what's more, MRSA strains are known to exhibit resistance to the entire β -lactam class of antibiotics due to this altered target mechanism^[48].

The plant extracts, on the other hand, showed strong antibacterial activity. *Origanum vulgare* displayed the largest zone of inhibition (20 mm) on the dicloxacillin plate, followed by *Thymus vulgaris* (18 mm), suggesting significant antibacterial activity. This implies that even resistant bacterial strains can be defeated by the bioactive substances included in these extracts. However, the zones of inhibition for plant extracts (7–8 mm) on the erythromycin plate were similar to the antibiotic control, suggesting that the effectiveness of the extracts may differ based on the resistance mechanisms implicated, such as ribosomal alteration or efflux-mediated resistance^[49].

The MIC results further supported the AST findings by demonstrating a clear concentration-dependent antibacterial effect. Both *Thymus vulgaris* and *Origanum vulgare* exhibited inhibitory activity at higher concentrations, with zones of inhibition gradually decreasing as the concentration was reduced. The minimum inhibitory concentration for both extracts was determined to be 12.5 mg/mL, as no inhibition was observed at lower concentrations (6.25 mg/mL and below). This confirms that the antibacterial activity of the plant extracts is dose-dependent and effective within a defined concentration range^[50].

Phytochemical screening indicated that the plant extracts' antimicrobial activity was caused by a variety of phytochemicals, including phenolics, flavonoids, and terpenoids. Commonly present in thyme and oregano, compounds like thymol and carvacrol are known to damage bacterial cell membranes, increase permeability, and interfere with vital cellular functions, ultimately resulting in bacterial cell death. Plant extracts continue to be effective against resistant bacteria because these mechanisms are very different from those of β -lactam antibiotics, which target cell wall production^[50].

A larger concentration or synergistic interaction of active chemicals is suggested by the somewhat higher activity seen in *Origanum vulgare*. Confirming that the antibacterial activity of the plant extracts is both considerable and concentration-dependent, the concordance between the AST and MIC data adds to the findings' dependability^[51].

Overall, the study shows that plant extracts of *Thymus vulgaris* and *Origanum vulgare* show potential antibacterial action against multidrug-resistant *Staphylococcus aureus*, while traditional medicines like oxacillin show decreased performance due to resistance mechanisms. These results demonstrate the potential of chemicals derived from plants as supplemental or alternative therapeutic agents in the treatment of diseases resistant to antibiotics^[52].

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CONCLUSION

The current study shows that ethanolic extracts of *Origanum vulgare* and *Thymus vulgaris* have strong antibacterial activity against *Staphylococcus aureus* that is resistant to many drugs. The bacterial isolate's resistance is confirmed by the study's reduced efficacy of oxacillin, which is probably caused by β -lactam resistance mechanisms such as the *mecA* gene expressing PBP2a. Plant extracts, on the other hand, demonstrated significant zones of inhibition, especially against isolates resistant to dicloxacillin, demonstrating their efficacy against resistant infections.

A rich composition of physiologically active substances, including as flavonoids, phenolics, and terpenoids, which are known to have antimicrobial properties through processes different from those of traditional antibiotics, was discovered using phytochemical screening. With effective inhibition seen at 12.5 mg/mL, the MIC data further demonstrated that these extracts' antibacterial activity is concentration-dependent.

All things considered, the results demonstrate the medicinal potential of *Origanum vulgare* and *Thymus vulgaris* as organic antibacterial agents. These plant extracts could be used as supplemental or alternative therapies to treat infections that are resistant to antibiotics. However, prior to their use in pharmaceutical formulations, more research involving the isolation of active chemicals, toxicity assessment, and clinical validation is necessary.

REFERENCES

1. World Health Organization. Global Action Plan on Antimicrobial Resistance. Geneva: WHO; 2015.
2. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. London: Review on Antimicrobial Resistance; 2016.
3. Dadgostar P. Antimicrobial resistance: implications and costs. *Infect Drug Resist.* 2019;12:3903–3910.
4. Aslam B, Wang W, Arshad MI, et al. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist.* 2018;11:1645–1658.
5. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health.* 2015;109(7):309–318.
6. Laxminarayan R, Sridhar D, Blaser M, et al. Achieving global targets for antimicrobial resistance. *Science.* 2016;353(6302):874–875.
7. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Microbiol Spectr.* 2016;4(2).
8. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials and antibiotic resistance. *Microb Pathog.* 2019;129:110–122.
9. Lee AS, de Lencastre H, Garau J, et al. Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers.* 2018;4:18033.
10. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections. *Clin Microbiol Rev.* 2015;28(3):603–661.
11. Marchese A, Orhan IE, Daglia M, et al. Antibacterial and antifungal activities of thymol. *Food Chem.* 2016;210:402–414.
12. Sharifi-Rad J, Sureda A, Tenore GC, et al. Biological activities of essential oils. *Molecules.* 2017;22(1):70.
13. Nazzaro F, Fratianni F, De Martino L, et al. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals.* 2017;10(4):86.
14. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of phytochemicals. *Microb Drug Resist.* 2018;24(2):256–265.
15. de Oliveira JR, Camargo SEA, de Oliveira LD. Thymol and carvacrol antimicrobial mechanisms. *J Appl Microbiol.* 2020;129(3):523–533.
16. Lagha R, Bouslama L, et al. Essential oils against MRSA biofilms. *Pharmaceuticals.* 2020;13(2):29.
17. Kwiatkowski P, et al. Antibiofilm activity of plant-derived compounds. *Int J Mol Sci.* 2020;21(3):1015.
18. Balouiri M, Sadiki M, Ibensouda SK. Methods for antimicrobial susceptibility testing. *J Pharm Anal.* 2016;6(2):71–79.
19. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 31st ed. 2021.
20. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine MIC. *Nat Protoc.* 2008;3:163–175.
21. Burt S. Essential oils: antibacterial properties and mechanisms. *Int J Food Microbiol.* 2015 update;94:223–253.
22. Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components. *Front Microbiol.* 2014;5:38.
23. Yap PSX, Yip BC, Ping HC, Lim SHE. Essential oils as antibacterial agents. *Evid Based Complement Altern Med.* 2014;2014:1–10.
24. Hemeg HA. Nanotechnology and plant extracts as antimicrobial agents. *Saudi Pharm J.* 2017;25(1):123–130.
25. Ghosh S, et al. Natural antimicrobial compounds from plants. *Biotechnol Rep.* 2020;28:e00585.
26. Silva NCC, Fernandes Júnior A. Biological properties of medicinal plants. *J Venom Anim Toxins Incl Trop Dis.* 2010;16(3):402–413.
27. Vázquez-Sánchez D, et al. Essential oils vs bacterial biofilms. *Food Sci Technol Int.* 2015;21(8):559–570.
28. Nostro A, Papalia T. Antimicrobial activity of carvacrol. *Recent Pat Anti Infect Drug Discov.* 2016;11(1):28–35.
29. Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol.* 2016;37:174–181.
30. Honório VG, et al. Antimicrobial activity of oregano oil against *S. aureus*. *Front Microbiol.* 2015;6:1223.
31. Brożyna M, et al. Antimicrobial activity of thyme essential oil. *Front Microbiol.* 2025;16:1668594.
32. Lei T, et al. Antibacterial effects of oregano essential oil. *Front Pharmacol.* 2025;16:1579283.
33. De Rose E, et al. Synergistic antimicrobial effects of oregano oil. *Front Cell Infect Microbiol.* 2025;15:1684624.
34. Zam W, et al. Antimicrobial activity of thyme and oregano extracts. *Int J Med Microbiol.* 2022.
35. Kifer D, et al. Monoterpenes antimicrobial activity. *J Antibiot.* 2016;69(7):528–531.
36. Bunse M, et al. Essential oils pharmacological effects. *Front Pharmacol.* 2022;13:837580.
37. Rani N, Singh T, Singh M, Gautam T, Gupta SK, Rajput T, et al. Qualitative phytochemical screening and evaluation of bioactive compounds in selected medicinal plants: *Punica granatum*, *Aloe vera* and *Citrullus lanatus*. *J Med Plants Stud.* 2026;14(2):280–285.
38. Belwal S, Vikas, Sah P, Annu, Gupta VK. Antibacterial activity of *Withania somnifera* in combination with antibiotics, against human pathogenic Gram-negative bacteria. *Studies in Science of Science.* 2025.
39. Greek thyme essential oil antimicrobial study. *Int J Food Sci Technol.* 2022;57(11):7076–7085.
40. Essential oils in food preservation and antimicrobial applications. *Procedia Food Sci.* 2015;5:108–111.

41. Essential oils and antimicrobial resistance modulation. *Front Microbiol.* 2021;12:678.
42. Plant-derived antimicrobials against resistant pathogens. *Microorganisms.* 2021;9(6):1197.
43. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *PT.* 2015;40(4):277–283.
44. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: are synergistic combinations of plant extracts effective? *Phytochem Rev.* 2017;16:473–490.
45. O'Neill J. Rapid diagnostics: stopping unnecessary antibiotic use. *Rev Antimicrob Resist.* 2016.
46. Tacconelli E, Carrara E, Savoldi A, et al. Discovery of new antibiotics—priority pathogens. *Lancet Infect Dis.* 2018;18(3):318–327.
47. Rice LB. Federal funding for antimicrobial resistance. *J Infect Dis.* 2016;213(2):197–199.
48. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of essential oils—review. *J Food Sci Technol.* 2017;54:611–623.
49. Sakkas H, Papadopoulou C. Antimicrobial activity of basil, oregano, and thyme essential oils. *Microb Pathog.* 2017;106:35–41.
50. Soković M, Glamočlija J, Marin PD, et al. Antibacterial effects of essential oils. *Food Chem.* 2015;185:321–328.
51. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant *S. aureus*: overview. *Clin Microbiol Rev.* 2019;32:e00034-18.
52. Foster TJ. Antibiotic resistance in *Staphylococcus aureus*. *FEMS Microbiol Rev.* 2017;41:430–449.