ORIGINAL ARTICLE

# Determination of Phytochemical Properties and Antimicrobial Activities of *Oregano vulgare* against MRSA

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## Abstract

**Introduction:** The focus of this study is to test the antibacterial activity of one such essential oils derived from *Oregano vulgare (O. Oregano*) culinary herbs belongs to *Lamiaceae* family, against the MRSA nosocomial infections and also determine the phytochemical properties of the herb. Our study is based on the MRSA nosocomial infection. The major concern of the nosocomial infection with MRSA is that majority of the cases were not curable with any drug due to the evolution of the abuse of antibiotics. Many bacteria not only *Staphylococcus aureus* have become resistant in the recent past to the multiple antibiotics. **Materials and Methods:** The phytochemical properties analysis of the *Oregano* essential oil procured from the local market was determined by the standard biochemical methods. The MRSA clinical specimens were procured from the nosocomial infected patients. Antibacterial activity was carried out. **Results and Discussion:** *O. vulgare* essential oil procured from the local market shown jubilant antibacterial activity results [Table 1] against all the clinical isolates with an average disk diffusion of 21 mm zone of inhibition diameter obtained from performing the Kirby–Bauer technique with an average MIC value of 0.88 µ/ml and an average MBC value of 1.13 µ/ml. The best susceptibility of MRSA clinical isolate was observed in nasal swab sample with a zone diffusion of 27 mm with MIC of 0.5 µ/ml and MBC of 0.75 µ/ml toward *O. vulgare* essential oil. **Conclusion:** Our study concludes that phytochemical compounds present in *O. vulgare* essential oil analyzed using biochemical tests and these phytochemical compounds act as an effective remedy toward the MRSA clinical isolates and shown significant results in the study when compared to the standard antibiotics for the same clinical isolates which shown variable subsidized results. The susceptibility result values of *O. vulgare* essential oil shown that it may require lower dosage values when compared to that of the standard antibiotics for the same clinical isolates. This study recommends the use of natural essential oils from the plant source as an alternative toward the chemical antibiotics with more detailed studies need to be done in near future with the expectations that many dangerous infections can be cured with these types of phytochemical compounds.

**Key words:** Phytochemical properties, antimicrobial activity, *Oregano vulgare,* Essential oil, MRSA, *Staphylococcus aureus*

## INTRODUCTION

In today's modern society, medicine has significantly relied on the use of antibiotics. Antibiotics are naturally occurring or artificially prepared by chemical substances capable of killing or inhibiting the bacterial growth.[1] However, the resistant strains of the bacteria emerge due to the abuse of chemical antibiotics giving rise to pathogens such

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**Table 1:** Comparative chart of MRSA isolate's susceptibility toward *O. vulgare* essential oil

| Specimen | *Oregano vulgare* essential oil | | |
|---|---|---|---|
| | Disk diffusion | MIC | MBC |
| Nasal sample | 27 mm S | 0.5 µ/ml | 0.75 µ/ml |
| Catheter sample | 26 mm S | 0.5 µ/ml | 0.75 µ/ml |
| Urine sample | 24 mm S | 0.5 µ/ml | 0.75 µ/ml |
| Groin sample | 23 mm S | 0.75 µ/ml | 1 µ/ml |
| Skin sample | 22 mm S | 0.75 µ/ml | 1 µ/ml |
| Abscess sample | 20 mm S | 1 µ/ml | 1.25 µ/ml |
| Ear sample | 19 mm S | 1µ/ml | 1.25 µ/ml |
| Throat swab sample | 18.5 mm S | 1 µ/ml | 1.25 µ/ml |
| Ulcer sample | 18 mm S | 1.25 µ/ml | 1.5 µ/ml |
| Wound sample | 18 mm S | 1.25 µ/ml | 1.5 µ/ml |
| Surgical sample | 17 mm S | 1.25 µ/ml | 1.5 µ/ml |
| Mean zone value for all the specimens | 21.1 mm | 0.88 µ/ml | 1.13 µ/ml |

as methicillin-resistant *Staphylococcus aureus* (MRSA) infections are strains of *Staphylococcus aureus* that became resistant to the antibiotic methicillin. Methicillin is commonly used to treat regular infections.[2] MRSA illustrates a group of genetically related strains that are the significant causes of infections such as in the skin and soft tissue in the hospital in the community among healthy individuals.[3] *S. aureus* is generally a normal flora but capable of causing diseases. *S. aureus* is Gram-positive cocci appears clusters and is non-spore forming, non-motile, mesophilic bacterium. *S. aureus* multiply quickly at optimum body temperature to produce the endotoxin that causes illness. Seven types (A, B, C, $C_2$, $C_3$, D, and E) of endotoxin are recognized. *S. aureus* is heat labile but the toxin is heat stable with high infective dose > 10 × 6 cfu/gm to produce toxin at 10 × 6 cfu/gm bacteria produce toxin and thermonuclease. A very small amount (100 nanogram) of toxin can cause illness. They are stable to low pH < 4.0 and to proteolysis by enzymes. This non-motile bacterium is one of the major causes for both the community-acquired infections as well as hospital-borne nosocomial infections. The focus of our study is based on the MRSA nosocomial infection. The major concern of the nosocomial infection with MRSA is that majority of the cases were not curable with any drug due to the evolution of the abuse of antibiotics. Many bacteria not only *S. aureus* have become resistant in the recent past to the multiple antibiotics.

The major concern of the nosocomial infection with MRSA is that majority of the cases were not curable with any drug due to the evolution of the abuse of antibiotics. Many bacteria not only *S. aureus* have become resistant in the recent past to the multiple antibiotics. The annual frequency of death from MRSA compared to human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is significantly higher than it exceeds the statistics.[4]

Essential oils (EOs) are organic compounds naturally derived from plants, usually by steam distillation. They can be *Oregano* EO from various sources such as herbs, flowers, trees, vegetables, spices, leaves, flowers, or bark. EOs and their components are widely used in medicines in the food industry, cosmetics, and fragrances due to their unique and beneficial effects.[5] In medicine, EOs have been inspected for their antibacterial, antifungal, antiviral, insecticidal, anticancer, and antioxidant properties.[6-8] EOs vary in high numbers and available for use, with many known beneficial antibacterial properties. Due to the odor and medicinal effects, the EOs are used for the numerous constituents that contribute to their special effects. The primary chemical components that account for the pleasant aromatic odors are primarily terpenes, monoterpenes, and linalool.[9]

EOs were found to be more effective against Gram-positive bacteria, including MRSA than Gram-negative bacteria. This evidence indicates that EOs could be a potential use and benefit against the resistant strains.[10] Although EOs are known for their antimicrobial properties, they are rarely used in medical fields.[11] Hence, the further introduction of natural sources such as EOs into the health system will open new doors to the constant battle against resistant strains. Not only will the health system benefit from this introduction but also it will also encourage the use of other natural sources in medicine.

The focus of this study is to test the antibacterial activity of one such EOs derived from *Oregano vulgare* culinary herbs belongs to the family *Lamiaceae* against the MRSA nosocomial infections and also determine the phytochemical properties of the herb.

## MATERIALS AND METHODS

### Materials

1. MRSA clinical isolates of *S. aureus* from nosocomial infected patients
2. Blood agar plates
3. Mueller-Hinton agar
4. Peptone
5. Standard antibiotic e-test strip

6. *Oregano vulgare* essential oil from the local market
7. Biochemical reagents were of analytical grade.

Different types of Hi-Media culture plates were used.

## Methodology

### *Phytochemical analysis of Oregano essential oil*

The phytochemical properties analysis of *Oregano* essential oil procured from the local market was determined by the following methodologies.[12-14]

1) Mayer's test: *Oregano* EOs were mixed with a drop of mercuric chloride and potassium iodide, respectively, resulting in the formation of a creamy substance indicating the presence of alkaloids.
2) Fehling's test: A 2 ml of Fehling's A and B reagents were mixed with *Oregano* essential oil in a test tube and heated slightly to observe brick red color indicating the presence of reducing sugar.
3) Iodine test: The presence of iodine in *Oregano* essential oil was determined by the addition of 2 ml of iodine solution to *Oregano* essential oil which results in the positive purple-colored test.
4) Salkowski's test: *Oregano* EOs were mixed with 2 ml of chloroform along with 2 ml of concentrated sulfuric acid in a test tube and gently shaken to observe a reddish-brown color which indicates the presence of steroids.
5) Ninhydrin test: *Oregano* EOs were mixed with 2 ml of ninhydrin solution and heated gently to observe a violet color indicating the presence of protein
6) $FeCl_3$ test: *Oregano* EOs were boiled with 10 ml of water in test tubes. A few drops of ferric chloride was added to the 10 ml of heated *Oregano* essential oil in a test tube to observe a blue-black coloration which indicates the presence of phenol
7) Libermann–Burchard's test: The mixture of 2 ml of acetic acid with 2 ml of chloroform was treated with *Oregano* essential oil in a test tube and few drops of concentrated sulfuric acid were added by placing the test tube on ice to observe the color change from violet to bluish-green which indicate the presence of glycosides
8) Benedict's test: In a test tube, 2 ml of Benedict's reagent was treated with *Oregano* essential oil and heated gently heated to observe the formation of orange-red precipitate which indicates the presence of reducing sugar
9) Keller-Kilani test: *Oregano* essential oil was treated with 2 ml of glacial acetic acid with 1–2 drops of ferric chloride solution in a test tube and 2 ml of conc. sulfuric acid was added to observe a brown ring at the interface which indicates the presence of cardiac glycosides.
10) Ammonia test: Dilute ammonia and conc. sulfuric acid treated with aqueous *Oregano* essential oil in a test tube to observe yellowish color formation indicating the presence of amino acids.

### Isolation and purification of *Staphylococcus aureus*

The MRSA clinical specimens were procured from the nosocomial infected patients such as nasal sample, catheter sample, urine sample, groin sample, skin samples, abscess sample, ear sample, throat swab samples, ulcer samples, wound sample, and from the surgical samples. The collected samples processed in the microbiology laboratory by following the standard aseptic microbiological technique by streaking on the blood enriched media at 37°C for overnight incubation.[12,13,15] The incubated isolates were identified and purified by performing the Gram's staining and the required biochemical reaction test in which the significant identification test was the catalase and coagulase tests which were positive for the bacterium.[16]

### Antimicrobial susceptibility testing

The antimicrobial susceptibility test for the isolated clinical specimens of MRSA against the standard antibiotics was determined using the modern rapid e-test methodology[15] where the isolates were inoculated on Mueller-Hinton agar plates separately and standard e-test plastic strips for the respective antibiotics were impregnated and incubated at 37ºC overnight to visualize the zone and ellipse.[15,17] The interaction of the ellipse is read as the minimum inhibitory concentration (MIC), whereas the zone as the susceptibility of the antibiotic toward the bacterium and results were tabulated for the interpretation. The traditional conventional standard antibiotic assay methods such as Kirby–Bauer disk diffusion method was employed to observe the susceptibility of the clinical specimens of MRSA standard disk prepared from *O. vulgare* essential oil procured from the local market where the bacterium isolates were inoculated separately on Mueller-Hinton agar plates along with the impregnated disks at 37°C for 24 h to observe the zone formation determining the sensitivity of the bacterium toward the disk.[17] The results were tabulated for the interpretation. The MIC values along with minimum bactericidal concentration (MBC) values for the efficacy antimicrobial activity of *O. vulgare* toward the bacterium were estimated by performing the standard tube dilution method where the isolates were inoculated separately in the different sets of dilutions of the acid in the peptone water and incubated at 37°C for 24 h to observe the no turbidity determining the sensitivity of the bacterium. The results were tabulated for the interpretation. The MBC was determined by inoculating each dilution of MIC dilutions onto the separate agar plates for each isolates and dilutions separately. The inoculation was incubated at 37°C for 24 h to observe the no growth determining the sensitivity of the bacterium. The results were tabulated for the interpretation.[15,17-19]

## RESULTS AND DISCUSSION

*O. vulgare* EO procured from the local market shown jubilant antibacterial activity results [Table 1] against all the

clinical isolates with an average disk diffusion of 21 mm zone of inhibition diameter obtained from performing the Kirby–Bauer technique[17,19] with an average MIC value of 0.88 µ/ml and an average MBC value of 1.13 µ/ml. The best susceptibility of MRSA clinical isolate was observed in nasal swab sample with a zone diffusion of 27 mm with MIC of 0.5 µ/ml and MBC of 0 .75 µ/ml toward *O. vulgare* EO. The clinical isolate sample of MRSA which shown the least susceptibility was the surgical sample with a zone diffusion of 17 mm with MIC of 1.25 µ/ml and MBC of 1.5 µ/ml toward *O. vulgare* EO, but still it is comparatively better than the studied standard synthetic chemical antibiotics for the same sample except susceptibility toward the vancomycin. The other MRSA clinical isolates such as catheter sample shown susceptibility toward *O. vulgare* EO with zone diameter of 26 mm in disk diffusion method with MIC of 0.5 µ/ml and MBC of 0.75 µ/ml, whereas the urine sample shown susceptibility toward *O. vulgare* EO with zone diameter of 24 mm in disk diffusion method with MIC of 0.5 µ/ml and MBC of 0.75 µ/ml where the groin sample shown susceptibility toward *O. vulgare* EO with zone diameter of 23 mm in disk diffusion method with MIC of 0.75 µ/ml and MBC of 1.0 µ/ml and the skin samples abscess sample, ear sample, throat swab samples, ulcer samples, wound sample, and surgical sample shown susceptibility toward *O. vulgare* EOs with zone diameter ranging from 17 mm to 22 mm in disk diffusion method with MIC of 0.75 µ/ml to 1.25 µ/ml and MBC of 1.0 µ/ml to 1.5 µ/ml, respectively, which were significantly far better than that of the standard chemical antibiotics results obtained [Tables 2 and 3]. Although the susceptibility of MRSA clinical isolates toward the standard antibiotic vancomycin shown significant constant results, comparatively, the study shown that the antibacterial effect of *O. vulgare* essential toward the isolates shown more better and promising susceptibility result values [Table 1]. The results of the susceptibility values of the other standard antibiotics toward the MRSA clinical isolates were far below the obtained susceptibility values of *O. vulgare* essential toward the isolates [Figure 1]. It was observed in the study that most of the standard antibiotic except vancomycin shown inconsistent susceptibility results as it shown antibacterial efficacy only to some samples not for others resulting in the non-reliable sensitivity of all the MRSA clinical isolates toward a specific antibiotic, except standard antibiotic vancomycin, the other standard antibiotics were found to be specific to a particular sample and the standard antibiotic methicillin shown resistant results from all the MRSA clinical isolates. This study shown that the effect of standard antibiotics toward the MRSA clinical isolates was limited with higher MIC and MBC values ranging from 0.5 µ/ml up to 2.5 µ/ml. The higher MIC and MBC values thus interpret that though the MRSA clinical isolates shown susceptibility toward the standard antibiotic even specific sample susceptible toward a specific antibiotic it requires higher proportion of the antibiotic dosage value to inhibit the infection or completely eradicate the infection. *O. vulgare* EO contradicting to the susceptibility values of the standard antibiotic shown encouraging average zone diameter of 21 mm in disk diffusion as all the MRSA clinical isolates were susceptible with the zone ranging from 18 mm to 27 mm. The MIC and MBC average values were significantly lower with that of the standard antibiotic toward the MRSA clinical isolates with average values of 0.88 µ/ml and 1.33 µ/ml, respectively. The MIC and MBC values of *O. vulgare* EO ranged between 0.5 µ/ml and 1.5 µ/ml for all the MRSA clinical isolates compared to that of the standard antibiotics which determine that the lesser dosage of *O. vulgare* EO is sufficient to inhibit or eradicate the infection completely. Hence, the naturally obtained antibacterial rich *O. vulgare* EO shown much better susceptibility results than that of the standard antibiotics toward the obtained MRSA clinical isolates. The study also been conducted to find out the reason behind its antibacterial efficacy of *O. vulgare* EO by means of some phytochemical analytical tests[12,13] [Table 4] such as Mayer's test, Fehling's test, iodine test, Salkowski's test, Ninhydrin test, ferric chloride test , Libermann–Burchard's

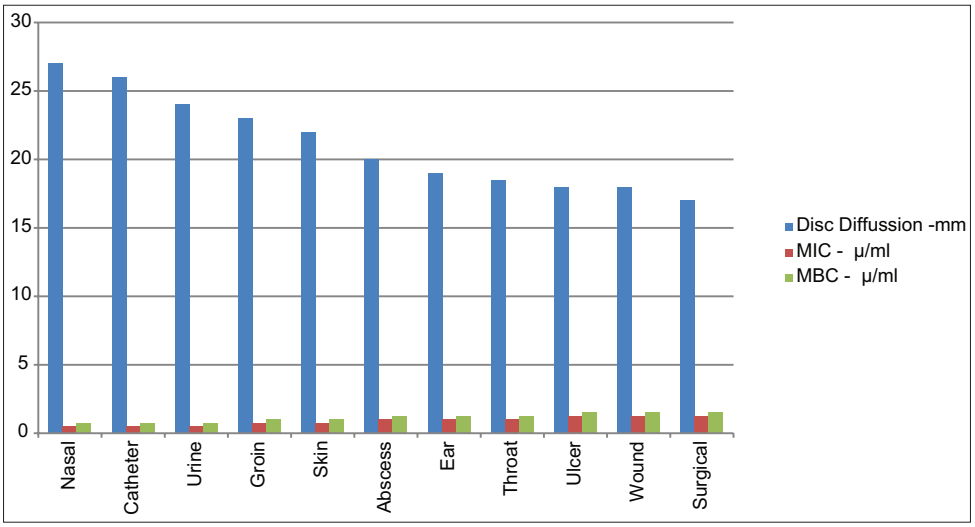


**Figure 1:** Comparative chart of MRSA isolate's susceptibility toward *O. vulgare* essential oil

**Table 2:** Comparative chart of MRSA susceptibility toward standard antibiotics diffusion for the e-test valuation

| Specimen | Antibiotics | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bactrim | Clindamycin | Minocycline | Doxycycline | Gentamycin | Penicillin | Methicillin | Vancomycin | S | I | R |
| Nasal sample | 3 mm R | 2 mm R | 4 mm R | 14 mm I | 2 mm R | 20 mm S | 5 mm R | 20 mm R | 1 | 1 | 6 |
| Catheter sample | 12 mm 1 | 3 mm R | 4 mm R | 3 mm R | 20 mm R | 14 mm I | 2 mm R | 21 mm S | 1 | 2 | 5 |
| Urine sample | 15 mm I | 12 mm I | 13 mm I | 4 mm R | 20 mm S | 20 mm S | 5 mm R | 22 mm S | 3 | 3 | 2 |
| Groin sample | 5 mm R | 2 mm R | 2 mm R | 4 mm R | 2 mm R | 10 mm I | 4 mm R | 23 mm S | 1 | 1 | 6 |
| Skin sample | 14 mm I | 13 mm I | 12 mm I | 4 mm R | 2 mm R | 20 mm S | 2 mm R | 20 mm S | 2 | 3 | 3 |
| Abscess sample | 21 mm S | 14 mm I | 12 mm I | 21mm S | 20 mm S | 2 mm R | 4 mm R | 22 mm S | 4 | 2 | 2 |
| Ear sample | 12 mm 1 | 14 mm 1 | 12 mm I | 14 mm I | 2 mm R | 9 mm I | 5 mm R | 21 mm S | 1 | 5 | 2 |
| Throat swab sample | 14 mm I | 12 mm 1 | 14 mm I | 20 mm S | 19 mm S | 11 mm I | 5 mm R | 20 mm S | 3 | 4 | 1 |
| Ulcer sample | 12 mm I | 22 mm S | 20 mm S | 10 mm I | 20 mm S | 2 mm R | 4 mm R | 20 mm S | 4 | 2 | 2 |
| Wound sample | 14 mm I | 13 mm I | 12 mm I | 14 mm I | 20 mm S | 4 mm R | 2 mm R | 19 mm S | 2 | 4 | 2 |
| Surgical sample | 11 mm I | 20 mm S | 19 mm S | 11 mm I | 22 mm S | 20 mm S | 10 mm I | 20 mm S | 5 | 3 | 0 |

**Table 3:** Comparative chart of MRSA e-test MIC (µ/ml) toward standard antibiotic dilution

| Specimen | Antibiotics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bactrim | Clindamycin | Minocycline | Doxycycline | Gentamycin | Penicillin | Methicillin | Vancomycin |
| Nasal sample | 0.75 µ/ml | 0.75 µ/ml | 1 µ/ml | 1 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 0.5 µ/ml |
| Catheter sample | 1 µ/ml | 1 µ/ml | 1µ/ml | 1µ/ml | 1.25 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 0.5 µ/ml |
| Urine sample | 1µ/ml | 1µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 0.5 µ/ml |
| Groin sample | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 0.75 µ/ml |
| Skin sample | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.5 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 0.75 µ/ml |
| Abscess sample | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.25 µ/ml | 1.5 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 1 µ/ml |
| Ear sample | 1.25 µ/ml | 1.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 1.5 µ/ml | 1.25 µ/ml | 2.5 µ/ml | 1 µ/ml |
| Throat swab sample | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 1.25 µ/ml | 2.5 µ/ml | 1 µ/ml |
| Ulcer sample | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 2.5 µ/ml | 1.25 µ/ml | 2.5 µ/ml | 1.25 µ/ml |
| Wound sample | 2.25 µ/ml | 2.25 µ/ml | 2.25 µ/ml | 2.5 µ/ml | 2.25 µ/ml | 1.25 µ/ml | 2.5 µ/ml | 1.25 µ/ml |
| Surgical sample | 2.25 µ/ml | 2.5 µ/ml | 2.5 µ/ml | 2.5 µ/ml | 2.5 µ/ml | 1.25 µ/ml | 2.5 µ/ml | 1.25 µ/ml |

**Table 4:** Phytochemical analysis of *Oregano* essential oil

| Test | Result | Compound present |
|---|---|---|
| Mayer's test | Creamy substance | Alkaloids |
| Fehling's test | Brick red color | Reducing sugar |
| Iodine test | Color purple | Iodine |
| Salkowski's test | Reddish-brown | Steroids |
| Ninhydrin test | Violet color | Protein |
| $FeCl_3$ test | Blue-black | Phenol |
| Libermann–Burchard's test | Violet to bluish-green color | Glycosides |
| Benedict's test | Orange-red precipitate | Reducing sugar |
| Keller-Kilani test | Brown ring at the interface | Cardiac glycosides |
| Ammonia test | Yellowish color | Amino acids |

test, Benedict's test, Keller-Kilani test, and ammonia test. These phytochemical analytical tests shown the presence of chemical compounds such as alkaloids, reducing sugar, iodine, steroids, proteins, phenols, glycosides, cardiac glycosides, and amino acids, respectively. Besides the presence of other chemical compounds in *O. vulgare* EO, the vital chemical compound responsible for its antibacterial properties is the presence of phenolic compounds. Thus, this study has shown that the phytochemical compounds present in *O. vulgare* EO prove to be an effective antibacterial compound toward the MRSA clinical isolates when compared to the standard antibiotics.

## CONCLUSION

The phytochemical compounds present in *O. vulgare* EO act as an effective remedy toward the MRSA clinical isolates and shown significant results in the study when compared to the standard antibiotics for the same clinical isolates which shown variable subsidized results. The susceptibility result values of *O. vulgare* EO shown that it may require lower dosage values when compared to that of the standard antibiotics for the same clinical isolates. Due to the abundant usage of antibiotics resulted in the emergence of the resistant organisms. Further the over dosage of the antibiotics can lead to more severe consequences. The WHO has already alarmed about the toxic effects due to the abuse of antibiotics. Hence, the need of the hour is to find out the non-toxic substances as an alternative toward the infection and one such small attempt toward a brighter future is this study about the antibacterial efficacy of *O. vulgare* essential toward the

MRSA clinical isolates which has shown promising results. This study recommends the use of natural EOs from the plant source as an alternative toward the chemical antibiotics with more detailed studies need to be done in near future with the expectations that many dangerous infections can be cured with these types of phytochemical compounds.[13]

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## CONFLICTS OF INTEREST

No conflicts of interest.

## CONTRIBUTION OF AUTHORS

All authors have made substantial contribution to the work and approved it for publication.

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