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Original article

# Antibacterial activity of *Illicium verum* essential oil against MRSA clinical isolates and determination of its phyto-chemical components

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ABSTRACT

*Objective:* The *Illicium verum* is an evergreen Chinese plant, from which a culinary spice named star anise is obtained and is predominantly used in Asian countries. Besides its flavor, it possess high medicinal value. *Illicium verum* possesses good antimicrobial properties and in the past utilized to treat versatile ailments of microbial origin. *Illicium verum* is commonly known as star anise.
*Methods:* This study is an attempt to resurrect the ancient forgotten traditional spice *Illicium verum* by analyzing its essential oils (EOs) for the phyto-biochemical properties using GC–MS, IR studies and various standard biochemical tests to record the presence of the chemical compounds based on its test reactions and also to measure its anti-microbial vulnerability testing towards the MRSA clinical isolates, by deploying the standard anti-microbial assay in the aseptic microbiological laboratory conditions.
*Results:* The data procured from the standard antimicrobial assay of *Illicium verum* essential oil were compared with that of the standard antibiotic e-strips towards the same set of MRSA clinical isolates. The antimicrobial assay test results of *Illicium verum* essential oil against the clinical isolates of the MRSA showed promising results when compared to that of standard antibiotic e-strips. The results showed that all the MRSA clinical isolates were susceptible to the tested drug with inhibition zone diameter ranging from 18 mm to 28 mm (Mean 24.05 mm). The MBC and MIC results confirmed that the tested extract was potent against the highly virulent Methicillin-Resistant Staphylococcus aureus (MRSA) clinical isolates with MBC and MIC of 0.97 μg/mL and 0.70 μg/mL, which was lower compared to MBC and MIC values of the standard antibiotics.
*Conclusion:* The results obtained from this study is a motivation to rediscover the ancient phytochemical compounds as an added asset to the standard available drugs in dealing with the upcoming hazardous diseases and pandemics. Thus, it relishes us to perform more of such studies to enhance the phyto-pharmacological industry.


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## 1. Introduction

The *Illicium verum* is an evergreen, medium-sized tree with star-framed fruits, is found all through southwestern Asian countries. Other than its usage in culinary arts, star anise is one of the basic components of the Chinese traditional medicine and is by and large known for its antiviral effects. The phytochemical molecule shikimic acid, a significant precursor in the pharmaceutical production of the anti-influenza medication oseltamivir (Tamiflu), is derived mainly from star anise, an antiviral remedy for influenza A and B. Moreover, the same plant has produced a number of additional compounds with a variety of biological activity. Apart from its antiviral qualities, star anise has antifungal, gastroprotective, antimicrobial, antioxidant, insecticidal, anthelmintic, antinociceptive, sedative, anti-inflammatory, expectorant and spasmolytic capabilities, as well as estrogenic effects. (Patra et al., 2020; Bisht et al., 2019; De et al., 2002; Muazzam et al., 2019). This study aimed to simplify information about the plant *Illicium verum's* standard credits, with an emphasis on its antibacterial capabilities and phytochemical components connected to its therapeutic indications. *Illicium verum* is generally called star anise, star aniseed, or Chinese star anise, and belongs to Schisandraceae family. It is an evergreen tree abundant in upper east Vietnam and southwest China. This tree is 6–8 m in height, dull gritty hued bark, green branchlets, glabrous; with all parts of the tree having a satisfying sweet-smell (Abdallah et al., 2013; Benmalek et al., 2013). The essential components that account for the awesome sweet-smelling aromas are essentially terpenes, monoterpenes and linalool (Muazzam et al., 2020; Bagewadi et al., 2019). In a previous study, EOs obtained from *I. verum* were evaluated for antiviral activity versus bovine herpes virus Type 1, in vitro and yielded promising results (Abdallah et al., 2013).

The focal point of this examination is to check antibacterial potential of *Illicium verum* EOs against the MRSA clinical isolates from nosocomial diseases and furthermore to know its phytochemical constituents. Our study is primarily focused on the difficult-to-treat MRSA nosocomial infections. Because of the inappropriate use of antibiotics, it has led to the creation of multi-drug resistant strains, the majority of instances of nosocomial illness with MRSA are not curable with standard antibiotics, which is a serious concern. Leading antimicrobial drug-resistant diseases include *Clostridium difficile*, *Mycobacterium tuberculosis, MRSA, Vancomycin-resistant Enterococci, Neisseria gonorrhea, Carbapenem-resistant Enterobacteriaceae.*

During the previous four decades, MRSA has progressed from a manageable nuisance to a major public health issue. MRSA is one of the most often acquired nosocomial infections. However, the - pathogens are increasingly circulating in the population and can cause serious community-acquired illnesses. Previous studies recommend that using natural essential oils derived from plants as an alternative to synthetic antimicrobials should be done on the basis that many of the negative effects associated with conventional antimicrobials may be avoided by using natural phytochemical substances (Muazzam et al., 2020; Maqbul et al., 2020; Chouksey et al., 2010).

EOs are liquids made up of a combination of volatile chemicals that may be collected from a variety of plant specimens. EOs include a variety of bioactive chemicals with antioxidative and antibacterial effects. A vast range of extracted EOs from plants are widely employed in industry, medicine, beauty products, and a wide range of daily items. These oils contain a variety of chemicals; they are a complex combination of volatile molecules with a wide range of chemical properties. What distinguishes and emphasizes them is their scent, which is typically pleasant and strong and evocative of the aroma of the plant, fruit, or wood from which these oils are derived. A complex spectrum of volatile chemicals with distinct fragrance notes and different sensory thresholds for their perception constitute the essence, which may be recalled as the scent of freshly cut vanilla or grass, sweet and cloying, among other aromatic tones that essential oil contains.

Essential oils have been reported to be more effective against Gram-positive microorganisms, including MRSA and *Methicillin Susceptible Staphylococcus aureus* (MSSA), than Gram-negative organisms. This shows that essential oils could benefit against the resistant strains. As a result, the use of essential oils will open up new possibilities in the fight against multidrug-resistant bacteria. The goal of this investigation is to investigate *Illicium verum EOs* antibacterial activity against MRSA nosocomial infections, as well as the phytochemical characteristics of the oil.

## 2. Materials and methods

### 2.1. Materials used

MRSA clinical isolates of *Staphylococcus aureus* from nosocomial infected patients, Blood agar plates, Mueller Hinton agar, Peptone, Standard antibiotic e- test strip, *Illicium verum* essential oil from the e-commerce platform (Old tree essentials), Standard Biochemical reagents used. Analytical grade chemicals were used for this research studies.

2.2. Fourier transform infra-red spectra of *Illicium verum* EO carried out using FTIR, Bruker spectrophotometer. GC-MS analysis was performed on a Shimadzu GCMS-QP2010 SE Standard Gas Chromatograph-Mass Spectrometer (Shimadzu Corp., Kyoto, Japan) equipped with a GCMS solution software. The GC-MS analysis was performed as per standard procedure mentioned in literature (Sitaram et al., 2011; Tsugawa and Fukusaki, 2020). Methodology for Phyto-bio synthetic investigation of the *Illicium verum* EO performed using Mayer's, Fehling's, Iodine, Salkowski's, Ninhydrin, Ferric, Libermann Burchard's, Benedict's, Keller-Kilani, Ammonia test. The phytochemical properties investigation of the *Illicium verum* EO acquired from the e-commerce platform (Old tree essentials) was done as previously described standard procedure (Gouse et al., 2017; Muazzam et al., 2020; Maqbul et al., 2020).

### 2.2. Microbiological processing of MRSA clinical samples

The MRSA clinical isolates were collected from the nosocomial patients which included isolates from nasal samples, catheter test, urine test, crotch test, skin tests, canker test, ear test, throat swab tests, ulcer tests, wound sample, and from the surgical samples. The gathered samples were handled in the microbiology lab by following the standard aseptic microbiological procedure of streaking on Blood improved media at 37 °C for overnight incubation. The brooded detaches were distinguished and cleansed by performing Gram's staining and the necessary biochemical response tests, with the catalase and coagulase tests being the most recognizable proof test. (Muazzam et al., 2019; Gouse et al., 2017; Muazzam et al., 2020; Maqbul et al., 2020; Maqbul et al., 2020).

### 2.3. Antimicrobial vulnerability testing

The antimicrobial vulnerability test for the segregated clinical samples of MRSA against the standard antibiotics were done by utilizing the cutting edge fast e-test system where the confines were immunized on Mueller-Hinton agar independently & standard e-test strips for individual anti-infection agents inoculated, at 37 °C incubated for short-term to determine inhibition zone. The connection of the oval is perused as MIC whereas, the zone as the weakness of the antibiotic on-to the bacterium and results

were organized for the translation. The customary ordinary standard anti-toxin measure strategies, for example, Kirby-Bauer circle dispersion strategy was utilized to notice the susceptibility of the clinical examples of MRSA standard plate ready from the *Illicium verum* essential oil, where the bacterium disengages were immunized independently on Mueller-Hinton agar plates alongside the impregnated circles at 37 °C for 24 h to notice the zone development deciding the affectability of the bacterium towards the plate. The outcomes were organized for the understanding. The MIC values alongside Minimum Bactericidal Concentration (MBC) values for *Illicium verum* towards the MRSA isolates was assessed by playing out the standard cylinder weakening strategy where the samples were inoculated independently in the various concentrations and incubated at 37 °C for 24 h. The outcomes were organized for the translation. The MBC was dictated by immunizing every weakening of MIC weakenings on different plates of agar for every disengages and weakening independently. The vaccinated were brooded at 37 °C for 24 h to notice the no development deciding the affectability of the bacterium. The outcomes were arranged for the understanding (Muazzam et al., 2019; Muazzam et al., 2020; Maqbul et al., 2020).

## 3. Results and discussion

### 3.1. GC–MS analysis

Fifty compounds were confirmed by searching NIST-EPA-NIH Mass Spectral Library (NIST 17) in ChemData. NIST. GOV (Fig. 1). These included Anethole, Eucalyptol, D-Limonene, Linalool, Squalene, Sitosterol, Piperine, etc. The volatile components like anethole, levomenthol, eucalyptol and estragole responsible for the characteristic aroma of star anise essential oil were also identified. The GCMS analysis results for star anise essential oil corroborate with previous reports (Zhang et al., 2015; Cu et al., 1990).

The IR spectrum (Fig. 2) shows peaks at 3022 $cm^{-1}$ indicate presence of aromatic benzene, peaks at 1607 to 1509 $cm^{-1}$ indicates framework of benzene ring and C-O-C stretching vibration at 1305 $cm^{-1}$.

The investigation was likewise directed to discover the potential phyto constituents that might be responsible for the antibacterial activity of *Illicium verum* essential oil. The phytochemical investigation revealed the presence of alkaloids, steroids, proteins, phenols, glycosides, cardenolides and amino acids (Table 1). Thus, the potent antimicrobial activity exhibited by *Illicium verum* EO can be attributed to the presence of phenolics and flavonoid content. Notably, an earlier study has proven that star anise ethanol fraction is higher in antioxidant activity compared to the non-polar petroleum ether fraction, which is due to the solubility of phenolic compounds in ethanol (Padmashree et al., 2007).

Medicinal plants have long been recognized as a source of compounds with medicinal promise, and they continue to be a valuable resource for finding new drug leads today, to treat several diseased conditions including nosocomial infections. *MRSA* and *A. baumannii* infections pose a major threat due to the rise of resistance to a variety of antibiotics. Because of their capacity to build biofilm on abiotic surfaces in hospitals, *A. baumannii* and *MRSA* have been linked to a number of nosocomial illnesses (Salem et al., 2021). They have been found to be resistant to a wide range of antimicrobials, prompting the World Health Organization (WHO) to identify them as one of the high priority microorganisms for the discovery and development of modern antimicrobials (Govindaraj Vaithinathan and Vanitha, 2018).

In the current study, *Illicium verum* essential oil showed encouraging antibacterial results (Table 2) against all the clinical isolates with a normal plate dissemination of 24.05 mm zone of inhibition with a MIC 0.70 & MBC of 0.97 μg/mL. The good clinical isolate MRSA vulnerability was seen in swab nasal test with inhibition zone of 28 mm with MIC of 0.25 μg/mL and MBC of 0.5 μg/mL. The clinical isolate test of MRSA which showed the least effectiveness was the surgical isolate with 18 mm zone of inhibition with MIC of 1 μ/mL and MBC of 1.25 μ/mL. However, these results were better compared to the standard synthetic antibiotics for similar isolates with the exception of vulnerability towards Vancomycin (Patra et al., 2020; De et al., 2002; Ohira et al., 2009; Maqbul et al., 2020). The other MRSA clinical isolates, for example, catheter test showed vulnerability towards the *Illicium verum* essential oil with zone width of 27 mm in circle dispersion strategy with MIC of 0.25 μg/mL and MBC of 0.5 μg/mL, while the urine test shown vulnerability towards the *Illicium verum* essential oil with zone width of 25 mm in plate dissemination technique with MIC of 0.25 μg/mL and MBC of 0.5 μg/mL, whereas, the crotch test shown vulnerability towards the *Illicium verum* essential oil with zone width of 24 mm in plate dissemination technique with MIC of 0.5 μg/mL and MBC of 0.75 μg/mL, and the skin tests, ear test, throat swab tests, ulcer tests, wound sample and surgical sample shown vulnerability towards the *Illicium verum* essential oil with zone breadth ranging from 18 mm to 23 mm in circle dispersion technique, with MIC of 0.5 μg/mL to 1 μg/ml and MBC of 0.75μg/mL to 1.25 μg/mL, which were better than that of the standard antibiotics results (Table 3 and Table 4). The vulnerability of MRSA clinical isolates towards the standard antibiotic Vancomycin showed consistent results, yet, relatively the antibacterial activity of *Illicium verum* essential oils towards the MRSA isolates showed all the more better and promising results (Table 2). The antibacterial activity against the clinical isolates displayed by *Illicium verum* essential oils was far more superior than other standard antibiotics employed in the assay (Chart 1). It was observed that majority of the standard antimicrobial agents with the exception of Van-

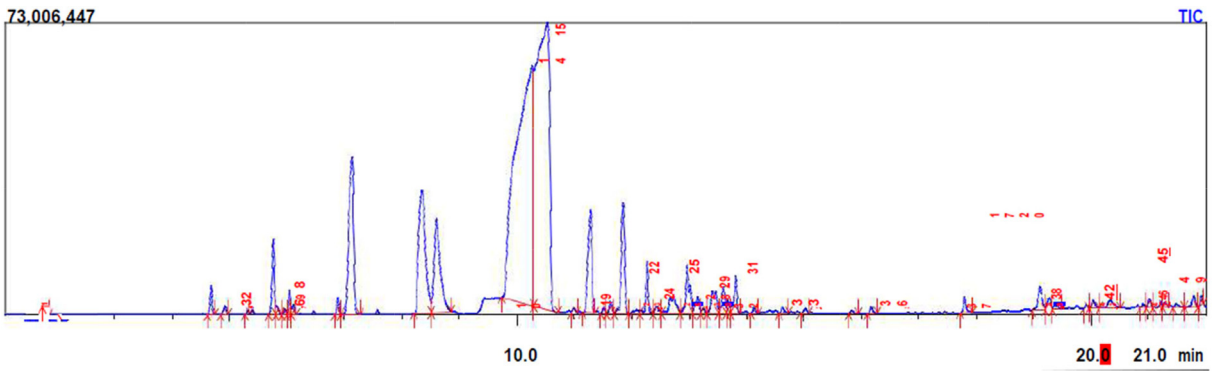


**Fig. 1.** GC–MS chromatogram of *Illicium verum* essential oil.

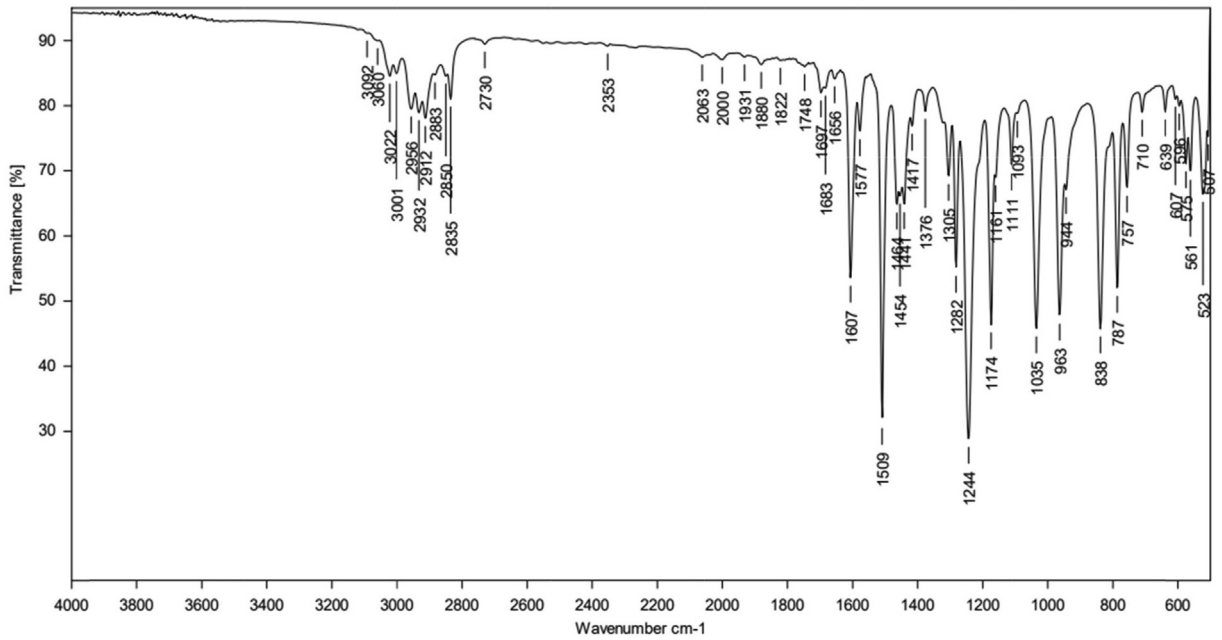


**Fig. 2.** IR spectra of *Illicium verum* essential oil.

**Table 1**
Phyto-bio synthetic investigation of the *Illicium verum* essential oil.

| Test | Result | Compound Present |
|---|---|---|
| Salkowski's test | Reddish brown | Steroids |
| Ninhydrin test | Violet color | Proteins |
| Ferric chloride test | Blue black | Phenols |
| 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 |
| Mayer's test | creamy substance | Alkaloids. |
| Fehling's test | Brick red color | Reducing sugar |
| Iodine test | Purple colour | Starch |

**Table 2**
Comparative chart of MRSA isolate's susceptibility towards *Illicium verum* essential oil.

| | *Illicium verum* Essential oil | | |
|---|---|---|---|
| SPECIMEN | Disc Diffusion (mm) | MIC (μg/mL) | MBC (μg/mL) |
| Nasal sample | 28 S | 0.25 | 0.50 |
| Catheter sample | 27 S | 0.25 | 0.50 |
| Urine sample | 25 S | 0.25 | 0.50 |
| Groin sample | 22 S | 0.50 | 0.75 |
| Skin samples | 22 S | 0.50 | 0.75 |
| Abscess sample | 21.0 S | 0.75 | 1.00 |
| Ear sample | 20 S | 0.75 | 1.00 |
| Throat swab samples | 19.5 S | 0.75 | 1.00 |
| Ulcer samples | 19 S | 1.00 | 01.25 |
| Wound sample | 19 S | 1.00 | 1.25 |
| Surgical samples | 18.0 S | 1.00 | 1.25 |
| Mean Zone value for all the specimens | 24.05 | 0.70 | 0.97 |

comycin, showed conflicting vulnerability result, with only a few MRSA clinical isolates showing significant susceptibility against the standard antibiotics. We found that the impact of standard antibiotics towards the MRSA clinical segregates were restricted with higher MIC and MBC values going from 0.75 μg/mL up to 2.75 μg/mL. The higher MIC and MBC values deduce that, even as MRSA clinical isolates have shown vulnerability to standard antibiotics, it may still need a higher antibiotic dose to control the infection and/or completely eliminate the disease. However, using a higher dose of antibiotic is linked with antibiotic-associated adverse drug reactions (Tamma et al., 2017). Certain patient-related risk factors may increase the likelihood of infection as well as the morbidity and mortality associated with infection, notably mycoplasma pneumonia, pneumococcal pneumonia, urinary tract infections, influenza virus infections, and other infections caused by multi-drug resistant organisms.

The results from the current study showed that all the MRSA clinical isolates were susceptible to the tested extract (*Illicium verum* essential oil) with inhibition zone diameter ranging from 18 mm to 28 mm (Mean 24.05 mm). The MBC and MIC results confirmed that the tested extract was potent against the highly virulent MRSA clinical isolates with MBC and MIC of 0.97 μg/mL and 0.70 μg/mL, respectively, which was lower compared to MBC and MIC values of the standard antibiotics. The MIC and MBC values of the *Illicium verum* essential oil ran between 0.25 μg/mL upto1.25 μg/mL for all the MRSA clinical isolates, confirming that a lesser dose of the *Illicium verum* essential oil is adequate to hinder or annihilate the disease totally. Henceforth, the antibacterial rich *Illicium verum* essential oil showed much desired susceptibility results compared to the standard antibiotic agents towards the acquired MRSA clinical isolates (Muazzam et al., 2019; Muazzam et al., 2020; Maqbul et al., 2020).

Previous studies have demonstrated that plants rich in phenolic compounds possess potent antioxidant activity and therefore exhibited significant antibacterial activity (Shan et al., 2007). Even

**Table 3**
Comparative chart of MRSA susceptibility towards standard antibiotics diffusion for the e-test valuation.

| SPECIMEN | ANTIBIOTICS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Bactrim (mm)* | *Clindamycin (mm)* | *Minocycline (mm)* | *Doxycyclin (mm)* | *Gentamycin (mm)* | *Penicillin (mm)* | *Methicillin (mm)* | *Vancomycin (mm)* | S | I | R |
| Nasal | 4 | 1 | 3 | 15 | 2 | 21 | 6 | 22 | | | |
| | R | R | R | I | R | S | R | S | 2 | 1 | 5 |
| Catheter | 13 | 4 | 2 | 3 | 22 | 12 | 3 | 22 | | | |
| | 1 | R | R | R | S | I | R | S | 2 | 2 | 4 |
| Urine | 14 | 13 | 12 | 3 | 21 | 21 | 6 | 23 | | | |
| | I | I | I | R | S | S | R | S | 3 | 3 | 2 |
| Groin | 5 | 02 | 02 | 4 | 02 | 10 | 4 | 23 | | | |
| | R | R | R | R | R | I | R | S | 1 | 1 | 6 |
| Skin | 10 | 12 | 10 | 1 | 4 | 22 | 1 | 22 | | | |
| | I | I | I | R | R | S | R | S | 2 | 3 | 3 |
| Abscess | 22 | 10 | 9 | 22 | 22 | 2 | 1 | 22 | | | |
| | S | I | I | S | S | R | R | S | 4 | 2 | 2 |
| Ear | 10 | 14 | 12 | 14 | 2 | 9 | 5 | 21 | | | |
| | I | I | I | I | R | I | R | S | 1 | 5 | 2 |
| Throat swab | 10 | 12 | 14 | 22 | 19 | 11 | 5 | 20 | | | |
| | I | I | I | S | S | I | R | S | 3 | 4 | 1 |
| Ulcer | 11 | 22 | 21 | 11 | 22 | 1 | 3 | 22 | | | |
| | I | S | S | I | S | R | R | S | 4 | 2 | 2 |
| Wound | 14 | 13 | 11 | 13 | 22 | 3 | 1 | 22 | | | |
| | I | I | I | I | S | R | R | S | 2 | 4 | 2 |
| Surgical | 10 | 20 | 20 | 12 | 20 | 21 | 9 | 22 | | | |
| | I | S | S | I | S | S | I | S | 5 | 3 | 0 |

**Table 4**
Comparative chart of MRSA e- test MIC (μ/mL) towards standard antibiotic dilution.

| SPECIMEN | ANTIBIOTICS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Bactrim* (μg/mL) | *Clindamycin* (μg/mL) | *Minocycline* (μg/mL) | *Doxycyclin* (μg/mL) | *Gentamycin* (μg/mL) | *Penicillin* (μg/mL) | *Methicillin* (μg/mL) | *Vancomycin* (μ/mL) |
| Nasal | 1.00 | 1.00 | 1.25 | 1.25 | 1.50 | 1.50 | 1.50 | 0.75 |
| Catheter | 1.00 | 1.00 | 1.25 | 1.25 | 1.50 | 1.50 | 1.50 | 0.75 |
| Urine | 1.25 | 1.25 | 1.25 | 1.25 | 1.50 | 1.50 | 1.50 | 0.75 |
| Groin | 1.25 | 1.25 | 1.25 | 1.25 | 2.25 | 2.25 | 2.25 | 1.00 |
| Skin | 1.25 | 1.25 | 2.25 | 2.50 | 2.50 | 2.50 | 2.50 | 1.00 |
| Abscess | 1.25 | 1.25 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 1.00 |
| Ear | 2.25 | 2.25 | 2.50 | 2.75 | 2.75 | 2.75 | 2.75 | 1.25 |
| Throat swab | 2.50 | 2.25 | 2.50 | 2.75 | 2.75 | 2.75 | 2.75 | 1.50 |
| Ulcer | 1.25 | 1.25 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 1.50 |
| Wound | 2.25 | 2.25 | 2.50 | 2.75 | 2.75 | 2.75 | 2.75 | 1.75 |
| Surgical | 2.50 | 2.25 | 2.50 | 2.75 | 2.75 | 2.75 | 2.75 | 1.75 |

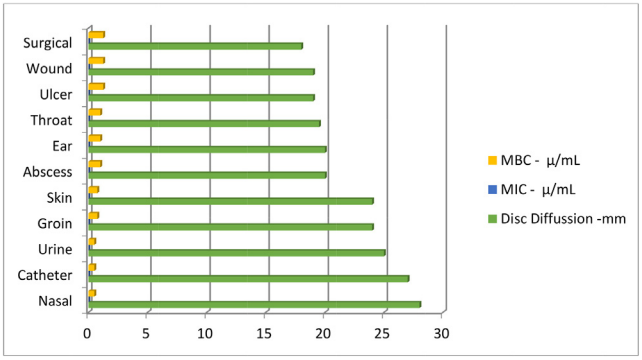


**Chart 1.** Comparative chart of MRSA isolate's susceptibility towards *Illicium verum* essential oil.

though the mechanism of the anti-bacterial potentials of polyphenols is not fully understood, there are several arguments that phenolic compounds can either affect the permeability of the cell membrane; induce intracellular enzymes hydrogen-binding and thus influence their proper function; produce permanent damage to the cytoplasmic membrane and cell contents coagulation, and/or loss of integrity of cellular membranes stiffness. Subsequently, this examination has shown that the phytochemicals present in the *Illicium verum* essential oil ends up being a powerful antibacterial components against the MRSA clinical isolates when contrasted with the standard antibiotics (Lenora et al., 2016; Maqbul et al., 2020; Maqbul et al., 2020).

## 4. Conclusions

The phytochemicals present in the *Illicium verum* essential oil can be used successfully to treat the multi-drug resistant and highly virulent MRSA. Recently, due to the irrational employment of antibiotics in the management of infectious diseases as well as patient non-compliance has led to the emergence of multi-drug resistant strains and further over utilization of the antimicrobials can lead to more serious outcomes (Abdallah et al., 2013; Benmalek et al., 2013; Muazzam et al., 2020). The WHO has effectively warned about the harmful impacts due to the misuse of antimicrobial agents, which not only has increased the incidence of antimicrobial resistance, but also led to antibiotic-induced adverse drug reactions. Hence, it is critical to discover nontox-

ic substances as treatment options for infectious diseases, and one such small step towards the more promising future is this investigation into the antibacterial viability of the *Illicium verum* essential oils against MRSA clinical isolates, which yielded promising results. This investigation suggests the utilization of herbal plant EOs as a therapeutic option for the management of infectious diseases caused by multi-drug resistant and highly virulent MRSA.

## Conflict of interest

The authors declare no conflict of interest.

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