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Antibacterial activity of Ajowan Essential Oil against Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from pus samples in local hospital in Amravati

Mankar SS[1*], Ukesh CS[1], Patil SD[1], Agrawal NK[2]

Email: - suwarnamankar99@gmail.com

1. ***Dept. of Microbiology, Shri Shivaji Science College Amravati, M.S. – 444603, India***
2. ***Dept. of Microbiology, B.S.Patel Arts, Commerce and Science College, Pimpalgaon Kale, M.S.-443403, India***

Abstract:-

In the present study the systematic isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the period of December 2021-April 2022 was carried out. Total 110 pus samples were collected from the local hospital of Amravati. Out of which 52(47.27%) pus samples were found to be positive for *Staphylococcus aureus*. Methicillin resistance was obtained in all the 52 pus samples of the *S.aureus* isolates. The sensitivities of Methicillin Resistant Staphylococci to various standard antibiotics were as follows: Vancomycin, Gentamycin, Tetracycline, Chloramphenicol, Streptomycin and Amoxyclav whereas resistant to Methicillin, Penicillin, Cefdinir, Cefotaxime ,Cefpirome ,Cefpodoxime ,Cefalexin and Imipenem respectively were used for comparative study with Ajowan Essential Oil (AEO).

Keywords –Ajowan, Essential oil, MRSA, Pus

1. Introduction:

The antibiotic resistance is a phenomenon as old as the advent of antibiotics. The development and spread of resistance to currently available antibiotics is a global concern (Moussaoui and Alaoui, 2016). Misuse of antibiotics has resulted in the emergence of resistance against them which is another problem affecting public health. The continual and indiscriminate use of antibiotics has encouraged the development of resistance in many species of bacteria which are pathogenic to humans. Bacteria have remarkable ability to adapt to adverse environmental conditions that lead to the emergence of resistant bacteria. This ever increasing bacterial resistance to antibiotics represents a serious problem for public health and highlights the urgent need for new drugs or combination therapies to treat the infections caused by resistant pathogens (Sienkiewicz *et al.*, 2017; Imane *et al.*, 2020).

Diabetes, obesity and advanced age influence the efficacy of the immune system and thus increase the risk of complications associated with wound infections. Colonization may impede wound healing,

depending on the status of the host immune system and the number and types of bacterial species present. Chronic wounds represent a major clinical problem because of the potential for serious complications, and the need for prolonged hospitalization for combination therapy greatly increases the cost of treatment (Sienkiewicz *et al.*, 2017).

Methicillin Resistant *Staphylococcus aureus* (MRSA) emerged as a nosocomial *pathogen in* the early 1960s .In 1970s an increasing number of large hospital outbreaks were reported in many countries including the USA, Europe, Japan and Australia (Tyagi *et al* .,2008). There has recently been a surge in the emergence and re-emergence of drug resistant bacteria such as MRSA that are recalcitrant to treatment (Buru *et al*., 2014). MRSA is the cause of wide-range of infectious complications which can be life –threatening. It has been implicated as a dominant hospital –acquired pathogens which causes substantial burden for health and economics in the world today. Hospital acquired infections are defined as infections that are likely to have seen strictly acquired in the hospital and which are not in incubationary phase upon admission of the patient in the hospital .Vancomycin is the reserved drug of choice for treatment of MRSA infections. However, recent evidence indicates the evolution of Vancomycin Resistant *Staphylococcus aureus* (VRSA). This is partly due to indiscriminate use of antibiotics for infectious diseases and evolutionary adaptations by pathogenic microorganisms enabling them to avert the deleterious effects of antimicrobials treatment (Buru *et al*., 2014).

There are approximately 20,000 plant species being used as ethno-medicines all over the world. The World Health Organisation (WHO) estimated that 80% of the global population is a panacea against communicable and non-communicable diseases (WHO, 2005).

Spices are one of the important food ingredients which play a cardinal role in food preparations. Over a hundred plant species are used as spices and condiments across the globe. They are aromatic, dried plant parts usually obtained from seeds, fruits, leaves, roots and bark, etc. A large number of spices also act as excellent preservatives which increase the shelf life of the food items by delaying the process of decaying. Moreover, spices being the rich reservoir of biologically active compounds also possess properties *viz.* antioxidant, antimicrobial, anti-inflammatory, antidiabetic and anticancer, etc. These properties of spices help in combating various ailments of the human body (Singh *et al.*, 2020).

In recent years, there has been a significant increased interest in natural products including essential oils (EOs) which have a wide range of antimicrobial activities (Saki *et al.*, 2020). Essential oils (EOs) are colorless liquids, mainly comprising the aromatic and volatile compounds naturally present in all parts of the plants including seeds, flowers, and peel, stem, bark and whole plants. EOs are secondary metabolites, and are important for plant defense mechanism, hence, they have various medicinal properties including antimicrobial activity. Generally, the medicinal plants used in Siddha and Ayurveda are the major sources of various volatile compounds,which are responsible for various biological activities, for example, the major volatile compounds such as alkenes, alcohol, and esters are characterized as major constituents of EOs showing significant pharmacological effects (Bhavaniramya *et al.*, 2019).

Ajowan(*Carum copticum Benth*. & Hook, syn: *Trachyspermum ammi)* (L) Sprague belong to family *Apiaceae (*formerly *Umbelliferae*) is an erect annual herb with striate stem, aromatic, grassy, annual plant originated in eastern regions of Perisa and India as well as in Iran, Pakistan and Egypt. It has white flowers and small brownish fruits. The most utilized part of ajowan is the small caraway like fruit, which is particularly popular in Indian savory recipes, savory pastries and snacks. In Ayurvedic medicines, it is used as a medicinal plant for its antispasmodic, stimulant, tonic and carminative properties (Paul *et al.*, 2011).

Therefore, the aim of the present study was to investigate the antibacterial activity of Ajowan essential oil (AEO) against clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from hospital pus samples.

2. Material and Methods:

Plant Material

The Ajowan seeds were purchased from the local market of Amravati, Maharashtra.

2.1 Extraction of Ajowan Essential Oil (AEO)

The ajowan seeds were subjected to hydrodistillation for 3 hrs in Clevenger type apparatus. For this purpose, 40 g of dry ajowan seeds samples were powdered and mixed with 400 ml distilled water.The Ajowan Essential Oil (AEO) was separated from water and dried over anhydrous sodium sulphate, then stored in dark bottles in a refrigerator at 4˚C until prior to use (Lalami, *et al.*, 2019).

2.2 Antibacterial activity:

Test Organisms

In the period of December 2021-April 2022, A total of 110 pus samples were screened .Out of that 52 clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were found from pus samples derived from patients of the local hospital in Amravati .On the basis of morphological, cultural and biochemical characterization the microorganism were screened. The isolates were grown on Nutrient Agar Medium and incubated at 37˚C for 24 hr.

Disc Diffusion Assay

The antibacterial activity of Ajowan Essential Oil (AEO) was conducted by using the disc diffusion method as per CLSI guidelines. The bacterial inoculum was adjusted to 0.5 McFarland standard was inoculated in Muller Hinton Agar plates. After this the sterile Whatman paper discs (6mm) in diameter were impregnated with 10µl of Ajowan Essential Oil were placed asceptically on the surface of the inoculated media agar plate. The plates were incubated at 37˚C for 24 hrs. After the incubation, The Zone diameter measured with the antibiotic susceptibility zone diameter measurement scale (HiMedia Pvt. Ltd., Mumbai, India). Each experiment was performed in triplicate and the diameter of each of the plate was measured in mm. The average diameter of zone of inhibition was recorded. The zones of inhibition were compared with each other and with antibiotic control.

The selected isolates were further screened for their antimicrobial activity as per CLSI guidelines (2014) .In all 14 Standard antibiotics such as Penicillin –G(2 units/disc), Gentamicin (10mcg), Tetracycline (30 mcg), Vanomycin (10mcg), Methicillin (5mcg), Streptomycin (10 mcg), Chloroamphenicol (30 mcg), Cefdinir (5 mcg), Cefotaxime (30 mcg), Cefpirome (30 mcg),Cefpodoxime (30 mcg), Cefalexin (30 mcg) ,Amoxyclav (30 mcg), Imipenem (10mcg) , from Himedia Laboratory (Mumbai, India) were used for comparative study with Ajowan Essential Oil.

3. Result:

Total 110 samples were received, out of which 52(47.27%) pus samples were found to be positive for *Staphylococcus aureus*. On the basis of morphological, cultural and biochemical characterization of samples

was confirmed to be *S.aureus* as per the Standard of Bergey's Manual of Bacteriological Identification and Methicillin resistance was obtained in all the 52 pus samples of the *S.aureus* isolates.

In the present study, Ajowan Essential Oil (AEO) tested had showed strongest antibacterial activity against all the 52 clinical isolates of Methicillin Resistant *Staphylococcus aureus* with zone of inhibition ranging from (40mm to 75mm) in diameter. The AEO had showed greater zone of inhibition than the standard antibiotics used. In this study, antibiotics from seven groups such as Beta-lactamase Inhibitor, Cephalosporins, Carbapenems, Aminoglycosides, Glycopeptides, Tetracycline, and Chloroamphenicol were used. All the isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were found fully (100%) resistant to four groups of antibiotics they were Beta-lactamase Inhibitor group (Penicillin, Methicillin and Amoxyclav), Cephalosporins group (Cefalexin, Cefdinir, Cefotaxime, Cefpirome, and Cefpodoxime) and Carbapenems group (Imipenem) with no zone of inhibition.

All the isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were found to be sensitive towards remaining three groups of antibiotics they were Glycopeptides group (Vancomycin) had showed reduced zone of inhibition ranging from (11mm to 14 mm) in diameter. The Aminoglycosides group (Gentamicin, Streptomycin) Gentamicin had showed moderate zone of inhibition ranging from (15 mm - 20mm), whereas Streptomycin had showed reduced zone of inhibition in the range of (10mm-14mm) in diameter. The Tetracycline group (Tetracycline) showed sensitive zone of inhibition ranging from (22 mm-26 mm) as well as Chloroamphenicol group (Chloroamphenicol) had also showed highest zone of inhibition ranging from (21mm-28 mm) in diameter respectively.

4. Discussion:

Most of the medicinal plants possess antimicrobial activity due to presence of the essential oil. The nature, structural composition, and the functional groups present in the essential oils play an important role in determining the antimicrobial activity. Essential oil contain a variety of volatile molecules such as terpenes and terpenoids, phenol derived aromatic and aliphatic compounds, which might have bactericidal, virucidal and fungicide consequences (Aktar *et al.*, 2014).

In the present study, Ajowan Essential Oil (AEO) demonstrated remarkable antibacterial activity against all the 52 isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) with an inhibition zone diameter (IZD) ranging from (40mm-75mm) respectively. Ajowan Essential Oil had showed larger inhibition zone diameter than standard antibiotics referred in this study. The ajowan oil had showed strong antibacterial activity against *Staphylococcus aureus* with an inhibition zone ranging from (21.13± 0.35 mm) in diameter, as well as ajowan oil had showed larger growth inhibition zone diameter in comparison to synthetic antibiotics reported by (Upadhyay *et al.,* 2010). The test isolates Methicillin Resistant *Staphylococcus aureus* (MRSA) with antimicrobial resistance pattern of 77 (82%) sensitive and 17 (18%) resistant against Ajowan oil reported by (Kaushik *et al.,* 2014). The *Carum copticum* (Ajowan) oil were tested for antibacterial activity against selected MDR *S. aureus* isolates from three sampling groups. Out of five essential oils, *Carum copticum* was found to be the most effective followed by *Zingiber officinale and Cuminum cyminum* with inhibition zone diameter ranging from (35.7 ± 1.7 mm) reported by Raja *et al*.,2016).

In the present study the most common pathogen isolated from pus samples was *Staphylococcus aureus*. The similar results were reported by (Mudassar *et al.,* 2018; Rai *et al.,* 2017; Tiwari *et al.,* 2010 ; Tyagi *et al*., 2008; Chauhan *et al*., 2005). In our study all the 52 (47.27 %) isolates were found to be Methicillin Resistant *Staphylococcus aureus* (MRSA), similar, results were reported by (Saba and Srinivasagam, 2018) 14(17.5 %) of *S.aureus* (MRSA) similar reported by (Tyagi *et al*., 2008; Chauhan *et al.,* 2005). High antibiotic resistance were seen by MRSA to penicillin, all the isolates were found fully (100%) resistant (R) to penicillin, and the similar results were reported by (Khanam *et al.,* 2018) MRSA had showed

strong resistance to penicillin (84%). All MRSA isolates had shown sensitivity pattern towards Gentamicin in the present study, in contrast to this result (Vazirian *et al.,* 2018) stated that all of the clinically isolated MRSAs were resistant to Gentamicin. The sensitivity pattern of MRSAs towards Gentamicin was stated by (Rai *et al.,* 2017) 122 (76%) sensitivity, similar to the present study. In this study, antibiotic sensitivity pattern of all isolates of MRSA was observed against Streptomycin, similar result was stated by (Mazumder *et al.,* 2014).

All the isolates of MRSA had showed fully (100%) resistant towards Amoxyclav and Imipenem similar result was stated by (Zahra *et al.,* 2019). In the present study, all the isolates of MRSA had showed fully resistant (100%) pattern towards antibiotics of Cephalosporins group (Cefalexin, Cefdinir, Cefotaxime, Cefpirome, and Cefpodoxime) with no zone of inhibition, in contrast to this report (Zahra *et al.,*2019) reported antibiotic sensitivity pattern of MRSA 7(30.4%) towards Cefotaxime. (Khanam *et al,* 2018) reported antibiotic sensitivity pattern of MRSA (42.5%) towards Cefalexin (Cephalexin). The strong antibiotic sensitivity pattern had shown by all the MRSA isolates against Tetracycline in this study, (Zahara *et al,* 2019) reported 50% sensitivity pattern of MRSA against Tetracycline. MRSA had reported to show highest sensitivity pattern against Chloramphenicol in the present study, in contrast to this result (Gadisa *et al.,* 2019) stated the resistant pattern of MRSA towards Chloramphenicol.

In present study, all the isolates of MRSA were showed reduced susceptibility to Vancomycin, similar result was reported by the Centres for Disease Control and Prevention (CDC, USA, 1997). All the isolates of MRSA were sensitive to Vancomycin reported by (Tyagi *et al*., 2008; Sujatha *et al.,* 2016; Subha and Srinivasagam, 2018). The (Hamamatsu *et al.*, 1997) Japanese workers reported of three *S.aureus* isolates fully resistant to Vancomycin.

The report on MRSA by (Tyagi *et al,* 2008) documented a high endemicity of MRSA at AIIMS hospital, New Delhi. This poses a serious problem for drug therapy because the treatment options have been restricted to potentially toxic antimicrobials like Vancomycin, leading to increased mortality and morbidity. The World Health Organization published in 2017 a report listing the most dangerous multidrug-resistant bacteria to which new antibiotics should be urgently discovered (Atki *et al.*,2019).

The discovery of new bacterial agents was mainly based on natural products, there has been an increased interest in bioactive compounds provided by plants as an alternative to the common antibiotics Essential oils (EOs) account for a source of very promising natural compounds for producing new antibacterial drugs (Atki *et al.,* 2019) Spices are aromatic plants characterized by their characteristic aroma by the virtue of their essential oil (EO) Spices have been screened for extensively for their antimicrobial properties (Patil *et al.,* 2016). Many studies have reported a strong antibacterial of Ajowan (*Trachyspermum ammi*) has been documented frequently. Chemical composition of Ajowan oil exhibited the presence of thymol, γ-terpinene and O-cymene without cravacol as the main component of Ajowan oil. Antimicrobial activities of oil are apparently attributable to high phenolic compounds such as thymol and cravacol (Hassanshahian *et al*., 2014). Ajowan oil traditionally was used as diuretic, carminative, and antihelmentic which contain 40-50 % of thymol. Some biological effects of ajowan such as antiviral, anti-inflammatory, antifungal, antipyretic, antifilarial, analgesic, antinociceptive and antioxidant activity have been confirmed. Limonene and Terpinol the other major component also present in the Ajowan oil in 10-30% (Mazumder *et al.,* 2014).

5. Conclusion:

In conclusion, the present study has shown, more resistant against *S.aureus* and also multi-drug resistant against four different groups of antibiotics. On the other hand plant essential oil tested in this study had potential antibacterial activity against the Methicillin Resistant *Staphylococcus aureus* (MRSA). Ajowan

Essential Oil might be considered as alternative antibacterial drug for treatment against wound infection (pus forming microorganisms).

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