

Isolation And Identification of Staphylococcus Aureus from Human Oral Cavity Infection and Properties of Some Essential Oils as Anti-Bacteria

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Abstract

Background: The most prevalent human pathogen, *Staphylococcus* sp., is in charge of several chronic and serious diseases especially oral dental diseases. A growing body of research shows that persisters are linked to treatment failure and recurrence of persistent illnesses. While several essential oils were said to have activity as antimicrobial effects on growing *Staphylococcus* spp., the effects of essential oils on *Staphylococcus* sp. richer in persisters have not been studied. **Methodology:** In this investigation, 35 *Staphylococcus aureus* isolates were obtained from patient infections' oral cavities and were thoroughly characterized and identified using standard bacteriological techniques, biochemical tests, and the VITEK-2 system compact. Evaluation of the antibacterial capabilities of essential oils to the same MDR under research is done using susceptibility tests for various antibiotics on *S. aureus* MDR. Different essential oil concentrations obtained from sources such as medicinal plants and herbs are employed as antibacterials. **Results:** When compared to medicines, these essential oils showed antibacterial efficacy against clinical bacterial isolates of MDR *S. aureus*.

Keywords: essential oil, antimicrobial, *Staphylococcus aureus*, MDR.

1. Introduction

Due to its moisture, oral cavity of patient's, nutrient content (like lipid) and periodontal of patient's temperature, oral cavity of human serves as a good growth medium for a variety of dental bacterial infections that affect pathogenic different microorganisms. These infections include tooth decay, dental plaque, dentin carbohydrate, and protein infections [1]. Hypersensitivity, acid erosion, hyperdontia, calculus malocclusion, acute necrotizing ulcerative patient's gingivitis, dental abscess, tooth impaction, dental fluorosis, etc. are among the conditions that can occur in the mouth. Numerous oral disorders, including endodontic infections, peri-implantitis, periodontitis, even dental caries and oral mucositis may be caused by *Staphylococcus aureus* [2-5].

Due to its combination of invasiveness, toxin mediated the virulence factors and antibiotic multi-resistance the *Staphylococcus* sp. is most significant coagulase positive bacteria from the family of staphylococci [6]. It is a Gram-positive, non-motile, grape-like cluster-forming organism that does not produce spores. *S. aureus* strains that have evolved antibiotic resistance. The term "methicillin-resistant *S. aureus*" (MRSA) refers to *S. aureus* bacterial strains that have developed mechanisms of resistance to beta-lactam drugs, including amoxicillin, penicillin, methicillin, oxacillin, cephalosporins and ampicillinetc. [8]. Due of *S. aureus*' propensity to develop antibiotic resistance, clones that express different antimicrobial resistances have been widely disseminated. MRSA strains are responsible for a number of bacterial illnesses that can be contracted

outside of hospitals and can result in death [9-11].

Essential oils have a significant impact, It is natural for plants to function as antibacterial, antifungal, insecticidal agents and antiviral. Plants may also naturally fend against some herbivores by lowering their hunger for plants with these traits. human resources for health some essential oils include some compounds that may be utilized as antibacterial additions, and public health agencies have acknowledged essential plant oils as healthy safe material[12]. Essential oils' (EOS) effectiveness against pathogens in the food sector has been documented in several research [13,14].

Antibacterial medicines have been used widely, which has led to rising levels of bacterial multi-resistance and their severity [15,16], that which in turn necessitates further escalating bacterial multi-resistance in a different invasion cycle [17]. In order to prevent increasing patients mortality [18] and the financial burden on both society and patients, it is crucial to use the right antibacterial drugs [19]. Antibacterial agents have, however, also been applied irrationally in the United States [20]. High mortality [21] and higher medical expenses are frequent negative effects of these flaws in the sensible use of antibacterial medicines [19].

The purpose of this study is to isolate, identify oral cavity Staph. aureus, as well as to test the antibacterial properties of essential oils in order to try to use them to lessen the risk of bacterial infections in humans by combining them with mouthwash and toothpaste.

2. Materials & Methods

Collection of Samples

The diverse oral cavity samples were used to acquire the

40 specimens. 35 *S. aureus* were found in the bacterial samples, which were collected. From several male and female patients between (15-45 year) in Al-Hilla city, bacteria samples were taken. Various culture medium, including blood agar and plates of Macconkey agar, were utilized for 24-48 hrs at 37°C as part of standard microbiological protocols to isolate and purify these bacteria isolates. All of the isolates were validated by the compact system of Vitek- 2 (Biomérieux).

Solution using and Media

Hi-Media, from (Mumbai- India) are provided the agar of Mueller-Hinton media. Three concentrations (100, 50 and 25%) of essential oils of Marjoran oil and Peppermint oil were provided by the (Zhengzhou Co. of Dongyao, Ltd. from China). Aztronam (ATM-30 µg/ml), Amoxicilin (AMC-30 µg/ml), Methicillin (ME-5 µg/ml), Ceftriaxane (CRO-30 µg/ml), and Trimethoprim (TMP-5 µg/ml) were some of the many antibiotic disks that were acquired from (Bioanalyse, Turkey).

Antibiotic susceptibility test

The Kirby-Bauer disk diffusion methods were employed to assess antibiotic susceptibility in accordance with CLSI 2021 criteria. Four *S. aureus* isolates were tested for drug resistance using five different antibiotics: azitronam, amoxidlin, methicillin, ceftriaxone, and trimethoprim (chosen at randomly from the total bacterial isolate). The percentage of resistant isolates among all discovered bacterial isolates served as an expression for the findings. MDR is characterized as having three or more antimicrobial classes of resistance. MDR is represented by the bacterial strains.

Antibacterial Properties of Eos

Biochemical test	<i>Staph. aureus</i> (n=35)
Catalase test	+
Oxidase test	-
Coagulase test	+
Clumping factor	+
Hemolysis	Beta
Mannitol salt agar	+
Bacitracin	-
Lactose fermentation	+
Mannitol fermentation	+
Ribose fermentation	+

All 35 isolates are *Staph. aureus* according to the table 2.



Figure 1: *Staphylococcus aureus* growth on mannitol salt agar as a selective and differential growth

On nutritional agar slants, oral pathogens collected from infections of the oral cavity were tested for the antibacterial activities of EOs. While evaluating antibiotic activity, the guidelines of the Clinical and Laboratory Standards Institute were adhered to [22]. Using a disk diffusion experiment, triplicates were utilized in EOs concentration dilutions of 100% and 50% in solvent to investigate the antibiotic sensitivity and antibacterial activity of EOs [*Origanu mmajoruna* (Marjoran oil) and *Mentha piperita* (Peppermint oil)] against the study microorganisms. The isolates underwent a 15-minute at-room-temperature incubation followed by an overnight incubation at 37 °C. The inhibition zone encircling the well was visible following an incubation period. The breadth of the inhibitory zone can measure using a digital Vernier calipers. [23]

3. Results and their Discussion

All of 35 isolates *Staph. aureus* that isolated from both male and female (15-45 year), identified using standard of microbiological techniques, and all isolates were identified using the Vitek-2 compact system, Table (1).

Gender (5-45 Y)	No. of specimens	Percentages %
Male	20	57.14%
Female	15	42.85%
Total	35	100%

Staph. aureus was isolated and identified by biochemical tests with different morphological, physiological and biochemical characteristic as in Table (2).

medium (ferment mannitol, product acidic and turn the phenol red to yellow on the agar)

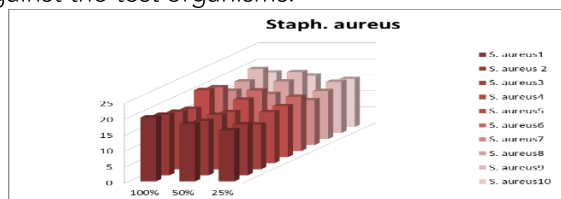
A modified Kirby- Bauer disc diffusion technique was used to examine each *Staph aureus* susceptibility to antibiotics. Selective antibiotics are frequently used to treat infections caused by *Staph. aureus* to show their impact on different populations, as seen in the Table- 3 . [24, 28]

Bacterial Isolate	ATM-30 µg/ml	AMC-30 µg/ml	ME-5 µg/ml	CRO-30 µg/ml	TMP-5 µg/ml
<i>Staph. aureus</i> 1	(R)	(R)	(R)	(R)	(R)
<i>Staph. aureus</i> 2	(R)	(R)	(R)	(S)	(S)
<i>Staph. aureus</i> 3	(R)	(R)	(R)	(R)	(S)
<i>Staph. aureus</i> 4	(R)	(R)	(R)	(R)	(R)
<i>Staph. aureus</i> 5	(R)	(R)	(R)	(S)	(S)
<i>Staph. aureus</i> 6	(R)	(R)	(R)	(S)	(S)
<i>Staph. aureus</i> 7	(R)	(R)	(R)	(R)	(S)
<i>Staph. aureus</i> 8	(R)	(R)	(R)	(R)	(S)
<i>Staph. aureus</i> 9	(R)	(R)	(R)	(S)	(R)
<i>Staph. aureus</i> 10	(R)	(R)	(R)	(R)	(R)

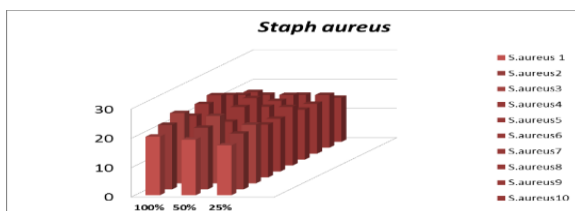
ATM= Aztronam, AMC= Amoxicilin, ME= Methicillin, CRO= Ceftriaxane and TMP= Trimethoprim
The US Food and Drug Administration (FDA), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the Clinical Laboratory Standards Institute (CLSI) documentation and breakpoints were used to produce lists of antibiotic susceptibility tests. Five antibiotics were selected to examine the isolated bacteria's susceptibility after the sample bacteria's antibiotic sensitivity was investigated ATM= Aztronam, AMC= Amoxicilin, ME= Methicillin, CRO= Ceftriaxane and TMP= Trimethoprim, as in Table-3.

PEO as Antibacterial

EOs evaluated their activity against robust broad-spectrum of multidrug resistant bacteria. Different antibiotics' effects on bacterial isolates were compared. As can be seen from the results in Figures 1 and 2, not all identified bacteria under study were successfully combatted by the medicines used (antibiotics). As EOs levels fell, EOs (Marjoran oil and Peppermint oil) showed a marked decrease in inhibitory zone width that even surpassed the effects of several antibiotics. The highest inhibition zone are 22 mm against *S. aureus* NO. 6, Figure (3) by the action of Peppermint oil was seen at a con. of 100% against the test organisms.



Figure(2): Antibacterial action of Marjoran oil on 10- isolates of *S. aureus*



Figure(3): Antibacterial action of Peppermint oil on 10- isolates of *S. aureus*

We shown in the figures (3) that all of the bacterial isolates included in the study are antibiotic resistant. By using the disc diffusion method in comparison to *S. aeruse* isolates, the antibacterial activity of the studied essential oils employed in our investigation was evaluated. Our research revealed that the essential oils employed in the study had varying degrees of antibacterial activity when compared to pathogenic microorganisms. It was also demonstrated that Peppermint oil has more antibacterial properties than Marjoran oil.

Elaissi et al., [25] examined the relationship between chemical composition and antibacterial activity in numerous *Mentha piperita* species. Limonene, 1,8-cineole, -pinene spathulenol and p-cymene were found to be the primary chemical constituents. *S. aureus* and *E. coli* had the strongest antibacterial activity, and a link between the concentrations of active components in essential oils

and their antibacterial effects was found. The study by Vaghasiya and Chanda showed comparable outcomes that are consistent with our findings. [26]. *Citrobacter freundii* was shown to be the most vulnerable bacteria to the antibiotic and antifungal effects of Peppermint essential oil, whereas *Proteus vulgaris* was found to be the most resistant. The hydrophobicity of essential oils and their components is a critical characteristic that enables them to partition the lipids of bacterial cell membrane and mitochondria, causing cell structures to be disrupted and made more permeable. [27].

4. Conclusion

The results of this study show that EOs has a substantial inhibitory and antibacterial effect on certain pathogenic isolates of bacteria from oral cavity infection. Because of PEs' potent capacity to prevent bacterial development, it is strongly advised that PEs be used as a less expensive alternative antibacterial agent, particularly with materials used to produce toothpastes and mouthwash. Despite the fact that a lot of essential oils have strong antibacterial properties, certain oils' specific antibacterial properties do not offer a comprehensive picture of how to use essential oils to prevent the spread of infectious illnesses. To find out if they are effective at preventing the growth of bacteria, fungus, parasites, and viruses, more research is required.

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