RESEARCH ARTICLE

Study on Essential Oils having Antimicrobial Activity Against *Staphylococcus aureus* and *Staphylococcus epidermidis* Isolated from Oral Cavity Infection

Ali A. Fadhil[1], Noora M. Hameed[2]*, Zainab H. Ridha[3], Ola A. Mahdi[4], Fadhil F. Sead[5], Doaa A. Hamad[6], Ayat H. Adhab[7]

[1]*Department of Medical Lab Techniques, College of Medical Technology, Al-farahidi University, Baghdad, Iraq*
[2]*Department of Anesthesia Techniques, Al–Nisour University College, Baghdad, Iraq*
[3]*Al-Manara College for Medical Sciences, Amarah, Iraq*
[4] *Department of Anesthesia Techniques, Al-Mustaqbal University College, Babylon, Iraq*
[5]*College of Dentistry, the Islamic University, Najaf, Iraq*
[6] *Department of Nursing, Hilla University College, Babylon, Iraq*
[7]*Department of Pharmacy, Al-Zahrawi University College, Karbala, Iraq*

Received: 14th February, 2022; Revised: 13th April, 2022; Accepted: 27th May, 2022; Available Online: 25th June, 2022

ABSTRACT

Background: *Staphylococcus sp.* is the most dominant human pathogen, responsible for a variety of chronic and severe infections. There is mounting evidence that persisters are associated with treatment failure and relapse of persistent infections. While some essential oils were reported to have antimicrobial activity against growing *Staphylococcus sp.*, the activity of essential oils against *Staphylococcus sp.* enriched in persisters has not been investigated.

Materials and Methods: In this study, we collected 100 isolates of *Staphylococcus sp.* from the oral cavity of patient infections that are fully characterized & identified by standard bacteriological procedures, using a biochemical test and VITEK2 system compact. Susceptibility tests for different antibiotics are used to evaluate the medical device reporting (MDR) of *S. aureus* and *S. epidermidis* and evaluate the anti-bacterial properties of essential oil to the same MDR under study. Different concentrations of essential oil from a source like medical plants and herbs are used as anti-bacterial.

Results: These essential oils expressed antimicrobial activity against clinical isolates of MDR *S. aureus* and *S. epidermidis* compared with antibiotics.

Conclusion: It is highly recommended to use EOs as an economical alternative anti-bacterial agent, especially with materials that make toothpaste and mouthwash, because of their effective ability to inhibit bacterial growth.

Keywords: Antimicrobial, Essential oil, MDR, *Staphylococcus* sp.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.2.17

How to cite this article: Fadhil AA, Hameed NM, Ridha ZH, Mahdi OA, Sead FF, Hamad DA, Adhab AH. Study on Essential Oils having Antimicrobial Activity Against *Staphylococcus aureus* and *Staphylococcus epidermidis* Isolated from Oral Cavity Infection. International Journal of Pharmaceutical Quality Assurance. 2022;13(2):178-181.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The human oral cavity acts as a growth medium for several types of dental infections which occur in pathogenic microorganisms due to its moistures, patient's oral cavity such as tooth decay, periodontal temperature, and nutrient content such as lipid, disease, dental ache, dental plaque, dentin carbohydrate and protein.[1] There are hypersensitivity, dental abscess, dental calculus, hyperdontia, malocclusion, acid erosion, acute necrotizing ulcerative gingivitis, dental fluorosis, tooth impaction, etc. *Staphylococcus aureus* is a putative pathogen of many oral diseases, such as oral mucositis, periodontitis, peri-implantitis, endodontic infections, and even dental caries.[2-5]

Staphylococcus sp. is a Gram-positive, non-motile, non-spore-forming grape-like cluster and the most important coagulase-positive pathogen from staphylococci due to a combination of toxic mediated virulence, invasiveness, and antibiotic resistance.[6] Some strains of *S. aureus* have developed drug resistance. Methicillin-resistant *S. aureus* (MRSA),[7] is the strain of *S. aureus* that obtained resistance to beta-lactam antibiotics, which incorporate such as penicillin, amoxicillin, ampicillin, methicillin, oxacillin, cephalosporins,

**Author for Correspondence: noora.anesth@nuc.edu.iq*

etc.[8] The propensity of *S. aureus* to acquire antibiotic resistance has prompted the worldwide dissemination of clones expressing various antimicrobial resistance. Several hospital and nonhospital bacterial diseases are caused due to methicillin-resistant *Staphylococcus aureus* (MRSA) strains and sometimes lead to death.[9-11]

An important role of essential oils in nature is the protection of plants by acting as antifungal, anti-bacterial, antiviral, and insecticidal agents and also protection against herbivores by reducing the appetite of herbivores for plants with such properties. Health and human services public health services have recognized essential oils as safe substances, and some essential oils contain a compound that can be used as anti-bacterial additives.[12] Efficacy of essential oils (EOS) has been reported in several studies against pathogens food industry.[13,14]

The widespread application of anti-bacterial agents has increased bacterial resistance levels and severity,[15,16] which in turn demands greater aggravating bacterial resistance in a vicious cycle.[17] Thus, it is essential to select appropriate anti-bacterial agents to avoid increased patient mortality[18] and the economic burden on patients and society.[19] However, In the United States, irrational application of anti-bacterial agents has also been observed.[20] These deficiencies in the rational use of anti-bacterial agents are often accompanied by adverse consequences, including high mortality[21] and increased medical costs.[19]

This study aims to isolate and identification of bacteria contamination in the oral cavity and use essential oil as an anti-bacterial to prove their capability to reduce bacteria to attempt to use these oils to reduce human infection by bacteria by mixing with toothpaste and mouth wash.

MATERIALS AND METHODS

Collection of Samples

The 75 specimens were collected from the different samples of the oral cavity. Bacterial samples were collected, including 40 of *S. aureus* (N = 40) and 35 of *S. epidermites* (N = 35). Samples of bacteria were collected from different patients in Al-Hilla city. Standard microbiological techniques were used to isolate and purify these samples for bacterial species identification, including utilizing various culture media such as blood ager and Macconkey agar plates for 24–48 hours at 37°C. Vitek 2 compact system (Biomérieux) verified all of the isolates.

Solution and Media

Mueller-Hinton agar and Mueller-Hinton media were obtained from Hi-Media, Mumbai, India. Essential oil of Eucalyptus oil and Nigella sativa oil with two concentrations (50 and 100%) were supplied from (Zhengzhou Dongyao Co., Ltd., China). Different antibiotic disks Aztreonam (ATM-30), Amoxicillin (AMC-30), Methicillin (ME-5), Ceftriaxone (CRO-30), and Trimethoprim (TMP-5) were purchased from (Bioanalyse, Turkey).

Antibiotic Susceptibility Test

The Clinical Laboratory Standard Institute (CLSI) recommendations of 2016 were used to evaluate antibiotic susceptibility using the Kirby-Bauer disk diffusion techniques. Aztreonam, Amoxicillin, Methicillin, Ceftriaxone, and Trimethoprim were among the five antibiotics used to evaluate the drug resistance of three *S. aureus* and *S. epidermidis* isolates (chosen at random from the total isolate). The results were expressed as the rate of resistant strains among all detected bacterial strains. MDR is defined as resistance to three or more antimicrobial classes. The bacterial strains represent MDR.

Anti-bacterial Properties of EOs

The anti-bacterial properties of EOs were evaluated against certain human pathogens that were obtained from oral cavity infections kept on nutritional agar slants. The Clinical and Laboratory Standards Institute's recommendations were followed while testing antimicrobial activity.[22] Antibiotic sensitivity and EOs (Eucalyptus oil and Nigella sativa oil) against bacteria under study are tested using a disk diffusion assay, with triplicates used in dilutions of concentration of EOs (50 and 100%) in a solvent. In the first step, the isolates were incubated for 15 minutes at room temperature, then incubated at 37°C overnight. After a time of incubation, the inhibition zone was seen surrounding the well. A digital Vernier caliper was used to determine the width of the inhibitory zone.[23]

RESULTS AND DISCUSSION

A total of 75 isolates of *S. aureus* and *S. epidermidis* were isolated and identified by standard microbiological procedure, then by VITEK 2. The rate of *S. aureus* from the oral cavity was 40 (53.33%), and the rate of *S. epidermidis* from the oral cavity was 35 (46.66%). All isolates were confirmed by Vitek 2 compact system (Biomérieux), Table 1.

Antibiotic sensitivity testing was done on each kind of bacteria using a modified Kirby-Bauer disc diffusion method. To demonstrate their effect on distinct groups, selective antibiotics are often employed against *S. aureus* and *S. epidermidis* infection, as indicated in the Figures 1 to 4.[24]

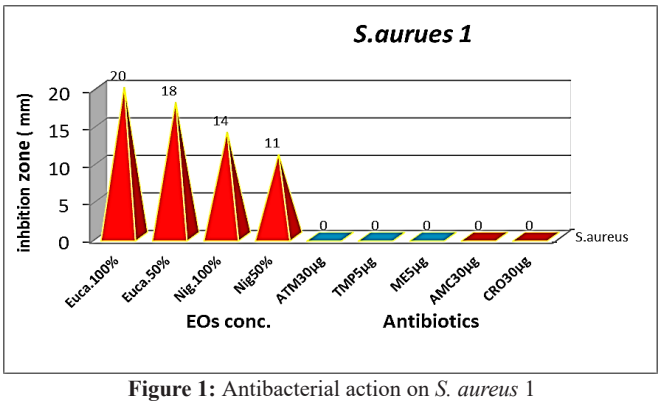

Figure 1: Antibacterial action on *S. aureus* 1

Table 1: Distribution of *S. aureus* and *S. epidermidis* from the different clinical samples

Source of Isolate	*No. of specimens*	*Percentages %*
S. aureus	40	53.33%
S. epidermidis	35	46.66%
Total	75	100%

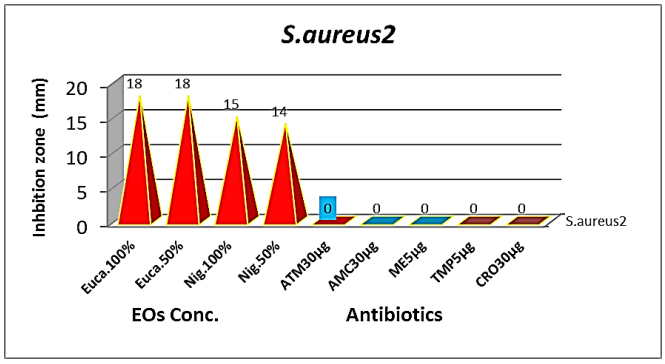

Figure 2: Antibacterial action on *S.aureus 2*

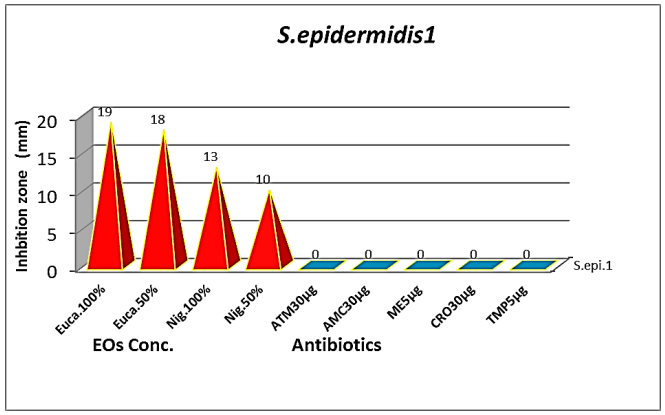

Figure 3: Antibacterial action on *S. epidermidis* 1

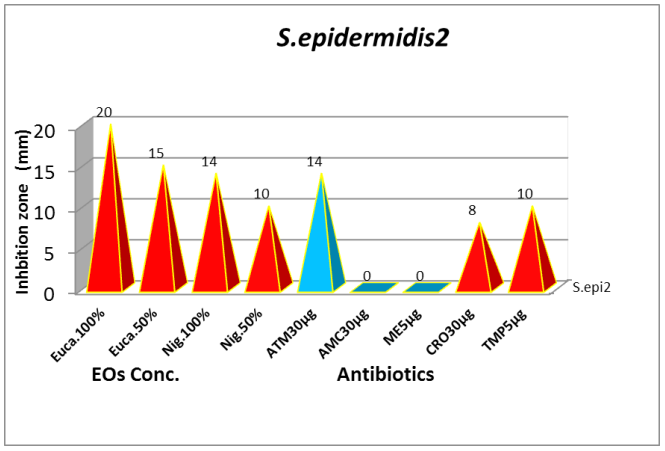

Figure 4: Anti-bacterial action of *S.epidermidis* 2

Anti-bacterial Activity of EO

EOs shows that powerful broad-spectrum anti-bacterial activity against multi-drug bacteria is tested. The effects of different antibiotics on bacterial isolates were compared. The result in Figures 1 to 4, showed that the selected antibiotics were not effective against all isolated bacteria under study. EOs demonstrated a distinct inhibitory zone width decrease as EOs concentration dropped, which even outperformed the action of certain antibiotics. The greatest zone of inhibition against the test organisms was found at a con. of 100%; maximum zone of inhibition of 20 mm appeared against *S.aureus* 1 Figure 1 and *S.epidermitdis* 2 Figure 4 by the action of Eucalyptus oil.

From the figures, we showed that all bacterial isolates are resistant to antibiotics used under study. The assessment of the antimicrobial activity in essential oils used in our study was determined by the disc diffusion method compared to *S. aureus* and *S. epidermidis* isolates. Our study showed that all essential oils that were used displayed a differing level of antimicrobial activity compared to pathogenic bacteria. Also showed that Eucalyptus oil has activity as anti-bacterial more than Nigella sativa oil.

Elaissi *et al.*[25] investigated the anti-bacterial activity of several Eucalyptus species and their correlation with chemical composition. The main chemical compounds were determined to be 1,8-cineole, spathulenol, α-pinene, p-cymene, and limonene. The most potent anti-bacterial activity was recorded against *S. aureus* and *E. coli*, while the correlation between the levels of active compounds in essential oil and the anti-bacterial activities was noticed. Similar results, which are by our findings, were demonstrated in the study of Vaghasiya and Chanda.[26] The authors investigated the antimicrobial and antifungal properties of eucalyptus essential oil and concluded that the most susceptible bacterium was *Citrobacter freundii*, while the most resistant was *Proteus vulgaris*.

Essential oils and their components have an important characteristic for their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable.[27]

CONCLUSION

EOs has a significant inhibitory and anti-bacterial impact on selected pathogenic bacterial isolates from oral cavity infection, according to the findings of this investigation. It is highly recommended to use EOs as an economic alternative anti-bacterial agent, especially with materials that make toothpaste and mouthwash because of their effective ability to inhibit bacterial growth. Although a certain number of essential oils show good anti-bacterial activity, some oils' narrow anti-bacterial activities do not provide a complete picture of the usage of essential oil against the occurrence of infectious diseases. Nevertheless, further study is necessary to investigate their efficacy in inhibiting the growth of bacteria, fungi, parasites, and viruses.

REFERENCES

1. Mohapatra SB, Pattnaik M, Ray P. Microbial association of dental caries. Asian Journal of Experimental Biological Sciences. 2012.
2. Gibson J, Wray D, Bagg J. Oral staphylococcal mucositis: A new clinical entity in orofacial granulomatosis and Crohn's disease. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2000;89(2):171-176.
3. Heitz-Mayfield LJ, Lang NP. Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. Periodontology. 2010;53(1):167-181.
4. Poeschl PW, Crepaz V, Russmueller G, Seemann R, Hirschl AM, Ewers R. Endodontic pathogens causing deep neck space infections: clinical impact of different sampling techniques and antibiotic susceptibility. Journal of Endodontics. 2011;37(9):1201-1205.

5. Passariello C, Puttini M, Iebba V, Pera P, Gigola P. Influence of oral conditions on colonization by highly toxigenic Staphylococcus aureus strains. Oral Diseases. 2012;18(4):402-409.
6. Loir LY, Baron F, Gautier M. Staphylococcus aureus and food poisoning. Genetics and Molecular Research Journal. 2003;2(1):63-76.
7. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: A multicentre study. Indian Journal of Medical Microbiology. 2006;24(1):34-38.
8. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clinical Microbiology Reviews. 2010;23(3):616-687.
9. Bannerman T, Peacock S. Staphylococcus, Micrococcus, and other catalase positive cocci. In: Murray, P., Baron, E., Jorgensen, J., Landry, M., Pfaller, M. (Eds.), Manual of Clinical Microbiology 9th ed. ASM Press, Washington, DC, 2007. pp. 390-411.
10. Moussa IM, Al-Qahtani AA, Gassem MA, Ashgan MH, Ismail DK, Ghazy AI, Shibl AM. Pulsed-field gel electrophoresis (PFGE) as an epidemiological marker for typing of methicillin-resistant Staphylococcus aureus recovered from King Saudi Arabia (KSA). African Journal of Microbiology Research. 2011;5(12):1492-1499.
11. Peters PJ, Brooks JT, McAllister SK, Limbago B, Lowery HK, Fosheim G, Guest JL, Gorwitz RJ, Bethea M, Hageman J, Mindley R, McDougal LK, Rimland D. Methicillin-resistant Staphylococcus aureus colonization of the groin and risk for clinical infection among HIV-infected adults. Emerging Infectious Diseases. 2013;19(4):623-629.
12. Stefanakis MK, Touloupakis E, Anastasopoulous E, Ghanotakis D, Katerinopoulous HE, Makirdis P. Anti-bacterial activity of essential oils from plants of the genus Origanum. Food control. 2013;34:539-546.
13. McCarthy RR, Mooij MJ, Reen FJ. *et al*., A new regulator of pathogenicity (bvIR) is required for full virulence and light microcolony formation in Pseudomonas aeruginosa. Microbiology. 2014;160:1488-1500.
14. Dou Y, Zhang Q, Liao ZJ. Investigation on the drug resistance of Pseudomonas aeruginosa in our burn ward in the past 11 years. Zhonghua Shao Shang za zhi= Zhonghua Shaoshang Zazhi= Chinese Journal of Burns. 2004 Feb 1;20(1):6-9.
15. Gallini A, Degris E, Desplas M, Bourrel R, Archambaud M, Montastruc JL, Lapeyre-Mestre M, Sommet A. Influence of fluoroquinolone consumption in inpatients and outpatients on ciprofloxacin-resistant Escherichia coli in a university hospital. Journal of antimicrobial chemotherapy. 2010 Dec 1;65(12):2650-2657.
16. Vernaz N, Huttner B, Muscionico D, Salomon JL, Bonnabry P, López-Lozano JM, Beyaert A, Schrenzel J, Harbarth S. Modelling the impact of antibiotic use on antibiotic-resistant Escherichia coli using population-based data from a large hospital and its surrounding community. Journal of Antimicrobial Chemotherapy. 2011 Apr 1;66(4):928-935.
17. Hsu LY, Tan TY, Tam VH, Kwa A, Fisher DA, Koh TH. Surveillance and correlation of antibiotic prescription and resistance of Gram-negative bacteria in Singaporean hospitals. Antimicrobial agents and chemotherapy. 2010 Mar;54(3):1173-1178.
18. Zillberberg M.D. Shorr A.F., Mice KST, *et al*., Multi-drug resistance, inapproprirate initial antibiotic therapy and mortality in Gram negative severe sepsis and septic shock: a retrospective cohort study Crit Care. 2014;18:596.
19. Lipsky BA, Napolitano LM, Moran GJ, Vo L, Nicholson S, Chen S, Boulanger L, Kim M. Economic outcomes of inappropriate initial antibiotic treatment for complicated skin and soft tissue infections: a multicenter prospective observational study. Diagnostic Microbiology and Infectious Disease. 2014 Jun 1;79(2):266-272.
20. Meeker D, Linder JA, Fox CR, Friedberg MW, Persell SD, Goldstein NJ, Knight TK, Hay JW, Doctor JN. Effect of behavioral interventions on inappropriate antibiotic prescribing among primary care practices: a randomized clinical trial. Jama. 2016 Feb 9;315(6):562-570.
21. Marquet K, Leisenborgs A, Bergs J. *et al*. Incidence and outcome of in appropriate in hospital empiric antibiotics for severe infection : a systematic review and meta analysis. Crit Care. 2015;19:63.
22. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8. Texas: Clinical and Laboratory Standards Institute; 2012.
23. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
24. Hamzah AF, Abd-Alsahib WH, Mahdi SS. Isolation and identification new bacterial strains isolated from different sources of Al-Rafidiyah oil field in Iraq. Catrina: The International Journal of Environmental Sciences. 2020 Jun 1;21(1):15-22.
25. Elaissi A, Rouis Z, Mabrouk S, Salah KBH, Aouni M, Khouja ML, Farhat F, Chemli R, Harzallah-Skhiri F. Correlation Between Chemical Composition and Anti-bacterial Activity of Essential Oils from Fifteen Eucalyptus Species Growing in the Korbous and Jbel Abderrahman Arboreta (North East Tunisia). Molecules. 2012;17:3044–3057.
26. Vaghasiya Y, Nair R, Chanda S. Antibacterial and preliminary phytochemical and physico-chemical analysis of Eucalyptus citriodora Hk leaf. Natural product research. 2008 Jun 15;22(9):754-762.
27. Abdulazeem L, Alrubaei HA, Al-Alaq FT, Al-Mawlah YH, Obed ASH. The Effect of Essential oils on Multi-drugs Resistant *Pseudomonas aeruginosa* (MDRPA). Annals of Tropical Medicine & Public Health SPe118, 2019;22(I).