Research Article

Antibacterial Activity of Tea Tree Oil against Clinical Isolates of *Staphylococcus aureus*

Shabana Bowsiya*[1], Dr. Naveen Kumar[1]

1. BDS Student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Allied Technical Sciences, India.
2. Senior Lecturer, Department of Orthodontics, Saveetha Dental College, Saveetha University, Chennai, India.

***Corresponding author's E-mail:** shabanafouziya97@yahoo.com

Received: 18-12-2019; **Revised:** 24-01-2020; **Accepted:** 02-02-2020.

ABSTRACT

Staphylococcus aureus is one of the important bacterial pathogen causing a wide spectrum of infections. Many studies have been conducted to explain the structures and pathogenic mechanisms by which *S. aureus* is able to cause serious infections. Our present study was indented to determine its antibacterial activity of tea tree oil against *S. aureus*. Anti-bacterial activity of tea tree oil was tested against *S. aureus* isolates by minimum inhibitory concentration method. Media containing various concentrations of essential oil were poured over the sterile petri dishes and allowed to dry. Media without essential oil was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for overnight. The lowest concentration of the essential oil that completely inhibited the growth of isolates was considered as MIC. The MIC of tea tree oil was appeared to be 0.125% for *S. aureus*. The tea tree oil is found to have antibacterial activity against *S. aureus*. However, its irritant properties have been evaluated before it is formulated for medicinal purpose.

Keywords: *Staphylococcus aureus*, MIC, tea tree oil, *Melaleuca alternifolia*, essential oils

INTRODUCTION

Staphylococcus aureus [*S. aureus*] is one of the important bacterial pathogen causing a wide spectrum of infections.[1] Many studies have been conducted to explain the structures and pathogenic mechanisms by which S. aureus is able to cause serious infections.[2] The ability of S. aureus to produce biofilm enables this organism to withstand the host immune response and is considered to be the cause of many chronic or persistent infections, as the biofilm creation protects bacteria from phagocytosis and antimicrobial agents.[3] Another concern related to this pathogen is increasing resistance to oxacillin and many other antibiotics, but also circulation of multidrug resistant isolates within the hospital environment. [4] Staphylococcal pathogenesis is multifactorial, involving a combination of adherence and biofilm formation. [5]

Many complementary and alternative medicines have increasingly gaining importance in recent decades. Tea tree oil is known to possess antibacterial[6-10], antifungal[11-17], anti-viral[18] and anti- protozoal properties.[19, 20, 21] The crushed leaves of tea tree oil was earlier used for treating coughs and cold and also for skin ailments. [22, 23] Tea tree oil has various application in dentistry. [24] It is primary used for its antimicrobial and anti-inflammatory properties.[25, 26] It has been used for endodontic treatments. Studies reveal that tea tree oil is as effective as sodium hypochlorite.[27, 28] Tea tree oil, the volatile essential oil derived mainly from the Australian native plant Melaleuca alternifolia. It is extracted from its leaves by steam distillation yielding about 1.8%.[29] The active component in tea tree oil is terpinen-4-ol. [30] It is employed widely for its antimicrobial properties; Tea tree oil is used as the active ingredient in many topical formulations used to treat cutaneous infections. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments.[31] Most bacteria are susceptible to TTO at a concentration 1.0% or less, and at 2% commensal organisms such as *Enterococcus fecalis*, staphylococci are found to be susceptible. [32, 33] The antibacterial activity of TTO is assumed to be based on its hydrocarbons and lipophilicity [25]. Loss of intracellular material and the inability to maintain homeostasis, and inhibition of respiration following treatment with TTO are consistent with a mechanism of action involving the loss of function and membrane integrity. [31] Thus, our present study was indented to determine its antibacterial activity of tea tree oil against S. aureus.

MATERIALS AND METHODS

Bacterial isolates

A total of 20 clinical isolates of S. aureus were collected from different clinical specimens of patients attending Saveetha Medical Collage and hospital. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy medium and stored at 4ºC until further use.

Antibiotic susceptibility test

It was determined for the isolates to the following antibiotics such as penicillin, tetracycline, cotrimoxazole, erythromycin, clindamycin, ciprofloxacin and linezolid. These antibiotics were procured from Himedia, Mumbai. This was performed by Kirby-bauer disc diffusion method as per CLSI guidelines. [34]

Detection of antibacterial activity of tea tree oil against clinical isolates of S. aureus

Anti-bacterial activity of tea tree oil was tested against S. aureus isolates by minimum inhibitory concentration method. Mueller Hinton broth was supplemented with 0.002% [V/V] tween 80 [HiMedia, Mumbai] to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oil such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in Mueller Hinton Agar [MHA]. Media containing various concentrations of essential oil were poured over the sterile petri dishes and allowed to dry. Media without essential oil was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for overnight. The lowest concentration of the essential oil that completely inhibited the growth of isolates was considered as MIC. [35]

RESULTS AND DISCUSSION

Sample wise distribution of clinical isolates of S. aureus

Of 20 clinical isolates of S. aureus, 8/20 [40%] were obtained from pus, 6/20 [30%] were from wound, 4/20 [20%] and 2/20 [10%] were from blood and sputum respectively [Figure 1].

Antibiotic susceptibility pattern

We have observed a varied pattern of sensitivity among one S.aureus isolates. There was complete resistance observed for penicillin [100%], 9/20[45%] isolates were shown to the resistant to erythromycin, 6/20[30%] were to cotrimoxazole, 4/20[20%] were to linezolid followed by 3/20[15%] were resistant to ciprofloxacin and clindamycin respectively [Table 1] [Figure 2].

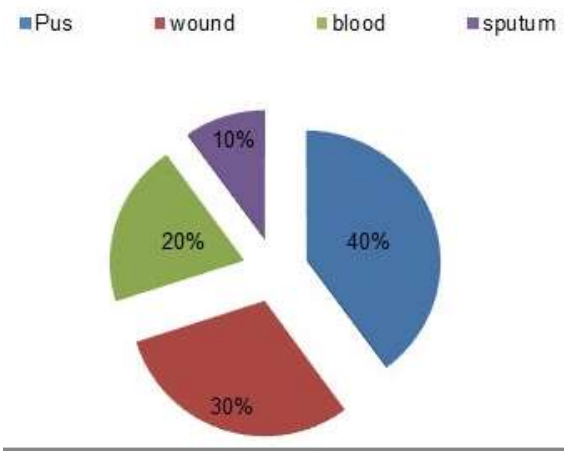

Figure 1: Pie chart showing the sample wise distribution of S. aureus

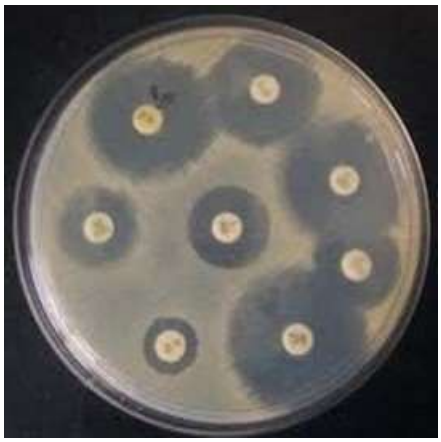

Figure 2: Representative picture showing antibiotic sensitivity pattern of *S. aureus*

Table 1: Results of antibiotic susceptibility pattern of *S. aureus*

Antibiotics	Sensitive [%]	Intermediate [%]	Resistant [%]
Penicillin	0	0	20[100]
Erythromycin	14[70]	4[20]	2[10]
Clindamycin	15[75]	2[10]	3[15]
Ciprofloxacin	9[45]	8[40]	3[15]
Tetracyclin	14[70]	4[20]	2[10]
Cotrimoxazole	10[50]	4[20]	6[30]
Linezolid	10[50]	6[30]	4[20]

Result of antibacterial activity of tea tree oil against clinical isolates of *S. aureus*

We have observed that, clinical isolates of S. aureus were inhibited from 0.125-1% of tea tree.

The MIC of tea tree oil was appeared to be 0.125% for S. aureus.

Dilutions of Tea tree oil	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
No. of organisms	0	0	6 [30%]	8 [40%]	3 [15%]	3 [15%]	0

Study conducted by Prakasam et al from Chennai in 2014 demonstrated that, Acinetobacter strains were inhibited from 0.06 to 0.25%, 0.25-1% and 0.125-1% for clove, peppermint and eucalyptus oils respectively. In clove oil, 14/50 [28%] isolates were inhibited at 0.06%, 25/50 [50%] at 0.125% and 11/50 [22%] at 0.25% of clove oil. In peppermint oil, 34/50 [68%] isolates were inhibited at 0.25%, 12/50 [24%] and 4/50 [8%] were at 0.5% and 1% concentrations of peppermint oil respectively. In eucalyptus oils, 10/50 [20%] isolates were inhibited at 0.125%, 18/50 [36%] at 0.25%, 16/50 [32%] and 6/50 [12%] were at 0.5% and 1% respectively. Thus, the MIC of clove oil was found to be 0.06%, 0.25% for peppermint oil and 0.125% for eucalyptus oil [31].

In contrast, in our study, we used tea tree oil against S. aureus isolates. 30% of isolates were inhibited at 0.125%, 40% were at 0.25%, 15% were at 0.5% and 15% were at 1% of essential oil. Thus, the MIC of tea tree oil against S. aureus was found to be 0.125%. In a study done by Penfold et al., tea tree oil was found to be 11 times more active that phenols [36]. Several studies done by Cox, et al [37] and Hada T. et al [38], TTO against S. aureus resulted in leakage of potassium ions and inhibited respiration. Further treatment with TTO also sensitized the cells to sodium chloride [39] and altered its morphology was also seen under electron microscopy [40]. On examining the effects of terpinen-4-ol, α-terpineol, and 1, 8-cineole on S. aureus it was found that none induce autolysis but cause leakage of 260-nm-light-absorbing material and to render cells susceptible to sodium chloride [39]. TTO is highly active during the stationary phase of S. aureus, and less sensitive during the growth phase [41]. Generally, antimicrobial agents cause gross membrane damage and cell lysis [42, 43] as this has been observed in various essential oils such as rosewood, thyme and oregano [44]. However, this mechanism of whole cell lysis is not observed in TTO. This explains the delayed lysis of S. aureus when treated with TTO [45]. Electron microscopy showed inability to lyse S. aureus by TTO but cause membrane damage and by appearance of mesosomes and loss of cytoplasmic contents. Similar mechanisms are seen on treatment with vancomycin [46], defensins [47] and betane [48]. TTO has found various applications in dentistry. 0.2% of TTO as mouthwash against two other active agents on the flora of 40 volunteers once daily for 7 days was found to reduce the bacterial load compared to placebo treatment[49]. Another study, comparing 2.5% TTO gel, 0.2% chlorhexidine gel and placebo gel suggested that TTO gel has significantly reduced gingival index and papillary bleeding index scores[50].

CONCLUSION

Antibiotic-resistant bacteria has become a major health concern worldwide. Particularly, *Staphylococcus aureus,* both methicillin-resistant and -sensitive, are of concern in their ability to cause difficult skin and underlying tissue infections [39]. Tea tree oil is found to have antibacterial activity against S. aureus. However, its irritant property has been evaluated before it is formulated for medicinal purpose. Due to the extended drug resistance in S. aureus, it can be used as an alternative medicine. Various mouth rinses are used for treating periodontal disease, but these have side effects [51]. Hence, using a natural product like tea tree oil is more advantageous. However, long term study on tea tree oil is necessary to have a clear understanding about its antimicrobial efficacy.

REFERENCES

1. Arciola CR, Baldassarri L, Montanaro L. Presence of ica A and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections. The Journal of Clinical Microbiology. 39[6], 2001, 2151–2156.
2. O'Neill E, Pozzi C, Houston P, Smyth D, Humphreys H, Robinson DA, O'Gara JP. 2007. Association between methicillin susceptibility and biofilm regulation in Staphylococcus aureus isolates from device-related infections. The Journal of Clinical Microbiology. 45[5], 2007, 1379–1388.
3. Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol. 3[12], 2005, 948–958.
4. Martín-López JV, Pérez-Roth E, Claverie-Martín F, Díez Gil O, Batista N, Morales M, Méndez-Alvarez S.. Detection of Staphylococcus aureus Clinical Isolates Harboring the ica Gene Cluster Needed for Biofilm Establishment. The Journal of Clinical Microbiology. 40[4], 2002, 1569–1570.
5. Klug D, Wallet F, Kacet S, Courcol RJ. Involvement of adherence and adhesion Staphylococcus epidermidis genes in pacemaker lead-associated infections. The Journal of Clinical Microbiology. 41 [7], 2003, 3348–3350.
6. Atkinson, N., and H. E. Brice. 1955. Antibacterial substances produced by flowering plants. Australas. J. Exp. Biol. 33, 1955, 547-554.
7. Beylier, M. F. Bacteriostatic activity of some Australian essential oils. Perfum. Flavourist, 4, 1979, 23-25.
8. Low, D., B. D. Rawal, and W. J. Griffin. Antibacterial action of the essential oils of some Australian Myrtaceae with special references to the activity of chromatographic fractions of oil of Eucalyptus citriodora. Planta Med. 26, 1974, 184-189.
9. Walsh, L. J., and J. Longstaff. The antimicrobial effects of an essential oil on selected oral pathogens. Periodontology. 8, 1987, 11-15.

10. Carson, C. F., and T. V. Riley. Antimicrobial activity of the essential oil of Melaleuca alternifolia. Lett. Appl. Microbiol. 16, 1993, 49-55.
11. Bassett, I. B., D. L. Pannowitz, and R. S. Barnetson. A comparative study of tea-tree oil versus benzoylperoxide in the treatment of acne. Med. J. Aust. 153, 1990, 455-458.
12. Christoph, F., P. M. Kaulfers, and E. Stahl-Biskup. A comparative study of the in vitro antimicrobial activity of tea tree oils with special reference to the activity of β-triketones. Planta Med. 66, 2000, 556-560.
13. D'Auria, F. D., L. Laino, V. Strippoli, M. Tecca, G. Salvatore, L. Battinelli, and G. Mazzanti. In vitro activity of tea tree oil against Candida albicans mycelial conversion and other pathogenic fungi. J. Chemother. 13, 2001, 377-383.
14. Griffin, S. G., J. L. Markham, and D. N. Leach. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Essent. Oil Res. 12, 2000, 249-255.
15. Nenoff, P., U.-F. Haustein, and W. Brandt. Antifungal activity of the essential oil of Melaleuca alternifolia [tea tree oil] against pathogenic fungi in vitro. Skin Pharmacol. 9, 1996, 388-394.
16. Rushton, R. T., N. W. Davis, J. C. Page, and C. A. Durkin. The effect of tea tree oil extract on the growth of fungi. Lower Extremity. 4, 1997, 113-116.
17. Southwell, I. A., A. J. Hayes, J. Markham, and D. N. Leach. The search for optimally bioactive Australian tea tree oil. Acta Hort. 344, 1993, 256-265.
18. Bishop, C. D. Antiviral activity of the essential oil of Melaleuca alternifolia [Maiden & Betche] Cheel [tea tree] against tobacco mosaic virus. J. Essent. Oil Res. 7, 1995, 641-644.
19. C. F. Carson, K. A. Hammer, T. V. Riley. Melaleuca alternifolia [Tea Tree] Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin. Microbiol. Rev. 19(1), 2006, 50-62.
20. Mikus, J., M. Harkenthal, D. Steverding, and J. Reichling. In vitro effect of essential oils and isolated mono- and sesquiterpenes on Leishmania major and Trypanosoma brucei. Planta Med. 66, 2000, 366-368.
21. Viollon, C., D. Mandin, and J. P. Chaumont. Activités antagonistes, in vitro, de quelques huiles essentielles et de composés naturels volatils vis á vis de la croissance de Trichomonas vaginalis. Fitoterapia. 67, 1996, 279-281.
22. Low, T. Bush medicine. Harper Collins Publishers, North Ryde, NSW, Australia, 1990.
23. Shemesh, A., and W. L. Mayo. Australian tea tree oil: a natural antiseptic and fungicidal agent. Aust. J. Pharm. 72, 1991, 802-803.
24. Prasanna Neelakantan, Nithya Jagannathan, Nabeel Nazar "Ethnopharmacological approach in Endodontic Treatment: A Focused Review", Int. J. Drug Dev. & Res., 3[4], Oct-Dec 2011, 68-77.
25. C. F. Carson, K. A. Hammer, T. V. Riley. Melaleuca alternifolia [Tea Tree] Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin. Microbiol. Rev. 19 (1), January 2006, 50-62.
26. Hart, P. H., C. Brand, C. F. Carson, T. V. Riley, R. H. Prager, and J. J. Finlay-Jones. Terpinen-4-ol, the main component of the essential oil of Melaleuca alternifolia [tea tree oil], suppresses inflammatory mediator production by activated human monocytes. Inflamm. Res. 49, 2000, 619-626.
27. Sadr Lahijani MS, RaoofKateb HR, Heady R .The effect of German chamomile [Marticariarecutitia L.] extract and tea tree [Melaleucaalternifolia L.] oil used as irrigants on removal of smear layer: a scanning electron microscopy study. IntEndod J, 39, 2006, 190-95.
28. Milind Parle, NitinBansal. Herbal medicines: Are they safe? – Natural Product Radiance 2006, 5, 6- 14.
29. Dhinahar.S, lakshmi.T. Role of botanicals as antimicrobial agents in management of dental infections – a review. International Journal of Pharma and Bio Sciences. 2 [4], 2011, 690-704.
30. Brophy, J. J., Davies, N. W., Southwell, I. A., Stiff, I. A. & Williams, L. R. Gas chromatographic quality control for oil of Melaleuca terpinen-4-ol type [Australian tea tree]. Journal of Agricultural and Food Chemistry. 37, 1989, 1330–5.
31. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia [tea tree] oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 19[1], 2006, 50-62.
32. Banes-Marshall, L., P. Cawley, and C. A. Phillips. In vitro activity of Melaleuca alternifolia [tea tree] oil against bacterial and Candida spp. isolates from clinical specimens. Br. J. Biomed. Sci. 58, 2001, 139-145.
33. Hammer, K. A., C. F. Carson, and T. V. Riley. Susceptibility of transient and commensal skin flora to the essential oil of Melaleuca alternifolia [tea tree oil]. Am. J. Infect. Control. 24, 1996, 186-189.
34. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Tests; Approved Standards; Doucement M2-A9, 9th ed., Vol 26. Wayne, PA, CLSI; 2015.
35. Gopinath Prakasam, Manju Bhashini, Lakshmipriya, Srivani Ramesh S. In-vitro antibacterial activity of some essential oils against clinical isolates of Acinetobacter baumannii. Indian J Med Microbiol. 32, 2014, 90-91.
36. Penfold, A. R., and R. Grant. The germicidal values of some Australian essential oils and their pure constituents, together with those for some essential oil isolates, and synthetics. Part III. J. R. Soc. New South Wales. 59, 1925, 346-349.
37. Cox, S. D., C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warmington, and S. G. Wyllie. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia [tea tree oil]. J. Appl. Microbiol. 88, 2000, 170-175.
38. Hada, T., Y. Inoue, A. Shiraishi, and H. Hamashima. 2003. Leakage of K+ ions from Staphylococcus aureus in response to tea tree oil. J. Microbiol. Methods. 53, 2003, 309-312.
39. Carson, C. F., B. J. Mee, and T. V. Riley. Mechanism of action of Melaleuca alternifolia [tea tree] oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. 48, 2002, 1914-1920.
40. Reichling, J., A. Weseler, U. Landvatter, and R. Saller. Bioactive essential oils used in phytomedicine as antiinfective

agents: Australian tea tree oil and manuka oil. Acta Phytotherapeutica. 1, 2002, 26-32.

41. Corre, J., J. J. Lucchini, G. M. Mercier, and A. Cremieux. Antibacterial activity of phenethyl alcohol and resulting membrane alterations. Res. Microbiol. 141, 1990, 483-497.
42. Denyer, S. P., and W. B. Hugo. 1991. Biocide-induced damage to the bacterial cytoplasmic membrane. Soc. Appl. Bacteriol. Tech. Ser. 27, 1991, 171-187.
43. Russell, A. D., A. Morris, and M. C. Allwood. Methods for assessing damage to bacteria induced by chemical and physical agents. Methods Microbiol. 8, 1973, 95-182.
44. Horne, D. S., M. Holm, C. Oberg, S. Chao, and D. G. Young. Antimicrobial effects of essential oils on Streptococcus pneumoniae. J. Essent. Oil Res. 13, 2001, 387-392.
45. Gilbert, P. The revival of microorganisms sublethally injured by chemical inhibitors. Soc Appl Bacteriol Symp Ser. 12, 1984, 175-197.
46. Sanyal, D., and D. Greenwood. An electron microscope study of glycopeptide antibiotic-resistant strains of Staphylococcus epidermidis. J. Med. Microbiol. 39,1993, 204-210
47. Shimoda, M., K. Ohki, Y. Shimamoto, and O. Kohashi. Morphology of defensin-treated Staphylococcus aureus. Infect. Immun. 63, 1995, 2886-2891.
48. Beveridge, E. G., I. Boyd, I. Dew, M. Haswell, and C. W. G. Lowe. Electron and light microscopy of damaged bacteria. Soc. Appl. Bact. Ser. 27, 1991, 135-153.
49. Groppo, F. C., J. C. Ramacciato, R. P. Simoes, F. M. Florio, and A. Sartoratto. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. Int. Dent. J. 52, 2002, 433-437.
50. Soukoulis, S., and R. Hirsch. The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. Aust. Dent. J. 49, 2004, 78-83.
51. Jaiganesh Ramamurthy, Lakshmi T. Pharmacological aspects of tea tree oil [tto] and its role in dentistry- a comprehensive review. International Journal of Pharma and Bio Sciences. 2(4), 2011, 572-581.

Source of Support: Nil, **Conflict of Interest:** None.