

In Vitro Comparison Of Antimicrobial Activity Of Nigella Sativa Methanolic Extract And Triclosan On Streptococcus Mutans

Belgaumi U.I¹, Patil SR², Datkhile K³, Durgawale P⁴, Shete A⁵, Gandhi J⁶

¹Associate Professor, Department of Oral Pathology and Microbiology, School of Dental Sciences, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

²Professor and Head of the Department, Department of Microbiology, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

³Department of Molecular Biology and Genetics, Krishna Vishwa Vidyapeeth, Karad, Maharashtra India, India.

⁴Department of Molecular Biology and Genetics, Krishna Vishwa Vidyapeeth, Karad, Maharashtra India, India.

⁵Department of Pharmacy, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

⁶Department of Pharmacy, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

Corresponding author

Dr. Uzma Iqbal Belgaumi

Associate Professor, Department of Oral Pathology and Microbiology, School of Dental Sciences, , Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

Email - uibelgaumi@gmail.com

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Abstract

Introduction –

The use of Triclosan, a common antimicrobial additive to dentifrices and many other consumer care products has been increasingly questioned for its safety in the recent years. In spite of a ban imposed on its use by Food and Drug Administration (FDA), it continued to feature as an active ingredient in some leading dentifrices for very long before it was finally withdrawn. During its use it gained widespread popularity due to its broad-spectrum nature and efficacy as a plaque control agent but eventually many global companies replaced Triclosan from their products with various alternative substances. However some of these alternatives too, pose similar risks like development of antibiotic resistance and potential toxicities.

Nigella sativa (*N. sativa*), which is known for its potent antimicrobial applications may be a reliable alternative in prevention of common oral diseases like dental caries and its role as triclosan alternative deserves further study.

Objectives –

The aim of the present research paper is evaluation and comparison of in vitro antimicrobial activity of *N. sativa* methanolic extract and Triclosan against *S. mutans* (MTCC 0497),

Material and methods- *N. sativa* extract was prepared using a Soxhlet Apparatus with Methanol as solvent. Antimicrobial efficacy of *N. sativa* extract and Triclosan was evaluated using MIC, MBC, Disk Diffusion and Time kill assay.

Results -

MIC for Triclosan and *N. sativa* extract against *S. mutans* was 0.4 µg/ml and 12.5 µg/ml, respectively. Triclosan showed no growth at 100µg/ml, however *N. sativa* extract showed no growth at a much lower value of 25 µg/ml. The maximum zone of inhibition seen

at 75µl/ml for Triclosan was 40±2mm and for *N. sativa* extract it was 28±1mm. Time kill assay for Triclosan at 60 minutes showed no growth while *N. sativa* extract did the same in half the time with no growth at 30 minutes.

Conclusion- The results of the present study using *N. sativa* extract showed promising results in comparison to the broad-spectrum antimicrobial, Triclosan, which has been banned due to its numerous adverse effects. *N. sativa* extract is a safe, effective alternative that may be used to replace this potentially toxic and harmful product from oral care products.

Keywords – *Nigella sativa*, Triclosan, Methanolic extracts, *Streptococcus mutans*

Introduction

Dental caries, is a microbial, biofilm-mediated disease that can result in a considerable global burden of disease.¹ Though dental caries may be a multispecies biofilm associated disease the role of *S. mutans* is indisputable due to its ability to synthesize extracellular polymers, the ability to produce acids and to thrive in low pH conditions.²

Chemical measures to prevent dental caries involve the use of various antimicrobial substances. Chlorhexidine, Fluorides and Triclosan are examples of such antimicrobials. Triclosan was a broad-spectrum antimicrobial that gained popularity due to its efficacy in controlling dental plaque and gingival inflammation.³

However due to the possibility of development of antimicrobial resistance, carcinogenicity and toxicity, FDA banned Triclosan in consumer products in 2016, however, it continued to remain in some oral care products until 2019.⁴ This ban on the marketing of triclosan and triclocarban in antibacterial soaps resulted in a decrease in the number of triclosan-containing products in the market with an associated increase in antibacterial alternatives (e.g., benzalkonium chloride, benzethonium chloride, chloroxylenol, chlorhexidine) and also in the cases of allergic and irritant contact dermatitis that resulted from the use of these products. Triclosan marketing ban instigated increased exposure to triclosan alternatives which do not improve effectiveness and may cause harm by contributing to antibiotic resistance and the development of allergic conditions.⁵

Dental ethnopharmacology is a growing branch that explores various plant extracts in prevention of oral diseases and provides additive advantages of being safer, effective, and preventing further development of antimicrobial resistance.⁶

The present article evaluates the antimicrobial activity of *N. sativa* extract and compares its efficacy to Triclosan so that the potential of its incorporation in developing safer and effective oral care products may be explored for future safer product development.

Materials and methods

Seeds of Indian *Nigella sativa* were obtained from the local market, screened manually, cleaned and dried. Triclosan and other chemicals were procured from Sigma Aldrich, India. A clinical strain of *Streptococcus mutans* (MTCC 0497) is obtained from Microbial Type Culture Collection (MTCC, *Nigella sativa*)

Extraction of essential oil

Dried seeds of *Nigella sativa* were ground to a fine powder and subjected to extraction at 70°C using methanol as solvent in a Soxhlet extractor. The extract was stored in an amber coloured airtight container at 4°C till further use.

Antimicrobial Activity

The in-vitro antimicrobial efficacy of *Nigella sativa* methanolic extract and Triclosan against *Streptococcus mutans* was analyzed through determination of the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) Disk Diffusion and Time kill assay using standard methodology. All microbiological investigations were done in triplicates. **Results** –

The MIC and MBC of *N. Sativa* extract against planktonic *S. mutans* (MTCC 0497) was determined as per the CLSI (Clinical and Laboratory Standards Institute) protocol. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented observable bacterial growth. By subculturing on agar plates, the MBC was established as the lowest concentration of the drug that yielded no colony growth. All antimicrobial investigations were performed in triplicates. The mean of MIC and MBC are mentioned in chart 1 and 2

Chart 1 - MIC of *N. Sativa* extract and Triclosan against *S. mutans*

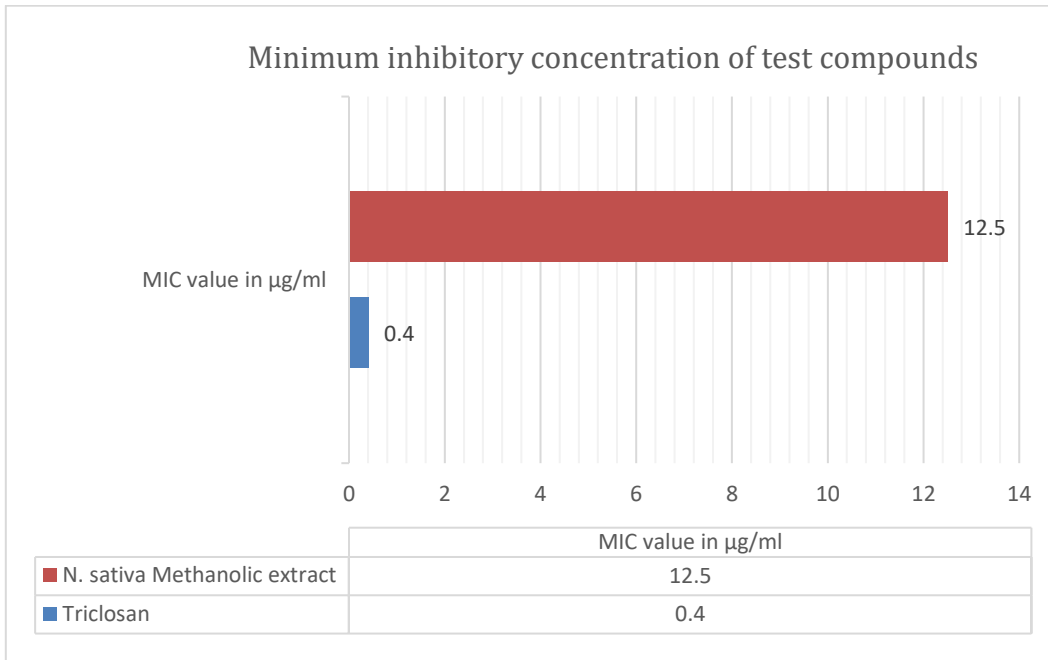
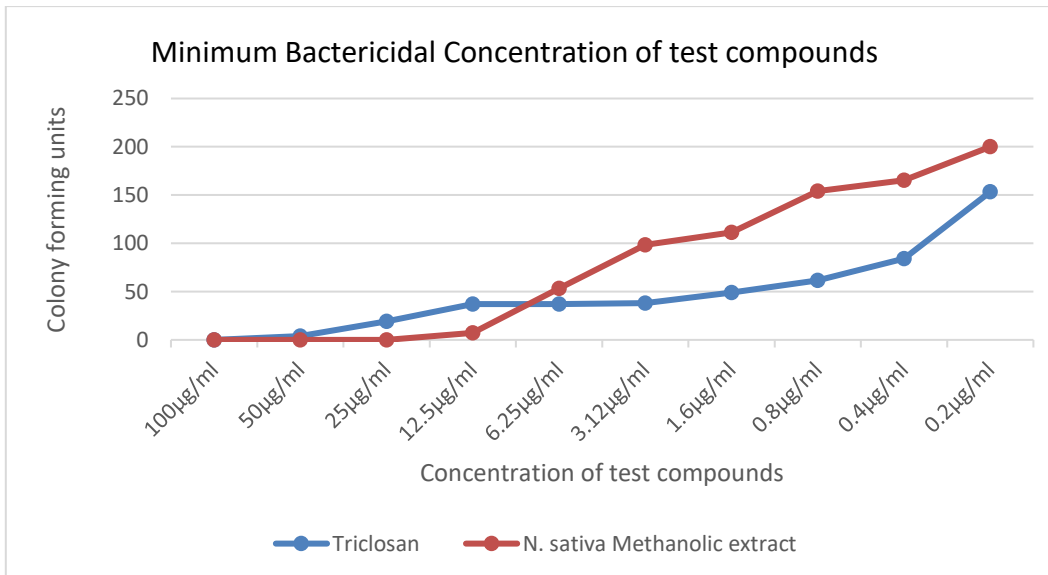
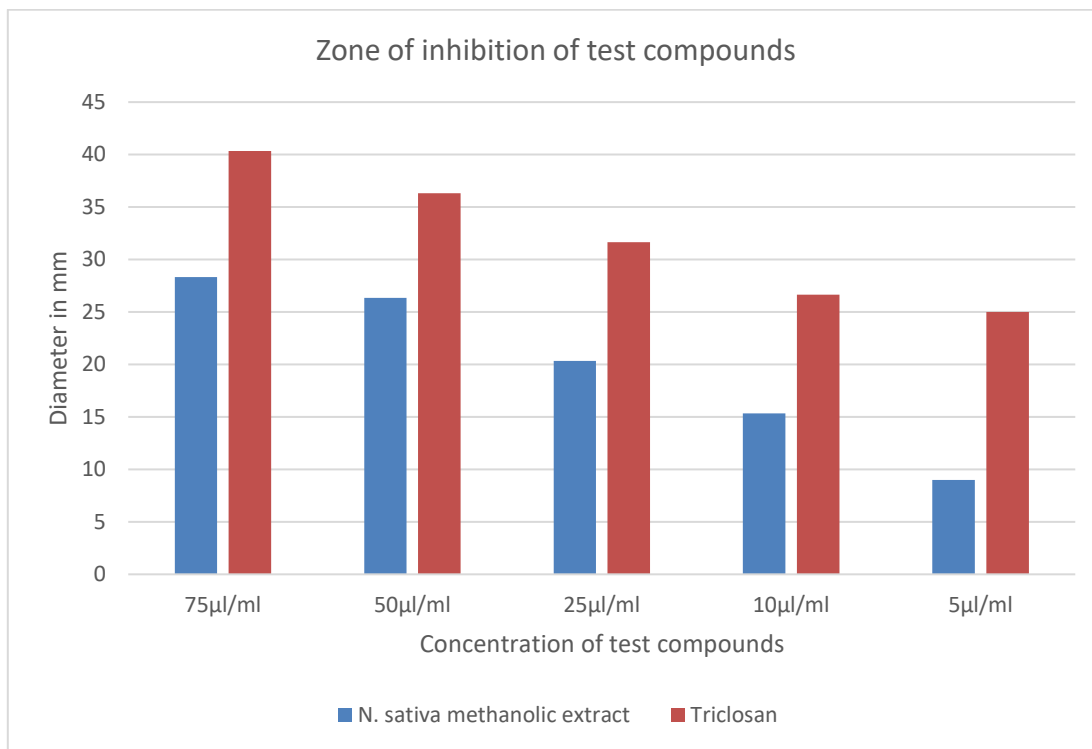


Chart 2- MBC of *N. Sativa* extract and Triclosan against *S. mutans*



We found that all concentrations of the *N. sativa* extract inhibited the growth of *S. mutans* (MTCC 0497), The inhibition zones for *S. mutans* increased from 9 mm to 28.33mm with increase in concentration from 5µg/mL to 75µg/mL Similarly the zone of inhibition of Triclosan also increased and was much larger than the zone of inhibition of *N. sativa* extract which is illustrated in chart 3.

Chart 3- Zone of inhibition N. sativa extract and Triclosan against S. mutans using well diffusion



Time kill curve was plotted at baseline, 5, 10, 30 and 60 mins to evaluate the time taken by the test compounds to inhibit the growth of *S. mutans* and is depicted in chart 4 and Fig 1.

Fig 1 - Time kill assay values of N. sativa extract and Triclosan against S. mutans

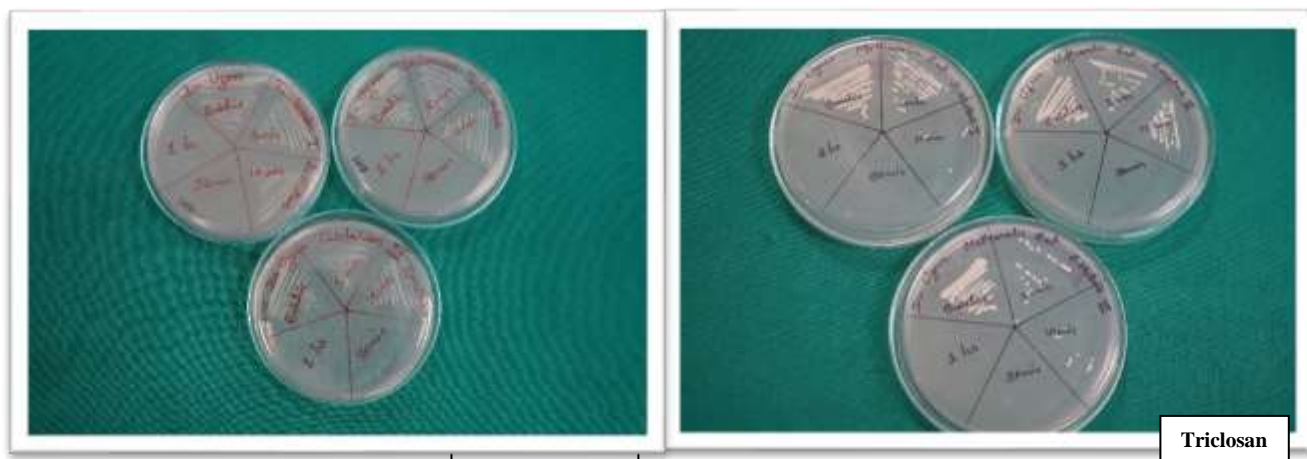
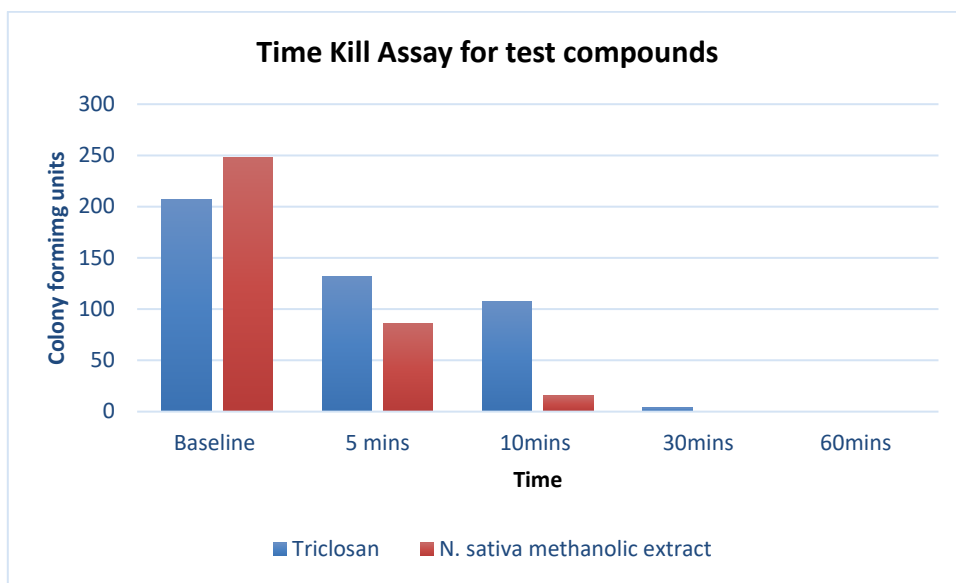


Chart 4 - Time kill assay values of N. sativa extract and Triclosan against S. mutans



Discussion -

Triclosan, a 5-chloro-2-(2,4-dichlorophenoxy) phenol, is a synthetic, broad spectrum, antimicrobial agent, is commonly used in numerous consumer products, medical devices, and household products. It is resistant to degradation and persists as an environmental pollutant. Evidence also suggests that it has been found to accumulate in human body fluids, has serious adverse effects on endocrine function especially on thyroid hormone, it can also cause oxidative injury resulting in decreased cellular lifespan and can be genotoxic and carcinogenic. It is also known to contribute to development of antibiotic resistance.^{7,8}

As awareness about safety and antimicrobial resistance are gaining a global voice it is time to reflect on safety of oral care products for individuals and the society at large. As oral health care providers, we regularly prescribe oral care products with active antimicrobials. It is vital that we evaluate our contribution to drug related toxicities, use of carcinogenic substances and possible emergence of superbugs. The present research was hence aimed at finding alternative to Triclosan that is safer and efficacious.

Antibiotic resistance poses a major public health threat. A short-term, in vitro exposure to triclosan increases triclosan resistance in susceptible *E. coli* isolates leading to development of (Multi Drug Resistance) MDR or cross-resistance phenotypes.⁹ In another study it was found that 0.2 mg/L of triclosan induced multi-drug resistance in wild-type *Escherichia coli* after a 30-day exposure.¹⁰

After the FDA ruling, triclosan and triclocarban were phased out in favor of alternative products like benzalkonium chloride, benzethonium chloride, chloroxylenol, and chlorhexidine gluconate. Though these substitutes are thought to be safe, their health impacts are poorly defined. In the quest for antibiotic alternatives, plant specific active compounds and secondary metabolites are gaining popularity in consumer products.¹¹

Herbal alternatives in this scenario are proving to be potent competitors due to their efficiency and safety. Data from a systematic review a meta-analysis on comparison of herbal toothpaste on 899 participants revealed that herbal tooth paste were superior to non-herbal tooth paste (SMD 1.95, 95% CI (0.97–2.93)) in plaque reduction.¹²

N. sativa also called Black Cumin is a widely used medicinal plant belonging to the family Ranunculaceae, known to possess a treasure trove of medicinal properties.¹³ Promising outcomes have been exhibited by *N. Sativa* against many MDR bacteria including resistant *S. aureus*, *P. aeruginosa*, *Salmonella*, *H. pylori*, and *E. coli*.^{14,15} At concentration levels of 4-16 g/ml, *N. sativa* seed extract activity against *S. aureus* was found to be better than a combined effect of amyl metacresol and 2,4-dichlorobenzyl alcohol, and similar to chlorquinaldol.¹⁶

N. sativa extract also showed better results than chlorhexidine gluconate in treatment of dental infections caused by *S. mutans* and other dental pathogens.¹⁷ In vitro antibacterial potential of *Spilanthes oleracea* and propolis, *Nigella* seed oil, and an ethanolic extract of black garlic evaluated showed that *Nigella* seed oil killed more than 60% of the oral pathogens.¹⁸

Methanolic extract of *N. sativa* seed showed a 22.3 mm zone of inhibition at 0.2 mg mL⁻¹ against *S. mutans* (MTCC 0497), while *L. acidophilus* (MTCC 10307) was more sensitive to *S. aromaticum* bud methanolic extract at 11.3 mm zone of inhibition at concentration 0.1 mg mL⁻¹.¹⁹

In our study, we found that *N. sativa* extract even at much lower concentration of 75µl/ml in comparison to earlier studies the zone of inhibition was 28.33 mm and at the MIC concentration of 12.5 µl/ml the zone of inhibition was 20.33 mm against *S. mutans* (MTCC 0497), Though the zone of inhibition of Triclosan against *S. mutans* (MTCC 0497) was higher than *N. sativa* extract the results of Minimum Bactericidal Concentration and Time kill assay showed better results with the extract. The result from the current study suggests that *N. sativa* methanolic extract is an effective, alternative to triclosan and should be further studied.

Conclusion

N. sativa methanolic extract can be used as a potent antimicrobial against *S. mutans* with a comparable efficacy to triclosan but without its many associated adverse effects on the human body and to the environment. Herbal alternatives should be included in the commercial oral health care products due to their beneficial and synergistic activity with conventional products.

List of Abbreviations

1. *S. mutans* – *Streptococcus mutans*
2. *N. Sativa*- *Nigella sativa*
3. MTCC- Microbial Type Culture Collection
4. ATCC- American Type Culture Collection
5. MIC- Minimum Inhibitory Concentration
6. MBC- Minimum Bactericidal Concentration
7. MDR- Multi Drug Resistance
8. CLSI- Clinical and Laboratory Standards Institute
9. CFU- Colony Forming Units

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