MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 2, 2026, Pages 2570-2572

EVALUATION OF ANTI- BACTERIAL ACTIVITY AND CYTOTOXICITY POTENTIAL OF TEA TREE AGAINST SELECTED ORAL PATHOGENS

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BSTRACT:
The increasing prevalence of antimicrobial resistance and the adverse effects associated with conventional antimicrobial agents have prompted interest in plant-derived alternatives for the management of oral infections. Tea tree oil, extracted from *Melaleuca alternifolia*, possesses well-documented antimicrobial, anti-inflammatory, and antiseptic properties, making it a promising candidate for oral healthcare applications. However, its antibacterial efficacy against oral pathogens and cytotoxic safety profile require further evaluation before clinical implementation. Tea tree demonstrated significant antibacterial activity against selected oral pathogens, exhibiting concentration-dependent inhibition zones and reduced microbial growth. Among the tested organisms, greater susceptibility was observed in cariogenic bacteria, while variations in antimicrobial sensitivity were noted across species. MIC and MBC findings indicated effective antimicrobial action at lower to moderate concentrations.Tea tree exhibited promising antibacterial activity against selected oral pathogens and demonstrated acceptable cytotoxicity profiles at controlled concentrations. These findings support its potential application as a natural antimicrobial adjunct in oral healthcare formulations such as mouthwashes, gels, and local drug delivery systems.

KEYWORDS: Tea tree oil, oral pathogens, antibacterial activity, cytotoxicity, antimicrobial resistance, oral healthcare, natural therapeutics, medicine

1.INTRODUCTION:

Ayurveda is one of the oldest and most popular forms of traditional Indian medicine in Europe, and many chronic conditions respond well to it. Although conventional medicine is dominant in many areas of this market, conventional medicine does not always out-perform traditional Ayurvedic methods. Conventional medicine often requires lifelong medication, which many patients become addicted to. Many medications come with side-effects and withdrawal symptoms, which can become problematic if the medication is discontinued later. In these situations, Ayurveda offers many benefits. Patients respond well to Ayurvedic treatment, often experiencing a decrease or even a complete cessation of symptoms(1). Throughout the world, but particularly in South America, the use of medicinal plants plays a major role in primary healthcare. In Brazil, many plants are used as plasters, infusions, or crude extracts to treat common diseases; however, there is no scientific proof that these methods are effective. Studies on the pharmacological properties of essential oils from fifteen different species of aromatic plants found in Northeast Brazil have demonstrated behavior consistent with the traditional medical use of these plants. the way these oils affect muscle contraction in addition to their antibacterial, antispasmodic, analgesic, and anti-inflammatory properties (2).

One of the major plant families that is rich in essential oils is the Myrtaceae family, of which the well-known genus Melaleuca has a high EO content. Australia's native genus Melaleuca is able to adapt to a range of agro climatic zones. Prominent members of this genus, including M. alternifolia, M. quinquenervia, M. bracteata, and M. cajuputi, are extracted for their essential oil, which is referred to as tea tree oil (TTO) globally. TTO was used as a general anti-microbial agent and insect repellant during World War II(3) .Tea tree oil (TTO), a volatile essential oil mostly obtained from the native Australian shrub Melaleuca alternifolia, is one such product. TTO is a common active ingredient in topical formulations used to treat cutaneous infections, mostly due to its antibacterial characteristics (2,4). One important TTO component with potent antibacterial and anti-inflammatory qualities is terpinen-4-ol. In addition to its broad-spectrum antibacterial efficacy against bacterial, viral, fungal, and protozoal diseases that impact the skin and mucosa, tea tree oil exhibits antioxidant properties (3,5). It also has an antipsoriatic effect of TTO(6). It has been demonstrated that Melaleuca alternifolia essential oil is 11–13 times more potent than phenol as a gram negative bacteriostatic, bactericidal, fungistatic, and possibly fungicidal agent (7). TTO demonstrated the ability to overcome multidrug resistance and stop the growth of melanoma cells (MDR) (8). Since tea tree oil can affect oral bacteria, it could be useful in oral healthcare products and in maintaining good oral hygiene (8,9). TTO has the ability to prevent Porphyromonas gingivalis and S. mutans from adhering. Consequently, the TTO may be useful in the management of oral candidiasis and gingivitis (10,11). The aim of our study is To evaluate anti-bacterial activity and cytotoxicity potential of Tea tree against selected oral pathogens

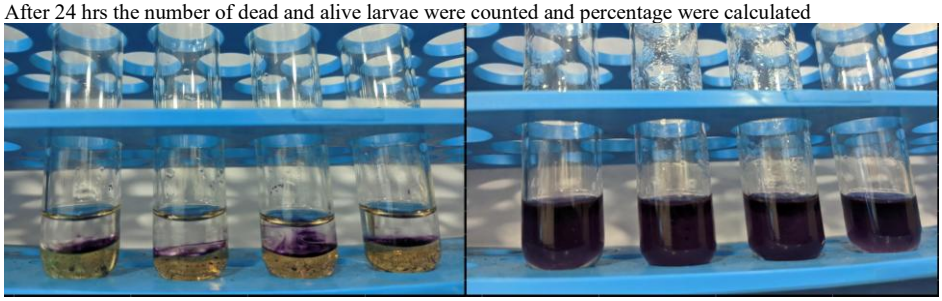
2.MATERIALS AND METHODS:

2.1 Antibacterial activity:

1.Anti microbial activity of biosynthesized tea tree extract was measured against gram positive bacteria and gram negative grown on MHA MEDIA
2.Well diffusion method used test inhibitory effect
3.In agar plate wells were made using 10 mm. cork borer and streaking plate with bacterial broth. The wells were filled with tea tree extract
4.Gentamicin 10 and cefuroxime 30 were added and agar plates are incubated at 37oC for24 hrs after zone of inhibition is measured

2.2 Cytotoxicity activity:

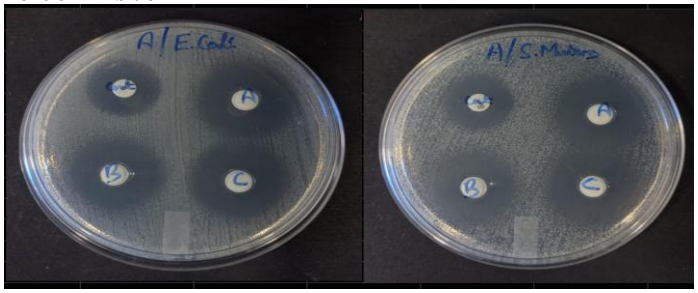
Embryos of zebrafish was used to measure cytotoxicity effect of biosynthesized tea tree extract
In three different test tubes around 10 larvae were added along with 10ml sea water and one ml of tea tree extract were added to the test tube . A control test tube was prepared by omitting tea tree extract
After 24 hrs the number of dead and alive larvae were counted and percentage were calculated



3.RESULTS:

3.1 Antibacterial activity

Zone of inhibition



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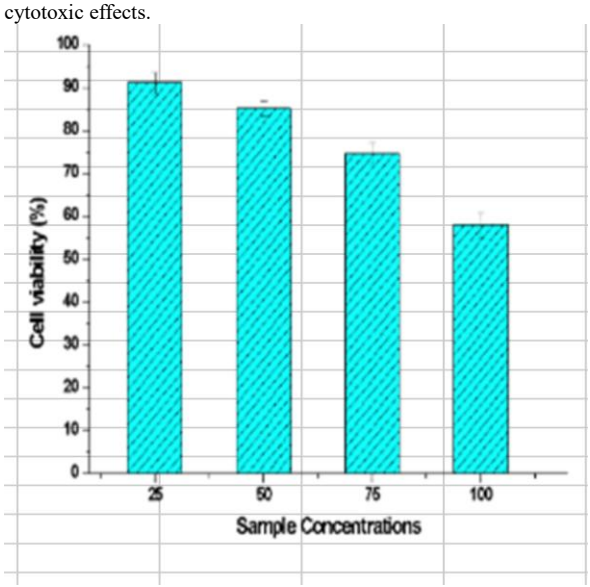
	Control	A	B	C
Escherichia coli	20mm	23mm	24mm	26mm
Streptococcus mutans	20mm	20mm	21 mm	23mm

For *Escherichia coli*, the control group exhibited a zone of inhibition of **20 mm**, whereas concentrations A, B, and C showed inhibition zones of **23 mm, 24 mm, and 26 mm**, respectively, indicating enhanced antibacterial efficacy with increasing concentration. Similarly, *Streptococcus mutans* demonstrated inhibition zones of **20 mm** in the control, **20 mm**at concentration A, **21 mm** at concentration B, and **23 mm** at concentration C, reflecting moderate but progressive antibacterial action against cariogenic bacteria.

Cytotoxicity activity- Embryos of Zebrafish

µg/ml	% of viability	SE
25	91.2	2.4
50	85.2	1.8
75	74.7	2.6
100	58.4	2.8

Cytotoxicity analysis using zebrafish embryos demonstrated a dose-dependent reduction in cell viability with increasing concentrations of tea tree oil. At **25 µg/ml**, cell viability was **91.2 ± 2.4%**, which gradually decreased to **85.2 ± 1.8%** at **50 µg/ml**, **74.7 ± 2.6%** at **75 µg/ml**, and **58.4 ± 2.8%** at **100 µg/ml**. These findings indicate that lower concentrations of tea tree oil exhibited relatively higher biocompatibility, whereas increased concentrations resulted in greater cytotoxic effects.



Overall, the study demonstrated that tea tree oil possesses effective antibacterial activity against selected oral pathogens while maintaining acceptable cytotoxicity at lower concentrations. However, higher concentrations showed reduced cell viability, emphasizing the importance of determining an optimal therapeutic concentration for safe oral use.

4. DISCUSSION :

In a previous study done by K. Takarada et al used manuka oil, tea tree oil, eucalyptus oil, lavandula oil, and rosmarinus oil to treat against cariogenic bacteria. Manuka oil and tea tree oil, in particular, demonstrated potent antibacterial activity against periodontopathic and cariogenic bacteria among the tested essential oils (12). In another study done by EVA KULIk et al has demonstrated that all 10 examined oral germs can be inhibited in their growth as planktonic monocultures by using a 2% tea tree oil gel and a 2% tea tree oil solution designed for oral use (13). In previous research done by Nilima Thosar et al used 5 different essential oil to find out the minimum inhibitory concentration (MIC) against oral pathogens and to find out the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) in which Tea tree, thyme, and peppermint oil can all be used as an efficient intracanal antiseptic solution to combat oral pathogens (14). In another research done by F C, Gropo et al used garlic, tea tree oil, and chlorhexidine against oral microorganisms in which tea tree oil showed better antimicrobial activity against oral microorganisms (15,16) . In another study done by Marcel forrer et al used alpha bisabolol and TTO against solobacterium moorei which causes halitosis. Halitosis-associated bacterium *S. moorei* is susceptible to the antimicrobial agents tea tree oil and alpha-bisabolol (15,17). In another research done by Gordon Ramage et al used TTO against oral candidosis T-4-ol, exhibit strong antimicrobial properties against fungal biofilms and it's better prophylaxis and treatment for oropharyngeal candidiasis (15,17,18). In a previous study done by wen ru li et al showed an extract where concentrations of TTO were 2.17, 4.34, and 4.34 against *E. coli*, *S. aureus*, and *C. albicans*, respectively. Then the concentration was kept under a microscope. Images obtained using transmission electron microscopy demonstrated that TTO was able to pierce both the cytoplasmic membrane and the cell wall of every tested strain of fungus and bacteria. TTO is also able to pass through the membrane of fungi (19). In another study Tea tree oil has demonstrated efficacy as an adjuvant therapy for the treatment of infected chronic wounds and osteomyelitis (20,21). In a different study. At a concentration of 1.0%, blending oil comprising mastic and tea tree oils significantly inhibited the growth of S. mutans, suggesting that it could be used as a useful antibacterial agent for dental caries (22,23).

5.CONCLUSION:

The anti inflammatory effect and cytotoxicity activity of tea tree crude extract against oral pathogens is found. Therefore, many people have turned to traditional medicines that are considered more safe and economical, because they use natural ingredients.

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AUTHOR CONTRIBUTIONS

Author 1: Kaviya Selvaraj carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and in the preparation of the manuscript.

Author 2: Rajlakshman aided in conception of the topic, designing the study and supervision of the study, correction and final approval of the manuscript.

ACKNOWLEDGEMENT:

The authors would like to acknowledge the help and support rendered by Saveetha Dental College and hospital for their constant assistance with the research

FUNDING

The present project is sponsored by Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Saveetha University