EVALUATION OF ANTIBACTERIAL AND HEAMOLYTIC ACTIVITY OF MELALEUCA ALTERNIFOLIA

Samudhrasri S
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Saveetha University, Chennai-600 077, India
Email:152001039.sdc@saveetha.com

DR. S Rajeshkumar
Professor
NanoBiomedicine Lab
Department of Pharmacology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,Chennai.
rajeshkumars.sdc@saveetha.com

CORRESPONDING AUTHOR:
DR. S Rajeshkumar
Professor
NanoBiomedicine Lab
Department of Pharmacology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,Chennai.
rajeshkumars.sdc@saveetha.com

INTRODUCTION:

Medicinal plants have been used for centuries as a primary source of healthcare and continue to play a significant role in modern medicine. The growing interest in natural products has led to increased research on plant-derived compounds due to their therapeutic efficacy, safety, accessibility, and lower incidence of adverse effects compared to synthetic drugs. In developing countries, a substantial proportion of the population still relies on traditional herbal remedies for the prevention and treatment of various diseases. Consequently, medicinal plants have become an important reservoir of bioactive compounds for the discovery and development of novel pharmaceutical agents.(1,2)

Among the numerous medicinal plants investigated, Melaleuca alternifolia, commonly known as the tea tree, has attracted considerable scientific attention. Although the term “tea tree” is also used for several species belonging to the genera Melaleuca and Leptospermum, M. alternifolia is

the primary commercial source of tea tree oil (TTO). Native to Australia, this plant has been traditionally used by Indigenous Australians for treating wounds, skin infections, and respiratory ailments.(1)

Tea tree oil is an essential oil obtained by steam distillation of the leaves and terminal branches of M. alternifolia. The oil consists of a complex mixture of over 100 components, predominantly monoterpenes and related alcohols.(1–3) The major bioactive constituents include terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, α-terpineol, and p-cymene. Among these, terpinen-4-ol is considered the principal component responsible for most of the biological activities of tea tree oil, particularly its antimicrobial and anti-inflammatory effects.

Over the past few decades, tea tree oil has gained widespread recognition for its broad spectrum of pharmacological properties. Numerous studies have demonstrated its potent antimicrobial activity against a variety of bacteria, fungi, viruses, and protozoa. In addition, tea tree oil exhibits significant antioxidant properties by scavenging free radicals and reducing oxidative stress, which contributes to cellular protection and tissue repair. Emerging evidence also suggests its potential anticancer activity through mechanisms involving induction of apoptosis, inhibition of tumor cell proliferation, and modulation of inflammatory pathways.(4)

The increasing prevalence of antimicrobial resistance and the limitations associated with conventional therapeutic agents have further highlighted the need for alternative and complementary treatment strategies. (5)Natural products such as tea tree oil represent promising candidates due to their multifaceted biological activities and relatively favorable safety profile. Furthermore, the incorporation of tea tree oil into pharmaceutical, cosmetic, and oral healthcare formulations has expanded its clinical relevance and commercial value.(6)

Given its diverse therapeutic applications and growing scientific evidence, Melaleuca alternifolia continues to be an important subject of research. This review aims to provide a comprehensive overview of the phytochemical composition, biological activities, mechanisms of action, and potential therapeutic applications of tea tree oil, with particular emphasis on its antimicrobial, antioxidant, and anticancer properties.

AIM: The aim of this study is to evaluate the antibacterial and haemolytic activity of melaleuca alternifolia

MATERIALS AND METHODS:

Antimicrobial Activity

The antimicrobial activity of Melaleuca alternifolia (tea tree) oil was evaluated against two bacterial strains, Escherichia coli and Staphylococcus aureus, using the agar well diffusion method. Mueller–Hinton Agar (MHA) was employed as the culture medium for determining the antibacterial efficacy of the test samples.

The Mueller–Hinton Agar was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes. The sterilized medium was poured into sterile Petri plates under aseptic conditions and allowed to solidify at room temperature. Fresh bacterial cultures of E. coli and S. aureus were prepared and uniformly inoculated onto the surface of the agar plates using sterile cotton swabs to obtain a confluent lawn culture.

Wells of approximately 9 mm diameter were aseptically punched into the agar using a sterile polystyrene tip. Different concentrations of tea tree oil extract (25 µL, 50 µL, and 100 µL) were carefully dispensed into the respective wells. The plates were then incubated at 37°C for 24 hours under aerobic conditions.

Following incubation, the antimicrobial activity was assessed by measuring the diameter of the clear zones of inhibition surrounding each well. The zones of inhibition were measured in millimeters (mm) using a calibrated ruler or Vernier caliper. Larger inhibition zones were considered indicative of greater antibacterial activity. All experiments were performed in triplicate, and the mean values were recorded.

Haemolytic Activity

The haemolytic activity of the selected peptides was assessed using human red blood cells (RBCs) to evaluate their cytotoxicity and biocompatibility. Fresh human blood was collected in sterile tubes containing anticoagulant and centrifuged at 1,500 rpm for 10 minutes to separate the erythrocytes. The packed red blood cells were washed three times with sterile phosphate-buffered saline (PBS, pH 7.4) to remove plasma and other cellular components.

A 2% (v/v) suspension of washed erythrocytes was prepared in PBS. Serial dilutions of the selected peptides were prepared in sterile PBS and mixed with equal volumes of the erythrocyte suspension. The mixtures were incubated at 37°C for 1 hour under gentle shaking conditions.

Following incubation, the samples were centrifuged at 3,000 rpm for 10 minutes to pellet intact erythrocytes. The supernatants were carefully collected, and the release of hemoglobin was quantified spectrophotometrically by measuring the absorbance at 540 nm. PBS-treated erythrocytes served as the negative control (0% haemolysis), while erythrocytes treated with 1% Triton X-100 served as the positive control (100% haemolysis).

The percentage of haemolysis was calculated using the following equation:

% Haemolysis = [(Absorbance of sample − Absorbance of negative control) / (Absorbance of positive control − Absorbance of negative control)] × 100

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. The haemolytic assay was performed to assess the safety profile of the selected peptides and determine their suitability for potential biomedical applications.

RESULTS:

ANTIMICROBIAL ACTIVITY:

FIGURE :1

Zone of Inhibition				
	Control	A	B	C
Escherichia coli	20mm	23mm	24mm	26mm
Streptococcus mutans	20mm	22mm	23mm	25mm

The results demonstrated that the tea tree oil extract exhibited antibacterial activity against both tested microorganisms. An increase in the concentration of the extract resulted in a corresponding increase in the zone of inhibition, indicating a dose-dependent antimicrobial effect.

For Escherichia coli, the control group showed a zone of inhibition of 20 mm, while concentrations A, B, and C produced inhibition zones of 23 mm, 24 mm, and 26 mm, respectively. The highest concentration (100 µL) exhibited the greatest antibacterial activity, producing a 6 mm increase in inhibition compared to the control.

Similarly, against Streptococcus mutans, the extract produced zones of inhibition of 22 mm, 23 mm, and 25 mm at concentrations of 25 µL, 50 µL, and 100 µL, respectively, compared with 20 mm in the control group. The maximum inhibition was observed at 100 µL, indicating enhanced antibacterial efficacy at higher concentrations.

Overall, Escherichia coli appeared slightly more susceptible to the tea tree oil extract than Streptococcus mutans, as evidenced by the larger inhibition zones at all tested concentrations. These findings suggest that Melaleuca alternifolia possesses significant antibacterial properties and may serve as a potential natural antimicrobial agent against both Gram-negative and Gram-positive oral pathogens. The concentration-dependent increase in antimicrobial activity further supports its therapeutic potential in oral healthcare and infection control applications.

Figure:2

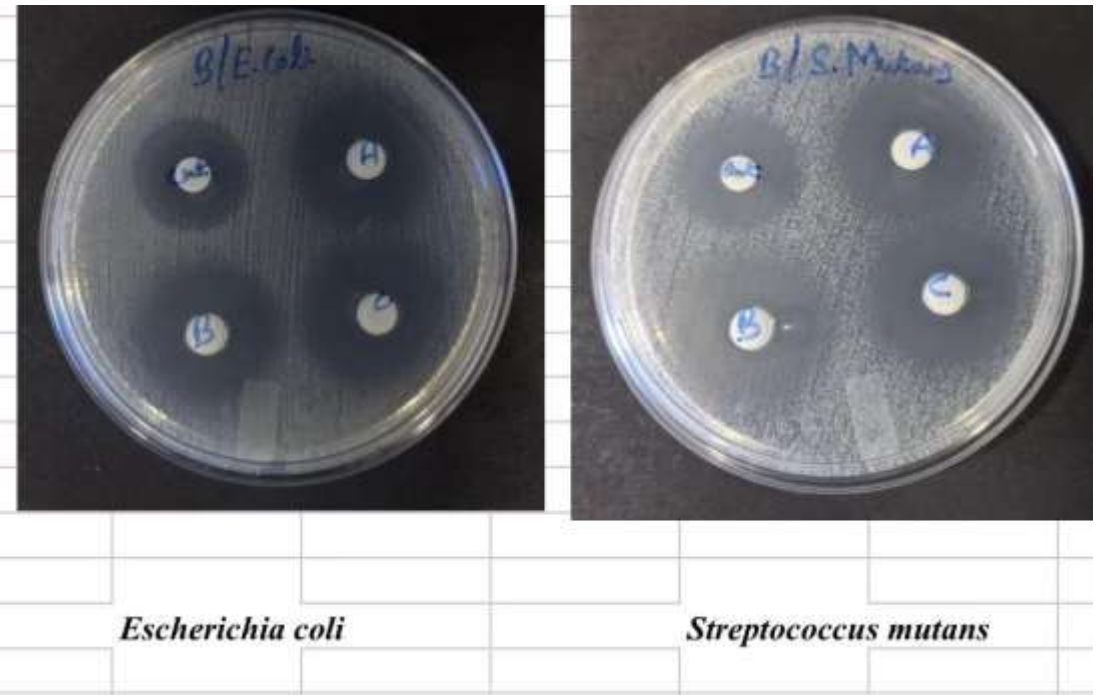

Figure:2 Agar well diffusion assay showing the antibacterial activity of Melaleuca alternifolia extract against Escherichia coli (left) and Streptococcus mutans (right). Wells A, B, and C represent increasing concentrations of the extract (25 µL, 50 µL, and 100 µL, respectively). The clear zones surrounding the wells indicate inhibition of bacterial growth, with the largest zones observed at the highest concentration tested.

HEMOLYTIC ACTIVITY :

Figure:3

Haemolytic activity		
Sample conce	RBC cell lysis	St.Er
25	0	0
50	8	1.1
75	14.2	1.2
100	18.2	1.6

Figure:3 Haemolytic activity of Melaleuca alternifolia extract at different concentrations. The percentage of red blood cell (RBC) lysis increased with increasing extract concentration, showing

a dose-dependent haemolytic effect. No haemolysis was observed at 25 µL, while the maximum haemolysis (18.2%) was recorded at 100 µL. The low level of haemolysis indicates good biocompatibility and suggests that the extract is relatively safe for biomedical applications at the tested concentrations.

The haemolytic activity assay was performed to evaluate the cytotoxic effect of Melaleuca alternifolia extract on human red blood cells (RBCs). The percentage of RBC lysis increased progressively with increasing concentrations of the extract, indicating a concentration-dependent haemolytic effect.

Figure:4

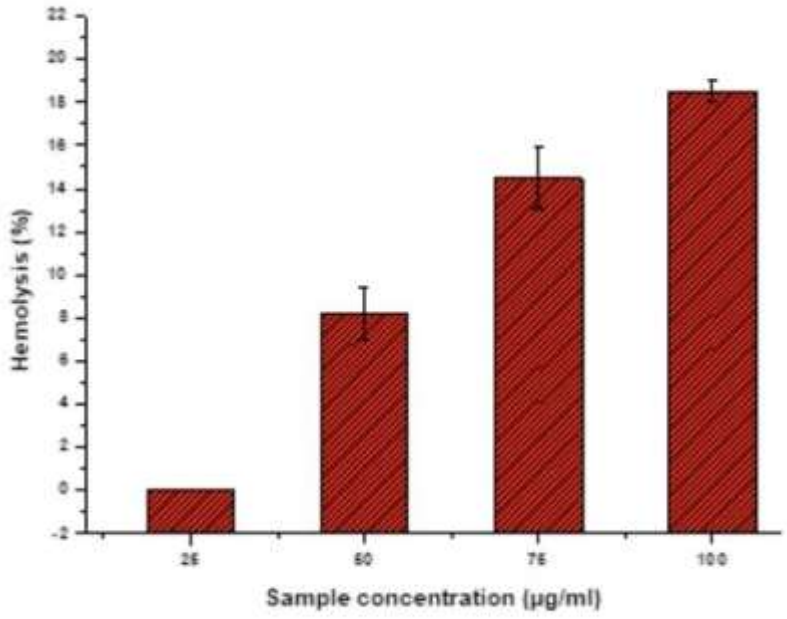

Figure 4: Haemolytic activity of Melaleuca alternifolia extract at varying concentrations. The percentage of red blood cell lysis increased progressively from 0% at 25 µg/mL to 18.2% at 100 µg/mL, demonstrating a concentration-dependent haemolytic effect. Error bars represent standard error values. The relatively low haemolysis observed at all tested concentrations indicates good biocompatibility of the extract.

DISCUSSION:

The present study evaluated the antimicrobial and haemolytic activities of Melaleuca alternifolia (tea tree oil) at different concentrations. The findings demonstrated that both antibacterial efficacy and haemolytic activity increased in a concentration-dependent manner.(5,7)

The antimicrobial assay revealed that increasing the concentration of tea tree oil resulted in a corresponding increase in the zone of inhibition against both Escherichia coli and Streptococcus mutans. The highest concentration tested (100 µL) produced the largest zones of inhibition, indicating enhanced antibacterial activity at elevated concentrations. These results suggest that the bioactive constituents present in tea tree oil exert stronger inhibitory effects on bacterial growth when present in greater amounts.Among the tested microorganisms, Escherichia coli exhibited slightly greater susceptibility to the extract than Streptococcus mutans, as evidenced by the larger zones of inhibition at all tested concentrations(8). This finding indicates that tea tree oil possesses effective antibacterial activity against diverse bacterial species and supports its potential application in both medical and oral healthcare settings.(9)

The haemolytic assay demonstrated that red blood cell lysis increased progressively with increasing concentrations of the sample. No haemolysis was observed at the lowest concentration (25 µL), whereas the highest concentration (100 µL) produced a haemolytic activity of 18.2%. This concentration-dependent increase suggests that higher concentrations of tea tree oil have a greater ability to interact with and disrupt erythrocyte membranes. Essential oil constituents are known to interact with lipid bilayers, and at elevated concentrations, these interactions may alter membrane stability, leading to increased haemolysis.(10)

Despite the increase in haemolytic activity, the maximum haemolysis observed remained below 20%, indicating relatively low cytotoxicity toward human red blood cells. The low haemolytic effect at therapeutically relevant concentrations suggests a favorable safety profile and good biocompatibility of the extract. These findings are particularly important because an ideal antimicrobial agent should exhibit potent activity against microorganisms while causing minimal damage to host cells(11).

Overall, the present study demonstrates that Melaleuca alternifolia possesses significant antibacterial activity against Escherichia coli and Streptococcus mutans, with efficacy increasing as the concentration increases(10,12). Although haemolytic activity also increased in a dose-dependent manner, the observed levels remained relatively low, indicating acceptable biological safety. The combination of strong antimicrobial activity and limited cytotoxicity highlights the

potential of tea tree oil as a natural therapeutic agent for pharmaceutical, biomedical, and oral healthcare applications. Further studies involving additional microbial strains, toxicity assessments, and clinical investigations are warranted to fully establish its therapeutic potential.

CONCLUSION:

The present study demonstrated that Melaleuca alternifolia (tea tree oil) possesses significant antibacterial activity against Escherichia coli and Streptococcus mutans, with antimicrobial efficacy increasing in a concentration-dependent manner. The highest concentration tested exhibited the greatest zone of inhibition, highlighting the strong antibacterial potential of tea tree oil against both Gram-negative and Gram-positive bacteria. In addition, the haemolytic assay revealed a gradual increase in red blood cell lysis with increasing concentrations; however, the overall haemolytic activity remained relatively low, indicating acceptable biocompatibility and limited cytotoxicity.

The findings suggest that tea tree oil is a promising natural antimicrobial agent with potential applications in pharmaceutical, biomedical, and oral healthcare formulations. Its ability to effectively inhibit bacterial growth while maintaining relatively low toxicity toward human cells supports its suitability for further therapeutic development.

In conclusion, Melaleuca alternifolia represents a valuable source of bioactive compounds with considerable antimicrobial potential. Continued research and technological advancements are expected to facilitate the development of innovative tea tree oil-based products for the prevention and treatment of microbial infections, thereby contributing to the growing demand for safe, effective, and naturally derived therapeutic agents.

REFERENCES

1. Helmy AK, El-Houssiny AS, Hegazi AG, Sidkey NM. Antimicrobial potential of different bee product-loaded carboxymethyl chitosan nanoparticles against multidrug-resistant clinical pathogens: a comparative in vitro study. BMC Biotechnol [Internet]. 2026 May 29;26(1). Available from: http://dx.doi.org/10.1186/s12896-026-01159-5

2. Mafe AN, Makut MD, Owuna JE, Nkene IH, Edo GI, Salisu SM, et al. Development, functional profiling, and probiotic evaluation of indigenous LAB from Nigerian fermented foods: Antimicrobial mechanisms, in vivo validation, and applications in food preservation and mycotoxin risk reduction. World J Microbiol Biotechnol [Internet]. 2026 May 30;42(6). Available from: http://dx.doi.org/10.1007/s11274-026-05051-4

3. Jiang T, Prioult G, Quann E. Microbial Biotransformation of Polyphenols and Bioactive

Substrates: Implications for Metabolite-Guided Synbiotics. J Nutr. 2026 May 28;101621.

4. Chamnanvinijchai N, Techawatcharathep N, Ngerndee N, Teerawongwiwat S, Anukul B, Jumjitvi P, et al. Phytosphingosine disrupts dual-species oral biofilms and attenuates LPS-induced pro-inflammatory responses in human gingival fibroblasts. Sci Rep [Internet]. 2026 May 30; Available from: http://dx.doi.org/10.1038/s41598-026-55714-3

5. Jarošíková R, Sojáková D, Antalová S, Tůma P, Polák J, Dubský M, et al. Tissue and Serum Concentrations of Time-Dependent Antibiotics in Infected Diabetic Foot Ulcers by Bolus or Continuous Administration: The Randomised DFIATIM Trial. Diabetes Metab Res Rev. 2026 Jul;42(5):e70185.

6. Wu W, Jiao P, Gu Y, Lu H, Fan C, Sun Z, et al. Choline counteracts pregnancy undernutrition-associated colitis in sheep by restoring microbiota driven bile acid metabolism. NPJ Biofilms Microbiomes [Internet]. 2026 May 30; Available from: http://dx.doi.org/10.1038/s41522-026-01027-2

7. Narduzzi M, Pelosi C, Vinciguerra V, Antonelli C, Vettraino AM. In Vitro Evaluation of Thymus serpyllum Essential Oil Against Paracoccus marcusii IBR3: A Potential Natural Protection Strategy for Historic Mural Paintings. Environ Microbiol Rep. 2026 Jun;18(3):e70368.

8. Nemaguvhuni FM, Traore AN, Razwinani M, Samie A. Synthesis, Characterisation, and Evaluation of the Biological Activities of Medicinal Plants-Based Gold Nanoparticles. Adv Exp Med Biol. 2026;1507:155–77.

9. Magna LR, Carreteiro MM, Couto GJ, de Carvalho L, Nogueira MH, Braga ML, et al. Bactericidal and anti-virulence effects of Melaleuca alternifolia essential oil against foodborne pathogens: in vitro and milk matrix assay. Lett Appl Microbiol [Internet]. 2026 Mar 6;79(3). Available from: http://dx.doi.org/10.1093/lambio/ovag030

10. Do DN, Trinh-Le MT, Nguyen-Thi HY, Pham-Vu MC, Tran TV, Le XT. Green enrichment of α-terpineol from niaouli oil residue molecular distillation. RSC Adv. 2026 May 13;16(28):25557–68.

11. Nguyen XN, Tu HL, Phan C. Comparative Preparation and Characterization of β-Cyclodextrin Inclusion Complexes With Two Melaleuca Essential Oils for Postharvest Mango Preservation. J Food Sci. 2026 Mar;91(3):e70998.

12. Yang F, Liao Y, Guo Y, Nie R, Fu Y, Chen B, et al. Behavioral Responses and Contact Toxicity of Australian Tea Tree Oil and Its Major Constituents Against the Asian Citrus Psyllid, Kuwayama. Insects [Internet]. 2026 Mar 24;17(4). Available from: http://dx.doi.org/10.3390/insects17040355