Journal of Carcinogenesis
Covering Genesis to Therapeutics
Journal of Carcinogenesis, ISSN: 1477-3163
2023; Vol 22: Issue 2, https://carcinogenesis.com/

Cytotoxicity and Anti Oxidant Effect of Malaleuca Alternifolia

Samudhrasri S[1], DR. S Rajalakshmanan Eswaramoorthy[2]

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

[2]Professor, NanoBiomedicine Lab, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai, rajalakshamanane.sdc@saveetha.com

CORRESPONDING AUTHOR: DR. S Rajalakshmanan Eswaramoorthy

rajalakshamanane.sdc@saveetha.com

ABSTRACT

Since ancient times, medicinal plants have been extensively used in traditional healthcare systems for the prevention and treatment of various diseases. In developing countries, a large proportion of the population continues to depend on traditional medicine due to its accessibility, affordability, and perceived safety. Natural products derived from medicinal plants have gained significant attention as potential therapeutic agents because they contain a wide variety of bioactive compounds with pharmacological properties. Consequently, medicinal plants have become an important source for the discovery and development of novel drugs used in the treatment of infections, cancer, inflammatory disorders, and other chronic diseases.(1)

How to Cite: Samudhrasri S, DR. S Rajalakshmanan Eswaramoorthy (2023) Cytotoxicity and Anti Oxidant Effect of Malaleuca Alternifolia. Journal of Carcinogenesis, Vol.22, No.2, 233-238

1. INTRODUCTION

Since ancient times, medicinal plants have been extensively used in traditional healthcare systems for the prevention and treatment of various diseases. In developing countries, a large proportion of the population continues to depend on traditional medicine due to its accessibility, affordability, and perceived safety. Natural products derived from medicinal plants have gained significant attention as potential therapeutic agents because they contain a wide variety of bioactive compounds with pharmacological properties. Consequently, medicinal plants have become an important source for the discovery and development of novel drugs used in the treatment of infections, cancer, inflammatory disorders, and other chronic diseases.(1)

In recent years, there has been growing interest in natural antioxidants obtained from aromatic and medicinal plants. Oxidative stress, resulting from the excessive production of free radicals, plays a crucial role in the pathogenesis of numerous chronic diseases, including cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and cancer. Plant-derived antioxidants help neutralize free radicals and protect biological systems from oxidative damage. Furthermore, the antioxidant activity of medicinal plants is often associated with additional biological functions, such as anti-inflammatory, antimicrobial, and anticancer effects.(1,2)

Among the various medicinal plants investigated, Australian tea tree (Melaleuca alternifolia) has emerged as one of the most valuable species because of its broad spectrum of biological activities. Tea tree oil, obtained by steam distillation of the leaves and terminal branches of M. alternifolia, is rich in terpenes and terpene alcohols, particularly terpinen-4-ol, which is considered the major active component. The oil has been widely recognized for its potent antimicrobial properties against a variety of bacteria, fungi, and viruses. In addition to its antimicrobial activity, tea tree oil exhibits significant antioxidant and anti-inflammatory effects, making it a promising natural therapeutic agent(1–3).

Several studies have demonstrated that tea tree oil possesses the ability to inhibit the growth of pathogenic microorganisms and reduce oxidative stress by scavenging free radicals. Recent investigations have also highlighted its potential anticancer activity through the induction of apoptosis and inhibition of tumor cell proliferation. Due to these diverse pharmacological properties, tea tree oil has found applications in pharmaceutical, cosmetic, and healthcare products.(1–4)

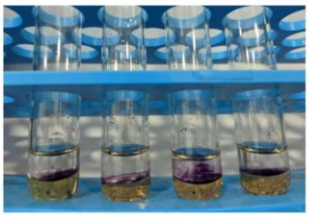
Given the increasing demand for natural alternatives to synthetic antimicrobial and antioxidant agents, the evaluation of

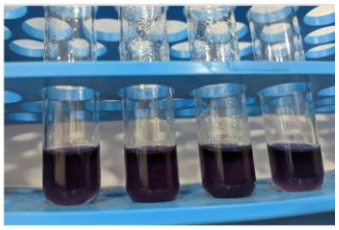
tea tree oil and its therapeutic potential has become an important area of research. Therefore, the present study aims to investigate the biological activities of tea tree oil, with particular emphasis on its antimicrobial and antioxidant properties, and to explore its potential applications in the prevention and management of various diseases(1–5).

2. AIM:

The aim of the study is to evaluate the cytotoxicity and anti oxidant effect of malaleuca alternifolia(Australian tea tree).

3. MATERIALS AND METHODS:





Cytotoxicity Assessment Using Zebrafish Embryos
The cytotoxic potential of the biosynthesized tea tree extract was evaluated using zebrafish (Danio rerio) embryos/larvae as an in vivo model. Zebrafish are widely used in toxicological studies due to their genetic similarity to humans, rapid development, transparency during embryonic stages, and ease of maintenance.

Healthy zebrafish larvae were collected and distributed into experimental test tubes. Approximately 10 larvae were placed in each of three test tubes containing 1 mL of sterile seawater. To the experimental groups, 1 mL of the biosynthesized tea tree extract was added, while the control group contained only seawater without the addition of tea tree extract. The larvae were maintained under standard laboratory conditions throughout the experimental period.

After an incubation period of 24 hours, the larvae were observed under suitable illumination, and the numbers of live and dead larvae were recorded. Larval viability was assessed based on the presence of heartbeat, spontaneous movement, and normal morphology. The results obtained were used to evaluate the cytotoxic effect of the biosynthesized tea tree extract.

Antioxidant Activity Assessment
The antioxidant activity of the biosynthesized tea tree extract was evaluated using two widely accepted methods: the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the Nitric Oxide (NO) scavenging assay.

DPPH Radical Scavenging Assay
The DPPH assay was performed to determine the free radical scavenging ability of the tea tree extract. Different concentrations of the extract were prepared and mixed with DPPH solution. The reaction mixture was incubated in the dark at room temperature for a specified period to allow the reaction between the antioxidant compounds and DPPH radicals.

Following incubation, the decrease in absorbance was measured spectrophotometrically at 517 nm. Ascorbic acid was used as the standard antioxidant for comparison. Higher percentages of inhibition indicated stronger antioxidant activity.

4. RESULT

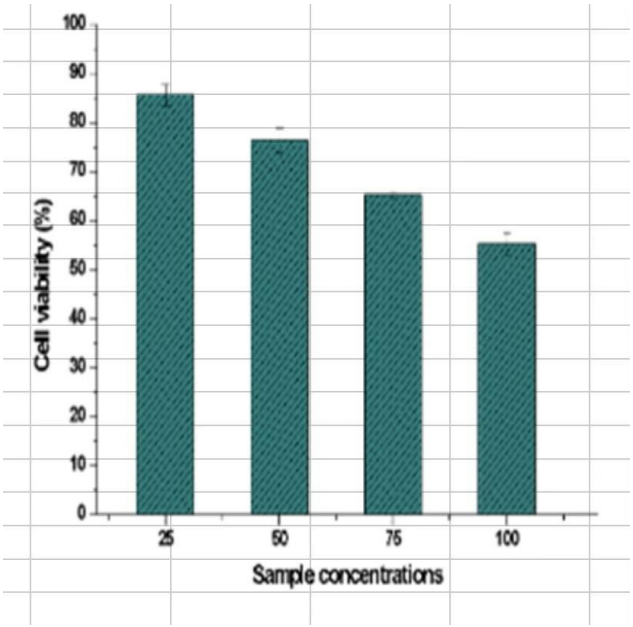
Sample concentration		
µg/ml	% of viability	SE
25	85.6	2.2
50	76.4	2.5
75	65.3	0.6
100	55.3	2.2

Table 1: The above table represents the cytotoxic activity of malaleuca alternifolia

The cytotoxic activity of the biosynthesized tea tree extract was evaluated using the zebrafish larval model at different concentrations (25, 50, 75, and 100 µg/mL). The percentage viability of zebrafish larvae was recorded after 24 hours of exposure, and the results are presented in Table 1.

The findings demonstrated a concentration-dependent decrease in larval viability with increasing concentrations of tea tree extract. At the lowest concentration of 25 µg/mL, the extract exhibited minimal toxicity, with a viability of 85.6 ± 2.2%. As the concentration increased to 50 µg/mL, viability decreased to 76.4 ± 2.5%. Further reduction in survival was observed at 75 µg/mL, where viability was recorded as 65.3 ± 0.6%.

Despite the observed reduction in viability at higher concentrations, more than 50% of the larvae remained viable even at 100 µg/mL, indicating a moderate level of cytotoxicity. The biological activity of tea tree extract may be attributed to the presence of bioactive phytochemical constituents, including terpenes and phenolic compounds, which are known to exert both therapeutic and cytotoxic effects depending on their concentration.



In the cytotoxic assay it is observed that as the sample concentration increases the cell viability decreases and is least observed as 55.3% at 100 µg/ml concentration.

DPPH assay			STD Ascorbic acid	
centration (µg/	Samples	S.Er	Std	S.Er
25	16.5	1.8	27.5	1.5
50	24.8	2.2	38.6	2
75	38.5	2.4	51.2	1.8
100	53.2	2.7	68.2	2.2

Table 2: The table represents the DPPH Assay for

Malaleuca alternifolia

The antioxidant activity of the biosynthesized tea tree extract was evaluated using the DPPH free radical scavenging assay. The results demonstrated a concentration-dependent increase in radical scavenging activity, indicating the antioxidant potential of the extract.

At a concentration of 25 µg/mL, the tea tree extract exhibited 16.5 ± 1.8% inhibition, whereas the standard ascorbic acid showed 27.5 ± 1.5% inhibition. As the concentration increased to 50 µg/mL, the scavenging activity of the extract increased to 24.8 ± 2.2%, compared to 38.6 ± 2.0% for ascorbic acid. Further increases were observed at 75 µg/mL and 100 µg/mL, where the extract exhibited 38.5 ± 2.4% and 53.2 ± 2.7% inhibition, respectively. The corresponding values for ascorbic acid were 51.2 ± 1.8% and 68.2 ± 2.2%.

Table 3: The above table represents Nitric oxide assay for Malaleuca Alternifolia.

The nitric oxide scavenging activity of the biosynthesized tea tree extract was also assessed and compared with standard ascorbic acid. Similar to the DPPH assay, the extract demonstrated a concentration-dependent increase in nitric oxide radical inhibition.

Nitric oxide assay			STD Ascorbic acid	
Concentration (µg/	Samples	S.Er	Std	S.Er
25	19.5	2.4	28.5	2.4
50	33.6	2.7	42.6	2.1
75	44.4	2.5	50.2	2.5
100	55.4	2.6	70.5	1.8

5. DISCUSSION

Natural antioxidants derived from medicinal plants have gained considerable attention because of their ability to neutralize free radicals and reduce oxidative stress, which is implicated in the development of numerous chronic diseases.(1–6) Tea tree (Melaleuca alternifolia) essential oil is recognized as a rich source of bioactive compounds with antioxidant, antimicrobial, and anti-inflammatory properties. The present study evaluated the antioxidant potential of biosynthesized tea tree extract using DPPH and nitric oxide scavenging assays and demonstrated significant free radical scavenging activity in a concentration-dependent manner.

Previous studies have reported that the antioxidant activity of M. alternifolia essential oil is stronger than that of α-tocopherol, a well-known natural antioxidant. Consistent with these findings, the results of the present study revealed that tea tree extract exhibited appreciable antioxidant activity against both DPPH and nitric oxide radicals. The percentage inhibition increased progressively with increasing concentrations of the extract, indicating enhanced free radical scavenging capacity at higher dose(1–7)s.

The antioxidant properties observed in this study may be attributed to the presence of various bioactive phytoconstituents found in tea tree oil, including monoterpenes, sesquiterpenes, phenolic compounds, and terpene alcohols such as terpinen-

4-ol. These compounds possess the ability to donate electrons or hydrogen atoms to unstable free radicals, thereby converting them into more stable molecules and preventing oxidative damage to cellular components.(1–8)

The DPPH assay demonstrated the ability of the extract to scavenge stable free radicals, while the nitric oxide assay indicated its potential to neutralize reactive nitrogen species. Excessive production of reactive oxygen and nitrogen species can lead to lipid peroxidation, protein degradation, DNA damage, and cellular dysfunction. (1–9)Therefore, the free radical scavenging activity exhibited by the tea tree extract suggests its potential role in protecting biological systems against oxidative stress-induced damage.

The observed antioxidant activity is likely due to the inherent activity of phenolic constituents present in the essential oil. Phenolic compounds are known to inhibit or reduce the rate of aerobic oxidation of organic substances by interrupting free radical chain reactions. In addition, terpenoid compounds present in tea tree oil may act synergistically with phenolic compounds, further enhancing the overall antioxidant capacity of the extract.

Although the antioxidant activity of the tea tree extract was lower than that of the standard antioxidant ascorbic acid, the extract demonstrated substantial radical scavenging potential, particularly at higher concentrations. These findings support previous reports on the therapeutic value of M. alternifolia and highlight its potential as a natural antioxidant source for pharmaceutical, nutraceutical, and biomedical applications.(1–10)

Overall, the present study confirms that biosynthesized tea tree extract possesses significant antioxidant activity, which may be attributed to its rich composition of phenolic and terpene compounds. Further studies are required to isolate and characterize the specific active constituents responsible for the observed antioxidant effects and to explore their potential clinical applications.

6. CONCLUSION

The findings of the present study demonstrate that Melaleuca alternifolia (tea tree) essential oil possesses significant biological activity, particularly with respect to its cytotoxic and antioxidant properties. The cytotoxicity assessment using the zebrafish larval model revealed a concentration-dependent reduction in larval viability, indicating the biological efficacy of the extract while maintaining acceptable levels of survival at lower concentrations. These results suggest that tea tree extract exhibits moderate cytotoxic potential and may serve as a promising candidate for further pharmacological investigations.

The antioxidant evaluation carried out through DPPH and nitric oxide scavenging assays confirmed the strong free radical scavenging ability of the tea tree extract. The antioxidant activity increased progressively with increasing concentrations, highlighting its effectiveness in neutralizing reactive oxygen and nitrogen species. The observed antioxidant potential can be attributed to the presence of bioactive phytochemicals such as phenolic compounds, terpenes, and terpene alcohols, which play a crucial role in preventing oxidative damage and maintaining cellular integrity.

Taken together, the results of this study indicate that M. alternifolia essential oil is a valuable natural source of bioactive compounds with considerable therapeutic potential. Its combined cytotoxic and antioxidant activities suggest possible applications in the development of pharmaceutical, nutraceutical, cosmetic, and healthcare products. Furthermore, due to its natural origin and broad spectrum of biological properties, tea tree oil may serve as an alternative to synthetic antioxidants and antimicrobial agents.

REFERENCES

[1] Mickienė R, Bakutis B, Baliukonienė V. Antimicrobial activity of two essential oils. Ann Agric Environ Med. 2011;18(1):139–44.
[2] Olivero-Verbel J, Quintero-Rincón P, Caballero-Gallardo K. Aromatic plants as cosmeceuticals: benefits and applications for skin health. Planta. 2024 Nov 5;260(6):132.
[3] Yang Y, Tang D, Zhang R, Liu M, Feng T. Integrating stress responses and secondary metabolism: the regulatory functions of histone deacetylases in medicinal plants. Front Plant Sci. 2026 May 28;17:1845669.
[4] Hong Y, Chen Y, Chen Z, Lin L, Wu J, Xu W, et al. Diversity patterns of medicinal plants along elevational gradients across forest layers in Meihua Mountains, Fujian. Front Plant Sci. 2026 May 29;17:1856248.
[5] Matsumoto S, Kasuga J, Kadowaki M, Ito M. Optimizing nitrogen fertilization for shaded cultivation of the medicinal plant Panax ginseng: implications for photosynthesis, root growth, and carbon accumulation in two-year-old plants. J Nat Med [Internet]. 2026 Jun 16; Available from: http://dx.doi.org/10.1007/s11418-026-02057-y
[6] Khadka D, Chen B, Xie Y, Shui K, Zhang X, Liu Z, et al. Ethnomedicinal plants used by Guoshan Yao minority communities in the foothills of Nanling Mountain, Guangdong, China. J Ethnobiol Ethnomed [Internet]. 2026 Jun

15; Available from: http://dx.doi.org/10.1186/s13002-026-00916-2

[7] Qiu L, Chen X, Luo Z, Jiang M, Chen H, Hou B, et al. Synthetic biology as a game-changer for endangered medicinal orchids: biosynthesis, engineering and conservation. Nat Prod Rep [Internet]. 2026 Jun 2; Available from: http://dx.doi.org/10.1039/d6np00027d

[8] Behtash F, Pashaei M, Beizaei Z, Seyed Hajizadeh H, Eghlima G, Karatas R, et al. Species-specific performance and nutritional responses of radish and red beet to zinc-manganese in sand culture. Sci Rep [Internet]. 2026 Jun 16; Available from: http://dx.doi.org/10.1038/s41598-026-58576-x

[9] Sathiyaseelan A, Zhang X, Wang MH. Enhancing the Antioxidant, Antibacterial, and Wound Healing Effects of Oil by Microencapsulating It in Chitosan-Sodium Alginate Microspheres. Nutrients [Internet]. 2023 Mar 7;15(6). Available from: http://dx.doi.org/10.3390/nu15061319

[10] Romano N, González A, Sciammaro L, Puppo MC, Quiroga A, Gómez-Zavaglia A. Structure-function relationships governing the multimodal antioxidant protection of quinoa protein hydrolysates. Food Chem. 2026 Jun 15;522:150073.