Griffith UNIVERSITY
Queensland, Australia

TECHNICAL REPORT

INVESTIGATION OF THE TEA TREE OIL VAPOUR FROM GELAIR AIR-CONDITIONING BLOCKS PERFORMANCE ON INACTIVATION OF AIRBORNE MICROORGANISMS

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7 May 2025

CONTENTS

1. INTRODUCTION

Tea Tree Oil (TTO), primarily obtained through steam distillation of the leaves of *Melaleuca alternifolia*, a plant native to Australia, is among the most widely used essential oils (Savla et al., 2020). Its disinfectant properties have been well-documented in scientific literature, showing effectiveness against a broad spectrum of microorganisms. These include both aerobic and anaerobic bacteria (Dzia, 2016; Hayley & Palombo, 2009; Carson et al., 2006; Wilkinson & Cavanagh, 2005; Salari et al., 2006), various fungal species (Hammer et al., 2000; Oliva et al., 2003; Li et al., 2016), and even viruses (Schnitzler et al., 2001; Cermelli et al., 2008; Garozzo et al., 2011, Pyankov et al., 2012).

Tea Tree Oil (TTO) is a complex and heterogeneous essential oil, with its chemical composition varying significantly depending on factors such as cultivation conditions, geographic origin, and harvesting methods (Moudachirou et al., 1999; Linge et al., 2024). These variations can directly influence the oil's efficacy, especially when used for antimicrobial purposes. The primary bioactive compound responsible for its antimicrobial properties is terpinen-4-ol, typically comprising 35–45% of the oil (ISO 4730: 2017). Another key constituent, 1,8-cineole, is generally present at lower concentrations (1–6%) but can also impact the oil's effectiveness and potential for irritation. In addition, components such as α-terpineol, terpinolene, and both α- and γ-terpinene are frequently detected and may act synergistically to enhance the overall antimicrobial activity of the oil (May et al., 2000; Li et al., 2021).

This complex composition contributes to TTO's broad-spectrum antimicrobial properties, enabling it to act against a wide range of bacteria, fungi, and viruses. As such, TTO has found growing use in healthcare settings, personal care products, and even in agricultural and veterinary applications. Its natural origin aligns well with increasing public demand for non-toxic, environmentally sustainable alternatives to synthetic disinfectants. Moreover, as global concern mounts regarding antimicrobial resistance (AMR), naturally derived substances like TTO offer a promising route for reducing reliance on conventional antibiotics and disinfectants, particularly in non-clinical environments.

In summary, TTO's potent antimicrobial activity, coupled with its renewable nature and growing societal interest in green alternatives, positions it as a promising candidate in the fight against microbial threats. Nevertheless, careful consideration of its chemical variability, potential toxicity, and formulation challenges is essential to fully harness its potential across diverse applications.

Griffith University has been engaged by Gelair Pty Ltd to undertake research focused on determination of antiviral activity of its Gelair Air-conditioning Blocks known as Gelair ABs produced by the company. The Gelair ABs have been successfully used around the world in 65 countries. As

agreed, the research has been focused on the investigation of inactivation of the Influenza A strain Puerto Rico/8/34 (PR/8) in aerosolised form when treated by vapour released by Gelair™ Air Conditioning Blocks (Gelair™ ABs) . All experimental procedures and virological analyses were conducted in Physical Containment Level 2 (PC2) laboratories by Professor Mahalingam's research group at the Institute for Glycomics, Griffith University.

2. MATERIALS AND METHODS

2.1. Microorganisms

The Influenza A strain Puerto Rico/8/34 (PR/8) was kindly provided by Professor Carl Feng, Faculty of Medicine and Health, University of Sydney. To prepare the viral suspension for aerosolization, the PR/8 strain was propagated in Madin-Darby Canine Kidney (MDCK) cells using maintenance medium comprising Eagle's Minimum Essential Medium (EMEM; ATCC), supplemented with 5% foetal calf serum (Bovogen Biologicals), 100 U/mL penicillin, 100 µg/mL streptomycin, and 1 µg/mL TPCK-treated trypsin (Merck), and incubated at 37°C with 5% CO_2.

MDCK cells, obtained from ATCC, were cultured in EMEM supplemented with 10% foetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1 µg/mL TPCK-treated trypsin, under the same incubation conditions. On completion, the final suspension was added to the nebulizer for following aerosolization to the rotational aerosol chamber used in all experiments (see description below). All viral preparations and experimental procedures were conducted within a Class II biological safety cabinet (Model BH 2000, Biolab, Australia) to ensure containment and prevent the release of viral aerosols into the laboratory environment.

2.2. Gelair product

Gelair™ Air Conditioning Blocks (Gelair™ ABs) were used in the experiments. Gelair™ ABs consist of a specially formulated and patented gel matrix block impregnated with Tea Tree Oil which delivers controlled and sustained release of high quality Australian Tea Tree Oil vapour for up to 12 weeks depending on air-conditioner use.

Tea tree oil is extracted from *Melaleuca alternifolia* strains by steam distillation of the leaves. TTO contain more than 100 separate components, mostly monoterpenes, sesquiterpenes and their alcohols, and while some components may induce allergic effects when applied to skin in rare cases, it is generally considered to possess a low toxicity (Carson et al. 2006), especially in dilute form. TTO can be

regarded as semi-volatile, however accurate volatility information is difficult to obtain, based on the variable multicomponent nature of TTO. TTO has a boiling range boiling range of about 100°C to 220°C.

2.3. Experimental setup

The laboratory setup used for this series of experiments is shown in Fig 1. A rotational toroid shaped 141 liters aluminium aerosol chamber (600mm diameter and 500mm long) was designed to keep materials in airborne form over extended periods of time (Pyankov et al. 2012). The optimal rotation speed was found to be around 7 rpm. The axis was designed to be stationary enabling to host pipelines used for aerosol charging and monitoring.

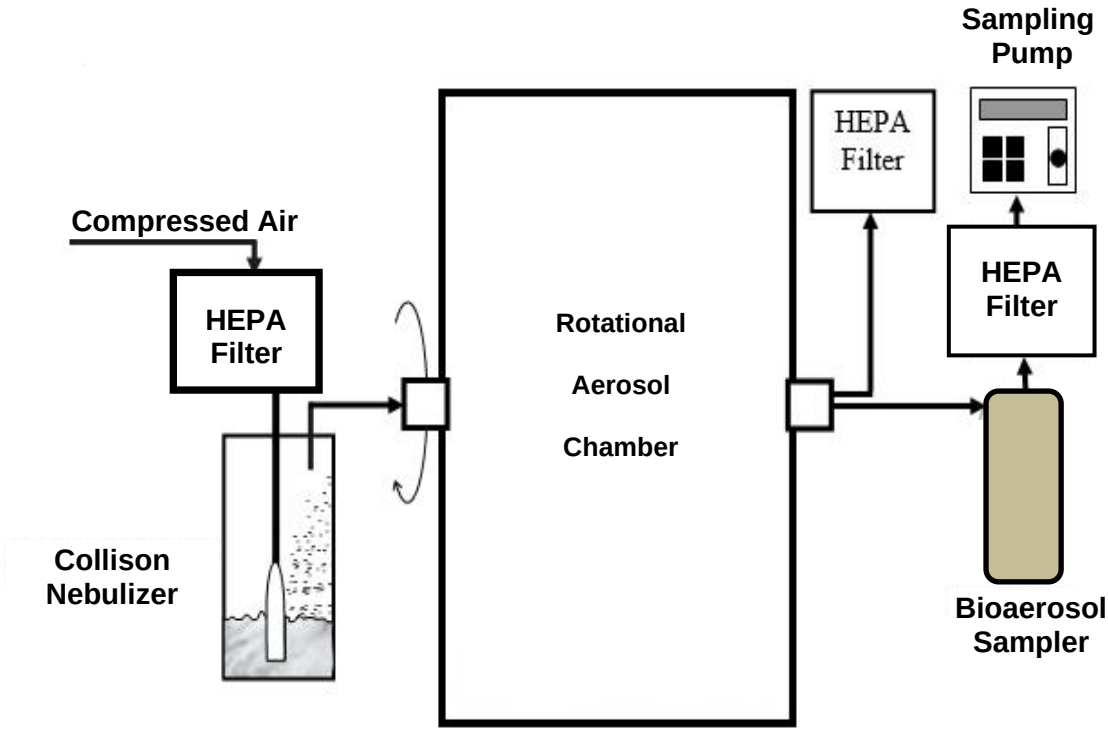

Figure 1. *Laboratory setup*

To eliminate any possibility of contamination of the laboratory space, the aerosol chamber along with all other parts of the laboratory setup was placed into the Class II biological safety cabinet (Model BH 2000, Biolab, Australia), as is seen in Fig. 2.

A small piece of AB gel of 0.25 kg with the product cloth covering was strategically placed on a stationary shelf installed inside a rotating aerosol chamber (Fig. 3). The chamber was then sealed and operated continuously for two hours to allow for thorough dispersion of Tea Tree Oil vapour throughout

the internal airspace, ensuring uniform exposure conditions for subsequent experiments. Then, the viral suspension, prepared according to the procedures described above, was aerosolised by the Collison nebuliser. The aerosol was supplied into the chamber over approximately 2 minutes achieving concentration equilibrium, and then the generator was stopped.

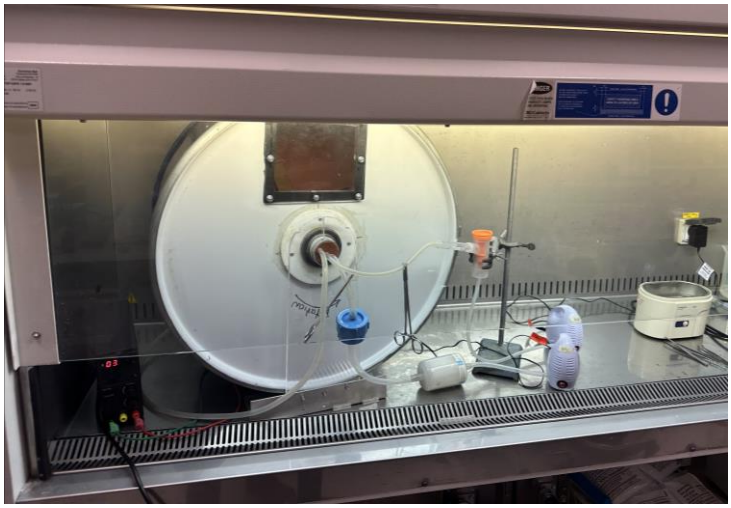

Figure 2. *Placement of the laboratory setup in the BSC*

The aerosol samples were collected by the 99.9% efficient wettable filter after 0, 30 and 60 min at the sample flow rate of 2.0 L/min over 120 seconds of sampling time. To minimise viral inactivation due to desiccation, the collecting filter media was wetted by EMEM and the sampling time shortened to 2 minutes. The temperature and RH were kept at levels of 25°C and 50 to 55%, respectively throughout all experimental runs.

Figure 3. *Location of Gelair™ AB inside rotational aerosol chamber*

Upon completion of sampling, the collecting filter was removed, placed into 5 mL of the EMEM media and sonicated in the sonic bath over 5 min ensuring all collected viruses to be transferred to the liquid. Then, 200µL aliquot of the collected virus suspension was used for Plaque Assay described below.

Considering that at each sampling point approximately 4 litres of air were removed from the chamber (200 litres) and replaced with HEPA filtered air of the same quantity, the following equation was

used to correct each particular result to account for such dilution:

$$C_t = C_{Mt} \times 1.031^n, \quad (1)$$

where C_t is corrected microbial concentration at time t, C_{Mt} is the actually measured microbial concentration at time t, and n is the time related point number (at 0 min, n= 0; at 30 min, n=1; etc.).

To isolate the specific effect of the product on viral inactivation and eliminate potential confounding factors, the entire experimental procedure was conducted under two controlled conditions: (1) in the presence of the product (4 repeats) and (2) in its absence (4 repeats). By comparing the outcomes from these two scenarios, any observed differences in viral inactivation could be attributed solely to the activity of Tea Tree Oil (TTO) vapour released by the product.

2.4. Plaque Assay of Influenza A strain in the Samples Collected from Aerosol Chamber

MDCK cells were grown to a density of $2.5x10^5$ cells per in 12-well tissue culture plates and incubated overnight at 37°C in a humidified atmosphere with 5% CO_2. Following incubation, the culture medium was aspirated, and cells were washed twice with sterile phosphate-buffered saline (PBS). Each well was then inoculated with 200 µL of virus suspension containing 1 µg/mL TPCK-treated trypsin, applied in serial dilutions. Plates were incubated for 1 hour at 37°C with 5% CO_2, with gentle rocking every 20 minutes to ensure even distribution and adsorption of the virus.

After viral adsorption, 1 mL of overlay medium was added to each well. The overlay consisted of serum-free EMEM supplemented with 1% bovine serum albumin (BSA), 100 U/mL penicillin, 100 µg/mL streptomycin, 1 µg/mL TPCK-treated trypsin, and 1% low-melting-point (LMP) agarose. The plates were then incubated for 3 days at 37°C with 5% CO_2 to allow for plaque development.

On day 3, the overlay was carefully removed, and each well was stained with 1 mL of 0.1% crystal violet in 3.7% formaldehyde for 20 minutes at room temperature. The stain was then aspirated, and wells were rinsed thoroughly under running tap water and left to air dry. Plaques were counted to determine viral titre, expressed as plaque-forming units per millilitre (PFU/mL), calculated using the following formula:

$$C\left(\frac{PFU}{ml}\right) = \frac{N}{D \times V}, \quad (2)$$

where N is the number of plaques counted, D is the dilution factor, and V is the volume of the viral suspension (ml) added to the wells.

3. RESULTS

The results for microbial inactivation of Influenza A strain Puerto Rico/8/34 (PR/8) aerosols are shown in Figure 4. All results were normalised according to Equation (1). The error bars show standard deviation of 4 experimental runs.

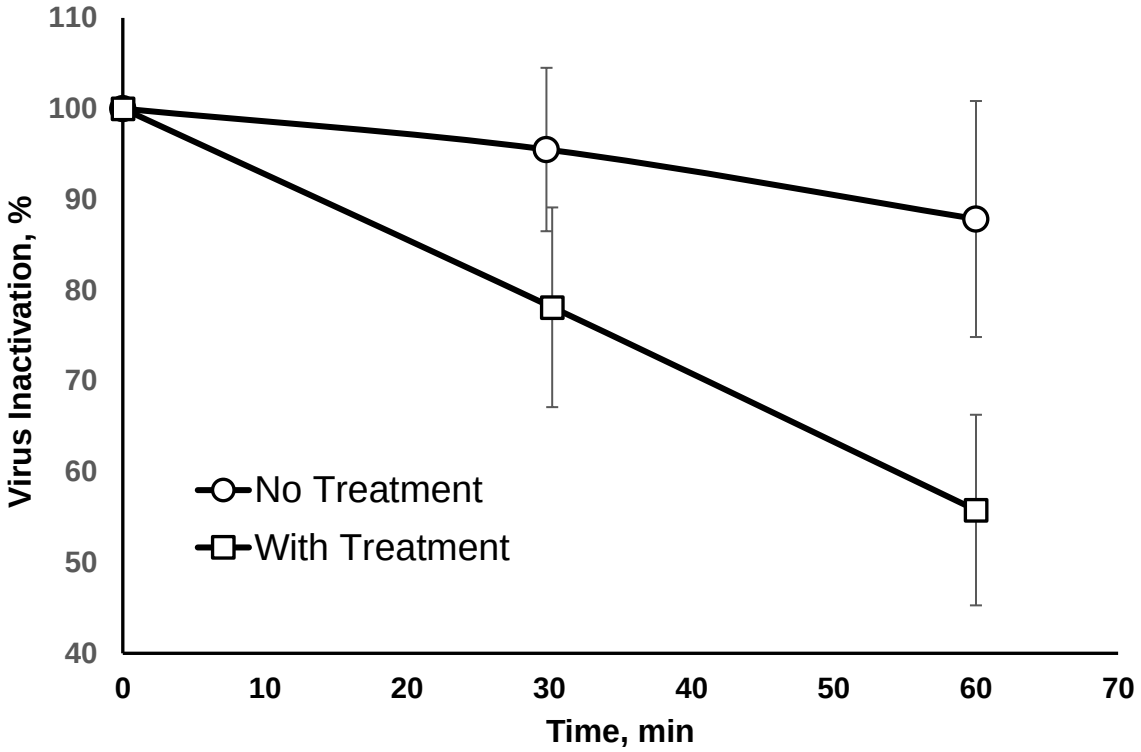

Figure 4. *Microbial inactivation of Influenza virus.*

As illustrated in the results, the natural inactivation of the virus under the "No Treatment" condition was relatively slow, with only 4.5% and 12.3% viral reduction observed after 30 and 60 minutes of the process run, respectively. In contrast, a significantly faster rate of viral inactivation was observed under the "With Treatment" condition, where the product containing Tea Tree Oil (TTO) was present. In this case, only 78.12% of the virus remained viable after 30 minutes, and 55.7% remained viable after 60 minutes, indicating a notable enhancement in antiviral activity due to TTO vapour exposure.

To quantify the sole contribution of TTO to viral inactivation, the efficiency (E%) was calculated using the standard inactivation efficiency formula:

$$E(\%) = 1 - \frac{C_{WT}}{C_{NT}}, \qquad (3)$$

where C_{WT} is the viral concentration obtained for "With Treatment" scenario and C_{NT} is the concentration of virus in absence of the TTO. Using this approach, the calculated TTO-specific inactivation efficiencies were 18.2% after 30 minutes and 36.5% after 60 minutes. These findings indicate that TTO vapour markedly improves the rate of viral inactivation, highlighting its strong potential for use in air sanitation. Moreover, the findings align well with existing literature, which supports TTO's effectiveness against viruses such as Influenza A and B, SARS (including SARS-CoV-2/COVID-19), RSV, and herpes (Wong and Strappe, 2024). The Gelair™ ABs typically release TTO vapour over several weeks, which suggests they may provide sustained inactivation of the Influenza A virus.

4. CONCLUSIONS AND RECOMMENDATIONS

This study assessed the efficiency of Gelair™ Air Conditioning Blocks (Gelair™ ABs) in inactivation of airborne Influenza A (PR/8) virus aerosols. Under untreated conditions, the virus exhibited only modest levels of natural decay over time, however the application of TTO vapour significantly accelerated viral inactivation. After 30 minutes of exposure to the TTO-containing product, the viable viral concentration decreased to 78.12% and further dropped to 55.7% after 60 minutes. These figures indicate that TTO vapour contributes meaningfully to disrupting viral integrity or viability in airborne form.

By applying a standard inactivation efficiency calculation, the TTO-specific contributions to viral inactivation were quantified as 18.2% at 30 minutes and 36.5% at 60 minutes. This analysis underscores that the antiviral activity observed is not solely due to natural decay but can be directly attributed to the presence of TTO vapour in the environment.

Overall, these findings suggest that TTO vapour holds significant promise as a natural antiviral agent capable of reducing the viability of aerosolised influenza virus in indoor air. This could have practical implications for public health, particularly in the context of airborne disease control and prevention in enclosed or poorly ventilated environments. The integration of TTO-based treatments into air purification systems or surface sprays may offer a complementary approach to existing infection

control measures, especially during seasonal influenza outbreaks or pandemics involving airborne respiratory viruses.

REFERENCES

Carson, C. F., Hammer, K. A. & Riley, T. V. (2006). Melaleuca alternifolia (Tea Tree) Oil: A Review of Antimicrobial and Other Medicinal Properties. *Clin. Microbiol. Rev.* 19: 50–62.

Cermelli, C., Fabio, A., Fabio, G. & Quaglio, P. (2008). Effect of Eucalyptus Essential Respiratory Bacteria and Viruses. *Curr Microbiol.* 56: 89–92.

Dzia, A. W. (2016). Evaluation of the tea tree oil activity to anaerobic bacteria-in vitro study. *Acta Poloniae Pharmaceutica*, 73: 389.

Garozzo, A., Timpanaro, R., Stivala, A., Bisignano, G. & Castro, A. (2011). Activity of Melaleuca alternifolia (tea tree) oil on Influenza virus A/PR/8: Study on the mechanism of action. *Antiviral Research.* 89: 83–88.

Hammer, K. A., Carson, C. F. & Riley, T. V. (2000). Melaleuca alternifolia (tea tree) oil inhibits germ tube formation by Candida albicans. *Med. Mycol.* 38: 355–362.

Hayley, L. A. & Palombo, E. A. (2009). Activity of Essential Oils Against Bacillus subtilis Spores. *J. Microbiol. Biotechnol*. 19(12): 1590–1595.

Li, W. R., Li, H. L., Shi, Q. S., Sun, T. L., Xie, X. B., Song, B., & Huang, X. M. (2016). The dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi. *Applied microbiology and biotechnology*, 100: 8865-8875.

Li, X., Shen D., Zang, Q., Qiu, Y., & Yang, X. (2021). Chemical Components and Antimicrobial Activities of Tea Tree Hydrosol and Their Correlation With Tea Tree Oil. *Natural Product Communications*. 16: 9. doi:10.1177/1934578X211038390

Linge, K. L., Cooper, L. & Downey, A. (2024). Comparison of Approaches for Authentication of Commercial Terpinen-4-ol-type Tea Tree Oils Using Chiral GC/MS. *J. Agric. Food Chem.* 72 (15): 8389-8400.

May, J., Chan, C.H., King, A., Williams, L. & French, G.L. (2000). Time-Kill Studies of Tea Tree Oils on Clinical Isolates. *J. Antimicrob. Chemother*. 45 (5): 639 – 643.

Moudachirou, M., Gbenou, J. D., Chalchat, J. C., Chabard, J. L. & Lartigue, C. (1999). Chemical composition of essential oil of eucalyptus from Benin: Eucalyptus citriodora and E. camaldulensis. Influence of location, harvest time, storage of plants and time of steam distillation. *Journal of Essential Oil Research*. 11: 109-118.

Oliva, B., Piccirilli, E., Ceddia, T., Pontier, E., Aureli, P. & Ferrini A. M. (2003). Antimycotic activity of Melaleuca alternifolia essential oil and its major components. *Lett Appl Micobiol*. 37: 185–187.

Pyankov, O.V., Usachev, E., Pyankova, O. & Agranovski, I.E. (2012). Inactivation of Airborne Influenza Virus by Tea Tree and Eucalyptus Oils. *Aerosol Science and Technology*. 46: 1295 – 1302

Salari, M. H., Amine, G., Shirazi, M. H., Hafezi, R. & Mohammadypour, M. (2006). Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. *Clin Microbiol Infect* 12: 194–196.

Savla, K., Le, J. T. & Pucker, A. D. (2020). *Tea tree oil for Demodex blepharitis*. Cochrane Database of Systematic Reviews, (6).

Schnitzler, P, Schön, K. & Reichling, J. (2001). Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie*. 56(4): 343-7.

Soylu, E. M., Kurt, Ş., & Soylu, S. (2010). In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. *Int J Food Microbiol*, 143(3): 183-189.

Wilkinson, J. M. & Cavanagh, H. M. (2005). Antibacterial activity of essential oils from Australian native plants. *Phytother Res*. 19: 643–646.

Wong, R. & Strappe. P. (2004). *Virucidal effects of tea tree oil. AgriFutures Australia publication no. 24-237*, Wagga Wagga NSW Australia.