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Antiviral activity of tea tree and eucalyptus oil aerosol and vapour

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ABSTRACT

Our previous studies demonstrated high antiviral efficiency of natural disinfectants, i.e. tea tree oil (TTO) and eucalyptus oil (EUO) on the filter surface. The TTO aerosol challenge as disinfectant showed its high antiviral potential. The main aim of this study was to investigate the antiviral activity of TTO and EUO aerosols in range of concentrations against Influenza A virus and *E. coli* phage M13. It was found that both tested oils aerosols possess strong antiviral action and capable of inactivating model viruses with efficiency of more than 95% within 5–15 min of exposure. Additionally, the TTO and EUO vapors were also challenged for their antiviral activity. The use of natural disinfectants like TTO and EUO in aerosol form as well as in vapour phase looks very promising for further development of virus inactivating procedures and technologies for air quality applications.

1. Introduction

Bioaerosols in occupational and residential environments could potentially make a considerable impact on human and animal health. These are generally complex mixtures consisted of microorganisms and their constituents. Live microorganisms could cause severe infections in humans. Since there is some unavoidable presence of hazardous bioaerosols in indoor and outdoor environments, the issue of effective bioaerosol deactivation is becoming increasingly important research subject. The effective ways to minimize infection risks of direct exposure to airborne pathogens are the air filtration, exposure to UV light and bioaerosol deactivation with chemical reagents. A range of bioaerosol deactivation approaches and technologies are presented in the literature for last decades. Some filtration enhancing procedures could be realised in a way of utilization of unipolar ions (Huang et al., 2008), electrostatic charging of the filter media (Raynor & Chae, 2004), coating of fibers with liquids (Agranovski & Braddock, 1998; Boskovic et al., 2007), and others. The photocatalytic microbial decomposition (Grinshpun et al., 2007; Vohra et al., 2006), infrared thermal decomposition (Damit et al., 2011) as well as using chemicals directly delivered into the air or applied onto the filter surface (Huang et al., 2010; Pyankov et al., 2008) show an effective bioaerosol deactivation. Despite the variety of proposed approaches, some new searches for new antimicrobial agents, which are effective against bioaerosols and possess minimal hazard for human health, is also a crucial way for indoor air quality control applications.

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A potential use of essential oils obtained from higher plants as disinfectants in liquid form was clearly showed in recent studies (Carson et al., 2006; Cermelli et al., 2008; Garozzo et al., 2011; Hammer et al., 2000; Hayley & Palombo, 2009; Oliva et al., 2003; Reichling et al., 2009; Salari et al., 2006; Schnitzler et al., 2001; Wilkinson & Cavanagh, 2005). As previously shown, the essential oils are usually heterogeneous mixture of various chemicals (Antonelli & Fabbri, 1999; Carson et al., 2006; Guillen & Cabo, 1996), with considerable batch to batch alteration of constituents (Kawakami et al., 1990; Moudachirou et al., 1999). An antimicrobial activity of essential oils' vapour phase was also intensively studied over last decades. However, most of studies were focused on antibacterial and antifungal activities of essential oils vapour (Bouaziz et al., 2009; Gocho, 1991; Smith-Palmer et al., 1998; Inouye et al., 2001; Inouye et al., 2006), whereas antiviral aspect was investigated quite poorly (Reichling et al., 2009). In addition, there are no studies available on treatment of airborne virus by such disinfectants.

Recently, we investigated an antiviral efficiency of tea tree oil (TTO) and eucalyptus oil (EUO) engaged into microbial inactivation on the filter surface, and reported significant antiviral efficiency of both substances (Pyankov et al., 2012).

Considering current strong interest towards natural disinfectants, the present study is logical continuation of our previous investigations with the focus on the assessment of antiviral activity of essential oils (TTO and EUO) aerosols as well as their vapour phases.

2. Materials and methods

2.1. Cells and virus

Madin–Darby canine kidney (MDCK) cells, from the American Type Culture Collection (Bethesda, MD), were kept in a humidified 5% carbon dioxide atmosphere at 37 °C and grown in Dulbecco's Modified Eagle Medium (DMEM) High Glucose 1x medium (GIBCO, USA) supplemented with 15 mM Hepes (pH = 7.4), 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL of penicillin, 100 μg/mL of streptomycin.

Influenza virus A strain NWS/G70C (H11N9) (Air et al., 1987) was obtained from Commonwealth Scientific and Industrial Research Organization, Australia and was propagated in MDCK cells in virus maintenance medium composed of DMEM containing 15 mM Hepes (pH = 7.4), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 1 μg/mL of tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin (Worthington Biochemical, Freehold, NJ).

To prepare viral suspension for aerosolization, MDCK cells infection was performed with virus at a multiplicity of infection of 0.05 per cell. After 1 h of adsorption at 37 °C, the cells were washed twice with phosphate-buffered saline (PBS), placed in medium, and then incubated at 37 °C with 5% CO_2. Virus was harvested when the cytopathic effect was visually evident, usually 3–4 days after inoculation. The virus working stock solution was prepared as cellular lysate using virus maintenance medium, sonicated 5 min in ultrasound bath and then centrifuged ($5000 \times g$ for 30 min at 4 °C) at concentration 5×10^7 PFU/mL. Final suspension was diluted with virus maintenance medium to 5×10^6 PFU/mL and used for aerosolization.

Bacteriophage M13 (ATCC 15669-B1) along with the host *E. coli* strain NM522 (Agilent Technologies, Inc., USA), were used as a second model system for bioaerosol study. The microorganism was recovered from freeze-dried and propagated on plates with soft-agar/host overlay. Host strain of *E. coli* was cultured in 2YT medium broth (Bacto tryptone 16 g; yeast extract 10 g; NaCl 5 g; distilled water 1 L). To prepare viral stock, a log-stage host cells culture was infected with phage from a single plaque and incubated overnight at 37 °C with constant agitation. Overnight phage culture was centrifuged at high speed twice to remove cells, and then chloroform to prevent microbial growth was added. Final phage suspension was diluted to 2×10^7 PFU/mL and used for aerosolization. Phage suspension for aerosolization as well as phage samples diluents were made of PBS.

2.2. Oils

Tea tree oil (Felton Grimwade & Bosisto's Pty Ltd, Oakleigh South, Australia) is originated from *Melaleuca alternifolia* strains (native to New South Wales, Australia). Pharmaceutically graded eucalyptus oil (Felton Grimwade & Bosisto's Pty Ltd, Oakleigh South, Australia) originated from *Eucalyptus polybractea*, containing more than 70% cineole (eucalyptol), was also used in the experiments. The light mineral oil (LMO) was used for negative control scenario due to its biologically inactive nature (Sigma-Aldrich, USA).

3. Experimental setup and procedure

3.1. Aerosol generation

Oil aerosols were generated by a Collison nebulizer (BGI Inc., Waltham, MA) from undiluted oil stock and its concentration in the chamber was controlled by the device operation time (2, 5, 10, 15 and 30 s). Considering the oil flowrate of 0.1 g/min (confirmed by weighing of the device before and after nebulisation) and the aerosol chamber volume of 200 L, the corresponding concentrations of oil were 16.7, 41.7, 83.3, 125 and 250 μg/L of the air in the chamber. Viral