

# Protective Essential Oil Attenuates Influenza Virus Infection: An in Vitro Study in MDCK Cells

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## Abstract

**Background:** Influenza is a significant cause of morbidity and mortality. The recent pandemic of a novel H1N1 influenza virus has stressed the importance of the search for effective treatments for this disease. Essential oils from aromatic plants have been used for a wide variety of applications, such as personal hygiene, therapeutic massage and even medical practice. In this paper, we investigate the potential role of an essential oil in antiviral activity.

**Methods:** We studied a commercial essential oil blend, On Guard™, and evaluated its ability in modulating influenza virus, A/PR8/34 (PR8), infection in Madin-Darby canine kidney (MDCK) cells. Influenza virus was first incubated with the essential oil and infectivity in MDCK cells was quantified by fluorescent focus assay (FFA). In order to determine the mechanism of effects of essential oil in viral infection inhibition, we measured hemagglutination (HA) activity, binding and internalization of untreated and oil-treated virus in MDCK cells by flow cytometry and immunofluorescence microscopy. In addition, the effect of oil treatment on viral transcription and translation were assayed by relative end-point RT-PCR and western blot analysis.

**Results:** Influenza virus infectivity was suppressed by essential oil treatment in a dose-dependent manner; the number of nascent viral particles released from MDCK cells was reduced by 90% and by 40% when virus was treated with 1:4,000 and 1:6,000 dilutions of the oil, respectively. Oil treatment of the virus also decreased direct infection of the cells as the

number of infected MDCK cells decreased by 90% and 45% when virus was treated with 1:2,000 and 1:3,000 dilutions of the oil, respectively. This was not due to a decrease in HA activity, as HA was preserved despite oil treatment. In addition, oil treatment did not affect virus binding or internalization in MDCK cells. These effects did not appear to be due to cytotoxicity of the oil as MDCK cell viability was only seen with concentrations of oil that were 2 to 6 times greater than the doses that inhibited viral infectivity. RT-PCR and western blotting demonstrated that oil treatment of the virus inhibited viral NP and NS1 protein, but not mRNA expression.

**Conclusions:** An essential oil blend significantly attenuates influenza virus PR8 infectivity in vitro without affecting viral binding or cellular internalization in MDCK cells. Oil treated virus continued to express viral mRNAs but had minimal expression of viral proteins, suggesting that the antiviral effect may be due to inhibition of viral protein translation.

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