

Investigating the antiviral activity of volatile compounds from *Nigella sativa* against coronaviruses

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The recent emergence of three major coronaviruses and presence of coronaviruses circulating in bats suggests that spillover of new pandemic-potential coronaviruses into humans is likely in future. The development of pan-coronavirus antivirals will be crucial to combat this. Here, we investigated the antiviral activities of *Nigella sativa* (black cumin) oil extracts from various global locations against seasonal human coronaviruses OC43 and 229E, and SARS-CoV-2 pseudoviruses.

Coronavirus-infected cells were directly treated with oil extracts and antiviral activity determined by quantifying viral titres. In diffusion assays, oils were incubated in microwell plates with virus in adjacent wells to investigate the effect of diffused volatile compounds. After incubation over a range of times, infectivity was determined.

Our diffusion assay results indicate that volatile compounds present in *Nigella sativa* extracts show antiviral activities against coronaviruses, with no cytotoxic effect on cells. Significant inhibition of infection was observed after 12 hours incubation, with the most potent oils showing a $\geq 4 \log_{10}$ reduction in OC43 infectivity at 24 hours. Interestingly, direct treatment of infected cells with oils showed limited antiviral efficacy, suggesting that the vapour phase may offer higher concentrations of the bioactive compounds without compromising cell viability. We also identified key volatile compounds present in the oil vapour phase; evaluation of the antiviral activity of these volatiles in isolation and in synergy are ongoing.

Overall, this work provides a first step towards identifying novel pan-coronavirus antiviral compounds that can be formulated as sprays or inhalers for direct delivery to the site of infection.