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Nutraceuticals: An Alternative Treatment for Influenza Virus
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Abstract
Influenza virus is a contagious respiratory pathogen that infects hundreds of thousands of people a year, making it a serious global health concern. The virus has a rapid mutation rate and has developed resistance to current antiviral agents, making it a difficult target for effective treatment. There is an increasing need to identify new treatments for Influenza. Recently, we have tested nutraceuticals as an effective alternative for blocking Influenza. We have tested Jamaican Sorrel, Black Currant Berries, and Manuka Honey together with Bee Pollen for antiviral activity. We have demonstrated that these nutraceuticals block the 2009 Pandemic California Influenza strain between a concentration range of 1:8-1:16 dilution. Stock solutions of these nutraceuticals are extracted with water to create a roughly 300 millennialsolute. They are then neutralized with HEPES buffer, 1N HCl and 1N NaOH.

Methods & Materials

Virus and Cell Culture
We use the A/CA/0709 strain for the CPE assay. MDCK cells are maintained in Dulbecco Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum, 0.5% streptomycin and penicillin, and 0.02% HEPES buffer.

Extraction and Isolation
Powders from the plant products are boiled and cooled until the insoluble portion of the powder is collected. The remaining solution is then centrifuged and the supernatants are collected. This process is repeated three times. The supernatants are added together and boiled down to about 50 ml. Next, the raw extract is filtered through a coffee filter and syringe system. It is then standardized to 280 millimoles and to a pH of 7.4 (± 0.2) by the addition of 50% of 1M HEPES buffer, as well as 1M H2C and 1N NaOH accordingly. After standardizing the pH and osmolarity the extract is autoclaved.

Cytopathic Effect Assay
MDCK cells are seeded at 2 x 10⁴ cells per well in 96-well plates in 5% FBS/DMEM and incubated for 48 h (37°C, 5% CO₂). The ground virus is agitated with a neutralized solution containing neutralizing antiserum and 2% DMEM. The infected cells are plated on 37°C, 5% CO₂ for one hour allowing the virus to adsorb and attach to the cells. The virus is removed and the cells are inoculated with a nutraceutical solution to a final stock dilution. The plates are incubated with 5% CO₂ at 37°C for 48 h. The cells are then stained with crystal violet and the optical density is measured at 590 nanometers in a spectrophotometer. The virus is considered for inhibition when a 100% reduction of viral replication is reached.

Results

Jamaican Sorrel

Black Currant Berries

I:1 Manuka Honey & Bee Pollen

Conclusions
We conclude that the administration of Jamaican Sorrel, Black Currant Berries, and the 1:1 mixture of Manuka Honey and Bee Pollen have antiviral activity against the 2009 pandemic California strain of influenza virus. Jamaican Sorrel, Black Currant Berries, and the mixture of Manuka Honey and Bee Pollen should be considered for further testing and the development of new antiviral compounds.

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References