Cell-based experimental evidence to the anti-COVID-19 potential of Ashwagandha and honeybee propolis ingredients

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Background - The COVID-19 pandemic emerged in December 2019 by a novel strain of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has led to new endeavours in repurposing of existing drugs, anti-COVID-19 vaccine and drug development. Natural products, due to their general safety and wider availability, have attracted research and public attention. In this study, we report anti-COVID potential of compounds from honeybee propolis and Ayurvedic herb, Ashwagandha. Effect of active ingredients was studied on human cell surface receptors (ACE-2:Angiotensin Converting Enzyme-2/Spike protein and TMPRSS2:Transmembrane Protease Serine 2), critical for virus infection and virus main protease (M^{pro}, essential for virus replication), through molecular simulations and *in vitro* experiments.

Methods - Structure-based computational analyses were performed to predict the effect of honeybee propolis (CAPE: Caffeic Acid Phenethyl Ester and ARC: Artepillin C), and Ashwagandha (Withanolides) ingredients on virus-host cell surface receptors. Cell-based assays were used to investigate the effect of these compounds on the expression level of the target proteins and virus replication.

Results - Ashwagandha-derived nine withanolides were tested *in silico* for their potential to target and inhibit (i) ACE-2 and TMPRSS2 receptors (ii) viral main protease M^{pro}. We found that most withanolides possess capacity to bind to ACE-2, TMPRSS2 and M^{pro}. On the other hand, CAPE and ARC showed stable interactions at the active site of ACE2 and M^{pro}. ARC, but not CAPE, showed stable interaction with TMPRSS2. Human cells treated with withanolides, CAPE or ARC showed downregulation of both the receptors. Furthermore, cell- and PCR-based SARS-CoV-2 replication assays endorsed their antiviral activity.

Conclusion - The findings suggest that the Ashwagandha-withanolides and honeybee propolis-derived compounds, CAPE, and ARC, could be helpful in the reduction of viral replication/infection, and hence warrant further experimental and clinical attention.