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Efficacy of natural compounds from *Tinospora cordifolia* against SARS-CoV-2 protease, surface glycoprotein and RNA polymerase.

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Short title: Efficacy of *Tinospora cordifolia* against SARS-CoV-2

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Abstract

Background: Antiviral activity of natural compounds from *Tinospora cordifolia* (Amritaballi) were evaluated for their efficacy against SARS-CoV-2 targets involved in virus attachment and replication. **Materials and Methods:** The binding efficacy (binding affinity, K_i and IC_{50} values) of natural compounds from *Tinospora cordifolia* were tested using Insilco tools against four key SARS-CoV-2 targets i.e., 1) surface glycoprotein (6VSB) and 2) Receptor binding domain (6M0J) both responsible for attachment of the virus to host cell, 3) RNA dependent RNA polymerase (6M71) and 4) main protease (6Y84) responsible for replication of the virus in the host cell.

Results: Berberine, Isocolumbin, Magnoflorine and Tinocordiside showed high binding efficacy against all the four key SARS-CoV-2 targets. Tinocordiside and Isocolumbin showed IC_{50} value of $< 1 \mu\text{M}$ against both 6Y84 and 6VSB.

Conclusion: At least four natural compounds from *Tinospora cordifolia* showed high binding efficacy against SARS-CoV-2 targets involved in attachment and replication of the virus. Hence validating the merit of using *Tinospora cordifolia* in the clinical management of infection caused by SARS-CoV-2.

Key Words: Protease inhibitors, RNA polymerase inhibitors, Coronavirus, Covid-19, Antiviral drugs, SARS-CoV-2, Viral entry.

Introduction

SARS-CoV-2 strain of coronavirus, which was first reported from the Wuhan region of China has caused a major disruption to everyday living worldwide.^[1, 2] Recent reports suggest that the patients recovering from the illness caused by SARS-CoV-2 (COVID-19)^[3] don't develop effective immunity against SARS-CoV-2. Considering this poor immunity against SARS-CoV-2 in the recovered patients,^[3] it is unlikely that a vaccine based approach or using hyperimmune serum will be beneficial in treating the patients with active infection. Also the currently available antiviral drugs (both approved and in clinical trials) are reported to have limited efficacy against SARS-CoV-2.^[2, 4, 5] Hence there is an urgent need for identifying effective antiviral drugs against SARS-CoV-2.^[6]

Natural products have been viable and sustainable source of antimicrobial agents in the past and several natural compounds are reported to have antiviral properties.^[5, 7-9] Recently we have shown the efficacy of neem and tulsi based natural compounds against the key SARS-CoV-2 targets.^[10] *Tinospora cordifolia* (TC), a deciduous, extensively-spreading, climbing herbaceous vine of the family Menispermaceae is considered an essential herbal plant in the Indian system of medicine.^[11, 12] It is referred to by the following common names, Chakralakshanika (Sanskrit), Gurcha (Hindi) and Amritaballi (Kannada). The phytoconstituents of TC are reported to have antioxidant, antibacterial, antifungal, anti-diabetic, hypolipidemic, antimalarial, hepatoprotective and anticancer activities.^[11-15] Besides this, the plant extract is also shown to have wound healing, and immunomodulating properties.^[15, 16] The leaf extract of TC is reported to have antimicrobial activity against gram-positive and gram-negative bacteria, clinically relevant fungal infections and malarial parasites.^[13, 15, 16] The antimicrobial properties of TC extracts is attributed to its phytoconstituents such as furanolactone, tinosporon, jateorine and columbin.^[15-17] The silver nanoparticles from *Tinospora cordifolia* were reported to be effective against chikungunya virus.^[17] Phytochemicals from TC are also reported be effective inhibitors of H1N1 viral proteins.^[5] TC is also a vital component of Shilajatu Rasayana (an Ayurvedic preparation), which was reported to synergistically improve efficacy of anti-retroviral therapy in HIV positive patients.^[18] Considering the positive reports of antiviral activity of *Tinospora cordifolia*, in this study the efficacy of some selected natural compounds reported in *Tinospora cordifolia* extracts were screened using Insilco pharmacology against three SARS-CoV-2 targets (Surface

glycoproteins (6VSB, 6M0J); RNA dependent RNA polymerase (6M71) and Main Protease (6Y84)).

Materials and Methods

The 3D structure of SARS-CoV-2 targets (surface glycoproteins (6VSB; 6M0J), RNA dependent RNA polymerase (6M71) and main protease (6Y84)) were downloaded as PDB files from the protein data bank (<https://www.rcsb.org/>) and were optimized for molecular docking in the Chimera software.^[19]

The structures of selected natural compounds in *Tinospora cordifolia* (Berberine, Tinocordiside, CordifolisideA, Jatrorrhizine, Magnoflorine, Isocolumbin, Sinapic acid, Syringin and Palmatine) were accessed from PubChem database and processed into PDB file format and minimised for molecular docking using the Chimera software.^[19, 20] Molecular docking was performed to evaluate the binding efficacy of these compounds against the SARS-CoV-2 targets using AutoDock Vina (version 1.5.4) and the docked protein-ligand complex were visualised using the Chimera and PyMOL v 1.8.2.0 software^[19-22]. AutoDock-MGLTools was employed to visualize and modify the receptor and ligand structures to PDBQT file formats. The PDBQT file formats of the ligand and receptor were used for molecular docking using the AutoDock Vina program. Ligands were docked individually to the receptor with grid coordinates and grid boxes of specific sizes for each receptor centralised in the AutoDock-MGLTools. The output file was saved in the PDBQT format and the ligand-receptor binding affinity estimated as negative Gibbs free energy (ΔG) scores (Kcal/mol), were documented based on AutoDock Vina scoring function. Post-docking analyses were visualized using PyMOL and Chimera, giving details of the sizes, locations of binding sites, hydrogen-bond interactions of the docked ligand in various conformations.

Simulation of dose response curves: Dose-response curves were modelled based on nonlinear regression analysis approach by specifying IC_{50} values as independent variable and response (% inhibition) as dependent variable. IC_{50} values were estimated from the binding affinity values using the following formula $IC_{50} = \exp(\Delta G/RT) (1 + ([S]/K_m))$. Where ΔG = Binding affinity (Kcal/mol), $RT = 298K$, S = substrate concentration, K_m =Michaelis constant. The increase in the ligand dose increases results in sequential changes to the response (% receptor inhibition) eventually achieving minimum or maximum response limits. As the 20 to

80% response is linear, this was modelled using four-point logistic increments of IC_{50} values (0.5x, 1x, 1.5x and 2x) and averaged. The resulting equation from this was $y = 26.362x - 158.85$ ($R^2 = 0.9967$). This one standard deviation increase and decrease of the IC_{50} values was estimated from this equation. The resulted three IC_{50} values estimated were used to calculate the $IC_{5, 20, 60, 80}$ and 100 values by employing a five-parameter logistic equation^[23]. The means of the $IC_{5, 20, 60, 80}$ and 100 values obtained (in x axis) were plotted against the % inhibition response (in y axis) to obtain the simulated dose response curves.

Results

Berberine and Isocolumbin showed superior binding affinity against all the four SARS-CoV-2 targets (6VSB, 6M0J, 6M71 and 6Y84) (Figure 1). Berberine, Isocolumbin, Magnoflorine and Tinocordiside had similar but best binding affinity against SARS-CoV-2 protease (6Y84) (Figure 1). Berberine and Isocolumbin had optimal binding affinity against SARS-CoV-2 surface glycoprotein (6VSB) as well as RNA polymerase (6M71) (Figure 1). Berberine, Tinocordiside and Isocolumbin had better binding affinity against SARS-CoV-2 receptor binding domain (6M0J) (Figure 1). All the natural compounds tested except Sinapic acid had binding efficacy (K_i and IC_{50} values) against the SARS-CoV-2 targets at a therapeutically feasible concentration (Figure 2). Tinocordiside and Isocolumbin showed IC_{50} value of $< 1 \mu M$ against both 6Y84 and 6VSB (Figure 2) suggesting their high efficacy in preventing SARS-CoV-2 attachment to host cells and replication.

Discussion

The natural compounds from *Tinospora cordifolia* tested in this study showed therapeutically feasible efficacy against SARS-CoV-2 targets involved in attachment of the virus to host receptor (6VSB, 6M0J) and its replication (6M71, 6Y84). Although antiviral properties of *Tinospora cordifolia* has been reported before against H1N1 and Chikungunya Virus,^[5, 17, 18] to best of our knowledge, this is the first study to report pharmacological activity of *Tinospora cordifolia* against coronavirus in general and SARS-CoV-2 in specific. Interestingly the reported efficacy (Binding affinity (Kcal/mol), K_i (μM) and IC_{50} (μM) values) of Favipiravir (-6.24 ± 0.35 , 26.17 ± 1.81 , 52.32 ± 3.62), Lopinavir/Ritonavir (-6.97 ± 0.45 , 7.72 ± 0.22 , 15.44 ± 0.43) against 6Y84,

efficacy of Remdesivir (-7.69 ± 0.29 , 2.28 ± 0.16 , 4.55 ± 0.33) against 6M71 and efficacy (Binding affinity (Kcal/mol), Ki (μM) and IC₅₀ (μM) values) of STGYC (-6.10 ± 0.27 , 33.39 ± 1.57 , 66.79 ± 3.14) against 6VSB was either similar or inferior to the natural compounds from *Tinospora cordifolia*.^[6, 10]

6VSB and 6M0J are responsible for viral attachment, while 6Y84 (main Protease) and 6M71 (RNA dependent RNA polymerase) are involved in viral replication.^[2, 6, 24]

Hence *Tinospora cordifolia* is likely to be efficacious in preventing SARS-CoV-2 attachment to the host cells and as well prevent replication of the virus. Several natural compounds from *Tinospora cordifolia* are reported to have anti-bacterial and immunostimulant properties, these pharmacological effects together with their specific antiviral effects against the SARS-CoV-2 may merit its use in the treatment of COVID-19. The validation of the pharmacological effects of *Tinospora cordifolia* against SARS-CoV-2 is timely as currently effective therapeutics to treat COVID-19 are not available. *Tinospora cordifolia* being a natural product with well-established safety profile through its time tested use in Ayurveda,^[13, 15] can be quickly adapted for clinical use to manage COVID-19.

Conflict of interest: none

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Figure legends

Figure 1: Binding location and affinity (Kcal/mol) of various confirmation of natural compounds (red) from *Tinospora cordifolia* against SARS-CoV-2 targets (surface glycoproteins (6VSB), receptor binding domain (6M0J), RNA dependent RNA polymerase (6M71) and main protease (6Y84)). The table represents the mean \pm SD of binding affinity (Kcal/mol) of nine different confirmation of natural compounds against the specific SARS-CoV-2 target.



