Ayurveda botanicals in COVID-19 management: An *in silico*-multitarget approach

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Abstract

The Coronavirus disease (COVID-19) caused by the virus SARS-CoV-2 has become a global pandemic in a very short time span. Currently, there is no specific treatment or vaccine to counter this highly contagiousdisease. Presently, existing anti-virals and disease-modifying agents are being repurposed to manage COVID-19. There is an urgent need to find a specific cure for the disease and global efforts are directed at developing SARS-CoV-2 specific anti-viralsand immunomodulators.The objective of this study is to explore the immunomodulatory and anti-SARS-CoV-2 potential of key phytoconstituents from Ayurveda based Rasayana drugs, *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi) and *Asparagus racemosus* (Shatavari) using *in silico* approaches like network pharmacology, and molecular docking. The SWISS-ADME tool was used to predict the pharmacokinetic and pharmacodynamic (PK-PD) interactions and drug likeliness potential. Using these approaches we propose a library of phytomolecules with potential to be developed as phytopharmaceuticals for COVID 19 management.The plant extracts were prepared as per Ayurvedic procedures and a total of 31 phytoconstituents were identified using HPLC and MS studies. The network pharmacology model shows that these phytoconstituents possess the potential to modulate several immune pathways. Amongst the three botanicals*Withania somnifera* was found to be the most potent immunomodulator through its potential to modulate T cell differentiation, NK cell cytotoxicity as well as T cell, B cell and NOD-like receptor signalling pathways.Molecular docking studies showed thatseveral phytoconstituents possess good affinity for the Spike protein, Main Protease and RNA dependent RNA polymerase of SARS-CoV-2 suggesting their application for the termination of viral life cycle. Further, predictive tools indicate that there would beneficial herb-drug pharmacokinetic-pharmacodynamic interactions with concomitantly administered drug therapy. We thus make a compelling case to evaluate the potential of these *Rasayana* botanicals in the management of COVID-19 following rigorous experimental validation.

Introduction

The, SARS-CoV–2 virus is responsible for causing the ongoing Coronavirus disease (COVID–19) pandemic[1]. Higher infectivity as compared to the SARS-CoV virus reported in 2003 and absence of a definite cure are the worrisome aspects of SARS-CoV–2.[2]. In the view of rapid spread in short period of time, the number of people infected globally is enormous (~ over 2 million), and this poses a tremendous challenge to healthcare systems.COVID–19 has an age related skewed distribution of morbidity and an overall lethality although the numbers are changing as the disease is progressing [3]. The Centre for Disease Control and Prevention (CDC) reported that, COVID–19 patients with co-morbidities such as chronic lung diseases (e.g. asthma, COPD), hypertension, obesity, Type 2 Diabetes Mellitus (T2DM) are vulnerable to a higher mortality rate[4].

The SARS-CoV–2 primarily attacks lung alveoli for its replication. The Spike protein of the virus binds to Angiotensin Converting Enzyme–2 (ACE–2) receptors on the surface of type-II pneumocytes of alveolar lining, which are then internalised and +ssRNA is released[5]. With the help of host ribosomal machinery and the RNA-dependent RNA polymerase (RdRp) enzyme SARS-CoV–2 synthesizes its polyproteins and multiplies its +ssRNA. The new copies of SARS-CoV–2 are released into the alveolar sac by destroying the infected pneumocytes. The inflammatory mediators released after pneumocyte damage recruit immune cells at the infected site. Macrophages release inflammatory cytokines into the blood leading to vasodilation of blood vessels increasing capillary permeability of endothelial cells. Neutrophils release reactive oxygen species (ROS) and proteases to destroy viruses which also damage normal pneumocytes and generate cellular debris in alveolar space. Theseinflammatory and immune responses result into alveolar consolidation leading to increased respiratory rate followed by cough. The systemic inflammatory response acts as messengers to hypothalamus to increase body temperature[6].In some patients,the cytokine response goes out of control leading to excessive collateral damage to organs with a possible progression to death[7,8].

Presently there is no cure for the disease however, the treatment is symptomatic. In some countries, patients are being treated using existing combinations of antivirals used for other viral infections[9]. Clinical evidence explaining the efficiency of these antivirals against SARS-COV–2 are limited and indefinable [10]. Therefore, for developing specific therapies as well as for boosting speed and scale of clinical evaluation WHO launched Solidarity clinical trial on April 8, 2020. This includes screening of four study treatments in comparison with standard of care. Based on available experimental data, remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta–1α, and chloroquine or hydroxychloroquine are chosen study drugs [11]. For patients with co-morbidities, it is inevitable to take daily medication along with COVID–19 managing drugs. Therefore, it is required to have safe pharmacotherapy for COVID–19 that can be co-prescribed with WHO solidarity trial drugs and commonly prescribed drugs such as anti-hypertensive, anti-asthmatic and anti-diabetic. Administering plasma of a recovered patient to the critically ill COVID–19 patients seems promising[12].Currently there is limited evidence on the safety and efficacy of Hydroxychloroquine which is being used in many countries for COVID–19 treatmentunder emergency circumstances[13,14].There is a great rush to find vaccines and therapeutics against SARS-CoV–2. Several pharmaceutical companies have announced clinical trials for drug and vaccine candidates. However, it may take a long time to reachthe community.

Traditional medicine systems such as Ayurveda, have a holistic approach of consideringmind-body-physiologyto deal with disease conditions[15]. The Ayurvedic philosophy suggests delivering “a group of phytoconstituents” that holds potential to act on multiple targetsof SARS-CoV–2 and also of immune pathways to give adaptogenic and immunomodulatory effect[16,17,18]. In Ayurveda, "*Rasayana* botanicals” are used for rejuvenation by boosting the immune system and alleviating disease condition[19,20,21]. Of several known botanicals *Asparagus racemosus* (AR), *Tinosporacordifolia* (TC), and *Withaniasomnifera* (WS) are known to modulate the immune system and possess antiviral activities[19,20,22,23,8].

Therefore, the ideal COVID–19 therapy should show (a) anti-viral properties against SARS-COV–2, (b) be safe for concomitantly administered drugs like anti-hypertensive, anti-diabetic, anti-asthmatics, and drugs those are used in respiratory tract infections (c) should modulate immune system with rejuvenation ability (mainly for cardio-respiratory and nervous system) (d)should show therapeutic adjuvant activity with drugs used in WHO Solidaritytrial and COVID–19 associated comorbidities.

In this work, phytochemicals present in AR, TC and WS were identified using chromatographictechnique. A network pharmacology model was used to identify and depict the interactions of bioactives with molecular targets in the immune system to unravel their immunomodulatory role. Further, the phytoconstituents were docked to three molecular targets of SARS-CoV–2 to assess the potential for antiviral activity. Further, predictive tools were used to assess the potential of interactions between phytoconstituents and commonlyprescribeddrugs.In summary, we present a compelling case that Ayurvedic *Rasayana* botanicals have the potential to be used as anti-SARS-CoV–2 agent having immunomodulatory and therapeutic adjuvant activity for COVID–19management.

Results

1. Extraction of test materials and phytochemical analysis: The percent yield of hydro alcoholic extract of WS, TC, and AR were found to be 10%, 6.6%, and 30% respectively. The percent yield of water extract of WS, TC, and ARwere 12%, 07% and 20% respectively.

Total of 31 phytoconstituents were identified from these plant extracts using HPLC and LCMS. The AR extract showed the presence of Asparagamine A, Asparanin A, Isoagatharesinol, Muzanzagenin, Rutin, Shatavarin-I,Shatavarin-IV, Shatavarin-IX, Shatavarin-VI, Shatavarin-VII, and Shatavarin-X. The TC extract showed the presence of 20-Hydroxy Ecdysone, Berberine, Columbamine, Columbin, Magnoflorine, Menisperine, Syringin, Tinocordiside, Tinosporaside and Tinosporide. WS extract showed the presence of 12-Deoxywithastramonolide, 27-Hydroxywithanone, Ashwagandhanolide, Withacoagin, Withaferin, Withanolide-A, Withanolide-B, Withanone, Withanoside-IV, Withanoside-V. The structures of 31 phytoconstituents are given in Supplementary (S1) Table.These phytoconstituents were used for the further studies

2. Molecular Docking: To identify if any of the 31 phytoconstituents might possess therapeutic potential against SARS-CoV–2, they were docked to three important drug targets of the virus, the Spike protein, the Main Protease and the RNA dependent RNA polymerase. Molecular docking was performed using the protocol as described in the methods section.

2.1 Several phytoconstituents are predicted to possess good affinity for the Main Protease (Mpro): Therewere84crystal structures of the Main Protease (Mpro) in the PDB at the time of writing this manuscript. The largest share of the deposition is a series of Mpro crystal structures obtained by fragment screening (Fearon, D., Unpublished). This indicates that the binding site of Mprois druggable. The Mprois known to be functional as ahomodimer and has a heart-like shape. The protein has one active site per monomer. The active site contains the catalytic cysteine-Cys145, thatperforms the proteolysis reaction. The structure, PDB ID: 5R84, solved at a resolution of 1.83Å was chosen for this study. In this structure, the protease is co-crystallized with the fragmentcyclohexyl-N-(3-pyridyl) acetamide (Z31792168) which is seen to bind in theS1-S3pocket of the protease (Figure 1a). The S1 pocket is characterized with the presence of His163, Glu166, S2 with Cys 145 and S3 is known as the aromatic wheel and includes Phe181 and His41. Thus, in our studies weplaced the docking grid over the S1-S3 pocket. Initially, the co-crystallized ligand was separated and docked to check if Autodock was able to reproduce the binding mode. The docked pose showed an RMSD of 0.46 Å with thecrystal structure pose and adocking score of –6.2 kcal/mol(Figure 1A and B). A detailed study of interactions revealed that the carbonyl group of Z31792168shows a H-bond with Glu166 in the S1 pocket. The cyclohexylmoiety of the ligand has hydrophobic interactions with Met165, His41, His164, Arg188 andGln189 residue in the S3 pocket. Thepyridine ring scaffold shows hydrophobic interactions with Phe140 and His163 in the S1 pocket. All these interactions are in line with the crystallographic pose.

Thereafter, 31 phytoconstituents were docked to the binding site. The results are compiled in Table 1.It is a frequent observation that compounds with a docking score better than –6 kcal/mol have a higher probability of being active *in vitro* and *in vivo*and hence use this score as a cutoff [24]. In our study we observed that, 18 out of 31 phytoconstituents have a docking score better than –6 kcal/mol.The best docking score –9.9 kcal/mol was observed for Ashwagandhanolide. Many other phytoconstituents like Withacoagin, Withaferin and Withanoneare observed to have docking scores close to the –9 kcal/mol. However, a point to be noted is that some of these

phytoconstituents are large molecules and hence the docking scores may appear to be inflated due to size and the proportionately larger number of interactions. Ligand efficiency is a measure of the activity corrected for the ligand size[25]. A calculation of the ligand efficiency indicates that the co-crystallized ligand, Z31792168, has a ligand efficiency of −0.4. Among the phytoconstituents, the highest ligand efficiency, −0.3, is observed forColumbin. Several other phytoconstituents have comparative ligand efficiency namely, Tinocordiside, MagnoflorineandIsoagatharesinol.Columbin and Tinocordisidenot only have docking scores close to −8 kcal/mol but also have high ligand efficiencies. Similarly, Withacoagin, Withaferin, Withanolide-A, B and Withanone have good docking scores and ligand efficiencies. Interestingly, all compounds also follow Lipinski Rules which helps to distinguish between drug like and non-drug like molecules. A detailed study of interactions of M^{pro} with Columbin(Figure 1 c and d) revealed that the ligand interacts with His163 in the S1 pocket, and several residues in the S2 and S3 pockets in a manner like Z31792168. The detailed interactions of Tinocordiside (Figure 1 E and F) reveal that the Hydoxymethyl group forms hydrogen bonds with Glu166 and the keto group forms hydrogen bond with His163 in the S1 pocket. The ligand forms three additional hydrogen bonds with Gln189, Thr190 andArg188. Additionally, hydrophobic interactions are observed with amino acids Phe140, Asn142, His164, Met165 and Pro186.

2.2 Several phytoconstituents are also predicted to possess good affinity for theRNA dependent RNA polymerase (RdRp):For the RdRp of SARS-CoV−2 the PDB ID: 6M71 was chosen for this study26. This is a cryoEM structure solved at a resolution of 2.9 Å. Residues 367 to 920 of the structure form the RdRp domain. The authors report that a structural comparison of theSARS-CoV−2RdRp with that of Poliovirus (PDB ID: 3OL6) and HCV indicates that the polymerase domain adopts a conserved structural architecture. The residues Arg553, Lys545 and Arg555 form the NTP entry channel while residues Asp760 and Asp761 coordinate divalent cations that stabilize the phosphate group (the cations are absent from PDB ID: 6M71 as it is an apo structure). We placed our grid over the entire RNA binding site based on the structure of Poliovirus RdRp that is co-crystallised with RNA.

Remdesivir is an ATP analog and is being evaluated as a potential treatment for SARS-CoV−2 as an inhibitor of RdRp based on results obtained for MERS and SARS-CoV[27].

Remdesivir is a prodrug whose active metabolite is GS−441524 (PubChem CID: 44468216). GS−441524 was docked to the RNA binding site. The docked results (Figure 2 a and b) indicate that the active metabolite forms hydrogen bonds with Asp760 which is an important active site residue and with Lys621 and Tyr619.The position of the active metabolite is that of the NTP entry channel. The docking score for the metabolite is −4.28 and the ligand efficiency is −0.2. Next, 31 phytoconstituents were docked to the binding site. The results are compiled in Table 1. Withanolide-B, Withacoagin, Withanone, Ashwagandhanolide and Muzanzagenin are predicted to possess docking scores ranging from ~ −9 to 10 kcal/mol. A total of twenty-one phytoconstituents have a docking score better than −6 kcal/mol. Calculation of ligand efficiency reveals that the top binders include Muzanzagenin, WithaolideB, Withacoagin and Magnoflorine. There are sixteen compounds with ligand efficiency better than the active metabolite. However, it is to be noted that the GS−441524 is a chain terminating nucleotide analog, while these compounds would be pure blockers. The detailed interactions of Muzanzagenin with RdRp (Figure 2 C and D) reveal two hydrogen bonds with the critical residues Asp760 and Asp761, and non-polar interactions over the NTP entry channel. The binding pose and interactions of Withanolide-B(Figure 2 E and F) also show interactions with the residues in NTP entry channel and the cation coordinating residues.

2.3 Phytoconstituents from WS are predicted to possess good affinity for theSpikeprotein:The entry of coronaviruses into cells is owing to the interaction between the clove shaped trimeric Spike viral protein with the human ACE2 receptors on the cell surface. Since this is the first and crucial point of contact between the viral protein with the human receptor this is a sought-after target for design of vaccine and therapeutics28. There are several structures of the SARS-CoV2 Spike protein with the human ACE2 protein. We chose the structure PDB ID: 6M17 for our docking studies29. This is a Single Particle cryoEM structure solved at a resolution of 2.9 Å. This structure represents the Receptor Binding Domain (RBD) of Spike protein with full length human ACE2. The Spike protein RBD interacts with the Peptidase Domain (PD) of the ACE2 in a 1:1 ratio. The interactions between the RBD and PD are mediated by residues Gln24, Lys417, Tyr453, Gln474, Phe486, Gln498, Thr500 and Asn501 of RBD. These residues are divided into three clusters, two clusters at each end and one in the center of the RBD. We placed the docking grid around all of these residues in an attempt to find molecules that could disrupt the contacts between RBD and PD.

The docking results with 31 phytoconstituents reveal that the compounds bind in various areas of the interface region of RBD. The top scoring compound is Ashwagandhanolide with a docking score of −10 kcal/mol. Ten phytoconstituents have a docking score better than −6 kcal/mol. Withacoagin, 27-Hydroxywithanone and Withanolide-B are predicted to have docking scores of −7.6, −7.6 and −7.4 kcal/mol respectively. The ranking based on ligand efficiency places Withacoaginat the top with a value of −0.23. Several compounds including Withanolide-B have a ligand efficiency of −0.22. Detailed analysis of the top docked pose of Withacoagin (Figure 3 A and B) indicates that it binds over middle and terimnal cluster of residues that interact with PD. The ligands form hydrogen bonds with Ser494, Tyr495 and Arg403 and vdW interactions with Phe497, Asn501, Gln493, Tyr453, Leu455 and Phe456.Withanolide-B only interacts with the terminal cluster of

residues and shows hydrogen bonds with Thr500 and Asn501 and non-polar interactions with Tyr453, Ser494, Tyr495, Arg403, Tyr505, Gly496 and Gln498(Figure 3 C and D).

Table 1: Docking details of the phytoconstituents from AR, TC and WS to the main drug targetsof SARS-COV-2

Ligand	Heavy atoms	Main Protease		Spike Glycoprotein (RBD)		RNA dependent RNA polymerase	
		Docking Score	Ligand efficiency	Docking Score	Ligand efficiency	Docking Score	Ligand efficiency
Asparagamine A	28	-7.1	-0.25	-5.4	-0.19	-6.3	-0.22
Asparanin A	52	-6.3	-0.12	-5.8	-0.11	-7.5	-0.14
Isoagatharesinol	21	-5.8	-0.28	-4.6	-0.22	-5.0	-0.24
Muzanzagenin	32	-7.6	-0.24	-7.1	-0.22	-9.3	-0.29
Rutin	43	-5.3	-0.12	-3.3	-0.08	-3.6	-0.08
ShatavarinI	74	-1.1	-0.01	-1.7	-0.02	-3.3	-0.04
ShatavarinIV	62	-2.7	-0.04	-6	-0.1	-6.3	-0.10
ShatavarinIX	63	-0.6	-0.01	-5.4	-0.09	-6.7	-0.11
ShatavarinVI	62	-4.9	-0.08	-4.6	-0.07	-7.5	-0.12
ShatavarinVII	62	-6.6	-0.11	-5.7	-0.09	-6.3	-0.10
ShatavarinX	66	-0.4	-0.01	-4	-0.06	-5.2	-0.08
20-Hydroxy Ecdysone	34	-6.4	-0.19	-4.8	-0.14	-5.7	-0.17
Berberine	25	-6.3	-0.25	-5	-0.2	-5.7	-0.23
Columbamine	25	-5.9	-0.23	-4.6	-0.18	-5.4	-0.22
Columbin	26	-7.9	-0.30	-5.8	-0.22	-6.8	-0.26
Magnoflorine	25	-7.0	-0.28	-5.2	-0.21	-6.6	-0.27
Menisperine	26	-6.9	-0.27	-5.2	-0.2	-6.2	-0.24
Syringin	26	-5.3	-0.20	-3	-0.11	-4.2	-0.16
Tinocordiside	28	-8.1	-0.29	-5.1	-0.18	-6.6	-0.24
Tinosporaside	35	-7.9	-0.23	-5.7	-0.16	-6.6	-0.19
Tinosporide	27	-4.3	-0.16	-4.6	-0.17	-4.4	-0.16
12-Deoxywithastramonolide	34	-8.1	-0.24	-6.9	-0.2	-7.9	-0.23
27-Hydroxywithanone	35	-8.6	-0.25	-7.6	-0.22	-7.6	-0.22
Ashwagandhanolide	69	-9.9	-0.14	-10	-0.14	-10.2	-0.15
Withacoagin	33	-8.8	-0.27	-7.6	-0.23	-8.8	-0.27
Withaferin	34	-8.8	-0.26	-6.9	-0.2	-8.5	-0.25
WithanolideA	34	-8.5	-0.25	-6.7	-0.2	-8.2	-0.24
WithanolideB	33	-8.3	-0.25	-7.4	-0.22	-8.9	-0.27
Withanone	34	-8.8	-0.26	-7.1	-0.21	-8.9	-0.26
WithanosideIV	55	-5.6	-0.10	-4.6	-0.08	-5.8	-0.11
WithanosideV	54	-6.1	-0.11	-5.1	-0.1	-6.0	-0.11

3. Network pharmacological analysis of Rasayana Botanicals associated with immune pathways:Rasayana botanicals have immunomodulatory potential and help in rejuvenation of body homeodynamics30,31. To explore this, we have followed network pharmacology approach.

The constructed network represents total 19 bioactives from *Rasayana* botanicals associated with 306 unique human protein targets. Of these, 53 protein targets were found to be involved in 20 immune pathways referred as immune targets (Figure 4).The distribution of immune targets amongst *Rasayana* botanicals has been shown in Venn diagram (Figure 5b) AR, TC, and WS showed association with 18, 19, and, 20 immune pathways through involvement of 19, 25, and, 27 immune targets respectively (Figure 5c).The 7 common targets associated with all three *Rasayana*botanicals are Bcl−2-like protein 1 (*BCL2L1)*,, glycogen synthase kinase−3 beta (*GSK3B)*,, Interleukin−2 (*IL2)*,, prostaglandin G/H synthase 1 and 2 (*PTGS1* and *PTGS2)*,, prothrombin (*F2)*,, and signal transducer and activator of transcription 3

(*STAT3)*.. These immune targets are found to be involved in 11 different immune pathways including chemokine and specific receptor signaling (NOD-like, C-type lectin, BCR, and TCR), immune cell differentiation (Th1, Th2, and Th17), platelet activation and coagulation cascade, and intestinal IgA production.

The compiled data showed 137, 192, and 109 human protein targets of 9bioactives of AR, and 10 bioactives of TC, and WS each respectively. The immune pathway data retrieved 1104 unique protein targets from 20 different human immune pathways of KEGG database (data not showed). Further data analysis showed the association of AR, TC, and WS bioactives with all immune pathways through several protein targets (Figure5). The bioactives i.e. shatavarins of AR, tinosporides of TC, and withanolides of WS play crucial role in immunomodulation[32,8,20].According to traditional medicine or Ayurveda principles, the synergistic effect of bioactive combination in an extract strengthens physiological immunity superior than a single molecule[33]. We analysed the data of bioactive-target association and mapped against immune pathways to explore the synergism principle by network pharmacology approach [34]. The analysis showed involvement of AR in Th17 cell differentiation with 24 combinations of 8 bioactives and 6 targets. AR was also found to be involved in IL–17 signaling with 13 combinations of 9 bioactive and targets. Data analysis also showed association of TC with chemokine signalling with 13 combinations of 7bioactives and 5 targets. TC was found associated with few other pathways such as NOD-like receptor signaling, leukocyte trans-endothelial migration, and NK cell mediated cytotoxicity by moderate bioactive-target associations.

The analysis also retrieved multimodal involvement of WS in immunomodulation through various pathways. It is found to regulate chemokine signaling at a greater extent with maximum 66 combinatorial associations of 9 withanolides with 11 immune targets. This is followed by potential of WS in FC-gamma R-mediated phagocytosis and receptor signaling (NOD-like and c-type lectin) by 54, 55, and 46 bioactive-target associations. WS was also found to modulate T cell differentiation, NK cell cytotoxicity and signalling pathways of IL–17, TCR and BCR etc. The multidimensional correlation with immune pathways underlines the importance of WS in managing the immunopathology of COVID–19 by improving T cell, B cell and NK cell function and hence anti-viral immunity.

Predicting Herb-Drug Interactions:

Swiss-ADME, chemoinformatics platform was used to predict pharmacokinetics and drug likeliness potential of the 31 phytoconstituents. The Swiss-ADME pharmacokinetic data which also includes the effect of drug metabolising enzymes (CYPs) and transporters(Pgp) on the 31 phytoconstituentswas used to predict the herb-drug interactions if any. It showed that phytochemicals of AR except, Isoagatharesinolmay not inhibit any of the major CYP isoforms. Few TC phytochemicals may act as inhibitor of CYP1A2, CYP2D6 and CYP3A4 whereas few WS phytochemical may inhibit CYP2C9 (Table 2). Bioavailability RADAR analysis showed, 9 phytoconstituents of TC are orally bioavailable and lie within pink region of graph (supplementary S1 Appendix). Out of 11 phytochemicals of AR only Muzanzagenin and Asparagamine-A was found to be orally bioavailable. For WS, except Ashwagandhanolide, Withanoside V and Withanoside IV all other analyzed phytochemicals were found to be orally bioavailable. Muzanzagenin and Asparagamine A from AR, all analyzed phytochemicals of TC except 20-β-Hydroxy Ecdysone and Tinosporaside and all analyzed phytochemicals of WS except Ashwagandhanolide, Withanoside V and Withanoside-IVhavedrug-likeness properties. Except Syringin, AsparagamineA and Isoagatharesinolall the phytomolecules are the substrate of P-gp transporter. Muzanzagenin, Asparagamine A from AR and Columbamine, Berberine,Magnoflorine, Menisperine from TC showed ability to penetrate the blood brain barrier (BBB).Log Kp (Kp in cm/s) is skin permeation coefficient that assesses ability of test molecule to permeate through skin. Higher negative value indicates lesser skin permeability. This is directly correlated with molecular size and lipophilicity of compounds [35]. Log Kp values shown by all analyzed phytochemicals indicate lower skin permeability. These properties need to be considered during phytopharmaceutical development. However, these predictions may not be the same for extracts as they contain multiple phytoconstituents.

COVID–19 pathogenesis mainly involves respiratory system and afterwards it leads to multiple organ failure based on patient related factors: sex, age, disease, individualization (PRF: SADI). As discussed earlier, diabetic, obese, asthamatic, geriatric, hypertensive population is more prone for COVID–19[36]. These associated comorbidities of COVID–19 are being continually treated along with anti-SARS-COV–2 therapy.Here, we propose the use of *Rasayana* botanicals for COVID–19 prophylaxis (both pre and post COVID–19) as well as anti-SARS COV–2 activity. This can lead to the chances of HDIs, which maybe harmful/beneficial/fatal[37]. Therefore, with the help of Swiss-ADME data (Table 2) and available published literature (Supplementary S2 Table)the probable pharmacokinetic HDI were explored. This showed that, whole plant extracts of these botanicals (especially those that are prepared according to the Ayurvedic procedure)do not inhibit main CYP isoforms such as, CYP1A2, CYP2C9, CYP2D6 and CYP3A4. Various extracts of AR, TC and WS have IC_{50} values >100 µg/mL for these CYP isoforms (Supplementary S2 Table).This indicatesthat athigher concentrations (generally more than therapeutic dose), these extracts may produce pharmacokinetic HDI *in vivo*[38].Based on the above results, these phytoconstituents or *Rasayana* botanicals may produce beneficial

pharmacokinetic-pharmacodynamic interactions *in vivo,* with the anti-viral and disease-modifying drugs that are currently being prescribed for COVID–19.

Table 2: *In silico* pharmacokinetic analysis of AR, TC, and WS phytochemicals

Herbs	Phytoconstituents	Predicted oral bioavailability[§]	Predicted Drug-likeness[¥]	Pharmacokinetics				
				CYP[£]	P-gp substrate	BBB permeability	GI absorption	Log Kp (cm/s)
Asparagus racemosus	Muzanzagenin	Orally bioavailable	Y	N	Y	Y	High	-6.94
	Asparanin A	Not orally bioavailable because polarity, solubility and size axes lie outside pink region	N	N	Y	N	Low	-8.48
	Asparagamine A	Orally bioavailable	Y	N	N	Y	High	-6.95
	Isoagatharesinol	Not orally bioavailable because saturation parameter does not lie within pink area	Y	CYP2D6, CYP3A4 only	N	N	High	-6.43
	Rutin	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.26
	Shatavarin I	Not orally bioavailable because of higher polarity, molecular weight and flexibility	N	N	Y	N	Low	-13.47

	Shatavarin IV	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.53
	Shatavarin VI	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.53
	Shatavarin VII	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.97
	Shatavarin IX	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.99
	Shatavarin X	Not orally bioavailable because of higher polarity, molecular weight and flexibility	N	N	Y	N	Low	-11.23
Tinospora cordifolia	Berberine	Orally bioavailable	Y	CYP1A2, CYP2D6, CYP3A4 only	Y	Y	High	-5.78
	Columbamine	Orally bioavailable	Y		Y	Y	High	-5.94

	Menisperine	Orally bioavailable	Y		Y	Y	High	-6.30
	Magnoflorine	Orally bioavailable	Y	CYP1A2, CYP3A4 only	Y	Y	High	-6.44
	Tinopsoraside	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-9.13
	Tinosporide	Orally bioavailable	Y	CYP2D6 only	Y	N	High	-7.64
	Tinocordiside	Orally bioavailable	Y	N	Y	N	High	-8.56
	Syringin	Orally bioavailable	Y	N	N	N	Low	-9.50
	Columbin	Orally bioavailable	Y	N	Y	N	High	-6.95
	20-β-Hydroxy Ecdysone	Orally bioavailable	N	N	Y	N	High	-8.91
Withania somnifera	Withacoagin	Orally bioavailable	Y	CYP2C9 only	Y	N	High	-6.29
	Withanolide B	Orally bioavailable	Y		Y	N	High	-5.76
	Withastramonolide-12-Deoxy	Orally bioavailable	Y		Y	N	High	-6.35
	Withanoside IV	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-10.37
	Withanolide A	Orally bioavailable	Y	N	Y	N	High	-6.86
	Withanone	Orally bioavailable	Y	N	Y	N	High	-7.01

	Withaferin A	Orally bioavailable	Y	N	Y	N	High	-6.45
	27-hydroxy Withanone	Orally bioavailable	Y	N	Y	N	High	-7.60
	Withanoside V	Not orally bioavailable because of higher molecular weight and polarity	N	N	Y	N	Low	-9.79
	Ashwagandhanolide	Not orally bioavailable as four axes namely of lipophilicity, size, polarity and solubility lies outside pink area	N	N	Y	N	Low	-6.95

Note: § Predicted oral bioavailability from RADAR graph (Supplementary file: S1 Appendix)

£ Predicted CYP Inhibitionfor CYP1A2, CYP2C9, CYP2C19, CYP2D6and CYP3A4

¥Predicted Drug-likeness (Results of Lipinski, Ghose, Veber, Egan and Muegge rules)

Discussion

The understanding of pathophysiology of COVID−19 is emerging with increasing prevalence over the globe[39]. Severe infections of SARS-CoV−2 lead to mortality due to severe acute respiratory syndrome accompanied with hypoxia followed by organ failure[6]. A wide variation in the patient population rangingfrom asymptomatic, to mild or moderate cases and severe cases (some showing relapse) is reported. In general, we need to have drugs that are best prophylactic (pre and post COVID−19), immunomodulatory and adaptogenic in nature along with anti-SARS-CoV−2 action. Using *in silico* approaches, the present study shows that the selected *Rasayana*botanicals may have these all the actions and may be effective for management of COVID−19(Figure06).

In the following sections we discuss in detail how these *Rasayana* botanicals and their phytoconstituents can be potential drugs for the effective management of COVID−19.

Immune-bolstering activity: SARS-CoV−2 attacks pulmonary pneumocytes in alveolar sac for viral replication[7]. Typically, the immune response to viruses involve cytotoxic T-cells.The viral-infected host cells are recognised through binding of T-cell receptors (TCR) to MHC-I molecules. In case of viruses that escape from TCR recognition, physiological immune surveillance employs NK cells to eliminate virus infected cells. Cytotoxicity triggered by cytokines and other cytotoxic mediatorsinduce infected cells to undergo apoptosis[40]. Ouranalysis showed that, withanolides of WS may augment TCR signallingpathway by modulating NF-kappa-β regulators and theta type protein kinase C (PK-C). Withanolides may also involve in NK cell mediated cytotoxicity by modulating beta and gamma type PK-C.Rutin of AR and 20-hydroxyecdysone of TC may also help in destroying infected cells by regulating cytotoxicity mediator TNF-α. Magnoflorine and menisperine of TC found to modulate apoptosis through caspase proteins. They may involve in mediating innate immunity by regulating proteolytic cascade of complement system through coagulation factors. Shatavarins of AR were found to modulate antigen processing and

presentation through heat shock proteins.Withanolides may interact with integrin protein and interleukins to intensify non-inflammatory intestinal IgA secretion to neutralize virus.It has also been reported that Withanone and Withaferin A may disrupt interactions between viral S-Protein and host receptor angiotensin converting enzyme (ACE) by binding to it [22].

Cytokine storm and inflammation: If the body's defence system or prophylactic treatment fails to control the viral entry and its clearance, the body activates strong inflammatory response leading to cytokine storm. Briefly, the internalization of SARS CoV–2 eventually leads to secretion of proinflammatory cytokines to a large extent[41]. This leads to engagement of immune cells at the infected site. The present data retrieved extensive potential of WS to interfere with chemokine signalling through beta and delta type PK-C and chemokine receptors. Similarly, IL–17 plays crucial role in acute and chronic inflammatory responses. The IL–17 signalling can be mitigated predominantly through heat shock protein by AR, glycogen synthase by TC, and prostaglandin synthase by WS.AR derived saponins and TC extract showed anti-inflammatory property by modulating pro-inflammatory cytokines along with other inflammatory modulators[42,43]. WS extract also modulated cytokine expressions by inhibiting MAPK/NF-κB pathway[44].This pathogenic reaction may become more complicated in co-morbidities like diabetes, asthma, hypertension, and obesity where inflammasome is already present[45]. This further activates this inflammatory cascade and COVID–19 progression. It has been reported that the *Rasayana* botanicals containing phytoconstituents like Withaferin-A have an ability to treat the inflammasome[46,47,19,48].

Increased vascular permeability: The macrophage-mediated inflammatory cytokines cause contraction of endothelial cells of blood vessels which leads to increase in vascular permeability[49]. This increases migration of immune cells to occupy alveolar space. The proteins involved in trans-endothelial leukocyte migration are found to modulate by withanolides through our analysis.Integrin alpha-L (ITGAL) is one of the factor involved in transendothelial migration of leukocytes associated with COVID–19 pathogenesis [50]. Present data mining suggested ITGAL as putative target of 20-hydroxyecdysonefrom TC.Withaferin-A may inhibit and downregulate vascular permeability factor i.e. VEGF[51,52]and protects vascular barrier integrity inhibiting hyperpermeability induced by high-mobility group box 1 protein[53]. WS aqueous extract may inhibit histamine mediated endothelial contraction to avoid venular intercellular gaps[54,55].

Alveolar consolidation: The engagement of immune cells and extent of cytokine secretion consolidate alveolar space. Production of surfactants by pneumocytes intensifies severity leading to dyspnea or shortness of breath[56].Withaferin A reduced accumulation of M1 type macrophages characterized by IL–6 and TNF-α[57]. The *Rasayana* botanicals affect physiological inflammatory response by downregulating proinflammatory mediators. This is accompanied with preventing immune cell accumulation at infected site i.e. alveolar space[58]. Therefore, *Rasayana* botanicals may evade alveolar consolidation keeping pneumocytes engaged in O_2 transport as usual.

*Systemic inflammation-induced pyrexia:*In COVID 19, the cytokine storm (CS) results in excessive and uncontrolled release of pro-inflammatory mediators and cytokines.Clinically, it commonly presents as systemic inflammation and these inflammatory mediators act as a messengers to hypothalamus to increase body temperature through prostaglandinsecretion[59]. Few studies have suggested that withaferin A may circumvent pyrexia by downregulating COX–2 expression with simultaneous decrease in prostaglandin production[60,61].The newer understanding of SARS CoV2 pathogenesis is coming out gradually. Recent computational evidence has suggested, haemoglobin disassociation andiron imbalance by SARS-CoV–2 infection. This was supported by clinical observations of reduction in haemoglobin and increased erythrocyte sedimentation rate. This induces hypoxia leading to organ failure [62].A systematic *in vivo* study of TC extract showed significant increase in levels of haemoglobin and erythrocyte count to maintain systemic iron homeostasis[63]. WS showed prophylactic effect in pulmonary hypertension characterised by hypoxia and inflammation[64]. The connection of hypoxia and inflammatory signals is well established throughHIF1α gene[65].Asparagus polysaccharides potentially inhibited HIF1α signalling which might ameliorate fatal systemic inflammation[66]. TC also exhibited anti-inflammatory effects in systemic models significantly [67]. Our data analysis also suggests probable action of AR, TC, and, WS in hematopoietic cell lineage through multiple protein targets involving integrin alpha–4 and CD38.It has now been clearly established that the adverse clinical outcomes in COVID–19 patients is associated with elevated IL–6 levels[68]. A case report using Tocilizumab(an anti-IL6 receptor antibody) defined an approach of mitigating risk of disease progression to target IL–6[69,70].WS aqueous extract attenuated IL–6 in arthritis model to reduce inflammatory response. Active components of WS (withanone and withaferin A) and AR (shatavarin-IV) also inhibited production of IL–6 in *in vitro* system. It has also been reported that aqueous extract of TC protects against inflammation associated anaemia by modulating hepcidin expression, IL–6 and other pro-inflammatory cytokine cascade[63].There is a clinical evidence of COVID–19 from Wuhan, China indicating that the immunocompromised patients are at high risk of developing fatal respiratory syndrome[71]. In view of this, immunomodulating drugs are being considered beneficial for COVID–19 management[72]. The Ayurveda-based *Rasayana* botanicals are well known for their immunomodulatory activities[73]. The adaptogenic and regenerative properties of *Rasayana* botanicals help to maintain physiological homeostasis [74]. The effects of improving antibody titre, modulating systemic Th1/Th2 immunity underline the potential of AR as an immunoadjuvant [75]. TC and its phytoconstituents are capable immunomodulators

through their multimodal actions [76,23]. The significant immune-boosting potential of WS in several model systems makes it one of the best possible therapeutic adjuvantsfrom traditional medicine[77,78,20].

Predicted beneficial Herb-Drug Interactions: COVID–19 is a viral pandemic disease with no specific cure available so far. Few drugs and drug combinations are still under investigation for their efficacy in managing this fatal infection [79,80].Current treatment involves combination of previously available antiviral agents[81]. COVID–19 patients having co-morbidities like T2DM, hypertension, asthma, obesity, etc. are also being treated with these drugs along with their ongoing prescriptions[4]. Along with modern therapeutic agents, patients are also being treated with drugs from traditional medicinal system like Ayurveda [82,37]. Thus, while using such diverse treatment regime, there is need for designing and executing detailed DDI (drug-drug interactions) and HDI (herb-drug interaction) studies for proposing safe, efficacious and beneficial combinations. DDI and HDI can be predicted by PK-PD pathways (with special focus on drug metabolising enzymes and transporters) of particular drug. Cytochrome P450 system (CYP's) are major enzymes involved in catalyzing biotransformation of administered drugs. CYP1A2, CYP3A4,CYP2C9, and CYP2D6are involved in metabolism of around 80–90% of drugs [83]. Pharmacokinetic profiles of plant extracts considered in this study are not well established [84]. Data fromour *in silico*analysis shows some phytochemicals may or may not inhibit main drug metabolizing enzymes (Table 2). This inference is also being supported by published *in vitro* studies on human liver microsomes using whole plant extracts of AR, TC, and WS (S2 Table).IC_{50} values for all the extracts are > 100 µg/mL which seems to be a higher concentration than used *invivo)*[85,38]. Thus we speculate that*in vitro* studies on human liver microsomes using whole plant extracts of AR, TC, and WS may not show any inhibition of these main CYP isoforms.However, there is a need to explore *in vitro - in vivo* HDI for rationalizing theiruse in pharmacotherapeutic management of COVID–19 [86]. We also recommend that HDI studies should be planned focusing on drugs used in the management COVID–19and its associated comorbidities.The Supplementary S3 Table, includes pharmacokinetic data of WHO Solidarity trial drugs and prescribed drugs (representative) for COVID–19 associated comorbidities (e.g. hypertension, asthma and T2DM). This data shows that, there is overlap for drugmetabolizing enzymes especially among the allopathic drugs, indicatingpossiblerisky DDIs.Therefore, careful clinical validation is required. For instance, WHO Solidarity trial drugs (Remdesivir; Lopinavir/Ritonavir; Lopinavir/Ritonavir with Interferon beta–1a; and Chloroquine or Hydroxychloroquine) are being metabolized by CYP1A2, CYP3A4, CYP2C9, and/or CYP2D6. The commonly prescribed drugs for hypertension (Propranolol, Metoprolol, Telmisartan, Losartan) and T2DM (Glimepiride, and Pioglitazone) shares the same drug metabolizing enzymes (CYP1A2, CYP3A4, CYP2C9, and CYP2D6). This raises the concern for careful pharmacotherapeutic management. On the other hand, it can be predicted that there are very less chances of pharmacokinetic mediated HDI with the studied *Rasayana* botanicals as no such overlap has been found in our *in silico* studies as well as published *in vitro data.*

In this study we explored the potential of Ayurveda based Rasayana drugs in COVID 19 management using *in silico* approaches and propose a library of phytomolecules with the potential to be developed as phytopharmaceuticals. Appropriate pharmaceutics developability assessment needs to be done if any of these phytomolecules are considered for phytopharmaceutical development. The *in-silico* pharmacokinetic data also shows that, Muzanzagenin is the main drug-like molecule from AR and it has also showed docking score of > –6.0 kcal/mol against all three protein targets of SARS-CoV–2. Majority of the key phytoconstituents show good oral bioavailability. All phytochemicals of TC were found to be drug-like substances. In case of WS phytochemicals, except Ashwagandhanolide, Withanoside V and Withanoside IV all were found to be drug-like molecules. Though, Ashwagandhanolide has shown highest docking score for all 3 SARS-CoV–2 protein targets its drug-likeness is an issue of concern and needs further detail investigations. On the other hand, the network pharmacology and docking data showed that there are very high chances of beneficial pharmacodynamic HDI for the effective pharmacotherapeutic management of COVID–19. At the same time, we cannot forget that the drug concentration plays the important role in both *in-vitro* and *in vivo* studies, therefore effective and safe clinical extrapolation based on experimentally validated data are warranted.

In conclusion, this study provides leads for clinical application of rasyana botanicals in prophylaxis because of their potential in inhibiting the replication of SARS-CoV–2. These botanicals can also be used as adjunct or mainstream treatment for COVID–19, when the disease is manifested with its symptom. The activities on immune mechanisms provide a sound logic for use of these botanicals in treatment. Some of the phytoconstituents have a possible role in arresting disease progression and preventing organ failure by reducing inflammatory responses. The adjunct use of these botanicals poses another question of herb drug interaction and possible reduction in therapeutic effects offered by modern medicines such as remdesvivir, HCQS and other drugs. However, the data suggests there is no harmful HDI.

Materials And Methods

1. Preparation of extracts and phytochemical characterization:The standardized extracts of roots of WS, AR and stem of TC prepared according to Ayurveda principles were provided by Pharmanza (India) Pvt. Ltd as part of an on-going AYUSH Center of Excellence (AYUSH-CoE) project. The optimized process of extraction of three selected botanicals was followed as per the protocol developed by AYUSH-CoE,

CCIH, Savitribai Phule Pune University85. Briefly, the hydro-ethanolic (70:30) and water extracts of theseRasayana botanicals were prepared using1:4 ratio of test material and respective solvent.

The extracts were chemically characterized and standardized using HPLC (quantitative) and mass spectrometry (qualitative). HPLC grade solvents were used for the analysis. The HPLC and MS analysis were done with the help of Pharmanza Herbals Pvt. Ltd., Gujarat, Bioanalytical Technologies Pvt. Ltd., Pune and Central Instrumentation facility (CIF), Savitribai Phule Pune University, Pune.

2. Molecular docking: A series of 31 phytoconstituentsidentified byHPLC and MS analysis including 11 phytoconstituents from AR, 10 from TCand 10 from WSwere considered in this study. All 31 compounds were downloaded from Pubcheminstructure data file i.e. (.sdf) format including 3D coordinates87. Three drug targets of SARS-CoV–2 were considered namely, the Receptor Binding Domain of Spike Protein (RBD), the Main Protease (Mpro) and the RNA dependent RNA polymerase (RdRp). Docking was performed with AutoDock 4.2.6 assembled in the PyRx 0.8 Virtual Screening Tool 88,89. For the Main protease, the crystal structure of the protease complexed with 2-cyclohexyl-{N}-pyridin–3-yl-ethanamide (GWS) (PDB ID: 5R84 Chain A, resolution 1.83Å) was considered for structural studies (Fearon, D. et al, Unpublished). A grid box was prepared with dimensions 60×60×60Åcentred on the protein around residues which interacted with co-crystallized ligand GWS representing pockets S1-S3, with grid spacing of 0.375Å. For the RNA-dependent RNA polymerase, the PDB ID: 6M71 Chain A, resolution 2.90 Å, was considered for structural studies26. A grid box of dimensions 100×100×100Å with grid spacing of 0.375Å was centred around the RNA binding site. The cryoEM structure of Receptor Binding Domain of Spike protein with ACE2-B0AT1 complex (PDB ID: 6M17 Chain E, resolution 2.90 Å) was considered for structural studies29. A grid box was prepared with dimensions 90×90×90Å and spacing 0.375Å and was centred on the protein residues: Lys417, Tyr453, Gln474,Phe486, Gln498, Thr500 and Asn501. Prior to docking, all compounds were subjected to energy minimization using the inbuilt OpenBabel module in Pyrx90. 50 docking runs were performed for each phytochemical with each drug target, with a population size of 150 and 250000 energy evaluations. All the other algorithm parameters were kept default. The final pose of the phytoconstituents was selected based on its highly suitable docking score.

3. Network pharmacology: The network pharmacology approach was followed to explore the role of Rasayana botanicals i.e. AR, TC, and WS in immunomodulation. Briefly, the qualitatively confirmed 31 phytochemicals (referred as bioactives) as mentioned earlier were queried in PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to obtain structural data files (.sdf)87. These files were uploaded to BindingDB database(https://www.bindingdb.org/bind/index.jsp) with ≥0.7score to retrieve putative protein targets of uploaded bioactives. The score represents structural similarity between queried molecule and available ligand in database wherein score 1 depicts maximum 100% structural similarity91. Collected protein targets were searched inUniProt database (http://www.uniprot.org/) to acquire universally accepted gene names with unique UniProt IDs92. The compiled targets were filtered for Homo sapiens (human) targets for further data analysis.

Simultaneously, KEGG pathway (https://www.genome.jp/kegg/pathway.html) database was mined to obtain proteins/genes involved in twenty human immune pathways[93]. The universally accepted gene names were collected as mentioned before. The entire data was collected and analysed in Microsoft Excel to identify putative immune-associated protein targets of bioactives of AR, TC, and WS. The network of available data was constructed with eloquent representation using Cytoscape 3.7.2 (https://cytoscape.org/) software[94].

4. Predicting Herb-Drug Interactions:

Theonlinetool, Swiss-ADME (http://www.swissadme.ch/)was used to predict pharmacokinetics, physicochemical properties, and drug likeness of queried compounds[95]. Swiss ADME requires Canonical SMILES of test molecule as input data. The PubChem database was minedto acquire canonical SMILES of of 11, 10, and 10 phytochemicals of AR, TC, and WS respectively[95].

Considering the COVID–19 complex pathogenesis there is concomitant administration of multiple drugs/herbs which may leads to risk of herb-drug interactions (HDIs)[37].Therefore, the probable pharmacokinetic-pharmacodynamic HDI were explored based on data generated from Swiss-ADME and available literature.

Declarations

Author contributions:

Contributor Role	Role Definition
Conceptualization	SB, MJ, PCG, GT
Data Curation	SB, MJ, AS, VB, SW, GT, SS
Formal Analysis	SB, MJ, AS, VB, SW, PCG,SS
Funding Acquisition	PCG, MJ, SB, GT
Investigation	SB, MJ, AS, VB, SW, PCG, GT, SS
Methodology	SB, MJ, AS, VB, SW, SS
Project Administration	PCG, SB
Resources	MJ, PCG, GT, SB
Supervision	PCG, SB, MJ
Validation	SB, MJ, AS, VB, SW, PCG, GT, SS
Visualization	SB, MJ, AS, VB, SW, PCG, GT, SS
Writing – Original Draft Preparation	SB, MJ, AS, VB, SW, PCG, SS
Writing – Review & Editing	SB, MJ, AS, VB, SW, PCG, GT, SS

Competing interests: Authors declares no competing interest.

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Figures

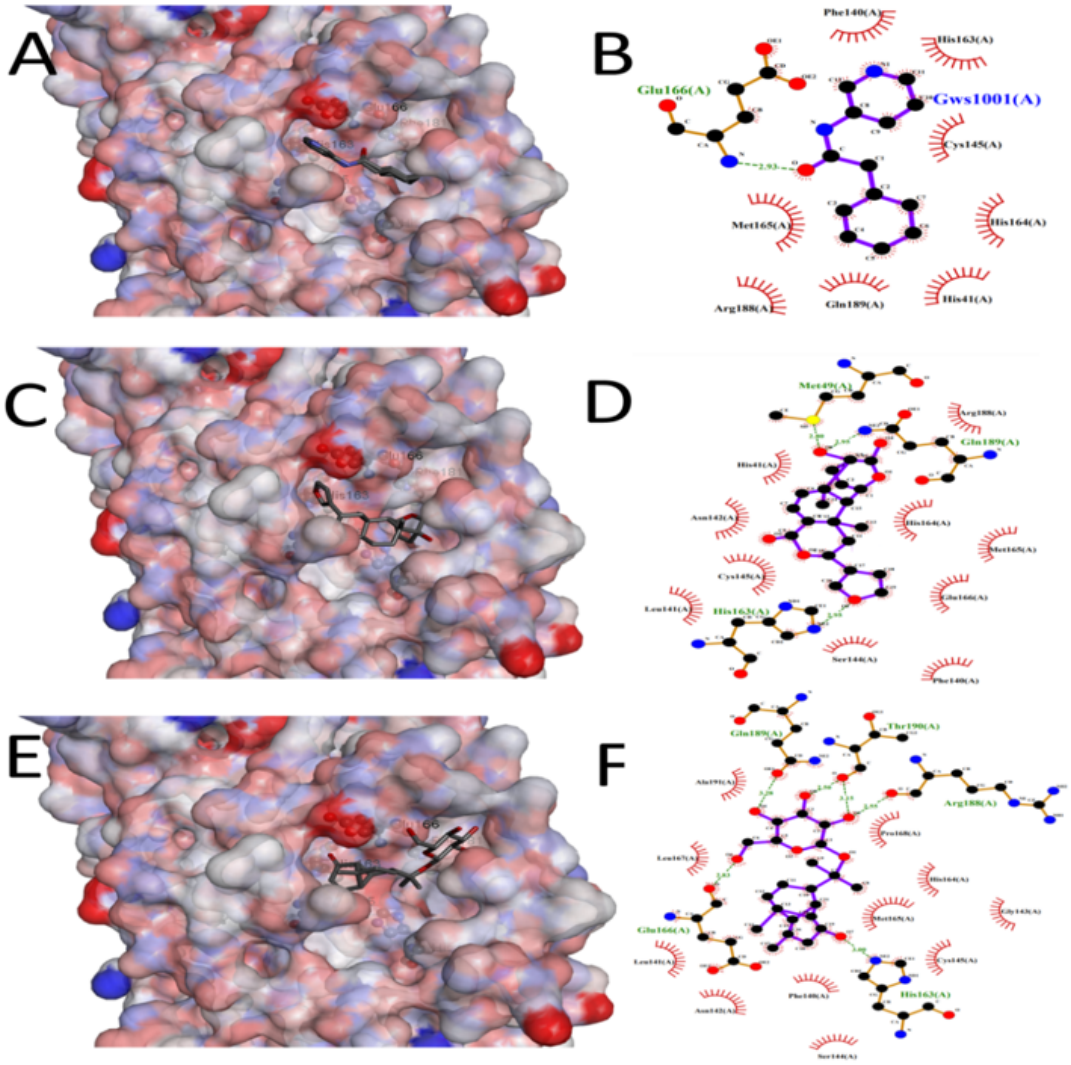

Figure 1

Interaction of (A and B) cyclohexyl-N-(3-pyridyl)acetamide (Z31792168), (C and D) Columbin and (E and F) Tinocordiside with Mpro. In the left panel the protein is shown in a surface representation colored by atom while the ligand is shown in stick representation. The right panel is a 2D interaction plot of the receptor with the inhibitors.

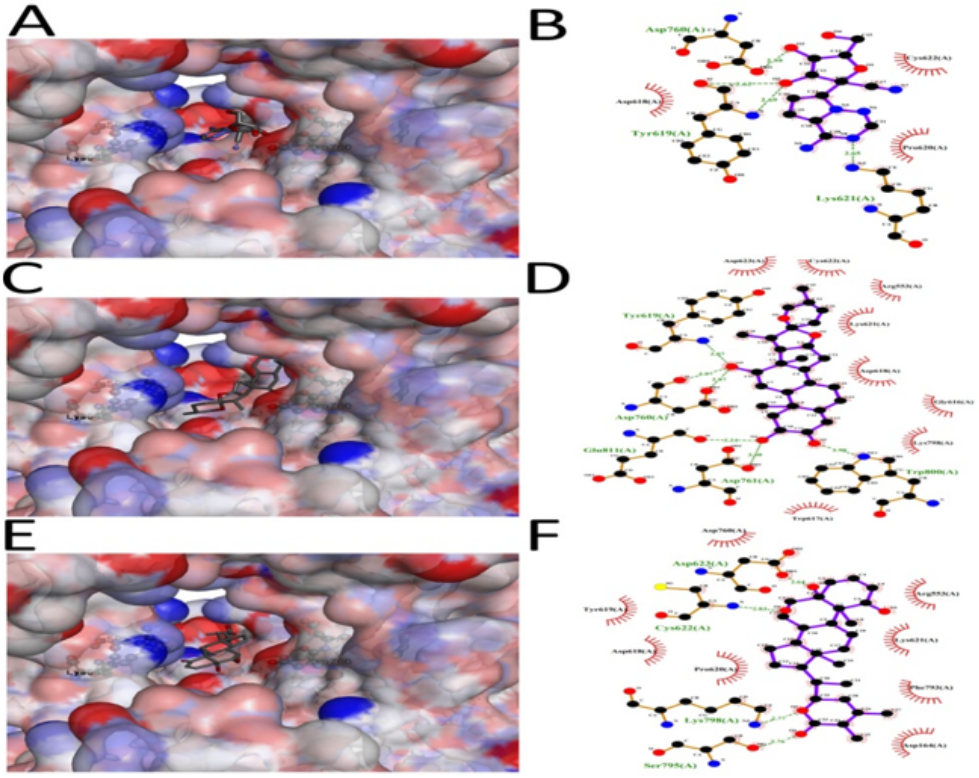

Figure 2

Interaction of (A and B) Remdesivir, (C and D) Muzanzagenin and (E and F) Withanolide-B with RdRp. In the left panel the protein is shown in a surface representation colored by atom while the ligand is shown in stick representation. The right panel is a 2D interaction plot of the receptor with the inhibitors.

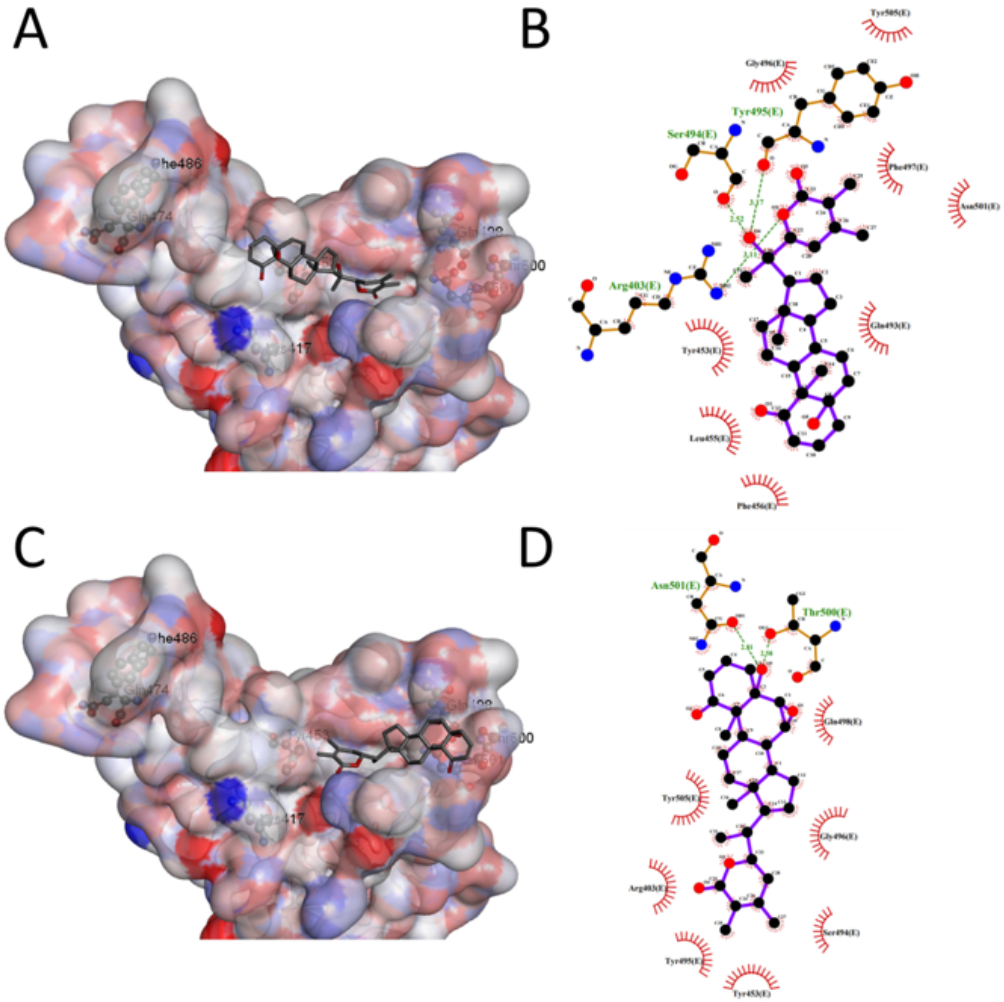

Figure 3

Interaction of (A and B) Withacoagin and (C and D) Withanolide-B with Spike protein. In the left panel the protein is shown in a surface representation colored by atom while the ligand is shown in stick representation. The right panel is a 2D interaction plot of the receptor with the inhibitors.

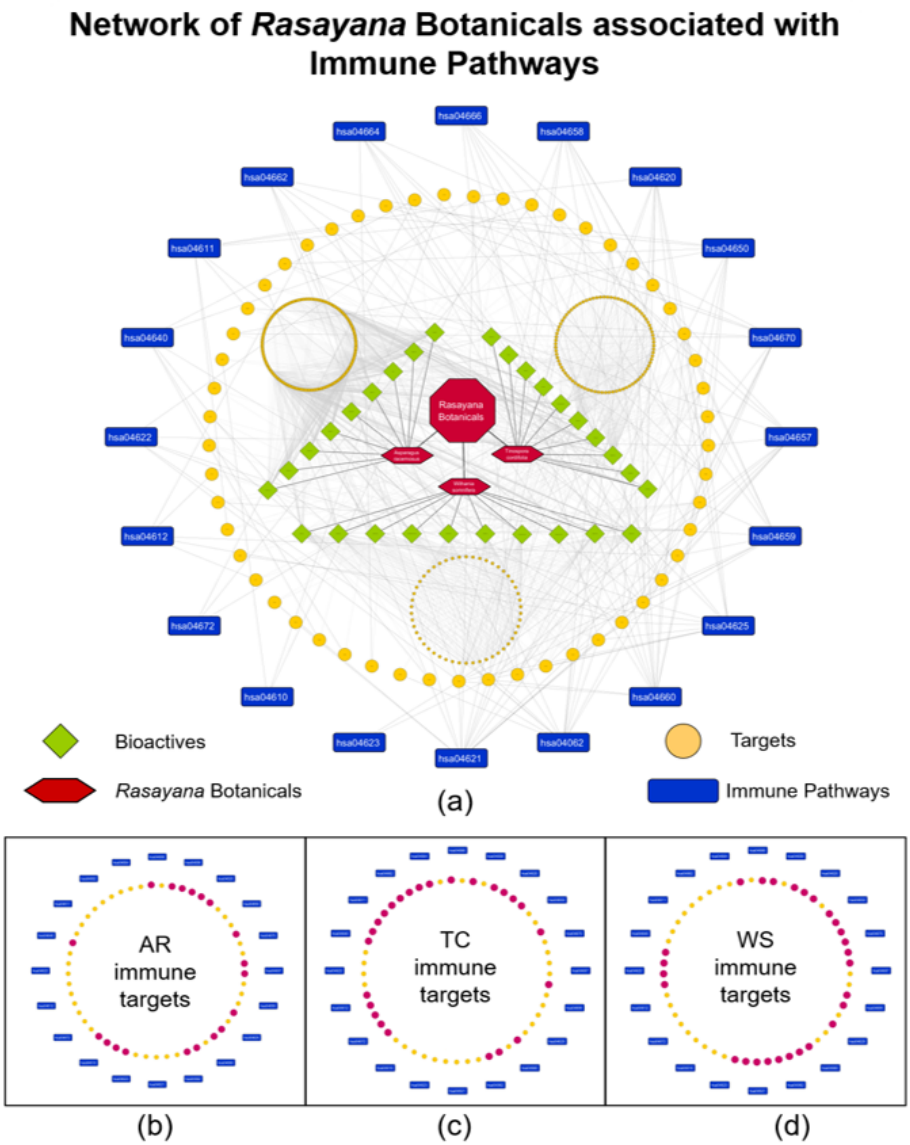

Figure 4

Network of Rasayana Botanicals associated with Immune Pathways. Figure(4a) depicts association of Rasayana botanicals with several immune pathways (outer circle). Outer big circle of targets represents immune targets whereas inner small circles represents other human targets of AR, TC, and WS bioactives (green diamonds). Figure (4b), (4c), and, (4d) depicts immune targets of AR, TC, and WS highlighted in red colour.

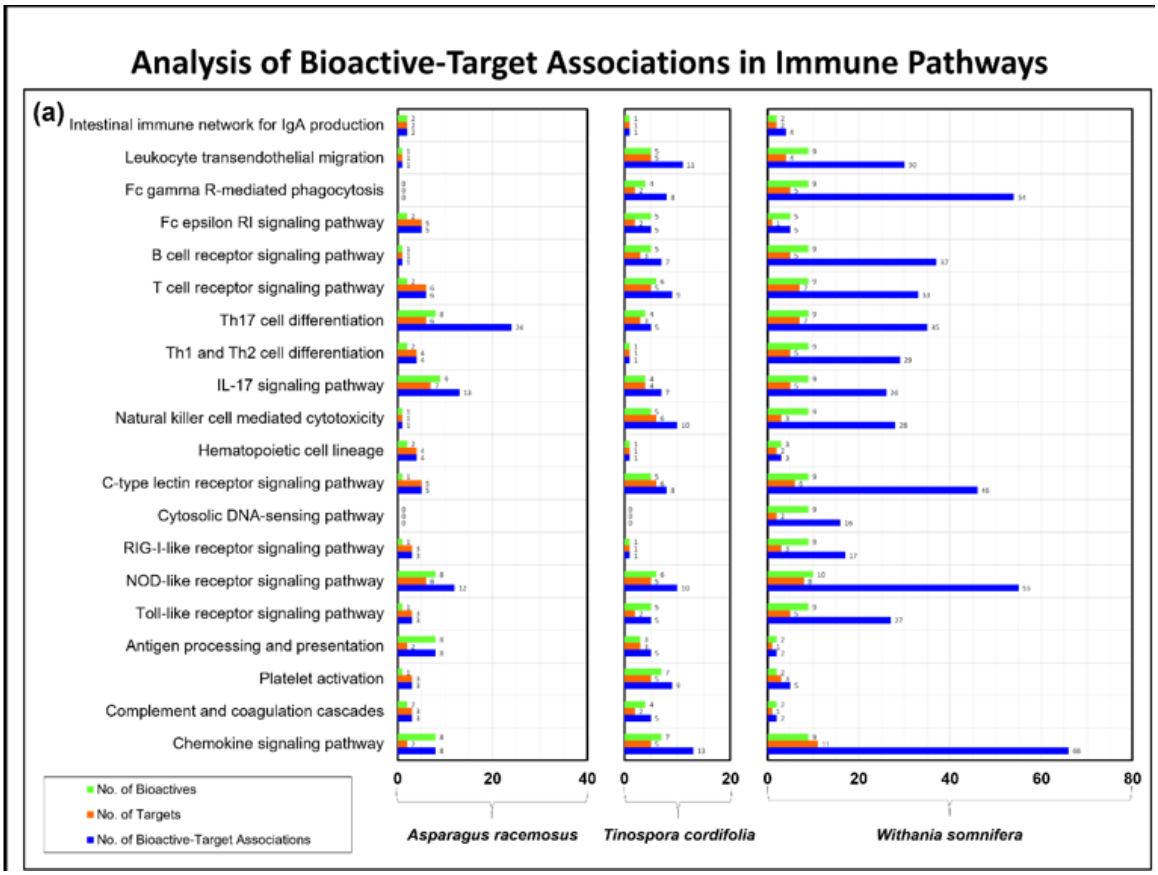

Figure 5

Analysis of Bioactive-Target Associations in Immune Pathways (a) The figure depicts the involvement of AR, TC, and WS in pathways of human immune system. The represented association is number of connecting combinations between bioactives and their targets in a particular pathway. (b) The figure represents number of individual and common immune targets of AR, TC, and WS. (c) The table lists all immune targets of AR, TC, and WS.

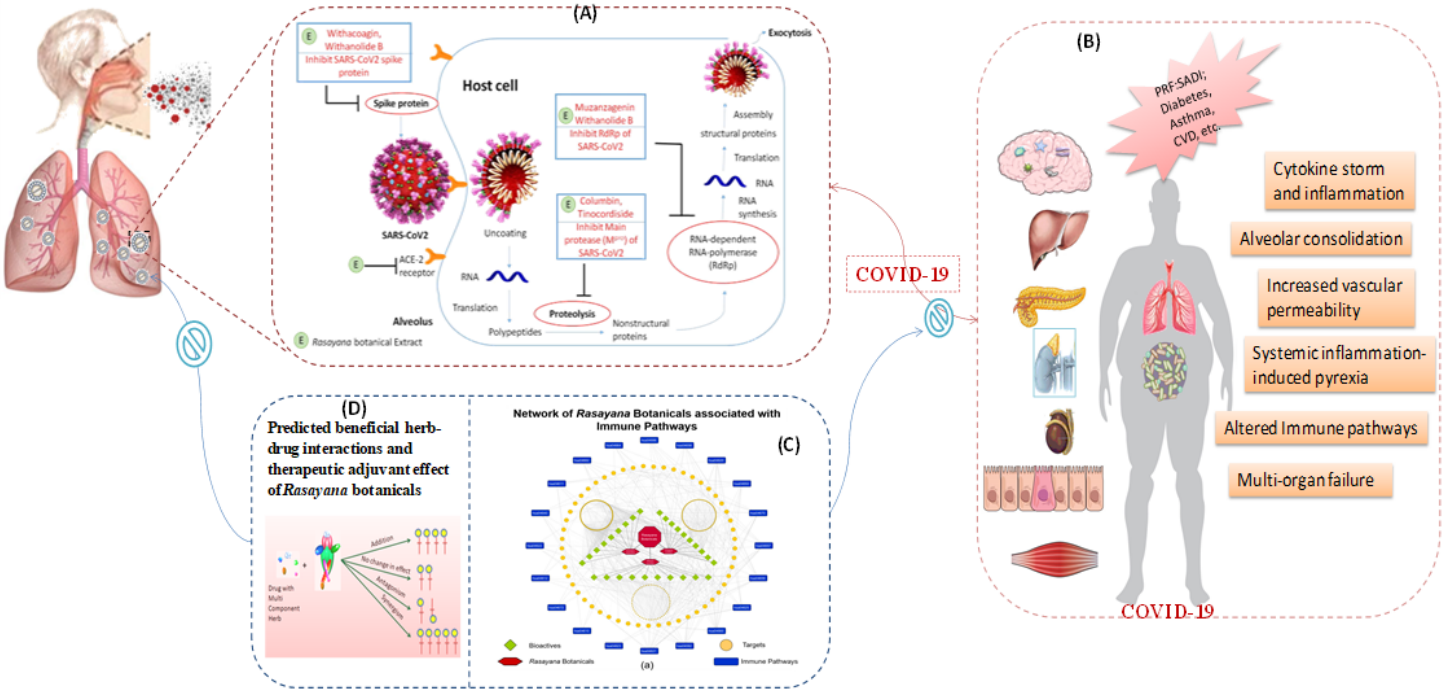

Figure 6

Predicted role of Ayurvedic Rasayana botanicals in the management of COVID-19. Once the SARS-CoV-2 virus passesthe respiratory tract, it enters the lung cells with the help of its Spike protein coupled with ACE-2 receptors (these are present almost all over the body;enabling its spread tomultiple organs and their failure in later stages of the disease). Rasayana botanical extract constituents ( ), Withacoagin and Withanolide B may inhibit COVID-19 entry by disrupting interactions between viral spike protein and host ACE-2 receptor. Rasayana botanicals may inhibit viral replication through inhibition of coronavirus main protease (Mpro) and RNA-dependent RNA-polymerase (RdRp) Part A: The Mpro is can be inhibited by Columbin and Tinocordiside.RdRp can be inhibited by Muzanzagenin and Withanolide-B thereby inhibiting RNA synthesis of SARS-CoV-2. This may lead to its life cycle arrest. Part-(B): The patient related predisposition factors such as sex, age, diseases, individualization (PRF: SADI) and COVID-19 pathophysiology involved in the multiple organ failure and death. Part (C): the multi-targeted immunomodulatory and adaptogenic potential of Rasayana botanicals as predicted by network pharmacology approach. Part-(D) shows higher probability for the beneficial pharmacokinetic-pharmacodynamic herb-drug interactions37if co-prescribed with WHO Solidarity trial drugs and drugs for COVID-19 associated comorbidities. It is interesting to note that above mentioned phytoconstituents are predicted to have good docking score, ligand efficiency, oral bioavailability, and drug likeliness. This makes them potential molecules for rapid drug discovery and development based on multi-targeted and reverse pharmacology approach for COVID-19 management. However, these in silicopredictions need in vitro – in vivo validation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S3TablePKdataofWHOSolidaritytraildrugsandCOVID19comorbidities2.docx
- S1appendixRADARgraphsfor31PhytochemicalsofARTCandWS2.docx
- S1TableStructureandDockingdetailsofthephytoconstituentsfromASTCandWSweretothebindingsite2.docx
- S2TableInvitroCYPinhibitionData2.docx