Withanolides from *Withania somnifera* as an immune booster and their therapeutic option against COVID-19

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Abstract

Aim: The present study aimed to investigate the withanolides as an immune system booster and anti-viral agents against the coronavirus.

Materials and Methods: Reported withanolides from *Withinana somnifera* were retrieved from the open-source database i.e. ChEBI, PCIDB and Dr. Duke's Phytochemical and Ethnobotanical Databases. Their protein-based targets were predicted using DigepPred and the protein-protein interaction was evaluated using STRING. Similarly, the drug-likeness score of individual compounds was predicted using MolSoft and intestinal absorptivity was predicted using the boiled-egg model. The network among the compounds, proteins, and modulated pathways was constructed using Cytoscape and the docking was performed using autodock4.0.

Results: Withanoloid Q was predicted to modulate the highest number of proteins, showed positive human intestinal absorption and had the highest druglikeness score. Similarly, combined network interaction identified withanolide Q to target the highest number of proteins; RAC1 was majorly modulated and regulating Fluid shear stress and atherosclerosis as a majorly regulated pathway. Similarly, Withanolide D and Withanolide G were predicted to have the better binding affinity with PLpro, Withanolide M with 3clpro, and Withanolide M with spike protein based on binding energy and number of hydrogen bond interactions.

Conclusion: Among the multiple withanolides from *Withania somnifera,* withanolide-D, -G, -M, and -Q were predicted as a lead hit based on druglikeness score, modulated proteins, and docking score to boost immune system and inhibit the COVID infection.

Introduction

At present CoV Disease (COVID-19); a positive-sense RNA has spread throughout the world leading to deaths of millions of people [1] affecting the pulmonary gas exchange in the lungs. It has been identified that subjects with the lower immune systems are at more risk factors to be affected by COVID-19 which is more prevalent in the pediatrics, geriatrics, and the subjects suffering from infectious and non-infectious diseases [2]. Multiple preventative approaches are suggested to avoid COVID-19 infection like “social distancing”; however, it is not applicable in all the subjects who are continuously working in the health field like doctors and nurses. Much research is undergoing developing the vaccine against COVID-19, however, the process of identifying new drug molecules/vaccines is time-consuming. Thus it is important to identify some other alternative method as prophylaxis to protect its health from this pandemic condition.

Recently many investigations are undergoing utilizing the multiple traditional folk medicines against COVID-19 [3-5]. Many academic researchers are reporting multiple phytoconstituents against COVID-19

3C-like protease (3CLpro), papain-like protease (PLpro), and spike protein **[6].** However, the tendency of the virus to mutate itself gets increased if the right dose-right time-right drug is not chosen. So, it may be difficult to treat this condition if the molecules are used blindly against this agent. In this case, the conventional "*single drug-single protein-single disease*" principle may fail. So, the approach could be slightly different. One should think as "*can we boost the immune system of the subject with reported anti-viral agents?"* Our research team believed, if an immune system booster can act as an anti-viral agent, it may contribute to the management of this condition. In this case, the principle of polypharmacology may be applicable which suggests "*multi component-multi protein interaction"*.

Based on the above concept, we attempted to investigate *Withania somnifera* as an immune system booster in COVID-19 infection and its binding affinity to three reported targets i.e. 3CLpro, PLpro, and spike protein in COVID-19 infection. *Withania somnifera* is itself is an anti-viral agent **[7]** and also recorded as the immune booster [8] in the ayurvedic medicine and multiple scientific literatures. Further, it composes withanolides as major components that are popular in increasing the immune system [9], are anti-viral [10]**,** and act as anti-inflammatory [11]. Hence, these triplex effects of withanolides may contribute to managing COVID-19 infection which can be evaluated by GO gene analysis and network pharmacology approach. Hence, the present study aims to investigate the network pharmacology of withanolides from *Withania somnifera* as an immune booster and anti-viral agent against COVID-19 by targeting 3CLpro, PLpro and spike protein

Materials And Methods

Mining of Withanolide from *Withania somnifera*

Withanolides from *Withania somnifera* were retrieved from three open source databases i.e. ChEBI, PCIDB, and Dr. Duke's Phytochemical and Ethnobotanical Databases by querying keyword "*Withania somnifera*". The bioactives were then further queried in PubChem or Chemspider database to retrieve their SMILES and 3D structures.

Protein-based target prediction of bioactives and their enrichment analysis

Protein-based targets of each compound were predicted by querying SMILES of each ligand molecule at Probable activity (Pa)>0.5 in Digep-Pred [12]. The up-regulated and down-regulated proteins were then queried on STRING [13] for *Homo sapiens* and probably modulated pathways were identified concerning the KEGG pathway (https://www.genome.jp/kegg/) database. The modulated pathways involved in the immune system modulation were identified from published literature. Similarly, the biological processes, molecular function, and modulated cellular component of the combined action were also predicted via GO gene analysis.

Network Construction and analysis

The network between phytoconstituents, proteins, and modulated pathways was constructed using Cytoscape [14] version 3.5.1. Any duplication was eliminated before the analysis of the network. The network was treated as directed and command "*network analyzer*" was used to analyze the network based on "*edge count*" by mapping the node size and color as "*low values to small sizes*" and "*low values to bright colors*" for both settings.

Prediction of druglikeness score and human intestinal absorptivity

Druglikeness score of an individual molecule was predicted using MolSoft (https://molsoft.com/mprop/). Similarly, a boiled egg [15] was used to predict the human intestinal absorptivity of bioactives via SWISS ADME [16].

Anti-viral PASS prediction of bioactives

By querying the SMILES of each ligand molecule in PASS [17] at Probable activity (Pa)> probable inactivity (Pi), anti-viral activity was recorded for a compound using keyword "anti-viral" as its probable biological spectrum. Records were queried for their probable pharmacological spectrum against multiple viruses like Herpes, HIV, Hepatitis B, Rhinovirus, Rhinovirus, and Influenza.

In silico molecular docking

Three protein molecules i.e. 3clpro (PDB: 6LU7), PLpro (PDB: 4M0W) and spike proteins (homology modeled target, accession number: AVP78042.1 as query sequence and PDB: 6VSB as a template using SWISS-MODEL [18] were chosen as target molecules against COVID-19. The complexed ligand/water molecules were removed using Discovery studio 2019 **[19]** and saved in .pdb format. The retrieved .mol/.sdf files of ligand molecules from ChemSpider/PubChem were converted into .pdb format using Discovery Studio 2019 and saved into .pdb format. The energy of each ligand molecule was minimized using the mmff94 force field **[20]** and conjugate gradients as an optimization algorithm and converted into .pdbqt format. Docking was carried using autodock4.0 **[21]** to obtain 10 different confirmations at exhaustiveness 8. After docking, pose scoring the minimum binding energy was chosen to visualize the ligand-protein interaction in Discovery studio 2019.

Results

Withanolides and their target prediction

Based on the availability of structures of bioactives, 17 withanolides were chosen for the study. Among them, Withanolide Q was predicted to modulated the highest number of proteins i.e. 12 in which 5 were down-regulated (CHEK1, NR3C1, PRKCA, PROS1, ESR2) and 7 were up-regulated (TNFRSF1A, PLAT, RAC1, VDR, FKBP5, AR, GH1). The down-/up-regulated proteins of each compound are summarized in **Table 1**.

Prediction of druglikeness score and human intestinal absorptivity

Prediction of human intestinal absorptivity identified Withanolide E, Withanolide F, Somniferawithanolide, Withanolide Q, Withanolide M, Withanolide D, Withianoloide R, Withanolide I, Withanolide L, and Withanolide N to be absorbable from g.i.t (Figure 1). Further, druglikeness prediction identified Withanolide Q to possess the highest druglikeness score i.e. 0.75 (**Figure 2)**.

Enrichment and network analysis

Enrichment analysis identified the prime modulation of Fluid shear stress and atherosclerosis with the highest number of gene sets i.e. 4 (CCL2, PLAT, RAC1, TNFRSF1A) at lowest false discovery rate i.e. 0.0012. The modulation of genes also identified the regulation of multiple pathways which are having direct/indirect relation with infectious/non-infectious diseases. Further, pathways related to the modulation of the immune system like TNF, Fc epsilon RI, p53, PI3K-Akt, IL-17, mTOR, NOD-like receptor, Chemokine, Rap1 and NF-kappa B signaling pathway and Cytokine-cytokine receptor interaction were also predicted (Table 2). Similarly, GO analysis identified the highest number of modulated genes i.e. 17 from membrane-bound organelle and cytoplasm as a cellular component, molecular function with 19 genes as binding, and stimulus respondent (19 genes) as biological processes **(Figure 3)**. The network interaction of bioactives and modulated pathways with respective genes is represented in **Figure 4.**

Anti-viral PASS prediction of bioactives

All the bioactives were predicted as the anti-viral against Rhinovirus, six (Withanolide -D, -M, -N, -O, -P and -Q) for influenza, four (Withanolide-D, -O, -N, -Q,) and Withanolide Q against Hepatitis and HIV. Similarly, three bioactives (Withanolide -N, -O and -Q,) were predicted as undefined anti-viral agents **(Figure 5).**

In silico molecular docking

Withanolide G and Withanolide D were predicted to possess the highest binding affinity with PLpro with binding energy -8.9 kcal/mol; however, Withanolide G was predicted for the highest number of hydrogen bond interactions i.e. 6 by interacting with ARG167, GLN233, and TYR208. Similarly, Withanolide I was predicted for its highest hydrogen bond interactions i.e. 7 with ARG131, ASP289, THR199, LEU287, TYR239, ASN238 though it possessed -9.1 kcal/mol compared to Withanolide J (-9.3 kcal/mol). Likewise, Withanolide M was predicted to possess the highest binding affinity with spike protein by interacting with 4 hydrogen bond interactions with CYS566, THR525, LYS523. However, Withanolide H was predicted to interact with spike protein via 6 hydrogen bonds with ASN211, ASN185, THR254, SER67, THR94, ALA259 though it scored comparatively higher binding energy (-8.5 kcal/mol) compared to Withanolide H (Table 3). **Figure 6** represents the interaction of lead hit based on binding energy with the respective protein.

Discussion

It is well reported that that subjects with infectious and non-infectious diseases are having a lower-immune system in which the complete homeostasis [22-24] could be due to the improper supply of oxygen/nutrients and imbalanced metabolism of endo-/exogenous components in the cell to produce

ATP. In this condition, if the subject is infected with COVID-19, the risk for mortality is very high as the natural defence system may not function. Thus, regular consumption of immune boosters may contribute to managing the risk of COVID-19 infection as prophylaxis. Based on this concept, we attempted to investigate the withanolides as an immune booster in COVID-19 infection and their affinity to act as anti-viral by acting over the recorded targets of COVID-19.

Withania somnifera has been identified as an immune system booster [8] and it is used in the management of multiple infectious and non-infectious diseases [7, 25, 26]**.** Hence, an attempt was made to identify the possible role of this agent against COVID-19 management. Initially, we mined the bioactives of "*Withania somnifera*" from three different databases, predicted the probably modulated targets at the minimum probable activity of 0.5, and evaluated the gene-set enrichment analysis. During target prediction, we identified Withanoloid Q to possess the highest affinity to regulate the maximum proteins. Further, it also scored the highest druglikeness score i.e. 0.75 which was based on "*Lipinksi's rule of five*" reflecting its probability to get absorbed from the human intestine. Further, to confirm this we also predicted the absorptivity of bioactives in the boiled egg model which also supported the absorptivity of Withanoloid Q from the human intestine. Further, PASS prediction also identified the anti-viral efficacy against multiple strains of organisms. However, during the docking study, this compound was not predicted as a lead hit against 3CLpro, PLpro, and spike protein. This result suggests, Withanoloid Q may not be individually used as an immune booster in COVID-19. This is because, though it possesses the anti-viral efficacy, possess the higher efficacy to get absorbed into the systemic circulation, the efficacy to bind with the targets is comparatively lower than other molecules. Now, management of COVID-19 with withanoloids can be explained via the poly-pharmacology approach.

Previously, it has been demonstrated that how a single compound can modulate multiple proteins and regulate numerous pathways via the concept of gene-set enrichment analysis and network pharmacology approach [27-30]. The same principle can be applied via the "*multi compound-multi protein*" interaction in COVID-19 management. In the present study, the combined network interaction of bioactives identified 54 different pathways. In this prediction, we identified the pathways related to infectious diseases (Tuberculosis, Hepatitis B, Influenza A, Herpes simplex infection, Salmonella infection) and non-infectious diseases (Pathways in cancer, Non-alcoholic fatty liver disease, Prostate cancer) in which is immune system is compromised. Further, few pathways like Rap1 signaling pathway, Chemokine signaling pathway, NF-kappa B signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, Cytokine-cytokine receptor interaction, p53 signaling pathway, TNF signaling pathway, and MAPK signaling pathway were identified which have their direct role in boosting the immune system.

Rap1 plays an important role in chemokine-induced polarization and regulating cell spreading and adhesion of T cells and B cells respectively [31]. In the present study, the combined synergistic effect identified the modulation of this pathway by regulating PRKCA and RAC1. **Chemokines** signaling pathway controls immune cell migration and positioning in tissues and also controls the release of immune cells from bone marrow during infection and homeostasis [32] which has been modulated by regulating CCL2 and RAC1. **PI3K** has been reported to activate **immune** by upregulating IL-10 and

inhibiting proinflammatory cytokines [33]; which has been reported to be modulated via GH1, PRKCA, and RAC1. The contribution of p53 has been identified as the regulation of multiple pathways of inflammation, cytokine production, and pathogen sensing. Further, it also responds to cell apoptosis in viral infection and is also responds to innate and adaptive immunity. Further, it has been reported to play an important role in dead cell clearance, regulating autoimmune disorder, and also acts as a regulator in immune checkpoint [34]**.** In the present study, withanolides from *Withinana somnifera* have been identified to regulate p53 signaling by regulating CASP8 and CHEK1 which could contribute to maintain the cell apoptosis during viral infection and removing dead cell; minimizes the inflammation in human cells. Previous report suggests the inhibition of the MAPK pathway leads to the diminished immune system as it regulates the production of chemokines like ILs, TNFα and is triggered by MAPK, JNK, and ERK pathways [35]**.** Further, production of these chemokines is well reported to an allergic reaction and eliminates extracellular pathogens [36]**.** In the present study, the MAPK pathway has been regulated via the modulation of CD14, PRKCA, RAC1, TNFRSF1A which would be helpful in the production of chemokines and eliminating the coronavirus infection.

3C-like protease (3CLpro) possess a role to alter ubiquitin system and also incorporates the viral polypeptides leading to the alteration of functional proteins [37]; which was majorly targeted by Withanolide I and Withanolide J. Further, PLpro processes the function of pp1a and pp1ab into the replicase proteins to regulate viral life cycle [38]; majorly inhibited by Withanolide D and Withanolide G. Likewise, spike protein utilizes angiotensin-converting enzyme 2 as a receptor to enter inside the cell [39, 40]**;** modulated by Withanolide H and Withanolide M. These results reflect one single molecule may not be applicable to inhibit the multiple proteins due to their multiple affinities which also suggest the concept of utilizing “multi compound-multi protein” interaction theory in managing these infections.

Conclusion

The present study utilized the system biology approach to identify the probably modulated pathways to boost the immune system by withanolides in COVID-19 infection. Although the study identified the lead hit molecule via docking study against COVID-19, the utilization pattern may be limited as multiple components were able to have different affinities against the different protein molecules. Hence, the utilization of multiple components can produce the synergistic/additive effect against the COVID-19 infection and may also regulate the immune system in a better way rather than a single lead hit molecule. Further, the findings of present study are knowledge-based and CPU processing which needs to be further proven via experimental analysis.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Foundation project

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Authors Contribution

Pukar Khanal made the concept, designed the study, reviewed literature, carried out the work and drafted the manuscript. BM Patil and Ismail Pasha have equally contributed in refining the protocol and final drafting of the manuscript. Sharad Chand and Yadu Nandan Dey have equal contribution in reviewing the final manuscript.

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Tables

Table 1: Protein-based targets of withanolides from *Withania somnifera*

S. No.	Bioactives	Downregulation	Upregulation
1	Somniferawithanolide	CHEK1	RAC1, TNFRSF1A, VDR, CAT, CD14, NPPB, PLAT
2	Withanolide F	CHEK1, CCL2	NPPB, VDR, FKBP5, RAC1, PLAT
3	Withanolide H	CHEK1, CCL2	TNFRSF1A, RAC1, NPPB, VDR
4	Withanolide I	CHEK1, CCL2	NPPB, RAC1, VDR
5	Withanolide J	CHEK1, CCL2	NPPB, VDR, FKBP5, PLAT, RAC1
6	Withanolide K	CHEK1, CCL2	NPPB, VDR, FKBP5, RAC1, PLAT
7	Withanolide L	CHEK1, NR3C1	FKBP5, VDR, PLAT, AR, NPPB
8	Withanolide M	CHEK1	PLAT, VDR
9	Withanolide P	CHEK1, CCL2	NPPB, VDR, FKBP5, PLAT, RAC1
10	Withanolide Q	CHEK1, NR3C1, PRKCA, PROS1, ESR2	TNFRSF1A, PLAT, RAC1, VDR, FKBP5, AR, GH1
11	Withanolide R	CHEK1, PRKCA	RAC1, PLAT, CYP3A4, VDR, AR
12	Withanolide D	CASP8, CHEK1	VDR, PLAT
13	Withanolide E	CHEK1, CASP8, CCL2	NPPB, VDR, PLAT
14	Withanolide G	CHEK1, CCL2	NPPB, RAC1, VDR, PLAT
15	Withanolide N	CHEK1, NR3C1	TNFRSF1A, VDR, RAC1, FKBP5, PLAT, NPPB, AR
16	Withanolide O	CHEK1	VDR, FKBP5, PLAT, NPPB, KRT18, RAC1, AR
17	Withanolide S	CHEK1, CCL2	NPPB, RAC1, VDR, FKBP5, KRT18, PLAT, AR

Table 2: Combined gene set enrichment analysis of Withanolides from *Withania somnifera*

m ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (labels)
5418	Fluid shear stress and atherosclerosis	4	133	0.0012	CCL2, PLAT, RAC1, TNFRSF1A
5014	Amyotrophic lateral sclerosis (ALS)	3	50	0.0013	CAT, RAC1, TNFRSF1A
5130	Pathogenic Escherichia coli infection	3	53	0.0013	CD14, KRT18, PRKCA
5152	Tuberculosis	4	172	0.0013	CASP8, CD14, TNFRSF1A, VDR
4010	MAPK signaling pathway	4	293	0.0032	CD14, PRKCA, RAC1, TNFRSF1A
4071	Sphingolipid signaling pathway	3	116	0.0032	PRKCA, RAC1, TNFRSF1A
4620	Toll-like receptor signaling pathway	3	102	0.0032	CASP8, CD14, RAC1
4668	TNF signaling pathway	3	108	0.0032	CASP8, CCL2, TNFRSF1A
4933	AGE-RAGE signaling pathway in diabetic complications	3	98	0.0032	CCL2, PRKCA, RAC1
5142	Chagas disease (American trypanosomiasis)	3	101	0.0032	CASP8, CCL2, TNFRSF1A
5200	Pathways in cancer	5	515	0.0032	AR, CASP8, ESR2, PRKCA, RAC1
4915	Estrogen signaling pathway	3	133	0.0035	ESR2, FKBP5, KRT18
4932	Non-alcoholic fatty liver disease (NAFLD)	3	149	0.0045	CASP8, RAC1, TNFRSF1A
4215	Apoptosis - multiple species	2	31	0.0048	CASP8, TNFRSF1A
5164	Influenza A	3	168	0.0055	CCL2, PRKCA, TNFRSF1A
5167	Kaposi's sarcoma-associated herpesvirus infection	3	183	0.0064	CASP8, RAC1, TNFRSF1A
5168	Herpes simplex infection	3	181	0.0064	CASP8, CCL2, TNFRSF1A
5203	Viral carcinogenesis	3	183	0.0064	CASP8, CHEK1, RAC1
4961	Endocrine and other factor-regulated calcium reabsorption	2	47	0.0077	PRKCA, VDR
5134	Legionellosis	2	54	0.0096	CASP8, CD14
5416	Viral myocarditis	2	56	0.0098	CASP8, RAC1
4370	VEGF signaling pathway	2	59	0.0103	PRKCA, RAC1
4060	Cytokine-cytokine receptor interaction	3	263	0.0126	CCL2, GH1, TNFRSF1A
4115	p53 signaling pathway	2	68	0.0126	CASP8, CHEK1
4664	Fc epsilon RI signaling pathway	2	67	0.0126	PRKCA, RAC1
4610	Complement and coagulation cascades	2	78	0.0149	PLAT, PROS1
5132	Salmonella infection	2	84	0.0166	CD14, RAC1
4666	Fc gamma R-mediated phagocytosis	2	89	0.0178	PRKCA, RAC1
4064	NF-kappa B signaling pathway	2	93	0.0184	CD14, TNFRSF1A
4151	PI3K-Akt signaling pathway	3	348	0.0184	GH1, PRKCA, RAC1
4657	IL-17 signaling pathway	2	92	0.0184	CASP8, CCL2
4972	Pancreatic secretion	2	95	0.0184	PRKCA, RAC1
5146	Amoebiasis	2	94	0.0184	CD14, PRKCA
5215	Prostate cancer	2	97	0.0184	AR, PLAT
5231	Choline metabolism in cancer	2	98	0.0184	PRKCA, RAC1
5145	Toxoplasmosis	2	109	0.0204	CASP8, TNFRSF1A
4670	Leukocyte transendothelial migration	2	112	0.021	PRKCA, RAC1
4380	Osteoclast differentiation	2	124	0.0248	RAC1, TNFRSF1A

4650	Natural killer cell mediated cytotoxicity	2	124	0.0248	PRKCA, RAC1
4210	Apoptosis	2	135	0.0277	CASP8, TNFRSF1A
4145	Phagosome	2	145	0.0297	CD14, RAC1
4310	Wnt signaling pathway	2	143	0.0297	PRKCA, RAC1
5161	Hepatitis B	2	142	0.0297	CASP8, PRKCA
4150	mTOR signaling pathway	2	148	0.0299	PRKCA, TNFRSF1A
4217	Necroptosis	2	155	0.032	CASP8, TNFRSF1A
4621	NOD-like receptor signaling pathway	2	166	0.0356	CASP8, CCL2
5010	Alzheimer's disease	2	168	0.0356	CASP8, TNFRSF1A
5202	Transcriptional misregulation in cancer	2	169	0.0356	CD14, PLAT
4360	Axon guidance	2	173	0.0361	PRKCA, RAC1
4062	Chemokine signaling pathway	2	181	0.0385	CCL2, RAC1
4510	Focal adhesion	2	197	0.0434	PRKCA, RAC1
5205	Proteoglycans in cancer	2	195	0.0434	PRKCA, RAC1
4015	Rap1 signaling pathway	2	203	0.0451	PRKCA, RAC1
4810	Regulation of actin cytoskeleton	2	205	0.0451	CD14, RAC1

Table 3: Binding energy, number of hydrogen bonds and residues of withanolides from *Withania somnifera* with PLpro, 3clpro and spike protein

Ligand	PLpro (4M0W)			3clpro (6LU7)			Spike protein		
	BA	NHB	HBR	BA	NHB	HBR	BA	NHB	HBR
rawithanolide	-8.3	3	TYR208, ARG167	-7.8	6	LEU271, MET276, ASN277, GLY278, TYR239	-7.5	-	-
olide D	-8.9	4	HIS273, TRP107, HIS290	-8.8	2	LEU287, LYS137	-8.4	2	THR831, ASP320
olide E	-8.1	3	THR309, GLU308	-8	2	THR199, ASN238	-8.2	1	CYS566
olide F	-8.6	1	MET207	-7.8	-	-	-7.4	2	ASP815, ASP820
olide G	-8.9	6	ARG167, GLN233, TYR208	-9	3	TYR239, LYS137, LEU287	-7.8	1	ASP815
olide H	-8.3	1	ARG167	-8.8	3	ASN238, THR199	-8.5	6	ASN211,ASN185, THR254, SER67, THR94, ALA259
olide I	-8.7	2	MET207, ARG167	-9.1	7	ARG131,ASP289, THR199, LEU287,TYR239, ASN238	-9.7	2	THR525, CYS566
olide J	-8.6	2	MER207, ARG167	-9.3	4	ASN238, ASP289, THR199	-8.7	1	ASN61
olide K	-8.6	2	ARG167, GLU204	-8.5	4	SER158, ARG105	-8.7	4	THR47, ASP46, GLN825, CYS823
olide L	-8.1	2	GLU308, LYS218	-8.3	5	ASN238, THR199,ARG131	-8.6	2	TYR59, ARG268
olide M	-8.3	2	LYS158, TYR265	-9.1	5	LYS137, ARG131,LEU287, THR199, TYR239	-9.9	4	CYS566, THR525, LYS523
olide N	-7.4	2	LYS95, ARG139	-7.6	2	GLN110, PRO108	-8.8	3	THR525, CYS566
olide O	-8.3	3	GLU204, THR171, ARG167	-7.8	1	SER158	-8.3	1	ASN61
olide P	-8.2	2	ARG167	-7.7	-	-	-8.2	-	-
olide Q	-8.2	4	ASN147, ARG139	-7.9	2	THR199, ARG131	-8.8	2	CYS566
olide S	-7.8	4	GLU308, SER310, THR309	-7.9	2	LEU141, GLY143	-8.1	-	-
noloid R	-8.2	4	THR201, HIS176, ASN129, GLN175	-9.1	5	ARG131, LYS137,THR199, ASN133, ASP197	-7.8	1	THR831

BE: Binding energy in Kcal/mol, NHB: Number of hydrogen bonds, HBR: Hydrogen bond residues

Figures

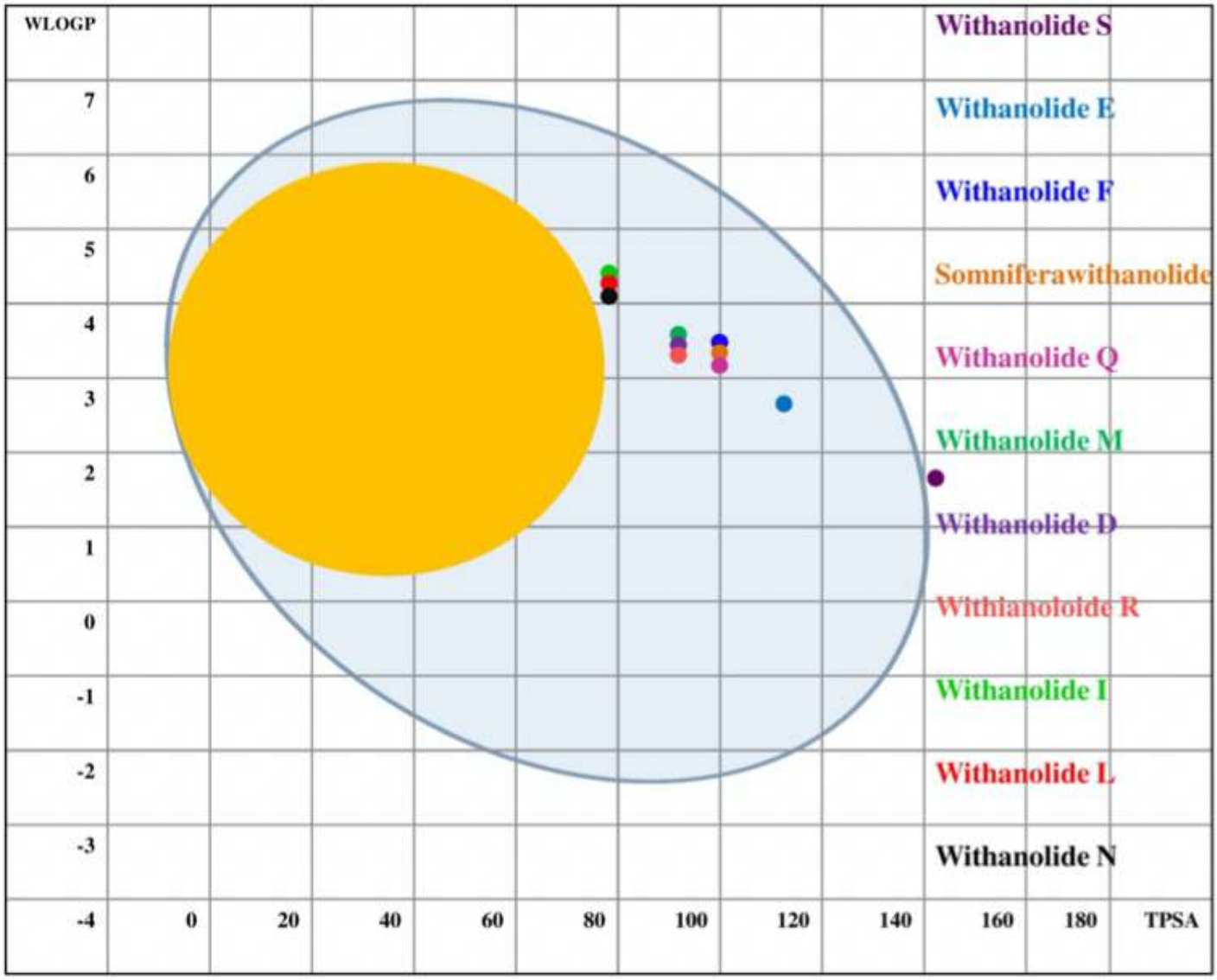
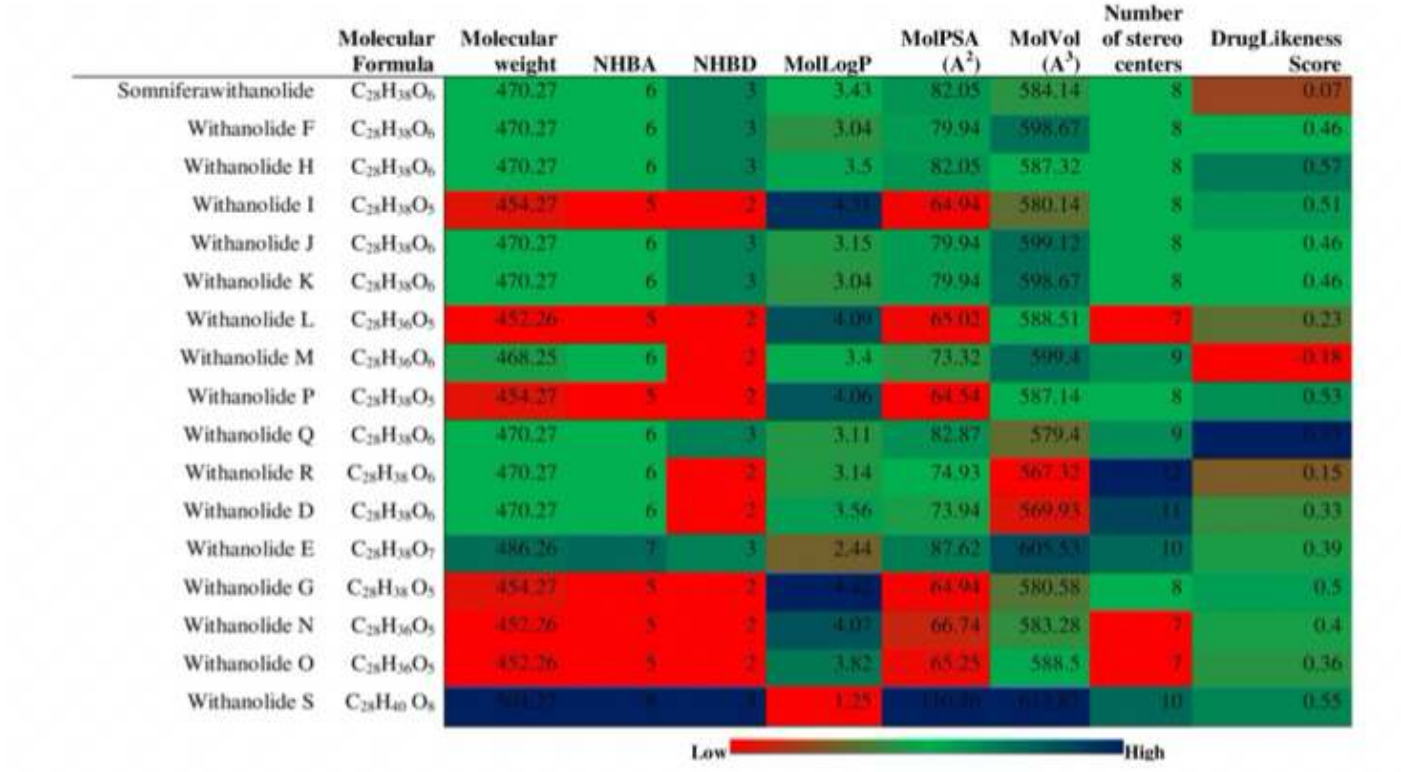


Figure 1

Boiled egg representation of withanolides for human intestinal absorption.



	Molecular Formula	Molecular weight	NHBA	NHBD	MolLogP	MolPSA (A^2)	MolVol (A^3)	Number of stereo centers	DrugLikeness Score
Somniferawithanolide	$C_{28}H_{38}O_6$	470.27	6	3	3.43	82.05	584.14	8	0.07
Withanolide F	$C_{28}H_{38}O_6$	470.27	6	3	3.04	79.94	598.67	8	0.46
Withanolide H	$C_{28}H_{38}O_6$	470.27	6	3	3.5	82.05	587.32	8	0.57
Withanolide I	$C_{28}H_{38}O_5$	454.27	5	2	4.31	64.94	580.14	8	0.51
Withanolide J	$C_{28}H_{38}O_6$	470.27	6	3	3.15	79.94	599.12	8	0.46
Withanolide K	$C_{28}H_{38}O_6$	470.27	6	3	3.04	79.94	598.67	8	0.46
Withanolide L	$C_{28}H_{36}O_5$	452.26	5	2	4.09	65.02	588.51	7	0.23
Withanolide M	$C_{28}H_{36}O_6$	468.25	6	2	3.4	73.32	599.4	9	-0.18
Withanolide P	$C_{28}H_{38}O_5$	454.27	5	2	4.06	64.94	587.14	8	0.53
Withanolide Q	$C_{28}H_{38}O_6$	470.27	6	3	3.11	82.87	579.4	9	0.33
Withanolide R	$C_{28}H_{38}O_6$	470.27	6	2	3.14	74.93	587.32	11	0.15
Withanolide D	$C_{28}H_{38}O_6$	470.27	6	2	3.56	73.94	569.93	11	0.33
Withanolide E	$C_{28}H_{38}O_7$	486.26	7	3	2.44	87.62	605.53	10	0.39
Withanolide G	$C_{28}H_{38}O_5$	454.27	5	2	4.4	64.94	580.58	8	0.5
Withanolide N	$C_{28}H_{36}O_5$	452.26	5	2	4.07	66.74	583.28	7	0.4
Withanolide O	$C_{28}H_{36}O_5$	452.26	5	2	3.82	65.25	588.5	7	0.36
Withanolide S	$C_{28}H_{40}O_6$	472.28	6	3	1.25	110.38	603.38	10	0.55

Low High

Figure 2

Druglikeness score of withanolides from Withania somnifera

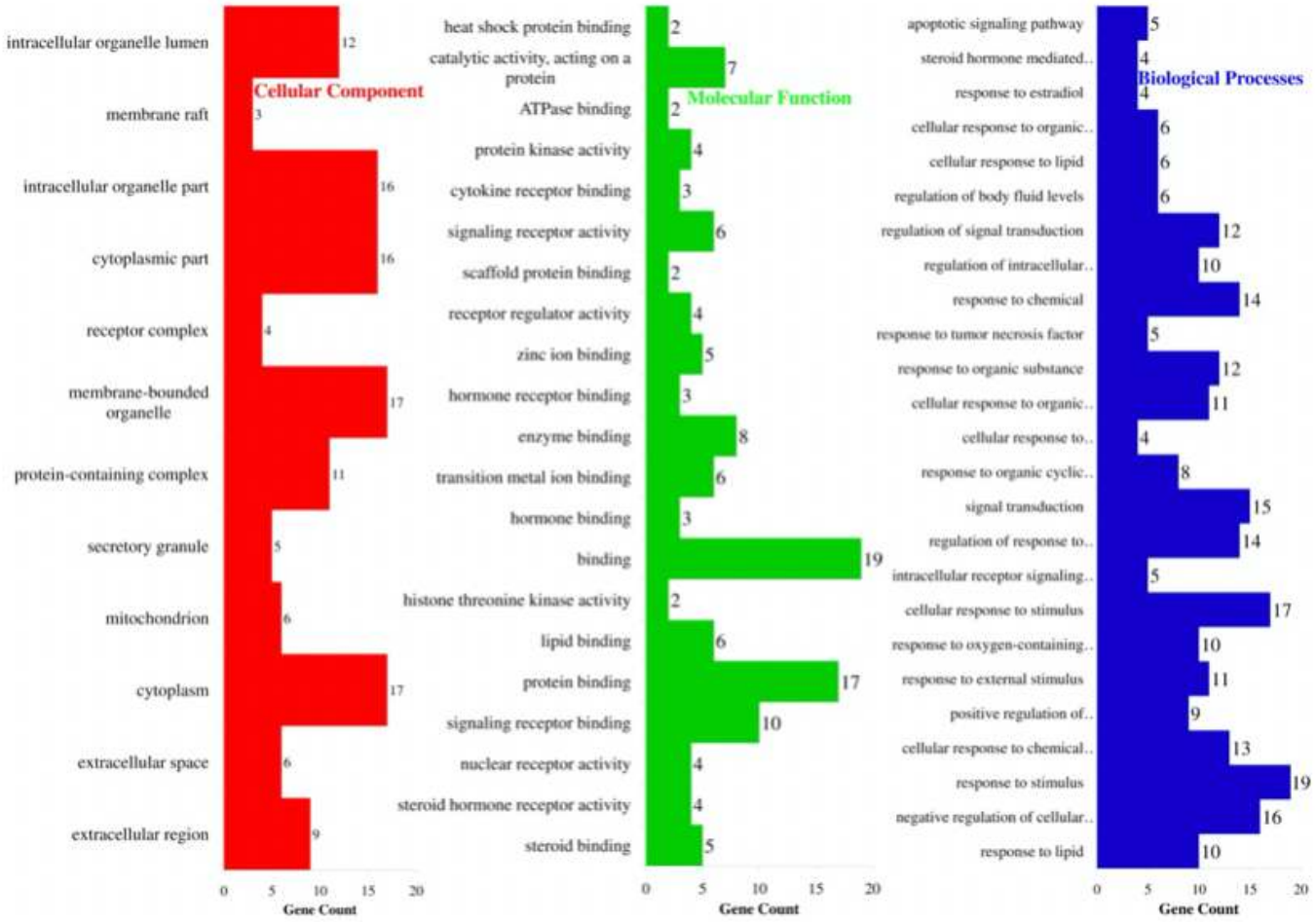


Figure 3

GO analysis of combined network for cellular component, molecular function and biological processes.

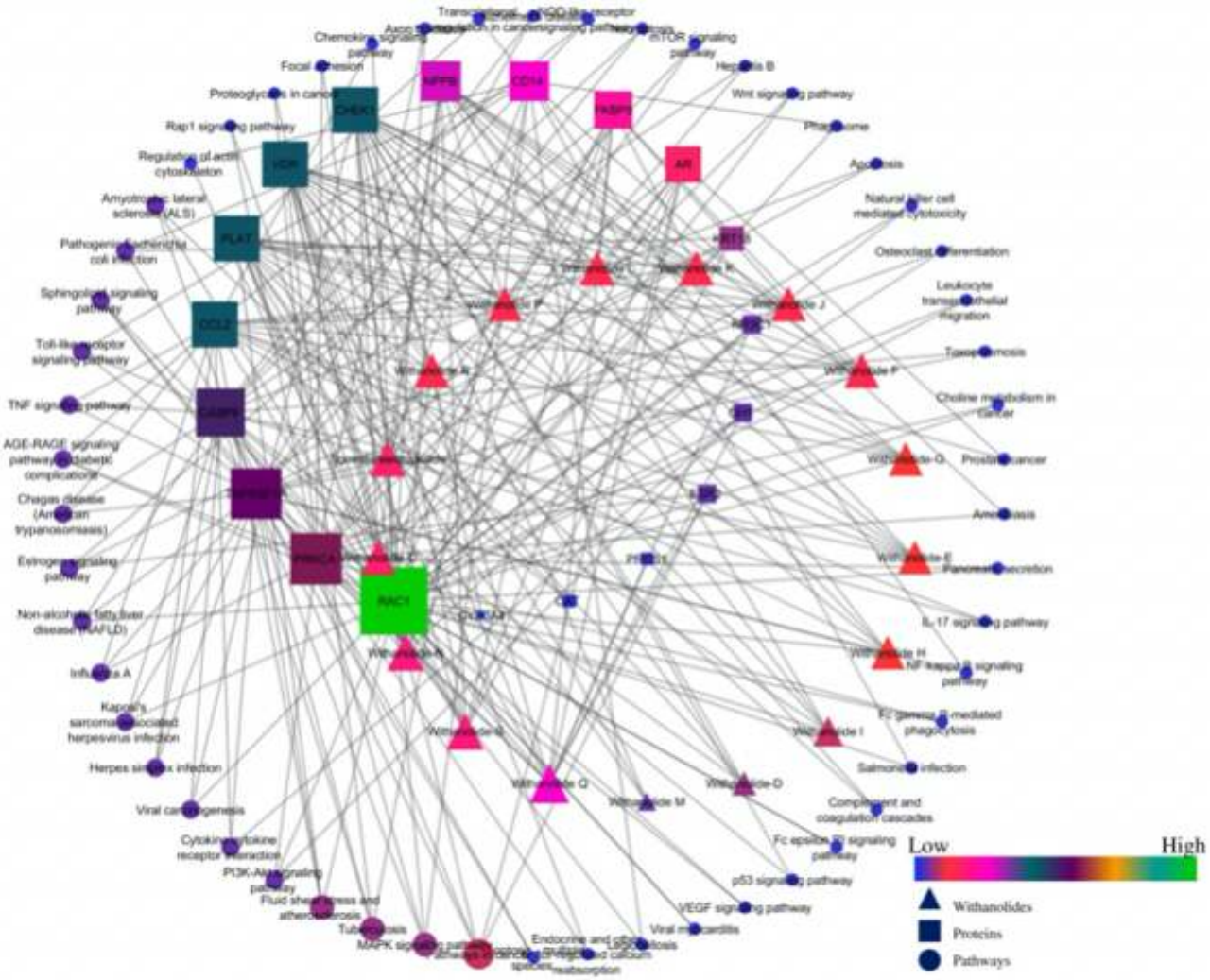


Figure 4

Network interaction of withanolides, regulated proteins and respective pathways.

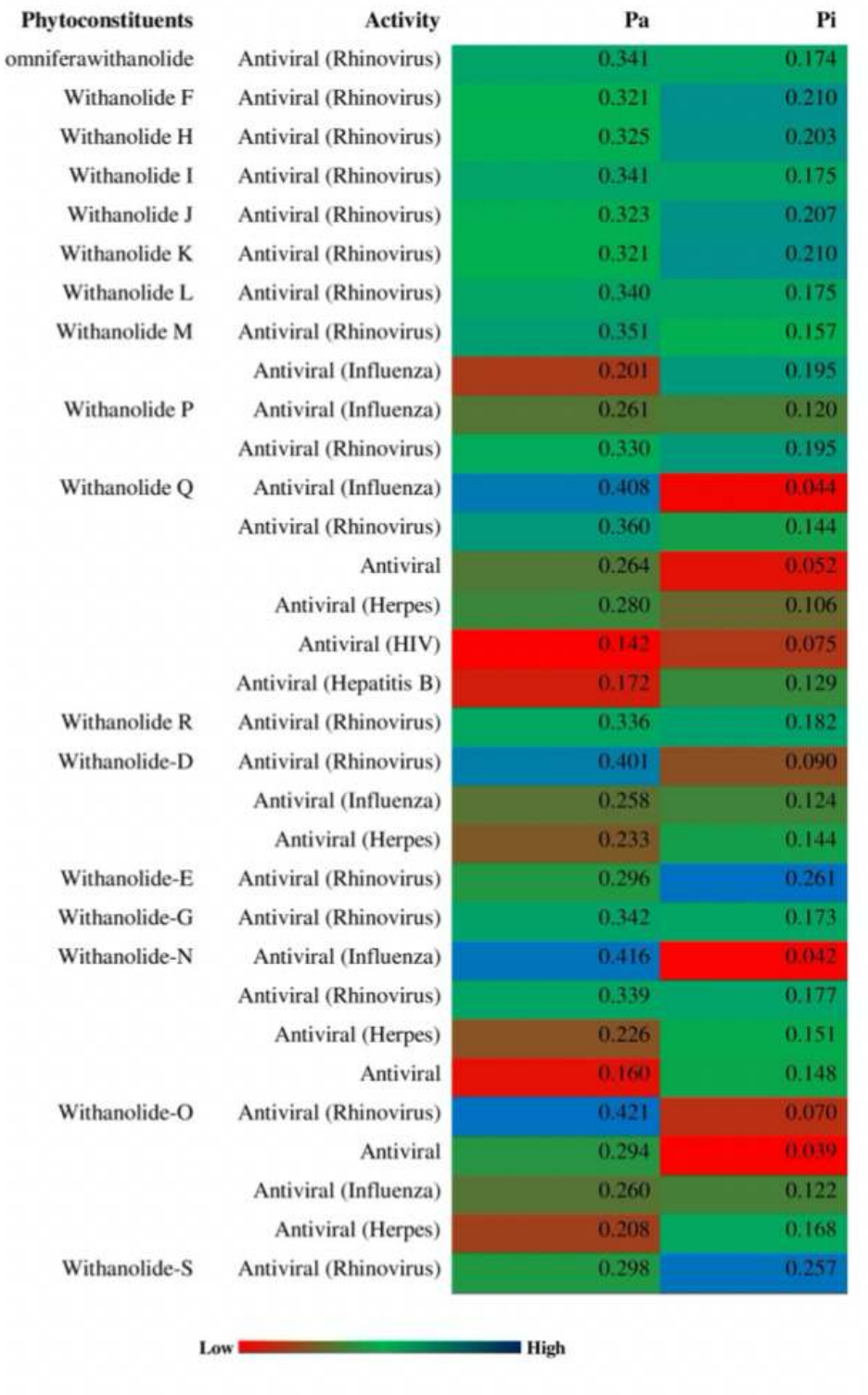


Figure 5

PASS analysis of antiviral activity of withanolides from Withania somnifera Pa: probable activity, Pi: probable inactivity

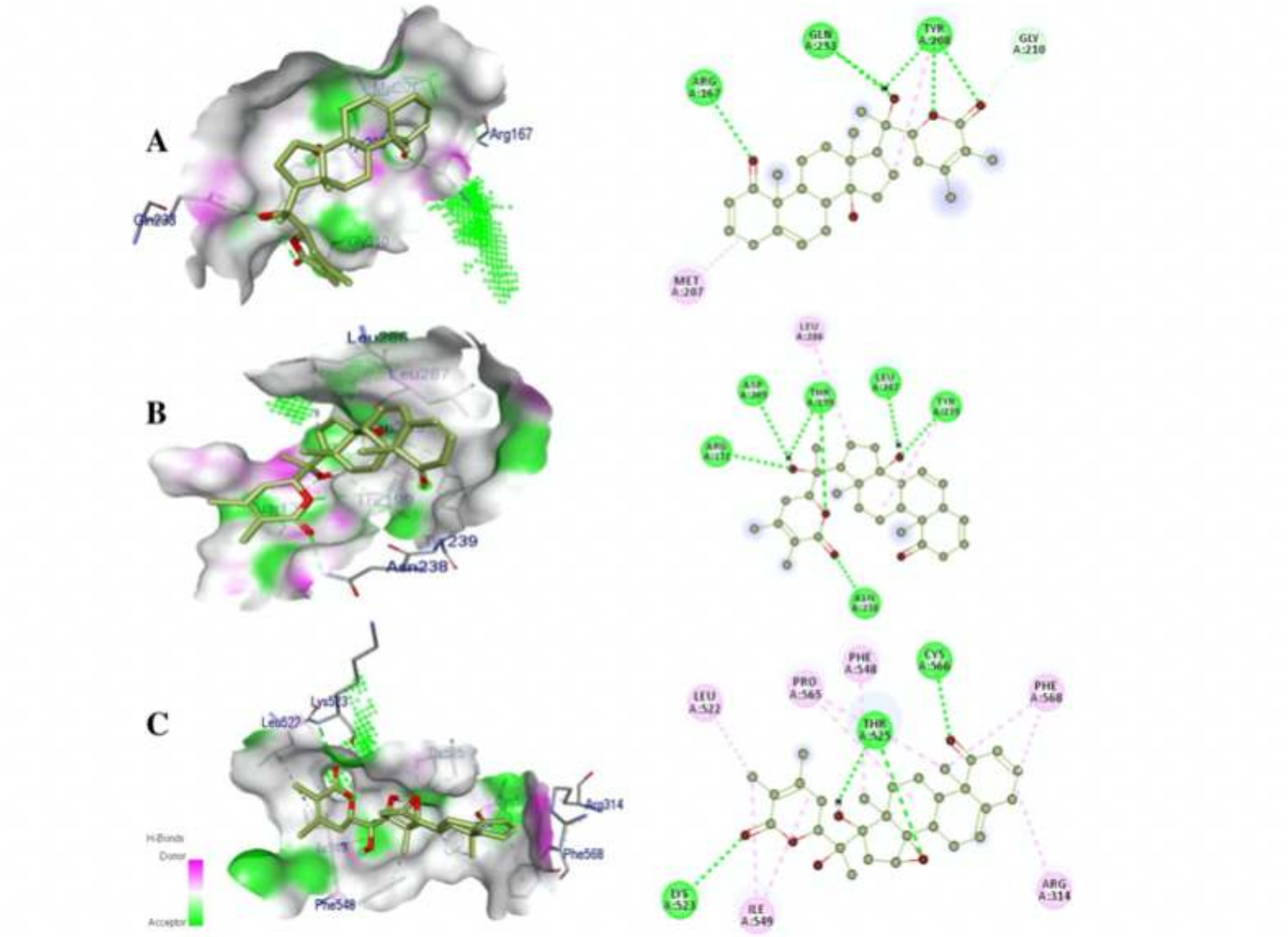


Figure 6

Interaction of (a) withanolide G, (b) withanolide I and (c) withanolide M with PLpro, 3clpro, and spike protein respectively