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RESEARCH ARTICLE

Multi-Targeted Prediction of the Antiviral Effect of *Momordica charantia* extract based on Network Pharmacology

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Abstract

The fruits of *Momordica charantia* (bitter gourd) have been well known for centuries as a natural remedy for the treatment of various ailments. In this study, we aimed to explore the metabolites present in both varieties of small and big bitter gourds and the multitarget mechanism of *M. charantia* in antiviral infection by utilizing network pharmacology. The study design involves the identification of the compounds in both varieties of the bitter gourd by Agilent QTOFLC-MS/MS system, followed by screening for ADME to analyze the possible mechanism of action, disease association, protein-protein interactions and major pathways involved therein. Several databases were used, including IMPAT, Binding DB, Swiss Target Prediction, STRING, DAVID, and KEGG databases, and algorithms were used to gather information. To visualize the network, Cytoscape 3.2.1 was used. As a result, a total of 22 and 27 compounds were detected in small and big bitter gourds, respectively. The molecules from *M. charantia* provide an antiviral response through the involvement of pathways like the Toll-like receptor pathway, the PI3/AKT pathway, NF-kappa B signalling pathway, and cytokine-cytokine receptor interaction. Moreover, the core target genes, termed "Hub Genes" were also identified through Cyto-hubba. The main mechanisms of *M. charantia* were acquired by investigating the enrichment of each cluster through functional association clustering analysis. Our results exposed the mechanism of *M. charantia* against viral infection through multi-component, multi-target, and multi-pathway study combinations.

Keywords: Antiviral, Hub Genes, LC-MS, *Momordica charantia*, Network Pharmacology, Pathway Analysis

1. Introduction

Medicinal plants play a pivotal role in the management and prevention of various infectious and non-infectious diseases. The medicinal plant performs a dynamic role in the development of new drugs. The threat of viral infection to human health has grown significantly. The emergence of new viruses with increasing virulence and the rapid spread of several known viruses are a great challenge facing the human population. Worldwide, viral infections are the main cause of morbidity and mortality. Apart from infections caused by the human immunodeficiency virus (HIV-1 and HIV-2), a new viral infection caused by SARS-CoV-2 has emerged to challenge human survival. To cure these viral infections, a variety of medicinal plants and their extracts have been used traditionally and as a natural remedy since ancient times throughout the world[1]. Combinatorial chemistry and targeted drug design have been developed in the past century for the development of new drugs. However, medicinal plants provide a safe, non-toxic, and multitargeted approach to treating

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various viral infections with broad-spectrum antiviral effects. *Momordica charantia* (Family Cucurbitaceae) is one such common consumable medicinal plant that has been preowned for centuries because of its numerous pharmacological and nutritional properties. The fruits of these plants are bitter, hence they are famously known as bitter gourds, bitter melons, or bitter squash. The plant contains a diverse range of phytochemicals, including steroids, triterpenes, saponins, alkaloids, and flavonoids. All these phytochemicals possess various therapeutic properties like bactericidal, anti-fungal, anti-viral, anti-tumorous, anti-carcinogenic, anti-fertility, anti-parasitic, and hypoglycaemic properties[2,3].

Currently, research on drug discovery is highlighted towards a systematic and multi-pharmacological approach as it promises a potential therapeutic solution to complex diseases and drug resistance issues. The approach to network pharmacology involves the identification of effective compounds from a medicinal plant[4].

Network pharmacology is an important field, evolving as frontier research in the field of drug discovery and drug development with the integration of pharmacology and Bioinformatics. To discover a drug having high potency and fewer adverse effects, the concept of network pharmacology is constructed by targeting multiple nodes that are intertwined, which generates information more sharply than each node[5]. Network pharmacology plays a pivotal role in establishing a relationship between plant components and disease targets. With the development of plant metabolite databases such as PubChem, IMPPAT, etc., researchers can now build a relationship between disease targets and components to scientifically extract the existing scientific data. Through the network pharmacology approach, "compound proteins/gene-disease" pathways can be established, which can help us to elucidate the complexity of the disease, drugs, and therapeutic targets from a systemic viewpoint. This concept offers a method for methodically revealing the correlation between multi-component targets and multi-pathways. Network pharmacology not only explains the occurrence of diseases but also their development from the perspective of systems biology and biological network balance. Thus, with this new field, researchers were able to elucidate the interactions between several compounds and disease targets rather than the concept of "single disease, single target" for investigating the activities of *M. charantia*[6-8].

M. charantia L. possesses various pharmacological benefits that include treatment of respiratory problems, diabetes, fever, HIV, AIDS, cancer, prevention, and treatment of many other viral infections[9-11]. Some of the experimental studies showed potential clinical activity and further studies need to be required to advocate its use. Many potent antiviral activities of *M. charantia* L. have been reported. Many studies reported on this plant showed that it strongly inhibits the growth of several viruses, including herpes simplex virus, hepatitis B virus and the human immunodeficiency virus. As far as we know, no comprehensive study has been conducted to scientifically describe the antiviral activity of *M. charantia* compounds, so our research focused on building a network that conveys the interactions between plant ingredients and viral protein targets in order to validate the antiviral activity of the active ingredients.

The main objective of this study is to explore the combinatorial approach of LC-HRMS and network pharmacology for the identification of compounds from small and big *M. charantia* and their associated pharmacological mechanisms related to viral infection. The primary chemical constituents of both varieties of *M. charantia* were investigated based on the network analysis approach. Phytochemicals of both varieties of *M. charantia* were done by LC-HRMS analysis. The network pharmacology approach is the best way to study the multicomponent synergistic mechanism. To explore the cellular mechanism involved behind the therapeutic effects of *M. charantia* in viral infection, the study of target compound-target-protein interaction, protein-protein interaction, and pathway analysis is very much important.

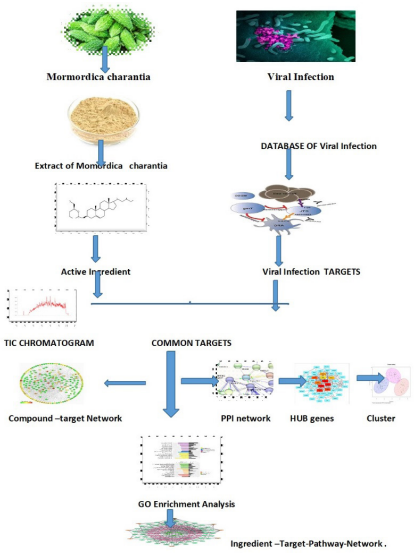

Figure 1. Workflow for *Momordica charantia* against viral infection.

2. Materials and Methods

Fresh *Momordica charantia* fruit samples were collected and washed properly. The fruits were then shade-dried and powdered. The powdered material was weighed, defatted with hexane, and finally extracted with methanol. The plant extract obtained after methanolic extraction was concentrated under a reduced vacuum by using a rotary evaporator at 40°C to obtain the methanolic extract. The extract was lyophilized for complete water removal and stored at 4°C. 10mg of methanolic extract was then weighed and dissolved in 1 ml LCMS grade methanol. The extract was then filtered through a 0.2-µm PTFE membrane filter and was subjected to liquid chromatography-mass spectrometry screening for compound identification. The solvents used for extraction are laboratory grade while LCMS grade solvents are used to carry out LCMS analysis.

3. Study Design

Chemical constituents of two varieties of *M. charantia* were recovered from public databases, namely, the IMPPAT database (Indian Medicinal Plants, Phytochemistry and Therapeutics), the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP, http://sm.nwsuaf.edu.cn/lsp/tcmsp.php) and reported literature. The phytochemicals were revealed through the application of four phases of pharmacokinetics parameters called Absorption, Distribution, Metabolism and Excretion (ADME) criteria. Genes related to antiviral infection were selected from human disease databases. Cytoscape 3.2.1 software is a platform-independent open-source Java application for visualizing complex networks. It helps to build graphical displays, conduct analysis, and allow editing. The software uses bioinformatics to examine and analyze the association between phytochemicals and target proteins and the pathways involved. Moreover, there are several network analysis plug-ins are available that can help us to examine the correlation between multi-component and multiple targets, which in turn helps to identify the key nodes of the network. This could help us to identify the basic mechanism by which *M. charantia* demonstrated its antiviral effect and could be useful in the treatment of a variety of infectious diseases. In such networks, the nodes denote the compounds and the edges denote the interactions between the nodes.

3.1 Chemical Databases

Various databases like IMPPAT (https://cb.imsc.res.in/imppat/home), ChEMBL (https://www.ebi.ac.uk/chembl/), PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider (http://www.chemspider.com/) have been used to retrieve the molecular weight, molecular formula and 2D chemical structure of *M. charantia*. SMILES formats of the enlisted 2D chemical structures were generated using Chemdraw Professional 15.0.

3.2 LC-HRMS Fingerprinting of *Momordica charantia*

The LCMS study was carried out on an ultra-high performance LC (UPLC), an Agilent 1290 Infinity II LC system that is coupled with Agilent. The UPLC system was assembled with a Diode Array Detector (DAD) and autosampler. The Chromatographic separation was achieved on an Agilent ZORBAX SB-C18 column (2.1 × 100 mm, 1.8 µm) as the stationary phase and a suitable LC gradient program has been developed by using acetonitrile and water (0.1% formic acid) as a mobile phase. The mass spectrometry parameters like drying gas (N2) flow, drying gas temperature, capillary voltage, skimmer voltage, nozzle voltage, and fragment voltage have also been optimized. The data acquisition was accomplished using Agilent Mass Hunter Acquisition software (Agilent Technologies, Santa Clara, CA, USA).

3.3 ADME Screening of *Momordica charantia* Ingredients

Most of the prominent active compounds fail to become drugs because of their poor kinetic characteristics (mainly oral bioavailability). Therefore, screening of ADME parameters plays a very crucial role in the drug discovery process (24-25). Drug Likeness (DL) is a qualitative concept that is utilized in the drug design process. It predicts the structural features and molecular properties of a lead molecule that will help us to optimize the pharmacokinetics and pharmaceutical features of those lead molecules to improve their solubility and chemical properties.

The oral bioavailability of any drug mainly depends on its difference in metabolism in the liver, but the variability in its absorption and distribution is equally important too. Compounds with OB > 30% will be potentially recognized as active ingredients. The majority of the compounds have a high absorption potential. Caco-2 permeability is a

parameter that measures the permeability rate of any drug/lead across polarized Caco-2 intestinal epithelial cells. This parameter will help us to predict the *in vivo* absorption of drugs in the intestine. Analysis of oral bioavailability limits F (20% and 30% bioavailability), Caco2 and Human Intestinal Absorption (HIA) indicators were performed for *M. charantia* fruit components by ADMETlab.

3.4 Network Pharmacology Analysis

To better exhibit the mechanism of action of two varieties of *M. charantia* in viral infection, we constructed four networks, namely, the drug compounds and compound target network, the drug compound-target-pathway network, the PPI network, and the hub gene analysis network.

3.5 Target Gene Prediction

The interaction between target proteins/genes with each active compound was obtained from the Swiss target prediction and binding database server. To obtain the interactivity between each active compound and its target genes in the Swiss target prediction server, first input the SMILES of query molecules in the Swiss target prediction server, then by choosing humans from the "organisms" parameter, and then press the search button to get the interacted target genes. While in the case of binding databases, the protein targets for identified *M. charantia* compounds of two varieties were predicted using "Find My Compound Targets". After this, the target genes related to the viral infection obtained from the two databases for the two varieties of bitter gourd were selected for further analysis. Moreover, the antiviral targets associated with M. *charantia* were retrieved from Gene Cards (https://www.genecards.com) and DisGeNET (https://www.disgenet.org/). The "Homo sapiens" specific UniProt ID for the selected protein targets was obtained from the UniProt database, a freely accessible protein database (https://www.uniprot.org), which provides information regarding protein sequence and their functional information[12,13].

3.6 Identification of Targets for Viral Infections

Gene Cards (https://www.genecards.com), DisGeNET (https://www.disgenet.org/), and Mala Cards (https://www.malacards.org) databases were used for retrieving antiviral targets. Cytoscape software has been used to construct and visualize the network between the active compounds and their corresponding target genes. The software particularly visually integrates the network with expression profiles and links the network to databases of functional annotations[14,15]. To elucidate the mechanism of action of small bitter gourd and big bitter gourd, network visualizations of "compound target" and "compound-target pathway network maps" were generated with the help of the visualization software, Cytoscape 3.2.1[16,17]. Moreover, this software was used to create, edit, visualize and analyze the networks. In these networks, the nodes represent the compounds and the edges represent the interactions between the nodes.

3.7 Protein-Protein Interaction (PPI) Network

A Protein-Protein Interaction (PPI) provides you with a statistical representation of the physical contacts between proteins in the cell. PPI networks are generally modelled via graphs in which the nodes correspond to proteins and the edges correspond to the interacting proteins. The PPI network was constructed by using an online free biological database known as STRING, which is known as Search Tool for the Retrieval of Interacting Genes/Proteins) database (http://stringdb.org, version 11.0 with the organism set as "Homo sapiens"[8]. To construct the PPI network, the PPIs of the target genes were screened with confidence >0.40. The STRING database is usually used to search for interactions between known proteins and predicted proteins[9,18] but the available information on protein-protein associations is incomplete and exhibits varying levels of annotation granularity and reliability. The STRING database aims to collect, score and integrate all publicly available sources of protein-protein interaction information, and to complement these with computational predictions. Its goal is to achieve a comprehensive and objective global network, including direct (physical. For the STRING analysis, the criteria included interaction sources from experiments, databases, co-expression, text mining, gene fusion, neighbourhood, and co-occurrence. A minimum interaction score of 0.70 or high-confidence interactions was considered. The top 10 genes showing the highest degree of confidence were sorted out and their protein network was built by using Cytoscape 3.2.1.

The PPI network systematically summarizes the interactions of *M. charantia* fruit targets correlated with viral infection treatment. The constructed PPI network showed that 36 viable protein target nodes were attached through 135 edges. The average node degree and local clustering coefficient were found to be 7.5 and 0.548, respectively. Interactions between proteins are indicated by a PPI enrichment p-value of less than 1.0 e^{-16}. The target proteins are partially biologically connected as a group, which indicates the enrichment is significant.

The novel Cytoscape plug-in *Cytohubba* was used to explore the important nodes in a biological network, which will rank the nodes in a network by their network features. The DEGREE algorithm was used to generate gene rankings, which were then chosen. The predicted top 10 contributing hub genes in small *M. charantia* that were considered crucial targets of *M. charantia* against viral infection are CASP3, CDk2, CXCL3, CCl3, IL1B, CASP, EGFR, CDK1, IL2, IL6. The top 10 genes predicted contributing hub genes in big *M. charantia* are IL1B, CCL3, CXCL8, EGFR, IL2, TNF, STAT3, CASP3, NOS2, and IL6.

3.8 DAVID Pathway Analysis

For pathway analysis, the identified targets corresponding to the individual compounds were subjected to pathway analysis using datasets for annotation. (DAVID) (https://david.ncifcrf.govt), a free online bioinformatics resource[19] is a Visualization and Integrated Discovery tool. Through DAVID, one can identify the cellular components, biological processes, molecular functions, and biological pathways that are associated with the subjected genes.

3.9 Gene Ontology (GO) and Pathway Enrichment Analysis

The targets in the network were imported into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for elucidating the mechanism of action. The main aim of Gene Ontology (GO) is to perform enrichment analysis on gene sets and analyze the biological process. KEGG is a database collection that deals with drugs and chemical substances, diseases, genomes, and biological pathways. For two varieties of *M. charantia,* to study biological processes and signaling pathways, GO and KEGG pathway enrichment was performed.

3.10 Cluster Analysis

Cluster analysis or topological modules are the regions of densely connected molecular complexes that have the property of a pure network in large Protein-Protein Interaction (PPI) networks. Proteins that belong to the same cluster show their synergistic effect on disease progression. In the current study, functional annotation cluster analysis for the target proteins in complex bioinformatics networks has been formed.

4. Results and Discussion

4.1 Analysis of Chemical Compounds

The LC-MS-based approach was employed to carry out the metabolite profiling of *M. charantia*. The Total Ion Chromatogram (TIC) obtained after LC-MS analysis of a methanolic extract of both big and small bitter gourds is illustrated in Figures 1 (a) and (b). We processed the chromatogram through MassHunter B.08 software and by comparing their molecular formulae and integrating with plant-specific customized libraries, we have identified 22 and 27 compounds, respectively, in small and big *M. charantia* (shown in Tables 1 and 2). The retention time, experimental and calculated m/z score, error in parts per million (ppm), and a molecular formula of the identified compounds were expressed through mass spectroscopic data (Figure 2).

The error in the mass tolerance limit of 5 ppm has been set for compound identification. The chemical compounds of *M. charantia* are complex and mainly contain the following chemical constituents: charantin, cucurbitacin, diosgenin, elaeostearic acid, gentisic acid, goyaglycosides, goyasaponins, karounidiols, momorcharasides, momordenol, momordicillin, momordicinin, momordicoside, momordin and petroselinic acid. Potential target genes and associated compounds of *M. charantia* are expressed in Tables 1 and 2.

Table 1. Results of Liquid Chromatography Electrospray Ionisation Tandem Mass Spectrometry (LC-ESI-MS/MS) analysis of small *Momordica charantia*

Sl no	Name of Compound	Formula	m/z	Mass	Rt	Score	PPM error
1	Charantin	$C_{18}H_{30}O_2$	279.2310	278.2235	8.06	92.73	-1.87
2	Charantoside IV	$C_{29}H_{36}O_{15}$	683.2179	624.2046	2.66	64.96	-6.11
3	Diosgenin	$C_{29}H_{46}O_2$	485.3635	426.3495	16.96	98.93	-2.78
4	Elaeostearic acids	$C_{30}H_{46}O_2$	439.3568	438.3495	7.05	97.28	-2.11
5	Galacturonic acids	$C_{30}H_{46}O_3$	455.3509	454.3437	6.2	77.59	-3.4
6	Gentisic acid	$C_{30}H_{48}O_2$	485.3636	440.3654	16.96	99.13	1.76
7	Goyaglycoside E	$C_{30}H_{48}O_3$	457.3676	456.3602	7.2	97.79	1.07
8	Karavilagenin E	$C_{30}H_{48}O_4$	473.3606	472.3534	7.93	92.52	-0.37
9	Karaviloside III	$C_{36}H_{56}O_7$	601.4100	600.4023	5.62	88.14	-5.97
10	Karounidiols	$C_{36}H_{56}O_7$	601.4090	600.4016	4.36	97.63	0.03
11	Kuguacin C	$C_{36}H_{60}O_{10}$	653.4263	652.4183	5.44	90.82	-3.15
12	Kuguacin J	$C_{37}H_{60}O_9$	671.4130	648.4235	5.44	94.27	-4.36
13	Momordenol	$C_{37}H_{62}O_8$	679.4385	634.4404	15.23	77.02	-0.67
14	Momordicin	$C_{40}H_{56}O_2$	569.4375	568.4306	6.75	60.30	-5.87
15	Momordicinin	$C_{42}H_{68}O_{13}$	780.4668	780.4668	5.62	97.65	**-0.64**
16	Momordicoside Q	$C_{44}H_{68}O_{14}$	821.4636	820.458	4.44	73.72	**-0.56**
17	Myristic acid	$C_6H_{10}O_7$	217.0301	194.0428	3.05	50.17	-4.42
18	Pipecolic acid	$C_{10}H_{16}N_4O_7$	305.1064	**304.1009**	2.43	62.4	-8.22
19	Taiwacin A	$C_6H_{11}NO_2$	130.0863	129.0791	1.02	87.04	-6.56
20	Verbascoside	$C_{35}H_{60}O_6$	611.4089	**576.4393**	27.1	81.78	**-1.32**
21	Vicine	$C_7H_6O_4$	153.0187	**154.0259**	4.85	97.13	-2.06
22	Zeaxanthin	$C_{27}H_{42}O_3$	459.3043	414.3069	18.65	10.95	**-4.6**

Table 2. Results of Liquid Chromatography Electrospray Ionisation Tandem Mass Spectrometry (LC-ESI -MS/MS) analysis of big *Momordica charantia*

Sl no	Name of Compound	Formula	m/z	Mass	Rt	Score	PPM error
1	Charantin	$C_{18}H_{30}O_2$	279.2310	278.2235	8.06	92.73	**-1.87**
2	Charantagenin D	$C_{37}H_{60}O_9$	671. 4146	648.4261	7.87	64.38	-2.12
3	Charantoside IV	$C_{29}H_{36}O_{15}$	**683.2179**	624.2046	2.66	**64.96**	-6.11
4	Diosgenin	$C_{29}H_{46}O_2$	485.3635	426.3495	16.96	98.93	-2.78
5	Elaeostearic acids	$C_{30}H_{46}O_2$	**439.3568**	438.3495	7.05	97.28	-2.11
6	Galacturonic acids	$C_{30}H_{46}O_3$	**455.3509**	454.3437	6.2	77.59	-3.4
7	Gentisic acid	$C_{30}H_{48}O_2$	485.3636	**440.3654**	16.96	99.13	1.76
8	Goyaglycoside E	$C_{30}H_{48}O_3$	457.3676	456.3602	7.2	97.79	1.07
19	Lycopene	$C_{40}H_{56}$	571.41	536.4352	8.9	40.29	1.98
10	Karavilagenin E	$C_{30}H_{48}O_4$	473.3606	472.3534	7.93	92.52	-0.37
11	Karaviloside III	$C_{36}H_{56}O_7$	601.4100	600.4023	**5.62**	88.14	**-5.97**

(*Continued*)

Table 2. (Continued)

Sl no	Name of Compound	Formula	m/z	Mass	Rt	Score	PPM error
12	Karounidiols	$C_{36}H_{56}O_7$	601.4090	600.4016	4.36	97.63	0.03
13	Kuguacin C	$C_{36}H_{60}O_{10}$	653.4263	**652.4183**	5.44	90.82	-3.15
14	Karavilagenin E	$C_{30}H_{48}O_3$	457.3676	456.3602	7.2	97.79	-2.10
15	Kuguacin J	$C_{37}H_{60}O_9$	671.4130	648.4235	**7.33**	94.27	-4.36
16	Momordenol	$C_{37}H_{62}O_8$	679.4385	634.4404	15.23	77.02	-0.67
17	Momordicin	$C_{40}H_{56}O_2$	569.4375	568.4306	6.75	60.30	-5.87
18	Momordicinin	$C_{42}H_{68}O_{13}$	780.4668	780.4668	5.62	97.65	**-0.64**
19	Moimordin	$C_{41}H_{64}O_{13}$	809.428	764.4313	11.51	56.24	-1.56
20	Momordicoside Q	$C_{44}H_{68}O_{14}$	821.4636	820.458	4.44	73.72	**-0.56**
21	Myristic acid	$C_6H_{10}O_7$	217.0301	194.0428	3.05	50.17	-4.42
22	Momordicosides	$C_{47}H_{80}O_{19}$	974.5278	948.5270	2.08	63.47	-6.54
23	Pipecolic acid	$C_{10}H_{16}N_4O_7$	305.1064	**304.1009**	2.43	62.4	-8.22
24	Petroselinic Acid	$C_{18}H_{34}O_2$	281.2481	282.2554	8.6	83.12	-2.20
25	Taiwacin A	$C_6H_{11}NO_2$	130.0863	129.0791	1.02	87.04	-6.56
26	Verbascoside	$C_{35}H_{60}O_6$	611.4089	**576.4393**	27.1	81.78	**-1.32**
27	Vicine	$C_7H_6O_4$	153.0187	**154.0259**	4.85	97.13	-2.06

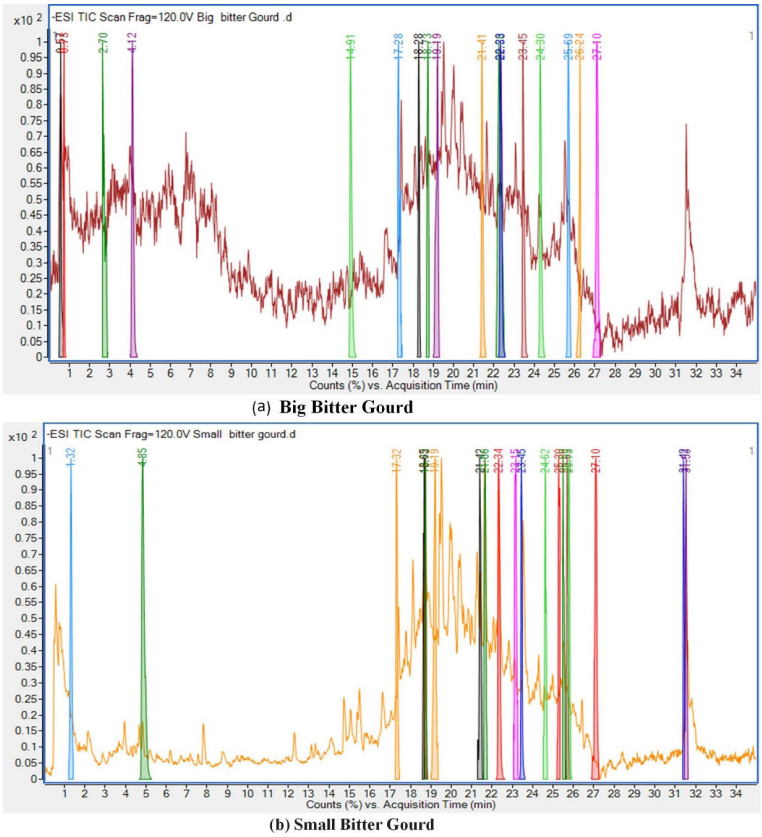

Figure 2. LCMS analysis of **(a)** Big Bitter Gourd, **(b)** Small Bitter Gourd.

4.2 ADME Screening of *Momordica charantia* Ingredients

The ADME properties of the metabolites of *M. charantia* are being analyzed. The specific information on each of the identified components is represented in Table 3. The present study was undertaken to screen the components along with their pharmacokinetic properties like Drug Likeness (DL), F (20% bioavailability), F (30% bioavailability), Caco2, and Human Intestinal Absorption (HIA) indicators. The compounds show a high probability of absorption.

Table 3. ADME Profile of *Momordica charantia* compounds

Compound	DL	CACO2	HIA	F20	F30
Pipecolic acid	0.55	-4.836	0.719	0.7	0.545
Gentisic acid	0.56	-5.099	0.466	0.716	0.542
Cucurbitacins		-5.172	0.63	0.26	0.296
Diosgenin	0.55	-4..843	0.782	0.522	0.366
Vicine	0.17	-6.328	0.242	0.289	0.303
Momordin	0.11	-5.944	0.433	0.279	0.307
Momordicin	0.55	-5.01	0.781	0.495	0.285
Momordenol	0.55	-4.735	0.927	0.541	0.461
karounidiols	0.55	-5.109	0.874	0.538	0.406
Zeaxanthin	0.17	-5.149	0.77	0.614	0.532
Gypsogenin,	0.85	-5.397	0.701	0.327	0.344
Elaeostearic acids	0.85	-4.730	0.78	0.491	0.259
Verbascoside	0.17	-6.809	0.18	0.396	0.199
Goyasaponins	0.11	-4.698	0.271	0.201	0.241
Petroselinic acid	0.85	-5.151	0.794	0.453	0.266
Momordicinin	0.55	-4.696	0.840	0.597	0.429
Charantin	0.17	-5.777	0.293	0.253	0.340
Lycopene	0.17	-4.851	0.750	0.721	0.478
Momordicosides	0.17	-6.327	0.271	0.272	0.31
Kuguacin J	0.55	-5.15	0.857	0.245	0.31
Kuguacin C	0.55	-5.38	0.772	0.482	0.320
Karavilagenin E	0.55	-5.128	0.771	0.478	0.325
Goyaglycoside E	0.17	-5.796	0.193	0.275	0.320
Charantoside IV	0.55	-5.232	.209	0.299	0.305
Taiwacin A	0.55	-5.802	0.276	0.226	0.219
Momordicoside Q	0.17	-5.83	0.262	0.243	0.316
Karaviloside III	0.55	-5.789	0.253	0.257	0.345
Charantagenin D	0.55	-5.791	0.259	0.255	0.351
Karounidiols	0.55	-5.109	0.874	0.538	0.406
Myristic acid	0.85	-4.636	0.8	0.453	0.291

4.3 Compound-Target-Pathway-Network Analysis of *M. charantia*

Network pharmacology is considered a powerful tool for the exploration of drug targets. The identified compounds from *M. charantia* can act on one or more targets in the current study, and most targets have a synergistic therapeutic effect. A compound-target-pathway combination network was constructed to establish this potential synergistic relationship, as shown in Figures 3 and 4 which represent the Venn diagram where 21 overlapping compounds are present in *M. charantia*.

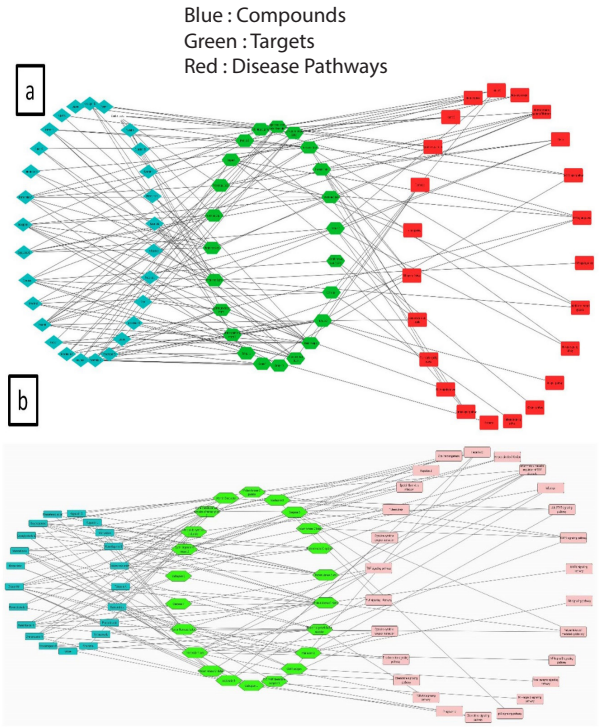

Figure 3. Compound-Target-Pathway analysis of *M. charantia*.

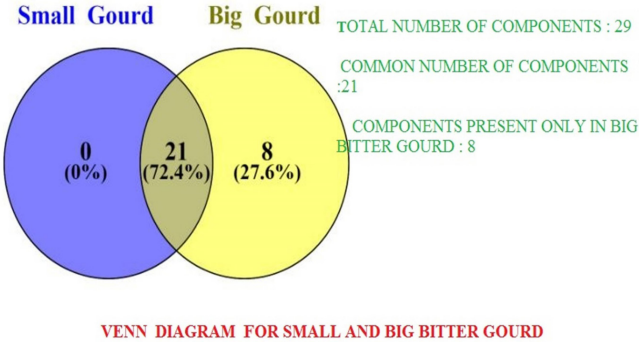

Figure 4. Venn Diagram for small and big bitter gourd compounds: 21 Overlapping compounds present both varieties of Bitter Gourd.

Through this network analysis, we could easily identify the multiple targets and multiple pathways that are associated with the *M. charantia* compounds, which will help us to justify the mechanism associated with the antiviral effect. For example, charantoside IV acted on 7 targets (such as STAT3, CTSD, CTSL, CASP7, CASP3.IL-2, CDK2), charantagenin D acted on 4 targets (IL-2, CTSL, EGFR, CDk2), while both charantoside IV and charantagenin D commonly acted on CDK2. Based on the network topological analysis, we have listed the top 9 phytoconstituents in descending order according to their major contributions to treating viral infection: gentisic acid, diosgenin, goya glycoside E, mormordicin, karoundiols, vicine, kuguain J, charantin, karavilagenin E.

The UniProt IDs of the targets were put into STRING (http://string-db.org/, version10.5). The "functional association" is the basic interaction unit in STRING, which provides a common tool for predicting protein-protein interaction. The STRING analysis summarized the interactions of *M. charantia* targets associated with viral infection treatment. The PPI network exhibits 24 viable protein target nodes in small *M. charantia* connected by 103 edges. The average node degree is 8.58 and the average local clustering coefficient is found to be 0.738, while in the case of big *M. charantia*, the network exhibits 26 viable protein target nodes that are attached by 109 edges. The average node degree and average local clustering coefficient were found to be 8.38 and 0.723, respectively. The PPI enrichment p-value is found to be less than $<1.0\ e^{-16}$ which suggests that the proteins have more interactions among themselves (Figures 4a and 4b). The target proteins are at least partially biologically connected as a group, as indicated by this significant enrichment.

Nodes that have important biological functions generally have a high connectivity degree (≥5) and are labelled as hub genes. The protein targets were ranked depending on their degree values and a network relationship among these top 10 targets by cytoHubba as shown in Figures 5a and 5b. CASP3, EGFR, IL6, IL2, CDK1, CDK2, CXCL8, CCL3, IL8, and CASP7 were the top 10 hub genes in the case of small *M. charantia*. In the case of big *M. charantia,* the top 10 hub genes were CXCL8, STAT3, TNF, CXCL8, CCL3, CASP3, IL6, NOS2, IL2, and IL1B. Thus, the mentioned proteins are generally considered the core targets in the Protein-Protein Interaction network and are closely linked with the components of *M. charantia* in the treatment of viral infection. The target proteins identified are also involved in the inflammatory response, Influenza A, Hepatitis C, Hepatitis A, Viral carcinogenesis, Apoptosis, Pathways of cancer, and Herpes simplex virus infection. The node's color was displayed in descending order of degree value, in a gradient from red to yellow. The ranking of PPI core targets of big and small *Momordica charantia* by degree method is represented in Table 4 (Figure 6).

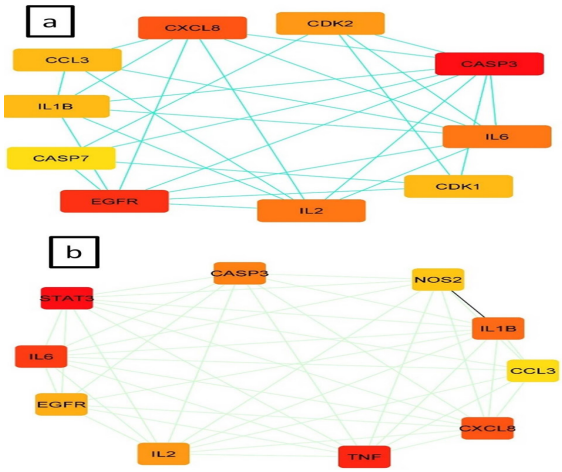

Figure 5. **(a)** Hub genes for Big bitter gourd, **(b)** Hub genes for Small bitter gourd.

Table 4. Ranking of PPI Core Targets of Big and Small *Momordica charantia* by Degree Method

Sl No	Targets of BMC	Degree	Targets of SMC	Degree
1	STAT3	36	CASP3	34
2	EGFR	36	EGFR	24
3	CASP3	32	CXCL8	18
4	TNF	30	IL6	14
5	IL6	26	IL2	14
6	IL2	25	CDK2	13
7	CXCL8	23	IL1B	12
8	IL1B	18	CDK1	12
9	CCL3	16	CCL3	12
10	NOS2	16	CASP3	11

BMC: Big *M. charantia*; **SMC**: Small *M. charantia.*

Most of the compounds, such as elaeostearic acid, kuguacin C, momordicin, and kuguaicin J interact with the Tumour Necrosis Factor (TNF), which is a cytokine - a small protein used by the immune system for cell signalling that activates the cytokine-cytokine receptor interaction, T cell receptor signalling pathway, and TNF signalling pathway. Moreover targets such as cathepsin L, cathepsin D, interleukin-2, interleukin-6, interleukin-8, epidermal growth factor, and vitamin D receptor interact with most compounds and give protection against viral infection.

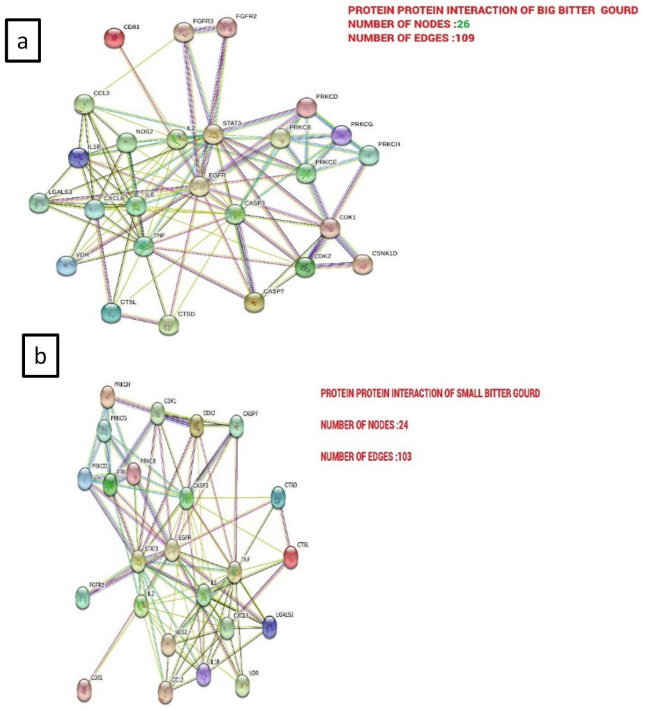

Figure 6. The Protein-Protein Interaction (PPI) network and significant module constructed by the STRING database and Cytoscape 3.7.2. Node, target proteins; lines, interactions between proteins. PPI of the common targets of **(a)** Big Bitter Gourd and **(b)** Small Bitter Gourd related to antiviral infection.

4.3.1 Gene Ontology (GO) and Enrichment Analysis

Gene ontology analysis has been commonly used for the functional analysis of genes. It mainly describes the role of gene products in the biological functions of cell function and molecular function. The top scores represent the most highly valued genes in the list of genes (as shown in Tables 5 and 6). The apex 10 terms involved in the three categories of gene ontology process designated as molecular function, cellular component and biological process, which are represented by Figures 7(a), 7(b) and 7(c), respectively.

GO enrichment analysis showed that the biological process was enriched with the response to an organic substance, positive regulation of molecular function, response to an organic substance, cellular response to oxygen-containing compounds, regulation of cell proliferation, etc.

The target genes responsible for protein kinase C activity, signalling receptor binding, calcium-independent protein kinase C activity, cytokine activity, calcium-dependent protein kinase C activity, and protein binding and receptor regulator activity were expressed at the molecular level. At the cellular level, they were related to the extracellular space, cyclin-dependent protein kinases, and the immunological synapse.

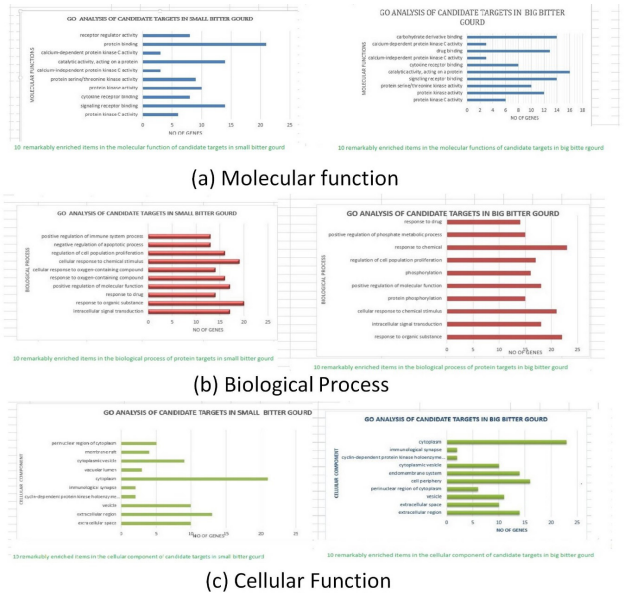

Figure 7. Go analysis for Big Bitter and Small Bitter Gourd was done under the categories of **(a)** Molecular function, **(b)** Biological process, **(c)** Cellular Function.

Table 5. The Top 10 most significant terms in GO enrichment analysis in Small *Momordica charantia*

ID	Description	P-adjust	Count
Biological Process			
GO:0035556	Intracellular signal transduction	8.17E-11	17
GO:0010033	Response to organic substance	1.12E-10	20
GO:0042493	Response to drug	1.76E-10	14
GO:0044093	Positive regulation of molecular function	1.76E-10	17
GO:1901700	Response to oxygen-containing compound	1.76E-10	16
GO:1901701	Cellular response to oxygen-containing compound	1.76E-10	14
GO:0070887	Cellular response to chemical stimulus	2.96E-10	19
GO:0042127	Regulation of cell population proliferation	4.87E-10	16
GO:0043066	Negative regulation of the apoptotic process	1.08E-09	13
GO:0002684	Positive regulation of the immune system process	1.22E-09	13
Molecular Function			
GO:0004697	Protein kinase C activity	3.00E-11	6
GO:0005102	Signalling receptor binding	3.18E-08	14
GO:0005126	Cytokine receptor binding	7.51E-08	8
GO:0004672	Protein kinase activity	9.95E-08	10
GO:0004674	Protein serine/threonine kinase activity	9.95E-08	9
GO:0004699	Calcium-independent protein kinase C activity	8.94E-07	3
GO:0140096	Catalytic activity, acting on a protein	8.94E-07	14
GO:0005515	Calcium-dependent protein kinase C activity	1.34E-06	3
GO:0097367	Protein binding	1.64E-06	21
GO:0030545	Receptor regulator activity	1.64E-06	8
Cellular Component			
GO:0005615	Extracellular space	7.54E-05	10
GO:0005576	Extracellular region	0.00015	13
GO:0031982	Vesicle	0.0136	10
GO:0000307	Cyclin-dependent protein kinase holoenzyme complex	0.0217	2
GO:0001772	Immunological synapse	0.0217	2
GO:0005737	Cytoplasm	0.0217	21
GO:0005775	Vacuolar lumen	0.0217	3
GO:0031410	Cytoplasmic vesicle	0.0217	9
GO:0045121	Membrane raft	0.0217	4
GO:0048471	Perinuclear region of cytoplasm	0.0217	5

Table 6. The Top 10 most significant terms in GO enrichment analysis in Big *Momordica charantia*

ID	Description	P-adjust	Count
Biological Process			
GO:0010033	Response to organic substance	6.75E-12	22
GO:0035556	Intracellular Transduction Signal	1.38E-11	18
GO:0070887	Cellular response to chemical stimulus	2.07E-11	21
GO:0006468	Protein phosphorylation	4.27E-11	15

(*Continued*)

Table 6. (Continued)

ID	Description	P-adjust	Count
GO:0044093	Positive regulation of molecular function	4.27E-11	18
GO:0016310	Phosphorylation	8.62E-11	16
GO:0042127	Regulation of cell population proliferation	1.59E-10	17
GO:0042221	Response to chemical	1.59E-10	23
GO:0045937	Positive regulation of the phosphate metabolic process	1.59E-10	15
GO:0042493	Response to drug	2.61E-10	14
Molecular Function			
GO:0004697	protein kinase C activity	5.18E-11	6
GO:0004672	protein kinase activity	1.15E-09	12
GO:0004674	protein serine/threonine kinase activity	9.25E-09	10
GO:0005102	signalling receptor binding	4.05E-08	14
GO:0140096	catalytic activity, acting on a protein	4.05E-08	16
GO:0005126	Cytokine receptor binding	5.25E-08	8
GO:0004699	Calcium-independent protein kinase C activity	9.93E-07	3
GO:0008144	Drug binding	1.37E-06	13
GO:0004698	Calcium-dependent protein kinase C activity	1.45E-06	3
GO:0097367	Carbohydrate derivative binding	2.02E-06	14
Cellular Component			
GO:0005576	extracellular region	0.00013	14
GO:0005615	extracellular space	0.00013	10
GO:0031982	Vesicle	0.0057	11
GO:0048471	Perinuclear region of cytoplasm	0.0098	6
GO:0071944	Cell periphery	0.0098	16
GO:0012505	Endomembrane system	0.0108	14
GO:0031410	Cytoplasmic vesicle	0.0108	10
GO:0000307	Cyclin-dependent protein kinase holoenzyme complex	0.0135	2
GO:0001772	Immunological synapse	0.0135	2
GO:0005737	Cytoplasm	0.0135	23

4.3.2 KEGG Pathway Annotation

KEGG pathway annotation showed that in both small and big *M. charantia*, the enriched target genes were 27 and involved in 131 pathways associated with the immune system, cytokine-cytokine receptor interaction, influenza A, hepatitis B, TNF signalling pathway, MAPK signalling pathway and PI3K-AKT signalling pathway.

Since the potential targets for the viral infection disease were identified, the pathways associated with influenza A and the immune system were chosen. For instance, the B-cell receptor, the Fc epsilon RI, the Toll-like receptor and the T-cell receptor signalling pathways are engaged in the regulation of the immune system and signalling pathways like NF-κ Band TNF are involved in inflammation. Moreover, the MAPK, PI3K/AKT, and NF-kappa B signalling pathways are also identified in this analysis. The PI3K/AKT signalling pathway is important for cell survival, cell growth, metabolism, differentiation, and apoptosis[17,18]. **KEGG** (Kyoto Encyclopedia of Genes and Genomes) pathway analysis revealed that the active phytoconstituents of *M. charantia* act on the gene nodes within the cAMP and PI3K/AKT signalling pathways (Figures 8(a) and 8(b)). These data follow the multiple effects that *M. charantia* has on different signalling and cellular pathways.

Figure 8. KEGG Pathway analysis of **(a)** Big Bitter Gourd, **(b)** Small Bitter Gourd.

4.4 DAVID Pathway Analysis

To investigate the mechanisms of *M. charantia*, the UniProt IDs list of 27 common genes was uploaded into the DAVID 6.8 bioinformatics resource. The pathways that are associated with the viral infection like cytokine-cytokine receptor interaction and Influenza A pathway were being selected in Figures 9(a) and 9(b).

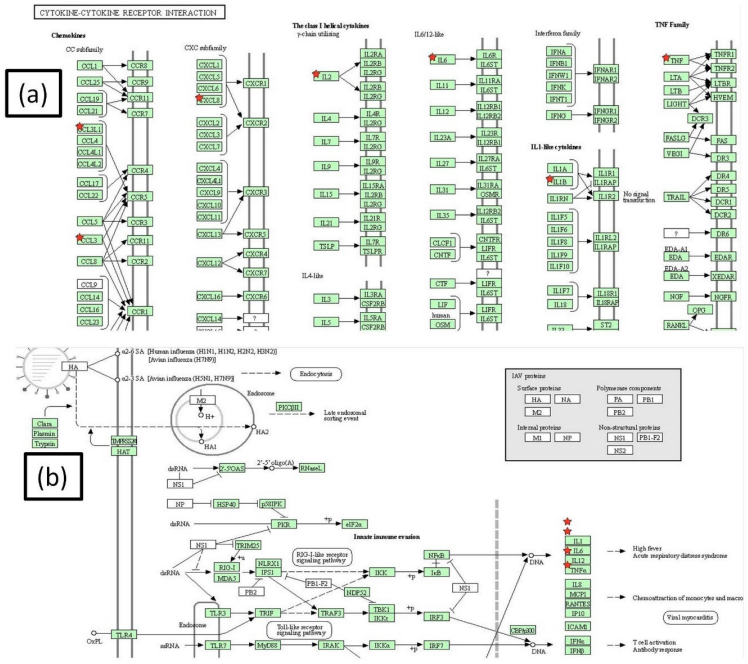

Figure 9. DAVID pathway analysis: **(a)** cytokine-cytokine receptor interaction, **(b)** Influenza A pathway; the stars indicate the targets where the molecules interact.

4.5 Cluster Analysis

In large PPI networks, clusters or topological modules are the regions where molecular complexes are connected densely. Functional association clustering analysis found 18 clusters for big *M. charantia* with the highest cluster score of 3.84 and 17 clusters for small *M. charantia* with the highest cluster scoring value of 5.75. The main mechanisms of *M. charantia* are acquired by investigating the enrichment of each cluster. The cluster analysis is represented in Figures 9 and 10. It was observed that through the network enrichment analysis, some important activities responsible for the antiviral effect include pathways like Protein Kinase C activity, Jak-STAT signalling pathway, P13K-AKT signalling pathway, Toll-Like Receptor Signalling Pathway, and Signal Transduction. Some important clusters of Small Bitter Gourds and Big Bitter Gourds were represented in Figures 10 and 11.

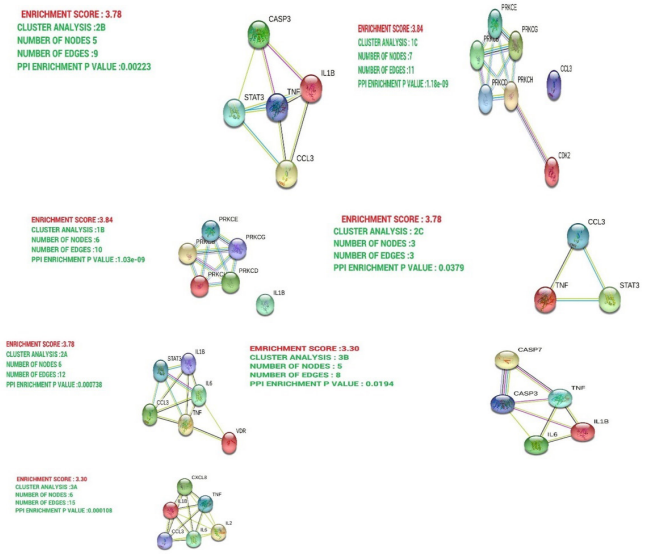

Figure 10. Cluster analysis of Small Bitter Gourd.

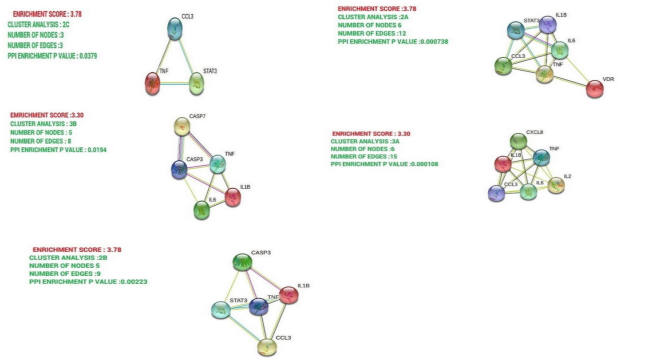

Figure 11. Cluster analysis of Big Bitter Gourd.

5. Conclusion

M. charantia has been described as a versatile plant and it has been studied extensively for its medicinal properties to treat a variety of diseases. Identification of compounds by LC-HRMS analysis followed by a new discipline called network pharmacology to understand drug actions and interactions with multiple targets provided a new perception of the mechanism of *M. charantia* in the treatment of viral infection. A total of 29 active compounds in both varieties of *M. charantia* were screened and hit by active 30 potential targets related to viral infection. Our results exposed the mechanism of *M. charantia* against viral infection through multi-component, multi-target, and multi-pathway studies.

Moreover, the results indicated that *M. charantia* was found to be powerful in the treatment of viral infection by regulating key pathways, including the Toll-like receptor, P13K-AKT signalling pathway, influenza, viral carcinogenesis, the Jak-Stat signalling pathway and the NF-kappa B signalling pathway.

These results provide significant information for further pharmacological investigations of *M. charantia*. The method of network pharmacology analysis helps to understand the mechanisms of *M. charantia* to combat viral infections and promote further drug research and development.

Moreover, the study provides a good foundation for carrying out further studies on specific pathways and provides evidence to support the safe and effective clinical use of *M. charantia.*

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