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Research Article

A NETWORK PHARMACOLOGY-BASED APPROACH OF TRADITIONAL INDIAN MEDICINAL PLANT *SOLANUM NIGRUM* LINN AGAINST HEPATOCELLULAR CARCINOMA

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ABSTRACT

Solanum nigrum (SN) has been widely used in traditional folk medicine to cancer treatments, but the defined target is not clear. To identify the potent targets for the treatment of HCC using various strategies, 23 active compounds were collected by IMPPAT and TCMSP databases based on their DL and ADME properties. PharmMapper, SEA, STITCH and DRAR-CPI followed to CTD and PHARMGKB were used for target fishing. 25 targets were retrieved to analyze GeneMANIA and DAVID. Cytoscape 3.6.1 was used for the network's constructions compound-target, target-pathway, compound-target-pathway and target organ networks were developed. For target validation, molecular docking GLIDE, AutoDock Vina and iGEMDOCK were used. Majorly, 21 targets were located in the liver, are considered as primary targets for *in silico* docking. The study concludes 5 potent targets and 4 active compounds via 4 signaling pathways involving HCC metabolism. SN could be potentially beneficial in treating HCC.

Keywords: *Solanum nigrum,* Network pharmacology, Hepatocellular carcinoma (HCC), Peroxisome proliferator-activated receptors (PPARs), Hyperin

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading cancers cause worldwide. Development of HCC includes many risk factors such as hepatitis B virus, hepatitis C virus, non-alcoholic fatty liver disease, alcohol consumption and diabetes. Metastasis of HCC is the major threatening that causes many deaths. Recent research is focused to find potent targets for treating HCC. Target therapy is the advancement in this field, yet there is a limitation in success rates due to multidrug resistance. It's an urgent need to discover effective targets for the treatment of HCC [1, 2].

Solanum nigrum Linn (SN) is Solanaceae family a common plant found throughout India, which is traditionally used for folk medicine to treat various purpose including pain, inflammation, fever, asthma, cough, ulcer wound, skin diseases, leprosy, hemorrhoids, dropsy, liver disorders, hepatoprotective agent, anticancer, antioxidant, neuroprotective, cytoprotective and antimicrobial [3,4]. SN is believed in Chinese traditional medicine to treat many types of cancer such as liver cancer, lung cancer, breast cancer, stomach cancer, bladder cancer and colon cancer [5]. SN reported with different chemical constituents, which was involved in medicinal properties, secondary metabolites like alkaloids, saponins, steroid, glycoprotein, flavonoids, saponins, tannins, glycosides, carbohydrates, proteins, phytosterol and Coumarins [6]. The presence of Quercetin in SN involved cancer prevention. It is a natural flavonoid found in plants, has been extensively used for its biological activities includes malignant cells growth in leukemia, breast, hepatic, ovarian, colorectal, gastric and endometrial cancers. It controls cancer cell growth by regulating specific pathways [7]. Studies reported in SN using animal models could be used to predict the pathway and targets were involved in cancer proliferation. AKR1B10 is generally, expressed in a normal condition in the colon, small intestine and liver. In carcinogenic cases, the level of AKR1B10 was elevated through the regulation of fatty acid synthesis and reactive carbonyls by AKR1B10 detoxification to enhance the growth and proliferation of cancer cells and overexpressed in Hepatocellular carcinoma (HCC) also involved in various cancer such as pancreatic cancer,

breast cancer and lung cancer [8]. Still, the specific individual compounds or targets involving in the HCC prevention are unclear maybe it could have a synergistic effect against HCC. We use network pharmacology to identifying potential targets and components via multiple signal pathways. This is the reason why we apply the computational approach construct network pathway and discover the effective components in SN against HCC.

Network pharmacology is a powerful tool to analyze the massive biological data. It deals with the multidrug-multitarget approach with a wide verity of diseases that provides knowledge about the effects and side effects of drugs. Also, it serves to document and analyze the clinical prescriptions of traditional medicine practitioners [9]. Improved *in silico* approaches can accelerate the drug discovery process through predict the pharmacokinetic model, metabolic and toxicity functions [10].

In the present study, we develop a network pharmacology approach to identify the active components and targets of SN against HCC through computational screening by evaluating their ADME properties and drug-likeness (DL) using IMPPAT and TCMSP server, potential targets further chose by an inverse docking method using PharmMapper, SEA, STITCH, DRAR-CPI and target mining for CTD and PHARMGKB. Target's genes were submitted to GeneMANIA and DAVID webservers to know the gene functions and pathways. The pharmacological data were integrated into four networks, compound-target, target-pathway, compound-target-pathway and target organ. This systematic overview of the network pathway provides potential targets and mechanisms of action in SN against HCC. Further, the progress validated through *in silico* molecular docking to find out the potent targets for the treatment of HCC using GLIDE v2017, Auto Dock Vina and iGEMDOCK v2.1.

2. MATERIAL AND METHODS

2.1. Compound selection

SN compounds were collected through Indian and Chinese traditional medicinal databases such as Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) (https://cb.imsc.res.in/imppat/home) and the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) (http://ibts.hkbu.edu.hk/LSP/tcmsp.php).

The compound was chosen based on the physiochemical properties, molecular descriptors, drug-likeness (DL), oral bioavailability (OB) and Tanimoto coefficient (DL ≥ 0.18) [11].

2.2. Computational Target Fishing and Data Mining

All selected compounds from SN structural data were retrieved from the Pub chem (https://pubchem.ncbi.nlm.nih.gov/) and IMPPAT. Active components were identified and compared with PharmMapper (lilab.ecust.edu.cn) [12], the Similarity Ensemble Approach (SEA), (http://sea.bkslab.org/) [13]. STITCH (http://stitch.embl.de/) [14] and Drug Repositioning and Adverse Reaction via Chemical-Protein Interactome (DRAR-CPI), (http://cpi.bio-x.cn/drar/) [15]. It automatically identifies the best mapping poses of query molecules the database annotated with *p*-value including the database like Target Bank, Binding DB, potential drug target databases and Drug Bank. Further, Comparative Toxicogenomic Database (CTD), (http://ctdbase.org/) and Pharmacogenomics knowledge (PHARMGKB), (http://www.pharmgkb.org) [16] were applied for target mining process.

2.3. GeneMANIA analysis

GeneMANIA (https://genemania.org) is a web-based server that provides information about gene lists, associated genes link and gene functional assays [17]. This server is a reliableweb interface, searching for the organism has changed to the homo sapiens, genes name was entered into the search bar to predict. The output files were retrieved.

2.4. Analysis of Gene Ontology (GO)

The targets gene of SN were performed by GO enrichment analysis using the Database for Annotation, Visualization and Integrated Discovery 6.8 server (DAVID), (http://david.abcc.ncifcrf.gov) is employed in the network biology were consolidated biological process information, it is used as analytical tools to identify the gene or proteins in systematically. Go and KEGG pathway information was collected based on the *p*-value of less than 0.05 [18].

2.5. Network/ Pathway Construction

To investigate relationships between the active ingredients of SN and HCC, we constructed four visualized networks namely, Compound Target network (C-T), Target Pathway network (T-P), Compound-Target-Pathway network (CTP) and Target-

Organ (T-O) network using Cytoscape 3.6.1. Cytoscape is a useful bioinformatics tool to visualize biological networks and data integration. The platform work with additional plugins like Network Analyzer and CentiScaPe 1.2 was installed from the Cytoscape app store. The metabolism pathway information further mapping through the KEGG database (Kyoto Encyclopedia of Genes and Genomes), (http://www.genome.jp/kegg/). Targets and location tissue were identified using BioGPS bank (http://biogps.org) [19].

2.6. *In silico* molecular docking validation

In silico docking is an efficient work field to identify the interaction between the compound and target proteins. The active compounds of SN and their potential targets were docked using Schrodinger- GLIDE v.2017, Auto Dock Vina and iGEMDOCKv2.1. Compounds structures were collected from PubChem and the target proteins were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/) used as ligand and targets, respectively for the molecular docking. Standard drug Sorafenib tosylate was used for the comparison [20].

3. RESULTS

3.1. Screening for active compounds

SN structural data were achieved from IMPPAT and TCMSP database, which resulted in 46 and 39 compounds, respectively. Common compounds of which were eliminated and gained 78 potential bioactive candidates. The resulted inactive compounds were followed Lipinski's rule of five and DL index≥ 0.18 on further, analysis. Finally, 23 active compounds were selected for further investigation (Table 1).

3.2. Drug-Target prediction

The 23 pharmacophores of SN were used to predict the potential targets by PharmMapper, SEA, STITCH, and DRAR-CP. SEA measures a similar reference target. For each reference, target SEA measures the similarity between the query compound and each reference compound and sums these numbers in Tanimoto Coefficients between ECFP4 fingerprints. To quantify the significance of this sum, SEA compares it to a background model fit with simulated targets made with compounds sampled at random from ChEMBL. Based on the disease specificity, 25 targets were mined by CTD and PHARMGKB (Table 2).

Table 1: Bioactive compounds and ADME properties of SN

No	Name	OB (%)	DL
C01	Syringaresinol	3.29	0.72
C02	2,4-Dihydroxycinnamic acid	0.56	0.596
C03	2-Amino-4,8-naphthalenedisulfonic acid	0.11	0.52
C04	2-Aminohexanedioic acid	0.56	0.394
C05	Beta-D-xylopyranose	0.55	0.295
C06	D-Galactose	0.55	0.29
C07	Flavylium perchlorate	0.55	0.53
C08	Hirsutrin	1.86	0.77
C09	Hyperin	6.94	0.77
C10	I-ascorbic acid	0.56	0.36
C11	lignoceric acid	14.90	0.33
C12	Medioresinol	0.55	0.81
C13	Nicotinamide	0.55	0.55
C14	Pinoresinol	4.25	0.52
C15	Pterosin B glucoside	16.07	0.46
C16	Quercetin	46.43	0.28
C17	Quercetin-3-gentiobioside	3.45	0.64
C18	Scopoletin	0.55	0.54
C19	Sitosterol	36.91	0.75
C20	Solanaviol	15.63	0.44
C21	Solatubin	17.12	0.76
C22	Tigogenin	14.09	0.81
C23	Tomatidenol	10.68	0.81

3.3. Analysis by GeneMANIA

Target genes (25) were input into the GeneMANIA to predict the pathway information, shared protein domains, localization, and genetic interactions. Overall, outputs showed a total of 45 genes and established 306 links. 60.45% of target genes displayed co-expression, 22.94% co-localization, 9.81% shared protein domains, 3.42% genetic interactions and 3.38% involved in pathway 3.38% (Fig. 1).

3.4. GO enrichment analysis for targets

The resulted target genes of HCC were used for GO enrichment analysis by DAVID. The interaction network was categorized in the Benjamini-Hochberg method ($p< 0.05$). Targets were involved in the biological process (BP, 63%), molecular function (MF, 21%), and cellular component (CC, 16%) respectively (Fig. 2).

Table 2: Putative targets information of SN

ID	PDB ID	Gene name	Target
T01	4D1N	NOS1	Nitric oxide synthase 1
T02	1ZUA	AKR1B10	Aldo-keto reductase family 1-member B10
T03	1YUC	NR0B2	Nuclear receptor subfamily 0 group B member 2
T04	2FLU	NFE2L2	Nuclear factor erythroid 2-related factor 2
T05	4H1S	NT5E	5'-nucleotidase
T06	2PEI	GCLC	Glutamate-cysteine ligase catalytic subunit
T07	1C9Y	OTC	Ornithine carbamoyl transferase, mitochondrial
T08	1BAS	FGF2	Fibroblast growth factor 2
T09	1M7M	ADA	Adenosine deaminase
T10	1E3G	AR	Androgen receptor
T11	1BJ1	VEGFA	Vascular endothelial growth factor A
T12	1IAR	IL 4	Interleukin 4
T13	1BJX	P4HB	Protein disulfide-isomerase
T14	3PM0	CYP1B1	Cytochrome P450 1B1
T15	5O0X	NOX4	NADPH oxidase 4
T16	5C65	SLC22A8	Solute carrier family 22 member 8
T17	1I7G	PPARA	Peroxisome proliferator-activated receptor alpha
T18	4QUV	DHCR7	7-dehydrocholesterol reductase
T19	1Z10	CYP2A6	Cytochrome P450 2A6
T20	1E6F	IGF2R	Cation-independent mannose-6-phosphate receptor
T21	5NJ3	ABCG2	ATP-binding cassette sub-family G member 2
T22	3MBG	GFER	FAD-linked sulfhydryl oxidase ALR
T23	2C17	CYP17A1	Steroid 17-alpha-hydroxylase/17,20 lyase
T24	3GKH	NPC1	Niemann-Pick C1 protein
T25	2VDX	SERPINA6	Corticosteroid-binding globulin

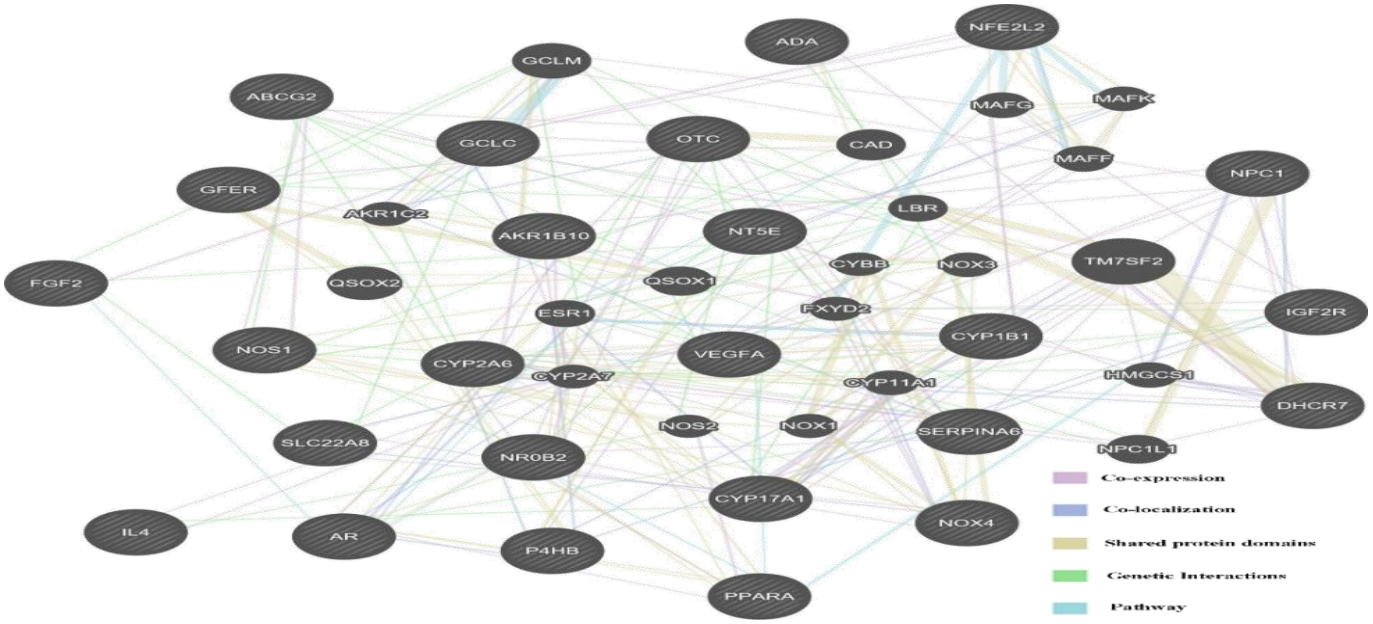

Fig. 1: A network analysis of potential targets using GeneMANIA.

Black nodes: target proteins; Grey circles: genes associated with query genes; Coloured lines: interactions

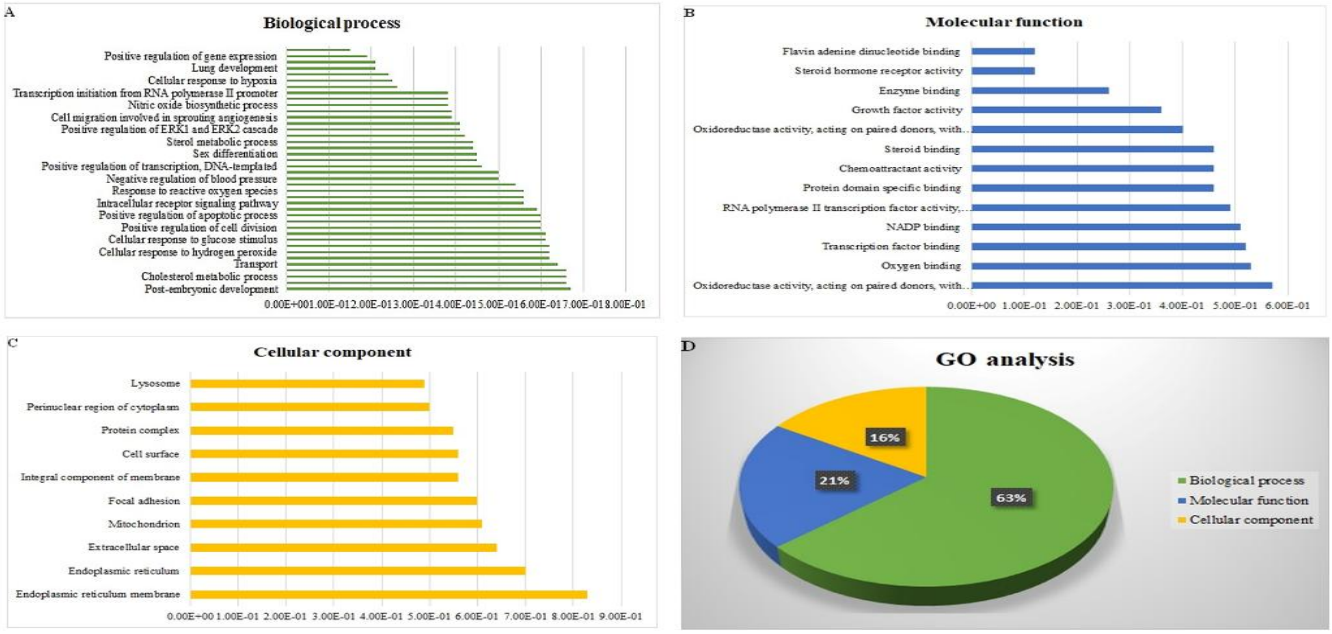

Fig. 2: Go enrichment analysis of the SN targets (A) Biological process; (B) Molecular function; (C) Cellular component; (D) Go analysis percentage chart

The top five biologicalprocessesare categorized post-embryonic development, cholesterol metabolic process, transport, cellular response to hydrogen peroxide and a glucose stimulus. KEGG pathways resulted in bile secretion, metabolic pathways, and arginine biosynthesis.

3.5. GO enrichment analysis for targets

3.5.1. Compound-Target (C-T) Network

System pharmacology wasassociated with signaling pathways to produce the pharmacodynamics model. It helps to visualize and interpret the interaction between active compounds and their targets. The construction of the C-T network provides the topological relationship of the compound and targets (Fig. 3). C-T network shows 60 interactions between 23 compounds and 25 targets. Compound C05, C10, C20, C21, C23, did not interact with any active targets. As the example of compound Quercetin (C16, degree 5), it has already been proved to treat cancer and synergistic effects in chemotherapy, This screening methods helps to identify the potential active compounds from SN related to HCC.

3.5.2. Target-Pathway (T-P) Network

The 25 potential targets of SN interact with 96 KEGG pathways and the T-P network was generated (Figure 4). The major pathwayswere related to carcinogenesis, fluid shear stress and atherosclerosis, hepatocellular carcinoma, metabolic pathways, metabolism of xenobiotics by cytochrome P450, microRNAs in cancer, ovarian steroidogenesis, pathways in cancer, protein processing in the endoplasmic reticulum, PI3K-Akt signaling pathway, steroid hormone biosynthesis andtryptophan metabolism.

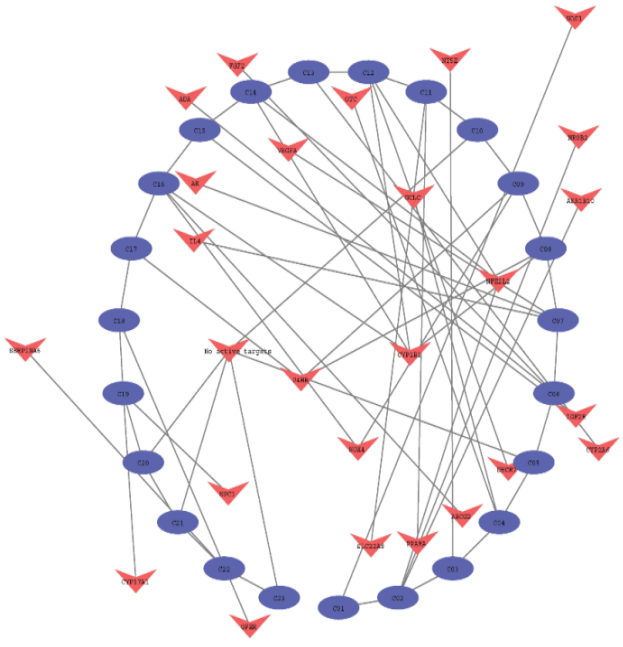

Fig.3: Compound-Target (C-T) network

Blue circle: Active compounds; Red inverted triangle: target proteins; Grey edges: interaction between compound and protein

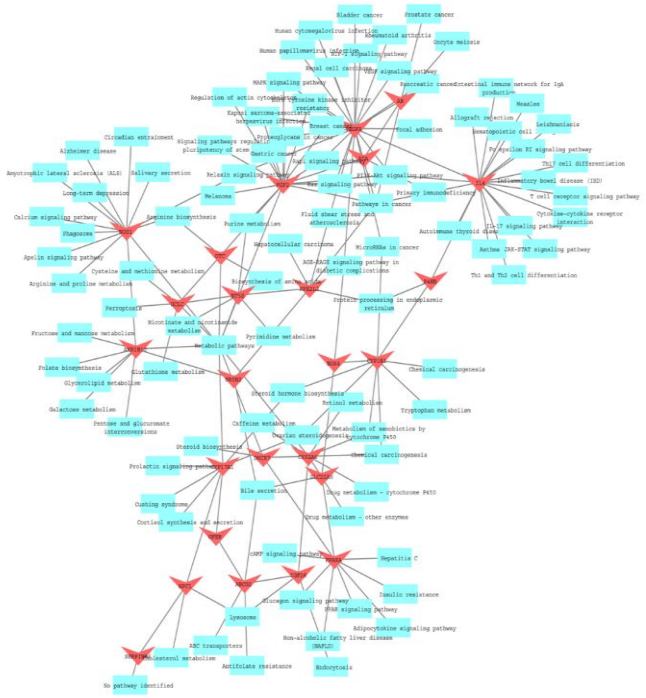

Fig. 4: Target-Pathway (T-P) network

Red inverted triangle: target proteins; Cyan square: Pathway; Grey edge: interaction between targets and pathway

3.5.3. Compound-Target-Pathway (CTP) Network

The compound-target-pathway interaction networks consisted of 148 nodes corresponding to 23 compounds and its targets, pathways in 215 edges (Fig.5).

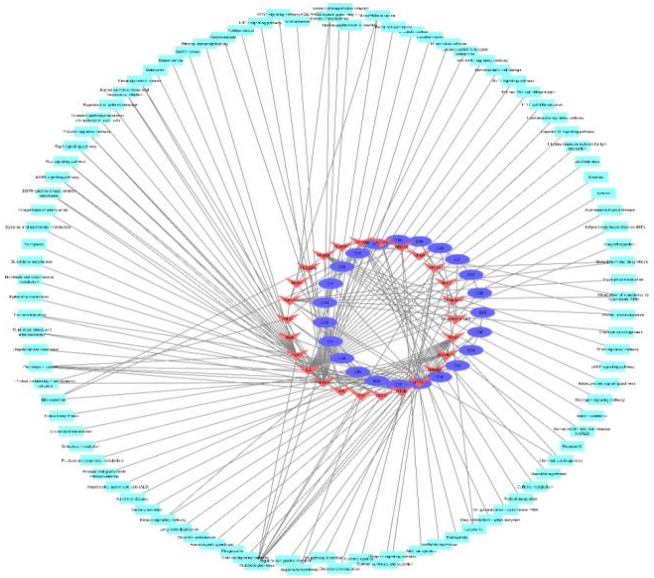

Fig. 5: Compound-Target-Pathway (CTP) network

Blue circle: Active compounds; Red inverted triangle: target proteins; Cyan square: Pathway; Grey edge: interaction between compound, targets, and pathway

NOS1, AKR1B10, NT5E, GCLC, OTC, FGF2, DHCR7, CYP2A6 and CYP17A1exhibited more connections with a metabolic pathway (degree=9) and NFE2L2, FGF2, AR, VEGFA and IL 4 involved pathways in cancer (degree=5). Compounds of SN associated with targets in HCC were majorly involved in PPAR, calcium, the PI3K-Akt, FGFR, VEGFand MAPK signaling pathways (Fig. 6), consistent with the SN compounds regulating various cancerous pathways such as breast cancer, gastric cancer, prostate cancer, pancreatic cancer and bladder cancer.

3.5.4. Target-Organ (T-O) Network Analysis

System-level detection of organ or tissue location helped to understand the HCC progression and development. The expression pattern and location of 25 targets were identified using BioGPS datasets. The Target Organ (T-O) network consisted of 109 nodes and 1309 edges corresponding to targets and organs (Fig. 7). The targets were connected with 84 different organs and tissues.

Specifically, 21 targets presented in the liver accounting for 84% of all the targets. These are the primary targets (Table 3) likely to be applied to the HCC treatment. 15 targets wereoverexpressed in the colon, 13 targets in the kidney, the pancreas, the uterus and 11 targets in the Uterus corpus. 18 targets were located in a white blood cell.Thismay involve in the pathological process of metastatic cancer as a bridge with various organs. Especially, targets NPC1, SLC22A8, P4HB, CYP2A6, DHCR7, PPARA, VEGFA, CY1B1, IL4 and IGF2R locatedat more than 80 organs. Therefore, cancer in the liver can spread intoreciprocally the other organs via the bloodstreamin the body

3.6. *In silico* validation-Molecular docking

Molecular docking was used to identify the interactions of 18 natural compounds of SN against 21 HCC targets.For the careful selection of targets, various ligand-protein docking programs were used such as GLIDE, AutoDock Vina and iGEMDOCK. Standard drug Sorafenib tosylate was used for the comparison study (Figure 8, Table 4), interactions of proteins and ligands were documented. These *in silico* studies revealed 5 potent targets from 25 proteins and 4 active compounds from 23 compounds of SN for the effective treatment of HCC.

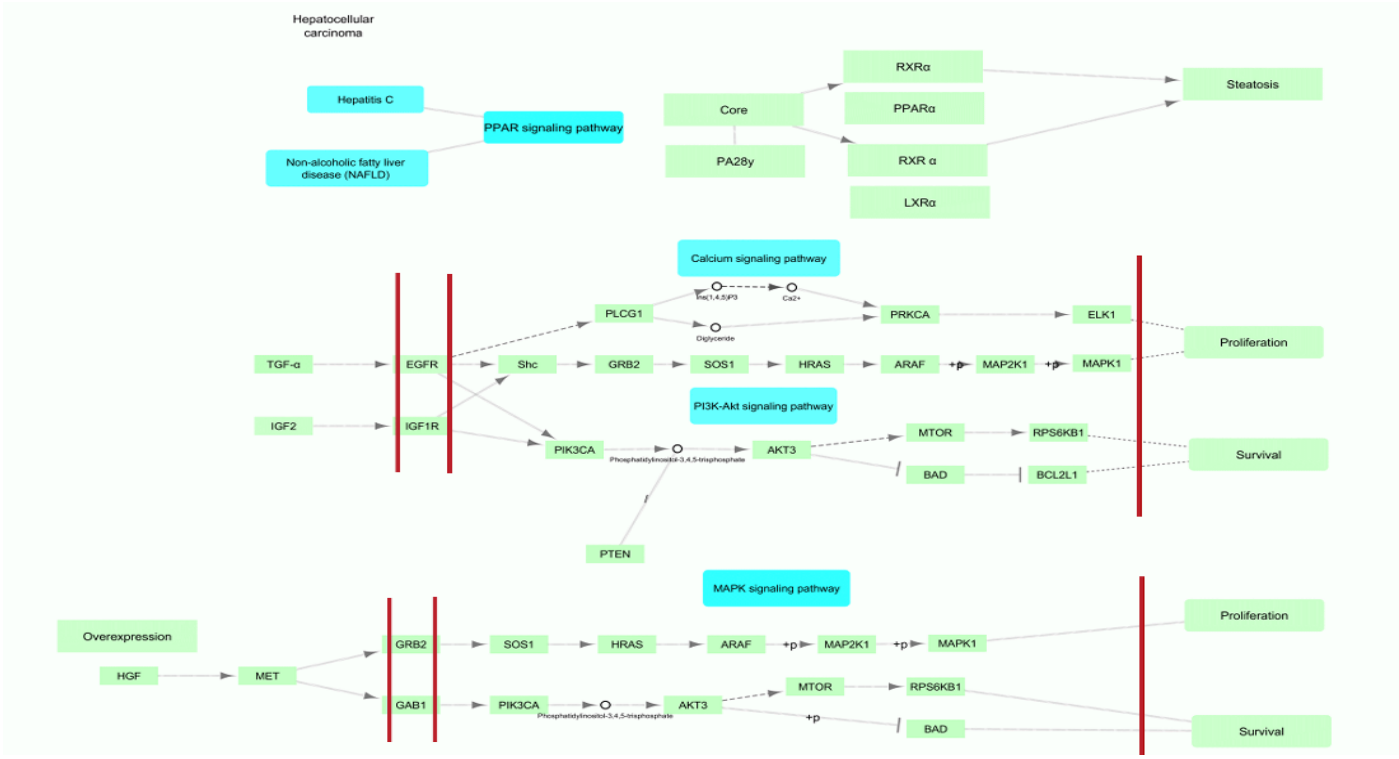

Fig. 6: Distribution of SN targets on the HCC compressed pathway

Cyan square: Relevant pathway

Table 3: Suggested targets for the treatment of HCC

ID	Gene name	PDB ID	Targets
T01	NOS1	4D1N	Nitric oxide synthase 1
T02	AKR1B10	1ZUA	Aldo-keto reductase family 1-member B10
T03	NR0B2	1YUC	Nuclear receptor subfamily 0 group B member 2
T07	OTC	1C9Y	Ornithine carbamoyltransferase, mitochondrial
T08	FGF2	1BAS	Fibroblast growth factor 2
T09	ADA	1M7M	Adenosine deaminase
T10	AR	1E3G	Androgen receptor
T11	VEGFA	1BJ1	Vascular endothelial growth factor A
T12	IL 4	1IAR	Interleukin 4
T13	P4HB	1BJX	Protein disulfide-isomerase
T14	CYP1B1	3PM0	Cytochrome P450 1B1
T15	NOX4	5O0X	NADPH oxidase 4
T16	SLC22A8	5C65	Solute carrier family 22 member 8
T17	PPARA	1I7G	Peroxisome proliferator-activated receptor alpha
T18	DHCR7	4QUV	7-dehydrocholesterol reductase
T19	CYP2A6	1Z10	Cytochrome P450 2A6
T20	IGF2R	1E6F	Cation-independent mannose-6-phosphate receptor
T22	GFER	3MBG	FAD-linked sulfhydryl oxidase ALR
T23	CYP17A1	2C17	Steroid 17-alpha-hydroxylase/17,20 lyase
T24	NPC1	3GKH	Niemann-Pick C1 protein
T25	SERPINA6	2VDX	Corticosteroid-binding globulin

Table 4: Potential targets and compounds of SN for HCC treatments

ID	Gene name	PDB ID	Targets	No	Bioactive compounds
T02	AKR1B10	1ZUA	Aldo-keto reductase family 1-member B10	C09	Hyperin
T03	NR0B2	1YUC	Nuclear receptor subfamily 0 group B member 2	C16	Quercetin
T17	PPARA	1I7G	Peroxisome proliferator-activated receptor alpha	C17	Quercetin-3-gentiobioside
T18	DHCR7	4QUV	7-dehydrocholesterol reductase	C18	Scopoletin
T22	GFER	3MBG	FAD-linked sulfhydryl oxidase ALR		

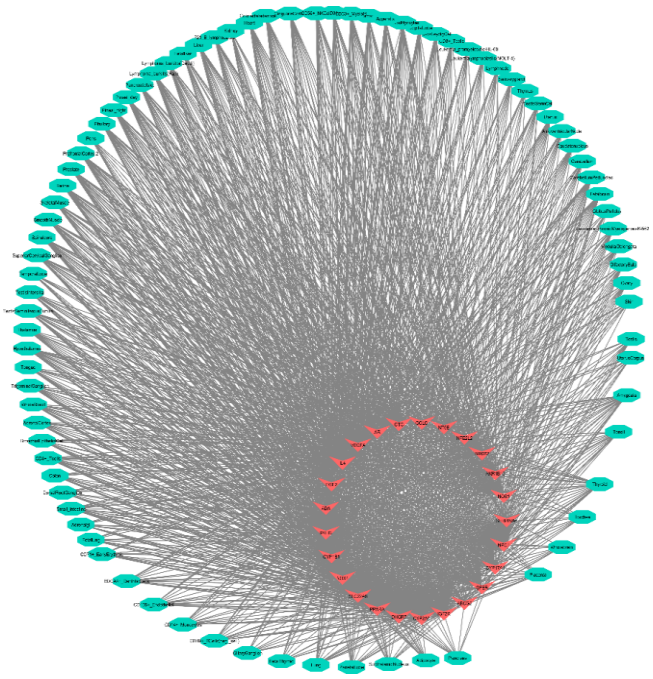

Fig. 7: Target-Organ (TO) network of SN

Red inverted triangle: target proteins; Green octagon: organs

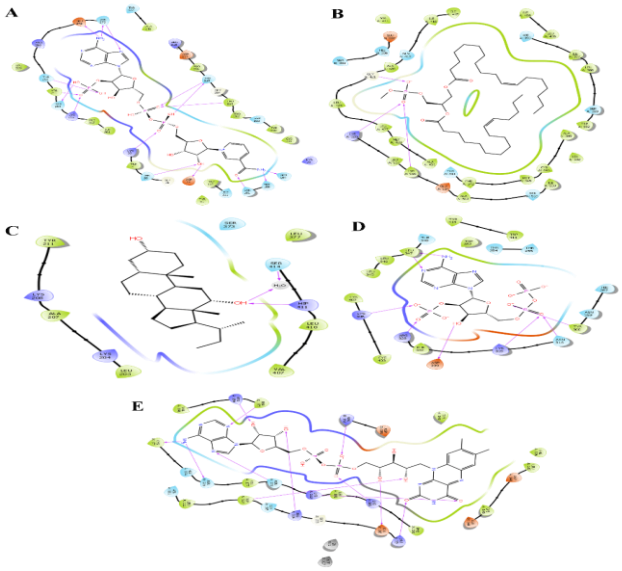

Fig. 8: H-Bond interaction of ligand and protein

(A)Hyperin with 1ZUA; (B) Quercetin-3-gentiobioside with 1YUC; (C) Quercetin with 1I7G; (D) Scopoletin with 4QUV; (E)Sorafenib tosylate with 3MBG

4. DISCUSSION

Network pharmacology is an approach to drug design that encompasses systems biology, network analysis, connectivity, redundancy, and pleiotropy. It used to identify the interaction of biological systems, drugs and diseases from a network perspective [21]. Computation methodologies are more efficient to save time, money and labor. Computational target fishing is one of the major concerns in the drug design and discovery to address medical needs [22]. In recent, Network pharmacology unlocks many therapeutic ideas to improve the safety and efficacy of existing medications and it well explored in Traditional Chinese Medicine (TCM) [23]. A computational approach of molecular docking was initiated in the 1980s, a small molecular weight natural compounds or drug-like compounds were binding with protein active sites and served as a major key methodology in the molecular drug design. It includes structural optimization and identifying biological activity through scoring functions by protein-ligand docking [24].

Identifying the ADME properties is an essential step in new drug development. Druglike is commonly referred by Lipinski's rule of 5, as it follows the guideline in drug lead optimization such as molecular weight (MW), physicochemical properties, aromatic rings and finds the suitable pharmacokinetics and safety [25]. It helps to identify the ADMET profile of the compound and filter out compounds with undesirable properties, especially those with poor ADMET profiles. In the systematic pharmacology-based analyses DL ≥ 0.18 was approved and permissible criteria for the compound selection. In drug discovery, the most important steps are target identification; it provides the mechanism of action of bioactive compounds with their interacting proteins

[26]. Many *in silico* methods, servers have been designed to predict the targets [27].

In the present work, we consolidated 23 potential bioactive compounds from the herb SN in the criteria of (DL ≥ 0.18). After mapping the 25 targets were chosen related to HCC. The GeneMANIA analysis was predicted the co-expression of genes and shared protein domains based on the 25 targets. It provides an idea about the targets and their interacting proteins functions for HCC treatment as well as with GO and pathway analysis. The constructed biological networks showed that SN compounds performed multiple targets, suggesting various pharmacological effects.

The systematic result of the C-T network displayed an average degree per compound of 5 and 4 per target, respectively and 4 of them adjust more than 5 targets. The Compound-Target-Pathway network analysis displays 23 compounds from the top 18 drug pairs through the PEA algorithm, connected with the different tissue location. Target-Organ pathway shows 21 potential targets mainly located in the liver and whole blood. 4 major pathways were highly associated with hepatocellular carcinoma; PPAR signaling pathway, Calcium signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway. Involving in various parts of cell proliferation and survival, these study modules are demonstrated and interpreted the mechanism of SN for the treatment of HCC. The most common targets Identified between all known bioactive compounds from SN and cancer targets (HCC) are Aldo-keto reductase family 1-member B10, Nuclear receptor subfamily 0 group B member 2, Peroxisome proliferator-activated receptor-alpha, 7-dehydrocholesterol reductase FAD-linked sulfhydryl oxidase ALR used for molecular docking

Aldo-keto reductase family 1-member B10 (AKR1B10) is a protein superfamily a group of NADs(P)H dependent oxidoreductase involved in cancer therapeutics [28]. Nuclear receptor subfamily 0 group B member 2 (NR0B2) is a superfamily of NR (nuclear receptor) was involved in transcriptional regulation of gene networks, which modulated by NR0B2 were regulate the metabolic pathways, carcinogenesis, tumor progression, and apoptosis in the liver. Peroxisome proliferator-activated receptor alpha (PPARα) is a member of the PPAR family, It consists of three groups PPARα, PPARδ and PPARγ. PPARα is expressed in various tissue and organ such as liver and brown adipose tissue, heart, kidney and skeletal muscle. PPARα-regulated the cell proliferation of breast cancer, renal cancer and liver diseases, also involving other biological processes [29].

In the PPAR signaling pathway, HCC has been associated with Hepatitis C and non-alcoholic fatty liver disease (NAFLD) leading to the Steatosis, is a chronic infection of hepatitis B and C virus in modulating PPARα and PPAR γ activity in PPAR pathway. Peroxisome proliferator-activated receptors (PPARs) have subtypes of PPAR α, β, γ. PPARs involving the regulation of lipid and carbohydrate metabolism and inflammatory responses, it shows the importance in NAFLD and HCC treatment used as suitable therapeutic targets [30]. Recent reports revealed that PPARγ is actively involving in cell growth inhibition and anti-metastasis, also synthetic PPARγ agonists, thiazolidinediones (TZDs), reported anti-tumor effects on HCC [31].

PI3K/Akt signaling pathway is essential for cell proliferation, invasiveness, angiogenesis and development and progression of HCC. PI3K/AKT/mTOR are important pathways to activated in HCC cell proliferation and survival. HCC cell survival was reported by different signaling pathway, mTOR signaling, ERK signaling, NF-κB signaling, and the p53 signaling pathways [32]. The pathway includes major targets of serine/threonine kinases c-Raf and B-Raf, the receptor tyrosine kinases VEGFR2, VEGFR3, platelet-derived growth factor receptor (PDGFR), FLT3, Ret and c-kit [33].

The mitogen-activated protein kinase (MAPK) is the main pathway in cancer cell survival for humans. The negative regulator of Sprouty and DUSP1 proteins where are down-regulated in HCC tumors in the MAPK pathway. The target proteins were involved in the MAPK pathway is VEGFA and FGF2. The VEGF pathway is expressed VEGF mRNA in liver tumors and also it increases the hepatocarcinogenic process in normal liver. It's played a vital role in HCC invasion and metastasis [34]. VEGF expression induces liver cancer. FGFR signaling is one of the largest families of growth factors that include 20 different FGFs and it is associated with tumorigenesis. Interestingly, the SN compounds also regulating various pathways related to breast cancer, gastric cancer, prostate cancer, pancreatic cancer, and Bladder cancer [35].

Asia is the most populated land in the world it hosts 60% of the world's current human population. The highest prevalence of HCC distributed in Asia. In the world, the highest liver cancer rate recorded in China, according to the cancer registry [36]. It consists, more

than 80% of HCC patients, recorded chronic hepatitis B virus (HBV) infection. In India, HBV, HCV infection and alcohol consumption are the main causes of HCC [37]. HCC accompanies and spreads the infections into the other organs in the body in the colon, kidney, pancreas, uterus and uterus corpus. SN is commonly used in TCM to treat various cancers and other illness. Hence, to treat the disease-specific to compound or targets provide effective treatment and rapid control.

This study was reliable for the traditional concept of "multiple drugs and targets with multiple effects." Network pharmacology is a valuable and convenient technique to approach scientifically with traditional knowledge in the field of drug discovery. Network pharmacology plays a role to understand the botanical and efficient components for the drug development process by a holistic approach. The study predicted 4 bioactive compounds and 5 potent targets from SN for the effective candidate against HCC, provided a neat and clear understanding to the mechanism of action of the candidates, and the therapeutic values by target fishing. Together, the study provides a systematic view against the hepatocellular carcinoma mechanisms of *Solanum nigrum* L from a network-based perspective.

5. REFERENCES

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