

Network Pharmacology Integrated Pharmacokinetics Approach to Decipher the Mechanism of Shankhapushpi Exerted Nootropic Activity

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Abstract Shankhapushpi herbs are widely recognized in traditional ayurvedic medicinal practices for their exceptional nootropic activity. Additionally, Shankhapushpi is known to ameliorate neurological, nootropic, and behavioral disorders like Alzheimer's, dementia, schizophrenia, and attention deficit hyperactivity disorder. Network pharmacology has been widely used to decipher the molecular mechanism of action of complex therapeutic formulations. In this work, network pharmacology integrated pharmacokinetics strategy was employed to understand the memory enhancement activity of Shankhapushpi. Chemical space of Shankhapushpi was identified by data mining and drug-likeness screening (oral bioavailability ($OB \geq 0.5$), Blood-Brain Barrier, and Gastro Intestinal permeability) further, the target identification of the screened chemical space was performed by constraint-based database prediction (similarity parameter ≥ 0.85). Genemania was employed to construct, annotate and analyze a protein-protein (P-P) interaction network of the identified Shankhapushpi nootropic targets. Further, a constraint-based ($P \leq 0.05$) comparative gene ontology and enrichment analysis of the (P-P) network was conducted using DAVID ($FDR \leq 0.02$) and Genemania ($FDR \leq 0.02$) to identify the nootropic pathways perturbed by the Shankhapushpi chemical space. DisGeNet and KEGG databases were queried to identify the diseases related to the identified shankhpushpi nootropic targets, and a gene-disease network was constructed. Finally, statistical network analysis results indicated the involvement of Dopaminergic activity, 5-hydroxytryptamine activity, mitogen-activated protein kinase cascade, histone deacetylase activity as the pivotal mechanisms behind the Shankhapushpi exerted nootropic activity. Finally, the Shankhapushpi herbs, phytochemicals, targets, pathways, diseases identified were organized and mapped into various networks for complete visualization, comprehension, and analysis of the Shankhapushpi network biology.

Keywords: Shankhapushpi, Nootropic activity, network pharmacology, gene ontology, neurodegenerative diseases, Ayurveda

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1. Introduction

Indian traditional medicine (Ayurveda, Unani) has been the backbone of the modern clinical practice in India and other Asian countries for centuries now. Ayurvedic formulations have been used to treat conditions and diseases for thousands of years and is still an efficient, holistic alternative for modern medicine. Among different ayurvedic formulations, Shankhapushpi has been in the spotlight of recent pharmaceutical research for many of its natural therapeutic properties. Charaka Samhita, an ancient ayurvedic text, explains the use of Shankhapushpi as a 'Medhya Rasayana' or 'Nervine tonic' for boosting memory and cognitive function. Shankhapushpi has been known to possess nootropic, antidepressant, anticonvulsant, tranquilizing, anxiolytic, and sedative properties [1].

Different plants are used as Shankhapushpi in various parts of India, the commonly known Shankhapushpi botanicals are, *Clitoria ternatea* Linn (Butterfly pea, Aparajitha), *Convolvulus pluricaulis* Choisy, *Evolvulus alsinoides* Linn and *Canscora decussata* [2]. Although the original Shankhapushpi source is ambiguous, numerous research works prove the efficacy of all the four above mentioned Shankhapushpi sources. Experimental Studies using Elevated plus maze and step-down passive avoidance models on rodents conducted by Jai Malik et al. show significant nootropic (memory enhancing), antidepressant, and anxiolytic activity of Shankhapushpi [3]. Furthermore, studies on Shankhapushpi (Ethanol extract) treated Sprague-Dawley rats by Alok Nahata et al. reported the reversal of scopolamine-induced amnesia along with positive anticholinergic activity [4]. Additionally, Shankhapushpi has been extensively employed to treat Alzheimer's disease, insanity, epilepsy,

nervous debility, anxiety attacks, mood disorders, Attention deficit hyperactivity disorder, amnesia, dementia, hypertension, convulsions, immunomodulation, spermatogenic disorders, hepatoprotection, and postmenopausal osteoporosis [3-6]. The nootropic (memory and cognition enhancement) activity of Shankhapushpi is of paramount pharmaceutical interest, but due to its complexity, the exact molecular mechanism of action of Shankhapushpi has not been completely understood as yet.

An extensive literature review on memory formation and retrieval elucidates numerous cascades of genes and their synergistic regulation with high speculation and ambiguity. The key challenge in studying memory enhancement activity is the identification and mapping of associated targets and genes, which, when perturbed, results in long term potentiation or long term synaptic plasticity and retrieval [7]. For studying complex models such as these, A multi-target multi-gene computational approach is necessary. Network pharmacology is an emerging discipline of computational systems biology capable of simplifying the study of complex systems comprising of multi-molecules, multi-targets, and multi-gene facets [8]. Network pharmacology utilizes redundancy, pleiotropy, connectivity, network analysis, and systems biology for simplifying the interpretation of complex biological systems. Furthermore, enrichment of the constructed networks increases the understanding of the system from various perspectives (pathway analysis, diseaseome analysis, Gene ontology (co-expression, physical interaction, co-dependency, etc.)).

The present study aims to establish the pharmacological mechanism of action all known Shankhapushpi sources with respect to their nootropic activity in the human model by utilizing a pharmacokinetics integrated Network pharmacology approach.

2. Materials and Methods

2.1. Identification of Chemical Space

An extensive database search was performed using the Knapsack [9] and Dr. Duke's database [10] for the identification of the Shankhapushpi (four botanicals) phytochemical compounds. Additional information regarding the physical properties, chemical properties, and identifiers of the phytochemicals were collected from the PubChem database [11].

2.2. Pharmacokinetic Assessment and Drug-likeness Screening

The identified Phytochemicals were further analyzed for their pharmacokinetic desirability. Swiss-ADME [12], Admet-SAR [13] online tools were utilized for this purpose. Oral bioavailability ($OB \geq 0.5$ (Swiss-ADME-scale)) and BBB (Blood Brain Barrier) penetration probability were considered as the most significant screening parameters considered for screening. ADME Screened (Set-2) and total (Set-1) phytochemicals were grouped separately for further analysis.

2.3. Target Identification and Clustering

The targets of the Shankhapushpi phytochemicals (sets 1 and 2) were obtained from the Drug bank [14], Binding DB [15], ChEMBL database [16] and were grouped separately (ADME-unscreened set-1 and ADME-screened 2). The targets were selected based on a minimum similarity parameter constraint of 0.85, and the targets with score 1 were selected for improving the accuracy of prediction. The associated genes of the predicted targets were obtained from the UniProt database [17]. Additional information like gene identifiers, Annotation scores, Gene names, and primary gene functions were also collected. Finally, the ambiguous points and duplicates in the data were cleaned and the data was curated for network construction. Further, an extensive literature survey was performed for the purpose of identifying the underlying target-gene cascades and pathways related to general nootropic activity [18]. These identified nootropic cascades, and their associated genes were identified and tabulated. The set of genes identified from the literature survey was overlapped with the set of genes obtained by database search and druglikeness screening (set-2). A final intersection gene subset comprising of nootropic genes targeted by Shankhapushpi, was generated for further analysis.

2.4. Genemania Analysis

Possible gene interactions of the genes in the intersection gene subset were predicted for the human model using default parameters in the Genemania online database [19], and the top twenty associated genes for the Homo sapiens model were predicted and listed. Finally, two gene clusters, namely, target database (Target set-2 derived genes) and target database with Genemania predicted gene clusters (Target set-2 derived genes with Genemania predicted genes), were created for comparative GO-Enrichment analysis.

2.5. Comparative Gene Ontology and Enrichment Analysis

Comparative Gene ontology and network enrichment analysis of the gene clusters was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) [20] online tool and the Genemania tool. The clustered gene sets were separately subjected to GO enrichment analysis using the DAVID tool with Expression Analysis Systematic Explorer (EASE) scores ($P \leq 0.05$) as the primary screening parameter. The EASE screened terms were subjected to functional annotation clustering with Benjamini scores ($FDR \leq 0.05$) as the secondary screening parameter for both DAVID and Genemania tools [21]. The qualifying GOBP (Gene Ontology-Biological processes) were listed. The Genemania tool scores the gene ontology terms based on the Q value. Top 10 GOBP terms were retrieved from DAVID, and 10 pathway annotations were retrieved from Genemania.

Figure 1. Pictorial illustration of the Shankhapushpi botanicals-phytochemicals network. The Shankhapushpi Botanicals are represented as triangular pink nodes and their associated phytochemicals are represented as blue square nodes.

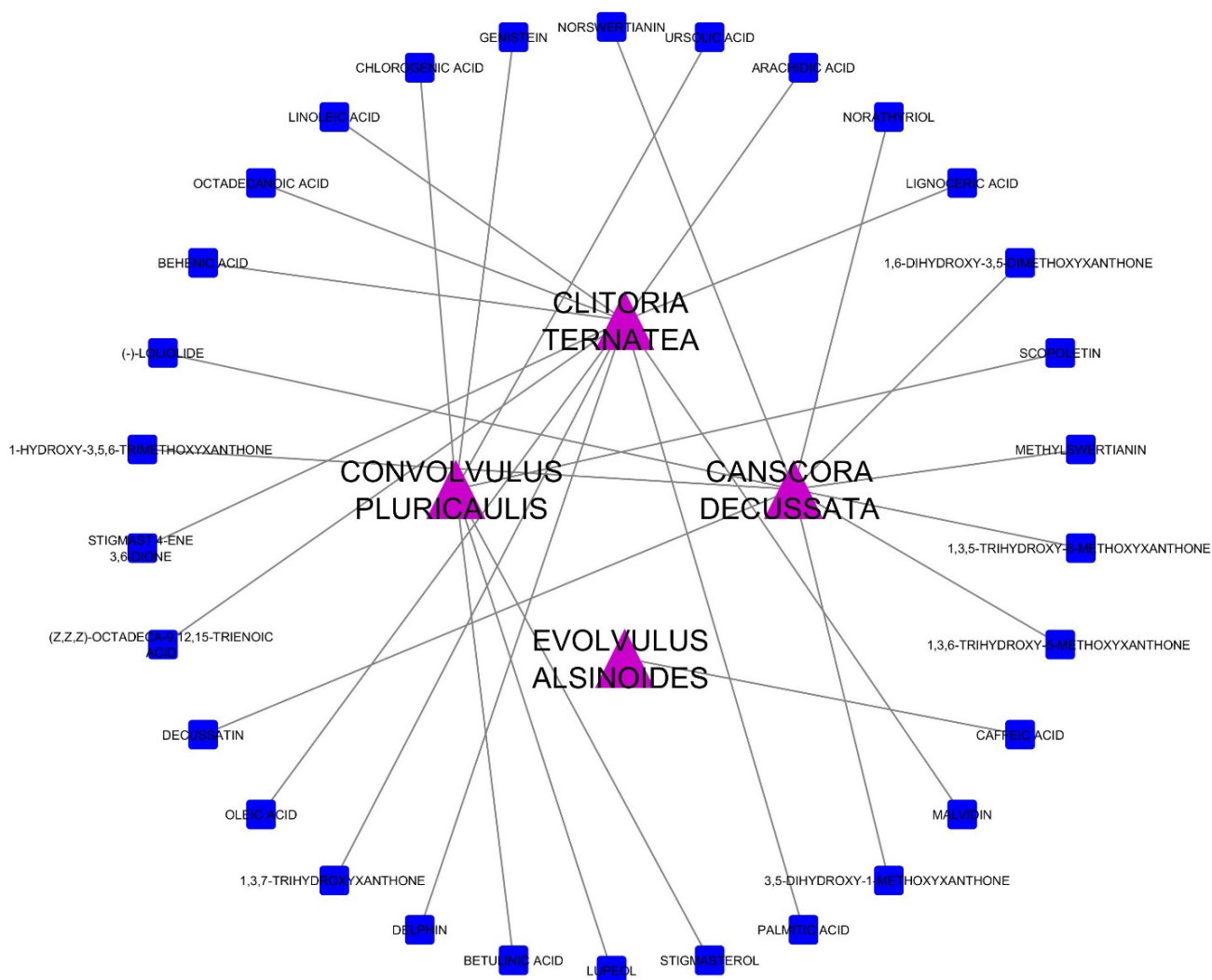


Figure 2. Pictorial illustration of the Shankhapushpi botanicals and their associated druglikeness screened phytochemicals network. The Shankhapushpi Botanicals are represented as triangular pink nodes and their associated drug-likeness screened phytochemicals are represented as blue square nodes.

Pharmacokinetic screening is a crucial step in the drug development process. Proper pharmacokinetic screening of compounds will yield better candidates with lower drug-drug interaction. The hippocampal cellular cytosolic concentration of the phytochemicals is crucial for eliciting a response from targets associated with nootropic activity (Nootropic targets). Hence, BBB permeation constraint is crucial for the validity of this study, as the majority of nootropic target proteins are located past the Blood-Brain Barrier. The Shankhapushpi botanicals-complete unscreened phytochemicals network is illustrated in Figure 1.

Gastrointestinal permeability and matrix solubility of the phytochemical bioactives also play a significant role in drug design. In the context of this study, Ayurvedic medicines are administered orally; hence, the oral bioavailability of the phytochemicals was assessed. A total of 30 desirable phytochemical candidates (set-2) with a molecular weight range of 180-627 g/mol were obtained from the pharmacokinetic screening procedure. Finally, the Shankhapushpi Botanicals-phytochemical network was constructed for the visualization of the Shankhapushpi chemical space. The constructed network comprised of 446 edges and 67 nodes and is illustrated in Figure 2.

3.2. Target Prediction and Analysis of Phytochemical – Target Network

The database search predicted a total of 223 putative targets for the 63 phytochemicals (set-1) with a similarity score higher than 0.85. Two phytochemical – target networks were constructed for the phytochemical sets with their respective predicted targets. The phytochemical (set-1) – total target network was constructed for understanding the complete range of phytochemical – target interaction of the Shankhapushpi botanicals. The constructed network comprised of 2278 edges and 286 nodes. The nodal degree of the network was found to be 3.53 (number of target nodes/number of phytochemical nodes). The degree of a node corresponds to its promiscuity and is directly proportional to its significance in the network [25]. The phytochemical (set-1) – targets network is illustrated in Figure 3. Thirty drug-likeness screened phytochemicals (set 2) shared 200 targets out of the 223 targets predicted for the 63 phytochemicals (set 1). This statistic sufficiently unveils the multi-component multi-target synergistic therapeutic potential of the components. The drug-likeness screened set-2 phytochemicals, and the 200 predicted (set-2) targets

network was constructed for understanding the nootropic activity of Shankhapushpi. The network contained 446 edges and 230 nodes. The constructed phytochemical (set-2) – target network is illustrated in Figure 4.

An extensive literature survey was conducted for mining the targets and genes that play a significant role in nootropic activity. It was evident that 5-hydroxytryptamine (serotonin receptor activity), hippocampal cAMP (3',5'-cyclic adenosine monophosphate) pathway, Dopaminergic gene cascade, Histone deacetylase activity, mitogen-activated protein kinase cascade, N-methyl-D-aspartate receptor activity, and acetylcholinesterase activity were the

dominant gene cascades and pathways associated with memory consolidation, long and short term potentiation, Long term synaptic plasticity and Nootropic activity in general. The proteins associated with these pathways were identified and clustered. The (set-2) targets were overlapped with the set of targets obtained from the literature survey. The intersection of these protein sets revealed 14 nootropic targets obtained from the Shankhapushpi phytochemical (set-2) targets. The predicted targets of phytochemical sets 1 and 2 that were found to be unrelated to nootropic activity were also retained for network construction and presentation of the obtained data.

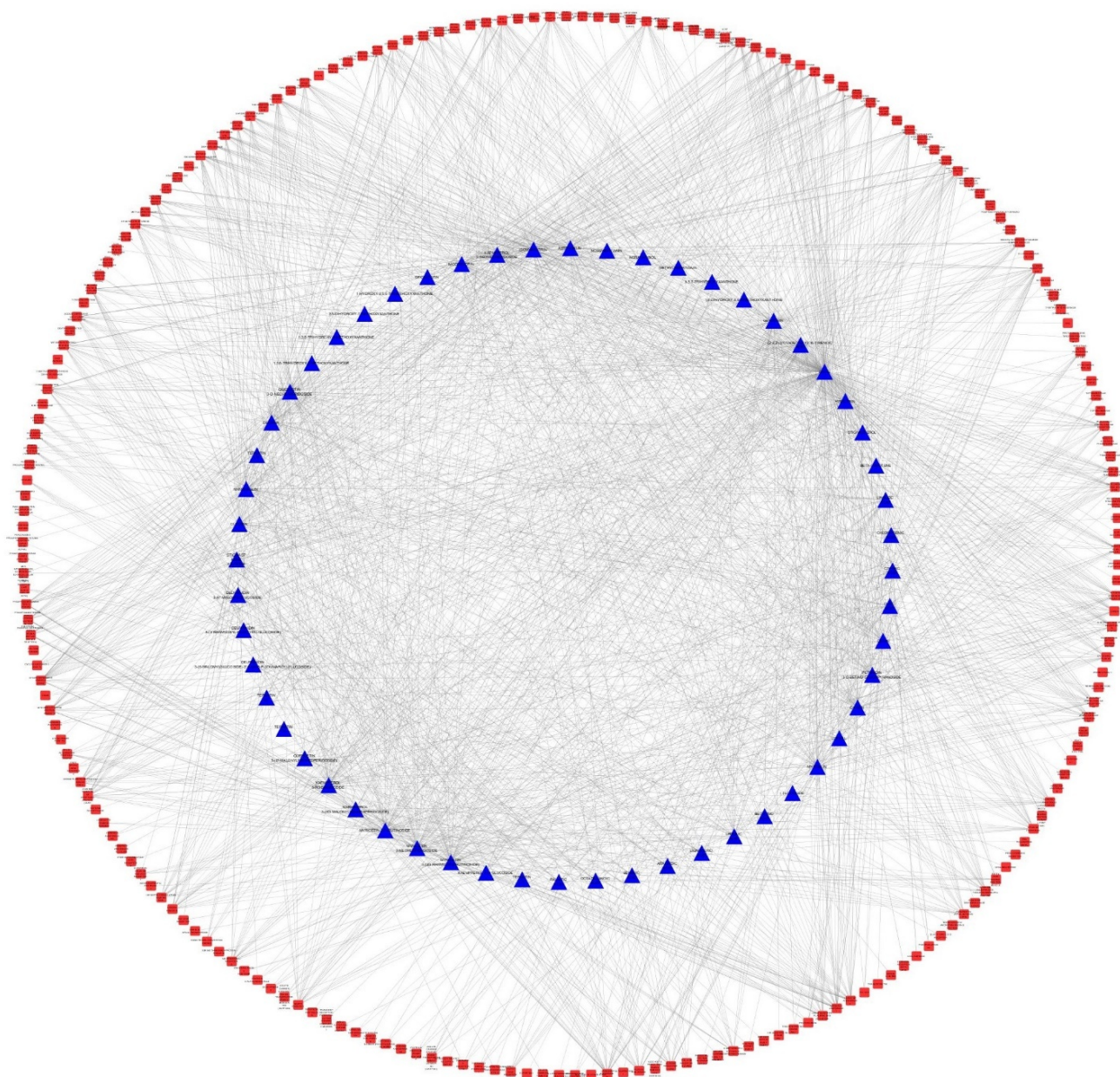


Figure 3. Pictorial illustration of the complete Shankhapushpi phytochemicals-targets network. The phytochemicals are represented as triangular blue nodes and their predicted targets are represented as red square nodes.

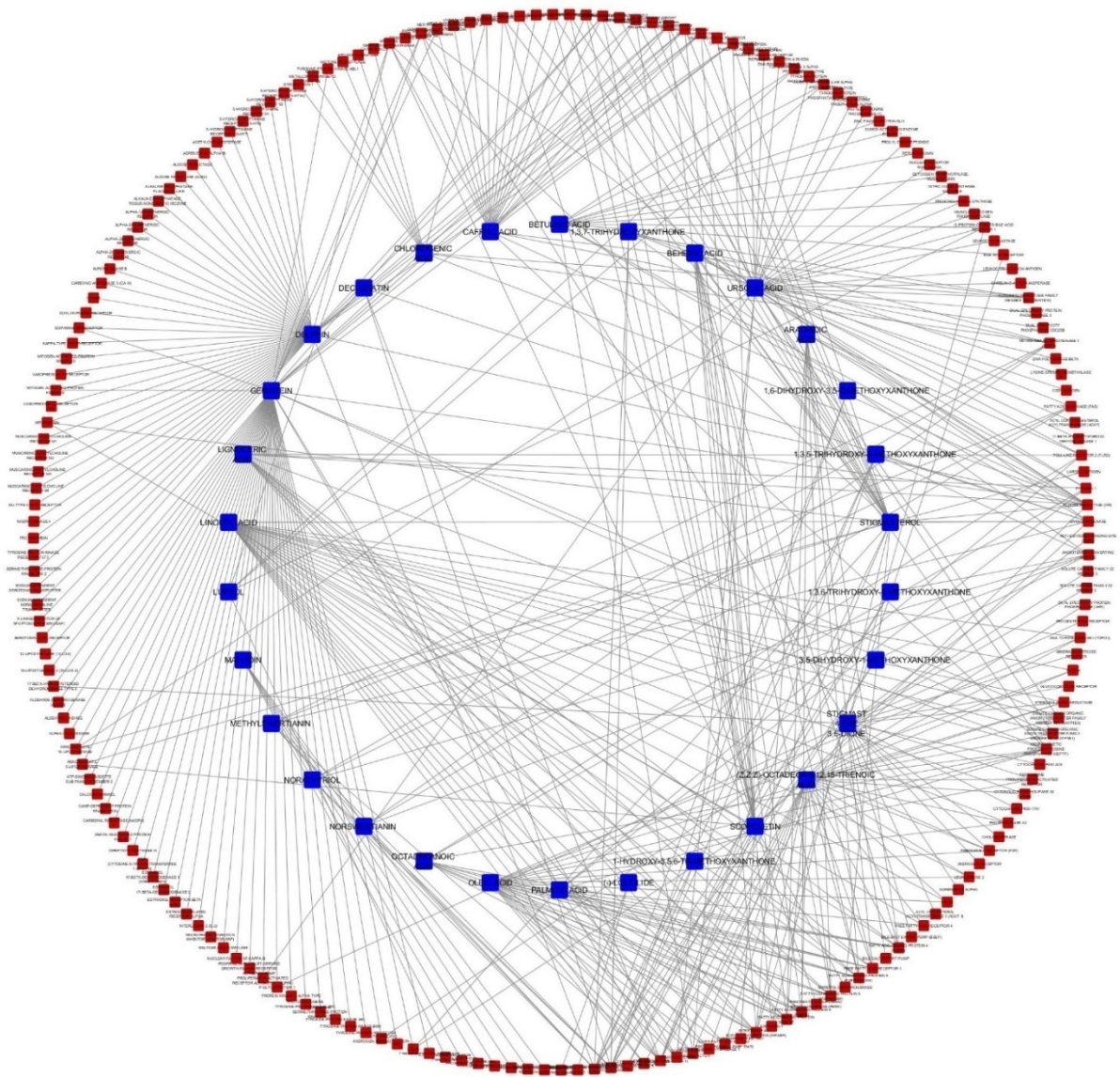


Figure 4. Pictorial illustration of the druglikeness screened Shankhapushpi phytochemicals-targets network. The druglikeness screened phytochemicals are represented as triangular blue nodes and their predicted targets are represented as red square nodes.

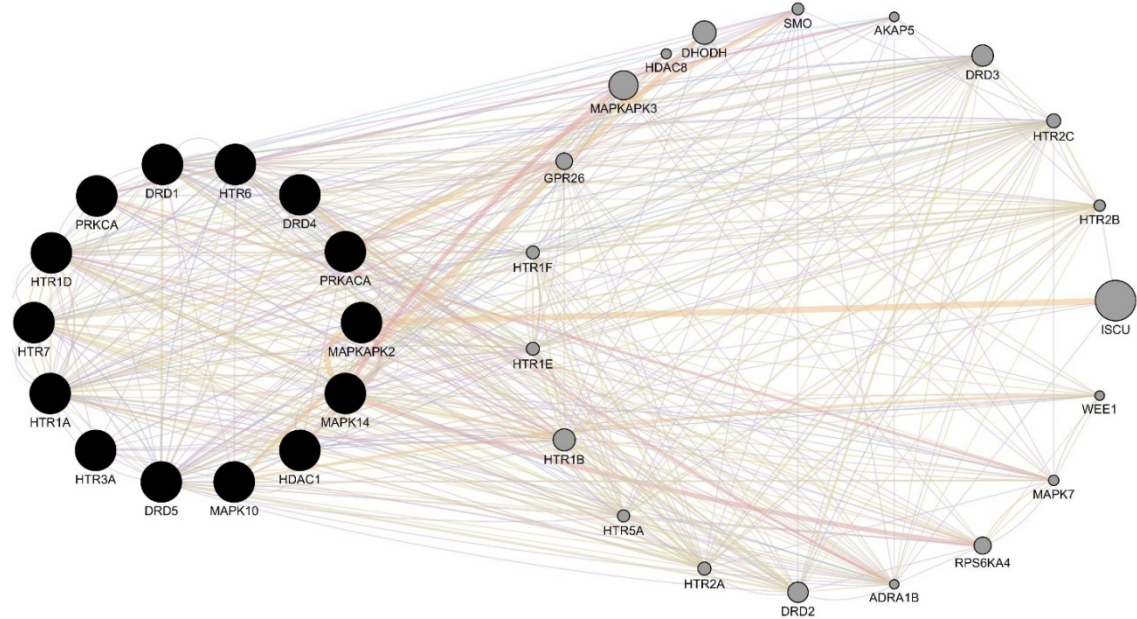


Figure 5. Genemania predicted protein-protein interaction network illustrating the Shankhapushpi nootropic targets. The input target proteins are represented as circular black nodes and their predicted targets are represented as circular grey nodes.

3.3. Protein-protein Interaction Network and Genemania Analysis

The 14 previously identified nootropic targets were input into the Genemania online tool for the construction of the protein-protein interaction network for the human model. A total of 20 associated proteins were predicted. The constructed protein-protein network is depicted in Figure 5. The black nodes represent the input targets, and the grey nodes represent the predicted targets. The Genemania result statistics depicting the scores of co-expression, physical interactions, and shared protein domains for the predicted network was obtained. The Genemania predicted GOBP terms are scored based on previously published literature regarding individual protein-protein interactions of the queried target genes. The network analyzer tool of Cytoscape was utilized for computing the reliability of the constructed P-P network. The following network statistics were computed; clustering coefficient of 0.722, network heterogeneity score of 0.358, average promiscuity score of 16.647 for a network with a total of 34 nodes, and 566 edges. Clustering coefficient score distributions are used to determine the modular structure of the networks. A low network heterogeneity score of 0.358 indicates a minimal deviation from empirical data suggesting the synergistic strength of the predicted genal nodes in the network. The degree of a node is very crucial in determining the prominence of a node in the network. Dopamine D5 receptor (DRD5) (n=50), 5-Hydroxytryptamine receptor 1A (HTR1A) (n=49), 5-Hydroxytryptamine receptor 1D (HTR1D) (n=45) and Dopamine receptor D1 DRD1(n=44) were the input nodes with the highest nodal degree. It can be inferred that these protein targets and their associated genes play a pivotal role in the natural nootropic activity exerted by the Shankhapushpi botanicals in the human model. The network analysis of the predicted protein-protein interaction network is illustrated in Figure 6. The nodal degree distribution of the Shankhapushpi targeted nootropic genes are tabulated in Table 1 and is illustrated in Figure 7.

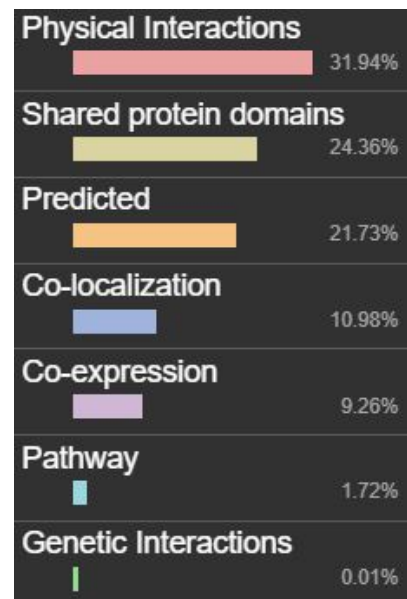


Figure 6. Genemania analysis of the predicted Protein-protein interaction network illustrating various genal parameters in the network

Table 1. Nodal degree distribution of the Shankhapushpi nootropic targets

Sl. No	Target Name	UniProt ID	Degree
1	Dopamine Receptor D5	P21918	50
2	5-Hydroxytryptamine Receptor 1A	P08908	49
3	5-Hydroxytryptamine Receptor 1D	P28221	45
4	Dopamine Receptor D1	P21728	44
5	5-Hydroxytryptamine Receptor 6	P50406	44
6	5-Hydroxytryptamine Receptor 7	P34969	35
7	Dopamine Receptor D4	P21917	34
8	cAMP-Dependent Protein Kinase	P17612	28
9	Mitogen-Activated Protein Kinase 14	Q16539	22
10	Mitogen-Activated Protein Kinase Activated Protein Kinase 2	P49137	17
11	Mitogen-Activated Protein Kinase 10	P53779	17
12	Protein Kinase C Alpha Type	P17252	16
13	Histone Deacetylase	Q13547	15
14	5-Hydroxytryptamine Receptor 3A	P46098	13

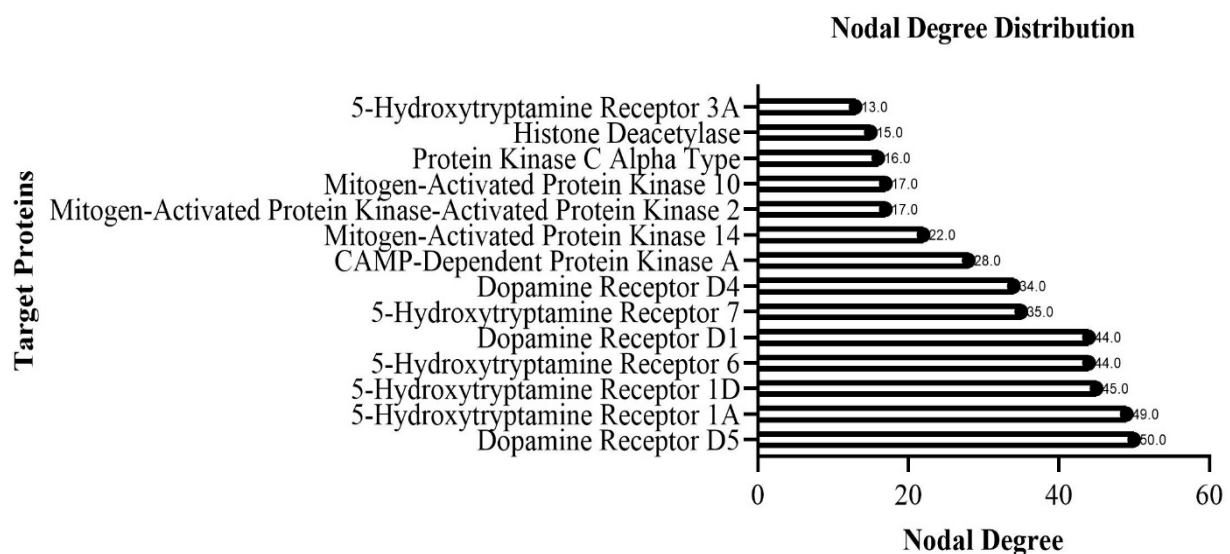


Figure 7. Nodal degree distribution of the Shankhapushpi nootropic genes

3.4. Comparative Gene Ontology and Enrichment Analysis of the Predicted Proteins

Comparative Gene ontology and enrichment analysis of the predicted protein-protein interaction network was performed using the DAVID and Genemania tool. Comparative Go-enrichment analysis is beneficial for validating the prediction of the gene ontology-biological processes (GOBP) terms. Genemania and DAVID employ different algorithms for GOBP prediction. The top 10 terms with $FDR \leq 0.02$ (DAVID) and $FDR \leq 0.02$ (Genemania) were selected. The distribution analysis of the DAVID database predicted Gene Ontology, and Enrichment terms are tabulated in Table 2 and are illustrated in Figure 8 and Figure 9. Serotonin binding activity, signal transduction, G protein-coupled receptor pathway, neuroactive ligand-receptor interaction, neurotransmitter receptor activity were the top predicted terms from both DAVID and Genemania databases. Further, KEGG enrichment of the network was performed

for the GOBP terms for the prediction of underlying pathways. Dopaminergic activity, acetylcholine esterase activity, cAMP pathway, regulation of mitogen-activated protein kinase activity, and associative learning were the top predicted terms from both online database tools. The predicted terms between the online database tools displayed high similarity, thereby validating the authenticity of the performed gene ontology enrichment analysis. The genes underlying the predicted terms were tabulated for further analysis. Form analysis, it is evident that different variants of the 5-Hydroxytryptamine receptor proteins, dopaminergic proteins, mitogen-activated protein kinases, and histone deacetylase proteins were involved with the KEGG enriched pathways in high frequencies. Therefore, it can be stated with certainty that the nootropic activity exerted by Shankhapushpi metabolites is majorly due to their interaction with the members of the serotonin, dopaminergic, mitogen-activated protein kinase pathways. The FDR/Benjamini score distribution analysis of the Genemania predicted Gene Ontology and Enrichment terms are tabulated in Table 3 and is illustrated in Figure 10.

Table 2. P Score, and False discovery rate/Benjamini Scores distribution of the DAVID predicted Gene Ontology and Enrichment Analysis Terms

Sl. No	Gene Ontology Terms	Count	P Score	FDR/Benjamini Score
1	Serotonin Binding Activity	10	1.30E-24	1.30E-22
2	Neurotransmitter Receptor Activity	11	9.30E-22	4.90E-20
3	G-Protein Coupled Serotonin Receptor Activity	11	2.30E-21	8.00E-20
4	G-Protein Coupled Receptor	19	4.90E-17	4.40E-15
5	Serotonin Receptor Signaling Pathway	8	9.70E-17	5.70E-14
6	Serotonergic Synapse	14	2.30E-16	2.60E-14
7	Neuroactive Ligand-Receptor Interaction	17	2.80E-15	1.60E-13
8	Chemical Synaptic Transmission	13	1.50E-14	3.90E-12
9	Release of Sequestered Calcium Ion into Cytosol	8	1.20E-12	2.10E-10
10	G-Protein Coupled Receptor Signaling Pathway, Coupled to Cyclic Nucleotide Second Messenger	8	2.90E-12	3.80E-10

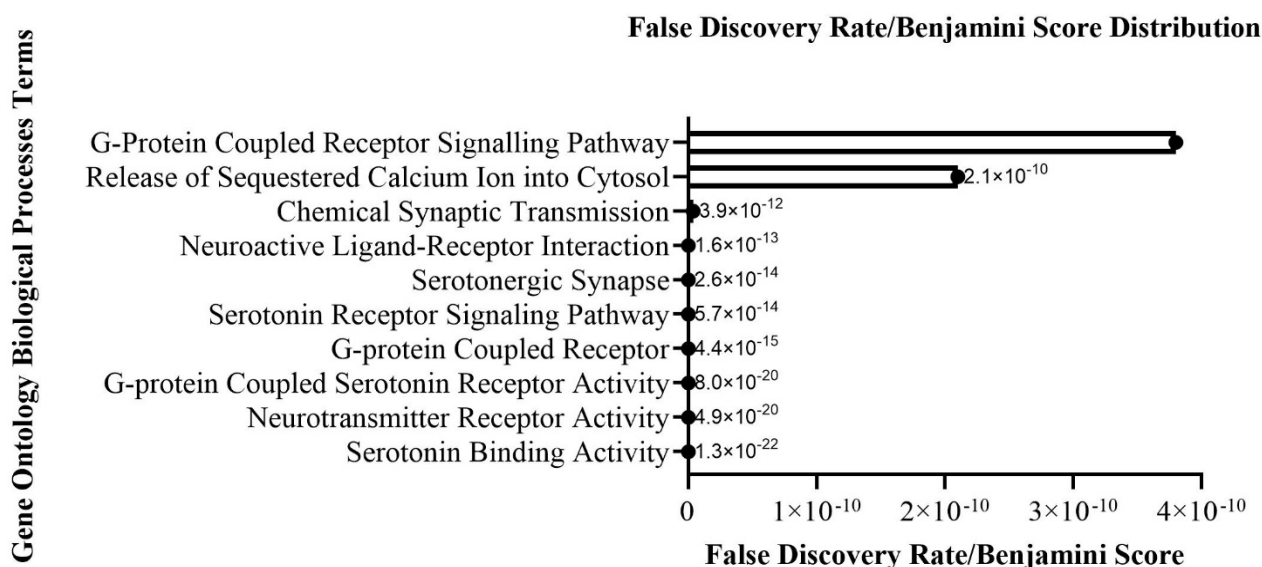


Figure 8. False discovery rate/Benjamini Score distribution of the predicted Gene Ontology and enrichment analysis terms from the DAVID tool

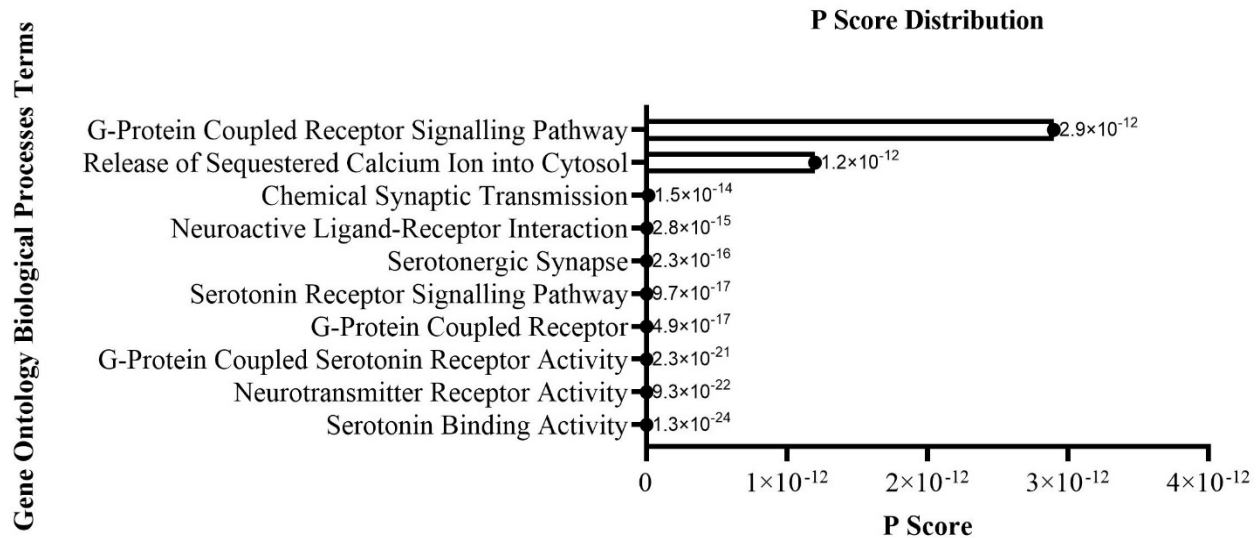


Figure 9. P Score distribution of the predicted Gene Ontology and Enrichment analysis terms from the DAVID tool

Table 3. False discovery rate/Benjamini score distribution of the Genemania predicted Gene ontology and enrichment analysis terms.

Sl. No	Gene Ontology Terms	FDR/Benjamini score
1	Serotonin Receptor Signaling Pathway	6.2E-24
2	G-Protein Coupled Receptor Activity	6.9E-23
3	Regulation Of cAMP Metabolic Process	2.7E-09
4	Dopaminergic Synaptic Transmission	7.1E-09
5	Positive Regulation of MAPK Cascade	7.6E-09
6	Stress Activated MAPK Cascade	1.1E-04
7	Visual Learning	1.1E-04
8	Associative Learning	1.8E-04
9	Activation of Protein Kinase Activity	2.0E-04
10	Histone Deacetylase Activity	5.8E-03

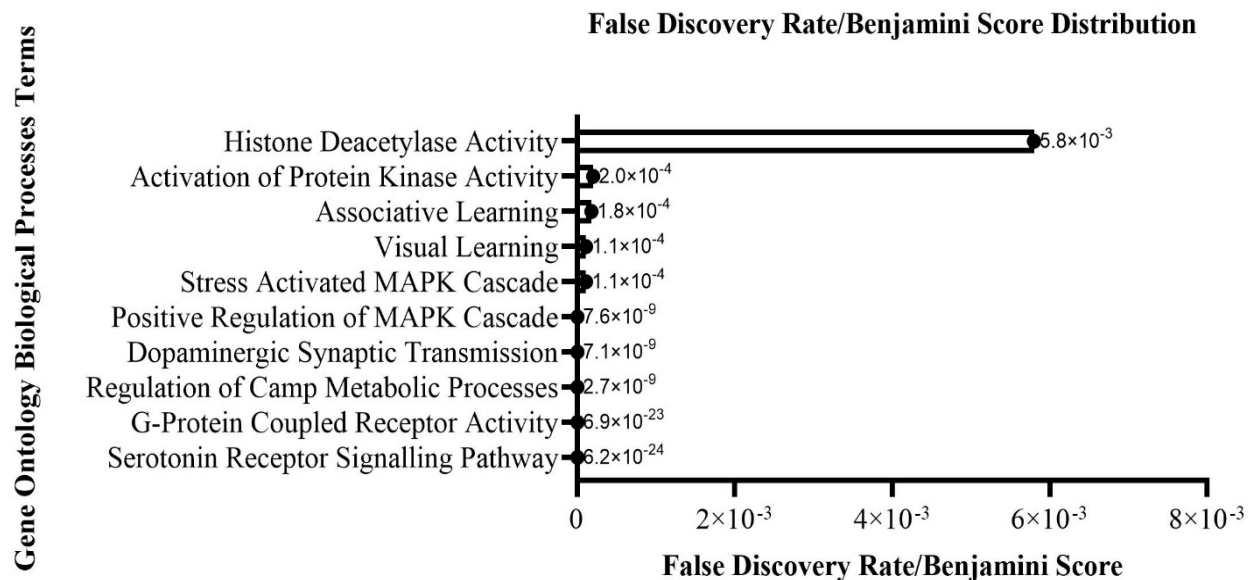


Figure 10. False Discovery Rate/Benjamini Score distribution of the Genemania predicted Gene Ontology and enrichment analysis terms

3.5. Analysis of the Target-pathways and Target-disease Networks

Pathways associated with the thirty-four (predicted and input) genes from the Genemania predicted protein-protein network were queried in the KEGG pathway database. A total of 560 unique pathways were predicted. A target-pathway network was constructed comprising of

594 nodes and 872 edges. The pathways to genes ratio (GR = 16.47) and the network heterogeneity score (2.732) were found to be very high; therefore, the pathways associated with nootropic activity in the network were separately illustrated in [Figure 11](#) for clear visualization. The network centralization score was computed to be 0.159. The nodes associated with dopaminergic activity (Dopamine receptor-D1, Dopamine receptor-D2,

Dopamine receptor-D3, Dopamine receptor-D4, and Dopamine receptor-D5,) were the nodes with the highest nodal degrees. Histone deacetylation activity associated nodes (Histone deacetylase 1 and Histone deacetylase 8), mitogen-activated protein kinase cascade activity associated genes (Mitogen-activated protein kinase 14 and mitogen-activated protein kinase 7), serotonin receptor activity associated nodes (Hydroxytryptamine receptor 1B, Hydroxytryptamine receptor 2B, Hydroxytryptamine receptor 2A) were also the nodes with higher nodal degrees. The unfiltered target-pathway network comprising of all the pathway nodes is illustrated in Figure 12.

The diseases associated with the genes were obtained

from the DisGeNET database. The target-diseases network was constructed comprising of 68 nodes and 154 edges and is illustrated in Figure 13. The diseases associated with memory and cognition, neurodegenerative diseases, depression, and anxiety disorders were grouped separately. A network centralization score of 0.412 was predicted. A total of 16 nootropic diseases, 11 neurodegenerative diseases, and 41 depression and anxiety disorders associated with the predicted pathways were identified. A consolidated network comprising of Shankhapushpi botanicals-phytochemicals-targets-pathways is illustrated in Figure 14. Another consolidated network comprising of Shankhapushpi botanicals-phytochemicals-targets-diseases is illustrated in Figure 15.

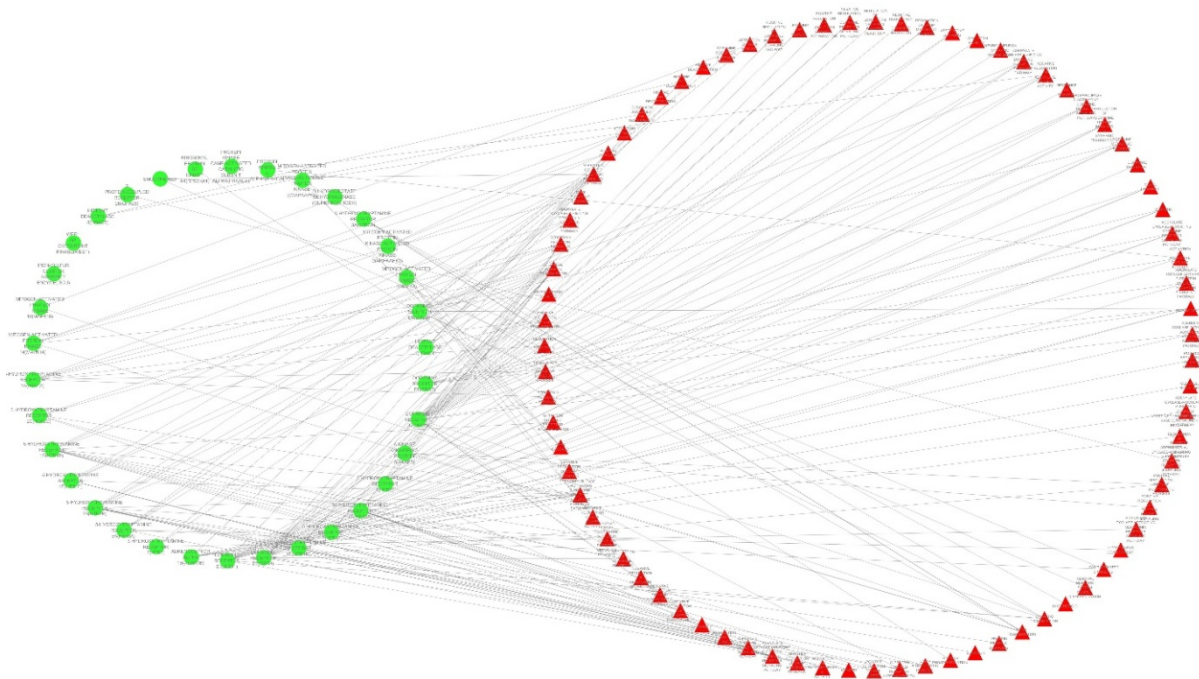


Figure 11. Pictorial representation of the Shankhapushpi nootropic genes and their associated nootropic activity related pathways. The Shankhapushpi nootropic genes are represented as circular green nodes and their predicted pathways are represented as triangular red nodes

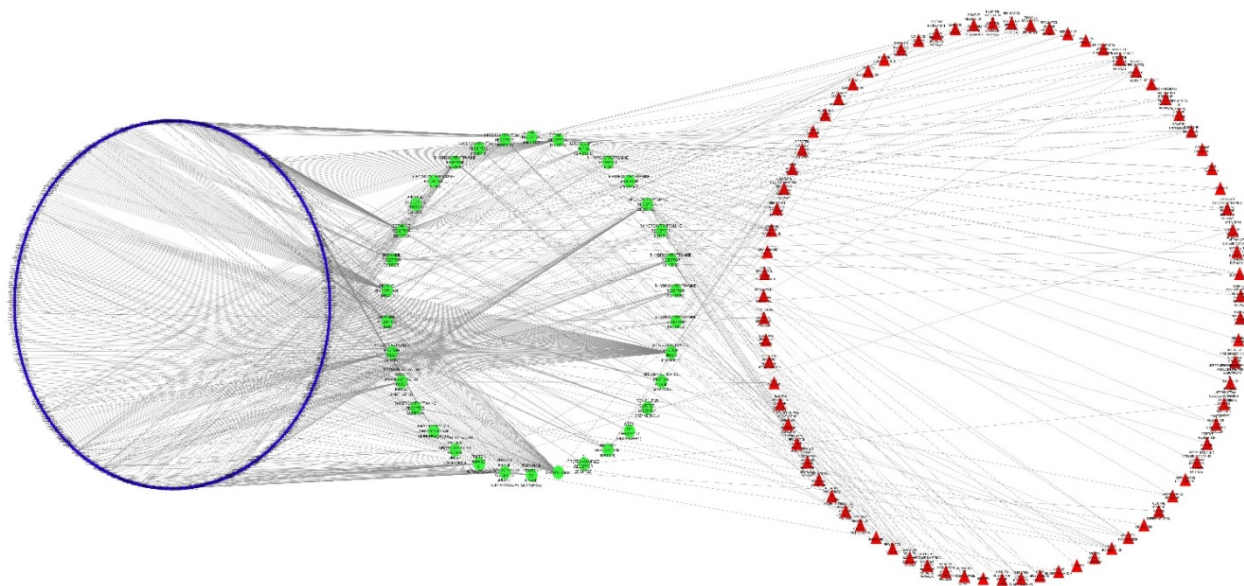


Figure 12. Pictorial representation of the Shankhapushpi nootropic genes and their associated nootropic activity related pathways. The Shankhapushpi nootropic genes are represented as circular green nodes and their predicted nootropic pathways are represented as triangular red nodes. The predicted pathways unrelated to nootropic activity are represented as blue nodes

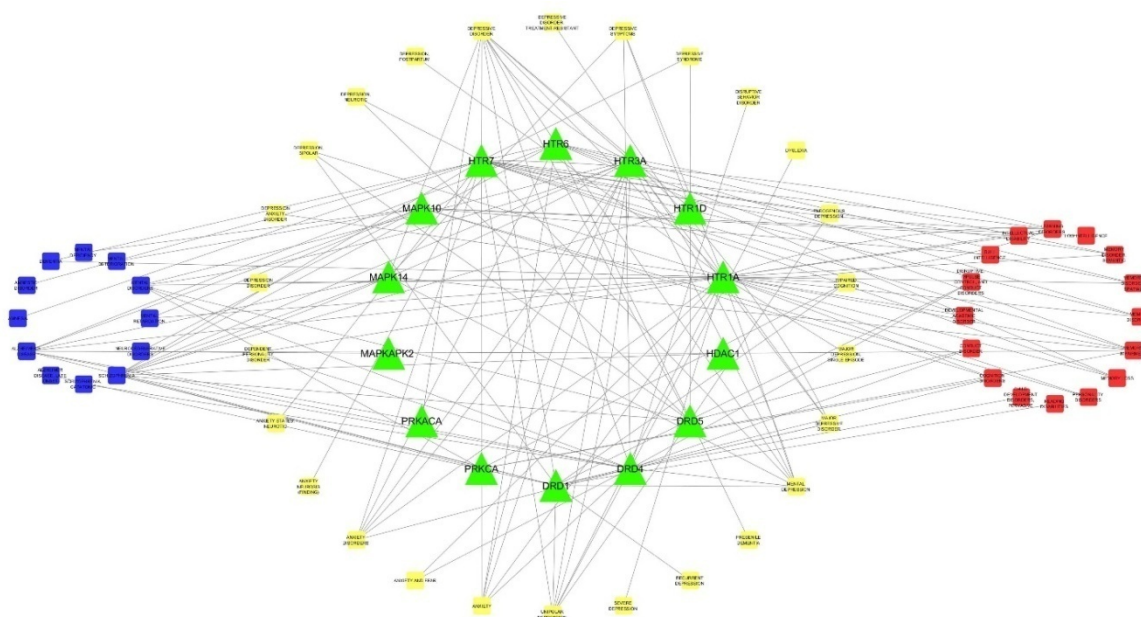


Figure 13. Pictorial illustration of the Shankhapushpi nootropic genes (represented as green triangular nodes) – nootropic disorders (represented as red square nodes), depression and anxiety disorders (represented as yellow square nodes), and, neurodegenerative diseases (represented as square blue nodes).

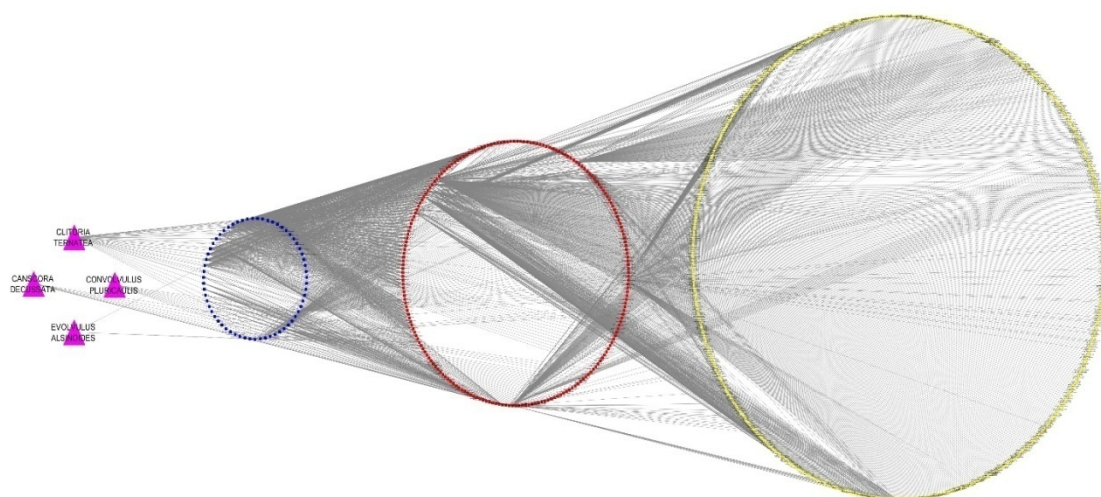


Figure 14. Consolidated network comprising of the Shankhapushpi botanicals (represented as triangular pink nodes), total Shankhapushpi phytochemicals (represented as square blue nodes), targets (represented as square red nodes) and associated pathways (represented as square yellow nodes).

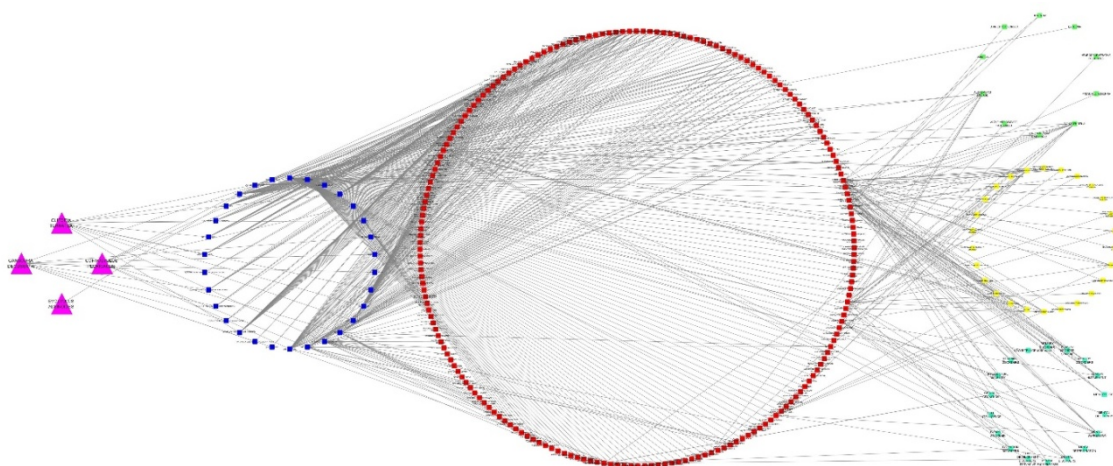


Figure 15. Consolidated network comprising of Shankhapushpi botanicals (represented as triangular pink nodes), total druglikeness screened Shankhapushpi phytochemicals (represented as square blue nodes), targets (represented as square red nodes), neurodegenerative diseases (represented as green nodes) depression and anxiety disorders (represented as yellow nodes) and nootropic disorders (represented as light blue nodes).

4. Discussion

Network pharmacology analysis plays an essential role in understanding the mechanism of action of traditional medicinal formulations. Developing potent drugs for complex, incurable diseases like Alzheimer's, Dementia, Schizophrenia, and amnesia requires equally complex drug designing strategies that involve the robust perturbation of multiple nodes that play pivotal roles in the pathogenicity of the disease. For eliciting robust perturbation of pivotal nodes, understanding the significance of every node and combinations of nodes in the network that can be perturbed to elicit a therapeutic outcome has to be understood. Impact of perturbation has to be lucid; hence, Quantitative and qualitative knowledge of the system has to be lucid from various perspectives like the side effects caused, the extent and effect of perturbation (positive and negative regulation), etc [7].

Shankhapushpi is a traditional medicinal formulation with exceptional nootropic potential. Recent studies conducted by A.Nahata et al, elucidates that the Shankhapushpi has the potential to reverse scopolamine-induced amnesia [4]. Natural nootropic drugs such as Shankhapushpi offer attractive advantages over their synthetic analogs for treating memory-related disorders. Memory related disorders are often linked to targets and gene cascades that occur in the brain past the blood-brain barrier (BBB). Designing synthetic BBB permeable nootropic drugs requires enormous resources, iterations of calculations, risk assessment, etc. A Shankhapushpi derived synthetic nootropic drug would be devoid of all these computational concerns. Hence, A network pharmacological analysis of the Shankhapushpi botanicals in the human model to establish the nootropic action of the herbs is highly beneficial.

By the use of network pharmacology, the nootropic action of the herbs was established. It was identified that Shankhapushpi herbs interact with target members associated with the dopaminergic pathway. Studies conducted by P.Gong et al, suggest that working memory is directly associated with higher dopamine levels. It is also explained that the equilibrium action of the dopamine receptor D2 receptor-mediated facilitation and dopamine receptor D3 receptor-mediated inhibition is crucial for increasing the working memory in a sample of healthy Chinese Han population [26]. Furthermore, the studies of R.Bernabeu et al, elucidates that the dopamine receptor D1 and dopamine receptor D5 are involved in the activation of the hippocampal 3',5'-cyclic adenosine monophosphate signaling pathways, which is crucial for modulating a late consolidation phase of inhibitory avoidance learning in male Wistar rodents [27]. Recent studies on memory acquisition clearly explains that the activation of dopamine receptor D1 and dopamine receptor D2 results in the hippocampal acquisition of visual and spatial memory during encoding [28,29]. Also, the studies conducted by M.Pantoni et al, elucidates that weak dopaminergic transporter inhibition results in a response that is similar to that of a fear-induced short and long term memory enhancement effects without the side effects caused by psychostimulant addiction. From a cursory analysis of the network, it can be speculated that the Shankhapushpi metabolites, with their highly

promiscuous interactions with various dopaminergic receptors, could activate hippocampal dopamine receptor D1 and dopamine receptor D5 and weakly inhibit dopamine transporters thereby eliciting a combinatorial short and long-term nootropic effect.

The present study also stresses the importance of serotonin receptors as a significant player in the Shankhapushpi effected nootropic activity. Studies conducted by C.Bailey et al, postulates that repeated serotonin pulses in the sensory neuron activates the catalytic subunit of the Protein Kinase A by stimulating the synthesis of 3',5'-cyclic adenosine monophosphate. Protein kinase A catalytic subunit upon activation from 3',5'-cyclic adenosine monophosphate then phosphorylates the 3',5'-cyclic adenosine monophosphate response element-binding protein (CREB) transcription factor that activates the CREB inducible genes [30]. Shankhapushpi metabolites interact with a variety of serotonin receptors, especially 5-Hydroxytryptamine receptor 6 and 5-Hydroxytryptamine receptor 7. Recent studies by S.D.Schmidt et al, indicate that activation of the 5-Hydroxytryptamine receptor 6, 5-Hydroxytryptamine receptor 7, and 5-Hydroxytryptamine receptor 5A engage in the reconsolidation process of contextual fear conditioning memory [31]. The Shankhapushpi metabolites, upon activating serotonin receptors, could ipso facto activate the cascade of protein-protein interactions leading to the activation of the CREB pathway. Also, the interaction of Genistein (Shankhapushpi metabolite) with the 3',5'-cyclic adenosine monophosphate (cAMP)-dependent catalytic subunit of Protein kinase A (PRKACA) is noteworthy in this context. Studies by C.Alberini conceptually establishes the prominence of the 3',5'-cyclic adenosine monophosphate response element-binding protein (CREB) pathway activation in long term potentiation and synaptic plasticity [32].

Furthermore, the work by C.Alberini also explains the role of Mitogen-activated kinases in the activation of CREB genes. The analysis of the constructed metabolite-target network indicated the interaction of astragalin, clitorin, cyanin, delphin, hirsutrin, isomyricitrin, kaempferol 3-neohesperidoside, kaempferol-3-robinobioside, kaempferol-7-o-glucoside, manghaslin, myricetin-3-(2g-rhamnosylrutinoside), myricetin-3-neohesperidoside, myricetin-3-o-rutinoside, myrtillin, nicotiflorin, oenin, petunidin-3-o-beta-d-glucopyranoside, quercetin-3-o-neohesperidoside, rutin, genistein, loliolide with various Mitogen-activated protein kinases including the mitogen-activated protein kinase activated protein kinase 2. The findings of P.C.Orban et al, explains that mitogen-activated protein kinase cascade is involved in CREB pathway activation by interaction with Mitogen-activated protein kinase activated protein kinase 2 (MAPKAPK2) [33]. MAPKAPK2 is known to activate the 3',5'-cyclic adenosine monophosphate response element-binding protein (CREB) pathway, thereby activating the expression of the CREB induced immediate early genes. Activational expression of the immediate early gene of the CREB resulting in the expression of the late response CREB gene cascade is the backbone of long term synaptic potentiation and memory consolidation [34,35,36]. All stress associated learning like active avoidance paradigm and contextual fear conditioning

serve as stimuli to the mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2). Network analysis has established that the metabolite Loliolide interacts with MAPKAPK2, it can be speculated that the interaction results in the activation of the MAPKAPK2 in the hippocampus contributing to the Shankhapushpi nootropic activity.

The activational expression of the early response genes results in the synthesis of CCAAT/enhancer-binding protein beta (C/EBP), is required for the activational expression of the late response genes. The late response genes are involved in the CERB linked long-term synaptic plasticity and long-term memory. Upon activation, the CERB induced genes undergo decondensation (decoiling) for further transcription with the activity of histone acyltransferases. For the activity of HAT, Histone deacetylase (HDAC) proteins play an inhibitory effect, thereby downregulating the expression of CERB genes. Thus, hippocampal histone deacetylase inhibitors play an essential role in synaptic plasticity and long term potentiation [37]. Studies on memory enhancement by histone deacetylase 2 inhibition conducted by N.Grissom and F.Lubin extrapolate theories that histone deacetylase 1 and histone deacetylase 2 proteins work in synergy and that the overexpression of histone deacetylase 1 would result in a decrease of histone deacetylase 2 expression [38]. From network analysis, it was found that Chlorogenic acid interacts with histone deacetylase 1 protein. Understanding the mechanism of action of chlorogenic acid on the histone deacetylase 1 protein is an exciting future scope of this work. It would provide a clear understanding of the histone deacetylase inhibitory activity dependent nootropic activity along with the histone deacetylase 1 inhibition induced nootropic activity exerted by the Shankhapushpi herbs.

Acetylcholine esterase inhibition has been an established strategy for enhancing memory and cognition. J.Malik et al, have hypothesized that acetylcholine esterase inhibition could be the prominent mechanism of action behind the nootropic activity of the Shankhapushpi botanicals [3]. It was found that Delphin and 16 other Shankhapushpi metabolites interact with acetylcholine esterase. It can be hypothesized that these metabolites elicit inhibitory effects on the enzyme resulting in memory enhancement. Interestingly, Shankhapushpi metabolites through nodal relationships in the consolidated network, displayed interactions with pathways associated with neurodegenerative diseases like Amnesia, Alzheimer's disease, Dementia, and Schizophrenia. The network could be used to design potent drugs against these diseases. Previous studies conducted by Hornick et al, on the action of scopoletin proved that scopoletin causes a surge of presynaptic acetylcholine release, thereby enhancing long term potentiation and nootropic activity. Scopoletin has been found by spatial chemical profiling in *E.Alsinoides* [4].

5. Conclusion

The present study has adequately established the pharmacological network of the Shankhapushpi herbs in the human model. The significance of the interactions of

the Shankhapushpi metabolites with dopaminergic receptors, mitogen-activated protein kinases, 5-Hydroxytryptamine receptors, and histone deacetylases have been clearly illustrated. The predicted gene-gene interaction network and its analysis has laid a foundation for understanding the Shankhapushpi nootropic activity. The network also provides sufficient insight regarding the future scope of work on the Shankhapushpi nootropic activity. From the statistical data analysis of the consolidated network, it can be unequivocally stated that *Clitoria ternatea* Linn is the Shankhapushpi herb with the highest nootropic activity. Target validation studies using Surface plasmon resonance, Molecular docking, Molecular dynamics simulation and Cell-based assays with solvent-based Shankhapushpi extracts are necessary for quantitative measurement of individual nodal contributions to the Shankhapushpi exerted nootropic activity.

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Supporting Data

The network (.cys) files and the data supporting this study are available upon request from the author.

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Conflict of Interest

The author declares that there are no competing interests with any entity or individual regarding this study.

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