Network pharmacological evaluation of *Withania somnifera* bioactive phytochemicals for identifying novel potential inhibitors against neurodegenerative disorder

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ABSTRACT

Neurodegenerative disorders are illnesses that are responsible for neuronal cell death and resulting in lifelong cognitive problems. Due to their unclear mechanism, there are no effective drugs available for the treatment. For a long time, herbal drugs have been used as a role model in the field of the drug discovery process. *Withania somnifera* (Ashwagandha) in the Indian medicinal system (Ayurveda) is used for several neuronal disorders like insomnia and memory loss for decades. This study aims to identify active components of *Withania somnifera* (WS) as potential inhibitors for the treatment of neurodegenerative diseases (ND). To fulfill this objective, Network pharmacology approach, gene ontology, pharmacokinetics analysis, molecular docking, and molecular dynamics simulation (MDS) studies were performed. A total of 77 active components in WS, 175 predicted neurodegenerative targets of WS, and 8085 ND-related targets were identified from different databases. The network analysis showed that the top ten targets APP, EGFR, MAPK1, ESR1, HSPA4, PRKCD, MAPK3, ABL1, JUN, and GSK3B were found as significant target related to ND. On the basis of gene ontology and topology analysis results, APP was found as a significant target related to Alzheimer’s disease pathways. Molecular docking results found that Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine showed the best binding affinities $-$5.55, $-$4.73, $-$4.04, and $-$4.11 Kcal/mol. Further, MDS results suggested that Isopelletierine and Nicotine could be used as potential inhibitors against APP protein and could be useful for the treatment of Alzheimer’s disease.

1. Introduction

Neurodegenerative diseases collectively form a group of diverse illnesses that are marked by the gradual deterioration and subsequent loss of neurons (Aldewachi et al., 2021; Przedborski et al., 2003). The deposition of misfolded proteins is the most significant cause of numerous neurodegenerative diseases (Dash et al., 2021; Gandhi et al., 2019). Given the surge in age-associated afflictions among the global population, the number of people suffering from neurodegenerative disorders is on the rise. The most common neurodegenerative disorders are Alzheimer’s, Parkinson’s, Huntington’s, Multiple Sclerosis, and Amyotrophic Lateral Sclerosis, mostly resulting in impaired memory, shrunken intellect, and hindered body movement (Gitler et al., 2017; Skovronsky et al., 2006). In certain instances, the pathways leading to these conditions aren’t very well understood (Przedborski et al., 2003). The most common way of combating these disorders in the current times involves the usage of some drugs, combined with physiotherapy, a healthy diet, and exercise. Unfortunately, these treatments relieve the symptoms but do not slow down the damage that is being afflicted to the nerve cells (Petkova, 2017). Presently, no definite cure is available for neurodegenerative disorders. Nonetheless, many natural plants and their components have been known to have a considerable effect against these diseases (Pohl and Lin, 2018; Zschucke et al., 2013). One such plant is *Withania somnifera*, also known as ‘Ashwagandha’ (in Sanskrit) or the Indian Winter Cherry. In India, it is a common medicinal herb. For thousands of years, it has been used as an adaptogen, nerve tonic, anti-stress, and rejuvenating agent. Its effect as a memory-enhancing tonic, as well as against cognitive deficiencies, is widely known. Preparations made from its roots, leaves, and seeds are predominantly used in the Unani and Ayurvedic medicinal systems. Also called ‘SattvicKaphaRasayana’, it is a marvelous natural Rasayana that is centuries-old and is being used to combat neuronal illnesses (Blaylock and Maroon, 2012; Umadevi et al., 2012).

The herb is known to restore the activity of mitochondria and endothelia along with reducing inflammation and oxidative stress. Because of these actions, Ashwagandha can be employed as a potential treatment alternative for various neurodegenerative disorders. However, its efficacy is still in
the preclinical and clinical research stages. The plant extracts of Ashwagandha have no reported significant toxicity or side effects, so they can be utilized reliably in the case of humans (Dar and Ahmad, 2020; Kulkarni and Dhir, 2008; Singh et al., 2011). Over the last decade, the dominance of the notion that the aim of developing drugs is to build exquisitely selective drug components that function on a particular disease target has triggered more incidences of drug failure in late-stage clinical studies. On the other hand, many successful medications work by modulating different proteins instead of a particular target. One such approach is that of network biology. Rather than looking for ‘disease-causing mutations,’ network biology indicates that the approach should be to look for disturbances in the disease-causing network or the pathway that is ultimately leading to it (Kuboyama et al., 2014). Network pharmacology moves the study paradigm away from the existing ‘one drug, one target’ model and towards a modern ‘multi-component, multitarget’ model. Such a concept is termed Polypharmacology. Polypharmacology’s aim is to find a compound that has the desired biological profile over several targets, and whose combined modulation can cause the state of the disease to change. Network pharmacology models help to find out how and where one target in the disease network controls the phenotype of the disease, making the switch between its active and inactive states. By using this approach, drugs tend to become less susceptible to drug resistance and result in fewer side effects. Although herbs possessing medicinal properties are complex by nature, their effectiveness is dependent on multi-target action through different active components. As a result, they can be investigated for developing drugs based on network pharmacology (Hopkins, 2008; Zhang et al., 2013). In this study, we selected W. somnifera to study its active components as potential drug candidates against neurodegenerative diseases using a network pharmacology approach. The flowchart of the study is represented in Figure 1.

2. Material methodology

2.1. Identification and retrieval of active components of W. somnifera

The biologically active phytochemicals of W. somnifera were identified through literature review and downloaded in Structure Data File (SDF) format from the PubChem database (Kim et al., 2016). These phytochemicals were further subjected for the assessment of pharmacokinetics parameters.

2.2. Adme/T analysis of active components

In order to prevent false discovery, all retrieved compounds were passed through ADMET (Absorption, distribution, metabolism, excretion, toxicity) analysis. This analysis was done using SwissADME, an open-access online tool to predict drug-like properties of the components (Daina et al., 2017). Toxicity profiles like carcinogenicity and mutagenesis of compounds were assessed using the AdmetSAR online tool (Cheng et al., 2012).

2.3. Prediction of W. somnifera targets and ND targets

Targets of W. somnifera specific to neurodegenerative diseases were retrieved from different databases such as GeneCards, STITCH, BATMAN-TCM, Drugbank, and literature review (Liu et al., 2016; Safran et al., 2010; Szklarczyk et al., 2016). The known current therapeutic targets of neurodegenerative diseases were retrieved using GeneCards and NCBI Gene database (Brown et al., 2015). For better representation of targets network, Gephi 0.9.2, a visualization software, was used to construct their network (Bastian et al., 2009). Further, Venn diagram analysis was done to find out the common targets in neurodegenerative disease and W. somnifera targets using the Venny tool.

2.4. Target network construction and topological analysis

Selected target genes were imported in Cytoscape plugin, GeneMANIA (Multiple Association Network Integration Algorithm) to construct their network based on their functional relations (Smoot et al., 2011). The resulting network of genes was then analyzed based on their co-expression, genetic interaction, physical interaction, pathway, colocalization, predicted, and shared protein domains. Further, the CytoNCA plugin was used to study the topological parameters such as betweenness centrality (BC), degree centrality (DC), and closeness centrality (CC) of the resulting network (Tang et al., 2015).

2.5. Gene ontology (GO) and pathway enrichment analysis of targets

GO is a way to depict detailed information of genes and their products in terms of molecular function (MF), biological process (BP), and cellular component (CC) (Thomas, 2017). The PANTHER (Protein Analysis Through Evolutionary Relationships) tool was used for GO analysis of selected target genes with their role in different KEGG pathways (Mi et al., 2017).

2.6. Active site prediction of the target protein

Active sites are the position at which ligand molecules bind to the target protein. These sites are significant components of the protein surface that determine the kinds of interactions between protein and ligand (Li et al., 1998). CASTp server was used to identify the binding pockets of 2fk1 before proceeding for docking studies (Tian et al., 2018). For the identification of cavities, protein coordinates were used as an input for the prediction of cavities.
2.7. Identification of binding affinities of protein and ligands

The most popular molecular docking programs, Autodock tools (ADT) was used for the binding affinity analysis of target protein (2fk1) with selected ligands such as Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine (Morris et al., 2012). PDB formats of both ligand and target protein were converted to Autodock readable format (.pdbqt) in ligand and protein preparation step followed by grid parameter file and docking parameter file generation. Using the commandline interface, molecular docking was performed to calculate the binding site score. Additionally, to compare the feasibility of the results of docking, 2-PMAP (2-((pyridine-2-ylmethyl)amino)-phenol), a drug effective against APP target, was used as a reference (Asuni et al., 2014; Greig et al., 2014). The resulting non-covalent docking interactions between target protein and ligands in detail, PLIP (Protein-Ligand Interaction Profiler) web tool was used (Salentin et al., 2015).

2.8. Molecular dynamic (MD) simulation (MDS)

MDS study of docked complexes was performed using GROningen MACHine for Chemical Simulations (GROMACS 2019.6) package with the standard CHARMM36 all-atom force field (Huang and MacKerell, 2013). The top five protein-ligand complexes, such as Anahygrine, Cuscohygrine, Isopelletierine, Nicotine, and 2-PMAP, were selected for their conformational and stability analysis. PRODRG server was used to generate topology files of ligand file (Schüttelkojf and Van Aalten, 2004). For solvation of complex, TIP3P water model in a dodecahedron box with boundaries 1.2 nm was opted. Counter ions (NaCl) were added to the complex in order to neutralize the system charge. In order to minimize system energy, the steepest descent algorithm without constraints was run for 5000 steps (Hirshman and Whitson, 1983). Equilibration of five complexes was done under NVT and NPT conditions for a period of 5000 ps with 300 K temperature and coupling constant \( \tau_s = 0.1 \) ps. The Berendsen weak-coupling method was used for maintaining the temperature and pressure of the system. To constrain the bond length of heavy atoms, Linear Constraint Solver (LINCS) algorithm was used (Hess et al., 1997). Finally, a 100 ns production run was performed for each system. Van der Waals interactions were maintained using the Lennard-Jones potential, and the particle-mesh Ewald method was used for the electrostatic interactions (Cheadham et al., 1995). For the entire simulation time, every 2ps final coordinate trajectories were saved. GROMACS modules such as gmx_rmsd, gmx_rmsf, and gmx_gyration were used to analyze the MD trajectories. Further, the QTgrace tool was utilized for the interpretation of MD results (QTGrace download SourceForge.net).

3. Results and discussion

3.1. Retrieval of active components of W. somnifera

After going through the literature review, a total of 77 active components of W. somnifera were identified and downloaded in .sdf format. Compounds along with their PubChem ID and literature information are given in Table S1 (supplementary material).

3.2. Assessment of physicochemical properties and drug-likeness properties

Different physicochemical, pharmacokinetic, medicinal chemistry, and toxicity parameters were observed for all compounds. In the first step, these compounds were filtered on the basis of their physicochemical properties such as Molecular weight (MW), H-bond donors (HBD), H-bond acceptors (HBA), aqueous solubility (Log S), log P, number of rotatable bonds (nRot), topological polar surface area (TPSA) and lipophilicity. Lipinski’s rule of five was taken into consideration. According to this rule, components should have MW
Table 1. Results of physicochemical properties, drug-likeness, lipophilicity, and medicinal chemistry of selected active compounds.

| CID       | Mol.Wt. | ROTB(n) | HBA(n) | HBD(n) | ESOL LogS | Bioavailability Score | Lipinski’s Violations | TPSA | CLogP | PAINS Alert | Brenk Alert | Lead likeness | Synthetic accessibility |
|-----------|---------|---------|--------|--------|-----------|-----------------------|-----------------------|------|-------|------------|-------------|-----------------|----------------------------|
| 12306778  | 224.34  | 4       | 3      | 1      | -1.53     | 0.55                  | 0                     | 32.34 | 1.6   | 0          | 0           | 1               | 2.62                       |
| 1201543   | 224.34  | 4       | 3      | 0      | -1.6      | 0.55                  | 0                     | 23.55 | 1.5   | 0          | 0           | 1               | 2.6                        |
| 92987     | 141.21  | 2       | 2      | 1      | -0.81     | 0.55                  | 0                     | 29.1  | 1.12  | 0          | 0           | 1               | 1.79                       |
| 89594     | 162.23  | 1       | 2      | 0      | -1.89     | 0.55                  | 0                     | 16.13 | 1.48  | 0          | 0           | 1               | 2.05                       |
| Inhibitor | 200.24  | 3       | 2      | 2      | -2.74     | 0.55                  | 0                     | 45.15 | 1.83  | 0          | 1           | 1               | 1.69                       |

Table 2. Results of pharmacokinetic parameters and toxicity profile of selected active compounds.

| CID       | GI Absorption | BBB Permeant | P-gp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | Ames mutagenesis | Carcinogenicity | Hepatotoxicity |
|-----------|---------------|--------------|----------------|------------------|------------------|------------------|------------------|------------------|-----------------|----------------|----------------|
| 12306778  | High          | Yes          | No             | No               | No               | No               | No               | No               |                |                |                |
| 1201543   | High          | Yes          | No             | No               | No               | No               | No               | No               |                |                |                |
| 92987     | High          | Yes          | No             | No               | No               | No               | No               | No               |                |                |                |
| 89594     | High          | Yes          | No             | No               | No               | No               | No               | No               |                |                |                |
| Inhibitor | High          | Yes          | No             | Yes              | No               | No               | No               | No               |                |                |                |

≤ 500, logP ≤ 5, HBD ≤ 5, HBA ≤ 10 or else its absorption and bioavailability would be poor. Veber’s filter was considered to check if compounds have TPSA ≤ 140 and nRot ≤ 10 or not.

These parameters were analyzed for 77 compounds, and 46 compounds were found to be in the acceptable range. In the second step, the pharmacokinetics of these compounds were studied on the basis of their gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeation, P-glycoprotein (P-gp), and Cytochrome P450 (CYP) inhibition. These parameters are helpful in determining drug-likeness properties of compounds. Compounds that were having high GI absorption, BBB permeation, P-gp non-substrates, and non-inhibitors of CYP were selected. In this study, we are targeting neurodegenerative disease, so to reach the brain cells and bind the target, the drug must cross the Blood-Brain Barrier. P-gp non-substrate components were chosen so that drug efflux by P-gp would not become an issue and compounds would be having high absorption and bioavailability. CYP non-inhibitor components were selected as CYPs are responsible for drug metabolism, and their inhibition could result in prolonged drug-drug interaction thus turning out to be lethal. Only four compounds having CID’s 12306778 (Anahygrine), 1201543 (Cuscohygrine), 92987 (Isopelletierine), and 89594 (Nicotine) were found in the permissible range.

In the third step, the medicinal chemistry of these compounds was analyzed based on pan assay interference compound (PAINS) alert, Brenk alert, and their synthetic accessibility. There were no PAINS and Brenk alerts for these four compounds. This confirmed that these compounds don’t contain any substrates that could show false positive results in bioassays and could be responsible for their toxicity, instability, or poor pharmacokinetics. The synthetic accessibility of all these compounds was found to be <10, which indicated that their synthesis would not present any challenge. At the last step, their toxicity profile was evaluated based on their mutagenesis and carcinogenic properties. Ames mutagenesis test is an indicator of whether a compound has the potential to cause mutations at the genetic level inside the target organism. Carcinogenicity indicates the ability of the compound to cause cancer and hepatotoxicity averse the compound’s ability to damage the liver. All four compounds showed negative results for these tests, which indicated that these compounds were safe to use further.

ADME profile of Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine was compared with 2-PMAP. All these components showed a comparable ADME profile with 2-PMAP and were found to be having the advantage of not being a different CYP isoform’s inhibitor (Table 1 and 2).

The BOILED-Egg or Egan’s Egg model prediction was observed based on WLogP vs TPSA (Figure 2). The yellow region in the graph indicated the BBB permeation capability of molecules lying in this part, and the white area indicates high GI absorption (HIA). Components represented as red circles depicted that these were non-substrates of Pgp (Pgp-).

Generated bioavailability radar provided an overall idea of the drug-likeness of the compounds. This was based upon 6 physicochemical parameters: lipophilicity (XLogP range between −0.7 – 5.0), size (MW lie within 150-500 g/mol), polarity (TPSA within 20-130), solubility (Log S < 6), saturation (>−0.25) and flexibility (nRot < 9). All four molecules of this study were found within the colored region of radar depicted their good bioavailability and drug-likeness properties (Figure 3).

3.3. Targets prediction results

In this study, two categories of targets (Targets related to neurodegenerative disease and targets related to W. somnifera) were retrieved from different public databases (Table 2). For detailed information of these targets, refer to Tables S2 and S3 (supplementary material). According to literature and GENEcards, BATMAN-TCM, NCBI, STITCH databases, total of 175 targets were found related to W. somnifera, and 8085 targets were found related to neurodegenerative diseases. For network representation of all these targets, refer to Figure S1.
After performing Venn diagram analysis of both categories targets, a total of 145 common molecules were included for further studies (Figure 4).

3.4. Gene–Gene interaction analysis

A Cytoscape plugin, GeneMania was used to construct the gene–gene interaction network of targets. Out of 175 targets, a total of 165 targets were recognized by GeneMania, an interaction network of similar targets shown in Figure 5.

Genes were denoted by circular nodes, and colored edges represented their different correlations. Larger circles denoted that these genes were most correlated to other genes in this network. For detailed information of gene-gene interactions, refer to Table S4 (supplementary material).

Furthermore, topological parameters, Betweenness Centrality (BC), Degree Centrality (DC), and Closeness Centrality (CC) of each node were observed. DC is the measure of direct connections of a node in the network, and a higher degree indicates the high impact of that node. On the basis of the highest degree score, we selected the top 10 nodes of the network. These nodes were Amyloid-beta precursor protein (APP), Epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 1 (MAPK1), estrogen receptor 1 (ESR1), heat shock protein family A (Hsp70) member 4 (HSPA4), protein kinase C delta (PRKCD), mitogen-activated protein kinase 3 (MAPK3), ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1), Jun proto-oncogene, AP-1 transcription factor subunit (JUN), and glycogen synthase kinase 3 beta (GSK3B) (Table 3). For topology analysis results of all 165 targets, refer to Table S5 (supplementary material).

3.5. Gene ontology (GO) and pathway enrichment analysis

Several kinds of enriched Biological Processes (BP), Molecular Functions (MF), Cellular components (CC), Protein class, and pathways were observed for ten key targets (Figure 6). In the...
case of BP, targets were found to be enriched in different processes such as cellular process, biological regulation, developmental process, metabolic processes, multicellular organismal process, response to stimulus, and signalling. For CC, targets were found to be enriched in the cellular, anatomical entity, intracellular and protein-containing complex. In the case of MF, targets were found to be involved in binding molecular function, catalytic activity, molecular function regulator, and molecular transducer activity. Targets were observed to be enriched in different protein classes like a gene-specific transcriptional regulator, protein modifying enzyme, protein-binding activity modulator, and the transmembrane signal receptor.

Pathways analysis of targets were found to be enriched in 5HT2 type receptor-mediated signaling pathway, Alpha-adrenergic receptor signaling pathway, Alzheimer disease-amyloid secretase pathway, Alzheimer disease-presenilin pathway, Apoptosis signaling pathway, Axon guidance mediated by Slit/Robo, Huntington disease, Inflammation mediated by chemokine and cytokine signaling pathway, Insulin/IGF pathway-mitogen activated protein kinase/MAP kinase cascade, Insulin/IGF pathway-protein kinase B signaling cascade, Muscarinic acetylcholine receptor 1 and 3 signaling pathway, PI3 kinase pathway, Parkinson disease, Ras Pathway, T cell activation, Wnt signaling pathway, and p38 MAPK pathway. For more detailed results of all the categories, refer to Table S6 (supplementary material).

APP (Amyloid precursor protein) with the highest degree (41.0) in the network was selected for molecular docking. According to GO & Pathway enrichment analysis, APP was found to be involved in the Alzheimer disease-amyloid secretase pathway and, Alzheimer disease-presenilin pathway. For a detailed analysis of the involvement of APP in these pathways, refer to Figure S2 (supplementary material).

3.6. Identification of binding site

Total six binding pockets were calculated for the target protein. On the basis of the highest volume and surface area, binding pocket one was selected as the best active site for the ligand (Figure 7). The active site residues of the best pocket are shown in Table 4. This predicted site was used for molecular docking with ligands.

3.7. Binding affinity analysis of target protein with ligands

To achieve a pharmacological effect, the drug substance must bind with the protein active site residues, and this is
the basic step for potential drug development. Binding affinity scores of the target protein with four ligands 12306778 (Anahygrine), 1201543 (Cuscohygrine), 92987 (Isopelletierine), 89594 (Nicotine), and 14936865 (2-PMAP) were observed to be 5.55, 4.73, 4.04, 4.11, and 3.73 respectively (Table 5). More the negative binding energy shows the highest binding affinity. Docking score of reference molecule, 2-PMAP was found to be the lowest binding energy (-3.73) in comparison to among four ligands (Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine). Hydrophobic interactions, hydrogen bonding, and salt bridges were found to be involved in interactions between the target protein and ligands (Figure 8). To gain more insight into these interactions refers to Table S7 (supplementary material). The chemical structures of these five compounds are shown in Figure 9.

3.8. Molecular simulation dynamics results

MD trajectories such as RMSD and RMSF were observed for docked complexes. In the case of APP-Nicotine complex, the RMSD value decreased for the first 20 ns, after this remained constant for almost 30 ns, next increased slightly and remained constant till 40 ns, thereafter for almost 60 ns decreased gradually; afterward a slight increase was observed till 70 ns and later on remained almost stable till 100 ns. For APP-Isopelletierine complex, RMSD value decreased till 27 ns and then almost stable till 55 ns; after that, a slight increase was observed till 70 ns and afterward remained almost constant till 100 ns. For APP-Cuscohygrine complex, RMSD value decreased gradually till 20 ns, then increased gradually till 25 ns and again decreased till 40 ns, next a gradual increase was observed till 45 ns and after this decrease till 60 ns, afterward gradually increased till 70 ns and later on remained almost stable till 100 ns with a little
bit of fluctuations. In the case of APP-Anahygrine complex, the RMSD value increased till 20 ns and further decreased till 40 ns, next gradually increased till 60 ns and decreased till 70 ns, again an increase in value was observed till 80 ns and then decreased till 100 ns. For APP-2-PMAP complex, RMSD value was observed to be decreasing a bit and a slight increase till 20 ns, remained nearly constant till 40 ns, afterward decreased a little bit for 50 ns and then increased a bit and remained almost constant till 80 ns and thereafter showed a slight increase till 100 ns.

The average RMSD values for APP-Nicotine, APP-Isopelletierine, APP-Cuscohygrine, APP-Anahygrine, and APP-2-PMAP were found to be approximately 0.86 nm, 0.81 nm, 1.04 nm, 1.07 nm, and 1.03 nm, respectively (Figure 10(a)). The RMSD plot depicted that APP-Nicotine complex and APP-Isopelletierine complex had less deviations (<0.87 nm) during the whole simulation time. On the other hand, APP-Cuscohygrine, APP-Anahygrine, and APP-2-PMAP complexes showed larger deviations (>1 nm). These values indicated that APP-Nicotine and APP-Isopelletierine complexes were more stable in comparison to the other three complexes.

The radius of gyration plot was also analyzed for each complex to understand the compactness (Figure 10(b)). The average Rg value of APP-Nicotine, APP-Isopelletierine, APP-Cuscohygrine, APP-Anahygrine, and APP-2-PMAP complexes was observed to be 1.167 nm, 1.165 nm, 1.166 nm, 1.165 nm, and 1.29 nm, respectively. The plot clearly indicated that the 2-PMAP complex had a higher Rg value and showed huge fluctuations during the whole simulation time. While the Nicotine, Isopelletierine, cuscohygrine and Anahygrine complexes had low Rg values and showed very little fluctuations during the whole simulation period. Therefore, the APP-2-PMAP complex had less rigidity in comparison to other complexes.

Further, the RMSF graph was also plotted of all five complexes to see the behavior of residues. The graph showed that all exhibited almost similar patterns of fluctuation during the whole simulation time (Figure 10(c)). The average RMSF values of APP-Nicotine, APP-Isopelletierine, APP-Cuscohygrine, APP-Anahygrine, and APP-2-PMAP complexes were observed as 0.070 nm, 0.074 nm, 0.070 nm, 0.075 nm, and 0.078 nm, respectively. These values indicated that the APP-2-PMAP complex had higher conformational fluctuations in comparison to other complexes. All these results thus coincided with docking results.

4. Discussion

Neurodegenerative diseases (ND) are devastating and affecting millions of people worldwide. Several reasons such as age, genetics, and environmental factors like stress are considered as underlying causes of these diseases (Gitler et al., 2017; Skovronsky et al., 2006). ND exact mechanism is still unclear due to their unclear mechanism, there are no specific

Table 4. CytoNCA results of the gene-gene interaction network.

| S. No. | Target | Degree | Betweenness | Closeness |
|-------|--------|--------|-------------|-----------|
| 1.    | APP    | 41     | 4741.673    | 0.028898254 |
| 2.    | EGFR   | 29     | 1814.4824   | 0.02880576  |
| 3.    | MAPK1  | 25     | 622.9579    | 0.02862254  |
| 4.    | ESR1   | 25     | 1175.0629   | 0.028679546 |
| 5.    | HSPA4  | 22     | 1518.7238   | 0.028742515 |
| 6.    | PRKCD  | 18     | 620.5449    | 0.028696962 |
| 7.    | MAPK3  | 18     | 253.06743   | 0.028520498 |
| 8.    | ABL1   | 17     | 641.16486   | 0.028702412 |
| 9.    | JUN    | 16     | 259.8645    | 0.02862254  |
| 10.   | GSK3B  | 15     | 274.61465   | 0.028639618 |

Table 5. Table showing the dimensions of all binding pockets of 2fk1 and constituent residues of pocket 1.

| Pocket ID | N_mth | Area_sa | Area_ms | Vol_sa | Vol_ms | Length | cnr |
|-----------|-------|---------|---------|--------|--------|--------|-----|
| 1         | 1     | 16.571  | 50.260  | 6.987  | 51.708 | 23.204 | 19  |
| 2         | 1     | –0.234  | 17.985  | 1.890  | 14.275 | 12.284 | 9   |
| 3         | 1     | 6.525   | 40.683  | 0.886  | 29.726 | 12.451 | 8   |
| 4         | 1     | 2.799   | 18.583  | 0.541  | 14.013 | 7.512  | 11  |
| 5         | 1     | 0.645   | 14.991  | 0.091  | 9.707  | 5.323  | 6   |
| 6         | 1     | 0.019   | 13.592  | 0.000  | 6.585  | 0.456  | 3   |

Active site residues of pocket ID 1

| PHE A135, ASP A167, TYR A168, GLY A169, GLU A183, PHE A184, VAL A185 |

Note: N_mth: number of mouth openings for the pocket, Area_sa, and Area_ms: The Solvent Accessible and Molecular Surface area of the pocket or cavity, Vol_sa, and Vol_ms: The SA and MS volume of the pocket or cavity, Length: Length sums the arcs of the pocket, cnr: cnr is the total count of the corner points.

Table 6. Entry ID, docked energy and interacting residues of finally selected ligands.

| Ligand’s name | Entry ID | Docked Energy (KCal/mol) | Interacting residues |
|---------------|----------|--------------------------|---------------------|
| Anahygrine    | 12306778 | –5.55                    | THR152A, VAL153A, GLU156A |
| Cuscohygrine  | 1201543  | –4.73                    | THR152A, VL153A, GLU156A, GLU160A |
| Isopelletierine| 92987    | –4.04                    | THR152A, VAL153A, GLU156A |
| Nicotine      | 89594    | –4.11                    | THR152A, VAL153A, GLU156A, GLU160A |
| 2-PMAP        | 14936865 | –3.73                    | PHE135A, LEU136A, HIS137A, GLN138A, THR157A |

(2-[(2-pyridinylmethyl)amino]-phenol)
drugs available for their cure. Several studies have reported that herbal drugs are helpful in treating such diseases and enhance general health and wellbeing. There is a renowned herb in the Indian medicinal system, *W. somnifera* (WS) which is a powerful rejuvenator and has been used from centuries in treating the number of diseases. This herb has been proven to cure stress problems as well as treatment of ND (Blaylock and Maroon, 2012; Mohanty et al., 2021; Pohl and Lin, 2018; Syed et al., 2021). One study found the effectiveness of leaf extract of WS in restoring the outgrowth of neurites and protection of brain cells from apoptosis. They also reported that it suppresses ataxia in mouse models (Gupta and Kaur, 2019). Dar et al. 2020 found that its role in protecting from neurodegeneration via restoring the functions of mitochondria and by alleviating the apoptotic death and oxidative stress in cells (Dar and Ahmad, 2020). To uncover the therapeutic benefits of WS phytochemicals related to ND, the network pharmacology (NP) approach was used to find out the significant targets. In the current study, a total number of 77 active components of WS, 175 WS targets, and 8085 ND-specific targets were identified from different public databases. Total 145 targets were found to be common in both categories. Gene-Gene interaction network was constructed for these targets. Topological analysis of the network
revealed top ten significant targets such as Amyloid-beta precursor protein (APP), Epidermal growth factor receptor (EGFR), mitogen-activated protein kinase 1 (MAPK1), estrogen receptor 1 (ESR1), heat shock protein family A (Hsp70) member 4 (HSPA4), protein kinase C delta (PRKCD), mitogen-activated protein kinase 3 (MAPK3), ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1), Jun proto-oncogene, AP-1 transcription factor subunit (JUN), and glycogen synthase kinase 3 beta (GSK3B). APP was found to have the highest Degree, Betweenness, and Closeness scores, such as 41.0, 4741.673, and 0.028898254, respectively. Gene Ontology and pathway enrichment analysis of these key targets speculated the involvement in different cellular components, biological processes, molecular functions, protein classes, and cellular pathways. The topmost key target, APP (Amyloid precursor protein) was found to be enriched in Alzheimer disease-amyloid secretase pathway and Alzheimer disease-presenilin pathway. By pathway analysis, it was elucidated that the cleavage of amyloid precursor protein sequentially by beta-secretase and presenilin gamma-secretase results in the generation of amyloid-beta fragments. These fragments when get aggregate into senile plaques that leads to death of neuron cells as well as disease pathogenesis.

The result suggested that APP target could be used as a possible target for the treatment of Alzheimer’s disease. Pharmacokinetic properties and toxicity analysis of active components were helpful in assessing their drug-likeness. Only four active components, such as Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine, were found as drug-like candidates. In the predicted active site of the protein, several residues like PheA 135, AspA 167, TyrA 168, GlyA 169, GluA 183, PheA 184, and ValA 185 were predicted on the basis of the highest solvent accessible surface area score. These four compounds showed the best binding affinities —5.55, —4.73, —4.04, —4.11, and —3.73, respectively, with APP. Hydrogen bond, hydrophilic bond, hydrophobic bond, and salt bridge were involved in the interaction of ligand and protein with binding site residue Thr152A, Val153A, Glu156A, Glu160A, Phe135A, Leu136A, His137A, Gln138A, and Thr157A.

The 2-PMAP drug has been reported as an inhibitor of amyloid precursor protein (APP) and amyloid-β (Aβ) secretion (Asuni et al., 2014). This drug was selected as a reference
drug in this study. Docking results clearly showed that components of WS (Anahygrine, Cuscohygrine, Isopelletierine, and Nicotine) had greater binding affinity than reference drug (2-PMAP) with APP target. Further, MD simulation results suggested that Nicotine and Isopelletierine were more stable in comparison to 2-PMAP.

In conclusion, these findings will facilitate the identification of WS compounds as inhibitors of APP protein to suppress amyloid β protein production and thus provide a potential alternative for the treatment of Alzheimer’s disease. Further in-vitro studies of these active components against APP could be a future perspective.

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Disclosure statement

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Author’s contributions

SS conceived and designed this study. SP performed analysis and wrote the manuscript. AG and PC helped in performing analysis. AC and SS did the proofreading.

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