

A Network Pharmacology Based Approach to Decipher the Pharmacological Mechanisms of *Salvia officinalis* in Neurodegenerative Disorders

Sheikh Sana Nazir

ssananazir_sch@jamiahamdard.ac.in

Jamia Hamdard <https://orcid.org/0009-0001-5609-9519>

Divya Goel

Jamia Hamdard Faculty of Pharmacy

Divya Vohora

Jamia Hamdard Faculty of Pharmacy <https://orcid.org/0000-0003-1852-9191>

Research Article

Keywords: *Salvia officinalis*, Neurodegenerative disorders, Network pharmacology, Alzheimer's disease, Parkinson's disease, Huntington's disease

Posted Date: September 6th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3288079/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Metabolic Brain Disease on April 23rd, 2025. See the published version at <https://doi.org/10.1007/s11011-025-01599-6>.

Abstract

The present study aimed to assess the pharmacological mechanism of *Salvia officinalis* in Neurodegenerative disorders using a network pharmacology approach followed by molecular docking analysis. Phytoconstituents of *S.officinalis* were obtained from various databases, followed by the screening of active ingredients using the Swiss ADME web tool. Potential targets of active ingredients were identified using PubChem & SwissTargetPrediction. Genes related to Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) were gathered using online databases. Besides, the correlation between active ingredient targets and disease-associated genes was linked. Networks were constructed, visualized, and analyzed using Cytoscape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis were performed using DAVID database. Decisively, Autodock was used for molecular docking. The results of network analysis identified 9 key active ingredients based on topological analysis of the active ingredient - candidate targets network. Also, the analysis revealed a shared target of 9 key active ingredients of *S. officinalis* that interacted with 133 AD-related targets whereas only 6 active ingredients interacted with 85 and 58 targets of PD and HD respectively. The core genes from the network were AKT1, BACE1, CASP3, MAPK1, TNF, and IL6. Furthermore, GO and KEGG enrichment analysis showed that FOXO, TNF, MAPK, PI3K-Akt, Rap 1, and neurotrophin signalling pathways as enriched, which were further validated by molecular docking suggesting the protective role of *S. officinalis* in neurodegenerative diseases. Our research reveals the therapeutic benefits of *S. officinalis*, which might play a crucial role in modulating neurodegenerative diseases.

1. Introduction

Neurodegenerative disorders (NDDs) are incurable and debilitating conditions that affect around one billion people worldwide. These are characterized by progressive degeneration and/or the death of nerve cells. Depending upon the occurrence, the two most prevalent NDDs are Alzheimer's Disease (AD) and Parkinson's Disease (PD) (Bulck et al., 2019). In addition, there is Huntington's Disease (HD) which is caused by a CAG triplet repeat expansion in Huntingtin (HTT) gene, which encodes an expanded polyglutamine stretch in the HTT protein (Ross and Tabrizi, 2011). NDDs such as AD, PD and, HD are the main cause of dementia worldwide (Davis et al., 2014; Fischer and Maier, 2015).

Over the past decades, the philosophy of drug design has relied on the "one gene, one drug, one disease" paradigm which focuses on designing maximally selective ligands that act on individual drug targets (Hopkins, 2008). However, in real biological systems, it has been appreciated that many effective drugs act on multiple proteins rather than single targets. Developing a new drug is a lengthy, expensive, and time-consuming process (Zhang et al., 2012). Despite the dramatic increase in global spending on drug discovery and development, the approval rate for new drugs is declining. This unproductivity in drug approval can be overcome by the novel concept of network pharmacology, which is built on the fundamental concept that drugs modulate multiple targets (Zhou et al., 2016). NDDs are complex and multi-factorial in origin (Tan et al., 2015), and multiple stages along the disease pathway may need to be

manipulated simultaneously for effective treatment of the disease. For mono-factorial diseases, the drug is directed toward a single target, however, for complex diseases, the hypothesis of a multi-target strategy is considered more superior and efficient (Chandra and Padiadpu, 2013). Due to the component diversity, higher multi-target activity, and lower toxicity, herbs become an important source for developing multitarget drugs. Herbal medicines have received extensive attention as potential treatments for NDDs. The multiple components of herbs could hit multiple targets (Ke et al., 2016).

Salvia officinalis commonly known as Sage, culinary sage, garden sage, and common sage, is a multipurpose culinary herb belonging to Lamiaceae family. It was earlier used for treating cognitive impairment, Alzheimer's disease besides being used in traditional Indian Siddha medicine, for the treatment of asthma, bronchitis, migraine, and rhinitis (Hamidpour et al., 2014; Ghorbani and Esmailizadeh, 2017). It has been reported that *S. officinalis* exhibits CNS acetylcholine receptor activity, along with nicotinic and muscarinic binding properties (Wake et al., 2000). It also exhibits potential antioxidant properties, an effect that is presently relevant to the treatment and prevention of Alzheimer's disease and other neurodegenerative disorders. In various randomized placebo-controlled trials, it improved mood and cognitive functions (Akhondzadeh et al., 2003; Kennedy et al., 2006).

Hence, the current study aims to identify the potential phytoconstituents from *S. officinalis*, recognize their targets involved in NDD, and report the probable pharmacological mechanism in its management using a network pharmacological approach. The study establishes a basis in favour of its therapeutic application against neurodegeneration and considering it for further studies.

2. Materials and Methods

2.1. Assessing the phytoconstituents of *S. officinalis*

All the phytoconstituents of *S. officinalis* were mined from available literature and publicly available databases: Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) (<https://cb.imsc.res.in/>) and Dr. Duke's Phytochemical and Ethnobotanical database (<https://phytochem.nal.usda.gov/>).

2.2. Screening the active ingredients of *S. officinalis*

Data of three ADME-related parameters, including Drug Likeness (DL), Gastrointestinal (GI) absorption, and ability to cross Blood-Brain Barrier (BBB) was retrieved from the SwissADME database (<http://www.swissadme.ch/>), using the canonical SMILE values for each compound. The "Rule-of-five" proposed by Lipinski, used to determine whether a molecule is well absorbed or not was implicated for screening. The rules are Molecular Weight ($MW \leq 500$), octanol/water partition coefficient ($ClogP \leq 5$), no. of Hydrogen Bond Donors (HBD) ≤ 5 , no. of hydrogen bond acceptors (HBA) ≤ 10 . If a compound violates two or more rules it may not be orally active (Tian et al., 2015). The screening criteria were defined as follows: GI absorption defined as "high", the ability to cross BBB defined as "yes" and DL (Lipinski) defined as "Yes, 0 or 1 violations" (Oh et al., 2021). The results obtained after the screen were considered as potential active ingredients.

2.3. Prediction of Compound related targets

The targets of active ingredients were obtained using SwissTargetPrediction (<http://www.swisstargetprediction.ch/>). Predicted targets limited to “Homo sapiens” and “Probability > 0” were obtained..

2.4. Identification of targets related to NDDs

Targets related to NDDs such as AD, PD, and HD were obtained from DisGeNet (<http://www.disgenet.org/>), Therapeutic Target Database (TTD) (<http://bidd.nus.edu.sg/group/cjttd/>), Genecards (<http://www.genecards.org/>) and Malacards (<http://www.malacards.org/>). Target gene names collected from the above databases were uniformly standardized against UniProt knowledgebase (<http://www.uniprot.org/>).

2.5. Screening the targets of *S. officinalis* associated with NDDs

The targets of active ingredients were mapped to NDD related target genes to obtain candidate targets of *S. officinalis* that act against NDDs. Mapped Targets with probability value ≥ 0.1 were selected for further analysis. Moreover, targets that were common to AD, PD, and HD were analysed and visualized using Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) webtool.

2.6. Network Construction and Topological analysis

An online tool Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) was used to construct a Protein-Protein Interaction network among the targets. This network consisted of nodes and edges representing targets of their intermolecular interactions, respectively. Furthermore, the lists of AD, PD, and HD-related genes were retrieved from various databases. The targets related to NDD were those matched with *S. officinalis* key active ingredient targets. To demonstrate and visualize the interaction of *S. officinalis* active ingredients with targets associated with NDDs, a network was constructed using Cytoscape software (<https://cytoscape.org/>).

Topological analysis was used to detect proteins that can act as essential drug targets (Panagiotou and Taboureau, 2012). Key nodes in the network were found, using NetworkAnalyzer plugin of cytoscape. Key nodes were identified with the following topological parameter criterion: 1) Nodes with “degree”, “betweenness centrality” (BC), and “closeness centrality” (CC) values higher than the corresponding average values; and 2) Nodes with “average shortest path length” (ASPL) values lower than the corresponding average values. Nodes with higher degree/betweenness centrality/closeness centrality and lower average shortest path length were considered important nodes in the network and such high degree nodes were termed as “hubs”.

2.7. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

GO and KEGG pathway enrichment analysis was performed using an online biological knowledgebase and analysis tool - Database for Annotation, Visualization Integrated Discovery (DAVID) database (<https://david.ncifcrf.gov/>). GO and KEGG terms with a p-value of < 0.05 were considered significant.

2.8. Molecular docking Analysis

Active ingredients having the highest degree and targets associated with AD, PD and HD were selected for docking. Targets having a crucial role in the inflammation, apoptosis, and other risk factors related to the pathobiology of NDD were selected. Additionally, majority of the targets involved were also the hub nodes in the PPI network besides played a significant role in KEGG signalling pathways, were also used for docking. The 2D structure files for active ingredients were obtained from the PubChem database and minimized using MMFF94 forcefield by Avogadro. The crystal structure files of target proteins were acquired from the RCSB Protein Data Bank (PDB database, <http://www.rcsb.org/>). We used PyMOL 2. 3. 4 software to remove ligand on the binding sites. Furthermore, the AutoDock Tool was used to prepare, run, and analyze the docking simulations. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and analysed using autodock to predict the binding affinity of molecules with target. After docking the ligand-protein 2D interactions were visualized using Maestro (Schrodinger).

3. Results

3.1. Active ingredients of *Salvia officinalis*

A total of 163 phytoconstituents of *S. officinalis* were obtained from IMPPAT, Dr. Duke's Phytochemical and Ethnobotanical database, and available literature. Out of 163 phytoconstituents, 47 were screened out based on ADME properties such as DL, GI absorption and ability to cross BBB. Most of these constituents showed optimal pharmacokinetic properties and were considered to be potential active ingredients of *S. officinalis*. The detailed characteristics of potential active ingredients of *S. officinalis* were shown in Supplementary Table 1 (Table S1).

3.2. Screening the targets of *S.officinalis* active ingredients

Targets of 47 active ingredients with "Probability > 0 " were obtained from SwissTarget prediction database.

3.3. Screening the targets of NDD and construction of PPI network

Targets related to AD, PD and HD were obtained from DisGeNet, TTD, Genecards and Malacards. A total of 1284-AD-related, 914-PD-related, 410-HD related target genes were obtained. Among these targets 251 targets were common to AD, PD and HD (Fig. 1a). Disease target network was constructed, nodes with high confidence ≥ 0.700 were selected. Disconnected nodes were removed from the network. The network was then analysed using NetworkAnalyser plugin of Cytoscape, topological parameters calculated; nodes with "degree", BC and CC values greater than and ASPL value lower than average of

the corresponding values were selected, as they represented key targets in the disease network. The network consisted of 59 nodes and 653 edges. In (Fig. 1b) TP53, AKT1, HSP90AA1, EGFR, STAT3, CASP3, JUN, TNF, MAPK1, BDNF, APP, IL6 were recognised as important NDD related targets in disease target network of NDD (Supplementary Table 2: Table S2).

3.4. Candidate targets of *S. officinalis* with therapeutic effect against NDDs

Candidate targets associated with NDD were obtained by mapping the targets of active ingredients with the targets related to AD, PD and HD. Furthermore, common targets were screened based on probability value ≥ 0.1 for study. A total of 142 targets of *S. officinalis*, were associated with AD, whereas 102 targets of *S. officinalis* were associated with PD and 75 targets, associated with HD.

3.5. Construction of *S. officinalis* active ingredients and NDDs target network

“Active ingredients-candidate targets” network for AD (Fig. 2a) (Supplementary table 3: Table S3), PD (Fig. 2b) and HD (Fig. 2c) (Supplementary table 4 & 5 respectively: Table S4 & S5) was constructed using Cytoscape software. In these networks each active ingredient was connected to more than one target and each target was shared by multiple active ingredients. Thus, demonstrating multi-target and multi-component activity of *S. officinalis*.

3.6. Topological analysis and construction of key active ingredient and NDD target network

Topological analysis of Active Ingredient-Candidate target was done after analysing the network using NetworkAnalyser plugin of cytoscape. The key nodes in the network were identified. LA28: Linoleic Acid, AA17: Abietic Acid, V15: Viridiflorol, MR18:7-Methylrosmannol, C13: Carnosol, S30: Salviol, E04: Eugenol, PCA24: p-coumaric acid, C20: 2-coumarinate, with degrees of 51,42,37,36,35,25,16,14 and 11 respectively, represented the key active ingredients, these 9 active ingredients shared 133 AD related targets. In PD active ingredient-candidate target network, (Fig. 2b) topological analysis revealed, 6 key active ingredients (LA22: Linoleic acid, V12: Viridiflorol, AA13: Abietic Acid, C11: Carnosol, MR14:7-Methylrosmannol, S24: Salviol), with degrees of 29,27,26,23,21 and 19 respectively, these 6 active ingredients shared 85 PD-related targets. In HD active ingredient-candidate target network, (Fig. 2c) topological analysis revealed 6 active ingredients (LA21: Linoleic acid, V10: Viridiflorol, MR13:7-Methylrosmannol, C09: Carnosol, AA11: Abietic acid, S22: Salviol), with degrees of 21,19,16,16,15 and 11 respectively representing key active ingredients. These 6 active ingredients shared 58 HD-related targets. AD, PD and HD related targets were analysed and duplicate targets were removed to construct an integrated network of key active ingredients of *S.officinalis* and their NDD related targets (Fig. 3).

3.7. PPI analysis of key active ingredient related targets against NDDs

To explore the relationship between 133 AD-related, 85 PD-related and 58 HD-related targets of *S. officinalis*, protein-protein interaction (PPI) analysis was performed using STRING database (high confidence ≥ 0.700). Targets that were common between AD, PD and HD were analysed and visualised using Venny 2.1 tool. 45 common targets related to NDD were obtained (Fig. 4a). PPI network was constructed and it consisted of 41 nodes and 147 edges (4 disconnected nodes were eliminated from the 41-node network) (Fig. 4b). Topological analysis was performed by NetworkAnalyzer plugin of cytoscape and 6 key NDD-related targets i.e, TP53, AKT1, EGFR, MAPK1, ESR1, MAPK14 were obtained (Fig. 4c).

3.8. Gene Ontology and KEGG Enrichment Analysis

45 NDD-related targets were further analysed to elaborate the neuroprotective role of *S. officinalis* against NDDs. Top 20 highly enriched GO terms under BP included regulation of transcription, signal transduction, response to drug, apoptotic processes, regulation of cell proliferation, response to hypoxia (Fig. 5a). In the CC category, the target proteins were primarily distributed in cytoplasm followed by cytosol and nucleus (Fig. 5b). In the MF category, target proteins were mainly involved in protein binding (Fig. 5c).

To identify pathways that are enriched and likely to be involved in neuroprotective effects of *S. officinalis*, KEGG enrichment analysis of 45 NDD-related targets was performed using DAVID database with a p value cut off < 0.05 . Top 20 pathways based on the number of enriched genes and p value were selected and represented as bar plots respectively (Fig. 5d). KEGG analysis of 45 NDD-related targets revealed that the targets were closely related to pathways in cancer, FoxO signalling pathway, TNF signalling pathway, MAPK signalling pathway, PI3K-Akt signalling pathway, Rap1 signalling pathway, Neurotrophin signalling pathway etc (Fig. 5d).

In addition, we identified targets related to NDD that were shared between multiple pathways and constructed a target-pathway network (Fig. 6a). MAPK1, AKT1, RAF1, MAPK10, BRAF, TP53, TNF, IL6, CASP3 were among the targets that act via multiple pathways, thus were considered as highly enriched genes. Top 20 pathways shared 31 targets, out of which 24 targets participated in more than 1 pathway, thus key active ingredients of *S. officinalis* may act via multiple pathways to exert neuroprotective effect.

Moreover, to figure out the mutual effect of disease, pathways, *S. officinalis* active ingredients and their NDD related targets an intrinsic network was set up, by selecting NDD related pathways that included pathways in cancer, FoxO signalling pathway, TNF signalling pathway, MAPK signalling pathway, PI3K-Akt signalling pathway, Rap1 signalling pathway, and Neurotrophin signalling pathway (Fig. 6b). Thus, this network describes the effect of Sage active ingredients on NDD related targets and their effect via modulation of multiple pathways. Pathway analysis of this network revealed pathways in cancer, Foxo signalling pathway, TNF, MAPK PI3K-Akt, Rap-1, neurotrophin, pathways as highly enriched. Further AD, PD and HD related genes modulated by *S. officinalis* active ingredients were also shown in Fig. 7.

Finally, NDD related pathways were analyzed. In AD-pathway, a total of 17 target proteins were involved including those that are involved in amyloidogenesis (BACE), neuronal growth and survival (MAPK1), inflammation (TNF), apoptosis (CASP3, CASP8). Besides APH1A, APH1B, PSEN1, PSEN2, PSENEN were also among the targets of *S. officinalis* active ingredients (Fig. 7a).

3.9. Molecular Docking between key active ingredients and potential targets.

From the main active ingredients- candidate targets network of AD (Fig. 2(a)) and topological analysis of the network, LA28-Linoleic acid, AA17-Abietic acid, V15-Viridiflorol, MR18-7-Methylrosmannol, C13-Carnosol, S30-Salviol, E04-Eugenol, PCA24-p-coumaric acid, C20-2-coumarinate had the highest number of targets related to AD. Targets such as AKT1, BACE1, CASP3, MAPK1, TNF, IL6 were selected because they not only represented the key nodes of the PPI network, but also played an important role in KEGG signalling pathways. Furthermore, these targets also played crucial roles in inflammation, oxidative stress and apoptotic processes, which are associated with pathology of NDDs. The results of molecular docking of nine active ingredients to six NDD related targets were shown in Table 1. Molecular docking was followed by binding energy analysis. Binding energy (B.E) indicates whether the ligand molecule effectively binds to the receptor, with lower energy values suggesting better binding capacity of the ligand and receptor. Inhibition constant (K_i) is reflective of the binding affinity, smaller value indicating better affinity.

Table 1
Molecular docking of NDD related key targets with *S.officinalis* key active ingredients

Ligands	Target	PDB	Binding Energy (Kcal/mol)
Linoleic acid	AKT	4EKL	-4.12
Linoleic acid	BACE1	6JT4	-4.27
Linoleic acid	CASP3	3ITN	-5.23
Linoleic acid	MAPK1	6RQ4	-4.71
Linoleic acid	TNF	5UUI	-3.62
Linoleic acid	IL6	1ALU	-5.78
Abietic acid	AKT	4EKL	-7.95
Abietic acid	BACE1	6JT4	-7.17
Abietic acid	CASP3	3ITN	-8.12
Abietic acid	MAPK1	6RQ4	-7.63
Abietic acid	TNF	5UUI	-6.9
Abietic acid	IL6	1ALU	-8.39
Viridiflorol	AKT	4EKL	-6.17
Viridiflorol	BACE1	6JT4	-5.6
Viridiflorol	CASP3	3ITN	-5.59
Viridiflorol	MAPK1	6RQ4	-6.37
Viridiflorol	TNF	5UUI	-5.52
Viridiflorol	IL6	1ALU	-6.53
7-Methylrosmanol	AKT	4EKL	-7.46
7-Methylrosmanol	BACE1	6JT4	-7.32
7-Methylrosmanol	CASP3	3ITN	-6.48
7-Methylrosmanol	MAPK1	6RQ4	-6.64
7-Methylrosmanol	TNF	5UUI	-6.23
7-Methylrosmanol	IL6	1ALU	-7.37
Carnosol	AKT	4EKL	-7.75
Carnosol	BACE1	6JT4	-6.73

Ligands	Target	PDB	Binding Energy (Kcal/mol)
Carnosol	CASP3	3ITN	-6.44
Carnosol	MAPK1	6RQ4	-6.35
Carnosol	TNF	5UUI	-6.34
Carnosol	IL6	1ALU	-6.7
Salviol	AKT	4EKL	-7.77
Salviol	BACE1	6JT4	-6.57
Salviol	CASP3	3ITN	-6.33
Salviol	MAPK1	6RQ4	-7.4
Salviol	TNF	5UUI	-6.28
Salviol	IL6	1ALU	-6.47
Eugenol	AKT	4EKL	-5.69
Eugenol	BACE1	6JT4	-4.54
Eugenol	CASP3	3ITN	-4.26
Eugenol	MAPK1	6RQ4	-4.5
Eugenol	TNF	5UUI	-4.87
Eugenol	IL6	1ALU	-4.5
p-coumaric acid	AKT	4EKL	-5.46
p-coumaric acid	BACE1	6JT4	-4.5
p-coumaric acid	CASP3	3ITN	-6.35
p-coumaric acid	MAPK1	6RQ4	-5.45
p-coumaric acid	TNF	5UUI	-5.54
p-coumaric acid	IL6	1ALU	-5.77
2-Coumarinate	AKT	4EKL	-5.91
2-Coumarinate	BACE1	6JT4	-5.09
2-Coumarinate	CASP3	3ITN	-5.66
2-Coumarinate	MAPK1	6RQ4	-5.44
2-Coumarinate	TNF	5UUI	-5.38
2-Coumarinate	IL6	1ALU	-5.81

Among these targets Linoleic acid showed the highest binding affinity with IL6, CASP3 and MAPK1, with a binding energy of -5.78, -5.23, -4.71 respectively. Abietic acid showed highest binding affinity with IL6, CASP3, AKT1, with a binding energy of -8.39, -8.12, -7.95 respectively (Table 1). Viridiflorol showed highest binding affinity with IL6, MAPK1, AKT1. 7-Methylrosmanol showed highest binding affinity with AKT1, IL6, BACE1. Carnosol showed highest binding affinity with AKT1, BACE1, IL6. Salviol showed highest binding affinity with AKT1, MAPK1, BACE1. Eugenol with AKT1, TNF, BACE1. p-coumaric acid with CASP3, IL6, TNF. 2-coumarinate with AKT, CASP3, MAPK1 (Table 1). Docking results also demonstrate that linoleic acid and abietic acid interacted with key NDD related targets via hydrophobic and polar interactions. Viridiflorol, 7-methylrosmanol, carnosol, salviol, eugenol, p-coumaric acid and 2-coumarinate interacted with NDD related targets via hydrogen bonds, polar and hydrophobic interaction (Supplementary data 6: Table S6). The results indicated that these active ingredients showed potential binding affinities with key NDD related targets (particularly AD) which could be considered as an in-silico approach for validation of ingredient-target interactions.

4. Discussion

Based on network pharmacological approach, we decoded the molecular mechanism of action of *S. officinalis* against NDDs. Initially we explored and identified various phytoconstituents of *S. officinalis*, characterized their pharmacokinetic properties and screened the phytoconstituents on the basis of their G.I absorption, drug-like properties and BBB accessibility. The targets of active ingredients were identified and mapped with NDD related targets. Thus, targets of *S. officinalis* acting upon AD, PD and HD were identified.

We identified 9 chemical compounds, including Linoleic acid, Abietic acid, Viridiflorol, 7-Methylrosmanol, Carnosol, Salviol, Eugenol, p-coumaric acid, and 2-coumarinate as potential active ingredient of *S. officinalis* after performing topological analysis of the active ingredient - candidate targets network. Among these, many of them have been widely reported to possess anti-oxidant, anti-inflammatory properties with a protective effect against neurodegeneration (Lee et al., 2011; Trevizan et al., 2016; Lopresti, 2017; Kang, Zhang and Yuan, 2018).

Linoleic acid, a polyunsaturated fatty acid, showed a protective effect against A β -42 cytotoxicity and exhibited a strong anti-oxidant effect on Drosophila NDD model (Lee et al., 2011). Another active ingredient abietic acid exhibited anti-inflammatory effects by inhibiting NF- κ B and MAPK signalling pathways in non-small-cell lung cancer (NSCLC) cell lines and human osteoarthritis chondrocytes (Kang, Zhang and Yuan, 2018; Liu et al., 2019). Carnosol, a phenolic diterpene from sage showed a protective effect in mouse primary cortical neuronal cultures from H₂O₂ induced cell death (Fischedick et al., 2013). It is the most effective antioxidant from *S. officinalis*, possesses high anti-oxidant activity, and can either stimulate endogenous anti-oxidative defence system or scavenge reactive species (Hamidpour et al., 2014). Production of nerve growth factor, a neurotrophin important for neuronal growth, survival and maintenance has also been shown to be enhanced by carnosol (Lopresti, 2017). Progression of NDDs is reportedly being suppressed by carnosol via modulation of inflammation and oxidative stress signalling

pathways (Kashyap et al., 2017). Carnosol was reported to activate PPAR γ (Christensen et al., 2010), which plays an important role in inflammatory responses. p-coumaric acid, a phenolic acid has shown anti-oxidant activity (Syta et al., 2018) and neuroprotective role in rat model of embolic cerebral ischemia (Guven et al., 2015) and in an in vitro study p-coumaric acid attenuated β Amyloid 25 – 35-Induced toxicity through regulation of the NF- κ B signalling Pathway in PC12 Cells (Yoon et al., 2014) p-coumaric acid also protects neurons against injury induced by 5-S-cysteinyl-dopamine *in vitro*, 5-S-cysteinyl-dopamine possess strong neurotoxic effects and may contribute to the progression of PD (characterized by progressive and selective loss of dopaminergic neurons in substantia nigra) (Vauzour, Corona and Spencer, 2010). Therefore the above components indicate their effectiveness for treating NDDs.

Furthermore, active ingredient-candidate targets network analysis revealed 9 active ingredients of *S. officinalis* interact with 133 AD-related targets whereas only 6 active ingredients of *S. officinalis* interact with 85 targets of PD and 58 HD-related targets. The intersection of the three lists identified 45 therapeutic targets of *S. officinalis* related to AD, PD and HD (Fig. 4a). The PPI analysis of these targets revealed key targets associated with NDD and these predicted interactions imply that the active ingredients of *S. officinalis* interact with proteins that have known functions in neurological disorders, which might describe its therapeutic potential. KEGG pathway analysis revealed FOXO signalling, TNF signalling pathways as highly enriched.

Mammalian forkhead transcription factors of the forkhead box class O (FoxO) are considered as promising targets for disorders of the nervous system, such as AD, PD, and HD. FoxO proteins are expressed selectively in the nervous system and play a crucial role in oxidative stress, autophagy, and apoptotic processes. They can lead to the induction of autophagy and promote cell survival, during oxidative stress to clear the presence of toxic proteins like α -synuclein and A β (Maiese, 2015).

Furthermore, GO and KEGG enrichment analysis was performed for AD, PD, and HD-related targets. Considering this, we recognized a subset of proteins, AKT1, BACE1, CASP3, MAPK1, TNF, and IL6 which exert their response by distinctive biological pathways. Molecular docking followed by binding energy calculation revealed that active constituents of *S. officinalis* showed a significant binding affinity with these targets. Likewise, these targets play crucial roles in inflammation, oxidative stress, and apoptotic processes, which are associated with the pathology of NDDs (Jellinger and Stadelmann, 2001; Spittau et al., 2012; Fischer and Maier, 2015; Rai et al., 2019a). For example, AKT (also known as protein kinase B), a regulatory protein acts as a regulator of cell survival, transcription, metabolism, and progression of the cell cycle. Various isoforms of AKT are significantly expressed in the nervous system and play a crucial role in neuroprotection. It has been reported that active phosphorylated Akt aids in cell survival (Chong et al., 2012; Rai et al., 2019b). In the PI3K-Akt signalling pathway, Akt plays a central link where it gets translocated from the cell membrane to the nucleus and affects transcriptional regulators and factors upon activation, for instance, mTOR, NF- κ B, GSK-3 β , CREB, caspase, and BCL2 (Gao et al., 2021). Therefore its targeting has been proposed as a practical approach to suppress ND progression (Rai et al., 2019a). Various studies have reported the neuroprotective role of polyphenolic anthocyanins by

reducing oxidative stress and improves memory dysfunction, via regulation of the PI3K/Akt/Nrf2 pathway (Ali et al., 2018).

Caspase-3 (CASP3) is a key pro-apoptotic protease involved in neuronal programmed cell death (D'Amelio, Sheng and Cecconi, 2012). CASP3 is one of the common targets of *S. officinalis* based on the results of PPI. Our network pharmacology analysis also revealed PI3K/Akt signalling pathway is enriched which, along with MAPK signalling regulates cell growth and survival. Furthermore, *S. officinalis* targets caspase, which is one of the transcription regulators of PI3K/Akt pathway, maintaining cell survival through upregulating Bcl-2. p38 MAPK signalling and its association with the neurodegenerative disease have been studied in past few years. MAPK signalling is involved in a wide variety of physio pathological processes, such as cell growth, apoptosis, differentiation & development (Venigalla and Turner, 2012). It is a potential regulator of inflammation and target to break the unwanted amyloid-beta toxicity cycle (Kheiri et al., 2018). Moreover, MAPK1 is involved in the phosphorylation of tau protein (Lee and Trojanowski, 2006). Drugs that target the p38 MAPK signalling pathway directly, might demonstrate a valuable strategy for the treatment of AD (Gao et al., 2021). Additionally, p38 MAPK is well-characterized oxidative stress-responsive pro-death signalling pathways, appears to participate in neuronal cell death in PD models (Karunakaran and Ravindranath, 2009). TP53, TNF, PTGS2 or COX, and IL6 the inflammatory cytokines are also a common target of *S. officinalis* identified by our study. TP53 or p53, acts as a tumour suppressor and has been called “the guardian of the genome”. It plays an important role in many areas of cellular physiology and biology, ranging from cellular development and differentiation to cell cycle arrest and apoptosis. A substantial increase in p53 level and activity seems to be a common feature of all neurodegenerative diseases (Szybinska and Lesniak, 2017). Hence, inhibition of p53 may present an ideal target to restore neuronal functions. p53 functionally interacts with cellular or viral factors and this interaction leads to mitochondrial deregulation and activation of the caspase pathway, which could promote cell dysfunction and death (Chang et al., 2012). Moreover the neuroprotective role of p53 in *Drosophila* model of tau-mediated neurodegeneration was also described (Merlo et al., 2014). PTGS2 or COX-2 (prostaglandins) play a regulatory role in inflammation. Cyclooxygenases are key mediators of inflammation. Role of COX-2 in neuroinflammation and neurodegeneration is well studied. Inhibition of COX-2 remains a valuable target as a potential neuroprotective treatment strategy aimed at halting or slowing the progression of disease (Teismann, 2012). TNF a cytokine and its type 1 receptor (TNFRI) are associated with cerebral neuroinflammation and regulate amyloid formation through secreted enzymes. Designing drugs to reduce the production of TNF α , such as P38 MAPK, and TNF receptor antagonist inhibitors might prove an effective treatment strategy to treat cognitive impairment and other neurodegenerative diseases (Gao et al., 2021). Our study also shows inflammation-related pathway, including TNF signaling, HIF-1 pathways as enriched, indicating that the anti-inflammatory action of *S. officinalis* could play a significant role in the treatment of NDD. The β -secretase (BACE1) cleaves amyloid precursor protein leading to the production of amyloid-beta in the brain and its aberrant activity is associated with AD. Therefore, it is a potential target for the reduction in A β aggregation via inhibition of BACE1 activity (Cole and Vassar, 2007; Hamidpour et al., 2014). Recently, a study reported that fucosterol shows non-competitive inhibition against β -

secretase which was further confirmed by network pharmacology and in silico analysis (Jung et al., 2016; Hannan et al., 2019).

Our network pharmacology study revealed that *S. officinalis* interacted with β -secretase, suggesting its effective anti-amyloidogenic activity. Furthermore, KEGG pathway analysis also demonstrates that signalling pathways involved in neuronal growth and survival such as FOXO signalling, cAMP signalling, and neurotrophin signalling are enriched suggesting the protective role of *S. officinalis* in NDDs.

5. Conclusion

Here we report for the first time that *S. officinalis* active ingredients interacted with NDD related proteins such as Akt1, BACE1, Caspase-3, MAPK1, TNF and IL-6, which are involved in several cellular and molecular pathways related to neuronal growth and survival. We further validated these proteins through molecular docking. Due to the complex pathogenesis of NDDs, we propose that the active ingredients of *S. officinalis* may act by modulating different pathways and interact with key NDD related targets thus demonstrating the multi-component, multi-target and multi-pathway function of Sage. Our study provides a molecular basis for the use of *S. officinalis* active ingredients as therapeutic components against NDDs requiring further investigations.

Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of interest:

The authors declare no conflict of interest.

Availability of data and material:

Data used in the current study, is available from the first author on reasonable request.

Code availability:

Not applicable.

Authors contribution:

Sheikh Sana Nazir collected, analysed and interpreted the data. Divya Goel helped in analysis of the data and writing of the manuscript. Dr.Divya Vohora planned, supervised and approved the final manuscript.

Ethics approval:

Not applicable.

Consent to participate:

Not applicable.

Consent for Publication

All authors have given consent for the publication.

Abbreviations

DL Druglikeness

BBB Blood Brain Barrier

TTD Therapeutic Target Database

GO Gene Ontology

BP Biological process

CC Cellular Component

MF Molecular Function

BC Betweenness Centrality

CC Closeness Centrality

ASPL Average Shortest Pathlength

KEGG Kyoto Encyclopedia of Genes and Genome

AD Alzheimer's Disease

PD Parkinson's Disease

HD Huntington's Disease

NDD Neurodegenerative disorders

PPI Protein Protein Interaction

B.E Binding Energy

References

1. Akhondzadeh, S. *et al.* (2003) 'Salvia officinalis extract in the treatment of patients with mild to moderate Alzheimer ' s disease : a double blind , randomized and placebo-controlled trial', *Journal of Clinical Pharmacy and Therapeutics*, pp. 53–59.
2. Ali, T. *et al.* (2018) 'Natural dietary supplementation of anthocyanins via PI3K/Akt/Nrf2/HO-1 pathways mitigate oxidative stress, neurodegeneration, and memory impairment in a mouse model of Alzheimer's disease', *Molecular neurobiology*, 55(7), pp. 6076–6093.
3. Bulck, M. Van *et al.* (2019) 'Novel approaches for the treatment of Alzheimer's and Parkinson's disease', *International Journal of Molecular Sciences*, 20(3). Available at: <https://doi.org/10.3390/ijms20030719>.
4. Chandra, N. and Padiadpu, J. (2013) 'Network approaches to drug discovery', *Informa healthcare*, pp. 7–20.
5. Chang, J.R. *et al.* (2012) 'Role of p53 in neurodegenerative diseases', *Neurodegenerative Diseases*, 9(2), pp. 68–80. Available at: <https://doi.org/10.1159/000329999>.
6. Chong, Z.Z. *et al.* (2012) 'A critical kinase cascade in neurological disorders: PI3K, Akt and mTOR', *Future Neurology*. NIH Public Access, pp. 733–748. Available at: <https://doi.org/10.2217/fnl.12.72>.
7. Christensen, K.B. *et al.* (2010) 'Activation of the nuclear receptor PPAR γ by metabolites isolated from sage (*Salvia officinalis* L.)', *Journal of Ethnopharmacology*, 132(1), pp. 127–133. Available at: <https://doi.org/10.1016/j.jep.2010.07.054>.
8. Cole, S.L. and Vassar, R. (2007) 'The Alzheimer's disease β -secretase enzyme, BACE1', *Molecular neurodegeneration*, 2(1), pp. 1–25.
9. D'Amelio, M., Sheng, M. and Cecconi, F. (2012) 'Caspase-3 in the central nervous system: beyond apoptosis', *Trends in Neurosciences*, 35(11), pp. 700–709. Available at: <https://doi.org/https://doi.org/10.1016/j.tins.2012.06.004>.
10. Davis, M.Y. *et al.* (2014) 'The Co-Occurrence of Alzheimer's Disease and Huntington's Disease: A Neuropathological Study of 15 Elderly Huntington's Disease Subjects', *Journal of Huntington's Disease*, 3(2), pp. 209–217. Available at: <https://doi.org/10.3233/JHD-140111>.
11. Fishedick, J.T. *et al.* (2013) 'Structure activity relationship of phenolic diterpenes from *Salvia officinalis* as activators of the nuclear factor E2-related factor 2 pathway', *Bioorganic and Medicinal Chemistry*, 21(9), pp. 2618–2622. Available at: <https://doi.org/10.1016/j.bmc.2013.02.019>.
12. Fischer, R. and Maier, O. (2015) 'Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF', *Oxidative medicine and cellular longevity*, 2015.
13. Gao, X. *et al.* (2021) 'A Network Pharmacology Approach to Estimate Potential Targets of the Active Ingredients of Epimedium for Alleviating Mild Cognitive Impairment and Treating Alzheimer's Disease', *Evidence-based Complementary and Alternative Medicine*, 2021. Available at: <https://doi.org/10.1155/2021/2302680>.
14. Ghorbani, A. and Esmaeilzadeh, M. (2017) 'Pharmacological properties of *Salvia officinalis* and its components', *Journal of Traditional and Complementary Medicine*, pp. 1–8. Available at: <https://doi.org/10.1016/j.jtcme.2016.12.014>.

15. Guven, M. *et al.* (2015) 'The Neuroprotective Effect of Coumaric Acid on Spinal Cord Ischemia/Reperfusion Injury in Rats', *Inflammation*, 38(5), pp. 1986–1995. Available at: <https://doi.org/10.1007/s10753-015-0179-0>.
16. Hamidpour, M. *et al.* (2014) 'Chemistry , Pharmacology , and Medicinal Property of Sage (Salvia) to Prevent and Cure Illnesses such as Obesity , Diabetes , Depression , Dementia , Lupus , Autism , Heart Disease , and Cancer', *Journal of Traditional and Complementary Medicine*, 4(2), pp. 82–88. Available at: <https://doi.org/10.4103/2225-4110.130373>.
17. Hannan, A. *et al.* (2019) 'Deciphering Molecular Mechanism of the Neuropharmacological Action of Fucosterol through Integrated System Pharmacology and In Silico Analysis', *Marine Drugs*, *mdpi*, pp. 1–21. Available at: <https://doi.org/10.3390/md17110639>.
18. Hopkins, A.L. (2008) 'Network pharmacology: the next paradigm in drug discovery', *Nature chemical biology*, 4(11), pp. 682–690. Available at: <https://doi.org/10.1038/nchembio.118>.
19. Jellinger, K.A. and Stadelmann, C. (2001) 'Problems of cell death in neurodegeneration and Alzheimer's disease', *Journal of Alzheimer's Disease*, 3(1), pp. 31–40.
20. Jung, H.A. *et al.* (2016) 'Kinetics and molecular docking studies of fucosterol and fucoxanthin, BACE1 inhibitors from brown algae Undaria pinnatifida and Ecklonia stolonifera', *Food and Chemical Toxicology*, 89, pp. 104–111.
21. Kang, S., Zhang, J. and Yuan, Y. (2018) 'Abietic acid attenuates IL-1 β -induced inflammation in human osteoarthritis chondrocytes', *International Immunopharmacology*, 64(April), pp. 110–115. Available at: <https://doi.org/10.1016/j.intimp.2018.07.014>.
22. Karunakaran, S. and Ravindranath, V. (2009) 'Activation of p38 MAPK in the substantia nigra leads to nuclear translocation of NF- κ B in MPTP-treated mice: implication in Parkinson's disease', *Journal of neurochemistry*, 109(6), pp. 1791–1799.
23. Kashyap, D. *et al.* (2017) 'Mechanistic insight into carnosol-mediated pharmacological effects: Recent trends and advancements', *Life Sciences*, 169, pp. 27–36. Available at: <https://doi.org/10.1016/j.lfs.2016.11.013>.
24. Ke, Z. *et al.* (2016) 'Drug discovery of neurodegenerative disease through network pharmacology approach in herbs', *Biomedicine & Pharmacotherapy*, 78, pp. 272–279. Available at: <https://doi.org/10.1016/j.biopha.2016.01.021>.
25. Kennedy, D.O. *et al.* (2006) 'Effects of Cholinesterase Inhibiting Sage (Salvia officinalis) on Mood , Anxiety and Performance on a Psychological Stressor Battery', *Neuropsychopharmacology (Nature Publishing Group)*, pp. 845–852. Available at: <https://doi.org/10.1038/sj.npp.1300907>.
26. Kheiri, G. *et al.* (2018) 'Role of p38/MAPKs in Alzheimer's disease: implications for amyloid beta toxicity targeted therapy', *Reviews in the Neurosciences*, 30(1), pp. 9–30.
27. Lee, M.J. *et al.* (2011) 'The effects of hempseed meal intake and linoleic acid on drosophila models of neurodegenerative diseases and hypercholesterolemia', *Molecules and Cells*, 31(4), pp. 337–342. Available at: <https://doi.org/10.1007/s10059-011-0042-6>.

28. Lee, V.M.-Y. and Trojanowski, J.Q. (2006) 'Tau focused drug discovery for Alzheimer's disease and related Neurodegenerative Tauopathies', in *Alzheimer: 100 Years and Beyond*. Springer, pp. 333–336.
29. Liu, X. *et al.* (2019) 'Abietic acid suppresses non-small-cell lung cancer cell growth via blocking IKK β /NF- κ B signaling', *OncoTargets and Therapy*, 12, pp. 4825–4837. Available at: <https://doi.org/10.2147/OTT.S199161>.
30. Lopresti, A.L. (2017) 'Salvia (Sage): A Review of its Potential Cognitive-Enhancing and Protective Effects', *Drugs in R and D*, 17(1), pp. 53–64. Available at: <https://doi.org/10.1007/s40268-016-0157-5>.
31. Maiese, K. (2015) 'FoxO Proteins in the Nervous System'. Available at: <https://doi.org/10.1155/2015/569392>.
32. Merlo, P. *et al.* (2014) 'P53 prevents neurodegeneration by regulating synaptic genes', *Proceedings of the National Academy of Sciences of the United States of America*, 111(50), pp. 18055–18060. Available at: <https://doi.org/10.1073/pnas.1419083111>.
33. Oh, K.K. *et al.* (2021) 'A network pharmacology study on main chemical compounds from *Hibiscus cannabinus* L. leaves', *RSC Advances*, 11(19), pp. 11062–11082. Available at: <https://doi.org/10.1039/d0ra10932k>.
34. Panagiotou, G. and Taboureau, O. (2012) 'The impact of network biology in pharmacology and toxicology', *SAR and QSAR in Environmental Research*, 23(3–4), pp. 221–235. Available at: <https://doi.org/10.1080/1062936X.2012.657237>.
35. Rai, S.N. *et al.* (2019a) 'The role of PI3K/Akt and ERK in neurodegenerative disorders', *Neurotoxicity research*, 35(3), pp. 775–795.
36. Rai, S.N. *et al.* (2019b) 'The Role of PI3K/Akt and ERK in Neurodegenerative Disorders', *Neurotoxicity Research*. Springer New York LLC, pp. 775–795. Available at: <https://doi.org/10.1007/s12640-019-0003-y>.
37. Ross, C.A. and Tabrizi, S.J. (2011) 'Huntington's disease: From molecular pathogenesis to clinical treatment', *The Lancet Neurology*, 10(1), pp. 83–98. Available at: [https://doi.org/10.1016/S1474-4422\(10\)70245-3](https://doi.org/10.1016/S1474-4422(10)70245-3).
38. Spittau, B. *et al.* (2012) 'IL6 protects MN9D cells and midbrain dopaminergic neurons from MPP⁺-induced neurodegeneration', *Neuromolecular medicine*, 14(4), pp. 317–327.
39. Sytar, O. *et al.* (2018) 'Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants', *Saudi Journal of Biological Sciences*, 25(4), pp. 631–641. Available at: <https://doi.org/10.1016/j.sjbs.2016.01.036>.
40. Szybinska, A. and Lesniak, W. (2017) 'P53 dysfunction in neurodegenerative diseases - The cause or effect of pathological changes?', *Aging and Disease*, 8(4), pp. 506–518. Available at: <https://doi.org/10.14336/AD.2016.1120>.
41. Tan, E.K. *et al.* (2015) 'Neurodegeneration: Etiologies and New Therapies', *BioMed Research International*, p. 2. Available at: <https://doi.org/http://dx.doi.org/10.1155/2015/272630>.

42. Teismann, P. (2012) 'COX-2 in the neurodegenerative process of Parkinson's disease', *BioFactors*. Wiley-Blackwell, pp. 395–397. Available at: <https://doi.org/10.1002/biof.1035>.
43. Tian, S. *et al.* (2015) 'SC', *Advanced Drug Delivery Reviews* [Preprint]. Available at: <https://doi.org/10.1016/j.addr.2015.01.009>.
44. Trevizan, L.N.F. *et al.* (2016) 'Anti-inflammatory, antioxidant and anti-Mycobacterium tuberculosis activity of viridiflorol: The major constituent of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk.', *Journal of Ethnopharmacology*, 192, pp. 510–515. Available at: <https://doi.org/10.1016/j.jep.2016.08.053>.
45. Vauzour, D., Corona, G. and Spencer, J.P.E. (2010) 'Caffeic acid, tyrosol and p-coumaric acid are potent inhibitors of 5-S-cysteinyl-dopamine induced neurotoxicity', *Archives of Biochemistry and Biophysics*, 501(1), pp. 106–111. Available at: <https://doi.org/10.1016/j.abb.2010.03.016>.
46. Venigalla, R.K.C. and Turner, M. (2012) 'RNA-binding proteins as a point of convergence of the PI3K and p38 MAPK pathways', *Frontiers in immunology*, 3, p. 398.
47. Wake, G. *et al.* (2000) 'CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory', *Journal of ethnopharmacology*, 69(2), pp. 105–114.
48. Yoon, J.H. *et al.* (2014) 'P -coumaric acid and ursolic acid from corni fructus attenuated β -amyloid25-35-induced toxicity through regulation of the NF- κ B signaling pathway in PC12 cells', *Journal of Agricultural and Food Chemistry*, 62(21), pp. 4911–4916. Available at: <https://doi.org/10.1021/jf501314g>.
49. Zhang, A. *et al.* (2012) 'Predicting new molecular targets for rhein using network pharmacology', *BMC Systems Biology*.
50. Zhou, W. *et al.* (2016) 'Systems Pharmacology in Small Molecular Drug Discovery', *International Journal of Molecular Sciences*, pp. 1–16. Available at: <https://doi.org/10.3390/ijms17020246>.

Figures

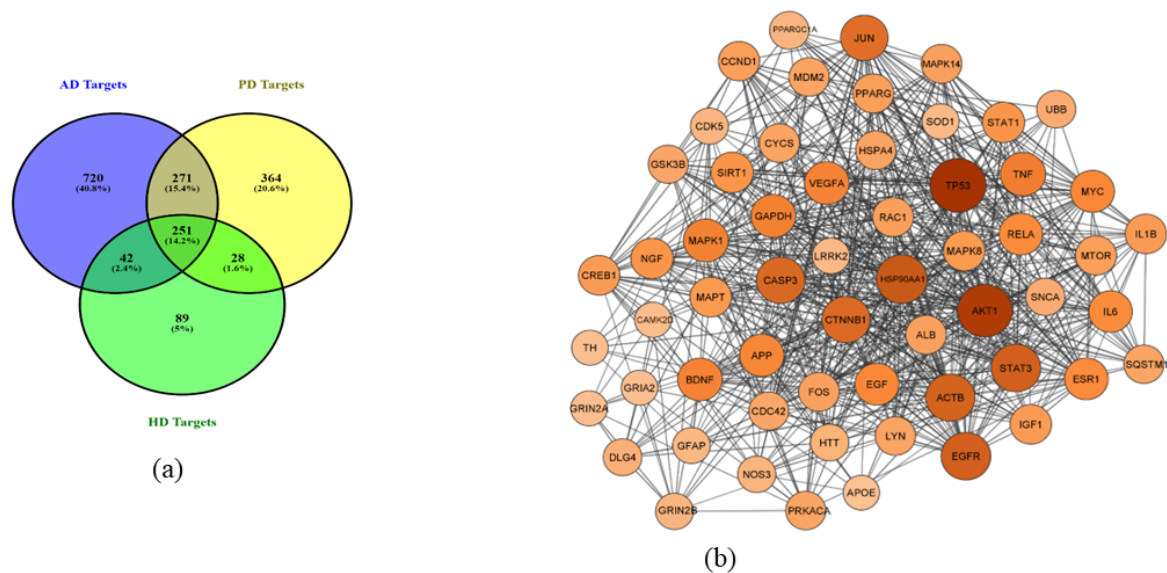


Figure 1. PPI network of NDD related targets (a) Venn diagram representing common targets between AD, PD and HD (b) NDD target network.

Brown round nodes represent NDD-related targets, and size of nodes is directly proportional to degree centrality by topological analysis.

Colour of nodes are displayed in a gradient manner, darker the node, greater the degree and more important it is in the network.

Figure 1

See image above for figure legend.

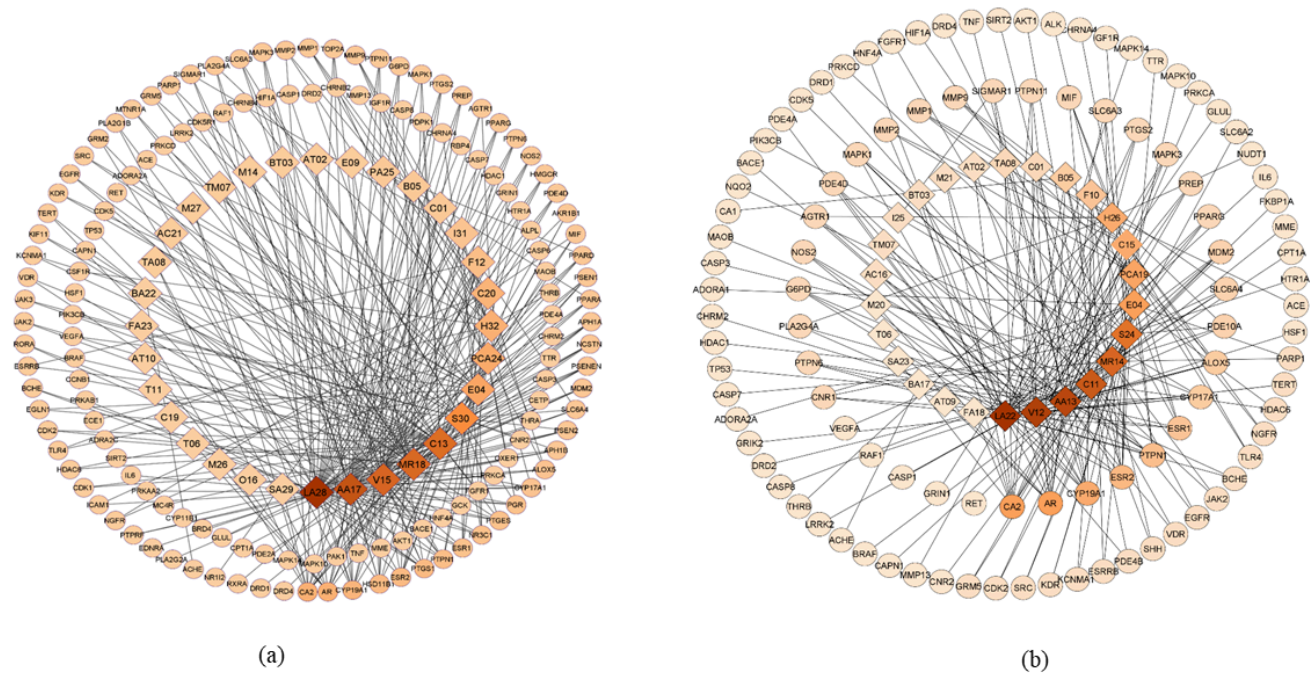


Figure 2: *S.officinalis* active ingredients- candidate target network of NDD

(a) Active ingredients- candidate targets network of AD (b) Active ingredients- candidate targets network of PD (c) Active ingredients- candidate targets network of HD

Figure 2

See image above for figure legend.

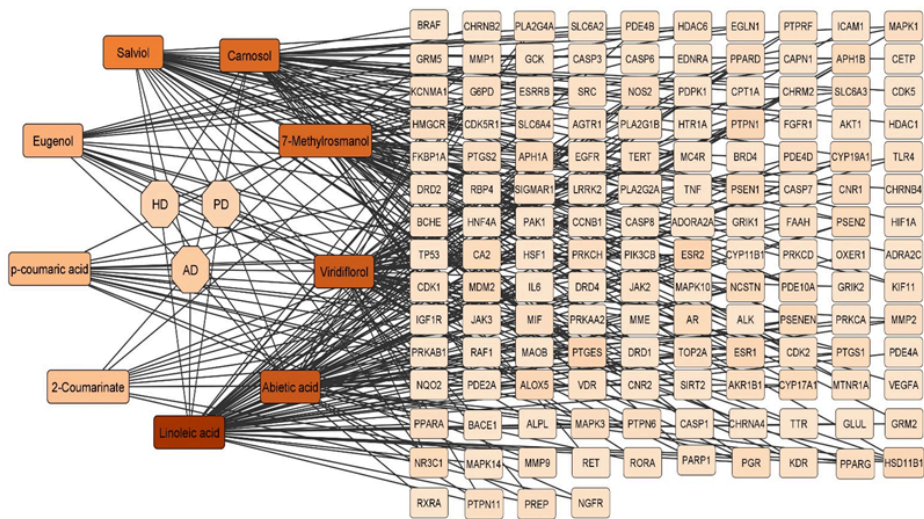


Figure 3. Integrated network of *S. officinalis* key active ingredients and their NDD related targets. i.e, nine key active ingredients of *S. officinalis* and their AD related targets. Six key active ingredients of *S.officinalis* and their PD, HD related targets. Six rectangular, darker coloured nodes represent key active ingredients that are common to AD,PD and HD while remaining three active ingredients are AD-related and lighter nodes represent NDD related targets.

Figure 3

See image above for figure legend.

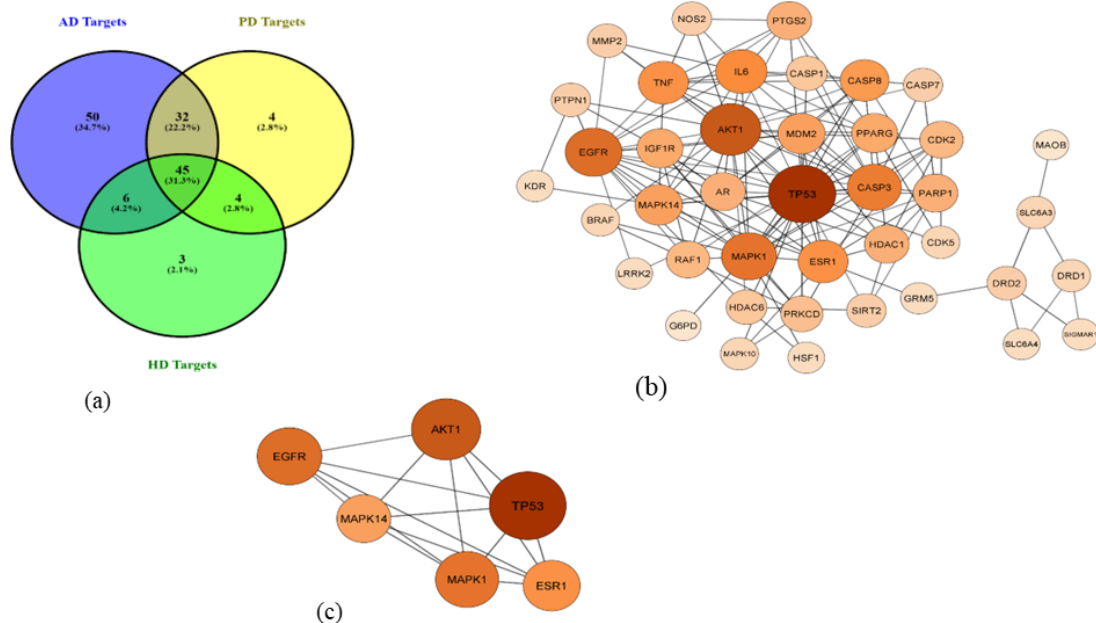


Figure 4. PPI network of key active ingredients -related targets against NDDs.

(a) Venn diagram showing intersection or common AD, PD and HD related targets of *S. officinalis* (b) PPI network of 41 NDD-related targets. Nodes represent target proteins, and edges represent interactions among targets. Larger and darker the node more important it is in the network (c) Key NDD-related targets of *S. officinalis* active ingredients.

Figure 4

See image above for figure legend.

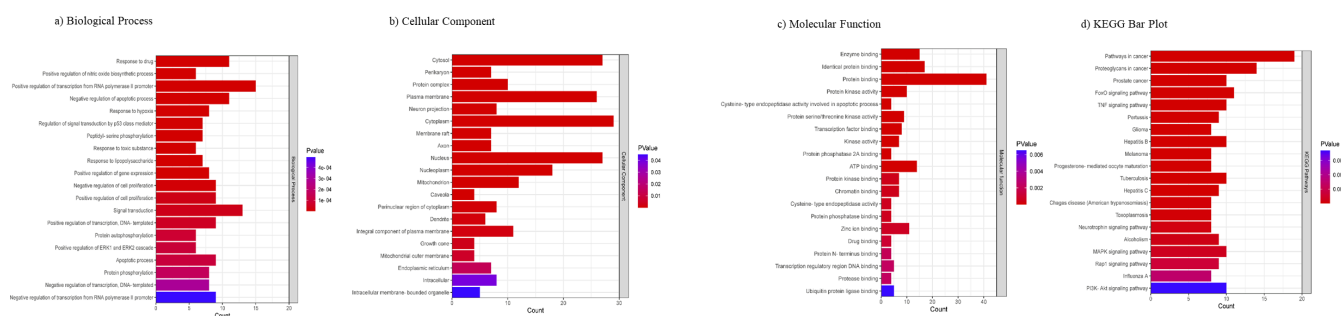


Figure 5. GO enrichment analysis and KEGG enrichment analysis of 45-NDD-related target genes.

Figure 5

See image above for figure legend.

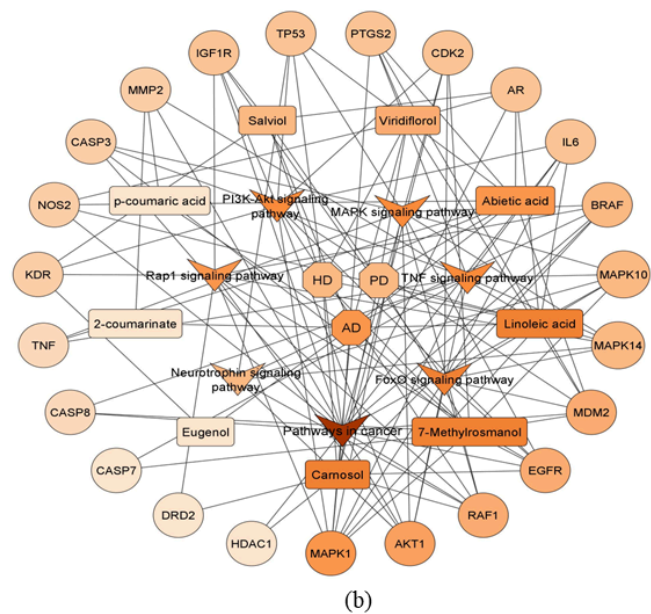
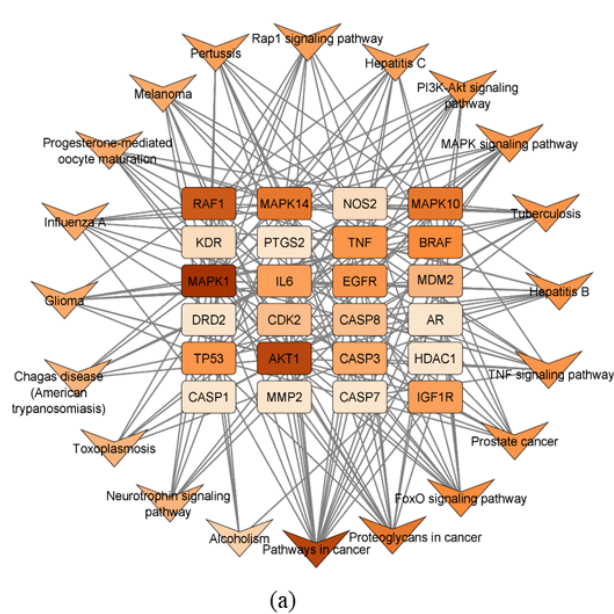
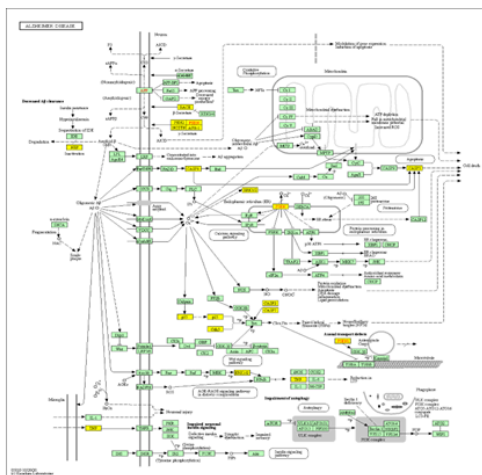


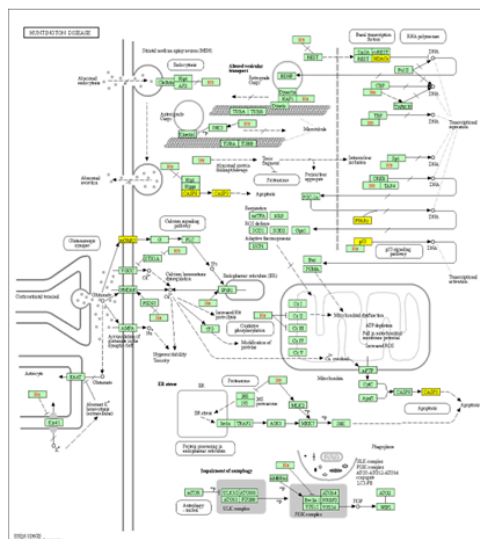
Figure 6. NDD target-pathway network. (a) Network of 20 pathways and their shared targets. (b) Integral network of *S. officinalis* active ingredients, AD, PD, HD related targets and their NDD related pathways.

Figure 6

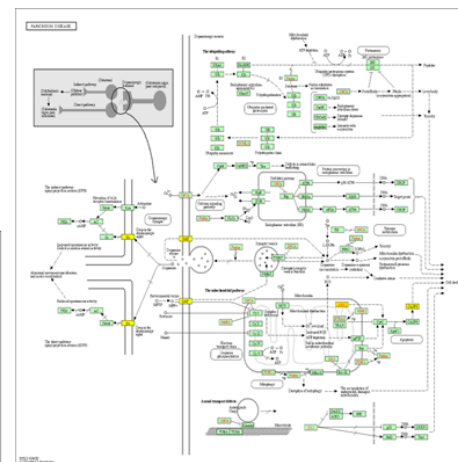
See image above for figure legend.



(a) Alzheimer's Disease pathway



(c) Huntington Disease pathway



(b) Parkinson's Disease pathway

Figure 7. Pathways of NDDs in KEGG pathway analysis

Figure 7

See image above for figure legend.