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Research article

Evaluation of Rhus Tox, a homeopathic formulation for treatment of Parkinson's and Alzheimer's disease: a network pharmacology and *ex-vivo* approach

G Mahalakshmi Priya[1], D Sujatha*[1], P Mayuri[2], T Charmila[3]

[1] Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India
[2] Department of Physical Sciences, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India
[3] DBT-Builder Project (Level – 1), Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India

Corresponding author: D. Sujatha, ✉ drsujathasai@gmail.com, **Orcid Id**: https://orcid.org/0000-0003-1374-7077

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India

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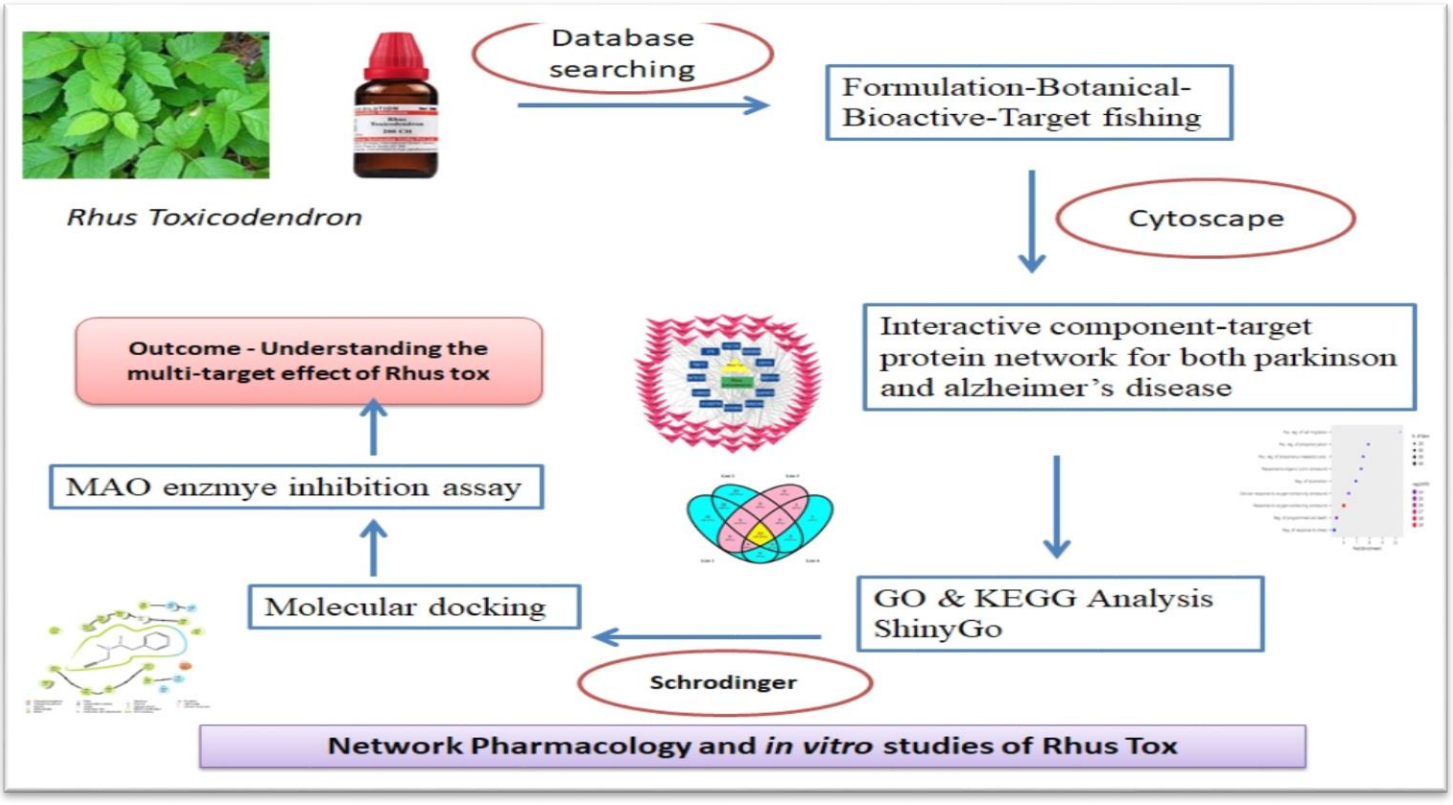
ABSTRACT

Rhus toxicodendron, commonly known as Rhus tox, is a potent homeopathic medication widely used in the treatment of rheumatoid arthritis and is claimed to have potential benefits for neurodegenerative disorders. Given the ability of homeopathic medications to address the root causes of diseases, our study aimed to evaluate Rhus tox for Parkinson's Disease (PD) and Alzheimer's Disease (AD) using *in-silico*, *ex-vivo*, and network pharmacology approaches to obtain comprehensive information on its multi-component and multi-target effects.



Phytoconstituents of Rhus tox were collected from PubMed and screened for ADMET properties. Targets for these constituents were predicted using the Binding Database and DisGeNET, resulting in 61 targets for Parkinsonism and 85 targets for alzhiemer's disease. Protein-protein interactions (PPIs) were analysed using the STRING database. A Venn diagram was constructed for all these targets, and 5 out of 13 common targets were selected for docking studies. From these analyses, kaempferol and quercetin were identified as promising neuroprotective agents with good blood-brain barrier (BBB) permeation. The docking studies validated these predictions as most of the constituents showed good binding affinity with EGFR, SNCA and MAO-B. It was further validated using MAO inhibition assay, in which Quercetin was found to be highly efficacious, with an IC_{50} value of 32.69 µg/mL among the other flavonoids studied for MAO inhibition assay. The Rhus Tox also showed MAO inhibition assay similar to the standard seligiline.

To conclude, we can propose that Rhus Tox primarily acts on the dopaminergic pathway, serotonergic pathway, and EGFR signaling pathway. Further *in vitro and in vivo* studies are needed to confirm the mechanism action and efficacy of Rhus Tox for treatment of neurodegenerative diseases.

Keywords: Alzheimer's disease; Monoamine oxidase; Network pharmacology; Parkinsonism; Rhus Tox.

INTRODUCTION

Rhus Tox is a homeopathic formulation extracted from the plant *Rhus toxicodendron* (also called Toxicodendron pubescens and Toxicodendron radicans) that belongs to Anacardiaceae family [1]. It has been reported that, Rhus Tox is widely used to treat cramps, strains, sprains, restless leg syndrome, flu, viral infections and arthritis [2]. Though, existing literature claims Rhus Tox prevents herpes virus outbreak, inflammations and back pains etc., there is a need to establish evidence for the activities mentioned in the aforesaid literatures [3-6]. Noteworthy, homeopathic Rhus Tox remedies for neurodegenerative diseases were also not discussed earlier. An extensive research study has to be carried regarding the mechanism of effectiveness of Rhus Tox in treating specific conditions, and its side effects. This would help to scientifically validate the therapeutic use of Rhus Tox. Hence, in this work we would like to evaluate the homeopathic formulation Rhus Tox against Parkinson's disease (PD) and Alzheimer's disease (AD) using network pharmacology approach and ex-vivo studies.

Neurodegenerative diseases are conditions more prevalent in geriatric population, and characterized by progressive neuronal death leading to a gradual decline in brain functions. These diseases are often associated with a wide range of clinical symptoms, such as cognitive decline (Alzheimer's disease), loss of locomotors functions (Parkinson's disease) etc. In brief, 6.5 million Americans are living with Alzheimer's Disease (AD) in which 73% of people are of age 75 and above. It is estimated, this number could grow to 13.8 million by 2060. Even the deaths have doubled between 2000 and 2019 [7, 8]. Likewise, Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. More than 10 million people are living with PD in the whole world. In the past 25 years the incidence of PD has been doubled. In 2019 5.8 million disabilities are caused due to PD and 3, 29,000 deaths [9, 10]. Currently, there is no treatment to completely cure the root cause of these neurodegenerative disorders. The available treatments just rectify the symptoms. So there is a need of research in this area to systematically elucidate the mechanism of neurodegenerative diseases and search for safe and effective agents against AD and PD.

In search for new therapeutic agents, the technology advancements have really changed the picture of drug discovery process. We can make use of all the existing databases, software's to screen the drugs and predict the targets with the help of bioinformatics, artificial intelligence, Computer assisted drug designing etc [11]. This can reduce the harm caused to animals and also we can skip unethical practices with the improved efficiency and increased throughput of the models. So many alternative models to animal testing's have emerged such as *in-silico*, *in-vitro*, *in-chemico*, *ex-vivo* techniques [12]. Recently, *in-silico* approaches have been attracting considerable interest because of their potential to accelerate drug discovery in terms of time, labor, and costs [13]. On the other hand, Network Pharmacology helps us to understand complex drug actions and interactions with multiple targets. Network Pharmacology is combination of System Biology, Bioinformatics, network science, *in-silico* pharmacology and other related disciplines. The drug-target network can help us to understand the mechanisms of action of approved and experimental drugs. Molecular docking studies are performed as validation for network pharmacology [14]. Hence, in the present study, we choose *in-silico*, *ex-vivo* and Network Pharmacology approach to evaluate the effectiveness of Rhus Tox, and homeopathic formulation against PD & AD.

MATERIALS AND METHODS

All the chemicals used in the study were of analytical grade and obtained from SRL chemicals, Bombay, India.

Construction of Network

Acquisition of the Bio Actives

The botanicals and the phytochemical (bioactives) constituents reported in the Rhus Tox was identified by search using NCBI PubMed database (https://pubmed.ncbi.nlm.nih.gov/). The 3D-structures and canonical smiles of the phytoconstituents were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The 3D-structures were then saved in standard SDF format. ADMETlab2.0

(https://admetmesh.scbdd.com/) was used to predict the physicochemical properties, medicinal chemistry details, various pharmacokinetic parameters, toxicological predictions of the collected phytoconstituents of *Rhus toxicodendron*. The selected parameters in the current study to measure the drug likeliness (DL) index of the phytoconstituents were Blood brain barrier (BBB) permeation, HIA (Human intestinal absorption), carcinogenicity, mutagenicity and TPSA (Topological polar surface area)

Target Fishing

The process of identifying possible protein targets for a molecule based on their structure is called 'Target fishing'. In the present study, the protein targets of phytoconstituents were searched using the binding data base and DisGeNET. Totally 61 potential targets for PD and 84 potential targets for AD were identified.

Construction of Network

Cytoscape 3.9.1 (https://cytoscape.org/), an open-source bioinformatics software platform was used for visualizing the complex molecular interaction networks [15]. Here, in order to construct a 'Formulation-Botanical- Bioactive-Target' network the data was imported in three different excel sheets and three individual networks were constructed. The Individual networks included Formulation – Botanicals Network, Botanicals – Bioactive Network and Bioactives – Targets Network. At the end all the three networks were merged then to form the overall network explaining the interaction of botanicals, bioactives with the targets important in PD and AD.

The constructed network was analysed for the network's topological features like degree, betweenness-centrality, closeness-centrality etc., it depicts the possible complex molecular mechanisms underlying anti-neurodegenerative activity among the potential targets ranked by degree, betweenness and closeness.

Protein-protein Interactions

The STRING (Search Tool for the Retrieval of Interacting Genes) (https://stringdb.org/) is a database of known and predicted protein-protein interactions. The obtained "potential therapeutic targets of Rhus Tox for Parkinson's disorder and Alzheimer's disease" were imported into STRING database for generating the Protein-Protein Interaction network with a high confidence level (0.7) and the species defined as human [16]. More the degree value means more connection. Among them, top 20 potential therapeutic targets with highest degree selected for further study.

GO and KEGG Pathway Enrichment Analysis

ShinyGo is the graphical enrichment tool for gene analysis (http://bioinformatics.sdstate.edu/go/) which was employed for Gene Ontology (GO) functional analysis and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO Functional analysis was performed for enrichment analysis of the gene sets in the aspects like biological process analysis, molecular function analysis, and cellular component analysis. In contrast to GO analysis, KEGG pathway enrichment analysis have an advantage of in depth information on interactions of genes (or) gene products in different physiological pathways, but a limitation of less comprehensive coverage of genes. The list of target genes were imported into ShinyGO. The top 20 enriched pathways with least P-value (or) highest -log10 (P value) were selected and the bubble charts were saved.

$$\text{Rich factor} = \frac{\text{Number of input genes annotated in a pathway}}{\text{Total number of genes annotated}}$$

Venn Diagram

To select the targets for docking Venn diagram was constructed using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to get common targets among 61 targets of PD, 84 targets of AD, among them top 20 targets each of PD and AD were located from string database.

Molecular Docking

In the present study, to assess the binding affinities of specific phytochemicals with specific target proteins Glide-standard precision module of Schrodinger suite-Maestro 13.0 was used. We have selected the best five targets obtained from the Venn diagram to carry on the molecular docking studies. Receptor grids were generated with default setting and the grid generation panelled of Schrodinger (maestro 13.0) which permits specifying the location and size of the protein's active site for ligand docking. A cubic box with a boundary box (14 A0 ×14 A0 × 14 A0) was specified for the receptors using co-crystallized ligand molecule as the centroid. Each hub potential target was docked with all selected phytoconstituents of RT and the known standard drug chosen for that particular protein. The obtained results of the docking were exported to an excel sheet which include many parameters like – docking score, interacting residues, etc. The four important criteria followed in the analysis of the docking results were docking score, Docking pose, Number of interactions and the types of interactions.

MAO-B Inhibition Assay by Using Goat Liver Homogenate

This assay was performed for Quercetin, Rutin, Gallic acid, Tannic acid, Selegiline (standard), and Rhus Tox (homeopathic formulation). Quercetin, rutin, tannic acid, Gallic acid were the bioactives reported to be present in the Rhus Tox. Goat liver was obtained from the slaughter house and stored in ice cold phosphate buffered saline (PBS) and homogenized in PBS. The obtained homogenate was subjected to centrifugation at 1000g at 4°C for 15 min. Supernatant was collected, stored at -70°C, protein estimation was performed using Erba Total Protein kit and that supernatant was used as a source of MAO enzyme.

DNPH (2, 4-dinitrophenylhydrazine) spectrometry is based on the oxidation of Benzylamine to aldehydes which is catalyzed by MAO-B. These aldehydes were derivatized with DNPH, which were converted to quinones by addition of sodium hydroxide.

Then the DNPH derivatives with large conjugated structures were directly measured spectrometrically at 465 nm (Guili Huang et al., 2016). To 200µL of MAO protein liver homogenate, 1.1mL of ice cold PBS and test compounds (10 -160 µg/ml) were incubated for 20 min at 37°C. To the above mixture 200 µL of 0.016 M Benzylamine in buffer was added and incubated for another 1 hour. The reaction was initiated by addition of 400µL of 2 M DNPH in 1 M HCl and incubated for 40 min at room temperature. The reaction was stopped by adding 2 mL of 1.25 M NaOH containing 5g/L of Triton-X-100 and incubated for 30 min. The absorbance was read at 465nm and the % inhibition of MAO-B was calculated using

$$\%\text{Inh} = \frac{Abs.\,of\ control - Abs.\,of\ test}{Abs.\,of\ control} \times 100$$

RESULTS AND DISCUSSION

Acquisition of Bio Actives

In this study, we investigated the active constituents of *Rhus toxicodendron* by mining the PubMed database. Twenty-two active constituents were identified and screened for their ADMET properties using ADMETlab2.0, with a specific focus on blood-brain barrier (BBB) penetration. Subsequently, twelve constituents exhibiting favourable BBB penetration were selected for further analysis to predict their targets and construct a network. The exclusive active components of Rhus Tox were listed in Table 1.

Table 1: ADMET screening of active constituents of *Rhus toxicodendron*

Active Constituent	Pub Chem IDs	BBB Penetration	HIA	TPSA (0-140)	DL Index (>0.18)	Carcinogenecity	Mutagenecity
Urushiol	44144477	Yes	Yes	40.46	0.17	No	No
Fisetin	5281614	Yes	Yes	111.13	0.55	No	Yes
Gallo tannic acid	16129778	Yes	No	777.98	0.17	No	No
Kaempferol	5280863	Yes	Yes	111.13	0.55	No	Yes
Quercetin	5280343	Yes	Yes	131.36	0.55	No	Yes
Urushiol	5478167	Yes	Yes	131.36	0.55	No	No
Myricetin	5281672	Yes	Yes	151.59	0.55	No	No
Cardanol	11266523	Yes	Yes	20.23	0.55	No	No
Cardol	76617	Yes	Yes	40.46	0.55	No	No
3-n- pentadecylcatechol	152135	Yes	Yes	40.46	0.55	No	No
3- (tridecafluoroundecyl)-catechol	125480	No	Yes	40.46	0.55	No	No
3- (nonafluoropentadecyl)-catechol	125481	Yes	Yes	40.46	0.55	No	No
Gallic acid	370	Yes	Yes	97.99	0.56	No	No
Rhamnose	25310	No	No	90.15	0.55	No	No
Rutin	5280805	Yes	No	269.43	0.17	No	Yes

BBB – Blood brain Barrier; Dl – Drug likeliness; HIA – Human gastro intestinal absorption; TPSA- Topological surface area

Note: Data of all the 22 phytoconstituents was not given as other phytoconstituents were unable to produce any results in ADMET Lab 2.0 or SwissADME

Prediction of Potential Targets

In our investigation, we employed the Binding Database (BDB) and DisGeNET to predict potential targets associated with Parkinson's disease (PD) and Alzheimer's disease (AD) for the 12 selected constituents of Rhus toxicodendron. A total of 629 targets of Rhus Tox, 219 targets of PD and 271 targets of AD were obtained. After the targets of each group were intersected with the targets of PD, and AD respectively, we identified that Rhus tox is acting on 61 targets related to PD and 85 targets related to AD (Supplementary data).

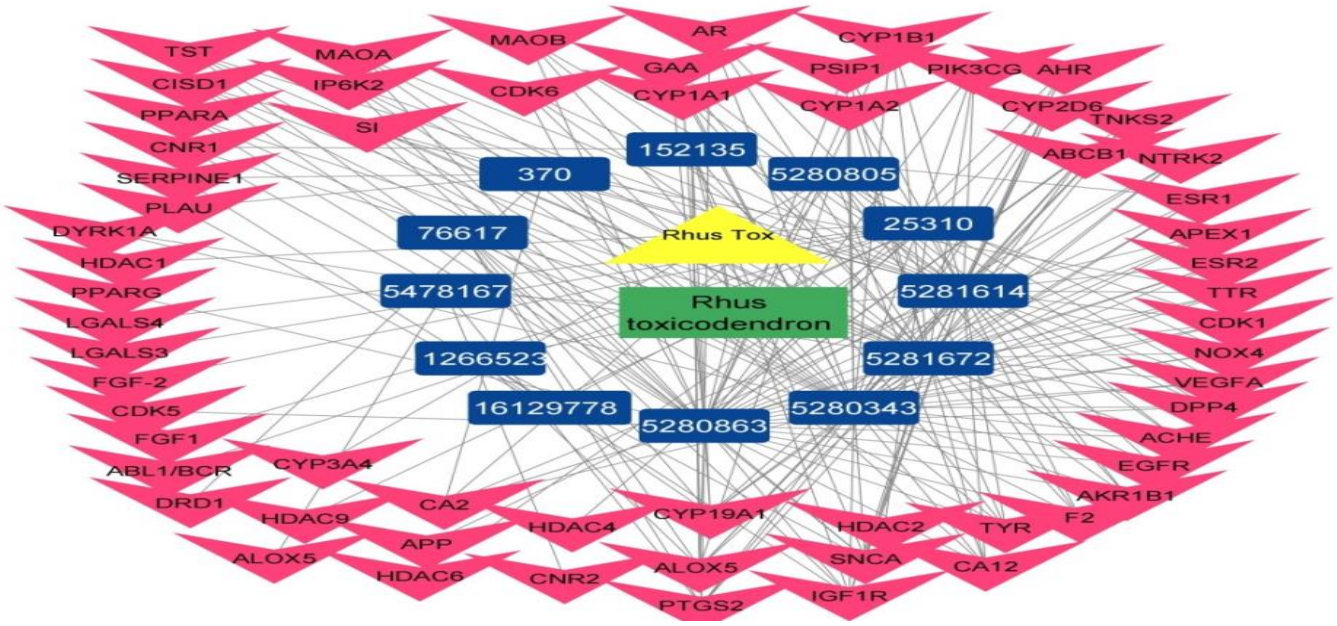
Interactive Network - Construction and Evaluation

After the potential targets were obtained, the mode of association between active components with potential targets was developed as interactive networks. Those networks of "Formulation-Botanical-Bioactives-Targets (For-Bot-Bio-Tar)" for both PD and AD were developed utilizing the Cytoscape software and shown in Figure 1 & Figure 2.

Protein-protein Interactions

Potential targets have many interactions and each target is involved in numerous functional pathways. At first, we established the PPI networks to explore the interactions between potential targets using String database. Figure 3 represents PPI network of potential targets of *Rhus toxicodendron* in treatment of Parkinson's disease (61). When the 61 predicted targets of PD from the binding database and DisGeNET were uploaded in String database, a network is displayed which shows the interactions between target proteins. The proteins which are highlighted in yellow colour are top 20 proteins sorted by their degree ranging from 30 to 6. The PPI network of Rhus Tox with the PD disease targets consists of 82 nodes and 539 edges. Similarly, figure 4 represents PPI network of potential targets of *Rhus toxicodendron* in treatment of Alzheimer's disease (85) and the top 20 are highlighted in green colour. The proteins which are highlighted in green colour were top 20 proteins sorted by their degree ranging from 47 to 9. Whereas, the PPI network for Rhus Tox with the AD disease targets consisted of 57 nodes and 305 edges.

Figure 1: Formulation-Botanicals-Bioactives-Targets network of Rhus Tox an homeopathic formulation for Parkinson's Disease



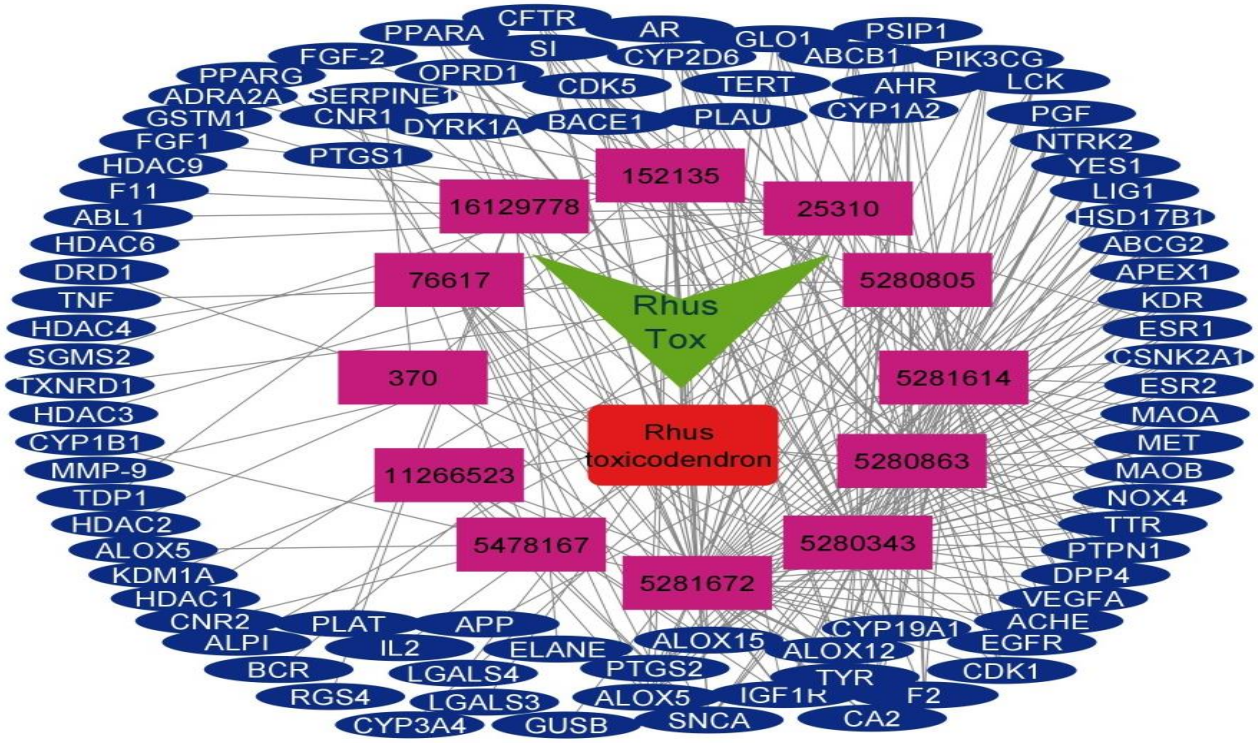
Rhus Tox, an homeopathic formulation

Rhus toxicodendron, a botanical present in Rhus Tox

PubChem IDs of active constituents present in *Rhus toxicodendron*

Targets on which the active constituents act

Figure2: Formulation-Botanicals-Bioactives-Targets network of Rhus Tox a homeopathic formulation for Alzheimer's disease



Rhus Tox, an homeopathic formulation

Rhus toxicodendron, a botanical present in Rhus Tox

PubChem IDs of active constituents present in *Rhus toxicodendron*

Targets on which the active constituents act

Figure 3: PPI network of potential targets of *Rhus toxicodendron* in treatment of Parkinson's disease [61] and the top 20 are highlighted in yellow colour

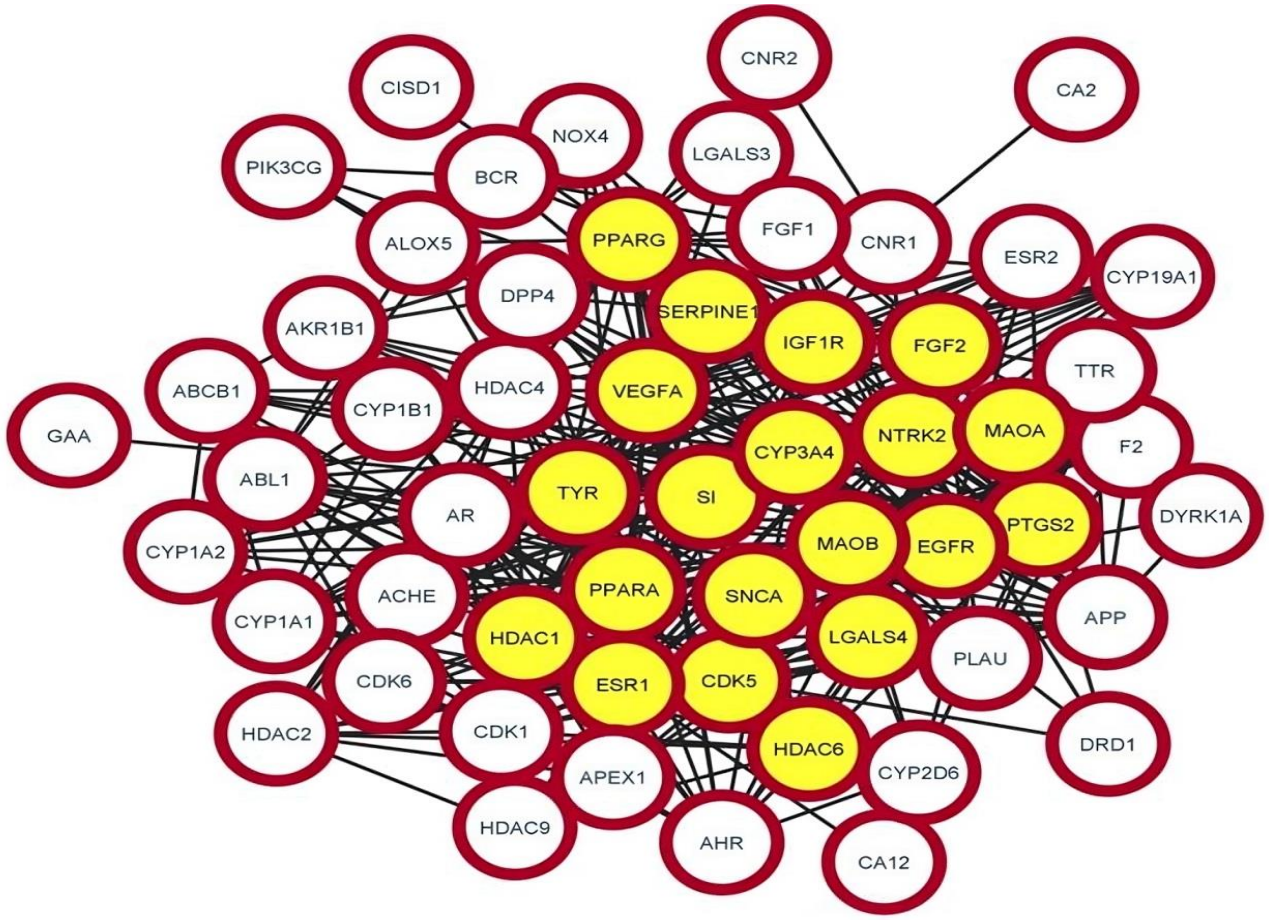
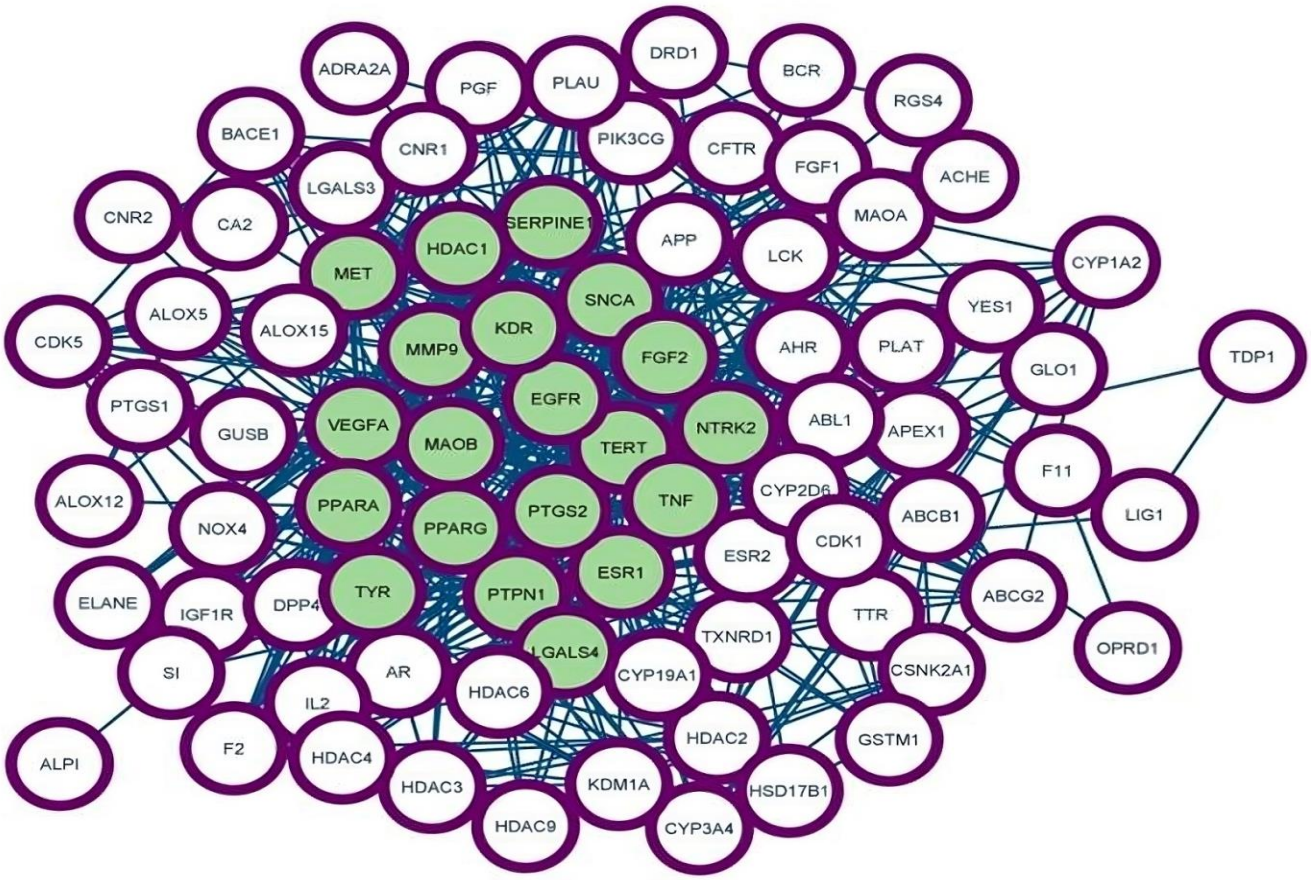


Figure 4: PPI network of potential targets of *Rhus toxicodendron* in treatment of Alzheimer's disease [85] and the top 20 are highlighted in green colour



Among the 12 constituents, 4 active components with degree ≥20 and 2 active components of Rhus Tox with degree ≥15 were identified to be the key active components in PD and AD respectively; the results were listed in Table 2.

Table 2: Bioactives with Node degrees ≥15 from Cytoscape network analysis

Bioactives with Node degrees ≥15 for Parkinson's Disease	Bioactives with Node degrees ≥15 for Alzheimer's Disease
Kaempferol (43)	Myricetin (52)
Quercetin (42)	Quercetin (51)
Myricetin (42)	Kaempferol (49)
Fisetin (40)	Fisetin (46)
	Rutin (17)
	L-Rhamnose (15)

Protein-protein Interactions

Potential targets have many interactions and each target is involved in numerous functional pathways. At first, we established the PPI networks to explore the interactions between potential targets using String database. Figure 3 represents PPI network of potential targets of *Rhus toxicodendron* in treatment of Parkinson's disease (61). When the 61 predicted targets of PD from the binding database and DisGeNET were uploaded in String database, a network is displayed which shows the interactions between target proteins. The proteins which are highlighted in yellow colour are top 20 proteins sorted by their degree ranging from 30 to 6. The PPI network of Rhus Tox with the PD disease targets consists of 82 nodes and 539 edges. Similarly, figure 4 represents PPI network of potential targets of *Rhus toxicodendron* in treatment of Alzheimer's disease (85) and the top 20 are highlighted in green colour. The proteins which are highlighted in green colour were top 20 proteins sorted by their degree ranging from 47 to 9. Whereas, the PPI network for Rhus Tox with the AD disease targets consisted of 57 nodes and 305 edges.

GO and KEGG Analysis

To perform GO and KEGG analysis 85 genes of AD and 61 genes of PD were entered in the ShinyGO 0.76.2 and species was specified as Homo sapiens. For GO studies results were saved as bubble charts for top 10 pathways and for KEGG important pathways related to the current diseases were selected. Figure 5, 6 and 7 represents GO for molecular function terms, cellular component terms and biological process terms.

GO items with counts greater than 20 of Rhus Tox were shown in figure 5, 6 and 7, with a maximum of 10 items included in each analysis. The biological process (BP) results showed that cellular response to oxygen-containing compound had the highest count; the molecular function (MF) results were mainly focused on signalling receptor binding and the cellular components (CC) results mainly related to neuron projection. Top 20 KEGG pathways of Rhus Tox were sorted by *P* value and shown in figure 8, and the results were mainly concerned in PI3K-Akt signalling pathway and serotonergic synapse.

Figure 5: Gene Ontology - Molecular Function terms

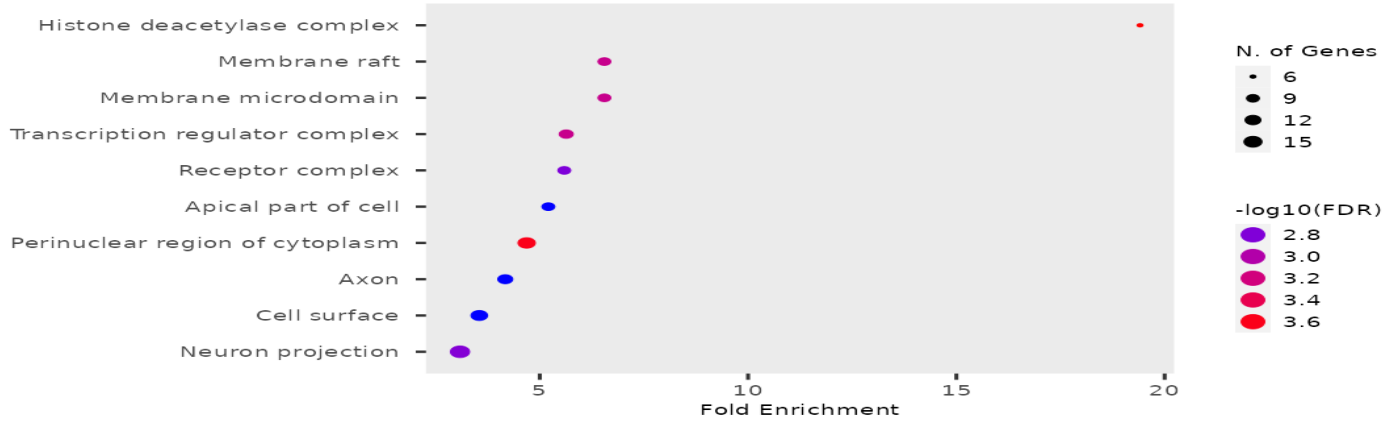


Figure 6: Gene Ontology - Cellular Component terms

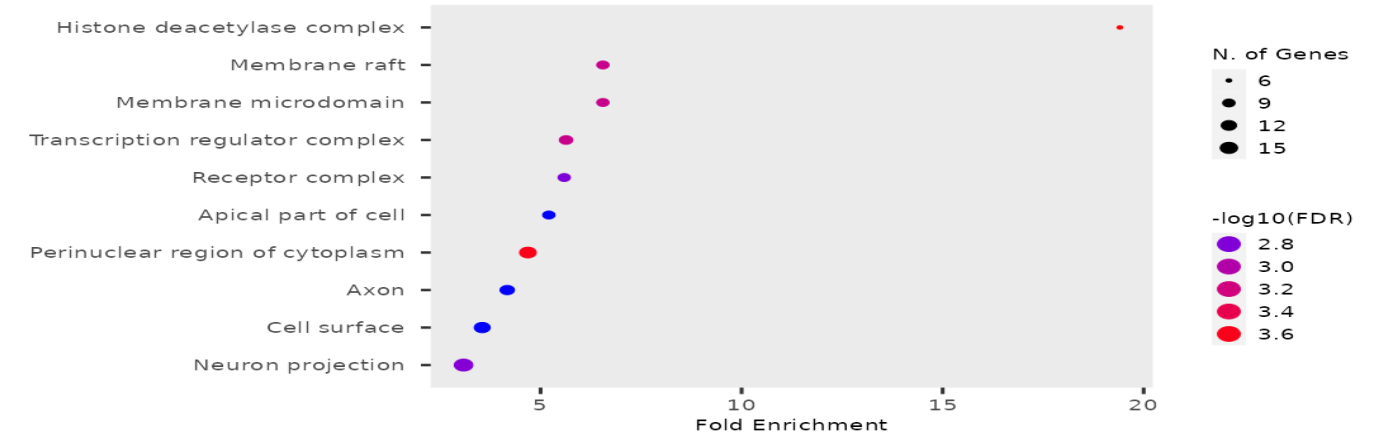


Figure 7: Gene Ontology - Biological Process terms

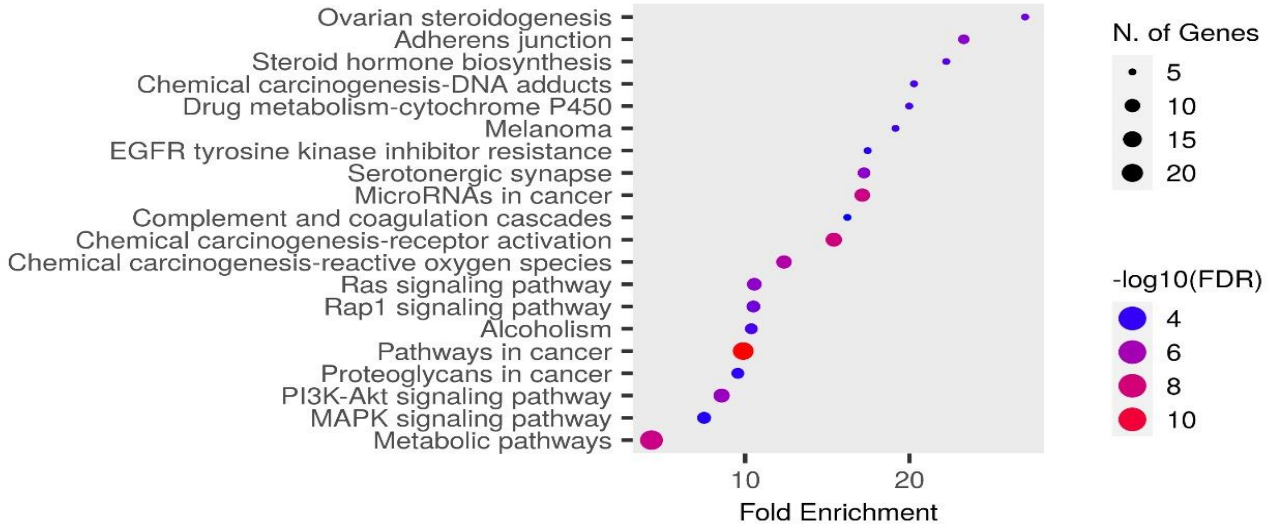


Figure 8: Top 20 KEGG Pathways sorted by *P* values**.**

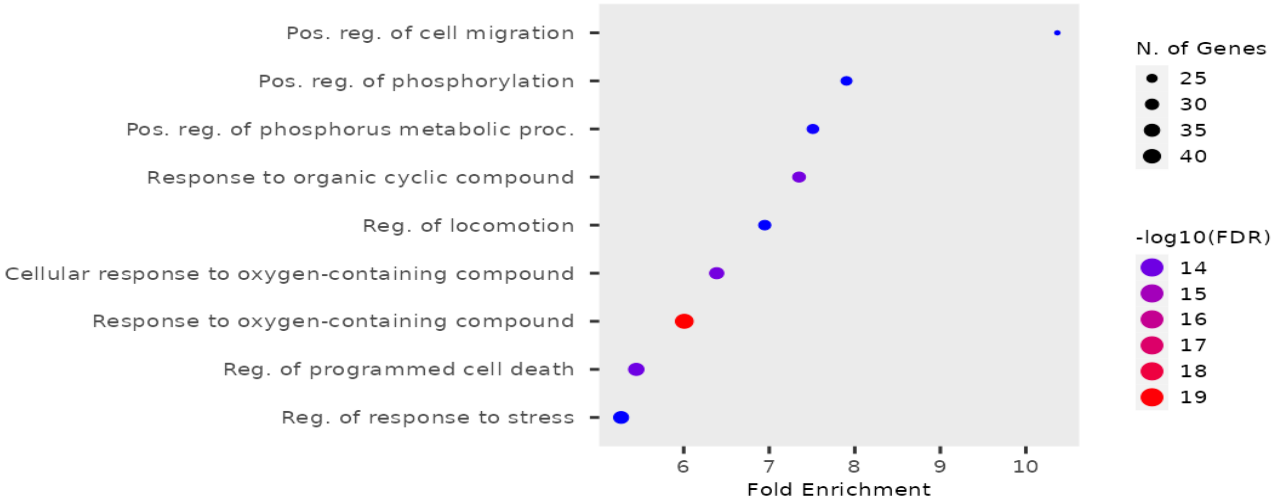


Figure 9: Venn diagram of targets for active compounds of *Rhus toxicodendron* for the treatment of Parkinson's Disease and Alzheimer's Disease

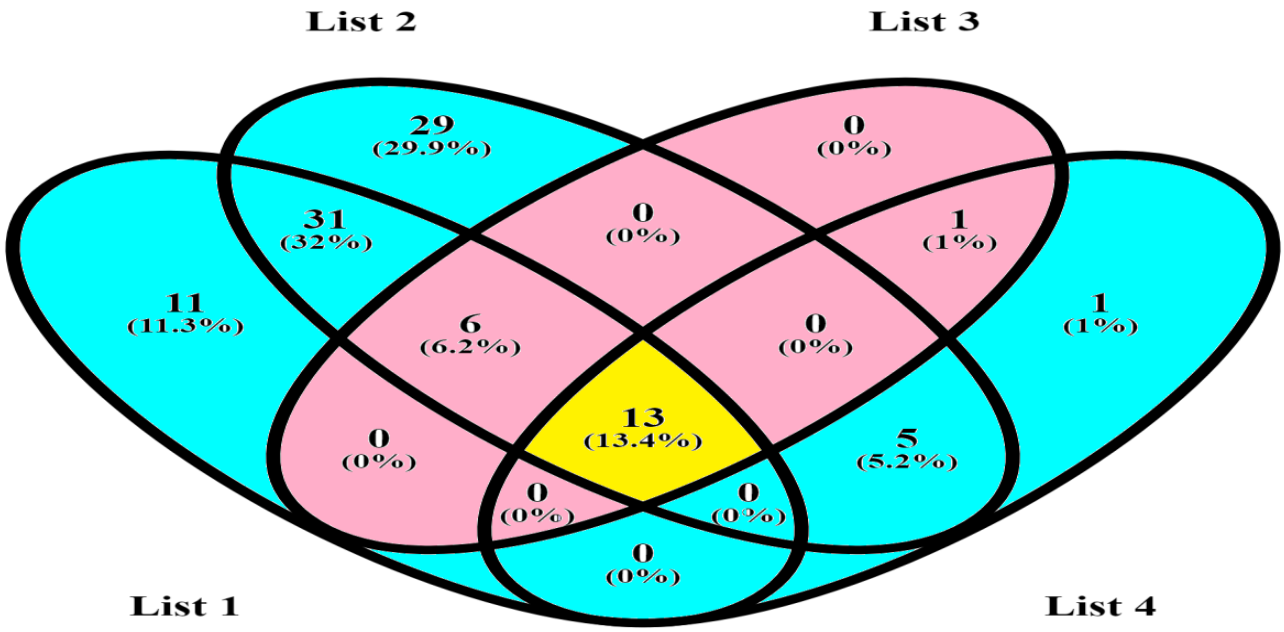
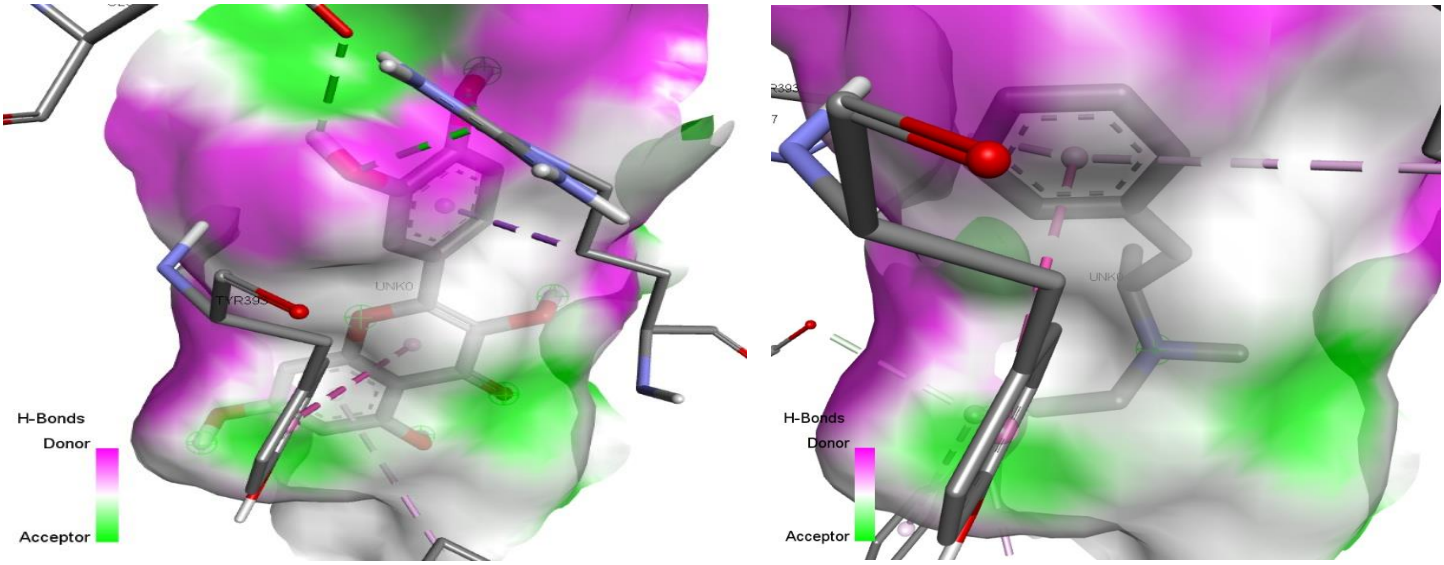


Figure 10: Molecular docking poses of bioactives with MAOB and SNCA (Binding energies were given in the bracket)



A B

[A = Docking of MAOB-Quercetin (-7.6 kcal/mol); B = Docking of MAOB-Selegiline (-5.0 kcal/mol); C = Docking of SNCA Rutin (-9.1 kcal/mol); D = Docking of SNCA-Selegiline (-5.7 kcal/mol)]

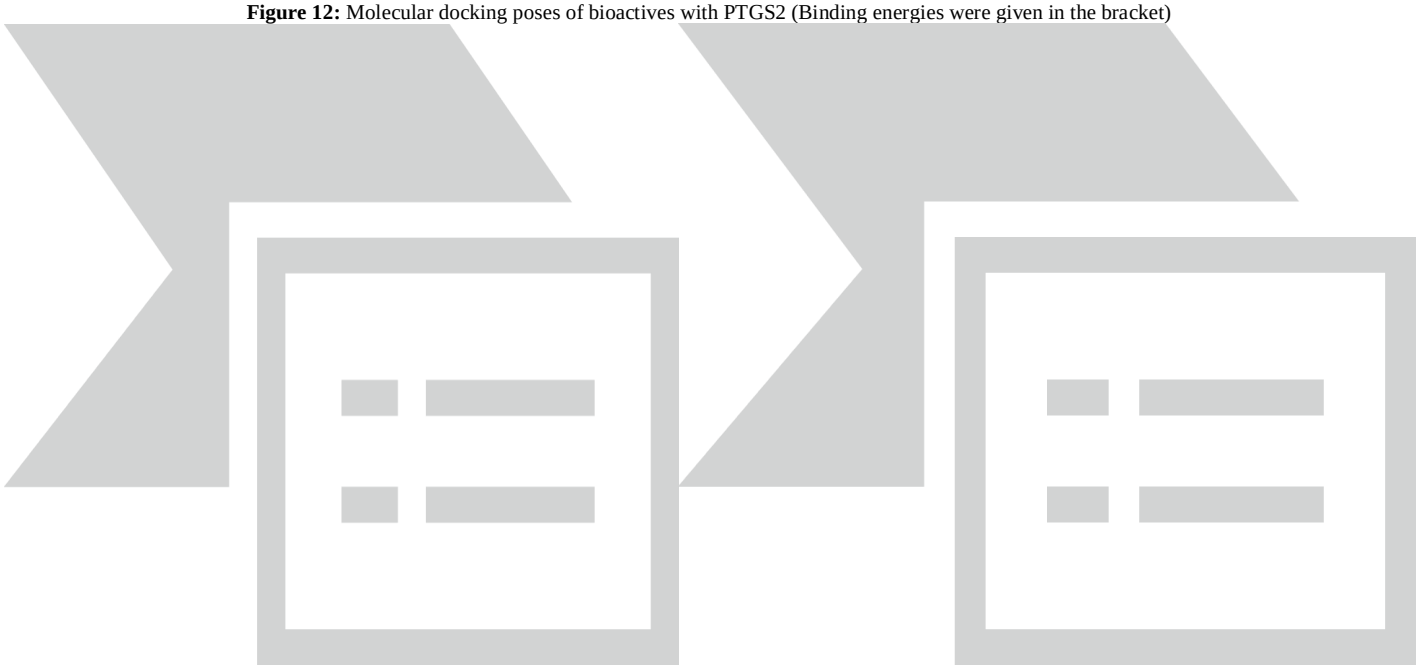
Figure 11: Molecular docking poses of bioactives with EGFR and NTRK2 (Binding energies were given in the bracket)

E F

G H

[E = Docking of EGFR-Fisetin (-9.3 kcal/mol); F = Docking of EGFR-Selegiline (-6.8 kcal/mol); G = Docking of NTRK2- Myricetin (-6.7 kcal/mol); H = Docking of NTRK2-Selegiline (-5.1 kcal/mol)]

Figure 12: Molecular docking poses of bioactives with PTGS2 (Binding energies were given in the bracket)



I J

[i = Docking of PTGS2- Fisetin (-7.61); j = Docking of PTGS2- Selegiline (-4.8)].

Table 3: MAO-B inhibition assay of various flavonoids, rhus tox and selegiline

Concen tration (µg/mL)	% Inh of Selegiline	% Inh of Quercetin	%Inh of Rutin	%Inh of Tannic acid	%Inh of Gallic acid	%Inh of Rhus Tox (200 Ch)
10	47.96±3.18	41±1.12	34.89±1.03	28.37±1.79	2.94±0.28	27.18±6.15
20	63.68±0.62	49.49±2.49	45.88±0.95	32.25±0.71	17.94±4.52	37.16±1.39
40	64.94±0.56	55.33±1.69	56.41±0.88	39.73±2.06	28.75±1.25	47.22±1.67
80	71.20±4.18	58.8±5.39	58.24±0.34	42.30±0.57	37.76±2.64	53.03±1.47
160	73.36±5.77	67.46±0.91	76.49±1.12	46.81±1.69	45.54±1.13	60.03±4.00
IC $_{50}$ Values	**15.68[a]**	**32.69[a]**	**38.69 [a]**	**171.60 [a]**	**168.67 [a]**	**78.75 [b]**

All the values were given in mean ± SD of triplicates; a = dose given in µg/ml; b= dose given in µL

Venn Diagram

Figure 9 represents Venn diagram which was constructed using Venny 2.1.0 for four groups of targets, that includes both targets of Parkinson's disease (61) & Alzheimer's disease (85), from which we obtained top 20 targets each of Parkinson's disease and Alzheimer's disease using STRING database. Out of 20 targets, we found 13 targets as common targets on which bioactives of Rhus Tox were noticed to network and interact.

Molecular Docking

In the present study, to assess the binding affinities of specific phytochemicals with specific target proteins Glide - standard precision module of Schrodinger suite-Maestro 13.0 was used. Among the 13 common targets obtained after Venn diagram 5 targets such as MAO-A, SNCA, EGFR, NTRK2, PTGS2 based on their established role in both PD and AD diseases. Selegiline was used as standard to compare the binding affinities of the 12 bioactives. Figure 10, 11 and12 (a-j), represents the docking poses and scores of drugs with highest docking score and standard drugs with the respective targets. The four important criteria followed in analysis of docking results were docking score, docking pose, number of interactions and the types of interactions. Upon analysis of the docking data, we identified that Kaempferol, Fisetin, Quercetin, myrecitin had shown better docking score than the standard selegilline.

MAO-B Inhibition Assay

In the MAO-B inhibition assay, the IC_{50} values of the Quercetin, Rutin, Gallic acid, Tannic acid, and Rhus Tox were comaparble to that of standard Selegiline. The values were mentioned in the Table 3.

DISCUSSION

In the present study Rhus Tox, an homeopathic formulation was evaluated for its effects on PD and AD. Even tough, homeopathic formulations were claimed to be effective in curing the root cause with less adverse effects, their mechanism of actions are not clear. The pathology behind PD and AD are entirely different but neuronal cell death is common. Hence, to get clear picture of the mechanism of actions against neuronal cell death, the network pharmacology approach was used.

Initially, the 21 phytoconstituents of Rhus Tox were identified from PubMed then they were screened for BBB permeation, DL index, carcinogenicity, mutagenicity, TPSA, HIA using ADMETlab2.0 and SwissADME. This screening of ADME properties helps us to understand the safety and efficacy of drug candidate [17]. ADMET indicates Absorption, Distribution, Metabolism, Excretion and Toxicology. With ADMETlab2.0, we can predict multiple physicochemical properties, medicinal chemistry properties, ADME properties, toxicity endpoints, and toxicophore rules which help in identifying druggability of the lead compounds. [18].

BBB Permeation indicates the ability of the drug candidate to be able to pass through blood brain barrier and for drugs acting on CNS this is the most important phenomenon to be checked [19]. In some of the earlier works done by network pharmacology approach they used these ADMET criteria to filter down the active constituents [20-22]. In our study out of 21 phytoconstituents, 12 satisfied the above criteria in which BBB permeability was considered more prominent.

For these 12 phytoconstituents targets were predicted by target fishing method using the binding data base and DisGeNET. Binding data base is a publicly accessed database consisting of approximately 20,000 protein-ligand interactions [23].

After necessary data cleaning steps, 61 targets were predicted for PD and 85 targets were predicted for AD on which the bioactives of Rhus Tox were predicted to act. Simultaneously, protein-protein interactions were performed using STRING database for the above targets and top 20 targets were selected for both PD and AD.

The effectiveness of any traditional medicinal system is demonstrated by the synergistic effects of bioactives on the potential targets. However, experimentally validating these effects is quite extensive and costly affair, which can slow the progress in observing the relationships between bioactives and targets. KEGG enrichment analysis can identify significant signalling pathways involved in these synergistic effects. Consequently, KEGG enrichment analysis gradually narrows down the targets which can be further probed.

According to the results of the KEGG pathway enrichment analyses, bioactives of Rhus tox was mainly focused on PI3K-Akt signalling. The importance of PI3K-Akt cell survival pathway on mitochondrial damage-mediated apoptosis, promotion autophagy of amyloid, neuroprotective role is well established [24]. The activation of the PI3K-Akt pathway is also involved in the neuroinflammatory response and oxidative stress [25]. The KEGG analysis also indicated the effect of bioactives of Rhus tox on other potential pathways such as dopaminergic pathway, serotonergic pathway and EGFR signalling pathway. It is already established in the literature that these pathways play a major role in Neurodegeneration [26-29]. The GO analysis has highlighted the specific role of bioactives of Rhus tox on response to oxygen-containing compound, signalling receptor binding, neurone projection in molecular, cellular and biological processes. The effect on histone deacetylase was also noted but not as prominent as the other functions. These are very crucial findings which testify the antioxidant and neuroprotective effect of Rhus Tox. In particular, the signalling receptor binding effect supports the neuroprotection via PI3K-Akt cell survival pathway as observed in KEGG analysis.

From Venn diagram 13 targets were found to be common in both PD and AD on which bioactives of Rhus tox were acting. Among them 5 targets such MAO-B, SNCA, EGFR, NTRK2, PTGS2 were selected for further docking based on their importance in neuro-degeneration as reported in literature.

In order to support the above studies MAO-B inhibition assay was performed *in-vitro* for the available flavonoids like rutin, quercetin, gallic acid, tannic acid and Rhus Tox as per the reported method [30]. The results confirm the prediction that individual phyto-constistuents and Rhus Tox also has comparable MAO-B inihibition property similar to Selegilline which might be one of the mechanism

behind the neuroprotection offered by the Rhus Tox. The involvement of Rhus Tox on other targets such as SNCA, EGFR, NTRK2 and PTGS2 needs to be further confirmed which can give better understanding of the neuroprotection offered by Rhus Tox against AD and PD.

CONCLUSION

In conclusion, as Kaempferol and Quercetin are good neuroprotective agents and has very good BBB permeation they were able to bind to all the targets selected. Fisetin, Rhamnose, Gallic acid could also bind to multiple targets with good binding energy indicates that they can also be potential act as anti-neurodegenerative agents but BBB permeation is very less when compared with Quercetin and Kaempferol. From the KEGG analysis we can conclude that Rhus Tox might be acting on the dopaminergic pathway, Seretonergic pathway and EGFR signaling pathway. The docking studies validated these predictions as most of the constituents showed good binding affinity with EGFR, SNCA and MAO-B. Further studies are required targeting these pathways and phytoconstituents to establish their efficacy in the *in- vitro* and *in-vivo* models will give better treatment options to treat neurodegenerative diseases.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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