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ORIGINAL ARTICLE / ÖZGÜN MAKALE

UNVEILING THE THERAPEUTIC POTENTIAL OF *GINKGO BILOBA*: A NETWORK PHARMACOLOGY APPROACH FOR PARKINSON'S DISEASE

GİNKGO BİLOBA'NIN TEDAVİ POTANSİYELİNİN ORTAYA ÇIKARILMASI: PARKİNSON HASTALIĞINA YÖNELİK AĞ FARMAKOLOJİSİ YAKLAŞIMI

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ABSTRACT

Objective: *The aim of the current study is to identify the major phytoconstituents in Ginkgo biloba that could modulate the role of major therapeutic targets involved in the pathogenesis of Parkinson's disease using approaches in network pharmacology.*
Material and Method: *The phytoconstituents in Ginkgo biloba and their therapeutic targets and the targets of Parkinson's disease were identified using various online databases and software. The identified phytoconstituents were subjected to evaluation of several pharmacokinetic properties and druglikeness study. The phytoconstituents with favourable pharmacokinetic and druglikeness properties and targets with better topological parameters were subjected to molecular docking study and MMGBSA analysis.*
Result and Discussion: *This study identified the presence of 125 major phytoconstituents in Ginkgo biloba and out of 125 phytoconstituents, 30 phytoconstituents passed the pharmacokinetics and druglikeness property. The therapeutic targets for these selected phytoconstituents were found to be 468 and the disease targets in PD were found to be 2033. The common targets between phyto-targets and disease targets were found to be 44 targets. Out of 44 common targets, 5 top proteins CNR1, HPGDS, AR, RXRA and HDAC1 were identified on the basis of the topological parameters such as degree centrality and betweenness centrality in the Cytoscape 3.9.1 software. The docking studies and MMGBSA analysis revealed that beta-eudesmol has better interaction with the top 5 therapeutic targets.*
Keywords: *Ginkgo biloba, network pharmacology, parkinson's disease, schrödinger, sitoscape*

ÖZ

Amaç: *Mevcut çalışmanın amacı, ağ farmakolojisindeki yaklaşımları kullanarak Parkinson hastalığının patogenezinde yer alan ana terapötik hedeflerin rolünü modüle edebilen Ginkgo biloba'daki ana bitki kaynaklı bileşenleri belirlemektir.*
Gereç ve Yöntem: *Ginkgo biloba'daki bitki kaynaklı bileşenler ile bunların terapötik hedefleri ve Parkinson hastalığının hedefleri, çeşitli çevrimiçi veritabanları ve yazılımlar kullanılarak belirlendi. Tanımlanan bitki kaynaklı bileşenlerin, çeşitli farmakokinetik ve ilaç benzeri özellikleri değerlendirildi Uygun farmakokinetik ve ilaç benzeri özelliklere sahip bitki kaynaklı bileşenler ve daha iyi topolojik parametrelere sahip hedefler, moleküler yerleştirme çalışmasına ve MMGBSA*

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analizine tabi tutuldu.
Sonuç ve Tartışma: *Bu çalışma ile Ginkgo biloba'da 125 ana bitki kaynaklı bileşenin varlığı saptandı ve 125 bitki kaynaklı bileşenden 30'u uygun farmakokinetik ve ilaç benzeri özellik gösterdi. Seçilen bu bitki kaynaklı bileşenler için 468 terapötik hedef ve Parkinson için 2033 hastalık hedefi bulundu. Fito-hedefler ile hastalık hedefleri arasındaki ortak hedefler 44 hedef olarak bulundu. 44 ortak hedeften, Cytoscape 3.9.1 yazılımındaki derece merkeziliği ve arasındalık merkeziliği gibi topolojik parametrelere dayanılarak 5 üst protein CNR1, HPGDS, AR, RXRA ve HDAC1 tanımlandı. Yerleştirme çalışmaları ve MMGBSA analizi, beta-eudesmol'ün ilk 5 terapötik hedefle daha iyi etkileşime sahip olduğunu ortaya çıkardı.*
Anahtar Kelimeler: *Ağ farmakolojisi, cytoscape, Ginkgo biloba, parkinson hastalığı, schrödinger*

INTRODUCTION

The current approach in drug discovery is to design highly selective ligands to either activate or inhibit a specific target that is proven to play a crucial role in the pathogenesis of a specific disease. This approach is based on the assumption that a ligand that exhibits a high degree of specificity towards a single target would be safe and effective with minimal adverse effects [1]. This 'one gene, one drug, one disease' approach towards discovery of novel drugs is challenged by the recent developments in the field of systems biology and network pharmacology. Network pharmacology aims to discover drug leads and understand mechanism of action of potential drugs through interaction with multiple therapeutic targets [2]. Network pharmacology is considered to be an essential method for development of phytochemicals as potential therapeutic agents because of the potential of natural phytoconstituents to act on multiple targets in various signalling pathways [3]. Network pharmacology elucidates the interaction between drug and targets; drug and drug and the impact of potential drugs on biological pathways and networks. Through comprehensive analysis of biological pathways and networks, network pharmacology can reveal the underlying mechanisms of drug action, identify potential therapeutic targets, predict adverse effects and help in drug discovery and development. *Ginkgo biloba*, native to China, is a large ancient tree reaching a height of 20-35 m that belongs to the family, Ginkgoaceae. Phytochemical analysis of *Ginkgo biloba* has revealed the presence of terpene trilactones such as ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J and bilobalide, flavonol glycosides biflavones, proanthocyanidins, alkylphenols, phenolic acids, 6-hydroxykynurenic acid, 4-*O*-methylpyridoxine and polyprenol [4,5]. *Ginkgo biloba* has been used medicinally for around 2000 years mostly in China but also in other parts of the world. Several studies have revealed the potency and efficacy of *Ginkgo biloba* in various ailments like Alzheimer's disease, epilepsy, peripheral vascular disease, ischemia induced oxidation, liver injury, hyperlipidaemia, depression, tardive dyskinesia, generalized anxiety, diabetes, inflammation and cancer [6]. *Ginkgo biloba* has also been reported in literature to exerts its beneficial effects in various in vitro and in vivo animal models of Parkinson's disease [7]. Parkinson's disease is a complex neurological condition that involves the loss of dopaminergic neurons in the substantia nigra as well as the emergence of Lewy bodies and Lewy neurites. The prominent hallmarks of the disease include resting tremors, stiffness, bradykinesia, and postural instability, along with a number of non-motor indications, including autonomic dysfunction, sleep issues, exhaustion, constipation, depression, cognitive decline, and loss of smell or taste, among others [8]. The present study aims to identify the main phytochemicals of the *Ginkgo biloba* that modulate the considerable therapeutic targets of Parkinson's disease using network pharmacology and computational approaches such as molecular docking, MMGBSA, and molecular dynamics.

MATERIAL AND METHOD

Identification and Retrieval of Phytoconstituents of *Ginkgo biloba*

The phytoconstituents of the plant, *Ginkgo biloba* were identified and retrieved from Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) Dr. Duke's Phytochemical and Ethnobotanical databases and through comprehensive literature survey. The chemical structure of the phytochemicals present in *Ginkgo biloba* was obtained in SDF format from PubChem [9].

Evaluation of Drug-likeliness and BBB Permeation

The phytochemicals present in *Ginkgo biloba* were analysed for their drug likeness and permeation through blood brain barrier (BBB) using SwissADME. For prediction of drug-likeness and BBB permeability, SwissADME received input in the Canonical SMILES format of phytochemicals. The Canonical SMILES format of phytochemical compounds was given as the input in SwissADME for prediction of drug-likeness and permeation through BBB. The potential toxicity of the phytochemicals was predicted using AdmetSAR. The compounds that pass the BBB, have one or less than one violation of Lipinski rule of five and exhibited no toxicity were considered for further analysis [10].

Prediction of Phytochemical Targets and Disease Targets

The therapeutic targets of the retrieved phytochemicals present in *Ginkgo biloba* were predicted using SwissTargetPrediction. A list of potential targets was obtained by giving the Canonical SMILES format of phytochemicals compounds as input in SwissTargetPrediction.[11]. The disease targets were obtained using DisGeNet database. The list of therapeutic targets involved in the pathogenesis of Parkinson's disease was obtained by typing the name of the disease (Parkinson's disease) in the DisGeNet database followed by retrieval of the disease-gene associations [12].

Identification of Common Targets

The common targets between the phytochemical targets and Parkinson's disease targets were identified using Venny2.1.0 tool. A Venn diagram was constructed to identify the common targets between the phytochemicals and the Parkinson's disease targets. The list of phytochemical targets and Parkinson's disease targets were given as input to Venny tool in two separate lists. Venny tool analyses the list of phytochemical targets and Parkinson's disease targets and provides a list of the common targets in the results box.

Target Network Construction and Topological Analysis

A network of common targets based on their functional relationship were constructed using GeneMania (Multiple Association Network Integration Algorithm), a Cytoscape plugin [13]. The topological parameters, betweenness centrality (BC) and degree centrality (DC) were determined using CytoNCA to identify the top 5 essential therapeutic targets involved in Parkinson's disease [14].

Molecular Docking

The structure of the top 5 proteins CNR1 (6N4B), HPGDS (1IYH), AR (2YHD), BCHE (6ESJ), HDAC1 (4BKX) were downloaded in the PDB format from the RCSB Protein data bank (PDB). Further, the 2D structures of the selected ligands were obtained from the PubChem online database and downloaded in sdf format. The 3D structure of the protein was prepared using Schrodinger Maestro protein preparation wizard [15,16]. The 2D structures of all the selected phytoconstituents were transformed into a minimal 3D structure using the LigPrep wizard of Schrodinger maestro. For precise tautomer enumeration and to determine the protonation state in biological status, the potential ionisation state was created using Epik at the target pH of 7.0 ±2.0. By maintaining certain chiralities, stereoisomers might be created with a maximum of 32 per ligand. OPLS3 force field settings were employed and the processes that followed used the lowest penalty state. The receptor grid files were generated by using the default options of the Receptor Grid generation function of Schrodinger maestro. The ligand required for docking was determined to create the grid around it. The centroid of the ligand served as the centre of the grid box, and "Dock ligands similar in size to the Workspace ligand" was selected as the grid size. The top 5 selected target proteins were docked with the chosen phytoconstituents using the extra precision (XP) glide of Schrodinger maestro. It takes into account the penalties applied to non-cis/trans amide bonds. The docking procedure was validated through measurement of root mean square deviation (RMSD) [15,16]

Molecular Mechanics with Generalised Born and Surface Area Solvation (MMGBSA)

MMGBSA was used to calculate the binding free energy of the top 5 target-phytoconstituent docked complexes. The binding free energy was calculated using the OPLS 2005 force field, VSGB solvent model, and rotamer search methods [17]. The binding free (ΔGbind) energy calculated using the below formula:

$$\Delta Gbind = Gcomplex - (Gprotein + Gligand)$$

where Gcomplex, Gprotein, and Gligand are the free energies of the complex, protein, and ligand respectively.

RESULT AND DISCUSSION

Identification and Retrieval of Phytoconstituents of the Plant *Ginkgo biloba*

Network pharmacology is a distinctive *in-silico* approach that allows targeting multiple therapeutic targets involved in the pathogenesis of a disease instead of a single target. It has enabled a paradigm shift from the classical existing 'one disease–one target–one drug' dogma to the current 'multicomponent, multi-target' approach [18]. In the current study, network pharmacology analysis was adopted to identify the potential phytochemicals present in *Ginkgo biloba* that can modulate the therapeutic targets involved in the pathogenesis of PD.

The current principle of 'one disease–one target–one drug' in drug design and discovery is challenged by large scale functional genomic studies. These findings ascertain that modulation of single proteins in a network do not affect the disease networks to a significant degree whereas modulation of multiple proteins might offer significant therapeutic benefits. Single gene knock-out studies have revealed that knockout of single genes have little or no effect at all on the phenotype of an organism[19-21]. For instance, a systematic single gene deletion study in yeast has discovered that only 15 % of the single gene deletions result in defects in the fitness whereas the remaining deletions have no significant effect on yeast[22]. These findings reveal that a biological pathway is highly robust, redundant, and has alternative signalling routes. These properties of a disease network emphasize that instead of modulating the function of a single protein in a pathway, network pharmacology recommends that modulating the functions of multiple proteins in a disease network would offer significant therapeutic benefits.

A total of 125 phytoconstituents reported to be present in *Ginkgo biloba* were identified and retrieved from Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) and Dr. Duke's Phytochemical and Ethnobotanical database. The list of phytoconstituents in *Ginkgo biloba* is presented in the supplementary file.

Drug-likeness of the Retrieved Phytochemicals Compounds

Physicochemical, drug-likeness, various pharmacokinetic, and toxicity properties of 125 phytochemicals found in Ginkgo biloba were estimated using Swiss ADME and AdmetSAR. Out of 125 selected phytochemicals, 30 phytochemicals passed the drug- likeness and blood-brain barrier BBB permeation. The complete list of the 30 phytochemicals that passed pharmacokinetic prediction is provided in the supplementary file and represented in Figure 1. The results of the pharmacokinetic properties of the top five phytoconstituents are represented in Tables 1, 2, and 3.

Table 1. Physicochemical properties and drug-likeness of selected phytoconstituents

Phyto-constituent	Physicochemical properties					Drug-likeness	
	Mol.Wt.	Number of rotatable bonds (ROTB)	Number of Hydrogen Bond acceptors (HBA)	Number of Hydrogen Bond Donors (HBD)	Aqueous Solubility (LogS)	Bioavailability score	Lipinski's violations
Beta-Eudesmol	222.37	1	1	1	-3.51	0.55	0

Table 1 (*continue*). Physicochemical properties and drug-likeness of selected phytoconstituents

Phyto-constituent	Physicochemical properties					Drug-likeness	
	Mol.Wt.	Number of rotatable bonds (ROTB)	Number of Hydrogen Bond acceptors (HBA)	Number of Hydrogen Bond Donors (HBD)	Aqueous Solubility (LogS)	Bioavailability score	Lipinski's violations
Linoleic acid	280.45	14	2	1	-5.05	0.85	1
Elemol	222.37	3	1	1	-3.8	0.55	0
Linolenic acid	278.43	13	2	1	-4.78	0.85	1
Myristic acid	228.37	12	2	1	-4.31	0.85	0

Table 2. Lipophilicity and medicinal chemistry of selected phytoconstituents

Phytoconstituent	Lipophilicity		Medicinal chemistry		
	Topological Polar Surface Area (TPSA)	Partition Coefficient (CLogP)	PAINS alert	Brenk alert	Lead-likeliness
Beta-Eudesmol	20.23	3.61	0	1	2
Linoleic acid	37.3	5.45	0	1	2
Elemol	20.23	3.77	0	1	2
Linolenic acid	37.3	5.09	0	1	2
Myristic acid	37.3	4.45	0	0	3

Table 3. Pharmacokinetic and toxicity profile of selected phytoconstituents

Phytoconstituent	Pharmacokinetic parameters							Toxicity	
	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Ame's mutagenicity	Carcinogenicity
Beta-Eudesmol	High	Yes	No	No	Yes	No	No	No	No
Linoleic acid	High	Yes	Yes	No	Yes	No	No	No	No
Elemol	High	Yes	No	No	Yes	No	No	No	No
Linolenic acid	High	Yes	Yes	No	Yes	No	No	No	No
Myristic acid	High	Yes	Yes	No	No	No	No	No	No

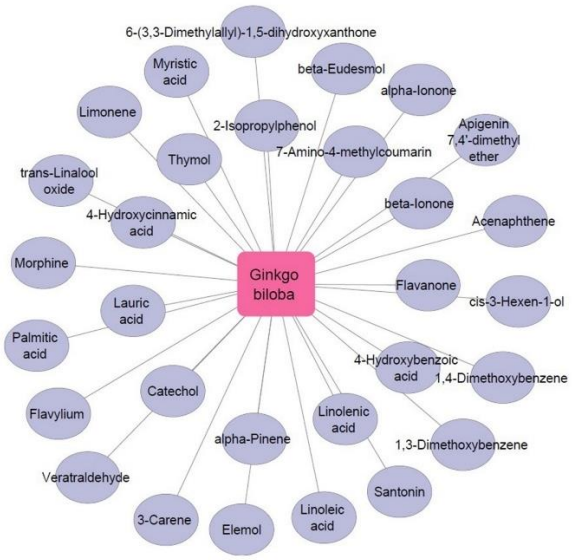

Figure 1. Pictorial representation of selected phytoconstituents of *Ginkgo biloba*

The figure represents the major phytoconstituents present in *Ginkgo biloba.*

Prediction of Phytochemical Targets and Disease Targets

The therapeutic targets associated with the phytoconstituents present in *Ginkgo biloba* and Parkinson's disease were retrieved from SwissTargetPrediction and DisGeNET databases respectively. A total of 468 targets was associated with phytoconstituents present in *Ginkgo biloba* and 2033 targets were associated with Parkinson's disease. A Venn diagram analysis of the targets revealed that 44 targets are common between phytoconstituents targets and Parkinson's disease targets. The list of phytoconstituent targets, disease targets and common targets are provided in the supplementary file. The results are represented in Figure 2.

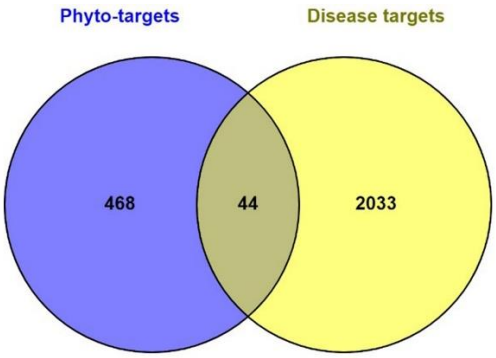

Figure 2. Common Targets between *Ginkgo biloba* and Parkinson's disease (The figure represents the total number of phyto-targets, disease targets and common targets)

Target Network Construction and Topological Analysis

A protein-protein interaction network of selected common targets was constructed using GeneMania, a Cytoscape plugin. The topological parameters like Degree Centrality (DC), Betweenness Centrality (BC) and Closeness Centrality (CC) of the network were analyzed to determine the most important targets in the network. The top five proteins in the network were selected based on Degree Centrality (DC), Betweenness Centrality (BC) and Closeness Centrality (CC). Degree centrality measures the number of connections a node (protein/target) makes with other nodes in a network. Betweenness centrality determines the influence of a node (protein/target) in controlling the interaction between a pair of nodes (protein/target) passing through this node in the network. Closeness centrality measures the inverse of average distance of a node from all other nodes in a network. It is widely accepted that nodes with higher degree, betweenness and closeness centrality values may represent important targets and play a crucial role in a biological network. The targets selected based on topological analysis were: CNR1 (Cannabinoid receptor 1), HPGDS (Prostaglandin-D synthase), AR (Androgen receptor), BCHE (Butyrylcholinesterase) and HDAC1 (Histone deacetylase 1). The network is represented in Figure 3 and the results are given in Table 4.

Table 4. Topological analysis of the target proteins

Protein	**Topological Parameters**		
	Degree Centrality (DC)	**Betweenness Centrality (BC)**	**Closeness Centrality (CC)**
BCHE (6ESJ)	**7**	457.0333	0.172691
CNRI (6N4B)	**7**	450.4714	0.167315
HPGDS (1IYH)	**7**	407.8857	0.17623
AR (2YHD)	**7**	165.4762	0.173387
HDAC1 (4BKX)	**7**	96.66429	0.172

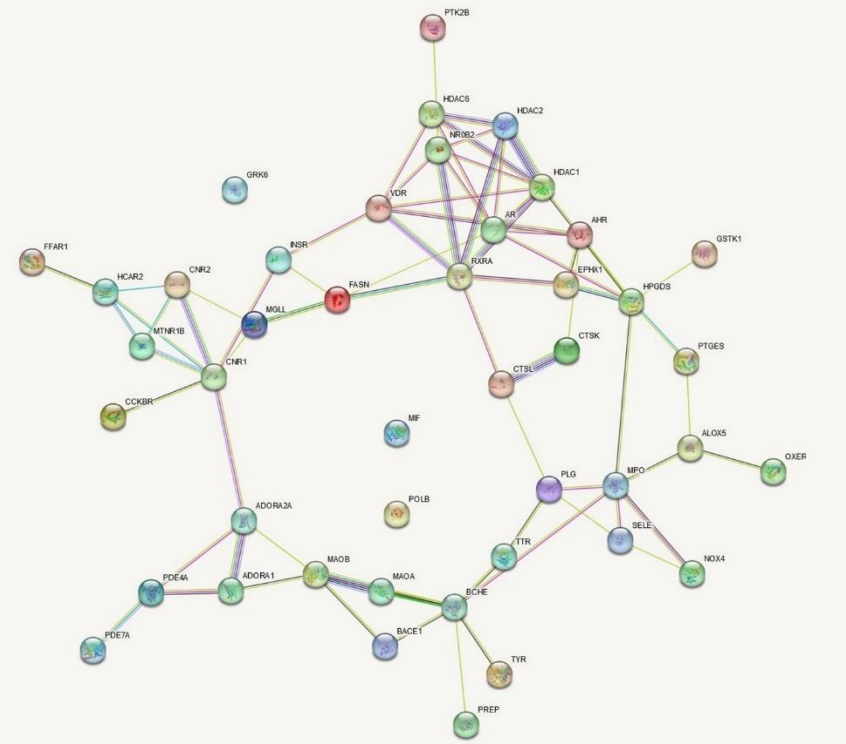

Figure 3. Protein Interaction Diagram of the Common targets

Proteins are represented by circular nodes in the PPI diagram and their interactions are represented by lines called edges. The purple edges represent interactions determined by using curated databases and the pink edges represent experimentally determined protein-protein interactions. The green, red and blue edges represent predicted determined protein-protein interactions based on gene neighbourhood, gene fusions and gene co-occurrence respectively. The yellow and black edges represent protein-protein interactions based on text mining and co-expression respectively.

Molecular Docking

The binding interactions between the top 5 phytoconstituents selected based on pharmacokinetic profile and the top 5 proteins selected based on topological parameters were studied using molecular docking studies. Molecular docking studies revealed the binding interactions and binding mode of the selected phytoconstituents with the target proteins. Among the 5 selected phytoconstituents, beta-eudesmol exhibited favourable docking scores with the five selected target proteins. Beta-eudesmol exhibited maximum docking scores with four out of five targets compared to other phytoconstituents. The docking scores of beta-eudesmol are CNR1 (-4.54 kcal/mol), HPGDS (-5.894 kcal/mol), AR (-4.73 kcal/mol) and BCHE (-6.30 kcal/mol). Beta-eudesmol exhibited the second maximum docking score with HDAC1 (-1.613 kcal/mol). Beta eudesmol forms eight hydrophobic interactions with PHE 155, LEU 209, ILE 212, ILE 216, ILE 227, VAL 228, ALA 233 and ALA 236 and two polar interactions with THR 210 and THR 229 with 6N4B. It forms seven hydrophobic interactions with TYR 8, PHE 9, MET 11, MET 99, TRP 104, CYS 156, LEU 199 and one polar interaction with THR 159 with 1IYH. Beta-eudesmol with 2YHD forms seven hydrophobic interactions with LEU 712, VAL 713, VAL 716, VAL 730, MET 734, ILE 737, AND MET 894 and two polar interactions with GLN 733 and GLN 738. With 6ESJ, beta-eudesmol forms two hydrogen bonds with TRP 82 and TYR 440 and six hydrophobic interactions with ALA 328, PHE 329, TYR 332, TRP 430, MET 434 and MET 437 and three polar interactions with SER 79, THR 120 and HID 438. With 4BKX, beta-eudesmol forms two hydrophobic interactions with LEU161 and LEU 164. The dock score of beta-eudesmol with 6N4B, 1IYH, 2YHD and 6ESJ was -4.545, -5.894, -4.731 and -6.308 kcal/mol and the dock score of respective reference compounds were -4.569, -4.464, -2.307 and -10.806 kcal/mol respectively. Root mean square deviation

(RMSD) was measured to validate the accuracy of docking results. The value of RMSD calculated between the reference and docked pose was found to be 1.21 Å, 0.98 Å, 1.32 Å and 0.90 Å for 6N4B, 1IYH, 2YHD and 6ESJ respectively. The results of the molecular docking studies are given in Table 5 and 2D interaction diagrams are represented in Figure 4.

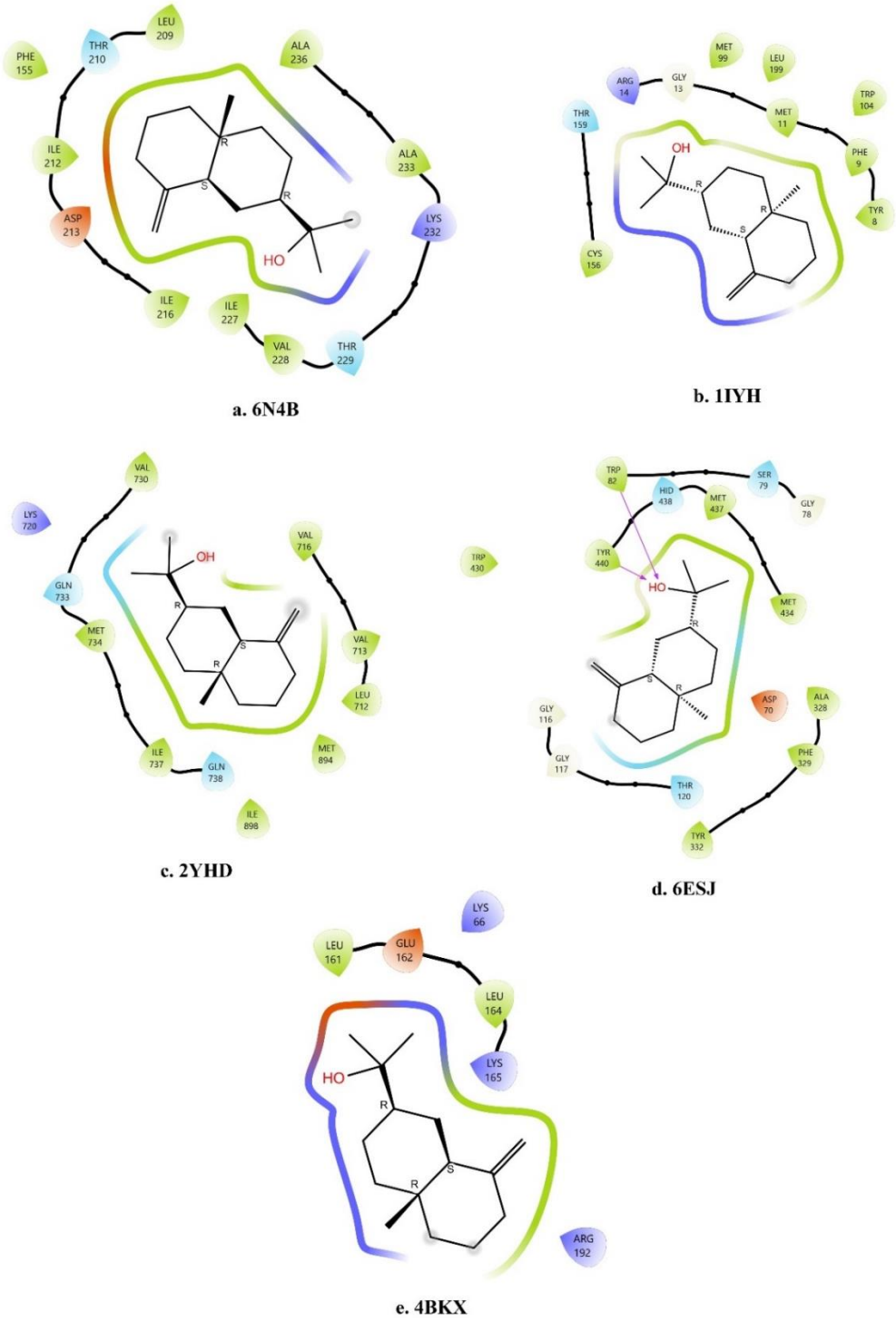

Figure 4. 2D Interactions diagrams of the top 5 proteins with beta-Eudesmol (The figure represents the 2D interaction diagrams of beta eudesmol with the top five therapeutic targets selected based on topological parameters. Figures a-e represent the 2D interaction diagrams of 6N4B, 1IYH, 2YHD, 6ESJ and 4BKX respectively)

Table 5. Dock Score of the selected Phytoconstituents

Phytoconstituents	Dock Score (kcal/mol)				
	6N4B	**1IYH**	**2YHD**	**6ESJ**	**4BKX**
Beta-Eudesmol	-4.545	-5.894	-4.731	-6.308	-1.613
Linoleic acid	-3.889	-5.827	-4.154	-4.524	-2.304
Elemol	-2.418	-5.646	-3.499	-6.273	-2.2
Linolenic acid	-4.552	-4.052	-2.261	-4.43	-2.078
Myristic acid	-1.625	-3.09	-2.05	-3.656	-1.888
Co-crystal	-4.569	-4.464	-2.307	-10.806	

Molecular Mechanics with Generalised Born and Surface Area Solvation (MMGBSA)

The binding free energy of top 5 phytoconstituents based on pharmacokinetic profile and top 5 proteins based on topological parameters were studied using determined MMGBSA technique. Beta-eudesmol showed better binding free energy with all the proteins especially it showed maximum binding free energy with CNR1 and HDAC1 and second best with HPGDS and BCHE. Linoleic acid also exhibited favourable binding free energy with CNR1, BCHE and HDAC1. The results of the MMGBSA studies are given in Table 6.

Table 6. Binding Free Energy of the Target Proteins

Phytoconstituent	Binding Free Energy (ΔGbind)				
	6N4B	**1IYH**	**2YHD**	**6ESJ**	**4BKX**
Beta-eudesmol	-73.71	10.22	-21.97	-73.71	21.01
Linoleic acid	-52.39	-4.16	-5.84	54.85	7.13
Elemol	-30.03	4.49	-41.1	-129.3	47.2
Linolenic acid	-59.75	-6.79	-36.19	29.61	-0.42
Myristic acid	-38.27	-17.68	-6.45	48.6	9.69
Co-crystal	-50.12	25.42	-38.17	-192.14	

Ginkgo biloba extract (EGb761) has been proven in the past to improve cell viability, reduce apoptosis and protect mitochondrial membrane potential, increased tyrosine hydroxylase positive cells, bcl-2 positive cells and decreased the caspase-3 positive cells on PC12 cells injured by paraquat [23]. A similar *in vitro* study carried out by Yang et al., on PC12 cells using MPTP as a neurotoxic agent also proved that leaf extract of *Ginkgo biloba* prevents apoptosis in Parkinson's disease [24]. *Ginkgo biloba* dropping pill is a leaf extract preparation of *Ginkgo biloba* which has been reported in the literature for its antioxidant and neuroprotective properties. Yu et al., studied the neuroprotective effect of *Ginkgo biloba* dropping pill in PD using 6-OH model in zebra fish and MPTP model in mice. The results of this study showed that *Ginkgo biloba* dropping pill prevented the loss of dopaminergic neurons in zebra fish and improved cognitive abilities and decreased damage to the dopaminergic neurons in the mice probably through Akt/GSK3β pathway [25]. Patricia Rojas et al., have proven that *Ginkgo biloba* extract prevents the mice form MPTP induced PD probably through regulation of copper levels in the corpus striatum, midbrain and hippocampus [26]. In another study, Patricia Rojas et al., have proven that *Ginkgo biloba* extract prevents the mice form MPTP induced PD through up regulation of tyrosine hydroxylase (Th), vesicular monoamine transporter 2 (Vmat2), dopamine transporter (Dat), dopamine D2 receptor (Da-d2r), and transcription factors (Pitx3 and Nurr1) related to dopamine neurotransmission [27]. Kuang et al., have proven that A53T transgenic mice fed and treated with *Ginkgo biloba* extract improves locomotor activity, levels of superoxide dismutase and glutathione peroxidase, expression of tyrosine hydroxylase and dopamine transporters and inhibits the expression of methane dicarboxylic aldehyde [28]. All the above finding ascertains the neuroprotective effect of *Ginkgo biloba* extract in various *in vitro, in vivo* and transgenic models of PD. In the current, an attempt is made to identify the phytoconstituents of *Ginkgo biloba* that are vital for its neuroprotective action and its potential therapeutic targets using network pharmacology and molecular docking studies.

The network pharmacology study revealed the presence of 125 major phytoconstituents in *Ginkgo biloba*. All 125 phytoconstituents were subjected to prediction of pharmacokinetic and druglikeness studies. Out of 125 phytoconstituents in *Ginkgo biloba*, 30 phytoconstituents showed favourable pharmacokinetic like the ability to penetrate the blood brain barrier and druglikeness property. The therapeutic targets for these selected phytoconstituents were found to be 468 and the disease targets in PD were found to be 2033. The common targets between phyto-targets and disease targets were found to be 44 targets. Out of 44 common targets, 5 top proteins CNR1, HPGDS, AR, RXRA and HDAC1 were identified on the basis of the topological parameters such as degree centrality and betweenness centrality in the Cytoscape 3.9.1 software. Further, the top 5 proteins were docked with 5 potential phytochemicals such as beta-eudesmol, linoleic acid, elemol, linolenic acid and myristic acid. Molecular docking was performed using the XP ligand docking in Maestro Schrodinger. The docking studies reveals that beta-eudesmol has the highest docking scores in most of the proteins. The complexes of protein-ligand were subjected to MM-GBSA studies to determine the stability of the protein-ligand complex. MM-GBSA assay revealed that beta-eudesmol forms stable complexes with maximum number of proteins.

Consistent with our findings, *Eucalyptus citriodora* L. leaf extract that contains beta-eudesmol as one of its major phytoconstituent delayed the loss of climbing ability and attenuated the oxidative stress in transgenic Drosophila melanogaster[29]. In a related study, beta eudesmol has been reported to control the hallucinations associated with PD on 1-(2,5-Dimethoxy-4- odophenyl)-2-aminopropane induced Head Twitch Response in Mice[30]. α-synuclein, mitochondrial dysfunction, oxidative stress, neuroinflammation and deficiency of trophic factors have been reported to impair neurite outgrowth leading to consequences like impaired neuroplasticity and a compromise in synaptic function[31,32]. Beta-eudesmol has been reported by Yutaro et al., to induce neurite outgrowth at concentrations of 100 and 150μM in rat pheochromocytoma (PC-12) cells mediated by MAPK pathway[33].

In conclusion, the current study reveals that beta-eudesmol present in *Gingko biloba* could act as a potential lead molecule in the management and prevention of Parkinson's disease through activation of multiple therapeutic targets.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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