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IN SILICO DOCKING ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS FROM TRADITIONAL MEDICINAL PLANTS: UNVEILING POTENTIAL ANXIOLYTIC ACTIVITY AGAINST GABA, DOPAMINE D2, DOPAMINE D3, AND SEROTONIN RECEPTORS

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Abstract

In today's society, anxiety is one of the most prevalent mental illnesses affecting kids and teenagers. It is composed of neurological, cognitive, and behavioral elements. The primary active components of many traditional Indian medicinal herbs, including Nigella sativa, Syzygium aromaticum, Convolvulus prostratus (shankhpushpi), Withania somnifera, and Punica granatum, will be docked in silico for a phytochemical constituent analysis in this work. The anxiolytic action of these ingredients, which include 4-hydroxycinnamic acid, delphinidin, kaempferol, taraxerone, eugenol, carvacrol, anaferine, and pellentine, will be evaluated. The phytochemical components are obtained using the PubChem chemical database. The GABA (PDB ID: 4COF), dopamine D2 (PDB ID: 6LUQ), dopamine D3 (PDB ID: 3PBL), and serotonin (PDB ID: 6VRH) receptors—which are implicated in anxiety—are the targets for the anxiolytic effect that are chosen from the Protein Data Bank. For in silico docking, Molegro Virtual Docker (MVD) was used. The parameters used in docking include Rerank score, MolDock score, and hydrogen bond interactions. The dock score and binding patterns of the phytochemical components are compared to the reference drugs. The phytochemical component employed in drug development showed greater potential, maximum affinity, similar binding patterns, and a higher MolDock score than standard pharmaceuticals. The phytoconstituents under study support the anxiolytic activity claims of their parent plants and have the potential to be leaders in anxiolytic action.

Keywords: Withania Somnifera, Convolvulus Prostrates, Syzygium Aromaticum, Nigella Sativa, Anxiety.

INTRODUCTION

In the modern world, anxiety is a common mental disorder that affects children and adolescents. It is defined by behavioral, cognitive, and neurobiological aspects. Anxiety disorders affect an estimated 264 million people worldwide, or 3.6% of the total population, according to estimates from the World Health Organization. Furthermore, 2.6 percent of males and 4.6 percent of women worldwide suffer from anxiety. 1-2 Anxiety is a condition of the central nervous system (CNS) marked by a

depressed emotional state that results in symptoms such as fear, discomfort, and other reactions to perceived internal or external stimuli. The precise process that causes anxiety is yet unknown. Numerous modulatory mechanisms have been linked to one or more neurotransmitter systems. The most often considered neurotransmitter systems are the noradrenergic and serotonergic. It is well acknowledged that both an underactive serotonergic system and an overactive noradrenergic system play a part. The dysregulation of physiological arousal and the emotional perception of this arousal result from these systems being directed and controlled by other neural circuits and pathways in other parts of the brain. Many think it developed as a result of reduced serotonin system activity and increased noradrenergic system activity. Thus, the first-line therapies for it are serotonin-norepinephrine reuptake inhibitors (SNRI) and selective serotonin reuptake inhibitors (SSRI).3-5 The manner that many anxiety spectrum illnesses react to benzodiazepine medication has also been connected to disruption of the gamma-amino butyric acid (GABA) system. There has been much speculation on the relationship between anxiety and fear and corticosteroid regulation. Certain neuronal pathways may be altered by corticosteroids, which influences not just stress-related behavior but also how the brain processes anxiety-inducing stimuli.

Cholecystokinin is a neurotransmitter that has long been thought to have a part in mood regulation. Due to the complicated regulation of these neurotransmitters, changes made to one system often result in changes made to another, including sophisticated feedback systems. GABA and serotonin are examples of inhibitory neurotransmitters that reduce stress; these neurotransmitters are now important therapeutic targets.6-9 Tricyclic antidepressants (TCA), serotonin reuptake inhibitors (SSRI), GABA agonists, and monoamine oxidase inhibitors (MAOI) are a few medications used to treat anxiety disorders. Through the amplification and regulation of neurotransmitters, these medications ultimately alter the chemistry of neurons. Among the many unfavorable side effects of our conventional pharmacology for treating anxiety are psychomotor imbalances, dependence, and sexual dysfunction.

It is important to identify phytoconstituents since they may one day be developed into reasonably priced, secure, and efficient anxiolytic medications.10-12 A sizable fraction of people worldwide choose to use complementary and alternative medicines to manage their mental health issues. Most mental health patients believe that these drugs are reasonably priced and have fewer adverse effects, most of which go unnoticed. Moreover, it is now evident that traditional medicines are still used in our culture and customs, especially in African communities. Many studies have examined the potential of medicinal plants to lower anxiety.13-14 Convolvulus prostratus Forssk, also known as Shankhpushpi, is primarily known for its neuroprotective, nootropic, and neuro-modulatory effects. It also has many other therapeutic properties, including as immunomodulatory, antidiabetic, cardioprotective, and antibacterial properties. The pomegranate, Punica granatum L., has several medicinal uses that may be linked to anti-inflammatory, antioxidant, and anti-carcinogenic mechanisms. Antioxidants are abundant in cloves. These substances help your body fight against free radicals, which damage cells and have the potential to cause illness. Antioxidants found in cloves assist your body in eliminating free radicals, which may lower your risk of diabetes, heart disease, and certain forms of cancer. Because of its antioxidant qualities, the herb Nigella sativa is utilized in medicine. Rats with chronic cerebral hypoperfusion-induced loss of spatial cognition also benefit greatly from nigella sativa seeds. Additionally, Nigella sativa reduces anxiety in scopolamine-induced neurotoxicity and

improves learning and memory impairments. 15-18 Ashwagandha, also known as Withania somnifera, is an Ayurvedic herb that has recently acquired recognition for its use in the treatment of cancer, microbial infections, neurological illnesses, anxiety, and immunomodulation. Aiming for any objective connected to a sickness.19-20 Based on computer analysis, the molecular docking research examines the effectiveness of each candidate generated during the first phase of the process. Most academics today use powerful computer algorithms to choose "hit" or "lead" choices. Indeed, natural substances—often referred to as phytochemicals—have a wide range of biological impacts. This makes figuring out how potent each individual is in a random experimental trial difficult and time-consuming. In this case, molecular docking is a more useful method for determining the potency of any desired natural compounds before carrying out a randomized experimental investigation. Indeed, the general agreement at this point is that molecular docking is an advanced and affordable technique that might potentially assist steer clear of the "hit-and-trial" or haphazard approach to drug screening. Molecular docking is used as an early guiding technique in contemporary drug development to save time, since it is not viable to offer therapeutic options for human use without doing extensive pharmacological and experimental research. When everything is said and done, molecular docking is a simple technique that might be useful in the drug development process. The results of in silico prediction are equivalent to those of in vitro and in vivo tests, as supported by scientific evidence. 21-25 In this study, in silico molecular docking research was carried out on phytoconstituents with several targets linked to anxiety in order to discover and generate a novel medication. After the phytoconstituent docking investigations, an estimate of the binding free energy was produced. Further studies on its physicochemical, drug-likeness, and ADMET profiles were conducted to ensure its safety and efficacy in the treatment of anxiety.

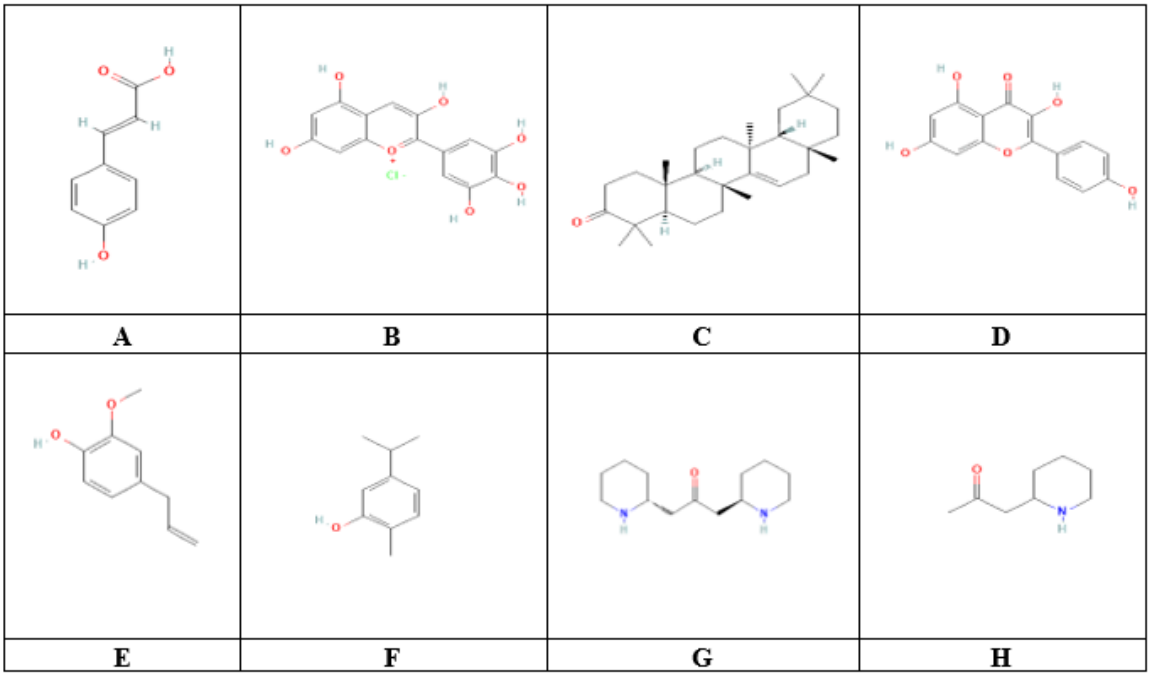

Figure 1: Chemical structure of A) 4-hydroxycinnamic acid, B) Delphinidin, C) Taraxerone, D) Kaempferol, F) Eugenol, G) Carvacrol, H) Anaferine, I) Pelletierine

MATERIALS AND METHODS

Physicochemical and Drug-Likeness Properties

The physicochemical properties of various phytoconstituents were mainly obtained from PubChem, since understanding the molecule's physicochemical properties is the first step to allow it to be transformed into a drug-like molecule 26. The drug-likeness properties as described in Lipinski's rule of five were calculated using DruLiTo, offline open-source software. DruLiTo is an open-source virtual screening tool in which drug likeliness descriptors such as Molecular weight (MW), log P, Alog P, H-bond acceptor (HBA), H-bond donor (HBD), Total Polar surface area (TPSA), Atom Molar Refractivity (AMR), number of rotatable bonds (nRB), number of atom, number of acidic groups, rotatable bond count (RC), Number of Rigid bond (nRigidB), nAtom Ring, and Number of Hydrogen Bonds (nHB) parameters can be predicted 27. The 3D Structure of the ligands was retrieved from the PubChem online database. The generated Ligands were then saved in the Standard Database format (SDF) 28. All the prepared ligands were then tested for drug likeliness properties using the software. The calculations were based on various drug likeliness rules like Lipinski's rule, Veber rule, BBB rule, CMC-50, etc. Overall, compounds that do not violate Lipinski's rule of five are predicted to have superior folding, polarity, and molecular size and to have more potential therapeutic effects 29.

ADME Properties

The Swiss ADME web server was used to predict the ADME properties (http://www.swissadme.ch/). This website allows you to compute physicochemical descriptors as well This website allows you to compute physicochemical descriptors as well as to predict ADME Parameters, pharmacokinetic properties, drug like nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery 30.

Toxicity Estimation

The Toxicity Estimation Software Tool (TEST) was developed to allow users to easily estimate the toxicity of chemicals using Quantitative Structure Activity Relationships (QSARs) methodologies 31. LC50 threshold was calculated using TEST (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test) software based on predictions from each model and the consensus average of the component models (32). The hierarchical technique, the single-model method, the group contribution method, the consensus method, and the nearest neighbour method are the QSARs methodologies used in this study effort.

A compound can be imported into the software using the following methods a) Using the provided molecular structure drawing tool, b) Importing from an MDL mol file, c) Searching by CAS number, SMILES string, or name. T.E.S.T. allows the user to estimate the value for several toxicity endpoints:

- Oral rat LD50 (amount of chemical in mg/kg body weight that is lethal to 50% of rats after oral ingestion).
- Developmental toxicity (binary indication of whether or not a chemical can interview with normal development of humans or animals).
- Ames mutagenicity (a compound is positive for mutagenicity if it induces revertant Colony growth in any strain of Salmonella typhimurium).

In silico studies of anxiolytic compounds

In the docking method, ligand structure and orientation inside a specified binding site were predicted. The two main goals of docking research are precise structural modeling and accurate activity prediction.

The process of docking is typically represented as a series of steps, each of which adds a new degree (or layers) of complexity 33. Docking methods are first used to place tiny molecules in the active site of a cell.

In order to anticipate biological activity, these algorithms are enhanced by scoring functions that assess interactions between molecules and prospective targets (34). Four human targets associated with anxiety were chosen to investigate the phytoconstituents anxiolytic effects based on an in silico molecular docking approach. Table 1 summarizes the targets and the criteria for selection used in the present investigation. As per the requirements, the retrieved three-dimensional (3D) crystal structure of selected targets was from the protein data bank (PDB) with individual PDB IDs.

Table 1: Targets in Anxiety

Disorder	Targets	Reason for Selected Targets	References
Anxiety	GABA (Gamma-amino butyric acid)	Low levels of GABA activity slowdowns central nervous system and leads to anxiety	(36)
	Dopamine D2	D2-receptor binding are *associated to symptom reduction after psychotherapy in social anxiety* disorder	(37)
	Dopamine D3	Dopamine (D3) can modulate the BLA GABAergic system, thus linking fear/anxiety states	(38)
	Serotonin	Associated with reduced serotonin binding to the receptors of the postsynaptic neurons	(39)

The receptors GABA (PDB Id: 4COF), dopamine D2 (PDB Id: 6LUQ), dopamine D3 (PDB Id: 3PBL), and serotonin (PDB Id: 6VRH), which are responsible for anxiety and are selected as the targets for anxiolytic action, were chosen as the targets for docking investigations. The target for the disease was first chosen, and then the 3D structures of numerous targets were obtained from the protein data bank in.pdb format (https://www.rcsb.org).

It is commonly known that the PDB file format cannot provide bond order information and that PDB files frequently feature incorrect or missing assignments of explicit hydrogen. As a result, the MVD was used to assign the appropriate bonds, bond orders, hybridization, and charges.

MVD's integrated cavity detection technique was used to determine the possible binding locations of both targets. A subset zone of 25.0 Å around the active side cleft used as the study area for the search space of the simulation used in the docking investigations. The replacement water molecules received a score of 0.50 when the water molecules are also taken into account 35.

The major phytochemical constituents are identified from the selected medicinal plants namely 4-hydroxylcinnamic acid, Kaempferol, Taraxerone, Delphinidin, Anaferine, Pelletierine, Eugenol, and Carvacrol the 3D structures of the active constituents are retrieved from PubChem Chemical databases and saved in. mol format.

The ligands are imported to the Workspace and preparation is done for docking studies. The Docking scores of the active Constituents are compared against the Standard drugs such as benzodiazepine, pramipexole, cariprazine and paroxetine obtained from the drug bank in mol format (https://pubchem.ncbi.nlm.nih.gov/).

As per docking software, both target and ligand structures were saved in dot PDB (.pdb) file format for a docking study using the software (Molegro virtual Docker 6.0 offline open-source software) (40). The molecular docking investigation was conducted using Molegro Virtual Docker 6.0, and the findings were compared (http://molexus.io/molegro-virtual-docker/, accessed on 26 September 2022), MVD 2013.6.0.1– 2013-12-13 academic license).

Analysis

Pose Organizer was used to see the returned postures from the docking engine. Pose organiser has the ability to dynamically load postures from a docking run, allowing users to explore thousands of ligands. More sophisticated re-raking calculations combined with binding affinity measurements were made while many energy terms and interactions were simultaneously examined 41.

When changing positions, electrostatic interactions and hydrogen bonds were dynamically updated. Selected ligands' MolDock scores were compared to those of the reference drug. The ligands with the highest binding affinity to the target protein are those with the lowest binding energy. The top ligands and potential lead molecules for a treatment for anxiety were those whose ligands displayed the highest MolDock scores 42.

RESULTS

Physicochemical, Drug-Likeness Properties and ADME properties

All the phytoconstituents from various medicinal plants that are 4-hydroxycinnamic acid, Delphinidin, Kaempferol, Taraxerone, Eugenol, Carvacrol, Anaferine and Pelletierine appears to follow all the five rules of Lipinski’s drug-likeness criteria (Table 2).

According to the data acquired from DruLiTo and Swiss ADME software, 4-hydroxycinnamic acid, Delphinidin, Kaempferol, Taraxerone, Eugenol, Carvacrol, Anaferine and Pelletierine also passed Veber’s rule, the blood-brain barrier (BBB) likeness rule was passed by all except 4-hydroxycinnamic acid and Taraxerone, the constituents also passed the Ghose filter except the phytoconstituents 4-hydroxycinnamic acid, Taraxerone and Carvacrol as shown in table 2.

The GI absorption was high in all the constituents except Taraxerone which showed low GI absorption. Only Taraxerone cannot cross the Blood Brain Barrier (BBB).

Eugenol, Carvacrol, Delphinidin and Kaempferol may produce the inhibition of CYP 1A2 as showed in table 2. All of the above findings indicate that all have a good potential drug-like molecule and a useful therapeutic agent against a variety of disorders including anxiety.

Table 2: Physicochemical, drug-likeness and ADME properties of anxiolytic compounds

Property	Anaferine	Carvacrol	Eugenol	Pelletierine	Delphinidin	Kaempferol	Taraxerone	4-Hydroxy cinnamic acid
Molecular weight (g/mol)	224.34	150.22	164.20	141.21	303.24	286.24	424.70	16.16
Hydrogen bond donors	2	1	1	1	6	4	0	2
Hydrogen bond acceptors	3	1	2	2	7	6	1	3
Rotatable bonds	4	1	3	2	1	1	0	2
Log P (Partition coefficient, Predicted value)	2.78	2.24	2.37	1.90	-2.79	1.70	4.55	0.95
Molar refractivity	74.01	48.01	49.06	45.37	78.20	76.01	133.92	45.13
Topological polar surface area in Å²	41.13	20.23	29.46	29.10	134.52	111.13	17.07	57.53
Lipinski's rule of five	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose filter	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Veber's rule	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
BBB likeness rule	Yes	Yes	Yes	Yes	Yes	Yes	No	No
GI absorption	High	High	High	High	High	High	Low	High
BBB Permeability	YES	YES	YES	YES	YES	YES	NO	YES
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.85

Toxicity Estimation

The endpoint of the oral rodent LD50 is the measure of the compound (chemical mass per rodent body weight) that destroys half of the rodents when administered orally. The oral rodent LD50 directed in four methods for the selected compound and the discoveries were relatively assessed. All phytoconstituents have been shown to have an acceptable toxicity limit as shown in Table 3 for drug production and preclinical and clinical appraisal. Developmental toxicity was performed in four approaches with all of the chosen compounds and the findings were comparatively analysed. Toxicity is indicated by a predicted value greater than 0.5. Except Anaferine all the other phytoconstituents shows developmental toxicity. Ames Mutagenicity was conducted in four methods for all of the chosen compounds and the findings were comparatively analysed in Table 3. Toxicity is indicated by a predicted value greater than 0.5. All the phytoconstituents except Kaempferol are not mutagens based on the results on the Ames mutagenicity as predicted by TEST software as shown in Table 3.

Table 3: Predicted value for Oral rat LD50 - Log10 (mol/kg), Developmental toxicity, Ames Mutagenicity

Method	Endpoint	Anaferine	Carvacrol	Delphinidin	Eugenol	4-Hydroxy cinnamic acid	Kaempferol	Pelletierine	Taraxerone
Consensus	Oral rat *LD50*	2.95	2.18	-	1.85	1.77	2.15	1.67	2.30
	Developmental toxicity	0.35	0.77	-	0.82	0.60	0.75	0.63	0.83
	Mutagenicity	-0.14	0.41	-	0.25	0.32	0.62	0.43	0.10
Hierarchical clustering	Oral rat *LD50*	2.78	2.21	-	1.94	1.78	2.24	1.86	2.73
	Developmental toxicity	0.28	0.9	-	0.87	0.56	0.98	0.82	0.78
	Mutagenicity	-0.26	0.48	-	0.16	0.3	0.58	0.53	0.21
Single model	Oral rat *LD50*	2.95	2.18	-	-	1.77	2.15	1.67	2.30
	Developmental toxicity	0.35	0.77	-	0.60	0.60	0.76	0.63	0.83
	Mutagenicity	-0.14	0.41	-	-	0.32	0.62	0.43	0.10
Group contribution	Oral rat *LD50*	2.95	2.19	-	-	1.77	2.15	1.57	2.30
	Developmental toxicity	0.35	0.77	-	-	0.60	0.76	0.63	0.83
	Mutagenicity	-0.14	0.41	-	-	0.32	0.62	0.43	0.10
Nearest neighbor	Oral rat *LD50*	3.13	2.15	-	1.76	1.76	2.07	1.49	1.87
	Developmental toxicity	N/A	1.00	-	1.00	0.67	N/A	0.67	1.00
	mutagenicity	0.00	0.33	-	0.33	0.33	0.67	0.33	0.00

In-silico studies of anxiolytic compounds

The ability of the phytoconstituents to bind with the targets is given in terms of MolDock Score. The MolDock Score is used as the parameter for analysing the docking results. The phytoconstituents are ranked according to their MolDock Score; rerank score and hydrogen bond interaction. The pose of the ligand which has least MolDock score shows a strong affinity towards its enzyme target. The ligand having the most elevated MolDock and re rank score shows a strong affinity towards its target receptor. In-silico docking analysis was performed for all 8 phytoconstituents such as 4-hydroxycinnamic acid, Delphinidin, Kaempferol, Taraxerone, Eugenol, Carvacrol, Anaferine and Pelletierine and Compared with Marketed drugs using Molegro virtual Docker on GABA (PDB ID: 4COF), Dopamine D2 (PDB ID: 6LUQ), Dopamine D3 (PDB ID: 3PBL) and serotonin (PDB ID: 6VRH) receptors.The pose is represented in ball and stick model along with the molecular weight and the amino acids in protein are represented in stick frame model with the residue numbers. As per the MVD software, the docking score is always expressed in a negative value, where a higher negative value indicates a better potency. The MolDock score of the ligands Eugenol, Carvacrol, Taraxerone, Pelletierine, Delphinidin, Anaferine, 4-hydroxy Cinnamic acid, Kaempferol and Benzodiazepine against GABA receptor was found to be -53.0226, -47.3339, -76.5405, -46.6556, -71.9564, -57.4265, -58.7002, -65.6419 and -37.7307 respectively shown in Table 4. For GABA MolDock score of Taraxerone, shows -76.5405 followed by Delphinidin shows -71.9564 which is higher than the other ligands and marketed drug benzodiazepine -37.7307, the docking pose seen in figure 1.

Table 4: Docking study of ligands on GABA receptor (PDB ID: 4COF) based on MolDock score

Name	Ligand	MolDock Score	Rerank Score	HBond
Eugenol	3314	-53.0226	-45.9211	-6.58917
Carvacrol	10364	-47.3339	-41.1804	-4.479
Taraxerone	92785	-76.5405	-56.3844	0
Pelletierine	92987	-46.6556	-42.0722	-4.02252
Delphinidin	128853	-71.9564	-61.329	-12.616
Kaempferol	5280863	-65.6419	-59.9399	-4.35857
Anaferine	443143	-57.4265	-47.1977	0
4-Hydroxy cinnamic acid	637542	-58.7002	-50.2235	-6.84347
Benzodiazepine	134664	-37.7307	-37.7199	-1.22276

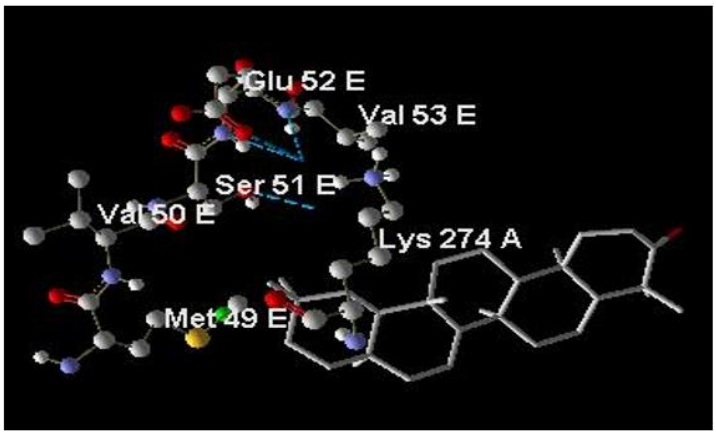

Figure 1: Docked View of Taraxerone against GABA Receptor Using Molegro Virtual Docker (MVD)

The MolDock score of the ligands Eugenol, Carvacrol, Taraxerone, Pelletierine, Delphinidin, Anaferine, 4-hydroxy cinnamic acid, Kaempferol and pramipexole against Dopamine D2 receptor was found to be -29.0959, -41.9634, -76.2877, -30.5221, -58.338, -59.0293, -58.3074, -46.7278 and -35.9252 respectively as shown in Table 5. For Dopamine D2 MolDock score of Taraxerone, shows -76.2877 followed by Anaferine shows --59.0293 which is higher than the score of marketed drug Pramipexole shows -35.9252, the docking pose seen in figure 2.

Table 5: Docking study of ligands on the D2 receptor (PDB ID 6LUQ) based on MolDock score

Name	Ligand	MolDock Score	Rerank Score	HBond
Eugenol	3314	-29.0959	-26.1966	-5
Carvacrol	10364	-41.9634	-36.4331	-2.5
Taraxerone	92785	-76.2877	-55.2484	0
Pelletierine	92987	-30.5221	-28.5189	-0.294833
Delphinidin	128853	-58.338	-50.6027	-2.28845
Anaferine	443143	-59.0293	-51.1436	0
4-Hydroxy Cinnamic Acid	637542	-58.3074	-49.2945	-4.31952
Kaempferol	5280863	-46.7278	-41.7466	-5.85606
Pramipexole	119570	-35.9252	-28.6598	-3.22329

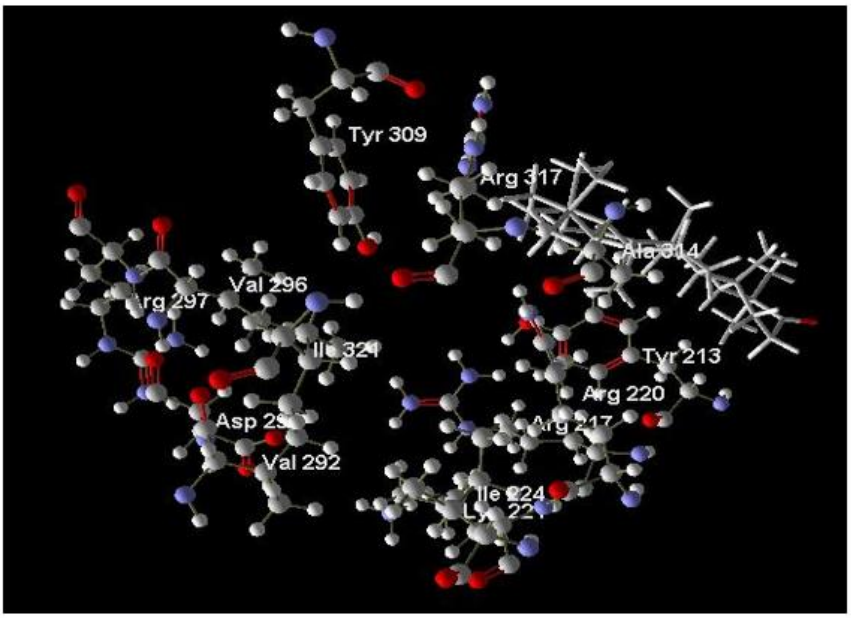

Figure 2: Docked View of Taraxerone against Dopamine D2 Receptor Using Molegro Virtual Docker (MVD)

The phytoconstituents such as Eugenol, Carvacrol, Taraxerone, Pelletierine, Delphinidin, Anaferine, 4-hydroxy cinnamic acid, Kaempferol and Cariprazine as standard drug, in silico docking analysis was performed against Dopamine D3 receptor was found as -68.999, -57.675, -92.8193, -60.3203, -70.0025, -76.5438, -74.4535, -55.326 and -103.838 respectively as shown in Table 6. For Dopamine D3 MolDock score of Taraxerone, shows -92.8193 followed by Anaferine shows -76.5438 when compared to the score of marketed drug Cariprazine shows -103.838, the docking pose seen in figure 3.

Table 6: Docking study of ligands on the D3 receptor (PDB ID 3PBL) based on MolDock score

Name	Ligand	MolDock Score	Rerank Score	HBond
Eugenol	3314	-68.999	-56.7551	-1.15184
Carvacrol	10364	-57.675	-49.4966	0
Taraxerone	92785	-92.8193	-66.3937	0
Pelletierine	92987	-60.3203	-52.9604	0
Delphinidin	128853	-70.0025	-7.01669	-4.56461
Anaferine	443143	-76.5438	-65.441	-0.00764301
4-Hydroxy Cinnamic Acid	637542	-74.4535	-49.7164	-5.11077
Kaempferol	5280863	-55.326	-51.9549	-2.5
Cariprazine	11154555	-103.838	-80.0919	0

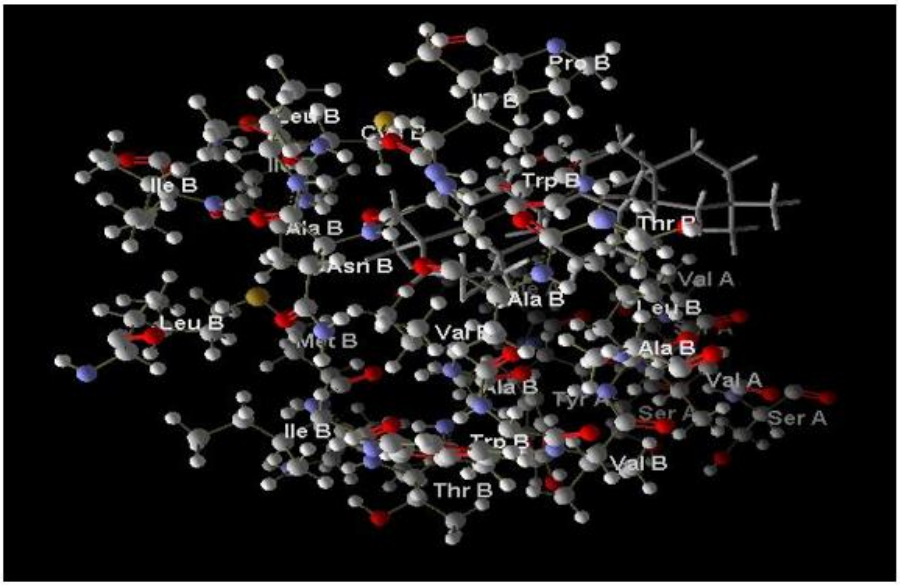

Figure 3: Docked View of Taraxerone against Dopamine D3 Receptor Using Molegro Virtual Docker (MVD)

The MolDock score of the ligands Eugenol, Carvacrol, Taraxerone, Pelletierine, Delphinidin, Anaferine, 4-hydroxy cinnamic acid, Kaempferol and paroxetine against serotonin receptor was found to be -65.8526, -63.1008, -71.8009, -57.4526, -76.8073, -73.6193, -78.0614, -81.0347 and -95.7425 respectively as shown in Table 7. For serotonin MolDock score of Kaempferol, shows -81.0347 followed by 4-hydroxy cinnamic acid shows -78.0614 which is nearer to the score of marketed drug paroxetine shows -95.7425, the docking pose seen in figure 4.

Table 7: Docking study of ligands on the serotonin receptor (PDB ID: 6VRH) based on MolDock score

Name	Ligand	MolDock Score	Rerank Score	HBond
Eugenol	3314	-65.8526	-56.5977	-2.5
Carvacrol	10364	-63.1008	-55.4394	-4.84736
Kaempferol	5280863	-81.0347	-73.5435	-5.633
Taraxerone	92785	-71.8009	-57.6135	0
Pelletierine	92987	-57.4526	-50.1954	-4.06586
Delphinidin	128853	-76.8073	-61.93	-13.2872
Anaferine	443143	-73.6193	-61.3025	-1.34695
4-Hydroxy Cinnamic Acid	637542	-78.0614	-66.2985	-7.48804
Paroxetine	43815	-95.7425	-71.7945	-2.35043

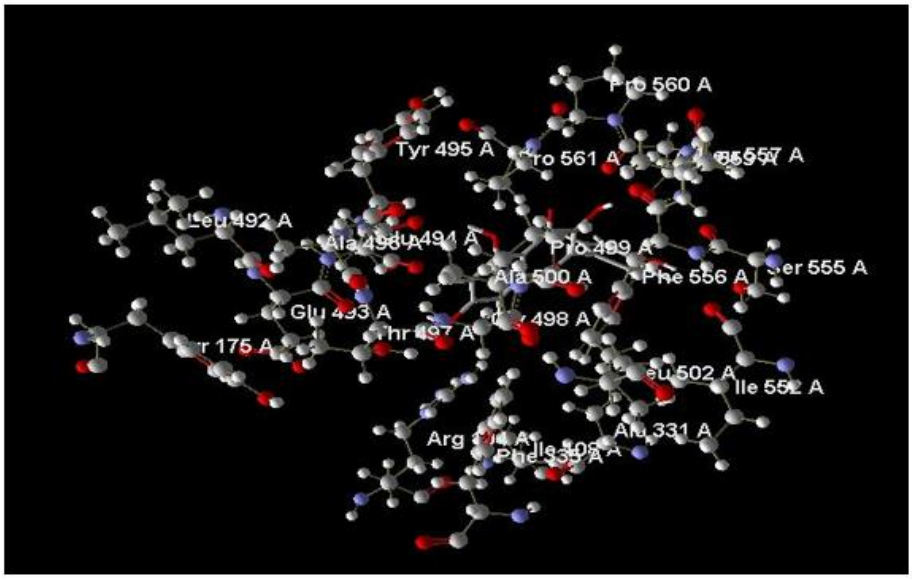

Figure 4: Docked View of Kaempferol against Serotonin Receptor Using Molegro Virtual Docker (MVD)

DISCUSSION

Benzodiazepines facilitate the inhibitory actions of GABA by binding to γ-amino butyric acid. Research has shown Benzodiazepine to cause sedation, psychomotor, cognitive impairment, Respiratory arrest, visual disturbances, incontinence and digestive disturbances 43. In neonates, less than 1% of patients experience laryngospasm and/or bronchospasm, ventricular arrhythmias including ventricular bigeminy or premature ventricular contractions, vasovagal syncope, bradycardia, or tachycardia 44. Pramipexole is a selective dopaminergic agonist with a minor agonistic activity at D2 receptors also used in treatment of anxiety. The adverse effects of pramipexole are attributed to both peripheral and central dopaminergic stimulation. They also cause Hallucinations and psychotic-like behavior, Dyskinesia and Postural deformity 45. Cariprazine significantly reduced drinking latency in the novelty-induced hypophagia test in wild-type mice, further confirming its antianhedonic-like effect and showing that it also has anxiolytic-like activity. But also shows some serious side effects that include orthostatic hypotension, Neuroleptic malignant syndrome, Low white blood cell count, High blood sugar and diabetes, Tardive dyskinesia 46. As an SSRI class drug, paroxetine's mechanism of action is to block the serotonin reuptake transporter (SERT) and thus increase the concentration of synaptic serotonin. It is used to treat depressive disorder, obsessive-compulsive disorder, and social anxiety disorder 47. Many of the side effects of paroxetine are dose-dependent. The side effects include drowsiness, tachycardia, vasodilation, sleep disturbance, and sexual side effects. The negative side effects of these pharmaceuticals promote the development of herbal medicines in complementary medicine and advise taking herbs regularly to prevent disorders like anxiety and other mental abnormalities that may be prevented by a healthy lifestyle 48.

In silico research has the power to quicken the pace of discovery while lessening the demand for expensive lab work and clinical trials 49. The benefit of using computational methods is that they can deliver new drug candidates more quickly and for less money which include Drug likeness, Toxicity estimation and Molecular docking

to choose the best drug candidate and carried to perform in vitro, in vivo studies easily 50. The 'drug likeness properties' of the phytoconstituents was evaluated according to the 'The Lipinski rule of five' and to develop them as potential lead compound for anti-anxiety activity. All the phytoconstituents are Eugenol, Carvacrol, Taraxerone, Pelletierine, Delphinidin, Anaferine, 4-hydroxy cinnamic acid; Kaempferol passes the drug likeness properties. All substances have been shown within limit toxicity of Oral LD50 which can be further taken for drug production and preclinical and clinical appraisal.

The phytochemical constituents Taraxerone which is present in medicinal plant Convolvulus prostratus Frossk (shankhpushpi) shows MolDock score of -76.5405, -76.2877 and -92.8193 against GABA, Dopamine D2 and Dopamine D3 receptor which is higher and nearer than to the standard drug benzodiazepine -37.7307, Pramipexole -35.9252 and Cariprazine shows -103.838 respectively. Taraxerone exhibits a good modulatory effect on the immune system and proves to be a potent drug for the treatment of many allergic disorders. Taraxerone is used as anti-parasitic, antifungal, allopathic, antibacterial (which is comparable to the activity of ampicillin against Escherichia coli and other strains), antioxidant, antitumor, and antiviral against herpes simplex viruses 51. It can prevent catalase and superoxide dismutase, and reduce glutathione concentration. The inhibitory effect of Taraxerone on nitric oxide generation was significantly more effective than that of caffeic acid and/or Gallic acid. Taraxerone exhibited comparable antioxidant capacities with butylated hydroxyl toluene (BHT) by the DPPH (p=0.117) and FRAP (p= 0.179) assays 52. Convolvulus prostratus Forssk, one such cognitive booster herb is mainly endowed with neuroprotective, nootropic and neuro modulatory activities 53. Besides, it also possesses several other therapeutic properties, antidiabetic and cardio protective activities 54. Therefore, maximum chances of Taraxerone to show anti-anxiety active since it is active constituent of Convolvulus prostratus Forssk.

For serotonin MolDock score of kaempferol, shows -81.0347 which is nearer to the score of marketed drug paroxetine shows -95.7425. Kaempferol has therapeutic effects on inflammation associated diseases 55, including allergies, arthritis, diabetes, cardiovascular diseases, cancers and neurological regression by inhibiting protein kinases and transcription factors 56. If there are chances to work on in vitro and in vivo activity of Kaempferol against anxiety disorder, more chances to get a good drug candidate without any side effects for the treatment of anxiety.

The eight phytoconstituents were docked against GABA, Dopamine D2, Dopamine D3 and Serotonin receptors. Taraxerone showed highest binding affinity when compared with standard drugs against GABA and Dopamine D2 receptor can be a good drug candidate and possess potential anxiolytic activity against anxiety disorder. Additional research can be done to determine the taraxerone's in-vitro and in-vivo anxiety activity as well as the pharmacokinetic characteristics of the phytoconstituents to learn about their absorption, distribution, metabolism, and excretion.

CONCLUSION

In the current work, we have included 4-hydroxycinnamic acid, delphinidin, kaempferol, taraxerone, eugenol, carvacrol, anaferine, and pellentine as eight phytoconstituents to evaluate their affinity for GABA, dopamine D2, dopamine D3, and serotonin receptors. Herbal treatments made from plants are becoming more and

more popular as a consequence of the introduction of synthetic drugs, which have a wide range of positive and negative therapeutic effects. In recent decades, several therapies for a variety of illnesses have either been approved or are undergoing clinical studies. Plant-derived compounds are essential for the treatment and prevention of many ailments, even though synthetic chemistry now controls the pharmaceutical research and manufacturing sector. In this study, eight ligands were investigated in order to determine the main ligand against anxiety disorder. The selection of the ligand was based on evaluating its receptor-binding affinity and contrasting its potency with that of the drugs used in the business. According to the study's findings, tartacerone may be used to treat anxiety if the intention is to inhibit GABA and dopamine D2 function. In vitro and in vivo experimental animal models of anxiety disorders may be used in future research to assess the efficacy of a potential treatment.

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