Current Trends in Biotechnology and Pharmacy
Vol. 15 (6) 83 - 89, 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2021.6.15

Molecular Docking Analysis of Natural Compounds Against Serotonin Transporter (SERT)

Surbhi Sharma[1], Shweta Dang[1]

[1]Department of Biotechnology,JIIT, NOIDA, 201309, India
*Corresponding author: Shweta.dang@jiit.ac.in

Abstract

Depression is one of the leading threats to the global health characterized by low mood and sadness and even loss in interest in daily routine activities. For treating depression, inhibition of serotonin transporter (SERT) can be considered as an effective way by the inhibitory ligands. Some of the commercially used drugs such as paroxetine, fluoxetine and sertraline and many more work on the similar principle of inhibiting the SERT receptor. There are various natural compounds which have been reported to show antidepressant effect with minimal side effects. In the present study, certain natural compounds and phytochemicals have been explored through the method of computer aided technique such as molecular docking. Docking was performed between the selected list of natural compounds and SERT and also with a standard drug paroxetine. Out of the compounds taken in the study, 6 compounds showed binding energy comparable to standard drug paroxetine and amongst them Withaferin A showed the highest binding affinity score (-8.74kcal/mol). Results predict that these compounds can be equivalently used as chemically synthesized in form of certain drug formulation against the treatment of depression after further experimental studies.

Keywords *Depression; Serotonin; Docking; Binding Energy; Natural Compounds*

Introduction

Depression and anxiety related disorders are one of the prevalent disorders and major cause of significant public health problem affecting more than 100 million people worldwide [1]. Depression these days is considered to be serious threat as it is known to be the primary reason for cognitive dysfunctioning leading to disease burden in world [2]. Depression most often occurs as comorbidity with certain other mental disorders like anxiety, bipolar disorder, anxiety deficit hyperactivity disorder (ADHD) [3]. It is recognized by low and sad mood, lack of interest in living life, lowered self-esteem, hopelessness and felling of unhappiness. In worst cases, a person may develop suicidal tendencies, directing the cause of more than one million deaths annually causing high morbidity rate [4]. According to the World Health Organization Global Burden of Disease, depression is predicted to become primary cause long-term incapacity by 2030 [2]. Although there is no such known reason for depression but scientist and researchers believe that genetic and environmental factors generally impact the development of these disorders [5].

Various studies show that pathological reason for depression and anxiety are disturbances in the level of neurotransmitters such as noradrenaline, dopamine, and serotonin [3]. Thus, the reliable and effective medication for depression is the intake of drugs that inhibit the reuptake of monoamines and enhance the level of monoamines in the synaptic cleft [6]. Moreover, drugs which inhibit the monoamine oxidase enzyme and hence, increasing the concentration of monoamines in the pre synaptic neurons have also proven to be effective antidepressant [7]. Serotonin is known for maintaining the chemical balance of the brain and modulating mood and cognitive behavior, memory, learning and various physiological processes. Serotoninergic neurotransmission plays important role in maintaining normal functioning of brain however, due to imbalance in serotonin concentration or transport defects, depressive psychological conditions can be caused [8]. As an effective treatment, targeting serotonin transporter (SERT) with inhibitory effects is considered as an effective approach to achieve antidepressant activity [9]. There are several FDA approved antidepressants drugs targeting serotonin receptor (SERT) known as selective serotonin reuptake inhibitors (SSRIs) includes drugs like paroxetine, fluvoxamine, citalopram and sertraline. The available synthetic SSRIs have large number of side effects for instance indigestion, feeling anxious, weight gain, sexual disfunctioning, sleeping disorders and can also show late onset of action [10]. Thus, there is a need to develop the therapeutics which could provide more effective treatment with reduced side effects. Recently, there has been inclination of natural compounds like curcumin, quercitin, naringenin, green tea bioactive compounds such as Epigallocatechin gallate (EGCG), active ingredients of lavender, withania somnifera and various other naturally derived compounds for the development of therapeutics for various psychiatric disorders such as depression, they also exhibit lesser side effects as compared to chemically synthesized drugs [11,12]. According to various experiments, phytomedicines have shown progressive results and known to be effective remedies than standard drugs used for psychiatric indications [12-14].

Development of drugs requires *in-vitro* studies prior to *in-vivo* experiments, which is quiet extortionate, time consuming and intensive work to perform for a number of less explored drug candidates. Hence, computer aided drug discovery techniques has been widely applied approach, as it improves the virtue of drug discovery and development [15]. Molecular docking is reported to be a very effective and important tool for predicting ligand and inhibitor binding with protein [16]. The software is based on the technique of fitting ligand into binding sites of target protein and generating ligand-receptor complex through the computational methods [17]. Docking can be extensively used for screening, analyzing and ranking the compounds virtually on the basis of score as they predict the binding efficiency of the ligand with the targeted protein and

how it inhibits the protein [18]. Types of bonds impacts on docking between ligand and receptor are - intramolecular (bond angle, bond width, and dihedral angle) along with intermolecular (H-bonding, hydrophobic bonds, electrostatic, dipolar, and van der Waals) forces [17]. Many docking studies have been performed where scientist and researchers have selected the natural compound and performed docking with the brain receptors such as serotonin transporter (SERT), 5-hydroxytryptamine receptors or Neurokinin 1 receptor (NK1R). A study was performed by Somayeh Zare *et al.*, in which derivatives of phenyl piperidine were selected as antagonists for dual NK1R and inhibitor of SERT and then QSAR (quantitative structure–activity relationship) analysis was performed. Molecular docking studies of all the derivatives on SERT and NK1R were performed, results were obtained and validated. Results validation implies that there were some important amino acids (Glu33, Arg26 and Asp395 for SERT and, Phe5, Lys7, Ala30, Tyr82 ans Asp31 for NK1R) at the active site cavity which led to effective interactions with the receptors [19]. In a study by Ravichandran Srividhya et al., bioinformatics tools were used and acetylcholine, donepezil and EGCG were docked with the enzyme acetylcholine esterase. EGCG was found to form good docking interaction with acetylcholine esterase. Thus, it was theorized that EGCG has neuroprotective efficiency and therefore helps in the cognitive functions of the brain [20]. In another study by Sabitu Babatunde Olasupo cheminformatics modeling, molecular docking was performed with some derivatives of Phenyl piperidine which have been reported to act as potential inhibitors of SERT to explain the interactions and binding efficiency between ligands and receptor to anticipate antidepressant effects of selected ligands [8].

In the present study, molecular docking were executed to analyze the interactions formed between the ligand and 3D- structure of the receptor. So as to perform this, various natural compounds and derivatives were docked with the receptor (PDB ID: 3GWW) which was obtained from pubchem and protein data bank (PDB) respectively. As a result, the details obtained from the following work performed can be considered as a definitive work template and rational for compound selection which could be further modified to develop a more targeting inhibitor of the serotonin transporter (SERT) and can be used as efficacious and novel antidepressant agent.

2. Materials & Computation Methods

2.1 Retrieval of Protein Structure

The 3D structure of Serotonin transporter (SERT) receptor of PDB code-3GWW has been retrieved through Protein Data Bank (PDB) using the address (http://www.rcsb.org/pdb) to study the interactions of various shortlisted natural compounds (ligands) towards the active site of the receptor along with their binding energy [21].

2.2 Ligand Selection & Retrieval

Through literature survey we identified ten natural compounds which belong to the class of Flavonoids, Isoflavones, Catechine, Phenolics families and can be used for the treatment of mental disorders such as depression [12]. We also selected an Food and Drug Administration (FDA) approved chemical drug known as paroxetine reported for the cure of depression [22]. The 3D structures of the selected natural compounds and paroxetine were obtained from the source i.e. PubChem database [23] and downloaded in SDF format. After downloading these are preferably changed into the PDB format by Open Babel version 2.4.1 software as PDB format is compatible to the docking software environment [24].

2.3 Prediction of Protein Active sites

Active Site of the SERT protein is predicted by the help of online CASTp webserver [25]. It provides the information of the amino acids that are involved in the active site of the protein molecule as well as active site's area, volume and pocket prediction. Further, mapping of active site residues on protein macromolecule was done in AutoDock tool version 4.2 to verify where these residues are exactly present.

2.4 Molecular Docking Procedures

Molecular docking is a software based technique through which we can analyze the interaction and binding efficiency between the targeted protein molecule and desired ligand. It also predicts the best conformation of the ligand which is known as "pose". The docking studies were implemented out by molecular docking tool known as AutoDock version 4.2 [26] Docking procedures involves some of the following steps- Such as ligand and protein molecule preparation, setting up grid around the active sites of protein and saving it as grid parameter file, preparing docking parameter file and then running autogrid and autodock and later, analyzing DLG files to get results.

In the present study, the crystal 3D structure of SERT (PDB ID: 3GWW) were obtained from protein data bank. For the preparation of protein molecule, firstly all the H_2O molecules were removed, and then the HETATM were selected & deleted. Polar hydrogen's were added and after then Kollman charges were identified and the protein file was saved as PDBQT file using MGLTOOLS 1.5.6 for further docking and analysis. List of natural compounds were selected as ligands for docking process as given in table1 along with their pubchem ID. All the ligands were prepared by choosing as input file and detecting the roots, choosing the torsions and saved as PDBQT format in AutoDock tool [27].

Table1. The 10 Natural compounds of phyto origin were obtained from literature survey.

Natural Compounds	Pubchem ID
Linalyl acetate	Pubchem CID- 8294
Linalool	Pubchem CID- 6549
L-Theanine	Pubchem CID- 439378
EGCG	Pubchem CID- 65064
Naringenin	Pubchem CID- 932
Resveratol	Pubchem CID- 445154
Withaferin A	Pubchem CID- 265237
Hypericin	Pubchem CID- 3663
Hyperforin	Pubchem CID- 441289
Piperine	Pubchem CID- 638024

In AutoDock 4.2, three different search algorithms can be performed, while in the present study the most commonly applied, Lamarckian Genetic Algorithm (LGA) was carried out [28]. In Lamarckian Genetic Algorithm, energy evaluations of maximum number (2,500,000) with 27000 maximum Generations of 150 population size were selected with 0.02 gene mutation rate along

with 0.8 crossover speed were also applied. Thereafter, grid maps of protein receptor were calculated and grid box was generated by AutoGrid tools. The formation of grid box was done in a manner so as all the active sites and also the surrounded area. A grid box of 54×54×54 in x, y, and z axis was created and placed in the middle of ligand in the protein ligand complex 0.375 Å spacing for 3GWW. The value of points for 3GWW was approximately 27.086, 23.513 and 23.2802. AutoDock Tools generates both grid file and docking parameter file known as gpf and dpf respectively which are confirmed through docking procedure [8, 19].

Cluster analysis was conducted for the results generated for all natural compounds in AutoDock tools as dpf files. For the comparative validation studies, structure of ligand paroxetine drug (Pubchem CID-43815) was docked with SERT (3GWW) which was treated in the same way as other ligands, was visualized and analyzed [8,19]. The existence of ligand-receptor interactions were determined and analyzed by the results of docking generated using AutoDock tools program (ADT, Version 1.5.6), Discovery Studio Visualizer 2020 [29] and PLIP (fully automated protein–ligand interaction profiler) [30]. These softwares visualize the bond such as hydrogen bonding, hydrophobic interactions, π-π stacking & π-cationic produced through the docking procedure.

3. Results & Discussion

3.1 Protein Structure Visualization

The structure of protein which was downloaded from PDB server with PDBID- 3GWW was visualized using VMD software version 1.9.3 and Discovery Studio Visualizer 2020 The structure consists of A chain with sequence length of 515 amino acids. The structure has some already bound ligands which were removed before docking procedures. Fig. 1(i) represents part the 3D structure of protein and 1(ii) shows the docking pose of its ligand fluoxetine.

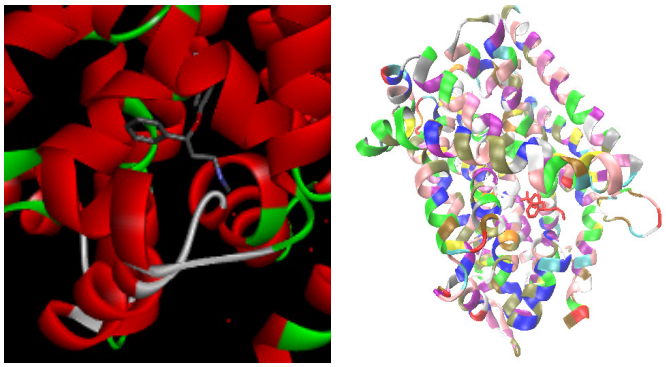


Fig.1 (i) Structure of the protein receptor with PDB ID -3GWW & (ii) Docking structure of bounded ligand (fluoxetine) to protein structure.

3.2 Active site prediction: Residues involved

The active sites of the targeted protein (3GWW) were determined by using CASTp web server. The shaded light blue color part is representing the active site residue. Many amino acid residues from chains- A are associated in binding with ligand as in fig.2. These amino acids residues are mapped on the structure which is helpful in determining the exact active site in the macromolecule. To further get the exact active site in relation to our studies, we observed the molecule in Discovery Studio Visualizer 2020 and mapped the only residues which were bonded to fluoxetine (antidepressant) in our protein model. We obtained the active site residues which were bonded to fluoxetine as ARG30, ASP401, LEU25, PHE253, ILE 111, and ALA319 as shown in fig.3. Similar residues were also observed from the literature provided for this structure.

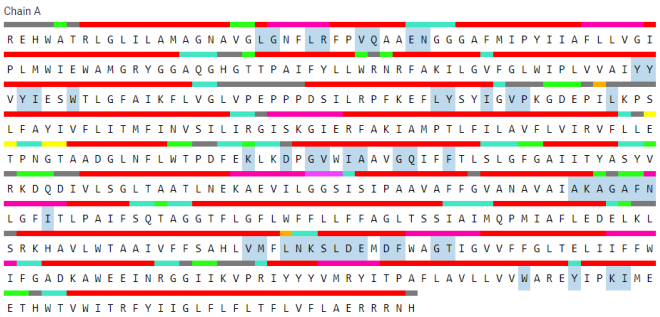


Fig.2 Active site residues predicted through CASTp. These are also mapped over protein structure to see the exact location of residues.

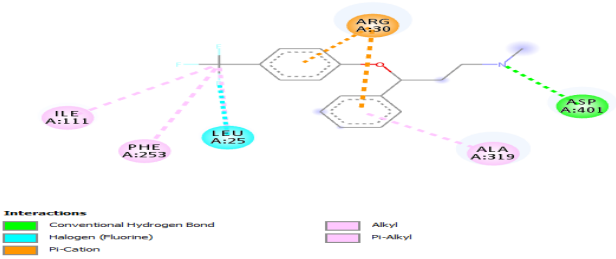


Fig.3 Amino acid representing active site of 3GWW and types of interaction formed.

3.3 Docking Studies

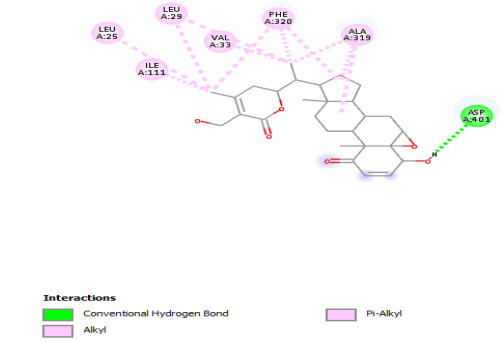
Docking was carried out for the selected ligands including all natural compounds and chemical drug paroxetine. The docking was run by considering the active sites of the protein molecule (PDB ID 3GWW) so as to explicate the precise ligand –receptor conformation and their binding interaction and efficiency. Active site plays an important role as this will determine that the selected compounds will bind to similar residues and would result into similar inhibitory effects. After running autodock, the generated ligand – receptor complex displays several numbers of poses and varied conformations. Different conformations have different binding energies (ΔG_{bind}) amongst which best pose can be selected as per the highest rank of binding energy. The bonding between residues of active site and protein consists of many types of interactions such as hydrogen bonding, hydrophobic bonding, Vander Waals forces, and various other interactions as predicted by Discovery Studio Visualizer and PLIV automated software. The binding affinity of all the ligands docked with protein molecule were in the range from − 5.05 to − 8.75 kcal/mol. Binding energy along with the corresponding types of interaction of the involved amino acid residues with the ligands are presented in Table2.

Most of the selected compounds gives ΔG_{bind} ranging between − 6.53 to − 8.75 kcal/mol, amongst which compound Withaferin A reflects the maximum binding efficiency i.e. -8.75 kcal/mol for best pose in docking with SERT receptor. The Withaferin A compound binding energy was compared to the standard drug (Paroxetine)

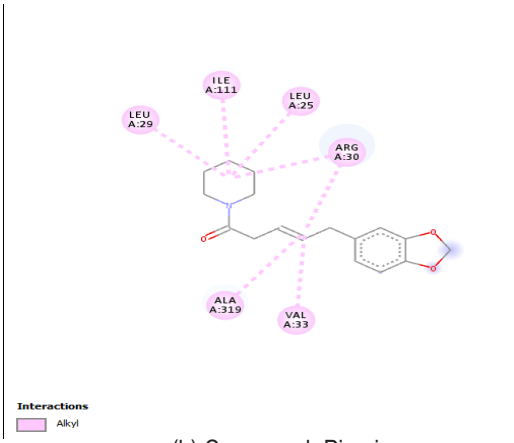
Table.2 Binding Energy and Amino acids residues involved in interactions with the Ligands and standard drug.

S.No	Name of Natural Compound/Standard Drug	Binding Energy(kcal/mol)	Amino acid residues Showing Interaction
1	Withaferin A	-8.75	ASP401,LEU25,LEU29,PHE320,ALA319,VAL33,ILE111
2	Piperine	-6.80	ARG30,LEU25,LEU29,ALA319,VAL33,ILE111
3	Hyperforin	-6.80	ARG30,ALA319,VAL33,ASP404,PHE405,ILE475,TYR471,TRP467
4	Naringenin	-6.62	ALA319,PHE320,LLEU162,VAL393,LEU400,SER399
5	Resveratol	-6.60	ARG30,LEU25, LEU29, ALA319,VAL33,ILE111,GLY26PHE253
6	Hypericin	-6.53	ARG30,ALA319,VAL33,GLU37,PHE405,TRP467,THR409,ILE249
7	Paroxetine (Standard Drug)	-8.05	ARG30,LEU25,LEU29,ALA319,PHE320,VAL33,GLN34, PHE253

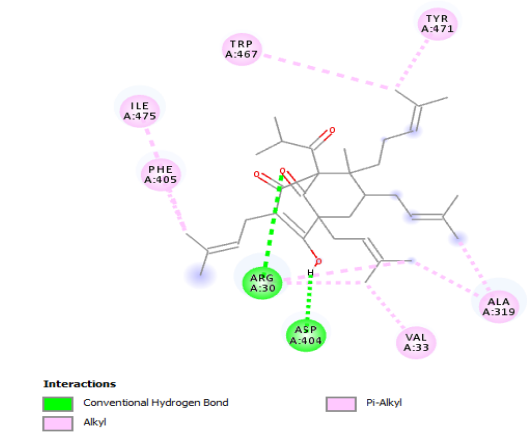
and it was observed that paroxetine has comparable binding energy (-8.05 kcal/mol) for its best pose than Withaferin A. This suggests that the selected compounds can also be considered as antipsychotic agents as they show good binding energy and have also proven to show lesser side effects as per literature and compound Withaferin A, does shows promising binding results from all other compounds as well as from standard drug paroxetine, so it can be said that it can act as better inhibitor for serotonin transporter (SERT) and can be used as an antidepressants targeting mood disorder such as depression and anxiety. All docking poses of selected ligands were precisely visualized and there after examined by Discovery Studio Visualizer software. The structure displays that mostly all the natural compounds get bonded to the active site region of protein. The structure of 2D interactions of receptor with 6 ligands which showed good binding (ΔG_{bind} − 6.53 to − 8.75 kcal/mol) and standard drug are shown in Figure 4. Ligand Withaferin A showed



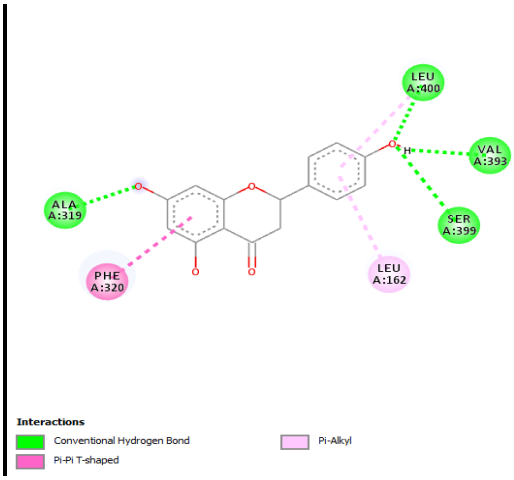
(a) Compound- Withaferin A



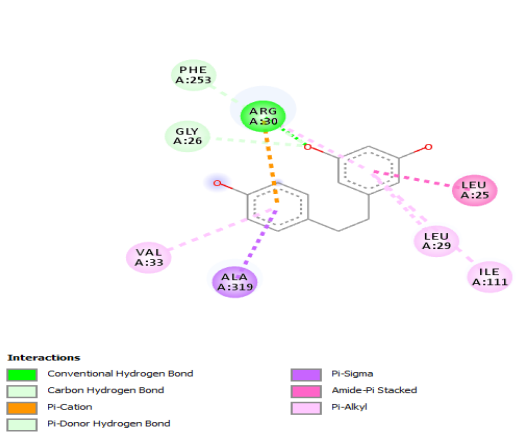
(b) Compound- Piperine



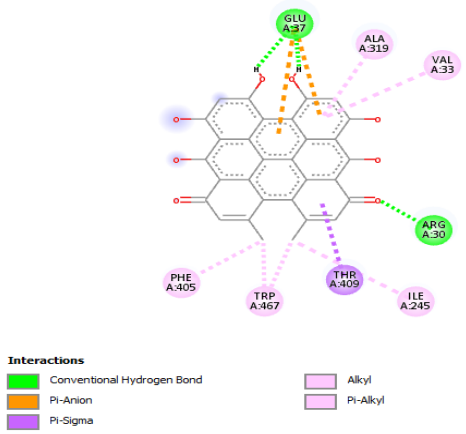
(c) Compound- Hyperformin



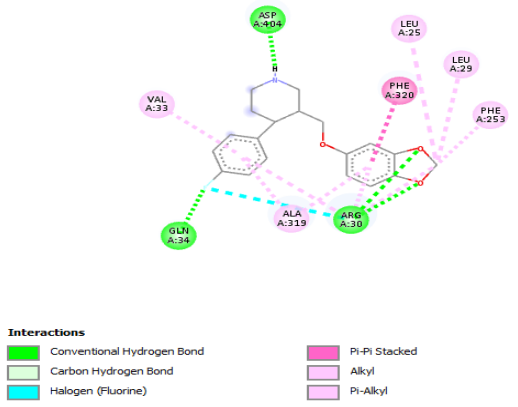
(d) Compound- Naringenin



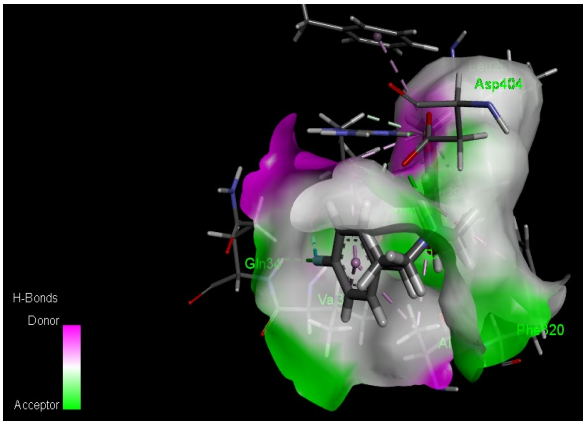
(e) Compound- Resveratol



(f) Compound- Hypericin



(g) Standard Drug- Paroxetine



(h) Docked structure of receptor interaction with Paroxetine

Fig4. (a, b, c, d, e, f and g) 2D structures of the amino acids of the receptor interacting with ligands and type of bonds formed between them and (h) Docked structure of receptor interaction with Paroxetine (standard Drug) Paroxetine representing hydrogen bond interactions.

the maximum binding energy i.e. -8.75 kcal/mol and is bonded to the amino acids residues present in the active sites, named as ASP401, LEU25, ALA319,ILE111 and also with VAL 33, LEU29 and PHE320 present in the surrounding of the active site residues. Hence, it can be concluded that ligand Withaferin A can be used as potential inhibitor of serotonin transporter (SERT).

4. Conclusion

Drug discovery is a very time consuming and expensive process as it demands extensive studies & research and millions to develop a safe and effective and bringing it to the market [17]. To reduce the effort in wet lab and to make it cost effective process, it is of great application to study the drug candidates and their binding towards receptor molecule using computer aided drug discovery techniques such as molecular docking software [31]. Drugs interact with receptors with high specificity and interactive manner. In this study, we docked the natural compounds with the serotonin transporter (SERT) receptor and standard drugs paroxetine which is commercially used for mental disorders such as depression by the help of AutoDock tool. Depression is considered to be the most prevalent and widely growing mental health concern worldwide. Large number of people is getting affected and many are resulting into suicides [3]. For the treatment, use of antidepressants such as Selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are currently used. These drugs have various side effects which can result into several other problems in patients such as weight gain, sexual dysfunction and sleep disturbances. [32]. Hence, it is important that researchers and scientists discover the natural compounds with more potential and minimum or no side effects at all and are also economical.

The hypothesis suggested in this study involves the screening and molecular docking for 10 naturally occurring ligands against SERT structure for observing its binding affinities and interactions virtually. As a result, the molecular docking studies shows the receptor (3GWW) & ligands (natural compounds) interactions with adequate binding energy ranges from -5.05 to -8.75kcal/mol. The screened compound Withaferin A has been predicted to show the highest binding affinity towards the SERT receptor and with binding energy - 8.75kcal/mol whereas Paroxetine energy is -8.05kcal/mol. As compound Withaferin A has higher binding affinity scores than paroxetine and rest all other compounds also have nearby binding energies it can be hypothesized that the phytochemicals

such as Withaferin A and various others can also be considered as an inhibitor against SERT for treatment for mental disorders rather than chemically synthesized drugs. Although to explore their antidepressant properties and better understanding of their onset of action and mechanism further experimental studies are required to be performed before using them for depression and anxiety disorders.

Acknowledgement

Authors would like to thank Jaypee Institute of Information & Technology, Noida for basic help and support.

References

1. F. Lin et al. (2017) Mechanism exploration of arylpiperazine derivatives targeting the 5- HT2A receptor by in silico methods. Molecules 22, no. 7, p. 1064.
2. Rinwa P, Kumar A, Garg S. (2013) Suppression of neuroinflammatory and apoptotic signaling cascade by curcumin alone and in combination with piperine in rat model of olfactory bulbectomy induced depression. PLoS One 8:e61052.
3. Surbhi Sharma and Shweta Dang (2020) Neuropsychological Disorders and their Nanocarriers. Current Pharmaceutical Design 26, 1-10.
4. Tong G-F, Qin N, Sun LW. (2017) Development and evaluation of Desvenlafaxine loaded PLGA-chitosan nanoparticles for brain delivery. Saudi Pharm J 25(6): 844-51.
5. O. Berton and E. J. Nestler, (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nature Reviews Neuroscience 7, no. 2, pp. 137–151.
6. R.H. Belmaker, G. Agam (2008) Major depressive disorder, N. Engl. J. Med. 358, 55–68.
7. F. Lopez-Munoz, C. Alamo, (2009) Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. Curr. Pharm. Des. 15, 1563–1586.
8. Sabitu Babatunde Olasupo , Adamu Uzairu et al. (2020) Profiling the antidepressant properties of Phenyl piperidine derivatives as inhibitors of serotonin transporter (SERT) via Cheminformatics Modeling, Molecular docking and ADMET Predictions. Scientific African vol 9, e00517.
9. Choudhury et al. (2018) Neurochemicals, behaviours and psychiatric perspectives of neurological diseases. Neuropsychiatry (London). vol. 8, no. 1, pp. 395–424.
10. Ferguson JM. (2001) SSRI antidepressant medications: adverse effects and tolerability. Primary care companion to the Journal of clinical psychiatry 3 (1):22-27.
11. Seyed Mohammad Nabavi et al (2015) Natural products, micronutrients, and nutraceuticals for the treatment of depression: A short review .Nutritional Neuroscience vol. 20, 180-194.
12. Gihyun Lee and Hyunsu Bae1 (2017) Therapeutic Effects of Phytochemicals and Medicinal Herbs on Depression, Hindawi BioMed Research International Vol 2017, Article ID 6596241, 11 pages.
13. Priyanka Dhiman (2019) Quercetin and Related Chromenone Derivatives as Monoamine Oxidase Inhibitors: Targeting Neurological and Mental Disorders, Molecules 24(3), 418.
14. M. Victoria Ruiz-Pérez (2012) Structural Perspective on the Direct Inhibition Mechanism of EGCG on Mammalian Histidine Decarboxylase and DOPA Decarboxylase, J. Chem. Inf. Model. 52, 1, 113–119.
15. Zhang S (2011) Computer-aided drug discovery and development. Methods Mol Biol 716: 23-38.
16. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. Curr Comput Aided Drug Des 7(2): 146- 157.
17. Rahul Ravichandran and Raji Sundararanjan (2017) in silico-based Virtual Drug Screening and Molecular Docking Analysis of Phytochemical-derived Compounds and F D A Approved Drugs against BRCA1 Receptor, J Cancer Prev Curr Res 8(2): 00268.
18. Morris GM, Wilby ML, (2008) Molecular Docking. Methods Mol Biol 443: 365-382.
19. Somayeh Zare et al. (2017) A comparative QSAR analysis and molecular docking studies of phenyl piperidine derivatives as potent dual NK1R antagonists/serotonin transporter (SERT) inhibitors, Computational Biology and Chemistry vol 67.
20. Ravichandran Srividhya et al. (2012) Impact of epigallo catechin-3-gallate on acetylcholine-acetylcholine esterase cycle in aged rat brain. Neurochemistry International vol 60, Issue 5, 517-522.
21. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN (2000) The Protein Data Bank. Nucleic Acids Research. 28(1): 235-242.
22. Michel Bourin (2001) Paroxetine: A Review. CNS Drug Reviews Vol. 7, No. 1, pp. 25–47.
23. Kim S, Thiessen PA, Bolton EE et al. (2016) PubChem Substance and Compound databases. Nucleic Acids Res 44(D1).
24. Boyle ONM, Banck M, James CA, Morley C, Vandermeersch T, et al. (2011) Open Babel: An open chemical toolbox. J Cheminform 3: 33.
25. Dundas J, Ouyang Z, Tseng J, Binkowski A, Turpaz Y (2006) CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic Acids Res 34.
26. Stefano Forli (2016) Computational protein-ligand docking and virtual drug screening with the AutoDock suite, Nat Protoc. 11(5): 905–919.

27. Morris GM, Huey R, Olson AJ (2002) Using AutoDock for Ligand-Receptor Docking. Current Protocols in Bioinformatics: John Wiley & Sons, Inc.

28 Morris GM, Goodsell DS, et al. (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function Journal of computational chemistry. 19(14):1639-62.

29. Accelrys (2016) Discovery Studio Visualizer Software. Version 16.1.0.15350.

30. Sebastian Salentin, PLIP: fully automated protein–ligand interaction profiler, Nucleic Acids Research, vol 43, W443–W447.

31. Assia Belhassan et al. (2019) Study of novel triazolo-benzodiazepine analogues as antidepressants targeting by molecular docking and ADMET properties prediction. Heliyon 5 e02446.

32. James M. Ferguson, (2001) SSRI Antidepressant Medications: Adverse Effects and Tolerability, Prim Care Companion J Clin Psychiatry 3(1): 22–27.