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Identification of potential monoamine oxidase inhibitor from herbal source for the treatment of major depressive disorder: An in- silico screening approach

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ABSTRACT

Major depressive disorder (MDD) is a mental disorder characterized by pervasive and persistent low mood associated with lack of interest, pleasure and self-esteem. The major reason for the occurrence of MDD. Monoamine oxidases (MAO) are belongs to the class of enzymes involved in the oxidative deamination of biogenic amines such as the neurotransmitters dopamine, norepinephrine and serotonin. The MAO- A activity is predominant in the brain particularly in the pathogenesis of MDD.MAO-A expression is elevated in patients with major depressive episodes MDE secondary to MDD. Current treatment strategy for MDD is achieved through MAO-A inhibitor (MAOIs) but often patient suffered with side effects like hypotension, anxiety, dizziness, weight gain and impotence. In the present study phytoconstituents like arecoline, apigenin, curcumin, kaempferol, luteolin and quercetin was selected for virtual screening against the target MAO-A enzyme. Computational biology tools like docking will be helpful in optimizing the leads and its binding efficacy towards amino acid residue present in the target enzyme. The energy value of docking between the target and phytoconstituents under investigation is compared with standard drug brofaromine which is was a potent inhibitor of enzyme MAO-A. Results obtained from the study projects that all the selected lead shows MAO-A inhibition potential in which luteolin,kaempferol and quercetin shows significant binding similar to that of standard drug. It was concluded that phytoconstituents from traditional medicine with interesting biological properties and structural diversity, have often served as valuable lead drug candidate for the treatment of MDD replacing the chemically synthesized drugs.

Keywords: Major depressive disorder, Phytoconstituents, Docking, brofaromine, Monoamine oxidase.

INTRODUCTION

Major depressive disorder (MDD) is a kind of psychiatric illness in which mood and behavioural pattern of the affected individual are impaired for long period of time.In the year 2000, the World Health Organization (WHO) identified MDD as the fourth ranked cause of disability and premature death in the world. The disorder is common in the United States, with a life time prevalence rate of 17 percent and a recurrence rate of more than 50 percent.It was further estimated that life time prevalence of MDD in child and adolescents was about 15-20%.[1].

Even oxidative damage to the proteins and nucleic acid in the CA1,CA3 dentate gyrus regions of hippocampus in brain may ultimately leads to MDD and other bipolar disorders[2].

Monoamine oxidase enzyme (MAO) found abundant in the central nervous system (CNS) on the outer mitochondrial membrane. It is exist in two forms MAO- A and MAO-B furtherMAO is of critical importance in CNS since too little or too much of these neuronal enzymes can affect the health of the individual[3].

Increase level of MAO activity ultimately leads to aging and other neuro degenerative disorders. Increased oxidation of dopamine, epinephrine, tyramine and tryptamine by MAO-A and MAO-B may generates free radicals which is capable of damaging the mitochondrila DNA of nearby dopaminergic neurons [4].

Central dopaminergic and noradrenergic systems play essential roles in controlling several forebrain functions. Consequently, perturbations of these neurotransmissions may contribute to the pathophysiology of neuropsychiatric disorders [5].

Inhibition of enzyme MAO significantly elevates the level of vital neurotransmitters which is essential for the clinical manifestation of the patients with MDD. Literature reviews are extensively supports that 5HT deficiency plays a role in depression [6].

Recent studies shows that monoamine oxidase-A inhibitor (MAOAI) plays substantial role in the current treatment strategy of MDD because antidepressants like MAOAI raise the levels of multiple monoamines which improves the essential brain monoamines and significantly alleviates the symptoms of MDD [7,8] . Selective serotonin reuptake inhibitors (SSRIs) are also used for the treatment of MDD but the major drawback behind SSRIs are it can only elevate the extracellular serotonin.

The leads of Central Nervous System (CNS) active medicinal plants, that have emerged besides Rawolfiaserpentina, Mucunapruriens for Parkinson's disease, Ocimumsantum as an antistress agent, Withaniasomnifera as anxiolytic, Centellaasiatica and Bacopamonneria for learning and memory disorders. Bacopamonneria and Ginkgo biloba for Alzheimer's disease. The study related to Alzheimer's disease (A.D) is focused towards the traditionally used rejuvenating and neurotonic agents[9]. The recent trends in the pharmacological studies are based on the biochemical and molecular mechanism that leads to the development of CNS active principles from the herbal drugs.

Phytoconstituents like arecoline, apigenin, curcumin, kaempferol, luteolin and quercetin have remarkable clinical efficacy in indian system of traditional medicine for the treatment of various diseases and disorders and also become the integral part of the man kind since years together. Curcumin has been shown to exert its activity against Alzheimer's disease through destabilization of amyloid beta protein [10]. Apigenin [11] ,kaempferol[12]and quercetin [13]are well established anti-epileptic agents.Similarly rececent studies shows that luteolin[14,15] and quercetin[16] act as a potent neuroprotective agents against multiple sclerosis. Arecoline is a naturally occurring alkaloid has a tendency to bind with muscarinic and nicotinic receptor [17]**.**

The challenge lies in optimizing the phytochemical lead molecule towards brain target is blood brain barrier. Achieving sufficient concentration of drug near the target seems to highly important hence the lead will become a hit only if it can able to cross or penetrate the blood brain barrier (BBB) system. Now days it's become mandatory for a computational biologist to look for BBB crossing potential of a drug candidate. The drug molecule has tendency to cross BBB is considered as BBB (+) and one which fails to cross is considered to be BBB (-).

Due to the rising ethical issues on the usage of laboratory animals against screening of drugs made researcher to acquire alternate high precision techniques like virtual screening. Molecular Docking continues to hold great promise in the field of Computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein. So result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 - 80% of ligands correctly [18]**.**

The main aim of the present study is to investigate the MAO inhibition potential of the selected leads molecule against MAO-A as protein target and to find out the hit with high inhibition potential and that can be utilized for the clinical management of MDD after through optimization.

MATERIALS AND METHODS

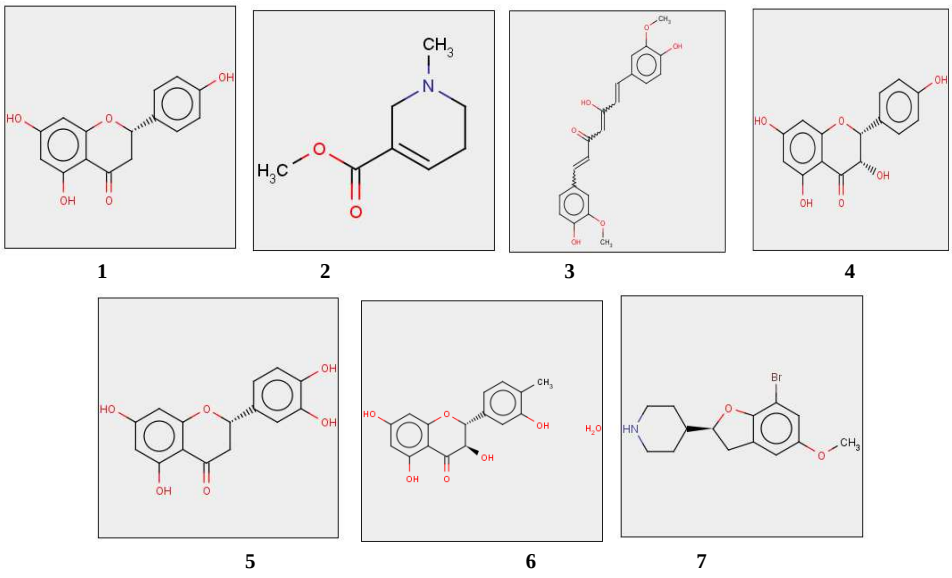
I.Software's required

Various computational tools and software's are used to analyze the protein MAO-A structure and to study the binding energy properties with Arecoline,Apigenin, Curcumin ,Kaempferol ,Luteolin ,Quercetin ,Brofaromine . Monoamine oxidase A (MAO-A) enzyme with pdb code 2Z5X sequence was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents at the active site 3D space of enzyme of interest MAO-A using online DOCKING SERVER web tool module.

II.Ligand preparation

The ligands such as Arecoline ,Apigenin, Curcumin ,Kaempferol ,Luteolin ,Quercetin and Brofarominewere built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

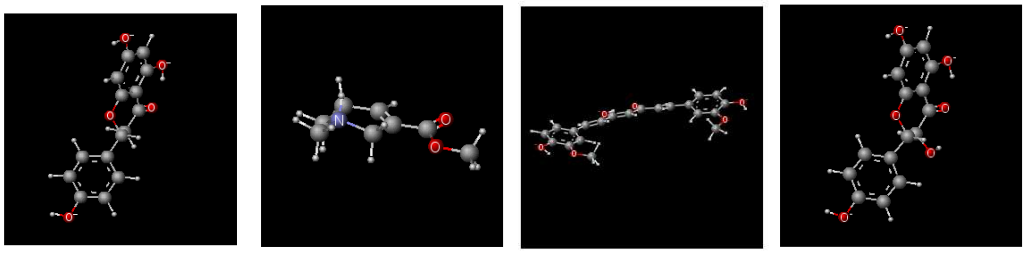
Figure 1 Showing 2D Structure of lead 1.Arecoline 2.Apigenin3.Curcumin 4.Kaempferol 5.Luteolin 6.Quercetin and 7.Brofaromine



1 **2** **3** **4**

5 **6** **7**

Figure 2 Showing 3D Structure of lead 1. Arecoline 2.Apigenin3. Curcumin 4.Kaempferol 5.Luteolin 6.Quercetin and 7.Brofaromine



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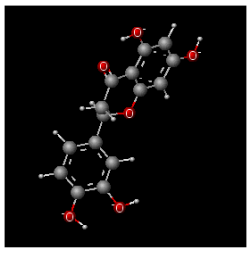

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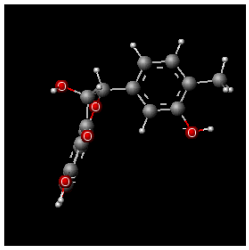

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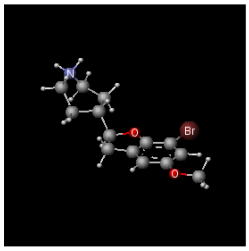

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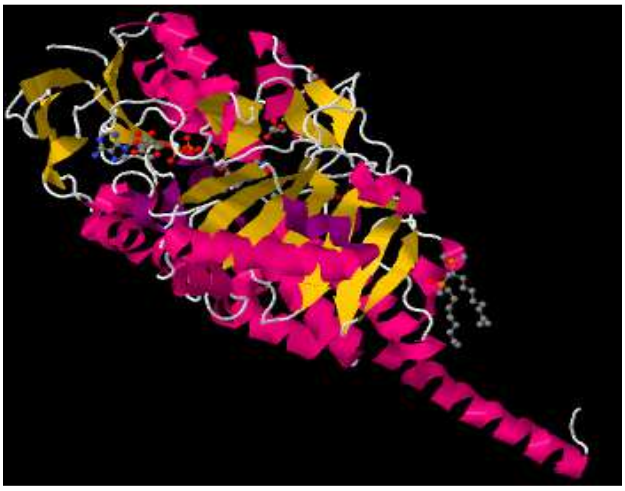
Table 1 Ligand Properties

Compounds	*molar weight*	*Molecular Formula*	*H Bond Donor*	*H Bond Acceptor*	*Rotatable bonds*	*Log P*	*pKa*
Arecoline	155.19	$C_8H_{13}NO_2$	0	3	2	0.17	6.84
Apigenin	270.24	$C_{15}H_{10}O_5$	3	1	1	1.22	8.23
Curcumin	368.38	$C_{21}H_{20}O_6$	2	6	8	2.85	7.8
Kaempferol	286.23	$C_{15}H_{10}O_6$	4	6	1	1.9	6.44
Luteolin	286.24	$C_{15}H_{10}O_6$	4	6	1	01.5	6.63
Quercetin	304.252	$C_{15}H_{10}O_6$	5	7	1	1.5	7.15
Brofaromine	310.18	$C_{14}H_{16}BrNO_2$	1	3	2	3.1	7.4

III. Protein preparation

The target protein human monoamine oxidase A (PDB Code: 2Z5X) was retrieved from protein Data Bank (www.rcsb.org) and crystallographic water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. As shown in Figure 3.

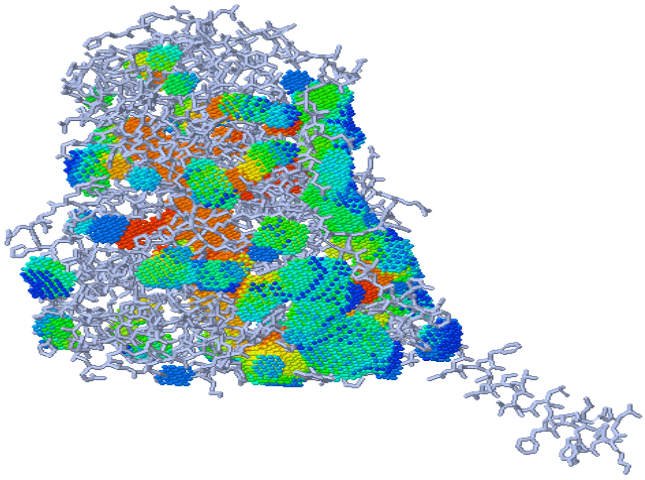
Figure 3 Target protein human monoamine oxidase A (PDB Code: 2Z5X)



IV. Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein.The potential ligand binding sites in MAO-A target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [19]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations[20, 21]. As shown in Figure 4.

Figure 4 Showing the possible ligand binding pockets on the surface of target enzyme MAO-A. Pockets calculated by GHECOM



V. Docking Methodology

Docking calculations were carried out using Docking Server[22, 23]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Arecoline ,Apigenin, Curcumin ,Kaempferol ,Luteolin ,Quercetin ,Brofaromineand their binding affinity towards the MAO-A (PDB 2Z5X)

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [24]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [25].

VI. Blood brain barrier crossing potential study

In order to predict the potential of the lead in crossing BBB,all the molecule were screened online by using BBB predictor. Molecule which crosses BBB considered as BBB (+) and one which fails to cross was found to be BBB (-).

RESULTS AND DISCUSSION

I. Docking scores

Interaction of ligand with the active amino acid residue of the protein plays a significant role in computer aided drug designing. In the present work, MAO-A enzyme which plays key role in the pathogenesis of MDD was docked with some of the important phytoconstituents derived mostly from the plant source. The different score such as binding free energy, inhibition constant, intermolecular energy and electrostatic energy values represented in Table 2.

The results showed that all the selected compounds showed binding energy ranging between -7.66 kcal/mol to -3.19 kcal/mol when compared with that of the standard brofaromine with binding free energy of -7.52 kcal/mol.Electrostatic energy (-0.29 kcal/mol to -0.02 kcal/mol) of the ligands also coincide with the binding energy. All the selected compounds contributed significant Monoamine oxidase-A inhibitory activity because of its structural parameters.

The docking calculations of all the six lead compounds at the active sites of MAO-A revealed that the compounds bound to the active site of enzyme with lower docking (D energy) when compared with standard brofaromine. The

best conformation was exhibited by luteolin with binding energy -7.66 Kcal/mol. The second best score was ranked by compound apigenin with binding energy -7.62 Kcal/mol when compared with standard brofaromine with binding free energy of -7.52 kcal/mol

Table 2 Summary of the molecular docking studies of compounds against MAO-A Enzyme

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki mM*/µM	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol
Arecoline	-4.36	382.54	-0.20	-5.27
Apigenin	-7.62	2.61	-0.08	-8.15
Curcumin	-3.19	12.99	-0.25	- 8.95
Kaempferol	-5.15	4.63*	-0.15	-3.98
Luteolin	-7.66	2.42	-0.07	-8.03
Quercetin	-4.36	636.60	-0.29	-5.17
Brofaromine	-7.52	3.06	-0.02	-8.12

Inhibition constant is directly proportional to binding energy. Inhibition constant ranges from (636.60 µM to 4.63 mM). Thus from the report it was clearly evident that all the phytoconstituents having promising MAO-A inhibition activity when compared to standard brofaromine with inhibition constant 3.06 µM.

Intermolecular energy of all six compounds ranging between -8.95 to -3.98 kcal/mol. Intermolecular energy is also directly proportional to binding energy. It was found intermolecular energy of all the selected compounds coincide with the binding energy.

II. Hydrogen bond interaction

By enlarging this interaction analysis the hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interaction of the compounds (Fig 5 - 11) was analyzed for possible involvement of hydrogen bond formation with amino acid residues on receptor protein surface.

Fig 5 :Hydrogen bond interaction between MAO-A2Z5X with Arecoline

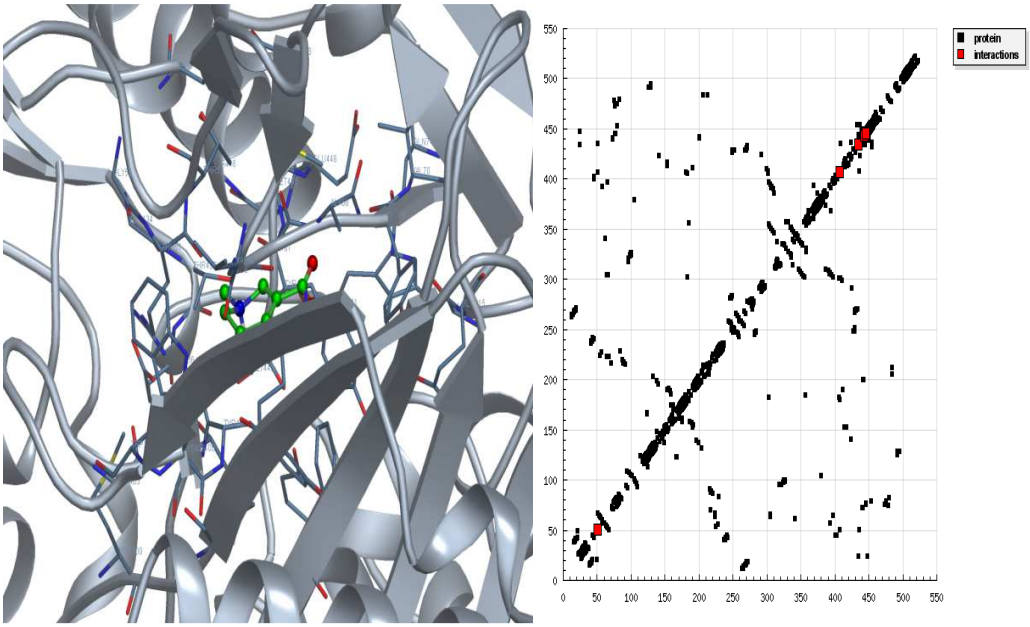


Fig 5: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Arecoline involved in hydrogen bond formation with aminoacids residues on the protein like 51 ARG,407 TYR,435 THR,443 GLY,445 MET. Total interaction surface of about 478.59.

Fig 6:Hydrogen bond interaction between MAO-A2Z5X with Apigenin

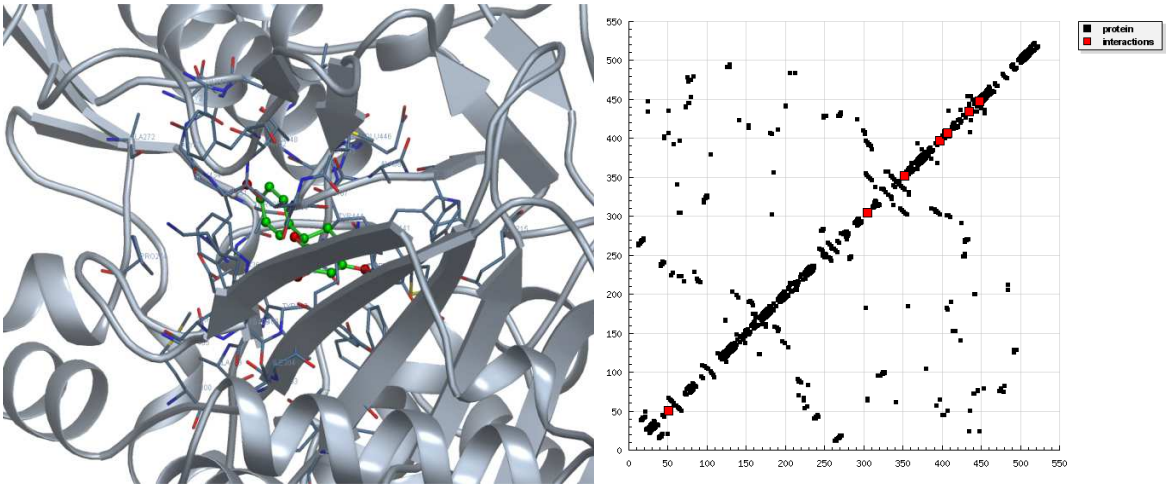


Fig 6: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Apigenin involved in hydrogen bond formation with aminoacids residues on the protein like 51 ARG,305 LYS,352 PHE,397 TRP,406 CYS,407 TYR,435 THR,448 ALA. Total interaction surface of about 670.36.

Fig 7:Hydrogen bond interaction between MAO-A2Z5X with Curcumin

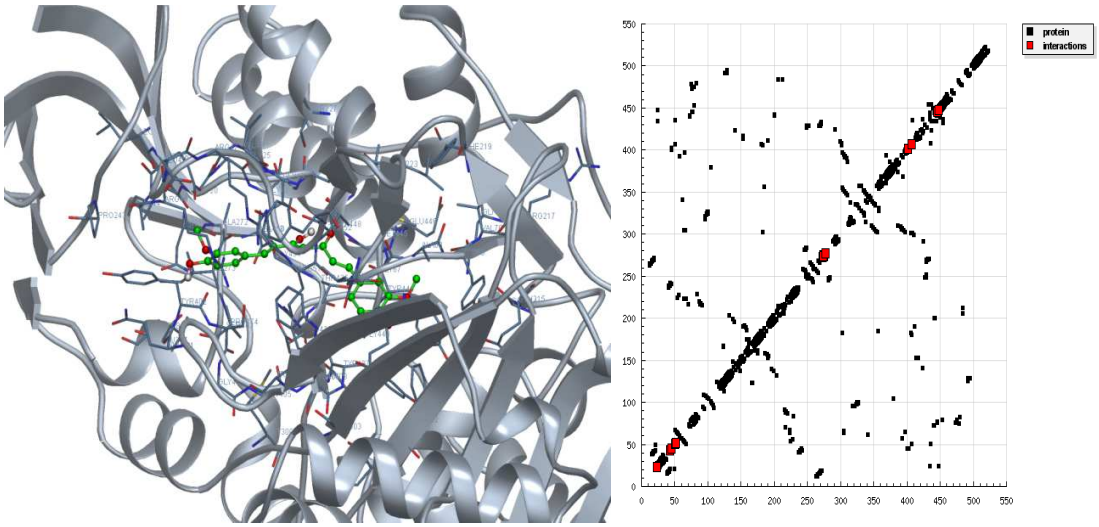


Fig 7: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Curcumin involved in hydrogen bond formation with aminoacids residues on the protein like 23 ILE, 43 GLU,45ARG,51 ARG,52THR, 273 ILE,274 PRO,277 LEU,402 TYR,407 TYR,445 MET,448 ALA.. Total interaction surface of about 978.61.

Fig 8:Hydrogen bond interaction between MAO-A2Z5X with Kaempferol

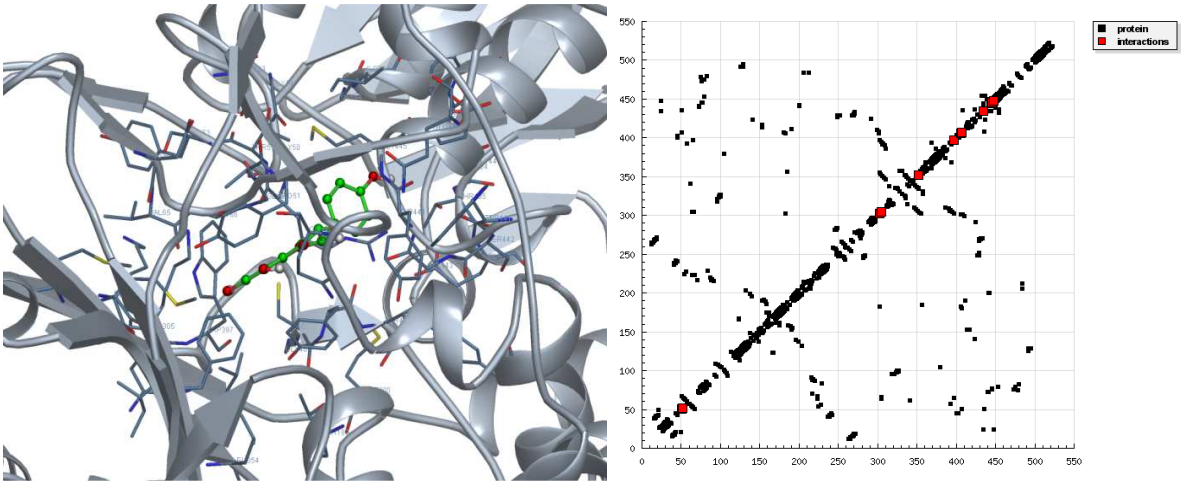


Fig 8: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Kaempferol involved in hydrogen bond formation with aminoacids residues on the protein 51ARG,52 THR,303 VAL,305 LYS,352 PHE,397 TRP,406 CYS,407 TYR,435 THR,444 TYR,445 MET,448 ALA. Total interaction surface of about 624.69.

Fig 9:Hydrogen bond interaction between MAO-A2Z5X with Luteolin

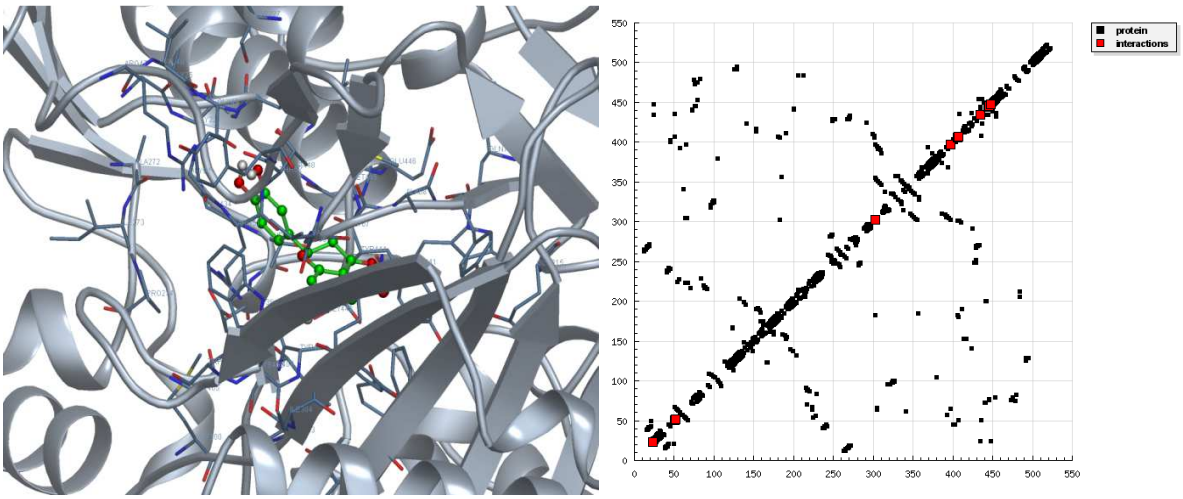


Fig 9: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Luteolin involved in hydrogen bond formation with aminoacids residues on the protein 23 ILE,51 ARG,52 THR,303 VAL,397 TRP,406 CYS,407 TYR,435 THR,444TYR, 445 MET,448 ALA .Total interaction surface of about 693.38.

Fig 10:Hydrogen bond interaction between MAO-A2Z5X with Quercetin

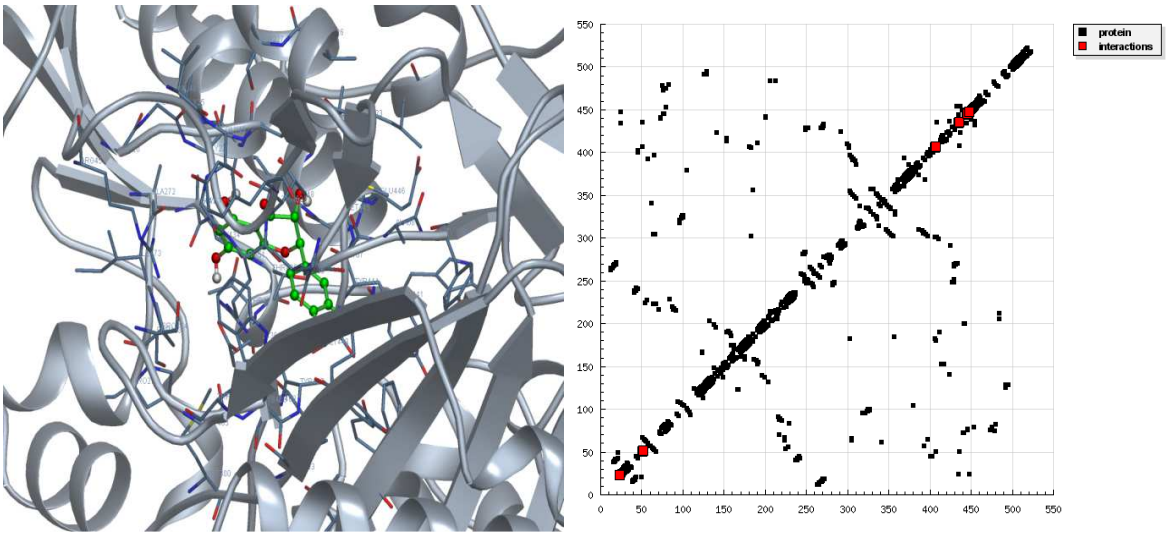


Fig 10: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Quercetin involved in hydrogen bond formation with aminoacids residues on the protein 23 ILE,51 ARG,52 THR,406 CYS,407 TYR, 435 THR,436 GLU,445 MET,448 ALA .Total interaction surface of about 666.93.

Fig 11:Hydrogen bond interaction between MAO-A2Z5X with Brofaromine

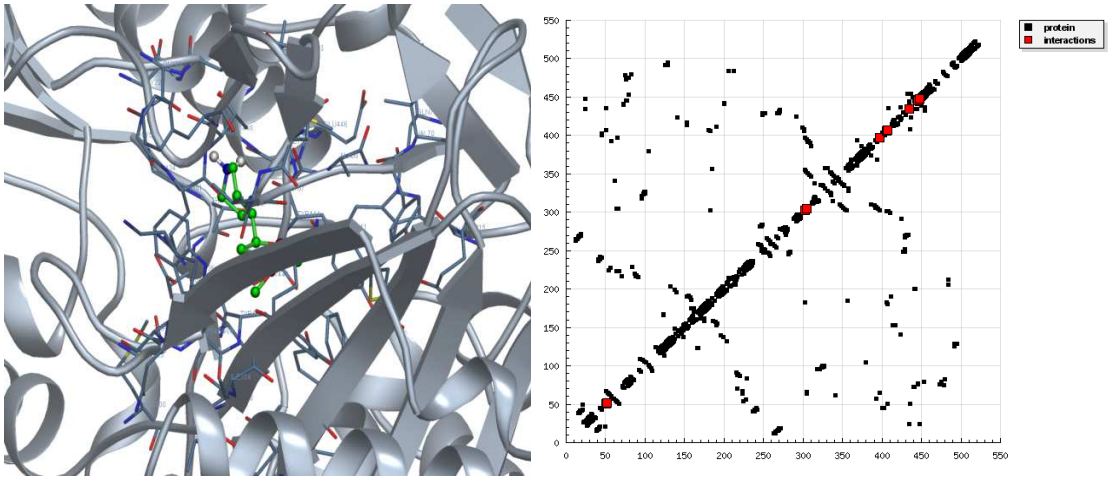


Fig 11: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme MAO-A (2Z5X) and the ligand Brofaromine involved in hydrogen bond formation with aminoacids residues on the protein 51 ARG,52 THR,303 VAL,305 LYS,397 TRP,406 CYS,407 TYR,435 THR,445 MET,448 ALA .Total interaction surface of about 622.36.

The result obtained from the hydrogen bond interaction study shows that the phytoconstituents such as luteolin,kaempferol and quercetin possess great MAO-A enzyme inhibition activity by binding with the active site pocket on target protein. Further these compounds may have a direct action on target enzyme by binding to the potentially active amino acid residue in the same way as that of the standard brofaromine as listed in the Table 3.

Table 3 Interaction of lead compounds with active site amino acid residue of MAO-A Enzyme

Compounds	Target binding Amino acid residue
Arecoline	51 ARG,407 TYR,435 THR,443 GLY,445 MET
Apigenin	51 ARG,305 LYS,352 PHE,397 TRP,406 CYS,407 TYR,435 THR,448 ALA
Chlorogenic acid	22 GLY,24 SER,43 GLU,45ARG,51 ARG,273 ILE,274 PRO,277 LEU,403 SER,407 TYR, 445 MET,448 ALA.
Curcumin	23 ILE, 43 GLU,45ARG,51 ARG,52THR, 273 ILE,274 PRO,277 LEU,402 TYR,407 TYR,445 MET,448 ALA.
Kaempferol	51ARG,52 THR,303 VAL,305 LYS,352 PHE,397 TRP,406 CYS,407 TYR,435 THR,444 TYR,445 MET,448 ALA
Luteolin	23 ILE,51 ARG,52 THR,303 VAL,397 TRP,406 CYS,407 TYR,435 THR,444TYR, 445 MET,448 ALA
Quercetin	23 ILE,51 ARG,52 THR,406 CYS,407 TYR, 435 THR,436 GLU,445 MET,448 ALA
Brofaromine	51 ARG,52 THR,303 VAL,305 LYS,397 TRP,406 CYS,407 TYR,435 THR,445 MET,448 ALA

III. Permeability toward BBB

Results obtained from the BBB prediction study shows that all the phytochemical leads selected for the study will have tendency to cross the BBB and thereby increased drug concentration may achieved on the target side and may improves the effecting binding of the lead with the target enzyme in-vivo.The results are tabulated in in the Table 4.

Table 4 BBB crossing potential and BBB score of lead molecule

Name of the Lead	BBB (+) / BBB (-)	BBB Score
Arecoline	BBB (+)	0.841
Apigenin	BBB (+)	0.184
Curcumin	BBB (+)	0.325
Kaempferol	BBB (+)	0.226
Luteolin	BBB (+)	0.154
Quercetin	BBB (+)	0.086
Brofaromine	BBB (+)	0.288

CONCLUSION

In conclusion, the results obtained from the current investigation clearly demonstrated the in silico molecular docking studies of brofaromine and selected phytoconstituents with MAO-A enzyme exhibited binding interactions and warrants further studies needed for the development of potent MAO inhibitors for the treatment of MDD. Further computational screening shows that all these leads have high potential in crossing BBB and reach the target site with marginal bioavailability upon administration.

These results clearly indicates that the leads especially luteolin,kaempferol and quercetin shows similar binding sites and interactions with MAO-A enzyme compared to the standard drug brofaromine. This in silico studies is actually an added advantage to screen the potential lead against MAO-A inhibition activity. Now a day's phytoconstituents from the natural derivatives may serve as therapeutic leads in the development of clinically effective MAO inhibitor. Further investigations on the above compounds on preclinical and clinical studies are necessary to develop potential drug entity for the treatment of mental disorders like MDD.

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