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VIRTUAL SCREENING OF *GINKGO BILOBA* FOR THERAPEUTIC POTENTIALS AGAINST PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder that affects 2% of the population older than 60 years. Monoamine Oxidase B (MAO-B) inhibitors improve the symptoms of Parkinson's disease and can delay the progress. Inhibition of MAO-B, further prevent breakdown of dopamine in the brain and reduce the motor symptoms associated with PD. *Ginkgo biloba* has a number of therapeutic properties and contains phytonutrients that helps in improvement of neurological disorders. In present study, phytonutrients of *Ginkgo biloba* namely Myricetin, Quercetin, Isorhamnetin, Kaempferol, Ginkgolides A-C, and Ginkgolide J were selected for Molecular docking against Monoamine Oxidase-B enzyme. The Molecular Docking studies were performed using Autodock 4.2 and interaction between MAO-B and compounds were analyzed. The efficiency of the compound was screened based on the binding energy existing between the protein and inhibitor. The docking studies show that the phytochemicals of *Ginkgo biloba* against MAO-B were quite effective. The potential compound can be subjected to further clinical trials and can be an alternative in the future treatment of Parkinson's disease.

Keywords: Virtual Screening, Molecular Docking, Auto Dock, *Ginkgo biloba,* Phytonutrients.

1. INTRODUCTION

The age dependent neurodegenerative diseases include Parkinson's disease and Alzheimer's disease (Arumo *et al*., 2003), which are caused by genetic and environmental influences (Jenner and Olanow, 1998) and lead to the accumulation of protein aggregation thereby causing oxidative stress and inflammation (Behl, 1999). Abnormal action of the monoamine oxidase B isoform has been associated with neurological dysfunctions including parkinson´s disorder and alzheimer´s disorder whereas the monoamine oxidase A isoform seems to be associated with psychiatric considerations including depression and cardiac cellular degeneration (Bortolato *et al*., 2008). MAO-B inhibitors are used for the treatment of Parkinson's disease and for symptoms associated with Alzheimer's disease (Binda *et al*., 2004; Terud and Langston, 1989). In the present work, our purpose was to distinguish correct poses of inhibitor in the binding pocket of monoamine oxidase B and to predict the affinity between the inhibitor and monoamine oxidase B.

In other words, in this study docking procedure describes a process by which two molecules fit together in three-dimensional space (Kitchen *et al*., 2004). Computer aided drug design is an applicable method that can study these interactions and describe significant characteristics for monoamine oxidase binding site recognition (Delogu *et al*., 2011; Harkcom and Bevan, 2007). Extracts of *Ginkgo biloba* leaves produce reversible inhibition of rat brain monoamine oxidase (MAO) (White *et al*., 1996). Mao inhibition was due to the phytonutrients present in *Ginkgo biloba*. The computer aided drug design is an attempt to study the interaction of phytonutrients with MAO-B which in turn helps to treat the symptoms of Parkinson's disease.

The use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process (Schoichet, 2004; Koppen, 2009). There is a wide range of software packages available for the conduct of molecular docking simulations like, Auto Dock, GOLD, and FlexX (Collignon *et al*., 2011). Auto Dock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Dykstra, 2007). Docking is applied to predict the binding orientation of small molecular drug candidates to protein targets, subsequently predicting the affinity and activity of the drug candidates (Goodsell, 2009; Morris *et al*., 2009). Docking is often applied to predict binding affinities of drug candidates in virtual screening experiments and in considering structure-activity relationships to prioritize synthesis of new drugs (Wu *et al*., 2003). The present study deals with the examination of the

interactions between potentials from *Ginkgo biloba* and MAO-B protein by molecular docking method in order to calculate the minimum binding energy (kcal/mol) between them. Molecular docking determines the binding affinity between the protein and ligands which aims to determine the 3D conformation and binding interactions.

2. MATERIALS AND METHODS

2.1. Protein structure

The high-resolution crystal structure of monoamine oxidase-B, co-crystalized with its irreversible inhibitor 6-hydroxy-N-propargyl-1(R)-aminoindan, was obtained from the Protein Data Bank (PDB entry code 1S3E, 1.6A° resolution). The study was carried out on only one subunit of the enzyme protein (Yelekçi *et al*., 2007) shown in **Figure 1**. The water molecules were removed during modeling. The energy minimized protein structure was included prior to docking to accommodate hydrogen atoms.

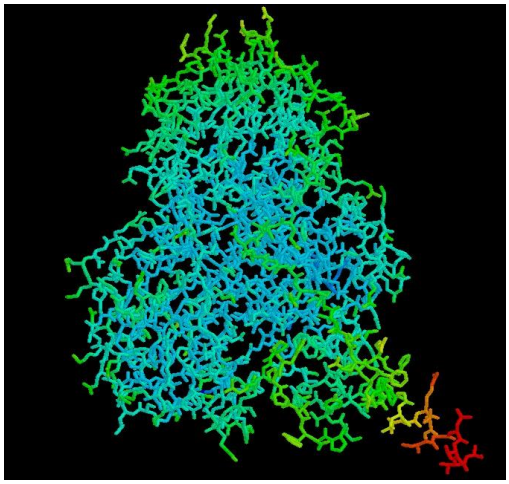


Fig. 1. 3D structure of Monoamine oxidase – B molecule (PDB ID: 1S3E)

2.2. Phytochemicals

The structures of phytochemicals namely Myricetin, Quercetin (Oyama *et al*., 1994), Ginkgolide A, Ginkgolide B, Ginkgolide C, Ginkgolide J, Bilobalide (Teris, 2002), kaempferol, Isorhamnetin (Xu *et al*., 2012) used in this study were retrieved from Pubchem compound database. The 2D structures of molecules were converted to 3D structures using Open Babel software (O'Boyle *et al*., 2011). These phytochemicals satisfied Lipinski's rule of 5 and ADME properties.

2.3. Binding site Prediction

The binding site in MAO-B was determined using Computer Atlas of Surface Topology of Proteins (CASTp) (Dundas *et al*., 2006). CASTp helps in identifying the geometric properties of protein pockets which are assumable positions on protein surface. The residues within the binding site were identified. Potential active site of protein calculated by CASTp in Fig. 2. Showed there are several pockets which fit in the role of active site.

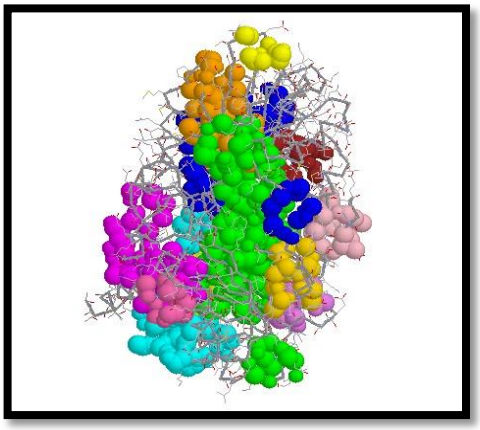


Fig. 2. Active sites predicted in the MAO-B using CASTp server

2.4. Molecular docking

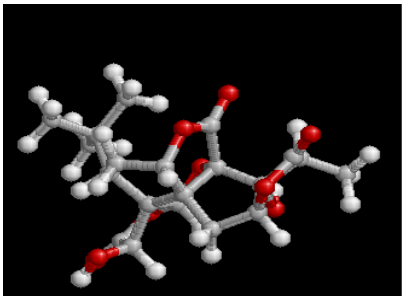
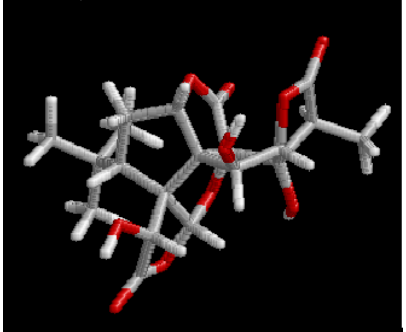
Molecular docking combined with a scoring function can be used to screen potential drugs insilico to identify molecules that are likely to bind to protein target of interest. To perform the docking model, the Auto Dock 4.2 suite molecular-docking tool was used and the methodology was followed (Gowthaman *et al*., 2008). AutoDock was employed to perform a docking simulation using a Lamarckian genetic algorithm (Morris *et al*., 1998). Auto Dock 4.0 is widely distributed molecular docking software which performs the flexible docking of the ligands into a known protein structure. The default parameters of the automatic settings were used. Each docking experiment consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations. The size of the grid box is key parameter in Auto Dock. The volume of the box was fixed to 27000Å to have large search space. The Auto Dock results indicated the binding position and bound conformation of the protein, as well as hydrogen bond interactions between the protein and ligand molecule. The docked conformation which had the minimum binding energy was selected to analyze the mode of binding.

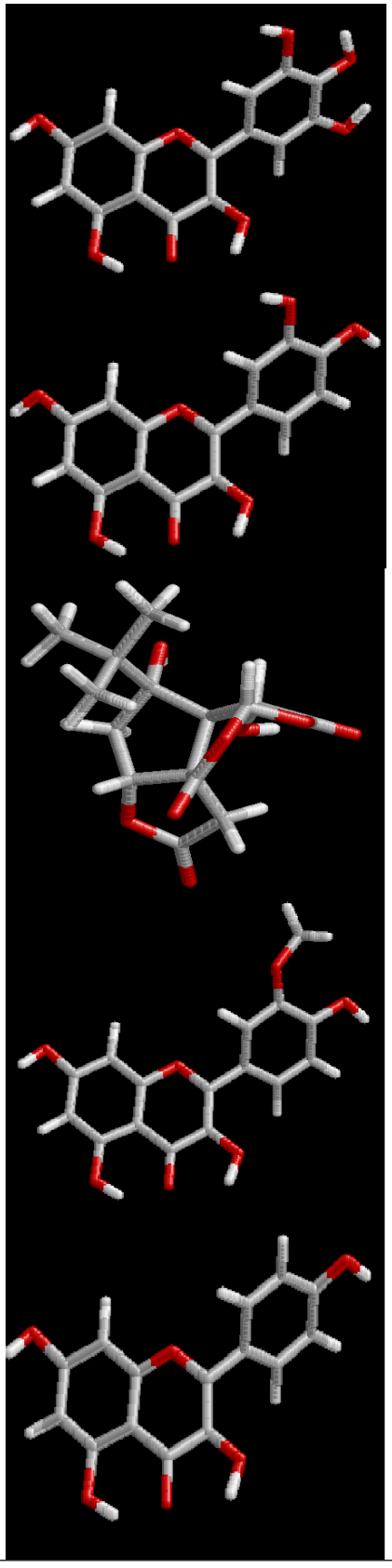
3. RESULTS AND DISCUSSION

The phytochemicals were docked using Auto dock 4.2 successfully. The interactions and binding energy of the phytochemicals are listed in Table 1. Good interactions were observed between the amino

acid residues of the protein and phytochemical molecules. The phytochemicals showed binding energy between -7.78 to -8.70 kcal/mol. The results were analyzed based on the binding energy of the complex. The number of H-bonds was calculated between atoms of protein-ligand docked complex. Quercetin and Kaempferol illustrate high affinity for the protein molecules with score of –8.70 and -8.68 shown in Fig. 3 and 4. Quercetin and Kaempferol showed six and five hydrogen bond interactions with MAO-B respectively. Quercetin illustrated interactions with GLN206, GLY434, SER59, TYR435 and TYR60 residues of the protein. Kaempferol showed five hydrogen bond interactions with SER59, TYR60, TYR188, and GLY434 residues of the MAO-B protein and bound to the active sites. The compounds were bound to the active site of the MAO-B receptor.

Table 1. Docking results of *Ginkgo biloba* phytochemicals with MAO-B protein.

Phytochemicals	Compound structure	Binding energy kcal/mol	Hbonds	Residues interacting with ligand (Hbonds)
Ginkgolide A		-8.33	2	SER59, LYS296
Ginkgolide B		-8.65	4	SER59, LYS296, GLN206, TYR398
Ginkgolide C		-7.78	3	SER59, LYS296, TYR398
Ginkgolide J		-8.57	3	LYS296, TYR398, TYR60

Myricetin		-8.06	3	ILE14, TYR398, TYR60
Quercetin		-8.70	6	GLN206, GLY434, SER59, TYR435, TYR60
Bilobalide		-8.42	2	SER59
Isorhamnetin		-8.50	2	TYR398, TYR60
Kaempferol		-8.68	5	SER59, TYR60, TYR188, GLY434

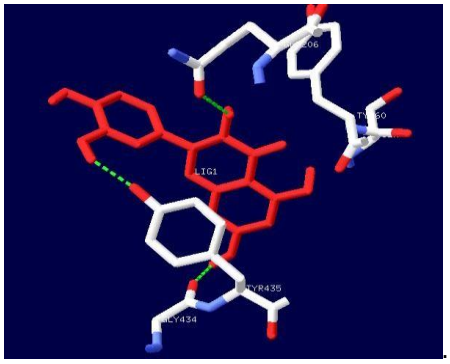


Fig. 3a. Quercetin interacting with residues of MAO-B protein

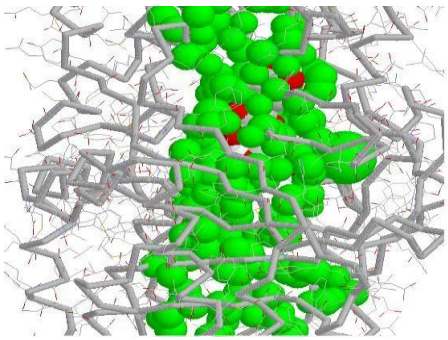


Fig. 3b. Quercetin bound to the active site of protein molecule.

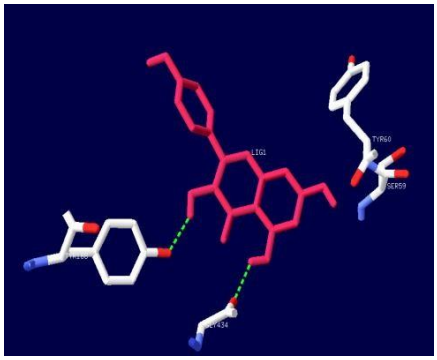


Fig. 4a. Kaempferol interacting with residues of MAO-B protein.

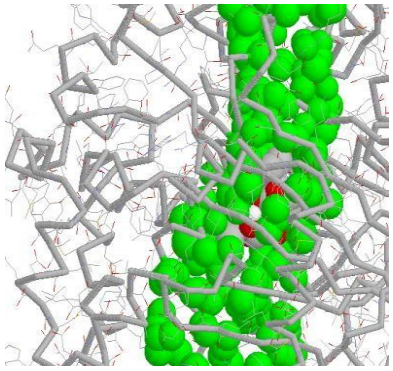


Fig. 4b. Kaempferol bound to the active site of protein molecule.

The screening of phytochemicals from *Ginkgo biloba* against MAO-B is carried out using molecular docking methods. Screening of Phytonutrients compounds showed the binding affinity towards MAO-B receptor. The Quercetin and Kaempferol were screened with least binding energy of – 8.70 and -8.68 and were selected as Lead molecule. The molecular docking of the two compounds showed the binding mode and interaction energy. H-bond pattern was analyzed and confirmed the inhibition of MAO -B target and show the molecular activity of phytochemicals. This work based on Insilico studies, concluded that Quercetin and Kaempferol possess better activity against MAO-B. Further In vivo studies on these compounds can be done to confirm the inhibition and used in the treatment of Parkinson's disease.

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