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Short Communication

Molecular docking studies of bacoside from *Bacopa monnieri* with LRRK2 receptor*

Chakresh Kumar Jain[1]**, Aman Gupta[1], Ashish Tewari[1], Vanashika Sharma[1], Vipul Surender Kumar[1], Ashwani Mathur[1] & Sanjeev Kumar Sharma[1]

[1] *Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62, Noida – 201301, Uttar Pradesh, India; e-mail: ckj522@yahoo.com*

Abstract: Bacosides, constituents of *Bacopa monnieri* (Linn.), are reported to be potential therapeutic saponins in the cure of Parkinson's disease (PD). However, detailed mechanism for control of PD by bacosides is not well documented. PD has been reported to be caused by genetic mutations in leucine-rich repeat kinase 2 (LRRK2) leading to higher kinase activity that has been identified as a major cause of familial PD. The LRRK2 was thus proposed as an important marker in the pathogenesis of PD. This suggests that inhibition of LRRK2 holds promise as a potential treatment for PD. Our study focuses on the possible application of bacoside A constituents as potential inhibitors of LRRK2. In this work, we have carried out the *in silico* molecular docking studies of bacoside A constituents with LRRK2, proposing their role as an inhibitor in PD. The study has revealed the significant interactions between bacosaponin and LRRK2 having ten H-bonds at receptor-ligand site with binding affinity –7.5 kcal/mol. Hence, amongst the studied triglycosidic saponins, bacosaponin was analyzed to be a better ligand, proposing it to be a major constituent in inhibiting enzymatic activities of mutated LRRK2.

Key words: Parkinson's disease; bacoside A; saponin; *Bacopa monnieri* L.

Abbreviations: LRRK2, leucine-rich repeat kinase 2; MVD, Molegro virtual docker; PD, Parkinson's disease; YFP, yellow fluorescent protein.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease that is characterized by the progressive loss of dopaminergic neurons in the substantia nigra leading to the major clinical and pharmacological abnormalities of PD (Dauer & Przedborski 2003; Schulz & Falkenburger 2004). Based on genetic, neuropathological, and biochemical data in patients and experimental animal models, dysfunction of the ubiquitin-proteasome pathway, protein aggregation, mitochondrial dysfunction, oxidative stress, activation of the c-Jun N-terminal kinase pathway, and inflammation have all been identified as important pathways leading to excitotoxic and apoptotic death of dopaminergic neurons (Schulz & Falkenburger 2004). Current PD medications treat symptoms without any significance on dopaminergic neuron degeneration. The main obstacle to developing neuroprotective therapies is a limited understanding of the key molecular events that provoke neurodegeneration (Dauer & Przedborski 2003). Most of the chemical drugs, used for treatment of symptoms of PD, *viz.* levodopa, dopamine agonists, catechol-o-methyltransferase inhibitors and nondopaminergic agents, are associated with side effects (Jankovic & Aguilar 2008).

Based on ancient Indian traditional medicine system, Ayurveda, some plants have specific influence on brain function (Vollala et al. 2010 and reference therein). *Bacopa monnieri*, one such popular herb well-known as a memory booster, belongs to the Scrophulariaceae family. Locally known as brahmi or Jalbrahmi in India, the herb has been in use for centuries in Ayurvedic medicine as a brain tonic, memory enhancer, anti-anxiety, cardio-tonic, antidepressant and anticonvulsant agent. Several clinical studies reporting about antiinflammatory, analgesic and antipyretic properties have lead to confirmation about beneficial actions of *B. monnieri*. The pharmacological effect of *B. monnieri* is due to alkaloids, saponins and sterols compounds. Various alkaloids have been isolated including 'brahmine', nicotine, herpestine and D-mannitol.

Ongoing research on *B. monnieri* extracts and isolated bacosides have lead to building up of information citing neuropharmacological effects. These pharmaco-

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** Corresponding author

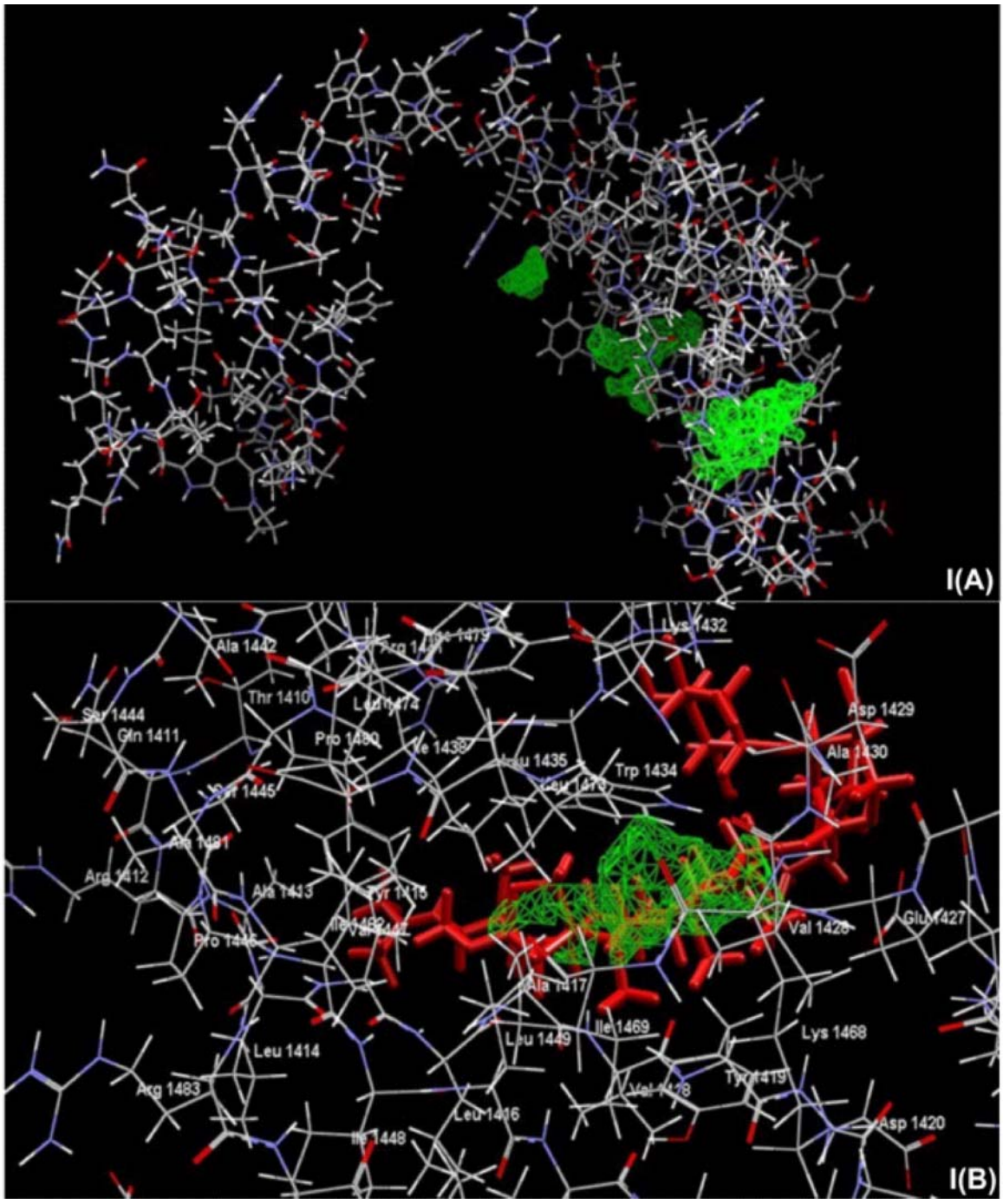

Fig. 1. Snapshots of docking of LRKK2 protein receptors (Parkinson's disease) and their respective drug Bacosaponin from *Bacopa monnieri*. Part I(A) – the active site or cavity in LRKK2 receptor; part I(B) – red colour represents Bacosaponin drug molecule.

logical actions are mainly attributed to the saponin compounds present in the extract of the plant. The initial chemical investigations described the occurrence of two saponins, designated as bacosides A and B (Sivaramakrishna at al. 2005). Several reports have emphasized the therapeutic application in PD of *B. monnieri* constituents. Neurodegeneration is the death of dopaminergic neurons which results in the cognitive and motor impairment. The disease has no complete cure till date (Singh & Dhawan 1997).

Recently, the neuroprotective effect of *B. monnieri* constituent in the treatment of PD has been demonstrated in the model system of *Caenorhabditis elegans* expressing human α-synuclein [NL5901 (Punc-54::alphasynuclein::YFP+unc-119)] (Jadiya et al. 2011). The mutation in leucine-rich repeat kinase 2 (LRRK2) leads to the implications of PD effecting the functionality of specifically α-synuclein and tau, thereby involved in the emergence of PD (Cookson 2010). The inhibition of LRRK2 has been reported one of the recent therapeutic strategy against PD and evidenced with protective effect in both the *in vitro* and *in vivo* models of LRRK2-induced neurodegeneration (Byoung et al. 2010).

Table 1. Output of molecular docking score with respect to energy, binding affinity and number of H-bonds interactions.

Molegro virtual docker			Ligand Scout		
Protein receptor	Drug (ligand)	Cavity volume (Å^3)	Mol dock score – energy (arbitrary unit)	Binding affinity) (kcal/mol)	Number of interactions (H-bond)
LRKK2	Bacopasaponin	53.736	−139.918	−7.5 approx.	10
	Bacopaside X	53.736	−118.869	−11.225	09
	Bacoside A3	53.736	−152.372	−4.90	06

Our work focuses on demonstrating the comparative binding pattern of different constituent of *B. monnieri* with LRKK2 as a drug target, hence understanding the possible interactions and/or mechanism of action and portraying bacoside A constituent as a potential inhibitor of LRRK2 in PD curing.

Material and methods

This study is primarily concerned with the *in silico* molecular docking of bacoside A, constituents from *Bacopa maoneri* L. with LRRK2 receptor (PDB code: 3D6T; Deng et al. 2008) proposing their role as an inhibitor in PD. These constituents are tested *in silico* for drug-likeness, toxicity and other pharmacological properties and then docked with the protein via sophisticated docking software.

The results were analyzed in terms of energies or "poses" to give the best five poses, which bind satisfactorily to the target protein. These molecules can then be further analyzed *in vitro* and *in vivo* for confirming, evaluating and testing the drug's properties. Following is a step-by-step working of the *in silico* drug design process carried out in this study.

Docking

The three-dimensional structure co-ordinates of LRRK2 receptor were retrieved from the Protein Data Bank (Berman et al. 2000). Docking was done using Molegro virtual docker (MVD; Thomsen & Christensen 2006) utilizing the largest cavity (on the basis of volume). MVD, commercial high-capacity docking software, was used to load the files representing the drug target and ligands. The MolDock scoring function (MolDock Score) used by MVD is derived from the piecewise linear potential scoring functions to approximate the energies, originally proposed by Gehlhaar et al (1995) and later extended by Yang et al. (2004). The target protein was analyzed for cavities, which can also be done online via castP software (Dundas et al. 2006). Fixing a suitable cavity, the ligand was docked to the particular region on the target and the docking result obtained was saved. The energy vs. conformations graph, the MolDock score representing the binding energy and the hydrogen bonding energy score are of particular importance in analyzing the docking result. The docking result was loaded onto the same workspace containing ligand and target cavity. This displays the ligand bound to the cavity in different conformations or "poses".

Study of ligand-substrate interaction

Interactions of different poses of ligands was investigated on LigandScout 3.03b software package (Wolber & Langer 2005). Pharmacophores corresponding to all the drugs were studied and differences between interactions of drug-drug targets were identified. Pharmacophores are ensemble of universal chemical features that characterize a specific mode of action of a ligand in the active site of the macromolecule in three-dimensional space. Chemical features are, e.g., hydrogen bonds, charge interactions and hydrophobic areas.

Results and discussion

Bacosides, constituent of *B. monnieri*, ares to be potential therapeutic saponins in the cure of PD, which has been reported to be due to genetic mutations in LRRK2 leading to higher kinase activity, being identified as a major cause of familial PD (Kruger 2008).The LRRK2 was thus proposed as important marker in the pathogenesis of PD. This suggests that inhibition of LRRK2 holds promise as a potential treatment for PD.

In this work, we have carried out the molecular docking studies of bacoside A constituents (Bacopasaponin, Bacopaside X and Bacoside A3) with LRRK2 receptor (Fig. 1) taking various parameter in consideration (binding affinity, Mol dock score) (Table 1). We have also performed Ligandscout, which revealed the significant interactions between bacoside A constituents and LRRK2 receptor (Fig. 2), proposing their role as an inhibitor in PD.

Bacopasaponin, Bacopaside X and Bacoside A3 ligands were docked with LRRK2 receptor. The binding affinity for these ligands was –7.5 approximately, –11.225 and –4.90 kcal/mol, respectively. The energy between ligands and the proteins is given in Table 1. Interaction between ligand and receptor was done by using a sophisticated interaction software Ligandscout which represents interaction in term of hydrogen binding. Bacopasaponin, Bacopaside X and Bacoside exhibited 10, 9 and 6 interaction with LRRK2 receptor, respectively as shown in Table 1 and Figure 2.

Our study has revealed the significant interactions between Bacopasaponin and LRRK2 having ten H-bonds at receptor-ligand site with binding affinity –7.5 kcal/mol approximately. Hence, amongst the studied triglycosidic saponins, Bacopasaponin was analyzed to be a better ligand, proposing it to be a major constituent in inhibiting enzymatic activities of mutated LRRK2, thus could be a potential drug for PD.

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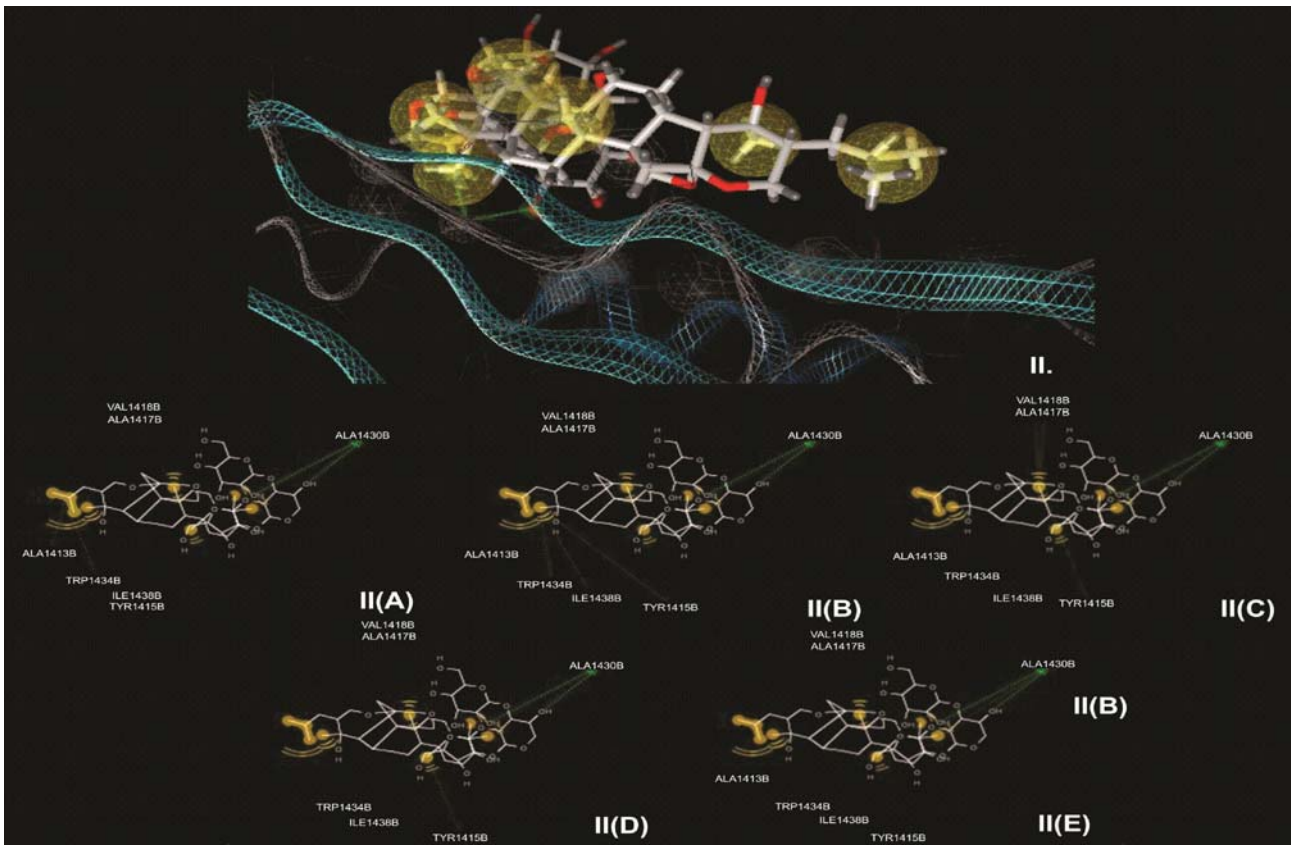

Fig. 2. The interaction of Bacopasaponin with LRKK2 protein showing pharmacophore. The individual parts II (A,B,C,D and E) represent H-bond interaction of pharmacophore with amino acid residues.

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