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Research Article

A POWERFUL BINDING OF PLANT BIOACTIVE COMPOUND SWERTIAMARIN TO OMPF PORINS RESEMBLING ANTIBIOTICS-AN IN SILICO STUDY

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ABSTRACT

Antibiotic resistance of the bacteria leads to Multi Drug Resistance (MDR) strain which is achieved mainly by the presence of Outer membrane Proteins (OMP) in the bacteria. Swertiamarin is an active compound isolated from Enicostemma axillare, a herb which was already proved to have antimicrobial activity. Also the herb was used to treat diseases like skin disease, intermittent fever, helminthiasis, tumors, diabetes mellitus, rheumatism, abdominal ulcer, hernia, swelling, itching, and insect poisoning. In this study, *in silico* analysis of swertamarin interaction was studied by docking the compound with *Salmonella typhi* and *Escherichia coli* OmpF porins. The molecular information helps in checking the efficiency of the compound as antibacterial agent and a way to design effective drugs from the compound.

INTRODUCTION

Around 25 to 30% of most of the organisms gene encode integral membrane proteins [1] and they are the key target of many pharmacological drugs. Porin is a major outer membrane protein (OMP) of most of the gram negative bacteria and a few gram positive bacteria.

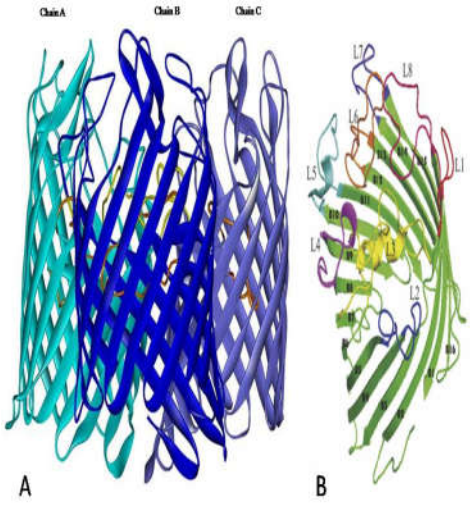

Figure [1(7)] A) Cartoon representation of OmpF homotrimer B) OmpF monomer showing loops and beta strands information

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Porins function in allowing the passive diffusion of small, polar molecules like water, ions, glucose, and other nutrients as well as waste products (600-700 Da). In particular, gram negative bacteria is deficient in expressing OmpF when exposed to antibiotics which leads to develop resistant against that antibiotics mainly β- lactam antibiotics [2,3,4,5,6] . The structure of OMPF is a homotrimer and each monomer forms water filled open channels in the outer membrane that allows the movement of small hydrophilic solutes such as amino acids, monosaccharides and ions[7] (Figure 1). Also, OmpF porins provide multi drug resistance to the bacteria by reducing the antibiotic permeability through altered pore properties which lowers the susceptibility of the bacteria for antibiotics [8]. There is always a need of efficient drugs to treat diseases. The study of OmpF antibiotic complexes will provide a better understanding about the molecular level interaction of the antibiotic with the porin and in future it helps in designing potent drugs to eradicate pathogenic diseases [8].Then *in silico* study of ompf antibiotic. India is endowed with a rich wealth of Medicinal plants. The increasing failure, chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants. Phytochemical is one such biologically active compound used effectively in treating human diseases in an idea to develop more effective and less toxic medicines. Plant derived drug serves as a prototype to increase body's natural resistance to diseases which is the expected remedy of any disease. *Enicostemma axillare* (Lam) A. Raynal (Gentianceae) is commonly known as 'Vellargu' in Tamil. It is an herb, 50cm inch in height

widely distributed throughout India up to 450 MSL. It is a very bitter plant and used in indigenous medicines in treatment of fever and as bitter tonic. And the other benefits of the plant in treating skin disease, intermittent fever, helminthiasis and tumors, diabetes mellitus, rheumatism, abdominal ulcer, hernia, swelling, itching, and insect poisoning have been reported[9,10]. Though the plant has so many medicinal properties, swertiamarin (Figure 2) is one identified potent compound isolated mainly from the aerial part of the plant [11,12] . Earlier, column chromatography over silica gel is performed to isolate swertiamarin but recently, Centrifugal Partition Chromatography is been successfully used for the separation of the compound from the crude extract of the plant [13,14]. In the present study, *S. typhi* OmpF (3NSG) and *E. coli* OmpF (2OMF) is docked with swertiamarin in order to find the antibacterial activity of the compound and the interacting residues involved in the influx of the compound through the porins [15]. This gives insight to the specificity of OmpF binding with swertiamarin in different organism porins namely *E. coli* and *S. typhi*.

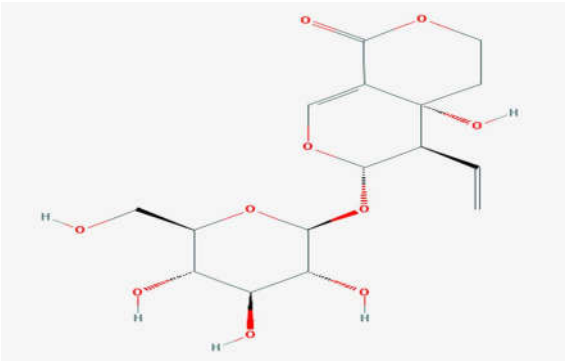

Figure 2 Structure of swertiamarin

MATERIALS AND METHODS

Selection of Target and Ligand

OmpF porin structures (3NSG and 2OMF) were retrieved from the protein databank (PDB) (www.rcsb.org/). Active site of the target protein was predicted using CASTp (http://sts.bioe.uic.edu/castp/). Swertiamarin (CID 442435) compound was retrieved from PubChem database (pubchem.ncbi.nlm.nih.gov).

Receptor Preparation and Docking

The heteroatom, water molecules and ligands were removed using Discovery Studio 4.1 before docking. Superimposition of 3NSG and 2OMF was done in SuperPose online server (http://wishart.biology.ualberta.ca/superpose/). The docking calculation for *S. typhi* OmpF and *E. coli* OmpF with ligand (swertiamarin) was performed with online Molecular docking server (http://www.dockingserver.com/web) and stand-alone AutoDock 4.0.1. A grid of 120, 120 and 120 points in x,y and z directions in AutoDock was built to cover the entire protein.

RESULTS

Molecular docking

Molecular docking was successfully completed between target receptor and the selected compound named swertiamarin (CID 442435).

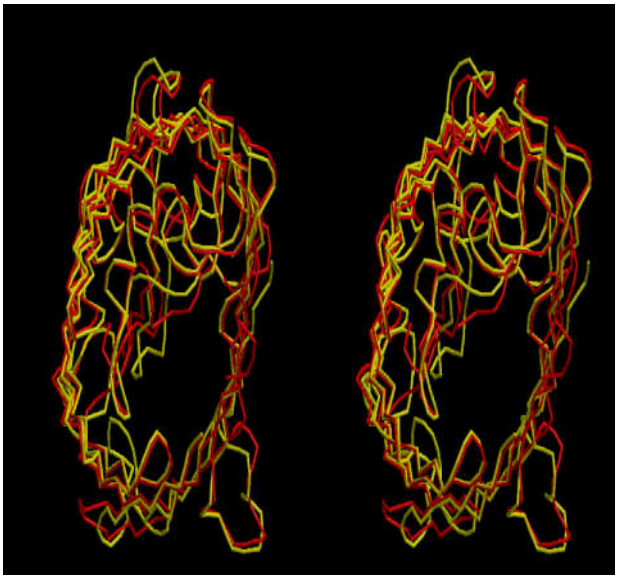

Figure 3 Superimposition of 2OMF and 3NSG by SuperPose server

Table 1 Active site residues calculated for 3NSG and 2OMF by CASTp server

Protein name	Predicted active site residues
E. coli OmpF (2OMF)	Tyr^{14}, Gly^{15}, Lys^{16}, Va^{l18}, Leu^{20}, Tyr^{22}, Asn^{27}, Gly^{28}, Glu^{29}, Ser^{31}, Tyr^{32}, Gly^{33}, Gly^{34}, Asn^{35}, Asp^{37}, Met^{38}, Tyr^{40}, Arg^{42}, Lys^{46}, Gln^{60}, Glu^{62}, Asn^{64}, Gln^{66}, Asn^{68}, Asn^{69}, Thr^{77}, Gly^{78}, Lys^{80}, Thr^{81}, Arg^{82}, Leu^{83}, Arg^{100}, Tyr^{102}, Tyr^{106}, Gly^{110}, Asp^{113}, Met^{114}, Leu^{115}, Pro^{116}, Glu^{117}, Phe^{118}, Gly^{119}, Gly^{120}, Asp^{121}, Ala^{123}, Tyr^{124}, Ser^{125}, Asp^{126}, Gly^{131}, Arg^{132}, Ala^{166}, Arg^{167}, Arg^{168}, Gln^{203}, Phe^{250}, Gln^{262}, Arg^{270}, Ser^{272}, Leu^{291}, Val^{292}, Asn^{293}, Tyr^{294}, Thr^{300}, Tyr^{302}, Asn^{304}, Lys^{305}, Asn^{306}, Met^{307}, Ser^{308}, TYR^{310}, Asn^{316}, Ile^{318}, Leu^{324}, Val^{326}, Gly^{327}, Val^{337}, Gln^{339}, Phe^{340}
S.typhi OmpF (3NSG)	Lys^{10}, Asp^{12}, Tyr^{14}, Gly^{15}, Lys^{16}, Val^{18}, Arg^{20}, His^{21}, Val^{22}, Trp^{23}, Thr^{24}, Thr^{26}, Asp^{28}, Ser^{29}, Lys^{30}, Asn^{31}, Ala^{32}, Asp^{33}, Gln^{34}, Thr^{35}, Tyr^{36}, Gln^{38}, Ile^{39}, Lys^{42}, Gly^{43}, Glu^{44}, Thr^{52}, Phe^{54}, Gly^{55}, Gln^{56}, Glu^{58}, Arg^{60}, Lys^{62}, Ala^{63}, Asp^{64}, Arg^{65}, Ala^{66}, Glu^{67}, Leu^{75}, Arg^{77}, Leu^{78}, Phe^{80}, Lys^{84}, Tyr^{85}, Ala^{86}, Glu^{87}, Gly^{89}, Ser^{90}, Asp^{92}, Arg^{95}, Asn^{96}, Tyr^{97}, Gly^{98}, Ile^{99}, Tyr^{101}, Asp^{102}, Glu^{104}, Ser^{105}, Tyr^{106}, Thr^{107}, Asp^{108}, Ala^{110}, Pro^{111}, Tyr^{112}, Phe^{113}, Ser^{114}, Gly^{115}, Glu^{116}, Thr^{117}, Gly^{119}, Gly^{120}, Ala^{121}, Tyr^{122}, Thr^{123}, Asp^{124}, Asn^{125}, Ser^{129}, Arg^{130}, Ala^{131}, Gly^{132}, Gly^{133}, Thr^{136}, Arg^{138}, Asn^{139}, Ser^{140}, Asp^{141}, Gly^{148}, Ser^{150}, Phe^{151}, Gly^{152}, Lys^{158}, Asn^{159}, Gln^{160}, Asp^{161}, Asn^{162}, His^{163}, Ser^{167}, Asn^{169}, Thr^{176}, Ala^{178}, Tyr^{179}, Glu^{180}, Thr^{187}, Lys^{217}, Asp^{219}, Tyr^{224}, Glu^{239}, Thr^{241}, Asp^{244}, Glu^{256}, Val^{258}, Gln^{260}, Gln^{262}, Arg^{268}, Ala^{270}, Ser^{272}, Val^{274}, Tyr^{293}, Gln^{295}, Thr^{299}, Tyr^{301}, Asn^{307}, Trp^{309}, Arg^{313}, Glu^{319}, Asn^{320}, Ser^{323}, Ser^{324}, Ser^{325}, Tyr^{326}, Val^{327}, Gly^{328}, Thr^{329}, Asp^{330}, Gln^{332}, Thr^{338}, Gln^{340}, Phe^{341}

Table 2 Docking calculations of *S. typhi* and *E.coli* OmpF porins

Protein (PDBID)	Binding Energy	Inhibition Constant Ki	Interaction surface	Binding residues	Common active site residues of predicted and actual binding residues
S. typhi OmpF 3NSG	-4.50 kcal/mol	499.77 uM	690.523	Tyr^{101}, Asp^{108}, Arg^{20}, Gly^{15}, Tyr^{14}, Arg^{130}, Arg^{77}, Glu^{56}, Glu^{58}, Gln^{38}, Tyr^{97}	Tyr^{101}, Asp^{108}, Arg^{20}, Gly^{15}, Tyr^{14}, Arg^{130}, Arg^{77}, Glu^{56}, Glu^{58}, Gln^{38}, Tyr^{97}
E. coli OmpF 2OMF	-5.14 kcal/mol	169.34 uM	658.387	Phe^{118}, Asp^{121}, Tyr^{124}, Leu^{291}, Val^{292}, Val^{100}, Gln^{80}, Tyr^{32}, Arg^{163}	Phe^{118}, Asp^{121}, Tyr^{124}, Leu^{291}, Val^{292}, Val^{100}, Gln^{80}, Tyr^{32}

The active sites of the target structures were predicted using CASTp server and the results were tabulated (Table 1). The binding energy (ΔG) was found to be -6.87 kcal/mol and -7.95 kcal/mol for *S. typhi* OmpF and *E. coli* OmpF respectively. Inhibition constant was calculated to be 499.77 uM and 169.34 uM for *S. typhi* OmpF and *E. coli* OmpF respectively (Table 2). Interacting residues in the receptor was studied with Discovery studio 4.1 (Table 3 and 4). Superimposition of 2OMf and 3NSG was done in SuperPose server showing 54.9% identity and 70.2% similarity (Figure 3).

Table 3 Structure information of the interacting residues in 3NSG

Interacting amino acids in *S. typhi* OmpF	**Structure information of the interacting residues in *S. typhi* OmpF (3NSG)**
Tyr^{101}, Asp^{108}	Loop L3
Arg^{130}	Beta strand β7
Arg^{20}, Gly^{15}, Tyr^{14}	Beta strand β2
Arg^{77}	Beta strand β5
Glu^{56}, Glu^{58}	Beta strand β4
Gln^{38}	Loop L1
Tyr^{97}	Beta strand β6

Table 4 Structure information of the interacting residues in 2OMF

Interacting amino acids in *E.coli* OmpF	**Structure information of the interacting residues in *E. coli* OmpF (2OMF)**
Phe^{118}, Asp^{121}, Tyr^{124}, Val^{100}	Loop L3
Gln^{80}	Beta strand β 5
Tyr^{32}	Loop L1
Leu^{291}, Val^{292}	Beta strand β15
Arg^{163}	Loop L4

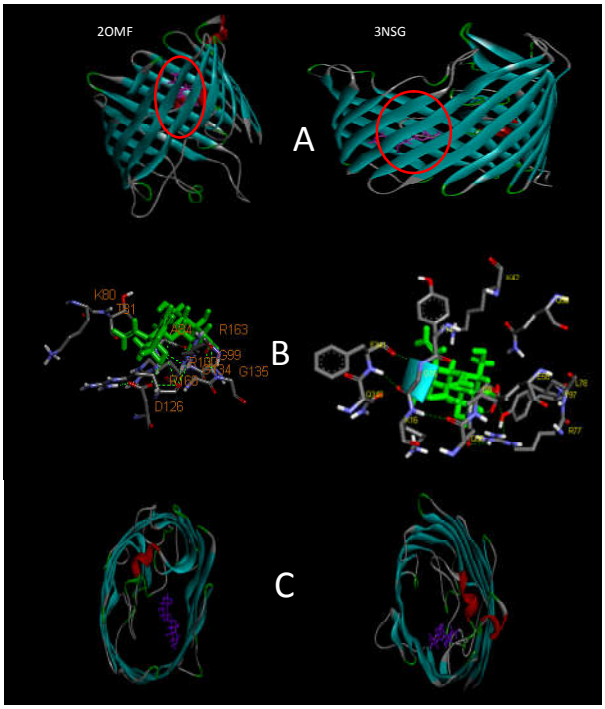

Figure 4 A) Side view of docked swertiamarin with 2OMF and 3NSG B) Docked pose of swertiamarin with 2OMF and 3NSG showing aminoacid residues C) Top view of docked swertiamarin with 2OMF and 3NSG

Binding of swertiamarin in an antibiotic pattern to S. typhi and E. coli OmpF

In *S. typhi* OmpF, the compound interacted with the following aminoacid residues Tyr^{101}, Asp^{108}, Arg^{20}, Gly^{15}, Tyr^{14}, Arg^{130}, Arg^{77}, Glu^{56}, Glu^{58}, Gln^{38} and Tyr^{97} in which Asp^{108}, Arg^{20}, Arg^{130}, Arg^{77} and Gln^{38} were reported binding residues in *E. coli* ompF with ampicillin [16], moxifloxacine [17] and enrofloxacin [18] (**Figure 4**). *S. typhi* and *E. coli* OmpF share higher percentage of sequence similarity [7]. In comparison with *E. coli* sequence, Leu^{20} (from strand β2) in *E. coli* sequence is replaced by Arg (from strand β2) and Met^{38} (from loop L1) in *E. coli* sequence is replaced by Glu (from loop L1) in *S. typhi* which on strong binding again with swertiamarin indicates the same efficiency of the replaced residues in binding with the compound. Leu^{20} and Gln^{38} are known for providing the hydrophobic interaction of the beta lactam antibiotics in *E. coli* OmpF. Interestingly[7] Asp^{108} (from loop L3), Arg^{20} (from strand β2) and Arg^{77} (from strand β5) residues in the beta strand, lines from extracellular to intracellular constriction zone and the binding of the compound to these residues in a particular fashion paves way for the translocation of the compound in to the porin.

In *E. coli* OmpF, swertiamarin interacts with Phe^{118}, Asp^{121}, Tyr^{124}, Leu^{291}, Val^{292}, Val^{100}, Gln^{80}, Tyr^{32} and Arg^{163} in which Tyr^{32}, Phe^{118}, Asp^{121}, Tyr^{124}, Leu^{291} and Val^{292} are common reported binding residues of *E. coli* OmpF with ampicillin [19]. Arg^{163} (from loop L4) is present only in *E. coli* OmpF and is known for binding with antibiotics which is replaced with His^{163} (from loop L4) in *S. typhi* OmpF [16]. Asp^{121} plays important role in the binding of colicin to OmpF (Bredin 2003) and the Asp^{121} mutant *E. coli* OmpF showed[20]~20% increase in carbenicillin susceptibility.

CONCLUSION

Even though porins belong to general diffusion category, they do not transport molecules without any specificity inside the channel. The molecule to be transported through the channel makes specific interactions with the residues in the constriction zone which actually paves way to the transport of the particular molecule. In this study, the interaction of swertiamarin with *E. coli* OmpF and *S.typhi* OmpF was studied to explore the binding configuration of a swertiamarin compound to both porins. Despite the fact that *S. typhi* OmpF and *E. coli* OmpF share around 70.2% similarity, the property of the porin in allowing the same swertiamarin molecule differs. Based on the results obtained from the interaction study swertiamarin a phytochemical exhibit almost similar pattern of binding configuration with that of well-known antibiotics. Since *E. axillare* is known for its medicinal properties, the activity of swertiamarin comparable with antibiotics shows the efficiency of the compound as antibacterial agent. Being a plant derived compound it is less harmful and the information on the compound binding serve as a key for next generation medicine since the compound has many chemotherapeutic properties in treating diseases.

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