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Molecular Docking Analysis - An aid for Selection of Promising Natural Plant Products against Diphtheria Toxin

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- Lipinski rule of five

Abstract

Molecular docking study was performed in order to identify Natural Plant Products that shows higher binding energy with Diphtheria toxin of the pathogen *Corynebacterium diphtheriae*, responsible for Diphtheria, a respiratory tract infection. Thirty herbals were selected based on their ethnopharmacological activities reported against different clinical problems. Five phytoconstituents from each herbal were docked with the tertiary chemical structure of Diphtheria toxin and compared with the binding potential obtained from substrate of the toxin, i.e., Nicotinamide adenine dinucleotide. Further, the potent Natural Plant Products were screened for compliance to the Lipinski rule of five with molinspiration tool. It was found that 20 Natural Plant Products from 14 herbals have shown E-value lower than Nicotinamide adenine dinucleotide. Only 03 phytoconstituents Cyanidin (*Sambucus nigra*), 3-hydroxyflavanone (*Oxycoccus palustris*) and 6-gingerol (*Zingiber officinale*) have shown no violation from Lipinski's rule of five.

ABBREVIATIONS

DT: Diphtheria Toxin; WHO: World Health Organization; EF-2: Elongation Factor- 2; NAD: Nicotinamide Adenine Dinucleotide; ADPR: Adenosine Diphosphate Ribose; NPPs: Natural Plant Products; SPF: Spherical Polar Fourier; MMDB: Molecular Modeling Database; SMILES: Simplified Molecular-Input Line-Entry System; PDB: Protein Data Bank

INTRODUCTION

Diphtheria, a serious malady caused by *Corynebacterium diphtheriae*, has been known to cause intense respiratory disease attributed towards its high transmissibility and toxin intervention(s) [1-3]. The infection is predominantly portrayed by the formation of a pseudo-membrane over tonsils and pharynx, ultimately reaching up to trachea. Subsequent inflammation of pseudo-membrane leads to dyspnoea [1]. The condition becomes more serious when the toxigenic strains produce DT that is absorbed in circulation and ultimately affect many tissues [1,4]. The main challenge in clinical management of Diphtheria is the limited prognostic as well as therapeutic interventions. However, the death rate significantly declined after the introduction of first line Diphtheria antitoxin in the early 1940's [5]. Asian nations with temperate environment are conducive for the easy growth and dissemination of *C. diphtheriae*, thereby making it an endemic in such geographical regions [6]. India, a South East Asian country, is considered as the hot zone of Diphtheria infection with more than 6,000 cases (~ 83.51% morbidity) recorded as per the disease surveillance report of WHO, 2015 [7]. Also an outbreak of Diphtheria is recorded by Integrated Disease Surveillance Programme in India in 2014 [8]. However, after the implementation of Expanded Programme on Immunization (EPI) by WHO in 1984, significant decline was observed in morbidity [7]. *C. diphtheriae* is known to be vulnerable to the first line anti-infection agents, *i.e.*, penicillin and erythromycin [6]. Other alternative second line chemotherapeutic modalities include amoxicillin, vancomycin, clindamycin, tetracycline, rifampin, kanamycin, gentamycin, imipenem, etc. These antibiotics are acting differently on physiological pathways and molecular mechanism of the bacteria, but none of them is targeting the DT, which is one of the major virulent factors to cause death of the host cell [9]. DT inhibits protein synthesis of eukaryotic cells by inactivating EF-2 during chain elongation process of protein synthesis. DT act catalytically by utilizing NAD for transferring ADPR moiety from NAD to EF-2, thereby inactivating EF-2 and ultimately kills susceptible cell [9]. Therefore, the agent that can prevent the action of DT at mechanistic level and ultimately inhibit the inactivation of EF-2 needs to be explored. Hence in the present study rationale based molecular docking approach is employed to identify potent NPPs that can bind more efficiently

with DT. The basis of this molecular docking analysis is evaluation of minimization of binding energy that is obtained by the correct conformation of the complex analyzed by using SPF correlation. Results obtained from the present study need further validation at both *in vitro* and preclinical levels.

MATERIALS AND METHODS

Receptor

The three dimensional crystal structure of receptor 'DT' was taken from MMDB (http://www.ncbi.nlm.nih.gov).

Ligands

One hundred and fifty NPPs from 30 herbals (~5 from each) and its substrate NAD were taken as ligands. The structural data format of ligands (NPPs and NAD) was obtained from PubChem and converted into SMILES formula by using Open Babel Graphical user interface program (http://openbabel.org/docs/dev/GUI/GUI.html) [10, 11]. The PDB file formats of all ligands were attained by online SMILES translator and structure generator (http://cactus.nci.nih.gov/translate/).

Molecular Docking: Molecular simulation was performed by using Hex 6.12 with PDB file pattern filter for both receptor and ligand. The parameters involved were shape + electro complementarity, 1 grid dimension and 180° range angle of receptor and ligand to calculate the linear relationship based binding energy of receptor-ligand complex [10, 11].

Virtual screening of leads: The lead molecules were screened for drug likeliness on the basis of "Lipinski's Rule of Five" by using Molinspiration Cheminformatics 2016.

RESULTS AND DISCUSSION

Molecular Docking: The tertiary structure of Diphtheria toxin (with PDB and MMDB ID as 1SGK and 57365, respectively) was retrieved from MMDB database. The predominant NPPs of 30 herbals were docked with Diphtheria toxin using Hex 6.12. It was found that 20 phytoconstituents of different categories from 14 herbals have shown lower E-value as compared to NAD (-300.05 Kcal/mol) (Table 1).

The ligands were also checked for conformity to the Lipinski rule of five, and the results are summarized in (Table 2). The rule states that a molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: (i) It should not have more than five hydrogen bond donors, (ii) it should not have more than 10 hydrogen bond acceptors, (iii) it should not have molecular weight greater than 500 Da, and (iv) it should not have an octanol–water partition coefficient greater than 5. Molecular properties of all NPPs were calculated by molinspiration, and it was found that 03 phytoconstituents namely Cyanidin (*Sambucus nigra*), 3-hydroxyflavanone (*Oxycoccus palustris*) and 6-gingerol (*Zingiber officinale*) have a good potential for eventual development as oral agents and can be potentially active drug candidates.

DISCUSSION

The objective of this study was to identify the NPPs that can bind efficiently with DT in comparison to that of NAD, substrate of DT. Many studies are ongoing for the discovery of novel drug in order to manage diphtheria based on various therapeutic rationale therapies. Discovery of antibiotics and anti-toxin is

Table 1: E-value of ligands < -300.05 Kcal/mol (NAD).

S. No.	Phytoconstituents	Herbal	Class	E vale(Kcal/mol)
1.	Crocin	*Gardenia jasminoide*	Carotenoid	-391.27
2.	Nimbin	*Azadiracta indica*	Alkaloid	-407.89
3.	Azadirachtin	*Azadiracta indica*	Limnoid	-429.03
4.	Vicenin	*Ocimum sanctum*	Flavanoid glycoside	-490.87
5.	Eriocitrin	*Mentha piperita*	Flavanone	-319.75
6.	Beta carotene	*Solanum lycopersicum*	Terpenoids	-359.57
7.	Betulinic acid	*Syzigium cumunii*	Terpenoids	-300.89
8.	Ecdysterone	*Achyranthes aspera*	Sterol	-317.97
9.	Furosin	*Emblica officinalis*	Tannin	-475.19
10.	Hesperidine	*Citrus limonum*	Flavanone	-471.63
11.	3- hydroxyflavone	*Oxycoccus palustris*	Flavanone	-304.83
12.	Cycloeucalenol	*Tabermontana coronaria*	Terpenoid	-396.77
13.	Lupeol acetate	*Tabermontana coronaria*	Terpenoid	-450.22
14.	Stigmasterol	*Tabermontana coronaria*	Sterol	-433.50
15.	Viminalol	*Tabermontana coronaria*	Terpene	-418.19
16.	6-gingerol	*Zingiber officinale*	Alkaloid	-357.77
17.	Beta-farnasene	*Zingiber officinale*	Terpene	-303.12
18.	Zingiberene	*Zingiber officinale*	Terpene	-309.76
19.	Spirostanol	*Tribulus terrestris*	Saponin	-420.3
20.	Cyanidin	*Sambucus nigra*	Flavanoid	-329.92

Table 2: Molinspiration Calculation of Properties for the Lipinski Rule of Five. Three NPPs (shown in bold) are drug able moieties.

S.No.	Phytoconstituents	n violation	n atoms	milogP <5	MW <500	nOH <10	nOHNH <5	nrotb
1.	**Cyanidin**	**0**	**21**	**-0.75**	**287.25**	**6**	**5**	**1**
2.	**3- hydroxylflavone**	**0**	**38**	**3.45**	**238.24**	**3**	**1**	**1**
3.	**6-gingerol**	**0**	**21**	**3.22**	**294.39**	**4**	**2**	**10**
4.	Betulinic acid	1	33	7.04	456.71	3	2	2
5.	Nimbin	1	39	3.55	540.61	9	0	8
6.	Ecdysterone	1	34	1.36	480.64	7	6	5
7.	Cycloeucalenol	1	31	7.62	426.73	1	1	5
8.	Lupeol acetate	1	34	8.71	468.77	2	0	3
9.	Stigmasterol	1	30	7.87	412.70	1	1	5
10.	Viminalol	1	31	8.08	426.73	1	1	0
11.	Beta-farnasene	1	15	5.84	204.36	0	0	7
12.	Zingiberene	1	15	5.12	204.36	0	0	4
13.	Spirostanol	1	30	6.12	416.65	3	1	0
14.	Beta-carotene	2	40	9.84	536.89	0	0	10
15.	Azadirachtin	2	51	1.42	720.72	16	3	10
16.	Eriocitrin	3	40	-1.68	564.60	14	10	4
17.	Hesperidine	3	43	-0.55	234.30	15	8	7
18.	Furosin	3	46	- 2.56	650.45	19	10	4
19.	Vicenin	3	40	-1.62	250.96	14	10	4
20.	Crocin	3	68	-2.20	976.97	24	14	20

hailed as one of the biggest achievement of science as this gave a ray of hope to win over the life threatening pathogens. The disease involving toxin mediated pathophysiology is difficult to treat, as discovery of chemotherapeutic agent targeting toxin directly is still illusive. One approach to treat such pathogenic strains could be in light of the utilization of promising herbals, with restorative NPPs is being practiced in various alternative therapies. However, this practice is not normally based on modern tools of drug development. Therefore, the goal of this study is to present a molecular docking approach to select convincing plants against DT by employing Hex 6.12, which uses SPF correlation in which only top ranked correct conformation have been identified as a complex [12]. The process involves targeting bioactivity parameter, i.e., DT as receptor in order to bind the selected phytomolecules with them and its comparison with the binding energy obtained by its substrate, NAD. Therefore, it can be assumed that NPPs of selected herbals could be selected as potent agents that can inhibit the action of DT further.

CONCLUSION

Our analyses have demonstrate that Molecular docking approach offers a convincing approach for the selection of promising herbal leads on the basis of binding energy as the NPPs have shown significant interaction with the selected toxin. Thus it can be used as potent herbal lead. Further studies are warranted to validate these results.

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