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RESEARCH ARTICLE

PHYTOCEHMICAL SCREENING OF *Catharanthus roseus* FOR ANTITUMOUR ACTIVITY AND DOCKING STUDIES BASED ON LIPINSKI'S RULE

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ABSTRACT

The aim of the study is that the extraction of phytochemicals from *Catharanthua Roseus* and the identification of antitumour agent based on docking studies The Phytochemical compounds were extracted and the effect of absorption were calculated based on Lipinski's rule. The list of phytochemicals was tested with Parameters and the effective ligand has been found. Finally we have observed that the ligand n-hexadecanoic acid has satisfied the conditions of Lipinski's rule and docked effectively with the receptor.

INTRODUCTION

Periwinkle *Catharanthus roseus* is one of the few medicinal plants which has found mention in the folk medicinal literature. Vincristine is an alkaloid derived from flowering periwinkle. Its use is limited because of its toxic effects, among them being neuro toxicity and Pharmacology The root bark contains the alkaloid Alstonine which has been used traditionally for its calming effect and its ability to reduce blood pressure. The anti cancer drug namely Vincristine and Vinblastine are produced from Periwinkle and it has Pharmaceutical Activities. [1] In *Catharanthus roseus* each tissue is known to produce a distinct spectrum of terpenoid indole alkaloids. Since the invaluable anti neoplastic bis indole alkaloids are restricted to the aerial parts of the plant and do not occur in its underground tissues, identification of the structural and regulatory factors operating distinctly in the shoot/leaf of the plant will bea necessity for modulation of bisindole alkaloid biosynthesis. [2] Identification of molecular markers of mono terpenoid indole alkaloid (MIA) accumulation in cell-suspension cultures of *Catharanthusroseus* was performed by two-dimensional poly acrylamide gel electrophoresis. Comparison of the protein patterns from alkaloid-producing and non-producing cells showed the specific occurrence of polypeptide restricted to cells accumulating MIAs. [3] TheMadagascar Periwinkle, *Catharanthus roseus* a valued medicinal plant was exposed to different concentrations of heavy metal slike, $CdCl_2$ and $PbCl_2$ with a view to observe their bioaccumulation efficiency. [4] *Catharanthus roseus* L (*C. roseus*) has been used to treat a wide assortment of diseases including diabetes. [5] The present study was conducted to find out the antibiogram of different extracts of two varieties of *Catharanthus roseus.* The plant parts, leaves, stems, roots and flowers were separately tested for their antibiogram by using different solvents like methanol, acetone and ethyl acetate.Among the three solvents used for antibiogram, ethyl acetate extracts of different plant parts were found to induce best antibiogram followed by methanol and acetone extracts. [6] The phytochemical screening of methanol and aqueous crude plant extracts revealed the presence of various secondary metabolites such as alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, glycosides, terpenoids and saponins. [7] Docking is frequently used to predict the binding orientations of small molecules drug candidates to protein targets in order to in turn predict the affinity and activity of the small molecule. The receiving molecule that primarily binds to a small molecule or another protein or a nucleic acid called receptor. A molecule that forms the complementary partner in the docking process called ligand. [8] The findings derived from the docking studies shows the possible involvement of systematic mechanism of drug designing process. [9] This progressive-docking procedure therefore substantially accelerates high throughput screening, especially when using high accuracy (slower) docking approaches and large-sized datasets, and has allowed us to identify several novel potent nonsteroidal SHBG ligands. [10]

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METHODOLOGY

Catheranthus roseus flowers were collected at various locations around Perambalur. The flowers were used for study and the flowers were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. The plant sample was subjected to GC-MS study for phytochemical analysis. About 2.0g of sample was soaked in 100ml methanol for 24 hours. The extract was filtered through what man no.1 and the filtrate was concentrated to dryness. The dried extract was diluted with GC methanol and was injected in to GC-MS.

Lipinski's Rule

The Lipinski's rule has some conditions. They are H Bond Donors should not more than 5 and hydrogen bond acceptors should not more

than 10, Molecular mass should be less than 500 Daltons and Log P should not be greater than 5. The phytochemical compounds were identified from GC-MS study and these compounds were tested with Lipinski's Rule. The compounds which are satisfied the condition of Lipinski's Rule and these compounds were selected for docking studies. There are four parameters which were calculated based on Lipinski's Rule. The H- Bond Donor, H-Bond Acceptor and Mol. weight retrieved form PubChem Compound database. The Log P value was calculated by ALOGPS tool. The effects of absorption for each compound were identified and the compound which shows the good absorption was selected for Docking with the receptor. Based on the parameters, n-Hexadecanoicacid shows good absorption effect and it was docked with the receptor ADAM17 by Hex tool.

RESULTS AND DISCUSSION

Fig 1. Snap Shot of *Catharanthusroseus* extract

DISCUSSION

The Plant *catharanthus roseus* was selected and subjected for GC-MS study. The phytochemical compounds were identified by GC-MS technique and the compounds were displayed in Table 1. The compounds were tested based on Lipinski's Rule and calculate the parameters such as H-Bond donor, H-Bond Acceptor, Molecular Weight and Log P values. The list of compounds and their values showed in Table 2. The Log P value was calculated by ALOGPS tool. The Log P value for n-Hexadecanoic acid is 7.23 and Molecular Weight is 256 Daltons. The H-Bond donor and H-Bond acceptor for n-Hexadecanoic acid is 1 and 2 respectively. Table 3 which represents the conditions of Lipinski's Rule and showed the effect of absorption for phytochemical compounds. In the Table 3, column A, B, C and D which represents H bond donor, Acceptor, molecular weight and Log P respectively. The effect of absorption of the phytochemical compounds represented in column 5. Among these phytochemical compounds, n- Hexadecanoic acid which satisfied the conditions of Lipinski's rule and it has a good absorption power. 2, 20-Cycloaspidospermidine-3-carboxilic acid, methyl ester, (2α,3,5,12,19, 20R)-6,7-dihydrovindolinine and 9, 12-Octodecanoic acid (Z,Z)- have the moderate absorption effect were calculated based on Lipinski's rule. The rest of the phytochemical compounds showed poor absorption effect. So, n-Hexadecanoic acid was selected for docking studies. Fig 2 which shows the structure of the Ligand n-Hexadecanoic acid. The structure for the Ligand and the Receptor was retrieved from PDB and submitted in to Hex tool. The structure for the target protein was showed in Fig 3. Both the structures were subjected to docking (Fig 4). Fig 5 which represents the docked structures of the ligand and the receptor. Table 4 and 5 which represents the docking

Table 1. Phytochemical Components of *Catharanthus roseus*

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: Formamide, N-(1,1,3,3-tetramethylbutyl)- Formula: $C_9H_{19}NO$ MW: 157	3.01	2029091	0.0891
2.	Name: Propanoic acid Formula: $C_3H_6O_2$ MW: 74	3.26	932676	0.0409
3.	Name: 1-[(1-Oxo-2-propenyl)oxy]-2,5-pyrrolidinedione Formula: $C_7H_7NO_4$ MW: 169	3.64	26239836	1.1516
4.	Name: Propanoic acid, 2-oxo-, methyl ester Formula: $C_4H_6O_3$ MW: 102	3.93	7919397	0.3476
5.	Name: Furfural Formula: $C_5H_4O_2$ MW: 96	4.78	29627170	1.3003
6.	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	5.25	72580608	3.1854
7.	Name: 2-Cyclopentene-1,4-dione Formula: $C_5H_4O_2$ MW: 96	5.72	16633169	0.7300
8.	Name: 4,4-Dimethyl-2-cyclopenten-1-one Formula: $C_7H_{10}O$ MW: 110	6.14	2902161	0.1274
9.	Name: 1,2-Cyclopentanedione Formula: $C_5H_6O_2$ MW: 98	6.59	14055879	0.6169
10.	Name: 2-Furancarboxaldehyde, 5-methyl- Formula: $C_6H_6O_2$ MW: 110	7.14	22440894	0.9849
11.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	7.42	16911342	0.7422
12.	Name: 2H-Pyran-5-carboxylic acid, 2-oxo- Formula: $C_6H_4O_4$ MW: 140	8.06	16282151	0.7146
13.	Name: Sulfone, 2-hydroxyoctyl t-butyl Formula: $C_{12}H_{26}O_3S$ MW: 250	8.54	11919311	0.5231

Continue……..

14.	Name: 1,3-Dioxol-2-one,4,5-dimethyl- Formula: $C_5H_6O_3$ MW: 114	9.27	7588300	0.3330
15.	Name: 2,5-Dimethyl-4-hydroxy-3(2H)-furanone Formula: $C_6H_8O_3$ MW: 128	9.45	24118686	1.0585
16.	Name: Maltol Formula: $C_6H_6O_3$ MW: 126	9.90	4408636	0.1935
17.	Name: 2-Furancarboxylic acid Formula: $C_5H_4O_3$ MW: 112	10.13	19996852	0.8776
18.	Name: Benzoic acid 2-methylpentyl ester Formula: $C_{13}H_{18}O_2$ MW: 206	10.84	1711042	0.0751
19.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	11.16	239762352	10.5228
20.	Name: 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- Formula: $C_6H_6O_4$ MW: 142	11.92	6156996	0.2702
21.	Name: 5-Acetoxymethyl-2-furaldehyde Formula: $C_8H_8O_4$ MW: 168	12.36	10557526	0.4634
22.	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	13.06	983837760	43.1790
23.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.93	17993902	0.7897
24.	Name: N-Nitroso-2,4,4-trimethyloxazolidine Formula: $C_6H_{12}N_2O_2$ MW: 144	14.37	36334348	1.5947
25.	Name: Phenol, 2,6-dimethoxy- Formula: $C_8H_{10}O_3$ MW: 154	14.55	5854684	0.2570
26.	Name: N-(2-Methoxyethyl)alanine Formula: $C_6H_{13}NO_3$ MW: 147	14.90	23781172	1.0437
27.	Name: Acetamide, N-(2,4-dihydroxyphenyl)- Formula: $C_8H_9NO_3$ MW: 167	16.33	13873897	0.6089
28.	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	18.16	18295928	0.8030
29.	Name: 3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$ MW: 180	18.32	27234660	1.1953
30.	Name: 1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)- Formula: $C_6H_{12}O_4$ MW: 148	22.19	214267152	9.4038
31.	Name: 1-Methyl-3-acetylindole Formula: $C_{11}H_{11}NO$ MW: 173	22.78	4469069	0.1961
32.	Name: Tetradecanoic acid Formula: $C_{14}H_{28}O_2$ MW: 228	23.33	7459621	0.3274
33.	Name: 2-Octanol, 2-methyl-6-methylene- Formula: $C_{10}H_{20}O$ MW: 156	24.55	3303595	0.1450
34.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	27.40	111822408	4.9077
35.	Name: Heneicosane Formula: $C_{21}H_{44}$ MW: 296	29.07	12554356	0.5510
36.	Name: 9,12-Octadecadienoic acid (Z,Z)- Formula: $C_{18}H_{32}O_2$ MW: 280	30.01	37609816	1.6506
37.	Name: Tricosane Formula: $C_{23}H_{48}$ MW: 324	31.68	11627792	0.5103
38.	Name: 2,20-Cycloaspidospermidine-3-carboxylic acid, methyl ester, (2à,3á,5à,12á,19à,20R)- Formula: $C_{21}H_{26}N_2O_2$ MW: 338 .6,7-Dihydrovindolinine	37.67	193413936	8.4886

Table 2. Drug parameters for Phytochemical Constituents based on Lipinski's Rule

S. No.	PHYTOCHEMICAL COMPONENT	H-BOND ACCEPTOR	H-BOND DONOR	MOLECULAR WEIGHT [g/mol]	SOLUBILITY (log P)
1.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyle-	4	1	144.12532	-0.78
2.	1,2,3,5-Cyclohexanetetrol,(1a,2a,3a,5a)-	4	2	148.15708	-0.49
3.	2,20Cycloaspidospermidine-3-carboxylic acid	3	1	338.44334	3.46
4.	n-Hexadecanoic acid	2	1	256.42408	7.23
5.	9,12, Octadecadienoic acid(Z,Z)-	2	1	280.44548	7.06
6.	N-Nitroso-2,4,4-trimethyloxazolidine	2	2	144.17168	-1.26
7.	1-[(1-Oxo-2-propenyl)oxy]-2,5-pyrrolidinedione	4	4	169.13478	0.06
8.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	3	1	128.12592	-0.14
9.	N-(2-Methoxyethyl)alanine	4	2	147.17232	1.11
10.	2-Furancarboxylic acid	3	1	112.08346	0.12
11.	Dodecanoic acid	2	1	200.31776	0.12
12.	2H-Pyran-5-carboxylic acid,	4	1	140.09356	0.68

Table 3. Effect of Absorption for Phytochemical Components

S. No.	PHYTOCHEMICAL COMPONENT	A	B	C	D	Absorption
1.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyle-	0	1	0	0	poor
2.	1,2,3,5-Cyclohexanetetrol,(1a,2a,3a,5a)-	0	1	0	0	poor
3.	2,20Cycloaspidospermidine-3-carboxylic acid,methyl ester,(2α,3β,5α,12β,19α ,20R)-	0	1	1	0	Moderate
4.	n-Hexadecanoic acid	1	1	1	1	Good
5.	9,12, Octadecadienoic acid(Z,Z)-	0	1	1	1	Moderate
6.	N-Nitroso-2,4,4-trimethyloxazolidine	0	1	0	0	Poor
7.	1-[(1-Oxo-2-propenyl)oxy]-2,5-pyrrolidinedione	0	1	0	0	Poor
8.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0	1	0	0	Poor
9.	N-(2-Methoxyethyl)alanine	0	1	0	0	Poor
10.	2-Furancarboxylic acid	0	1	0	0	poor
11.	Dodecanoic acid	0	1	0	0	Poor
12.	2H-Pyran-5-carboxylic acid, 2-oxo-	0	1	0	0	Poor

Table 4. Parameters in Receptor ADAM 17

S.No	Parameters in Receptor	Composition	
1	Atoms	5092	
2	Residues	836	
3	Net formal Charge	-15	
4	Formal Charged Residues	57+ve	72-ve
5	Score Value	60.75	

Table 5. Parameters in Ligand n-Hexadecanoic acid

S.No	Parameters in Receptor	Composition	
1	Atoms	1274	
2	Residues	186	
3	Net formal Charge	-2	
4	Formal Charged Residues	13+ve	15-ve
5	Score Value	340	

Table 6. Docking Parameters and Scores

S.No	Parameters in Receptor	Composition
1	E Min	-80.53
2	E Max	-49.96
3	E Ave	-54.74
4	Top 10 Average Energy	-73.37
5	Top 100 Average Energy	-64.86
6	Top 1000 Average energy	-55.39

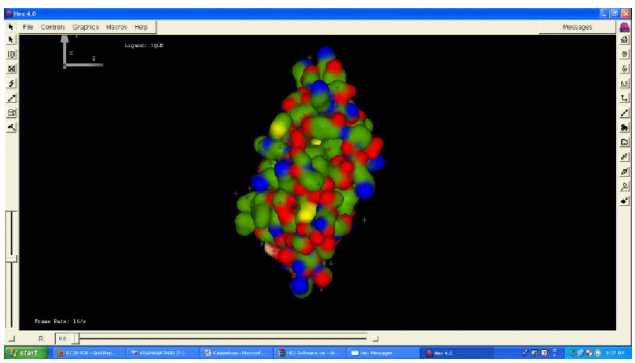

Fig. 2. Structure for the ligand n-Hexadecanoic acid

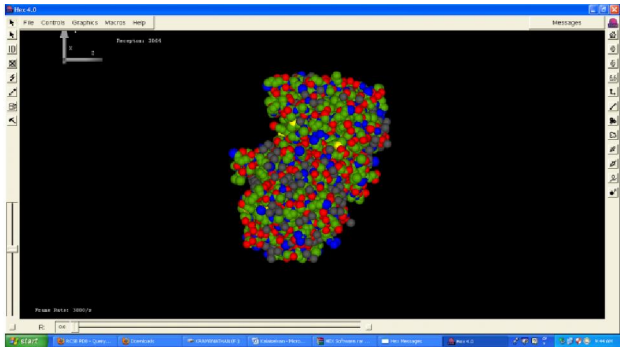

Fig. 3. Structure for the Receptor ADAM 17

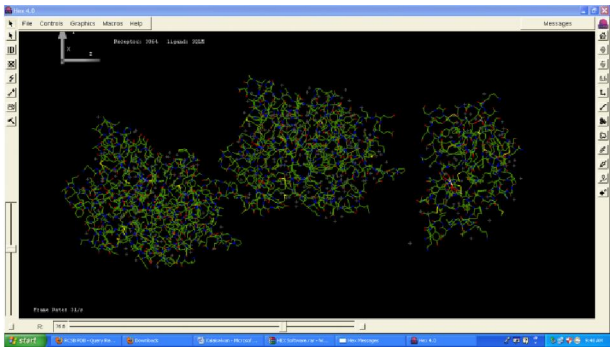

Fig. 4. Before docking the receptor and the ligand

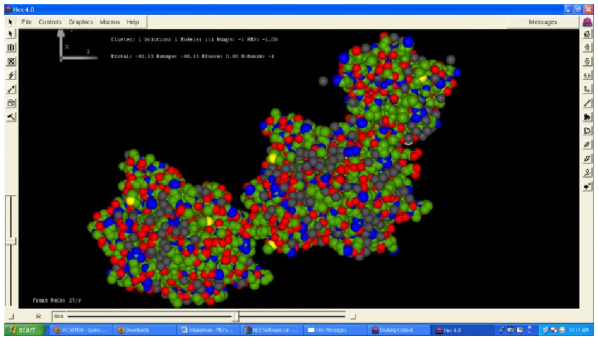

Fig. 5. After docking the receptor and the ligand

parameters for the receptor and the Ligand respectively. These parameters which shows Atoms, Residues, Net formal charge and score values. The average energy scores for docked structures were represented in (Table 6).

Conclusion

Phytochemical compounds of *Catharanthus Roseus* are extracted with the help of methanol and were subjected to GCMS study. It shows the number of phytochemical compounds present in the sample. The effect of absorption was calculated for these compounds based on Lipinski's rule. The Parameters such as H Bond Donor, Acceptor, Mol. Weight and Log P shows n-hexadecanoic acid has good absorption power. So, it is docked with the receptor by Hex tool. From these results we found that n-hexadecanoic acid is a sumptuous compound and addressed the problem of cancer by *Catharanthus Roseus.*

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