Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 12 (4), 792-795

Molecular docking studies of potential anticancer agents from *Thunbergia Fragrans* against Colorectal cancer mutant genes through *In silico* study

Research Article

Charankumar Chandrasekaran[1], Ramar K[2]*

1. Research Scholar, 2. Assistant Professor,
Department of Botany, National College (Autonomous), Tiruchirappalli, India.

Abstract

Colorectal cancer (CRC) is one of the deadly diseases which incidence rate will increase every year due to people lifestyle and food habit etc., Moreover, people's required a new therapeutic molecule to resolve this problem. Therefore plant-based chemical constituents are the best option due to the low side effects, easy availability and cost-effective manner. The flowering plant of *Thunbergia fragrans* Roxb belongs to the Acanthaceae family has a vast range of medicinal properties, anticancer activity is one among them. *Thunbergia fragrans* has reported to had chemical constituents of Palmitic acid, Cis-9-Hexadecenal and Campesterol which possess anticancer activity. For the beginning of TF chemical constituents were studied against the Colorectal cancer (CRC) mutant genes such as NRAS (PDB ID: 6ZIZ), Beta-Catenin (PDB ID: 6M93) – Oncogenes; APC (PDB ID: 3NMX), Smad2 (PDB ID: 1KHU) – Tumor Suppressor genes through insilico docking studies. AutoDock 4.2 tool was used to predict the interaction between ligand and receptor, Binding energy and Bond specification in a 3D space. Finally, the results revealed TF chemical constituents showed excellent binding energy against CRC mutant genes such as Palmitic acid against Beta-Catenin (-4.75) and APC (-4.01), Cis-9-Hexadecenal against NRAS (-1.92), Beta-Catenin (-3.96) and APC (-4.41), Campesterol against Beta-Catenin (-8.55) and APC (-8.85) respectively.

Key Words: *AutoDock 4.2, Binding energy, Oncogenes, Tumor suppressor genes, PDB, PubChem*.

Introduction

Colorectal cancer (CRC) is the leading cause of cancer death in both sexes which the incidence rate in men is 3rd and women are 2nd. In Asia, the incidence rate of CRC is increased in well-developed Asian countries such as Japan, Singapore, and South Korea (1). In the recent decade, researchers are focused on the biologically active molecule from natural sources which has a vast range of medicinal properties like Anticancer, Anti-inflammatory, and Antioxidant, etc., Among the natural sources, the plant-derived phytoconstituents are the finest ones due to their low side effects and easy availability (2, 3). *Thunbergia fragrans* Roxb is a flowering plant that belongs to the *Acanthaceae* family, mainly cultivated in tropics and subtropic regions. TF plant contained phytoconstituents are possessed various pharmacological activities such as anti-cancer, Anti-inflammatory, Antimicrobial, and Antidiabetic activity (4, 5). But also the identification, extraction, and purification of the biologically active molecule from a particular plant is the most difficult task. In this manner, computational tools are used to eradicate these problems (6). Among all computational work, docking is one of the best methods because of the identification of active site between Ligand and Receptor in a 3D space which helps to find the biologically active molecule (7). Furthermore, various docking tools are available in the market, AutoDock4.2 is the finest one because of the accuracy and docking score calculation (8, 9). The present investigation was to explore the anti-colon cancer activity of reported compounds of *Thunbergia fragrans* through insilico docking studies.

Aim and Objectives

- Identification of the colon cancer mutant genes (Oncogenes, Tumor suppressor genes)
- Retrieving the data from PubChem and PDB database.
- To reveal the anticancer potential of *Thunbergia fragrans* with insilico manner

Materials and Methods

Retrieval of Protein and file preparation

The 3D structures of Colorectal cancer mutant genes such as NRAS (PDB ID: 6ZIZ), Beta-Catenin (PDB ID: 6M93) – Oncogenes; APC (PDB ID: 3NMX), Smad2 (PDB ID: 1KHU) – Tumor Suppressor genes retrieved from the PDB database showed as Table 1. The excess water molecules and chains are detached from the protein with the help of the Discovery studio visualizer (Visualizing tool). The addition of Polar

*** Corresponding Author:**
Ramar K
Assitant Professor,
Department of Botany, National College (Autonomous), Tiruchirappalli 620001.
Tamilnadu. India.
Email Id: ramarbot@gmail.com

bonds, Kollman charges, and generating the docking parameters in protein structure was performed by the AutoDock 4.2 tool (10).

Retrieval of Ligand and file preparation

The 3D structures of Palmitic acid, Cis-9-Hexadecenal and Campesterol compounds were retrieved from the PubChem database. The setting of the number of torsions (1-6), the addition of aromaticity criteria and the setting of angle cutoff (7.5) was performed by the Auto Dock 4.2 tool. After the above-mentioned process, the docking parameter files are generated (11, 10).

Molecular docking

PDBQT files, Docking parameter files and grid parameter files were generated along with grid box dimensions (50x50x50). 10 runs were set for the default parameters. Lamarckian GA (4.2) genetic algorithm was used for the docking procedure. Cygwin software was used for the molecular docking coding and binding energy calculation (Every run) which also revealed the binding interaction of electrostatic bond, hydrogen bond, van der Waals force and polar bond (12). The interaction between ligand atom and receptor protein was revealed by Binding energy calculation.

Results and Discussion

Table 1: Colon cancer receptor details

S.NO	Receptor Name	Codon	PDB ID
1	NRAS (Oncogene)	Q61R	6ZIZ
2	Beta-Catenin (Oncogene)	S33	6M93
3	APC (Suppressor gene)	Cdc42	3NMX
4	Smad2 (Suppressor gene)	S464L	1KHU

Table 1 indicates colon cancer associated receptor details along with codon and PDB ID.

Table 2: *Thunbergia fragrans* associated ligand details

S.NO	Ligand	PubChem ID
1	Palmitic acid	985
2	Cis-9-Hexadecenal	5364643
3	Campesterol	173183

Table 2 represents the *Thunbergia fragrans* associated ligand details along with PubChem ID.

Table 3: Binding energies of Thunbergia fragrans associated phytoconstituents against colorectal cancer genes.

S.No.	Ligand	Receptor	Binding energy
1	Palmitic acid	NRAS	**9.08**
2		Beta-Catenin	-4.75
3		APC	-4.01
4		Smad2	**0.28**
5	Cis-9-Hexadecenal	NRAS	-1.92
6		Beta-Catenin	-3.96
7		APC	-4.41
8		Smad2	**115.55**
9	Campesterol	NRAS	**523.70**
10		Beta-Catenin	-8.55
11		APC	-8.85
12		Smad2	**126.42**

Table 3 represents the binding energy between ligand and receptor. Campesterol showed highest binding energy against Beta-Catenin (-8.55) and APC (-8.85) compare with Palmitic acid and Cis-9-Hexadecenal. Smad2 receptor showed lowest binding energy against all the three ligands.

Figure 1: The docked complex of Palmitic acid against colorectal cancer mutant genes.

A) Beta-Catenin, **B) APC**

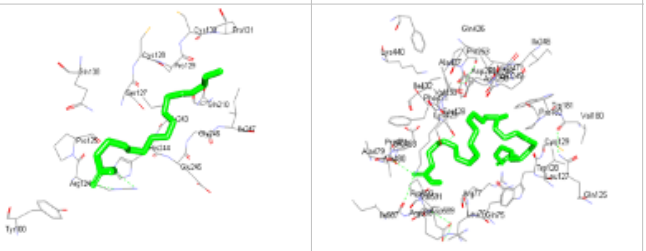

Figure 2: The docked complex of Campesterol against colorectal cancer mutant genes.

A) Beta-Catenin, **B) APC**

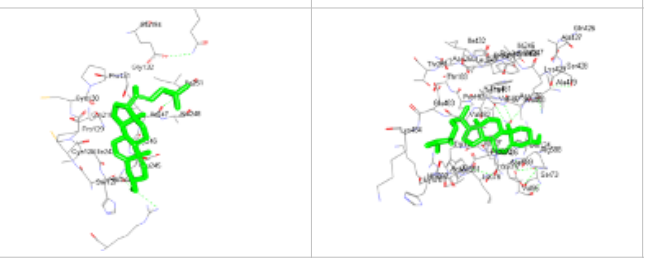

Figure 3: The docked complex of Cis-9-Hexadecenal against colorectal cancer mutant genes.

A) NARS, **B) Beta-Catenin,**

C) APC

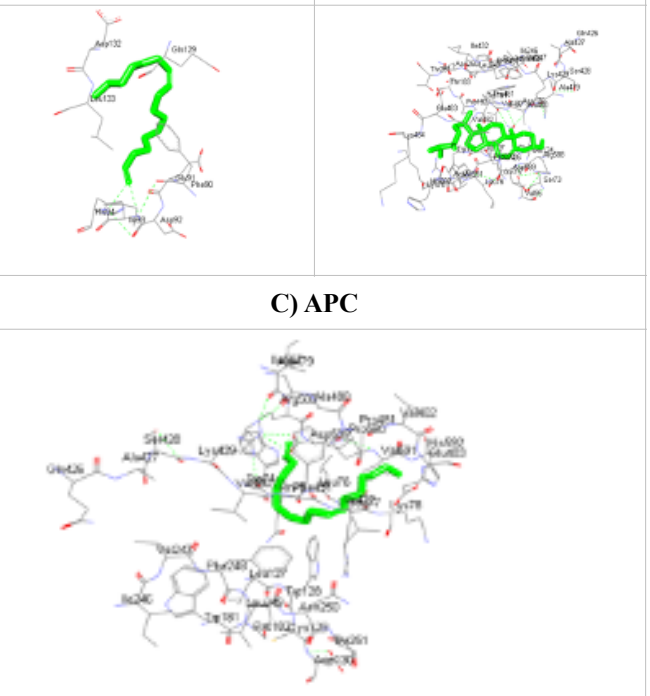

Table 4: Bond specification of Palmitic acid against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	10	-4.75	Hydrogen bond	ARG124	O	2.81

Table 4 represents the binding interaction between Palmitic acid (ligand) and Beta-Catenin (S33 codon) receptor. In the 10th run Palmitic acid showed the binding energy of -4.75 (Kcal/mol), bond length 2.81 (Å) between O-atom and ARG124 amino acid residue. Fig 1 (A) illustrate the three-dimensional molecular docking images of the Palmitic acid in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 5: Bond specification of Palmitic acid against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	7	-4.01	Hydrogen bond	LYS429	O	2.95

Table 5 represents the binding interaction between Palmitic acid (ligand) and APC (Cdc42 codon) receptor. In the 7th run Palmitic acid showed the binding energy of -4.01 (Kcal/mol), bond length 2.95 (Å) between O-atom and LYS429 amino acid residue. Fig 1(B) illustrate the three-dimensional molecular docking images of the Palmitic acid in the colorectal cancer mutant protein APC (Cdc42 codon).

Table 6: Bond specification of Cis-9-Hexadecenal against NRAS

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
NRAS	5	-1.92	Hydrogen bond	HIS94	O	2.89

Table 6 represents the binding interaction between Cis-9-Hexadecenal (ligand) and NRAS (Q61R codon) receptor. In the 5th run, Cis-9-Hexadecenal showed the binding energy of -1.92 (Kcal/mol), bond length 2.89 (Å) between O-atom and HIS94 amino acid residue. Fig 3(A) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein NRAS (Q61R codon).

Table 7: Bond specification of Cis-9-Hexadecenal against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	4	-3.96	Hydrogen bond	PRO125	O	3.18

Table 7 represents the binding interaction between Cis-9-Hexadecenal (ligand) and Beta-Catenin (S33 codon) receptor. In the 4th run, Cis-9-Hexadecenal showed the binding energy of -3.96 (Kcal/mol), bond length 3.18 (Å) between O-atom and PRO125 amino acid residue. Fig 3(B) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 8: Bond specification of Cis-9-Hexadecenal against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	7	-4.41	Hydrogen bond	LYS429	O	2.95

Table 8 represents the binding interaction between Cis-9-Hexadecenal (ligand) and APC (Cdc42 codon) receptor. In the 7th run, Cis-9-Hexadecenal showed the binding energy of -4.41 (Kcal/mol), bond length 2.95 (Å) between O-atom and LYS429 amino acid residue. Fig 3(C) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein APC (Cdc42 codon).

Table 9: Bond specification of Campesterol against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	3	-8.55	Hydrogen bond	ARG124	O	3.18

Table 9 represents the binding interaction between Campesterol (ligand) and Beta-Catenin (S33 codon) receptor. In the 3rd run, Campesterol showed the binding energy of -8.55 (Kcal/mol), bond length 3.18 (Å) between O-atom and ARG124 amino acid residue. Fig 2(A) illustrate the three-dimensional molecular docking images of the Campesterol in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 10: Bond specification of Campesterol against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	1	-8.85	Hydrogen bond	LYS429	O	3.06

Table 10 represents the binding interaction between Campesterol (ligand) and APC (Cdc42 codon) receptor. In the 1st run, Campesterol showed the binding energy of -8.85 (Kcal/mol), bond length 3.06 (Å) between O-atom and LYS429 amino acid residue. Fig 2(B) illustrate the three-dimensional molecular docking images of the Campesterol in the colorectal cancer mutant protein APC (Cdc42 codon).

Conclusion

The present study was to investigate the anti-colon cancer activity *of Thunbergia fragrans* with colon cancer mutant genes through an insilico manner. Binding energy, Electrostatic interaction, Bond length are calculated using Discovery visual studio. The above results revealed Cis-9-Hexadecenal has good binding energy against colorectal cancer mutant genes NRAS (-1.92), Beta-Catenin (-3.96), APC (-4.41) without Smad2. Palmitic acid and Campesterol showed good binding energy against Beta-Catenin (-4.75, -8.55), APC (-4.01, -8.85) respectively without NRAS and Smad2. Therefore *Thunbergia fragrans* have the potential to inhibit the colon cancer mutant genes.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

The authors declare that they have no funding support for this study.

References

1. Rawla P, et al, Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterology Review, 2019; 14(2): 89–103.
2. Purushothaman B et al, A comprehensive review on *Ocimum basilicum*. Journal of Natural Remedies, 2018; 18(3): 71-85. https://doi.org/10.18311/jnr/2018/21324.
3. Tungmunnithum D, et al, Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018; 5(3): 93.
4. Payamalle S et al, Chemical Properties and Fatty Acid Composition of *Thunbergia fragrans* Roxb. Seed Oil. Journal of the American Oil Chemist's Society, 2015; 93(2): 295–297.
5. Ismail L.D, Iridoid glycosides from *Thunbergia grandiflora*. Phytochemistry, 1996; 42(4): 1223–1225.
6. Waghulde S, et al, In silico docking and drug design of herbal ligands for anticancer property. Journal of Pharmacognosy and Phytochemistry, 2018; 6: 84-91.
7. Glen, R.C, Allen S.C, Ligand-Protein Docking: Cancer Research at the Interface between Biology and Chemistry. Current Medicinal Chemistry, 2003;10(9): 763-77.
8. Morris G.M et al, AutoDock4 and AutoDock Tools 4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 2009; 30(16): 2785-91.
9. Norgan A.P. Multilevel Parallelization of AutoDock 4.2. Journal of Cheminformatics, 2011; 3(1): 12.
10. Forli S, Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nature Protocols, 2016; 11(5): 905–919.
11. Danish Rizvi S.M et al, A simple click by click protocol to perform docking: autodock 4.2 made easy for non-bioinformaticians. EXCLI Journal, 2013; 12: 831-57.
12. Purushothaman B, et al, Molecular Docking Studies of potential anticancer agents from *Ocimum basilicum* L. against human colorectal cancer regulating genes: An insilico approach. Research J. Pharm. and Tech. 2019; 12(7): 3423-3427. https://doi.org/10.5958/0974-360X.2019.00579.1.

*****