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RESEARCH ARTICLE

Evaluating the Anti-Cancer Efficacy of Ethanolic Extract of *Erythrina variegate* Linn. – An In-silico Approach

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Abstract

Colorectal cancer is one of the deadly diseases and is ranked as third for men and second for women amongst all cancers in the world. Due to 6,08,000 deaths, it ranks as the fourth common cause of death due to cancer (WHO, 2018). The "ethnomedicinal studies" of the therapeutic values of plants evolves a systematic scientific evaluation. In the present study, the lead compounds hit on the GCMS analysis of the ethanolic extract of *Erythrina variegata* L. Leaf. The extract was studied for its anticancer potential in Insilco molecular docking. The results revealed that the 3-eicosyne have strong binding energies against the colorectal cancer mutated oncogene KRAS G12D and tumour suppressor gene p53 R249S in comparison to other compounds. Finally, it is confirmed that the active pharmaceutical ingredients have potent anti-Cancer activity and have great efficacy against the colorectal adenocarcinoma oncogenes and tumour suppressor regulating genes.

Keywords: Ethanolic Extract, Erythrina *variegata* Linn., Molecular Docking, GCMS

1. Introduction

Cancer is a common cause for mortality, and an over looming threat to mankind[1,2]. In developing countries like India, breast cancer is highly prevalent and in years to come the numbers may increase alarmingly due to environmental pollution, changes in lifestyle and inclusion of genetically modified food in the diet[3]. Plants are an important source of therapy for human ailments. Worldwide and more so in India, there are a number of tribal/ethnic groups who possess a vast knowledge about plant theraphy. The "ethnomedicinal studies" of the therapeutic values of these plants involve a systematic scientific evaluation[4]. A number of ethnomedicinal species which have exhibited cytotoxic effects on tumour/cancer cells[5] have been identified for the treatment of cancer. The anticancer properties by members of Fabaceae have been traced to the presence of phytochemicals such as isoflavones genistein, anthocyanins, b-sitosterol, and resveratrol. *Erythrina variegata* L. (Family, Fabaceae) also known as a coral tree is a medicinal plant that is widely distributed in the tropical and subtropical regions of the world. Finding the active pharmaceutical ingredients (API) from the plant is not an easy task[6].

Although the plant is being used in alternative medicines to treat cancer, very few investigations have evaluated the mechanism of action. Even though a number of benefits are present, the identification, extraction, and purification of the API from the plant parts are entirely difficult. To find out the novel API, Rational Drug Design (RDD) provides the facility but it involves a number of methods, among them docking is one of the best methods because of the process of fitting of a receptor and ligand in 3D space and finding the active site in it[7]. In addition to that,

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the identification of the molecular bonding of ligand (API) and the receptor (Protein) plays a major role in the selection, extraction, and purification of the API from the plant. In order to change this problem, computational biology is a promising area[8]. There are a number of docking tools available in the market among which Auto dock 4.2 is one of the most advanced automatic docking tool for grid generation, docking score calculation, and conformers evaluation. Our present study focused on the ligand receptor interaction of the bio active compound forms the ethanolic extract of *Erythrina variegata* L. which was docked against the oncogenic and tumour suppressor proteins of colon cancer mutated genes KRAS G12D and P53 R249S respectively.

2. Materials and Methods

2.1 Sample Preparation

The plant *Erythrina variegate* L.were collected from Thanjavur district and the leaves were shade dried and powdered using a grinder. Further the dried leaf, powder of *Erythrina variegate* L. plant materials were extracted with ethanol using a Soxhlet apparatus overnight. The extracts were then filtered through Whatman No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotary evaporator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in an airtight container in the refrigerator below 10°C.

2.2 GCMS Analysis

The identification and quantification of *Erythrina variegate* L. chemical constituents were evaluated by Gas chromatography coupled with Mass spectrometry QP2010 plus, Shimadzu, Japan equipped with RTX-5 MS GC capillary column (5% diphenyl / 95% dimethyl polysiloxane) of 0.5 µm diameter and 30 m length. GC working conditions: The temperature was kept between 40- 290° C with a gradual increase of 8° C/min. Column oven and injection temperatures were set at 100° C and 270° C respectively. Injection mode was set as split with a ratio of 20; Helium was used as carrier gas (mobile phase) with a flow rate of 1ml/min. MS working conditions: ion source and interface temperatures were set at 200 and 260° C. Solvent cut-off time was set as 4 min and detector voltage was set at 0.1 kV. Injection conditions: 1 µL injection volume; 10 µL injection syringes; injection temperature at 240° C; mass range at 20–300 m/z. The analytes were matched with the NIST and Wiley library for the similar hits of the basil chemical compositions[9].

2.3 Molecular Docking

The three-dimensional structures of the mutant proteins and ligands were retrieved from PDB and PubChem databases respectively. Then the PDBQT format files, Grid and Docking Parameter files were prepared. Grid box was set as 30x30x30 dimensions and the run was set as 10 and the mean binding energy of the compounds against the mutant proteins were studied[10].

3. Result and Discussion

3.1 GCMS Analysis

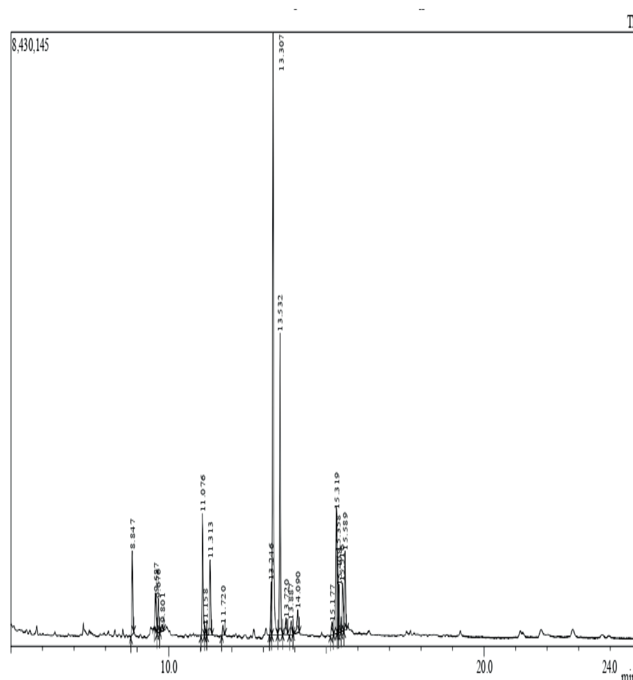

Figure 1. GCMS Chromatogram.

3.1.1 Peak Report

The various compounds present in the entire herb of *E. variegata* detected by the GC-MS are shown in Table 1. In the GC-MS analysis, 3-eicosyne, Butanoic acid, Phytol, Octadecenoic acid, Methyl stearate, Hexane, 2,4,4-trimethylcompounds were identified and highly in the ethanolic extract of *Erythrina variegata* L. leaves as mentioned in Table 1. The identification of compounds is based on the peak area, molecular weight and molecular formula. These compounds are responsible for pharmacological activities. Out of 20 compounds, 3 compounds have highest peak area.

Table 1. Compounds present in the entire herb of *E. variegata* detected by the GC-MS

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	8.847	2348786	4.18	1152027	4.71	2.04	Phenol, 3,5-bis(1,1-dimethylethyl)- (CAS) 3,5
2	9.587	1178370	2.10	511946	2.09	2.30	1,2-BENZOLDICARBONSAEURE, DI-(HE
3	9.676	1075627	1.91	463599	1.90	2.32	1,2-BENZOLDICARBONSAEURE, DI-(HE
4	9.801	288595	0.51	94926	0.39	3.04	Hexane, 2,4,4-trimethyl- (CAS) 2,4,4-Trimeth
5	11.076	3922163	6.98	1673580	6.85	2.34	1-Tetradecanamine, N,N-dimethyl- (CAS) DI-
6	11.158	317083	0.56	118967	0.49	2.67	Dodecane, 4,6-dimethyl- (CAS)
7	11.313	2466285	4.39	1025053	4.19	2.41	Pentanoicacid, 4-methyl-, methyl ester (CAS)
8	11.720	398745	0.71	148478	0.61	2.69	Hexadecanoic acid (CAS) Palmitic acid
9	13.246	1544544	2.75	730079	2.99	2.12	7,10-Hexadecadienoic acid, methyl ester(CAS
10	13.307	18918617	33.65	8260228	33.79	2.29	3-eicosyne
11	13.532	8818519	15.69	4143535	16.95	2.13	Butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester
12	13.720	627388	1.12	219124	0.90	2.86	(3-TERT-BUTYL-5-HYDROXYMETHYL-C
13	13.887	635645	1.13	189348	0.77	3.36	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- (
14	14.090	897833	1.60	317280	1.30	2.83	1,2-Benzenedicarboxylic acid, dibutyl ester(C
15	15.177	460610	0.82	174285	0.71	2.64	9-Octadecene, (E)- (CAS)
16	15.319	4306459	7.66	1732535	7.09	2.49	PHYTOL
17	15.358	2202194	3.92	1099958	4.50	2.00	17-Octadecenoic acid, methyl ester (CAS)ME
18	15.408	1462730	2.60	633946	2.59	2.31	9,12,15-Octadecatrienoic acid, methyl ester, (
19	15.516	1735473	3.09	667660	2.73	2.60	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-
20	15.589	2609401	4.64	1090064	4.46	2.39	METHYL STEARATE
		56215067	100.00	24446618	100.00		

3.2 Molecular Docking

Table 2. 3-eicosyne against KRAS G12D and p53 R249S

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid residue	Ligand Atom	Bond length (Å)
KRAS (G12D)	8	-5.05	Hydrogen bond	Asp12	O	2.66
p53 (R249S)	4	-5.03	Hydrogen bond	GLN99	O	2.69

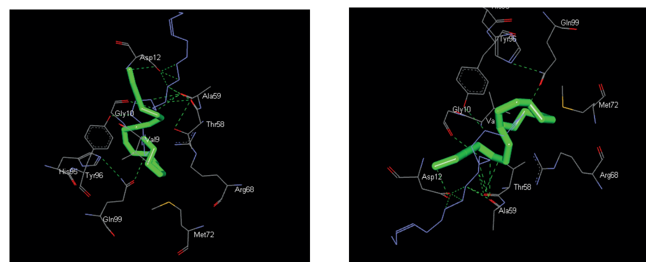

Figure 2. Docked complex of 3-eicosyne with KRAS G12D and P53 R249S respectively

The Table 2 represents the binding interactions of 3-eicosyne against the receptors KRAS G12D and P53 R249S, along with their bond specifications. Comparatively, 3-eicosyne showed high binding affinity against KRAS (G12D) followed by p53 (R249S); The 8th run of KRAS (G12D) had a binding energy of -5.05 and H-bond of length 2.66 Å between O-atom and Asp12 residue. whereas 4th run of p53 (R249S) had a binding energy of -5.03 and H-bond of length 2.69Å between O-atom and GLN99 residue. Figure 2 shows the three-dimensional molecular modelling pictures of the 3-eicosyne in the colorectal cancer mutant proteins (KRAS G12D and P53 R249S) respectively.

Table 3. Butanoic acidagainst KRAS G12D and p53 R249S

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid residue	Ligand Atom	Bond length (Å)
KRAS (G12D)	9	-2.35	Hydrogen bond	VAL173	O	2.61
p53 (R249S)	8	-3.12	Hydrogen bond	TYR96	O	2.99

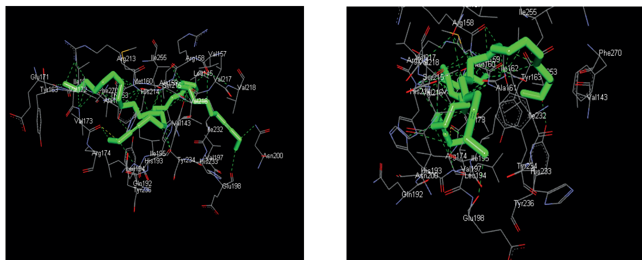

Figure 3. Docked complex of Butanoic acid with KRAS G12D and P53 R249S respectively.

The Table 3 represents the binding interactions of Butanoic acid against the receptors KRAS G12D and P53 R249S, along with their bond specifications. Comparatively, Butanoic acid showed high binding affinity against p53 (R249S) followed by KRAS (G12D); The 9th run of KRAS (G12D) had a binding energy of -2.35and H-bond of length 2.61Å between O-atom and VAL173 residue whereas the 8th run of p53 (R249S) had a binding energy of -3.12 and H-bond of length 2.99 Å between O-atom and TYR96 residue. Fig 3 shows the three-dimensional molecular modelling pictures of the Butanoic acid in the colorectal cancer mutant proteins (KRAS G12D and P53 R249S) respectively.

Table 4. Phytolagainst KRAS G12D and p53 R249S

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid residue	Ligand Atom	Bond length (Å)
KRAS(G12D)	7	-3.50	Hydrogen bond	SER89	O	3.02
p53 (R249S)	3	-4.50	Hydrogen bond	ALA11	O	3.08

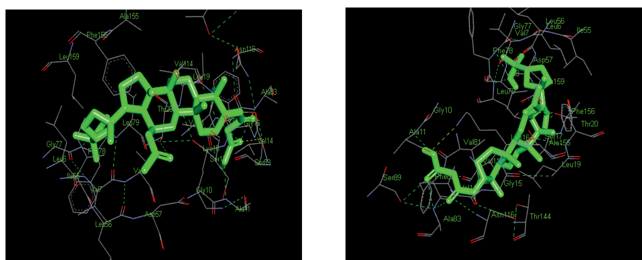

Figure 4. Docked complex of Butanoic acid with KRAS G12D and P53 R249S respectively.

The Table 4 represents the binding interactions of Phytol against the receptors KRAS G12D and P53 R249S, along with their bond specifications. Comparatively, Phytol showed high binding affinity against p53 (R249S) followed by KRAS (G12D); The 7th run of KRAS (G12D) had a binding energy of -3.50 and H-bond of length 3.02 Å between O-atom and SER89 residue whereas 3rd run of p53 (R249S) had a binding energy of -4.50 and H-bond of length 3.08 Å between O-atom and ALA11 residue. Fig 4 shows the three-dimensional molecular modelling pictures of the Phytol in the colorectal cancer mutant proteins (KRAS G12D and P53 R249S) respectively.

From the results, we conclude that the 3-eicosyne docked with KRAS G12D showedhighest binding energy compared to other compounds. Also,3-eicosyne docked with p53 R249S shows highest binding energy and good binding affinities. Finally, we conclude that these compounds from *Erythrina variegata* Linn. have strong inhibitory effects on the mutant oncogene and tumour suppressor gene of colorectal cancer.

4. Conclusion

This study was designed to evaluate the anti-colon cancer activity of ethanolic extract of *Erythrina variegata* Linn. compounds through *Insilco* studies. The GCMS results showed the 20 bioactive compounds present in the ethanolic extract of *Erythrina variegata* Linn. Here all the 3 phytoconstituents show higher peak area, especially 3-eicosyne has good affinities against oncogenic and tumour suppressor proteins KRAS G12D and P53 R249S respectively. The least binding energies of the interacted complexes revealed that they could have the strong anti-colon cancer potential by docking the receptors and that mutated protein receptors won't involve in cancer signalling pathways. Further the ethanolic leaf extracts

were taken to assess the cytotoxic, apoptotic induction ability.

5. References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA: A Cancer J Clin. 2007; 57(1):43–66. https://doi.org/10.3322/canjclin.57.1.43. PMid:17237035
2. Bray F, Moller B. Predicting the future burden of cancer. Nat Rev Cancer. 2006; 6(1):63–74. https://doi.org/10.1038/nrc1781. PMid:16372017
3. Sen U, Sankaranarayanan R, Mandal S, Ramanakumar AV, Parkin DM, Siddiqi M. Cancer patterns in eastern India: The first report of the Kolkata cancer registry. Int J Cancer. 2002; 100(1):86–91. https://doi.org/10.1002/ijc.10446. PMid:12115592
4. Jahan R, Hossain S, Seraj S, Nasrin D, Khatun Z, Ranidas P, et al., Centella asiatica (L.) Urb.: Ethnomedicinal uses and their scientific validations. Am Eur J Sustain Agric. 2012; 6:261–70.
5. Amri E. The role of selected plant families with dietary ethnomedical species used as anticancer. J Med Plants Stud. 2014; 2:28–39.
6. Martins J, Brijesh S. Anti-depressant activity of Erythrina variegata bark extract and regulation of monoamine oxidase activities in mice. J Ethnophar macol. 2019; 248. https://doi.org/10.1016/j.jep.2019.112280. PMid:31600560
7. Kapetanovic IM. Computer-Aided Drug Discovery and Development(CADDD): In silico-chemico-biological approach. Chem Biol Interact. 2008; 171(2):165–176. https://doi.org/10.1016/j.cbi.2006.12.006. PMid:17229415 PMCid:PMC2253724
8. Purushothaman B, Suganthi N, Jothi A, Shanmugam K. 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): 3423–7. https://doi.org/10.5958/0974-360X.2019.00579.1
9. Bruni R, Bellardi MG, Parrella G. Change in chemical composition of sweet basil (*Ocimum basilicum* L.) essential oil caused by alfalfa mosaic virus. Journal of Phytopathology. 2015; 164(3): 202–6. https://doi.org/10.1111/jph.12410
10. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a lamarckian genetic algorithm and empirical binding free energy function. J. Comput. Chem. 1998; 19:1639–62. https://doi.org/10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B