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IDENTIFICATION OF NARINGENIN-7-GLUCOSIDE FROM *H. ANTIDYSENTRICA* AS L858R INHIBITOR IN BREAST CANCER

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ABSTRACT

EGFR is an oncogene commonly overexpressed in breast cancer. It is associated with unfavourable prognosis. Drug resistance against tyrosine kinase inhibitors is a major drawback in the current line of treatment. Phytochemicals from Holarrhena antidysentrica were used for molecular based drug designing to target L858R mutation of EGFR leading to higher efficacy with minimal side effects. Molecular docking facilitates study of molecular and structural diversity in an organised manner. Using curated databanks, 150 phytochemicals were identified from the plant of interest and 52 compounds passed the SwissADME test for drug likeness. The phytochemicals were targeted against the native and mutant structure of EGFR retrieved from PDB. The identified phytochemicals to combat L858R mutation revealed 2 compounds including naringenin-7-glucoside that showed a good dock score and fit value as the novel outcome. It is found out that the naringenin-7-glucoside can act as an active antidote to breast.

Keywords: *H. antidysentrica, L858R mutation, EGFR, Docking, naringenin-7-glucoside*

1.0 INTRODUCTION

Breast cancer has overhauled lung cancer and became world's most commonly diagnosed cancer in 2020 (WHO, 2021). In spite of significant advances in disease detection and treatment, the control remains at bay. Numerous genes are involved in the progression and metastasis of breast cancer including BRCA1, BRCA2, PTEN and EGFR (Shiovitz and Korde., 2015, Masuda *et al.,* 2012).

EGFR is the foremost breast cancer marker, which is the primary target for specific inhibitors like gefitinib and erlotinib. It is a tyrosine kinase family member depending on enzymatic phosphorylation for signal transduction (Maennling *et al.,* 2019). The binding of Epidermal Growth Factor to EGFR activates the tyrosine kinase pathway leading to dimerization, phosphorylation and cell division (Wieduwilt and Moasser., 2008). An estimation of about 50% of triple negative breast cancer and inflammatory breast cancer overexpress EGFR (Masuda *et al.,* 2012). The higher expression of EGFR in breast cancer is mainly found in younger women and is inversely proportional to lower hormone levels. Over expression results in higher proliferation and genomic instability. It is associated with an increased risk of relapse and poor prognosis (Changavi *et al.,* 2015).

L858R mutation is found in EGFR at the 21st exon. The Leucine at the 858th amino acid is substituted by Arginine, which along with exon 19 deletion mutation accounts for about 90% of EGFR mutation in lung cancer (Zhuo *et al.,* 2017). L858R mutation has a frequency of 10.9% in TNBC (Kim *et al.,* 2017). Currently used drugs for targeting L858R mutation is solely used as first line treatment options with numerous serious side effects including loss of hearing, bleeding gums, anaemia, tachycardia, peripheral neuropathy, risks of blood clots, recurrence of cancer and is not 100% fruitful.

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Phytochemicals namely curcumin, quercetin, rutin, coumarin, and thymol exhibit anti-cancer activities in different cancer cell lines (Singh *et al.,* 2016). *Holarrhena antidysenterica,* also known as *Sinapis dichotoma* is used in Ayurveda for treating all kinds of arbuda tumours or cancer (Kulkarni *et al.,* 2014). Phytochemicals are emerging as an effective alternative against conventional synthetic drugs (Dutt *et al.,* 2019). They are more effective than the conventional drugs with minimal side effects (Mendie and Hemalatha, 2022., Ashraf, 2020).

Computer-aided drug discovery (CADD) plays a crucial function in identifying therapeutically significant small molecules for the past three decades (Sliwoski *et al.,* 2013). Docking predicts the preferred orientation of the complex by analysing the binding energy and dock score of the interacting molecule. Identification of binding design helps in rational drug designing and explicate essential biochemical practices (Madeswaran *et al.,* 2012, Mostashari-Rad *et al.,* 2019).

Therefore, targeting L858R mutation using phytochemicals will be a safer alternative with infinitesimal side effects.

2.0 METHODS

2.1 Identification of plants and compounds

Holarrhena antidysenterica L. was chosen for the study. Studies were not undertaken to identify the anticancer compounds. The primary and secondary metabolites of these plants were identified from Dr. Dukes Phytochemical and Ethnobotanical Database, Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) and by intensive literature review.

2.2 Retrieval of proteins and ligands

The 3-dimensional structure of 150 compounds obtained from the plant were retrieved from PubChem. The 3D structure of native and mutated EGFR was procured from PDB.

2.3 Drug-likeness evaluation

The drug-likeness of the compounds were evaluated using SwissADME based on Lipinski rule of five which stated no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular mass less than 500 Daltons and LogP less than 5.

2.4 Docking against L858R mutation

Phytochemicals of *H. antidysenterica* were docked against the L858R mutation of Epidermal Growth Factor Receptor where the Leucine at the 858th position is substituted by Arginine. Binding sites were determined from existing docked complexes. Molecular docking was carried out using the LigandFit protocol of Discovery studio 3.5.

2.5 Pharmacophore analysis and Fit value

The 3D and 4D QSAR pharmacophore analysis helps identify the steric and electronic features that is necessary to ensure the supra-molecular interactions with the L858R mutation of EGFR. The hydrogen bond donor, hydrogen bond acceptor and hydrophobic features were mapped on the training set of molecules.

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3.0 RESULT

3.1 Identification of plants and phytochemicals

A total of 150 compounds were identified from *H. antidysenterica*. Through extensive literature screening and using databases namely Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) and Dr. Dukes Phytochemical and Ethnobotanical Database, the phytochemicals were identified.

3.2 Database mining for protein and ligands

The 3D structure of the target protein EGFR was obtained from the Protein Data Bank. The mutant and native structure specifically, 4LQM and 4WRG were respectively considered for the study (Fig.1).

Figure 1:3D Structure of Epidermal Growth Factor Receptor

Using PDBSUM, the molecular structural details were established. The resolution of the structure 4LQM was 2.50Å. Procheck statistics revealed the number of residues in the most favoured region, allowed regions and disallowed regions. Most favoured region showed 238 residues with 0 residues in the disallowed region making the structure highly significant (Fig. 2A)

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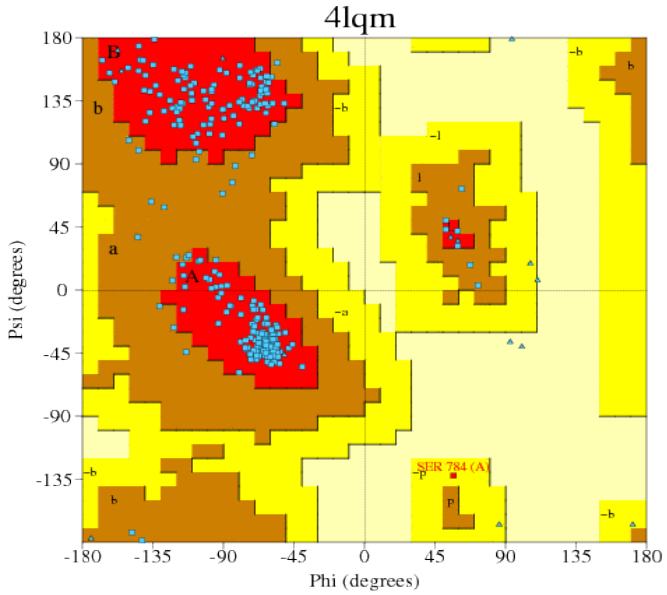

Figure 2a: Ramachandran plot of the L858R mutated EGFR showing the residues in allowed and disallowed region (4LQM)

4WRG was chosen as native structure. The resolution of this structure was 1.90Å. Most favoured region showed 224 residues with 0 residues in the disallowed region thus, making the structure highly significant (Fig. 2b).

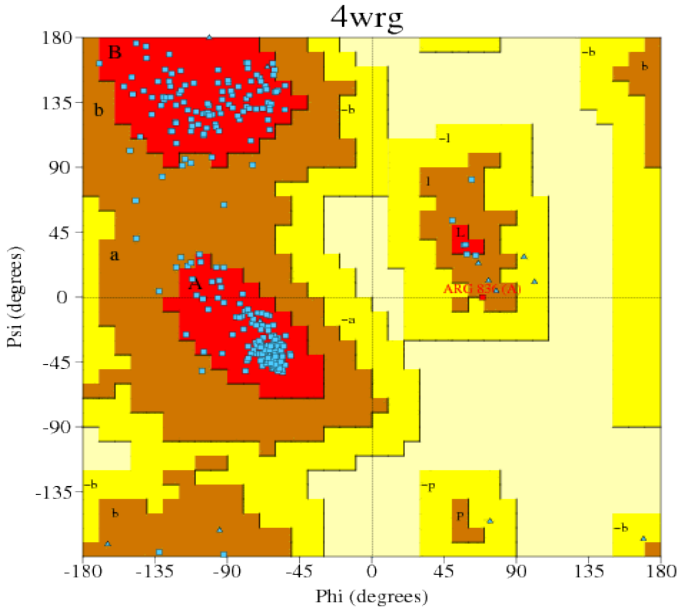

Figure 2b: Ramachandran plot of the native EGFR showing the residues in allowed and disallowed region (4WRG)

The 3D structures of the 150 ligands were acquired from PubChem.

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3.3 Drug-likeness evaluation

Drug-likeness evaluation (ADMET) depicts the disposition of the drug in humans. Out of 150, 52 compounds passed the ADMET test in SwissADME. The result of the ADMET revealed physicochemical properties of the identified phytochemicals, which includes the rules of five MW, iLOGP, HBAs and HBDs (table 1).

Table 1: Drug likeable phytochemicals from *H.antidysenterica*

Sl.No:	Compound	H bond donors	H bond acceptors	Molecular mass (g/mol)	Log P
1	3,5-dihydroxybenzoic acid	3	4	154.12	0.46
2	4-hydroxybenzoic acid	2	3	1.05	138.12
3	7a-Hydroxyconessine	1	3	3.5	372.59
4	Alpha-resorcylic-acid	0	4	6.45	496.9
5	Antidysentericine	1	2	3.63	356.54
6	Aricine	1	5	2.63	382.45
7	Betulinaldehyde	1	2	6.39	440.7
8	Betulinic acid	2	3	6.14	456.7
9	Conarrhimine	2	2	2.49	314.59
10	Conessidine	1	2	3.94	326.52
11	Conessimine	1	2	4.1	342.56
12	Conessine	0	2	4.36	356.59
13	Conimine	2	2	3.85	328.53
14	Ergostenol	1	1	7.46	400.68
15	Holadysenterine	3	4	2.69	390.56
16	Holadysine	1	2	4.25	329.52
17	Holadysone	2	4	2.44	344.44
18	Holamide	1	2	3.35	368.51
19	Holamine	1	2	3.88	315.49
20	Holantosine-A	3	7	2.86	493.68
21	Holantosine-B	1	6	3.77	475.66
22	Holantosine-C	3	7	2.95	493.68
23	Holantosine-D	1	6	3.79	475.66

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24	Holaromine	0	1	4.63	309.49
25	Holarosine A	2	7	3.44	517.7
26	Holarrhenine	1	3	3.49	372.59
27	Holarrhimine	3	3	2.9	332.52
28	Holonamine	1	3	2.77	325.44
29	Isoconessimine	1	2	4.11	342.56
30	Isoconessine	0	2	4.32	356.59
31	Kurchinin	1	2	5.01	300.44
32	Kurcholessine	2	3	4.69	298.46
33	Linoleic-acid	1	2	5.09	278.43
34	L-querbrachitol	5	6	2.16	194.18
35	Lupeol	1	1	7.28	426.72
36	Mitiphylline	2	7	3.79	531.72
37	N-3-methyl-holarrhimine	3	3	3.26	346.55
38	Naringenin 7-O-b-Dglucoside	6	10	0.23	434.39
39	Norholadiene	1	2	3.58	311.46
40	Pubadysone	1	3	3.09	326.46
41	Pubamide	2	3	2.28	341.44
42	Pubescimine	1	3	2.53	372.59
43	Pubescine	1	5	2.61	382.45
44	Pubescinine	1	3	3.22	367.48
45	Puboestrene	0	3	3.64	312.4
46	Regholarrhenine D	1	3	3.82	358.56
47	Regholarrhenine E	2	4	3.23	406.63
48	Regholarrhenine F	1	3	4.13	388.63
49	Schembl13324298	1	1	6.81	398.66
50	Stigmasterol	1	1	6.98	412.69
51	Syringic-acid	2	5	1.02	198.17
52	Ursolic acid	2	3	5.93	456.7

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3.4 Docking

The native and mutant structures of EGFR were docked against 52 compounds to analyse the dock score. Dock score is a computational score that is used to predict the affinity of ligand and protein once it is docked. The more the dock score, the better affinity (Pantsar and Poso., 2018). 13 compounds exhibited a dock score of more than 50 in mutant structure and less dock score in native structures. The conventionally used drugs docked showed a score of 57 and 63.2 in gefitinib and erlotinib respectively. Complexes which gave a dock score of more than 63 (score of conventional drug currently used in market) were considered for the 3D and 4D fingerprinting studies. Hence, the compounds naringenin-7-glucoside (81.15), pubescine (63.35) from *H. antidysenterica* were considered for further studies (Table 2, figure 3a-3b).

Table 2: Phytochemicals docked against L858R mutation of EGFR

Compounds	PubChem ID	Dockscore against 4QLM	Dockscore against 4WRG
Naringenin-7-glucoside	9910769	81.15	Fail
Pubescine	72313	63.35	Fail

Figure 3a: Docked complex of naringenin-o-glucoside and 4lqm

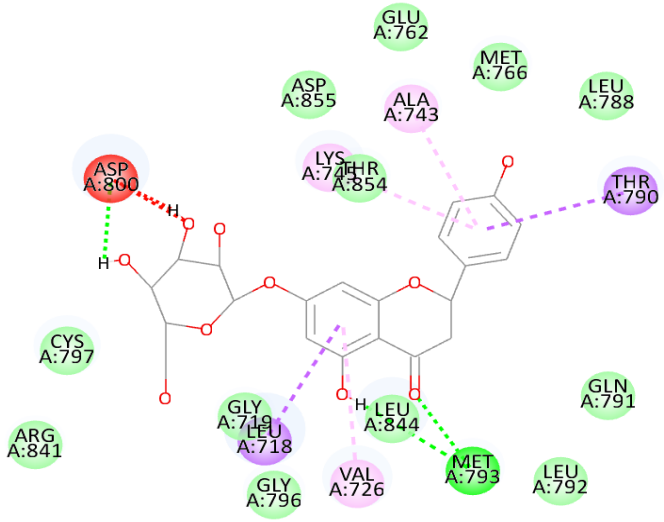

Figure 3b: Docked complex of pubescine and 4lqm

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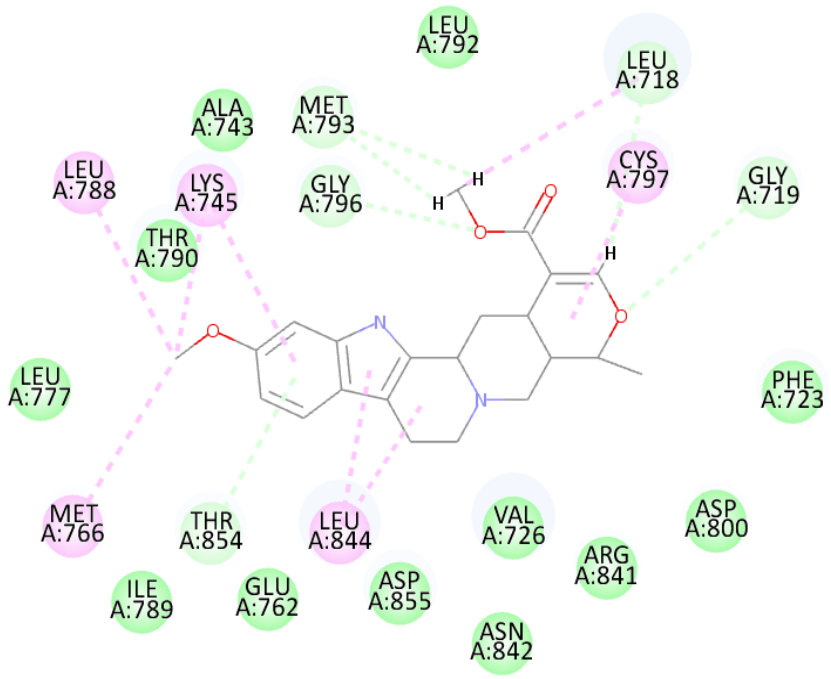

3.5 Pharmacophore analysis and Fit value

The 3D and 4D fingerprinting was exhibited in naringenin-7-glucoside, pubescine, 3-O-caffeoyl-D-quinic acid, 2,6-dihydroxybenzoic acid and herbacetin. The fit value was also calculated. Fit value indicates how well the features in the pharmacophore overlap the chemical features in the molecule (Gaurav and Gowtham, 2017). Naringenin-7-glucoside (prunin) had a fit value of 4.99 (table 3). Based on the fit value, position of attachment of atoms and availability in market, naringenin-7-glucoside (prunin) was considered for further studies.

Table 3: Fit value of the docked compounds

Compounds	PubChem ID	Fit value
Naringenin-7-glucoside	9910769	4.99
Pubescine	72313	7.99

4.0 DISCUSSION

Though, leaps and bounds of technological advancements are achieved every day in the field of drug discovery, breast cancer treatment and control remain at bay.

The occurrence of EGFR mutations in breast cancer patients is yet to be studied conscientiously. In TNBC, frequency of EGFR mutations falls under a wide range of 13-78% (Nakai *et al.,* 2016). Masuda *et al.* (2012) evaluated 70 patients where exon 19 deletion mutation and exon 21 missense mutation (L858R mutation) were determined in 11.8%. Studies reported that EGFR mutations including L858R and T790M mutation initially respond to drugs erlotinib and gefitinib. Over a period of 6-12 months, they develop resistance towards the same hence proving the necessity for a novel drug to overcome the resistance (Nguyen *et al.,* 2009, Qin *et al.,* 2019).

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In this study, EGFR exon 21 missense mutation was targeted for drug development against EGFR in breast cancer. Out of the compounds identified from *H. antidysenteric* 13 of them surpassed the dock score of conventionally used drugs erlotinib and gefitinib. After initial use of erlotinib, most patients developed folliculitis, diarrhoea, paronychia, fatigue and hair changes. Inconvenience persisted on long-term use and even when drug administration was ceased. Gefitinib can lead to interstitial lung disease in rare cases. In some cases, 5-Fu induce cardiotoxicity. All these can be fatal to the patient (Becker *et al.,* 2010, Focaccetti *et al.,* 2015).

In normal cells, the dimerization of EGFR leads to auto-phosphorylation by adenosine triphosphate binding to the ATP binding site in 745th position of EGFR leading to cell division. If ATP cannot bind, less EGF receptors will be activated and thereby, helps to inhibit cell division in tumour cells (Kannan *et al.,* 2018). In the present study, two compounds from *H. antidysenterica* showed a better dock score against L858R mutation in breast cancer.

The compounds naringenin-7-glucoside and pubescine from *H. antidysenterica* binds itself to the ATP binding site of EGFR at Lys-745 by pi-alkyl bond, the N lobe of Tyrosine kinase region of EGFR preventing phosphorylation and eventually cell division. The pi-alkyl bond is a strong ionic bond, which interacts between pi-electrons and alkyl group thus, making the docked complex stable thereby inhibiting tumour formation (Arthur *et al.,* 2021).

The docking based 3D and 4D pharmacophore analysis allows a comparison between the chemical interactions which describes the activity relationship of the docked complexes (Velázquez-Libera *et al.,* 2019). Fit value is directly proportional to the stability of docked complex. A fit value greater than 3.99 is significant. Hence, the compound naringenin-7-glucoside, with a fit value of 4.99 forms a stable complex.

Therefore, naringenin-7-glucoside can be a potent option against L858R mutation in breast cancer. It is a flavanone 7-O-beta-D-glucoside where (S)-naringenin is substituted by a beta-D-glucopyranosyl moiety by glycosidic linkage at position 7. Therapeutically, it functions as a metabolite, an antilipemic drug, a hypoglycemic agent and an antibacterial agent (Jung *et al.,* 2017, Choi *et al.,* 1991, Céliz *et al.,* 2010).

5.0 CONCLUSION

The efficacy of the plant based drug helps in eliminating the side effects caused by the synthetic drugs. *H. antidysenterica* is a plant with potent phytochemicals that exhibit anticancer activity. It inhibits the cancer pathway by binding to the L858R mutation in EGFR in breast cancer and prevent cell proliferation, invasion and metastasis. Through ADMET studies using SwissADME and Discovery Studio 3.5, naringenin-7-glucoside showed promising association with L858R mutation of EGFR. Hence, it is proved that the naringenin-7-glucoside could be administered as an effective inhibitor against breast cancer.

ABBREVETION

- ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity
- BRCA1: BRCA1 DNA repair associated
- BRCA2: BRCA2 DNA Repair Associated
- CADD: Computer Aided Drug Designing

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- EGFR: Epidermal Growth Factor Receptor
- HBA: Hydrogen Bond Acceptors
- HBD: Hydrogen Bond Donors
- iLOGP: Water coefficient
- IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics
- MW: Molecular weight
- PDB: Protein Data Bank
- PDBsum: Protein Data Bank sum
- PTEN: Phosphate and Tensin Homolog
- QSAR: Quantitative structure-activity relationship
- TNBC: Triple Negative Breast Cancer
- WHO: World Health Organization

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