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RESEARCH ARTICLE
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Identification of Herbacetin as L858R and T790M inhibitor in Breast Cancer

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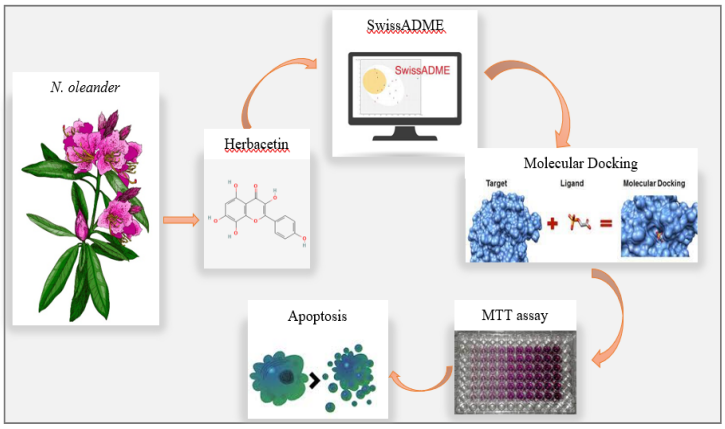
ABSTRACT

Epidermal Growth Factor Receptor plays a major role as an oncogene in breast cancer. Associated with unfavourable prognosis and relapse of disease, it is a major drug target for tyrosine kinase inhibitors. Currently used chemotherapeutic agents can only be used as a first line treatment option as it develops resistance against drug over a period of time. Phytochemicals identified from Nerium oleander were targeted against L858R and T790M mutation of EGFR in breast cancer. Among 203 phytochemicals identified, 93 passed the drug likeability test where the compounds can be used as drugs with minimal side effects. The phytochemicals were targeted against the native and mutant structure of EGFR retrieved from PDB. Among them, nine compounds including herbacetin expressed a good dock score and fit value against the mutations. Further, invitro cytotoxicity assay was conducted to confirm the result. Based on the dock score, fit value and the MTT results, herbacetin can be considered as an active inhibitor of L858R and T790M mutation.

Keywords: *Docking, EGFR, L858R mutation, Nerium oleander, T790M mutation*

GRPAHICAL ABSTRACT



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INTRODUCTION

The most frequent malignancy in women is breast cancer. Recent technological advancements led to an improvised treatment in various diseases, but cancer treatment and control remains an unattained goal. Unspecific drug target, common drugs for all patients, resistance against currently used drugs and interaction of several genes involved in cancer are some of the major reasons leading to uncontrollable cancer development. Targeting a particular gene using a specific drug can bring down the mortality rate in breast cancer (Housman et al., 2014).

Epidermal Growth Factor Receptor (EGFR) is a foremost breast cancer marker from the tyrosine kinase family. Inherited genes and its functional diversification have led this family to take up various roles in maintenance and development of cells and tissues. The EGFR protein dimerise and phosphorylates for signal transduction leading to cell division. EGFR consists of extracellular ectodomain where ligand binds to the receptor promoting dimerization, a transmembrane domain, followed by a short juxtamembrane section that forms an interconnection between the transmembrane domain and intracellular tyrosine kinase domain where the phosphorylation take place at the C terminal tail (Wieduwilt and Mosser, 2008). Phosphotyrosine activates downstream process including cellular differentiation and development (Hedger et al., 2015). Higher expression of EGFR in breast cancer is associated with higher proliferation of cells, genomic instability, increased relapse rate and poor prognosis (Levva et al., 2017; Connor et al., 2013).

Various mutations are associated with EGFR. L858R and T790M mutation occurring at the 21st and 20th exon together comprise about 90% of mutation in EGFR in lung cancer. The mutations together resist the tyrosine kinase inhibitors over a period of time (Teng et al., 2011). The T790M mutation is a gatekeeper EGFR mutation where the threonine is substituted with methionine at 790th amino acid of exon 20. The mutation alter the ATP binding site's conformation and boost ATP binding affinity, outcompeting the inhibitors and accelerating the progression of cancer. (Wang et al., 2016). Similarly, L858R mutation is found in the 21st exon of EGFR tyrosine kinase domain where the leucine at the 858th amino acid is substituted by Arginine. The mutations together accounts for carcinogenesis and tyrosine kinase inhibitor resistance against breast cancer (Budhiarko et al., 2017). Currently used drugs for targeting T790M and L858R mutation is used as first line treatment options with various drawbacks and side effects including risks of blood clots, bleeding gums, peripheral neuropathy, anaemia, loss of hearing, tachycardia, recurrence of cancer and does not always cure the disease completely.

Nerium oleander also known as Nerium odorum are used in Ayurveda for treating all kinds of arbuda tumours or cancer (Kulkarni et al., 2014). Phytochemicals has proved to be an efficient alternative with less or no side effects against conventional synthetic anticancer drugs (Singh et al., 2016). Therefore, targeting the EGFR mutations using natural products from plants will be a safer alternative with insignificant side effects.

MATERIALS AND METHODS

Plant identification

Nerium oleander is a plant used in Ayurveda against cancer. Scientific studies were not conducted to confirm and determine the compounds exhibiting anticancer activity. The primary and secondary metabolites of these plants were identified from Dr. Dukes Phytochemical and Ethnobotanical Database, Indian Medicinal Plants (https://phytochem.nal.usda.gov/phytochem/search), Phytochemistry and Therapeutics (IMPPAT, https://cb.imsc.res.in/imppat/) and by intensive literature review (Gupta et al., 2010; Sinha et al., 2016; Hase et al., 2017; Bhuvaneshwar et al., 2007; Farooqui and Tyagi., 2018; Al-Snafi., 2020; Zibbu and Batra., 2010).

Protein and ligand retrieval

The Protein Data Bank (PDB) is a structural archive of biological macromolecules (http://www.rcsb.org/pdb/). The three-dimensional structure of the mutant 4LQM (https://www.rcsb.org/structure/4LQM) and

native structure 4WRG (https://www.rcsb.org/structure/4wrg) of EGFR kinase domain was obtained from the Protein Data Bank. Similarly, the 3-D structures of the identified phytochemicals were acquired from a curated database, PubChem (https://pubchem.ncbi.nlm.nih.gov/), a repository for information on different chemical structures and associated biological activities.

ADME evaluation

Drug candidates should have good ADME characteristics and ideally not be toxic. Therefore, the identified compounds were assessed for their ADME profile using the SwissADME module offered in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss), which included drug-likeness, partition coefficient, solubility, and numerous other parameters (Daina et al., 2017).

The Lipinski rule of five was adopted for drug likeness which stated less than 5 H- bond donors, less than 10 H- bond acceptors, molecular mass less than 500 Daltons and LogP less than 5.

Docking

Using MetaPocket 2.0 and Discovery Studio, the target protein's active site was identified. The target files "4LQM.pdb" and "4WRG.pdb" were opened in the Discovery Studio's working window. Manual removal of the structure's heteroatoms, co-crystallized water molecules, and co-crystalized elements was done. Prior to docking, CHARMm Forcefield was put to the building. The molecule's active site was identified and selected. Under Docking option of the tool, LigandFit module was selected and the analysis was ran.

Cell line culture

The Vero and MCF-7 cell lines were maintained in a sterile environment and cultivated according to standard protocols in a CO2 incubator with 5% carbon dioxide at 390 C. The cells were cultivated in accordance with the needs of the experiment.

Cytotoxicity assay

Cytotoxicity assay measures the amount of cell damage exhibited when subjected to a compound. The MTT assay was conducted on the MCF-7 breast cancer cell line and Vero cell line.

The cytotoxic effect of compounds on the MCF-7 cell lines was evaluated in a quick colorimetric experiment using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and results were compared to untreated controls. For the screening experiment, the cells were incubated in 96-well plates at density of 10,000 cells per well with 100 mL of medium having 5% FBS. The cells were then incubated for 48 hours at 37 °C, 95% air, 5% CO2, and 100% relative humidity before the compound herbacetin was added. After the initial 48 hours, the compound was added and incubated for a further 48 hours at 37 °C, 5% CO2, 95% air, and 100% relative humidity. A triplicate of the treated and the control was maintained for accuracy.

Each well received 50 µL of MTT (5 mg/mL) in triple-distilled water after 48 hours, which was followed by a 4-hour incubation period at 37°C. After disposing the MTT medium, the formazan crystals were dissolved in 100 µL of DMSO, and the absorbance at 570 nm was then determined using a microplate reader. The percentage of cell inhibition was calculated using the formula below.

Cell inhibition (%) = 100- Absorbance (Sample)/Absorbance (control) x 100

RESULTS

Identification of phytochemicals

Identified 199 phytochemicals from N. oleander using databases and extensive literature review. Dr. Dukes Phytochemical and Ethnobotanical Database and Indian Medicinal Plants Phytochemistry and Therapeutics (IMPPAT) were the databases used for the study (Supp. Table 1).

Database mining for protein and ligands

The mutant and native structure, 4LQM and 4WRG respectively were considered for the study. 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. 1a).

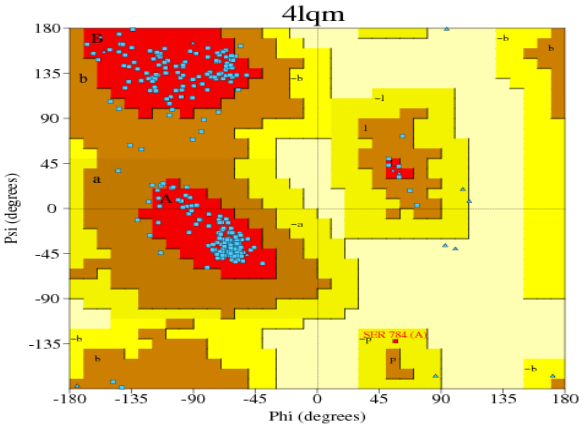


FIG. 1A: Ramachandran Plot for 4LQM (mutant EGFR)

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. 1b).

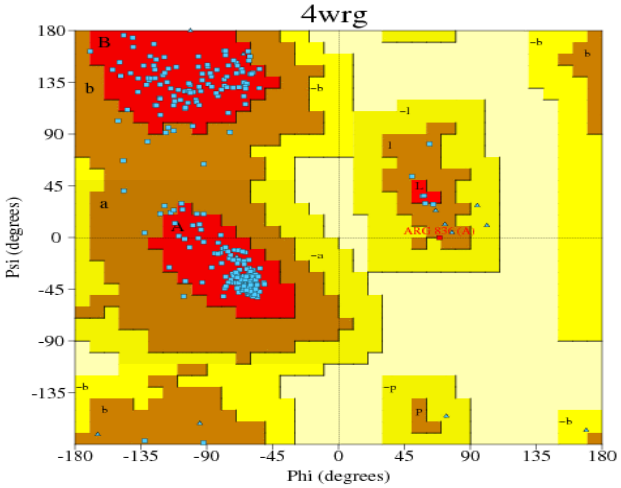


FIG. 1B: Ramachandran Plot for 4LQM (native EGFR)

The 3D structures of the 199 ligands were acquired from PubChem (Suppl. Table 1).

Drug-likeness evaluation

Drug-likeness evaluation (ADMET) depict the disposition of the drug in humans. Out of 199, 93 compounds passed the ADMET test in SwissADME. The ADMET revealed physicochemical properties of the identified phytochemicals, including the rule of five MW, iLOGP, HBDs and HBAs (Table 1).

TABLE 1: Drug likeable phytochemicals from Nerium oleander

Sl No	Compound	H bond donors	H bond acceptors	Molecular mass (g/mol)	Log P
1	1,15-pentadecanediol	2	2	4.15	244.41
2	16-deacetyloleandrin	3	8	2.41	534.68
3	17-chloro-7-heptadecene	0	0	6.66	272.9
4	2,3,5- trimethoxyamphetamine	1	4	1.89	225.28
5	2,6-dimethoxy-4-(-2- propenyl)-phenol	1	3	2.29	194.23
6	2,6-dimethoxyphenyl ester	0	4	1.44	182.17
7	21- hydroxypregna- 4, 6-diene- 3, 12, 20- trione	1	4	2.27	342.43
8	28-norurs-12-ene-3beta-ol	1	1	6.71	418.62
9	2-hydroxy-4-isopropyl-7- methoxytropone	1	3	1.88	194.23
10	2-methoxy-4-vinyl phenol	1	2	2.14	150.17
11	2-methoxy-5-methylphenol	1	2	1.82	138.16
12	2-methoxyphenol	1	2	1.4	124.14
13	2-methyl-z,z-3,13- octadecadienol	1	1	5.9	280.49
14	3beta, 27- dihydroxy- 12-oleanen- 28- oic acid	2	3	6.07	456.7
15	4- ethyl-2-methoxy-phenol	1	2	2.02	152.19
16	4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	0	2	6.7	372.58
17	4-tetradecyl este	0	3	4.91	286.45
18	5beta-pregnane	0	0	6.28	288.51
19	5-isopropyl-3,3-dimethyl-2- methylene-2,3-dihydrofuran	0	1	2.85	152.23
20	5-ter-butylpyrogallol	3	3	1.91	182.22
21	9,12-octadecadienoic acid	1	2	5.45	280.45
22	9-hexadecenoic acid	1	2	4.92	254.41
23	9-octadecenamide	1	1	5.32	281.48
24	Acetic acid, 3- hydroxy-6- isopropenyl-4,8a-dimethyl1,2,3,5,6,7,8,8aoctahydronapthalen-2-yl-ester	1	3	3.07	278.39
25	Adigoside	2	9	3.8	618.8
26	Adynerigenin	1	4	3.5	372.5
27	Adynerin	1	7	3.73	516.67
28	Alpha-amyrin	1	1	7.03	426.72
29	Alpha-d-galacturonate	4	7	-2.48	193.13
30	Arabinose	4	5	-1.85	150.13
31	Azafrin	3	4	5.38	426.59
32	Beta- anhydroepidigitoxigenin	1	3	4	356.5
33	Beta-sitosterol	1	1	7.24	414.71
34	Betulin	2	2	6.38	442.72
35	Betulinic-acid	2	3	6.14	456.7
36	Calotropin	3	9	1.92	532.62
37	Campesterol	1	1	6.92	400.68
38	Caoutchouc	0	0	1.83	68.12
39	Capric-acid	1	2	3	172.26

40	Caproic-acid	1	2	1.47	116.16
41	Caprylic-acid	1	2	2.23	144.21
42	Cardenolides	0	2	5.22	342.51
43	Chembl497040	1	2	6.5	454.73
44	Chembl524527	3	4	5.37	474.72
45	Choline	1	1	-1.38	104.17
46	Dammarane-type triterpene 15	0	0	8.95	414.75
47	D-diginose	2	4	-0.35	162.18
48	Deacetyloleandrin	3	8	2.37	534.68
49	Decanoic acid	1	2	3	172.26
50	Digitoxigenin	2	4	3.27	374.51
51	D-sarmentose	2	4	-0.28	374.51
52	Foliandrin/ oleandrin/ folinerin	2	9	2.9	576.72
53	Galactose	5	6	-2.33	180.6
54	Galacturonic acid	5	7	-2.41	194.14
55	Glucodigitoxigenin	5	9	1.73	536.65
56	Herbacetin	5	7	1.33	302.24
57	Hexanoic acid	1	2	1.47	116.16
58	Isoricinoleic acid	1	2	5.01	300.4
59	Neridienone a	1	3	2.92	326.43
60	Neridienone b	2	4	2.23	344.44
61	Neritaloside	3	10	2.55	592.72
62	Neriumogenin- a- 3 beta- d-digitaloside	3	8	2.71	528.63
63	Nerizoside	2	8	3.23	546.69
64	Ocotillol	2	3	5.96	460.73
65	Odoroside a	2	7	3.49	518.68
66	Odoroside- h	3	8	2.69	534.68
67	Odoroside b	2	7	3.49	518.68
68	Oleagenin	1	4	3.48	372.5
69	Oleanane-type triterpene 2	0	0	8.42	412.73
70	Oleanderolide	2	4	5.36	472.7
71	Oleandigoside	1	10	3.83	658.82
72	Oleandrigenin	2	6	2.84	432.55
73	Oleandrin	2	9	2.9	576.72
74	Oleandrose	2	4	-0.39	162.18
75	Oleic acid	1	2	5.71	282.46
76	P-cresol	1	1	1.76	108.4
77	Plumericin	0	6	1.24	290.27
78	Plumieride	5	12	-1.08	470.42
79	Pregna-5,16,20-triene-3beta,20-diol diacetate	0	4	4.68	398.54
80	Pregnanes	0	0	6.29	288.51
81	Proceragenin	2	4	5.17	470.68
82	Rhamnose	4	5	-1.42	164.16
83	S-2,6-diaminohexanoic acid	2	2	-2.78	147.2
84	Stigmasterol	1	1	6.98	412.69
85	Strospeside	4	9	1.54	550.68

86	Taraxasterane	0	0	8.42	412.73
87	Trans- isogenol	1	2	2.41	164.2
88	Tridecanedial	0	2	3.38	212.33
89	Ursolic acid	2	3	5.93	456.7
90	Uvaol	2	2	6.15	442.72
91	Uzarigenin	2	4	3.27	374.51
92	Z-10- methyl-11-tetradecen-1-olpropionate	0	2	5.47	282.46
93	A-d-glucofuranose	5	6	-2.02	180.16

Docking

The native and mutant structures of EGFR were docked against 93 compounds to analyse the dock score. 43 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. Hence, the compounds 2,6-dihydroxybenzoic acid (65.44), 10-formyfolic acid (71.75), alpha-D-Galacturonate (147.15), Herbacetin (63.24), galactose (68.97), galacturonic acid (77.64), herbacetin (70.92), S-2,6-diaminohexanoic acid (198.98) and thiamine (69.23) from N. oleander exhibited good results (Table 2; Figure 2a-2i).

TABLE 2: Phytochemicals docked against L858R mutation of EGFR

Compounds	**PubChem ID**	**Dockscore against 4QLM**	**Dockscore against 4WRG**
2,6-dihydroxybenzoic acid	9338	65.44	41.37
10-formyfolic acid	135405023	71.75	Fail
Alpha-D-Galacturonate	11883891	147.15	Fail
Calotropin	16142	63.24	Fail
Galactose	6036	68.97	35.47
Galacturonic acid	439215	77.64	36.14
Herbacetin	5280544	70.92	45.61
S-2,6-diaminohexanoic acid	5962	198.98	Fail
Thiamine	1130	69.23	Fail

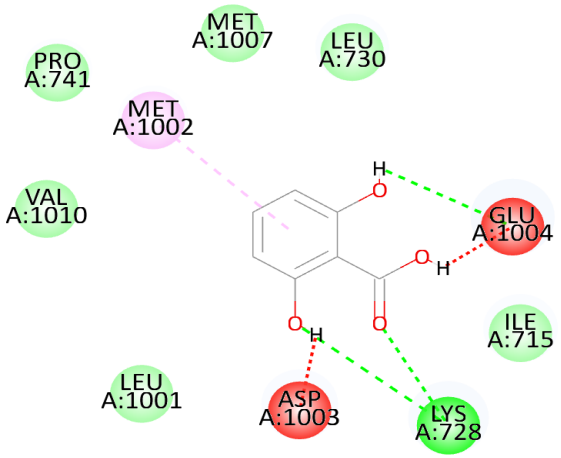


FIGURE 2A: Docked complex of 2,6-dihyroxybenzoic acid and 4lqm

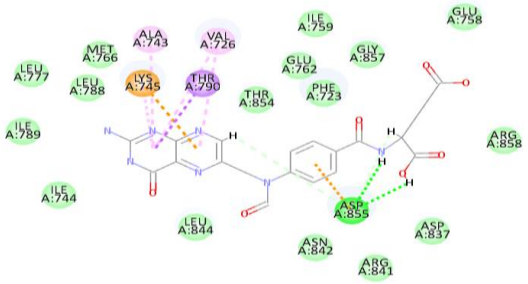


FIGURE 2B: Docked complex of 10-formyfolic acid and 4lqm

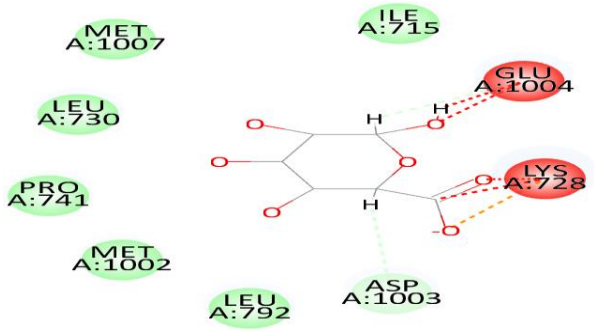


FIGURE 2C: Docked complex of Alpha-D-Galacturonate and 4lqm

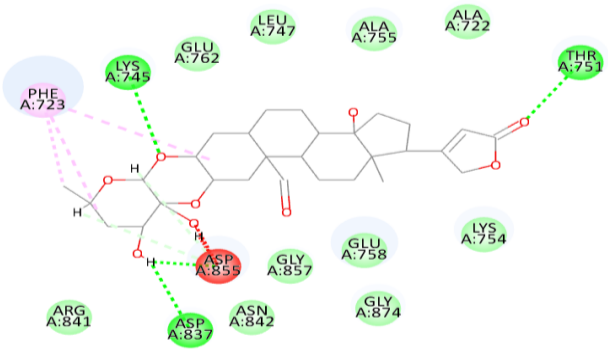


FIGURE 2D: Docked complex of Calotropin and 4lqm

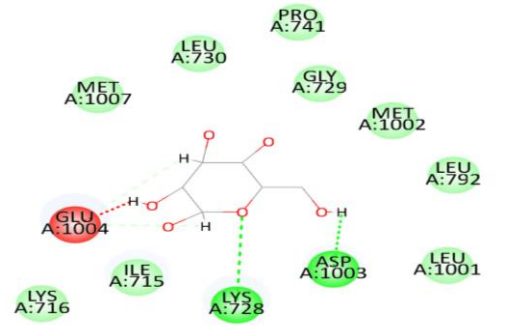


FIGURE 2E: Docked complex of Galactose and 4lqm

J Popul Ther Clin Pharmacol Vol 30(6):e267–e286; 05 April 2023.

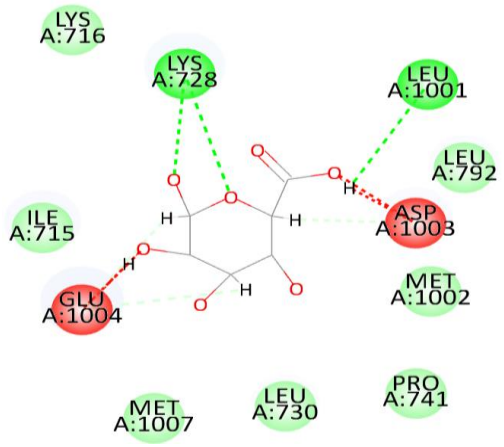


FIGURE 2F: Docked complex of Galacturonic acid and 4lqm

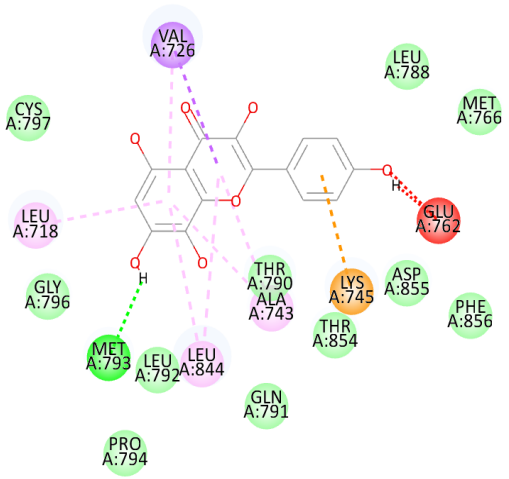


FIGURE 2G: Docked complex of Herbacetin and 4lqm

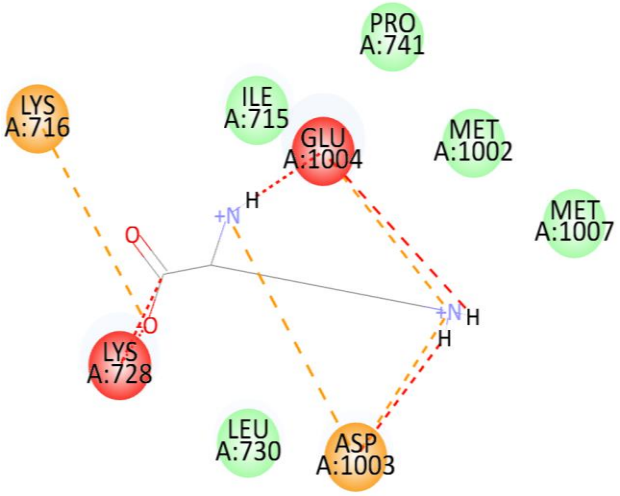


FIGURE 2H: Docked complex of S-2,6-diaminohexanoic acid and 4lqm

J Popul Ther Clin Pharmacol Vol 30(6):e267–e286; 05 April 2023.

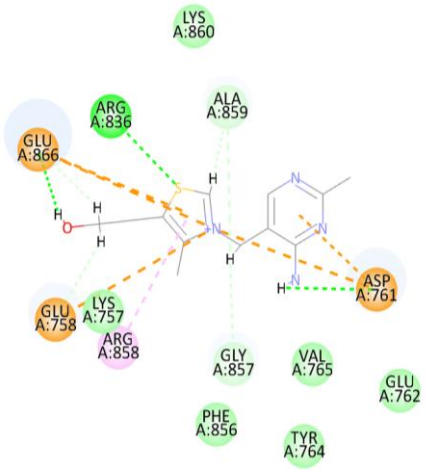


FIGURE 2I: Docked complex of thiamine and 4lqm

Fit value analysis

Fit value indicates the degree to which the pharmacophore's features and the molecule's chemical features overlap (Pal et al., 2019; Gaurav and Gowtham, 2017). Herbacetin had a fit value of 5.00 (Table 3). Based on the fit value, position of attachment of atoms and availability in market, herbacetin was considered for further studies.

TABLE 3: Fit value of the docked compounds

Compounds	PubChem ID	Fit value
2,6-dihydroxybenzoic acid	9338	3.00
10-formyfolic acid	135405023	7.99
Alpha-D-Galacturonate	11883891	3.99
Calotropin	16142	7.99
Galactose	6036	3.99
Galacturonic acid	439215	4.00
Herbacetin	5280544	5.00
S-2,6-diaminohexanoic acid	5962	3.00
Thiamine	1130	3.99

Cytotoxicity assay

The cytotoxicity activity of herbacetin was determined at various concentrations ranging from 100 µg/µL – 1000 µg/µL and the IC-50 value was analysed. The values were calculated after 48 hours using a dose-response inhibition curve. The IC-50 value of herbacetin on MCF-7 cell line was 404.23 µg/µL and on vero cell lines was 902.3 µg/µL (Table 4). Figure 2 shows the dose-response curve of herbacetin exposure to cancer cell lines and vero-cell lines. Figure 3 shows the effect of herbacetin on vero and cancer cell lines. The figure 3 clearly illustrates the cell death in MCF-7.

TABLE 4: IC50 values of compound on MCF-7 and vero cell lines

Sample	MCF-7 IC50 (µg/µL)	Vero cells IC50 (µg/µL)
Herbacetin	404.23	902.32

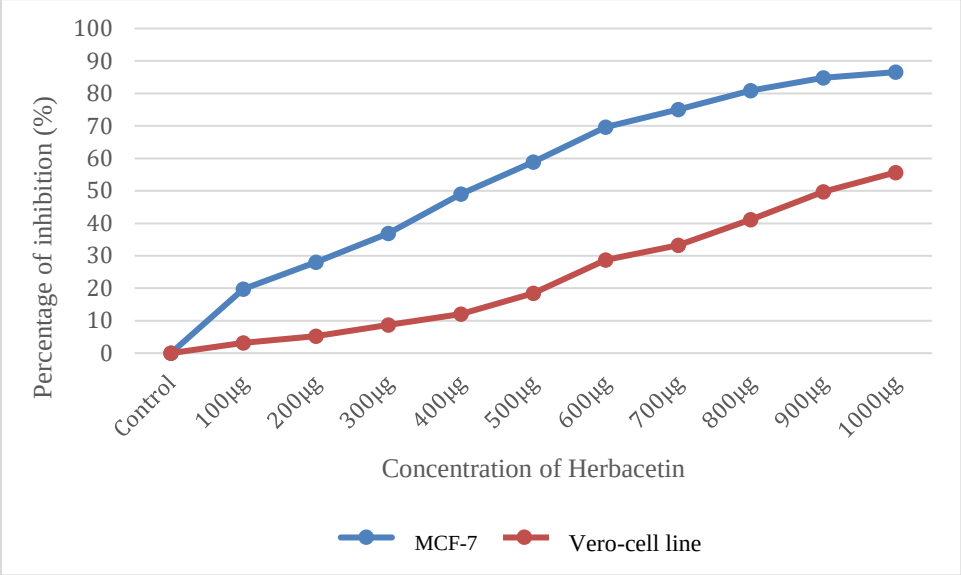
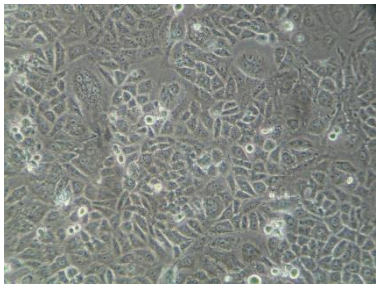
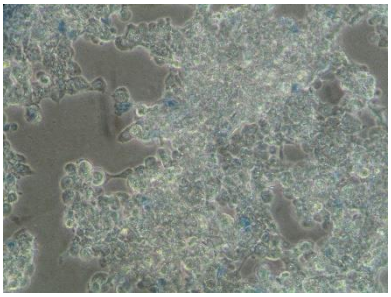


FIGURE 2: Dose dependant inhibition curve of herbacetin



Vero cell line with herbacetin

Scale- 5 micron



MCF-7 treated
Magnification – 20X

FIGURE 3: Cell lines treated with herbacetin

DISCUSSION

Despite the modern era's scientific improvements, breast cancer treatment and control remain elusive.

The incidence of EGFR mutations in breast cancer patients is yet to be studied conscientiously. Highly metastatic breast cancer with a bad prognosis is associated with up-regulation of EGFR (Ali and Wendt, 2017). The co-occurrence of the L858R and T790M mutations results in high treatment resistance (Ali et al. in 2017). While the 858th residue of EGFR is found at the A loop, the 790th residue is found in the depth of the ATP binding pocket (Yun et al., 2008; Kumar et al., 2008). Although these mutations are first treated by the medications, over time, a high resistance is built up, increasing the risk of a cancer recurrence. Therefore, when the inhibitor binds to the ATP binding pocket at the Lys-745th position, the progression and resistance against currently used drugs could be browbeaten (Shien et al., 2014; Tetsu et al., 2016).

In the present study, EGFR L858R and T790M mutation were targeted for drug development against EGFR in breast cancer. 43 compounds from N. oleander scored a dock score of more than currently used drugs erlotinib and gefitinib. Administration of these drugs results in various side effects like diarrhoea, cardiotoxicity,

hepatotoxicity, interstitial lung diseases, hair changes, folliculitis, paronychia and fatigue (Cersosimo 2006; Shahrokni et al., 2009; Becker et al., 2010).

In normal cells, EGFR dimerization results in autophosphorylation, which triggers cell division by attaching to the ATP-binding site in position 745 of the protein. Less EGF receptors will be activated if ATP cannot attach, which helps to prevent cell division in tumour cells (Kannan et al., 2018; Abe et al., 2006). Therefore, preventing autophosphorylation and ultimately inhibiting tumour growth will be accomplished by attaching the inhibitor to the ATP receptor site. Nine compounds from N. oleander shown improved dock scores in the current investigation were used to treat breast cancer with the T790M and L858R mutations.

Herbacetin from N. oleander binds to the ATP binding site by Lys-745. 10-formylfolic acid and herbacetin share a pi-anion bond with Lys-745. Alpha-D-Galacturonate, galactose, Galacturonic acid, S-2,6-diaminohexanoic acid and thiamine does not bind to the ATP binding site of EGFR hence making it an unstable compound.

Molecular docking studies can assist identify the locations of interaction and the common interaction between an inhibitor and the epidermal growth factor receptor (Nasab et al., 2018). The stability of the docked complex can be better understood by determining the fit value because the two are directly proportional to one another. With a fit value of 5.00, the compound herbacetin therefore forms a stable complex.

As a result, herbacetin may be a viable alternative for treating breast cancer with the L858R and T790M mutations. The compound is a dietary flavonoid having qualities that include anti-hyperlipidemia, anti-hyperglycaemia, antioxidant, anti-inflammatory, antiviral, antidiabetic, and anticholinesterase (Veeramani et al., 2018; Xiaohan et al., 2021; Wei et al., 2021).

The compound exhibited cytotoxic activity on MCF-7 breast cancer cell line against control and vero-cell line. Phytochemicals are known to show cytotoxic activity on cancerous cell line without affecting the normal cell lines. The aerial parts of the plant Scrophularia subaphylla exhibited cytotoxicity with an IC-50 of 300.8 ± 41.2 µg/mL on MCF-7 cell lines (Delazar et al., 2019). Similarly, the methanol and aqueous extracts of the plant Barleria hochstetteri showed an IC-50 value of 266.66 µg/ µL and 324.24 µg/ µL, respectively (Alkahtani et al., 2022).

CONCLUSION

Since the beginning of time, people have mostly employed plants as a source of medicine. Plant-based medications effectively eliminate the negative effects brought on by synthetic medications. Ayurveda, an antiquated system of medicine, uses N. oleander as an herb to treat a variety of illnesses, including cancer. The phytochemicals were docked against the L858R and T790M mutations of EGFR. Herbacetin shown a positive interaction with the mutations through ADMET tests utilising SwissADME and Discovery Studio 3.5 by attaching to its ATP binding site at Lys-745 thus prevent auto-phosphorylation. Its fit value, which is 5.00, indicates that the docked complex is stable. Through the use of the in-vitro cytotoxicity assay, it has been verified that the compound induces cell death to the MCF-7 breast cancer cell line (IC-50-404.23 µg/µL) without affecting the Vero-cell line. The compound herbacetin could therefore be used as a breast cancer inhibitor.

DECLARATION

None

REFERENCES

1. Abe, M., Kuroda, Y., Hirose, M., Watanabe, Y., Nakano, M., and Handa, T. (2006). Inhibition of autophosphorylation of epidermal growth factor receptor by small peptides in vitro. British journal of pharmacology, 147(4), 402–411. https://doi.org/10.1038/sj.bjp.0706634
2. Ali, R. and Wendt, M. K. (2017). The paradoxical functions of EGFR during breast cancer progression. Signal Transduction and Targeted Therapy 2:16042. https://doi.org/10.1038/sigtrans.2016.42
3. Alkahtani, S. A., Alshabi, A. M., Shaikh, I. A., Orabi, M. A., Abdel-Wahab, B. A., Walbi, I. A.,

... and Hoskeri, J. H. (2022). In Vitro Cytotoxicity and Spectral Analysis-Based Phytochemical Profiling of Methanol Extract of Barleria hochstetteri, and Molecular Mechanisms Underlying Its Apoptosis-Inducing Effect on Breast and Lung Cancer Cell Lines. Separations, 9(10), 298. . https:// doi.org/10.3390/separations9100298

4. Al-Sanfi, A.E. (2020). Bioactive ingredients and pharmacological effects of Nerium oleander. 2IOSR Journal of Pharmacy 10(9): 19-32.
5. Becker, A., van Wijk, A., Smit, E. F. and Postmus, P. E. (2010). Side-effects of long-term administration of erlotinib in patients with non-small cell lung cancer. Journal of Thoracic Oncology 5(9):1477–1480. https://doi.org/10.1097/JTO.0b013e3181e981d9
6. Bhuvaneshwari, L., Arthy, E., Anitha, C., Dhanabalan, K., and Meena, M. (2007). Phytochemical analysis and Antibacterial activity of Nerium oleander. Ancient science of life 26(4), 24–28.
7. Budhiarko, D., Putra, T. P., Harsono, A. T., Masykura, N., Tjindarbumi, D., Widjajahakim, G. and Utomo, A. (2017). Frequency of L858R and L861Q EGFR mutation in triple-negative, luminal and HER2 of Indonesian breast cancers patients. Annals of Oncology 28: 10. https://doi.org/10.1093/annonc/mdx678.005
8. Cersosimo, R. J. (2006). Gefitinib: an adverse effects profile. Expert Opinion on Drug Safety 5(3):469–479. https://doi.org/10.1517/14740338.5.3.469
9. Connor, A. E., Baumgartner, R. N., Baumgartner, K. B., Pinkston, C. M., John, E. M., Torres-Mejía, G., Hines, L. M., Giuliano, A. R., Wolff, R. K. and Slattery, M. L. (2013). Epidermal growth factor receptor (EGFR) polymorphisms and breast cancer among Hispanic and non-Hispanic white women: the Breast Cancer Health Disparities Study. International Journal of Molecular Epidemiology and Genetics 4(4):235–249.
10. Daina, A., Michielin, O., and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7, 42717. https://doi.org/10.1038/srep42717
11. Delazar, A., Asnaashari, S., Nikkhah, E., and Asgharian, P. (2019). Phytochemical analysis and antiproliferative activity of the aerial parts of Scrophularia subaphylla. Research in pharmaceutical sciences, 14(3), 263–272. https://doi.org/10.4103/1735-5362.258495Ali, R. and Wendt, M. K. (2017). The paradoxical functions of EGFR during breast cancer progression. Signal Transduction and Targeted Therapy 2:16042. https://doi.org/10.1038/sigtrans.2016.42
12. Farooqui, S., and Tyagi, T. (2018). Nerium oleander: It's application in basic and applied sciences: a review. International Journal of Pharmacy and Pharmaceutical Sciences 10(3): 1-4. http://dx.doi.org/10.22159/ijpps.2018v10i3.22505
13. Gaurav, A. and Gautam, V. (2017). Pharmacophore Based Virtual Screening Approach to Identify Selective PDE4B Inhibitors. Iranian Journal of Pharmaceutical Research 16(3):910–923.
14. Gupta, V., and Mittal, P. (2010). Phytochemical and Pharmacological Potential of Nerium oleander: A review. International Journal of Pharmaceutical Sciences and Research 3: 21-27. http://dx.doi.org/10.13040/IJPSR.0975-8232.1(3).21-27
15. Hase, G. J., Deshmukh, K.K., Pokharkar, R.D., Gaje, T.R., Phatanagre, N.D. (2017). Phytochemical Studies on Nerium oleander L. Using GC-MS. International Journal of Pharmacognosy and Phytochemical Research 9(6); 885-891. DOI number: 10.25258/phyto.v9i6.8195
16. Hedger, G., Sansom, M. S. and Koldsø, H. (2015). The juxtamembrane regions of human receptor tyrosine kinases exhibit conserved interaction sites with anionic lipids. Scientific Reports 5:9198. https://doi.org/10.1038/srep09198
17. Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers, 6(3), 1769–1792. https://doi.org/10.3390/cancers6031769
18. Kannan, S., Venkatachalam, G., Lim, H. H., Surana, U. and Verma, C. (2018). Conformational landscape of the epidermal growth factor receptor kinase reveals a mutant specific allosteric pocket. Chemical Science 9(23):5212–5222. https://doi.org/10.1039/c8sc01262h
19. Kiran, C., and Prasad, D. N. (2014). A review on: Nerium oleander Linn.(Kaner). International Journal of Pharmacognosy and Phytochemical Research, 6(3), 593-597.
20. Kulkarni, A. R., Reddy, R. G., Marlewar, S. S., Wadikar, S. S., Jangle, V. M. and Kulkarni, A. P. (2014). Cancer- An intergrative approach by Ayurveda. International Journal of Experimental Pharmacology 3(6):47-51.

21. Kumar, A., Petri, E. T., Halmos, B., & Boggon, T. J. (2008). Structure and clinical relevance of the epidermal growth factor receptor in human cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 26(10), 1742–1751. https://doi.org/10.1200/JCO.2007.12.1178
22. Levva, S., Kotoula, V., Kostopoulos, I., Manousou, K., Papadimitriou, C., Papadopoulou, K., Lakis, S., Koukoulias, K., Karavasilis, V., Pentheroudakis, G., Balassi, E., Zagouri, F., Kaklamanos, I. G., Pectasides, D., Razis, E., Aravantinos, G., Papakostas, P., Bafaloukos, D., Rallis, G., Gogas, H., … Fountzilas, G. (2017). Prognostic Evaluation of Epidermal Growth Factor Receptor (EGFR) Genotype and Phenotype Parameters in Triple-negative Breast Cancers. Cancer genomics & proteomics, 14(3), 181–195. https://doi.org/10.21873/cgp.20030
23. Nasab, R. R., Mansourian, M., Hassanzadeh, F., and Shahlaei, M. (2018). Exploring the interaction between epidermal growth factor receptor tyrosine kinase and some of the synthesized inhibitors using combination of in-silico and in-vitro cytotoxicity methods. Research in pharmaceutical sciences, 13(6), 509–522. https://doi.org/10.4103/1735-5362.245963
24. Pal, S., Kumar, V., Kundu, B., Bhattacharya, D., Preethy, N., Reddy, M. P., & Talukdar, A. (2019). Ligand-based Pharmacophore Modeling, Virtual Screening and Molecular Docking Studies for Discovery of Potential Topoisomerase I Inhibitors. Computational and structural biotechnology journal, 17, 291–310. https://doi.org/10.1016/j.csbj.2019.02.006
25. Shahrokni, A., Rajebi, M. R. and Saif, M. W. (2009). Toxicity and efficacy of 5-fluorouracil and capecitabine in a patient with TYMS gene polymorphism: A challenge or a dilemma? Clinical Colorectal Cancer 8(4):231–234. https://doi.org/10.3816/CCC.2009.n.039
26. Shien, K., Yamamoto, H., Soh, J., Miyoshi, S. and Toyooka, S. (2014). Drug resistance to EGFR tyrosine kinase inhibitors for non-small cell lung cancer. Acta Medica Okayama 68(4):191–200. https://doi.org/10.18926/AMO/52785
27. Singh, S., Sharma, B., Kanwar, S. S. and Kumar, A. (2016). Lead Phytochemicals for Anticancer Drug Development. Frontiers in Plant Science 7:1667. https://doi.org/10.3389/fpls.2016.01667
28. Sinha, S.N., and Biswas, K. (2016). A concise review on Nerium oleander L. - An important medicinal plant. Tropical Plant Research 3(2): 408–412.
29. Teng, Y. H., Tan, W. J., Thike, A. A., Cheok, P. Y., Tse, G. M., Wong, N. S., Yip, G. W., Bay, B. H. and Tan, P. H. (2011). Mutations in the epidermal growth factor receptor (EGFR) gene in triple negative breast cancer: possible implications for targeted therapy. Breast Cancer Research 13(2):R35. https://doi.org/10.1186/bcr2857
30. Tetsu, O., Hangauer, M. J., Phuchareon, J., Eisele, D. W. and McCormick, F. (2016). Drug Resistance to EGFR Inhibitors in Lung Cancer. Chemotherapy 61(5):223–235. https://doi.org/10.1159/000443368
31. Türkmenoğlu, B. and Güzel, Y. (2018). Molecular docking and 4D-QSAR studies of metastatic cancer inhibitor thiazoles. Computational Biology and Chemistry 76:327–337. https://doi.org/10.1016/j.compbiolchem.2018.07.003
32. Wang, S., Cang, S. and Liu, D. (2016). Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. Journal of Haematology and Oncology 9:34. https://doi.org/10.1186/s13045-016-0268-z
33. Wei, X., Zhao, Z., Zhong, R., and Tan, X. (2021). A comprehensive review of herbacetin: From chemistry to pharmacological activities. Journal of ethnopharmacology, 279, 114356. https://doi.org/10.1016/j.jep.2021.114356
34. Wieduwilt, M. J. and Moasser, M. M. (2008). The epidermal growth factor receptor family: biology driving targeted therapeutics. Cellular and Molecular Life Sciences 65(10):1566–1584. https://doi.org/10.1007/s00018-008-7440-8
35. Yun, C. H., Mengwasser, K. E., Toms, A. V., Woo, M. S., Greulich, H., Wong, K. K., Meyerson, M., and Eck, M. J. (2008). The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proceedings of the National Academy of Sciences of the United States of America, 105(6), 2070–2075. https://doi.org/10.1073/pnas.0709662105
36. Zibbu, G., and Batra, A. (2010). A Review on Chemistry and Pharmacological activity of Nerium oleander L. J. Chem. Pharm. Res. 2(6):351-358.

SUPPLEMENTARY TABLE 1: Details of the phytochemicals identified from Nerium oleander

Sl. No:	Phytochemicals	PubChem CID	References
	16-anhydro-deacetylnerigoside		Dr. Dukes Phytochemical Data Base
	9-d-hydroxy-cis-12-octadecanoic-acid	5282942	Dr. Dukes Phytochemical Data Base
	Adigoside	91809642	Dr. Dukes Phytochemical Data Base
	Adynerin	441840	Dr. Dukes Phytochemical Data Base
	Alpha-amyrin	73170	Dr. Dukes Phytochemical Data Base
	Beta-sitosterol	222284	Dr. Dukes Phytochemical Data Base
	Betulin	72326	Dr. Dukes Phytochemical Data Base
	Betulinic-acid	64971	Dr. Dukes Phytochemical Data Base
	Campesterol	173183	Dr. Dukes Phytochemical Data Base
	Caoutchouc	6557	Dr. Dukes Phytochemical Data Base
	Capric-acid	454075	Dr. Dukes Phytochemical Data Base
	Caproic-acid	596974	Dr. Dukes Phytochemical Data Base
	Caprylic-acid	379	Dr. Dukes Phytochemical Data Base
	Choline	305	Dr. Dukes Phytochemical Data Base
	Cornerin		Dr. Dukes Phytochemical Data Base
	Cortenerin		Dr. Dukes Phytochemical Data Base
	Deacetyloleandrin	76962086	Dr. Dukes Phytochemical Data Base
	Diacetyl-nerigoside		Dr. Dukes Phytochemical Data Base
	Foliandrin/ oleandrin/ folinerin	11541511	Dr. Dukes Phytochemical Data Base
	Gentiobiosyloleandrin		Dr. Dukes Phytochemical Data Base
	Pseudocuramine	7247	Dr. Dukes Phytochemical Data Base
	Quercetrin / quercitrin	5280459	Dr. Dukes Phytochemical Data Base
	Quercetrin-3-rhamnoglucoside / rutin	5280805	Dr. Dukes Phytochemical Data Base
	Rosaginin		Dr. Dukes Phytochemical Data Base
	Stearic acid	5281	Dr. Dukes Phytochemical Data Base
	Stigmasterol	5280794	Dr. Dukes Phytochemical Data Base
	Strospeside	21636336	Dr. Dukes Phytochemical Data Base
	Uzarigenin	92760	Dr. Dukes Phytochemical Data Base
	Urehitoxin		Dr. Dukes Phytochemical Data Base
	Galacturonic acid	439215	Gupta et al., 2010
	Rhamnose	25310	Gupta et al., 2010
	Arabinose	439195	Gupta et al., 2010
	Galactose	6036	Gupta et al., 2010
	Cardenolides n-1		Gupta et al., 2010
	Cardenolides n-2		Gupta et al., 2010
	Cardenolides n-3		Gupta et al., 2010
	Cardenolides n-4		Gupta et al., 2010
	Pregnanes	131269062	Gupta et al., 2010
	21- hydroxypregna- 4, 6-diene- 3, 12, 20- trione	16104852	Gupta et al., 2010
	20r-hydroxypregna-4,6-diene- 3, 12-dione		Gupta et al., 2010
	16beta, 17beta-epoxy- 12beta-hydroxypregna- 4, 6-diene- 3, 20- dione		Gupta et al., 2010
	Neridienone a	100630	Gupta et al., 2010
	Neridienone b	44418781	Gupta et al., 2010
	Neriucoumaric	17269060 (PMID)	Gupta et al., 2010
	Isoneriucoumaric acids	17269060 (PMID)	Gupta et al., 2010
	Oleanderoic acid	17265258 (PMID)	Gupta et al., 2010

	Kaneroside		Gupta et al., 2010
	Neriumoside		Gupta et al., 2010
	3β, 27- dihydroxy- urs- 18- en- 13, 28-olide/ Neriumin		Gupta et al., 2010
	3β, 22α, 28-trihydroxy-25-nor-lup-1 (10), 20 (29)-dien-2- one		Gupta et al., 2010
	Cis- karenin	6440661	Gupta et al., 2010
	Trans- karenin	101921671	Gupta et al., 2010
	Beta- anhydroepidigitoxigenin	10784500	Gupta et al., 2010
	Neriumogenin- a- 3 beta- d-digitaloside	10425146 (PMID)	Gupta et al., 2010
	Proceragenin	101281384	Gupta et al., 2010
	Ursane-type triterpene 1	595630 (Bioassay AID)	Gupta et al., 2010
	Oleanane-type triterpene 2	622106 (Bioassay AID)	Gupta et al., 2010
	Dammarane-type triterpene 15	415650 (Bioassay AID)	Gupta et al., 2010
	3beta, 27-dihydroxy- 12- ursen- 28-oic acid	377098 (Bioassay AID)	Gupta et al., 2010
	3beta, 13beta- dihydroxyurs- 11- en-28- oic acid	377094 (Bioassay AID)	Gupta et al., 2010
	3beta- hydroxyurs- 12- en- 28-aldehyde	377094 (Bioassay AID)	Gupta et al., 2010
	28- norurs- 12- en- 3beta- ol	101515392	Gupta et al., 2010
	Urs-12- en- 3beta- ol	146158198	Gupta et al., 2010
	Urs- 12- ene- 3beta-28-diol	15922573	Gupta et al., 2010
	3beta- hydroxy- 12- oleanen- 28- oic acid (oleanolic acid)	10494	Gupta et al., 2010
	3beta, 27- dihydroxy- 12-oleanen- 28-oic acid	377098 (Bioassay AID)	Gupta et al., 2010
	3beta- hydroxy-20 (29)- lupen- 28- oic acid (betulinic acid)	64971	Gupta et al., 2010
	20 (29)- lupene- 3beta, 28- diol (betulin)	72326	Gupta et al., 2010
	(20s, 24r)- epoxydammarane-3beta, 25-diol	14587770	Gupta et al., 2010
	20 beta, 28- epoxy- 28alpha-methoxytaraxasteran- 3beta- ol		Gupta et al., 2010
	20beta, 28- epoxytaraxaster- 21- en-3beta- ol	378066 (Bioassay AID)	Gupta et al., 2010
	28- nor- urs- 12- ene- 3 beta		Gupta et al., 2010
	17 beta- diol and 3 beta-hydroxyurs-12- en- 28- aldehyde		Gupta et al., 2010
	Alpha-neriursate	19851926 (PMID)	Sinha et al., 2016
	Beta-neriursate	19851926 (PMID)	Sinha et al., 2016
	Kanerodione		Sinha et al., 2016
	Kanerocin	17262420 (PMID)	Sinha et al., 2016

	D-sarmentose	5460676	Sinha et al., 2016
	D-diginose	5461039	Sinha et al., 2016
	Neridiginoside	9933955 (PMID)	Sinha et al., 2016
	Nerizoside	10816404	Sinha et al., 2016
	Neritaloside	44566654	Sinha et al., 2016
	Odoroside- h	205840	Sinha et al., 2016
	Isoricinoleic acid	5282941	Sinha et al., 2016
	Gentiobiosyl- nerigoside		Sinha et al., 2016
	Gentiobiosylbeaumontoside		Sinha et al., 2016
	Gentiobiosyl- oleandrin		Sinha et al., 2016
	8β- hydroxy-digitoxigenin		Sinha et al., 2016
	Δ16-neriagenin		Sinha et al., 2016
	Cardenolides	53957771	Sinha et al., 2016
	Oleandrigenin	9802865	Sinha et al., 2016
	Digitoxigenin	4369270	Sinha et al., 2016
	Adynerigenin	15558417	Sinha et al., 2016
	Neriagenin	51040390	Sinha et al., 2016
	P-cresol	2879	Hase et al., 2017
	2-methoxyphenol	460	Hase et al., 2017
	2-methoxyphenyl ester	133623	Hase et al., 2017
	Androsta-1,4-diene-3-on3,17-hydroxy-17-methyl	6432673	Hase et al., 2017
	[5,9-dimethyl-1-(3-phenyloxiran-2-yl)-deca-4,8- dienylidene]-(2-phenyl-aziridin1-yl) –amine	5362950	Hase et al., 2017
	4-hexyl-1-(7-methoxycarbonylheptyl) bicyclo[4.4.0]deca-2,5,7-triene	562334	Hase et al., 2017
	1h-cyclopropa[3,4]benz91,2-e]azulene-5,7b.9,9a-tetrol	596973	Hase et al., 2017
	Cyclopropene ,1-(3-acetoxy-1,1-dimethylhexan-5-onyl)- 2- isopropenyl	539232	Hase et al., 2017
	2-methoxy-5-methylphenol	14519	Hase et al., 2017
	2h-benzo[f]oxireno[2,3-e] benzofuran8-(9h)- one,octahydro-9-[[[(2- methoxyphenyl) methyl]amino]-methyl]-2,5adimethyl	3732816	Hase et al., 2017
	2-(1,2,3,4-tetrahydronaphthalen-1-yliden)hydrazine-1-carbothioamide	5372742	Hase et al., 2017
	A-d-glucofuranose	7067560	Hase et al., 2017
	4- ethyl-2-methoxy-phenol	14578531	Hase et al., 2017
	5-isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	586164	Hase et al., 2017
	3-methyl -1h-indazole	11062700	Hase et al., 2017
	4-(1h-1,2,3,4 – tetrazolr-1-yl)-benzeneaceticacid	841658	Hase et al., 2017
	2-methoxy-4-vinyl phenol	332	Hase et al., 2017
	2,6-dimethoxyphenyl ester	87333665	Hase et al., 2017
	A-ethyl-4-methoxybenzenemethanol	641444	Hase et al., 2017
	1h-1,2,3,4-tetrazole1,5-diamine, n(1)[(2-ethoxy3methoxyphenyl) methyl]		Hase et al., 2017
	Anobin	538430	Hase et al., 2017

	6,7-epoxypregn-4-ene9,11,18-triol-3,20-dione, 11,18-diacetate	539836	Hase et al., 2017
	Trans- isoeugenol	853433	Hase et al., 2017
	Decanoic acid	2969	Hase et al., 2017
	Oleic acid	445639	Hase et al., 2017
	4-tetradecyl ester	101033719	Hase et al., 2017
	5-tert-butylpyrogallol	597592	Hase et al., 2017
	2,3,5- trimethoxyamphetamine	602804	Hase et al., 2017
	2,6-dimethoxy-4-(-2- propenyl)-phenol	5352905	Hase et al., 2017
	1,15-pentadecanediol	518994	Hase et al., 2017
	2-hydroxy-4-isopropyl-7-methoxytropone	606603	Hase et al., 2017
	17-chloro-7-heptadecene	5364489	Hase et al., 2017
	2-methyl-z,z-3,13- octadecadienol	5364412	Hase et al., 2017
	Tridecanedial	544162	Hase et al., 2017
	L-(+)-ascorbic-acid-2,6-dihexadecanoate	54722209	Al-Snafi 2020; Zibbu and Batra, 2010
	Z-10- methyl-11-tetradecen-1-olpropionate	5365070	Al-Snafi 2020; Zibbu and Batra, 2010
	E,e,z-1,3,12-nonadecatriene5,14-diol	5364768	Al-Snafi 2020; Zibbu and Batra, 2010
	2-(9,12- octadecadienyloxy)- ,(z,z)-ethanol		Al-Snafi 2020; Zibbu and Batra, 2010
	9-hexadecenoic acid	5282745	Al-Snafi 2020; Zibbu and Batra, 2010
	Octadecanoic acid	5281	Al-Snafi 2020; Zibbu and Batra, 2010
	6,9,12,15-docosatetraenoic acid, methyl ester	5362672	Al-Snafi 2020; Zibbu and Batra, 2010
	9,12-octadecadienoic acid	3931	Al-Snafi 2020; Zibbu and Batra, 2010
	3-(tetradecyloxy)-1,2- propanediol	64674	Al-Snafi 2020; Zibbu and Batra, 2010
	1h-cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1ah)- pento	596973	Al-Snafi 2020; Zibbu and Batra, 2010
	Pregna-5,16,20-triene-3beta,20-diol diacetate	222820	Al-Snafi 2020; Zibbu and Batra, 2010
	Hydrocortisone acetate	5744	Al-Snafi 2020; Zibbu and Batra, 2010
	2-[1-hydroxy-2-(3-methyl phenyl)ethyl- cholestan-3-one		Al-Snafi 2020; Zibbu and Batra, 2010
	5-[(acetyloxy)methyl3a,4,6a,7,9,10, 10a,10boctahydro-3a,10a-dihydroxy2,10-dimethyl-,(3aa',6aa',10a'10aa',10ba')		Al-Snafi 2020; Zibbu and Batra, 2010
	13-docosenamide	5365369	Al-Snafi 2020; Zibbu and Batra, 2010
	9-octadecenamide	1930	Al-Snafi 2020; Zibbu and Batra, 2010
	6,10,14,18,22-tetracosapenaen2-ol,3-bromo-2,6,10,15,19,23- hexamethyl-,(all-e)	5367591	Al-Snafi 2020; Zibbu and Batra, 2010
	Azafrin	5281225	Al-Snafi 2020; Zibbu and Batra, 2010

	A-neooleana-3(5),12-diene	632542	Al-Snafi 2020; Zibbu and Batra, 2010
	Acetic acid, 3- hydroxy-6- isopropenyl-4,8a-dimethyl1,2,3,5,6,7,8,8aoctahydronapthalen-2-yl-ester	540542	Al-Snafi 2020; Zibbu and Batra, 2010
	Astaxanthin	5281224	Al-Snafi 2020; Zibbu and Batra, 2010
	16-deacetyloleandrin	76962086	IMPPAT
	Glucodigitoxigenin	15558776	IMPPAT
	Oleagenin	101967000	IMPPAT
	Proceragenin a	101281384	IMPPAT
	17beta-neriifolin	441867	IMPPAT
	28-norurs-12-ene-3beta-ol	44583863	IMPPAT
	5beta-pregnane	439513	IMPPAT
	Ac1l1ukb	10134	IMPPAT
	Alpha-d-galacturonate	11883891	IMPPAT
	Astragalin	5282102	IMPPAT
	Biosides	277994 (Bioassay AID)	IMPPAT
	Calcein	65079	IMPPAT
	Calotropin	16142	IMPPAT
	(3beta)-3,27-dihydroxyolean-12-en-28-oic acid	12001894	IMPPAT
	Chembl497040	44583866	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl497269	21580512	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl498254	44583862	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl500910	16083124	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl524080	44583858	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl524438	16083125	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Chembl524527	44583857	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Choline chloride	6209	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Herbacetin	5280544	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Hexanoic acid	8892	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Odoroside a	44425145	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Oleanderolide	11113483	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Oleandrine	11541511	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	S-2,6-diaminohexanoic acid	5460926	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Ursolic acid	64945	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018

	Taraxasterane	12306150	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Uvaol	92802	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Ocotillol	3850493	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	20s,28s)-28-methoxy-20,28-epoxytaraxasterane-3beta-ol	16083124	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Oleandrose	5461155	Bhuvaneshwar et al., 2007; Farooqui and Tyagi, 2018
	Plumericin	5281545	Kiran and Prasad, 2014
	Odoroside-b	91809650	Kiran and Prasad, 2014
	Odoroside-c	135318835	Kiran and Prasad, 2014
	Odoroside-d	135286767	Kiran and Prasad, 2014
	Odoroside-e		Kiran and Prasad, 2014
	Odoroside-f	120681	Kiran and Prasad, 2014
	Odoroside-g	21636339	Kiran and Prasad, 2014
	Odoroside-h	205840	Kiran and Prasad, 2014
	5α-adynerin		Kiran and Prasad, 2014
	Plumieride	72319	Kiran and Prasad, 2014
	Oleanderocinoic acid		Kiran and Prasad, 2014
	Oleandigoside	5461155	Kiran and Prasad, 2014
	Heptacosane-3-enyl-5-hydroxyhexanoate		Kiran and Prasad, 2014
	4- oxooctyl-2-hydroxyundecanoate	163041663	Kiran and Prasad, 2014
	B-d-digtialoside		Kiran and Prasad, 2014
	B-d-glucosyl crocetin	10368299	Kiran and Prasad, 2014