PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp3): 71-82
https://doi.org/10.14719/pst.4579

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Phytochemical analysis, therapeutic and molecular docking studies for the compounds of wild type and Senkambu variants of curry leaves targeting HER2 Kinase domain a potential gastric cancer receptor

Sandhya S[1], Jegadeeswari V[2] *, Jayakanthan M[3] & Caroline Nirmala R[3]

[1]Department of Spices and Department of Spices and Plantation Crops, HC and RI, amil Nadu Agricultural University, P N Pudur, Coimbatore 641 003, Tamil Nadu,India
[2]Department of Fruit Science, HC and RI (W), Dindugal Main Rd, near UCO Bank, Muthukulam, Navalurkottapattu, Tiruchirappalli 620 009, Tamil Nadu, India
[3]Department of plant Molecular Biology and Bioinformatics, CPMB and B, TNAU, Coimbatore 641 003, Tamil Nadu, India
*Email: : jegadeeswari.v@tnau.ac.in

OPEN ACCESS

ARTICLE HISTORY

Received: 13 August 2024
Accepted: 18 November 2024

Available online
Version 1.0 : 25 December 2024
Version 2.0 : 14 February 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Sandhya S, Jegadeeswari V, Jayakanthan M, Caroline Nirmala R. Phytochemical analysis, therapeutic and molecular docking studies for the compounds of wild type and Senkambu variants of curry leaves targeting HER2 Kinase domain a potential gastric cancer receptor. Plant Science Today. 2024; 11(sp3): 71-82. https://doi.org/10.14719/pst.4579

Abstract

Gastric cancer is the fifth most notable health concern globally. In recent years, molecular docking, a computational technique, has emerged as tool in drug discovery. The present investigation aimed to identify the major bioactive compounds in the wild-type curry leaves found in the Shevaroy Hills and the local Senkambu variant from Karamadai. Virtual screening of 40 ligands from Curry leaves of wild type and Senkambu type was identified through GC-MS profiling. These compounds were targeted against HER2 Kinase domain which is a potential receptor for Gastric cancer. Information regarding the binding site residues for the receptor was predicted using CASTp server. Molecular docking was performed for HER2 kinase domain with the predicted compounds through GC-MS profiling. The top 3 hits reported with least binding affinity for the target protein were considered for further interaction analysis using Biovia Discovery studio visualizer. Upon analyzing the interacted compounds, the Piperine from Wild type curry leaves was found to have good interaction with HER2 Kinase domain by forming two hydrogen bonds and binding score of -8.3 kcal/mol. The current study might guide the designing of analogues of piperine in the evolution of effective broad spectrum drug development in cancer therapy.

Keywords

bio-active compounds; curry leaf; docking; gastric cancer; piperine

Introduction

Gastric cancer ranks as the fifth most common cancer worldwide and the third leading cause of cancer-related deaths. Although there have been improvements in detecting and treating gastric cancer, the outlook for patients remains unfavourable, particularly in advanced cases. This disease shows significant geographic variation, with high incidence and mortality rates being recorded in Eastern Asia, Eastern Europe and South America, and lower rates in North America and certain parts of Africa. This variation is linked to diet, standard of living, and *Helicobacter pylori* infection, which is a major known risk factor, accounting 89% of non-cardia gastric malignancies. Salty, smoked foods and alcohol consumption and smoking increase the risk, while increased fresh food consumption decreases it. Hereditary account for about 10% of all cases, warranting further investigation into high-risk populations (1).

The human epidermal growth factor receptor 2 (HER2), part of the epidermal growth factor receptor (EGFR) family, is overexpressed in approximately 20-30% of gastric cancer cases. Overexpression of HER2 correlates with more aggressive tumours, worse outcomes and reduced survival in patients. Therefore, targeting the HER2 signalling pathway has emerged as a promising strategy for treating gastric cancer (2).

The HER2 kinase domain plays a vital role in drug development, with numerous HER2 inhibitors developed to combat HER-2-positive cancers. Among these is TAK–285, a compound that inhibits both HER2 and EGFR. This dual inhibitor has been investigated for its potential in treating various cancers, including as those of the breast, lung, stomach and prostate (3). The crystal structure of the HER2 kinase domain in complex with TAK-285 (PDB ID: 3RCD) provides valuable insights into binding interactions and can be used for the virtual screening of potential inhibitors.

In recent years, natural remedies and traditional medicinal plants have attracted increased attention due to their potential in treating various diseases, including cancer (4). Curry leaves (*Murraya koenigii*), part of the Rutaceae family, are widely used in Indian cuisine and traditional medicine for their antioxidant and pharmacological properties. Known as a "miracle plant," these small trees or shrubs grow up to 6 meters in height and are propagated through sensitive seeds and grafting techniques (5). The plant features pinnate leaves with 11-21 aromatic, glossy green leaflets, small white flowers, and black drupe fruits. India is the global leader in the production and consumption of curry leaves, with Andhra Pradesh and Tamil Nadu serving as key cultivation regions due to favourable climates, large cultivation areas, and well-developed infrastructure (6). Beyond culinary uses, recent research has highlighted the plant's potential in managing cancer, as its polyphenols and flavonoids can inhibit the proteolytic action of cancer cell proteasomes, ultimately inducing cell death (7). To explore natural compounds as complementary treatments, curry leaves show great promise as a potential avenue for developing more effective and accessible therapies for gastric cancer (8). In this study, we aim to identify potential HER2 kinase domain inhibitors from the phytochemicals present in wild-type and Senkambu varieties of curry leaves using GCMS analysis and virtual screening.

Materials and Methods

Extract preparation

Two ecotypes of curry leaf were used for this study: one from Yercaud in the Salem district (Wild Curry leaf) and another from Karamadai, Coimbatore district, Tamil Nadu (Senkambu). Leaves of both ecotypes were collected from their respective locations, shade-dried and subject to oil extration using hydro-distillation with a Clevenger apparatus. The extracted oil was analyzed using GC-MS (9).

Gas chromatography – Mass spectroscopy (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) equipment, manufactured by the Shimadzu and model QP2020 NX, is a single quadrupole apparatus used to analyse the derivatized samples. A sample volume of 1 µL was injected at a temperature of 250 °C. A sample volume of 1 µL was injected at temperature of 250 °C. The temperature program initiated at 40 °C for 2 min and then increased by 8 °C per min until reaching 320 °C, with a total runtime of 10 min. A splitting ratio of 1:30 was used for sample injection. The spectrometer was set with an ionization chamber temperature of 220 °C, a mass spectrum range of 40 m/z to 600 m/z, an interface temperature of 300 °C and a solvent cutoff at 6.0 min (10) (Table 1, 2 and Fig. 1, 2).

Table 1. Phytochemical profiling of curry leaves wild type by using GCMS

Peak	RT	Compound name	Probability %	Area %	Area
1	4.989	trans-Verbenol	34.6	0.511	7767947.0
2	5.489	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	52.1	1.222	18574506.0
3	5.694	à-Terpineol	10.3	0.583	8854532.0
4	6.825	1,4-dihydroxy-p-menth-2-ene	78.3	0.736	11188087.0
5	7.070	2-Cyclopenten-1-one, 3,4-dimethyl-	45.6	0.683	10373454.0
6	7.135	Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)-	18.0	0.767	11652054.0
7	7.460	4,4-Dimethylpent-2-enal	15.8	1.142	17349418.0
8	9.611	(1S,2S,4S)-Trihydroxy-p-menthane	96.8	1.852	28154132.0
9	10.912	(-)-Spathulenol	43.8	1.273	19348892.0
10	11.012	Caryophyllene oxide	69.1	4.039	61390676.0
11	11.077	Globulol	25.7	0.467	7095714.0
12	11.232	2-(4a,8-Dimethyl-2,3,4,4a,5,6-hexahydronaphthalen-2-yl)propan-1-ol	10.4	0.523	7951917.5
13	11.542	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	75.4	0.986	14990439.0
14	11.697	Neointermedeol	19.0	0.576	8758720.0
15	12.087	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5á-ol	32.2	0.746	11332339.0
16	12.452	trans-Z-à-Bisabolene epoxide	13.1	1.068	16239014.0

17	12.502	1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl	50.5	1.859	28259780.0
18	12.742	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	17.9	1.194	18140082.0
19	13.763	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	20.6	0.735	11167184.0
20	14.198	Longipinocarvone	36.2	0.572	8687007.0
21	15.563	6-epi-shyobunol	7.3	1.996	30330940.0
22	15.698	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	10.2	0.490	7454271.0
23	16.879	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	46.9	1.289	19589286.0
24	17.019	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	11.7	0.881	13386353.0
25	17.154	á-Santanol acetate	7.6	1.803	27406612.0
26	18.104	Oleoyl chloride	15.5	0.729	11073526.0
27	18.209	cis-Vaccenic acid	12.8	1.015	15432659.0
28	18.605	n-Hexadecanoic acid	68.0	0.685	10409497.0
29	21.161	9,12-Octadecadienoic acid (Z,Z)-	7.8	0.445	6760808.5
30	21.716	Oleic Acid	36.0	3.172	48209128.0
31	21.846	Ursodeoxycholic acid	15.1	7.301	110965040.0
32	22.291	4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-	45.3	2.237	34002672.0
33	22.606	Ethyl iso-allocholate	9.8	1.573	23914378.0
34	25.197	Squalene	9.1	1.398	21248230.0
35	25.702	Gamabufotalin	30.1	0.726	11034978.0
36	27.433	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	28.1	7.965	121059800.0
37	27.923	Piperine	48.3	0.542	8239795.5
38	28.233	(E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)hept-6-en-1-one	38.2	0.503	7642923.0
39	29.064	Piperine	57.8	20.041	304596000.0
40	29.329	(E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)hept-6-en-1-one	63.8	2.054	31211530.0

Table 2. Phytochemical profiling of Senkambu by using GCMS

Peak	RT	Compound name	Probability %	Area %	Area
1	3.344	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	21.9	6.014	1691162624.0
2	5.624	2-Cyclohexen-1-one, 4-(1-methylethyl)-	67.9	2.962	832996800.0
3	6.380	Benzaldehyde, 4-(1-methylethyl)-	58.8	0.634	178388928
4	7.065	p-Cymen-7-ol	69.6	0.894	251314304.0
5	7.520	Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)-	22.4	1.117	314156544.0
6	7.690	Bicyclo[2.2.2]octane-1,4-diol, monoacetate	9.0	0.405	113936136.0
7	8.115	1-Cyclohexene-1-methanol, à,à,4-trimethyl-	17.6	1.616	454469888.0
8	8.286	4-Hydroxynonenal	39.3	0.752	211330096.0
9	8.346	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à, 2á,4á	20.7	2.115	594685376.0
10	8.766	Caryophyllene	15.4	6.584	1851414528.0
11	8.886	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	20.9	0.450	126490256.0
12	8.961	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	9.0	0.580	163138000.0
13	9.131	7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)	38.0	1.521	427692160.0
14	9.196	Humulene	34.1	2.504	704184128.0
15	9.396	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	9.8	0.516	145170672.0
16	9.606	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	28.4	1.499	421561184.0
17	9.696	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	8.5	1.566	440364160.0
18	9.756	8-Oxabicyclo[4.3.0]nonane, 7,9-dimethyl-	7.1	0.974	273882048.0
19	10.341	(4aS,8R)-4a,8-Dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one	14.4	0.455	127902800.0
20	10.516	Caryophyllene oxide	66.8	1.957	550255680.0
21	10.756	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	26.1	0.546	153646288.0
22	11.192	trans-Z-à-Bisabolene epoxide	85.3	24.553	6904496640.0

23	11.352	1,3,4-Trimethyladamantane	46.9	1.854	521355744.0
24	11.497	Ledol	14.3	0.545	153194608.0
25	11.652	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	71.8	6.520	1833380992.0
26	11.762	Neointermedeol	30.6	1.462	410989184.0
27	11.932	Epicubenol	28.0	0.697	195971024.0
28	12.037	Aromadendrene oxide-(2)	17.0	1.060	298185888.0
29	12.142	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5á-ol	33.6	0.655	184171392.0
30	12.227	.tau.-Cadinol	33.9	0.628	176680480.0
31	12.317	á-Guaiene	7.4	0.370	104103328.0
32	12.592	Neointermedeol	48.2	4.594	1291910400.0
33	12.827	Caryophylla-4(12),8(13)-dien-5à-ol	29.9	2.741	770860288.0
34	13.793	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	11.6	0.468	131479832.0
35	14.233	Longipinocarvone	37.1	0.477	134116600.0
36	15.628	6-epi-shyobunol	8.2	0.931	261861856.0
37	21.906	9-Octadecenoic acid, (E)-	14.5	0.609	171277056.0
38	27.503	Thunbergol	10.7	0.427	120188360.0
39	28.308	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	7.0	0.384	107981552.0
40	29.424	Friedelan-3-one	6.7	0.436	122553840.0

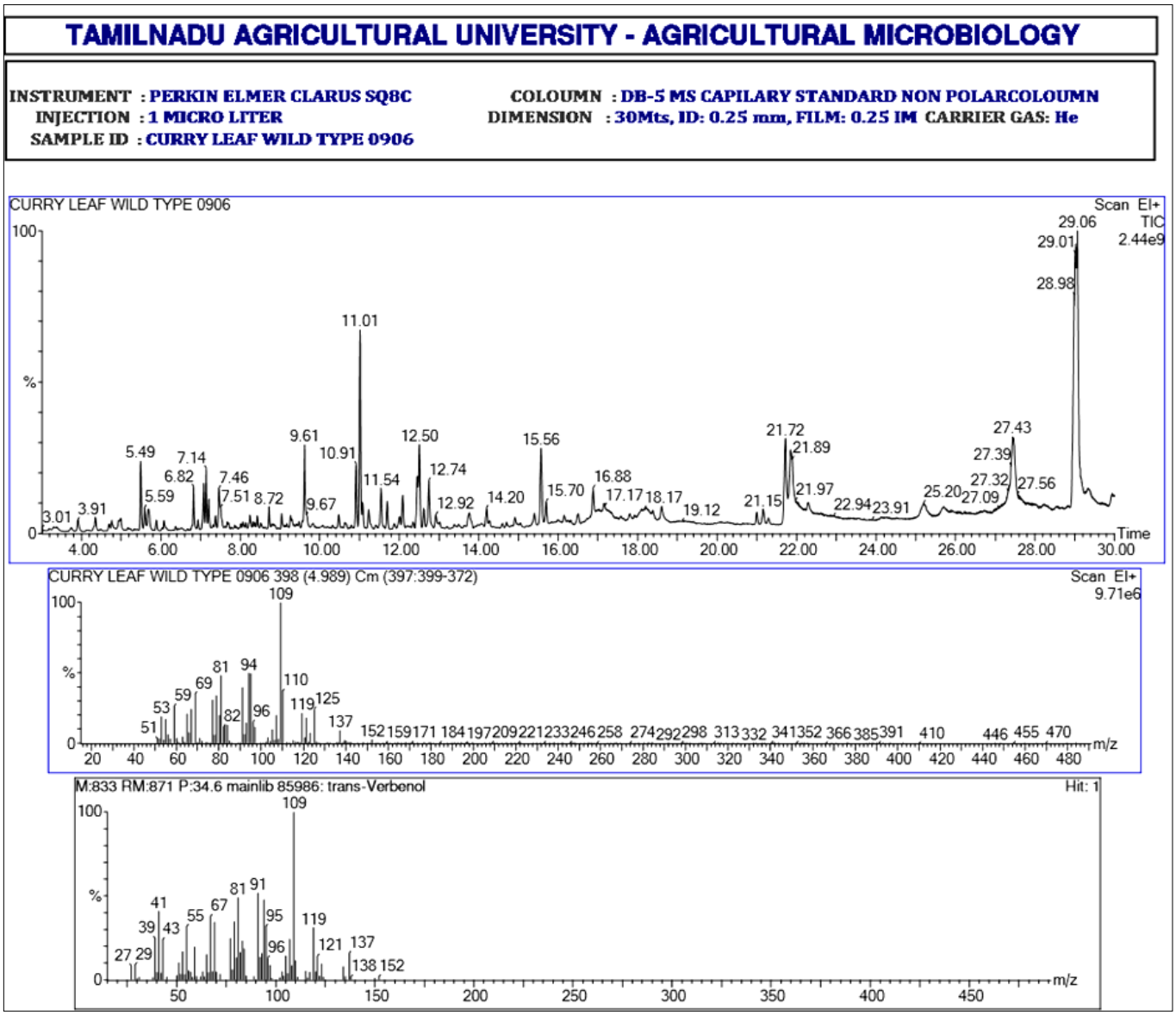


Fig. 1. Chromatogram of Wild type Curry leaf

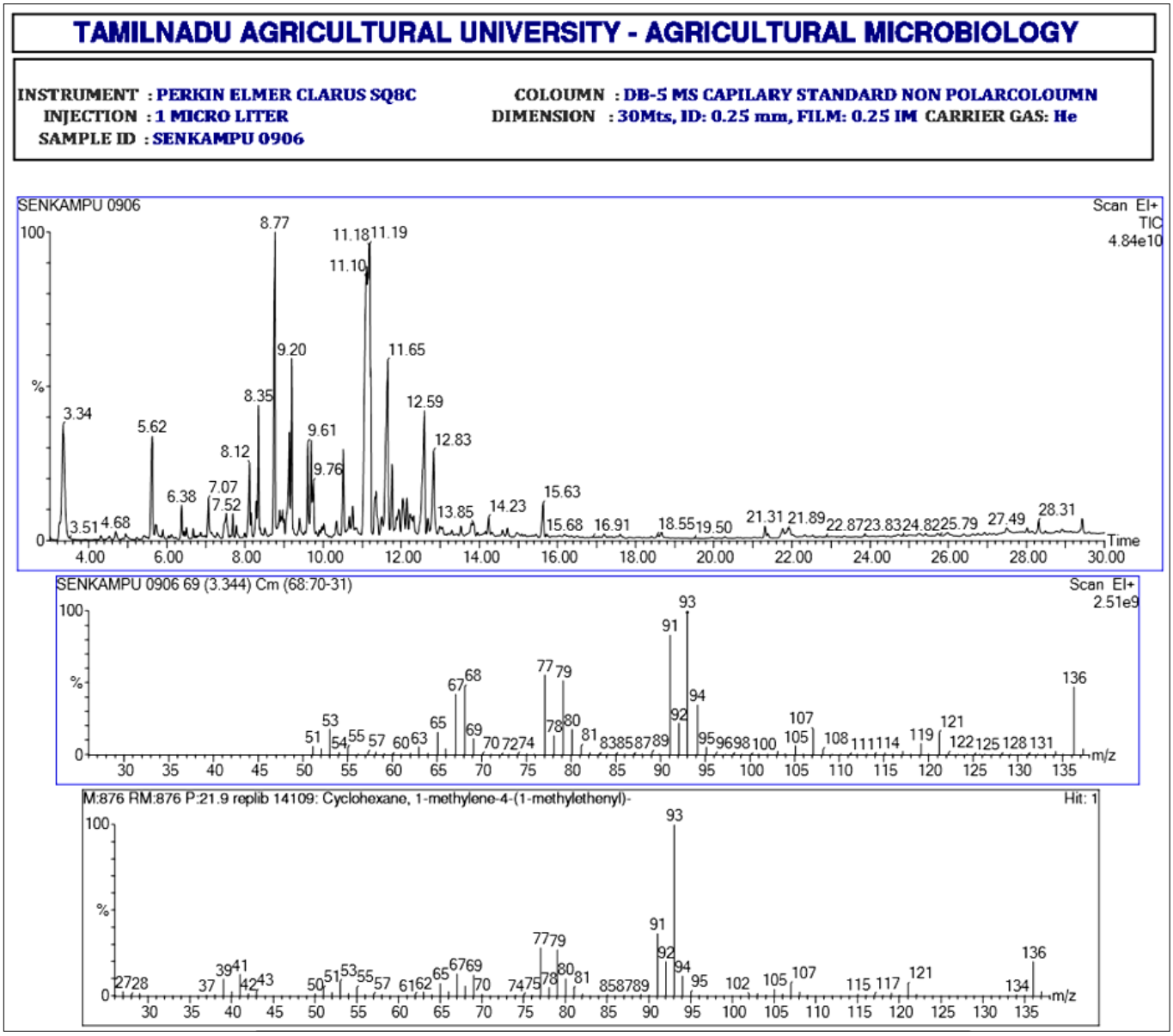


Fig. 2. Chromatogram of Senkambu type

Selection of Receptor

The domain of HER2 Kinase a 4-chain structure with a human sequence that is complexed with TAK-285 (3RCD) was selected as a possible receptor for molecular docking. Dual inhibitors targeting both human epidermal growth factor receptor (HER2) and epidermal growth factor receptor (EGFR) have been studied for treating malignancies in the breast, lungs, stomach, prostate and other organs (Fig. 3).

Protein preparation

Three-dimensional X-ray crystal structures of the target protein were obtained from the Protein Data Bank. The 3D structure of the HER2 Kinase domain protein (PDB ID: 3RCD) was retrieved from Protein Data Bank. The Protein Data Bank file was pre-processed to remove crystallographic water and add hydrogen atoms.

Active site prediction

The active sites of the protein structure were identified using the Computed Atlas of Surface Topography of Proteins (CASTp) version 3.0. This web-based platform was used to detect and quantify gaps in three- dimensional

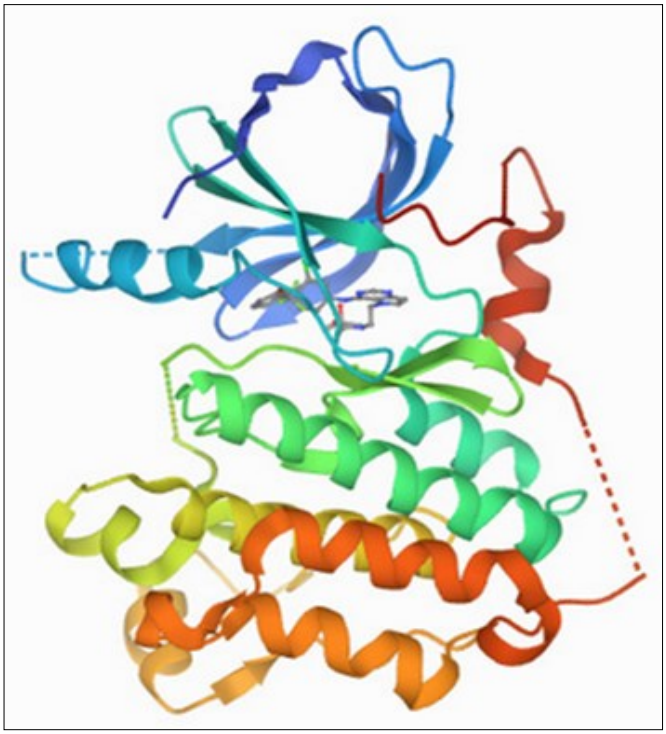


Fig. 3. 3D structure of HER2 kinase domain Complexed with TAK- 285

protein configurations. Binding site residues were predicted and the 3D protein structure was submitted to the CASTp service.

Selection of ligands

Compounds from curry leaves of wild-type and Senkambu variety were identified through GC-MS examination. Structures of 40 compounds from both varieties were retrieved from PubChem and CORINA and used for virtual screening against potential gastric cancer targets. Structure of 40 compounds of both varieties were retrieved from PubChem and CORINA and used for virtual screening against possible gastric cancer targets.

Virtual Screening

Virtual screening was performed using the AutoDock Vina module within the Python Prescription Virtual Screening tool (PyRx 0.8). The structure of the protein was prepared and the 40 ligand structures were entered into the PyRx tool along with the prepared macromolecule. The AutoDock Vina module in the PyRx tool was used to optimize the ligands and convert them into a pdbqt file. Binding sites were predicted using the CASTp server to build the grid (XYZ dimensions: 25x25x25) for the Auto Dock Vina during the virtual screening experiment, with an exhaustiveness setting value of 8 (11).

ADMET analysis

Assessing ADMET properties provides insights into the pharmacokinetic nature of the ligands. Swiss ADME, accessible via the SIB (Swiss Institute of Bioinformatics) webpage (https://www.sib.swiss), analyzed the ligands saved in Canonical SMILES format, which were then submitted for ADME estimation. Various factors, including the drug's aqueous solubility, blood-brain barrier penetration, gastrointestinal absorption, CYP 2D6 interaction, hepatotoxicity and plasma protein binding levels, were recorded and examined (12) (Table 3, 4).

Table 3. Curry leaves wild type – Swiss ADME

Sl. No.	Chemical name	Formula	M. wgt	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Log $P_{o/w}$(MLOGP)	Water Solubility Class	GI absorption	BBB permeant	CYP1A2 inhibitor	Lipinski
1	trans-Verbenol	$C_{10}H_{16}O$	152.23 g/mol	0	1	1	2.30	Soluble	High	yes	no	Yes, 0 violation
2	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	$C_{10}H_{18}O$	154.25 g/mol	1	1	1	2.30	Soluble	High	Yes	No	Yes, 0 violation
3	à-Terpineol	$C_{10}H_{18}O$	154.25 g/mol	1	1	1	2.30	Soluble	High	Yes	No	Yes, 0 violation
4	1,4-dihydroxy-p-menth-2-ene	$C_{10}H_{18}O2$	170.25 g/mol	1	2	2	1.38	Very Soluble	High	Yes	No	Yes, 0 violation
5	2-Cyclopenten-1-one, 3,4-dimethyl-	$C_7H_{10}O$	110.15 g/mol	0	1	0	1.24	Very Soluble	High	Yes	No	Yes, 0 violation
6	Ethanone, 1-(6-methyl-7-oxabicyclo [4.1.0]hept-1-yl)-	$C_9H_{14}O_2$	154.21 g/mol	1	2	0	107	Very Soluble	High	Yes	No	Yes, 0 violation
7	4,4-Dimethylpent-2-enal	$C_7H_{12}O$	112.17 g/mol	2	1	0	1.63	Very Soluble	High	Yes	No	Yes, 0 violation
8	(1S,2S,4S)-Trihydroxy-p-menthane	$C_{10}H_{20}O_3$	188.26 g/mol	1	3	3	0.64	Very Soluble	High	No	No	Yes, 0 violation
9	(-)-Spathulenol	$C_{15}H_{24}O$	220.35 g/mol	0	1	1	3.67	Soluble	High	Yes	No	Yes, 0 violation
10	Caryophyllene oxide	$C_{15}H_{24}O$	220.35 g/mol	0	1	0	3.67	Soluble	High	Yes	No	Yes, 0 violation
11	Globulol	$C_{15}H_{26}O$	222.37 g/mol	0	1	1	3.81	Soluble	High	Yes	No	Yes, 0 violation
12	2-(4a,8-Dimethyl-2,3,4,4a,5,6-hexahydronaphthalen-2-yl)propan-1-ol	$C_{15}H_{24}O$	220.35 g/mol	2	1	1	3.56	Soluble	High	Yes	No	Yes, 0 violation
13	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	138.21 g/mol	1	1	0	1.89	Very Soluble	High	Yes	No	Yes, 0 violation
14	Neointermedeol	$C_{15}H_{26}O$	222.37 g/mol	1	1	1	3.67	Soluble	High	Yes	No	Yes, 0 violation
15	10,10-Dimethyl-2,6-dimethylenebicyclo [7.2.0]undecan-5á-ol	$C_{15}H_{24}O$	220.35 g/mol	0	1	1	3.56	Soluble	High	Yes	No	Yes, 0 violation
16	trans-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220.35 g/mol	3	1	0	3.56	Soluble	High	Yes	No	Yes, 0 violation

17	1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl	$C_{15}H_{26}O$	222.37 g/mol	0	1	1	3.81	Soluble	High	Yes	No	Yes, 0 violation
18	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	$C_{15}H_{24}O$	220.35 g/mol	1	1	1	3.46	Soluble	High	Yes	No	Yes, 0 violation
19	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222.37 g/mol	7	1	1	3.86	Moderately Soluble	High	yes	Yes	Yes, 0 violation
20	Longipinocarvone	$C_{15}H_{22}O$	218.33 g/mol	0	1	0	3.56	Soluble	High	Yes	No	Yes, 0 violation
21	6-epi-shyobunol	$C_{15}H_{26}O$	222.37 g/mol	3	1	1	3.56	Moderately Soluble	High	Yes	No	Yes, 0 violation
22	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{22}O$	194.31 g/mol	3	1	0	3.04	Soluble	High	Yes	No	Yes, 0 violation
23	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)] dodecane-2,9-diol	$C_{15}H_{26}O_2$	238.37 g/mol	0	2	2	2.88	Soluble	High	Yes	No	Yes, 0 violation
24	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	$C_{26}H_{50}$	362.68 g/mol	12	0	0	8.27	Poorly Soluble	Low	No	No	Yes, 0 violation
25	á-Santanol acetate	$C_{17}H_{26}O_2$	262.39 g/mol	6	2	0	3.84	Soluble	High	Yes	No	Yes, 0 violation
26	Oleoyl chloride	$C_{18}H_{33}C_{IO}$	300.91 g/mol	15	1	0	4.91	Poorly Soluble	Low	No	Yes	Yes; 1 violation: MLOGP>4.15
27	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46 g/mol	15	2	1	4.57	Moderately soluble	High	No	Yes	Yes; 1 violation: MLOGP>4.15
28	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	14	2	1	4.19	Moderately soluble	High	Yes	Yes	Yes; 1 violation: MLOGP>4.15
29	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45 g/mol	14	2	1	4.47	Moderately soluble	High	Yes	Yes	Yes; 1 violation: MLOGP>4.15
30	Oleic Acid	$C_{18}H_{34}O_2$	282.46 g/mol	15	2	1	4.57	Moderately soluble	High	No	Yes	Yes; 1 violation: MLOGP>4.15
31	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392.57 g/mol	4	4	5	3.88	Soluble	High	No	No	Yes, 0 violation
32	4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-	$C_{20}H_{31}NO_3$	333.47 g/mol	11	4	1	2.67	Moderately Soluble	High	Yes	Yes	Yes ; 0 Violation
33	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.62 g/mol	6	5	3	3.46	Soluble	High	No	No	Yes; 0 violation
34	Squalene	$C_{30}H_{50}$	410.72 g/mol	15	0	0	7.93	Poorly soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
35	Gamabufotalin	$C_{24}H_{34}O_5$	402.52 g/mol	1	5	3	2.75	Soluble	High	No	No	Yes, 0 violation
36	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	$C_{16}H_{17}NO_3$	271.31 g/mol	4	3	0	2.14	Soluble	High	Yes	Yes	Yes; 0 violation
37	Piperine	$C_{17}H_{19}NO_3$	285.34 g/mol	4	3	0	2.39	Soluble	High	Yes	Yes	Yes; 0 violation
38	(E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)hept-6-en-1-one	$C_{19}H_{25}NO_3$	315.41 g/mol	7	3	0	2.94	Moderately soluble	High	Yes	No	Yes; 0 violation

Table 4. Senkambu type – Swiss ADME

Sl.No	Chemical name	Formula	M. wgt	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Log $P_{o/w}$(MLOGP)	Water Solubility Class	GI absorption	BBB permeant	CYP1A2 inhibitor	Lipinski
1	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	$C_{10}H_{16}$	136.23 g/mol	1	0	0	3.27	Soluble	Low	Yes	No	Yes; 0 violation
2	2-Cyclohexen-1-one, 4-(1-methylethyl)-	$C_9H_{14}O$	138.21 g/mol	1	1	0	1.89	Very Soluble	High	Yes	No	Yes; 0 violation
3	Benzaldehyde, 4-(1-methylethyl)-	$C_{10}H_{12}O$	148.20 g/mol	2	1	0	2.40	Soluble	High	Yes	Yes	Yes; 0 violation
4	p-Cymen-7-ol	$C_{10}H_{14}O$	150.22 g/mol	2	1	1	2.49	Soluble	High	yes	Yes	Yes; 0 violation
5	Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)-	$C_9H_{14}O_2$	154.21 g/mol	1	2	0	1.07	Very soluble	High	Yes	No	Yes; 0 violation
6	Bicyclo[2.2.2]octane-1,4-diol, monoacetate	$C_{10}H_{16}O_3$	184.23 g/mol	2	3	1	1.31	Very soluble	High	Yes	No	Yes; 0 violation
7	1-Cyclohexene-1-methanol, à,à,4-trimethyl-	$C_{10}H_{18}O$	154.25 g/mol	1	1	1	2.30	Very Soluble	High	Yes	No	Yes; 0 violation
8	4-Hydroxynonenal	$C_9H_{16}O_2$	156.22 g/mol	6	2	1	1.36	Very soluble	High	Yes	No	Yes; 0 violation
9	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à, 2á,4á	$C_{15}H_{24}$	204.35 g/mol	3	0	0	4.53	Moderately soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
10	Caryophyllene	$C_{15}H_{24}$	204.35 g/mol	0	0	0	4.63	Soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
11	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	$C_{15}H_{24}$	204.35 g/mol	3	0	0	4.63	Moderately soluble	low	No	No	Yes; 1 violation: MLOGP>4.15
12	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	204.35 g/mol	0	0	0	4.63	Soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
13	7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)	$C_{10}H_{16}O_2$	168.23 g/mol	1	2	0	1.38	Soluble	High	Yes	No	Yes; 0 violation
14	Humulene	$C_{15}H_{24}$	204.35 g/mol	0	0	0	4.53	Soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
15	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	204.35 g/mol	1	0	0	4.63	Moderately soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
16	Bicyclo[5.3.0]decane, 2-methylene -5-(1-methylvinyl)-8-methyl-	$C_{15}H_{24}$	204.35 g/mol	1	0	0	4.63	Moderately soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
17	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$	204.35 g/mol	1	0	0	4.63	Moderately soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
18	8-Oxabicyclo[4.3.0]nonane, 7,9-dimethyl-	$C_{10}H_{18}O$	154.25 g/mol	0	1	0	2.45	Soluble	High	yes	No	Yes; 0 violation
19	(4aS,8R)-4a,8-Dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one	$C_{12}H_{18}O$	178.27 g/mol	0	1	0	2.77	Soluble	High	Yes	No	Yes; 0 violation
20	Caryophyllene oxide	$C_{15}H_{24}O$	220.35 g/mol	0	1	0	3.67	Soluble	High	Yes	No	Yes; 0 violation
21	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	222.37 g/mol	7	1	1	3.86	Soluble	High	Yes	Yes	Yes; 0 violation
22	trans-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220.35 g/mol	3	1	0	3.56	Soluble	High	Yes	No	Yes; 0 violation
23	1,3,4-Trimethyladamantane	$C_{13}H_{22}$	178.31 g/mol	0	0	0	5.28	Moderately Soluble	Low	yes	yes	Yes; 1 violation: MLOGP>4.15
24	Ledol	$C_{15}H_{26}O$	222.37 g/mol	0	1	1	3.81	Soluble	High	Yes	No	Yes; 0 violation

25	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	138.21 g/mol	1	1	0	1.89	Very soluble	high	Yes	No	Yes; 0 violation
26	Neointermedeol	$C_{15}H_{26}O$	222.37 g/mol	1	1	1	3.67	Soluble	High	Yes	No	Yes; 0 violation
27	Epicubenol	$C_{15}H_{26}O$	222.37 g/mol	1	1	1	3.67	Soluble	High	Yes	No	Yes; 0 violation
28	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	220.35 g/mol	0	1	0	3.81	Soluble	High	Yes	Yes	Yes; 0 violation
29	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5á-ol	$C_{15}H_{24}O$	220.35 g/mol	0	1	1	3.56	Soluble	High	Yes	No	Yes; 0 violation
30	.tau.-Cadinol	$C_{15}H_{26}O$	222.37 g/mol	1	1	1	3.67	Soluble	High	Yes	No	Yes; 0 violation
31	á-Guaiene	$C_{15}H_{24}$	204.35 g/mol	0	0	0	4.63	Soluble	Low	No	No	Yes; 1 violation: MLOGP>4.15
32	Neointermedeol	$C_{15}H_{26}O$	222.37 g/mo	1	1	1	3.67	Soluble	High	Yes	No	Yes; 0 violation
33	Caryophylla-4(12),8(13)-dien-5à-ol	$C_{15}H_{24}O$	220.35 g/mol	0	1	1	3.56	Soluble	High	Yes	No	Yes; 0 violation
34	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	$C_{15}H_{26}O_2$	238.37 g/mol	0	2	2	2.88	Soluble	High	Yes	No	Yes; 0 violation
35	Longipinocarvone	$C_{15}H_{22}O$	218.33 g/mol	0	1	0	3.56	Soluble	High	Yes	No	Yes; 0 violation
36	6-epi-shyobunol	$C_{15}H_{26}O$	222.37 g/mol	3	1	1	3.56	Moderately soluble	High	Yes	No	Yes; 0 violation
37	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46 g/mol	15	2	1	4.57	Moderately soluble	High	No	Yes	Yes; 1 violation: MLOGP>4.15
38	Thunbergol	$C_{20}H_{34}O$	290.48 g/mol	1	1	1	4.65	Moderately soluble	High	No	No	Yes; 1 violation: MLOGP>4.15
39	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	306.48 g/mol	1	2	2	3.73	Moderately	High	Yes	No	Yes; 0 violation
40	Friedelan-3-one	$C_{30}H_{50}O$	426.72 g/mol	0	1	0	6.92	Poorly soluble	low	No	No	Yes; 1 violation: MLOGP>4.15

Interaction analysis

The binding interactions of the selected compounds with the HER2 kinase domain were analyzed using Biovia Discovery Studio Visualizer. The software visualizes the hydrogen bonds, hydrophobic interactions, and other non-covalent interactions between the ligands and the protein.

Results

GC-MS

Phytochemical profiling offers a comprehensive insight into the diverse array of bioactive compounds of curry leaves wild-type and Senkambu type. The GC-MS analysis identified nearly 40 compounds in both the samples, each with varying probabilities and abundances. In curry leaf of wild-type, prominent compounds included trans-Verbenol,3-Cyclohexen-1-ol, à-Terpineol and Caryophyllene oxide, others (Table 1). On the other hand, Senkambu type exhibited a distinct profile, with compounds including Caryophyllene, Caryophyllene oxide, and trans-Z- à-Bisabolene epoxide dominating the spectrum (Table 2). The comparative analysis of these two plant species has their unique chemical fingerprints and highlights their significance in traditional medicine and modern drug discovery.

Molecular Docking –Wild type and Senkambu

Swiss ADME datasets provide a detailed characterization of various chemical compounds, including their characteristics of molecules like molecular weight, number of rotable bonds, and acceptors of hydrogen bonds. and donors, Log P/oil- water partition coeffient (MLOGP), water solubility, blood-brain barrier (BBB), gastrointestinal (GI) absorption, permeability, CYP1A2 inhibition and Lipinski's rule of five violations. These properties are crucial for predicting the pharmacokinetic and pharmacodynamic behavior of compounds, which is essential for drug discovery and development. All the compounds screened from ADMET analysis were subjected to virtual screening. Details on the anticipated binding site residues made with the CASTp server. Using Biovia Discovery Studio Visualize, the top three hits with the highest binding affinity for the target protein are taken into consideration for additional interaction analysis.

Information about the binding site residues predicted by the CASTp server. The top 3 hits which exhibit highest binding affinity for the target protein, were selected for further interaction analysis using Biovia Discovery studio visualizer.

The results of molecular docking of wild-type curry leaves revealed promising interactions between selected compounds and their protein targets. Piperine exhibited strong affinity towards the protein target 3rcd, forming a conventional hydrogen bond with THR 793 and resulting (–8.3 kcal/mol binding energy). Similarly, gamabufotalin demonstrated notable interactions with Arg 814 and ASP

924, yielding a binding energy of –8.2 kcal/mol. Additionally, Tyr 923, Asp 924 and Ala 710 exhibited significant interactions with the molecule Pyrrolodine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-, with a binding energy of –7.9 kcal/mol (Table 5, 6).

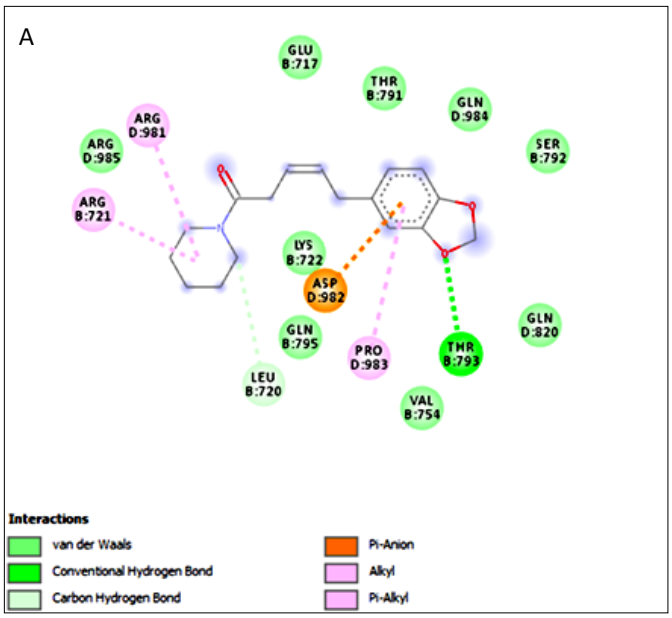
Table 5. Amino acids involved in interactions were visualized using Biovia Discovery Studio Visulaizer – Wild type

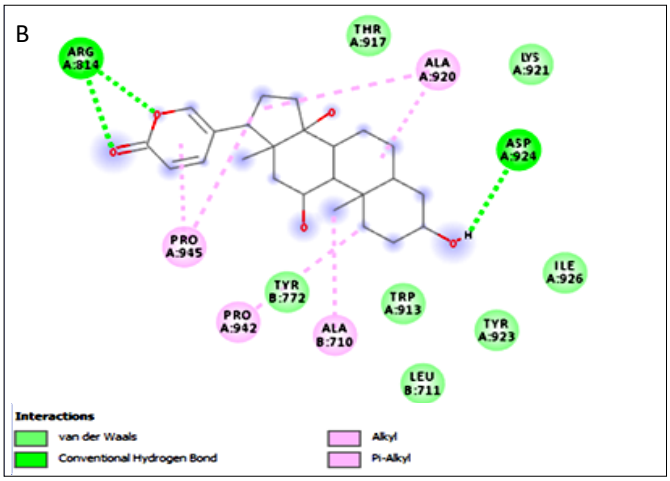
Sl. No.	Protein target	Best compound	Interacting amino acids involved in conventional hydrogen bond formation	Binding energy (kcal/mol)	No. of Hydrogen bonding
1.	3rcd	Piperine	Thr 793	-8.3	2
2.		Gamabufotalin	Arg 814, ASP 924	-8.2	3
3.		Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)-	Tyr 923, Asp 924, Ala 710	-7.9	3

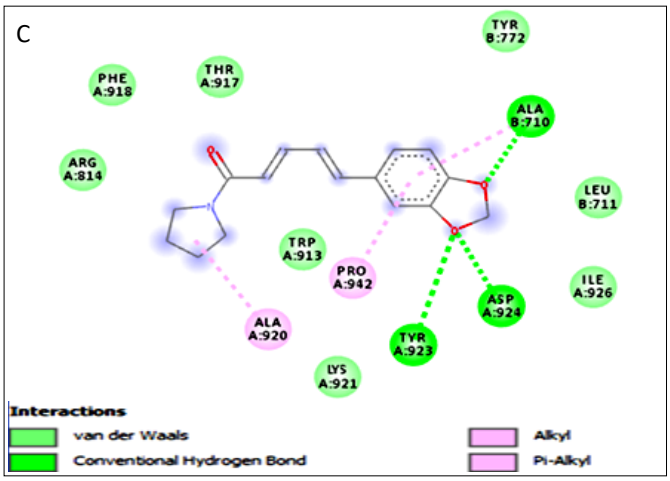
Table 6. Amino acids involved in interactions were visualized using Biovia Discovery Studio Visulaizer- Senkambu type

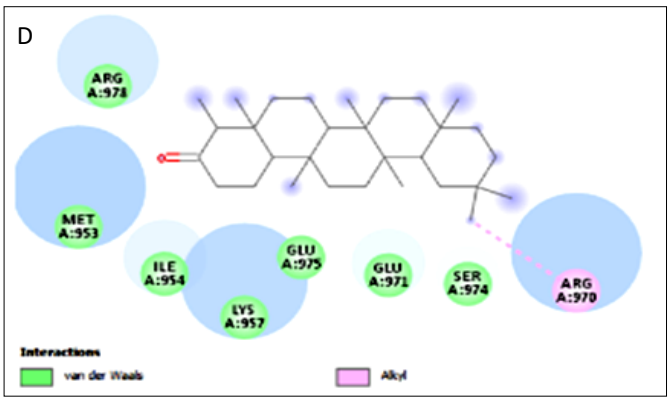
Sl. No.	Protein target	Best compound	Interacting amino acids involved in conventional hydrogen bond formation	Binding energy (kcal/mol)	No. of Hydrogen bonding
1.	3rcd	Friedelan-3-one	No hydrogen bond formation	-8.2	0
2.		Gamabufotalin	Arg 814, ASP 924	-7.4	3
3.		Ursodeoxycholic acid	Asp 769, Gln 943, Arg 814	-7.3	3

The results of molecular docking analysis of Senkambu revealed compelling interactions between the selected compounds and their protein targets. Friedelan-3-one, despite not forming conventional hydrogen bonds, showed a remarakable binding energy of – 8.2 kcal/mol and a considerable affinityu for the protein targets 3rcd. Gamabufotalin showed significant interactions with ASP 924 and Arg 814, yielding an energy of -7.4 kcal/mol. Ursodeoxycholic acid demonstrated notable interactions with Asp 769, Gln 943, and Arg 814, yielding a –7.3 kcal/mol binding energy. The interactions analysed for both wild type and Senkambu type has been given in the Fig. 4 (A-E).









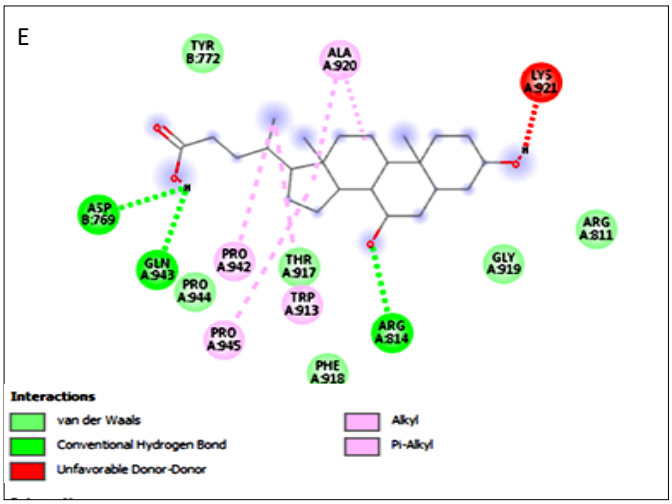


Fig. 4. Amino acids involved in interactions were visualized using Biovia Discovery Studio Visulaizer –Curry leaf wild type and Senkambu. (**A**)**.** Piperine (**B**).Gamabufotalin, (**C**) . Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl) -1-oxo-2,4-pentadienyl]-, (E,E), (**D**).Friedelan-3-one and (**E**). Ursodeoxycholic acid

Discussion

Upon analyzing the compounds, piperine from wild-type curry leaves was identified as a potential inhibitor for HER2 Kinase domain due to its hydrogen bond interactions and a good binding affinity of -8.3 k cal/mol. Similarly, it has been shown that piperine inhibits the growth of gastric cancer by inducing apoptosis, highlighting its potential as an anti-cancer agent for treating gastric cancer (13). Piperine shows great promise in preventing cancer due to its ability to halt the cell cycle, increase autophagy and apoptosis and disrupt redox homeostasis (14). Similar to the above results, piperine blocked cell migration by reducing STAT –3 activity, which led to the prevention of metastasis (15). The findings revealed that piperine can suppress IL-6, which effectively decreases the invasion of gastric cells (TMT-1) (16). In addition to the above results, piperine inhibits the development of human stomach cells and induces programmed cell death by targeting the P13K/Akt signaling pathway (17). It was shown that piperine efficiently suppressed the growth of breast cancer cells overexpressing HER2 and caused them to undergo apoptosis (18). This was achieved by inhibiting caspase-3 activation and PARP cleavage, ultimately resulting in the transcriptional downregulation of the HER2 gene expression. Additionally, piperine pretreatment resulted in increased sensitization to paclitaxel- induced cell death in these cells. The findings also suggest that Gamabufotalin interacts proficiently with essential amino acids that play a role in gastric cancer progression, indicating its potential as a suppressor of cancer cell growth and spread.

The consistent binding observed, even with minor variations in binding energy, emphasizes gamabufotalin's potential as a therapeutic option. These results reinforce its significance in targeting proteins essential to the progression of gastric cancer, justifying further investigation into its clinical efficacy. It has been demonstrated that gamabufotalin and apatinib work in concert to suppress the growth and spread of gastric cancer cells by downregulating VEGFR and MMP-9, improving treatment outcomes (19). This study confirmed that piperine has strong cytotoxic effect against gastric cancer cells, both in its solo form and when coupled with chemotherapeutics. Piperine promoted apoptosis and inhibited cancer cell proliferation, thereby increasing the efficacy of chemotherapy treatments. These findings imply that piperine may improve therapeutic outcomes in gastric cancer therapy (20).

Conclusion

In this study, potential HER2 kinase domain inhibitors were identified from the phytochemicals present in wild-type and Senkambu varieties of curry leaves using GC-MS analysis and virtual screening. Piperine from wild-type curry leaves exhibited the highest binding affinity, forming stable hydrogen bonds with a key residue in the binding site. ADMET analysis of the selected compounds revealed favorable properties for drug development. These findings highlight piperine as a potent phytochemical from curry leaves and a potential source of innovative therapeutic agents for stomach cancer that inhibit the HER2 kinase domain. Additionally, further *in vitro* and *in vivo* studies are required to validate the anticancer activity and mechanism of action of these compounds. This work establishes a foundation for developing targeted therapies using natural products for the treatment of stomach cancer. Moreover, structural modifications and formulation approaches could be explored to enhance the effectiveness and safety profiles of these natural products for future drug development endeavors.

Acknowledgements

Support from the Department of Agricultural microbiology for GC-MS analysis and Department of Plant Molecular Biology and Bioinformatics for molecular docking studies, Tamil Nadu Agricultural University, Coimbatore, is greatly acknowledged.

Authors' contributions

S carried out the research work. V guided to carry out the research work and GC-MS analysis work. M and R helped in molecular docking work

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Mamun TI, Younus S, Rahman MH. Gastric cancer-epidemiology, modifiable and non-modifiable risk factors, challenges and opportunities: An updated review. Cancer Treatment and Res Commun.2024;24:100845.https://doi.org/10.1016/j.ctarc.2024.100845
2. Scheck MK, Hofheinz RD, Lorenzen S. HER2-positive gastric cancer and antibody treatment: State of the art and future developments. Cancers. 2024;29(7):1336. https://doi.org/10.3390/cancers16071336
3. Takiuchi H, Ohtsu A, Fuse N, Goto M, Yoshida M, Dote N, et al. Phase I first-in-human study of TAK-285, a novel investigational dual HER2/EGFR inhibitor, in cancer patients. British J Cancer. 2012;106(4):666-72. https://doi.org/10.1038/bjc.2011.590
4. Ho JW, Leung Y, Chan C. Herbal medicine in the treatment of cancer. Curr Med Chem Anti-Cancer Agents. 2002;*2*(2):209-14. https://doi.org/10.2174/1568011023354164
5. Sandhya S, Jegadeeswari V, Shoba N, Jeyakumar P. A preliminary study to check the graft compatibility and success percentage of curry leaf (*Murraya koenigii* Spreng.). J Pharmaco and Phytochem. 2020;9(4):3479-83.
6. Noolu B, Ismail A. Anti-proliferative and proteasome inhibitory activity of *Murraya koenigii* leaf extract in human cancer cell lines. Discovery Phytomed- J Nat Prod Res and Ethnopharma. 2015;25(1):1-9. https://doi.org/10.15562/phytomedicine.2015.18
7. Balakrishnan R, Vijayraja D, Jo SH, Ganesan P, Su-Kim I, Choi DK. Medicinal profile, phytochemistry and pharmacological activities of *Murraya koenigii* and its primary bioactive compounds. Antioxidants. 2020;24(2):101. https://doi.org/10.3390

%2Fantiox9020101

8. Raghu BR. Diversity and distribution of curry leaf in India. J Horticultural Sci. 2020;15(1):1-8.
9. Vali DM, Venkatesan K, Senthil N, Selvi BS, Raveendran M. Comparative analysis of *Curcuma* species essential oil through gas chromatography-mass spectrometry. Medicinal Plants-Inter J Phytomed and Related Indust. 2022;14(4):632-36.
10. Santhanakrishnan VP, Shoba N, Varun E, Mohankumar S, Raveendran M. Aromatic profiling of *Murraya koenigii* leaves by Thermal Desorption Gas chromatography-Mass Spectroscopy (TD-GC-MS). Heliyon. 2023 Jul 1;9(7):
11. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31(2):455-61.
12. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 2017;7 (1):42717.
13. Shin SA, Lee HN, Choo GS, Kim SJ, Kim HJ, Park YS, et al. Induction of apoptosis and inhibition of growth in human gastric cancer by piperine. J Korean Society of Food Sci and Nutri. 2016;45(11):1589-94. https://doi.org/10.3746/jkfn.2016.45.11.1589
14. Zadorozhna M, Tataranni T, Mangieri D. Piperine: role in prevention and progression of cancer. Mol Biol Reports. 2019;46 (5):5617-29.
15. Turrini E, Sestili P, Fimognari C. Overview of the anticancer potential of the “king of spices” *Piper nigrum* and its main constituent piperine. Toxins. 2020;26:(12):747. https://doi.org/10.1186/s43088-022-00196-1
16. Xia Y, Khoi PN, Yoon HJ, Lian S, Joo YE, Chay KO, et al. Piperine inibits IL-1β-induced IL-6 expression by suppressing p38 MAPK and STAT3 activation in gastric cancer cells. Molecular and Cellular Biochem. 2015;398:147-56.
17. Chen H, Sheng H, Zhao Y, Zhu G. Piperine inhibits cell proliferation and induces apoptosis of human gastric cancer cells by downregulating phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Med Sci Monitor: Inter Med J Experimental and Clinical Res. 2020;26:e928403-1.
18. Tripathi AK, Ray AK, Mishra SK. Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials. Beni-Suef University J of Basic and Applied Sci. 2022;28(1):16. https://doi.org/10.1186/s43088-022-00196-1
19. Chen B, Zhao Y, Lin Z, Liang J, Fan J, Huang Y, et al. 2. Apatinib and gamabufotalin co-loaded lipid/prussian blue nanoparticles forsynergistic therapy to gastric cancer with metastasis. J Pharmaceutical Analysis. 2023; https://doi.org/10.1016/j.jpha.2023.11.011
20. Ramos IN, da Silva MF, Lopes JM, Cruz JN, Alves FS, do Rego JD, et al. Extraction, characterization and evaluation of the cytotoxic activity of piperine in its isolated form and in combination with chemotherapeutics against gastric cancer. Molecules. 2023;28(14):5587. https://doi.org/10.3390/molecules28145587