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Structure-Based In-silico and In-Vitro Investigations on *Carica Papaya's* Chemical Components as a Possible HER2 Breast Cancer Inhibitor

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Abstract
Background: A type of breast cancer referred to as human epidermal growth factor receptor 2 (HER-2) is characterized by a positive detection of the HER-2 protein, which encourages the growth of cancer cells. Approximately one in every five breast cancer cases is more aggressive due to the presence of extra copies of the genes responsible for producing the HER2 protein. Identifying plant compounds capable of reducing the expression of HER-2 proteins could offer potential treatment options for breast cancer patients, particularly in developing countries where herbal therapy is commonly used. The chemical composition of a Carica papaya leaf extract was analyzed using gas chromatography-flame ionization detector (GC-FID) to explore its potential for breast cancer treatments. The extract's antioxidant capacity was evaluated *in vitro* through the phosphomolybdate technique. The top ten compounds, determined by docking scores, underwent further investigations including administration, distribution, metabolism, elimination and toxicity (ADMET) assessments, pharmacophore modeling, and molecular docking.
Results: The GC-FID analysis revealed forty-three components in *Carica papaya* leaf extract. The extract demonstrated dose-dependent activity in ferric-reducing antioxidant power, superoxide scavenging activity, nitric oxide scavenging activity, and 1,1-diphenyl-2-picrylhydrazyl radical
(DPPH) radical scavenging activity compared to the standard. Binding affinity was found to be significant for ten different chemicals. Among them, naringenin, flavan-3-ol, ellagic acid, epicatechin, and lunamarine showed higher binding affinity compared to 5-fluorouracil (5-FU). Analysis of the two- and three-dimensional views indicated that the top compounds occupied the designated binding regions and primarily interacted through hydrogen and hydrophobic interactions.
Conclusion: Epicatechin emerged as the safest among all the substances, displaying no evidence of toxicity based on LD_{50} of 10,000 mg/kg and toxicity class 6. Further research on epicatechin may be warranted to validate its potential effectiveness in preventing breast cancer.
Keywords: Computational drug design, Breast cancer, Medicinal plants, *Carica papaya*, Epicatechin.

I. Introduction

Breast cancer is a well-known form of adenocarcinoma, the most common cancer in women and the second leading cause of death in women after lung cancer. Mortality outcomes are somewhat unclear and are often related to socioeconomic factors and lifestyle choices[1]. The condition is quite diverse, showing variations in histological grade, proliferation index (Ki67), immunohistochemistry, and clinical symptoms[2].

The Ki67 marker and the Bloom-Richardson scoring system serve as useful tools to predict the level of tumor aggressiveness. However, it is noteworthy that the same histological subtype may present differently at different ages, influenced by various factors related to fertility. These factors, such as impaired fertility, sexual dysfunction, and menopausal symptoms, may also influence the spread and treatment of the disease[3]. Therapy and outcomes for breast cancer are often dependent on the subtypes (hormone receptor-positive, HER-2, and triple-negative subtypes), and involve hormonal, radiotherapy, molecular, and chemotherapy interventions. Surgery has become increasingly advised, especially for metastatic situations [4]. The prognosis of breast cancer is also influenced by its stage, which correlates with the size of the primary breast tumor, involvement of axillary lymph nodes, and the presence of distant metastasis [5].

Medicinal plant products have shown promising results as anti-cancer agents. Their effectiveness is also reported as decreased toxicity in usage, and less recurrent resistance to hormonal targeting anti-cancer agents[6] (multidrug resistances as seen with several anti-cancer agents). These applications stem from the antioxidant and

anti-inflammatory attributes of natural plant products, along with their immunomodulatory characteristics. They possess the capability to induce anti-proliferative and anti-apoptotic effects on cancer cells. In doing so, they exhibit a chemo-preventative property, which can serve both prophylactic and therapeutic purposes, and they are deemed safe for long-term usage[7]. Constituents of medicinal plant products such as flavonoids, alkaloids, terpenoids, and coumarins are known for their antioxidant and anti-inflammatory properties, which are strong immunomodulatory properties needed to suppress or fight against cancer cells[8]. An example of such a plant is *Carica papaya* which belongs to the family Caricaceae and is commonly known as pawpaw, this plant contains bioactive compounds like steroids and flavonoids which act as endocrine disrupters for hormonal disorders, which are often the basis for the presentation of these cancer outcomes. These flavonoids in *Carica papaya* are reported to possess estrogenic and or anti-estrogenic properties, which are also chemo-preventative properties[9]. They can inhibit estrogen receptor-dependent (cell growth and proliferation) and possess the ability to induce oxidative stress and cancer induction through estrogen receptor signaling[10]. Several studies have provided evidence of the efficacy of medicinal plants such as *Carica papaya* in the development of anti-cancer drugs.

Chemotherapeutic drugs like Irinotecan, vinblastine, doxorubicin, oxaliplatin, melphalan, carboplatin, cisplatin, etc. are significantly effective against a wide range of cancers and have shown promising results alone or in combination with other cancer therapies[11]. However, these drugs have their limitations including limited bioavailability, toxicity, non-specificity, and fast clearance[12]. These drugs are frequently linked with adverse effects like high cytotoxicity, neutropenia, sensory neuropathy, cardiovascular toxicity, pulmonary and hematologic toxicity, gastrointestinal toxicity, diarrhea, and nephrotoxicity[11]. This led to the discovery of alternative treatments against cancer from medicinal plants with minimal or without side effects. This study was carried out for the identification of novel anticancer compounds from *Carica papaya* leaf extract through chemical profiling and in-silico computational modeling.

II. Materials And Methods

Chemical Reagents

All the chemicals and reagents used for the analysis were of analytical standard.

Plant Collection and Extraction

Fresh leaves of Carica papaya were collected from the *Mgbalukwu Inyimagu community* in Abakaliki LGA in Ebonyi State. The fresh leave was identified and voucher number (H-019) was assigned and stored in the University herbarium. The fresh leaves were properly washed under running water and allowed to dry under shade for 5-6 days. The dried leaves were pulverized into a fine powder, then 250g of the powdered leaves were soaked in 300ml of ethanol (70%), the mixture was left for 48 hours. Afterward, the extract was filtered, evaporated to dryness using a rotary evaporator, and stored in a clean bottle.

Extraction of phytochemical components from *Carica papaya* leaf extract

1 g of sample was accurately weighed and placed in a test tube containing 10 ml of 50% potassium hydroxide and 15 ml of ethanol. The test tubes were left to react in a water bath at 600 degrees Celsius for 60 minutes. After the reaction time had elapsed, the reaction product in the test tube was transferred to the recovery funnel. After adding 20 milliliters of ethanol, 10 milliliters of cold water, 10 milliliters of hot water, and 3 milliliters of hexane, the test tube was effectively washed and placed in a funnel. After mixing these extracts, they were washed three times using 10 ml of 10% ethanol aqueous solution. The mixture was dried using anhydrous sodium sulfate, and the solvent was removed. Samples were dissolved in 1000 µl of pyridine, then 200 µl was transferred to a test vial.

Quantification using Gas Chromatography – Flame Ionization Detector (GC-FID)

Phytochemical analyses were performed using a BUCK M910 gas chromatograph equipped with a flame ionization detector. The instrument used was a 15 m RESTEK MXT-1 column (15 m x 250 µm x 0.15 µm). The carrier gas was helium at a 5.0 Pa·s velocity, and the injector temperature was set at 280°C. The injector was designed to inject 2 µL of sample without splitting at a linear velocity of 30 cm/s and a flow rate of 40 mL/min. The oven was first set to 200°C. It was then heated to 330°C at a rate of 3°C/min and held at this temperature for 5 min. The detector operated at a temperature of 320°C. To determine phytochemicals, the ratio of the area of the detected phytochemical to the area and mass of the internal standard was used. Concentrations of various phytochemicals are expressed in µg/g.

In vitro analysis

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the extracts was determined by the phosphomolybdate method[13]. Aliquots (30 ml) of the test extracts at different concentrations (20, 40, 60, 80, and 100 mg/ml1) were mixed with

3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was placed in a test tube. The tubes were covered with aluminum foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture was cooled to room temperature and the absorbance of the solution was measured at a wavelength of 695 nm against a control sample containing 3 ml of reagent solution and a corresponding volume of dissolving solvent. Control samples were cultured under the same conditions as the test samples. To compare the activity of the extracts, ascorbic acid was used as a standard reference compound.

DPPH Spectrophotometric Assay

Spectrophotometric analysis of DPPH, the ability of natural leaf antioxidants to scavenge the stable free radical DPPH was determined according to the method (14).

Measurement of superoxide Scavenging Activity

The peroxide absorption capacity of the samples was evaluated according to the method (15). Superoxide anions were generated in samples containing 3.0 mL, 0.02 mL leaf sample (20 mg), 0.2 mL EDTA, 0.1 mL NBT, 0.05 mL riboflavin, and 2.64 mL phosphate buffer. A control tube containing dimethylsufoxide (DMSO) added instead of the sample was also installed. All tubes were shaken and the initial optical density was measured at a wavelength of 560 nm using a spectrophotometer (Genesys, 10-S, USA). The tube was illuminated with fluorescent light for 30 minutes. Absorbance was measured at 560 nm. The difference in absorption before and after illumination indicates superoxide anion scavenging activity.

Measurement of nitric oxide scavenging activity

The degree of inhibition of nitric oxide radical formation in vitro was monitored using the method described by (16). The reaction was started by adding 2.0 ml sodium nitroprusside, 0.5 ml PBS, and 0.5 ml (50 mg) leaf sample and incubating for 30 min at 25°C. Griess reagent (0.5 ml) was added and incubated for an additional 30 min. Control tubes were prepared without a sample. Absorbance was measured at a wavelength of 546 nm against reagent blanks in a spectrophotometer (Genesys 10-S, USA).

Ferric Reducing Antioxidant Property

Antioxidant properties that reduce iron content The reducing power of the extract was determined by (17). Approximately 0.25 ml of extract was mixed with 0.25 ml of 200 mM sodium phosphate buffer (pH 6.6) and 0.25 ml of 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 minutes, then 0.25 ml of 10% trichloroacetic acid was added, centrifuged at 2000 rpm for 10 minutes, and 1 ml of supernatant was mixed with 1 ml of distilled water, and 0.2 ml of distilled water. 1 ml of ferric chloride was added and the absorbance was measured at 700 nm.

In-silico evaluation of anti-cancer assays

Protein preparation

The crystal structure of the kinase domain of human HER2 (PDB ID:3PP0) was obtained from the Protein Data Bank (PDB) repository. Proteins were prepared using the Glide Protein Preparation Wizard panel (Schrádinger Suite 2021-2) where bond sequences were specified, hydrogens were added disulfide bonds were created, and missing side chains and loops were filled in using primes. Water molecules exceeding 3.0 A heteroatoms were removed and the structure was minimized using OPLS2005 and optimized using PROPKA. Subsequently, a receptor grid file was generated to define the binding pocket for the ligand[24].

Ligand preparation

Several compounds from *Carica papaya* were prepared for molecular docking using the Ligprep module (Schrödinger Suite 2021-2). A low-energy 3D structure with appropriate chirality was created. For each ligand structure, possible ionization states were generated at physiological pH 7.2–0.2. Stereoisomers of each ligand were calculated while maintaining the given chirality, while others were changed.

Receptor Grid Generation

The creation of a receptor mesh allows the location and size of the ligand docking site in the protein active site to be determined. The scoring grid is the cocrystallized ligand 03Q(2-{2-[4-({5-chloro-6[3-(trifluoromethyl) phenoxy] pyridin-3yl} amino)-5H-pyrrolo[3, 2- d]pyrimidin-5-yl]ethoxy}ethanol) using the Schrödinger Maestro 12.5 acceptor meshing tool. The van der Waals radius scaling factor (vdW) for nonpolar acceptor atoms was adjusted to 1.0 with a partial charge cutoff of 0.25.

Protein-ligand docking

The Schrödinger Maestro 12.8 sliding tool was used to perform molecular docking studies using the generated receptor mesh files. Prepared ligands were docked using standard precision (SP), with ligand sampling set to flexible and ligand sampling set to none (precision only). The radial scale factor vdW was scaled to 0.80 with a partial charge cutoff of 0.15 for the ligand atoms.

Pharmacological parameters

The test compounds' absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were determined using in silico integrated model predictions on the Swiss ADME and PROTOX-II servers, respectively.

Statistical analysis

The obtained data were expressed as mean and standard deviation. One-way analysis of variance (ANOVA) was performed with post hoc comparisons between the control group and each treatment group using the Dukan multiple comparison test to determine the statistical significance of differences. $P<0.05$ was considered statistically significant.

III. Result

Phytochemical components of Leaf Extract of *Carica papaya*

GC-FID analysis revealed the identification of twenty phytochemical substances from the chromatogram peaks depicted in Figure 1. Predominantly, these compounds consisted of polyphenols, with alkaloids, steroids, and tannins following in descending order of abundance. The identified compounds were classified into four categories of phytochemicals: polyphenolics, alkaloids, tannins, saponins, and steroids, as presented in Table 1.

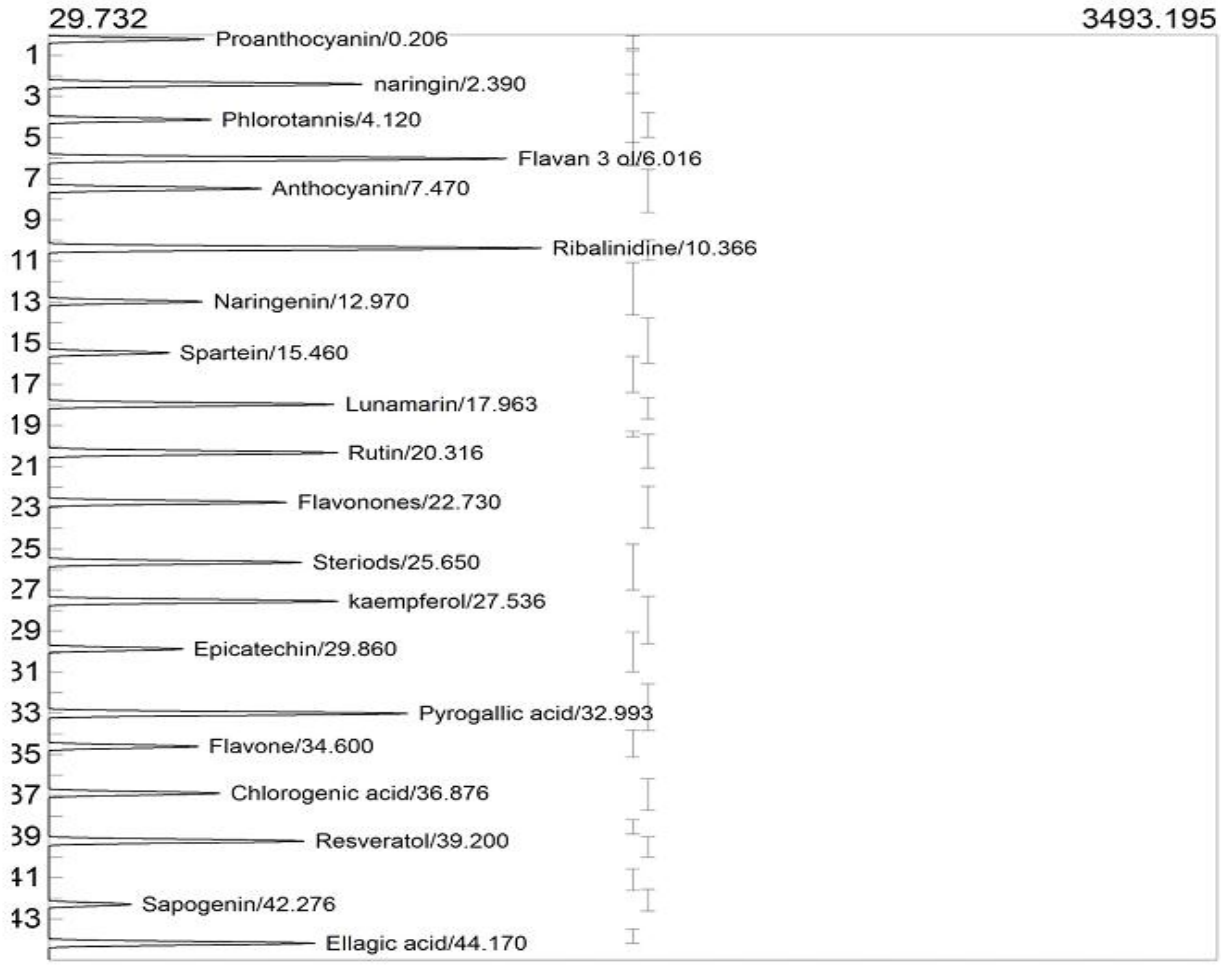


Figure 1: Chemical constituents of Carica papaya leaf extract

Table 1: Different Chemical Compositions of Ethanol Leaf Extract of *Carica papaya* and their medicinal properties.

Phytochemical Classes	Phytochemicals	Percentage Composition	Medicinal Properties
Polyphenolic compounds	Proanthocyanin	0.206	Possess antioxidative, anticancer, anti-obesity, anti-diabetic and antimicrobial effects[18]
	Naringin	2.390	Used in the treatment of osteoarthritis and osteoporosis[19]

	Flavan-3-ol	6.016	Antioxidant, anticarcinogen, cario-preventive, antimicrobial, anti-viral, and neuroprotective agents.
	Anthocyanin	7.470	Antioxidant properties[20]
	Ribalinidine	10.366	Free radical scavenging activity[21]
	Naringenin	12.970	Aids metabolism, antioxidants, antitumoral and anti-inflammatory.
	Rutin	20.316	Cardio-protective, neuro-protective, anti-diabetic, and nephroprotective[21]
	Flavanones	22.730	Used to support cardiovascular health, reduce inflammation, and improve skin health[22]
	Kaempferol	27.536	Cytotoxic, antioxidant, and anti-inflammatory[21]
	Epicatechin	29.860	Anticancer, anti-inflammatory, and antioxidant[21]
	Pyrogallic acid	32.993	No health benefits
	Flavone	34.600	Antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[22]
	Chlorogenic acid	36.876	Improves glucose tolerance and improves blood pressure in hypertension[23]
	Resveratrol	39.200	Antioxidant, anti-inflammatory, immunomodulatory, glucose and lipid regulatory, neuroprotective, and cardiovascular protective effects[24].
Alkaloids	Spartein	15.460	Anti-hyperlipidemia, anti-convulsant[21]
	Lunamarin	17.963	Radical scavenging activity[25]
Tannins	Phlorotannins	4.120	Antioxidant, anticancer, antidiabetic, antihuman immunodeficiency virus, antihypertensive
	Sapogenin	42.276	Hypocholesterolemia is immunomodulatory, anti-inflammatory, and cytotoxic[21]
	Ellagic acid	44.170	Antioxidant, antimutagenic, and anticancer properties[26]
Steroids	Steroids	25.650	Used to ease inflammation

Effect of Leaf Extract of *Carica papaya* on in vitro Antioxidant parameters

As depicted in Fig 2 (1-6), the ferric-reducing antioxidant power of the extract demonstrated dose-dependent behavior across varying concentrations. The highest activity percentages were recorded at 40mg/ml (55.521%), while the lowest inhibition percentage of Fe3+ was observed at 10mg/ml (38.037%). The extract exhibited an IC_{50} value of 53.359%.

For the superoxide scavenging activity, different concentrations of the extract were examined, revealing the highest inhibition at 20mg/ml with a scavenging activity of 65.106%. The extract's IC_{50} was determined to be 71.183%. Notably, the extract exhibited significant inhibition of nitric oxide, with the most effective inhibition observed at 80mg/ml, yielding nitric oxide scavenging activities of 5.502ug/g, 6.6966ug/g, 5.698ug/g, and 5.161ug/g for concentrations of 10mg/ml, 20mg/ml, 40mg/ml, and 80mg/ml respectively.

In the DPPH radical scavenging activity, both extract and reference molecules displayed decreasing percentages of free radical inhibition as concentrations increased. The extract exhibited its highest inhibition percentage (87.541%) at 10mg/ml, while the reference molecules reached 98.244%. The extract's IC_{50} was calculated to be 86.109%.

Furthermore, the hydroxyl radical scavenging activity and ABTS scavenging activity of the extract intensified with increasing concentrations, showing optimal scavenging activity at 100mg/ml. The IC_{50} for the extract's 50% scavenging activity was determined to be 54.35%.

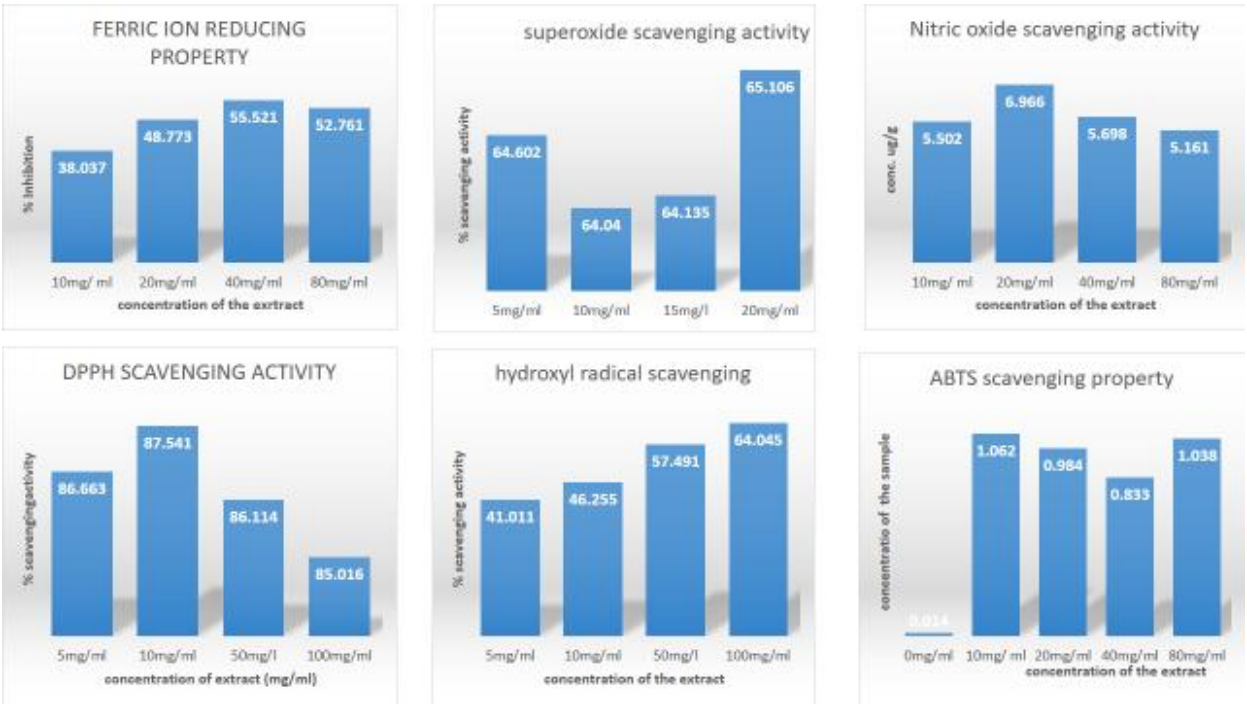


Figure 2: Effect of ethanol leaf extract of *Carica papaya* on 1. ferric ion reducing property, 2. superoxide scavenging activity, 3. Nitric oxide scavenging activity, 4. DPPH scavenging activity, 5. hydroxyl radical scavenging activity, and 6. ABTS scavenging property.

Molecular Docking Scores

Table 1 shows the docking scores for some compounds of *Carica papaya* leaf extract with their scores ranging from the highest to the lowest; naringenin (-8.882 kcal/mol), flavan-3-ol (-8.424 kcal/mol), ellagic acid (-8.412 kcal/mol), epicatechin (-8.370 kcal/mol), lunamarine (-8.044 kcal/mol), kaempferol (-8.038 kcal/mol), flavone (-7.888 kcal/mol), resveratoral (-7.484 kcal/mol) and anthocyanins (-7.407 kcal/mol).

Table 2: The Binding Affinity (kcal/mol) of the Top Ranked Bioactive Compounds of *Carica papaya* against the HER2 Protein Target.

Standard Drugs	PubChem	Docking Score
03Q (Reference compound	16736274	-11.485
Naringenin	439246	-8.882
Flavan-3-ol	3707243	-8.424
Ellagic acid	5281855	-8.412
Epicatechin	72276	-8.37
Lunamarine	442922	-8.044
Kaempferol	5280863	-8.038
Flavone	10680	-7.888
Resveratoral	445154	-7.484
Anthocyanins	145858	-7.407

Receptor-ligand complex pharmacophore modeling

The pharmacophore models of the standard ligand and five selected compounds are depicted in Figures 1 through 12. These models illustrate both 2D and 3D interactions, highlighting various features such as hydrogen acceptor (D), hydrogen donor (H), hydrophobic interaction (N), and aromatic ring (R). The phytochemical constituents

were subjected to docking with HER2, alongside a standard drug, 5-fluorouracil, to evaluate their comparative binding interactions.

Figure 1: 2D View of the Molecular Interactions of Amino-Acid Residues of HER2 with Standard Compound (O3Q)

Figure 2: 3D View of the Molecular Interactions of Amino-Acid Residues of HER2 with Standard Compound (O3Q)

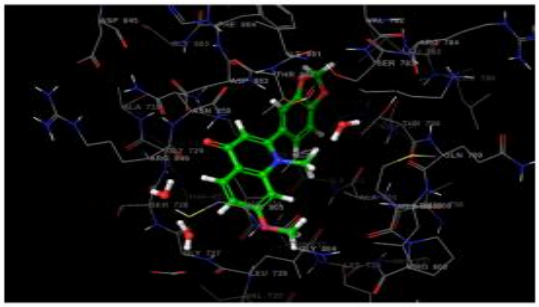


Figure 3: 2D View of the Molecular Interactions of Amino Acid Residues of HER2 with Naringenin

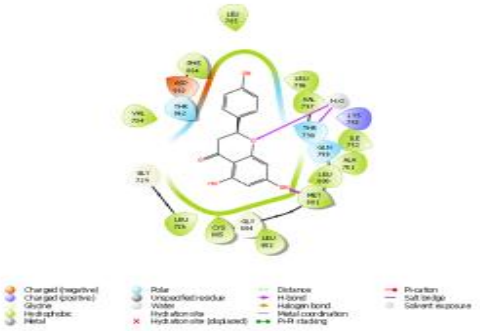


Figure 4: 3D View of the Molecular Interactions of Amino Acid Residues of HER2 with Naringenin.

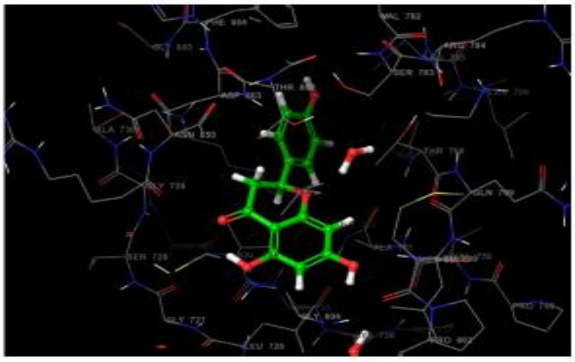


Figure 5: 2D View of the Molecular Interactions of Amino Acid Residues of HER2 with Flavan-3-ol.

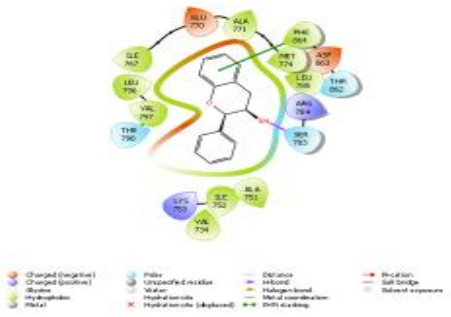


Figure 6: 3D View of the Molecular Interactions of Amino Acid Residues of HER2 with Flavan-3-ol.

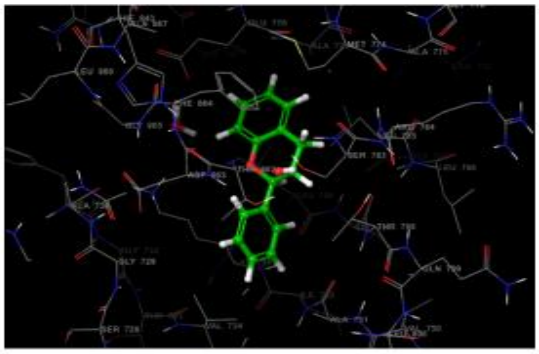


Figure 7: 2D View of the Molecular Interactions of Amino Acid Residues of HER2 with Ellagic Acid

Figure 8: 3D View of the Molecular Interactions of Amino Acid Residues of HER2 with Ellagic Acid

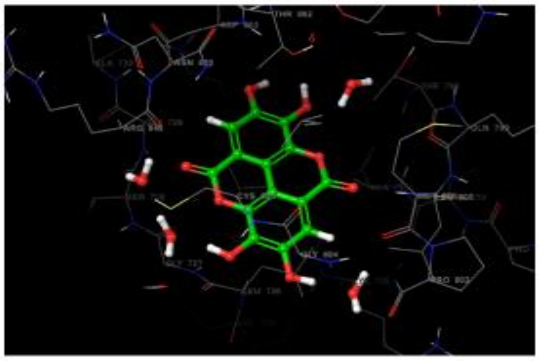


Figure 9: 2D View of the Molecular Interactions of Amino Acid Residues of HER2 with Epicatechin

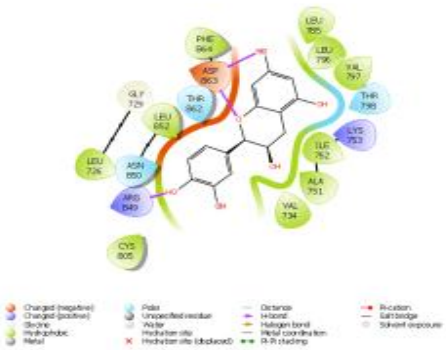


Figure 10: 3D View of the Molecular Interactions of Amino Acid Residues of HER2 with Epicatechin

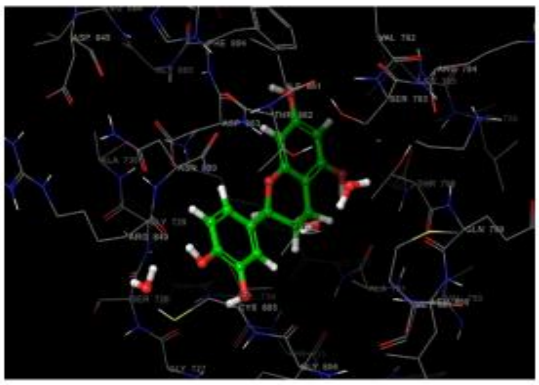


Figure 11: 2D View of the Molecular Interactions of Amino Acid Residues of HER2 with Lunamarine

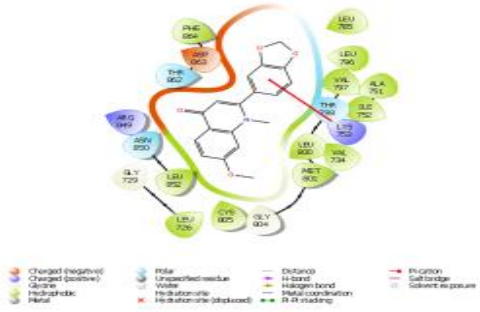
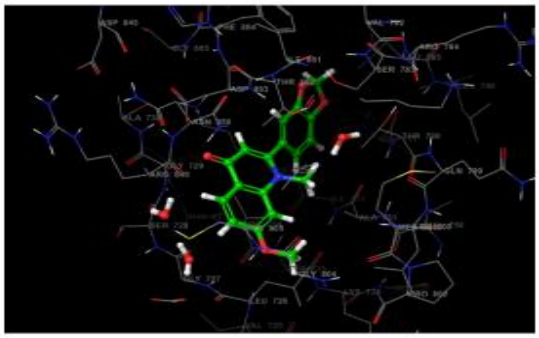


Figure 12: 3D View of the Molecular Interactions of Amino Acid Residues of HER2 with Lunamarine.



ADMET Properties of Ten Selected Hit Compounds

Tables 3 to 5 show the ADMET properties of ten selected hit compounds, demonstrating their lipophilicity, water solubility, drug-likeness bioavailability, toxicity as well as other parameters.

Table 3: The lipophilicity profile of the top 10 ranked phytochemical constituents of *Carica papaya* extract.

Molecules	**iLOGP**	**XLOGP3**	**WLOGP**	**MLOGP**	**Silicon-IT Log P**	**Consensus Log P**
03Q (Reference compound	1.75	2.52	2.19	0.71	2.05	1.84
Naringenin	2.52	2.84	2.4	2.54	2.98	2.66
Flavan-3-ol	0.79	1.1	1.31	0.14	1.67	1
Ellagic acid	1.47	0.36	1.22	0.24	0.98	0.85
Epicatechin	3.14	3.12	2.94	1.65	3.47	2.87
Lunamarine	1.7	1.9	2.28	-0.03	2.03	1.58
Kaempferol	2.55	3.56	3.46	2.27	4.04	3.18
Flavone	1.71	3.13	2.76	2.26	2.57	2.48
Resveratoral	-0.76	3.51	4.38	3.28	2.79	2.64
Anthocyanins	1.83	2.72	2.25	1.51	3.23	2.31
Flavonone	0.96	-0.42	-0.75	-1.05	-0.61	-0.38
Chlorogenic Acid	2.06	1.57	1.32	0.89	1.91	1.55
Ribalinidine	0.97	0.52	0.8	0.18	0.43	0.58

Table 4: **The water solubility profile of the top 10 ranked phytochemical constituents of *Carica papaya* extract**

Molecules	**ESOL Log S**	**ESOL Solubility (mg/ml)**	**ESOL Solubility (mol/l)**	**ESOL Class**
03Q (Reference compound	-3.49	8.74E-02	3.21E-04	Soluble
Naringenin	-3.49	7.35E-02	3.25E-04	Soluble
Flavan-3-ol	-2.94	3.43E-01	1.14E-03	Soluble
Ellagic acid	-2.22	1.74E+00	5.98E-03	Soluble
Epicatechin	-4.11	2.42E-02	7.83E-05	Moderately soluble
Lunamarine	-3.31	1.40E-01	4.90E-04	Soluble
Kaempferol	-4.09	1.80E-02	8.11E-05	Moderately soluble
Flavone	-3.62	5.51E-02	2.41E-04	Soluble
Resveratoral	-4.01	2.02E-02	9.77E-05	Moderately soluble
Anthocyanins	-3.46	8.30E-02	3.48E-04	Soluble
Flavanone	-1.62	8.50E+00	2.40E-02	Very soluble
Chlorogenic Acid	-2.91	3.42E-01	1.24E-03	Soluble
Ribalinidine	-1.44	4.55E+00	3.61E-02	Very soluble

Table 5: The Toxicity Profile of the Top 13 Ranked Phytochemical Constituents of *Carica Papaya*

Compound	LD50 (mg/kg)	Toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Naringenin	2000	4	Inactive	Inactive	Inactive	Inactive	Active
Flavan-3-ol	2500	5	Inactive	Inactive	Inactive	inactive	inactive
Ellagic Acid	2991	4	Inactive	Active	Inactive	inactive	inactive
Epicatechin	10000	6	Inactive	Inactive	Inactive	Inactive	Inactive

Lunamarine	400	4	Inactive	Active	Active	Active	Active
Kaempferol	3919	5	Inactive	Inactive	Inactive	Inactive	Inactive
Flavone	2500	5	Inactive	Active	Inactive	Inactive	Active
Resveratrol	1560	4	Inactive	Inactive	Inactive	Inactive	Inactive
Anthocyanins	2500	5	Inactive	Active	Inactive	Active	Inactive
Flavanone	2647	5	Inactive	Active	Inactive	Inactive	Inactive
Chlorogenic Acid	5000	5	Inactive	Inactive	Active	Inactive	Inactive
Ribalinidine	150	3	Inactive	Inactive	Active	Active	Inactive
Pyrogallic acid	300	3	Inactive	Active	Inactive	Active	Inactive

IV. Discussion

A study using high-performance liquid chromatography (HPLC) identified 43 different chemicals in the leaf extract of Carica papaya. Recent years have seen a rise in interest in the research of natural products' antioxidant activity. Several in vitro methods have been used to assess the antioxidant activity in natural products, including, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical[27], hydroxyl radical scavenging, superoxide radical scavenging activity, nitric oxide scavenging, FRAP ferric reducing/ antioxidant power assay. Using all of these techniques, the antioxidant activity of C. papaya leaf extracts was assessed in this investigation. The extracts all exhibited strong antioxidant activity, according to the results.

Because of their capacity to donate electrons, the extracts demonstrated a dose-dependent, substantial scavenging of DPPH free radicals[28]. The IC_{50} values demonstrate that *C. papaya* leaf extracts have anti-free radical activity. These findings therefore suggest that *C. papaya* extracts may shield biomolecules in vulnerable biological and food systems from the harmful effects of reactive radical species. Using the FRAP method, it was possible to observe that *C. papaya* leaf extracts could decrease ferric III iron. Reductases, which break the chains of free radicals and have an antioxidant effect, are mainly responsible for the reducing capabilities of plant extracts by hydrogen atom donation[29].

Important nitric oxide scavenging action has also been demonstrated in this investigation. Using nitroprusside, nitric oxide was produced and quantified using the Greiss reaction. In an aqueous solution at physiological pH, sodium nitroprusside spontaneously produces nitric oxide[28]. This nitric oxide then reacts with oxygen to produce nitric ions, which can be calculated using a Griess reagent.

The primary active oxygen species responsible for lipid oxidation and significant cellular damage are hydroxyl radicals. It is discovered that papaya leaf extracts from Carica have a concentration-dependent hydroxyl radical scavenging activity. Although H_2O_2 by itself is not extremely reactive, it occasionally has the potential to be harmful to cells due to its ability to produce hydroxyl radicals within them. As a result, H_2O_2 removal is crucial. Proteins, lipids, and DNA can all sustain oxidative damage at the hands of extremely reactive hydroxyl radicals. Methanol extract outperformed the other three extracts in terms of its ability to scavenge superoxide radicals produced in the riboflavin-NBT-light system *in vitro*. The results of this extract's antioxidant activity showed that it included significant antioxidants, and more research could result in the creation of powerful antioxidant agents from *C. papaya* leaves[29].

Molecular docking studies were conducted to investigate the binding interactions between the phytochemical constituents of *C. papaya* extracts and HER2. These studies were carried out utilizing the AutoDock Vina software[30]. All the phytochemical constituents were docked with HER2 along with a standard drug 5-fluorouracil to determine comparative binding interactions. From the results, binding affinity was found to be significant for 10 compounds. The top 5 compounds in order of binding affinity are Naringenin, Flavan-3-ol, Ellagic acid, Epicatechin, and Lunamarine in comparison with 5-FU. The 2D and 3D views show that the top compounds occupied the designated binding site and interacted majorly through hydrophobic and hydrogen contacts.

The 2D and 3D structure of Naringenin shows that the compound occupied the HER2 protein-ligand by interacting with the amino acid residues in the binding pockets of the protein ligand. Naringenin binds to methionine and threonine through hydrogen bonds, metal interactions are also seen among active amino acid residues in the binding pocket of the HER2 ligand. It has a docking score of -8.882kcal/mol. The 2D and 3D structure of Flavan-3-ol shows that the compound occupied the binding site by interacting with two amino acid residues of the protein-ligand. The compound interacted with serine through hydrogen bonds and phenylalanine through pi-pi stacking. It has a docking -8.424kcal/mol. From the 2D and 3D structure of Ellagic acid, it also reacts with the protein-ligand by binding to water molecules on the binding pockets, it also interacts with

methionine and threonine. Ellagic acid interaction with the protein-ligand shows a metal coordination between arginine and leucine, it has a docking score of -8.412kcal/mol. In the 2D and 3D structure of Epicatechin, the compound binds with protein ligands by interacting with aspartic acid through hydrogen and hydrophobic contact, the compound also makes hydrophobic contact with arginine. There is also a metal coordination between glycine and leucine and leucine and asparagine. Epicatechin has a molecular docking score of -8.37kcal/mol.

The binding affinity of lunamarine with the protein-ligand of HER2 is seen through interaction with the compound with lysine using pi-cation and also metal coordination is seen between glycine and leucine. The molecular docking score of lunamarine is -8044kcal/mol. The highest negative docking energy corresponds to better binding affinity. The molecular docking result showed that lunamarine has the lowest negative docking energy which makes it have a better affinity to inhibit the HER2 protein in breast cancer [31,32].

Induced fit docking of Naringenin showed the best ten poses of the compound. Each pose carried a different docking score and showed a better interaction of Naringenin with the HER2 protein ligand, a pose can show the compound making contact with active amino acid residues of the protein ligand not seen in a different pose. Induced fit docking of Naringenin also showed more metal coordination between the amino acid residues of the HER2 protein ligands. All the top five compounds in the order of binding affinity showed acceptable ADMET properties. The five top compounds were seen to have high GI absorption and were also seen to be soluble in the Ali class. Naringenin, Ellagic, and Lunamarine are positive CYPIA2 inhibitors while Flavan-3-ol and Epicatechin are negative inhibitors of CYPIA2. All the five top compounds in the other of binding affinity are negative CYP2CI9 inhibitors[33,34].

V. Conclusion

In conclusion, high-performance liquid chromatography (HPLC) identified 43 different chemical bioactives in the leaf extract of Carica papaya. These compounds present in leaf extract of Carica papaya exhibited high invitro antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical scavenging, superoxide radical scavenging activity, nitric oxide scavenging and FRAP ferric reducing/ antioxidant power assay.

The top 5 bioactive compounds found in the extract; Naringenin, Flavan-3-ol, Ellagic acid, Epicatechin, and Lunamarine subjected to molecular docking shows that Naringenin is inactive for hepatotoxicity, carcinogenicity, and immunotoxicity mutagenicity and active for causing cytotoxicity. Flavan-3-ol is seen to be inactive for the different toxicities. Ellagic acid is inactive for hepatotoxicity, immunotoxicity, mutagenicity, and cytotoxicity but is an active carcinogenic compound. Epicatechin is inactive for all the different toxicities. Lunamarine is inactive only for hepatotoxicity but is an active compound causing carcinogenic, immune-toxic, mutagenic, and cytotoxic effects. From the toxicity profile, Epicatechin looks to be the safest of all compounds and qualified for orally bioavailable drug candidates for inhibiting the activity of HER2 protein in breast cancer[35,36].

List of Abbreviations

03Q - (2-{2-[4-({5-chloro-6[3-(trifluoromethyl) phenoxy] pyridin-3yl} amino)-5H-pyrrolo[3, 2- d]pyrimidin-5-yl]ethoxy}ethanol).
2D – Two dimentional structure.
3D – Three dimentional structure.
5- FU – 5 fluorouracil
ABTS – 2,2' – azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation.
ADMET – Absorption, distribution, metabolism, excretion and toxicity.
ANOVA – Analysis of variance
CYP1A2 – Cytochrome P450 family 1 subfamily A member 2
CYP2C19 – Cytochrome P450 family 2 subfamily C member 19
DPPH - 1,1-diphenyl-2-picrylhydrazyl radical
DMSO – Dimethylsufoxide.
EDTA – Ethylene diaminetetraacetic acid
ESOL – Estimated aqueous solubility
FRAP – Ferric reducing antioxidant power
GC-FID Gas chromatography – flame ionization detector.
HER 2 – Human epidermal growth factor receptor 2
HPLC - High-performance liquid chromatography
IC50 – Half maximal inhibitory concentration
Ki67 – Proliferation index
PBS – Phosphate buffered saline.
PDB – Protein Data Bank

TAC – Total antioxidant capacity

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