Plumeria obtuse. L exhibits *In-silico* and *In-vitro* effects by investigation of MCF -7 and MDA-MB-231 cell line

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Abstract:

Background: Breast cancer is still a major health concern, with high fatality rates. Despite advances in treatment, difficulties like as drug resistance, harsh side effects and the limited efficacy of current medicines remain. This necessitates the development of novel therapeutic agents such as EEPO, and isolated compounds show promise in addressing drug resistance through their distinct mechanism of action, potentially increasing efficacy by specifically targeting cancer cells while minimizing side effects due to their more targeted approach.

Objective: The objective of this research project was to EEPO and is isolated compounds l anti-breast cancer agents, particularly to inhibit ERα and HER2, which are significant targets in breast cancer therapy.

Methods: Docking investigations yielded isolated compounds with promising inhibitory properties. Tin-silico ADME by QuickPort, followed by Molecular Docking by Glide model, MMGBSA by Prime model, the extracted compound of EPPO to be perfume phytochemical, and in vitro cell lines such as MTT Assay, MCF-7, and MDA-MB 231 cell line.

Results: In-silico ADME studies revealed that EEPO and isolate compounds to exhibited favorable drug-like properties. Molecular docking performed 25 isolated compounds of EEPO against PDB id 2IOG, the result showing 8.12 kcal/mol to -0.22kcal/mol, and PDB id 3PPO, the result showing 8.46 kcal/mol to -1.97 kcal/mol, when compared to the standard drugs Doxorubicin -7.3 kcal/mol and -9.79 kcal/mol. MTT Assay – MCF -7 Cell line study EEPO IC50 57.51 µg and Doxorubicin (std) IC50 7.46µg. MDA-MB-231 cell line study result showing IC50 76.54 µg and Doxorubicin (std)IC50 8.93 µg.

Conclusion: The most promising EEPO and its isolated compounds have strong anti-tumor activity, indicating their promise as a useful treatment agent for breast cancer.

Keywords: Anti-Breast Cancer, EEPO, Estrogen Receptor Alpha (ERα),HER2,Computational design, MCF -7, MDA-MB 231

1. Introduction:

Breast cancer is a kind of cancer that begins in the cells of the breast. It most commonly begins in the cells of the ducts (the tubes that carry milk to the nipple or the lobules (the glands that produce milk). While it primarily affects women, men can also develop breast cancer, though it's much less common. Breast cancer can be categorized into various types based on factors such as the specific cell types involved, molecular features, and receptor status. The primary classifications include histological types, where Ductal Carcinoma in Situ (DCIS) represents a non-invasive form confined to breast ducts, while Invasive Ductal Carcinoma (IDC) is the most prevalent type that invades surrounding tissues. Invasive Lobular Carcinoma (ILC) originates in the lobules and spreads to adjacent areas, alongside

several rare types like medullary, mucinous, tubular, papillary, and metaplastic carcinomas. Hormone receptor status further divides breast cancer into hormone-receptor positive, which includes estrogen and progesterone receptor-positive cases, and hormone-receptor negative, characterized by the absence of these receptors. Additionally, HER2 status distinguishes between HER2-positive cancers, which exhibit elevated levels of the human epidermal growth factor receptor 2, and HER2-negative cancers with normal levels. Triple-negative breast cancer (TNBC) is notable for lacking estrogen, progesterone, and HER2 receptors, making it more aggressive and less amenable to targeted therapies. Lastly, molecular subtypes identified through gene expression profiling include Luminal A, Luminal B, HER2-Enriched, and Basal-Like, with the latter often overlapping with TNBC. Inflammatory breast cancer, a rare and aggressive variant, is characterized by the obstruction of lymph vessels in the breast skin, resulting in redness, swelling, and a rash-like appearance.[1-3]

Risk factors for breast cancer encompass a range of genetic, hormonal, reproductive, and lifestyle elements. Genetic predispositions, particularly mutations in the BRCA1 and BRCA2 genes, are strongly linked to hereditary breast cancer, with additional contributions from mutations in genes such as PALB2, TP53, and ATM. Hormonal and reproductive factors, including early onset of menstruation, late menopause, hormone replacement therapy, and nulliparity or delayed childbirth, further elevate risk levels. Lifestyle choices, such as alcohol consumption, smoking, obesity, and insufficient physical activity, are also correlated with an increased likelihood of developing breast cancer, alongside dietary habits and environmental pollutant exposure, although the latter associations are less well established.

The likelihood of breast cancer is significantly influenced by age and gender. The incidence of breast cancer rises with advancing age, peaking among women aged 55 to 64, while the occurrence in women under 40 remains relatively low but escalates markedly as age increases. Although breast cancer primarily affects women, men are not immune, with male cases representing approximately 1% of all breast cancer diagnoses.

Survival rates for breast cancer have seen notable improvements over the years, largely attributed to advancements in early detection and treatment methodologies. In the United States, the five-year relative survival rate for breast cancer stands at approximately 90%, although this figure is influenced by the stage at which the cancer is diagnosed and other individual factors. Early-stage diagnoses, classified as localized cancers, exhibit significantly higher survival rates compared to those identified at more advanced stages, such as regional or distant cancers.

Screening programs play a crucial role in early detection, with mammography being the predominant method recommended for women beginning at ages 40 or 50, contingent upon specific guidelines. Additional screening techniques, including breast ultrasound and MRI, are employed for women identified as high-risk. For those with a substantial genetic predisposition, preventive measures such as prophylactic mastectomy or oophorectomy may be considered. Furthermore, lifestyle changes, including maintaining a healthy weight, reducing alcohol consumption, and engaging in regular physical activity, can contribute to

lowering breast cancer risk. Socioeconomic factors and access to healthcare significantly influence breast cancer outcomes, with women from lower-income backgrounds or those lacking insurance often encountering obstacles to screening and treatment. Health disparities are evident across various racial and ethnic groups, with African American women experiencing a higher incidence of aggressive breast cancer types and elevated mortality rates compared to their Caucasian counterparts.[4-6]

1.1. Molecular Biology of Breast Cancer

Breast cancer is a complex and heterogeneous disease characterized by the uncontrolled growth of malignant cells in the breast tissue. The molecular biology of breast cancer involves a detailed understanding of the genetic, epigenetic, and signaling pathways that contribute to its development and progression. This overview highlights key aspects of breast cancer biology, including genetic mutations, molecular subtypes, and signaling pathways.

1.2. Genetic Mutations and Risk Factors

Breast cancer often arises from genetic mutations that disrupt normal cellular processes. Several key mutations are associated with increased risk for breast cancer:

BRCA1 and BRCA2 Genes: Mutations in BRCA1 and BRCA2, which are tumor suppressor genes, are linked to hereditary breast and ovarian cancer syndrome. These genes encode proteins involved in DNA repair through homologous recombination. Mutations in these genes lead to defective DNA repair, increasing the likelihood of genomic instability and cancer development.

HER2 (Human Epidermal Growth Factor Receptor 2): Over expression of HER2, a receptor tyrosine kinase, is found in approximately 15-20% of breast cancers. HER2 amplification leads to constitutive activation of signaling pathways that promote cell proliferation and survival. HER2-positive breast cancers are often more aggressive but can be treated with targeted therapies like trastuzumab (Herceptin).

TP53 Gene: TP53 encodes the p53 protein, a critical tumor suppressor that regulates the cell cycle and induces apoptosis in response to DNA damage. Mutations in TP53 are common in many breast cancers and result in loss of function, contributing to tumor progression.(7-14)

1.3. Molecular Subtypes

Breast cancer is not a single disease but rather a collection of distinct molecular subtypes, each with unique genetic and molecular profiles. The classification of these subtypes is crucial for personalized treatment strategies:

- **Luminal A:** This subtype is characterized by estrogen receptor (ER) positivityϖ and low levels of HER2. Luminal A: tumors generally have a better prognosis and are often treated with hormone therapy.
- **Luminal B:** These tumors are also ER-positive but may have higher levels ofϖ HER2 and/or higher proliferation rates compared to Luminal A tumors. They may require a combination of hormone therapy and chemotherapy.
- **HER2-Enriched:** These tumors overexpress HER2 and are typically ER negative.
- They tend to be more aggressive but respond well to HER2-targeted therapies.
- **Triple-Negative Breast Cancer (TNBC):** TNBC lacks ER, progesteroneϖ receptor (PR), and HER2 expression. It is often more aggressive and has fewer targeted treatment options, making chemotherapy a common treatment approach.(15)

1.4. Signaling Pathways and Molecular Mechanisms

The signaling pathways involved in breast cancer is essential for developing targeted therapies:

- **Estrogen Signaling:** In ER-positive breast cancer, estrogen binds to the estrogen receptor, activating a cascade of signaling events that promote cell proliferation. Aromatase inhibitors and selective estrogen receptor modulators (SERMs) are used to block estrogen signaling in these tumors.
- **PI3K/Akt/mTOR Pathway:** This pathway is frequently dysregulated in breast cancer and plays a role in cell growth, survival, and metabolism. Mutations in PI3K or loss of PTEN (a negative regulator of this pathway) can lead to aberrant activation, contributing to tumorigenesis. Inhibitors targeting components of this pathway are being developed to treat breast cancer.
- **MAPK/ERK Pathway:** This pathway is involved in cell proliferation and differentiation. Mutations or dysregulation in this pathway can contribute to the aggressive behavior of some breast cancers. Sure! Here's a comprehensive overview of invasive and non-invasive cancer approaches, formatted to cover roughly two pages of content.[16-18]

1.5. Role of Medicinal Plant treats the Breast Cancer and its Mechanism of Actions

Breast cancer is the major cause of cancer-related morbidity and death in women worldwide. As traditional therapies often come with significant side effects, there is growing interest in complementary and alternative medicine, particularly the use of medicinal plants. Phytochemicals, including flavonoids, terpenoids, and other compounds, have shown promise in treating breast cancer. This paper explores the roles of these phytochemicals and their mechanisms of action.(19)

1.5.1. Medicinal Plants and Key Phytochemicals

a). Flavonoids:

Flavonoids, found in fruits, vegetables, and herbs, have biological activities beneficial in breast cancer treatment. Quercetin, found in onions, apples, and green tea, inhibits cell proliferation and induces apoptosis by modulating signaling pathways. Epigallocatechin gallate (EGCG) in green tea can inhibit cancer cell growth by downregulating anti-apoptotic protein expression and affecting estrogen metabolism.

b). Terpenoids:

Terpenoids, or isoprenoids, are phytochemicals with anti-cancer properties found in plants. Limonene, found in citrus fruits, has been shown to reduce tumor burden in breast cancer models by promoting apoptosis and inhibiting cell proliferation. Beta-caryophyllene, present in essential oils, has anti-inflammatory properties and can modulate cannabinoid receptors to induce apoptosis in cancer cells. These compounds impact breast cancer by activating death receptor pathways, modulating cell signaling pathways, inhibiting tumor growth, and enhancing the immune response against tumor cells.

c). Phenolic compounds:

Phenolic compounds, found in medicinal plants, have antioxidant and anti-inflammatory properties. Resveratrol, found in grapes and berries, inhibits breast cancer cell growth by interfering with estrogen receptor signaling. Ferulic acid, found in grains and vegetables, reduces cell proliferation and induces cell cycle arrest.[20-22]

2. PLANT PROFILE

Plumeria obtusa, sometimes known as the Singapore graveyard flower, is a Plumeria (Apocynaceae) species. Locally, it is known as Frangipani or Champa. Indigenous to Central America, Southern Mexico, and Southeast Asia. The leaves are dark green and lustrous. The leaves are elliptical and obovate. The apex is rounded. Flowers are white, with a golden center and rounded obovate petals. The seeds produce the white, red, pink, yellow, and multicolored flowers. Plumeria seed may be identified by identifying a pod that splits open on the tree. The plant is commonly cultivated for its attractive and fragrant flowers around the world, where a suitable warm environment prevails.

Figure 1: Photographs of *Plumeria obtuse*

2.2. Taxonomical Classification

Table: 1. Classification of *Plumeria obtusa*

Kingdom	**Plantae-Plants**
Sub kingdom	Tracheobionta-Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta - Floweringplants
Class	Magnoliopsida - Dicotyledons
Subclass	Asteridae
Order	Gentianales
Family	Apocynaceae – Dogbane family
Genus	*Plumeria* L.-plumeria
Species	*Plumeria obtuse* L. Singapore grave yard flower

Plumeria obtusa has numerous biological activities that could be beneficial for medicinal use. Its extracts show potential in various therapeutic areas, including antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, analgesic, cytotoxic, liver protective, antiviral, and anti-ulcer. These activities help neutralize harmful free radicals, reduce the risk of chronic diseases like cancer and heart disease, inhibit bacterial growth, and protect against fungal infections. Plumeria obtusa's antifungal properties could be effective against skin or systemic fungal infections. Its anti-inflammatory properties could help manage conditions like arthritis, regulate blood sugar levels, and act as a natural pain reliever. Its cytotoxic activity could potentially inhibit cancer cell growth, while its liver protective properties could protect against damage. Its antiviral activity could help fight viral infections, and its anti-ulcer properties could benefit digestive health.

3. MATERIAL AND METHODS:

3.1. Plant Materials:

Plumeria obtusa.l was collected from Perundurai, Erode District. The plant was authenticated by Dr. V. Aravindhan. Professor, Botany Department, Kongunadu arts & Science college, Coimbatore, Tamil Nadu, India. The plant was recorded in the institute's medicinal plant repository.

3.2. Preparation of *Plumeria obtusa.l* extract:

The aerial part of *Plumeria obtusa.l* has been dried in the shade and crushed in an electric blender to make coarse powder before being exposed to Soxhlet extraction (continuous hot extraction) using aqueous as the solvent. The extracts were concentrated using a rotary evaporator and tested for anti-helminthic action. A preliminary phytochemical screening was conducted to determine the presence of phytoconstituents in the extract. The preliminary test was followed by standard book kokate.

3.3. Determination of Total Flavonoids content of the plant extract:

Content of Plant Flower Mix 500 µl of plant extract with 1500 µl of 95% methanol. Add 100 µl of Aluminum chloride (10%) and Potassium acetate (1M) to generate a volume of 10ml. Add distilled water and agitate. The incubation period lasted 20-30 minutes at room temperature. The absorbance was measured at 415 nm using an UV spectrophotometer against a blank that contained all of the reagent but no sample. The total flavonoid was quantified in triplicates using the standard curve of quercetin solution (R2 =0.9684).

3.4. Determination of Total Phenol content of the plant extract:

Content of Plant Flower One ml of extracts from various plant parts was thoroughly combined with 10 ml of distilled water, followed by 1.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 4 ml of 20% sodium carbonate (Na2CO3) was added, diluted with distilled water to 25 ml, and stirred. The samples were then incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm using a spectrophotometer against a blank containing all of the reagents but not the sample. This method was carried out three times for each extract. The total phenols were measured using a standard curve of gallic acid solution generated in the same way (R2 = 0.998). [23-33].

3.5. In-silico Study:

3.5.1. Molecular Docking Studies:

3.5.1. a. Plant active compounds to be performed compounds:

Molecular docking is a computer approach used in drug development and structural biology. It predicts the ligand's preferred orientation relative to the receptor when the two molecules are linked to form a stable complex. This method simulates binding contacts between chemicals, including medication candidates and target proteins, to determine their degree of connection.[34-36]

3.5.1. b. Protein Preparation:

The protein structure was modeled using crystallographic data from the Protein Data Bank (PDB) for ERα (PDB ID: 2IOG) at 1.60 Å and HER2 (PDB ID: 3PP0) at 2.25 Å. Standard refining processes were used to address characteristics such as bond ordering, formal charges, the lack of hydrogen atoms, topological features, and incomplete or terminal amide groups in protein structures, resulting in a more realistic depiction. Conducting restrained energy minimization on the resulting protein structures using an impact refinement tool and the OPLS-3 force field to optimize the geometric configuration and reduce the protein's energy.[37]

3.5.1. c. Ligand Preparation

In ligand preparation, ChemDraw2021 software was used to create 2D structures of Schiff base substituted 9-anilinoacridine derivatives. Schrödinger Suite's LigPrep was used to convert 2D Structure Data File (SDF) representations into their equivalent 3D formats, ensuring that chiralities were accurately addressed. Geometry minimization was performed using the OPLS-3 force field, and compounds were filtered based on strict geometric parameters. This complete procedure improves molecular representations for further computational investigations, leading to the refining and optimization of ligands for receptor interactions. Following that, a low-energy ring conformation was created for each a ligand and the resulting optimized ligands were used for subsequent docking analysis.[38]

3.5.1. d. Receptor Grid Generation

The utilization of a grid facilitates the exclusion of undesirable residues in close proximity to the receptor volume or docking site, which could potentially interfere with ligand interactions. The Receptor Grid Generation panel within the Schrödinger suite was employed for this purpose. Grid points were strategically positioned based on specified residues within the binding pocket, specifically those engaged in the active site or interactions. The grid was generated with consideration for non-covalent interactions, including hydrogen bonds to aromatic hydrogens and halogens, adhering to predefined settings under the OPLS-3 force field.[39]

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3.5.1. f. Molecular Docking

Ligands were subjected to docking studies with protein active sites using the advanced molecular docking program Schrödinger suite. The binding site of both the proteins 2IOG and 3PP0 was utilized to begin a virtual workflow using the XP (Extra Precision) mode. These investigations aimed to ascertain the binding affinities of the compounds. The resulting data offer significant insights into the molecular interactions, aiding in the comprehension of binding mechanisms and affinities, crucial aspects in the context of drug discovery and molecular design. The study utilized the glide g-score as a quantitative measure, along with glide energy data and hydrogen bond analysis, to identify the most favorable binding position. The 2D interaction diagram of protein- ligand was generated using the Ligand Interaction script in Maestro, a tool recognized in the scientific community for molecular modeling and simulation. This visualization technique aids in comprehensively understanding the spatial relationships and interactions between the protein and ligand at the atomic level.

3.5.1. g. ADMET Prediction

The QikProp program from Maestro Schrodinger Suite was utilized to determine the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds, which encompass their pharmaceutical, physiological, biochemical, and molecular impacts. QikProp module was employed to forecast the physicochemical and pharmacokinetic characteristics of the compounds, concurrently evaluating the tolerability of the analogs in accordance with Lipinski's Rule of Five which stipulates that physicochemical properties must conform to predefined acceptable ranges: molecular weight less than 500, log P less than 5, Hydrogen bond acceptor≤10 and Hydrogen bond donor≤5. [41-42]

3.5.1. h. Binding Free Energy Calculation by Using Prime/MM-GBSA Approach

To calculate the binding free energy for the ligand-receptor complexes the Prime MM-GBSA module from the Schrodinger suite was utilized. To determine the relative energy of the complexes, we utilized the OPLS-3 force field. For the designed compounds MM-GBSA was performed for both ERα and HER2 proteins(72). The equation to calculate Binding free energy for a ligand and receptor is: $\Delta G_{bind} = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$ A more negative score indicates a stronger binding affinity. [43]

3.6. *Invitro* Breast Cancer study

3.6.1. MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)

3.6.1.a. Preparation of test solutions

For MTT assay, serial two-fold dilutions (6.25 – 100 µg) were prepared from this assay.

3.6.1.b. Cell lines and culture medium

MCF-7 cell line was procured from NCCS, stock cell was cultured in DMEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent.

Procedure

The monolayer cell culture was trypsinied and adjusted to 1.0 x 105 cells/mL using medium containing 10% FBS. In each well of the 96-well microtiter plate, 100 µL of diluted cell suspension (1 x 104 cells/well) was added. After 24 hours, a partial monolayer was created. The supernatant was removed, the monolayer was washed with medium, and 100 µL of various test sample concentrations were applied to the monolayer in microtiter plates. The plate was then incubated at 37°C for 24 hours in a 5% CO2 environment. After incubation, remove the test solutions and add 20 µL of MTT (2 mg/1 mL in PBS) to each well. The plate was incubated for 4 hours at 37°C in a 5% CO2 environment. The supernatant was removed, 100 µL of DMSO was added, and the plate was gently agitated to dissolve the generated formazan. The absorbance was measured using a microplate reader at 570 nm. The percentage of vitality was estimated using the following formula: percentage viability = Sample abs / Control abs x 100.[44-47]

3.6.1.c. Cell lines and culture medium

MDA-MB-231 cell line was procured from NCCS, stock cell was cultured in DMEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1 x 10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the

monolayer once with medium and 100 µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37^{o}C for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37^{o}C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula, % viability = Sample abs/Control abs x 100[48-50]

4. Result and discussion:

4.1 Phytochemical Studies

Phytochemical studies reveals that the Ethanolic extract of EEPO contains presence of Flavonoids, Terpenoids, Flavonoids, Steroids and saponins. **(Table no: 2)**

4.2. In-silico Studies:

4.2.1. 2IOG Vs EPPO isolated compounds was analyzed 25 compounds from EEPO against a target protein, revealing binding energies ranging from -8.12 kcal/mol to -0.22 kcal/mol. Some compounds exhibited competitive binding affinity compared to standard anticancer drugs. Key amino acid interactions, involving ARG-394, GLU-353, and PHE-404, were found to enhance anticancer activity.

4.2.2. 3PPO Vs EPPO isolated compounds also analyzed 22 compounds from EEPO against PDB ID: 3PPO, revealing competitive binding potential compared to standard anticancer drug Doxorubicin. Key amino acid interactions were identified, contributing to anticancer activity. MMGBSA binding free energy calculations indicated favorable binding and potential anticancer efficacy of the tested compounds.

4.2.3. ADMET Screening performed by Quikprop model result showing all the molecular weight 472.05 -170.25, obey the lipinski rule of 5 and log D values -.6.572 to 1.170, all the value of ADMET attributes are within the suggested range.

4.3. *Invitro* Studies:

4.3.1. MTT Assay – MCF -7 Cell line study

MTT assay – MCF-& cell line aganist ethanolic extract of *Plumeria obtusa.l invitro* assay was perform five different concentration Like 6.25 µg, 12.5 µg , 25 µg , 50 µg , 100 µg. all the sample to performed triplicate the assay procedure. The test compound result was showed at 6.25 µg, -96.25, 12.5 µg – 91.48, 25 µg- 67.84, 50 µg - 45.22, 100 µg- 22.61. The standard Compounds result was showed at 6.25 µg, -57.44, 12.5 µg –, 25 µg- 32.16, 50 µg - 27.41, 100 µg- 17.18. The ethanolic extract of *Plumeria obtusa* L.

exhibited a dose-dependent cytotoxic effect on MCF-7 cells, with higher concentrations showing reduced activity. The test compound demonstrated significantly greater cytotoxicity compared to the standard compound at all concentrations, indicating its potential as an effective anticancer agent. **(Table no: 10 & Fig. No. 35)**

4.3.2. MDA-MB-231 Cell line study

MDA-MB-231 Cell line against the ethanolic extract of *Plumeria obtusa.l invitro* assay was perform five different concentration Like 6.25 µg, 12.5 µg , 25 µg , 50 µg , 100 µg. all the sample to performed triplicate the assay procedure. The test compound result was showed at 6.25 µg, - 1.19, 12.5 µg – 2.8, 25 µg- 4.44, 50 µg - 2.2, 100 µg- 1.4. The standard Compounds result was showed at 6.25 µg, - 4.6, 12.5 µg – 2.9, 25 µg- 1.2, 50 µg - 1.8, 100 µg- 0.5. The ethanolic extract of *Plumeria obtusa* L. showed moderate cytotoxic activity against MDA-MB-231 cells, with dose-dependent variation. While the test compound exhibited cytotoxicity across different concentrations, its effect was slightly higher than the standard compound at 25 µg but lower at other concentrations. These findings suggest that *Plumeria obtusa* L. extract may have potential anticancer properties, but further studies are required to confirm its efficacy. **(Table no: 11 & Fig. No. 36)**

5. Discussion:

This study investigates the anti-breast cancer effects of EEPO by targeting the ERα and HER2 signalling pathways. These two pathways engage in reciprocal signalling, contributing to resistance against both targeted and hormonal therapies. In ER+/HER2+ breast cancer, a bidirectional communication occurs between the ER and HER2 pathways, often mediated through the RAS and PI3K pathways. EPPO and Isolated compounds have shown promise in cancer therapy by interfering with DNA replication and repair processes in cancer cells. In this research, *in-silico* and *Invitro* methods were used to examine the potential mechanisms of action of these newly designed derivatives. The compounds are proposed to induce DNA damage, triggering apoptosis specifically in cancer cells, while potentially minimizing harm to normal cells.

6. Conclusion:

The present study focused on evaluating the potential of EPPO and its isolated compounds as inhibitors of ERα and HER2, assessing their activity through both in- silico and in-vitro methods. The results showed that EPPO and its isolated compounds exhibit promising anti-breast cancer effects, demonstrating significant efficacy in inhibiting cell growth. These findings suggest that EPPO and its isolated compounds hold substantial potential as therapeutic agents for breast cancer. However, further research is needed to fully assess their anti-cancer activity and therapeutic viability.

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Phytochemical analysis of ethanolic extract of *Plumeria obtusa.l*

Initial Weight of *Plumeria obtusa.l* flower = 4.25 kg Initial weight of dried flowers = 475.25 g Weight of ethanol extract 14.36 g Total Yeild of ethanolic extract of *Plumeria obtusa.l* Flower = 3.1%

Table: 2. phytochemical analysis of ethanolic extract of *Plumeria obtusa.l*

S. no	Phytochemical constituents	Ethanolic extract -90%
1	Carbohydrates	+
2	Saponins	+
3	Alkaloids	+
4	Cardiac glycosides	+
5	Amino acids and proteins	-
7	Terpenoids	+
8	Flavonoids	+
9	Steroids	+

'+' indicates presence '-' indicates absence

4.2. *Insilico* Result

Table 3: Molecular docking ligand vs protein interaction – PDB ID 2IOG

S.NO	Compound Code	doc_G score	XP_HBond	glide_evdw	glide_ecoul	glide_energy
1	5280805	-12.58	-5.44	6.4	-16.66	-10.25
2	173183	-11.15	-0.25	-34.19	-1.179	-35.37
3	72319	-10.73	-2.4	-24.55	-6.11	-30.61
4	3604942	-9.72	-1.59	-35.96	-4.14	-40.11

5	155380	-9.08	-1.44	-43.7	-1.38	-45.09
6	5281543	-8.12	-0.31	-35.74	-0.98	-36.72
7	5280343	-8.1	-1.92	-35.69	-8.24	-43.93
8	399421	-7.98	-0.46	-15.75	-0.96	-16.71
9	5280443	-7.87	-1.23	-31.23	-5.76	-37.02
10	5281672	-7.81	-2.4	-35.35	-8.42	-43.78
11	5280863	-7.65	-1.73	-23.25	-6.51	-29.77
12	1794427	-7.55	-3.56	-39.14	-10	-49.14
13	5281545	-7.48	0	-31.03	-0.26	-31.29
14	689043	-7.1	-1.35	-21.72	-9.7	-31.42
15	44593505	-6.94	-2.4	-26.49	-1.99	-28.49
16	5280460	-6.63	-0.59	-25.99	0.19	-25.79
17	445858	-6.39	-0.66	-24.56	-1.37	-25.94
18	370	-6.26	-1.87	-9.17	-14.67	-23.81
19	72	-6.16	-1.32	-19.15	-9.25	-28.4
20	637542	-5.99	-0.53	-21.09	-4.85	-25.94
21	637775	-5.81	-0.48	-27.1	1.04	-26.05
22	10742	-5.39	-0.96	-18.86	-3.31	-22.17
23	135	-5.35	-0.7	-11.26	-11.38	-22.65
24	8468	-5.24	-0.44	-21.71	-2.2	-23.92
25	5312351	-5.22	-0.61	-18.22	1.68	-16.54
26	2733526(Tom - std)	-9.79	-0.1	47.86	-8.45	-47.56
27	31703 Doxorubicin (std)	-7.3	-0.54	42.65	-7.35	-43.63

2-D INTERACTION 2IOG VS LIGAND - TOP 10 COMPOUNDS (A N T I BREAST CANCER ACTIVITY)

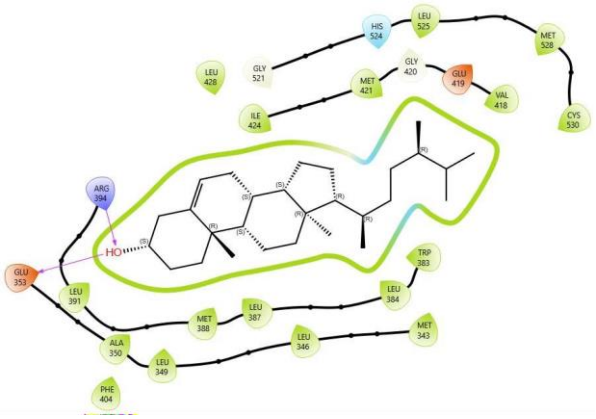


Figure 2: 173183 – 2IOG

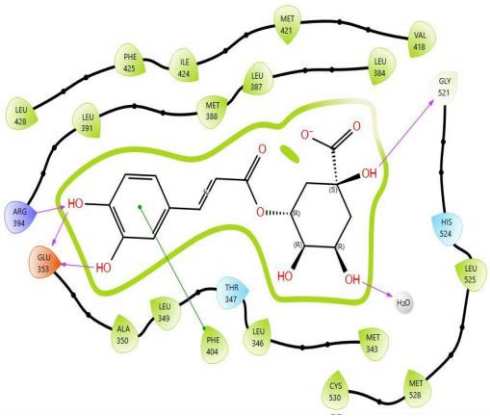


Figure 3:1794427 – 2IOG

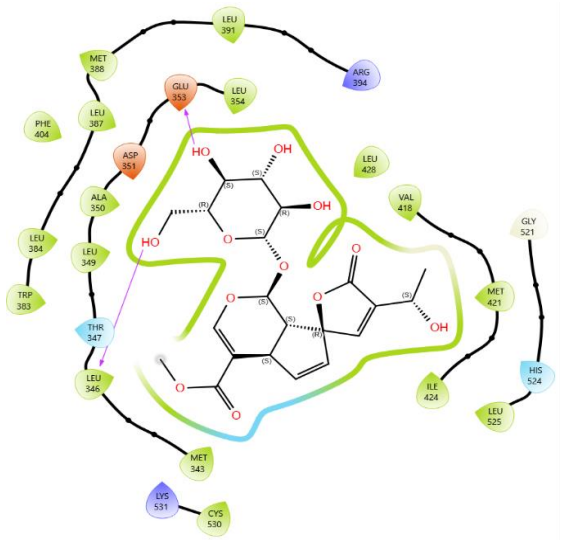


Figure 4:72319 – 2IOG

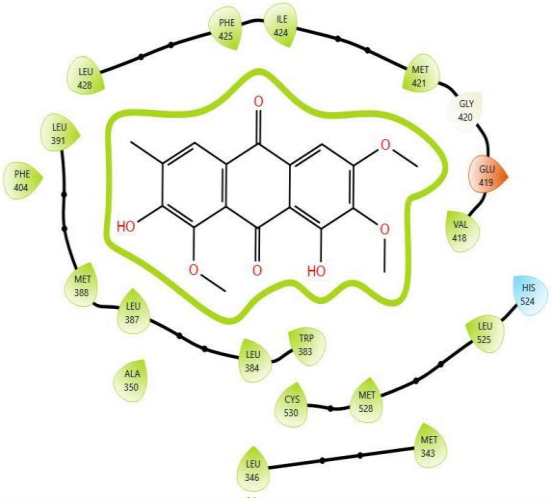


Figure 5:155380 – 2IOG

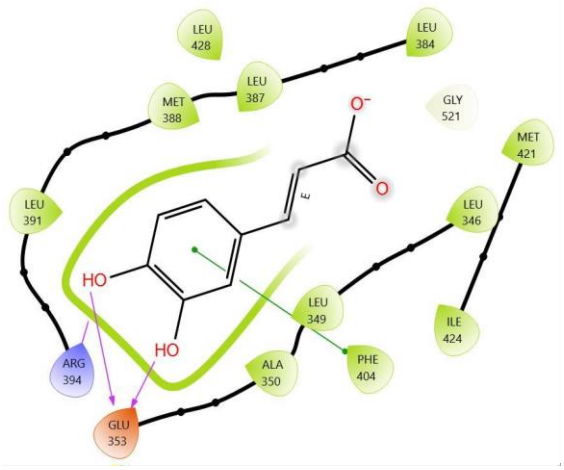


Figure 6:689043 – 2IOG

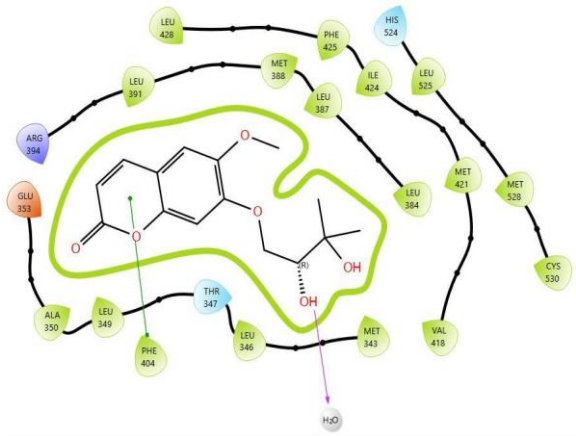


Figure 7:3604942 – 2IOG

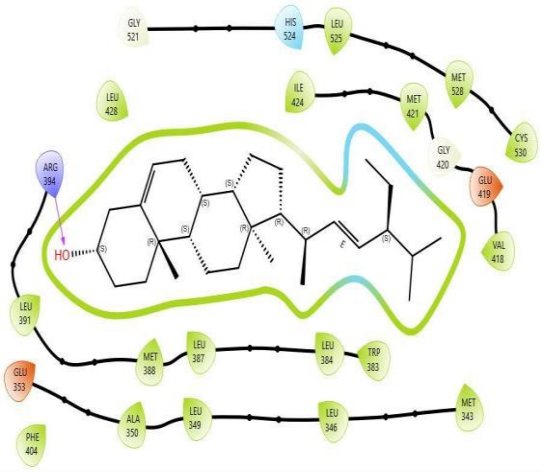


Figure 8:5280794 – 2IOG

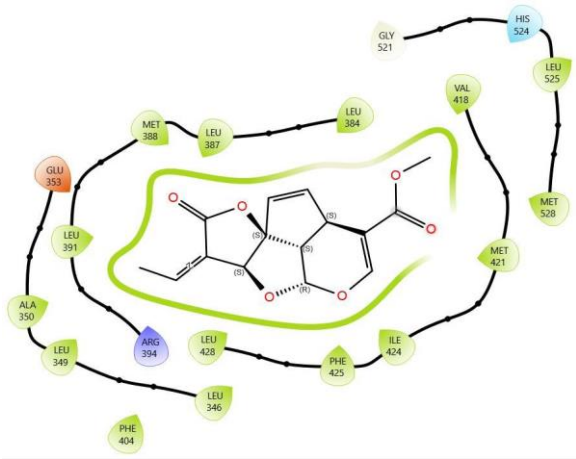
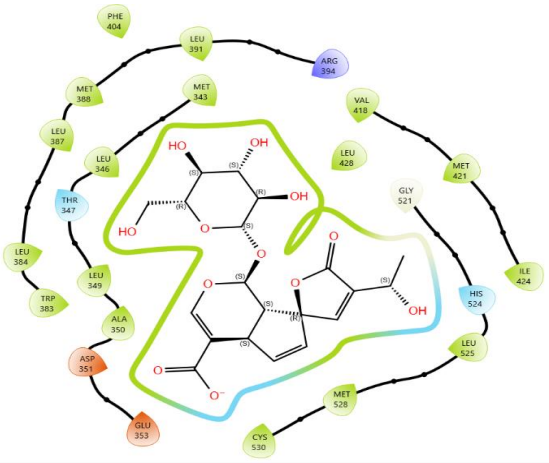


Figure 9:5281543 – 2IOG



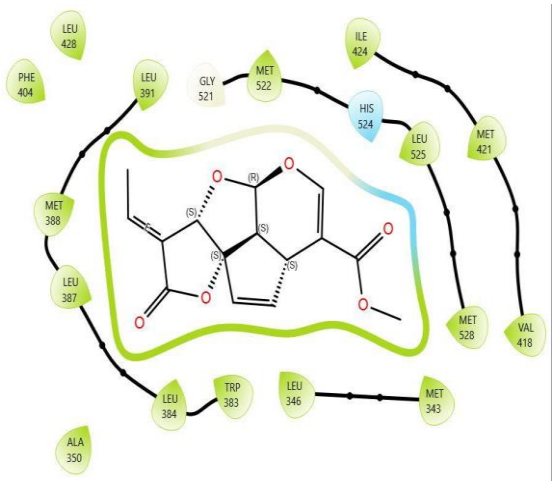


Figure 10:4459505 – 2IOG **Figure 11 :5281545 – 2IOG**

Table 4: PRIME MMGBSA ASSAY LIGAND VS 2IOG PDB

Compound	_dG_Bind	_Bind_vdW	r_Energy	r_Coul	r_Cov	r_effi
44593505	-5.8114	-32.4132	20.40486	1.888251	13.35	-1.3
3604942	-44.2115	-38.9903	11.2389	7.833839	5.37	-10.93
155380	-54.9486	-40.9201	7.37801	2.770651	3.31	-13.02
72319	-27.4139	-41.1089	21.74839	1.840959	15.55	-6.09
5280794	22.71732	11.1555	46.83983	-0.26052	27.21	5.16
399421	-17.9925	-19.4143	19.05472	-0.05788	14.02	-4.56
5281543	-46.1533	-38.947	2.211097	0.041317	1.158	-11.41
5280863	-8.03792	-33.335	5.485859	-0.21728	5.37	-1.98
5280343	-10.4208	-36.153	2.177387	1.587423	1.42	-2.54
1794427	-41.3472	-41.523	12.90909	0.441963	9.97	-9.8
5280443	-11.4354	-35.353	4.712088	0.129734	4.65	-2.86
5280805	84.01386	19.2179	67.62928	1.896225	43.48	17.64
5281545	-41.4745	-34.789	2.883634	-0.33096	2.56	-10.25
689043	-29.8301	-31.493	2.034947	3.178587	1.82	-8.36
5280460	-35.5166	-28.745	2.531234	-1.38082	4.69	-9.75
445858	-28.8631	-34.845	2.576021	0.337602	4.33	-7.93
5281672	-10.2464	-40.542	-2.60346	-8.1448	1.9	-2.47

637542	-27.6369	-29.31	0.553122	-0.01765	1.68	-7.93
72	-24.7341	-24.94	0.382998	1.222307	-0.2	-7.27
637775	-13.8508	-35.25	3.605743	-1.84555	7.88	-3.67
6440657	16.24707	-14.91	72.57937	19.60841	51.23	3.39
8468	-21.5555	-29.01	1.446691	-0.65909	2.41	-6.18
370	-25.0727	-26.7973	0.773116	1.519547	0.91	-7.19
5312351	-7.01029	-24.934	6.463573	1.26557	2.92	-2.01
10742	-16.8903	-33.043	0.993291	1.320472	1.95	-4.64
135	-20.0517	-23.768	0.317685	0.166416	1.06	-6.07

Table 5: Molecular Docking – ligand vs 3PPO

2D INTERACTION TOP 10 COMPOUNDS -3PP0 (BREST CANCER ACTIVITY)

S.No	Compound	g_doc_scor	g_evdw	g_ecoul	g_emodel	g-engy
1	5280443	-5.26	-37.2102	-0.458573	-54.2901	-37.6688
2	689043	-6.88	-26.0174	-8.71141	-53.3963	-34.7288
3	1794427	-7.94	-39.9114	-8.5327	-55.8875	-48.4441
4	445858	-6.34	-25.4358	-6.57037	-47.9697	-32.0062
5	370	-7.65	-13.3729	-18.2516	-53.3096	-31.6246
6	5281543	-6.63	-30.0203	3.87887	-30.3993	-26.1414
7	5280863	-5.69	-36.1221	-4.63633	-59.2864	-40.7585
8	5281672	-5.66	-36.0738	-6.57257	-61.7907	-42.6463
9	5312351	-5.21	-22.857	-6.5644	-41.3132	-29.4214
10	155380	-6.89835	-24.1918	-8.9391	-31.5386	-33.1309
11	3604942	-8.46855	-40.3153	-6.89131	-63.7845	-47.2067
12	637542	-6.20346	-25.2619	-5.43198	-46.8513	-30.6939
13	135	-7.15774	-11.6497	-16.8011	-46.0181	-28.4508
14	5281545	-6.3367	-27.6912	1.46277	-30.8185	-26.2285
15	6440657	-5.27565	-37.0483	-12.5408	-60.4448	-49.5892
16	72	-7.23823	-15.0069	-15.4291	-50.3037	-30.436
17	5280343	-5.74632	-38.1511	-4.65913	-62.3852	-42.8102

18	5280460	-6.81085	-28.3815	-5.99182	-48.0952	-34.3733
19	637775	-7.0636	-22.604	-9.50338	-50.3718	-32.1074
20	10742	-6.73593	-21.9612	-9.56246	-49.3283	-31.5236
21	8468	-6.74387	-19.3496	-10.6512	-47.661	-30.0009
22	15895318	1.97419	-30.1418	-9.46183	-26.4173	-39.6036

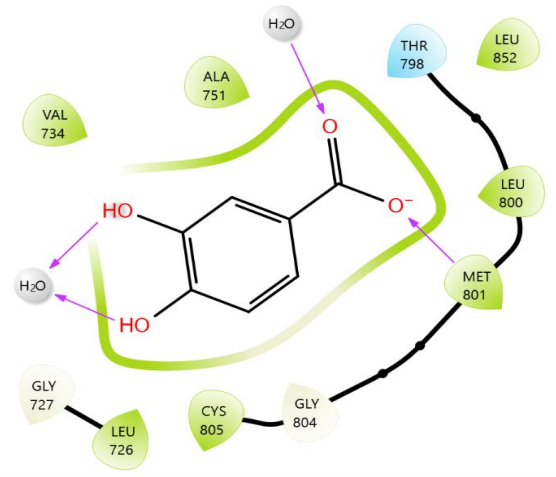


Figure 12:72 -3PPO

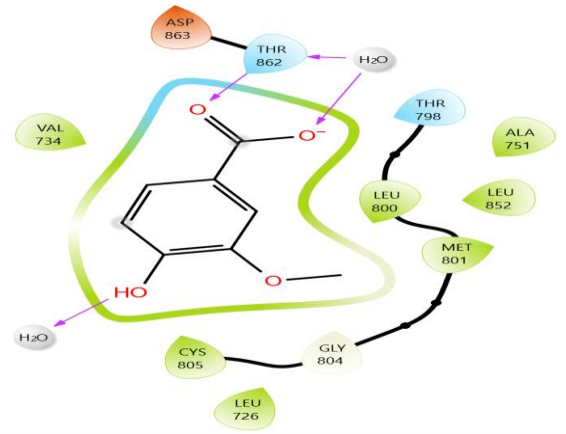


Figure 13: 8468 -3PPO

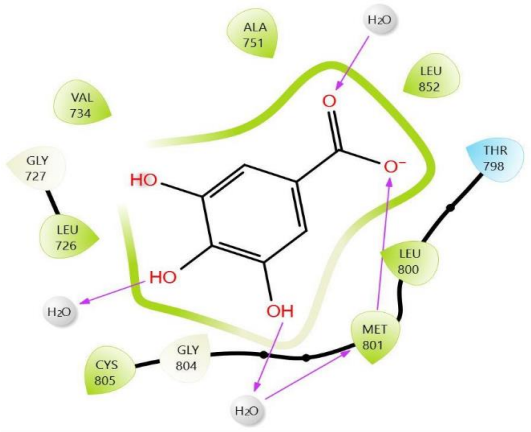


Figure 14:370 – 3PPO

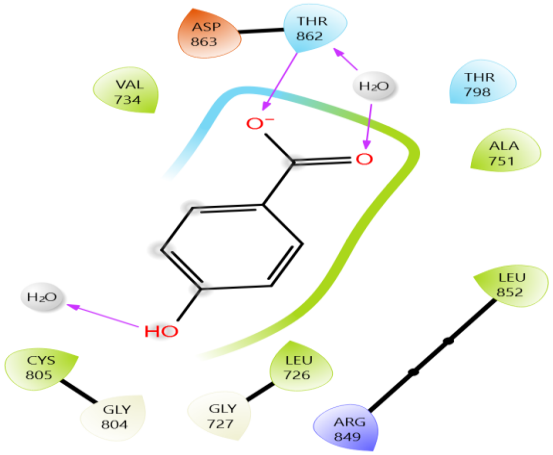


Figure 15:135 – 3PPO

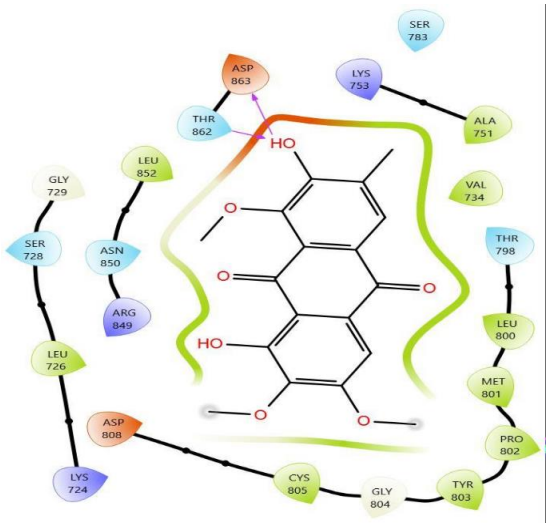


Figure 16:155380 – 3PPO

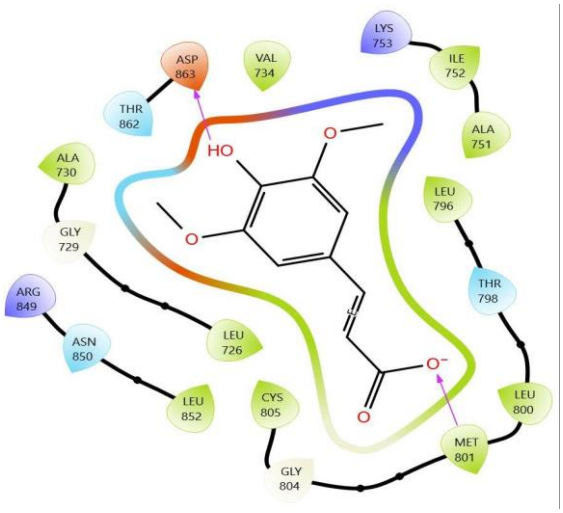


Figure 17:637775 – 3PPO

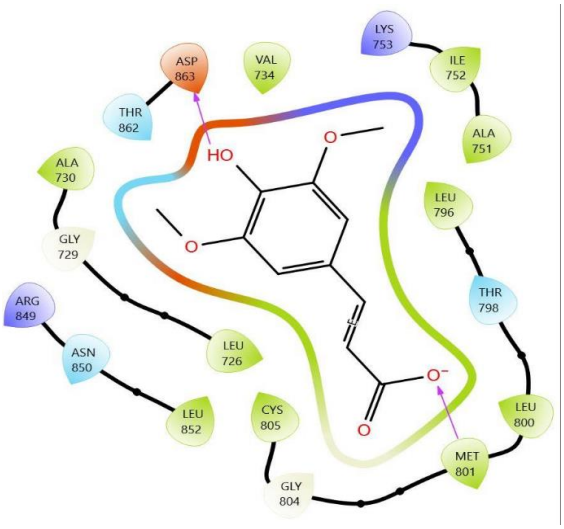


Figure 18:689043 -3PPO

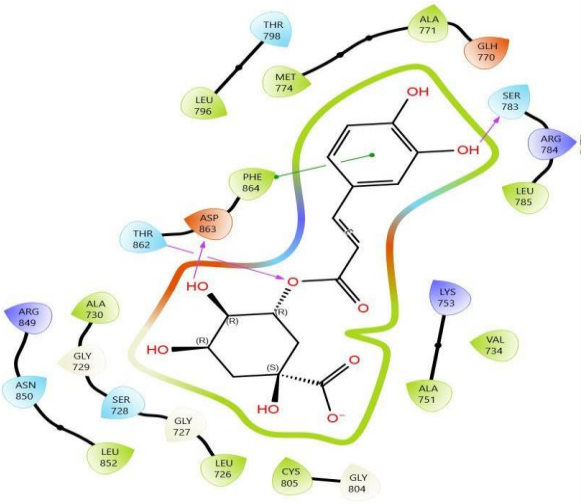


Figure 19:1794427 – 3PPO

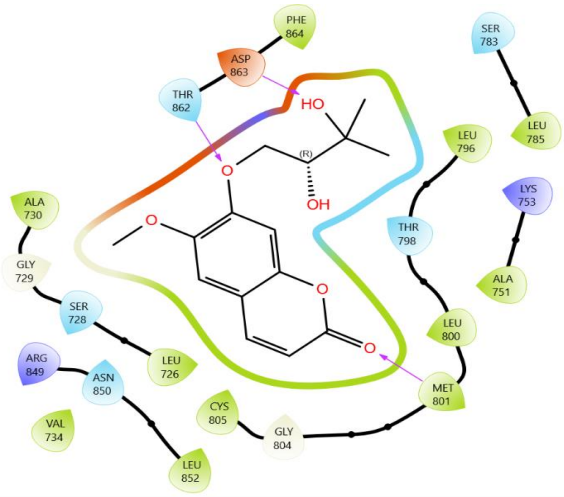


Figure 20:3604942-3PPO

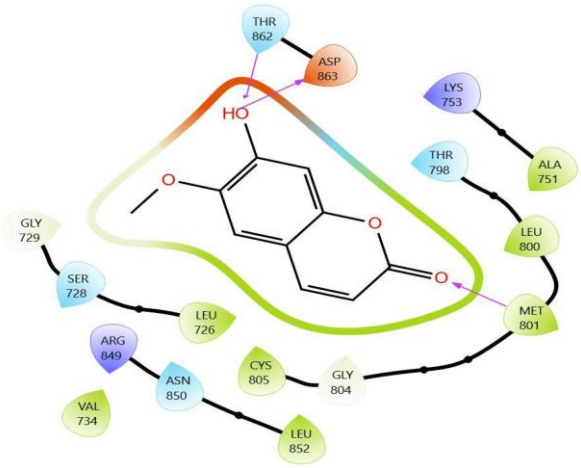


Figure 21: 5280460- 3PPO

Table 6: MMGBSA FOR LIGAND VS 3PPO

S.No	Compounds	_dG_Bind	_dG_Cov	dG_Bind_Hbond	_dG_Bind_Lip	_dG_Bind_vdW
1	3604942	-62.39	11.63	-2.55	-20.06	-47.03
2	1794427	-24.29	23.49	-2.55	-20.52	-49.51
3	370	-34.35	0.11	-2.95	-9.00	-20.61
4	72	-31.90	-0.09	-2.43	-9.12	-22.43
5	135	-23.83	0.46	-1.71	-8.66	-20.24
6	637775	-30.70	1.72	-1.68	-14.50	-29.00
7	155380	-40.38	9.04	-1.81	-18.03	-31.39
8	689043	-28.55	6.06	-1.39	-14.03	-34.67
9	5280460	-48.03	1.50	-1.81	-13.22	-31.72
10	8468	-27.90	1.81	-1.70	-10.55	-26.85
11	10742	-27.53	0.97	-1.84	-11.66	-29.60
12	5281543	-10.83	14.30	-0.61	-18.66	-21.52
13	445858	-24.27	7.75	-0.85	-16.30	-31.24
14	5281545	-16.58	15.44	-0.57	-18.00	-23.50
15	637542	-27.94	3.15	-0.86	-14.55	-34.97
16	5280343	-18.38	4.98	-2.14	-9.09	-42.73
17	5280863	-21.27	4.17	-1.87	-9.18	-41.98

18	5281672	-17.94	4.06	-2.28	-9.03	-42.10
19	6440657	-37.04	9.46	-3.10	-14.97	-42.41
20	5280443	-15.34	3.99	-1.21	-9.13	-44.23
21	5312351	-34.2	3.12	-1.23	-15.51	-34.76

Table 7: ADMET SCREENING TOP HIT COMPOUND AGAINST BREAST CANCER ACTIVITY

Molecule	mol_MW	dipole	Accpt HB	Q PlogPw	Q PlogKp	Rule of three
44593505	456.402	11.231	17.6	26.803	-6.572	1
12305768	472.707	6.787	5.4	11.407	-3.714	1
5280443	270.241	6	3.75	10.191	-3.948	0
64971	456.707	5.608	3.7	8.181	-3.09	1
689043	180.16	6.439	3.5	9.888	-4.506	1
173183	400.687	1.818	1.7	3.879	-1.747	1
1794427	354.313	4.196	9.65	20.544	-6.184	1
445858	194.187	6.391	3.5	8.052	-3.683	0
370	170.121	5.578	4.25	12.043	-5.501	1
5281543	290.272	2.947	8.4	10.172	-2.88	0
5280863	286.24	5.195	4.5	12.325	-4.602	0
92157	468.762	3.209	2	3.517	-2.041	1
259846	426.724	1.868	1.7	4.478	-1.949	1
5281672	318.239	7.215	6	16.502	-6.401	1
14414398	442.724	3.588	3.4	7.286	-2.331	1
5312351	170.251	6.49	2	3.534	-2.639	0
21671982	470.691	6.851	5.7	10.456	-3.679	1
155380	344.32	5.004	6.75	9.648	-3.325	0
3604942	294.304	6.465	6.45	10.799	-2.953	0
399421	414.607	7.586	1.7	4.333	-2.175	0
11541511	576.726	4.148	12.55	16.866	-3.885	1
10494	456.707	3.938	3.7	8.313	-3.081	1
12313704	454.692	4.598	4	7.373	-3.208	1
637542	164.16	5.679	2.75	7.798	-3.607	0

135	138.123	5.374	2.75	7.871	-3.717	0
5281545	290.272	3.343	8.4	10.245	-3.028	0
6440657	616.574	9.448	19.65	29.266	-5.745	2
72319	470.429	8.353	17.6	25.131	-5.718	1
72	154.122	4.307	3.5	9.96	-4.618	0
5280343	302.24	6.039	5.25	14.416	-5.505	1
5280805	610.524	6.43	20.55	36.188	-7.204	2
5280460	192.171	5.408	4	7.594	-3.065	0
637775	224.213	3.832	4.25	8.31	-3.754	0
5280794	412.698	2.407	1.7	3.91	-1.747	1

MTT ASSAY – MCF 7 CELL LINE RESULT

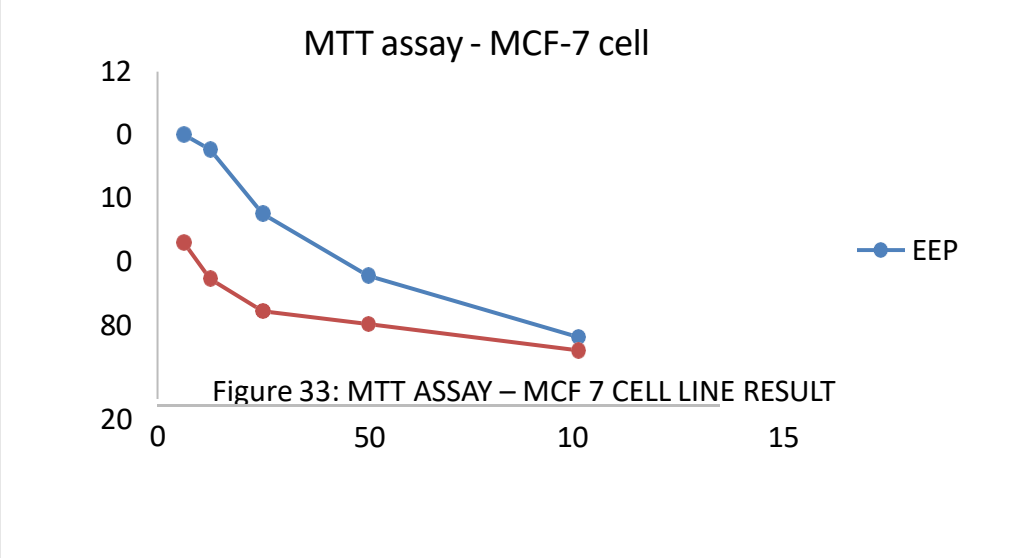


Figure 33: MTT ASSAY – MCF 7 CELL LINE RESULT

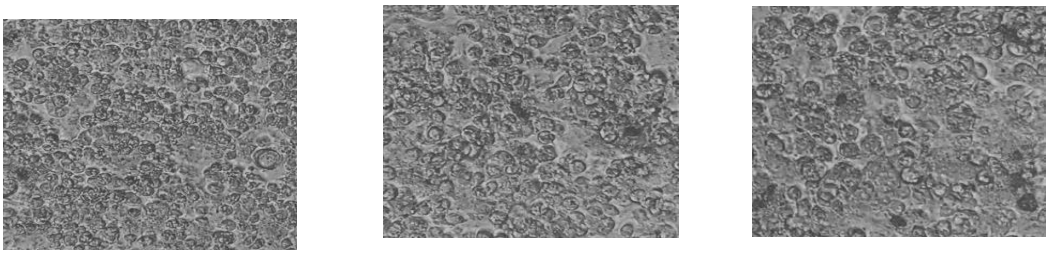
Table 8: MTT ASSAY – MCF-7 CELL LINE

MTT assay - MCF-7 cell line

			OD at 570 nm			% viability					IC50
Sample	Concentration		Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate	Mean	SD	(µg)
Control	0		1.314	1.329	1.337	100	100	100	100		
EECI	6.25		1.285	1.274	1.298	97.79299848	95.86155004	97.08302169	96.9125234	0.97694704	
	12.5		1.221	1.209	1.211	92.92237443	90.97065463	90.57591623	91.48964843	1.256376713	
	25		0.9	0.9	0.9	68.49315068	67.72009029	67.31488407	67.84270835	0.598627134	57.51
	50		0.6	0.6	0.6	45.66210046	45.14672686	44.87658938	45.22847223	0.399084756	
	100		0.3	0.3	0.3	22.83105023	22.57336343	22.43829469	22.61423612	0.199542378	
DOXORUBICIN	6.25		0.741	0.769	0.773	56.39269406	57.86305493	57.81600598	57.35725166	0.835662561	
	12.5		0.594	0.571	0.588	45.20547945	42.96463506	43.97905759	44.04972404	1.122092333	
	25		0.433	0.428	0.419	32.95281583	32.20466516	31.33881825	32.16543308	0.807713696	7.46
	50		0.376	0.36	0.355	28.61491629	27.08803612	26.55198205	27.41831148	1.07039048	
	100		0.226	0.249	0.233	17.19939117	18.73589165	17.42707554	17.78745279	0.829223882	

Fig. no. 22. Sample: EEPO (MCF-7 cell line)

Control **6.25µg** **12.5µg**



25µg **50µg** **100µg**

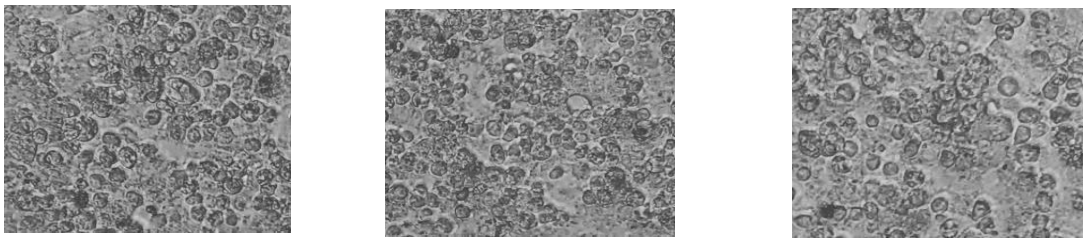


Fig. no. 23 Sample: DOXORUBICIN (MCF-7 cell line)

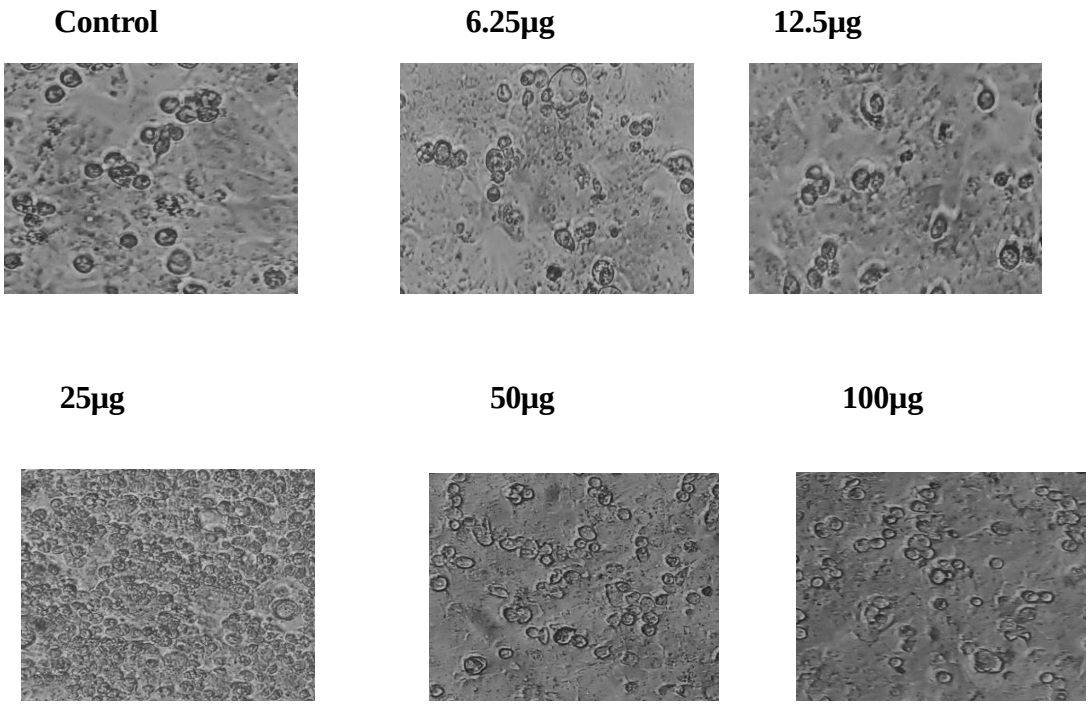


Table: 9. EEPO Vs DOXORUBICIN (MCF-7 cell line)

Conc.	**EEPO**	**DOXORUBICIN**
6.25	96.91252	57.35725166
12.5	91.48965	44.04972404
25	67.84271	32.16543308
50	45.22847	27.41831148
100	22.61424	17.78745279

Table 10 : MTT ASSAY – MDA-MB-231 CELL LINE

OD at 570nm %viability

Sample	**Concentration**	**Singlet**	**Duplicate**	**Triplicate**	**Singlet**	**Duplicate**	**Triplicate**		**Mean**	**IC50 (µg)**
Control	0	1.267	1.283	1.277	100	100	100	100		
EEPO	6.25	1.117	1.221	1.237	88.16101	95.16757599	96.86765863	93.3987	4.615	**76.54**
	12.5	1.1	1.122	1.178	86.819258	87.45128605	92.247455	88.8393	2.9684	

	25	0.964	0.947	0.966	76.085241	73.81137958	75.6460454	75.1809	1.2062	
	50	0.811	0.841	0.79	64.009471	65.54949337	61.8637431	63.8076	1.8512	
	100	0.427	0.443	0.445	33.701657	34.52844895	34.8472984	34.3591	0.5913	
DOXORUBICIN	6.25	0.658	0.697	0.68	51.933702	54.32579891	53.2498042	53.1698	1.1981	**8.93**
	12.5	0.514	0.592	0.543	40.568272	46.14185503	42.5215348	43.0772	2.828	
	25	0.474	0.38	0.38	37.411208	29.61808262	29.7572435	32.2622	4.4597	
	50	0.37	0.324	0.324	29.202841	25.25331255	25.3719655	26.6094	2.2468	
	100	0.227	0.212	0.248	17.916338	16.52377241	19.4205168	17.9535	1.4487	

Fig.no. 24 Sample: EECI (MDA-MB-231 cell line)

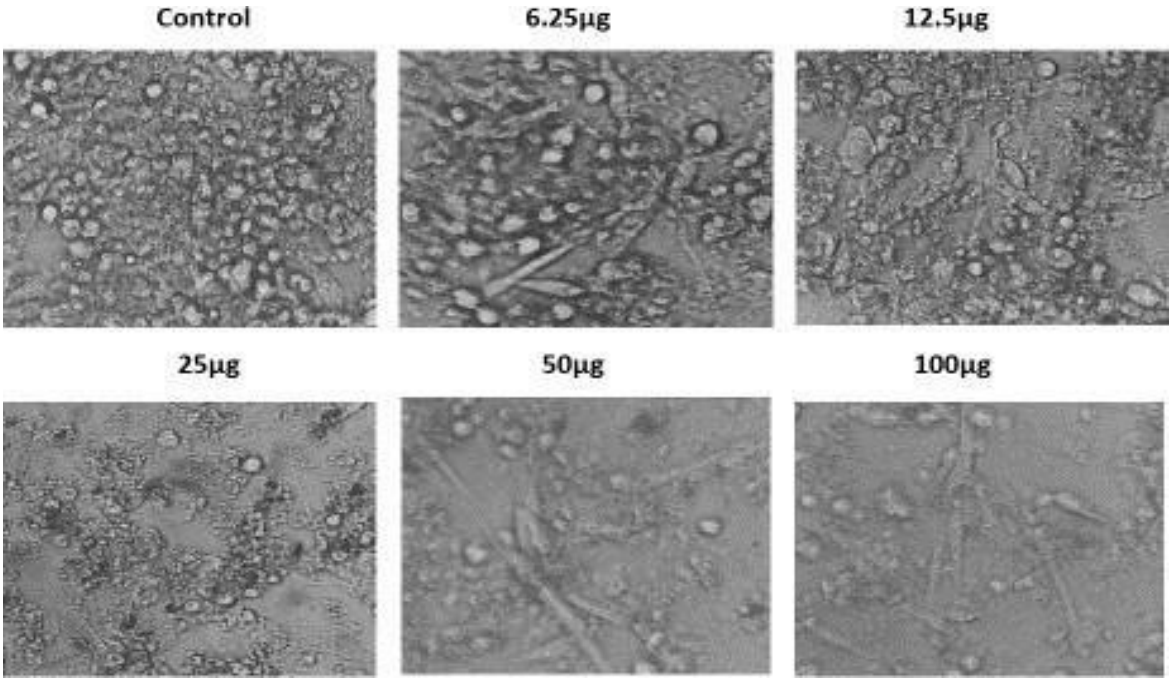
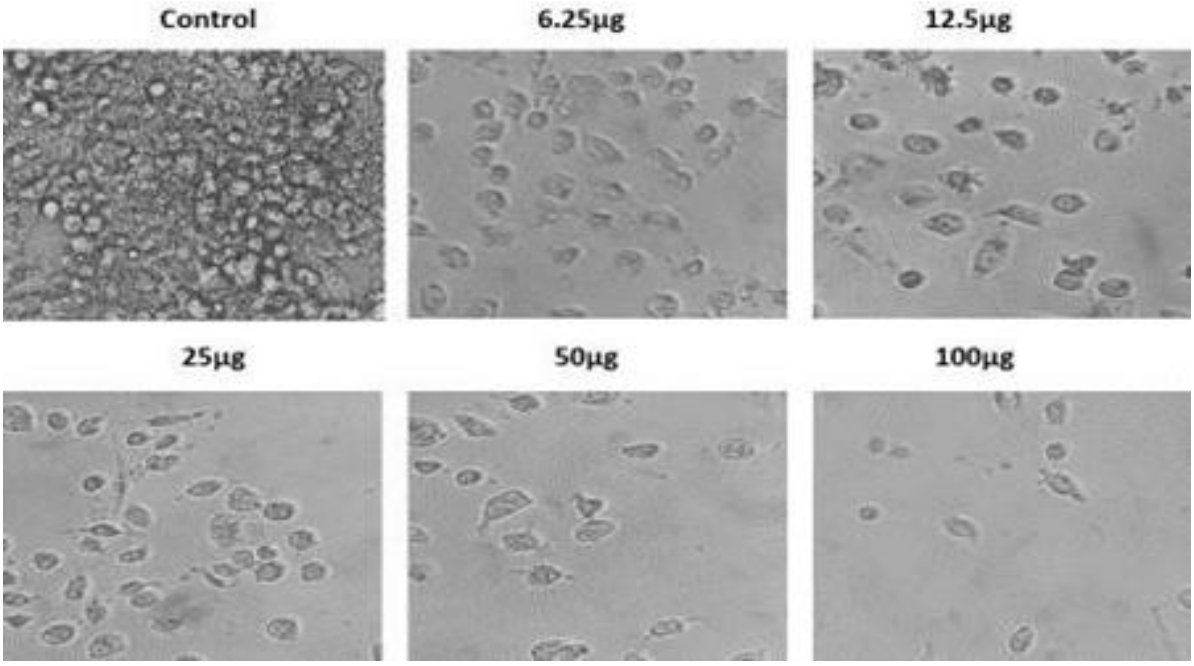


Fig.no. 25 Sample: DOXORUBICIN (MDA-MB-231 cell line)



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