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IN-SILICO ANALYSIS OF SOME PHYTOCHEMICALS AS POTENTIAL THERAPEUTIC INHIBITORS AGAINST BREAST CANCER

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Keywords:

Clitoria ternatea, Pharmacokinetics, ADMET, EGFR, Molecular docking

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ABSTRACT: Breast cancer continues to pose a serious hazard to women worldwide. (EGFR) was one of the first key targets for these new anticancer drugs to be discovered. Triple-negative breast cancer affects almost half of all women in the World. This study focused on the phytochemicals from a medicinal plant *Clitoria ternatea*; these medicinal plants possess more phytochemical compounds that effectively fight against cancer cells. The five compounds were selected and a molecular docking study was performed against EGFR protein. The Pharmacokinetics and ADMET properties of these ligands help identify the best hits. Myricetin exhibits the highest binding energy among these five ligand molecules and is the best ligand to inhibit EGFR protein. Hence, this compound may act as a promising agent for treating breast cancer. However, more *in-vivo* and *in-vitro* testing is needed to establish their safety and efficacy in this area.

INTRODUCTION: Breast cancer is a struggle for all women, and research into prevention techniques may be more effective than treatment strategies. According to WHO statistics estimates (2012), the risk of breast cancer has grown over the last 50 years and now accounts for 23% of all cancer deaths in Asia. Breast cancer chemotherapy is distinguished by the targeting of receptor functions such as ER (estrogen receptor alpha), PR (progesterone receptor), EGFR (epidermal growth factor receptor) and others [1]. EGFR targeted treatment has recently been proposed as a potential therapeutic approach in triple-negative breast cancer, although outcomes have been poor [2].

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Due to the increase in the Cancer rate worldwide, Millions of plant, animal, marine and microbe species are fascinating sources of novel medicinal candidate substances. Furthermore, there may be difficulty identifying, isolating, evaluating and collecting sufficient amounts of the active component in sample [3]. The members of the epidermal growth factor receptor [4] (EGFR) / ErbB family are among the most important cancer molecular targets found to date: EGFR (also known as ErbB1 and HER1), The most well-established therapeutic target in breast cancer is HER2, which is overexpressed in 20–25 percent of cases.

EGFR [5] has been related to a significant role in triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) [6]. *Clitoria ternatea,* is a popularly known plant from the Fabaceae family herbaceous plant. It has lately sparked considerable attention because of its potential applications in contemporary health and agriculture and as a source of natural food colorants

and antioxidants [7]. It is an ancient medicinal plant that has been used for millennia as a memory enhancer, nootropic, antistress, and sedative agent [8]. The flowers of this plant are also used in the treatment of snake-bit due to the presence of several phytochemicals constituents it also has several properties such as anti-viral. Anti-inflammatory, anti-microbial and is also used to prevent and treat several diseases [9]. *Clitoria ternatea's* extensive research indicated that the extract's main composition comprises mostly flavonols such as kaempferol [10], Quercetin [10], myricetin [10,] and some anthocyanin [11] molecules such as ternatin [12] and delphinidin [13]. Molecular docking is a computational methodology that can offer information regarding protein, nucleic acid, lipid, and ligand intermolecular interactions. The goal of molecular docking is to get an optimal configuration of proteins and ligands and also a relative position between proteins and ligands using the minimum amount of energy [14]. The purpose of the present study is to identify the best compound from the phytochemicals of *Clitoria ternatea* by performing molecular docking for the treatment of breast cancer.

MATERIALS AND METHODS:

Preparation of Protein Molecule: The protein molecule EGFR (PDB: 1M17) [4] was extracted from (https://www.rcsb.org/). The other substances, such as ligand and water molecules, were deleted from the structure. The pure protein molecule is saved, and the molecular docking was carried out using Autodock tools.

Selection of Ligands Molecules: Among the several phytochemicals, we have selected only [5] ligands, They were Ternatin, Delphinidin, Kaempferol, Quercetin, and myricetin [13]. These compounds were downloaded in SDF format from (https://zinc.docking.org/substances/home/) and were converted to Pdbqt format for docking against EGFR protein.

Pharmacokinetics, ADMET Properties of Selected Ligands Molecules: *In-silico* absorption, distribution, metabolism, excretion and toxicity (ADME) (http://www.swissadme.ch/) and toxicity from (ORISIS) (https://www.organic-chemistry. org/prog/peo/) 15 were performed on the compounds with the most promising structural backbones to identify which lead compounds exhibit drug-like characteristics and should be a focus for further investigation. It helps develop new natural compounds with the best pharmacokinetics and pharmacodynamic properties [16].

Molecular Docking Analysis: Auto dock tools were used to perform the molecular docking study. The grid spacing used for docking was 0.375Å, the grid points x, y, z was 40 x 40 x 40, and the grid center was at x= 23.245, y=-0.451, and z = 56.12. The grid box was constructed based on the dimensions mentioned above, and molecular docking was carried out using Perl. The results obtained were analyzed to determine the binding energy and the interacting atoms between protein and the ligand and were visualized using bio *via* discovery studio software [17].

RESULTS AND DISCUSSION: Protein structure EGFR (1M17) with 4-anilinoquinazoline inhibitor was used in this study with a resolution 2.60 Å. The pure protein structure was prepared without the ligand and water molecules, as shown in **Fig. 1**. The molecular properties of the selected protein was listed in **Table 1**. The 2D and 3D structures of five ligands selected from the Clitoria ternatea were shown in **Fig. 2**. The ligands' pharmacokinetics, ADME, and toxicity were listed in **Table 2**. The molecular docking studies were performed for the ligands with the protein, and the results were shown in **Fig. 3**. The binding energy of the five ligands and the interaction with residues were listed in **Table 3**.

From the results obtained, the five ligands binding energy and their interacting residues were Ternatin (-7.8Kcal/mol) VAL 702, ARG 817, ALA 719, ASP 831, PHE 699, THR 766, LYS 721, THR 830, LEU 764, GLU 738 AND MET 742. Delphinidin (-7.8 Kcal/mol) CYS 773, LEU 820, MET 769, CYS 751, MET 742, THR 830, ASP 831, LEU 764, LYS 721, ILE 720, ALA 719, THR 766,VAL. Kampferol (-8.0Kcal/mol) LEU 820, GLY 695, PHE 699, THR 766, MET 742, GLU 738, THR 830, LYS 721, ASP 831, VAL 702, GLY 772, MET 769, LEU 768, LEU 694. Quercitin (-8.6Kcal/mol) THR 766, LEU 820, ALA 719, PHE 699, ASN 818, ARG 817, ASP 831, LYS 721, VAL 702, GLU 738,MET 742, LEU 764, THR 830 and Myricetin (-8.7 Kcal/mol) LEU 820, CYS 773,

THR 830, ASP 831, PHE 832, GLY 772, MET 769, LEU 768, ALA 719, VAL 702, LEU 694, THR 766, LYS 721, GLU 738, MET 742, LEU 764 respectively. Myricetin molecule showed the highest binding energy when compared with the other ligand molecules. This molecule has obeyed Lipinski rule 5 and is the best molecule with the highest affinity. Hopefully, these findings will lead to the development of new breast cancer therapies.

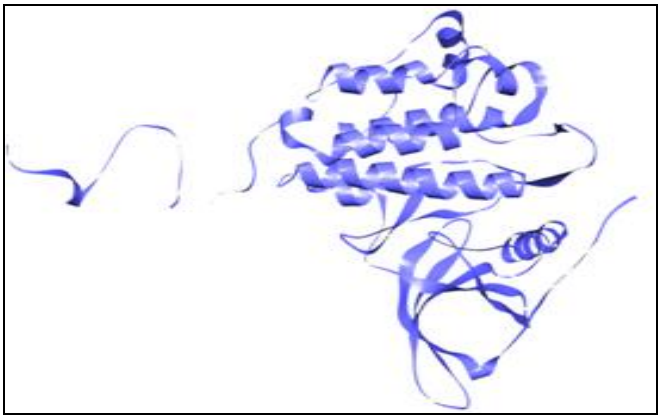


FIG. 1: THE STRUCTURE OF EGFR (IM17) PROTEIN

TABLE 1: THE MOLECULAR PROPERTIES OF THE SELECTED EGFR PROTEIN

Protein (EGFR) and its molecular properties	
Molecular properties	EGFR (1M17)
Classification	Transferase
Chain	A
Length	333
Source organism	Homo sapiens (Human)
Ligand	[6,7-bis(2-methoxy ethoxy) quinazoline-4-yl]-(3-ethynylphenyl) amine

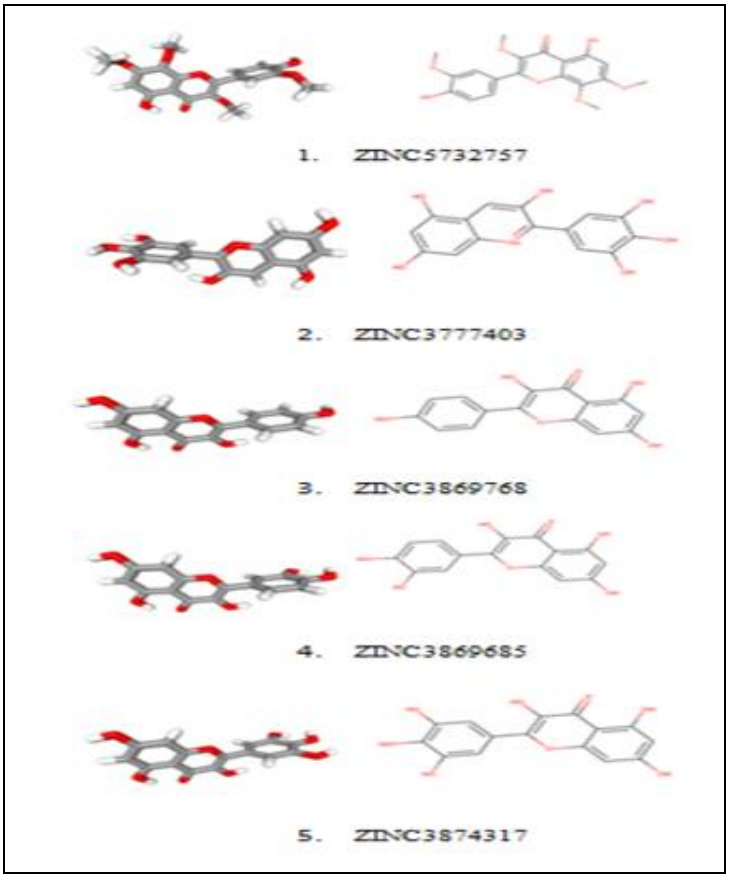
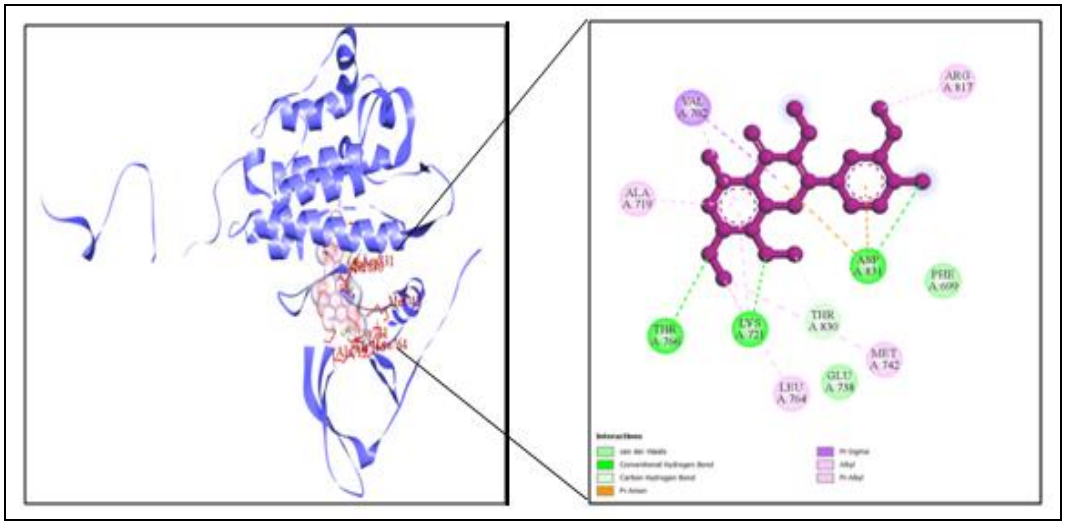
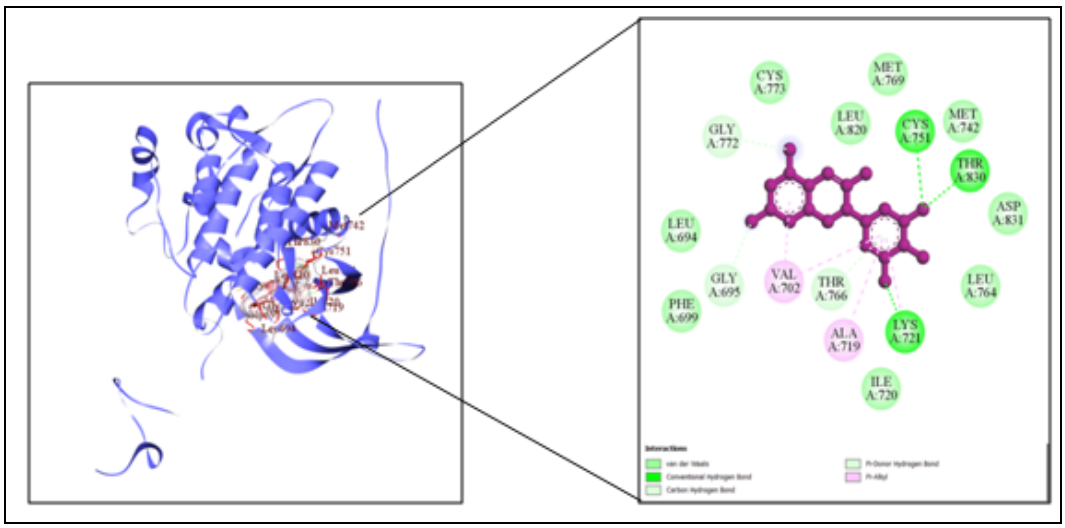


FIG. 2: THE 2D AND 3D STRUCTURES OF THE SELECTED LIGANDS FROM *CLITORIA TERNATEA*

TABLE 2: THE PHARMACOKINETICS, ADME AND TOXICITY PROPERTIES OF THE SELECTED LIGANDS

Pharmacokinetics, ADME Properties of the selected ligands								
Compound ID	**Mol. wt**	**Rotatable bonds**	**HBA**	**HBD**	**Molar refractivity**	**TPSA Å²**	**Lipinski rule**	**Bioavailability score**
ZINC5732757	374.34g/mol	5	8	2	97.93	107.59	Yes	0.55
ZINC3777403	303.24g/mol	1	7	6	78.20	134.52	Yes	0.55
ZINC3869768	286.24g/mol	1	6	4	76.01	111.13	Yes	0.55
ZINC3869685	302.24g/mol	1	7	5	78.03	131.36	Yes	0.55
ZINC3874317	318.24g/mol	1	8	6	80.06	151.59	Yes	0.55
Compound ID	**GI absorption**	**BBB Permeant**	**P-gp Substrate**	**CYP1A2 Inhibitor**	**CYP2C19 Inhibitor**	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**
ZINC5732757	High	No	no	yes	No	Yes	No	Yes
ZINC3777403	High	No	Yes	Yes	No	No	No	No
ZINC3869768	High	No	No	Yes	No	No	Yes	Yes
ZINC3869685	High	No	No	Yes	No	No	Yes	Yes
ZINC3874317	Low	No	No	Yes	No	No	No	Yes
Toxicity risks								
Compound ID		**Mutagenic**		**Tumorigenic**		**Irritant**		**Reproductive effect**
ZINC5732757		Yes		Yes		No		No
ZINC3777403		Yes		Yes		Yes		Yes
ZINC3869768		No		Yes		yes		Yes
ZINC3869685		Yes		Yes		No		No
ZINC3874317		No		Yes		Yes		Yes





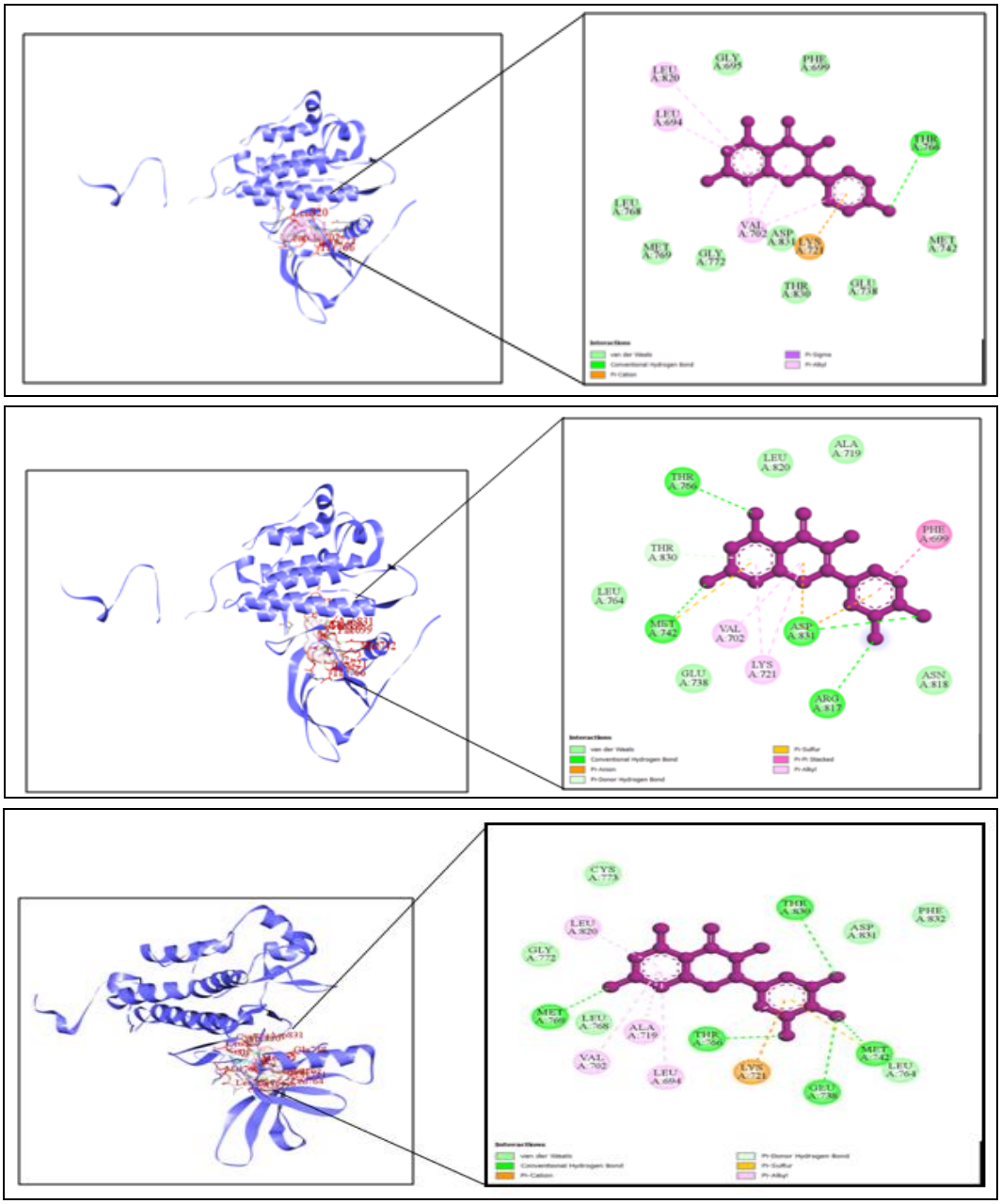


FIG. 3: THE VISUALIZATION OF THE LIGANDS DOCKED WITH A PROTEIN MOLECULE

TABLE 3: THE BINDING ENERGY AND INTERACTING RESIDUES OF THE LIGANDS WITH THE PROTEIN

S. no.	Zinc ID	Ligand Name	ΔG Binding energy (Kcal/mol)	Interacting residues.
1.	ZINC5732757	Ternatin	-7.8	VAL 702, ARG 817, ALA 719, ASP 831, PHE 699, THR 766,LYS 721, THR 830, LEU 764,GLU 738 AND MET 742
2.	ZINC3777403	Delphinidin	-7.8	CYS 773, LEU 820, MET 769, CYS 751, MET 742, THR 830, ASP 831, LEU 764, LYS 721, ILE 720, ALA 719, THR 766,VAL 702,GLY 695,PHE 699, LEU 694, GLY 772
3.	ZINC3869768	Kampferol	-8.0	LEU 820, GLY 695, PHE 699, THR 766, MET 742, GLU 738, THR 830, LYS 721, ASP 831, VAL 702, GLY 772, MET 769, LEU 768, LEU 694
4.	ZINC3869685	Quercitin	-8.6	THR 766, LEU 820, ALA 719, PHE 699, ASN 818, ARG 817, ASP 831, LYS 721, VAL 702, GLU 738,MET 742, LEU 764, THR 830
5.	ZINC3874317	Myricetin	-8.7	LEU 820, CYS 773, THR 830, ASP 831, PHE 832, GLY 772, MET 769, LEU 768, ALA 719, VAL 702, LEU 694, THR 766, LYS 721, GLU 738, MET 742, LEU 764

CONCLUSION: The current investigation identified a prospective medicine that focuses on the target protein (EGFR) as a distinct option to prevent breast cancer. *In-silico*-based assessments performed in this study were to provide for identifying novel photochemical compounds inhibitors against human EGFR. The protein-ligand complexes' binding affinity values and interaction residues revealed that these five natural phytochemicals have higher binding affinities. Hence, it is concluded that these phytochemicals from the plants play an important role. Further in-vitro experiments may help identify the best compounds and may be helpful in the treatment of breast cancer by using these phytochemicals as medicines.

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CONFLICT OF INTEREST: No conflict of interest is associated with this work.

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