



In silico analysis of secondary metabolites from *Abrus precatorius* L. leaves as a novel approach to breast cancer treatment

[Análisis *in silico* de metabolitos secundarios de *Abrus precatorius* L. como un enfoque novedoso para el tratamiento del cáncer de mama]

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Abstract

Context: Breast cancer, characterized by abnormal growth in breast tissue with potential invasion into other body parts, poses significant challenges in treatment due to the associated negative implications of conventional therapies like radiation, chemotherapy, and surgery. *Abrus precatorius*, a plant species from the *Leguminosae* family, offers an alternative herbal approach for cancer therapy due to its leaves' antioxidant and antiproliferative properties.

Aims: To evaluate the bioactivity of *A. precatorius* leaf compounds against the epidermal growth factor receptor (EGFR) protein within the breast cancer signaling pathway, employing a protein-ligand networking approach and molecular docking analysis.

Methods: Bioactive *A. precatorius* compounds were retrieved from PubChem, analyzed for similarity using SWISS ADME, anticancer potential using PASSOnline, docked using PyRX, visualized using Biovia discovery studio, and ADMET analysis using pkCSM.

Results: The bioactive compounds abrin, abrusoside A, glycyrrhizin, and stigmasterol, sourced from *A. precatorius* leaves, possess the potential to inhibit EGFR proteins implicated in breast cancer. Among these compounds, stigmasterol stands out as a bioactive compound with a binding affinity that surpasses that of the ATP as a control ligand and erlotinib as a reference drug.

Conclusions: *In silico* analysis highlights stigmasterol from *A. precatorius* as a promising EGFR inhibitor for breast cancer, showing superior binding affinity (-9.9 kcal/mol) compared to ATP (-8.7 kcal/mol) and erlotinib (-6.8 kcal/mol). Its excellent intestinal absorption (94.97%), good permeability (1.213), and moderate BBB permeability (0.771) support its potential for both central nervous system and peripheral targets, warranting further research.

Keywords: anticancer; epidermal growth factor receptor; herbal medicine; molecular docking; stigmasterol.

Resumen

Contexto: El cáncer de mama, caracterizado por un crecimiento anormal en el tejido mamario con posible invasión a otras partes del cuerpo, plantea importantes desafíos en el tratamiento debido a las implicaciones negativas asociadas de las terapias convencionales como la radiación, la quimioterapia y la cirugía. La especie *Abrus precatorius* ofrece un enfoque herbario alternativo para la terapia contra el cáncer debido a las propiedades antioxidantes y antiproliferativas de sus hojas.

Objetivos: Evaluar la bioactividad de los compuestos de las hojas de *A. precatorius* contra la proteína del receptor del factor de crecimiento epidérmico (EGFR) dentro de la vía de señalización del cáncer de mama, empleando un enfoque de red de proteína-ligando y análisis de acoplamiento molecular.

Métodos: Los compuestos bioactivos de *A. precatorius* se recuperaron de PubChem, se analizaron para determinar su similitud con SWISS ADME, su potencial anticancerígeno con PASSOnline, se acoplaron con PyRX, se visualizaron con Biovia Discovery Studio y el análisis ADMET con pkCSM.

Resultados: Los compuestos bioactivos abrina, abrusósido A, glicirricina y estigmasterol, obtenidos de las hojas de *A. precatorius*, poseen el potencial de inhibir las proteínas EGFR implicadas en el cáncer de mama. Entre estos compuestos destaca el estigmasterol como compuesto bioactivo con una afinidad de unión superior a la del ATP como ligando de control y el erlotinib como fármaco de referencia.

Conclusiones: El análisis *in silico* destaca el estigmasterol de *A. precatorius* como un prometedor inhibidor de EGFR para el cáncer de mama, mostrando una afinidad de unión superior (-9,9 kcal/mol) en comparación con el ATP (-8,7 kcal/mol) y el erlotinib (-6,8 calorías/mol). Su excelente absorción intestinal (94,97%), buena permeabilidad (1,213) y permeabilidad BBB moderada (0,771) respaldan su potencial tanto para el sistema nervioso central como para objetivos periféricos, lo que justifica más investigaciones.

Palabras Clave: acoplamiento molecular; anticancerígeno; estigmasterol; medicina herbaria; receptor del factor de crecimiento epidérmico.

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INTRODUCTION

Breast cancer is a complex and heterogeneous disease involving multiple tumor entities associated with unique histological patterns and various biochemical and clinical characteristics. Breast cancer can be classified in various ways, including clinical characteristics, expression of tumor markers, and type of histological characteristics (Weigelt and Reis-Filho, 2009). With an estimated 2.3 million new cases worldwide in 2020, breast cancer is a serious global health problem. With 2.3 million new cases (11.7%), breast cancer among women will outperform lung cancer as the most diagnosed cancer. The following are lung (16.4%), colorectal (10%), prostate (7.3%), stomach (5.6%), and lung (10%) cancers (Sung et al., 2021). Estimates for 2020 alone place breast cancer deaths at 685,000, making it the main cause of female cancer deaths worldwide. Due to demographic change, the number of cancers in transitional countries (64 to 95%) and countries (32 to 56%) is projected to increase by 47% from 2020 to 2040 to 28.4 million (Sung et al., 2021). However, this situation may be further exacerbated by increasing risk factors associated with them.

Epidermal growth factor receptor (EGFR) is often overexpressed in triple-negative breast cancer (TNBC), a molecular subtype of breast cancer that resembles aggressive clinical behaviors due to loss or reduction in the expression of estrogen, progesterone and Her2neu receptors (Salman et al., 2022). Studies have shown that the expression of EGFR is associated with drug resistance and the progression of cancer (Tang et al., 2022). In addition, the re-use of compounds approved by the US Food and Drug Administration (FDA) targeting EGFR and HER2 has shown growth inhibitory activity for TNBC cells (Balbuena-Rebolledo et al., 2021). In addition, new bispecific antibodies targeting EGFR and VEGFR2 show potent antitumor activity *in vitro* and in the TNBC model (Mohan et al., 2021). These results indicate that EGFR is a promising treatment for breast cancer, especially TNBC.

Erlotinib, an anti-EGFR drug, has shown promise in the treatment of triple-negative breast cancer (TNBC) (Fenn et al., 2020). In a study investigating the clinical relevance of biomarkers in TNBC, erlotinib was found to significantly reduce the proliferation of TNBC cells expressing EGFR (El Guerrab et al., 2016). Additionally, a case report described the successful use of erlotinib in a patient with metastatic breast carcinoma expressing an uncommon EGFR mutation (Singh et al., 2020). These findings suggest that erlotinib may be an effective targeted therapy for breast cancer patients with EGFR overexpression or specific

EGFR mutations. In contrast, the study examines the impact of prolonged erlotinib use in non-small cell lung cancer patients over a minimum period of six months, revealing a diverse range of adverse effects such as paronychia, fatigue, and hair changes, highlighting the intricate challenge of managing these side effects effectively (Becker et al., 2010).

Modern breast cancer therapy has improved patient survival rates but can also cause side effects such as lymphedema, neuropathy, changes in body composition, radiation therapy, and psychological symptoms (Tommasi et al., 2022). To address these issues, alternative approaches, such as using organic substances derived from plants like *Abrus precatorius*, have been explored. *Abrus precatorius* L. (family Leguminosae), popularly referred to as the saga plant in Indonesia, is a widely distributed plant species with a global presence. It exhibits the capacity to thrive in various environments, including forests, fields, and domestic premises (Adilah et al., 2022). The genus under consideration is relatively diminutive in scale, encompassing roughly 17 distinct species, and it exhibits a wide distribution across tropical and subtropical zones, with a particular prevalence noted in regions within Asia and Africa. Additionally, there is a likelihood that this genus has been introduced into the New World (Hassan et al., 2021). Research investigations have been undertaken with the purpose of discerning the chemical constituents of *A. precatorius*. The utilization of gas chromatography-mass spectrometry (GC-MS) has been prevalent in the realm of metabolomics research, serving dual purposes of ensuring the quality of natural product-based medications and examining the tolerance of plants to abiotic stress. The metabolomic profile derived from GC-MS analysis imparts invaluable insights pertaining to the pharmacological aspects of plants (Mildawati et al., 2022). The leaves of *A. precatorius* were found to contain stigmasterol, glycyrrhizin, abrusoside A, and abrine (Vasani and Thakkar, 2022). An investigation was conducted to examine the anti-proliferative properties of extracts derived from the leaves of *A. precatorius*, along with their impact on cellular mortality. The aforementioned study noted that the methanolic extract derived from *A. precatorius* leaves had the potential to trigger cell cycle arrest and apoptosis. These effects were believed to be, in part, mediated through mitochondrial pathways, resulting in the eventual demise of MDA-MB-231 breast cancer cells (Wan-Ibrahim et al., 2019).

In silico molecular docking, a computational methodology used in drug discovery and design offers a valuable tool for studying chemical compounds, binding affinity, and spatial arrangement with protein

targets (Chen et al., 2020). This research methodology provides a swift and cost-efficient means of examining chemical compounds' potential to bind with specific proteins, exerting inhibitory effects on their activity (Gholizadeh et al., 2023). Furthermore, the interaction of ligand-protein is still unknown; this study aims to delineate the mechanistic pathways governed by bioactive compounds found in *A. precatorius* leaves in their interactions with the EGFR protein, aiming to develop innovative avenues within alternative breast cancer treatment strategies.

MATERIAL AND METHODS

Sample preparation

The research's target molecules were abrin, abrusoside A, glycyrrhizin, and stigmaterol (Vasani and Thakkar, 2022). Canonical SMILES and 3D SDF files of ligands such as abrin (CID: 160511), abrusoside A (CID: 6857683), glycyrrhizin (CID: 14982), stigmaterol (CID: 5280794), ATP (CID: 5957), and erlotinib (CID: 176870) (Fig. 1). Ligand molecules commonly used as comparison ligands in molecular docking studies were retrieved from the PubChem compound server, accessible at <https://pubchem.ncbi.nlm.nih.gov/>. The detailed workflow of the study is illustrated in Fig. 2. PubChem is a specific database that contains data on many chemical substances, including synthetic and organic compounds. This dataset encompasses a range of critical elements, comprising a compound identifier (CID), bibliographical references, information regarding toxicity, physicochemical properties, as well as both two-dimensional (2D) and three-dimensional (3D) structural attributes. Furthermore, it encompasses the canonical depiction of chemical structures using the simplified molecular input line entry system (SMILES) notation (Kim, 2016). The ob-

jective of the sample production within PubChem for this research is to procure the 3D structure and canonical SMILES of the target compound (Table 1). Subsequently, the PyRx software was employed to enhance the flexibility of the ligands and transform the data structure format from structure-data file (SDF) to the protein data bank format (PDB) (Dhea Kharisma et al., 2018). The target protein is EGFR (1XKK), which was found in the RCSB PDB database <https://www.rcsb.org/>.

Drug-likeness analysis and activity prediction of biological compounds

The initial phase of our investigation involved procuring samples from the PubChem database, followed by a comprehensive evaluation of their potential as viable drug candidates, a process executed using the SWISS ADME web server (Wang et al., 2019). This analytical procedure was conducted with the overarching objective of computing and forecasting a myriad of critical parameters pertaining to the suitability of these compounds for drug development, including various aspects of pharmacokinetics. Subsequently, our study focused on discerning the physicochemical attributes and drug-likeness of compounds derived from *A. precatorius* leaves, employing the SWISS ADME server for this purpose. This meticulous examination was undertaken to gain insights into the compounds' viability as pharmaceutical agents. Furthermore, in a parallel endeavor, the biological activities associated with these compounds were forecasted utilizing the PASSOnline server, a platform known for its capacity to predict a diverse range of approximately 4000 biological activities, encompassing pharmacological effects, molecular interactions, gene expressions, enzymatic responses, and various other relevant facets (Dhea Kharisma et al., 2018).

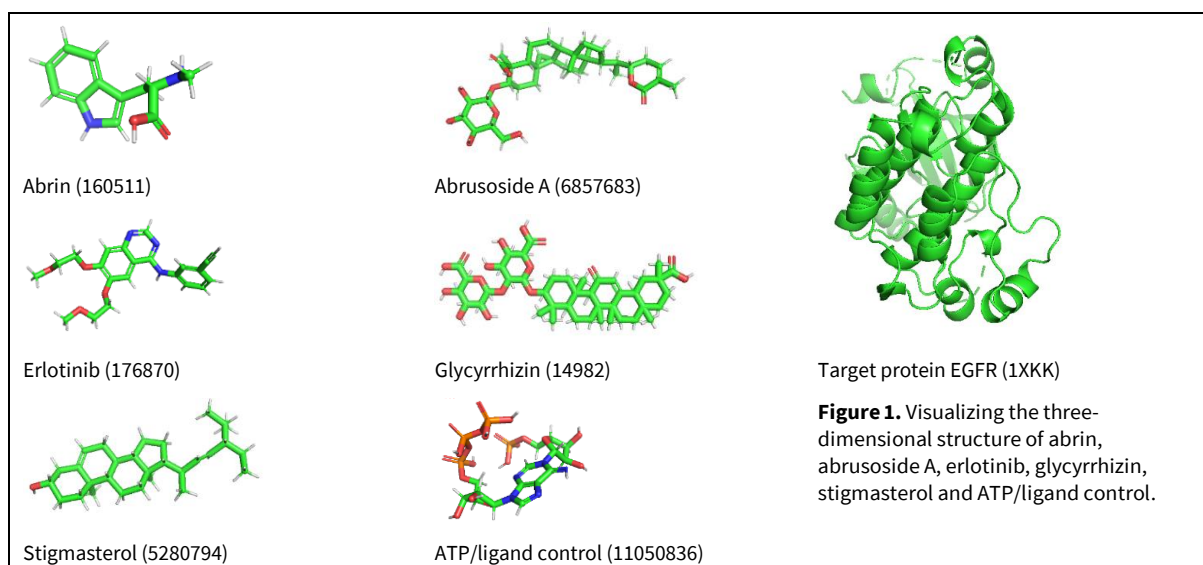


Table 1. Compound names, ID and canonical SMILES from *A. precatorius* leaves.

Compound name	Compound ID	Canonical SMILES
Erlotinib (reference drug)	176870	<chem>COCCOC1=C(C=C2C(=C1)C(=NC=N2)NC3=CC=CC(=C3)C#C)OCCOC</chem>
ATP	5957	<chem>C1=NC(=C2C(=N1)N(C=N2)C3C(C(C(O3)COP(=O)(O)OP(=O)(O)OP(=O)(O)O)O)N</chem>
Abrin	160511	<chem>CNC(CC1=CNC2=CC=CC=C21)C(=O)O</chem>
Abrusoside A	6857683	<chem>CC1=CCC(OC1=O)C(C)C2CCC3(C2(CCC45C3CC6C4(C5)CCC(C6(C)C(=O)O)OC7C(C(C(C(O7)CO)O)O)C)C</chem>
Glycyrrhizin	14982	<chem>CC1(C2CCC3(C(C2(CCC1OC4C(C(C(C(O4)C(=O)O)O)O)OC5C(C(C(C(O5)C(=O)O)O)O)C(C(=O)C=C6C3(CCC7(C6CC(CC7)(C)C(=O)O)C)C)C</chem>
Stigmasterol	5280794	<chem>CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C</chem>

Molecular docking and visualization

In order to assess the capacity of bioactive constituents derived from the leaves of *A. precatorius* to form interactions with the EGFR protein and subsequently compare their binding affinity with that of control compounds, a reference drug and control ligand using a molecular docking simulation was employed.

Molecular docking is a computational technique employed to calculate the binding energy related to the interaction between ligand molecules and the corresponding receptor (de Ruyck et al., 2016). Within the context of this study, the term "specific binding" denoted the comparative evaluation of binding energy between the query compounds and the control compounds, each interacting with the identical binding site (Dallakyn and Olson., 2015). The molecular docking simulations were executed using the VinaWizard tool, which is seamlessly accessible within the PyRx platform (Zidan et al., 2020). Moreover, for the three-dimensional visualization of the outcomes of the molecular docking simulations, the PyMol software was deployed (Kharisma and Ansori, 2020).

Molecular interaction

Through the utilization of the Discovery Studio 2.0 software, we undertook the task of identifying and subsequently comparing the interactions manifested within protein-ligand complexes, as yielded by the outcomes of molecular docking simulations (Susanto et al., 2018). The principal aim underpinning this analytical endeavor was to establish a comparative assessment between the bioactive ligands, reference drug, and control ligands, specifically concerning the core aim of this study was to assess their binding affinity to the binding site of the target protein, with a specific emphasis on gauging the level of resemblance between these affinities. The assorted forms of interactions examined encompassed phi bonds, hydro-

phobic bonds, and hydrogen bonds (Jamal et al., 2012). It is imperative to acknowledge that the presence of a lower frequency of adverse bond observations is conducive to the formation of molecular complexes characterized by stability.

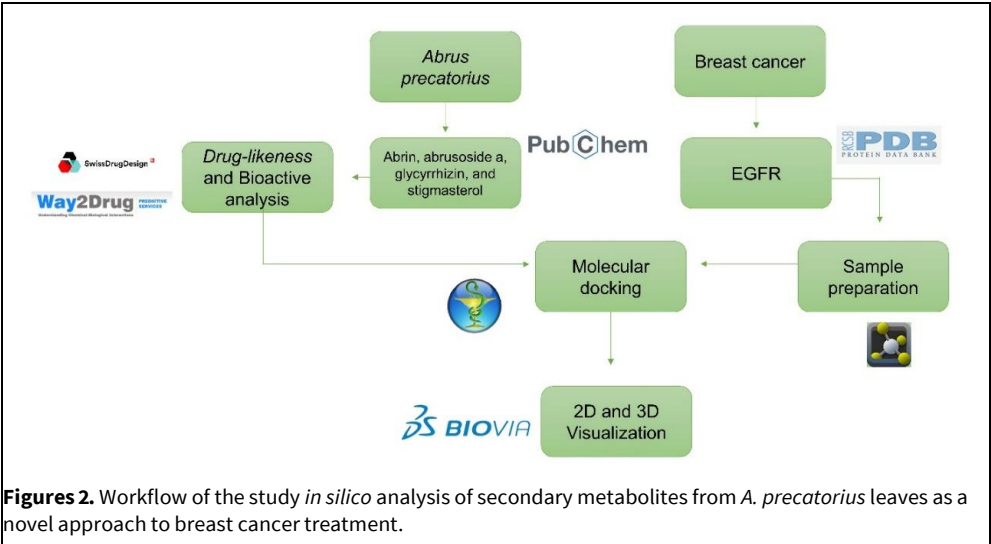
ADMET analysis

Once the main compound is identified, it's crucial to assess its properties related to ADMET to evaluate its effects on the human body. On the pkCSM website, physicochemical and pharmacokinetic predictions of active compounds were conducted. The ADMET profile analysis, which includes absorption, distribution, metabolism, excretion, and toxicity, was then performed by uploading the SMILE file or copying the SMILE code and selecting the prediction mode. Modern *in silico* ADMET studies rely on computational methods to select molecules with reasonable property values, allowing less competent candidates to be eliminated early on, and only desired ones to proceed to synthesis and biological testing. This approach ultimately leads to cost reduction by circumventing *in vivo* and *in vitro* testing (Ojha et al., 2021).

RESULTS

Analysis of drug-likeness and anticancer activity of bioactive compounds

The compounds designated as the subjects of investigation within this research were abrin (CID: 160511), abrusoside a (CID: 6857683), glycyrrhizin (CID: 14982), stigmasterol (CID: 5280794) and ATP for a ligand control also erlotinib for reference drug. PyMol software will be used to represent each sample as a three-dimensional stick structure (Fig. 1). The study focused on the EGFR target protein (PDB ID: 1XKK), and the data was extracted from the RCSB PDB database, available at <https://www.rcsb.org/>.



Following an evaluation based on the criteria relevant to drug similarity, it was expected that all chemical compounds originating from *A. precatorius* leaves would be deemed eligible candidates for drug molecules, as indicated by the findings in the drug similarity prediction results (Table 2). Further investigation is possible to ascertain the overall potential of all the chemicals found in *A. precatorius* leaves as breast anticancer agents. According to the biological activity prediction results, chemicals found in the leaves of *A. precatorius* exhibit anticancer properties when their medium probability is foreseen when the value of Pa exceeds 0.3, as illustrated in Table 3.

Molecular docking of bioactive compounds from *A. precatorius* leaves

Based on the results of molecular coupling simulations, the compounds of stigmasterol exhibit notably

lower binding energies than other compounds. This observation raises the possibility that the binding of stigmasterol to EGFR may exert an influence on the biological responses pertaining to the activity of breast cancer inhibitors (Table 4). Discovery Studio 2.0 was used to examine the molecular docking simulation interpretations. Green lines indicate the type of hydrogen bond interaction, orange bonds indicate alkyl bonds, purple bonds indicate van der Waals interactions and red bonds indicate unfavorable couplings. To determine the inhibitory potential against the EGFR protein, we examined the relationship between the bioactive chemicals found in the leaves of *A. precatorius* and the control substance. Corresponding interaction sites of the ligand-protein complex can be identified within A:CYS797, A:ASP855, A:LEU718, A:VAL726, A:LEU844, and A:GLY796, according to the results in Fig. 3.

Table 2. Chemical compounds of *A. precatorius* leaves are eligible candidates for drug molecules based on hydrogen bonding.

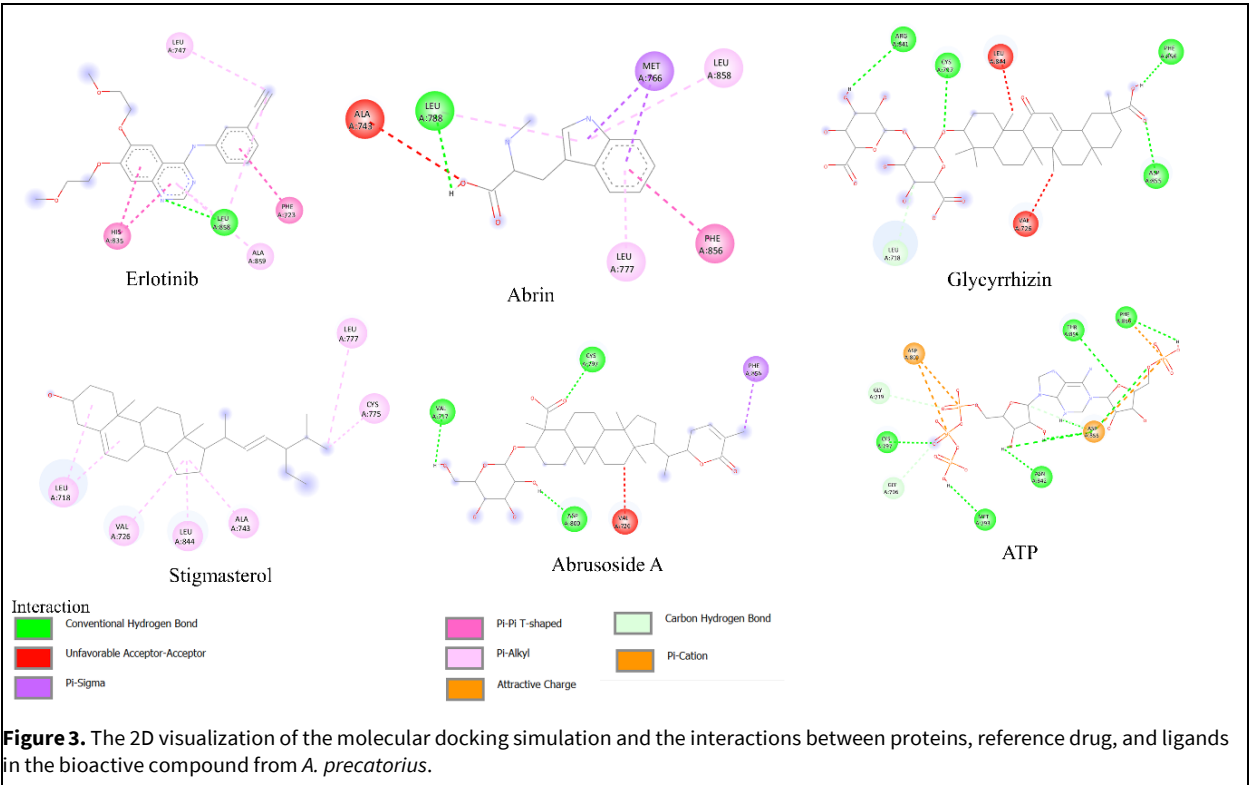
Bioactive compound	Binding energy (kcal/mol)	Number of hydrogen bond	Number of hydrophobic bonds	Amino acids involved in hydrogen bonding	Amino acids involved in hydrophobic bonding
Abrin	-7.3	1	6	LEU788	MET766, MET766, PHE856, LEU788, LEU858, LEU777
Abrusoside A	-7.7	4	1	CYS797, CYS797, VAL717, ASP800	PHE856
Stigmasterol	-9.9	-	7	-	LEU718, VAL726, ALA743, LEU844, LEU718, CYS775, LEU777
Glycyrrhizin	-4.1	5	-	CYS797, ASP855, ARG841, PHE856, LEU718	-
ATP	-8.7	15	-	CYS797, THR854, ASP855, ASP855, MET793, ASN842, ASP855, ASP855, PHE856. GLY719, GLY796, ASP855	-
Erlotinib (reference drug)	-6.8	1	8	ASP 871	LEU858, HIS835, HIS835, VAL765, VAL769, LEU828, LEU833, LEU858

Table 3. Predicting the biological activity of compounds obtained from *A.precatorius* leaves using the PASSOnline.

Compound	Pa	Pi	Biological activity
Abrin	0.472	0.005	Cancer-associated disorder treatment
Abrusoside A	0.517	0.017	Antineoplastic (breast cancer)
Glycyrrhizin	0.641	0.008	Antineoplastic (breast cancer)
Stigmasterol	0.261	0.067	Antineoplastic (breast cancer)
Erlotinib	0.791	0.003	Epidermal growth factor receptor kinase inhibitor

Table 4. Biological responses about the activity of breast cancer inhibitors of compounds derived from *A. precatorius* leaves.

Compound name	Molecular weight (g/mol)	High lipophilicity (LogP)	Hydrogen bond donors	Hydrogen bond acceptors	Molar refractivity
Erlotinib (reference drug)	393.44	1.48	1	6	111.40
ATP	507.18	-4.79	7	16	95.40
Abrin	218.25	-1.38	3	3	62.26
Abrusoside A	646.81	2.42	5	10	168.47
Glycyrrhizin	822.93	0.02	8	16	202.84
Stigmasterol	412.69	6.62	1	1	132.75



ADMET properties of bioactive compounds and erlotinib

Table 5 presents a total of 14 ADMET property parameters, classified into categories for toxicity, distribution, metabolism, excretion, and absorption. Drugs access the human body through four distinct path-

ways: absorption, distribution, metabolism (biotransformation), and elimination, collectively known as ADME regulation. Analogous to ADMET properties analysis, the pharmacokinetic approach is employed to determine if a medication can produce the necessary pharmacological effects (Zhong, 2017).

Table 5. ADMET properties.

ADMET properties	Abrin	Abrusoside A	Glycyrrhizin	Stigmasterol	Erlotinib (reference drug)
Water solubility (Log mol/L)	-2.171	-3.263	-2.892	-6.682	-4.403
CaCO ₂ Permeability (Log P app in 10 ⁻⁶ cm/s)	0.664	-0.39	-0.812	1.213	1.238
Intestinal absorption (% absorption)	100	41.123	0	94.97	95.549
VDss (log L/kg)	-1.782	-1.502	-0.615	0.178	-0.053
BBB Permeability (Log BBB)	-0.23	-1.191	-1.494	0.771	-0.67
CYP2D6 Substrate	No	No	No	No	No
CYP3A4 Substrate	No	Yes	Yes	Yes	Yes
CYP1A2 Inhibitor	No	No	No	No	Yes
CYP2C19 Inhibitor	No	No	Yes	No	Yes
Renal OCT2 Substrate	No	No	No	No	No
Total clearance	0.781	0.174	-0.304	0.618	0.591
AMES toxicity	No	No	No	No	No
Max. dosage tolerance (human) (log mg/kg/day)	0.694	-0.372	0.389	-0.664	-0.002
hERG I Inhibitor	No	No	No	No	No
hERG II Inhibitor	No	No	No	Yes	Yes

DISCUSSION

In order to assess the viability of a molecule for consideration as a prospective pharmaceutical candidate, an analysis of its suitability for drug development, commonly referred to as "drug-likeness analysis," is conducted. (Wang et al., 2019). Bioactive constituents derived from *A. precatorius* leaves must adhere to established criteria and specific guidelines to be classified as promising drug candidates.

Lipinski characteristics were applied in this analysis to ascertain prospective pharmaceutical candidates. The SWISS ADME web server is employed as a tool for identification and evaluation

(<https://www.swissadme.ch/>). The defined criteria include a molar refractivity that falls within the range of 40 to 130, a limited lipophilicity value not surpassing 5, no more than five hydrogen bond donors, a maximum allowance of 10 hydrogen bond acceptors, and a molecular mass below 500 Daltons (Daina et al., 2017). The rule of every parameter explains the molecule's solubility in fat or water, its molecular weight, its lipophilicity efficiency, and its capacity to serve as an agent for instigating particular biological responses (Wang et al., 2019). Utilizing the PASSOnline Server, which is accessible at <http://www.pharmaexpert.ru/passonline/>, the general potential of each chemical component was determined in this analysis. This system utilizes the

activation probability score (Pa) and inhibition score (Pi) For the purpose of predicting biological activity, encompassing pharmacological effects, interactions, and toxicity, for a set of 4000 chemical compounds (Rahmaningsih and Pujiastutik, 2019). Pa indicates the probability that a possible query compound is active, whereas the Pi score signifies the extent of inhibition (Susanto et al., 2018). The analysis's results must be retested to predict the mechanism where the bioactive compounds of *A. precatorius* leaves carry out these biological activities. Additionally, more research must be done to analyze the mechanism where the bioactive compounds of *A. precatorius* leaves act as breast anticancer agents.

Moreover, the suppression of EGFR protein activity by advantageous chemical compounds present in the leaves of *A. precatorius* significantly contributes to the reduction of breast cancer incidence. The molecular docking method was applied in this investigation to determine the bioactive chemicals found in *A. precatorius* leaves that bind to the EGFR protein. When molecules interact, binding affinity with negative energy is created, allowing the formation of a stable complex (Dhea Kharisma et al., 2018). Stigmasterol, as a ligand, exhibits a binding affinity characterized by a value of -9.9 kcal/mol, signifying its ability to establish bonds with the most pronounced negative energy when contrasted with the control ligand ATP, also erlotinib as a reference drug. However, when it comes to fulfilling Lipinski's criteria, stigmasterol falls short in terms of its lipophilicity (LogP).

Abrusoside A, with a molecular weight exceeding 500 Da, fails to meet one of Lipinski's criteria. In contrast, abrin satisfies all Lipinski's rules. However, glycyrrhizin differs as it fails to meet three criteria: its molecular weight exceeds 500 Da, it has more than ten hydrogen bond acceptors, and it has more than five hydrogen bond donors. Among these compounds, abrin, abrusoside A, and stigmasterol meet criteria akin to drug-like molecules, with no more than one violation of Lipinski's rules. Consequently, according to the SWISS ADME analysis (<https://www.swissadme.ch/>), stigmasterol and abrusoside A exhibit limited absorption potential within the gastrointestinal tract.

Stigmasterol, categorized as an unsaturated phytosterol belonging to the class of tetracyclic triterpenes, is the focal point of examination concerning its pharmacokinetic attributes. Pharmacokinetics encompasses the comprehensive examination of the processes involving the absorption, distribution, metabolism, and elimination of pharmaceutical compounds or other substances within the biological system (Bakrim et al., 2022). A comprehensive study was undertaken to thoroughly investigate the pharmaco-

kinetic properties of β -sitosterol, campesterol, and stigmasterol within the plasma of rats. The research endeavor involved the meticulous development and validation of a liquid chromatography technique integrated with atmospheric pressure chemical ionization tandem mass spectrophotometry. It was discerned that the method exhibited linearity across a concentration range spanning from 250 to 5000 ng/mL for β -sitosterol and campesterol, while for stigmasterol, this range extended from 50 to 2000 ng/mL (Kim et al., 2016).

The principal aim of another aspect of this study was to scrutinize the inherent potential for chemopreventive and anti-breast cancer characteristics within compounds derived from the leaves of *A. precatorius*. Through rigorous investigation, it was revealed that stigmasterol, in conjunction with various other compounds, exhibited remarkable effectiveness in countering breast cancer, thus underscoring its significance as a potential agent in the fight against this malignancy (Sofi et al., 2018). Furthermore, no data was procured from the literature regarding the specific binding affinity and the chemical interactions formed between stigmasterol and proteins.

Using Discovery Studio 2.0 software, the docked molecular ligand-protein complex was then analyzed to determine the type and location of the formed chemical bond connections (Fig. 4). The formation of molecular complexes is attributed to the presence of non-covalent interactions, such as hydrophobic, alkyl, hydrogen, Van der Waals, and π bonds (Jamal et al., 2012). Abrusoside A compound interacts within the EGFR protein domain, forming a hydrophobic bond at the PHE856 position and hydrogen bonds at positions CYS797, CYS797, VAL717, and ASP800. Abrin compound interacting with EGFR protein forms hydrogen bonds at the LEU788 position and hydrophobic bonds at the MET766, MET766, PHE856, LEU788, LEU858, and LEU777 positions. EGFR interacts with stigmasterol, which has the highest binding affinity of -9.9 kcal/mol, forming hydrophobic bonds at positions LEU718, VAL726, ALA743, LEU844, CYS775, LEU777, and LEU718. The study reveals that hydrogen bonding and optimizes hydrophobic interactions act synergistically to stabilize ligands at the target site, exerting a dual influence on both binding affinity and drug efficacy, with the significance of hydrogen bonds particularly emphasized in molecular docking visualization (Patil et al., 2010). These interactions play a pivotal role in eliciting biological responses within the target protein. In the context of this study, the term "reference molecule" refers to the ligand binding to the same domain as the query ligand (Kharisma and Ansori, 2020).

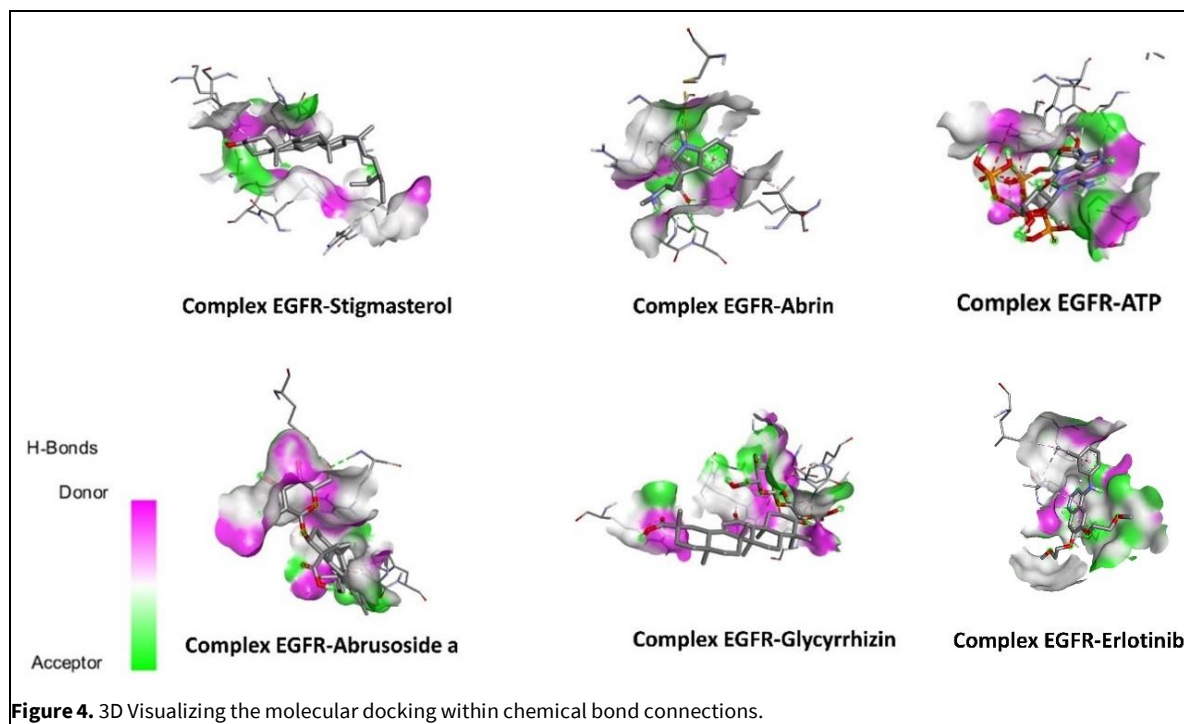


Figure 4. 3D Visualizing the molecular docking within chemical bond connections.

Because they contain the same bond as the control molecule, bioactive compounds derived from the leaves of *A. precatorius* are anticipated to operate as inhibitors of the EGFR protein. The bioactive component of the leaves of *A. precatorius* and the identified control share a similar contact site, which contributes to the stability of the molecular complex (Susanto et al., 2018).

Binding analysis showed that these molecules have the potential to efficiently bind to drug targets, particularly as inhibitors of the EGFR protein. Negative and low values of binding affinity signify a strong favorable binding between the protein and the ligand, especially when the ligand is in its most favorable confirmation. The observed ligands show potential as the best inhibitor candidates to inhibit breast cancer. The difference in affinity values is related to the formation of hydrogen bonds in the binding pocket. This study aims to help focus on drug design using an *in silico* approach to the EGFR target protein, which is recognized as a treatment target for breast cancer. Fig. 3 shows that the docked ligand can bind to the target protein on the same active side.

From the pkCSM website, abrin has a water solubility (Log mol/L) of -2.171, indicating low solubility in water. Its CaCO₂ permeability is 0.664, showing moderate permeability through cell membranes. Abrin has 100% intestinal absorption, indicating excellent absorption in the intestines. The volume of distribution (VDss) is -1.782, indicating limited distribution within the body. Its BBB permeability (Log

BBB) is -0.23, showing very low ability to cross the blood-brain barrier. Abrin is neither a substrate for CYP2D6 or CYP3A4 nor an inhibitor of CYP1A2 or CYP2C19 nor a renal OCT2 substrate. According to the AMES test, abrin has a total clearance of 0.781 and is non-toxic. The maximum tolerated dose in humans (Log mg/kg/day) is 0.694. Abrin is not an inhibitor of hERG I or hERG II.

Abrusoside A has a lower water solubility with a Log mol/L value of -3.263. Its CaCO₂ permeability is -0.39, indicating low permeability. Intestinal absorption is 41.123%, indicating moderate absorption. The volume of distribution (VDss) is -1.502, suggesting limited distribution. Its BBB permeability is -1.191, indicating a very low ability to cross the blood-brain barrier. Abrusoside A is not a substrate for CYP2D6 but is a substrate for CYP3A4. It does not inhibit CYP1A2 or CYP2C19 and is not a renal OCT2 substrate. Total clearance is 0.174, and it is non-toxic according to the AMES test. The maximum tolerated dose in humans is -0.372, and it is not an inhibitor of hERG I or hERG II.

Glycyrrhizin has a water solubility of -2.892 Log mol/L. Its CaCO₂ permeability is -0.812, showing low permeability. Intestinal absorption is 0%, indicating no absorption in the intestines. The volume of distribution (VDss) is -0.615, suggesting limited distribution. Its BBB permeability is -1.494, indicating a very low ability to cross the blood-brain barrier. Glycyrrhizin is not a substrate for CYP2D6 but is a substrate for CYP3A4. It inhibits CYP2C19 but not CYP1A2 and

is not a renal OCT2 substrate. Total clearance is -0.304, and it is non-toxic, according to the AMES test. The maximum tolerated dose in humans is 0.389. Glycyrrhizin is not an inhibitor of hERG I but is an inhibitor of hERG II.

Stigmasterol has very low water solubility with a Log mol/L value of -6.682. Its CaCO₂ permeability is 1.213, indicating good permeability. Intestinal absorption is 94.97%, showing very high absorption. The volume of distribution (VD_{ss}) is 0.178, suggesting a broader distribution. Its BBB permeability is 0.771, indicating a moderate ability to cross the blood-brain barrier. Stigmasterol is not a substrate for CYP2D6 but is a substrate for CYP3A4. It does not inhibit CYP1A2 or CYP2C19 and is not a renal OCT2 substrate. Total clearance is 0.618, and it is non-toxic according to the AMES test. The maximum tolerated dose in humans is -0.664. Stigmasterol is not an inhibitor of hERG I but is an inhibitor of hERG II.

Erlotinib, the reference drug, has a water solubility of -4.403 Log mol/L. Its CaCO₂ permeability is 1.238, indicating good permeability. Intestinal absorption is 95.549%, showing very high absorption. The volume of distribution (VD_{ss}) is -0.053, suggesting limited distribution. Its BBB permeability is -0.67, indicating a low ability to cross the blood-brain barrier. Erlotinib is not a substrate for CYP2D6 but is a substrate for CYP3A4. It inhibits CYP1A2 and CYP2C19 and is not a renal OCT2 substrate. Total clearance is 0.591, and it is non-toxic according to the AMES test. The maximum tolerated dose in humans is -0.002. Erlotinib is not an inhibitor of hERG I but is an inhibitor of hERG II.

CONCLUSION

Through *in silico* analysis utilizing PyRx 0.8 and Discovery Studio 2021 software, the mechanism of action of *A. precatorius* compounds inhibiting EGFR protein was explored. Bioactive components, including abrin, abrusoside A, and stigmasterol, exhibited drug similarity, except glycyrrhizin. With a binding affinity of -9.9 kcal/mol, stigmasterol outperformed ATP and Erlotinib, which registered -8.7 and -6.8 kcal/mol, respectively, underscoring its potential as a leading anticancer agent. *A. precatorius* holds promise as a breast cancer alternative treatment pending further research on compound isolation and *in vitro* and *in vivo* testing. Additionally, based on the ADMET properties, stigmasterol stands out as the most favorable compound, exhibiting excellent intestinal absorption (94.97%) and good permeability (CaCO₂ permeability of 1.213), indicating efficient absorption and cellular transport, along with moderate blood-brain barrier permeability (0.771), making it potentially

useful for both central nervous system and peripheral targets.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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