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In Silico Screening of Flavonoids from Aratiles (*Muntingia calabura* L.) for Potential Anti-Angiogenic Activity

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Abstract

In silico methods have been widely acknowledged by researchers as efficient and effective tools for accelerating drug discovery through computer modelling. This study explored the anti-angiogenic potential of flavonoids from Aratiles (*Muntingia calabura* L.) the first *in silico* screening of specific flavonoids against a panel of key angiogenic protein receptors, thereby providing a more precise evaluation of its anti-angiogenic potential than previously reported crude extract studies. PyRx software was used to assess each flavonoid ligand's binding score followed by BIOVIA Discovery Studio Visualizer Analysis to determine the binding site, docking pose, binding affinity score, and specific amino acid interactions of each flavonoid ligand within the receptor sites. Out of the forty (40) initial flavonoid ligands, twenty-three (23) were found to comply with Lipinski's Rule of 5, indicating favorable ADME properties (Absorption, Distribution, Metabolism, and Excretion). Using ProTox-3.0-2024 web server, seventeen (17) out of these twenty-three (23) flavonoid ligands have passed the toxicity screening being categorized as Class V- may be harmful and Class VI- non-toxic. The selected flavonoid ligands were then analyzed for their binding affinity to five (5) angiogenic receptors: Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) (Kent, 2012), Fibroblast Growth Factor Receptor 2 (FGFR-2), Transforming Growth Factor Beta Receptor 1 (TGF-βR-1), Insulin-like Growth Factor 1 Receptor (IGF-1R), and Endothelial Growth Factor Receptor (EGFR). Ultimately, flavonoid ligand 17 identified as 7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone showed strong binding interactions ranging from -9 to -11 kcal/mol for all five (5) angiogenic receptors. These values were almost equal to, and in some instances, higher than those of the control inhibitors. By and large, flavonoid ligand 17 from Aratiles demonstrated a promising anti-angiogenic potential and may serve as a lead compound for further development in anti-cancer therapy.

Keywords: Anti-angiogenesis, Flavonoids, *In silico*, *Muntingia calabura* L.

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Introduction

Traditional drug research and development (R&D), such as in vitro and in vivo approaches, is a laborious, expensive, and time-consuming process that involves a series of stages with only a 13.8% probability of success rate (Wong et al., 2018; Shaker et al., 2021). Many technologies have been developed to address these challenges, including Computer-aided Drug Design (CADD) (Tang et al., 2006). CADD involves using computational techniques, bioinformatics tools and models to identify drug-like molecules. It also analyzes and predicts the biological activity and physicochemical properties of potential drug candidates.

Recently, CADD strategies have gained popularity for their ability to enhance the production scale, save time, and cut costs associated with the experimental phase (Shaker et al., 2021). These approaches are often referred to as *in silico* methods which include molecular modeling and simulations performing biological studies (Osakwe, 2016) like binding and interaction patterns of the possible drug candidate (Roney & Aluwi, 2024). These minimize the use of live subjects, address ethical concerns and speed up the research time, reduces the need for costly laboratory experimentations (Beyleveld et al., 2013), as well as make predictions, offer theories, and aid discoveries and advancements in various fields like in medicine (Ekins et al., 2007). *In silico* techniques have been integrated int nearly every stage of drug R&D which has significantly transformed the pipeline and strategy for drug discovery (Tang et al., 2006).

Muntingia calabura L. or Jamaican cherry, locally called "Aratiles" in the Philippines, is a slender tree with a height of up to 12 meters (Dean, 2024; Mahmood, 2014). It is resistant to acidic, alkaline, and drought environments (Kathirvel & Sundar, 2025; Nasir et al., 2017; Osorno et al., 2024). It has a long history of usage in traditional folk medicine among the people of Peru, Cambodia, Colombia, Vietnam, and the Philippines.

The bark of Aratiles exhibits antibacterial, anti-inflammatory, and anti-ulcer characteristics. The flowers are traditionally used for their antispasmodic, antibacterial, and wound-healing properties. The leaves and fruits have been decocted to treat headaches, colds, gastric ulcers, prostate disorders, diabetes, and respiratory ailments, while the fruits themselves are consumed for their antioxidant, antimicrobial, antidiabetic, anticancer, anti-gout, and digestive-supportive properties due to their high vitamin, flavonoid, and anthocyanin levels (Jisha et al., 2020; Kathirvel & Sundar, 2025; Kuchekar et al., 2021; Lazatin, 2019; Saud et al., 2023; Tinrat & Jiraprasertwong, 2023; Zakaria et al., 2008).

One of the prevalent diseases in the Philippines is cancer, which has 188,976 new cases and 113,369 deaths in 2022. The first stage in the development of cancer is the formation of new blood vessels from existing blood vessels is known as angiogenesis (Ferlay et al., 2024; Zhang et al., 2024; Rad, 2015).

It usually supports body healing and helps supply nutrients but becomes a crucial and deadly process as cancer cells rely on nutrients, oxygen, and other essentials from the host to support their growth which eventually becomes a tumor or mass of cancer cells (Ferlay et al., 2024; Cooper, 2000).

Numerous studies suggest that flavonoids have characteristics that prevent the growth of angiogenesis (Khater & Osborn, 2020; Pandey et al. 2025; Subbaraj, et al., 2021; Wei & Zhang, 2024). Flavonoids are phytochemicals that are ubiquitously present as constituents of flowering plants involved in pigmentation, odor, and color of flowers. They also exhibit antiviral, anti-allergic, antibacterial, and anti-inflammatory properties (Abotaleb et al., 2018; Dias et al., 2021). Riganti & Contino (2019) indicated that flavonoids can increase the potency of anti-cancer drugs by affecting signaling pathways in the early stages of cancer and in the advanced stages like metastasis. Moreover, certain flavonoids act as inhibitors in the propagation of new cells by acting as a replacement for certain ligands such as Vascular Endothelial Growth Factor (VEGF) which binds to protein receptors to promote cancer growth (Liskova et al., 2020).

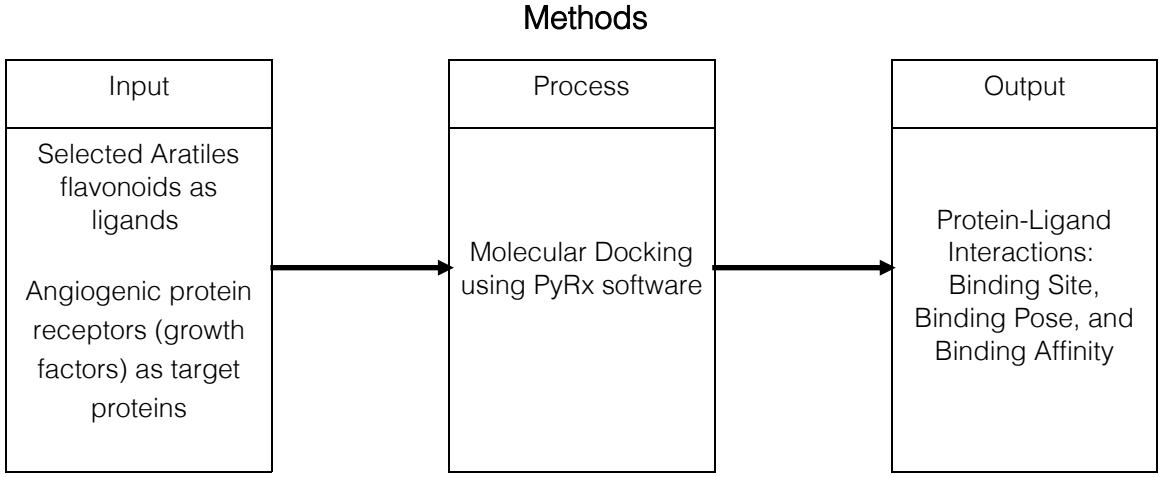
In the study of Nas (2020), he identified 40 flavonoids from *Muntingia calabura* aqueous leaf extract and evaluated their Absorption, Distribution, Metabolism, and Excretion (ADME) properties. ADME refers to the four key pharmacokinetic properties or bioavailability that help predict whether a compound will behave well in the body. It screens compounds that, despite good docking scores, would fail in real-world settings due to poor ADME properties and/or inability to reach the target subject of interest. This study builds on the study of Nas (2020) by utilizing these identified flavonoids and screened their potential anti-angiogenic properties *in silico*, thus, filling a gap in the targeted, ligand-based evaluation of *Muntingia calabura's* anti-angiogenic activity through the integration of CADD (Computer-Aided Drug Design). Protein-ligand "molecular docking" interaction is a key research area which is employed in nutraceutical research and has become a vital tool in drug discovery (Agu et al., 2023). Rather than studying crude extracts, the researchers mined forty flavonoid-ligand structures from Protein Data Bank (PDB) and Pubchem Database, filtered them based on pharmacokinetic and toxicity parameters, and conducted molecular docking simulations against five pro-angiogenic protein receptors.

This study generally aimed to determine the anti-angiogenic potential of flavonoids found in Aratiles (*Muntingia calabura* L.) via *in silico* screening. Specifically, it screened Aratiles' flavonoids that satisfied the Lipinski's rule of 5 and have acceptable values in terms of solubility and toxicity level, compared and ranked the different molecular docking results of the selected flavonoids with a well-studied angiogenic growth factor inhibitor, and analyzed the molecular interaction of the ligand with the highest binding affinity. Lipinski Rule of Five evaluates a compound's drug-likeness, along with toxicity screening. Numerous flavonoids have anti-angiogenic properties; however, they do not satisfy the Lipinski rule, which limits their potential for therapeutic application. The Lipinski rule sets the criteria for molecular properties consisting of molecular weight, hydrogen bond donors and acceptors, and lipophilicity, which are all key variables in predicting a compound's oral bioavailability (Pillai *et al., 2001).*

Aratiles is rich in phytochemicals such as flavonoids, triterpenoids, tannins, saponins, and glycosides (Ansori, 2021; Gilbis & Yasmin 2022; Nas, 2020; Pratiwi & Dewi, 2022; Stuart, n.d.). Due to the Aratiles' rich ethnomedicinal background, abundance, and availability, especially in the Philippines, it is a viable candidate in search of plant-based anti-angiogenic drugs. Therefore, exploring the potential of Aratiles-derived flavonoids as anti-angiogenic agents merits investigation. Figure 1 shows the framework of the study.

Figure 1

The framework of the study.

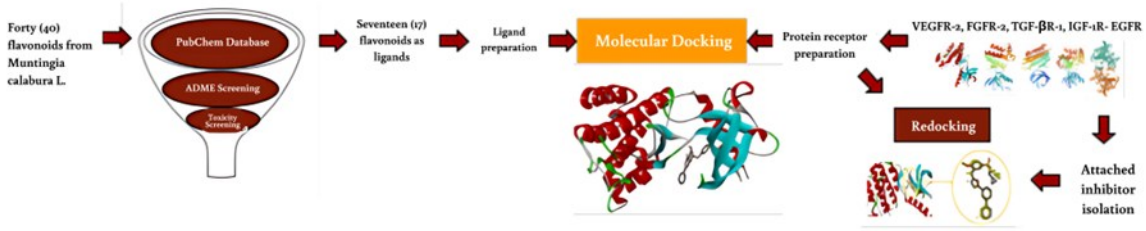


Research Design

All docking simulations (ligand-protein receptors) were performed using the same software to ensure consistency. Data analysis involved comparing docking scores to determine the flavonoids with the highest potential anti-angiogenic activity. The study follows the Structure-Based Drug Design (SBDD) which is a rational drug-discovery approach that uses the three-dimensional structure of a biological target to design or optimize small molecules that will bind with high affinity and specificity to that target. This study specifically used SBDD as the three-dimensional structures of the five angiogenic target proteins are known and available in the Protein Data Bank (PDB). These structural models were utilized for molecular docking simulations. Furthermore, by using SBDD, the study enables the design and evaluation of ligand molecules specifically selected flavonoids to fit precisely within the binding sites of the target proteins. This targeted approach ensures a systematic, accurate, and reliable assessment of the flavonoids' potential anti-angiogenic efficacy.

Figure 2

The Flowchart of the study.



Ligand Selection

The 40 distinct flavonoids found in *Muntingia calabura* L. based on scientific literature by Nas (2020), were the following: (1) Delphinidin-3-O-glucoside (CID: 443650), (2) Cyanidin-3-O-glucoside (CID: 197081), (3) Gallocatechin (CID: 65084), (4) Epigallocatechin (CID: 72277), (5) Catechin (CID: 73160), (6) Epicatechin (CID: 72276), (7) Naringenin (CID: 439246), (8) Rutin (CID: 5280805), (9) Quercetin (CID: 5280343), (10) Protocatechuic acid (CID: 72), (11) 4-Hydroxybenzoic acid (CID: 135), (12) Vanillic acid (CID: 8468), (13) Gentisic acid (CID: 3469), (14) Gallic acid (CID: 370), (15) Chlorogenic acid (CID: (16) 1794427), (17) Caffeic acid (CID: 689043), (18) p-Coumaric acid (CID: 637542), (19) Sinapic acid (CID: 637775), (20) Ferulic acid (CID: 445858), (21) 2′,4′-dihydroxy-3′-methoxydihydrochalcone (CID: 14157883), (22) 3'-Formyl-2',6', beta-trihydroxy-4'-methoxy-5'-methylchalcone (CID: 5319471), (23) 5′-hydroxy-7,3′,4′-trimethoxyflavanone (CID: 5272653), (24) 8-hydroxy-10-methoxy-5H-isochromeno[4,3-b] chromen-7-one (CID: 139030740), 7-hydroxyflavanone (CID: 1890), (25) 2′,4′-dihydroxychalcone (CID: 5376979), (26) 2′,4′-dihydroxydihydrochalcone (CID: 586491), (27) 5,7-dimethoxy-4-hydroxyflavone (CID: 161172), (28) 3,5-dihydroxy-6,7-dimethoxyflavone (CID: 13291608), (29) 5-hydroxy-7-methoxyflavone (CID: 4101463), (30) 3,7-dimethoxy-5-hydroxyflavone (CID: 5748697), (31) 3,5-dihydroxy-7-methoxyflavone (CID:5318691), (32) 5,7-dihydroxy-3,8-dimethoxyflavone (CID: 9972910), (33) galangin (CID:5281616), (34) chrysin (CID: 5281607), (35) 7-hydroxy-8-methoxyflavanone (CID: 71762131), (36) 4′-hydroxy-7-methoxyflavanone (CID: 676307), (37) 2′,4′-dihydroxy-3′-methoxychalcone (CID:10612087), (38) 5-hydroxy-3,7,8-trimethoxyflavone (CID: 21632881), (39) 2′,3′-dihydroxy-4′-methoxychalcone (CID: 5743235), and (40) 7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone (CID: 5403474).

The aforementioned flavonoids served as a starting point in screening for potential anti-angiogenic activity. To ensure the drug-likeness and effectiveness, Lipinski's Rule of Five test was performed as well as their bioavailability and solubility. Ultimately, the flavonoids were narrowed down to 23 based on the pre-screening results. Compounds with solubility values between -7 and -2.3 and bioavailability scores ≥ 0.55 were considered ideal and can be absorbed very well by the body (Long et al., 2009; Martin, 2005). The table below shows that seventeen (17) out of these (23) flavonoids met the safety standards (Table 1).

Table 1

Flavonoids Lipinski, Solubility, Bioavailability, and Toxicity Screening Process

Name of Flavonoids	Lipinski rule violation	Solubility	Predicted Toxicity Level	Bio-availability score	TOTAL violations
Acceptable values	*0*	*-7.1 - -2.3*	*Class VI or Class V (>50%)*	*≥ 0.55*	*0*
Naringenin	0	-3.11	IV (69.26%)	0.55	1
Sinapic acid	0	-2.55	IV (70.97%)	0.56	1
2′,4′-dihydroxy-3′-methoxydihydrochalcone	0	-3.94	IV (69.26%)	0.55	1
3'-Formyl-2',6',beta-trihydroxy-4'-methoxy-5'-methylchalcone	0	-4.05	IV (69.26%)	0.56	1
5′-hydroxy-7,3′,4′-trimethoxyflavanone	0	-4.18	V (70.97%)	0.55	0 (Ligand 1)
8-hydroxy-10-methoxy-5H-isochromeno[4,3-b]chromen-7-one	0	-3.82	V (69.26%)	0.55	0 (Ligand 2)
7-hydroxyflavanone	0	-3.33	IV (69.26%)	0.55	1
2′,4′-dihydroxychalcone	0	-3.27	V (69.26%)	0.55	0 (Ligand 3)
2′,4′-dihydroxydihydrochalcone	0	-3.27	IV (69.26%)	0.55	1
5,7-dimethoxy-4-hydroxyflavone	0	-3.96	V (70.97%)	0.55	0 (Ligand 4)
3,5-dihydroxy-6,7-dimethoxyflavone	0	-3.26	V (70.97%)	0.55	0 (Ligand 5)
5-hydroxy-7-methoxyflavone	0	-3.44	V (70.97%)	0.55	0 (Ligand 6)
3,7-dimethoxy-5-hydroxyflavone	0	-3.76	V (70.97%)	0.55	0 (Ligand 7)
3,5-dihydroxy-7-methoxyflavone	0	-3.58	V (70.97%)	0.55	0 (Ligand 8)
5,7-dihydroxy-3,8-dimethoxyflavone	0	-3.70	V (70.97%)	0.55	0 (Ligand 9)
Galangin	0	-3.36	V(70.97%)	0.55	0 (Ligand 10)
Chrysin	0	-3.38	V(70.97%)	0.55	0 (Ligand 11)
7-hydroxy-8-methoxyflavanone	0	-3.71	V (70.97%)	0.55	0 (Ligand 12)
4′-hydroxy-7-methoxyflavanone	0	-3.86	V (72.97%)	0.55	0 (Ligand 13)
2′,4′-dihydroxy-3′-methoxychalcone	0	-3.92	V (69.26%)	0.55	0 (Ligand 14)
5-hydroxy-3,7,8-trimethoxyflavone	0	-4.08	V (70.97%)	0.55	0 (Ligand 15)
2′,3′-dihydroxy-4′-methoxychalcone	0	-3.95	V (69.26%)	0.55	0 (Ligand 16)
7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone	0	-4.67	V (70.97%)	0.55	0 (Ligand 17)

Docking Procedure

Five protein receptors were screened and selected to serve as the specific target as they play significant roles in regulating angiogenesis. There were many protein receptors involved in the process of angiogenesis, but these five (5) were chosen based on their high resolution structures which is less than 2.5 angstroms. These target proteins have attached inhibitors or ligands in their structure. By isolating these inhibitors, the researchers set them as controls and served as the basis for the flavonoids binding effectiveness by re-docking them together with the 17 selected flavonoid ligands. The resulting scores were compared to the 17 flavonoids which act as ligands. The control ligands were originally attached to their specific target protein and were proven to have inhibitory effects based on their curated Protein Data Bank (PDB) profiles.

In the preparation of the protein receptors using PyRx, a strict adherence to the protocol was observed to ensure the structure was adequately optimized for molecular docking. The protein structures were first imported from PDB and cleaned by deleting water molecules and any unnecessary ligands systematically in order to avoid interference during docking using BIOVA software. In the case of proteins in which cofactors or metal ions are crucial to structural integrity or to binding, these were retained carefully and placed appropriately. The software was then employed to assign specific bond orders such that the atomic connectivity accurately mirrored the biological form. Polar hydrogens were next added explicitly, which was essential to accurately model hydrogen bonding interactions in the binding site. Simultaneously, partial charges were added with standard procedures, specifically the Kollman charges, which are crucial for computing the electrostatic interactions between the potential ligands and the receptor. In addition, there were no missing atoms or structural ambiguity features that had to be corrected, accompanied by an energy minimization step that re-optimized the protein conformation to its most stable form and maintained important structural features. This careful multi-step preparation process ensures that the receptor was thoroughly optimized, hence, offering a sound basis for high-quality docking experiments with PyRx.

Software Used

This study was conducted at the University of the Philippines High School in Iloilo, Computer and Science laboratories. All software used for screening and molecular docking was downloaded and installed on available computers.

All the ligand structures were acquired from the PubChem database. This is the largest repository, most easily accessible database for chemical and molecular information used to identify the structure of flavonoids. It is part of the National Library of Medicine, which is part of the US National Institutes of Health (Eth zurich, n.d.). The five (5) angiogenic protein receptors were acquired in Protein Data Bank (PDB). It is a database for the worldwide archive of 3D structure for biomolecules (i.e. proteins, DNA, and RNA).

SwissADME (ver. 2024) was used for the first screening of 40 flavonoids. It is a free web tool used to evaluate the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. ProTox-3.0 (ver. 3.0) was used to determine the toxicity levels of the selected flavonoids. It is a web server with high prediction accuracy that applies molecular similarity, pharmacophores, fragment propensities, and machine learning models to predict various toxicity endpoints. The twenty-three (23) flavonoids passed the screening and they further underwent toxicity screening to filter the flavonoids that proved to be safe for human consumption.

PyRx software (ver. 0.8) was used to dock the selected flavonoids to the five angiogenic proteins. From this, the researchers calculated their binding scores and demonstrated the interaction strength of each ligand with its respective protein receptor. This also acted as an application of virtual screening in computer-aided drug discovery, widely employed to assess diverse compounds at possible drug targets. The researchers used BIOVIA Discovery Studio Visualizer (ver. 2021) to examine the position of ligands as well as specific amino acid interactions between the ligands and the receptors. It is a free molecular modeling application that offers a suite of intuitive tools for viewing, comparing, and analyzing protein and small molecule data.

This study was performed through an *in silico* setting which means that all the data and tests were simulated using computers and software. The 17 (selected) flavonoid structures were acquired from the PubChem database and were converted to PDB format using OpenBabel (ver. 3.1.1).These structures were accessed using the BIOVIA Discovery Studio Visualizer along with the angiogenic growth factors; VEGFR-2 (PDB ID: 1Y6B), FGFR-2 (PDB ID: 8STG), TGF-βR-1 (PDB ID: 1VJY), IGF-1R (PDB ID: 3NW6), and EGFR (PDB ID: 6P1D) as objects of analysis.The flavonoids were minimized and further prepared with the target proteins using the Autodock Vina (ver. 1.1.2) utilized by PyRx software. In docking, the researchers applied the blind docking method which maximizes the grid box to include the whole structure of the uploaded target proteins (the specific x, y, and z coordinates were not recorded). This method also ensures that the PyRx software accurately predicts the original position of the control after redocking them as part of the 17 ligands. The exhaustiveness value is 8 (default value). The redocking of angiogenic inhibitors (control) attached to the target proteins was conducted to set a standard for evaluating the potential efficacy of the selected flavonoids. This method was utilized to observe potential differences in the flavonoids of Aratiles focusing on the binding sites and scores.

Data Analysis

The PyRx software analysis focused on docking poses, binding affinities, and amino acid interactions between the ligands and receptors. The more negative the value of the flavonoids to the docked protein, the more effective it is at binding to the specific receptor and the higher the possibility of inhibiting angiogenesis.

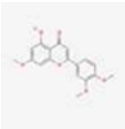
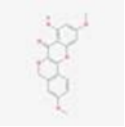
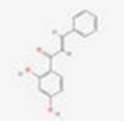
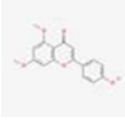
The collected data were tabulated and ranked to identify the highest binding affinity of the selected flavonoids docked to the five (5) pro-angiogenic target proteins (Agu et al., 2023; Nas, 2020; PyRx, n.d.). Only the top or standout flavonoids from each receptor were further analyzed based on their binding poses, the active sites, and specific amino acid interactions. Figure 2 shows the flowchart of the study.

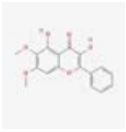
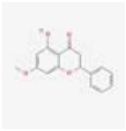
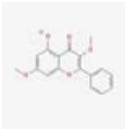
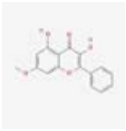
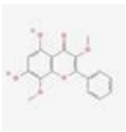
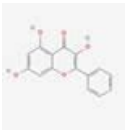
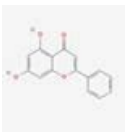
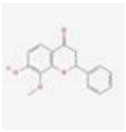
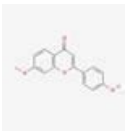
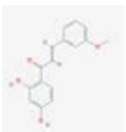
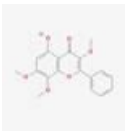
Results

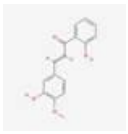
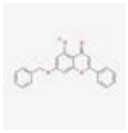
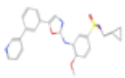
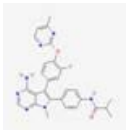
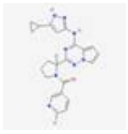
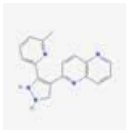
Table 2 shows the binding affinities of the 17 ligands with their corresponding binding affinities and ranking. The ligand 17 [7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone] has ranked first in all protein receptors, indicating a strong inhibitory potential. High binding affinity indicates that a compound, such as a flavonoid, strongly interacts with the target protein. This is typically reflected by a more negative binding energy value, suggesting a more stable and favorable binding. A strong interaction implies that the compound has the potential to effectively influence the biological activity of the protein, which is important in the development of medicinal drugs (BiologyInsights Team, 2025). This corroborates with the study of Nasir et al. (2017) wherein the methanolic extract of Aratiles demonstrated significant anti-cancer effects by slowing the growth of azoxymethane-induced colon cancer in rats, which may be attributed to the activity of flavonoids.

Table 2

Binding affinities of the 17 flavonoid ligands and the control with target proteins

Flavonoid Name*	PubChem ID	Chemical Structure	Docking scores (kcal/mol) and Top Five Ranking				
			VEGF	FGF	IGF	TGF	EGF
Ligand 1	5272653		-8.0	-7.6	-7.3	-9.6 (Rank 5)	-7.7
Ligand 2	139030740		-8.3	-8.6 (Rank 4.5)	-7.9 (Rank 4)	-9.4	-8.3
Ligand 3	5376979		-7.7	-7.7	-7.1	-9.2	-8.8
Ligand 4	161172		-7.9	-8.6 (Rank 4.5)	-7.2	-9.0	-8.2

Ligand 5	13291608		-8.1	-8.1	-6.9	-9.6	-8.3
Ligand 6	4101463		-8.4 (Rank 3.5)	-8.0	-7.7	-9.6	-9.2 (Rank 2)
Ligand 7	5748697		-8.0	-7.7	-7.0	-9.4	-7.6
Ligand 8	5318691		-8.2	-8.1	-7.3	-9.3	-8.9 (Rank 4.5)
Ligand 9	9972910		-7.8	-7.6	-7.0	-8.3	-7.5
Ligand 10	5281616		-8.2	-8.5	-7.8 (Rank 5)	-9.9 (Rank 4)	-8.9 (Rank 4.5)
Ligand 11	5281607		-8.4 (Rank 3.5)	-8.6 (Rank 4.5)	-8.2 (Rank 3)	-10.1 (Rank 3)	-9.1 (Rank 3)
Ligand 12	42607832		-8.4 (Rank 3.5)	-8.2	-7.0	-8.6	-8.1
Ligand 13	676307		-8.2	-9.3 (Rank 3)	-7.6	-9.3	-8.8
Ligand 14	10612087		-7.6	-7.7	-7.2	-8.2	-7.8
Ligand 15	21632881		-7.8	-7.9	-6.6	-8.2	-7.3

Ligand 16	5743235		-7.4	-7.9	-7.6	-9.6	-8.9
Ligand 17	5403474		-9.3 (Rank 1.5)	-9.4 (Rank 1.5)	-9.8 (Rank 1)	-11.1 (Rank 1)	-10.0 (Rank 1)
			Control Ligands				
Control ligand 1	15743202		-9.3 (Rank 1.5)	-	-	-	-
Control ligand 2	483424065		-	-9.4 (Rank 1.5)	-	-	-
Control ligand 3	25101485		-	-	-9.0 (Rank 2)	-	-
Control ligand 4	5287512		-	-	-	-10.6 (Rank 2)	-
Control ligand 5	145946092		-	-	-	-	-8.8 (Rank 6)

**Ligand chemical name:*
1= 5'-hydroxy-7,3',4'-trimethoxyflavanone;
2= 8-hydroxy-10-methoxy-5H-isochromeno[4,3-b]chromen-7-one;
3= 2',4'-dihydroxychalcone;
4= 5,7-dimethoxy-4-hydroxyflavone;
5= 3,5-dihydroxy-6,7-dimethoxyflavone;
6= 5-hydroxy-7-methoxyflavone;
7= 3,7-dimethoxy-5-hydroxyflavone;
8= 3,5-dihydroxy-7-methoxyflavone;
9= 5,7-dihydroxy-3,8-dimethoxyflavone;
10= Galangin;
11= 7-hydroxy-8-methoxyflavone;
12= 7-hydroxy-8-methoxyflavone;
13= 4'-hydroxy-7-methoxyflavanone;
14= 5-hydroxy-3,7,8-trimethoxyflavone;
15= 5-hydroxy-3,7,8-trimethoxyflavone;
16= 2',3'-dihydroxy-4'-methoxychalcone;
17= 7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone;

Control 1= 2-anilino-5-aryloxazole(5);
Control 2= N-{4-[(5P)-4-amino-5-{3-fluoro-4-[(4-methylpyrimidin-2-yl)oxy] phenyl}-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl] phenyl}-2- methylpropanamide;
Control 3= N-(5-cyclopropyl-1H-pyrazol-3-yl)-2-{(2S)-1-[(6-fluoropyridin-3-yl) carbonyl]pyrrolidin-2-yl}pyrrolo[2,1-f][1,2,4]triazin-4-amine;
Control 4= 2-[5-(6-methylpyridin-2-yl)-2,3-dihydro-1H-pyrazol-4-yl]-1,5- Naphthyridine;
Control 5= 10-benzyl-8-fluoro-5,10-dihydro-11H-dibenzo [b,e][1,4]diazepin-11-one

Table 3 shows the basic properties of 17 selected ligands and 5 control ligands, highlighting their chemical formula, molecular weight, and number of atoms. The selected flavonoid ligands generally range from 240 to 344 g/mol and 29 to 42 atoms. Contrastingly, the control ligands are larger, more complex molecules containing additional elements such as nitrogen, fluorine, and sulfur, with molecular weights up to 511 g/mol. These suggest that the selected ligands may have simpler structures and potentially better bioavailability, while the controls possess greater structural diversity that could influence binding specificity.

Table 3

Information of Selected Ligands

List of Ligands	PubChem CID	Chemical Formula	Molecular Weight	No. of Atoms
Ligand 1	5272653	$C_{18}H_{16}O_6$	328.31604	40
Ligand 2	139030740	$C_{18}H_{14}O_6$	326.30016	38
Ligand 3	5376979	$C_{15}H_{12}O_3$	240.25398	30
Ligand 4	161172	$C_{17}H_{14}O_5$	298.29006	36
Ligand 5	13291608	$C_{17}H_{14}O_6$	314.28946	37
Ligand 6	4101463	$C_{16}H_{14}O4$	270.27996	34
Ligand 7	5748697	$C_{17}H_{14}O_5$	298.29006	36
Ligand 8	5318691	$C_{16}H_{12}O_5$	284.26348	33
Ligand 9	9972910	$C_{17}H_{14}O_6$	314.28946	37
Ligand 10	5281616	$C_{15}H_{10}O_5$	270.23690	30
Ligand 11	5281607	$C_{15}H_{10}O_4$	254.23750	29
Ligand 12	42607832	$C_{16}H_{14}O_4$	270.27996	34
Ligand 13	676307	$C_{16}H_{12}O_4$	268.26408	32
Ligand 14	10612087	$C_{16}H_{14}O_4$	270.27996	34
Ligand 15	21632881	$C_{18}H_{16}O_6$	328.31604	40
Ligand 16	5743235	$C_{16}H_{14}O_4$	270.27996	34
Ligand 17	5403474	$C_{22}H_{16}O_4$	344.36004	42
		Control Ligands		
VEGF	15743202	$C_{25}H_{24}N_4O_4S$	476.54746	34
FGF	483424065	$C_{28}H_{26}FN_7O_2$	511.55014	63
EGF	25101485	$C_{20}H_{15}FN_2O$	318.34430	24
TGF	5287512	$C_{17}H_{13}N_5$	287.31862	22
IGF	145946092	$C_{22}H_{21}FN_80$	432.45354	32

A. Interactions of Ligand 17 and VEGFR-2 with Control Ligand

As shown in Figure 3, Ligand 17 functioned as an orthosteric inhibitor and interacted (-9.3 kcal/mol) with VEGFR-2 via hydrogen-bonding (Glu915, Cys917), pi-pi stacking (Phe1045) and hydrophobic interactions, resulting in blocking of angiogenesis through VEGFR-2 signaling. There were no major alterations in the control ligand's binding site before and after docking. The inhibitor/control ligand was 2-anilino-5-aryloxazole(5) bound in VEGFR-2 and had a redocked score of -9.3 kcal/mol, comparable to ligand 17 which has the highest binding scores of all tested flavonoids.

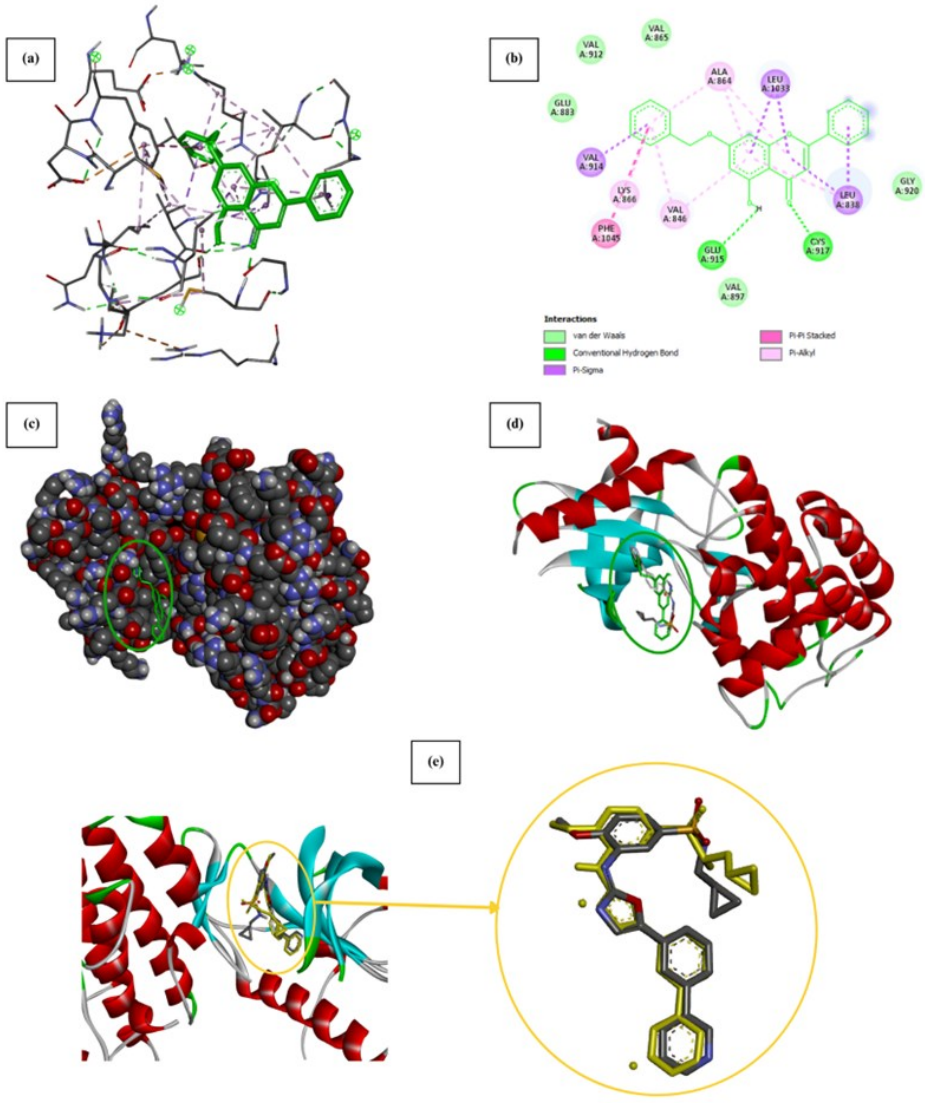


Figure 3

Ligand 17 interaction with VEGFR-2 (encircled stick figure): (a) 3D view with surrounding amino acids; (b) 2D interaction map; (c) Ligand 17 binding at the active site; (d) Ligand 17 and control ligand at the same binding site; (e) Redocking result compared to the original control ligand pose.

B. Ligand 17 and FGFR-2 Interactions with Control Ligand

In Figure 4, Ligand 17 acted as an orthosteric inhibitor in FGFR-2 (-9.4 kcal/mol), sharing the same binding pocket as the control ligand. It formed Pi-T stacking (Phe645) and hydrogen bonds (Phe557, Cys531), enhancing its stability. The binding site remained unchanged before and after docking. The inhibitor ligand bound in this protein receptor and used as the control ligand for FGFR-2 was N-(4-(4-amino-5-(3-fluoro-4-((4-methylpyrimidin-2-yl)oxy)phenyl)-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-6yl)phenyl) methacrylamide or RLY-4008 and had a redocked score of -9.4 kcal/mol, comparable to ligand 17 which had the highest binding scores of all tested flavonoids.

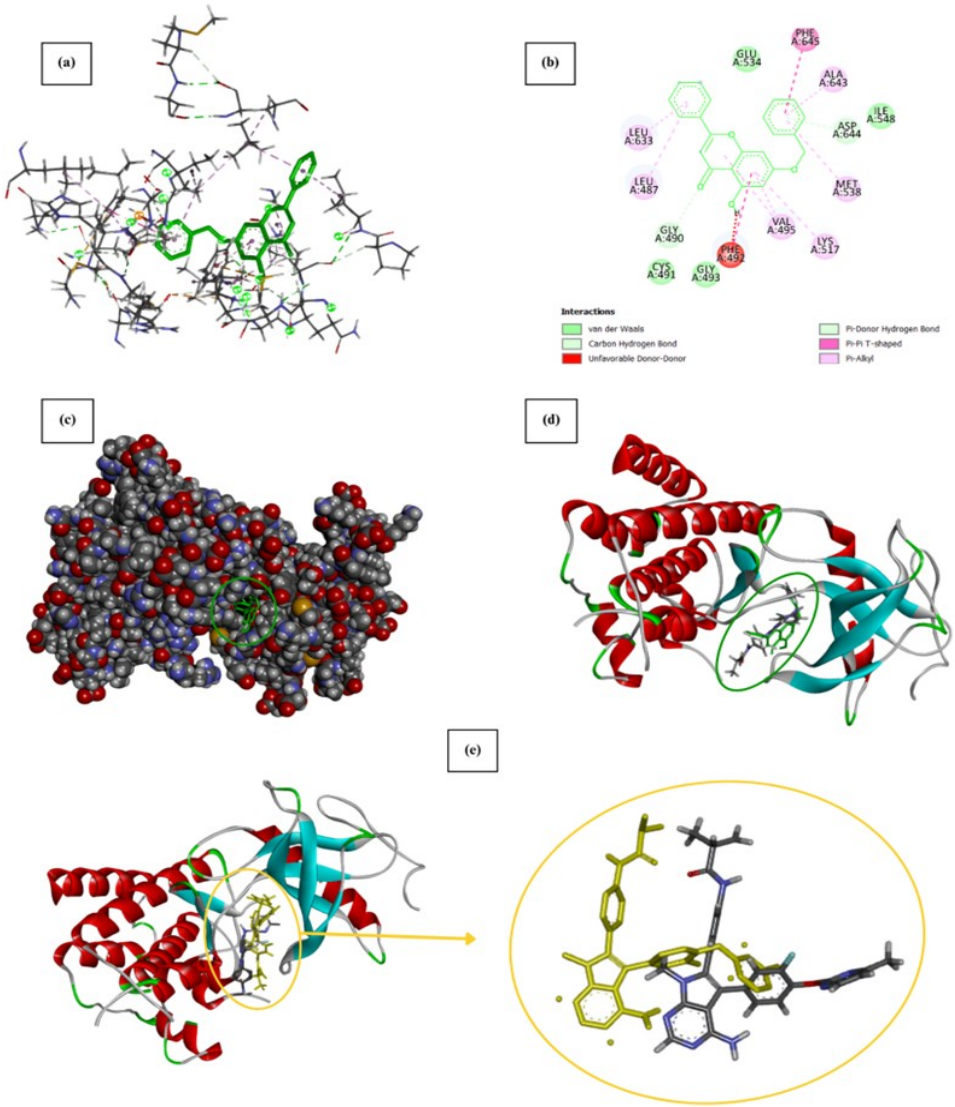


Figure 4

FGFR-2 interaction with Ligand 17 (encircled stick figure): (a) 3D view with surrounding amino acids; (b) 2D interaction map; (c) Ligand 17 binding at the active site; (d) Ligand 17 and control ligand at the same binding site; (e) Redocking result compared to the original control ligand pose.

C. Ligand 17 and TGF-βR-1 Interactions and Control Ligand

In Figure 5, Ligand 17 acted as an orthosteric inhibitor in TGF-βR1 with the highest binding score (-11.1 kcal/mol). It formed 13 hydrogen bonds and hydrophobic interactions (Leu278, Val219, Ala230), surpassing the control (-10.6 kcal/mol). This indicated a strong stability as a competitive inhibitor. The binding site remained unchanged before and after docking. The inhibitor ligand bound in this protein receptor and was also used as the control ligand for TGF-βR1 was 2-[5-(6-methylpyridin-2-yl)-2,3-dihydro-1H-pyrazol-4-yl]-1,5-naphthyridine. It had a redocked score of -10.6 kcal/mol, which was lower compared to ligand 17.

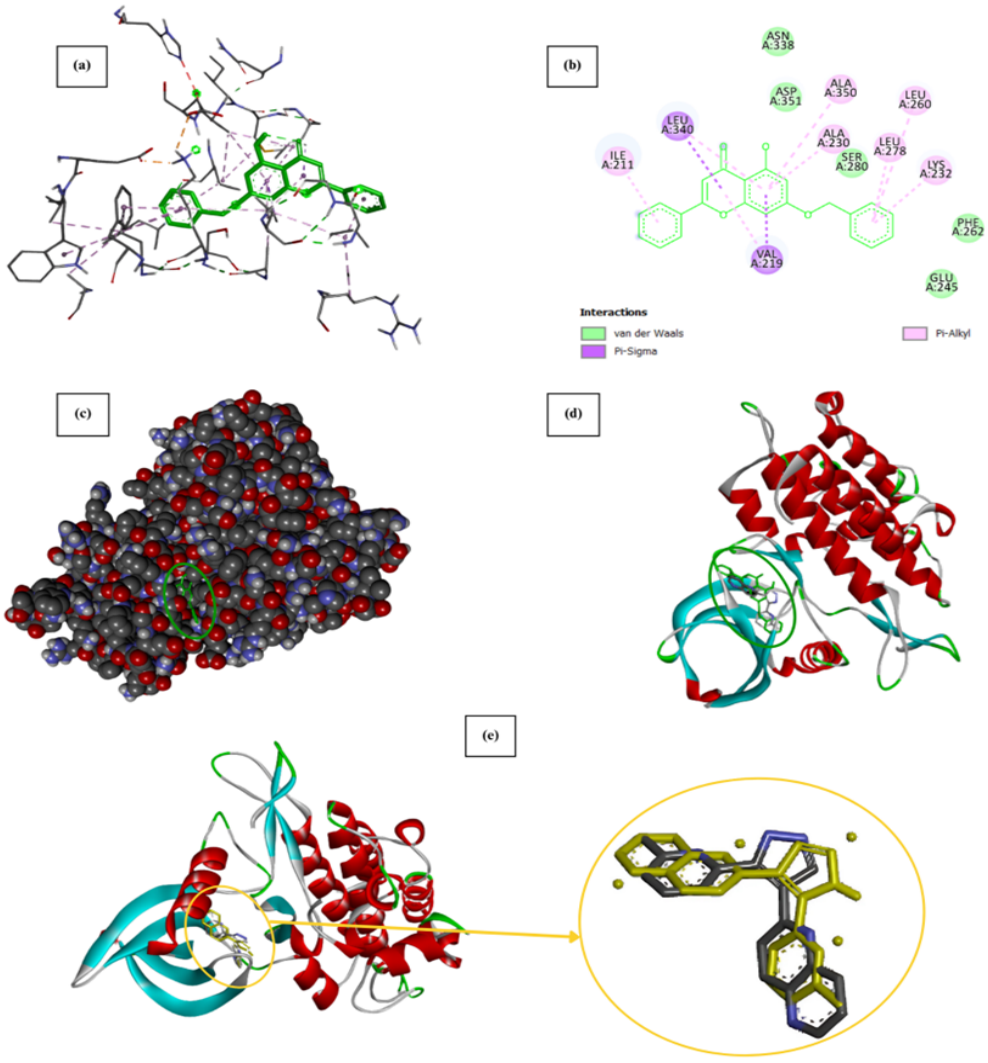


Figure 5

TGF-βR1 interaction with Ligand 17 (encircled stick figure): (a) 3D view with surrounding amino acids; (b) 2D interaction map; (c) Ligand 17 binding at the active site; (d) Ligand 17 and control ligand at the same binding site; (e) Redocking result compared to the original control ligand pose.

D. Ligand 17 and IGF-1R Interactions with Control Ligand

In Figure 6, Ligand 17 interacted with IGF-1R (-9.8 kcal/mol), forming hydrogen bonds (Asp1105, Gly1125) and pi-pi stacking (Phe1124, Tyr1135, His1103), enhancing its stability. The control, which was an allosteric inhibitor, scored -9.0 kcal/mol (weaker binding). The inhibitor ligand bound in this protein receptor and used as the control ligand for IGF-1R was N-(5-cyclopropyl-1H-pyrazol-3-yl)-2-{(2S)-1-[(6-fluoropyridin-3-yl) carbonyl]pyrrolidin-2-yl}pyrrolo[2,1-f][1,2,4]triazin-4-amine and had a redocked score of -9.0 kcal/mol, subpar compared to ligand 17 which has the highest binding scores of all tested flavonoids.

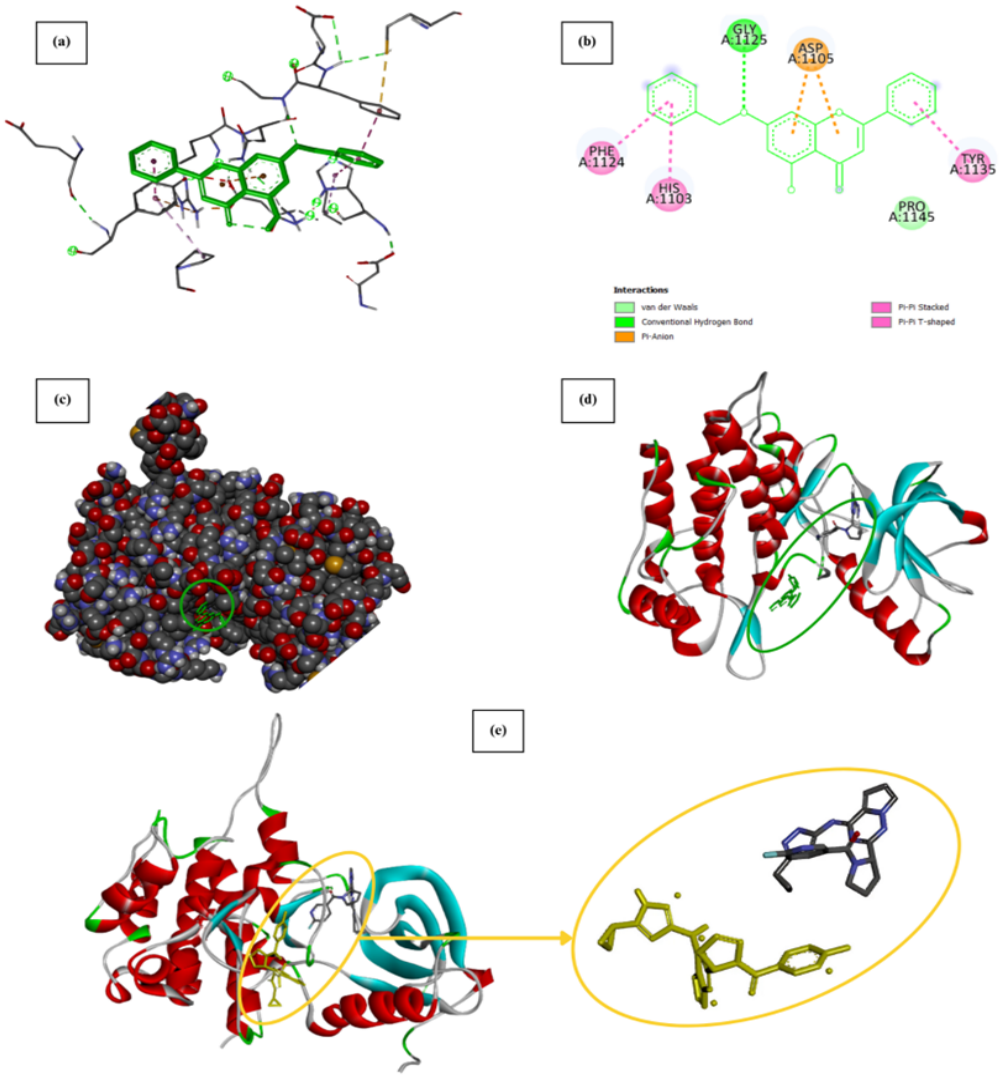


Figure 6

IGF-1R interaction with Ligand 17 (encircled stick figure): (a) 3D view with surrounding amino acids; (b) 2D interaction map; (c) Ligand 17 binding at the active site; (d) Ligand 17 and control ligand at different binding sites; (e) Redocking result compared to the original control ligand pose.

E. Ligand 17 and EGFR Interactions with Control Ligand

In EGFR, ligand 17 scored -10.0 kcal/mol, showing strong pi-cation and van der Waals interactions with Lys745 and Leu777, outperforming the control ligand (-8.8 kcal/mol). This suggests high binding affinity and excellent allosteric inhibition. Ligand 17's consistent high binding affinities indicated its strong potential as a competitive inhibitor across targets. The inhibitor ligand bound in this protein receptor and used as the control ligand for EGFR was 10-benzyl-8-fluoro-5,10-dihydro-11H-dibenzo[b,e][1,4]diazepin-11 -one and had a redocked score of -8.8 kcal/mol, subpar compared to ligand 17 which had the highest binding scores of all tested flavonoids. Figure 7 is shown below.

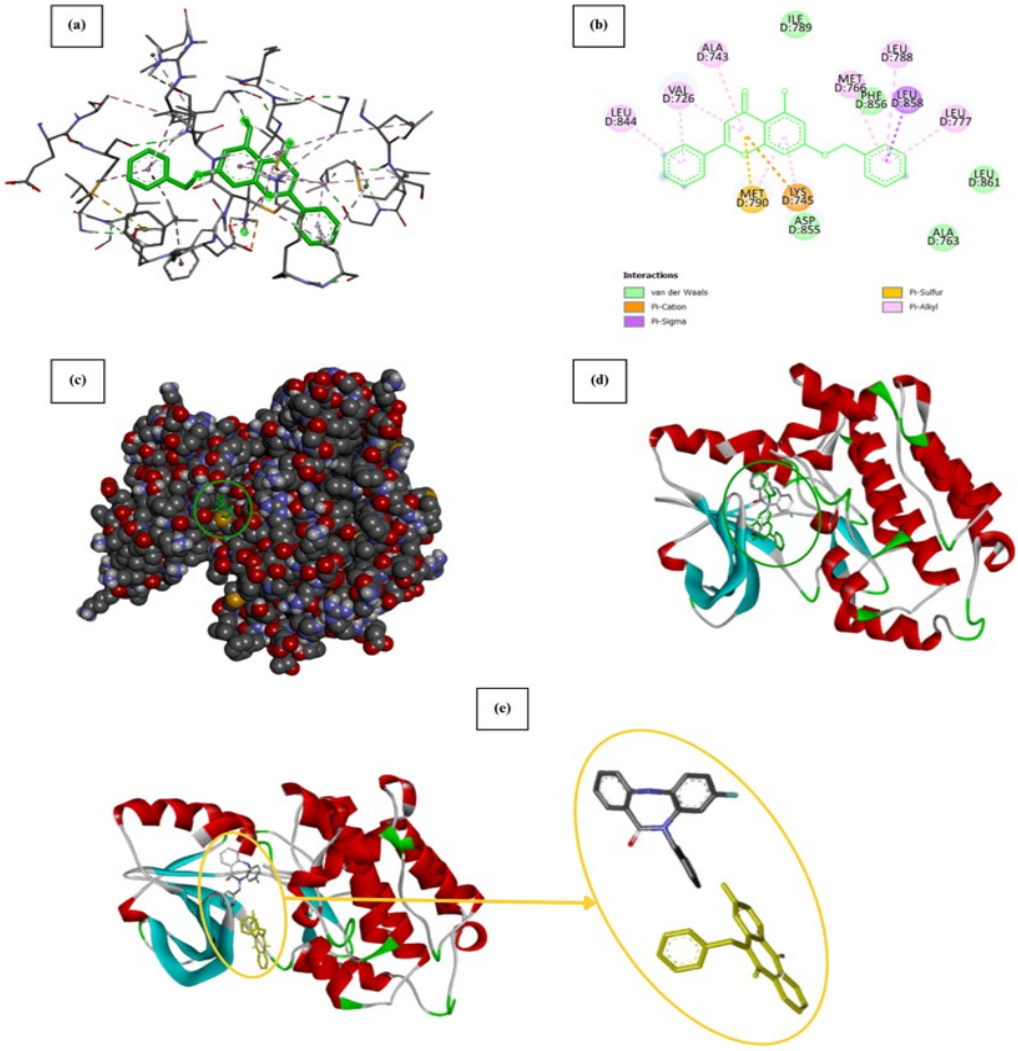


Figure 7

EGFR interaction with Ligand 17 (encircled stick figure): (a) 3D view with surrounding amino acids; (b) 2D interaction map; (c) Ligand 17 binding at the active site; (d) Ligand 17 and control ligand at the same binding site; (e) Redocking result compared to the original control ligand pose.

Table 4

Amino acid interactions of Ligand 17 with target proteins

Target proteins	Amino acids involved in the interaction with ligand 17
VEGFR-2	Glutamic acid, Valine, Alanine, Lysine, Phenylalanine, Cysteine, Leucine, Glycine
FGFR-2	Phenylalanine, Glutamic Acid, Aspartic Acid, Valine, Lysine, Leucine, Glycine, Cysteine
TGF-βR1	Isoleucine, Leucine, Asparagine, Aspartic Acid, Alanine, Serine, Lysine, Phenylalanine, Glutamic Acid
IGF1-R	Aspartic Acid, Glycine, Phenylalanine, Tyrosine, Histidine, Proline
EGFR	Leucine, Valine, Alanine, Isoleucine, Methionine, Lysine

Based on Table 4, each receptor exhibits a unique set of binding amino acids and some overlap across the targets. Interestingly, lysine and leucine were involved across multiple interactions, suggesting their potential in Ligand 17's binding mechanisms. This may be attributed to its anti-angiogenic properties.

Discussion

The flavonoid ligand 17 [7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone] acting both as an allosteric and orthosteric inhibitor, showed strong inhibitory potential against angiogenic receptors. According to *Raval et al.* (2019), binding affinity is how much of a drug is needed to attach to its target site. A higher affinity means less drug is needed, while a lower affinity means more is required. As such, ligand 17 shows strong binding affinities to several key receptors involved in angiogenesis and tumor survival, which could significantly impact the way tumors grow, resist treatment, and spread. Ligand 17 exhibits its highest binding affinity to TGF-βR1 (-11.1 kcal/mol), indicating strong potential to inhibit critical pathways involved in cancer progression. These include tumor cell proliferation, migration, and epithelial-mesenchymal transition (EMT) processes known to be driven by TGF-βR1 signaling (Wang *et al.*, 2021). Its binding to IGF-1R (-9.8 kcal/mol) and EGFR (-9.9 kcal/mol) indicates that it may also disrupt growth signals and anti-apoptotic pathways, both of which helps tumor cells survive and resist treatment (Uribe *et al.*, 2021; Weroha & Haluska, 2012). Binding to FGFR-2 (-9.4 kcal/mol) and VEGFR-2 (-9.3 kcal/mol), although slightly lower, remains a strong enough to potentially block new blood vessel formation (angiogenesis), thus, effectively starving tumors of nutrients and oxygen. These interactions highlight Ligand 17's ability to target multiple survival mechanisms that tumors use, making it a promising multi-target inhibitor that may overcome resistance commonly seen in single-pathway therapies. Nevertheless, these findings are based on *in silico* predictions, and therefore, biological validation through in vitro and in vivo studies are suggested to confirm its safety and effectiveness.

Moreover, Ligand 17 interacts with multiple target proteins involved in key cancer-related pathways, engaging different amino acids at either orthosteric, allosteric, or both sites. Orthosteric binding as seen with VEGFR-2, FGFR-2, and TGF-βR1 suggests that Ligand 17 may directly compete with natural ligands (like growth factors), potentially blocking signal initiation at the receptor's active site (Nussinov et al., 2012). This could effectively shut down primary pro-angiogenic signals such as VEGFR-2, FGFR-2, and TGF-βR1, which are essential for tumor vascularization and survival under nutrient-deprived conditions. Conversely, allosteric binding, observed in EGFR, implies that Ligand 17 could be modulating receptor activity in a more nuanced way, either enhancing or inhibiting downstream signaling without directly competing with the endogenous ligand (Nussinov et al., 2012). This kind of interaction offers a less disruptive way to influence pathway activity, which can lead to fewer side effects compared to direct inhibition at the active site. Additionally, allosteric modulators may reduce the likelihood of resistance, as they can remain effective even when mutations occur at the orthosteric site, which is an issue commonly seen with traditional inhibitors (Nussinov et al., 2012). Bitopic ligands, like Ligand 17 in its interaction with IGF-1R, may bind to both orthosteric and allosteric sites, allowing dual regulation of receptor activity. These bitopic ligands exhibit receptor expression-dependent pharmacology, ligand-biased signaling, and complex structure-activity interactions (Wenthur et al., 2011), offering a strategic advantage in targeting resistant cancer pathways.

The diverse set of amino acid interactions across all targets show that Ligand 17 forms multiple types of bonds, which may contribute to its broad and stable binding profile. Nonpolar amino acids such as valine, leucine, isoleucine, alanine, methionine, proline, and phenylalanine are found in all protein receptors such as VEGFR-2, FGFR-2, TGF-βR1, IGF1-R, and EGFR. Hydrogen bonding also occurs with polar amino acids such as serine, asparagine, glutamine, glycine, cysteine, and tyrosine, which are found in FGFR-2, TGF-βR1, and IGF1-R. These hydrophobic and hydrogen interactions are significant drivers in ligand-protein binding. According to Barquilha & Braga (2021), water molecules form ordered shells around nonpolar species, decreasing entropy. To reduce this energetic cost, nonpolar regions cluster together, displacing water and increasing entropy. This explains Ligand 17's preference for hydrophobic pockets, stabilizing its interaction with target proteins. Hydrogen bonds, furthermore, are vital non-covalent interactions that maintain the structure and function of biological molecules like DNA and proteins (Patil et al., 2010). They aid in protein folding, stability, and specific ligand binding. These bonds form when a hydrogen atom attached to an electronegative donor interacts electrostatically with an acceptor atom or other electronegative groups, such as pi bonds (Patil et al., 2010). Additionally, ionic interactions involve positively charged residues like lysine and histidine, along with negatively charged residues such as glutamic acid and aspartic acid, all of which are found in the listed proteins. Ionic interactions play a key role in ligand-protein binding by stabilizing the complex through electrostatic attraction between oppositely charged groups. Positively and negatively charged ions in the protein and ligand can attract or repel each other, causing the complex to bind or dissociate (Shana, 2023). Lastly, aromatic and π-related interactions encompass π–π stacking (between aromatic rings) and cation–π interactions (between a positive charge and an aromatic ring).

These interactions involve aromatic residues such as phenylalanine, tyrosine, and histidine, which are present in VEGFR-2, FGFR-2, TGF-βR1, and IGF1-R. Aromatic stacking is a well-established key component of ligand-protein interfaces. According to Brylinski (2017), the stability of these interactions vary across the gas phase, aqueous environments, and protein-like conditions. π-π stacking contributes significantly through favorable electron correlation, while cation-π interactions provide additional electrostatic stabilization. Together, these interactions enhance ligand-protein binding by improving molecular stability, specificity, and affinity within the binding site.

While the study demonstrated Ligand 17's strong inhibitory activity, it must be recognized that its activity may also be involved in tissue regeneration instead of acting strictly as anti-angiogenic agents. This phenomenon is particularly observed in bioactive flavonoids, which are known to interact with multiple signaling pathways. This raises an important consideration about Ligand 17's favorable docking results, as its real-world effects may vary on 1) cellular context - the type of cells involved, 2) concentration, and 3) microenvironmental factors. In some cases, flavonoids may inadvertently facilitate neovascularization which is a crucial process in tumor development and metastasis, instead of suppressing tumor growth. This process suggests a potential issue where the flavonoids present in aratiles may not consistently show anti-angiogenic effects when considering various biological conditions (Pan et al., 2021).

The findings of the study corroborate with Nasir et al. (2017) who reported that flavonoids from aratiles possess anti-cancer activity by inhibiting angiogenesis. Flavonoids are rich in antioxidants such as superoxide dismutase, catalase, and glutathione. Further, it acts on malondialdehyde, which is a cancer biomarker. This is crucial against the initial stage of cellular damage. Flavonoids such as quercetin and kaempferol also support the inhibitory action of Ligand 17 on VEGFR-2 and EGFR, making it a viable candidate for a drug application (Nasir et al., 2017). Ligand 17 anti-angiogenesis activity may be attributed to the benzyloxy and methoxy groups that increase its molecular stability and binding capacity (Sangande, et al. 2020). The discovery of rutin, a prominent flavonoid of aratiles, further supports its anti-angiogenesis activity since rutin is known for its anti-inflammatory, and anti-cancer activities (Cheen, 2022; Gautam et al., 2016; Volate et al., 2005).

The study's findings advocate the potential development of new drugs or natural treatments for conditions such as cancer and diseases associated with angiogenesis. However, it is important to recognize that these results are derived from computational predictions, which may not entirely reflect the complexity of biological processes occurring in living organisms, possibly resulting in some inaccuracies. Moreover, the research only included a limited selection of flavonoids from Aratiles, excluding other compounds that may also possess biological activity. Future research should aim to confirm these findings through in vitro and in vivo experiments, particularly focusing on Ligand 17.

Conclusion

This study highlighted the potential of flavonoids from aratiles (*Muntingia calabura* L.) as a source of natural compounds for anti-angiogenic treatment, specifically targeting the angiogenic mechanism essential to cancer progression. *In silico* by molecular docking is an effective approach in the early phase of drug discovery. Flavonoid ligand 17 exhibited the highest binding affinity against VEGFR-2, FGFR-2, TGF-βR1, IGF-1R, and EGFR in comparison with other tested ligands. Hence, it has the potential of blocking fundamental vascular pathways in tumors, making it a promising natural compound which can be developed as anti-cancer drugs in future cancer research.

While these *in silico* findings are promising and suggest that Ligand 17 may effectively target key receptors involved in angiogenesis and tumor survival, they represent only an initial step. High binding affinity and favorable interactions do not guarantee therapeutic efficacy or safety in the complexity of real biological systems. Factors such as receptor kinetics, cell-type specificity, and compensatory signaling pathways can significantly influence outcomes. Therefore, in vitro and in vivo validations are essential. Overall, these results highlight Aratiles as a promising natural source of flavonoids for cancer prevention and treatment.

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Declaration of AI

No artificial intelligence (AI) tools were used in the conduct and analysis of this research. Only standard *in silico* computational tools were utilized, namely PyRx for molecular docking, SwissADME for ADME screening, and ProTox-II for toxicity prediction. These tools are based on established cheminformatics algorithms and not on AI. All scientific content, analyses, interpretations, and conclusions were made solely by the authors.

Conflict of Interest

The authors declare no conflict of interest. This study was conducted using computational methods, with computers provided by the University of the Philippines Visayas. The research study was conducted independently, and no financial or personal relationships could have influenced the study's design, data collection, analyses, or interpretation.

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