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Application of *In-silico* Methodologies in Exploring the Antagonistic Potential of *Trigonella foenum-graecum* on Cyclooxygenase-2 (Cox-2) in Cancer Treatment

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Abstract

Cancer is a disease in which abnormal cells divide uncontrollably and destroy body tissue. This research is dispensed utilizing in-silico drug design. Cyclooxygenase-2 (Cox-2) has been used as a target protein of the molecular docking study and fenugreek phytoconstituents obtained from PubChem were docked against Cox-2's pocket (PDB ID: 5IKV). We used Maestro 12.8 and the Schrödinger Suite to conduct computer-based drug testing. To document compounds with the best inhibitory ability to act as cyclooxygenase antagonists in the treatment of cancer. Sixty (60) compounds described with fenugreek was docked to the active site of Cox-2 (5IKV). The results demonstrated that tricin to 2,5-dimethyl pyrazine, which are the best molecules docked at the active site of Cox-2, had -11.007 to -4.58 kcal/mol and an MM-GBSA score ranging from -31.06 to -24.52 respectively, which suggests the free binding energy posed a competitive binding energy when compared to the co-crystallized ligand, 2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid. Numerous drugs have been made available, but due to their common side effects, researchers are now searching for novel herbal plants that can be utilized as long-term treatments with minimal adverse effects. Thus, utilizing computational studies such as molecular docking, MM-GBSA, pharmacophore modeling, and the lead compounds' ADMETox characteristics were computed.
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Keywords: *Cancer, Cox-2, Molecular docking, Pharmacophore, MM-GBSA, and Fenugreek*

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List of Abbreviations

MM-GBSA	Molecular mechanics generalized Born surface area.
PDB	Protein Data Bank
ADME-Tox	Absorption, Distribution, Metabolism, Excretion, and Toxicity.
Cox-2	Cyclooxygenase-2
ROV	Rule of Five.
NSAIDs	Non-steroidal anti-inflammatory drugs

1. Introduction

Trigonella foenum-graecum (Fenugreek) is an herb that has been used in traditional medicine for thousands of years and is believed to have various health benefits (Mawer, 2023). Some preliminary studies have suggested that certain compounds found in fenugreek may have anti-cancer properties (Umesh *et al*., 2014). Fenugreek is a plant that is widely cultivated for its seeds, which are used as a spice in many different cuisines. The seeds have a slightly bitter and nutty flavor, and are commonly used in dishes such as curries, spice blends, and pickles (Britannica 2022). Fenugreek is known to contain a variety of phytoconstituents, including saponins, flavonoids, alkaloids, proteins, minerals, fiber, and steroids (Nagulapalli *et al*., 2017). The use of medicinal plants as a treatment for cancer has been a topic of interest for many years.

1.1 Cancer

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells (Olaku and Taylor, 2017). Cancer is a complex disease that develops over time as a result of the interaction between genetic and environmental factors (Knox 2010). Normally, our cells grow and divide in a controlled manner to maintain the health of the body. However, in some cases, cells can develop mutations that cause them to grow and divide in an uncontrolled manner, leading to the formation of a mass of abnormal cells known as a tumor (Weinberg 1996). Symptoms of cancer can vary depending on the type and stage of the disease but may include fatigue, weight loss, pain, skin changes, and changes in bowel or bladder habits (Saini *et al*., 2020). Diagnosis is typically made through a combination of medical imaging tests, biopsies, and blood tests. There is ongoing research into new and better ways to diagnose, treat, and prevent cancer, and many people with cancer can manage the disease and enjoy a good quality of life with the help of advances in medical technology and supportive care.

1.2 Cyclooxygenase-2 (COX-2)

Cyclooxygenase-2 (COX-2) is an enzyme that plays a key role in the production of prostaglandins, which are hormone-like substances involved in the regulation of pain, inflammation, and fever. COX-2 is produced in response to injury or inflammation and is believed to contribute to the development of pain and inflammation in some conditions (Minghetti 2004). In recent years, COX-2 has been the subject of much research due to its potential role in the development of certain types of cancer, particularly colon cancer (Hashemi *et al*., 2019). Some studies have also suggested that COX-2 may contribute to the development of cancer by promoting the growth of abnormal cells and suppressing the immune system's ability to fight cancer.

1.3 COX-2 inhibitors

If COX-2 contributes to the development of certain types of cancer, such as colorectal cancer, by promoting the growth of abnormal cells and suppressing the immune system's ability to fight cancer, therefore, blocking the production of COX-2 has been a target for the development of new cancer treatments (Noble *et al*., 2000). One example of a COX-2 inhibitor used as a cancer treatment is celecoxib (Celebrex), which has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of familial adenomatous polyposis, a genetic condition that predisposes individuals to the development of colorectal polyps and an increased risk of colorectal cancer (Noble *et al*., 2000). Another example is the development of COX-2 inhibitors in combination with chemotherapy for the treatment of various types of cancer, including colon, breast, and lung cancer (Shuangshuang *et al*., 2020). A study published in the Journal of Clinical Oncology in 2004 demonstrated that the combination of a COX-2 inhibitor with chemotherapy improved outcomes in patients with advanced colon cancer (Dawn *et al*., 2004).

1.4 *In-silico* study

In-silico study is becoming increasingly important in the field of biology and medicine for several reasons (Edelman *et al*., 2010). Apart from the fact that it is cost and time time-efficient, in-silico studies are an important tool for advancing our understanding of cancer and other diseases, and for the development of new treatments (Pitcher *et al*., 2020). Computer-aided research also complement traditional laboratory-based experiments and has the potential to significantly improve the speed and efficiency of medical research and drug development (Yu and McKerell 2017). In this research, computational tools have been used to screen the library of compounds characterized with fenugreek. Figure 1 depicts steps in molecular docking simulation.

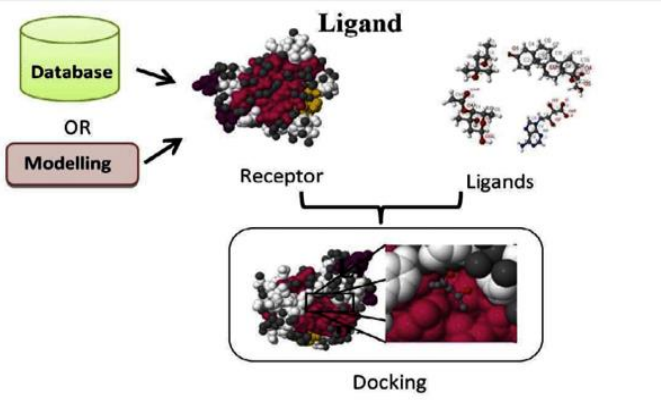

Figure 1: Simple workflow chart of molecular docking protocol (Molecular 2014).

2. Materials and methods

2.1 Molecular Docking

Computer-based drug testing was carried out using Maestro 12.8 and the Schrödinger Suite software (Schrodinger 2017). A total of 60 compounds reported with fenugreek were collected from an online database (PubChem) and docked to the active site of the enzyme to predict which substances had the best inhibitory potential to act as Cox-2 antagonists in the

treatment of cancer. We adhered to the standard molecular docking guidelines.

The secondary metabolites from the fenugreek plant (Fig. 2) were isolated in two-dimensional (2D) structures in SDF format, and it was found that they had anti-cancer properties (Olugbogi *et al*., 2022). Using the ligprep tool (Schrodinger 2021) to add hydrogen atoms, ionize at pH (7.2 0.2), and remove salt using Ep2i/UNEP/-Zk, the mined structures were transformed into three-dimensional structures (Schrodinger 2021). We used the OPLS4 force field to ionize and produce tautomeric states (Harder *et al*., 2016). When the number of stereoisomers each ligand produced was set to one, a total of 75 structures were constructed from 60 compounds.

2.2 Target Preparation

The X-ray crystallographic structure of Cox-2 complexed with an inhibitor was determined using the Protein Data Bank (PDB ID: 5IKV). The protein was created using the protein preparation wizard feature of Maestro's Schrodinger Suite. During the synthesis of proteins, employing Epik at pH 7.0 ± 2.0, bond orders were assigned, hydrogens were added, zero-order metal bonds, disulfide bonds, water molecules were removed, and het states were created. The protein was later reduced using the OPLS4 force field after the H-bond assignment was refined.

2.3 Generating a Receptor Grid

Using the receptor grid panel, the grid-generating job was configured and a receptor structure was specified. The receptor grid displays the region of the receptor where the ligand and protein interactions. On the binding site, the prepared protein grid was constructed using the Receptor Grid Generation tool (Glide Grid). The binding site was found by using the co-crystallized ligand (2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid] at the active site of 5IKV. The amino acid residues of the active site were automatically arranged into a cubic grid box. Three-dimensional coordinates of the created grid in terms of X, Y, and Z were 166.16, 185.21, and 190.89, respectively.

2.4 A Model to Produce E-Pharmacophore

An energy-optimized pharmacophore hypothesis was created using the crystal structure of the Cox-2 coupled to the 2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid at 2.508 resolution (E-pharmacophore). Using the Develop Pharmacophore from Protein-Ligand Complex option in the Phase module, the E-pharmacophore model was produced. The manual method was chosen in place of AUTO (E-pharmacophore). The 'SHOW FEATURES' setting was at its highest (More). A receptor-based excluded volume shell was then generated to resemble the receptor binding site (Figure 5) for the hypothesis settings, rejecting receptor atoms with surfaces closer than 2.00 to the ligand surfaces and capping the excluded volume shell thickness at 5.00 (Schrodinger 2016).

2.5 Virtual Screening Using E-Pharmacophores

On already-produced ligands, virtual screening with e-pharmacophores was done with LigPrep. The phase module of the Schrodinger suite's E-pharmacophore model (Dixon *et al*., 2006; Schrodinger 2020) was used to generate a list of medications with the required chemical characteristics for the best binding to Cox-2 (Fig. 6). Based on fitness ratings, the best hits were chosen.

2.6 Ligand Docking

Maestro 12.8 was docked using the Glide tool (Schrodinger 2017). The generated protein and manufactured chemicals were digitally screened to find the molecules with the lowest docking score using the crystal structure of Cox-2 (5IKV). The use of flexible Extra Precision (XP) docking approaches was made possible. The docking experiment, which employed the protein as a rigid body, left the rotatable bonds of the ligand open.

2.7 ADMET/Tox Screening

Using internet servers, the hit compounds' toxicity, drug-likeness, and pharmacokinetic profile were evaluated using swissADME (http://www.swissadme.ch) and Pro-Tox II (https://tox-new.charite.de/protox II).

2.8 MM/GBSA

The docked protein-ligand complex's binding free energy was calculated using the continuum solvent model of Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) (Mawer, 2023). To complete this research, Prime rotamer search techniques were combined with the OPLS4 force field and the VSGB solvent model.

Using Maestro 12.8's prime module, the expected binding free energies of the active androgen receptor inhibitors were determined. The MM-GBSA binding energy calculation method calculates binding affinity utilizing the energy characteristics of the free ligand, free receptor, and receptor-ligand complex (Schrodinger 2018; Genheden and Ryde 2015). The binding energies of the four hit ligands were estimated using the MM-GBSA method based on their XP docking glide scores.

The rotamer search approach, which makes use of the OPLS4 force field, VSBG solvent, and the rotamer search algorithm, was used to assess the relative free energy of the docked complexes. The binding free energy was calculated using the following equation.

$$\Delta G^{bind} = G^{complex}X - (G^{protein} + G^{Ligand})$$

3. Results and Discussion

It is estimated that more than 19.3 million new cancer cases emerged globally, and nearly 10 million people died from cancer in 2020 (Cao *et al*., 2021). Fenugreek is often used in combination with other herbs and supplements as part of a holistic approach to cancer treatment. It is essential to highlight that conventional cancer therapies like chemotherapy and radiation therapy should not be substituted with fenugreek. In this research, it has been demonstrated that fenugreek compounds inhibit the activity of the enzyme cyclooxygenase-2 (COX-2), which is responsible for the generation of prostaglandins that promote inflammation. Fenugreek may help reduce inflammation in the body and maybe offer treatment for illnesses like arthritis and digestive issues by suppressing COX-2 activity. Fenugreek has also been demonstrated to have anti-inflammatory and antioxidant effects, which may further contribute to its efficiency against COX-2.

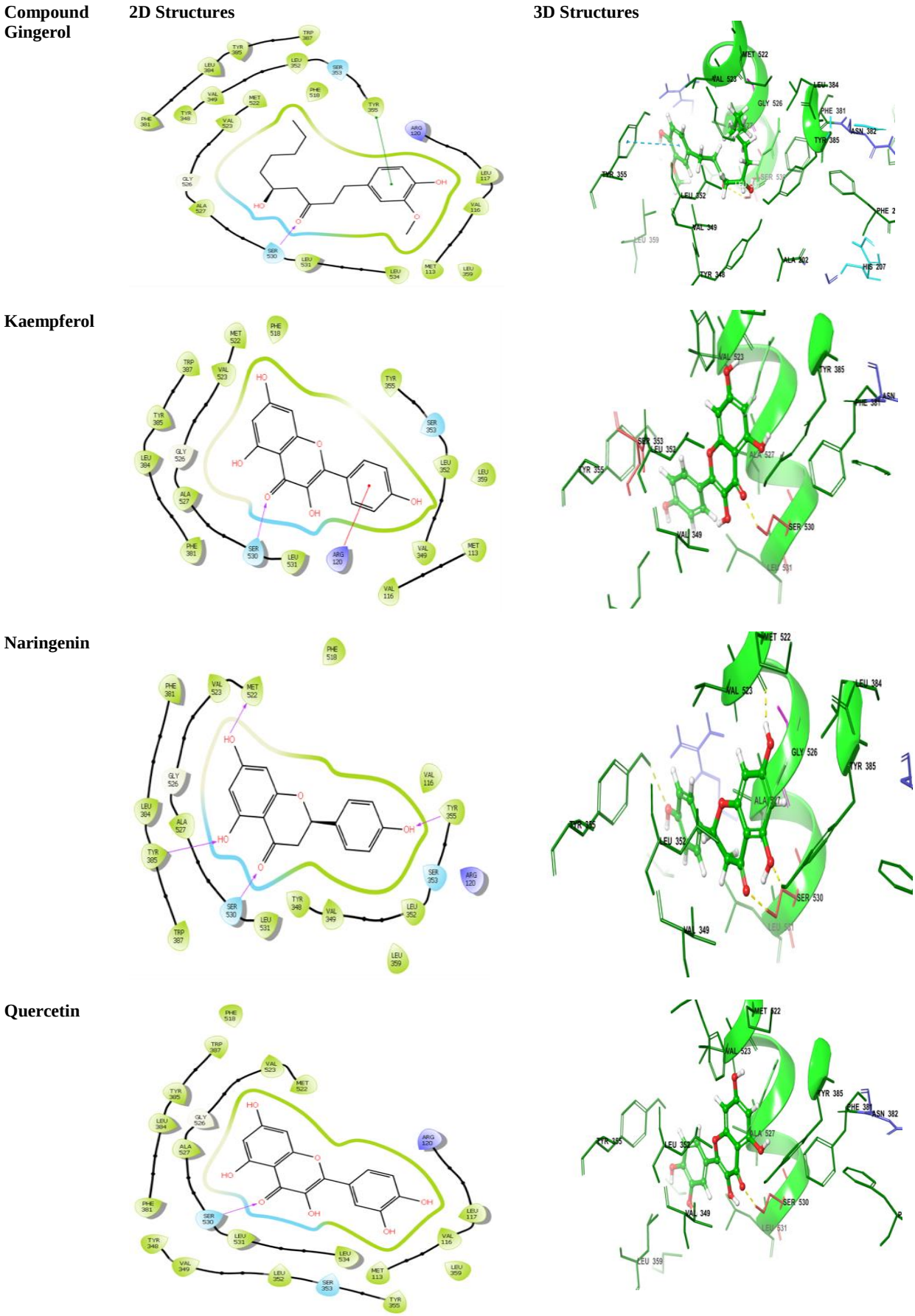

Figure 2: The top five compounds isolated from the fenugreek plant are shown in 2D and 3D structures, with the co-crystallize ligand 2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid docked against Cox-2's active site

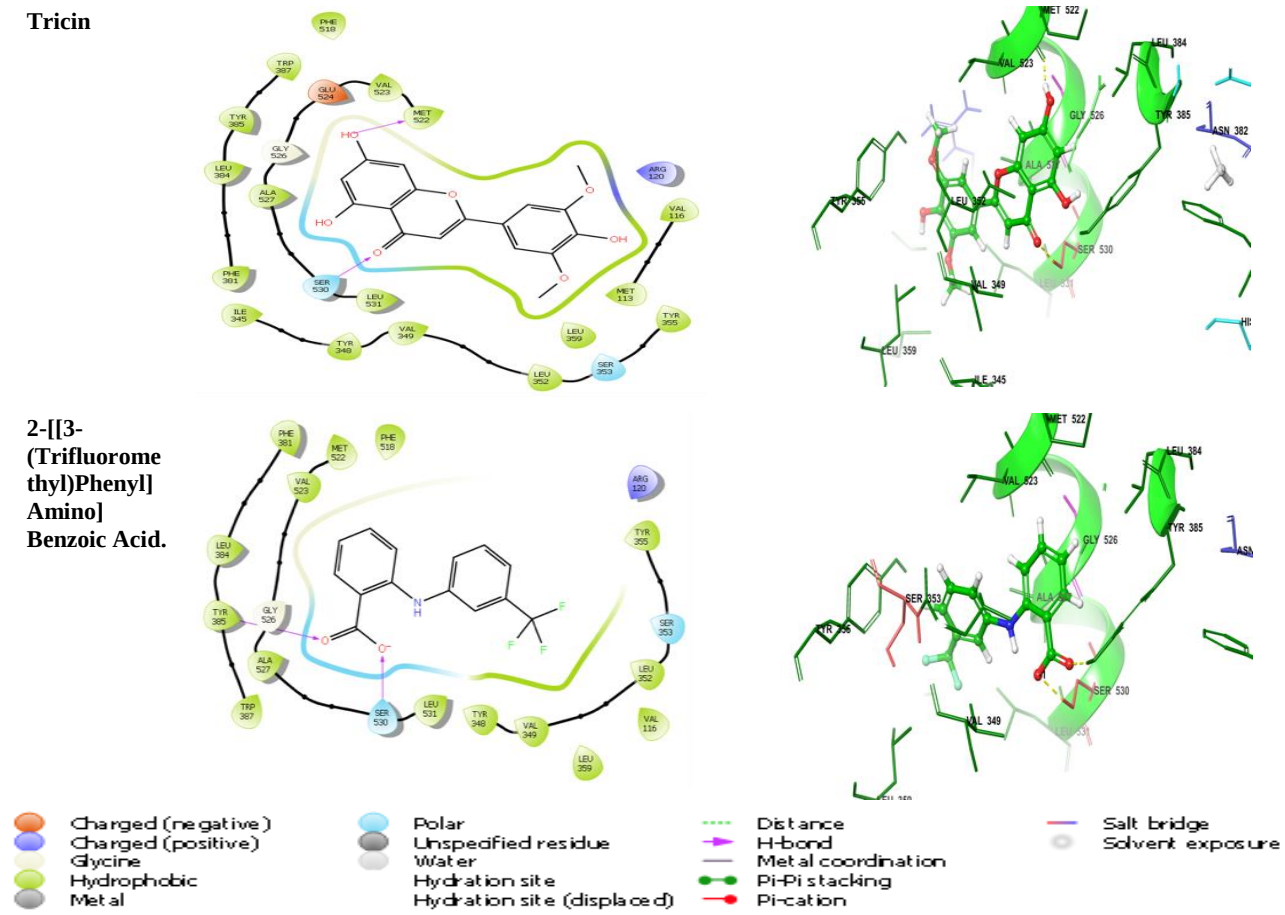

Figure 2: *Cont'd.*

COX-2 is often highly expressed in cancer cells. In the research to find suitable therapeutic substance, large epidemiological research comparing aspirin users and non-users found that non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase inhibitors may be useful in avoiding the initiation and progression of cancer (Ghosh *et al*., 2010). Most often, the argument has been, if computational experiments can reproduce experimental data. We got docking scores by using the maestro 12.8 and got the target from the online available database (cyclooxygenase (5IKV)) PubChem. Following the numerous health benefits documented by fenugreek, this study is based on the computational screening of the ligands as a potential Cox-2 inhibitor. Herbal medicines usually improve human health when used in moderation (Kaur *et al*., 2022).

Out of all the Phyto-compounds recovered from the plant in the current study, we found that Tricin, Naringenin, Quercetin, Kaempferol, and Gingerol demonstrated the strongest binding affinity with the cancer target. In a recent proof-of-concept study, molecular docking was proven to be a trustworthy stand-alone approach for predicting interactions. But we also present more data to support this claim.

The high binding energies of the lead compounds are a result of the interactions. From the 2D structure of the protein, we examine the binding affinities between the ligands and various amino acid residues. The relative attachment of gingerol to the receptor complex is mediated by both the pi-pi stack bond with TRY 355 and the hydrogen bond with serine residues SER 530. Kaempferol has a binding energy score of -10.109 kcal/mol due to hydrogen bond interactions, such as those involving SER 530 and the pi-cation interaction with ARG 120. One of the effective compounds, naringenin, is depicted in the image above with four hydrogen bonds with different amino acids. Examples are TYR 385, SER 530, MET 522, and TYR 355. A hydrogen bond is how quercetin interacts with SER530. Tricin, the most widely used chemical, demonstrates a hydrogen bond interaction between SER 530 and MET 522. Through hydrogen bonding, the common medicine communicates with SER 530 and TYR 385. According to the multiple interactions depicted in the illustration, the SER 530 pi-pi stack bond is shared equally by all of the hit compounds, whereas the TRY 355 pi-pi stack bond is shared by Gingerol, Naringenin, and the conventional treatment. The creation of molecules that are drug-like is greatly influenced by molecular interactions. These interactions comprise those between a drug's target protein and the drug's molecule as well as interactions between the drug's molecule and other molecules in the body, such as transporters or enzymes. The effectiveness and potency of medicine are significantly influenced by how well the drug molecule binds to its target protein. A pharmacological molecule that has a significant affinity for its target will attach to the protein tightly and may be more successful in changing the protein's function. A pharmacological molecule with an excessively high affinity may, however, also have unfavorable outcomes, such as off-target protein binding or an extended duration of action. A drug molecule must be able to interact with other molecules in the body in addition to its target protein to be

absorbed, distributed, metabolized, and removed. The pharmacokinetics and pharmacodynamics of a medication might be dramatically affected by these interactions, which are frequently mediated by enzymes or transporters. Overall, the design and optimization of druglike compounds must take the drug molecule's molecular interactions into account.

The computational approach MMGBSA (Molecular Mechanics Generalized Born Surface Area) is used to forecast the affinity of small compounds for binding to proteins. It combines molecular mechanics calculations, which predict the energy of a molecule based on its geometry and chemical properties, with generalized Born surface area calculations, which predict the energy associated with the interactions between molecules.

In drug discovery, MMGBSA is often used to predict the binding affinity of small molecule compounds to target proteins. This allows researchers to identify and optimize potential drug candidates based on their predicted ability to bind to and inhibit the target protein. Important details regarding the interactions between a small molecule and a protein, including the strength of the binding, the binding site on the protein, and the energetics of the interaction, may be learned via MMGBSA simulations. This knowledge may be utilized to understand the mechanisms of action of current medications as well as to direct the design of more powerful and selective drug candidates.

Overall, MMGBSA is a useful tool for the discovery and improvement of druglike compounds, assisting scientists in locating prospective drug candidates with the necessary binding affinity.

Prime MM-GBSA generates a large amount of energy. The energies for the ligand, receptor, and complex structures, as well as strain and binding energy differences, are reported by these features, which are split down into contributions from various terms in the energy expression. The MM-GBSA in the Prime module of the Schrodinger suite has previously been found to provide an accurate statistical post-docking analysis of docked complexes, with the lower the score, the higher the binding. Tricine, naringenin, quercetin, kaempferol, and gingerol have relative free binding energies of -31.06, -40.72, -36.25, and -42.75, respectively. The co-crystallized ligand's free binding energy is -43.55 as well (Table 1 and Figure 3). According to the MM-GBSA data, the two bioactive substances in question, Kaempferol, and Naringenin have a low and near binding energy to the reference molecule. Docking scores and MM/GBSA screening results for the hit compounds are shown graphically in Figures 3 and 4.

Table 1: Tabulated results of docking score, MM-GBSA, Fitness, and Ligand Energy scores. The compound's entry names are encoded in subsequent Tables.

Entry Name	Docking score	MM-GBSA	Fitness	Lig. Energy
Tricin (1)	-11.007	-31.06	1.997	-268.816
2-[[3-(TRIFLUOROMETHYL) PHENYL] AMINO] BENZOIC ACID (2)	-10.723	-43.55	2.669	-3.592
Naringenin (3)	-10.592	-40.72	0.284	-179.548
Quercetin (4)	-10.537	-36.25	0.24	-206.545
Kaempferol (5)	-10.109	-42.75	0.24	-222.101
Gingerol (6)	-8.703	-43	0.293	-27.734
Gentianine (7)	-7.841	-37.27	0.293	-0.72
Trigoforin (8)	-7.481	-46.17	0.102	-6.119
Scopoletin (9)	-7.149	-37.46	0.288	-5.639
Eugenol (10)	-7.137	-30.11	0.293	12.017
Zingerone (11)	-6.796	-31.29	0.287	-14.029
4-isopropyl-benzaldehyde (12)	-6.405	-31.21	0.293	6.894
Vanillin (13)	-6.323	-32.97	0.287	14.27
2,5-dimethyl pyrazine (14)	-4.58	-24.52	0.282	1.213

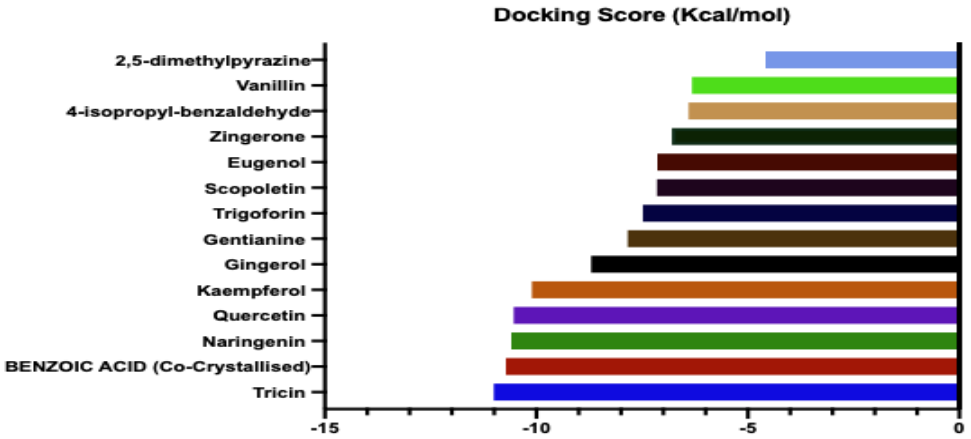

Figure 3: An illustration of the top fourteen compounds' molecular docking scores, together with the reference ligand 2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid (RED).

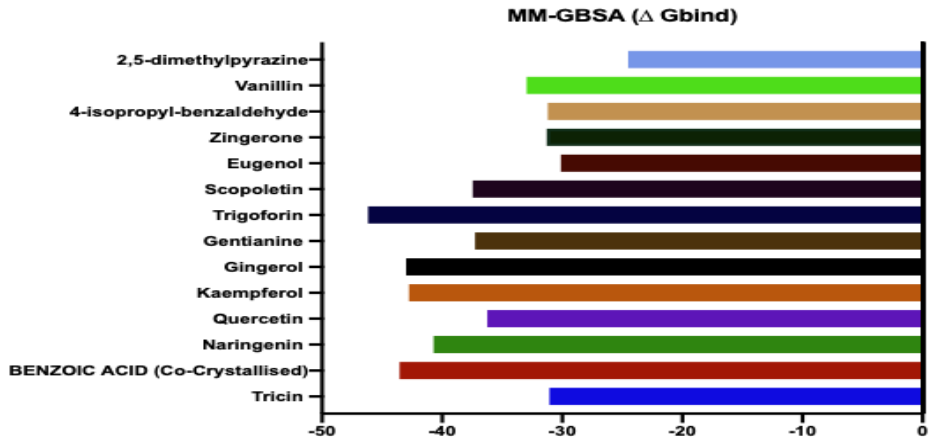

Figure 4: A graph depicting the top fourteen compounds' MM-GBSA scores, together with the reference ligand 2-[[3-(Trifluoromethyl) Phenyl] Amino] Benzoic Acid (RED).

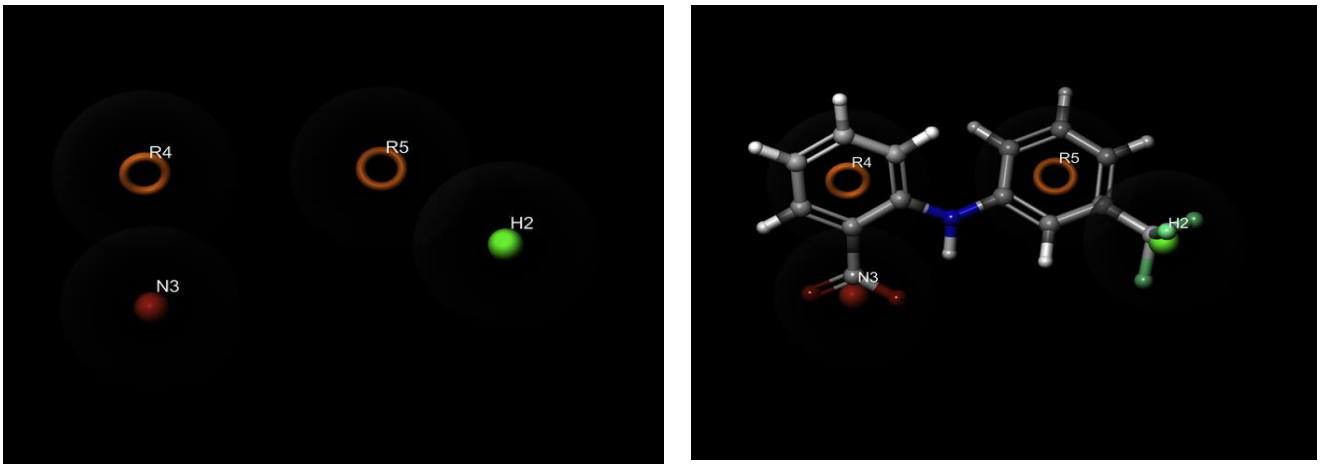

Figure 5: These reflect the reference ligand's pharmacophore and hypothesis.

Absorption, Distribution, Metabolism, Excretion, and Toxicity are referred to as ADMET. It is a phrase used to describe a substance's characteristics that might influence its potential as a drug. These characteristics can affect a substance's absorption, distribution, metabolization, elimination, and toxicity levels in the body. Due to their potential to impact a drug's safety and efficacy, ADMET characteristics are crucial factors to consider in the development of drug-like molecules. To find compounds with the appropriate qualities for usage as medications, researchers and pharmaceutical firms assess ADMET properties using a variety of approaches and technologies.

ADMETox screening was performed in maestro using QPlogHERG, QPPCaco, QPlogBB, QPPMDCK, and QPlogkhsa to filter the input hits and QikProp to identify Lipinski rule violations (i.e., Rule of Five and Rule of Three = 0). (Schrodinger 2020b). Tables 1, 2, 3, and 4 display these figures as well as how much the selected Phyto-ligands resemble pharmaceuticals. Table 5 further highlights the drug-like qualities of the medicinal plant against the target protein (5IKV). Although regulatory authorities are fascinated by ADME data, they are particularly interested in learning whether medications promote liver enzyme activity, which could affect drug-drug interactions. With a mandated and unchangeable panel of molecular, cellular, and whole animal testing, toxicology is typically a "box-ticking" process (Kaur *et al*., 2022).

A group of chemical characteristics known as a pharmacophore is necessary for a molecule to have biological action. For a chemical to interact with a target protein or receptor in a certain way, a hypothesized three-dimensional configuration of atoms or functional groups is required. By looking for molecules having the properties needed to interact with the target in the same way as the reference chemical, the determination of pharmacophores may be used to find and create novel compounds with potential therapeutic efficacy. As it can assist find compounds with the necessary biological activity while reducing the possibility of off-target effects, this can be a crucial stage in the drug discovery process.

The statistically significant pharmacophore hypothesis was applied to explore the compound library, and 13 compounds were mapped into the proposed hypothesis. Using ligand-based pharmacophore modeling, we produced a pharmacophore model containing one hydrophobic region, two aromatic rings, and one negative functional group. Using this pharmacophore model, 13 compounds were selected from a library of 60 compounds composed of fenugreek phytochemicals and the co-crystallized ligand. Fig. 5 displays the short list of substances that interacted with the four proteins in our receptors dataset and had traits in common with our pharmacophore model

Table 2: ADMETox research on the molecular weight, PISA, donor and accpt HBs, and QPlogPC16. As seen in Table 1, compound names are encoded using integers.

Entry Name	mol MW	PISA	donorHB	accptHB	QPlogPC16
1	330.293	177.738	2	5.25	10.228
2	281.234	266.458	1	1.5	7.849
3	272.257	246.824	2	4	9.409
4	302.24	210.536	4	5.25	10.418
5	286.24	252.7	3	4.5	9.992
6	294.39	100.058	1	4.2	9.557
7	175.187	125.805	0	4.5	5.605
8	188.226	122.109	0	2.5	5.864
9	192.171	166.875	1	4	6.321
10	164.204	133.768	1	1.5	5.643
11	194.23	101.465	1	3.5	6.547
12	148.204	123.657	0	2	5.378
13	152.149	109.722	1	3.5	5.323
14	108.143	94.402	0	2	3.594

Table 3: QPlogHERG, QPPCaco, QPlogBB, QPPMDCK, QPlogKp, and QPlogKhsa ADMETox research. As seen in Table 1, compound names are encoded using integers.

Entry Name	QPlogHERG	QPPCaco	QPlogBB	QPPMDCK	QPlogKp	QPlogKhsa
1	-4.772	129.598	-1.561	54.347	-4.073	0.001
2	-3.051	283.675	-0.202	709.927	-2.133	0.247
3	-4.664	137.603	-1.306	57.984	-3.972	-0.071
4	-4.6	20.635	-2.215	7.458	-5.509	-0.378
5	-4.843	57.24	-1.757	22.468	-4.595	-0.226
6	-4.455	601.222	-1.347	285.438	-2.38	0.084
7	-3.183	1454.217	-0.167	741.537	-2.6	-0.888
8	-3.708	2684.006	0.073	1438.183	-2.192	-0.088
9	-3.795	644.839	-0.566	307.885	-3.045	-0.48
10	-3.826	3030.792	-0.126	1640.043	-1.664	-0.138
11	-3.93	1031.241	-0.626	511.435	-2.592	-0.282
12	-3.635	1824.297	-0.186	947.466	-2.32	-0.224
13	-3.35	570.115	-0.647	269.51	-3.255	-0.608
14	-3.238	3905.166	0.229	2156.961	-1.973	-0.534

Table 4: Human Oral Absorption, QPlogPw, PSA, Rule of Five, and Rule of Three ADMETox studies. As seen in Table 1, compound names are encoded using integers.

Entry Name	HumanOralAbsorption	QPlogPw	PSA	RuleOfFive	RuleOfThree
1	3	10.605	114.323	0	0
2	3	5.696	57.09	1	0
3	3	10.093	98.604	0	0
4	2	14.188	138.775	0	1
5	3	12.164	117.234	0	0
6	3	5.619	75.213	0	0
7	3	6.269	52.014	0	0
8	3	4.413	38.432	0	0
9	3	7.596	70.073	0	0
10	3	4.013	30.062	0	0
11	3	5.983	59.123	0	0
12	3	3.542	37.245	0	0
13	3	6.452	67.05	0	0
14	3	3.391	24.898	0	0

Table 5: The bio-availability, pharmacokinetics properties, and cytochrome P450 metabolizing enzymes inhibitory potentials of selected phytochemical consistent of fenugreek (Target protein; 5IKV)

MODELS	Tricin	2- [3-(trifluoromethyl)phenyl]amino] benzoic acid	Naringenin	Quercetin	Kaempferol	Gingerol	Gentianine	Trigoforin	Scopoletin	Eugenol	Zingerone	4-isopropyl-benzaldehyde	Vanillin	2,5-dimethyl pyrazine
BLOOD BRAIN BARRIER	BBB-	BBB-	BBB-	BBB-	BBB-	BBB+	BBB+	BBB+	BBB+	BBB+	BBB+	BBB+	BBB+	BBB+
BIOAVAILABILITY SCORE	0.55	0.85	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
CYP1A2 INHIBITION	Inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor
CYP2C19 INHIBITION	Non-inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP2C9 INHIBITION	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP2D6 INHIBITION	Inhibitor	Inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP3A4 INHIBITION	Inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
GI ABSORPTION	High	Low	High	High	High	High	High	High	High	High	High	High	High	High
P-GLYCOPROTEIN SUBSTRATE	No substrate	Substrate	Substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate	No substrate
LD50 (mg/kg)	4000	800	2000	159	3919	2580	500	1691	3800	1930	2580	1320	1000	1020
TOXICITY CLASS	5	4	4	3	5	5	4	4	5	4	5	4	4	4
CARCINOGENICITY	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Active
MUTAGENICITY	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
CYTOTOXICITY	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
HEPATOTOXICITY	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

4. Conclusion

Cancer is indeed a major health concern worldwide. It is a leading cause of death and a major public health problem. There are many different types of cancer, and it can affect people of all ages. The good news is that there has been a lot of progress in the diagnosis and treatment of cancer, and many people with cancer can live long and healthy lives. This is largely due to advances in medical research, which have led to the development of new and more effective treatments. However, cancer remains a major public health challenge, and there is a need for continued research to better understand the disease and to develop even more effective treatments.

Concentrating on the Cox-2 pathway is a successful strategy for both solid tumor prevention and treatment. It is impossible to emphasize the multiple health benefits of fenugreek seed; nonetheless, in this study, we employed bioinformatics drug design to focus on the anti-cancer, detox, and inhibitory actions of the fenugreek plant.

The results suggest that Tricin, Naringenin, Quercetin, Kaempferol, and Gingerol are potential Cox-2 inhibitors that ought to be optimized as lead chemicals in additional pharmaceutical procedures.

However, there is a need for further *in vitro* experiments to further validate the inhibitory properties of the above-listed compounds obtained from fenugreek for the treatment of cancer.

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The study was approved by the Ethical unit of Molecular Biology and Simulation Center, Ado-Ekiti, Ekiti State, Nigeria with a reference number of MSERB/CADD/ NHNAS/2022/07.

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