Molecular Docking and ADMET Studies for Examining the Breast Cancer Specific Gene ETS2 against Plant Derived Natural Bioactive Compound of *Curcuma Longa*

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Abstract

An uncontrollably growing cell population brought on by a sequence of molecular events is referred to as "cancer." Breast cancer is a major worldwide health concern, demanding the development of new therapeutic approaches. . Recent World Health Organization (WHO) data state that breast cancer affects 2.1 million women annually and is the leading cause of cancer-related deaths among women. The ETS2 gene, which is linked in breast cancer growth, appears to be a good target for intervention. Molecular docking methods are widely employed in contemporary drug design to investigate the conformations of ligands that are adopted within the binding sites of macromolecular targets. We used molecular docking and ADMET (absorption, distribution, metabolism, excretion, and toxicity) analyses to look into the possible interaction between ETS2 and bioactive chemical obtained from Curcuma longa (turmeric).

We have determined the binding affinities and mechanisms of certain chemicals from Curcuma longa with the ETS2 protein by computational modeling. Our research reveals encouraging interactions that raise the possibility that these organic substances could function as ETS2 inhibitors. Moreover, ADMET tests offered insightful information on the drugs' pharmacokinetic and toxicity profiles, suggesting that more research should be done on them as potential treatments for breast cancer. This work emphasizes the use of in silico for drug discovery and the possibility of natural chemicals produced from plants as potential treatments for breast cancer that target ETS2. To validate these computational predictions and move the development of innovative anti-breast cancer medicines forward, more experimental validation is necessary.

Keyword: *ETS2, ADMET, Breast cancer, Curcuma longa, Molecular docking.*

INTRODUCTION

An uncontrollably growing cell population brought on by a sequence of molecular events is referred to as "cancer." The regular regulatory mechanisms in malignant cells are disrupted, making it difficult for the cells to proliferate and invade other tissues **[1]**. An estimated 14.1 million new cases of cancer were recorded in 2012, according to the International Agency for Research on Cancer (IARC). Of these cases, 8 million happened in economically developing nations, which are home to around 82% of the world's population. With 8.8 million recorded deaths in 2015, cancer ranks as the second most common cause of death globally. About one in six fatalities worldwide are attributable to cancer **[2]**. Global demographic trends predicted that by 2025, there will be around 420 million new instances of cancer yearly, indicating an increase in cancer incidence over time. Approximately 18 million cases of cancer were reported globally in 2018; roughly 9.5 million of these cases were in men and 8.5 million in women. The number of new cancer cases worldwide is predicted to increase to 21.7 million by 2030 **[3]**.

The most common disease in women in the world is breast cancer. Recent World Health Organization (WHO) data state that breast cancer affects 2.1 million women annually and is the leading cause of cancer-related deaths among women. Additionally, according to WHO estimates, 6,27,000 women died from breast cancer in 2018 . This suggests that 15% of all women's cancer-related fatalities occur as a result of breast cancer **[4].** Breast cancer is the most common disease diagnosed in this group, accounting for over one in ten new instances of cancer found in women. It ranks as the second most common cause of cancer-related deaths in women globally. Anatomically, the breast's milk-producing glands are situated in front of the chest wall. The breast is supported and connected to the chest wall by ligaments, and they rest on the pectoralis major muscle. There are 15–20 lobes arranged in a circle that make up the breast. The fat that covers the lobes determines the size and contour of the breasts. (Fig.2) Each lobe is made up of lobules, which are groups of glands that release milk in response to hormone stimulation. Every time breast cancer develops, it does so silently **[5].**

Curcumin, which is derived from turmeric, has been used for millennia for its medical benefits. Only recently, however, has research been done to identify the precise mechanism or mechanisms of action as well as the bioactive components of turmeric **[6]**. Worldwide, curcumin is being acknowledged and utilized in a variety of ways for its possible health advantagesThere are numerous ways to get curcumin, such as pills, capsules, ointments, energy drinks, soaps, and cosmetics **[7]**. The original member of the Ets transcription factor family is Ets1 (also referred to as ets, c-ets, c-ets-1, ets-1, or Tpl-1). In humans, 28 genes and in mice, 27 genes make up the Ets gene family**.** The primary isoform of Ets2 is 441 amino acids long in humans and 440 amino acids long in mice. The Ets domain, a ~85 amino acid winged-helix-turn-helix DNA binding motif, is how Ets2 and other Ets transcription factors attach to DNA **[8]**.

METHODOLOGY

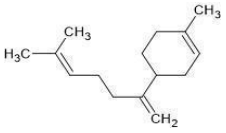
1. Retrieval of the bioactive compound: The major bioactive compounds are present in the curcuma longa known to have potential anti breast cancer properties. The bioactive compounds of curcuma longa were retrieved from Indian Medicinal Plants, Phytochemical And Therapeutics 2.0(IMPPAT 2.0). The largest database on phytochemicals of Indian medicinal plants to date. IMPPAT can be freely accessed **[9]**, and more data was retrieved from the PubChem like- PubChem ID, molecular formula,simplified molecular input line entry system(SMILES)**[10]** and 3D SDF files.

2. Physicochemical properties ,drug likeness, and pharmacokinetics predication Physically significant characteristics and significant descriptors for pharmaceutical use of the target curcuma longa were anticipated. The physicochemical properties(topological polar surface area(TPSA),molar refractivity, no.of hydrogen bond donor, no. of hydrogen bond acceptor,(log Po/w) lipophilicity) by using SwissADME to predict drug resemblance (RO5) **[11].** According to the published formula, % Abs = 109–(0.345*TPSA), the ligands percent absorption (%Abs) was computed. The ADME properties were retrieved from the SwissADME web Tools by using the SMILES. When ADME Properties were predicted show many data like GI Absorption, BBB permeant, P-gp substrate, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, LogKp(skin permeation).

3. Toxicity Prediction- An important part of the process of finding and developing new drugs is toxicity research. Ensuring safety and efficacy entails evaluating the possible harmful impacts of chemical substances on biological systems. The toxicological endpoints (Hepatotoxicity, Neurotoxicity, Nephrotoxicity, Respiratory Toxicity, Cardiotoxicity, Carcinogenicity, Cardiotoxicity, Immunotoxicity, Mutagenicity, Cytotoxicity, Ecotoxicity, Clinical toxicity, Nutritional toxicity), Toxicity class and the level of toxicity(LD_{50},mg/kg) of the studied of bioactive compound of curcuma longa were retrieved by using the ProTox-III webtools **[12].**

4. Molecular structure

Figure.1- The 2D structure of bioative compounds- The number of (1-30) stand for Beta-Bisabolene(1), 2-(4-Methylphenyl)propan-2-ol(2), Stigmasterol(3), Campesterol(4), Turmerone(5), Procurcumadiol(6), P-coumaric-acid(7), Niacin(8), Limonene(9), Norbixin(10), Bixin(11), Beta-carotene(12), L-alpha-curcumene(13), Isoborneol(14), Gamma-atlantone(15), Eucalyptol(16), Epiprocurcumenol(17), Eugenol(18), Curdione(19), Curcumin(20), Curcumenol(21), Curcumene(22), Cinnamic acid(23), Caffeic-acid(24), Borneol(25), Beta-pinene(26), Azulene(27), Ascorbic-acid(28), Arabinose(29), Bis-



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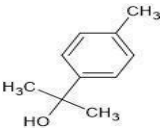

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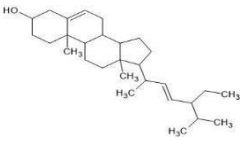

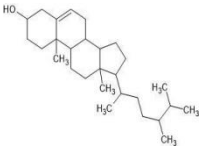

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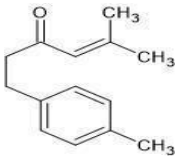

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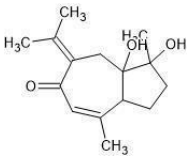

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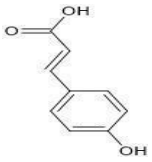

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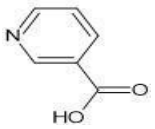

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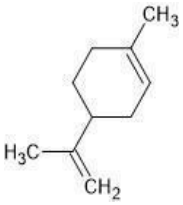

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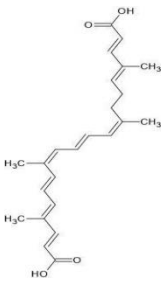

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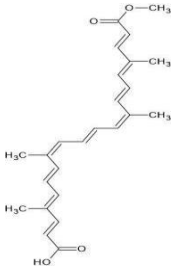

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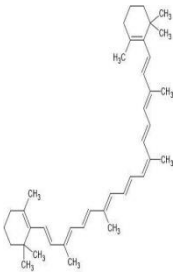

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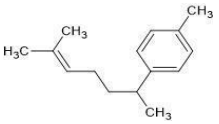

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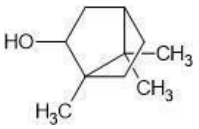

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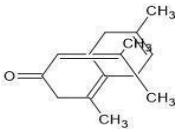

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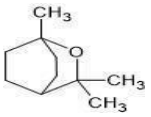

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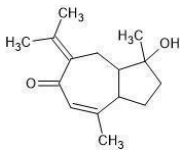

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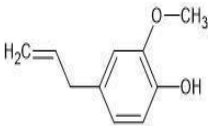

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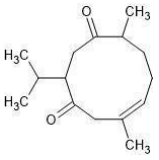

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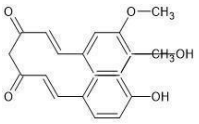

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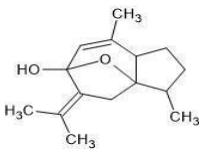

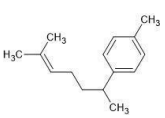

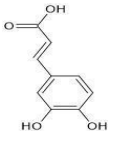

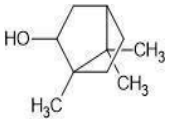

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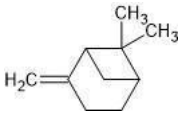

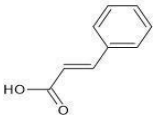

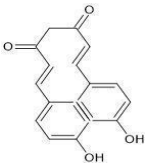

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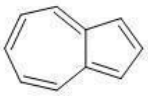

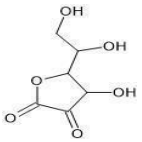

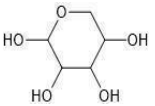

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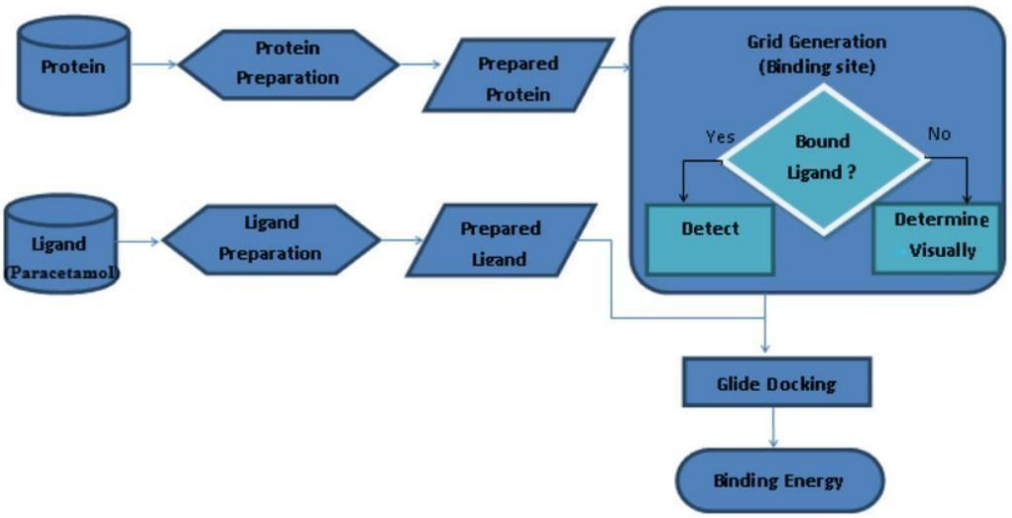
5. Computational details-



RESULT AND DISCUSSION

1. Analysis of Physicochemical properties and drug likeness-

Table. 1- The Physicochemical properties and drug likeness-

S NO.	Phytochemical Compound	Molecular Weight	No. of H-bond donor	No. of H-bond acceptor	No. of rotable bond	TPSA	% of abs.	Log Po/w
1	Beta-Bisabolene	204.35 g/mol	4	0	0	0.00 Å²	109	3.67
2	2-(4-Methylphenyl)Propan-2-Ol	150.22 g/mol	1	1	1	20.23 Å²	102.02	2.17
3	Stigmasterol	150.22 g/mol	1	1	1	20.23 Å²	102.02	2.17
4	Campesterol	400.68 g/mol	5	1	1	20.23 Å²	102.02	4.92
5	Turmerone	218.33 g/mol	4	1	0	17.07 Å²	103.11	3.21
6	Procurcumadiol	250.33 g/mol	0	3	2	57.53 Å²	89.15	2.3
7	P-Coumaric-Acid	164.16 g/mol	2	3	2	57.53 Å²	89.15	0.95
8	Norbixin	380.48 g/mol	10	4	2	74.60 Å²	83.263	3.96
9	Niacin	123.11 g/mol	1	3	1	50.19 Å²	91.68	0.86
10	Limonene	136.23 g/mol	1	0	0	0.00 Å²	109	2.72
11	L-Alpha-Curcumene	202.34 g/mol	4	0	0	0.00 Å²	109	3.5
12	Isoborneol	154.25 g/mol	0	1	1	20.23 Å²	102.02	2.29
13	Gamma-Atlantone	218.33 g/mol	3	1	0	17.07 Å²	103.11	3.2
14	Eucalyptol	154.25 g/mol	0	1	0	92.3 Å²	105.81	2.58
15	Epiprocurcumenol	234.33 g/mol	0	2	1	37.30 Å²	96.13	2.62
16	Eugenol	164.20 g/mol	3	2	1	29.46 Å²	98.83	2.37
17	Curdione	236.35 g/mol	1	2	0	34.14 Å²	97.22	2.8
18	Curcumin	368.38 g/mol	8	6	2	93.06 Å²	76.89	3.27
19	Curcumenol	234.33 g/mol	0	2	1	29.46 Å²	98.83	2.83
20	Curcumene	202.34 g/mol	4	0	0	0.00 Å²	109	3.5
21	Cinnamic Acid	148.16 g/mol	2	2	1	37.30 Å²	96.13	1.55
22	Caffeic-Acid	180.16 g/mol	2	4	3	77.76 Å²	82.17	0.97
23	Borneol	154.25 g/mol	0	1	1	20.23 Å²	102.02	2.29
24	Bixin	394.50 g/mol	11	4	1	63.60 Å²	87.05	4.74
25	Bis-Desmethoxycurcumin	308.33 g/mol	6	4	2	74.60 Å²	83.26	1.75
26	Beta-Pinene	136.23 g/mol	0	0	0	0.00 Å²	109	2.59
27	Azulene	128.17 g/mol	0	0	0	0.00 Å²	109	2.17
28	Ascorbic-Acid	176.12 g/mol	2	6	3	104.06 Å²	73.09	0.04
29	Arabinose	150.13 g/mol	0	5	4	90.15 Å²	77.89	-0.39

In this analysis, out of the thirty physico chemical compounds of Curcuma longa, only one (**27-Beta-carotene** the molecular mass is greater than 500 g/mol) of them do violate Lipinski's rules of five for oral bioavailability. In the above (table.1) mentioned parameters are molecular weight, no. of rotatable bond , TPSA, % of

Abs. and log p. (RO5) that a compound is likely orally active if it violates at most one of the molecular mass is not greater than 500 g/mol, hydrogen-bond-donor is not less than 5.

The TPSA value of a any bioactive compound is 140 $Å^2$ and above, then the percentage of absorption is very poor(<10% functional absorption), and when the TPSA value is 90 $Å^2$ and less, then the percentage of absorption is high(>90% functional absorption). The curcuma longa derivatives of TPSA value and the % of abs. demonstrated the preference of Norbixin(8), Curcumin(18), Caffeic-Acid(22), Bixin(24), Bis-Desmethoxycurcumin(25), Arabinose(30). It is found that the %Abn and TPSA values follow the order Bixin(24)> Bis Desmethoxycurcumin(25)> Norbixin(8)> Caffeic-Acid(22)> Arabinose(30)> Eucalyptol(14)> Curcumin(18). The highest percent absorption is 87.05(Bixin) and the better percentage of absorption is 76.89(Curcumin).

According to the Lipinski rules, the Log P value is not more than 5. The ligand is equally divided into the lipid and aq. Phase, table-1 the results show that there are only one (30) Arabinose(-0.39) that is more hydrophilic in nature. And the other compound they showed the lipophilic properties maintained in table -1.

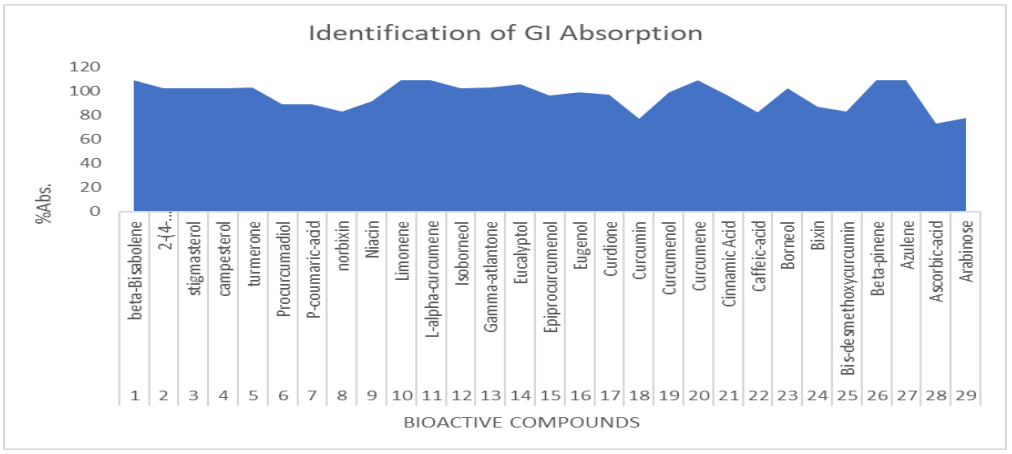


Figure.1.1. Graphic representation of GI absorption

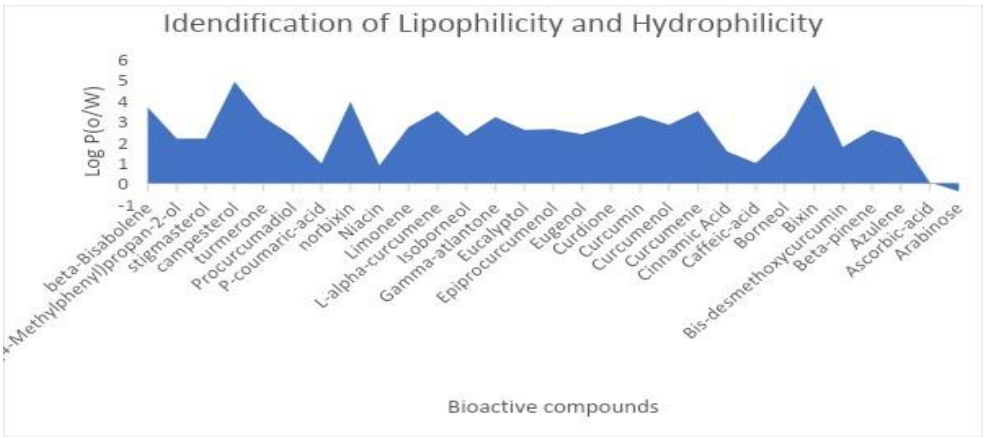


Figure.1.2. Graphic representation of Log P

2. Pharmacokinetic activity-

Table 1.2. The prediction of Pharmacokinetics properties of Bioactive compound-

S. No.	Bioactive compounds	GI Absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	LogKp (cm/s)
1	Beta-Bisabolene	0	0	0	0	0	1	0	0	-2.98 cm/s
2	2-(4-Methylphenyl)Propan-2-Ol	1	1	0	1	0	0	0	0	-5.80 cm/s
3	Stigmasterol	0	0	0	0	0	1	0	0	-2.74 cm/s
4	Campesterol	0	0	0	0	0	0	0	0	-2.50 cm/s
5	Turmerone	1	1	0	0	0	1	0	0	-5.27 cm/s
6	Procurcumadiol	1	1	0	0	0	0	0	0	-6.92 cm/s
7	P-Coumaric-Acid	1	1	0	0	0	0	0	0	-6.26 cm/s
8	Norbixin	1	0	1	0	0	1	0	0	-3.51 cm/s
9	Niacin	1	1	0	0	0	0	0	0	-6.80 cm/s
10	Limonene	0	1	0	0	0	1	0	0	-3.89 cm/s
11	L-Alpha-Curcumene	0	0	0	0	0	0	1	0	-3.71 cm/s
12	Isoborneol	1	1	0	0	0	0	0	0	-5.31 cm/s
13	Gamma-Atlantone	1	1	0	1	0	1	0	0	-3.96 cm/s
14	Eucalyptol	1	1	0	0	0	0	0	0	-5.30 cm/s
15	Epiprocurcumenol	1	1	0	0	0	0	0	0	-6.07 cm/s
16	Eugenol	1	1	0	1	0	0	0	0	-5.69 cm/s
17	Curdione	1	1	0	0	0	0	0	0	-5.86 cm/s
18	Curcumin	1	0	0	0	0	1	0	1	-6.28 cm/s
19	Curcumenol	1	1	0	0	0	0	0	0	-6.14 cm/s
20	Curcumene	0	0	0	0	0	0	1	0	-3.71 cm/s
21	Cinnamic Acid	1	1	0	0	0	0	0	0	-5.69 cm/s
22	Caffeic-Acid	1	0	0	0	0	0	0	0	-6.58 cm/s
23	Borneol	1	1	0	0	0	0	0	0	-5.31 cm/s

24	Bixin	1	0	1	0	0	1	0	0	-3.37 cm/s
25	Bis-Desmethoxycurcumin	1	1	0	1	0	1	0	1	-5.87 cm/s
26	Beta-Pinene	0	1	0	0	0	1	0	0	-4.18 cm/s
27	Azulene	0	1	0	1	0	0	0	0	-4.81 cm/s
28	Ascorbic-Acid	0	0	0	0	0	0	0	0	-8.46 cm/s
29	Arabinose	0	0	0	0	0	0	0	0	-9.36 cm/s

Whereas; High-1, low-0, yes-1, no-0

In the pharmacokinetic study, to evaluate the different parameters like Absorption, distribution, metabolism, excretion and the toxicity(ADMET) with the help of SwissADME wed tools(15). We found the various types data like GI absorption, BBB permanent, P-gp substrate, CYP1A2 inhibitor , CYP2C19 inhibitor , CYP2C9 inhibitor, CYP2D6 inhibitor , CYP3A4 inhibitor and LogKp (cm/s).

Absorption- The main functions of the GI system include waste product elimination, water and enzyme secretion, nutrition absorption, and food ingestion and digestion(17). To determine the absorption properties of the curcuma longa is, out of twenty nine bioactive compounds we find the twenty compounds have high GI absorption and remaining nine compounds have low GI absorption (table-3). The twenty high GI absorption of the bioactive compound are 2-(4-Methylphenyl)Propan-2-Ol(2), Turmerone(5), Procurcumadiol(6), P-Coumaric-Acid(7), Norbixin(8), Niacin(9), Isoborneol(12), Gamma-Atlantone(13), Eucalyptol(14), Epiprocurcumenol(15), Eugenol(16), Curdione(17), Curcumin(18), Curcumenol(19), Cinnamic Acid(21), Caffeic-Acid(22), Borneol(23), Bixin(24), Bis-Desmethoxycurcumin(25) and rest of compound are showed low GI absorption mention in in (table-3).

Distribution- We evaluate the different parameters for distribution are Blood Brain Barrier(BBB) and volume of distribution. We retrieved the pharmacokinetic properties by using the SwissADME web tools then they formed some compound are permeant the BBB like 2-(4-Methylphenyl)Propan-2-Ol(2), Campesterol(4), Procurcumadiol(6), P-Coumaric-Acid(7), Niacin(9), Limonene(10), Isoborneol(12), Gamma-Atlantone(13), Eucalyptol(14), Epiprocurcumenol(15), Eugenol(16), Curdione(17), Curcumenol(19), Cinnamic Acid(21), Borneol(23), Bis-Desmethoxycurcumin(25), Beta-Pinene(26), Azulene(27) and remaining compound do not have permeant Blood Brain Barrier.(Table-3)

Metabolism and Excretion: P-glycoprotein is also known as multi drug resistance. In this analysis we find only two compounds are active like Bixin(24), Norbixin(8), and whereas the rest of compounds are inactive(table-3).

The Cytochrome p450 is divided into various types such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. A medication's toxicity may arise from its inhibition of a CYP enzymatic pathway, which raises the amounts of other

medicines that are metabolized by the same system. To evaluate the inhibitor activity of CYP p450 .

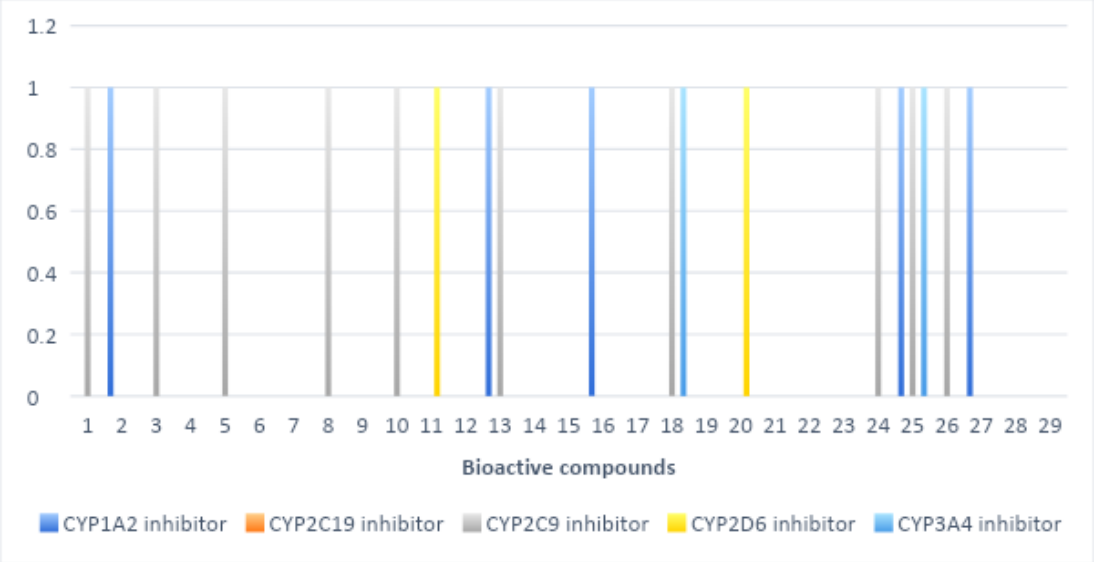


Figure.2.1. Graphic representation of inhibitors

The skin permeability (log kp cm/s)values ranged from -6.10 to -0.76 and the unit of Log kp is cm/s. When developing transdermal drug delivery, particularly for delivering anti-cancer drugs to skin cancer areas, skin permeability and drug uptake rate are crucial indicator parameters. The skin permeability, Kp values obtained for all the compounds considered in this study are in the range of -9.36cm/s to -2.50cm/s mansion in table-3. The most high skin permeation is Campesterol(4) and the low skin permeation is Arabinose(30).

3. Analysis of Toxicity-

Table-3. ProTox III predicted organ toxicity, toxicological endpoints, and acute toxicity

S. No.	Bioactive compound	Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Respiratory Toxic.	Cardiotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Ecotoxicity	LD 50 (mg/kg)	Toxicity Class
1	beta-Bisabolene	0	0	0	0	0	0	0	0	0	1	4400	5
2	2-(4-Methylphenyl)propan-2-ol	0	0	0	0	0	1	0	0	0	1	1020	4
3	stigmasterol	0	1	0	2	0	0	2	0	0	1	890	4
4	campesterol	0	1	0	2	0	0	2	0	0	1	890	4
5	turmerone	0	0	0	0	0	0	0	0	0	0	2920	5
6	Procurcumadiol	0	0	0	2	0	1	2	0	0	0	4000	5
7	P-coumaric-acid	0	0	1	0	0	1	0	0	0	0	2850	5
8	norbixin	0	0	1	0	0	0	0	0	0	0	4300	5
9	Niacin	2	1	1	2	0	0	0	0	0	0	3720	5
10	Limonene	0	0	0	0	2	0	0	0	0	1	4400	5
11	L-alpha-curcumene	0	0	0	0	0	0	0	0	0	1	2000	4
12	Isoborneol	0	0	0	1	0	0	0	0	0	1	500	4
13	Gamma-atlantone	0	0	0	0	0	0	0	0	0	1	4590	5
14	Eucalyptol	0	0	0	0	0	0	0	0	0	1	2480	5
15	Epiprocurcumenol	0	0	0	2	0	0	0	0	0	1	4000	5
16	Eugenol	0	1	0	0	0	0	0	0	0	0	1930	4
17	Curdione	0	0	0	0	0	0	1	0	0	0	5000	5
18	Curcumin	0	0	1	0	1	0	2	0	0	0	2000	4
19	Curcumenol	0	0	0	1	0	0	0	0	0	1	6000	6
20	Curcumene	0	0	0	0	0	0	0	0	0	1	2000	4
21	Cinnamic Acid	1	0	1	0	0	0	0	0	0	0	2500	5

22	Caffeic-acid	0	0	1	0	0	0	0	0	0	0	2980	5
23	Borneol	0	0	0	1	0	0	0	0	0	1	500	4
	Bixin	0	0	1	0	0	0	0	1	0	0	5600	6
25	Bis-desmethoxycurcumin	0	0	1	0	0	0	0	0	0	0	2560	5
26	Beta-pinene	0	1	0	0	0	0	0	0	0	2	4700	5
27	Azulene	0	1	0	0	0	2	0	0	0	2	3000	5
28	Ascorbic-acid	0	o	1	0	0	0	0	0	0	0	10600	6
29	Arabinose	0	0	2	0	0	0	0	0	0	0	23000	6

Where as; Inactive-0, active-1, toxic-2

The toxicological endpoints (hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutational toxicity, cytotoxicity, ecotoxicity, clinical toxicity, and nutritional toxicity) and the level of toxicity (LD50, mg/kg) and toxicity class of the twenty nine bioactive compound derivative were predicted by using the ProTox III tool. In the prediction of hepatotoxicity, only one bioactive compound, Niacin(9) showed high toxicity and remaining all bioactive compounds are inactive for hepatotoxicity mentioned in (table-4).

Neuroprotective drugs shield healthy tissue while maintaining anti-tumor activity, which helps to reduce the neurotoxicity brought on by cytotoxic agents. when we predicted the neurotoxicity then they found some compound showed less active like stigmasterol(3), campesterol(4), Niacin(9), Limonene(10), Eugenol(16), Beta-pinene(26), Azulene(27) and rest of compound inactive forneurotoxicity(table-4). In nephrotoxicity, one compound showed Arabinose(29) highly toxic and nine compounds showed low toxicity.

If the hazardous threshold is crossed during inhalation and subsequent absorption, inhaled chemicals may result in lung sickness. The results showed that stigmasterol(3), campesterol(4), Procurcumadiol(6), Niacin(9), Epiprocurcumenol(15), are highly toxic and low toxic are Isoborneol(12), Curcumenol(19), Borneol(23) and remaining all compound inactive for respiratory toxicity. The results predict that some compounds showed highly toxic like Limonene (10) and Arabinose(29) and the rest of compounds showed inactive for cardiotoxicity. It is also known as oncogenesis or tumorigenesis, carcinogenesis is the process by which healthy cells develop into malignant ones. In the prediction of Carcinogenicity were found Caffeic-acid(22) and Azulene(27) is more toxic other then 2-(4-Methylphenyl)propan-2-ol(2), Procurcumadiol(6), P-coumaric-acid(7) and remaining all bioactive compound are inactive for Carcinogenicity.

Stigmasterol(3), campesterol(4), Procurcumadiol(6) and Curcumin(22) are highly toxic in nature and the rest of all compounds are inactive for Immunotoxicity. All the bioactive compounds are inactive for mutagenicity except Bixin(24).

The toxicity were predicted by using the ProTox III tools. The (LD50) lethal dose values were found to be in the range from 500-23,000 (mg/kg) maintion in table-4. 2-(4-Methylphenyl)propan-2-ol(2), stigmasterol(3), campesterol(4), L-alpha-curcumene(11), Isoborneol(12), Eugenol(16), Curcumin(18), Curcumene(20), Borneol(23) were predicted as harmful (class IV), beta-Bisabolene(1), turmerone(5), Procurcumadiol(6), P-coumaric-acid(7), norbixin(8), Niacin(9), Limonene(10), Gamma-atlantone(13), Eucalyptol(14), Epiprocurcumenol(15), Curdione(17), Cinnamic Acid(21), Caffeic-acid(22), Bis-desmethoxycurcumin(25), Beta-pinene(26), Azulene(27) are may be harmful (classV) toxicity class and Curcumenol(19), Bixin(24), Ascorbic-acid(28), Arabinose(29) were predicted as non toxic (class VI) toxic classes. ccording to the findings, the majority of drug-like substances are more likely to have carcinogenic properties than any toxicological endpoint.

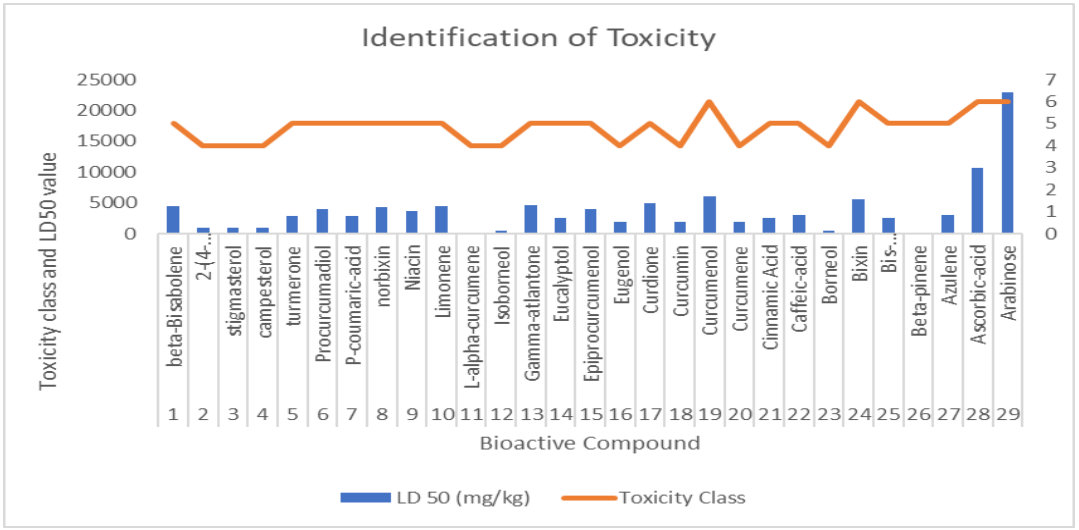


Figure.3.1. Graphic representation of LD50 and toxicity class

4. Evaluation of Molecular Docking and Visualization of Ligand- Protein interaction

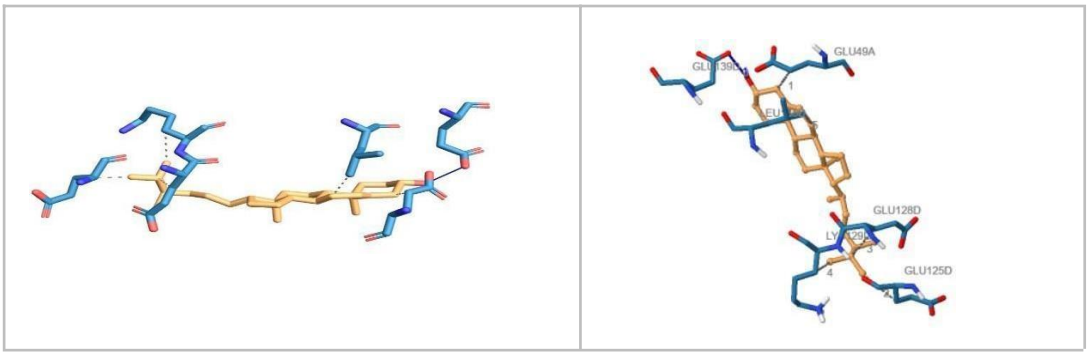
Table 3.4. Binding energy and Molecular docking interaction of ETS2 with phytoconstituent

S. No.	Bioactive Compound	Binding Energy (kcal/mol)	Hydrogen bond interaction	Hydrophobic interaction
1	Beta-Bisabolene	-5.58	….....	GLU 49A, GLN 52A, GLU 128D, ILE 131D, GLU 132D, LEU 135D, GLU 139D
2	Stigmasterol	-7.06	GLN 56B, HIS 53B.	GLU 49A, GLU 125D, GLU 128D, GLU 129D, LEU 135D, LYS 136E, GLU 139E, GLU 140E, LYS 143E
3	Campesterol	-6.1	GLU 139D	GLU 49A, GLU 125D, GLU 128D, GLU 129D, LEU 135D
4	Turmerone	-5.16	HIS 53B	GLU 49B, GLN 52B, GLU 128E, GLU 132E,LEU 135E LYS 136E, GLU 139E
5	Procurcumadiol	-5.64	GLN 52B, GLU 132E, LYS 136E	GLU 49B, GLN 52B, GLU 132E, LEU 135E
6	P-Coumaric-Acid	-4.47	GLN 39A, GLU 146D	…..
7	Norbixin	-6.39	GLU 135D, LYS129D	ARG 46A, GLU 49A, GLN 52A, GLU 128D, GLU 132D, LEU 135D
8	Niacin	-4.71	SER 35A, GLN 141F, LYS 154D.	GLN 39A.
9	Limonene	-4.31	…..	GLU 49C, GLN 52C, LEU 135F, LYS 136F, GLU 139F
10	L-Alpha-Curcumene	-5.24	….....	GLU 49A, GLN 52A, GLU 132D, LEU 135D, GLU 139D
11	Isoborneol	-4.14	HIS 53A	GLU 49A, GLN 52A, GLU 132D, LEU 135D
12	Gamma-Atlantone	-6.11	GLN 52A	GLU 49A, GLN 52A, GLU 128D, LEU 135D
13	Eucalyptol	-4.44	HIS 53B, GLN 56B	GLN 49B, GLN 52B, GLU 132E, LEU 135E,GLU 139
14	Epiprocurcumenol	-5.72	HIS 53C, GLU 132F, LYS 136F	GLU 49C, GLN 52C, GLU 132F, LEU 135F, LYS 136F
15	Eugenol	-3.81	GLN 56B, HIS 53B.	GLU 49B, GLN 52B, GLU 132E, LEU 135E, GLU 139E
16	Curdione	-5.46	HIS 53A	GLU 49C, GLN 52C, GLU 132F, LEU 135F, GLU 139F
17	Curcumin	-4.75	GLN 52B, GLN 56B, LYS 129E, LYS 136E	GLU 49B, GLN 52B, GLU 128E, GLU 132E, LEU 132E
18	Curcumenol	-5.78	HIS 53A, GLN 56A	GLU 49A, GLN 52A,ILE 131D, GLU132D, LEU 135D
19	Curcumene	-5.14	….....	GLU 49A, GLN 52A, GLU 128D, ILE 131D,GLU 132D, LEU 135D, GLU 139D
20	Caffeic-Acid	-4.59	ASN 43B, LYS 137D, GLN 141D	LYS 137D
21	Borneol	-4.13	ASN 43B, GLN 141D	LYS 137D, GLU 140D, GLN 141D
22	Bixin	-6.24	GLN 56B	ARG 46B, GLU 49B, GLU 52B, GLU 128E, GLU 132E
23	Beta-Pinene	-4.05	…….	GLU 49B, GLN 52B, ILE 131E, GLU 132E, LEU 135E
24	Azulene	-4.2	GLU 49C, GLN 52, GLG 132F, LEU 135F, LYS 136F, GLU 139F	GLU 125F
25	Arabinose	-3.23	ARG 46C, GLA 49, LYS 130E	…...

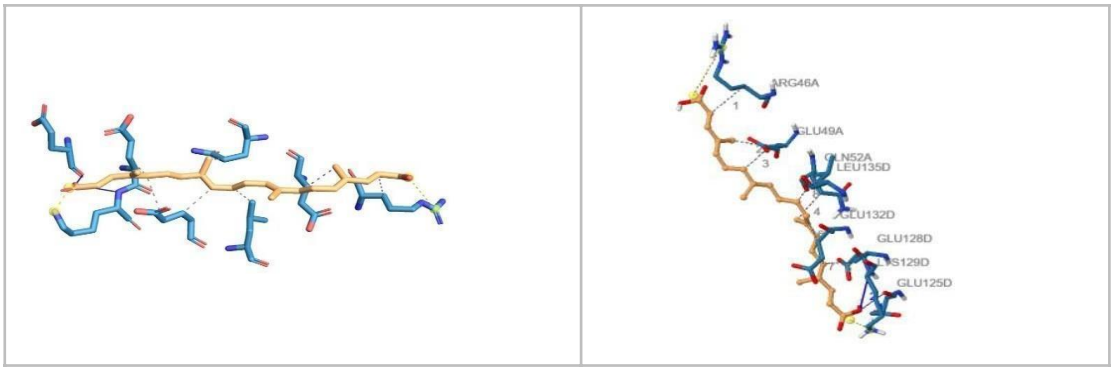
When we performed the docking process then they found only twenty five compounds have the best result for docking. Stigmasterol has the highest binding affinity with ETS2 of all the chemicals examined, according to the molecular docking results. This suggests that Stigmasterol is the most viable option for additional research as a potential therapeutic drug targeting ETS2 in breast cancer. The lowest binding energy of the bioactive compound ranges from -7.06 (kcal/mol) to -3.23 (kcal/mol) maintion in table-5. More negative binding free energy released after the complex formation during docking, the greater the stability of the complex formed .

An extensive variety of ligand poses were produced following the conclusion of the docking experiment for each of the twenty-eight bioactive compounds using AutoDock. An analysis of the optimal binding affinity ligand posture was conducted during the experiment. interactions involving salt bridges, hydrophobic bonds, bonds of hydrogen, and traces of amino acids are maintained in table-6. In the analysis of twenty five compounds we find the best interaction between bonds of hydrogen, hydrophobic bonds and other interactions.

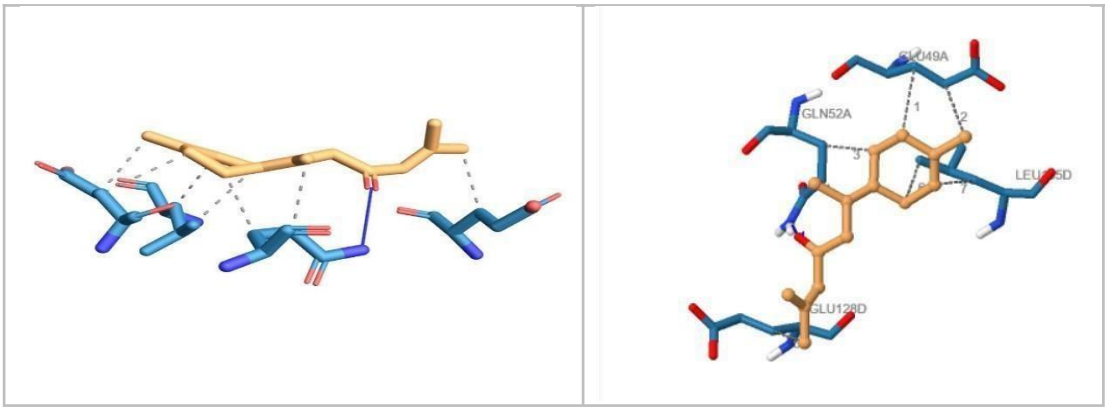
Campesterol forms bonds of hydrogen interaction with one traces of amino acids (GLU 139D), and thea water-resistant interaction with five traces of amino acids (GLU 49A, GLU 125D, GLU 128D, GLU 129D, LEU 135D). In the Norbixin compound forms bonds of hydrogen interaction with two traces of amino acids(GLU 135D, LYS129D), and water-resistant bond interaction with six traces of amino acids(ARG 46A, GLU 49A, GLN 52A, GLU 128D, GLU 132D, LEU 135D). Gamma-Atlantone form bonds of hydrogen interaction with only one trace of amino acids present (GLN 52A), and four traces of amino acids shown in water-resistant interaction are (GLU 49A, GLN 52A, GLU 128D, LEU 135D). The 2D and 3D ligand-protein interactions are represented in (figure-3.5)



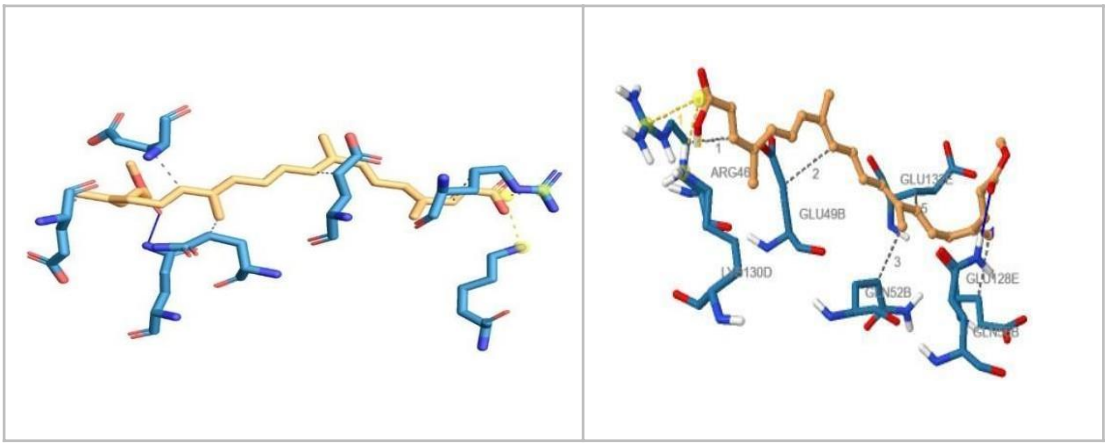
Campesterol



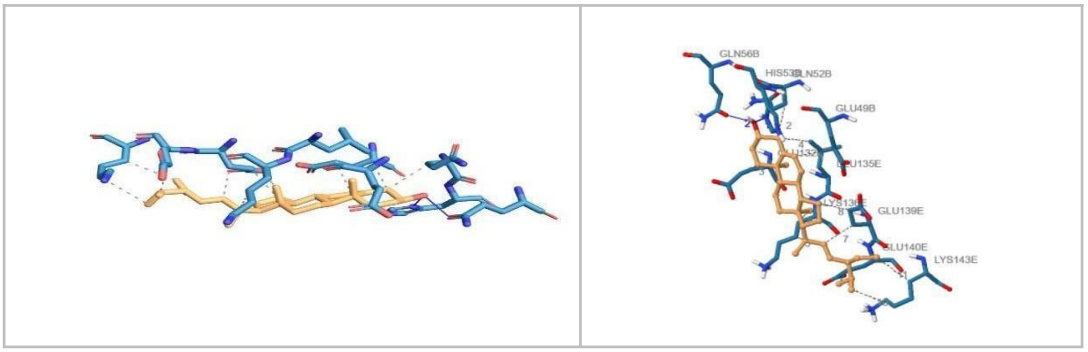
Norbixin



Gamma-Atlantone



Bixin



Stigmasterol

Where as;

- This colour is represented by ligands.
- This colour is represented by protein.
- This colour is represented by Aromatic ring center.
- This colour is represented by Hydrophobic interaction.
- This colour is represented by Hydrogen bonds.

Figure.4. The two-dimensional and three- dimensional structure representing the binding pose and ligand-protein interactions -The number of (1-5) stands for Campesterol, Norbixin, Gamma-atlantone, Bixin, Stigmasterol.

CONCLUSION

Conclusively, the physicochemical properties, drug-likeness, pharmacokinetic and toxicity profile of thirty phytoconstituents of *Curcuma longa* was investigated using insilico methods. Twenty nine phytoconstituents follow Lipinski's rule of five for oral bioavailability and one phytoconstituents do not follow Lipinski's rule of five. Tumerone is free from any of the predicted toxicological endpoints. The result showed that Stigmasterol (-7.06 kcal/mol), Norbixin (-6.3 kcal/mol), Bixin (-6.24kcal/mol), Gamma-atlantone(-6.11kcal/mol), Campesterol (-6.1kca/mol) have lowest binding energy and these compound tightly binds to targeted protein ETS2. The compounds with best binding energy show good ADMET properties but Stigmasterol ,Campesterol have low GI absorption and Norbixin, Bixin, Gamma-atlantone have high GI absorption. Norbixin, Gamma-atlantone and Bixin have toxicity classes respectively 5, 5 and 6. Overall study concludes that Norbixin, Bixin, Gamma-atlantone are potential agents for synthesis of breast cancer. These compounds for future in vitro and in vivo research based on the molecular docking, drug likeness, pharmacokinetics and toxicity profile.

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Conflict of Interest

All the authors declare that there is no conflict of interest.

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