

Original Article

Targeting Squamous Cell Carcinoma: In Silico Evaluation of Bioactive Ligands from Ethanolic Root Extract of Vitex NegundoAnitha K^{1,*}, Mageshwari Anand², Prasanth P¹, Sakti Kishore S¹, Sabarinathan R¹¹Department of Pharmaceutical Chemistry, Dr M.G.R Educational and Research Institute, Chennai, Tamil Nadu, India²Department of Pharmaceutical Chemistry, Madras Medical College, Park town, Chennai, Tamil Nadu, India

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ABSTRACT

Numerous pharmacological activities are present in Vitex negundo, a well-known medicinal plant used in traditional medicine. This study employs an in-silico approach to evaluate the potential of Vitex negundo against squamous cell carcinoma (SCC), a common form of skin cancer. Ethanol extraction of Vitex negundo roots was conducted to isolate bioactive compounds, followed by preliminary phytochemical screening. A virtual library of 56 ligands reported in literature from the ethanolic root extract was assembled and subjected to molecular docking studies targeting the epidermal growth factor receptor (EGFR), a key protein involved in SCC progression. Docking was performed using AutoDock 1.5.6, and subsequent screening for drug-likeness and toxicity was carried out via SwissADME and OSIRIS Toxicity Explorer. The top-scoring ligands demonstrated strong binding affinity to EGFR alongside favorable pharmacokinetic and safety profiles, indicating their promising therapeutic potential against SCC. These findings provide a foundation for further experimental validation and drug development involving Vitex negundo bioactives for skin cancer treatment.

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Introduction

Vitex negundo Linn, commonly known as Nigundi, is a large aromatic shrub belonging to the family Verbenaceae. It grows erectly to a height of 2–5 meters and is widely distributed throughout the tropical, semi-tropical, and warm temperate regions of India.

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Material and Methods**Biological target**

The selection of targets for this study was based on a thorough literature review of potential therapeutic targets for skin cancer. The X-ray crystallographic structures of these targets were obtained from the RCSB Protein Data Bank (PDB), taking into account factors like resolution and other relevant parameters. The Protein Data Bank is a resource for three-dimensional structural data of large biological molecules, including proteins and nucleic acids. Recent PDB enzyme targets with lower resolution were considered and further assessed based on their resolution values, R-free, R-value, and optimized crystal-ligand interaction details.

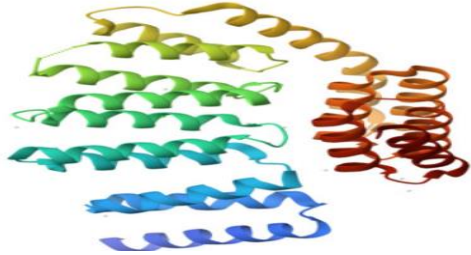
The following were some of the receptors for skin cancer [2].

Table 1: Biological target for skin cancer.

CTLA-4	JAK
MEK	PD-1
BRAF	EGFR

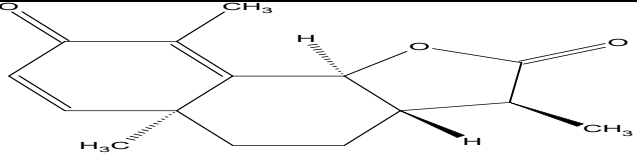
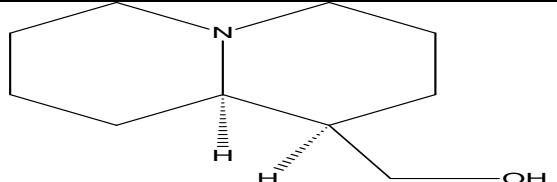
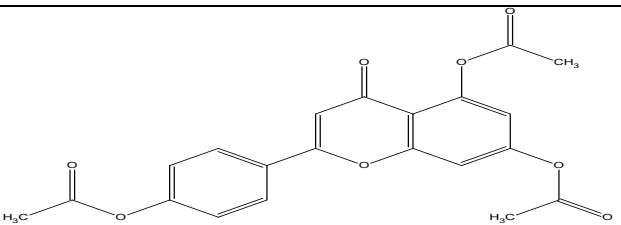
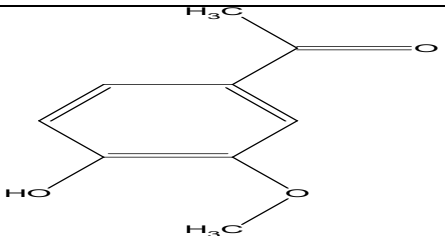
EGFR (Epidermal Growth Factor Receptor) is a transmembrane tyrosine kinase receptor that plays a crucial role in regulating cell proliferation, differentiation, migration, and survival. It is often overexpressed or dysregulated in various cancers, including non-melanoma skin cancers (NMSC) like squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), and to a lesser extent in melanoma [3].

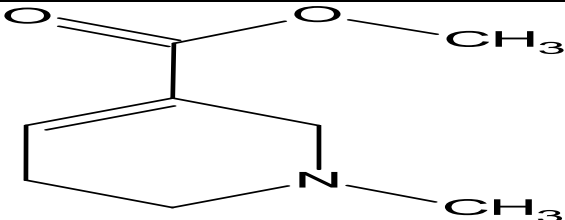
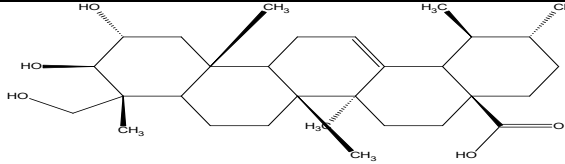
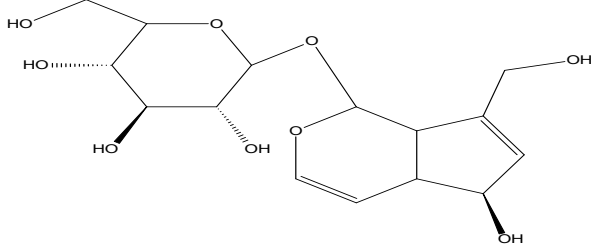
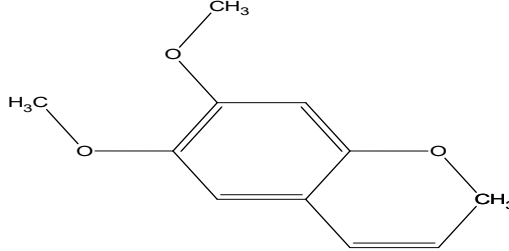
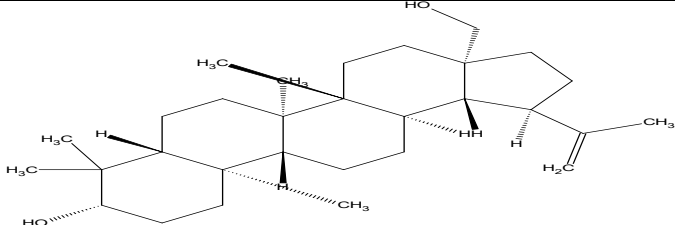
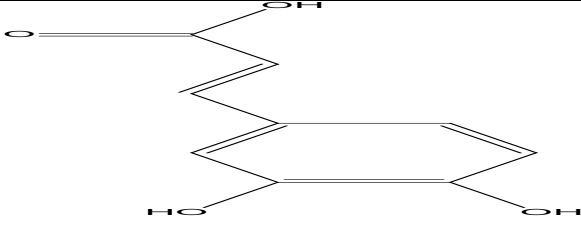
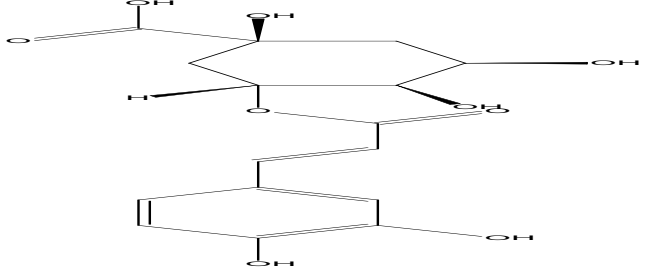
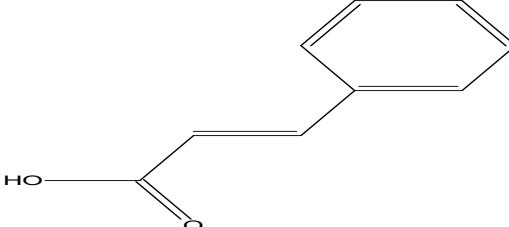
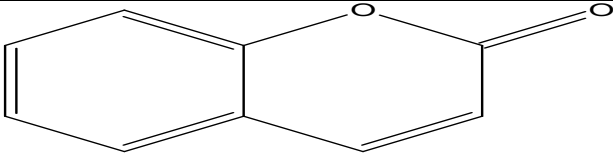
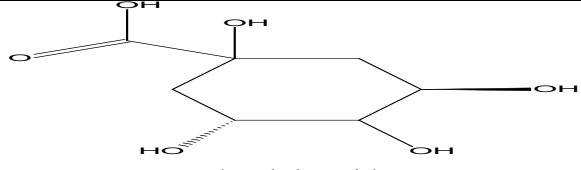
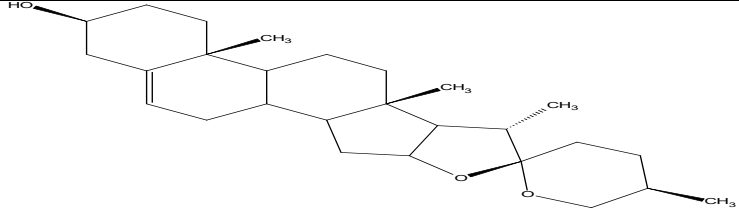
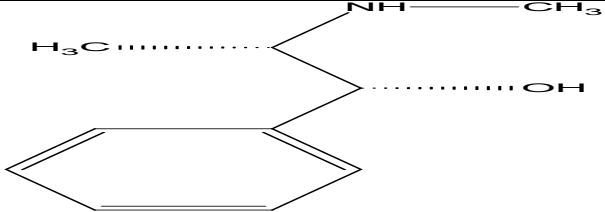
Table 2: PDB IDs of EGFR Target.

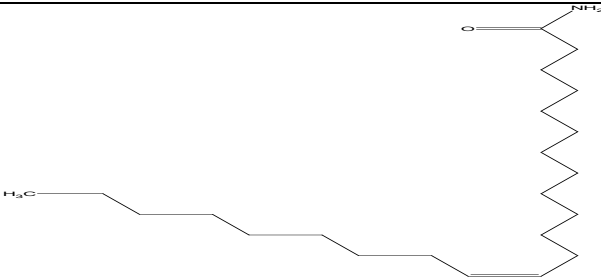
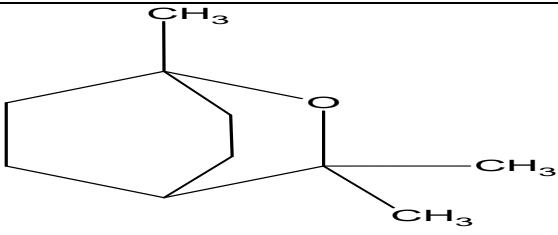
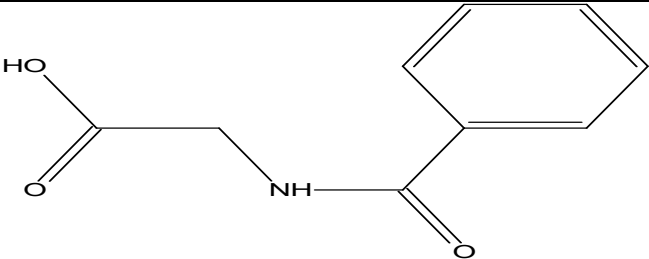
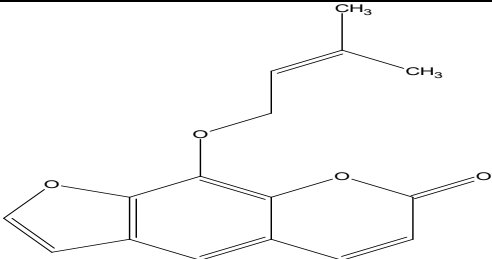
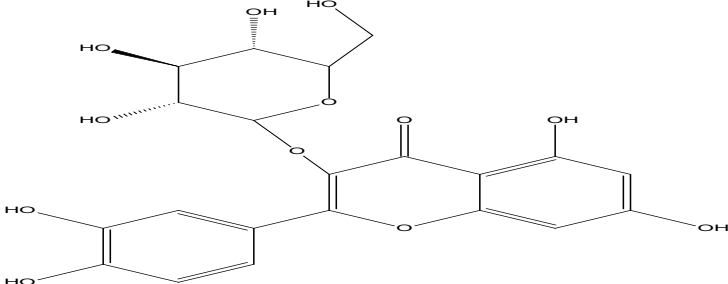
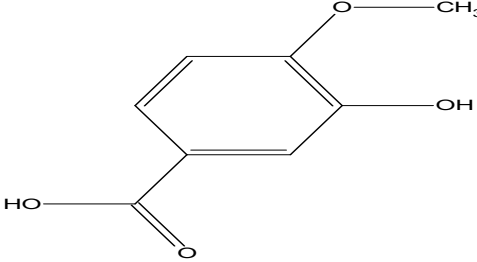
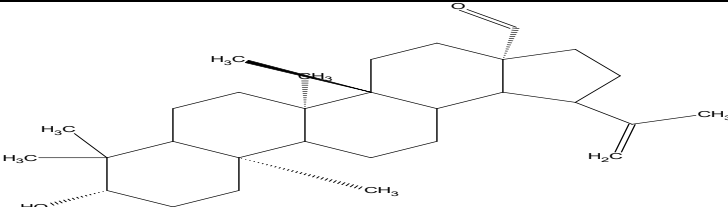
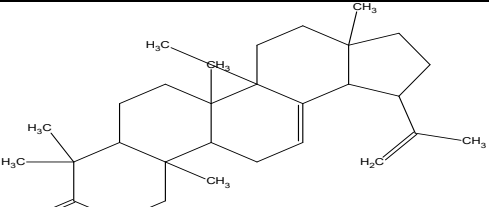
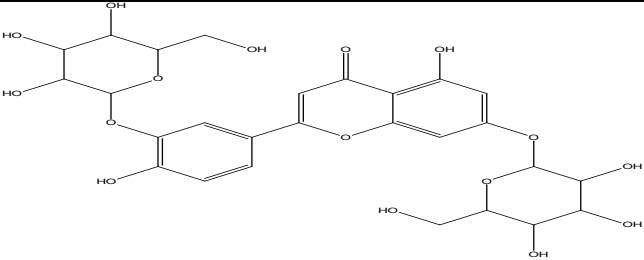
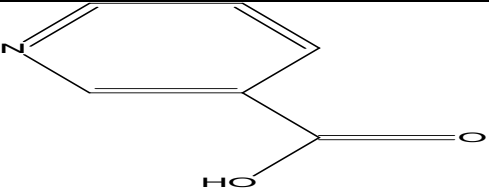
Target	PDB ID	Resolution (Å°)	5JJX 
EGFR	5JPZ	3.04	
	2ZV6	2.6	
	8KFD	1.8	
	7MRJ	2.12	
	5CTQ	2.6	
	5JJX	2	
	8HGK	1.9	

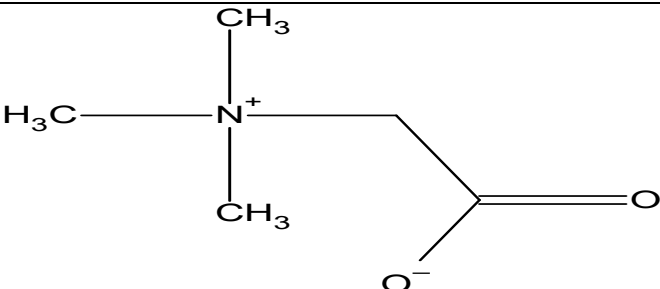
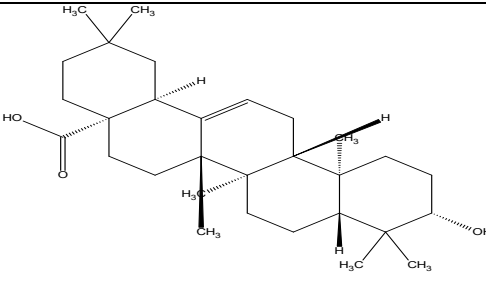
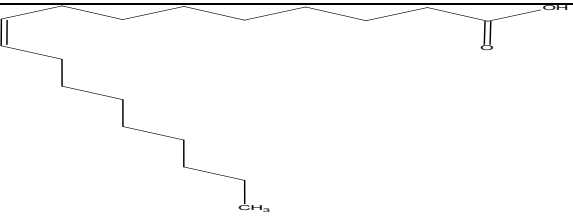
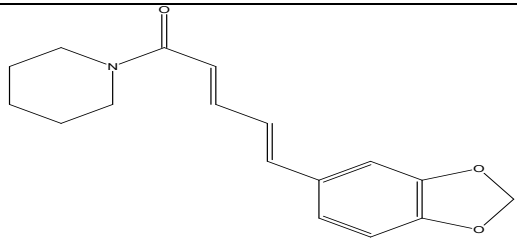
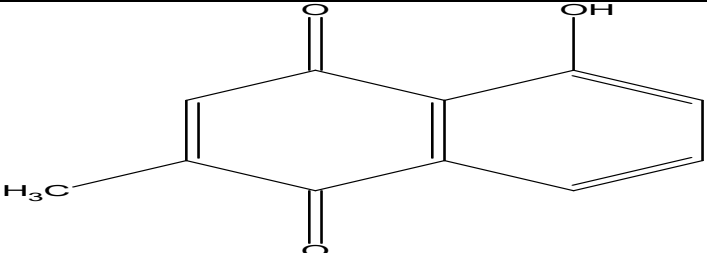
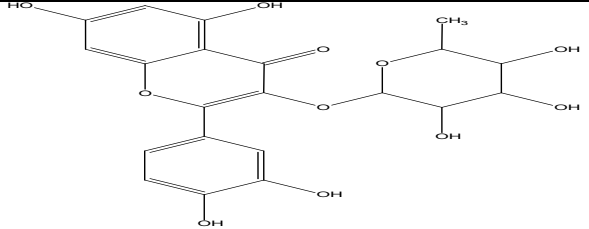
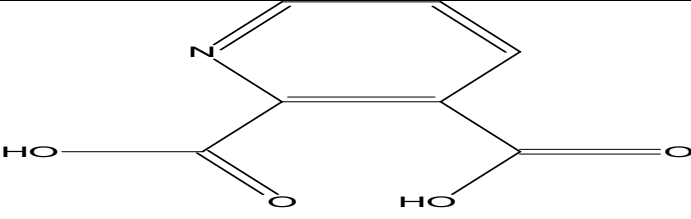
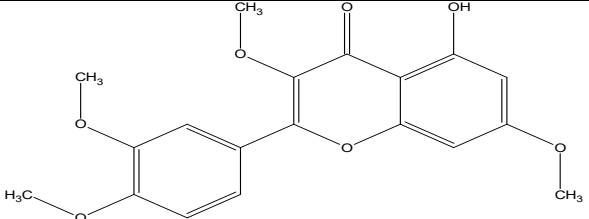
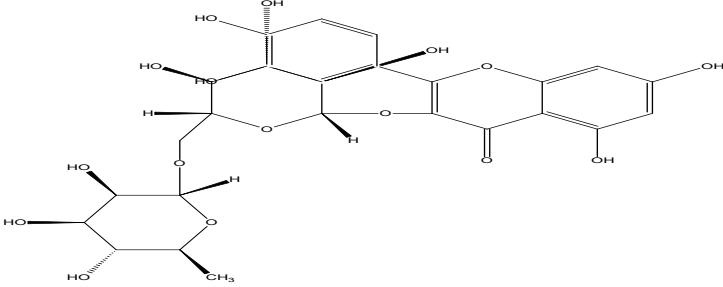
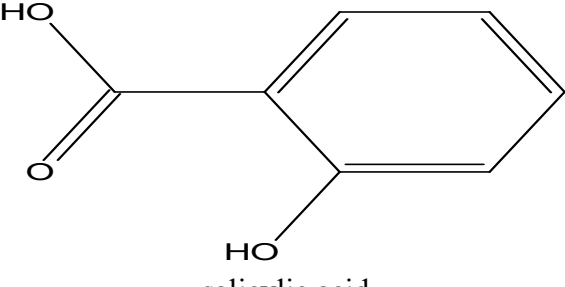
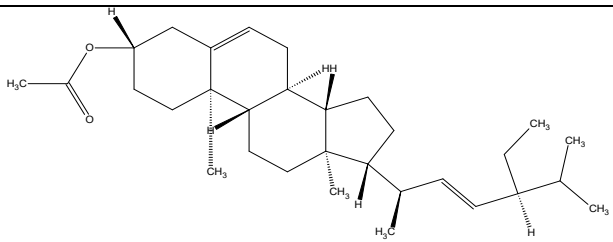
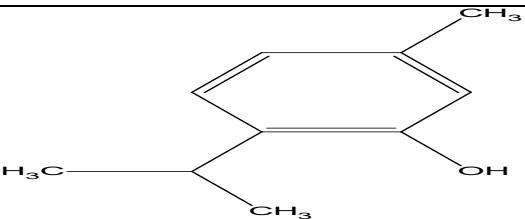
Ligand of Libraries

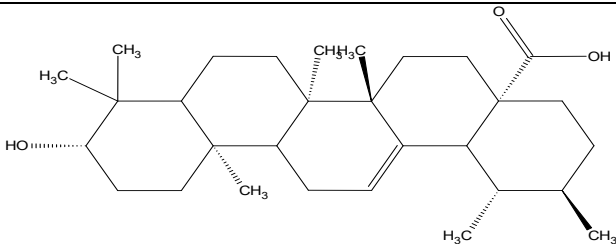
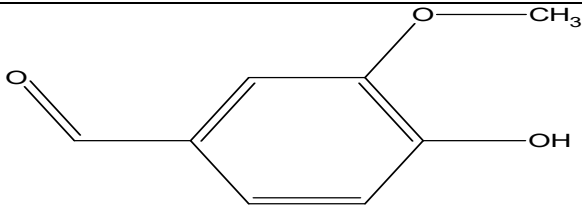
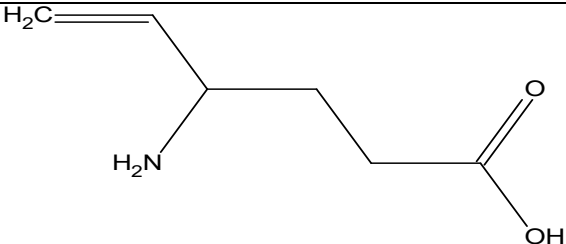
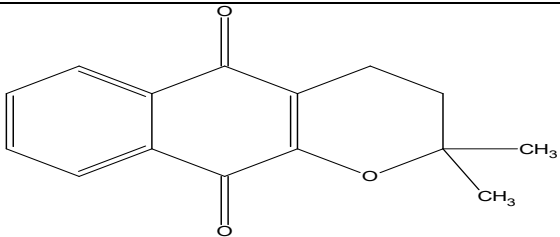
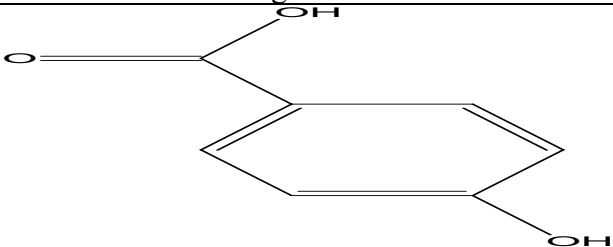
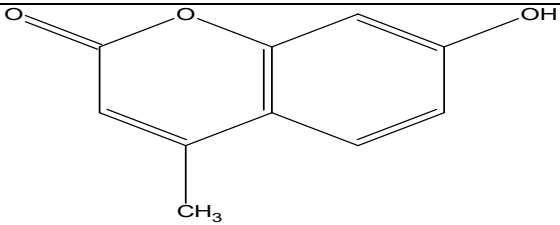
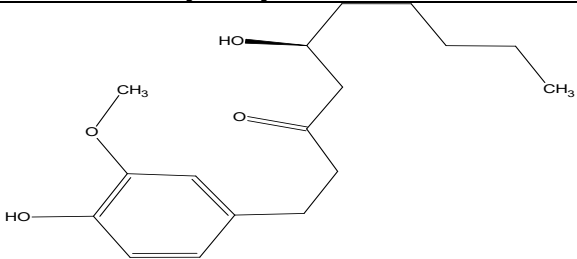
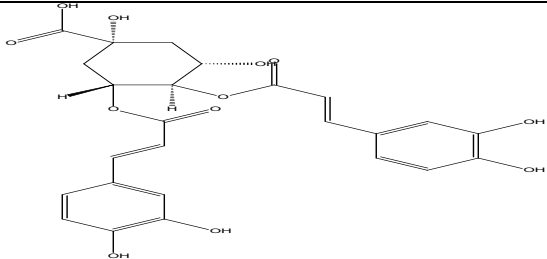
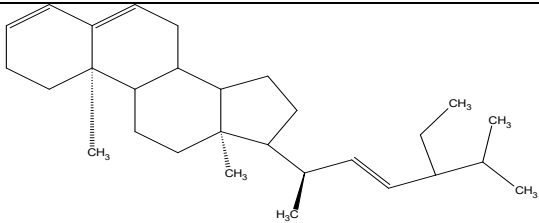
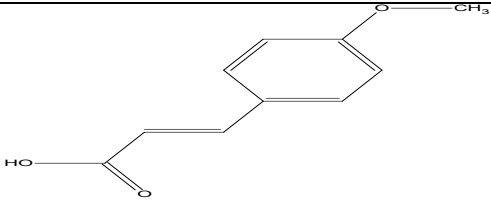
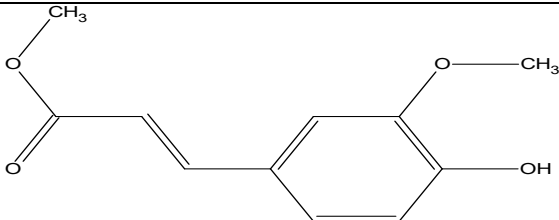
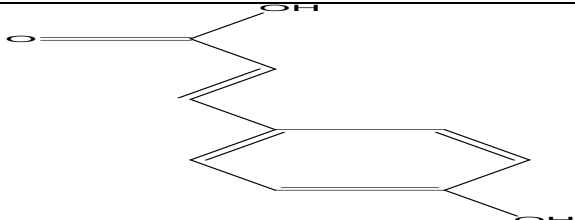
Table 3: Ligand of libraries.

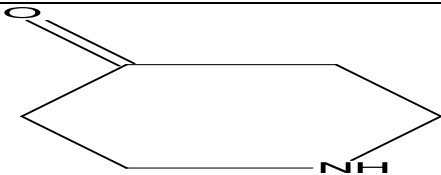
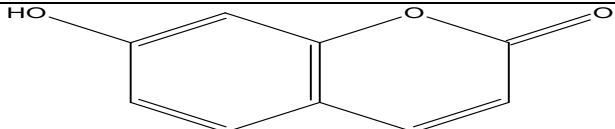
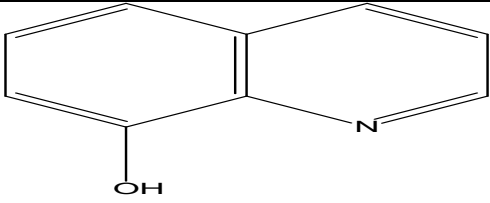
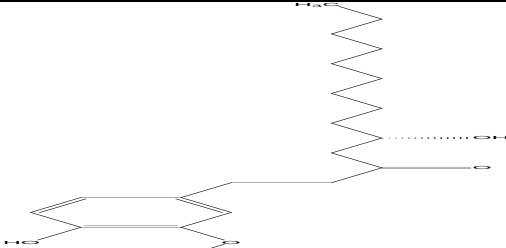
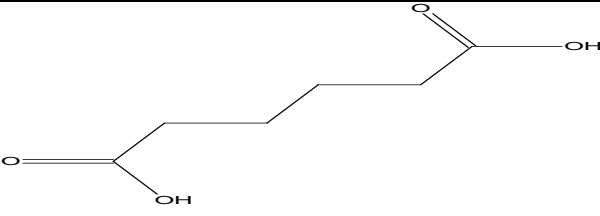
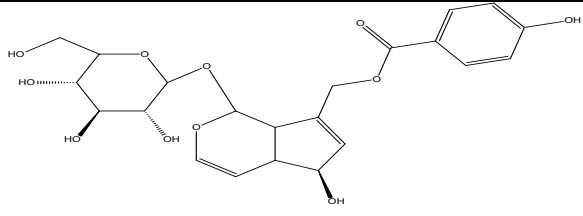
 (-)-alpha-santonin	 (-)-lupinine
 Apigenin triacetate	 Apocynin

 Arecoline	 Asiatic acid
 Aucubin	 b-asarone
 Botulin	 Caffeic acid
 chlorogenic acid	 cinnamic acid
 Coumarin	 d-guinic acid
 Diosgenin	 ephedrine

 Erucamide	 eucalyptol
 hippuric acid	 imperatorin
 Isoquercetin	 isovenillic acid
 Lup-20(29)-en-28-al,3beta-hydroxy	 Lupa-12,20(29)-dien-3-one
 Luteolin-3',7-diglucoside	 nicotinic acid

 (trimethylazaniumyl)acetate	 Oleanolic acid
 Oleic acid	 Piperine
 Plumbagin	 quercitrin
 quinolinic acid	 retusin
 Rutin	 salicylic acid
 Stigmasterol acetate	 thymol

 Ursolic acid	 vanillin
 Vigabatrin	 α-lapachone
 4-hydroxybenzoic acid	 4-methylumbelliferone
 [6]-gingerol	 4,5-dicaffeoylquinic acid
 (22E)-stigmasta-3,5,22-triene	 ϵ-4-methoxycinnamic acid
 ϵ-ferulic acid	 (E)-p-coumaric acid

 4-piperidone	 7-hydroxycoumarin
 8-hydroxyquinoline	 10-gingerol
 adipic acid	 agnuside

ADMET Studies

For evaluating the drug likeness pharmacokinetics and toxicity characteristics of the virtual library compounds will be subjected to the screening studies using online software such as SWISSADME, OSIRIS property explorer software, Molinspiration [4].

Lipinski's Rule

This rule provides a description of molecular qualities that are significant for the pharmacokinetics of a drug in the body. These properties include its absorptions, distributions, metabolism and excretion (ADME) values. Lipinski's rule says that an orally active drug has no more than one violation of the following criteria:

- Partition coefficient of log P less than 5
- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- Molecular weight under 500 daltons
- An additional rule was proposed by veber: Not more 10 rotatable bonds [5].

Drug likeness screening is a crucial step in drug design and discovery, aimed at assessing of newly designed compound to exhibit drug-like properties. These properties are defined by a set of qualitative criteria that differentiate drugs from other chemicals, emphasizing their effectiveness and safety as pharmaceutical agents.

In this study, drug likeness were evaluated for a series of bioactive ligands targeting the EGFR.

Various online software tools were employed to perform drug likeness screening, including Lipinski's rule of 5, Osiris online software and molinspiration

Lipinski's rule 5 widely used to guideline in medicinal chemistry that identified key physicochemical properties associated with oral bioavailability and permeation across biological membranes of molecular structure highlighting risk and features.

Additionally, SWISSADME provides insights into molecular properties and bioactivity profile of compounds. this screening process to lead candidates for future optimization and development, ultimately advancing the discovering effective therapeutic agent targeting against EGFR.

The in vitro determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt

yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol

or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

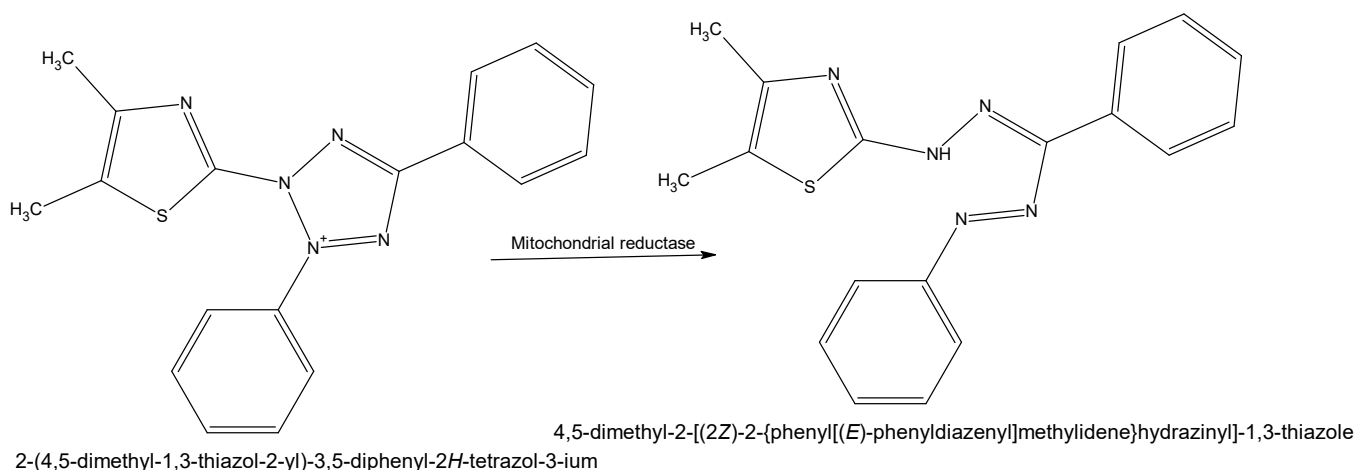


Figure 1: Formation of formazan in MTT assay.

Methods

1. MTT reagent (the solution is filtered through a 0.2 µm filter and stored at 2–8 °C for frequent use or frozen for extended periods)
2. DMSO (Dimethyl sulphoxide)
3. CO₂ incubator
4. Micro Plate reader
5. Inverted microscope
6. Refrigerated centrifuge

Preparation of test solutions

For MTT assay, serial two fold dilutions were prepared for this assay. Cell lines and culture medium cell line was procured from NCCS, stock cell was cultured in MEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO₂ at 37°C until confluent.

Procedure

The monolayer cell culture (A375 cells) was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1 x 10⁴ cells/well) was added. After 24

h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test sample was added on to the partial monolayer in microtiter plates. The plate was then incubated at 37°C for 24 h in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula [6].

$$\% \text{ viability} = \frac{\text{Sample abs}}{\text{Control abs}} \times 100$$

Results and Discussion

Ethanolic Root Extract

- The 40 g of dried coarsely powder dried root of Vitex negundo reflexed with 400 ml of ethanol in Soxhlet apparatus at 35°C for two days.
- The weight of the extract was found to be 1.93 g.

Table 4: Preliminary Test.

Test for amino acids

Test	Observation	Inference
Ninhydrin test	No purple or bluish color appeared	Negative

Test for steroids and triterpenoids test:

Test	Observation	Inference
Salkowski test	Formation of red colour in lower layer	Positive
Libermann Burchard test	No brown ring formed in upper layer	Negative

Test for glycosides:

Test	Observation	Inference
Keller-kellani test	No formation reddish brown ring	Negative
Baljet test	The colour changed from yellow to orange	Positive

Test for flavonoids:

Test	Observation	Inference
Lead acetate test	Yellow coloured precipitate	Positive
Sodium hydroxy test	Showed yellow colour which decolourized after addition of acid	Positive

Test for tannins:

Test	Observation	Inference
Ferric chloride solution test	Formation dark blue or greenish black colour	Positive
Lead acetate test	The formation of voluminous white precipitate	Positive

Test for carbohydrates:

Test	Observation	Inference
Molish test	Violet ring was formed	Positive
Fehling's test	Formation of brick red precipitate	Positive

Test for alkaloids:

Test	Observation	Inference
Dragendroff's test	Formation of orange-brown precipitate	Positive
Mayer's test	Formation of cream coloured precipitate	Positive

Test for saponins:

Test	Observation	Inference
Foam test	Foam formation present	Positive
Libermann Burchard's test	No colour change to green	Negative

Test for proteins:

Test	Observation	Inference
Millions test	Reddish colour precipitate is formed	Positive

Invitro Studies

Cytotoxicity of VN against A375 cells

A375 cell line was used for invitro studies against SCC activity and the result was shown below:

Table 5a: Absorbance readings at 570 nm for VN at various concentrations.

Concentration (µg/ml)	Replicate 1	Replicate 2	Replicate 3
0.02	1.563	1.547	1.522
0.2	1.487	1.507	1.462
2	1.391	1.386	1.374
20	1.253	1.297	1.279
200	0.223	0.267	0.199

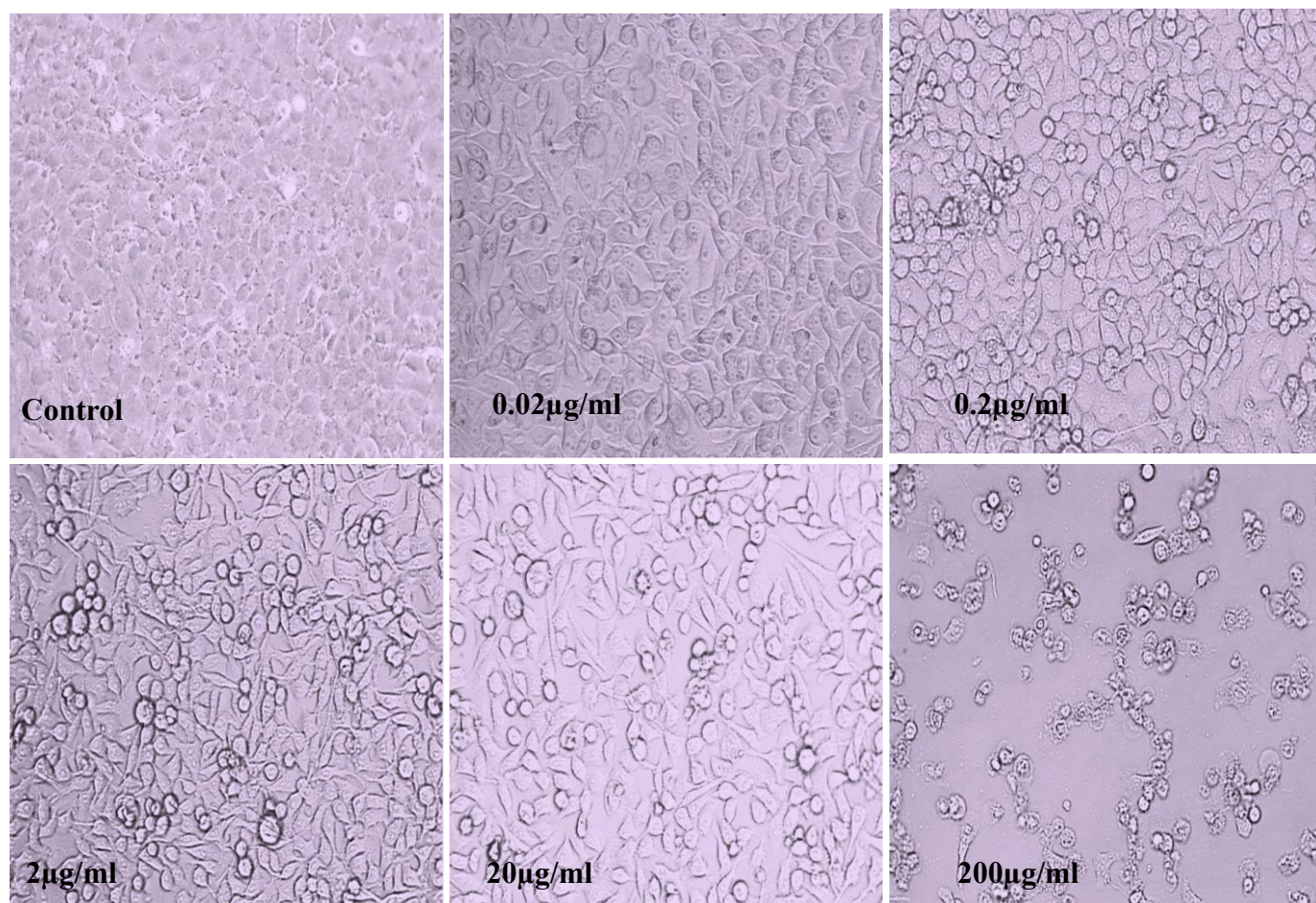


Figure 2: Cytotoxicity of VN against A375 cells.

Table 5b: Absorbance readings at 630 nm for VN at various concentrations.

Concentration (µg)	Replicate 1	Replicate 2	Replicate 3
0.02	0.124	0.121	0.125
0.2	0.12	0.122	0.123
2	0.118	0.115	0.112
20	0.107	0.111	0.106
200	0.098	0.101	0.096

Table 5c: Percentage cell viability of cells treated with VN.

Concentration (µg)	% Viability		
	Replicate 1	Replicate 2	Replicate 3
0.02	92.283027	91.4493373	89.589568
0.2	87.665669	88.200006	85.870043
2	81.637452	81.509198	80.932866
20	73.492946	76.0581445	75.224455
200	8.0162463	10.645575	6.6053869

Table 5d: Percentage cytotoxicity of VN with mean, SD, and SEM at different concentrations.

% Cytotoxicity			
Concentration (µg)	Mean	SD	SEM
0.02	8.89268918	1.37891863	0.796119
0.2	12.5480975	1.48656166	0.8582668
2	18.6404446	0.37576733	0.2169494
20	25.0748183	1.30852381	0.7554766
200	91.5775973	2.05048831	1.18385

Table 5e: Mean percentage viability and SD of VN treated cells.

Concentration (µg)	% Viability	
	Mean	SD
0.02	91.1073108	0.7759038
0.2	87.4519025	1.03300818
2	81.3595554	1.116738
20	74.9251817	3.15753868
200	8.42240274	0.62517421

Table 5f: Absorbance of untreated control cells at 570 and 630 nm wavelengths.

Untreated Control cells			
570-630nm			
1.567	1.595	Mean	1.559
1.583	1.517		
1.542	1.552		

Table 5g: Dose response analysis of VN cytotoxicity with IC₅₀ and Hill slope

log(inhibitor) vs. normalized response – Variable slope	
Best-fit values	
LogIC ₅₀	1.615
HillSlope	1.258
IC ₅₀	41.17
Standard Error	
LogIC ₅₀	0.09926
HillSlope	0.2864
95% Confidence Intervals	
LogIC ₅₀	1.400 to 1.829
HillSlope	0.6397 to 1.877
IC ₅₀	25.13 to 67.45
Goodness of Fit	
Degrees of Freedom	13
R ²	0.8854
Absolute Sum of Squares	1613
Sy.x	11.14
Number of points	
Analyzed	15

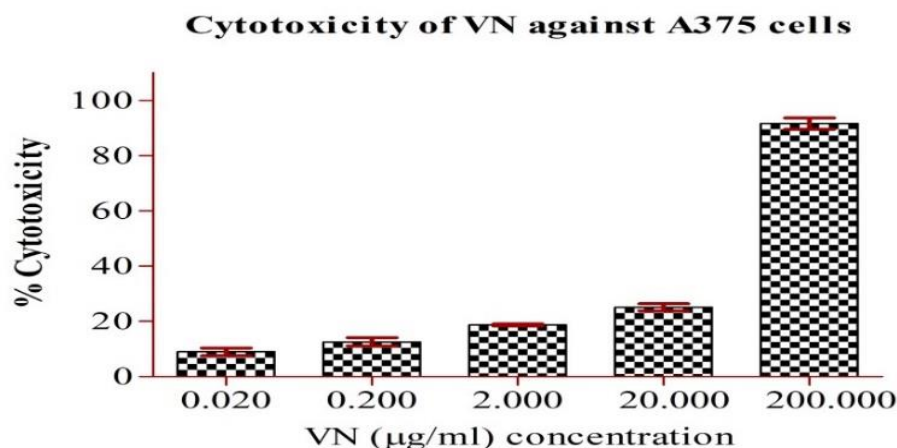


Figure 3: Dose-Dependent Cytotoxicity of VN Extract on Squamous Carcinoma.

Lead Optimization

The bioactive ligands were optimized by subjecting ADMET properties, Lipinski rule of five and toxicity prediction and docking studies. The data are show below

Toxicity Result

Table 6: Toxicity summary.

S. No.	Compound Name	Mutagenic	Tumorigenic	Gastric Irritant	Reproductive Risk
1	Betain	No	No	No	No
2	Arecolin	Yes	Yes	No	No
3	Hippuric Acid	No	No	No	No
4	D- (-)-Quinic Acid	No	No	No	No
5	Nicotinic Acid	No	No	No	No
6.	Quinolic Acid	Yes	No	Yes	Yes
7	Adipic Acid	Yes	No	Yes	No
8	Ephedrine	No	No	No	No
9	Vigabatrin	No	No	No	Yes
10	Cinnamic Acid	No	No	Yes	No
11	4-Piperidone	No	No	No	No
12	8-Hydroxyquinilone	Yes	Yes	Yes	No
13	4-Hydroxybenzoic Acid	Yes	No	No	No
14	Salicylic Acid	Yes	No	Yes	Yes
15	Isovanillic Acid	No	No	No	No
16	Aucubin	No	No	No	No
17	Chlorogenic Acid	No	No	No	No
18	Vanillin	Yes	No	Yes	Yes
19	Rutin	No	No	No	No
20	Plumbagin	Yes	No	No	Yes
21	Apocynin	Yes	No	No	No
22	Asarone	Yes	Yes	No	No
23	Imperatorin	Yes	No	No	No
24	Coumarin	Yes	Yes	No	Yes

Citation: Anitha K, Anand M, Prasanth P, et al. Targeting Squamous Cell Carcinoma: In Silico Evaluation of Bioactive Ligands from Ethanolic Root Extract of *Vitex Negundo*. *Int J Biomed Investig* 2025; 8(2): 173. doi: [10.31531/2581-4745.1000173](https://doi.org/10.31531/2581-4745.1000173)

25	Agnuside	No	No	No	No
26	Luteolin-3',7-Diglucoside	No	No	No	No
27	4,5-Dicaffeoylquinic Acid	No	No	No	No
28	Isoqercetin	No	No	No	No
29	(E)-Ferulic Acid	No	No	No	No
30	7-Hydroxycoumarine	Yes	No	No	No
31	Quercitrin	No	No	No	No
32	4-Methylumbelliferone	No	No	No	Yes
33	(-)-Alpha-Santonin	No	No	No	No
34	(E)-4-Methoxycinnamic Acid	No	No	Yes	Yes
35	(-)-Lupinine	No	No	No	No
36	Apigenin Triacetate	Yes	No	No	Yes
37	Alpha-Lapachone	No	No	No	No
38	Thymol	Yes	No	No	Yes
39	Piperine	No	No	No	Yes
40	Retusin	Yes	No	No	No
41	Asiatic Acid	No	No	No	No
42	(+/-)-Eucalyptol	Yes	No	No	Yes
43	(+/-)-[6]-Gingerol	No	No	No	No
44	10-Gingerol	No	No	No	No
45	Caffeic Acid	Yes	Yes	No	Yes
46	Diosgenin	No	No	No	Yes
47	Oleic Acid	Yes	Yes	Yes	No
48	(E) -P-Coumaric Acid	No	No	No	Yes
49	Ursolic Acid	No	No	No	No
50	Betullin	No	No	No	No
51	Oleanolic Acid	No	No	No	No
52	Lup20(29)-En-28-Al,3beta-Hydroxy	No	No	No	No
53	Lupa-12,20(29)-Dien-3-One	No	No	No	No
54	Erucamide	No	No	No	No
55	(22E)-Stigmasta-3,5,22-Triene	No	No	No	No
56	Stigamsterol Acetate	No	No	No	No

In silico Studies

Table 7: Docking summary.

Compound Name	Binding Energy	Inhibitory Constant (μm)
(-)-alpha-santonin	6.17	30.99
(-)-lupinine	3.85	1.52
(22E)-stigmasta-3,5,22-triene	6.4	20.19
(E)-4-methoxycinnamic acid	4.63	402.14
(E)-ferulic acid	5.39	112.62
(E)-p-coumaric acid	4.87	271.15
-[6]-gingerol	4.09	1.01
4,5-dicaffeoylquinic acid	5.75	61.2
4-hydroxybenzoic acid	4.86	275.82

Citation: Anitha K, Anand M, Prasanth P, et al. Targeting Squamous Cell Carcinoma: In Silico Evaluation of Bioactive Ligands from Ethanolic Root Extract of *Vitex Negundo*. *Int J Biomed Investig* 2025; 8(2): 173. doi: [10.31531/2581-4745.1000173](https://doi.org/10.31531/2581-4745.1000173)

4-methylumbelliferone	5.59	80.17
4-piperidone	5.34	120.8
7-hydroxycoumarin	5.11	181.0
8-hydroxyquinoline	5.74	62.17
10-gingerol	2.27	21.7
Adipic acid	4.02	1.14
Agnuside	6.88	9.12
Apigenin triacetate	7.63	2.54
Apocynin	4.86	273.84
Arecoline	5.71	64.85
Asiatic acid	7.58	2.79
Aucubin	5.83	53.11
b-asarone	4.78	315.54
Betulin	5.16	166.06
Caffeic acid	5.36	117.83
Chlorogenic acid	5.68	68.31
Cinnamic acid	4.99	219.39
Coumarin	6.27	25.15
d-guinic acid	5.24	143.8
Diosgenin	8.38	725.27
Ephedrine	4.29	720.25
Erucamide	0.66	330.66
-eucalyptol	6.24	55.02
Hippuric acid	5.64	73.51
Imperatorin	6.07	35.47
Isoquercetin	6.53	16.46
Isovenillic acid	4.88	264.33
Lup-20(29)-en-28-al,beta-hydroxy	7.52	3.09
Lupa-12,20(29)-dien-3-one	7.1	6.2
Luteolin-3',7-diglucoside	5.83	53.33
Nicotinic acid	5.22	150.2
Betain	5.16	166.06
Oleanolic acid	7.49	3.24
Oleic acid	1.88	41.99
Piperine	6.97	7.83
Plumbagin	5.68	68.39
Quercetin	7.46	3.43
Quinolinic acid	5.35	119.61
Retusin	6.89	8.94
Rutin	7.31	4.38
Salicylic acid	5.8	55.58
Stigmasterol acetate	6.15	11.31

Citation: Anitha K, Anand M, Prasanth P, et al. Targeting Squamous Cell Carcinoma: In Silico Evaluation of Bioactive Ligands from Ethanolic Root Extract of *Vitex Negundo*. *Int J Biomed Investig* 2025; 8(2): 173. doi: [10.31531/2581-4745.1000173](https://doi.org/10.31531/2581-4745.1000173)

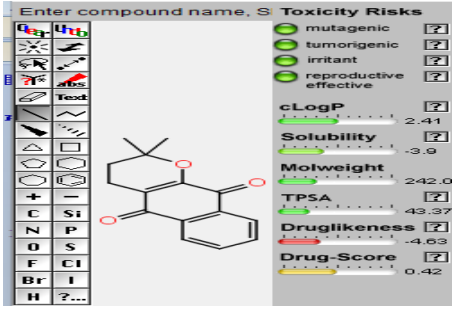
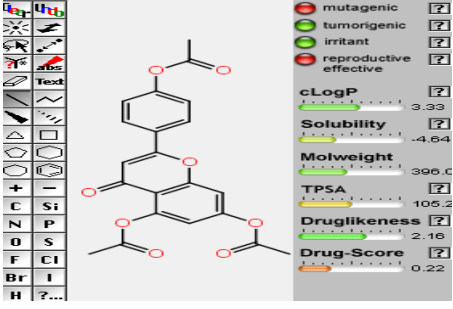
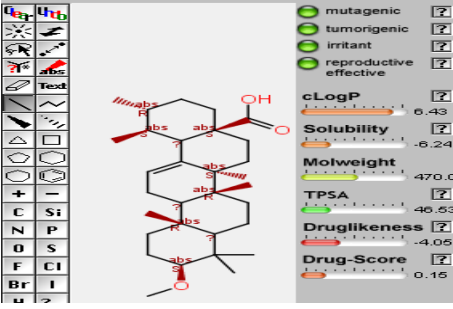
Thymol	4.12	954.98
Ursolic acid	7.42	3.66
Vanillin	4.85	278.81
Vigabatrin	4.43	569.95
Alpha-lapachone	7.86	1.72

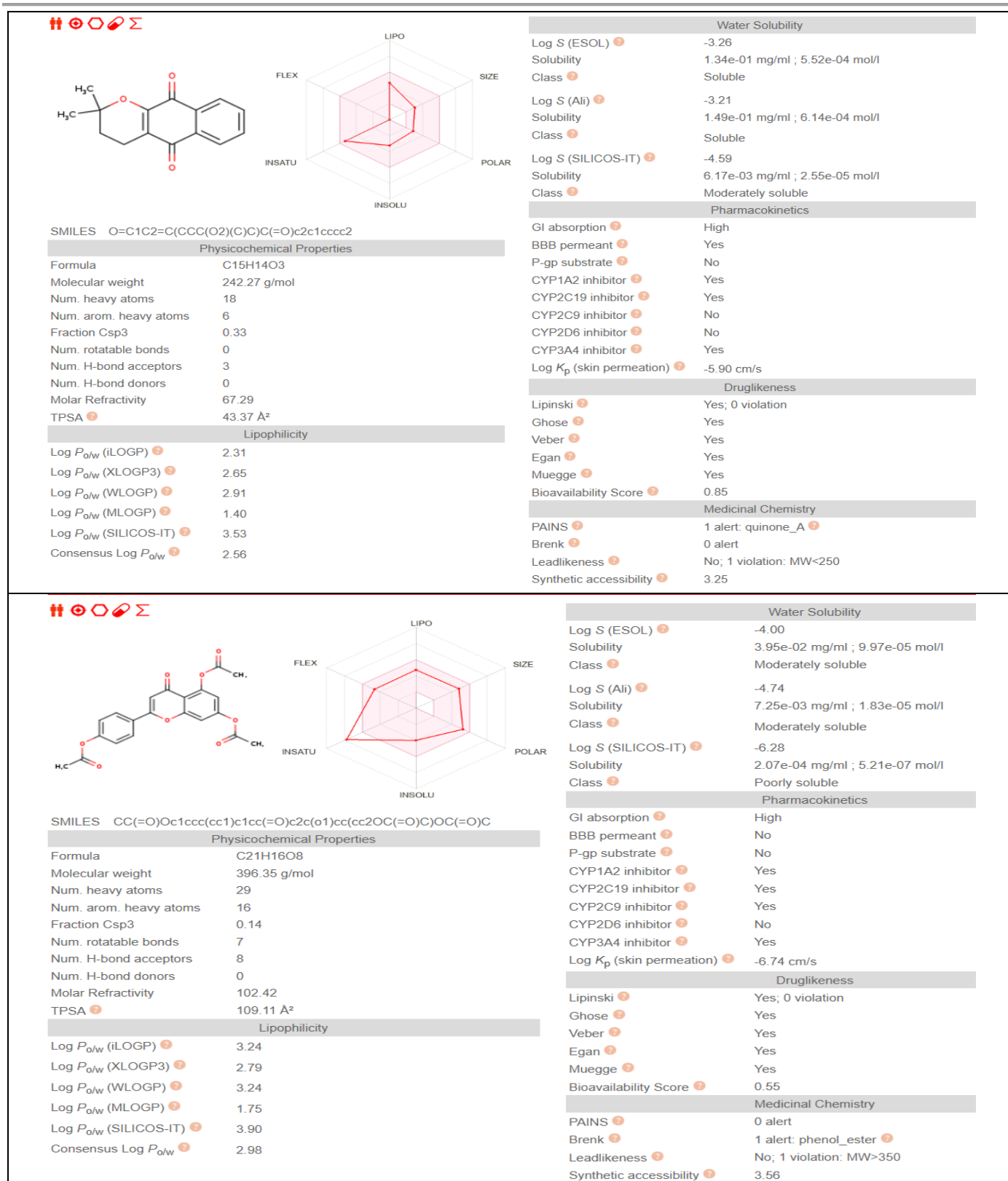
In-silico analysis revealed that certain bioactive ligands exhibit promising activity against squamous cell carcinoma (SCC) by targeting EGFR. Meanwhile,

the in vitro study also demonstrated significant anticancer activity. The top three ligands with the highest binding affinities are listed below.

Table 8: Top three ligands with the highest binding affinities.

Ligands	Binding energy	Inhibitory constant
Alpha-lapachone	-7.86	1.72
Apigenin triacetate	-7.63	2.54
Ursolic acid	-7.42	3.66

Ligands	Docking statement	Toxicity statement
Alpha-lapachone	<pre> binding_energy=-7.86 ligand_efficiency=-0.27 inhib_constant=1.72 inhib_constant_units=uM intermol_energy=-9.95 vdw_hb_desolv_energy=-9.95 electrostatic_energy=0.0 total_internal=-1.79 torsional_energy=2.09 unbound_energy=-1.79 filename=dock.dlg cIRMS=0.0 refRMS=16.02 rseed1=None rseed2=None </pre>	
Apigenin triacetate	<pre> binding_energy=-7.42 ligand_efficiency=-0.22 inhib_constant=3.66 inhib_constant_units=uM intermol_energy=-8.31 vdw_hb_desolv_energy=-8.31 electrostatic_energy=0.0 total_internal=-0.64 torsional_energy=0.89 unbound_energy=-0.64 filename=dock.dlg cIRMS=0.79 refRMS=19.15 rseed1=None rseed2=None </pre>	
Ursolic acid	<pre> binding_energy=-7.63 ligand_efficiency=-0.26 inhib_constant=2.54 inhib_constant_units=uM intermol_energy=-9.72 vdw_hb_desolv_energy=-9.72 electrostatic_energy=0.0 total_internal=-1.68 torsional_energy=2.09 unbound_energy=-1.68 filename=dock.dlg cIRMS=0.0 refRMS=15.67 rseed1=None rseed2=None </pre>	



Alpha-Lapachone

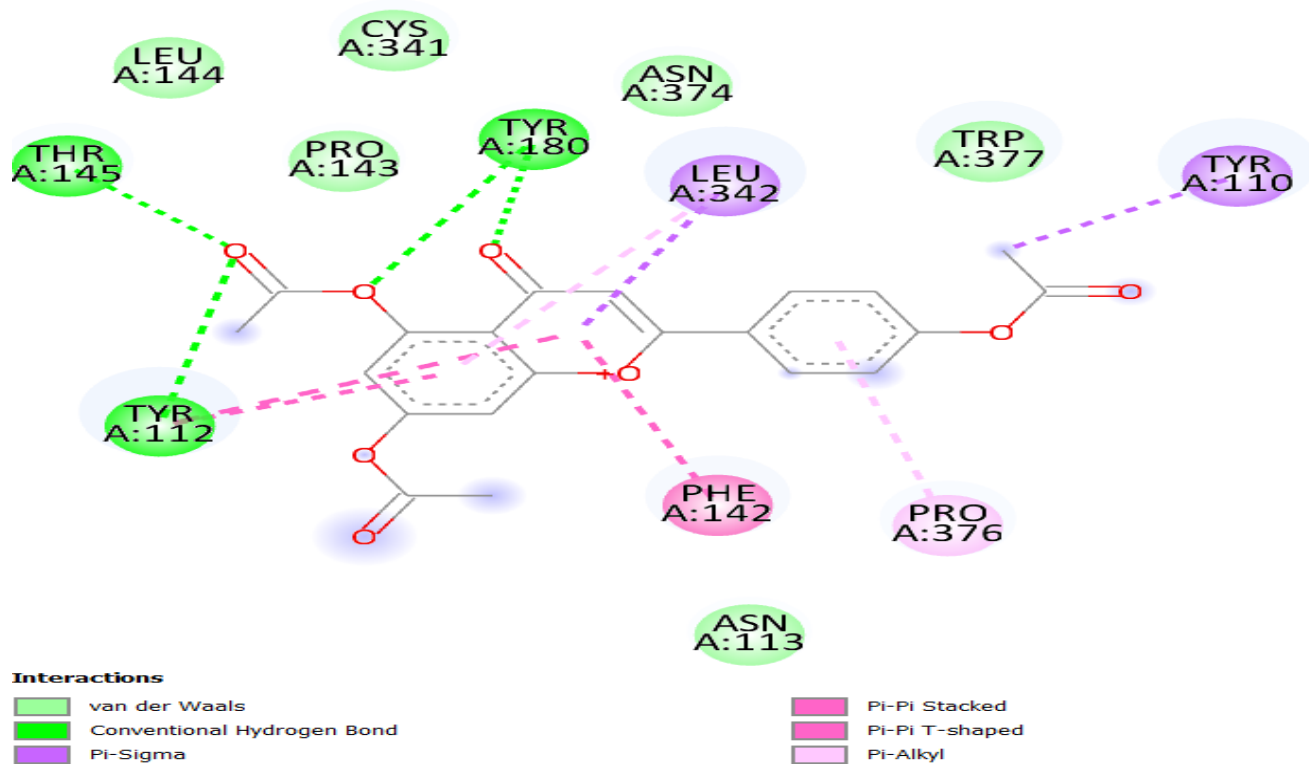
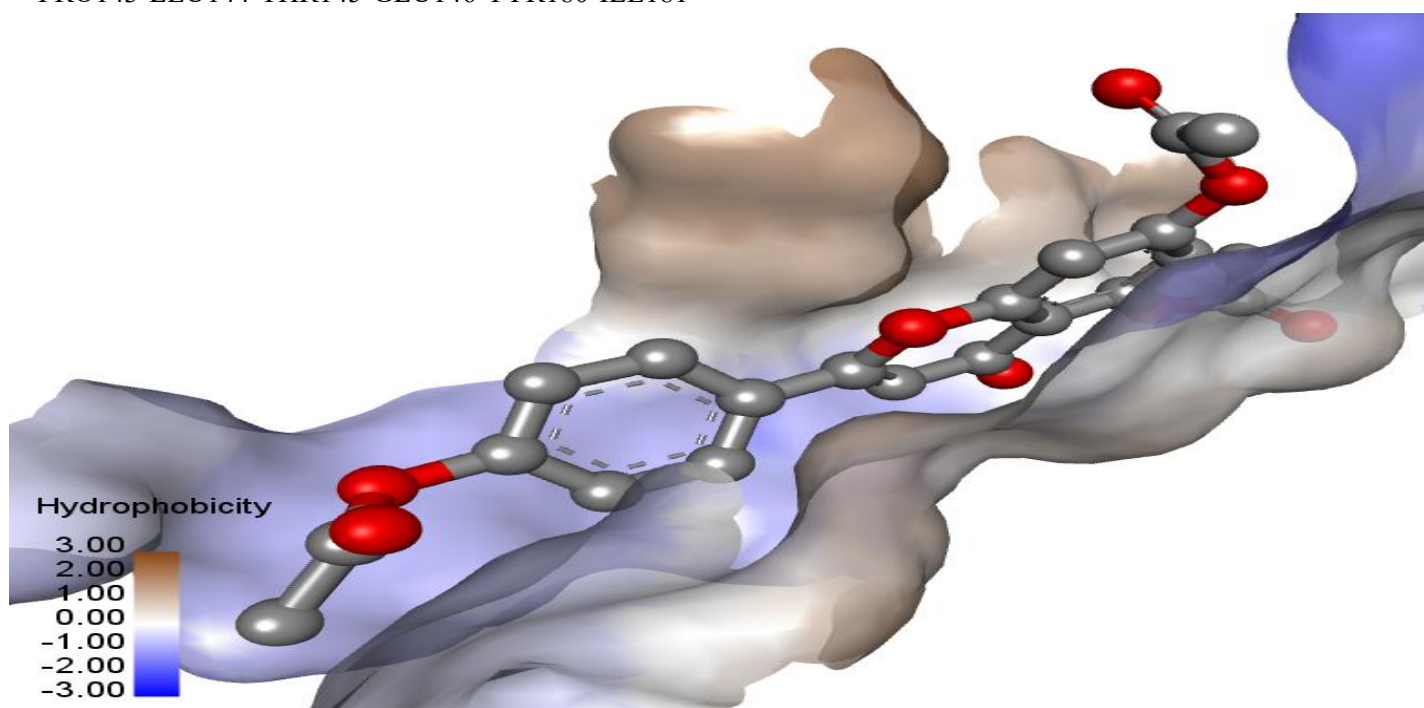


Figure 4: Receptor- Ligand interactions.

Contact residues

Chain A: TYR110 TYR112 ASN113 ILE141 PHE142
PRO143 LEU144 THR145 GLU146 TYR180 ILE181

CYS182 PRO183 LEU302 LEU303 ALA307 ARG309
TYR313 ARG335 VAL338 GLU339 CYS341 LEU342
ARG373 ASN374 CYS375 PRO376 TRP377



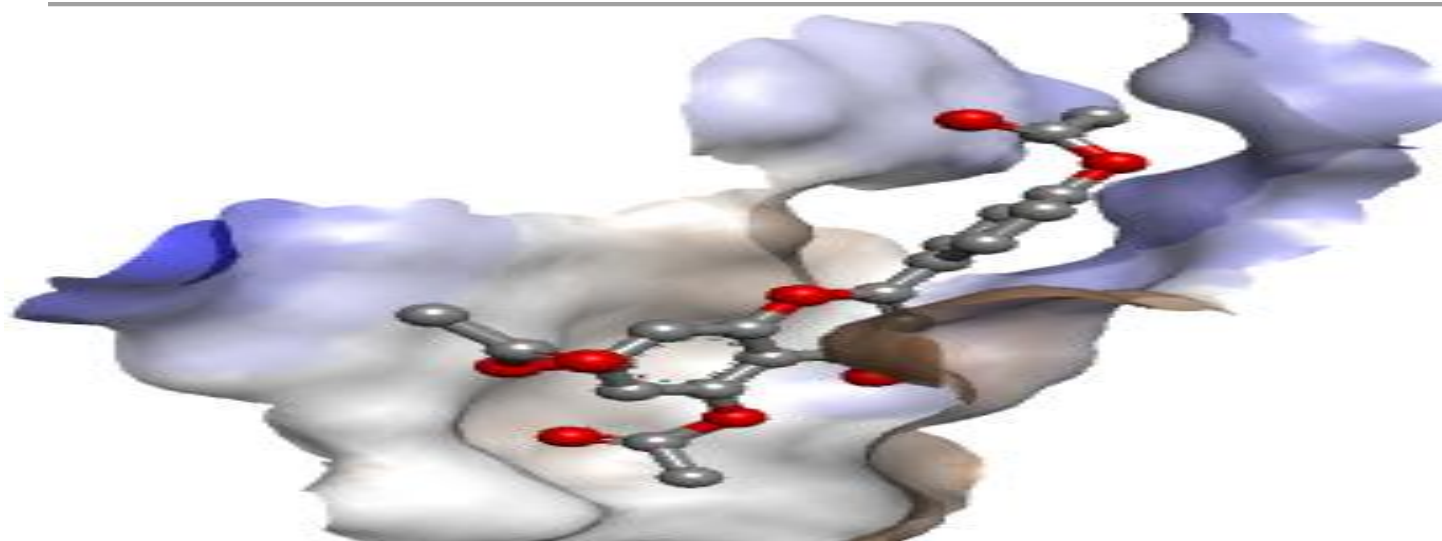


Figure 5: Hydrophobicity of Alpha-lapachone.

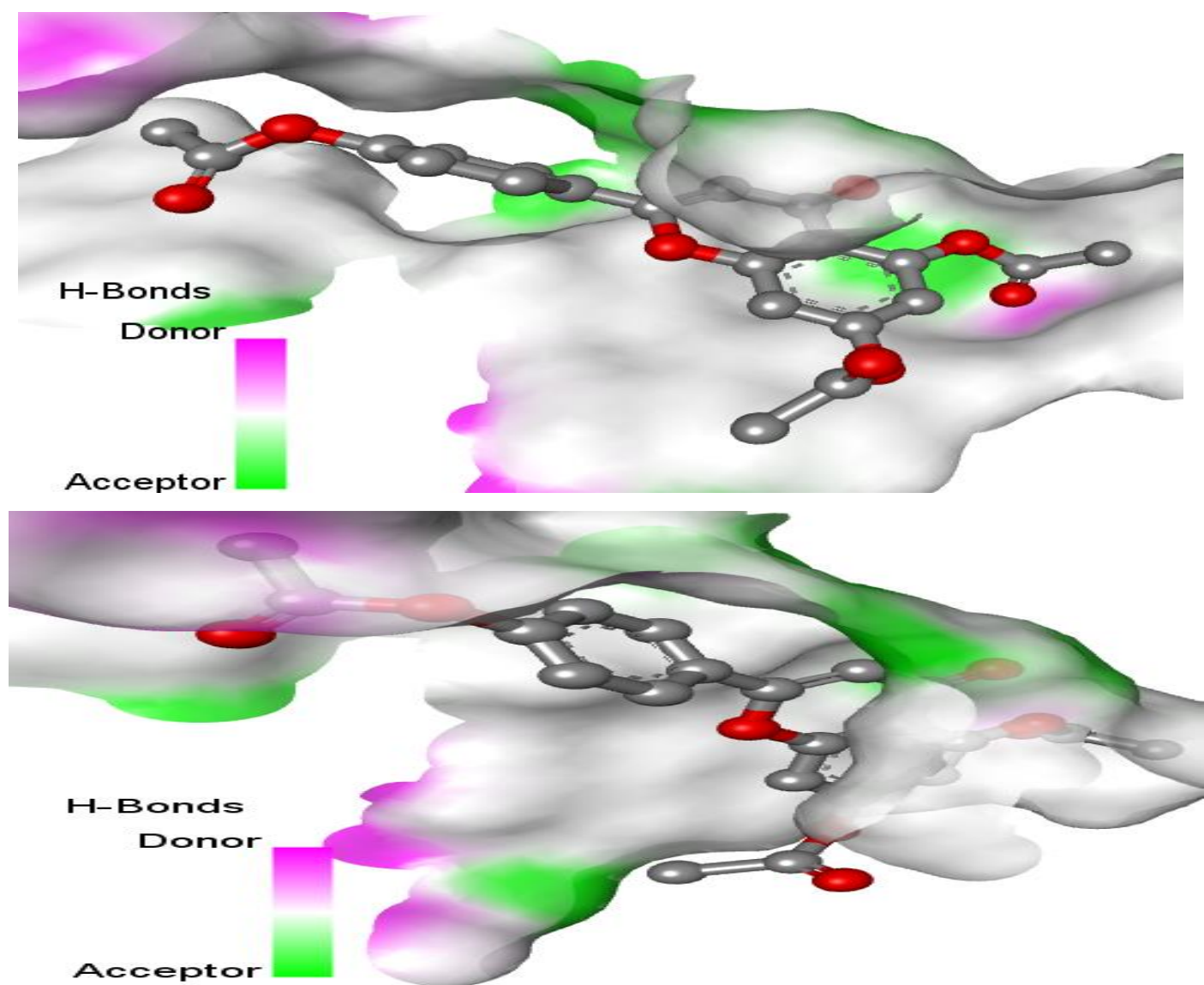
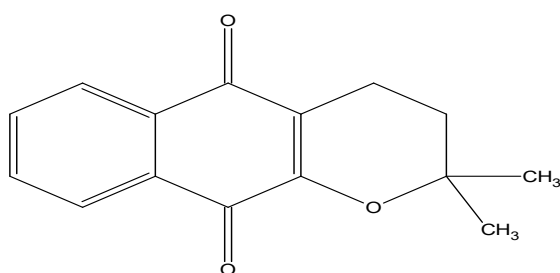


Figure 6: Hydrophobicity of Alpha-lapachone.

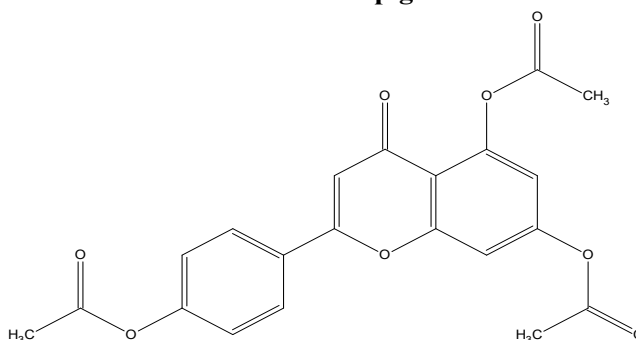
Table 9: Compounds may be useful for developing future skin cancer treatments.

Ligands	Binding energy	Inhibitory constant	Toxicity	GI absorption	BBB penetration
Alpha-lapachone	-7.86	1.72	Non-toxic	High	Yes
Apigenin triacetate	-7.63	2.54	Non-toxic	High	No
Ursolic acid	-7.42	3.66	Mutagenic	Low	No

Alpha-lapachone



Apigenin triacetate



Conclusion

Vitex negundo is a well-known medicinal plant used in traditional systems like Ayurveda, Siddha, and Unani. It has many healing properties such as anti-inflammatory, pain-relieving, antioxidant, and antimicrobial effects. The roots, leaves, and seeds of this plant contain helpful natural compounds that are useful in medicine, especially for cancer research.

In this study, we focused on testing the ethanolic root extract of *Vitex negundo* for its potential against Squamous Cell Carcinoma (SCC). We selected 56 compounds from research articles and tested them using in-silico tools like *AutoDock* (for docking), *OSIRIS* (for toxicity), and *SwissADME* (for drug-likeness). We also confirmed the presence of active compounds through basic lab tests.

Additionally, we checked the anti-cancer activity of the extract on A375 skin cancer cell lines. It showed strong anti-squamous cell carcinoma at higher dose of 200µ/ml with the IC₅₀ value (~41 µg/ml) suggests it's moderately potent.

Out of all, the top 3 compounds showed good results by binding well with EGFR, a protein linked to SCC. These compounds may be useful for developing future skin cancer treatments.

Among the top three ligands identified through in-silico screening:

Alpha-lapachone, a natural naphthoquinone derivative, demonstrated significant binding affinity against EGFR

in Squamous Cell Carcinoma (SCC) models. Despite its ability to cross the blood-brain barrier (BBB), its interaction profile marks it as a strong candidate for further exploration in skin cancer therapy.

Apigenin triacetate, a flavone-based compound, also showed notable inhibitory potential against EGFR in SCC. Its natural origin and favorable docking properties make it a promising lead molecule for designing effective anti-skin cancer drugs.

The study confirms that *Vitex negundo* root extract exhibits promising anti-squamous cell carcinoma activity both in-silico and in-vitro. Alpha-lapachone and Apigenin triacetate emerged as strong EGFR inhibitors with favorable drug-like properties. This research highlights the potential of plant-based molecules in cancer drug discovery. Further studies may lead to the development of effective herbal therapies for skin cancer, offering a cost-effective solution to benefit public health.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The authors did not receive any fundings from any private or government sources.

Ethical Approvals

This study does not involve experiments on animals or human subjects.

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