PLANT EXTRACTS IN CANCER THERAPY: A COMPREHENSIVE ANALYSIS OF ANTICANCER ACTIVITY AND MOLECULAR DOCKING PROFILES

Presented by:
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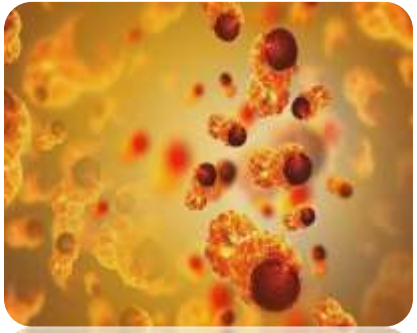


Selection, Collection and authentication of plant

Plant Name	*Annona squamosa* L.	*Annona squamosa* L.	*Aegle marmelos* L.
Image			
Selected Part	Leaves	Seeds	Leaves
Image			

Plant:
Annona squamosa
Collection site:
Danta,
Banaskantha
Collection Date:
2nd October 2021
Plant Part:
Leaves and Seeds

Plant:
Aegle marmelos
Collection site:
Sunshi,
Mehsana
Collection Date:
15th November 2021
Plant Part:
Leaves

Plant Identification done by **Dr. Hitesh Solanki** ,Department of Botany , Gujarat University Ahmedabad.

Pre treatment:

- Washing
- Treatment
  - Drying
  - Storage

EXTRACTION

- METHOD: **Maceration**
- Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 7 days with frequent agitation. (Azwanida, N. N 2015)
- **SELECTED SOLVENTS:**
  - **Methanol**
  - **Hexane**
  - **Water**

Cell Viability Test : MTT assay

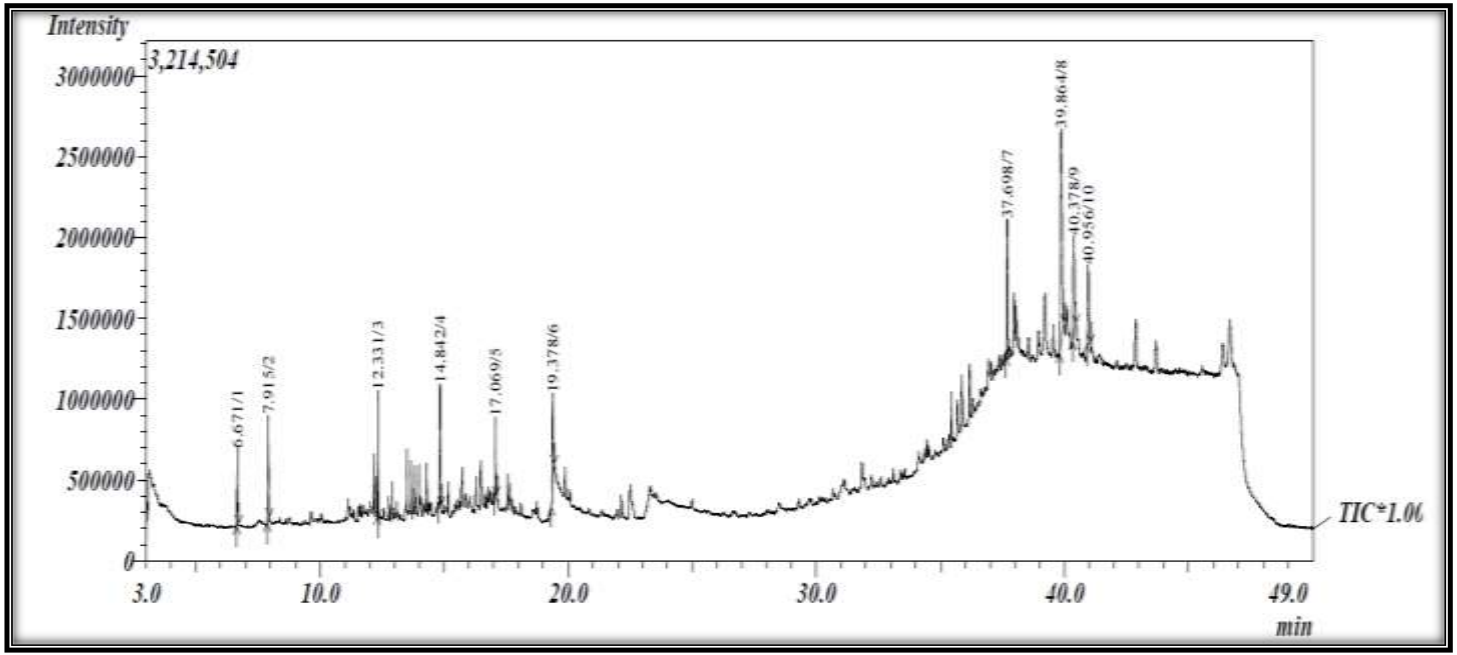
1. • Cells were seeded in well of 96-well plates (density:4 x10^5 cells/well) and complete media was added in each well of 96-well plate. It was incubated for 37˚C, 5% CO_2 for 24 hours.

2. • When cells reached 80% confluence, the culture medium was removed and replaced with 1 mL of fresh culture medium, and the cells were treated for 24 hours with various treatment groups of ALH, ALM, ALW, CLH, CLM, CLW, CSH, CSM, CSW, cisplatin (positive control), Negative control (without any treatment) at 5, 10, 20, 40 and, 80 µg/ml concentrations.

3. • After dosing period was over 10µl of MTT (0.5 mg/ml) was added into each well. Plate was incubated for further 3 to 4 hours at room temperature.

4. • Then 50 µl of dimethyl sulfoxide was added and the absorbance was recorded at 570 nm in spectrophotometer.[6,7]

Analytical study to identify phytoconstituents GC-MS/LC-MS



GC-MS Chromatogram for ALH

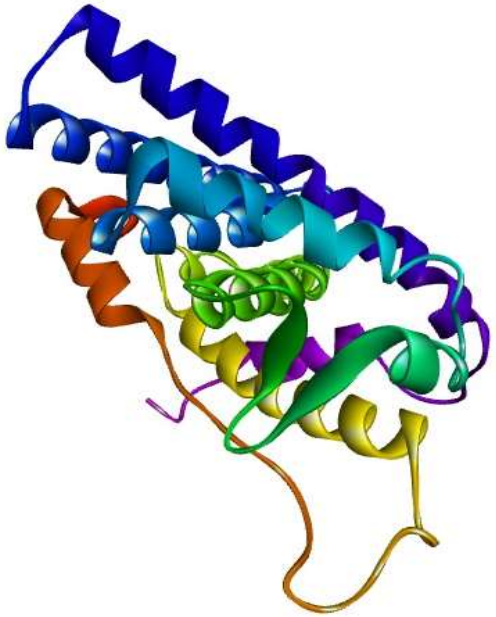
In Silico Analysis (MOLECULAR DOCKING)

- Molecular docking is a computational technique used to predict the preferred orientation of a ligand (small molecule) when bound to a receptor or target protein. The goal is to determine the binding affinity and the most favourable conformation of the ligand within the binding site of the protein.

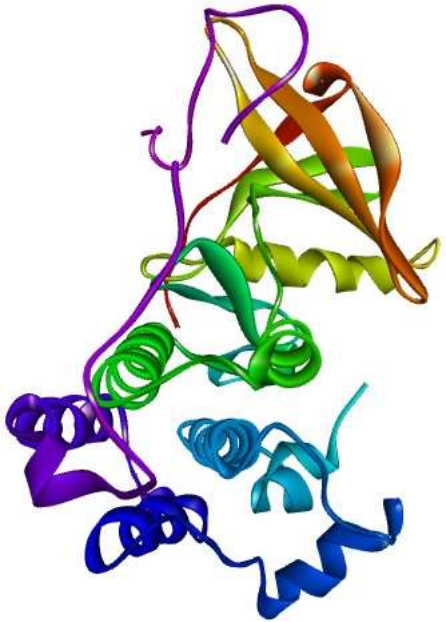
- In the present study, Docking-based virtual screening (DBVS) was conducted using the PyRx v0.8. It is a software tool that combines several open-source software, including AutoDock, AutoDock Vina, and Open Babel to carry out DBVS seamlessly. The following were the steps performed in the PyRx v0.8 for current *in silico* analysis.

- This method relies on two key approaches. Firstly, it utilizes force-fields to estimate the binding affinity between the protein and the ligand. Secondly, it explores the conformational space to identify various binding poses of the protein-ligand complex.

Target protein(Receptor) for docking study

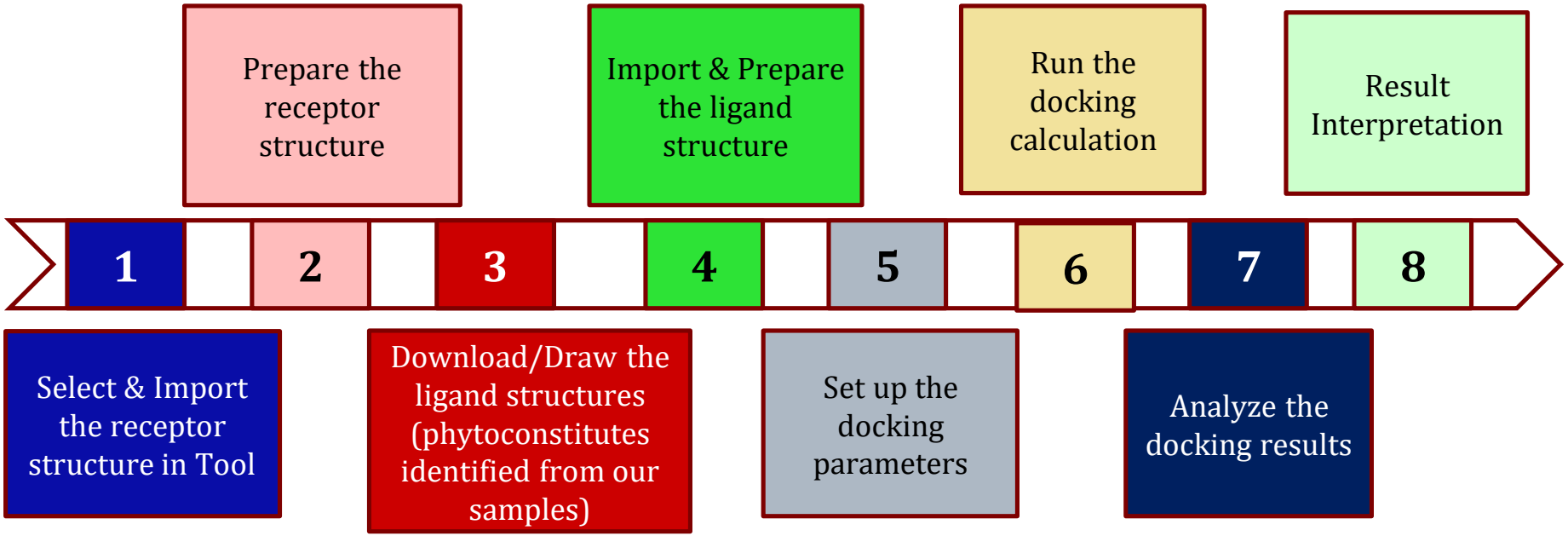


Human estrogen Alpha receptor (PDB id: 3ERT)

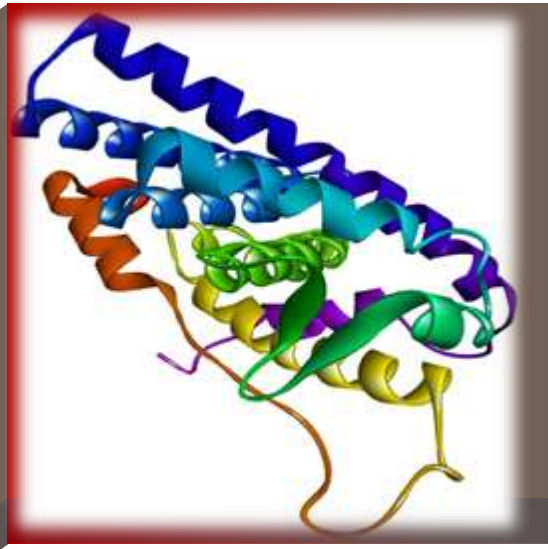


Epidermal growth factor receptor(PDB id:2J5F)

Steps for Molecular Docking

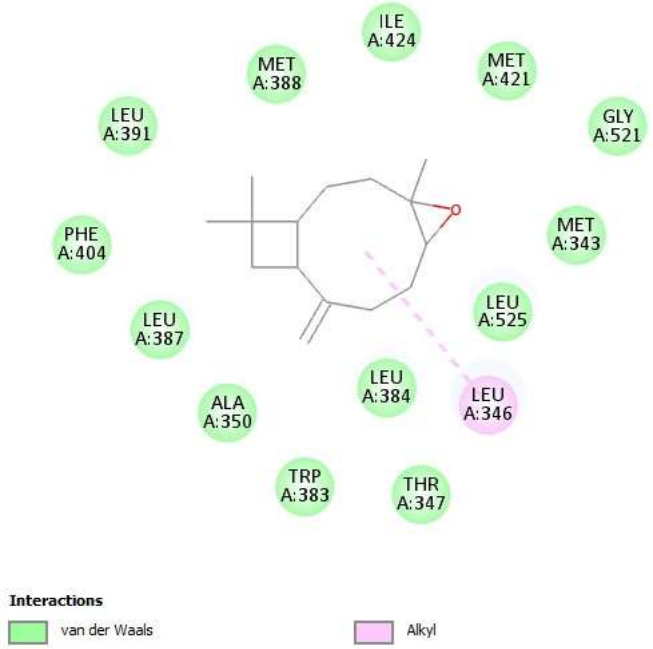


Molecular docking With Human estrogen Alpha receptor (PDB id: 3ERT)

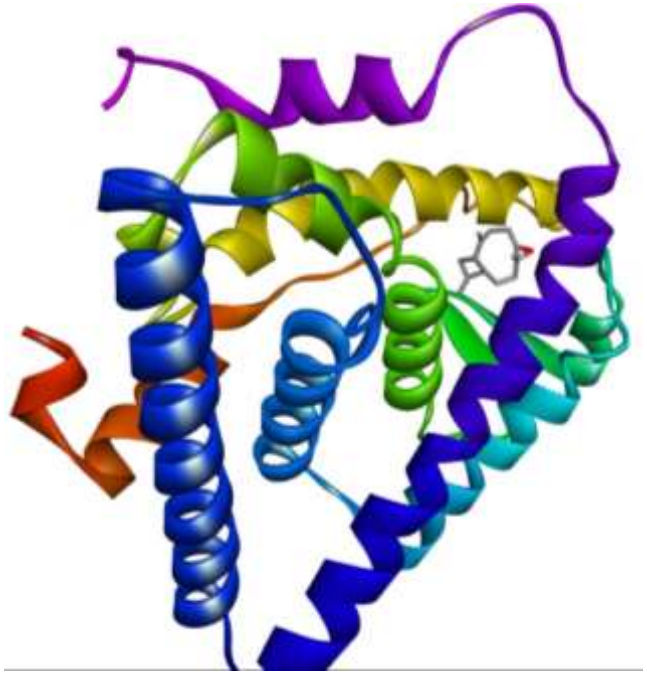


2D an 3D binding interaction of Caryophyllene oxide with 3ERT

Binding affinity: -8.2



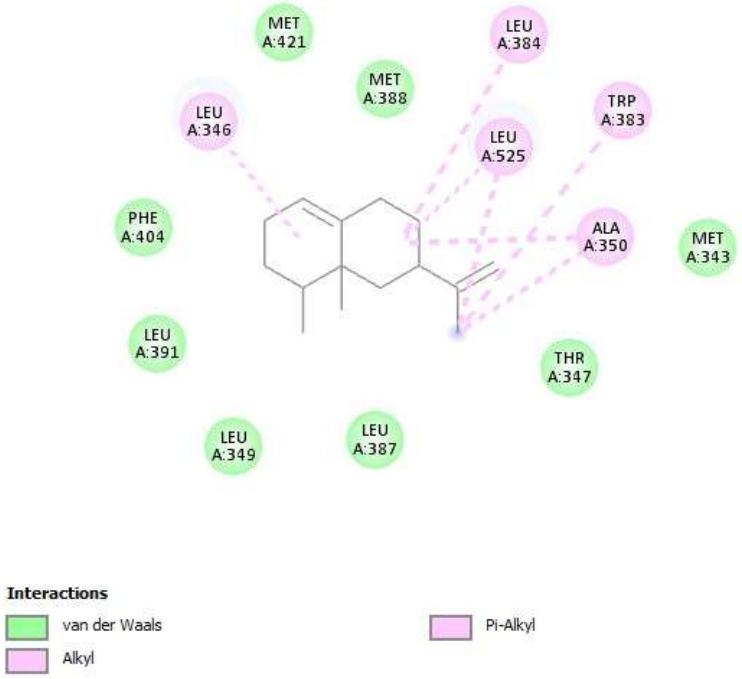
2D Interaction



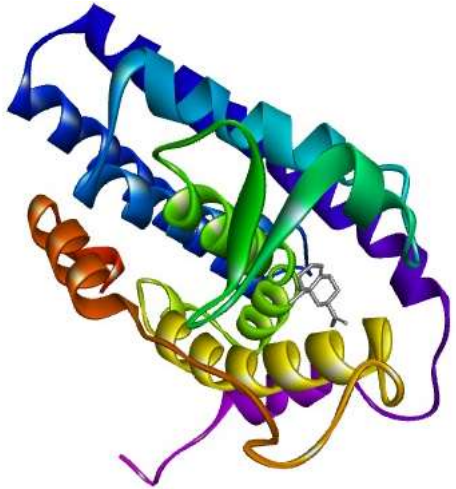
3D Interaction

2D an 3D binding interaction of Valencene with 3ERT

Binding affinity: -7.3



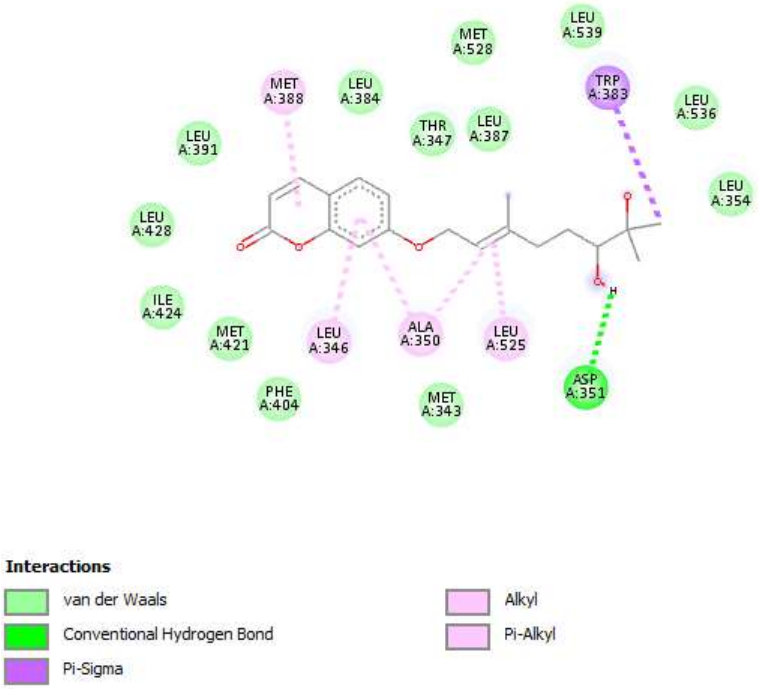
2D Interaction



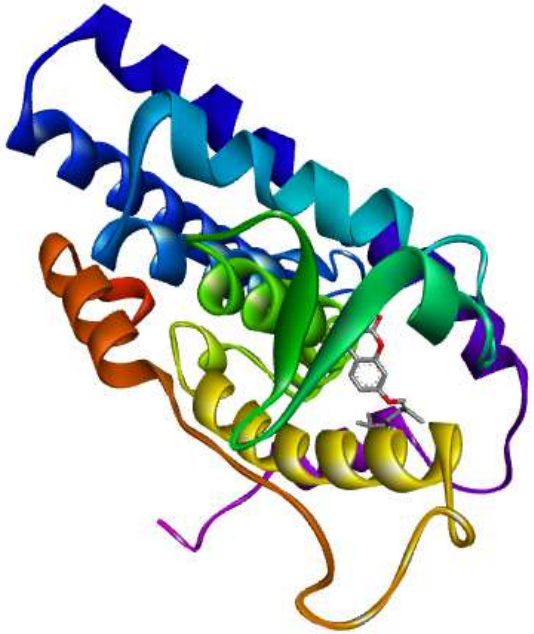
3D Interaction

2D an 3D binding interaction of Marmin with 3ERT

Binding affinity: -7.1



2D Interaction



3D Interaction

Selected phytochemical from Docking study

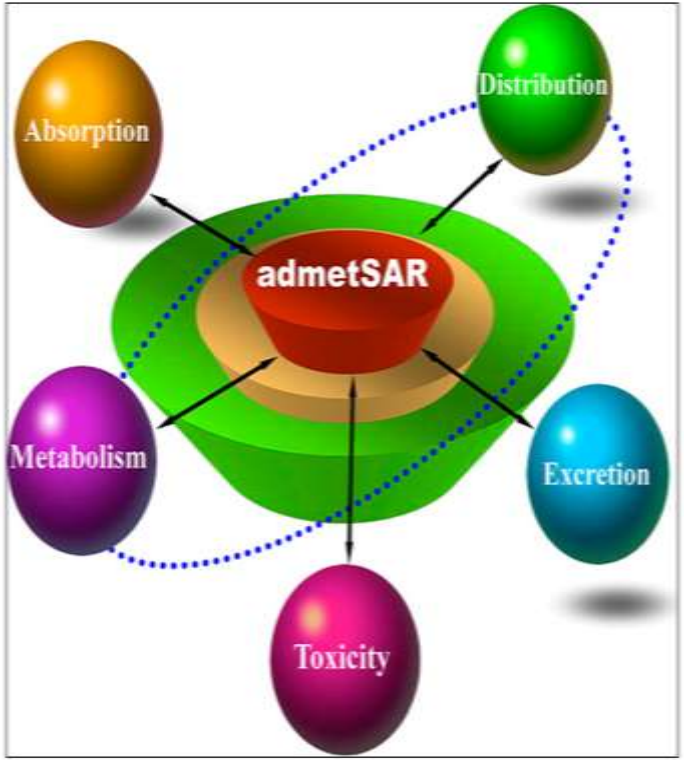
Extract	Name of Compound	Binding Affinity ERT	Binding Affinity EGFR
ALH	Caryophyllene oxide	-8.2	-7
	Clionasterol	-7.2	-8.3
	Marmin	-7.1	-7.5
	Valencene	-7.3	-6.9
	Rutin	-6.8	-6.9
	Aegeline	-6.8	-7.1

Drug likeness by ADME

- Drug likeness analysis is an important step in drug discovery and development processes. It involves assessing the potential of a compound to become a drug by evaluating its molecular properties and predicting its behavior in biological systems.

- ADMET refers to Absorption, Distribution, Metabolism, Excre- tion, and Toxicity. It contains the pharmacokinetic profile of a com- pound (drug molecule) and plays a significant role in determining its pharmacodynamics activities.

- **SwissADME** is used for current study it is a widely used computational tool developed by the Swiss Institute of Bioinformatics. It provides various features for drug likeness analysis, including the prediction of physicochemical properties, absorption, distribution, metabolism, and excretion (ADME), as well as toxicity risks.



Physicochemical properties of identified compound of ALH

Physicochemical properties							
Sr.no	Compound	Molecular weight (g/mol)	X log P3 -0.7 to 5	TPSA (Å) 20 to 130	Log S (ESOL) -6 to 0	Fraction Csp3 0.25 t0 1	Rotatable Bonds <9
1	Caryophyllene oxide	220.35	3.56	12.53	-3.45	0.87	0
2	Clionasterol	414.71	9.34	20.23	-7.9	0.93	6
3	Marmin	332.39	2.81	79.9	-3.52	0.42	7
4	Valencene	204.35	5.24	0	-4.34	0.73	1
5	Rutin	610.52	-0.33	269.43	-3.3	0.44	6
6	Aegeline	297.35	2.44	58.56	-3.16	0.17	7

Pharmacokinetic properties of identified compound of ALH

Pharmacokinetic properties							
Sr.no	Compound	Lipinski	BBB	HIA	PGP	Log K_p (skin permeation)	Bioavailability Score
1	Caryophyllene oxide	0	Yes	High	No	-5.12	0.55
2	Clionasterol	1	No	Low	No	-2.2	0.55
3	Marmin	0	No	High	No	-6.33	0.55
4	Valencene	1	No	Low	No	-3.83	0.55
5	Rutin	3	No	Low	Yes	-10.26	0.17
6	Aegeline	0	Yes	High	No	-6.38	0.55

Druglikeness properties of identified compound of ALH

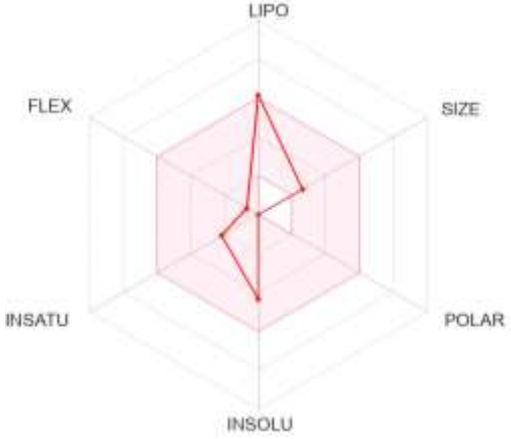
Druglikeness properties											
Sr.no	Compound	Lipinski		Ghose		Veber		Egan		Muegge		Bioavailability Score
		violations	follow	violations	follow	violations	follow	violations	follow	violations	follow	
1	Caryophyllene oxide	0	Yes	0	Yes	0	Yes	0	Yes	1	No	0.55
2	Clionasterol	1	Yes	3	No	0	Yes	1	No	2	No	0.55
3	Marmin	0	Yes	0	Yes	0	Yes	0	Yes	0	Yes	0.55
4	Valencene	1	Yes	0	Yes	0	Yes	0	Yes	2	No	0.55
5	Rutin	3	No	4	No	1	No	1	No	4	No	0.17
6	Aegeline	0	Yes	0	Yes	0	Yes	0	Yes	0	Yes	0.55

Bioavailability radar of ligands for druglikeness

Caryophyllene oxide

Clionasterol

Marmin



Valencene

Rutin

Aegeline

Screening of Anti cancer agent potency

Considered parameter:

- *In silico* analysis - Binding affinity with both target receptors
- ADME properties
- Drug likeness score
- *In vitro* analysis – MTT assay

Selected phytochemicals (after druglikeness)

Extract	Phytochemical	Parameter		
		Binding affinity for ERT	Binding affinity for EGFR	Drug likeness score >0.5
ALH	Caryophyllene oxide	-8.2	-7	High(0.8)
	Marmin	-7.1	-7.5	High(1.0)


Thank You