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Investigation of Novel Anticancer Compounds from *Wrightia tinctoria* Ethanolic Leaf Extract via GC-MS and Molecular Docking: A Study on Squamous Cell Carcinoma-Related Proteins

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Abstract: Squamous cell carcinoma (SCC) is a malignant tumor that develops from epidermal keratinocytes. Its prevalence is significantly higher in albinism due to the absence of melanin-mediated photoprotection. The aim of this investigation was to evaluate the anticancer effects of phytoconstituents obtained from *Wrightia tinctoria* leaves, with particular focus on their efficacy against SCC utilizing computational and molecular models. The collected *W. tinctoria* leaves were shade-dried, crushed, and solvent extracted. The initial phytochemical investigation was followed by gas chromatography-mass spectrometry (GC-MS), resulting in the identification of three important bioactive components: tryptanthrin, squalene, and hexadecanoic acid. The identified compounds were subsequently evaluated with molecular docking techniques against two important oncogenic proteins associated with SCC pathogenesis: EGFR (1M17) and KRAS (4OBE). The molecular docking investigations indicated high ligand-receptor binding affinities and favorable binding free energies, with tryptanthrin showing stronger interaction patterns and molecular stability in both target proteins' active regions. Furthermore, the ADMET (absorption, distribution, metabolism, excretion, and toxicity) study confirmed the pharmacokinetic appropriateness and drug-like properties of the selected substances. Overall, the *in silico* findings highlight the therapeutic potential of phytochemicals derived from *W. tinctoria* as prospective lead candidates for the development of targeted treatments for squamous cell carcinoma.

Keywords: Epidermal growth factor receptor, Squamous cell carcinoma, GC-MS, Kirsten rat sarcoma, Molecular docking, *Wrightia tinctoria*

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Introduction

Globally, non-melanoma skin cancers (NMSC), such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are on the rise, particularly among the elderly. There is significant diversity in

their prevalence among different areas, genders, and sociodemographic groups (Pan *et al*., 2025). Because melanin deficiency reduces the body's natural defenses against ultraviolet (UV) radiation, SCC is more common in those with albinism (Iloghalu *et al*., 2025). This particular type of cancer primarily affects people with lighter skin and is caused by keratinocytes found in the epidermis. Increased UV exposure, aging, male sex, weakened immunity, tobacco use, and genetic susceptibility are significant risk factors (Trosman *et al*., 2021; Catalano *et al*., 2024). It has been shown that moles can progress to multiple myeloma (MM), a disease that can spread and worsen over time, in around 25% of documented cases.

NMSC hardly ever migrates into the deeper tissues of the epidermis. Only when identified early it can be readily eradicated (Hasan *et al*., 2023). The main treatment for SCC is surgical excision, which may also involve reconstructive surgery and lymph node removal (Shambharkar *et al*., 2021). In the meantime, topical therapies especially those derived from plants are gaining popularity for treating superficial skin malignancies. Natural compounds found in botanicals that are isolated from different plant parts are highly regarded for their immune-regulating, anti-inflammatory, and antioxidant properties. They are also being investigated for their possible use in cancer treatment and prevention (Millsop *et al*., 2013).

Wrightia tinctoria (*W. tinctoria*), which is native to the Indian subcontinent, has been highly regarded in traditional medicine for a considerable time (Thiagarajan *et al*., 2024). The revival of interest in modern medicine can be attributed to the identification of various phytochemicals that possess therapeutic potential, such as steroids, triterpenoids, saponins, tannins, phenols, flavonoids, glycosides, alkaloids, and polyphenols. These compounds are linked to effects that include anti-inflammatory, anti-microbial, antioxidant, anticancer, antidiabetic, and anti-psoriatic properties (Kale *et al*., 2021). Historically, the bark and leaves have been utilized for the treatment of psoriasis, ulcers, diabetes, and digestive disorders (Srivastava, 2014).

Bioinformatics currently plays a crucial role in the screening of plant-derived compounds and the identification of potential drug targets (Mitra *et al*., 2022; Kalidass *et al*., 2024). Molecular docking specifically simulates the interactions between drugs and receptors, which assists in the process of drug design (Torres *et al*., 2019; Peluso and Chankvetadze, 2024). This study assesses the anticancer potential of *W. tinctoria* compounds through molecular docking to suggest alternative or supportive treatment strategies.

Epidermal growth factor receptor (EGFR) is a significant receptor tyrosine kinase (RTK). Additionally, this receptor and its ligands have been found to exhibit elevated expression levels in various tumors of epithelial origin, as well as in proliferative disorders of the epidermis, including psoriasis (Ruswanto *et al*., 2021). EGFR may serve as a more selective target in the search for anticancer drugs (Milano *et al*., 2008; Yamaoka *et al*., 2017). The mutant Kirsten rat sarcoma viral oncogene homolog (KRAS) protein is one of the most prevalent contributors to human cancer. Until recently, KRAS was regarded as an oncoprotein that could not be targeted by drugs. After a prolonged struggle, we are finally beginning to see some light at the end of the tunnel, as promising KRAS-targeted therapies are either in or nearing clinical trials. In recent years, with the promising advancements in RAS drug discovery, the scientific understanding of KRAS has significantly improved (Pantsar, 2020).

This study assesses the GC-MS analysis of phytochemical compounds derived from the ethanol extract of leaves of *W. tinctoria* and utilizes molecular docking techniques to identify the most promising drug target proteins, EGFR with PDB ID 1M17 and KRAS with PDB ID 4OBE.

Materials and Methods

Collection and Preparation of Samples

Healthy leaves of *W. tinctoria* were collected from Palakkad district of Kerala and were authenticated by Dr. S.S. Hameed, Scientist 'E' at the Botanical Survey of India, Tamil Nadu (No: BSI/SRC/5/23/2023/Tech–547). In the laboratory, the leaf samples were washed 2–3 times with running fresh water. The leaf material was then air-dried in the shade. After complete drying, the plant material (500 g) was ground using a mechanical grinder, and the powder was stored in small labeled plastic bags.

W. tinctoria was assessed and extracted using distilled water, ethanol, ethyl acetate, and hexane through Soxhlet's apparatus. The solvents were extracted from the extracts using a rotating vacuum evaporator, and the filtrate was stored in a desiccator for future use.

Phytochemical Analysis

The prepared extracts were analyzed for the presence of alkaloids, terpenoids, phenols, saponins, flavonoids, steroids, glycosides, carbohydrates, and proteins using the method described by Shankar *et al*. (2010) and Selvakumar and Singh (2016), with appropriate modifications made as necessary. The results are displayed as (+ve), indicating presence, and (-ve), indicating absence.

GC-MS Analysis of the Extract

The ethanol leaf extract was subjected to GC-MS for the determination of bioactive volatile compounds. The sample was analyzed using GC-MS with the Agilent model CH-GCMSMS02, employing MassHunter software. Helium served as the carrier gas, with the programming temperatures set as follows: 50℃ held for 1 min (run time 1 min), ramping at 5℃/min to 120℃ and holding for 1 min (run time 16 min), then increasing at 10℃/min to 210℃ with a hold for 1 min (run time 26 min), and finally rising at 10℃/min to 280℃ with a hold for 5 min (run time 38 min). The chemical components from the plant extract were identified by comparing the retention times of chromatographic peaks with mass spectral data from the NIST mass spectral library, as well as published literature.

Collection of Compounds

The dataset used in this study consisted of 36 compounds obtained from the ethanolic extract of *W. tinctoria*, as identified through GC-MS analysis. Only three compounds were deemed suitable for analysis based on their interaction with cancer targets and their use of the drug Imiquimod. This study assesses the GC-MS analysis of phyto-chemical compounds derived from the ethanol extract of *W. tinctoria* and employs molecular docking techniques to identify the most promising drug target proteins, EGFR with PDB ID: 1M17 and KRAS with PDB ID: 4OBE.

Prediction of potential bioactivity in silico for the ethanolic extract of W. tinctoria against targeted cancer proteins

Software and hardware

The RCSB Protein Data Bank (https://www.rcsb.org) and the PubChem database (https://pubchem.ncbi.nlm.nih.gov) were used in this investigation, as well as online applications such as ADMET Lab 2.0 and Swiss ADME. Utilizing PyRx and AutoDock Vina 0.8 (Dallakyan and Olson, 2015) software or, alternatively, using the BIOVIA Discovery Studio (RRID:SCR_015651) protein visualizer (Pawar and Rohane, 2021).

Preparation of Protein

The crystal structures of cancer-related proteins, namely EGFR (PDB-1M17) and KRAS (PDB-4OBE), were sourced from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) to facilitate docking studies (Figs. 1a, b). Preparing a selected protein involves altering its macromolecular structure to establish a more suitable arrange-ment for conducting a computational experiment (Opo *et al*., 2021).

Preparation of Ligands

The structures of three identified compounds obtained through GC-MS analysis of the ethanolic extract of *W. tinctoria*, along with the standard drug are illustrated in Figures 2 a-d. The

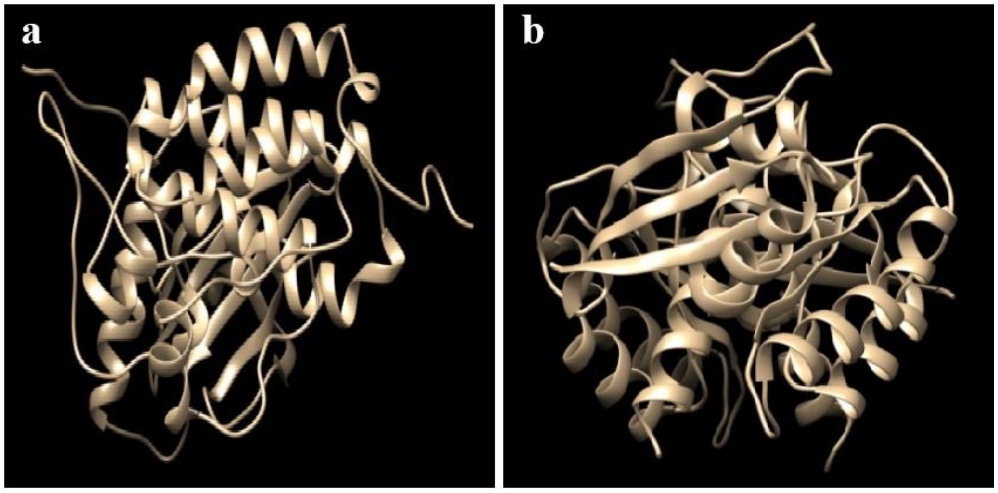

Fig. 1: (a) 3D structure representation of EGFR (PDB-1M17) and (b) KRAS (PDB-4OBE) protein.

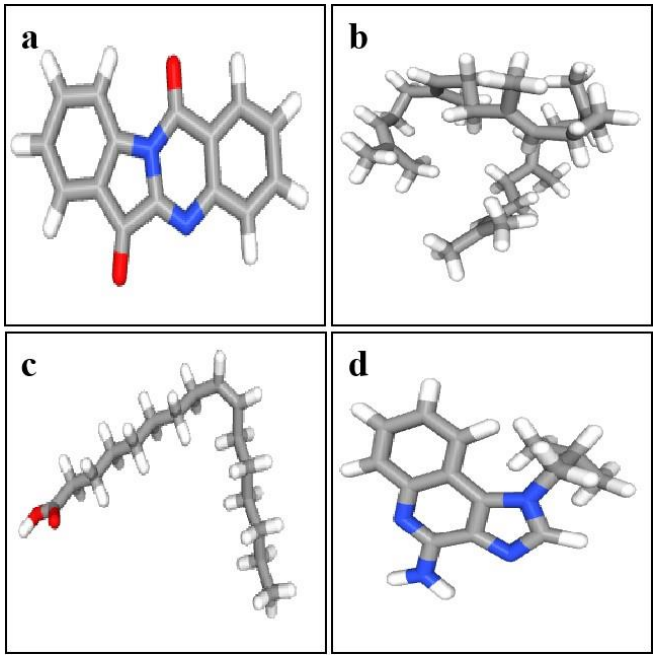

Fig. 2: 3D structure representation of (a) Tryptanthrine (PubChem CID: 73549), (b) Squalene (PubChem CID: 638072), (c) Hexadecenoic acid(PubChem CID: 5318393) and (d) standard drugs, Imiquimod (PubChem CID: 57469).

compounds tryptanthrine (PubChem CID: 73549), squalene (PubChem CID: 638072), and hexa-decenoic acid (PubChem CID: 5318393) were researched and located in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The 3D conformations of the ligands and the standard drug, Imiquimod (PubChem CID: 57469), were obtained. Ligands are utilized to investigate their potential interactions with the cancer target proteins EGFR (PDB: 1M17) and KRAS (PDB: 4OBE) through site-specific molecular docking. All structures were analyzed using PLIP v1.1.1. PLIP facilitates thorough detection and visualization of protein-ligand interaction patterns.

Table 1: Grid box parameters selected for skin cancer target protein, based on the binding site residues

Target Protein (PDB ID)	Active Site Residues	Grid Box Parameter	
EGFE-1M17	LYS 721,VAL 702,LEU 694,THR 766,ALA 719,MET 769,ARG 817,LEU 820,LYS 836,ARG 808,GLU 841,LYS 843,GLU 842,GLU 848,TYR 845,GLU 844,VAL 956,LEU 955,ILE 957,CYS 773,GLY 772,LEU 680,ASN 676,TYR 740,SER 744,GLU 685,ILE 682,ARG 681,LYS 782,ILE 756,PHE 699,GLU 710,LYS 713,GLU 939,LYS 946,LEU 764,GLU 738,MET 742,LEU 768,THR 830,CYS 751,LEU 820,ASP 831	32.785 x -3.738 x 58.328	48 x 68 x 58
KRAS-4OBE	ASP 30,VAL29,GLU 31,ASP33,PRO34,LLE36,THR35,GLU37,ALA59,THR58,ASP57,ALA11,LYS16,GLY12,TYR32,GLY13,SER17,VAL14,LYS16,GLY 15,ALA 18,LYS 117	3.611 x 10.972 x 15.750	60 x 58 x 64

Molecular Docking

The binding affinities of the hit compounds identified from the GCMS were predicted by docking them against the different targets associated with SCC (Mekala *et al*., 2021, 2022). Targeting the essential sites presents a viable approach to inhibit cancer and address its resistance (Ramalingam *et al*., 2023a). Molecular docking studies were conducted utilizing the AutoDock Vina program, integrated within the PyRx software (version 0.8), to forecast the binding orientation and affinity of ligand molecules to a target protein (Eberhardt *et al*., 2021). A grid box was established around the target protein to facilitate the docking calculations. The binding energies obtained from the molecular docking were represented in kilocalories per mole (kcal/mol). The energies presented offer an estimation of the binding strength between the ligand and the target protein (Table 1). Typically, lower binding energies suggest a stronger binding (Ramalingam *et al*., 2023b). The 3D structure of the SCC receptor was obtained from the Protein Data Bank, while the ligand was sourced from PubChem and converted into PDBQT files suitable for the docking process. Following the molecular docking, the results were visualized and analyzed with BIOVIA Discovery Studio Visualizer, which facilitates the examination and comprehension of the binding interactions established between the protein and the ligand, including a standard drug utilized in the study.

Evaluation of drug-like properties and ADMET characteristics

Evaluating the drug-likeness and ADMET profile of selected compounds at an early stage is crucial to prevent the waste of time and resources. The drug-like characteristics of the selected ligand molecules were assessed using *in silico* Swiss ADME. The essential molecular properties were evaluated in accordance with Lipinski's Rules of Five (RO5).

Study on in silico toxicity prediction

The toxicity of the compounds was predicted utilizing Pro Tox-II, a freely accessible web server (http://tox.charite.de/protox_II/). The tool employs models based on machine learning techniques to predict toxicological endpoints, pathways, and targets, in addition to acute (oral) and organ (liver) toxicities, all derived from the 2D chemical structures of the compounds.

In silico PASS Prediction Analysis

In silico PASS prediction is a computational method that anticipates the biological activities of

Table 2: Qualitative analysis of phytochemicals from *W. tinctoria* leaf extracts

S. No.	Phytochemical	Analyzing method	Aqueous extract	Ethanol extract	Hexane extract	Ethyl acetate extract
1	Alkaloids	Mayer's test	-	+	+	-
2	Terpenoids	Copper acetate test	+	+	+	-
3	Phenols	Ferric chloride test	+	+	-	-
4	Saponins	Foam test	+	+	-	-
5	Flavanoids	Sodium hydroxide test	+	+	-	-
6	Quinines	Borntrager's test	-	-	-	-
7	Steroids	Salkowski test	+	+	-	-
8	Glycosides	Keller-Kiliani's test	+	+	-	+
9	Carbohydrate	Molisch's test	+	+	-	+
10	Protein	Millon's test	+	+	+	-

Present (+), Absent (-)

a compound by utilizing the Prediction of Activity Spectra for Substances (PASS) algorithm. Utilizing PASS for *in silico* prediction offers two key advantages: it helps prioritize drug development initiatives and identifies the primary bioactivities of chemical compounds. To assess compounds as potential drug candidates, the Prediction of Activity Spectra for Substances (PASS) is utilized to forecast the possible pharmacological effects of a compound based on its structural information. This is achieved by examining the Pa score (probability of being active) and Pi score (probability of being inactive) through the input of the SMILES format.

Results and Discussion

Qualitative analysis of phytochemicals

Table 2 demonstrates the qualitative analysis of extracts from *W. tinctoria*, including water, ethanol, hexane, and ethyl acetate. The ethanol extract demonstrated the greatest abundance of phytochemicals, yielding positive results for alkaloids, terpenoids, phenols, saponins, flavonoids, steroids, glycosides, carbohydrates, and proteins. The water-based extract included a diverse array of phytochemicals, while the hexane extract showed positive results mainly for alkaloids, terpenoids, and proteins, indicating a greater affinity for non-polar compounds. The extract of ethyl acetate, on the other hand, exhibited a restricted range of phytochemicals, revealing only the presence of glycosides and carbohydrates. The results indicate that ethanol and aqueous extracts are superior in extracting a wide variety of phytochemicals, especially those that are polar and moderately polar, while hexane and ethyl acetate extracts demonstrate greater selectivity. The results indicate that the ethanolic extract contained a greater quantity of phyto-constituents in comparison to the other extracts.

The analysis of leaf extracts from *W. tinctoria* revealed the existence of steroids, flavonoids, fatty acid esters, and terpenes (Khan *et al*., 2021). It was recently reported that qualitative phytochemical analysis identified the presence of both primary and secondary metabolites, such as proteins, phenols, tannins, flavonoids, terpenoids, glycosides, saponins, and coumarins (Sailaja *et al*., 2024).

GC-MS Analysis

The components present in the ethanolic extract of *W. tinctoria* were determined using GC-MS analysis. A total of 36 compounds were recognized, as shown in the chromatogram (Fig. 3) and outlined in Table 3. The extract contained a

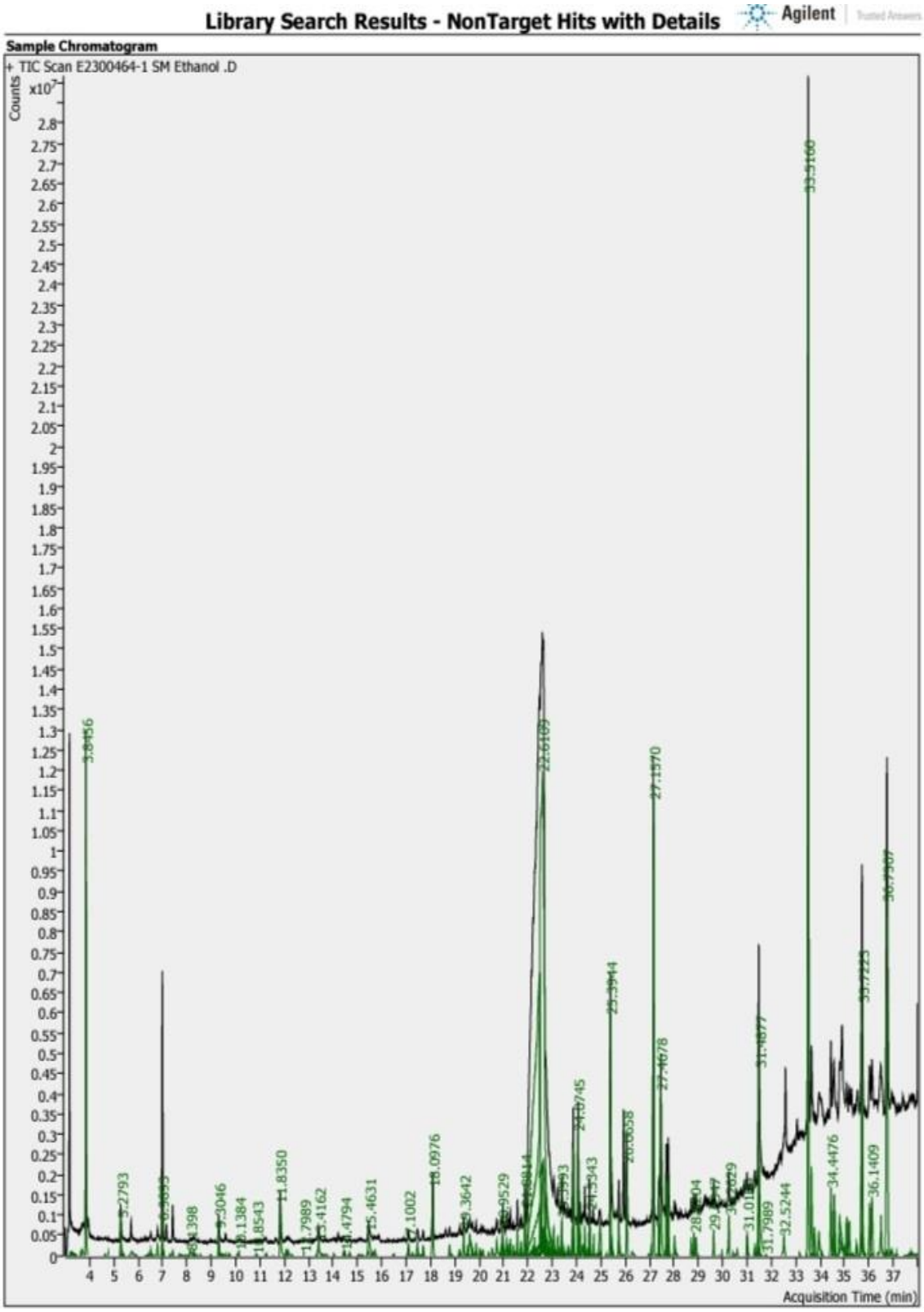

Fig. 3: GC-MS Chromatogram of ethanolic extract of *W. tinctoria.*

Table 3: Chemical profile of ethanolic leaf extract of *W. tinctoria*

Peak	RT/Min	Name of the compound	Molecular formula
1	5.2793	Oxime-methoxy-phenyl-	$C_8H_9NO_2$
2	6.9893	Aminobenzoic acid, TBDMS derivative	$C_{13}H_{21}NO_2Si$
3	8.1398	1,2-Propanediol,1-Phenyl	$C_9H_{12}O_2$
4	9.3046	Cyclotrisiloxane, Hexamethyl	$C_6H_{18}O_3Si_3$
5	10.1384	4-Piperidinone,2,2,6,6-tetramethyl	$C_9H_{17}NO$
6	10.8543	2,6,6-Trimethyl-2-cyclohexene-1,4-dione-	$C_9H_{12}O_2$
7	11.8350	Naphthalene	$C_{10}H_8$
8	12.7989	Isophthalaldehyde	$C_8H_6O_2$
9	13.4162	Cyclotetraasiloxane, Octamethyl-	$C_8H_{24}O_4Si_4$
10	14.4794	Decane, 3-ethyl-3-methyl-	$C_{13}H_{28}$
11	15.4631	Phenol, 5-ethenyl-2-methoxy	$C_9H_{10}O_2$
12	17.1002	(1S,4S,4As)-1-Isopropyl-4,7-dimethyl-1,2,3,4,4a,5-Hexahydronaphthalene	$C_{15}H_{24}$
13	18.0976	Bicyclononane,2-methylene-trimethyl-4-Vinyl	$C_{15}H_{24}$
14	19.3642	2,3,3,4,7-Pentamethyl-2,3dihydrobenzofuran	$C_{13}H_{18}O$
15	20.9529	Caryophyllene oxide	$C_{15}H_{24}O$
16	21.8814	Bornyl acetate	$C_{12}H_{20}O_2$
17	22.6109	2,3-Di-O-methyl-D-xylopyranose	$C_7H_{14}O_5$
18	23.3993	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$
19	24.0745	2-Hexadecen-1-01-37,11,15-tetramethyl-acetate	$C_{22}H_{42}O_2$
20	24.5343	Neophytadiene	$C_{20}H_{38}$
21	25.3944	n-Hexadecanoicacid	$C_{16}H_{38}O_2$
22	26.0658	Octadecanal	$C_{18}H_{36}O$
23	27.1570	Phytol	$C_{20}H_{40}O$
24	27.4678	9,12,15- octadecadienoic acid (z,z,z)-	$C_{18}H_{30}O_2$
25	28.8204	Triethylene glycol monododecyl ether	$C_{18}H_{38}O_4$
26	9.6347	4,8,12,16Tetramethylheptadecan-4-olide	$C_{12}H_{40}O_2$
27	30.2629	Cyclodexasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$
28	31.0100	Hexadecanoic acid,2-hydroxy-1-ethylester	$C_{19}H_{38}O_4$
29	31.4877	Tryptanthrine	$C_{15}H_8N_2O_2$
30	31.7989	Uracil, 1,3,6-trimethyl-5-	$C_{16}H_{17}N_3O_2$
31	32.5244	9,12,15-Octadecatrienoic acid, methyl ester,(z,z,z)-	$C_{19}H_{32}O_2$
32	33.5160	Squalene	$C_{30}H_{50}$
33	34.4476	1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-	$C_{30}H_{50}O$
34	35.7223	gamma.-Tocopherol	$C_{28}H_{48}O_2$
35	36.1409	12-Oleanen-3-yl acetate, (3.alpha.)	$C_{32}H_{52}O_2$
36	36.7507	dl-.alpha.- Tocopherol	$C_{29}H_{50}O_2$

varied selection of bioactive phytochemicals, mainly featuring terpenoids, fatty acids, phenolics, alkaloids, and sugar derivatives. Prominent terpenoids included caryophyllene oxide, bornyl acetate, phytol, neophytadiene, and squalene, referred to for their anti-inflammatory, anti-oxidant, and antimicrobial benefits. The identified fatty acids and esters, such as palmitic acid, linoleic acid, and 2-hexadecen-1-ol acetate, suggest possible emollient and antibacterial characteristics associated with lipids. Substances such as oxime-methoxy-phenyl and 5-ethenyl-2-methoxyphenol, in addition to alkaloids such as tryptanthrin and 4-piperidinone derivatives, exhibit antioxidant properties and may also possess cytotoxic traits. In a prior study, Jayamathi

Table 4: Docking scores of the three compounds from the ethanol extract of *W. tinctoria*against EGFR and KRAS cancer protein along with the standard drugs

Receptor	Ligand	Binding energy (kcal/mol)
EGFR (1M17)	Tryptanthrine (L1)	-8.3
	Squalene (L2)	-7.1
	Hexadecenoic acid (L3)	-4.2
	Imiquimod (D1)	-6.8
KRAS (4OBE)	Tryptanthrine (L1)	-8.7
	Squalene (L2)	-6.5
	Hexadecenoic acid (L3)	-4.5
	Imiquimod (D1)	-7.5

et al. (2012) discussed compounds that are related, including squalene and phytol. Tryptanthrin received a pharmacological assessment to determine its therapeutic significance (Srivastava, 2014). The GC-MS profile distinctly demonstrates the extract's promise for applications in medicinal, cosmetic, and nutraceutical sectors.

Protein-Ligand Interaction on Molecular Docking analysis

The prominent phytoconstituents (tryptanthrin, squalene, and hexadecanoic acid) exhibiting anticancer properties in the ethanolic extract of *W. tinctoria* were selected for molecular docking studies based on the GC-MS analysis. The molecular docking study resulted in the selection of conformations exhibiting optimal binding energy for protein-ligand interactions. The analysis of hydrogen bonds, hydrophobic, electrostatic, and Van der Waals interactions among tryptanthrin, squalene, hexadecanoic acid, and the oncogenic proteins EGFR and KRAS was performed utilizing Biovia Discovery Studio Client 2020 and AutoDock Vina software.

The standard medication administered was Imiquimod, targeting the oncogenic proteins EGFR and KRAS. The binding affinities of tryptanthrin (L1), squalene (L2), hexadecanoic acid (L3), and imiquimod (D1) to the receptor were evaluated with their binding affinities in kcal/mol and presented in Table 4. In comparison to Tryptanthrin (L1), the highest binding energy for this drug is recorded at -8.3 kcal/mol for EGFR and -8.1 kcal/mol for KRAS. Subsequently, squalene exhibited values of -7.1 kcal/mol and -6.5 kcal/mol, while hexadecanoic acid showed -4.2 kcal/mol and -4.5 kcal/mol. In comparison, imiquimod had values of -6.8 kcal/mol and -7.5 kcal/mol regarding EGFR and KRAS.

In molecular docking analyses concerning receptors 1M17 (EGFR) and 4OBE (KRAS), the ligand squalene (L2) predominantly demonstrated hydrophobic interactions. On the other hand, Tryptanthrin (L1) and hexadecanoic acid (L3) exhibited a combination of hydrogen bonding and hydrophobic interactions. The standard reference ligand, Imiquimod (D1), exhibited both types of interactions, as outlined in Table 5. Figures 4 and 5 demonstrate that ligands L1, L2, L3, and D1 effectively reached the active site of receptor 1M17 and maintained a stable position there. All these ligands inhibited the LSY721 binding domain, which may hinder the function of related oncogenic proteins.

Figures 6 and 7 illustrate the binding affinities of these compounds with the KRAS receptor (PDB: 4OBE), highlighting that L1, L2, L3, and D1 exhibited notable interactions. In the interactions observed, L1, L3, and D1 participated in both hydrogen bonding and hydrophobic interactions, whereas squalene (L2) was noted to engage with the receptor through hydrophobic relationships. Previous investigation has investigated similar docking approaches. Bayrakdar *et al.* (2024)

Table 5: Interaction of amino acid residues with ligands at receptor sites

Protein	Ligands	Amino acids involved and distance (Å)	
		Hydrogen-Binding Interaction	Hydrophobic Interaction
1M17	L1	766A Thr (2.58), 769A Met (1.98)	694A Leu(3.51), 702A Val (4.00), 721A Lys(3.62), 766A Thr(3.91)
	L2	————	694A Leu(3.67), 699A Phe(3.42), 699A Phe(3.63), 699A Phe(3.76), 699A Phe(3.74), 702A Val(3.59), 702A Val(3.68), 719A Ala(3.59), 721A Lys(3.70), 723A Leu(3.67), 735A Ile (3.82), 769A Met(3.67), 820A Leu(3.80), 820A Leu (3.63).
	L3	831A Asp(2.36)	694A Leu93.62), 699A Phe (3.56), 702A Val(3.87) 702A Val(3.73), 702A Val(3.67), 721A Lys(3.54), 820A Leu(3.64)
	D1	766A Thr (3.36), 767A Gln(2.93)	694A Leu(3.67), 702A Val (3.68), 702A Val (3.80), 702A Val (3.69)721A Lys(3.62)
4OBE	L1	161B Arg(2.42)	121A Thr(3.89), 142A Ile(3.83), 142A Ile(3.72), 142A Ile (3.90), 143A Glu(3.46), 154A Asp(3.93
	L2	----------	16ALys(3.72), 18A Ala(3.77),28A Phe(3.88),117A Lys(3.95),146A Ala(3.83)
	L3	123B Arg(3.84)	44A Val(3.67), 45A Val(3.56), 45A Val (3.77), 119B A sp(3.75).
	D1	116A Asn(2.22), 119A Asp(2.00)	28A Phe(3.90), 120A Leu(3.68), 147A Lys(3.78)

introduced a new derivative of acetylsalicylic acid, 2-[(4-acetylphenyl)carbamoyl]phenyl acetate, which shows promise as an anticancer agent targeting EGFR. Additionally, Pathania *et al*. (2014) explored a synthetic analogue of tryptanthrin, which demonstrated the ability to inhibit STAT3 signaling and trigger apoptosis in leukemia cells, thereby highlighting the therapeutic potential of tryptanthrin derivatives. In support of this, Rathod *et al*. (2025) indicated that a newly synthesized tryptanthrin Schiff base demonstrated superior efficacy compared to erlotinib in inhibiting EGFR, as validated by EGFR-TK assays. Recent research conducted by Magesh *et al*. (2023) revealed that squalene and 3-O-methyl-D-glucose, sourced from *W. tinctoria*, significantly inhibited the activity of cancer-related proteins like EGFR and MAPK in models of oral cancer. Furthermore, squalene is anticipated to engage close to the active site of Topoisomerase II alpha (Topo IIα), indicating its promise as a new inhibitor of Topo IIα (Pareek *et al*., 2024). Additionally, hexadecanoic acid, which is a long-chain saturated fatty acid, takes on a U-shaped conformation when positioned in protein pockets, enabling it to fit snugly within the binding site. This structural adaptation indicates that hexadecanoic acid could interact with surface pockets of the KRAS protein, akin to observations made with extracts from *Typhonium flagelliforme* (Lai *et al*., 2008; Fatima and Yee, 2014).

Analysis of ADMET

Table 6 provides an explanation of the phytochemical and pharmacokinetic (ADMET) characteristics of the evaluated compounds-- tryptanthrine (L1), squalene (L2), and hexa-decenoic acid (L3). Each of the three compounds is within the acceptable molecular weight range of less than 500 g/mol, indicating they may be suitable for drug development. Tryptanthrine (L1) demonstrates advantageous ADMET properties, involving 3 hydrogen bond acceptors, no donors, a moderate iLOGP of 1.95, no rotatable bonds, and a molar refractivity of 70.77-- all falling within the suggested ranges. Hexadecenoic acid (L3)

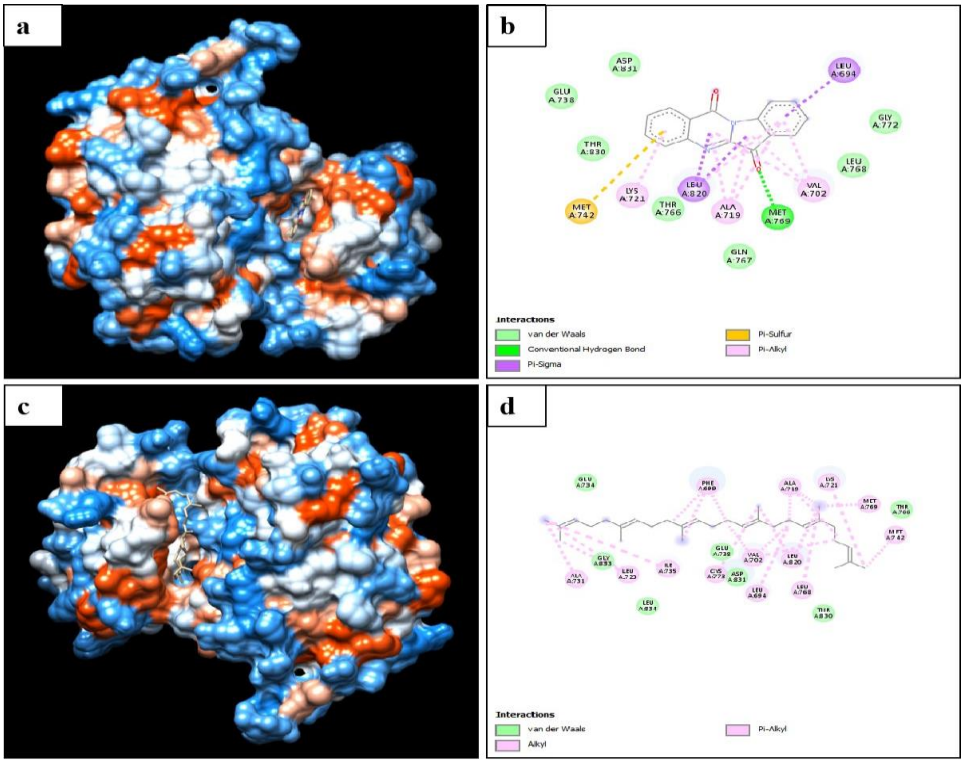

Fig. 4: Surface model of EGFR protein with L1-Tryptanthrine (a) and L2-Squalene (c) interaction and interaction pose of inhibitors illustrating hydrogen bonds, van der Waal's forces, alkyl, Pi-alkyl and Pi-sigma at the binding pocket of L1 (b) and L2 (d) with EGFR protein.

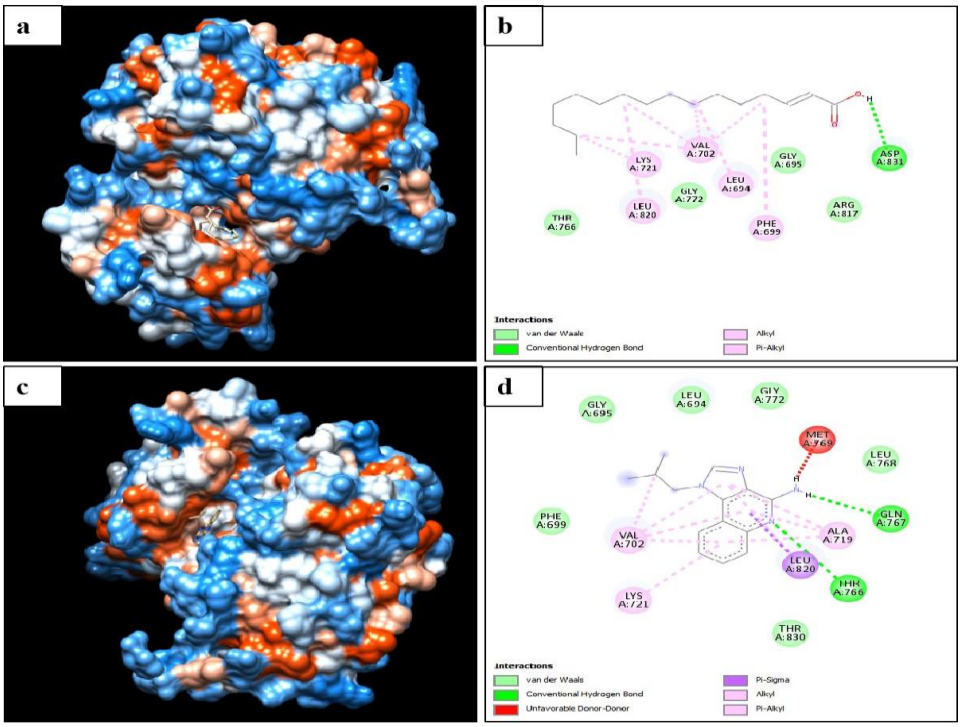

Fig. 5: Surface model of EGFR protein with L3-Hexadecenoic acid (a) and D- Imiquimod (c) interaction and interaction pose of inhibitors illustrating hydrogen bonds, van der Waal's forces, alkyl, Pi-alkyl and Pi-sigma at the binding pocket of L3 (b) and D (d) with EGFR protein.

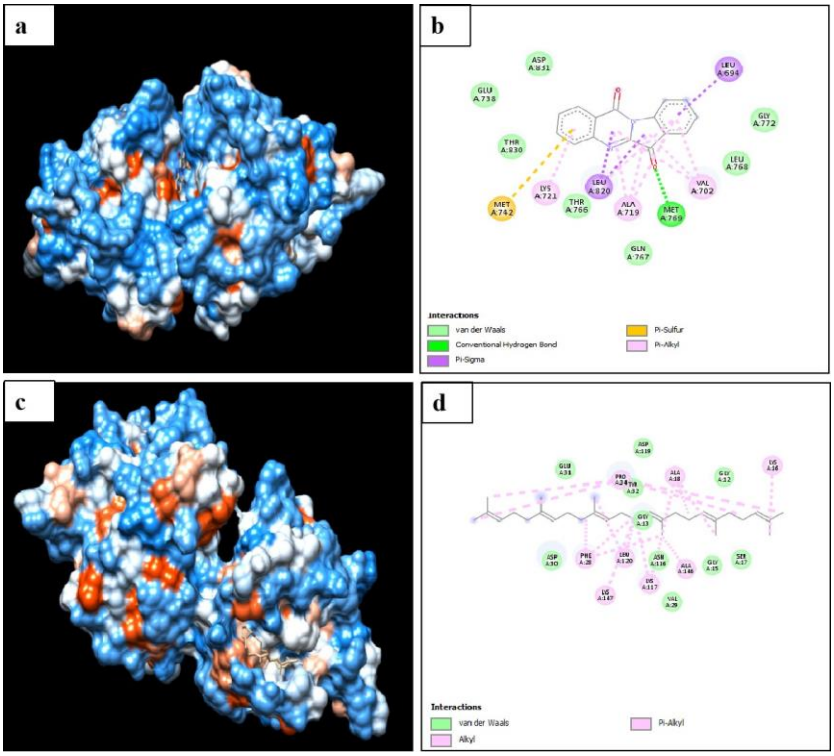

Fig. 6: Surface model of KRAS protein with L1-Tryptanthrine (a) and L2-Squalene (c) interaction and interaction pose of inhibitors illustrating hydrogen bonds, van der Waal's forces, alkyl, Pi-alkyl and Pi-sigma at the binding pocket of L1 (b) and L2 (d) with KRAS protein.

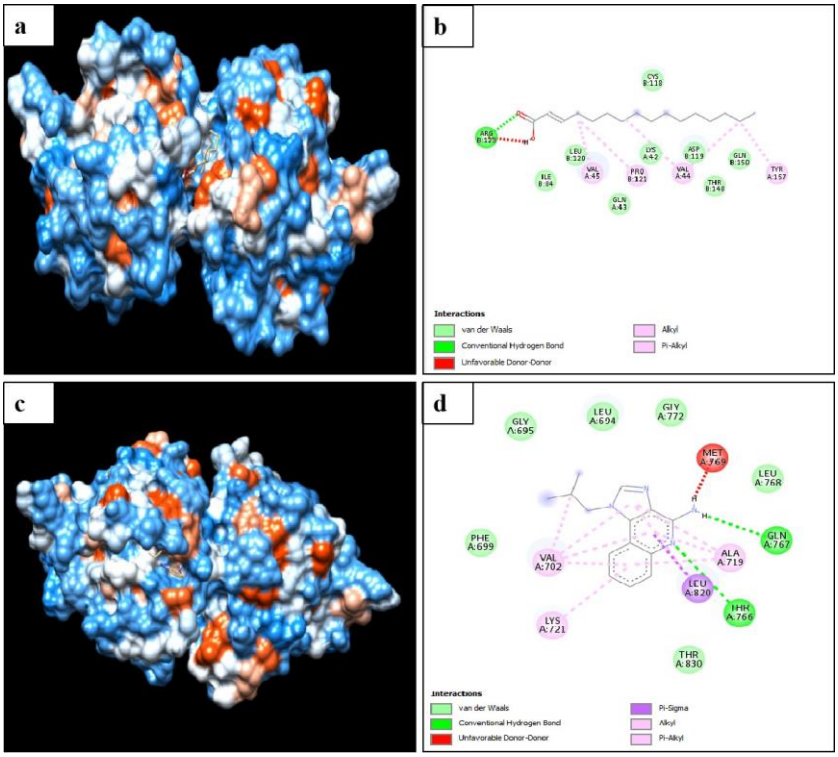

Fig. 7: Surface model of KRAS protein with L3-Hexadecenoic acid (a) and D- Imiquimod (c) interaction and interaction pose of inhibitors illustrating hydrogen bonds, van der Waal's forces, alkyl, Pi-alkyl and Pi-sigma at the binding pocket of L3 (b) and D1 (d) with KRAS protein.

Table 6: Phytochemical and pharmacokinetic properties of screened compounds (ADMET profiling)

Compound	Tryptanthrine	Squalene	Hexadecenoic acid
Ligand code	L1	L2	L3
Molecular formula	$C_{15}H_8N_2O_2$	$C_{16}H_{30}O_2$	$C_{16}H_{30}O_2$
Smile ID	C1([2H])c([2H])c([2H])c2c(c1[2H])nc1n(c2=0)c2c(c1=0)cccc2	(C)C)\C	CCCCCCCCCCCCC/C=C/C(=0)OC/C(=C\CC)\C=C(/CC/C=C/C(/CCC=C
Molecular weight	248.24 g/mol	410.4 g/mol	254.41 g/mol
HB acceptor	3	0	2
HB donor	0	0	1
iLOGP	1.95	0	3.81
nROT	0	15	13
Molar refractivity	70.77	143.88	80.32
Rule of 5	0	1	0

provides all required criteria, containing 2 hydrogen bond acceptors, 1 donor, an iLOGP of 3.81, and 13 rotatable bonds, along with a molar refractivity of 80.32, indicating favorable oral bioavailability. On the other hand, squalene (L2), while falling within the permissible weight and rotatable bond parameters, does exhibit a violation of Lipinski's rule because it lacks hydrogen bonding groups and has an unusually elevated molar refractivity (143.88), exceeding the acceptable limits. Overall, L1 and L3 show encouraging characteristics typical of potential drug employees, whereas L2 may require structural improvement or different methods of administration.

The assessment of drug-like qualities is crucial due to their correlation with dissolution and intestinal permeability (Lipinski, 2004; Malla *et al*., 2022; Aldossari *et al*., 2023). Bioactive chemicals are ineligible as drug moieties if they violate more than two of Lipinski's guidelines (2004). Drug degradation frequently results from the toxicity of the medications, primarily attributed to adverse consequences (Wang *et al*., 2015).

Study of in silico toxicity prediction

The computational prediction of natural substances' toxicities and drug score profiles is encouraging. The LD_{50} of the substances tryptanthrine (L1), squalene (L2), and hexadecenoic acid (L3) was predicted using the web application PROTOX. The chemicals used for present investigation were in the non-toxic range (above 1000 mg/kg) (Table 7). The blue-highlighted carcinogenicity, mutagenicity, cytotoxicity, cardiotoxicity, nephrotoxicity, and ecotoxicity were also anticipated; however, it was discovered that none of the selected compounds were either carcinogenic or mutagenic. The substances demonstrated both neurotoxicity and respiratory toxicity. The average probability of each class is shown by the orange dots or lines in the data, which were calculated from each model's training set data (Fig. 8). Determining the toxicities of compounds is a crucial step in the drug development process, and ProTox is a useful computational tool in this regard (Banerjee *et al*., 2018). Compared to animal experiments, computational methods examine chemical toxicities significantly more quickly since *in silico* methodologies yield results in a short amount of time (Drwal *et al*., 2014).

In silico pass prediction study

The *in silico* pass prediction study presented in Table 8 showed the anticipated impact of

Table 7: Effects of phytocompounds on Carcinogenicity, Mutagenicity, Cardiotoxicity, Nephrotoxicity, Respiratory toxicity, Cytotoxicity, Neurotoxicity and Toxicity prediction details by ProTox-II

Molecule	L1	L2	L3
Carcinogenicity	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Mutagenicity	Non-mutagenic	Non-mutagenic	Non-mutagenic
Cardiotoxicity	Inactive	Inactive	Inactive
Nephrotoxicity	Inactive	Inactive	Inactive
Respiratory toxicity	Active	Active	Active
Cytotoxicity	Inactive	Inactive	Inactive
Neurotoxicity	Active	Active	Active
Predicted LD_{50}(mg/kg)	1100mg/kg	5000mg/kg	1925mg/kg
Predicted toxicity class	4	5	4

LD_{50}>5000 indicates that the substance is non-toxic

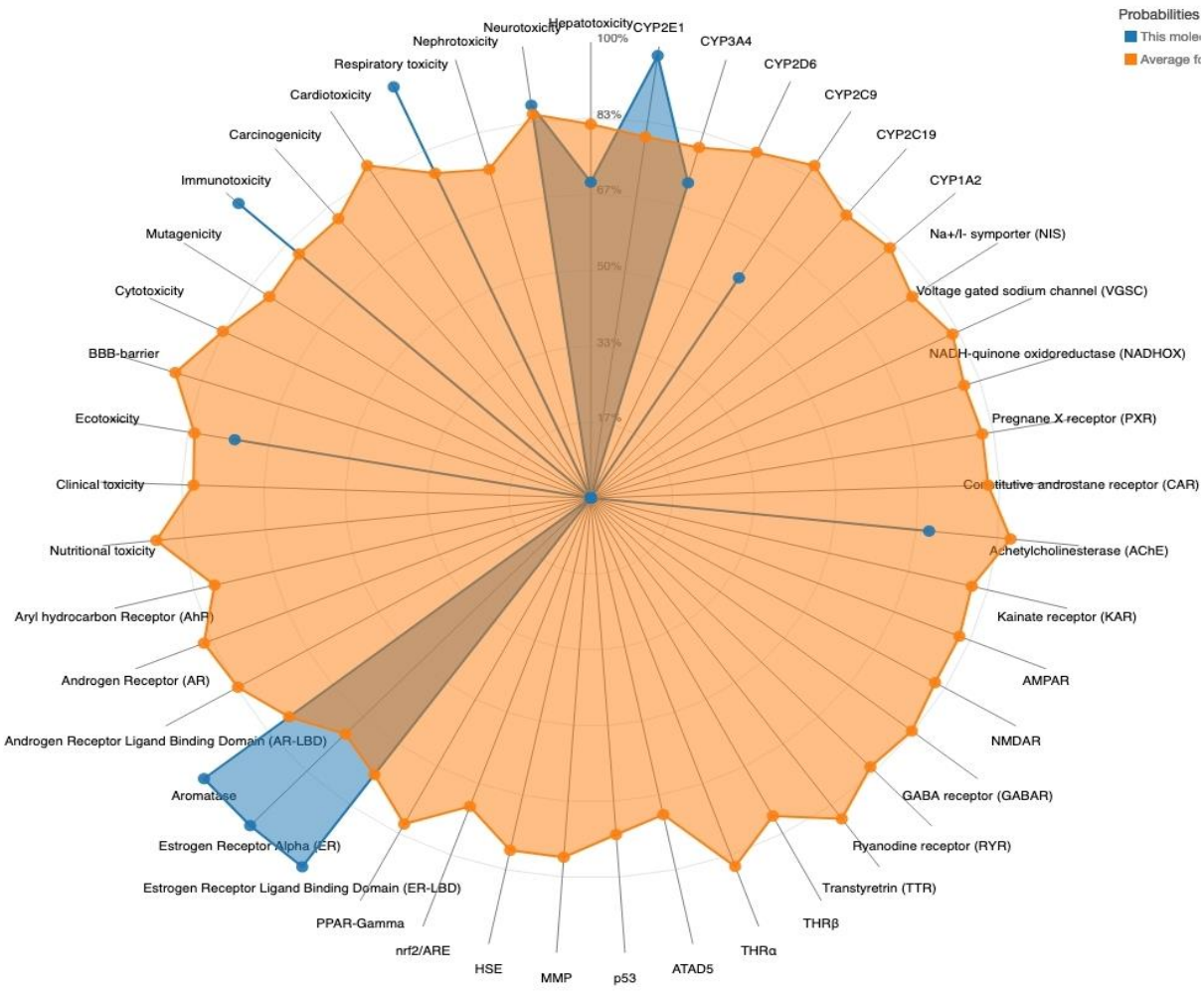

Fig. 8: Radar chart of toxicities of phytocompound L1, L2 and L3 and drug by PROTOX.

Table 8: Biological activity of Tryptanthrine, Squalene and Hexadecenioc acid by using *In silico* pass prediction study

Compound	Pa	Pi	Biological activity
Tryptanthrine	0.690	0.005	Antileukemic
	0.602	0.045	Antineoplastic
	0.544	0.045	Gastrin inhibitor
	0.109	0.042	Antifungal enhancer
	0.256	0.196	Anti allergic
Squalene	0.803	0.011	Antineoplastic
	0.701	0.016	Antiinflammatory,
	0.816	0.007	Hyperglycemic
	0.676	0.031	TP53 Enhancer
	0.612	0.009	Antipsoriatic
	0.793	0.005	Cell adhesion molecule inhibitor
Hexadecenioc acid	0.717	0.006	Preneoplastic conditions treatment
	0.727	0.005	Antiviral
	0.602	0.007	Antiulcerative
	0.715	0.004	Eye irritation
	0.475	0.007	Antihelmintic

tryptanthrine, squalene, and hexadecenoic acid on various biological activities, including anti-leukemic, antineoplastic, gastrin inhibition, antifungal enhancement, and anti-allergic effects. Squalene demonstrated effects on antineoplastic, anti-inflammatory, hyperglycemic, TP53 enhancement, and antipsoriatic properties and inhibition of cell adhesion molecules, while hexadecenoic acid exhibited effects on the treatment of preneoplastic conditions, antiviral activity, antiulcerative properties, eye irritation, and antihelminthic effects. Additionally, it includes the Pa and Pi values for each phytocompound, along with their corresponding target biological activities. Of all the compounds, tryptanthrine exhibited the highest Pa value in relation to antileukemic activity, while squalene demonstrated the highest Pa value concerning hyperglycemic effects. Additionally, hexadecenoic acid recorded the highest Pa value for antiviral activity. All three compounds demonstrated improved efficacy as anticancer agents in cancer treatment. This is a. Pa (probability—"to"be active") assesses the probability that the compound under investigation falls within the category of active compounds. Tryptanthrin is a recognized natural alkaloid that exhibits a wide range of biological activities, functioning as an anti-inflammatory, anticancer, antibacterial, antifungal, antiviral, antitubercular, and more (Zhou, 2024). Squalene has undergone extensive testing for its pharmacological effects, demonstrating properties such as anticancer, anti-inflammatory, antioxidant, and antidiabetic activities (Widyawati *et al*., 2023). The findings for tryptanthrine, squalene, and hexadecenoic reveal Pa values greater than Pi, suggesting biological activity and highlighting their particular effectiveness in anticancer properties (Shehzadi *et al*., 2016; Shah *et al*., 2022).

Conclusion

Wrightia tinctoria has significant medicinal potential due to its wide range of bioactive phytoconstituents with varying pharmacological properties. This study examined the phytochemical profiles of different solvent extracts and discovered that the ethanolic extract had a large concentration of secondary metabolites, indicating that ethanol is an excellent solvent for extracting bioactive compounds. The GC-MS analysis demonstrated its ability to detect and quantify significant phytocompounds with varying retention times. Three drugs with documented

anticancer efficacy were selected for molecular docking investigations, together with a reference drug targeting important molecular targets associated with SCC (EGFR and KRAS). Two of the evaluated substances, tryptanthrine and squalene, had higher binding affinities than the standard drug Imiquimod, indicating a stronger potential for interaction with the target oncogenic proteins. Overall, this study provides preliminary *in silico* evidence for the anticancer activity of chemicals derived from *W. tinctoria* against epidermoid carcinoma. However, further validation through broad *in vitro* and *in vivo* experimental models is required to confirm their therapeutic efficacy and safety profiles for future clinical trials

Ethical Statement

This study did not involve the use of animals, and therefore did not require ethical approval for animal experimentation.

Author Contributions

Mohan Sruthy: Conceptualization, methodology, interpretation and manuscript preparation; *Thangvael Muthu*: Conceptualization, editing and supervision; *Sidharth Kattumuchikkal*: Interpretation of results and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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