RESEARCH PAPER

Phytochemical profiling, Antioxidant, Anti-Inflammatory and Wound Healing Activities of *Tinospora cordifolia* with Molecular Insights into EGFR and KRAS Targeting in HCT116 Cells

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Abstract

The present study was aimed to study the phytochemical composition and biological activities of *Tinospora cordifolia* by GC-MS profiling, antioxidant assays, cyclooxygenase (COX) inhibition and molecular docking studies. GC–MS analysis of the active antioxidant fraction showed 26 compounds, mainly phytosterols, fatty acids, fatty acid esters and phenolic compounds. The major constituents were γ-sitosterol (45.23%), ethyl hexadecanoate (8.21%), ethyl oleate (7.86%), n-hexadecanoic acid (6.98%), butyl 9,12-octadecadienoate (6.55%), E, E, Z-1,3,12-nonadecatriene-5,14-diol (5.15%), β-sitosterol (2.33%) and stigmasta-3,5-diene (3.92%). The antioxidant evaluation showed 2 fractions with free radical scavenging activity of 52 and 58% respectively. The anti-inflammatory activity was further supported by the inhibition of cyclooxygenase (COX). It showed 76.67 ± 1.15 % inhibition and 66.6 % inhibition of albumin denaturation at 100µg. The scratch assay showed that the migration of HCT116 cells was significantly inhibited by *Tinospora cordifolia* extract at a concentration of 135 µg/mL, with wound closure reduced from 47.89% in the control to 13.23% after 24 h. This finding indicates that the extract has potent anti-migratory and potential anti-metastatic activity against colorectal cancer cells. The characterisation of the protein pocket determined the most favourable target for the interaction with ligand as epidermal growth factor (EGFR). Molecular docking analysis revealed that γ-sitosterol exhibits high binding affinity towards both EGFR (PDB: 1M17) and Kirsten Rat Sarcoma viral oncogene homolog (KRAS PDB: 6OIM) with binding energies of -8.325 and -8.323 kcal/mol, respectively. Importantly, the low average distance Root Mean Square Deviation (RMSD) on KRAS complex indicate a reliable and stable conformation of docking. These results suggest that γ-sitosterol might be a dual-target inhibitor of EGFR- and KRAS-mediated signalling pathways, which are responsible for the initiation and progression of colorectal cancer. The significant COX antagonistic property, together with the good anti-inflammatory and wound-healing characteristics and the presence of bioactive phytosterols, strongly suggest the therapeutic potential of *T. cordifolia* as an alternative source of therapeutic compounds for future drug discovery.

Keywords: Tinospora, COX, Wound Healing, EGFR, HCT116

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.INTRODUCTION

In Ayurvedic system, *Tinospora cordifolia* is considered as a Rasayana drug, referred to as "Amrita", because it increases resistance, life span, and stress tolerance. The plant belongs to the Menispermaceae family and is native to the Indian sub-continent (Singh and Devi, 2017). From phytochemical investigations, various components such as alkaloids, diterpenoid lactones, glycosides, steroids, phenolics, and polysaccharides have been documented to be present in *T. cordifolia* (Gupta *et al*., 2024). *Tinospora cordifolia* has antioxidant capacity due to methanolic, ethanolic and aqueous extracts, as well as having the ability for metal chelation and reduction. The use of *Tinospora cordifolia* as a source for the extraction of the alkaloid palmatine suggests that *T. cordifolia* has anti-cancer capability. Pyrrolidone, N-formylannonain, cordifolioside A, magnoflorine, tinocordiside and syringin are active components that have shown to have potent immunomodulatory (Bala *et al*., 2015) antioxidant (Bhawya and Anilakumar, 2010) and cytotoxic effects (Puttananjaiah *et al*., 2015). They contribute to its multiple physiological actions, including improving antioxidant functions through elevation of catalase, SOD and glutathione peroxidase levels (Priya *et al*., 2022). Recent studies still support the anti-cancer property of *T. cordifolia* through the cytotoxicity shown by *T. cordifolia* extracts from the leaves and stems of the plant, which include berberine. Studies performed by (Gotsurve *et al*., 2025) and accompanying *In silico*/*In vitro* studies further highlight the effectiveness of employing computational docking analysis in conjunction with other tests. New studies have highlighted the importance of using computational methods such as molecular docking in the understanding of the mechanism of action of bioactive plant constituents. For example, the combination of *in vitro* cytotoxicity studies with molecular docking has been used effectively in the search for new antitumor

agents from medicinal plants (Bachhar *et al*., 2024). Though the potential of *T. cordifolia* as an abundant source of biologically active phytoconstituents with cancer-fighting ability is well recognized, little information is available about their chemical properties and molecular interactions at the molecular level. This research attempts to fill the void by proposing that the essential phytochemical constituents of *T. cordifolia* have characteristic electronic and structural properties that determine their affinity for cancer proteins, thus making them effective against cancer cells.

MATERIALS AND METHODS

Collection of sample and Extraction process

The Plants belonging to the *Tinospora cordifolia* species are collected in the Cuddalore district (11.684689°/79.721434°), verified and authenticated at the Department of Botany, Annamalai University, Chidambaram. The stem was well-dried and finely ground, under blender mixer grinder and sieved. The fine powder was packed in to 25 x 80 mm porous cellulose thimble. The round bottom flask was taken with ethyl acetate and then built with column. The extraction procedure involves heating the setup for 5h at 50°C on a heating mantle. The solvent phase was extracted and then analyzed using GCMS.

Column purification

Column Chromatography and active fraction analysis

Column chromatography was done to isolate the plant constituents from the crude extract with 120 mesh silica 15x2.5 cm long. A small amount of 100 mg of dried extract was first dissolved in one mL ethylacetate and then loaded on the silica gel packed column. Elution was begins with 100 percent ethyl acetate. The compound was isolated using a mixed solvent system which had an carried out using the solvent ethylacetate contains hexane: and ethanol at 4:1:1. Fractions *Rf* were examined for scavenging using TLC method. Fractions examined under UV 365 and plates were treated with 0.05mM DPPH reagent and positive components were collected by preparative TLC. The fractions were subjected to quantitative DPPH assay.

GCMS analysis of fraction

Both fractions pooled together and redissolved in ethylacetate. A Shimadzu GC–MS QP2010 system with a BPX-5 capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) and electron impact (EI) ionization mode was used to analyze the samples. The carrier gas was helium (99.999% pure) flowing at a steady 1 mL/min. A split ratio of 10:1 was used to for injection volume of 10 µL first. The temperatures of the ion source and injector were kept at 200°C and 250°C, respectively. After setting the oven temperature at 60°C for one minute, it was raised to 300°C at a rate of 15°C per minute and kept there for thirty minutes. The range of the solvent interval was 0 to 45 minutes. With a scan range of m/z 35–500, mass spectra were captured in positive electron ionization mode at 70 eV.

Albumin denaturation inhibition assay

Protein Denaturation inhibition test

The extract ability to inhibit protein denaturation was evaluated using the albumin coagulation technique. In this study, heat-induced denaturation of bovine serum albumin was examined, and the extract's capability to inhibit this process served as a measure of anti-inflammatory activity. The reaction mixture (total volume of 3 mL) included 0.5 mL of 1% bovine serum albumin, 2 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.5 mL of the extract at concentrations of 25, 50, 75, and 100 µg/mL. The mixture was gently vortexed and then incubated at 37 °C in a water bath for 15 minutes to promote interaction, followed by heating at 70 °C for 5 minutes to facilitate protein denaturation. Upon reaching room temperature, the turbidity of every sample was assessed at 660 nm with a UV–Visible spectrophotometer **(**Derbel *et al*., 2023). The percentage inhibition of protein denaturation was calculated using the formula:

% Inhibition of denaturation = 100 × (1 − T/C),

COX enzyme inhibition assay

The stock of plant extracts was dissolved at 5 mg/mL with extracted solvent (99%) and different concentration (250 to 1000 µg/ml) working solution was prepared to estimate the inhibition activities according to the manufacturer's protocol. Indomethacin was used as a positive control in same dosages. 800 µl reaction buffer solution, 20 µl COX-2 enzyme, 100 µl plant extract were pre incubated 10min at 28^{o} C after that 30 µl LPS and 50 µl of Arachidonic Acid (AA) were added and the reaction terminated at 45 secs by 50 µl of 1 M HCl to terminate the COX-2 reaction. Stannous chloride (100µl) was introduced to transform prostaglandin H2 (PG-H2) into prostaglandin F2α (PG-F2α) **(**Mukhopadhyay *et al*., 2023). Once the solution turned yellowish green, the concentration of PG-F2α was assessed spectrophotometrically using a microtiter reader at 418 nm. The formula:

$$\text{COX-2 rate } (\%) = ((EC - ES)/(EC)) \times 100,$$

where EC is the value of PGE_2 measured in the Control which drug solution were not added, *ES* is the value of PGE_2 measured in samples or inhibitor added.

Wound healing test

Cell culture:

The Human Colorectal cell line (HCT116) was obtained from NCCS, Pune. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, HiMedia), supplemented with 10% Fetal Bovine Serum (Gibco), 1% Sodium Bicarbonate, and 1% Sodium pyruvate (HiMedia). The cells were maintained in a humidified 5% CO2 atmosphere at 37°C for further experiments.

Scratch assay:

Scratch Assay is a reliable assay used to measure fundamental cell migration characteristics based on the effective cytotoxic concentration of a sample drug. HCT116 cells were seeded (2×105 cells per well) in a 24-well plate and then incubated for 24 hours; a scratch was made in the middle by a 200µl pipette tip after 24 hours of incubation of cells to reach 90% confluency. The cells were washed with PBS to remove any floating cells after wound creation, and then the cells were treated with 50% of the IC_{50} concentration, 135 µg/mL of the drug (fraction), for another 24 hours, while the untreated cells served as a control. The cell's potential to move from the edge to the wounded area is observed for three time periods (0hr, 12hr, and 24hr). After creating a wound, the drug was added to the DMEM media supplemented with 5% FBS. 5% FBS ensures the proper supply of nutrients for cells to survive and eliminates cell proliferation to avoid false negative results.

HCT116 Cells were treated only with 50% of the IC_{50} concentration, which is 135 µg/mL. This minimizes the cytotoxic effect on cells, as the IC_{50} concentration (135 µg/mL) can be cytotoxic to 50% of the cell population. A reference line was drawn in the middle of the 24-well plates to ensure images were taken in the same field where they were created.

Molecular docking

Using AutoDock Vina, a computational docking investigation was carried out for all the phytoconstituents chosen as ligands with TNF-α as the target.

Protein preparation

The molecular docking technique was utilized to comprehend the binding interaction of the NIST matched compound against CDK4(PDB ID:7SJ3). The unwanted co-crystallized ligand and water molecules were removed and the enzyme was prepared using the QuickPrep tool module in the MOE program, saved as pdb and converted to PDBQT format by Autodock vina tools. AutoDock Tools was employed to set the size and the centre of the grid box. Partially charged atoms were allocated partial charges, and polar hydrogen was added to create a protonation state at physiological pH.

Ligand preparation

All constituents' three-dimensional (3D) structures were obtained from the PubChem database, which is accessible via the NCBI website (https://pubchem.ncbi.nlm.nih.gov/), using the UCSF Chimera program (v.1.14).

Molecular docking

To forecast the ligand molecule's binding affinity, molecular docking assesses the interactions between proteins and ligands and calculates the scoring function based on geometry. Together with the crystal structure of EPGF (PDB ID: 171M) and Crystal Structure of human KRAS (PDB 6OIM) were used molecular docking studies to examine the binding patterns of certain phytoconstituents. The AutoDock Vina feature in the UCSF Chimera program version 1.14 was utilized to carry out the molecular docking study. View Dock was used to investigate binding affinity. Using Discovery Studio 2020 Client, the final results were examined and displayed.

RESULTS AND DISCUSSION

From the TLC-DPPH bioautography test results, out of the six fractions, only two fractions, which are fractions 5 (Rf value of 0.68) and 6 (Rf value of 0.82), possessed antioxidant properties. This is because fraction 6 had a high degree of scavenging capacity, which was 58%, while fraction 5 possessed 52% scavenging capacity compared to other fractions, whose scavenging capacity was zero (Table 1). In the above experiment, the presence of yellow spots was an indication of the ability of antioxidants within those fractions to reduce the stable DPPH free radicals due to non polar to moderate polarity (Baliyan *et al*., 2022). The increased scavenging effects in fractions 5 and 6 indicate that the fractions contain compounds that can donate hydrogen and/or electron to neutralize free radicals. Based on previous literature on TLC-DPPH tests, antioxidant active zones with moderate to high *Rf* value often comprise phenolic acid and flavonoids, along with other polyphenolic compounds (Wang *et al*., 2021). The lack of effect in fractions 1–4 suggests that there is no or little amount of antioxidant active compound or/and the compounds have weak quenching capacity.

Table 1. TLC fraction bioassay on DPPH scavenging

Fraction	Rf value	DPPH scavenging	Percentage
1	0.034	-	0
2	0.40	-	0
3	0.48	-	0
4	0.56	-	0
5	0.68	+	52
6	0.82	+	58

GCMS spectrum of active fraction

GC-MS analysis of the extract responsible for the antioxidant property in *Tinospora cordifolia* indicated the existence of 26 compounds, thus suggesting a very complex phytochemical profile. Chromatograms displayed a number of prominent peaks which represented fatty acids, fatty acid esters, phytosterols, phenolic compounds, hydrocarbons, and derivatives of terpenes. Some of the prominent components include γ-sitosterol (27.54% and 17.69%), ethyl hexadecanoate (8.21%), ethyl oleate (7.86%), n-hexadecanoic acid (6.98%). The peak with the highest accumulation

rate was γ-sitosterol (45.23%), while β-sitosterol had a rate of 2.33%. Fatty acids and fatty acid derivatives like n-hexadecanoic acid, ethyl hexadecanoate, ethyl oleate, ethyl octadecanoate, and butyl 9,12-octadecadienoate were present in large amounts. Two phenolic antioxidants were isolated with retention times of 16.479 and 18.594 min. Different researches have proven the efficiency of sitosterols for their property of scavenging free radicals, preventing lipid peroxidation, and regulating the inflammatory mediators through NF-κB and COX-2 pathways (Babu and Jayaraman, 2020; Saha and Ghosh, 2012). The presence of n-hexadecanoic acid (palmitic acid) and its ethyl esters supports the results obtained in previous studies related to the extraction of *T. cordifolia*. Hexadecanoic acid and its derivatives are capable of inhibiting phospholipase A2 enzyme, resulting in lower oxidative stress *in vivo* (Das *et al*., 2025). Identification of the compound of stigmasta-3,5-diene indicates the increased pharmaceutical importance of the isolated compound because phyto-sterols were observed to exhibit antioxidative, anti-inflammatory, hepatoprotective, antilipidemic and anticancer properties by interfering with cellular redox mechanisms (El Omari *et al*., 2024).

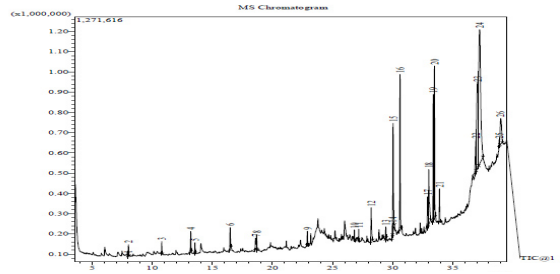


Figure 1. GCMS of DPPH positive fraction from ethyl acetate extract of *T. cordifolia* stem

Table 2. NIST matched compounds of active antioxidant fraction from *T.cordifolia*

Pea	Ret.	Area	Area	Compound Name
1	7.985	1502	0.006	Decanal
2	8.022	125931	0.488	Decane
3	10.78	120752	0.468	Undecane
4	13.20	261451	1.012	Naphthalene
5	13.55	100035	0.387	Dodecane
6	16.47	320010	1.239	2-Methoxy-4-
7	18.59	218262	0.845	Benzaldehyde, 3-
8	18.68	174914	0.677	Nonadecane
9	22.90	167688	0.649	Diethyl Phthalate
10	26.81	107607	0.417	Cyclohexasiloxane
11	27.17	104274	0.404	11-
12	28.20	625152	2.420	(3R,3aR,6R,8aR)-
13	29.42	130907	0.507	Tetradecanoic
14	29.97	122805	0.475	1,2-
15	30.03	1802838	6.980	n-Hexadecanoic
16	30.61	2119761	8.207	Hexadecanoic
17	32.90	473611	1.834	Ethanol, 2-(9,12-
18	33.00	1329111	5.146	E,E,Z-1,3,12-
19	33.37	1692070	6.551	Butyl 9,12-
20	33.47	2030815	7.863	Ethyl Oleate
21	33.89	415496	1.609	Octadecanoic
22	36.91	602588	2.333	beta-Sitosterol
23	37.04	4568999	17.689	gamma-Sitosterol
24	37.22	7112433	27.537	gamma-Sitosterol
25	38.86	91316	0.354	Eremophilone,8-
26	38.98	1011715	3.917	Stigmasta-3,5-
Total		2582903	100.000	

Anti-inflammatory activity of EAE of *T. cordifolia* crude

The protein denaturation inhibition was found to be concentration depndent in both extract and Standard (Fig 2). It was clear that the EAE of *Tinospora cordifolia* had shown a concentration-dependent effect on its anti-inflammatory activity. The mean percentage of inhibition rose progressively from 14.67 ± 2.31% at 25 µg/mL concentration to 66.67 ± 1.15% at 100 µg/mL concentration. It was observed that the standard errors for this particular experiment were quite small, ranging between 0.58 and 1.33, which indicates the reliability and reproducibility of experiments. On the other hand, for the standard drug used in this experiment, the percentage of inhibition was observed to be 32.00 ± 2.00%, 39.33 ± 1.15%, 47.00 ± 1.73%, and 62.67 ± 1.15% at concentrations of 25, 50, 75, and 100 µg/mL, respectively. In the low concentrations of 25 and 50 µg/mL, diclofenac displayed a better inhibitory effect than the extract. But with increasing concentration, the extract demonstrated increased efficiency and gave an inhibition percentage of 55.00 ± 1.00% for 75 µg/mL and 66.67 ± 1.15% for 100 µg/mL, compared to that of diclofenac (table 3). The high level of anti-inflammatory effect exhibited by EAE as the concentration increases could be explained by the presence of diterpenoid lactones (tinosporaside and cordifolioside), alkaloids, phenols, and flavonoids (Sharma *et al*., 2019; Singh *et al*., 2020). The results coincide with existing scientific literature showing that *T. cordifolia* is effective in terms of its anti-inflammatory and immune-modulating action. The capacity of the plant extract to produce greater inhibition compared to diclofenac makes it a valuable candidate for natural anti-inflammatory compounds.

Figure 2. Percentage of albumin denaturation inhibition

Table 3. Descriptive statistics on anti-inflammatory activity of extract and standard

Concentration µg	N total	Mean	Standard Deviation	SE of mean
EA25	3	14.66667	2.3094	1.33333
EA50	3	31.33333	1.1547	0.66667
EA75	3	55	1	0.57735
EA100	3	66.66667	1.1547	0.66667
DF25	3	32	2	1.1547
DF50	3	39.33333	1.1547	0.66667
DF75	3	47	1.73205	1
DF100	3	62.66667	1.1547	0.66667

COX enzyme Inhibition

This study proved that extract of *Tinospora cordifolia* has good anti-inflammatory effects due to inhibition of the cyclooxygenase enzyme. The level of inhibition increases with the increasing concentration, showing that there is dose-dependency. Despite having lesser effect when compared to aspirin, the extract was able to inhibit up to 76 % at a higher concentration level. The extract showed 20.33±1.52, 37.33±0.57, 65±1 and 76.66±1.15 % of COX inhibition whereas aspirin had 31±0.81,56±0.81,76±0.81 and 89±0.81 percentage COX inhibition among 25,50, 75 and 100µg/mL (Fig 3). There have been reports of a high binding affinity of several bioactive compounds such as berberine, tinosporaside, Dihydroberberine and β-sitosterol to COX-2 and inhibition of inflammation pathways (Jacob and Prakash, 2023). *In vivo* experiments conducted using *T. cordifolia* have shown remarkable reduction in the gene expression of COX-2 (Ghatpande *et al*., 2019).

Figure 3. COX enzyme inhibition ability of extract and standard aspirin

Wound healing potential of EAE of *T.cordifolia* stem

The effect of extract on anti-migration of HCT116 colorectal cancer cells was studied by employing the scratch wound healing test using the IC_{50} value of 135 µg/mL. In the initial 0 h time point, there was an identical scratch size between the control and experimental samples without any sign of cell migration. In the subsequent 12 h, 21.31% wound closure was observed in the control group while only 8.57% migration was seen in the experimental sample (Table 4). In the next 24 h, there was 47.89% wound closure for the control group while migration for the experimentally treated cells was only 13.23%. *Tinospora cordifolia* extract was found to successfully inhibit the migration activity of HCT116 colorectal cancer cell line in a dose of 135 µg/mL, which equals to the IC_{50}. The significantly lower closure rate observed for both time points suggests the inhibitory impact on cell movement and possibly the ability of the extract to function as an antitumor substance (Fig 4). Previous research had revealed that *Tinospora cordifolia* contains active substances such as diterpenoids, alkaloids, glycosides, and phenols which have the capacity to induce apoptosis, autophagy, and cell proliferation inhibition in the colorectal cancer cells (Deepa *et al*., 2019). One compound isolated from *Tinospora cordifolia*, called a clerodane furano diterpene glycoside, could induce autophagy and apoptosis in HCT116 cells (Sharma *et al*., 2018).

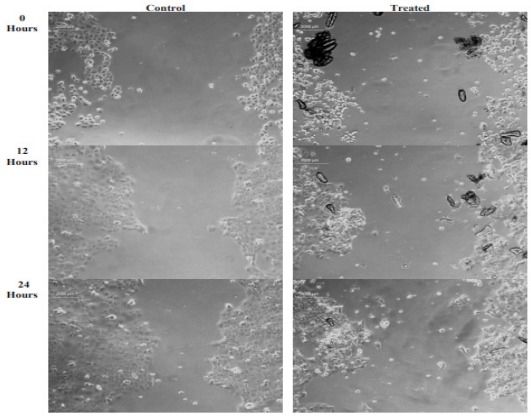


Figure 4. Microscopic observation of wound healing abilty of HCT116

Table 4. Percentage of Migration of HCT116 on scratch assay

Treatment	0 h	12 h	24 h
Control	0.00	21.31 ± 1.24	47.89 ± 2.16
T. cordifolia Treated (135 µg/mL)	0.00	8.57 ± 0.86	13.23 ± 1.12

Molecular docking of Gamma sitosterol

The assessment of the structural aspects for the chosen target proteins, namely Epidermal Growth Factor Receptor (EGFR) and KRAS, showed that there is a significant difference in their pocket properties (Table 5) The binding site in EGFR had a pocket score of 34.29, higher than the one found in KRAS, which was 20.07. This showed a better-defined and favorable ligand binding site in EGFR (Fig 5a). EGFR had a higher pocket volume (2662.9 $Å^3$) with 30 amino acid residues forming the

walls of the binding site. These results indicated a larger interaction surface where the ligand could interact in the active site. The large numbers and sizes of tunnels in EGFR could assist in the penetration of ligands into the active site. On the other hand, KRAS had a smaller pocket volume (980.3 Å³) with 18 residues contributing to its formation. Tunnel analysis showed that EGFR had three accessible tunnels measuring 12.48 to 28.35 Å in diameter, while KRAS had only one tunnel measuring 5.38 Å (Fig 5b).

γ-sitosterol showed affinity for binding to the target epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma viral oncogene homolog (KRAS). Binding energy calculated for the EGFR (Fig 6a) and KRAS (Fig 6b) suggested possibility of stable complex formation between the ligand and the receptor and hence possibility of inhibiting these oncogenes.The binding energies of -8.3 kcal/mol are believed to suggest a strong binding of the ligands with the proteins and are comparable to some of the phytochemical inhibitors that have been identified as effective against cancerous proteins. Phytosterols such as sitosterol are anti-cancer agents with regulatory effects on proliferation and apoptosis (Bao *et al*., 2022). In terms of the EGFR structure (PDB ID: 1M17), the γ-sitosterol was found to bind by hydrogen bonds with the residues Met and Gly and hydrophobic forces further stabilised the compound due to the residues Lys, Gln, Phe, Thr, Trp, and Pro. One of the receptor tyrosine kinases involved in the regulation of cellular proliferation and cell survival is the epidermal growth factor receptor (EGFR). The aberrations in EGFR pathway have been associated with the pathogenesis of colorectal cancer (Adhimoolam *et al*., 2024). Thus, it seems the possibility of γ-sitosterol binding in the kinase-binding pocket, and hence inhibiting EGFR pathwayThe binding energy for KRAS docking was -8.323 kcal/mol and the docking process was highly reliable with very low RMSD (0.549-1.440 Å) values. The hydrogen bond interactions with Pro and Ser amino acids, and the hydrophobic interactions between Lys, Leu, Pro and Phe were important contributors to the stabilisation of the complex. KRAS mutations are relatively common in the development of colorectal cancers, and are also known to cause failure of EGFR targeted drugs. Thus, any compound that targets KRAS will be of therapeutic importance (Arooj *et al*., 2025). In recent years, the computational use of phytochemicals has been demonstrated to be a good option to target KRAS.

Table 5. Protein structure evaluation for binding pockets

Target receptor	**score**	**No of residues**	**Volume**	**Tunnels**	**size Å**
EGFR(PDB 1M17)	34.29	30	2662.9 Å	3	12.48 to 28.35
KRAS (PDB 6OIM)	20.07	18	980.3 Å³	1	5.38

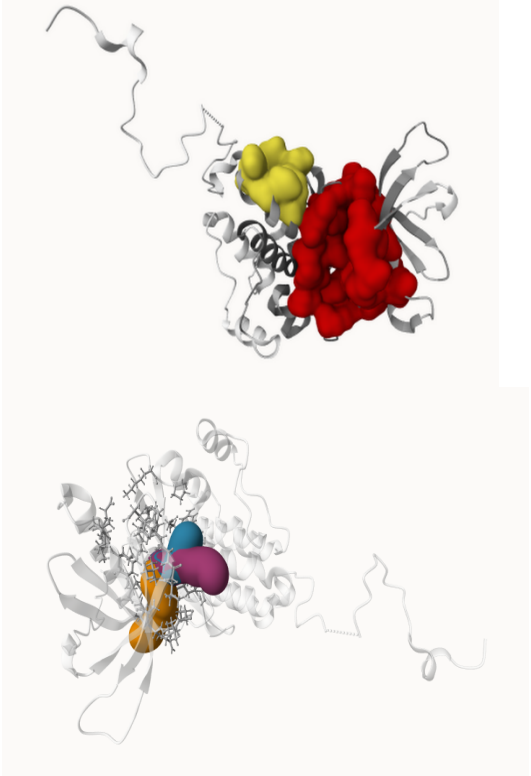
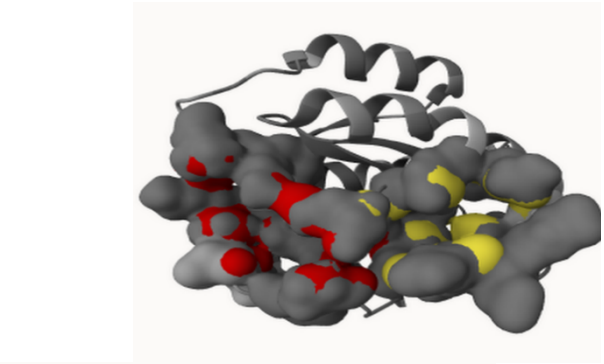


Figure 5 (a). Pocket and tunnel of EGFR



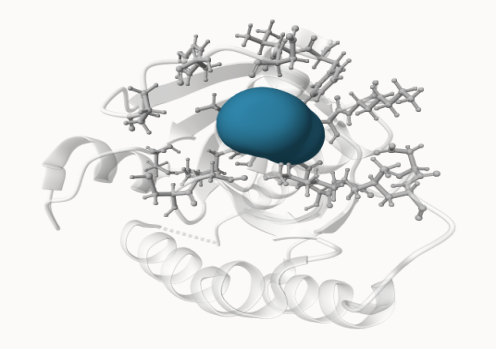


Figure 5 (b). Pocket and tunnel of KRAS

Table 6. Molecular interaction of gamma-Sitosterol

Receptor	Energy (kcal/mol)	RMSD/lb (Å)	RMSD/ub (Å)	Hydrogen bond	Hydrophobic
EGFR (PDB 1M17)	-8.325	3.435	7.136	Met, gly	Lys, gln, phe, thr, trp, pro
KRAS (PDB 6OIM)	-8.323	0.549	1.44	Pro, Ser	Pro, Lys (3), Leu, Phe

Figure 6 (a). Interaction of Ligand with EGFR

Figure 6 (b). Interaction of Ligand with KRAS

CONCLUSION

The findings indicate that γ-sitosterol could be involved in the biological activities of *T. cordifolia* through regulation of EGFR- and KRAS-linked signaling pathways in the development of colorectal cancer. The extract showed considerable free radical scavenging activity and was effective in inhibiting cyclooxygenase activity and protein denaturation which is a good indication of anti-inflammatory activity. The anti-migratory activities observed in the present study suggest *T. cordifolia* as a potential natural source of bioactive molecules for the development of multifunctional therapeutics. Further *in vivo* studies and mechanistic investigations are needed to validate its efficacy and explore its potential application in colorectal cancer management.

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