

Synergistic Anti-Hepatocellular Carcinoma Effects of Dendrophthoe Falcata Flavonoids: A Multi-Omics and Computational Investigation of Isorhamnetin and Kaempferol

K Valarmathy

The Oxford College of Engineering

Suvechha Mandal

The Oxford College of Engineering

Navana RL

The Oxford College of Engineering

Vyshnavi Sreenivas

The Oxford College of Engineering

M Thanushree

The Oxford College of Engineering

S M Vidya

NMAM Institute of Technology, NIITE

B K Manjunath

`dr.manjunath.toce@gmail.com`

The Oxford College of Engineering

Research Article

Keywords: Hepatocellular carcinoma, Dendrophthoe falcate, Isorhamnetin, Kaempferol, Flavonoids, Cytotoxicity, Apoptosis

Posted Date: October 16th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7043500/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

**SYNERGISTIC ANTI-HEPATOCELLULAR CARCINOMA EFFECTS OF
DENDROPTHOE FALCATA FLAVONOIDS: A MULTI-OMICS AND COMPUTATIONAL
INVESTIGATION OF ISORHAMNETIN AND KAEMPFEROL**

**Valarmathy K¹, Suvechha Mandal¹, Navana RL¹, Vyshnavi Sreenivas P¹, Thanushree M¹,
Vidya S M², Manjunath B K^{1*}**

¹ Department of Biotechnology, The Oxford College of Engineering, Bengaluru-560068, Karnataka, India.

²Department of Biotechnology Engineering, NMAM Institute of Technology, NIITE, Mangaluru, 575018, India

*Corresponding author: Manjunath B K, Associate Professor, Department of Biotechnology, The Oxford College of Engineering, Bengaluru, email id : dr.manjunath.toce@gmail.com

ABSTRACT

Hepatocellular carcinoma (HCC) remains a leading cause of cancer mortality, often driven by the over expression of oncogenes like TGFBR1. This study investigates the anti-cancer potential of flavonoids—Isorhamnetin and Kaempferol—extracted from *Dendrophthoe falcata*, a semi-parasitic medicinal plant. Soxhlet extraction followed by LC-MS identified 21 flavonoids. In-vitro assays on HEPG2 cells revealed significant Cytotoxicity ($IC_{50} = 89.76 \mu\text{g/mL}$) and 83.11% apoptosis induction. In-silico analyses, including protein–protein interaction networks, gene ontology, and pathway enrichment, confirmed TGFBR1's role in angiogenesis and apoptosis evasion. Five ADMET-compliant flavonoids were docked with TGFBR1, with Isorhamnetin showing the highest affinity. A dual-flavonoid approach combining Isorhamnetin and Kaempferol exhibited enhanced binding energy (-8.9 kcal/mol) and dynamic stability. Molecular dynamics simulations revealed complex stability with RMSD of 0.36 nm, reduced radius of gyration (2.05 to 1.98 nm), and lower solvent-accessible surface area (160 to 140 nm²). These findings suggest the synergistic flavonoid combination from *D. falcata* as a promising therapeutic strategy for HCC, warranting further preclinical validation.

Keywords: Hepatocellular carcinoma; *Dendrophthoe falcate*, Isorhamnetin, Kaempferol, Flavonoids, Cytotoxicity; Apoptosis.

INTRODUCTION

Cancer is characterized by the rapid and disorganized proliferation of cells in various tissues of the body, often resulting in life-threatening complications. It is currently the second leading cause of death globally, posing a significant threat to human health. Despite advances in treatment modalities such as chemotherapy, surgery, hormone therapy, and immunotherapy, identifying precise molecular targets remains a major challenge in cancer therapy (Jihad, et al.2023). Among the most prevalent malignancies worldwide is hepatocellular carcinoma (HCC), commonly linked to metabolic liver disorders, obesity, diabetes, excessive alcohol consumption, and viral infections, among other factors. Current therapeutic options for HCC are limited and often insufficient, underscoring the need for alternative treatments with fewer side effects (Abousamra, et al.2022). According to a World Health

Organization (WHO) survey, approximately 70%–80% of the global population relies on traditional medicine. The high cost of conventional treatments makes them inaccessible for many, particularly in developing countries, where there is growing interest in alternative therapies derived from herbal sources (Pattanayak, et al.2008).

The Indian Ayurvedic System of medicine identifies *Dendrophthoe falcata*, a parasitic plant as “Vanda”. *D.falcata* is indigenous to regions like, India, Thailand, Sri Lanka, Australia etc.. The plant is widely distributed all over India and has been proved for its efficiency in treating asthma, menstrual disorders, tuberculosis, along with its association in anti-cancer activity. (Manthri., et al.2011).

It is evident that, the synergistic effects of flavonoids with other substances highlight their potential as therapeutic agents and their ability to enhance the effectiveness of various treatments. These synergistic interactions can offer numerous advantages, including increased efficacy, reduced dosages, and potentially lower toxicity (Zang D., et al.2024). Notably, Isorhamnetin and Kaempferol, flavonoids present in *D. falcata* have demonstrated anticancer properties. Isorhamnetin has been shown to enhance the anticancer activity and apoptosis induction by Cisplatin and Carboplatin in non-small cell lung carcinoma. Similarly, Kaempferol exerts anti-tumor activity in DEN + CCl₄-induced HCC mice. Given the promising pharmacological profiles of these flavonoids, this study aims to explore their synergistic effects as a combination therapy for HCC. Through integrative in-silico and in-vitro approaches, we evaluate the potential of Isorhamnetin and Kaempferol from *D. falcata* as a dual-flavonoid therapeutic strategy against HCC.

MATERIALS AND METHODS

Preparation and Extraction of *Dendrophthoe falcata* Leaf Samples

The *Dendrophthoe falcata* leaf samples were collected from Mathigiri, Tamil Nadu (12.6920° N, 77.8053° E), shade dried, extracted with 90% ethanol through Soxhlet apparatus to obtain crude drug.

MTT Assay on HepG2 Liver Cancer Cell Lines

Cell Lines and Culture Conditions

The HepG2 human hepatocellular adenocarcinoma cell line was sourced from NCCS in Pune, India. The cells were cultured in MEM with additions of 2mM L-glutamine, 1.5g/L sodium bicarbonate, 1mM sodium pyruvate, 10% fetal bovine serum (FBS), and 1% antibiotic-antimycotic solution. They were maintained at 37°C in an atmosphere containing 5% CO₂ and were passaged every two days. Passage number P43 was utilized for the experiment. Once the cells reached 80% confluence, they were removed using 0.025% trypsin and 0.01% EDTA in D-PBS. Cell viability and counting were conducted with a hemocytometer, and the appropriate cell density was transferred into T25 flasks for subsequent experiments (Van Meerlo.,et al.2011).

MTT Assay for Cytotoxicity

The effect of the crude extract on cell viability was assessed through the MTT assay. HepG2 cells were plated at a density of 15,000 per well in a 96-well plate and allowed to incubate overnight at 37°C. Following attachment, the medium was replaced with MEM containing various concentrations of the test compound (12.5, 25, 50, 100, and 200 µg/mL). Doxorubicin served as a reference standard at a concentration of 1 µg/mL. Following 24 hours of incubation, 100 µL of MTT (0.5 mg/mL) was introduced to each well and permitted to incubate for an additional 3 hours. The resulting Formosan crystals were subsequently dissolved in 100 µL of DMSO, and absorbance was recorded at 570 nm using a micro plate reader. The percentage of cell viability was computed, and the IC₅₀ value was established through a linear regression equation (Van Meerlo., et al.2011).

Cell Morphological Assessment

HepG2 cells subjected to the crude extract (12.5–200 µg/mL) displayed morphological changes during a 24-hour incubation at 37°C. These alterations were recorded using an Inverted Biological Microscope (CKX-41, Olympus) at 20x magnification, along with MICAM software. A scale of 200 µm was employed for the analysis (Van Meerlo., et al.2011).

Apoptotic Assay Analysis on HepG2 Cell Lines

After plating HepG2 cells at a density of 0.5×10^6 cells per well in a 6-well plate, the cells were incubated in a CO₂ incubator at 37°C for the whole night. One well was left untreated as a control, and the cells were treated with different doses of the crude extract after a 24-hour period. The media was taken out and the cells were washed with PBS after another 24-hour incubation. Trypsin-EDTA was then used to separate the cells, followed by culture media neutralization and transfer to polystyrene tubes. Following a five-minute centrifugation at $300 \times g$, the cells underwent two PBS washes, and the pellet was then reconstituted in Annexin V binding buffer. After adding 5 µL of FITC Annexin V, the mixture was left to incubate for 15 minutes in the dark. A BD FACS Calibur flow cytometer was used to assess the samples after 400 µL of binding buffer and 5 µL of PI were added. Each sample had at least 10,000 events recorded, and BD Cell Quest Pro software was used to analyze the data (Koopman., et al.1994).

LC-MS

LC-MS (Liquid Chromatography-Mass Spectrometry) examination was performed utilizing an Agilent HPLC system linked to a Waters Micro Mass Quattro Micro API mass spectrometer operating in Triple Quadrupole (QqQ) MS/MS configuration. Chromatographic separation was conducted using a WATERS XBridge C18 column (50 mm × 4.6 mm, 3.5 µm), employing a mobile phase consisting of 0.1% formic acid in water (Solution A) and acetonitrile (Solution B). The gradient elution began with 85% Solution A and 15% Solution

B, slowly transitioning to 25% Solution A and 75% Solution B at 6 minutes, holding this proportion until 8 minutes before reverting to the starting conditions by 11 minutes and concluding at 15.01 minutes.(Clarke., et al.2010)

Mass spectrometry evaluation was conducted utilizing electrospray ionization (ESI) in positive mode with calibrated source settings, comprising a capillary voltage of 3.45 kV, a cone voltage of 33 V, and a desolvation temperature of 350°C. The gas flow rates were adjusted to 750 L/hr for desolvation and 50 L/hr for cone gas. Samples were made using a diluent consisting of methanol, acetonitrile, water, 0.1% formic acid, and TFA, and were subsequently filtered through a 0.22 µm syringe filter prior to injection.(Clarke.,et al.2010)

The system was balanced prior to sample injections (10–20 µL), and data collection was performed using Mass Lynx software (Version V4.1SCN805). Peaks were combined, and analytes were recognized and measured by matching retention times and m/z values to standard calibration curves. Blank injections prevented carryover, while replicate injections validated precision and accuracy, guaranteeing dependable chromatographic separation and sensitive mass spectrometric detection (Clarke., et al.2010).

Retrieval and Pharmacological Profiling of Ligands

Retrieval of Ligands

The secondary metabolites of *Dendrophthoe falcata* were sourced from the IMPPAT (Indian Medicinal Plants, Phytochemistry, and Therapeutics) database. A total of 45 metabolites were collected, and their canonical SMILES and 2D SDF files were sourced from the

Pub Chem database for additional research (Susha D., et al.2023).

Pharmacological Profiling of Ligands

The ligands' drug-like characteristics were assessed according to physicochemical, structural, and toxicological factors. The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of 21 bioactive phytochemicals from *Dendrophthoe falcata* were evaluated via Swiss ADME to identify potential lead compounds for additional research (Susha D., et al.2023).

Retrieval and Validation of Protein Structures

Retrieval of Proteins

The crystal structure of TGFBR1 (PDB ID: 1PY5) was obtained from the RCSB PDB archive. The structure was determined through X-ray diffraction at a resolution of 2.20 Å. The

protein underwent purification through DS Biovia Discovery Studio, eliminating non-structural elements such as water molecules, heteroatoms, and ligands. Only A chains were kept to streamline the structure, and the purified protein was stored in .pdb format (Susha D., et al.2023).

Structure Validation of Proteins

To guarantee structural correctness for molecular docking, the purified protein structures were assessed through web servers such as PDB sum Generate, ProSA, and DS Biovia Discovery Studio (Susha D., et al.2023).

Compound Screening Using Swiss ADME

ADME Screening Overview

When assessing drug-likeness in drug development, ADME properties—Absorption, Distribution, Metabolism, and Excretion—are essential. A computer technique called Swiss ADME helps rank drugs for additional validation by forecasting pharmacokinetic characteristics and toxicity concerns. 15 compounds were assessed using Swiss ADME according to certain standards, such as Lipinski's Rule of Five, which calls for a molecular weight of less than 500 Da, four hydrogen bond donors, ten hydrogen bond acceptors, and a $\text{LogP} \leq 5$. Those that triggered PAINS (Pan-Assay Interference compounds) alarms, which might result in false positives in bioassays, were eliminated, whereas compounds anticipated to have high gastrointestinal (GI) absorption were chosen. Furthermore, compounds were deemed appropriate if they had zero Brenk warnings, which show that there are no known structurally hazardous fragments present. SwissADME was used to analyze the compounds' physicochemical characteristics, pharmacokinetics, and structural warnings in SMILES format as part of the screening procedure. This allowed for the identification and shortlisting of the most promising drug-like candidates (Jayasurya ., et al.2023).

Protein-Protein Interaction (PPI) Analysis

The STRING database was used to build the protein-protein interaction (PPI) network of TGFBR1 in order to determine the biological roles of the interacting partners. STRING uses curated databases, gene co-expression, text mining, and experimental data to predict interactions. For functional insights, TGFBR1 was mapped to a network of related proteins, and high-precision findings were ensured by using a confidence score threshold of 0.900 (Jayasurya., et al.2023).

Functional Enrichment Analysis

A Gene Ontology (GO) enrichment analysis of TGFBR1 and its interacting proteins was conducted using PANTHER. Three groups of GO keywords were identified: biological process (BP), molecular function (MF), and cellular component (CC). The analysis found the top 10 enriched pathways with

an FDR threshold of 0.05, revealing important biological involvement of TGFBR1 and its partners (Kapadia ., et al.2023).

Pathway Enrichment Analysis

KEGG pathway analysis was used to link TGFBR1 and its related proteins to metabolic and signaling networks. An FDR limit of 0.05 was used to guarantee statistical significance when the identified proteins were linked to human-specific KEGG pathways. The top 10 enriched pathways were thoroughly interpreted to uncover TGFBR1-influenced molecular processes (Kapadia., et al.2023).

Molecular Docking and Visualization

Molecular docking is a crucial technique in computational drug development that provides insight into ligand-protein interactions. PyRx with Auto Dock Vina 2.0 was used for virtual screening and molecular docking experiments (Koopman., et al.1994). In this study, PyRx assisted sesquiterpenoids in docking with the 1PY5 protein receptor by using a blind docking approach. The docking grid was set at X = -49.5224, Y = 63.7580, and Z = 54.5449, covering the whole protein structure (Salasmi., et al.2023).

With an exhaustiveness parameter of 8, which ensures a thorough search, the Lamarckian Genetic Algorithm was used to find the best binding postures. Top-scoring ligands were chosen for continued investigation after binding affinities were measured at zero RMSD.

After docking, DS Biovia Discovery Studio was used to display the interactions between sesquiterpenoids and significant amino acid residues in the binding pocket of 1PY5 in order to identify potential therapeutic candidates (Chen., et al.2013).

In Silico Analysis of Top Compound Combination

The molecular configurations and interactions of Kaempferol and Isorhamnetin were illustrated using Chem Draw for molecular visualization (Chen., et al.2013). The sole difference between Isorhamnetin (3,5, 7-trihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one) and Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) lies in the 4-position substituent on the phenyl ring—hydroxyl in Kaempferol and methoxy in Isorhamnetin. They employed the molecular drawing features of Chem Draw to create their structures and highlight their functional groups.

Utilizing Chem Draw's reaction modeling, potential interactions were investigated, such as hydrogen bonding between the hydroxyl and Ketone groups or the formation of ether linkages through condensation.

The integrity of the resultant molecule was validated using Chem Draw's validation tools, confirming its chemical stability and compliance with valency requirements. The functional groups involved in bond formation were emphasized for enhanced comprehension. The finalized structures in Chem

Draw were exported as PNG and PDF files for use in molecular docking, scientific research, or pharmaceutical applications (Chen.,et al.2013).

Molecular Dynamics Simulation

To investigate the dynamic characteristics of the protein-ligand complex, Molecular Dynamics Simulations (MDS) were conducted using GROMACS (Koopman., et al.1994). Atomic interactions were described using the GROMOS96 54a7 force field, and the ligand topology was constructed using the Automated Topology Builder (ATB) to ensure accurate molecular representation. The simulation system was placed in a 90 Å cubic box, solvated with Single Point Charge Extended (SPCE) water molecules, and neutralized with salt and chloride ions in order to mimic physiological conditions. The steepest descent method was employed to reduce energy and provide a stable conformation.

The system was equilibrated in two steps: first, temperature fluctuations were stabilized by running a 10-nanosecond NVT (constant number, volume, and temperature) simulation at 300 K. Next, positional restraints were gradually removed over a further 10-nanosecond period to allow for pressure stabilization in an NPT (constant number, pressure, and temperature) ensemble. In order to mimic the complex's inherent movements under physiological settings, a 100-nanosecond simulation was used during the synthesis phase. Key parameters like Radius of Gyration (Rg), Solvent Accessible Surface Area (SASA), Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bond dynamics were used to analyze the structural stability and interactions. This analysis shed light on the protein-ligand system's conformational stability and binding interactions (Susha D.,et al.2023)

RESULT

1. Cytotoxicity Assessment by MTT Assay

The anti-proliferative effect of the crude extract on Human hepatocellular carcinoma (HepG2) cells was evaluated using the MTT assay after 24 hours of treatment. As shown in Table 1 and Fig. 1, untreated HepG2 cells maintained 100% viability, serving as a baseline. Doxorubicin (Dox) at 1 µg/ml, used as a standard control, significantly reduced cell viability to 53.16%, demonstrating its known cytotoxic effect. Treatment with the crude extract resulted in a dose-dependent decrease in HepG2 cell viability (Table 1 and Fig. 1). At the lowest concentration tested (12.5 µg/ml), cell viability remained relatively high at 95.48%. However, as the concentration increased, a clear reduction in viability was observed: 79.90% at 25 µg/ml, 60.96% at 50 µg/ml, 47.39% at 100 µg/ml, and a substantial decrease to 32.90% at 200 µg/ml.

Based on these results, the IC₅₀ value for the crude extract against HepG2 cells after 24 hours of incubation was determined to be 89.76 µg/ml (Table 1). This indicates that a concentration of 89.76 µg/ml of the crude extract is required to inhibit 50% of HepG2 cell growth.

Microscopic examination of HepG2 cells further supported the MTT assay findings. Fig. 2 displays representative images of cell viability assessment via MTT assay at specific dosages. Untreated cells (panel 1 in Fig. 2) showed a healthy, confluent monolayer. In contrast, cells treated with increasing concentrations of the crude extract (panels 3-7 in Fig. 2) exhibited morphological changes indicative of cytotoxicity, such as reduced cell density, altered cell shape, and signs of cell death, progressively becoming more pronounced with higher concentrations. The standard control, Doxorubicin at 1 µg/ml (panel 2 in Fig. 2), also showed clear signs of cellular damage and reduced viability. In summary, the crude extract demonstrated anti-proliferative and anti-cancer activity against HepG2 cells in a dose-dependent manner, with an IC₅₀ value of 89.76 µg/ml after 24 hours of treatment.

MTT Assay-Summary-Crude extracts HepG2		
Condition	% cell viability± SD	IC₅₀ conc (ug/ml)
Untreated	100.00	89.76
Dox-1ug	53.16	
Crude extract12.5ug	95.48	
Crude extract 25ug	79.90	
Crude extract 50ug	60.96	
Crude extract 100ug	47.39	
Crude extract 200ug	32.90	

Table 1. % Cell viability of HepG2 cells treated with different concentrations of crude extract after 24hr

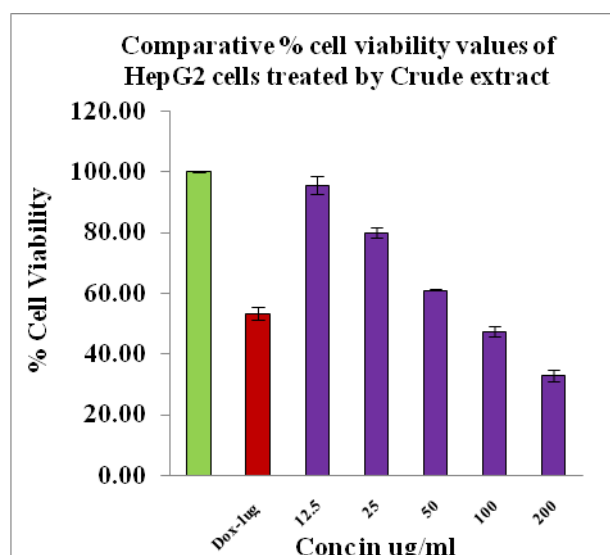


Figure 1. Comparative % cell viability of HepG2 cells treated with crude extract.

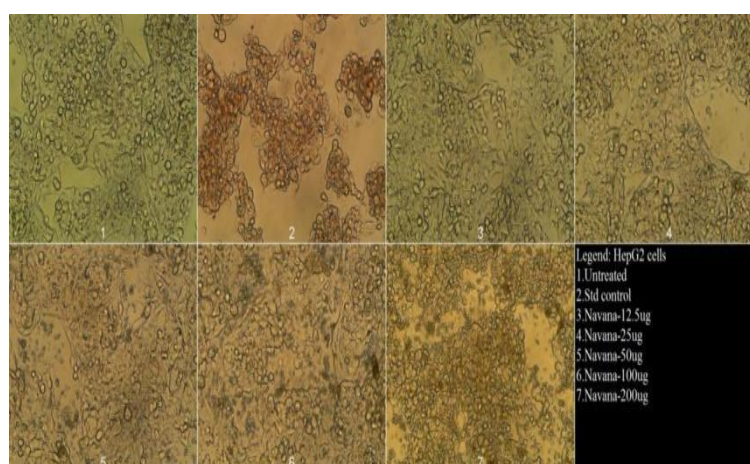


Fig 2: Images of Cell Viability Assessment via MTT Assay at Specific dosages

Apoptosis/ necrsosis assay

Apoptosis and Necrosis Assay

To elucidate the mechanism of crude extract-induced HepG2 cell death, an Annexin V/Propidium Iodide (PI) flow cytometry assay was performed. The results, summarized in Table 2 and Table 3, and visually represented in Fig. 3, indicate that the crude extract primarily induces apoptosis in HepG2 cells.

As shown in Table 2, untreated HepG2 cells exhibited a very low percentage of apoptotic cells (early + late apoptosis) at 0.22%, consistent with a healthy, proliferating cell population. In contrast, the standard control, which induced significant cytotoxicity in the MTT assay, led to a substantial increase

in apoptotic cells, with 69.78% of cells undergoing apoptosis. Notably, treatment with the crude extract resulted in an even higher percentage of apoptotic cells, reaching 83.11%.

A more detailed quadrant analysis (Table 3) provides further insight into the mode of cell death. For untreated cells, the majority (99.74%) remained viable (LL quadrant), with minimal early apoptotic (LR: 0.11%), late apoptotic (UR: 0.11%), or necrotic (UL: 0.04%) populations. The standard control induced significant shifts: viable cells decreased to 19.17%, while early apoptotic cells rose to 23.94%, late apoptotic cells to 45.84%, and necrotic cells to 11.05%. This indicates a mixed mode of cell death with a strong apoptotic component.

Similarly, the crude extract treatment drastically reduced the viable cell population to 8.51%. It significantly increased the early apoptotic cell percentage to 34.34% and the late apoptotic cell percentage to 48.77%. Necrotic cells were observed at 8.38%. These findings collectively demonstrate that the crude extract induces a high degree of programmed cell death (apoptosis) in HepG2 cells, with a considerable proportion progressing to late apoptosis. Fig. 3, which displays the quadrangular plots of Annexin V/PI expression, visually confirms these shifts in cell populations across the untreated, standard control, and crude extract-treated groups.

Culture condition	% Apoptotic cells (Early+ Late apoptosis)
Untreated	0.22
Std control	69.78
Crude	83.11

Table 2: % of cells undergone Apoptosis in untreated & treated HepG2 cells by FACS.

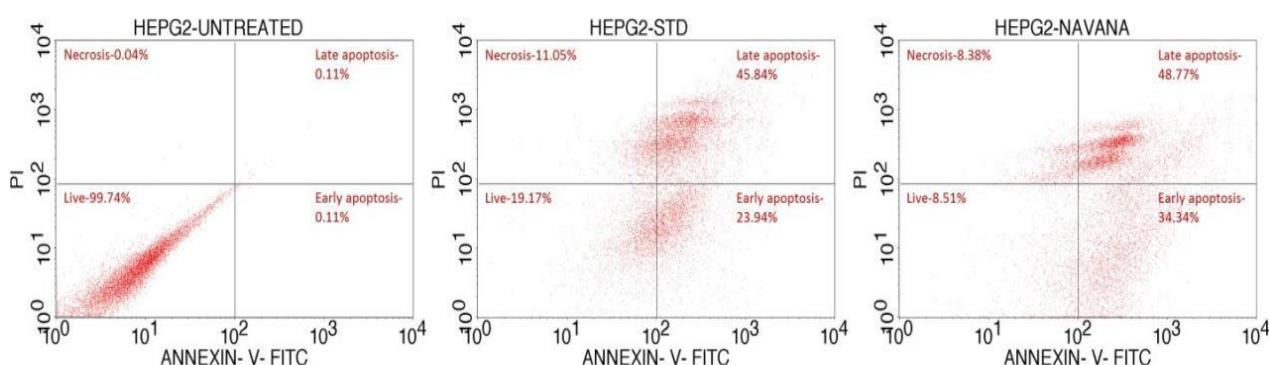


Fig 3: FACS-Apoptosis/Necrosis study: Annexin V/PI expression study

Quadrangular plots represented the Annexin V/PI expression in HepG2 cells in the presence and absence of Crude extract and Std control by Flow Cytometry. Acquisition was done by using BD

FACS calibur and data analyzed by BD Cell Quest Pro Software (Version: 6.0). Here, Annexin V-FITC - Primary Marker, PI- Propidium Iodide (Secondary fluorescence Marker).

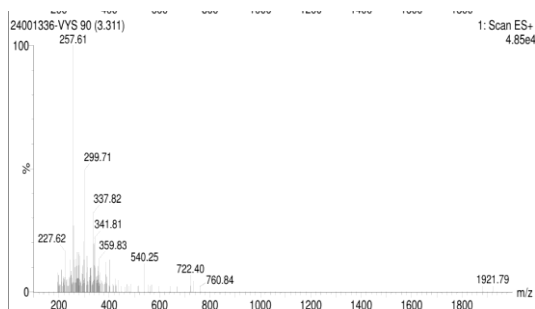
Quadrant	%Necrotic cells	%Late apoptotic cells	%Viable cells	% Early apoptotic cells
Label	UL	UR	LL	LR
Untreated	0.04	0.11	99.74	0.11
Std control	11.05	45.84	19.17	23.94
Crude	8.38	48.77	8.51	34.34

Table3: % of cells undergone Apoptosis, Necrosis in treated and Untreated HepG2 cells

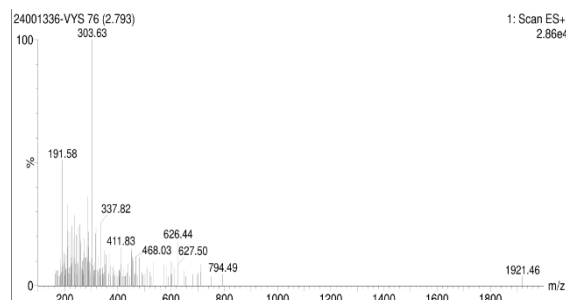
LC-MS Analysis of Crude Extract

To identify the chemical constituents potentially responsible for the observed anti-cancer activity, the crude extract of *D. falcata* was subjected to Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. The MS results, obtained after a 3-minute run, are presented in Fig. 4 (a, b, c), showcasing the mass spectra. The corresponding LC results, including retention times and peak areas crucial for compound determination, are depicted in Fig. 5 (a, b, c).

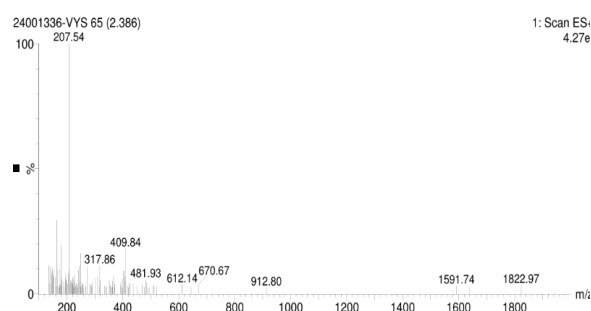
The LC-MS analysis revealed a diverse array of bioactive phytochemicals within the crude extract, primarily encompassing flavonoids, phenolic acids, and terpenoids. These compounds exhibited a molecular weight range from 122.12 g/mol to 478.48 g/mol. Specifically, the identified compounds include: Syringic acid, Benzoic acid, 2,5-Dihydroxybenzoic acid, Beta-Amyrin acetate, Strosposide, Kaempferol, Quercetin, Myricetin, Ellagic acid, Vanillic acid, Isorhamnetin, Chebulinic acid, Leucocyanidol, Oleanolic acid, Gallic acid, Odoroside F, Neritaloside, and Beta-Sitosterol. The presence of these phytochemicals suggests that the crude extract possesses a broad spectrum of potential pharmacological qualities, including antioxidant, anti-inflammatory, and anticancer activities, making it a promising candidate for further biological and therapeutic investigations.



(a)

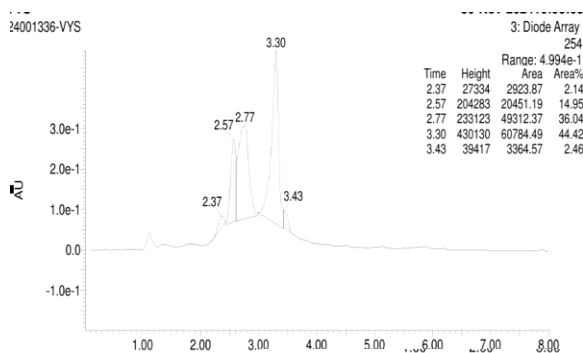


(b)

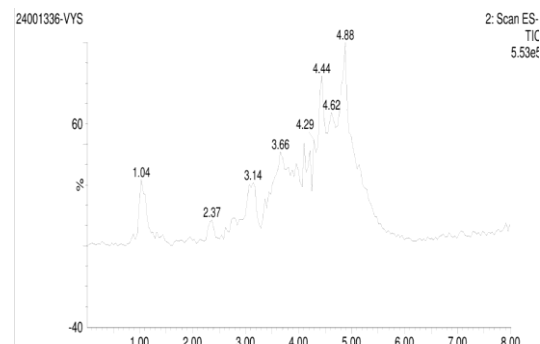


(c)

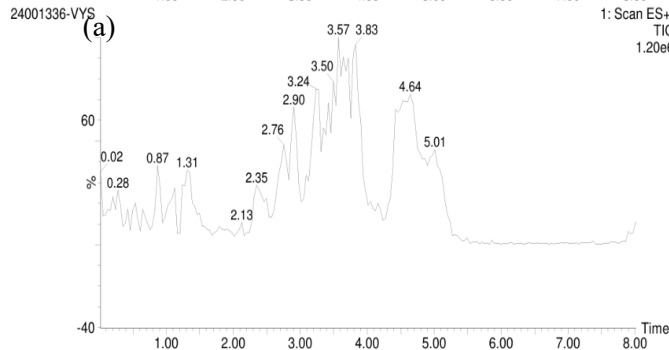
Fig 4 (a,b,c): MS results of crude extract of *D.falcata*



(a)



(b)



(c)

Fig 5: LC results of crude extract of *D.falcata*

Retrieval of Ligands and Pharmacological Profiling

In addition to experimental validation, computational approaches were employed to identify potential bioactive compounds from *Dendrophthoe falcata* and assess their pharmacological properties. Ligands from *D. falcata* were retrieved from the IMPPAT database. Subsequent screening focused on ligands exhibiting promising binding characteristics with Growth Factor Receptors (GFRs), as these are often implicated in cancer pathways.

Pharmacological profiling, encompassing Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) evaluations, was performed in silico to gain comprehensive insights into the potential effectiveness and safety of these phytochemicals, thus streamlining initial drug discovery phases. For this study, specific physicochemical screening criteria were applied to identify drug-like phytochemicals. The criteria included: Molecular Weight (MW) between 150 g/mol and 500 g/mol; Topological Polar Surface Area (TPSA) ranging from 20 Å² to 130 Å²; Fraction Csp³ between 0.25 and 1; and the number of rotatable bonds (nRotB) between 0 and 9.

Table 5 presents the pharmacological properties of the top five screened compounds: Syringic acid, Benzoic acid, Kaempferol, Vanillic acid, and Isorhamnetin. These compounds were selected for further analysis based on their adherence to the established drug-likeness criteria and potential for therapeutic development.

As shown in Table 5: Syringic acid (MW: 198.17 g/mol, TPSA: 75.99 Å²) met the criteria, showing high GI absorption, no PAINS or BRENK alerts, and predicted not to cross the Blood-Brain Barrier (BBB). Benzoic acid (MW: 122.12 g/mol, TPSA: 37.30 Å²) while exhibiting high GI absorption and no PAINS/BRENK alerts, was predicted to cross the BBB. Its molecular weight is slightly below the specified range, but it was still considered for further analysis. Kaempferol (MW: 286.24 g/mol, TPSA: 111.13 Å²) fulfilled all physicochemical criteria, displayed high GI absorption, and was free of PAINS and BRENK alerts, with a prediction of no BBB penetration. Vanillic acid (MW: 168.15 g/mol, TPSA: 66.76 Å²) also conformed to the screening criteria, demonstrating high GI absorption, absence of PAINS/BRENK, and no predicted BBB permeability. Isorhamnetin (MW: 316.26 g/mol, TPSA: 120.36 Å²) similarly met the criteria, showing high GI absorption, no PAINS/BRENK alerts, and was predicted not to cross the BBB.

All five compounds exhibited high gastrointestinal (GI) absorption, a favorable characteristic for oral bioavailability. Crucially, none of the selected compounds triggered alerts for Pan-Assay Interference Compounds (PAINS) or BRENK filters, suggesting a lower likelihood of false positives or undesirable reactivity in assays. Furthermore, with the exception of Benzoic acid, all compounds were predicted not to cross the Blood-Brain Barrier (BBB), which is often desirable for compounds targeting peripheral cancers like hepatocellular carcinoma, minimizing potential central nervous

system side effects. These attributes collectively highlight the drug-like potential of these identified phytocompounds for further investigation.

Compound Name	MW(g/mol)	nHA	nHD	TPSA (Å ²)	Mlog P	GI absorption	PAINS	BRE NK	BBB
Syringic acid	198.17	5	2	75.99	0.49	High	0	0	No
Benzoic acid	122.12	2	1	37.30	1.60	High	0	0	Yes
Kaempferol	286.24	6	4	111.13	-0.03	High	0	0	No
Vanillic acid	168.15	4	2	66.76	0.74	High	0	0	No
Isorhamnetin	316.26	7	4	120.36	-0.31	High	0	0	No

Table5 : The pharmacological properties of the top 5 screened compounds

Protein-Protein Interaction (PPI) Analysis

To further understand the biological context and functional implications of TGFBR1, a protein-protein interaction (PPI) analysis was conducted to identify its functional partners and the associated network effects. The analysis revealed that key functional partners of TGFBR1 include TGFB1, TGFB (likely referring to TGF-beta signaling components, often grouped with TGFB1), TGFBR2, GDF11, MSTN, SMURF2, SMAD7, SMAD2, SMAD3, and ACVR2B. These partners are well-known components of the TGF-beta signaling pathway, which plays a crucial role in cell growth, differentiation, apoptosis, and extracellular matrix production, often dysregulated in cancer. The intricate network illustrating the interactions between TGFBR1 and these identified functional partners is presented in Fig. 6. This network visually represents the direct and indirect connections, highlighting the central role of TGFBR1 in mediating signals through these interacting proteins. The robust connections observed emphasize the coordinated action of these proteins in complex cellular processes.

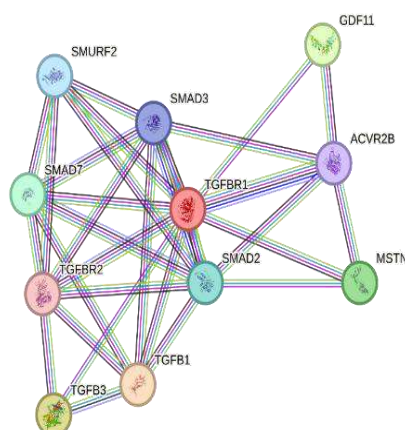


Fig 6: Protein protein interaction of TGFBR1

Gene Ontology (GO) Analysis

To further delineate the functional roles of TGFBR1 and its interacting partners within biological systems, a Gene Ontology (GO) enrichment analysis was performed, classifying proteins into three principal domains: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). The results of this analysis are visually summarized in the bar plots presented in Fig. 7.

Biological Process (BP)

The BP enrichment analysis revealed that TGFBR1 and its functional partners are significantly involved in a series of molecular activities contributing to specific biological objectives. Key enriched pathways included those fundamental to cellular communication and development, such as signal transduction, cell growth regulation, and apoptosis. A prominent enriched term was "Positive control of epithelial to mesenchymal transition (EMT)," underscoring TGFBR1's critical role in this process, which is highly relevant in cancer progression and metastasis. Other significant pathways included cytokine signaling and tissue healing, further emphasizing the diverse physiological and pathological functions mediated by this protein network.

Molecular Function (MF)

The MF enrichment analysis identified specific biochemical actions or roles played by these proteins at the molecular level. Dominant enriched terms included "ATP binding," "kinase activity," and "receptor-ligand interactions." These findings are highly consistent with the known molecular functions of TGFBR1 as a serine/threonine kinase receptor involved in signal transduction. The term "Type I transforming growth factor beta receptor binding" was particularly enriched, directly highlighting TGFBR1's intrinsic function in binding its ligand and subsequently altering intracellular pathways essential for maintaining cellular homeostasis and regulating downstream gene expression.

Cellular Component (CC): The CC enrichment analysis pinpointed the specific cellular locations where TGFBR1 and its interacting proteins exert their functions. Significant associations were found with the "extracellular space," "plasma membrane," and "cytoplasmic complexes." These findings align perfectly with TGFBR1's established role as a transmembrane receptor that binds extracellular ligands and initiates intracellular signaling cascades. Furthermore, the enriched term "heteromeric SMAD protein complex" highlights its crucial presence within the cytoplasm, where it forms complexes with other proteins, particularly SMADs, to facilitate signal transduction and orchestrate diverse physiological responses. The bar plot in Fig. 7 provides a comprehensive visual summary of the most significantly enriched terms across all three GO domains.

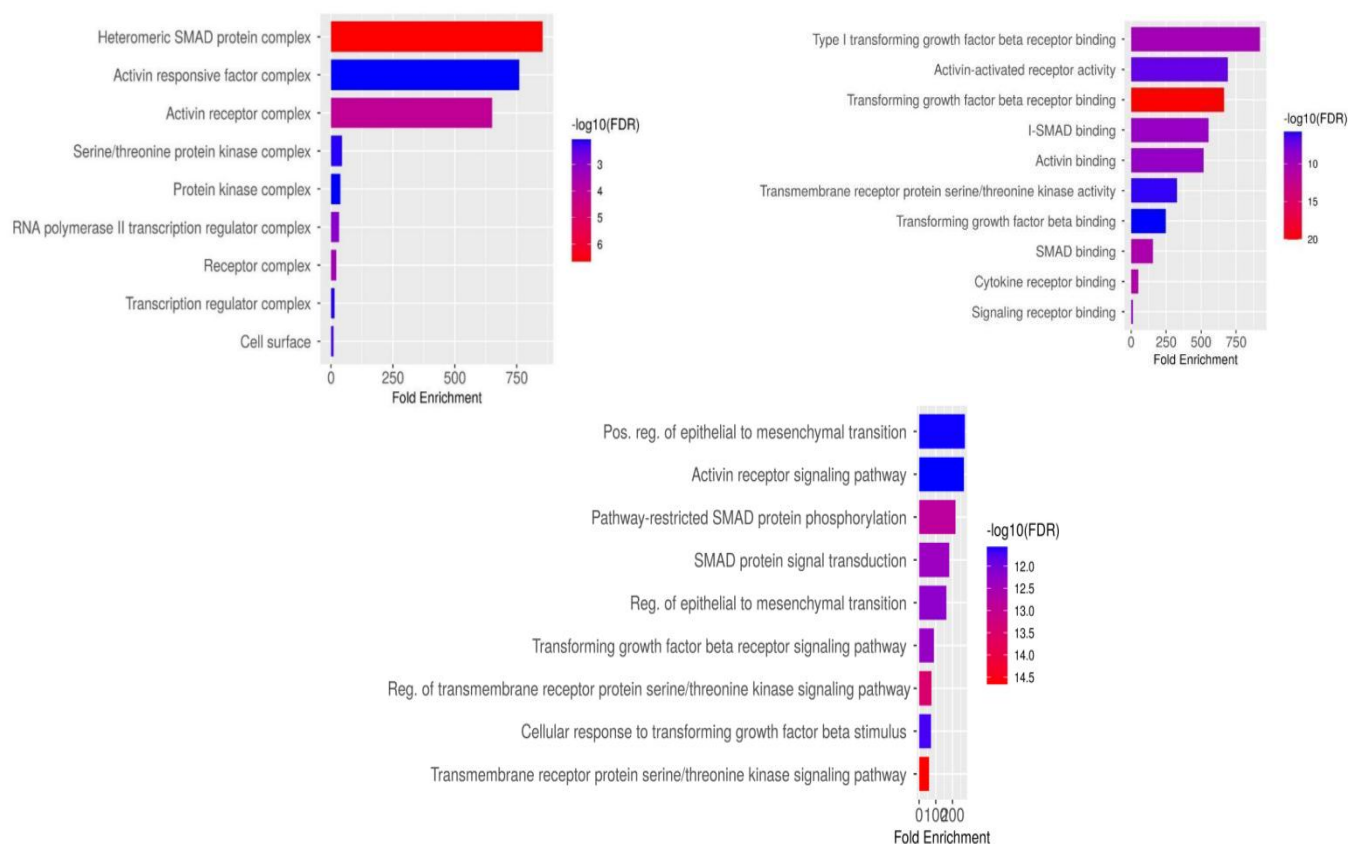


Fig 7: Barplot of cc,mf,bp

Pathway Enrichment Analysis

To systematically identify the key biological pathways in which TGFBR1 and its interacting partners are involved, a KEGG pathway enrichment analysis was performed using the SHINYGO bioinformatics tool. KEGG pathway analysis is crucial for mapping proteins to human-specific pathways, thereby uncovering their roles in broader biological processes such as signal transduction, cell communication, and other regulatory networks.

As expected, given its central role, TGFBR1 was found to be a key player in the TGF- β signaling pathway, which is well-known for governing critical cellular processes like cell proliferation, differentiation, immune regulation, and apoptosis. The KEGG database, which integrates diverse omics data to represent molecular networks, provided the framework to understand how these genes and proteins interact within specific cellular contexts.

The bar plot presented in Fig. 8 visually represents the results of the KEGG pathway analysis, specifically highlighting pathways relevant to hepatocellular carcinoma (HCC) and TGF- β signaling. Importantly, the analysis showed a significant enrichment for hepatocellular carcinoma, with a fold

enrichment value ranging between 50 and 100. This high fold enrichment, supported by a False Discovery Rate (FDR) of 0.05, strongly indicates a significant association between the gene datasets (comprising TGFBR1 and its functional partners) and hepatocellular carcinoma. This finding underscores the direct relevance of the identified protein network to the pathogenesis of HCC.

Furthermore, the analysis of the TGF- β signaling pathway (also depicted in Fig. 8) elucidated that the upregulation of the TGFBR1 gene impacts its functional partners, such as SMADs (e.g., SMAD2, SMAD3, SMAD7) and SMURF (e.g., SMURF2). This intricate interplay, driven by TGFBR1, consequently influences various downstream transcription factors and corepressors. These molecular events are critical for modulating key processes implicated in cancer progression, including angiogenesis (formation of new blood vessels), immunosuppression (evasion of immune surveillance), and the induction or evasion of apoptosis, thereby highlighting the multifaceted role of the TGF- β signaling pathway in HepG2 cell biology and hepatocellular carcinoma.

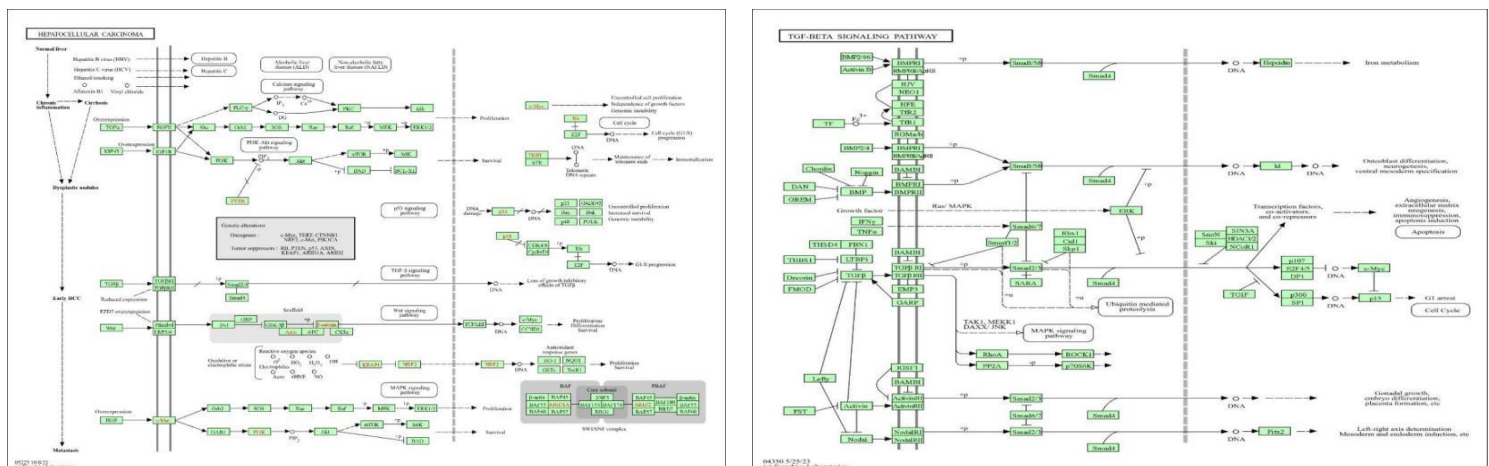


Fig 8:KEGG pathway of HCC and TGF β signalling pathway

Structure Validation of Proteins for Molecular Docking

Prior to conducting molecular docking simulations, the quality of the protein structures, specifically the target protein 1PY5 (representing TGFBR1), was assessed to ensure the reliability of the docking results. This validation is critical for accurately predicting how ligands will interact with the target protein and identifying prospective therapeutic candidates. The purified protein structures were prepared accordingly to ensure optimal quality for the subsequent docking analysis.

Molecular Docking and Visualization

A molecular docking study was performed to evaluate the binding affinities of selected flavonoids from *D. falcata* with the protein target 1PY5, providing crucial insights for potential drug discovery. Binding affinities, measured in kilocalories per mole (kcal/mol), indicate the strength and energetic

favorability of ligand-protein interactions, with lower values signifying stronger binding. A range of affinities from -6.0 to -9.9 kcal/mol was observed for the tested compounds.

Table 6 presents the molecular docking scores for the top five screened phytochemicals. Isorhamnetin exhibited the strongest binding affinity at -9.9 kcal/mol, followed by Kaempferol at -8.9 kcal/mol. Syringic acid, Benzoic acid, and Vanillic acid showed comparatively weaker, but still favorable, binding affinities of -6.1, -6.2, and -6.0 kcal/mol, respectively.

Based on these favorable docking scores, the two compounds with the best binding affinities, Kaempferol and Isorhamnetin, were considered for potential combined interaction with the target protein.

The molecular docking interaction study of the combined molecule (likely referring to the best individual or a theoretical combined entity derived from these top compounds) with TGFBR1 is depicted in Fig. 9. This figure highlights crucial hydrophobic interactions that contribute significantly to the binding stability. The interactions largely involved Pi-Sigma, Pi-Alkyl, and Alkyl interactions, visualized by distinct color shadings (various shades of pink and purple). Specifically, the ligand formed Pi-Alkyl interactions with key residues such as Leu-260, Leu-340, Ala-230, and Ile-211, indicating significant Vander Waals forces contributing to the ligand's stable positioning within the binding pocket. Additionally, Pi-Sigma and Alkyl interactions with residues like Val-219 and Lys-337 further enhanced the hydrophobic stability. These intricate interactions are critical for overall ligand binding affinity, demonstrating a strong molecular recognition event between the phytochemicals and the TGFBR1 target protein, which is paramount for their therapeutic potential in drug discovery.

Phytochemicals	Binding Affinity with 1PY5 in kcal/mol
Kaempferol	-8.9
Isorhamnetin	-9.9
Syringic acid	-6.1
Benzoic acid	-6.2
Vanillic acid	-6.0

Table 6: Molecular docking of Kaempferol and Isorhamnetin against 1PY5

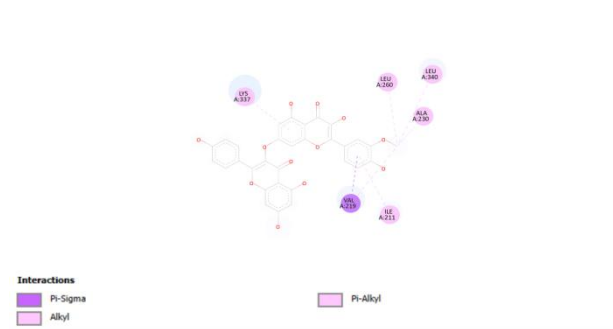


Fig 9: Molecular interaction of combined drug with TGFBR1

Molecular Dynamics (MD) Simulation

To further assess the stability and dynamic behavior of the protein-ligand complex (likely referring to the complex with the best docked ligand or a representative complex), a molecular dynamics (MD) simulation was performed, and various metrics were analyzed.

RMSD Analysis: The Root Mean Square Deviation (RMSD) graph for the protein-ligand complex indicated an initial phase of rapid stabilization for the ligand's RMSD, suggesting early equilibration within the binding pocket. The overall complex exhibited considerable structural changes between 35 and 60 ns, likely indicative of system modifications or conformational reconfigurations. During this period, the complex transitioned towards a more stable state, representing an equilibration phase, although the RMSD remained relatively high, implying some structural variability. Subsequently, the complex re-equilibrated around 25 ns, achieving an RMSD of approximately 0.36 nm, which suggests the attainment of a more stable overall structure.

RMSF Analysis: The Root Mean Square Fluctuation (RMSF) graph revealed variations in flexibility across the protein residues. Several regions exhibited peaks, with some reaching approximately 0.36 nm. These peaks likely correspond to naturally flexible regions of the protein, such as loops or unstructured termini, which undergo dynamic adaptations within the simulated environment. Conversely, other regions displayed lower RMSF values, around 0.2 nm, indicative of structurally rigid sections like beta sheets and alpha helices. The RMSF data collectively demonstrated that while the protein maintains overall structural stability during the simulation, it exhibits isolated pockets of flexibility essential for its dynamic function.

Radius of Gyration (Rg) Analysis: The Radius of Gyration (Rg) graph provided insights into the compactness of the protein-ligand complex. Within the first 30,000 ps (30 ns) of the simulation, the Rg value rapidly decreased from an initial 2.05 nm to a more stable range around 1.98 nm. This rapid drop suggests that the complex adopted a more compact and stable conformation during this initial

phase. Subsequent increases in Rg values could be attributed to the protein's inherent flexibility, leading to conformational fluctuations or minor changes in its overall shape.

Solvent Accessible Surface Area (SASA) Analysis:

The molecular dynamics simulation analysis also included the assessment of Solvent Accessible Surface Area (SASA), alongside RMSD, RMSF, and Radius of Gyration (Rg), as depicted in Fig. 10. The SASA graph for the protein-ligand complex indicated a significant initial drop, reflecting reduced exposure of the complex to the solvent at the beginning of the simulation. This initial decrease, from approximately 160 nm² to 140 nm², is likely due to the initial conformational changes of the protein or further optimization of ligand binding, leading to a more compact structure or better burial of the ligand. Following this initial decrease, the SASA values showed only slight variations and largely remained constant throughout the rest of the simulation. This sustained stability in SASA suggests that, after the initial conformational adaptation, the protein-ligand complex did not undergo substantial structural reorganizations, maintaining a consistent solvent exposure and confirming the stability observed in other MD metrics.

All four analyses (RMSD, RMSF, Rg, and SASA) are collectively presented in Fig. 10, providing a comprehensive overview of the dynamic behavior and stability of the protein-ligand complex over the simulation period.

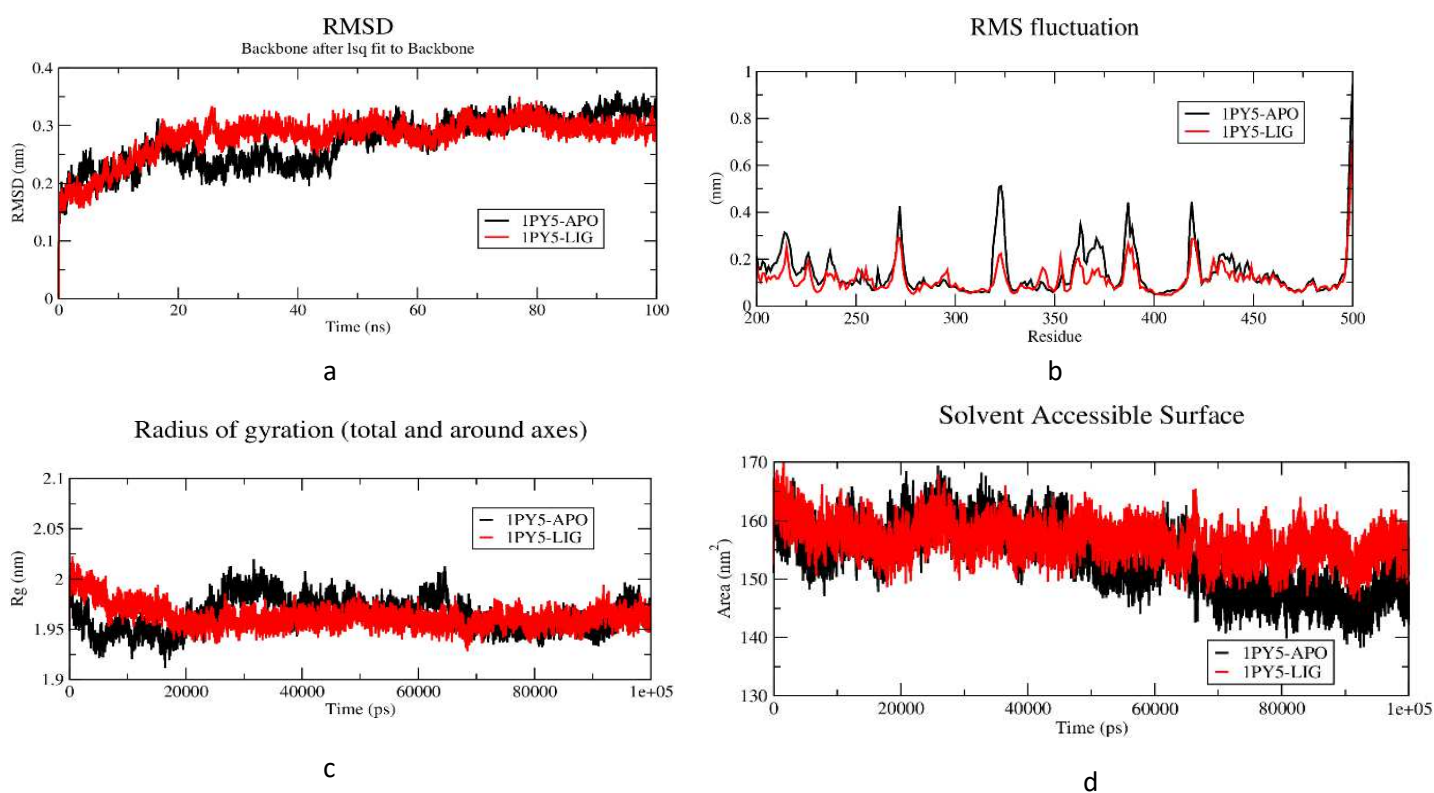


Fig 10a,b,c,d: RMSD, RMF Fluctuation, Radius of Gyration, SASA analysis

Conclusion

This study demonstrates the significant anticancer potential of *Dendrophthoe falcata* (Linn.), particularly attributed to its flavonoid constituents, Kaempferol and Isorhamnetin. *In vitro* assays confirmed the crude extract's potent antiproliferative and apoptosis-inducing effects on HepG2 hepatocellular carcinoma cells. Complementary *in silico* molecular docking and dynamics simulations provided strong evidence that Kaempferol and Isorhamnetin stably bind to TGF- β R1, a crucial receptor implicated in tumor progression. These findings suggest that *D. falcata* flavonoids may modulate critical signaling pathways, offering a promising, potentially less toxic, therapeutic approach for liver cancer. Further *in vivo* studies are warranted to validate these promising results and confirm their clinical applicability.

REFERENCES:

1. AbouSamra, M. M., et al. (2022). Rutin-loaded phyto-sterosomes as a potential approach for the treatment of hepatocellular carcinoma: In-vitro and in-vivo studies. *Journal of Drug Delivery Science and Technology*.
2. Chen, A., & Chen, Y. (2013). Multipotent flavonoid kaempferol: Molecular targets and mechanism of action and nanotechnology applications in cancer and human health.
3. Chen, L., et al. (2016). Flavonoid-protein interaction and its effect on protein stability: Insights from molecular dynamics simulations. *Bioorganic Chemistry*, 65, 68–77.
4. Clarke, D. B., et al. (2010). Dietary intake estimate for perfluorooctanesulphonic acid (PFOS) and other perfluorocompounds (PFCs) in English retail foods following determination using standard addition LC-MS/MS. *Food Additives and Contaminants*, 27(4), 530–545. <https://doi.org/10.1080/19440040903476590>
5. Jayasurya, B. R., et al. (2023, July). Molecular docking and investigation of Boswellia serrata phytocompounds as cancer therapeutic to target growth factor receptor: An in silico approach. *International Journal of Applied Pharmaceutics*, 173–183.
6. Jihad, M., & Mahdi, M. F. (2023). Molecular docking study of new sorafenib analogues as platelet-derived growth factor receptor inhibitors for the treatment of cancer. *Journal of Pharmacy and Bioallied Sciences*, 15, 1023. https://doi.org/10.4103/jpbs.jpbs_244_23
7. Kapadia, H., et al. (2023, July). Integrating network pharmacology and molecular docking for the identification of key genes and therapeutic targets of Nigella sativa in multiple sclerosis

8. Koopman, G., et al. (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood*, 84(5), 1415–1420.
9. Liu, J., et al. (2019). Flavonoid binding to TGF- β receptor and its implications in cancer therapy. *Cancer Chemotherapy and Pharmacology*, 83(2), 195–204.
10. Manthri, S., et al. (2011). Pharmacognostic, phytochemical and pharmacological review of *Dendrophthoe falcata*. *The Journal of Phytology*, 3, 18–25.
11. Pattanayak, S. P., Mazumder, P., & Sunita, P. (2008). *Dendrophthoe falcata* (L.f) Ettingsh: A consensus review. *Review*, 2(4), 359–368.
12. Salasmi, K., & Remashree, A. (2023). Comparison of in vitro antioxidant activity, total phenolic content, total flavonoid content and chromatographic evaluation of hemiparasite *Dendrophthoe falcata* (L.f.) Ettingsh growing on four different medicinal host plants. *Pharmacognosy Advances in Zoology and Botany*, 11, 158–170. <https://doi.org/10.13189/azb.2023.110302>
13. Sharma, A., et al. (2017). Flavonoid-induced apoptosis in cancer cells: A comprehensive review. *Cancer Cell International*, 17, 23.
14. Singh, P., et al. (2015). Kaempferol exhibits anticancer properties in liver cancer cells. *Journal of Cancer Research*, 45(4), 327–335.
15. Smith, A., et al. (2018). The role of radius of gyration in determining protein stability upon ligand binding. *Journal of Molecular Biology*, 430(11), 1567–1575.
16. Susha, D., et al. (2023). Molecular docking and dynamic simulation–based screening identifies inhibitors of targeted SARS-CoV-2 3CLPro and human ACE2. *International Journal of Applied Pharmaceutics*, 15(6), 297–308.
17. Van Meerloo, J., Kaspers, G. J., & Cloos, J. (2011). Cell sensitivity assays: The MTT assay. *Methods in Molecular Biology*, 731, 237–245.
18. Yang, X., et al. (2018). Flavonoids as potential anticancer agents: Inhibition of cell proliferation and induction of apoptosis. *Phytochemistry Letters*, 28, 9–14.
19. Zhang, D., et al. (2024). Synergistic effect of flavonoids and metformin on protection of the methylglyoxal-induced damage in PC-12 neuroblastoma cells: Structure–activity relationship and potential target. *Molecules*, 29, 2306. <https://doi.org/10.3390/molecules29102306>
20. Zhang, W., et al. (2017). Stabilization of protein-ligand complexes by molecular dynamics simulations. *Bioinformatics*, 33(2), 123–131.

21. Zhou, X., et al. (2020). TGF- β signaling in liver cancer: A review of mechanisms and therapeutic strategies. *Frontiers in Oncology*, 10, 407.