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Formation of Inclusion Complex of Curcumin and Tetrahydrocurcumin Prevents Angiogenesis by Inhibiting VEGF Activity: An *in-silico* Study

(Pembentukan Kompleks Rangkuman Kurkumin dan Tetrahidrokurkumin Menghalang Angiogenesis dengan Merencat Aktiviti VEGF: Suatu Kajian *in silico*)

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ABSTRACT

Curcumin and tetrahydrocurcumin (THC) are known for their anticancer properties, but limited solubility in water hinders their effectiveness against cancer. In this study, we conducted an *in silico* exploration of β-cyclodextrin's potential to form inclusion complexes with curcumin or THC. The aim of this study was to assess the potential of curcumin and THC inclusion complexes to inhibit vascular endothelial growth factor (VEGF) signaling pathway, a key element in carcinogenesis. The *in silico* analysis involved multiple stages, such as bioactive compound preparation, biological activity prediction, 3D structure retrieval of VEGF and VEGFR, protein-ligand docking, and visualization. The results of the study demonstrated that both the curcumin- and THC-inclusion complexes exhibit a lower requirement for binding free energy to interact with VEGFR compared to curcumin or THC molecules alone. When VEGFR binds with curcumin, the curcumin-inclusion complex, or the THC-inclusion complex before interacting with VEGF, there is a notable increase in the binding free energy for the VEGF-VEGFR interaction. Specifically, the presence of THC-inclusion complex demonstrates the highest binding free energy for the VEGF-VEGFR interaction. The molecular dynamic simulation study shows that when VEGFR binds with curcumin, curcumin-inclusion complex, or THC-inclusion complex, the fluctuation of amino acid residues in VEGFR decreases compared to the VEGFR protein structure before binding with these molecules. In conclusion, this study suggests that the formation of inclusion complexes holds considerable promise for enhancing the anticancer potential of curcumin and THC by augmenting their anti-angiogenic activity.

Keywords: Angiogenesis; cancer; curcumin; inclusion complex; tetrahydrocurcumin

ABSTRAK

Kurkumin dan tetrahidrokurkumin (THC) terkenal dengan sifat antikansernya, tetapi keterlarutan terhad dalam air menghalang keberkesanannya terhadap kanser. Dalam kajian ini, kami menjalankan penyelidikan *in silico* terhadap potensi β-siklodekstrin untuk membentuk kompleks rangkuman dengan kurkumin atau THC. Matlamat kajian ini adalah untuk menilai potensi kompleks rangkuman kurkumin dan THC untuk menghalang laluan isyarat faktor pertumbuhan

endoteliaum vaskular (VEGF), unsur utama dalam karsinogenesis. Analisis *in silico* melibatkan pelbagai peringkat seperti penyediaan sebatian bioaktif, ramalan aktiviti biologi, struktur 3D temuan semula VEGF dan VEGFR, dok protein-ligan dan visualisasi. Hasil kajian menunjukkan bahawa kedua-dua kompleks rangkuman kurkumin dan THC menunjukkan keperluan yang lebih rendah untuk mengikat tenaga bebas untuk berinteraksi dengan VEGFR berbanding dengan molekul kurkumin atau THC sahaja. Apabila VEGFR diikat dengan kurkumin sebelum kompleks rangkuman kurkumin atau kompleks rangkuman THC berinteraksi dengan VEGF, terdapat peningkatan ketara dalam tenaga bebas pengikat untuk interaksi VEGF-VEGFR. Khususnya, kehadiran kompleks rangkuman THC menunjukkan tenaga bebas pengikat tertinggi untuk interaksi VEGF-VEGFR. Kajian simulasi dinamik molekul menunjukkan bahawa apabila VEGFR mengikat dengan kurkumin, kompleks rangkuman kurkumin atau kompleks rangkuman THC, turun naik sisa asid amino dalam VEGFR berkurangan berbanding dengan struktur protein VEGFR sebelum diikat dengan molekul ini. Kesimpulannya, kajian ini mencadangkan bahawa pembentukan kompleks rangkuman berpotensi untuk meningkatkan potensi antikanser kurkumin dan THC dengan menambah aktiviti anti-angiogenik mereka.

Kata kunci: Angiogenesis; kanser; kurkumin; kompleks rangkuman; tetrahidrokurkumin

Introduction

Cancer is a non-communicable disease characterized by continuous and uncontrolled cell growth. This unrestrained cell growth can damage surrounding tissues and may spread from one organ or tissue to another. Globally, cancer stands as a leading cause of death, responsible for nearly 10 million deaths in 2020. The most prevalent types that year, in terms of new cases, were breast cancer (2.26 million cases), lung cancer (2.21 million cases), and colon and rectum cancer (1.93 million cases). The primary causes of cancer-related deaths in 2020 were lung cancer (1.80 million deaths), colon and rectum cancer (916,000 deaths), and liver cancer (830,000 deaths) (WHO 2020).

The Hallmarks of Cancer encompass distinctive features such as maintaining proliferative signals, avoiding cell death, enabling lasting replication, activating invasions and metastases, reprogramming energy metabolism, inducing genetic instability, triggering tumor inflammation, evading immune response, and promoting angiogenesis (Hanahan & Weinberg 2011). Notably, angiogenesis plays a crucial role in tumor growth and metastases, as the expansion of a tumor mass necessitates the development of new blood vessels to supply nutrients and oxygen (Lugano, Ramachandran & Dimberg 2020).

Currently, cancer treatment methods worldwide encompass a range of approaches, including surgery and chemotherapy. Despite their widespread use, established treatments like chemotherapy are associated with adverse effects such as hair loss, reduced appetite, allergies, diarrhea, and tingling sensations (Chan & Ismail 2014). Consequently, a multitude of ongoing research endeavors is dedicated to exploring the efficacy of natural compounds, such as curcumin and its derivative tetrahydrocurcumin (THC), as potential alternatives to conventional anticancer drugs. Researchers anticipate that these natural ingredients may offer heightened effectiveness while minimizing the occurrence of side effects (Hashem et al. 2022; Kooti et al. 2017; Shanmugam et al. 2016). This emerging field of study reflects a growing interest in harnessing the therapeutic potential of naturally occurring substances for cancer treatment, paving the way for more targeted and tolerable interventions.

Curcumin is the main bioactive compound of turmeric (*Curcuma longa*) (Tomeh, Hadianamrei & Zhao 2019). Curcumin has a rich history in Ayurvedic medicine spanning centuries. It garnered attention for its non-toxic nature and diverse therapeutic properties, encompassing antioxidant, analgesic, anti-inflammatory, and antiseptic activities. Curcumin has emerged as a promising candidate for its anti-cancer properties, influencing various biological pathways implicated in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis, and metastasis (Muninggar et al. 2019; Wilken et al. 2011). Several studies have highlighted the ability of curcumin to inhibit VEGF-mediated angiogenesis in various cancer types (Binion, Otterson & Rafiee 2008; Fu et al. 2015). THC demonstrates greater antioxidant activity than curcumin (Han et al. 2016; Liu et al. 2017). Despite THC's superiority over curcumin, its aqueous solubility remains an ongoing concern before clinical use, which has yet to be addressed. The poor solubility of compounds with limited absorption and low dissolution rates often leads to insufficient bioavailability and poor distribution in drug delivery (Kamalakkannan et al. 2010).

An inclusion complex occurs when one chemical compound, referred to as a 'host', binds to other compounds in its vicinity that have cavities. The use of β-cyclodextrin (β-CD) to form inclusion complexes has been explored to enhance the water solubility of bioactive compounds (Leclerq 2016). β-Cyclodextrin is preferred over other materials due to its hydrophobic cavity, accommodating various outer lipophilic molecules (Cid-samamed et al. 2022; Paramera, Konteles & Karathanos 2011). In general, the formation of inclusion complexes offers both dimensional and geometric advantages, thereby improving the solubility and stability of curcumin and THC in liquids (Chaudhary & Patel 2013). The addition of CDs can enhance the solubility and stability of curcumin and THC, consequently reducing the required dose and directly improving bioavailability and pharmacological effects (Martin-Del Valle 2004).

In this study, our objective was to investigate the potential of CDs to form inclusion complexes with curcumin or THC and to examine their effects on VEGF activity using *in silico* analysis. The advantages of *in silico* analysis include the ability to obtain optimal physical and chemical properties of ligands or drug compounds and the time efficiency derived from the use of various software simultaneously (Dewi et al. 2021; Wadood et al. 2013; Wahyuningsih et al. 2022). The primary aim of this study was to assess the potential of curcumin and THC inclusion complexes in inhibiting VEGF activity.

Materials and Methods

BIOACTIVE COMPOUNDS PREPARATION

Three bioactive compounds analyzed in this study, namely curcumin/CUR (CID 969516), tetrahydrocurcumin/THC (CID 124072), and β-cyclodextrin (CID 273016192), were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/).

BIOLOGICAL ACTIVITY PREDICTION

Human Intestinal Absorption (HIA) prediction and LD_{50} were determined using the Laboratory of Molecular Modeling and Design (http://lmmd.ecust.edu.cn). The web server employs cutting-edge machine learning techniques to construct predictive models encompassing key absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties crucial for drug discovery. Utilizing open-source cheminformatics and machine learning libraries, the algorithms and descriptors employed in model construction ensure transparency and reproducibility, which is an essential factor in evaluating the quality of the models (Patel et al. 2018; Yang et al. 2019). The assessment using this web server evaluated the bioactive compounds' ability to be absorbed by the gastrointestinal system into the bloodstream of the human body and the lethal dose when studying animal models. The assessment of bioactive compounds' absorption and toxicity profiles is indispensable in drug discovery as it contributes to the development of safe and effective therapeutic agents, streamlines the drug development process, and ensures regulatory compliance.

ANALYSIS MOLECULAR PATHWAY PREDICTION

The molecular pathway prediction of CUR and THC was conducted using the STITCH webserver (http://stitch.embl.de/). The STITCH webserver serves as both a search tool and a comprehensive resource for examining the interactions between chemicals and proteins. It enables users to investigate the network of chemical relations, particularly in relation to associated binding proteins (Kuhn et al. 2008). The analysis using this webserver is important to predict the signaling pathway affected by curcumin and THC. The expression profile and survival curve analysis of VEGF and VEGFR were performed using the GEPIA 2 webserver (http://gepia2.cancer-pku.cn/#index).

OBTAINING THE PROTEIN SEQUENCES OF VEGF AND VEGFR

The 3D protein structures of *Homo sapiens* vascular endothelial growth factor (VEGF) (ID: 1vpf) and vascular endothelial growth factor receptor (VEGFR) (ID: 3v2a) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). The complex structure of VEGFR and VEGF (ID: 3v2a) were separated by using UCSF Chimera software (https://www.cgl.ucsf.edu/chimera). Only chain R was used as VEFGR protein.

DOCKING

Docking analysis of CUR, THC, and inclusion complexes with VEGF and VEGFR was performed using Hex software (http://hex.loria.fr/dist800/index.php) (Macindoe et al. 2010).

VISUALIZATION AND ANALYSIS OF THE INTERACTIONS

The docking results were visualized using UCSF Chimera software and the ligand bond interactions

between bioactive compounds and proteins were analyzed using LigPlot+ software (LigPlot + v.2.2 - ligand-protein interaction diagrams) (Laskowski & Swindells 2011).

MOLECULAR DYNAMICS SIMULATION

Molecular Dynamics Simulation (MDS) using the CABS-flex 2.0 web server (http://biocomp.chem.uw.edu.pl/CABSflex2) was employed to assess the stability of VEGFR before and after binding with bioactive compounds. The protein structure input utilized default settings (mode: SS2, Gap: 3, Minimum: 3.8, Maximum: 8.0), with Global C-alpha restraints weight and global side-chain restraints weight both set to 1.0. For more advanced simulation options, the number of cycles was designated as 50, with cycles between trajectories also set to 50.

RESULTS

BIOLOGICAL ACTIVITY OF CURCUMIN (CUR) AND TETRAHYDROCURCUMIN (THC)

To evaluate the bioactive compounds' absorption potential in the gastrointestinal system, we conducted Human Intestinal Absorption (HIA) analysis. The HIA+ score for CUR was 0.9539, indicating a high absorption likelihood, while THC scored 0.8880. A HIA+ score above 0.9 suggests easy absorption of bioactive compounds (Cheng et al. 2012; Poerwosusanta et al. 2019), and in this context, CUR's higher score than THC aligns with its superior absorbability. The absorption rate of anticancer compounds significantly influences their bioavailability, therapeutic efficacy, onset of action, formulation, and the potential for minimizing resistance. A thorough understanding of absorption kinetics is essential for designing and optimizing anticancer therapies to maximize their therapeutic benefits. Lethal dose-50 (LD_{50}) represents the quantity of a compound administered at once, causing the death of 50% of test animals. CUR exhibited an LD_{50} of 2.5468 mg/mol, whereas THC's LD_{50} was 2.4132 mol/kg. The lower LD_{50} of THC compared to CUR suggests a higher potency for THC, emphasizing its potentially greater efficacy.

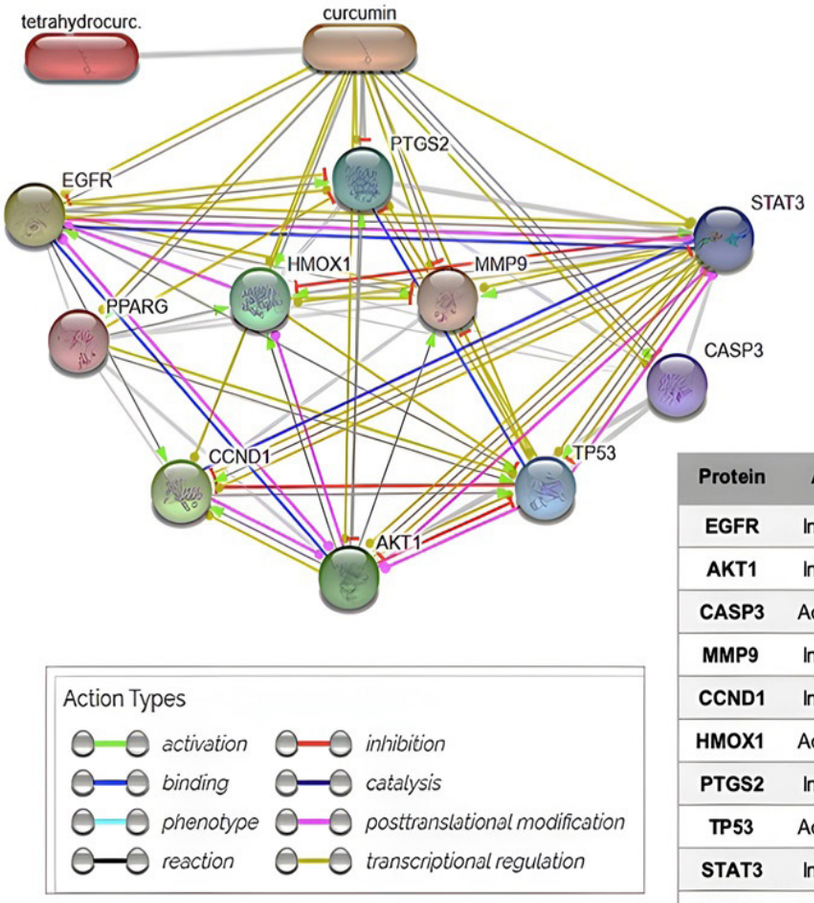
CURCUMIN AND TETRAHYDROCURCUMIN AS ANTI-ANGIOGENIC FACTORS VIA THE PI3K/AKT SIGNALING PATHWAY

In a previous study by Wang and Chen (2019), the antiangiogenic properties of curcumin derived from *Curcuma longa* were demonstrated, with a regulatory impact on various factors including VEGF, MMPs, and FGF. In the present study, we conducted a molecular pathway prediction analysis. The findings showed that both CUR and THC could influence multiple proteins, including epidermal growth factor receptor (EGFR), matrix metallopeptidase 9 (MMP9), serine/threonine-protein kinase (AKT1) and others (Figure 1). The tyrosine kinase activity of the EGFR induces pathways that govern various malignant characteristics of cancer cells. This receptor plays a key role in regulating the development and microarchitecture of intratumoral angiogenic vasculature, crucial for sustaining cancer cell intravasation (Minder et al. 2015). MMPs are instrumental in orchestrating angiogenesis and the complex interplay within cancer-related processes, encompassing angiogenesis, vasculogenesis, and lymphangiogenesis. Specifically, MMP-2 and MMP-9 contribute significantly to the dynamic remodeling of the ECM, exerting influence through proteolytic cleavages that release biologically active substances capable of modulating cellular regulation (Quintero-Fabian et al. 2019). Furthermore, AKT-1 enhances angiogenesis by phosphorylating and regulating downstream effectors that promote key aspects of angiogenic signaling. Expanding on the previously reported role of CUR in regulating VEGF activity, our results indicate that both CUR and THC possess the capability to inhibit AKT1, a downstream molecule in the VEGF/VEGFR signaling pathway.

HIGH VEGF EXPRESSION IN CANCER PATIENTS CORRELATES WITH POOR SURVIVAL

Vascular endothelial growth factor is an important factor in angiogenesis. The activity of VEGF mediated by the interaction with its receptor, VEGFR. Notably, our findings showed elevated VEGF expression in several cancers, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), rectum adenocarcinoma (READ), sarcoma (SARC), stomach adenocarcinoma (STAD), testicular germ cell tumor (TGCT), and thymoma (THYM).

Furthermore, we explored the correlation between VEGF expression and overall survival. Our analysis showed that high VEGF expression significantly correlated with a reduction in the percentage of survival in COAD



Protein	Action	Score
EGFR	Inhibition	0.987
AKT1	Inhibition	0.967
CASP3	Activation	0.959
MMP9	Inhibition	0.956
CCND1	Inhibition	0.967
HMOX1	Activation	0.966
PTGS2	Inhibition	0.964
TP53	Activation	0.962
STAT3	Inhibition	0.959
PPARG	Activation	0.957

FIGURE 1. The potential signaling pathways affected by curcumin and tetrahydrocurcumin

patients (Figure 2(A)-2(B)). In contrast to VEGF, VEGFR expression was only heightened in GBM, KIRC, PAAD, PCPG, READ, STAD, TGCT, and THYM patients compared to normal levels. Notably, high VEGFR expression did not show a significant correlation with the overall survival of COAD patients (Figure 2(C)-2(D)).

FORMATION OF INCLUSION COMPLEX AND ITS EFFECT ON VEGF-VEGFR INTERACTION

In this study, we fused THC and curcumin with β-cyclodextrin (β-CD) to form an inclusion complex which will serve as a basis to overcome the curcumin and THC insolubility issue. β-cyclodextrin itself is a cyclic oligosaccharide consisting of seven glucose units joined together. It possesses isomeric properties, is not hygroscopic, and maintains stability in its chemical structure. β-cyclodextrin was chosen to form the inclusion complex because it has a hollow structure inside. When this cavity is bound with other inclusion complexes, it enhances the stability of the bond. To form the inclusion complex, we initially conducted a docking analysis of β-CD with curcumin and β-CD with THC.

To assess the effectiveness of the inclusion complex in inhibiting Vascular Endothelial Growth Factor (VEGF) activity, we conducted docking analyses involving VEGF and VEGF Receptor (VEGFR). We explored the interactions between VEGF and VEGFR in the presence of curcumin, THC, or their respective inclusion complexes (curcumin-inclusion complex; THC-inclusion complex). The initial step involved docking curcumin, THC, or the inclusion complex with VEGFR. Utilizing molecular dynamics simulations, we assess the stability

of VEGFR both before and after interacting with bioactive molecules. The Root Mean Square Fluctuation (RMSF) value of VEGFR serves as a metric to characterize the protein's dynamic behavior, representing differences in flexibility among residues. A positive correlation exists between RMSF values and movement flexibility, with higher values indicating increased flexibility and lower values suggesting limited movement during simulations. Prior to binding with bioactive compounds, the RMSF range of VEGFR spans from 0.204 to 4.871 Å, showcasing notable fluctuations at residue positions 142, 152, 174, 283, 298, and 329. Following the binding of curcumin to VEGFR, the RMSF range expands to 0.173 - 5.643 Å, while the binding of curcumin-inclusion complex contracts it to 0.199 - 4.290 Å. Notably, the binding of THC to VEGFR significantly reduces the RMSF range to 0.125-3.542 Å. In the presence of the THC inclusion complex, the RMSF range of VEGFR is 0.122 - 4.252 Å, similar to the RMSF range observed with the VEGFR-curcumin inclusion complex (Table 1).

From the presented figure, it can be clearly observed that the fluctuation of amino acid residues in the VEGFR protein, when it binds to the curcumin-inclusion complex, THC, or THC-inclusion complex, has decreased compared to the structure of the VEGFR protein before binding with these molecules. Based on the RMSF data, the structure of VEGFR binding to the aforementioned molecules is more stable than the VEGFR protein before interacting with bioactive molecules (Figure 3).

Following docking analysis, THC exhibited a binding free energy of -189.63 kcal/mol with four hydrophobic bonds, while curcumin demonstrated a lower binding free energy of -192.62 kcal/mol and five hydrophobic bonds. This suggests that curcumin binds easily to VEGFR compared to THC. Further, the binding free energy of the THC-inclusion complex with VEGFR was -345.42 kcal/mol and characterized by three hydrophobic bonds, while the curcumin-inclusion complex exhibited a binding free energy of -368.10 kcal/mol, involving four hydrogen bonds. These findings indicate that the curcumin-inclusion complex and THC-inclusion complex binds more effectively to VEGFR than the curcumin or THC (Table 2), suggesting that the curcumin-inclusion complex and THC-inclusion complex exhibits enhanced potency in inhibiting the VEGF-VEGFR interaction by effectively blocking their binding through association with VEGFR.

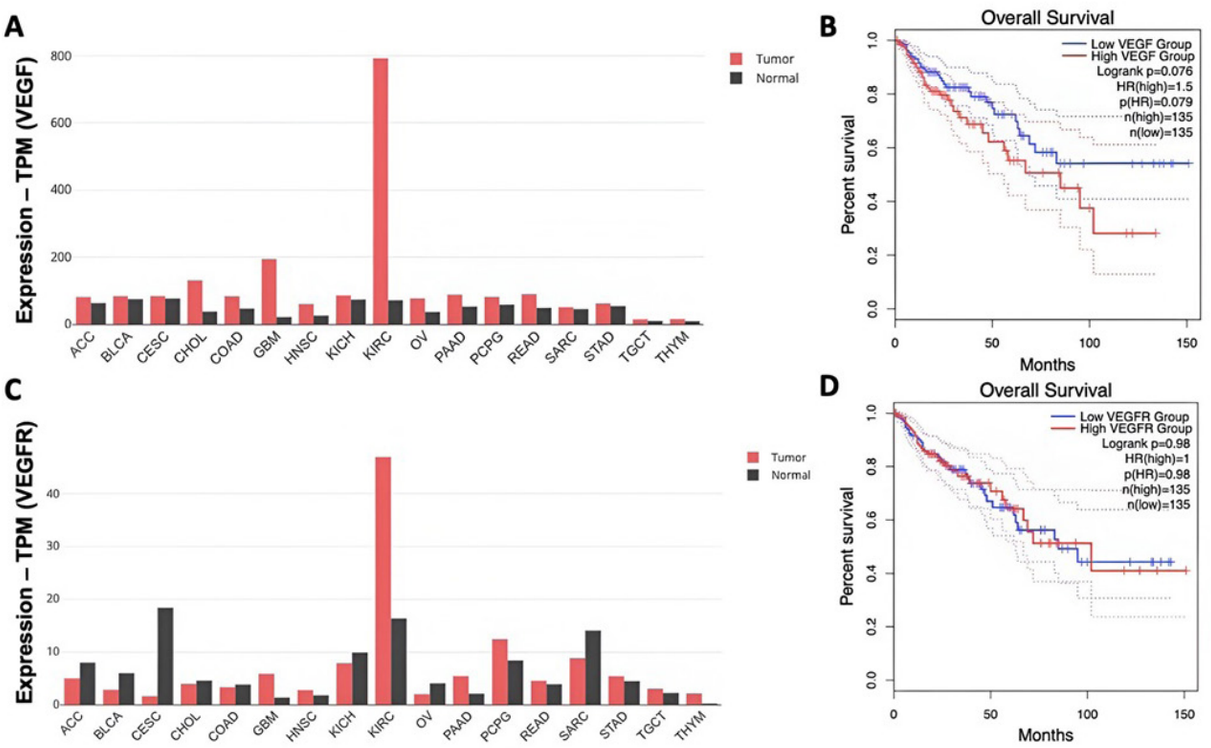


FIGURE 2. The expression of VEGF and VEGFR in several cancer types and its correlation with overall survival

For the next step, we conducted docking simulations involving VEGF and its receptor (VEGFR). Additionally, we explored the VEGF-VEGFR interaction in the presence of curcumin, THC, or their corresponding inclusion complexes (Figure 4(A)). The VEGF-VEGFR interaction exhibited a binding free energy of -530.62 kcal/mol, characterized by the formation of four hydrogen bonds and 39 residues involved in hydrophobic bonds interaction. Introducing curcumin to VEGFR increased the binding free energy to -484.71 kcal/mol, while the inclusion complex of curcumin further increased it to -468.19 kcal/mol. Conversely, the binding of THC to VEGFR resulted in a reduction of binding free energy for the VEGF-VEGFR interaction to -571.15 kcal/mol, but the THC inclusion complex significantly increased the binding free energy to -427.27 kcal/mol (Table 2; Figure 4). These results highlight the modulatory impact of the inclusion complex of curcumin and THC on the VEGF-VEGFR interaction, with potential implications for VEGF activity.

The interaction between Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGFR) was characterized by the formation of 5 hydrogen bonds and 39 residues involved in hydrophobic bonds interaction. The introduction of curcumin, curcumin inclusion complex, THC, and THC inclusion complex altered the number of both hydrogen and hydrophobic bonds. Furthermore, the molecules participating in the binding process were changed accordingly (Table 2). Moreover, when THC or the THC-inclusion complex is present, it induces a conformational change in the VEGF-VEGFR protein interaction (Figure 5).

TABLE 1. RMSF values and high fluctuated residues of VEGFR before and after binding with bioactive compounds

Ligand-Receptor	Binding free energy (kcal/mol)	Interaction (bond)	Residues involved in the interaction
THC-VEGFR	-189,63	Hydrophobic	His133; Met197; Met213; Phe199.
Curcumin-VEGFR	-192,62	Hydrophobic	Gln210; Gln132; His133; Met213; Tyr165
THC inclusion complex - VEGFR	-345,42	Hydrophobic	Val 135; Val136; Tyr137
Curcumin inclusion complex - VEGFR	-368,10	Hydrophobic	Val135; Tyr137; Val136, Leu313
VEGF - VEGFR	-530,62	Hydrogen	Glu42; Thr77; Gln89; Tyr25
		Hydrophobic	Ser310; Ser311; Asp257; Ile256; Gly255; Val218; Val219(2); Asn253; Val254; Lys286; Phe288; Asp276; Ala1195; Tyr194(3); Ser193; Tyr165; Ile215; Tyr137; Val217; Val216; Tyr190; Met191(2); Pro188; Glu140; Leu252; Glu284; Tyr224; Asn175; Asp173; Ile177; Tyr190; Val171; Arg169; Phe170; Lys168
VEGF - VEGFR, curcumin	-484,71	Hydrogen	Gln22; Thr77
		Hydrophobic	Phe288; Val218; Val219(2); Asn253; Val254; Lys286; Gly255; Ile256; Asp257; Ser311; Ser310; Asp276; Tyr137; Val217; Tyr194(3); Pro166; Tyr224; Glu140; Met191(2); Ser283; Glu284; Leu252; Ser193; Pro188, Tyr190; Arg176; Asn175; Lys168; Arg169; Glu167; Phe170; Asp173
VEGF - VEGFR, curcumin inclusion complex	-468,19	Hydrogen	Glu42; Asp41; Glu44; Tyr39; Gln89; His86; Pro85
		Hydrophobic	Asp276; Gly255; Ile256; Phe288; Lys286; Asn253; Val218; Ile215; Met197; Val216; Tyr165; Pro166; Ala195; Tyr194(2); Val217; Val219; Met191(2); Ser193; Leu252; Arg169; Phe170; Asn175; Tyr190
VEGF - VEGFR, THC	-571,15	Hydrogen	Glu44; Asp41; Glu42; Thr77
		Hydrophobic	Ile256; Gly255; Val218; Val219(2); Asn253; Val256; Ile215; Val216; Val217; Tyr137; Pro166(2); Tyr165; Ser193; Gly196; Ala195; Tyr194(2); Leu252; Glu284; Tyr224; Ser283; Ser193; Met191; Tyr190(2); Asp173; Asn175; Ile177; Met191; Agr169; Phe170; Lys168; Tyr194; Val171
VEGF - VEGFR, THC inclusion complex	-437,27	Hydrogen	Glu44; Asp41; Glu42; Tyr39; His86; Gln89
		Hydrophobic	Phe288; Ile256; Gly255; Asp276; Val218; Asn253; Lys286; Val219; Val217; Ala195; Tyr194(2); Val216; Ile215; Met197; Tyr165; Pro166; Leu252; Glu284; Ser193; Met191(2); Asn175; Phe170; Arg169; Tyr190

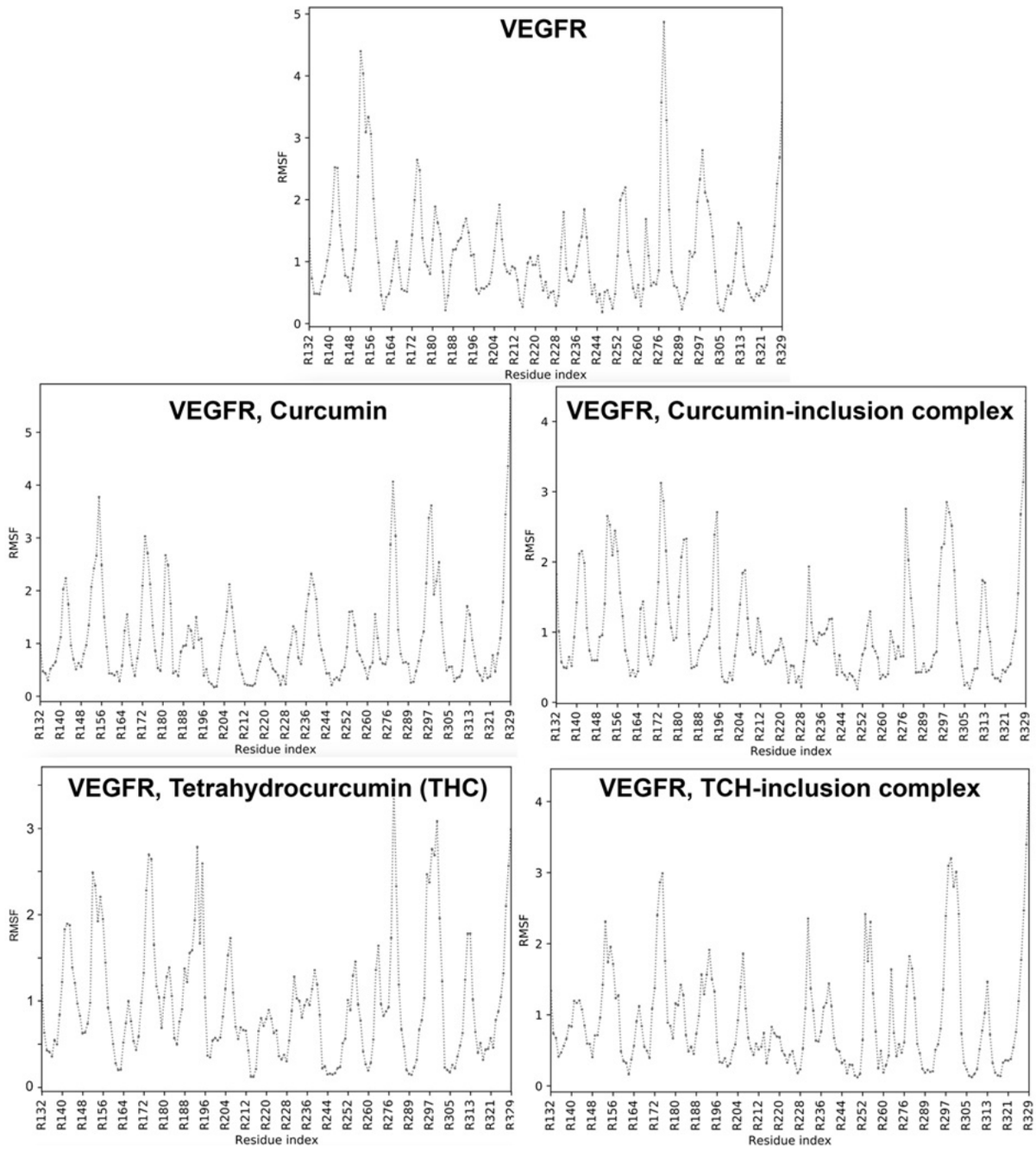


FIGURE 3. The fluctuation plot of VEGFR residues before and after binding with curcumin, THC, and its respective inclusion complex

TABLE 2. The docking involved VEGFR with bioactive compounds, VEGF-VEGFR, and VEGF with VEGFR that was already bound by curcumin, THC, or the inclusion complex

Molecules	RMSF range	High fluctuated residues position
VEGFR	0.204 - 4.871 Å	142, 152, 174, 283, 298, 329
VEGFR, curcumin	0.173 - 5.643 Å	155, 173, 181, 283, 298, 329
VEGFR, curcumin-inclusion complex	0.199 - 4.290 Å	142, 152, 173, 195, 277, 298, 329
VEGFR, THC	0.125 - 3.542 Å	142, 152, 174, 193, 283, 300, 329
VEGFR, THC-inclusion complex	0.122 - 4.252 Å	153, 175, 231, 253, 299, 329

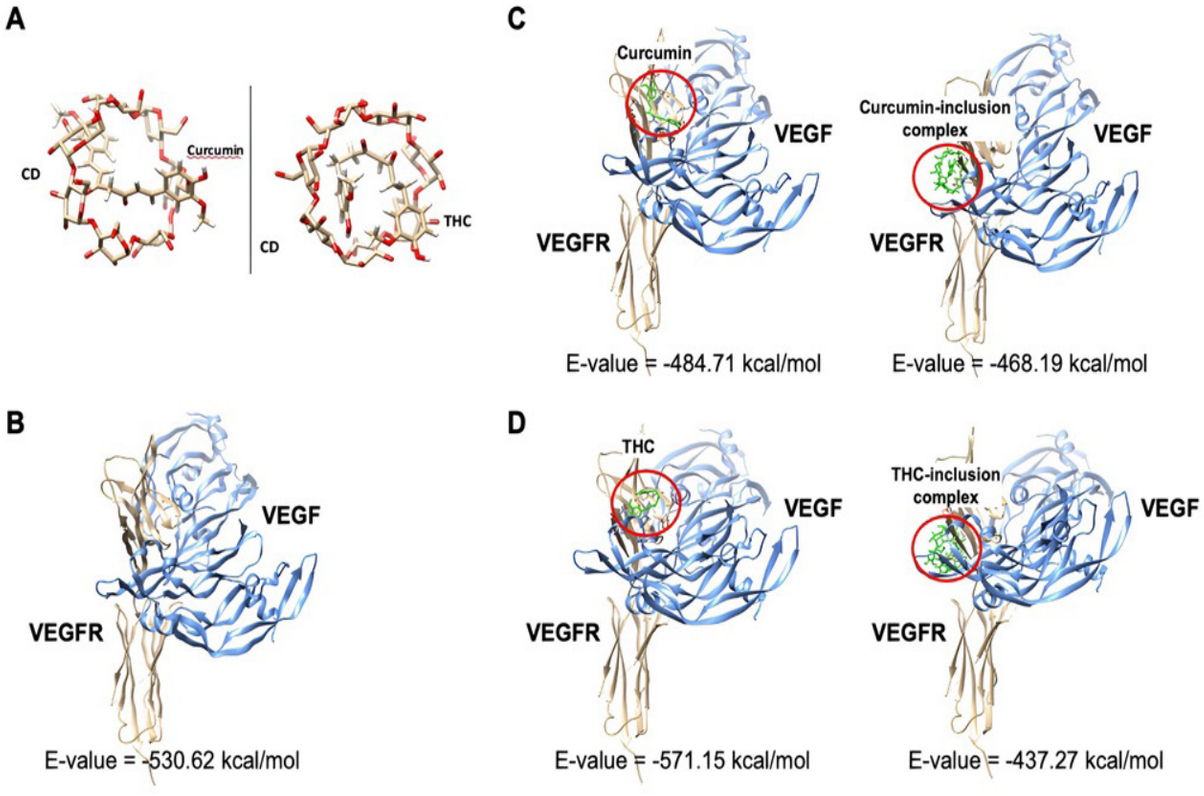


FIGURE 4. Curcumin inclusion complex and THC inclusion complex (A); Interaction between VEGF and VEFGR in the absence and presence of curcumin, THC, and inclusion complexes (B-D)

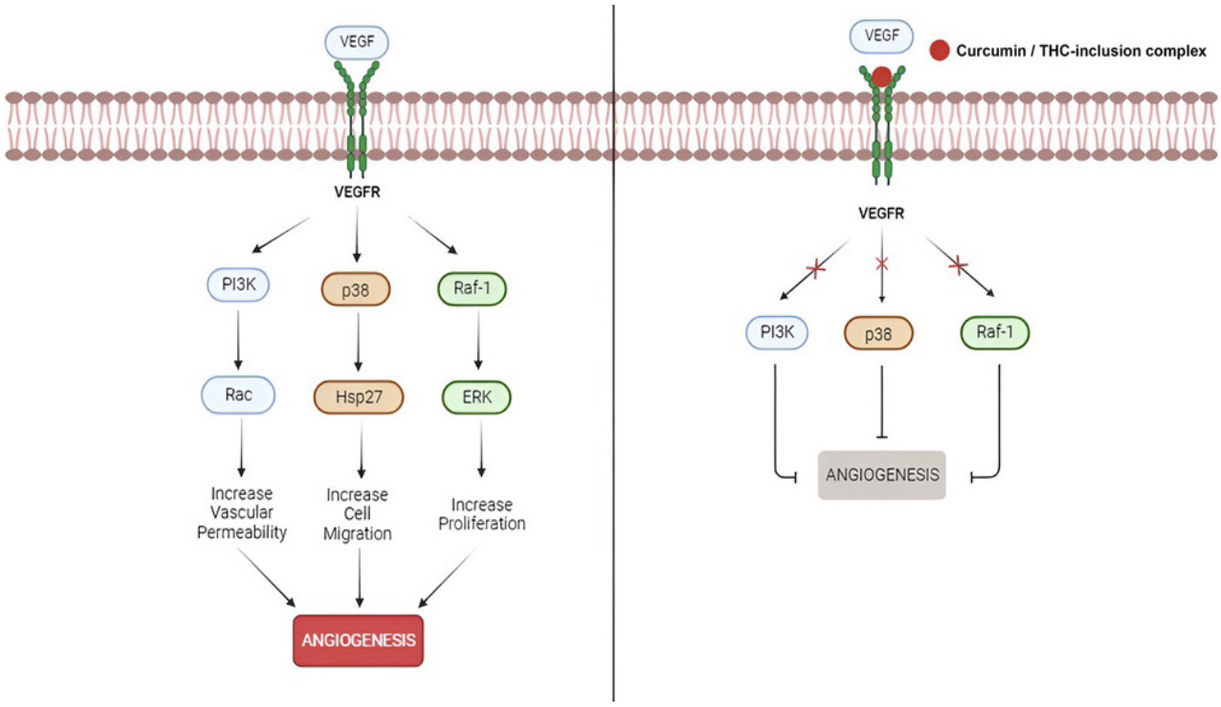


FIGURE 5. Schematic diagram illustrating the VEGF-VEGFR signaling pathway in the absence or presence of curcumin-inclusion complex

DISCUSSION

In cancer progression, angiogenesis assumes a pivotal role in tumor growth and metastases, as new blood vessels are essential for providing nutrients and oxygen to support tumor development. This study centers on Vascular Endothelial Growth Factor (VEGF) activity, a critical factor in angiogenesis through its interaction with VEGF Receptor (VEGFR). Targeting the inhibition of VEGF interaction with VEGFR represents a potential strategy for cancer therapy (Ferrara 2005). Our findings indicate elevated VEGF expression in many cancer patients compared to normal individuals (Figure 2), underscoring its significant role in carcinogenesis. VEGF is a key tumor-derived angiogenic factor that exerts multiple functions including stimulation of angiogenesis, vasculogenesis, inflammation and vascular permeability. VEGF as a therapeutic target has been validated in various types of human cancers. The VEGF family of growth factors and its receptors are pivotal components of the most crucial signaling pathways in tumor angiogenesis (Shibuya 2011). Elevated angiogenesis and VEGF expression have been observed across various human cancers, including colorectal cancer, breast cancer, non-small cell lung cancer, renal cell cancer, glioblastoma multiforme, and other tumors, compared to corresponding nonmalignant normal tissue. In patients with the highest levels of VEGF expression, survival outcomes were significantly poorer compared to those with negative or lower levels of VEGF expression (Niu & Chen 2010). Importantly, VEGF levels were found to be predictive of future metastases (Sopo et al. 2019), exhibiting independent prognostic value beyond nodal status and adjuvant chemotherapy (Niu & Chen 2010).

Building on a prior study by Binion, Otterson and Rafiee (2008) demonstrating curcumin's inhibition of VEGF-mediated angiogenesis through COX-2 and MAPK inhibition in human endothelial cells, supporting our study results that curcumin can also inhibit VEGF-VEGFR interaction by its binding to VEGFR. This suggests a potential hindrance to the activation of signaling pathways involved in angiogenesis. Intriguingly, THC exhibits a higher potential to inhibit VEGF-VEGFR interaction compared to curcumin, as evidenced by the increased binding free energy when VEGFR is bound to THC (Table 2; Figure 4). THC surpasses curcumin in terms of chemical stability, bioavailability, antioxidant, and

anticancer activity. Unlike curcumin, which undergoes autoxidation and exhibits pro-oxidant effects (Rege et al. 2012), tetrahydrocurcumin lacks pro-oxidant potential (Aggarwal, Deb & Prasad 2015). This notable distinction is primarily attributed to variations in their structures and the influence of the medium. Curcumin features double bonds in the heptane chain, while THC lacks such double bonds in its heptane chain (Rege, Varshneya & Momin 2021). The structural disparity between curcumin and THC may contribute to their respective abilities to inhibit VEGF binding to VEGFR.

Despite the anticancer potential of curcumin and THC, their solubility poses a major challenge in their application for cancer therapy. A study by Low et al. (2022) demonstrated that encapsulating curcumin in β-CD significantly improved its solubility, leading to enhanced effects in reducing colon cancer cell viability and migration while increasing apoptotic rates. These findings align with the notion that inclusion complexes may indeed manifest superior anticancer properties. In this study, we addressed the solubility issue by fusing curcumin or THC with β-CD to form inclusion complexes. Inclusion complexes can effectively address solubility issues by encapsulating hydrophobic compounds, such as curcumin and THC, within a carrier molecule. This process often involves the use of substances like β-CD, which have a hydrophilic exterior and a hydrophobic interior. β-CD composed of 7 glucopyranosyl units. This compound is cyclic oligoskarides that have a torus shape, formed from glucopyranosyl units connected through α-(1,4) bonds (Cheirsilp & Rakmai 2016). The hydrophobic substance, which may otherwise have poor solubility in water, is encapsulated within the hydrophobic cavity of the β-CD. This encapsulation enhances the solubility of the hydrophobic substance, making it more readily dispersible in aqueous environments (Chaudhary & Patel 2013). For cancer therapy, the solubility of drugs is a critical factor that directly impacts their bioavailability and therapeutic efficacy. Many anticancer drugs, including those derived from natural compounds like curcumin, often suffer from poor solubility, which can limit their effectiveness *in vivo* (Lee et al. 2013). By forming inclusion complexes, the solubility of these drugs can be significantly improved, leading to enhanced bioavailability and better distribution throughout the body.

Visualization of the docking results for curcumin inclusion complex and THC inclusion complex indicated successful binding to the cavity of β-cyclodextrin, confirming the formation of inclusion complexes (Figure 4(A)). Comparing the binding free energy and bonds formed during the interaction, THC inclusion complex exhibited a greater potential to inhibit VEGF-VEGFR interaction compared to curcumin inclusion complex. The VEGF signaling pathway initiates the activation of PI3K, p38, and Raf1, consequently enhancing vascular permeability, cell migration, proliferation, and ultimately driving angiogenesis (Simons 2012). However, in the presence of the curcumin- or THC-inclusion complex, the interaction between VEGF and VEGFR is disrupted, effectively inhibiting angiogenesis (Figure 5).

Conclusion

Curcumin- or THC-inclusion complex displayed superior efficacy in inhibiting the binding of VEGF to its receptor, VEGFR, compared to curcumin or THC molecules. This inhibitory effect was substantiated by a significant increase in binding free energy when VEGFR was pre-bound by curcumin- or THC-inclusion complex before VEGF interaction. This study suggests that the formation of inclusion complexes, particularly with THC, holds promise for enhancing the anticancer effectiveness of these compounds by enhancing their anti-angiogenic properties. However, additional *in vitro* and *in vivo* investigations are essential to validate the findings before considering their application in clinical settings.

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